Characterization of microbotryum lychnidis-dioicae secreted effector proteins that manipulate its host plant, Silene latifolia.

Ming-Chang Tsai

University of Louisville

Follow this and additional works at: https://ir.library.louisville.edu/etd

Part of the Biology Commons

Recommended Citation

This Doctoral Dissertation is brought to you for free and open access by ThinkIR: The University of Louisville's Institutional Repository. It has been accepted for inclusion in Electronic Theses and Dissertations by an authorized administrator of ThinkIR: The University of Louisville's Institutional Repository. This title appears here courtesy of the author, who has retained all other copyrights. For more information, please contact thinkir@louisville.edu.
CHARACTERIZATION OF MICROBOTRYUM LYCHNIDIS-DIOICAE
SECRETED EFFECTOR PROTEINS THAT MANIPULATE ITS HOST PLANT,
SILENE LATIFOLIA

By

Ming-Chang Tsai
B.S., TungHai University, Taiwan, 2000
M.S., San Jose State University, USA, 2011

A Dissertation
Submitted to the Faculty of the
College of Arts and Sciences of the University of Louisville
In Partial Fulfillment of the Degree of

Doctor of Philosophy in Biology

Department of Biology,
Division of Molecular, Cellular and Developmental Biology,
College of Arts and Sciences,
University of Louisville

August 2022
CHARACTERIZATION OF *MICROBOTRYUM LYCNOIDIS-DIOICAE*
SECRETED EFFECTOR PROTEINS THAT MANIPULATE ITS HOST PLANT,
*SILENE LATIFOLIA*

By

Ming-Chang Tsai
B.S., TungHai University, Taiwan, 2000
M.S., San Jose State University, USA, 2011

A Dissertation Approved on

July 25, 2022

By the following Dissertation Committee

_______________________________
Dr. Michael H. Perlin, Principal Advisor

_______________________________
Dr. David J. Schultz

_______________________________
Dr. James E. Graham

_______________________________
Dr. Michelle T. Barati

_______________________________
Dr. Mark Running
DEDICATION

This dissertation is dedicated to my beloved family
my parents and young brother
who support my research career without hesitation.
ACKNOWLEDGEMENTS

Back to early 2013 I was looking for a microbiology-related graduate program to apply to. Although I earned a MS degree in Nutrition, Food Science and Packaging program at San Jose State University in California at that time, I decided not to go to a career of dietitian, and I wanted to do biological research works. It was Dr. Michael Perlin who responded my e-mail after I contacted around 20 researchers in different universities. He kindly provided me information about his research works and his lab, and accepted my application in fall 2013. I am highly indebted to Dr. Michael Perlin for his guidance in the following years.

I would like to thank to my committee members Dr. David Schultz, Dr. James Graham, Dr. Michelle Barati, and Dr. Mark Running for their invaluable comments. Additionally, Dr. Schultz helped the maintenance of the chambers for cultivation of Arabidopsis thaliana, and Dr. Barati assisted me to take fluorescence images from plant tissues by a confocal microscope.

During my first three years in the program two former lab members Dr. Su San Toh (who sadly passed away due to cancer) and Dr. Margaret Wallen taught me laboratory techniques and skills. I appreciate their training very much. Then there was my long-time laboratory partner, Dr. Swathi Kuppireddy. We had worked on the same project for 5 years until she graduated in November, 2018. I continued the same framework of experiment design she established after she
left the program.

I also have to thank to people, whether our lab members or not, who provided special assistance to my project. They are Dr. Liang Bao who gave me suggestions on the *Agrobacterium* floral dipping method and *A. thaliana* growth, Dr. Susanna Martinez who provided the protocol of plant DNA extraction, Emma Lamb and Stevana Schauer who helped me conduct qRT-PCR, Rebecca Turney and Melissa Martinez who helped me growing *Arabidopsis*, and Otniel Alejandro Nava-Mercado (Alex) who provided the *Botrytis cinerea* fungal strain.

Finally, I would like to appreciate and thank to the cool former and current members of the Goat lab Dr. Lalu Vijaya Krishna Pillai, Dr. Sunitha Khanal, Dr. William Beckerson, Dr. Hector Mendoza, Joseph Paul Ham, Roxanne Leiter, Shikhi Baruri, and Rebecca Dangol, who together have created a cheerful environment to do research.

I have wanted to become a scientist ever since I read a brief biography of Albert Einstein when I was 10 years old. By the time I realized there was a job title called a scientist who could uncover the mysteries in nature. Today, I can tell the 10-year-old myself who was not afraid of holding a big land worm or catching a 2-inch-long green caterpillar “Hey young man, I made it! I am a scientist now!”
ABSTRACT

CHARACTERIZATION OF *MICROBOTRYUM LYCHNIDIS-DIOICAE*
SECRETED EFFECTOR PROTEINS THAT MANIPULATE ITS HOST PLANT, *SILENE LATIFOLIA*

Ming-Chang Tsai

July 25, 2022

The smut fungal species *Microbotryum lychnidis-dioicae* is an obligate phytopathogen colonizing the plant host, *Silene latifolia*. A significant feature of *M. lychnidis-dioicae* infection is that the fungus can replace pollen on the anthers of susceptible host plants with fungal teliospores, thus earning the fungus the name: anther smut disease of flowers. The fungus synthesizes and secretes effector proteins into the cells of the plant host during infection, and the protein-protein interactions may interfere with and modify metabolism, plant development, and gene expression of the host to allow fungal colonization. Two potential fungal effector proteins, MVLG_06175 and MVLG_05122, were identified by genome sequence analysis and prior expression studies. The yeast-two hybrid screening was used to identify their potential plant protein interactors. A potential plant protein interacting with MVLG_06175 was identified as CASP-
like protein 2C1 (CASPL2C1), while those interacting with MVLG_05122 were identified as COP9 signalosome subunit 5a and 5b (CSN5a/5b). CASPL2C1 might facilitate the polymerization of the Casparian strip by forming a protein scaffold at the endodermal cells where lignin deposits. CSN5a/5b could adjust the rate of protein ubiquitination and degradation by interacting with Cullin-RING E3 ubiquitin ligases (CRLs). CRLs is a large enzyme family labelling proteins with ubiquitin, and these proteins are subsequently recognized and degraded by the 26S proteasome.

*MVLG_06175* and *MVLG_05122* were tagged by generating fusion proteins with mCherry or cyan fluorescent protein (CFP), respectively, and then transformed into the model host plant *Arabidopsis thaliana*. Images taken from a confocal microscopy showed that fluorescence signals of MVLG_06175 form clustered granules or punctate regions at the tips of trichomes on leaves and in root caps, while those of MVLG_05122 formed a clear band structure at the base of leaf trichomes. These results indicate that the fungal MVLG_06175 might affect the formation of the Casparian strip in the roots and the synthesis of phytochemicals in the trichomes of host plants, and MVLG_05122 might modify the development of trichomes. There were no significant phenotype changes in *A. thaliana* transformed with MVLG_05122, while *A. thaliana* transformed with MVLG_06175 showed statistically smaller rosette diameter and leaf quantity. It is significant that both effector proteins located to the trichomes on leaves of model transgenic plants. Trichomes are defensive structures of plants protecting against both biotic and abiotic stresses. These findings suggested that *M. lychnidis-dioicae* might apply the two effector proteins to alter host metabolism related to
immune responses including the structure of trichomes in host plants.
# TABLE OF CONTENTS

ACKNOWLEDGEMENTS........................................................................................................ iv  
ABSTRACT........................................................................................................................... vi  
LIST OF TABLES.................................................................................................................. xiii  
LIST OF FIGURES................................................................................................................ xiv  

CHAPTER I: OVERVIEW OF THE BIOTROPHIC FUNGUS  
*MICROBOTRYUM LYCNI DIS-DIOICA E*............................................................... 1  
  Infection Cycle of *M. lychnis-dioicae*................................................................. 1  
  Dimorphism of *M. lychnis-dioicae*...................................................................... 4  
  Effector Proteins........................................................................................................ 5  
  The Purpose of the Study....................................................................................... 10  

CHAPTER II: IDENTIFICATION OF THE PLANT HOST PROTEINS POTENTIALLY INTERACTING WITH THE FUNGAL PROTEIN EFFECTORS DURING INFECTION......................................................................................... 12  
  Overview...................................................................................................................... 12  
  Introduction.................................................................................................................. 13  
  Results........................................................................................................................ 17  
    *In Silico* Identification of Effector Proteins...................................................... 17  
    Verification of Secretion by Yeast Secretion Trap Test................................. 18  
    MVLG_06175 Interacts with Two Fungal Proteins and One Plant Host Protein................................................................................................................................. 20  
    MVLG_05122 Interacts with One Plant Host Protein........................................ 22  
    Further Confirmation of Y2H Interactions: Swap of Fungal Effector
<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Genes. into the Opposite Vector</td>
<td>23</td>
</tr>
<tr>
<td>Discussion</td>
<td>26</td>
</tr>
<tr>
<td>Fungal Protein MVLG_06175 and Plant Protein CASPL2C1</td>
<td>26</td>
</tr>
<tr>
<td>Fungal Protein MVLG_05122 and Plant Protein CSN5a/5b</td>
<td>29</td>
</tr>
<tr>
<td>Materials and Methods</td>
<td>36</td>
</tr>
<tr>
<td>cDNA Library Construction</td>
<td>36</td>
</tr>
<tr>
<td>Ligation and Cloning of the Gene MVLG_06175 and MVLG_05122</td>
<td>37</td>
</tr>
<tr>
<td>Yeast Transformation</td>
<td>40</td>
</tr>
<tr>
<td>Yeast Secretion Trap Assay</td>
<td>41</td>
</tr>
<tr>
<td>Yeast Two-Hybrid Screening</td>
<td>43</td>
</tr>
<tr>
<td>CHAPTER III: CHARACTERIZATION OF THE FUNGAL EFFECTOR PROTEINS IN ARABIDOPSIS THALIANA, A HETEROLOGOUS PLANT HOST MODEL</td>
<td>47</td>
</tr>
<tr>
<td>Overview</td>
<td>47</td>
</tr>
<tr>
<td>Introduction</td>
<td>48</td>
</tr>
<tr>
<td>Results</td>
<td>51</td>
</tr>
<tr>
<td>Protein-protein Interactions between Fungal Effector Proteins and</td>
<td>51</td>
</tr>
<tr>
<td>Corresponding plant proteins of Arabidopsis thaliana</td>
<td>51</td>
</tr>
<tr>
<td>Determination of Phonotype Changes in Transgenic A. thaliana</td>
<td>54</td>
</tr>
<tr>
<td>Evaluation of Expression of MVLG_06175-mCherry, MVLG_06175 △ SP-mCherry, and MVLG_05122 △ SP-CFP in Transgenic Arabidopsis thaliana</td>
<td>57</td>
</tr>
<tr>
<td>Localization of M. lycnidis-dioicae Effectors in the Transgenic Plant</td>
<td>57</td>
</tr>
<tr>
<td>Tissues by Fluorescent Tags: MVLG_06175</td>
<td>59</td>
</tr>
<tr>
<td>Localization of M. lycnidis-dioicae Effectors in the Transgenic Plant</td>
<td>64</td>
</tr>
<tr>
<td>Tissues by Fluorescent Tags: MVLG_05122</td>
<td>64</td>
</tr>
<tr>
<td>Susceptibility Assay of Transgenic Plant toward Botrytis cinerea.</td>
<td>67</td>
</tr>
<tr>
<td>Infection</td>
<td>67</td>
</tr>
<tr>
<td>Discussion</td>
<td>70</td>
</tr>
</tbody>
</table>
Gibson Assembly to Construct MVLG_05122 Tagged with Cyan Fluorescent Protein Gene and MVLG_06175 Tagged with mCherry Protein Gene, as Well as CASPL2C1 and CSN5a/5b of Arabidopsis thaliana in the Prey Vector pGADT7

Agrobacterium Transformation through Electroporation

Arabidopsis and Growth Conditions

Floral Dipping Transformation of Arabidopsis thaliana Mediated by Agrobacterium

Test of Mendel’s Law of Segregation

Plant DNA Extraction

Plant mRNA Extraction

qRT-PCR

Fluorescence Images Taken by a Confocal Microscopy

Botrytis cinerea Infection Assay

CHAPTER IV: DISCUSSION AND FUTURE STUDY DIRECTIONS

Other Structures and Mechanisms Regulated by Plant Protein CASPL2C1

Other Structures and Mechanisms Regulated by Plant Protein CSN5a/5b

REFERENCES

APPENDIX

Yeast-Two Hybrid Sequencing and BlastX Results Showing MVLG_07305

Yeast-Two Hybrid Sequencing and BlastX Results Showing MVLG_06379

Yeast-Two Hybrid Sequencing and BlastX Results Showing CASP-like protein 2C1 (CASPL2C1)

Yeast-Two Hybrid Sequencing Results Showing Constitutive photomorphogenesis 9 (COP9) signalosome complex subunit 5a
and/or 5b (CSN5a/5b) ................................................................. 145
Sequencing Results of cDNA from A. thaliana Transformed with
MVLG_05122 Δ SP-CFP ................................................................. 176
Sequencing Result of cDNA from A. thaliana Transformed with
MVLG_06175 Δ SP-mCherry and MVLG_06175-mCherry .............. 177
CURRICULUM VITAE ................................................................. 181
LIST OF TABLES

Table 2.1: Determination of MVLG_06175 and MVLG_05122 as protein effectors.................................................................................................................. 18
Table 2.2: BLASTX outcome of yeast colony J7-2 on NCBI.................. 21
Table 2.3: BLASTX outcome of yeast colony J117 on NCBI............... 21
Table 2.4: BLASTX outcome of yeast colony A1 on NCBI................. 22
Table 2.5: BLASTX outcome of yeast colony 30-2 on NCBI.............. 23
Table 2.6: Total RNAs extracted from different developmental stages of the M. lychnidis-dioicae................................................................. 37
Table 2.7: Primers to acquire signal sequences of MVLG_06175 and MVLG_05122......................................................................................... 42
Table 3.1: Primers used to construct MVLG_06175-mCherry, MVLG_06175 ΔSP -mCherry, and MVLG_05122 ΔSP -CFP in pRI-101AN vector.................................................................................................................. 75
Table 3.2: Primers to acquire CSN5a/5b and CASPL2C1 from A. thaliana genome.................................................................................................................. 76
Table 3.3: Primers used for qRT-PCR to determine the expression of fungal effectors and fluorescent tags.......................................................... 86
Table 3.4: Primers to amplify cutinase A and α-shaggy kinase in qRT-PCR 88
Table S3.1: qRT-PCR result of MVLG_05122 in the transgenic A. thaliana.................................................................................................................. 175
Table S3.2: qRT-PCR result of CFP in the transgenic A. thaliana.......... 175
Table S3.3: qRT-PCR result of MVLG_06175 and mCherry in the transgenic A. thaliana................................................................. 176
LIST OF FIGURES

Figure 1: Life cycle of *Microbotryum lychnidis-dioicae* .................................................. 3
Figure 2.1: Yeast secretion trap assay .................................................................................. 19
Figure 2.2: Y2H spot test on *MVLG_06175* ................................................................. 24
Figure 2.3: Panels A and B, Y2H spot test ........................................................................ 25
Figure 2.4: Ubiquitination and degradation of the R receptor protein and NPR1 transcriptional coactivator. ................................................................. 33
Figure 2.5: A hypothetical model of the inhibition of CSN complex resulting from the interactions between *MVLG_05122* and CSN5a/5b. ................................ 35
Figure 3.1: Y2H spot test on protein-protein interactions between plant proteins of *A. thaliana* and fungal protein *MVLG_06175* and *MVLG_05122* .................................................................................. 53
Figure 3.2: Box-and-Whisker plot showing rosette diameter among transgenic *A. thaliana* ........................................................................................................ 55
Figure 3.3: Box-and-Whisker plot showing leaf quantity among transgenic *A. thaliana* ........................................................................................................ 56
Figure 3.4: PCR results of genomic DNA extracted from transgenic *A. thaliana* and wild type *Col-0* (WT). ................................................................. 58
Figure 3.5: Fold changes of gene construct expression compared with wild type *Col-0* (WT) in qRT-PCR ................................................................. 59
Figure 3.6: Localization studies of *MVLG_06175* in trichomes in 3-week-old *A. thaliana* ........................................................................................................ 62
Figure 3.7: Localization studies of *MVLG_06175* in roots in 2-week-old *A. thaliana* ........................................................................................................ 64
Figure 3.8: Localization studies of *MVLG_05122* in trichomes in 6 and 7-week-old transgenic *A. thaliana* ........................................................................................................ 65
Figure 3.9: Fluorescence images of trichomes in WT and transgenic *A. thaliana* expressing *MVLG_05122ΔSP-CFP* and CFP alone ............................................. 67
Figure 3.10: Ratios of relative expression values of CutA over those of SKII among four study groups................................................................. 69
CHAPTER I: OVERVIEW OF THE BIOTROPHIC FUNGUS MICROBOTRYUM LYCHNIDIS-DIOICAEX

Infection Cycle of *M. lychnidis-dioicae*

The smut fungus *Microbotryum lychnidis-dioicae* is an obligate plant parasite primarily infecting Caryophyllaceae species and resulting in smutted anthers (Kemler, Goker, Oberwinkler, & Begerow, 2006). In the current study the plant host is *Silene latifolia*. Similar to other smut fungal species, *M. lychnidis-dioicae* has a diphasic life cycle—the haploid and dikaryotic stages. When the fungus infects the *S. latifolia*, fungal proteins modify the metabolism and structure of the plant host to benefit the fungal parasitism and proliferation.

The infection starts as the diploid teliospore germinates and undergoes meiosis to yield haploid cells after landing on the host flower. The fimbriae of a haploid cell extend to search for a mate. When two haploid cells of opposite mating-types make contact with each other, they fuse to become dikaryotic hyphae via conjugation and start penetrating the host tissues. The diploid hyphae may reach to the roots, stay dormant during the winter, and rise to the flower next spring. When the hyphae reach the stamen, karyogamy happens and the dikaryotic hyphae become diploid fungal teliospores. The hyphae also utilize the plant protein machinery to turn the anther into a structure filled with fungal
teliospores, and the infection cycle restarts (Figure 1). Although female flowers do not have anthers, the fungus can inhibit the development of gynoecium while enhancing that of anther filament from a rudimentary stamen so that the teliospore sac still replaces the structures of anthers on female flowers (Uchida, Matsunaga, & Kawano, 2005). The SISUP gene of S. latifolia, a homolog of the floral development gene SUPERMAN (SUP), may be associated with the inhibition of the stamen. Although both male and female S. latifolia have the SISUP gene, this gene is expressed only in female flowers. It is significant that the expression of SISUP in female flowers is suppressed when the flowers are infected by M. lychnidis-dioicae (Kazama et al., 2009). Since the protein products of SISUP gene might inhibit the development of stamen in female flowers, one possibility is that M. lychnidis-dioicae impedes the transcription of the gene to enhance the growth of anther in female flowers.
Figure 1: Life cycle of Microbotryum lycnidis-dioicae (adapted from Perlin et al. (2015) with permission). The infection starts as the diploid teliospores land on the host plant. The spores germinate and undergo meiosis to become haploid cells. When two haploid cells of opposite mating type meet with each other, they fuse to turn into dikaryotic hyphae and penetrate into the tissue of the plant host. The hyphae may stay in the root stock during the winter, and return to the buds in the next spring. As the hyphae occupy the anther, the two nuclei inside hyphae fuse, and the hyphae develop into teliospores. These teliospores replace the pollen, ready to be picked up by pollinator species and transferred to a naïve flower; thus, the infection cycle starts again.
Dimorphism of *M. lychnidis-dioicae*

Although genetic recombination is considered an important mechanism to prevent the accumulation of deleterious mutations, the dimorphic structure of sex chromosomes was evolved from the accumulation of mutations resulting from the inhibition of genetic recombination around the sequences that determine compatibility (Bergero & Charlesworth, 2009). Fungal species do not develop into two different sexes, but rather, into two opposite mating type haploids to determine mating compatibility. The major difference between the gamete and the haploid is that the two mating types of haploids have equal influences on the future offspring; they experience identical life stages and invest equal nutrient supplement for the growth of hyphae (Abbate & Hood, 2010; Billiard, Lopez-Villavicencio, Hood, & Giraud, 2012). *M. lychnidis-dioicae* was identified as the first fungal species with heteromorphic chromosomes controlling mating types. The chromosomes are differentiated into a1 and a2 mating types and vary in size: the a1 mating type is encoded on a mating chromosome whose size ranges between 2.8 and 3.1 Mb, while the a2 mating chromosome is between 3.4 and 4.2 Mb (Hood, 2002). It is significant that the region restricted from recombination for the a1 and a2 mating type chromosomes could be around 1 Mb (Votintseva & Filatov, 2009). The large non-recombination area, as well as the dimorphic size of mating type chromosomes, is in agreement with the features of a sexual chromosome or allosome (Hood, Petit, & Giraud, 2013).
Effector Proteins

Phytopathogens infecting and colonizing plants include prokaryotic bacteria, eukaryotic fungi, oomycetes, and nematodes. They are classified into the necrotrophs which degrade living tissues of the plant hosts and absorb the nutrients, and biotrophs which colonize living tissues to grow and reproduce. The biotrophic pathogens can be further subdivided into the obligate biotrophic and hemibiotrophic pathogens (Selin et al., 2016). The obligate biotrophic pathogens are completely dependent on living plant hosts, while the hemibiotrophic pathogens initiate their life cycles as the biotrophic stage but end as the necrotrrophic stage. Phytopathogens have evolved dynamic genomes encoding protein products transported or extruded out of cells to facilitate the degradation of plant cell wall, absorption of nutrients, and/or modulation of the host. The corresponding genes are associated with synthesis of secondary metabolites such as mycotoxins and the effector proteins. Effector proteins, also referred to as small-secreted proteins (SSPs), have appeared as the primary molecular interactors with plant hosts to modify plant structure, metabolism, and defensive responses in order to benefit the lifecycle of phytopathogens in plant-microbe interactions. (Win et al., 2012). In some basidiomycete fungal species colonizing on maize, such as Ustilago maydis and Sporisorium reilianum, the genes encoding effector proteins are usually located on distinctive genomic regions and form dispersed small gene clusters (Schmidt & Panstruga, 2011).
The effector proteins often evolve at rapid rates, leading to high specificity for their plant hosts. There are also low degrees of conserved amino acid sequences among fungal effectors (Rouxel et al., 2011). Comparing effector proteins with non-effector proteins, the effector proteins carry higher percentages of cysteine residues but lower percentages of serine and tryptophan residues in the amino acid sequence based on the predicted models of EffectorP. Furthermore, a broader distribution of net protein charges is also a distinct feature of the fungal effector proteins (Sperschneider et al., 2016). Among the fungal effector proteins, they can be approximately separated into the apoplastic (extracellular) and cytoplasmic effector proteins based on their destinations in the plant host. The apoplastic effector proteins are secreted into the apoplast or xylem, while the cytoplasmic effector proteins enter the cytoplasm of the cells. The apoplastic effector proteins tend to contain multiple cysteine residues. However, this feature is not universal. The disulfide bridges between cysteine residues could reinforce the stability of effector proteins in the apoplast, an environment full of host proteases (Stergiopoulos & de Wit, 2009).

Bacterial phytopathogens evolved the type III secretion system to directly deliver effector proteins into the tissues of plant hosts. It is intriguing that certain Rhizobium species apply effector proteins to establish the symbiosis relationship with the legume plants. Rhizobial bacteria synthesize and release the Nod factors, mainly lipochitooligosaccharide molecules, into the soil. These chemical factors are recognized by leguminous receptors to stimulate plant nodule formation. However, some rhizobial species, such as Bradyrhizobium elkanii
USDA61, do not synthesize Nod factors. Rather, they utilize the type III secretion system (T3SS) to transfer specific effector proteins to alter the legume’s signaling transduction associated with nodulation in the plant tissues. This approach is similar to that of pathogenic bacteria to colonize the plant hosts. Since the symbiosis relationship can be considered a type of bacterial infection at the plant nodules, *B. elkanii* USDA61 might also use T3SS to deliver other effector proteins into the plant tissues to constantly suppress the plant immune system (Teulet et al., 2019; Ratu et al., 2021). With respect to eukaryotic phytopathogens, certain fungal and oomycete species have evolved special structures to deliver effector proteins into the hosts. When the hyphae penetrate the plant cell wall, some biotrophic fungal species and oomycetes secrete effector proteins into plant cells through the haustoria which develop at the terminal of hyphae. The haustorium is a specialized feeding structure surrounded by the plasma membrane of the plant cell (Lo Presti et al., 2015; Fawke, Doumane, & Schornack, 2015). Between the plant cell membrane and the pathogen cell wall there is the extra haustoria matrix. Pathogenic effector proteins are released into the matrix, and they may be translocated into the cytoplasm of the host cells by endocytosis while binding to receptors on the plant cell membrane. However, plant cells may also release proteasomes into the matrix to hydrolyze pathogenic effectors (Fawke, Doumane, & Schornack, 2015). Once the pathogenic effector proteins enter the cytoplasm of the host cells, they may start modifications of host metabolism and structures as well as trigger the plant immune responses.
The agricultural industry has been studying the effects of fungal effector proteins on crops. Examples of such effector proteins include the NIP1 protein of *Rhynchosporium secalis* which is secreted into barley (Rohe et al., 1995), specific recombinant fusion proteins of *Cladosporium fulvum* secreted into the leaf of tomato (van Esse, Thomma, van 't Klooster, & de Wit, 2006), and the SIX1 protein of *Fusarium oxysporum* secreted into tomato (van der Does et al., 2008). In smut fungal species, including *M. lychnidis-dioicae* of the current study, effector proteins are also identified to facilitate the infection. For instance, *Ustilago maydis* has 12 gene clusters which express effector proteins involved in virulence. The deletion of the gene clusters showed phenotype alterations in tumor formation, fungal development, and host penetration (Kamper et al., 2006).

Genes encoding effector proteins are host-specific and could vary among closely related fungal species (Schmidt & Panstruga 2011). Modifications in these genes could lead to the differentiation of saprophytic fungal species from multiple related pathogenic subspecies (Rep, 2005). Additionally, the differentiation of pathogenic species might occur as a family of effector proteins diverged to target different host plant proteins in response to the changes of the host defense system. Comparative genome analysis of the two related fungal pathogens infecting maize, *U. maydis* and *S. reilianum*, have revealed conserved genomic regions encoding fungal effector proteins along with some virulence clusters (Schirawski et al., 2010). After deleting these conserved regions individually, the researchers noticed the deletion resulted in different levels of virulence changes on maize by the two fungal species. They also demonstrated
that some of the common regions encoded very divergent fungal effector proteins, indicating the effectors were interacting with different host proteins.

To avoid plant host detection, effector proteins have to neutralize the plant immune system. The plant immune system in general contains two levels of defensive mechanisms. The first level of plant defense is the pathogen-associated molecular pattern (PAMP)-triggered immunity (PTI), resulting from the recognition of PAMPs. It occurs when the pattern recognition receptors (PRRs), located on the plasma membrane of the plant cells, recognize the conserved microbial membrane proteins and extracellular proteins as well as polysaccharide structures such as bacterial flagellin and fungal chitin. Once the presence of PAMPs is confirmed, mitogen activated protein kinase (MAPK) cascades deliver phosphates from PRRs to related transcriptional factors to initiate subsequent immune responses. Successful early colonization of phytopathogens depends upon avoiding or suppressing the plant host PTI and utilizing the plant protein machinery. As a result, some effector proteins of phytopathogens modify PTI-related signaling cascades, leading to effector triggered susceptibility (ETS) of the plant host to benefit the pathogenic infection. In response to the microbial strategy, plants utilize the second level of defense in which plant disease resistance (R) protein receptors, also known as NB-LRR (nucleotide-binding-leucine-rich repeat) receptors, attach to and recognize effector proteins and trigger effector triggered immunity (ETI), including the hypersensitive response (HR). HR is a form of programmed cell death (PCD) to prevent further spreading of phytopathogens within host tissues (Jones & Dangl, 2006; Bigeard,
Colcombet, & Hirt, 2015).

**The Purpose of the Study**

Complete genome sequencing and analysis *in silico* has provided a powerful tool to identify and compare new fungal virulence genes during infection. With respect to the genome of *M. lychnidis-dioicae*, there are 279 proteins that are predicted to be secreted. Of these, 71 proteins are composed of less than 250 amino acids. Additionally, the genes for 48 of the 71 protein effectors show significantly elevated transcription levels while infecting the plant host (Perlin et al., 2015), suggesting that they might play a key role in the invasion of the host plant, and thus, could be considered as protein effectors.

The purpose of the current study was to investigate the interactions between *M. lychnidis-dioicae* fungal effectors and plant host protein targets. When *M. lychnidis-dioicae* infects *S. latifolia*, the fungal effectors might play an important role in modulating the gene expression or protein function of the host flowers to enhance the fungal reproduction, such as altering anther growth to block pollen production and to allow fungal teliospore development there instead. The current study selected the fungal genes *MVLG_06175* and *MVLG_05122* to investigate possible target proteins of *S. latifolia* during infection. The genes were chosen because (1) they are only 357 and 522 base pairs, respectively, encoding very small predicted proteins, (2) the protein products were predicted to be secreted in the plant host, (3) their expression was highly upregulated during
infection, and (4) in comparison with other fungi (and the protein databases), they appeared to be unique to *M. lychnidis-dioicae* or to *Microbotryum* species. As a result, they might have a significant role to promote pathogenicity by regulating *S. latifolia* functions related either to plant defense or development.
CHAPTER II: IDENTIFICATION OF THE PLANT HOST PROTEINS POTENTIALLY INTERACTING WITH THE FUNGAL EFFECTOR PROTEINS DURING INFECTION

Overview

Biotrophic fungal species such as *Microbotryum lychnidis-dioicae* rely on hijacking cellular machinery of living plant hosts to complete the fungal reproduction cycle. They have evolved fungal effector proteins translocating to the plant host cells to attenuate immune responses and to utilize nutrients, structures and metabolism pathways without causing lethal damage to the host. The current study applied yeast two-hybrid screening (Y2H) to identify plant proteins of *Silene latifolia* potentially modified by fungal effector proteins MVLG_06175 and MVLG_05122. Potential host plant interactor proteins were identified for each: the Casparian strip membrane domain-like protein 2C1 (CASPL2C1) and COP9 signalosome subunit 5a/5b (CSN5a/5b) of the plant host were found to interact with MVLG_06175 and MVLG_05122, respectively. CASPL2C1 could be involved in the formation of the Casparian strip and transport across cell wall barriers in the plant, while CSN5a/5b is associated with ubiquitination of plant proteins, including those involved in the host defense response.
Introduction

The relationship between microbial parasitism and plant innate defense is a co-evolved arms race. Obligate biotrophic fungi including rust fungi and powdery mildew fungi have to depend on living host plants to complete the fungal life cycle. This is also true of the smut fungus *Microbotryum lychnidis-dioicae* in the current study. Different phytopathogenic fungal species develop different approaches to enter the host plant tissues. In the case of *M. lychnidis-dioicae*, it translocates its effectors via specialized hyphal structures. After two opposite mating-type haploid cells of *M. lychnidis-dioicae* conjugate with each other on the plant, they develop into dikaryotic fungal hyphae and form dome-shaped structures called appressoria on the plant surface. Most necrotrophic fungal species synthesize and secrete plant cell wall-degrading enzymes from appressoria to digest the cellulose-based plant cell wall. Some hemibiotrophic fungal species such as *Magnaporthe oryzae* and *Colletotrichum* spp. increase turgor pressure in appressoria, facilitating mechanical penetration of hyphae into the plant hosts (Lo Presti et al., 2015). Fungal species applying turgor pressure in the appressoria evolve septa to separate the appressoria and hyphae; this structure protects the hyphae from the potential damage caused by elevated turgor pressure. However, there are no septa observed in the appressoria of *M. lychnidis-dioicae*. Accordingly, the fungal appressoria may release lytic enzymes for degradation of cell wall and penetration of hyphae into the plant tissues (Schäfer, Kemler, Bauer, & Begerow, 2010). When the intercellular hyphae of
certain obligate biotrophic fungal pathogens enter adjacent plant cells, they form 
terminally specialized cell structures named haustoria which stay in between the 
cell wall and plasma membrane of plants to absorb nutrients and release protein 
effectors (Lo Presti et al., 2015; Garnica et al., 2014). The haustorium is not a 
universal feature among biotrophic fungi. For instance, the biotroph *Ustilago 
maydis* secretes effector proteins and absorbs nutrients from the invading hyphal 
tip (Bielska et al., 2014)

As previously described, the plant host initiates PAMP-triggered immunity 
(PTI) when the pattern recognition receptors (PRRs) on the cell membrane 
recognize conserved microbial structures of invasive pathogens. Subsequently, 
effector-triggered immunity (ETI) occurs when the plant disease resistance (R) 
receptor proteins recognize pathogenic effector proteins (Jones & Dangl, 2006; 
Bigeard, Colcombet, & Hirt, 2015). The immune responses of ETI are strongly 
associated with the plant defensive hormones. Plants have evolved the salicylic 
acid (SA) and jasmonic acid (JA) hormone signaling pathways to respond to the 
infection of biotrophic and necrotrophic pathogens, respectively (Thomma et al., 
1998). During the infection of biotrophic fungi, the R receptor proteins attach to 
fungal effector proteins, leading to the rise of the SA cellular concentrations at the 
infection sites and the subsequent ETI which triggers programmed cell death 
(PCD) to prevent further microbial colonization. Meanwhile, the elevation of SA 
levels in tissues distant from the infection sites induces the acquired resistance 
(SAR) that is used against many pathogenic species and lasts a longer period of 
time (Furniss & Spoel, 2015). These SA-induced immune responses are
mediated by a transcriptional cofactor NPR1 (Nonexpresser of PR gene 1), and the cellular NPR1 level is regulated by ubiquitination through Cullin-RING E3 ubiquitin ligases (CRLs) and degradation through the 26S-proteasomes (Yan & Dong, 2014).

There are some studies showing that the interactions between SA and JA pathways are antagonistic with each other. In tobacco leaves, the expression of pathogenesis-related genes (PRs) induced by increased SA levels is inhibited by the increased JA levels, and vice versa (Niki et al., 1998). The infection of Pseudomonas syringae pv tomato DC3000 on Arabidopsis thaliana demonstrates that the increase in SA levels results in the accumulation of the transcriptional cofactor NPR1 that suppresses JA levels (Spoel et al., 2003). The antagonism between SA and JA signaling pathways in the plant tissues might facilitate plants to spend resources on synthesis of the defensive hormones more effectively (Kazan & Lyons, 2014). However, the hypothesis of the antagonistic relationship raises a question of whether the plant become more susceptible to infection by necrotrophic pathogens while the JA signaling pathways is suppressed by the elevated SA concentrations during the infection of biotrophic pathogens, and vice versa. In fact, the relation between JA and SA signaling pathway may not always be antagonistic. A relatively recent study demonstrates that the cytoplasmic SA receptor NPR3 and NPR4 may also facilitate the degradation of JA transcriptional suppressor JAZ1s, leading to gene expression of the JA signaling pathway. The JA signaling pathway positively regulates the RPS2-mediated ETI, which is triggered by the binding of bacterial effector
proteins to the RPS2 (*resistance to Pseudomonas syringae* 2) receptor, one of the plant NB-LRR receptor proteins (Liu et al., 2016). Furthermore, both SA and JA signaling pathway could become activated around the microbial infection sites. The *pathogenesis-related gene 1* (PR1) is a gene associated with the SA pathway activity. Its promoter shows higher expression levels in cells surrounding the lesion zone caused by PCD, indicating upregulated gene expression of the pathway in the cells adjacent to the infection sites. Similarly, the *vegetative storage protein 1* (VSP1) is a gene associated with the JA pathway activity, but its promoter shows higher activities in cells slightly away from the lesion zone; there is a narrow spatial gap between the cells with higher activities of VSP1 and the lesion zone (Betsuyaku et al., 2017). These signaling pathways regulating plant immune responses are constantly interacting with microbial effector proteins, thus the cell around the lesion zone is the frontline of co-evolution between phytopathogens and plant hosts.

The current study sought to identify possible plant targets for putative effectors of *M. lychnidis-dioicae*. To do so, yeast two-hybrid analysis was carried out. The fungal genes *MVLG_06175* and *MVLG_05122* were cloned into the bait vector, and the prey vector encoded a cDNA library acquired from flower tissues of *S. latifolia* infected by *M. lychnidis-dioicae*. Both types of vectors were transformed into yeast cells to conduct yeast two-hybrid screenings to identify potential plant protein interactors of fungal protein effectors.
Results

*In Silico* Identification of Effector Proteins

*In silico* protein analysis of *M. lychnidis-dioicae* showed there are 279 fungal proteins containing a secretion signal sequence (Perlin et al., 2015) that did not otherwise appear to be localized to organelles or embedded in membranes. Of this group, 71 proteins we predicted to contain less than 250 amino acids and classified as small secreted proteins (SSPs) or effector proteins, and 46 of the 71 proteins were unique to the *Microbotryum* complex. Furthermore, 19 *Microbotryum*-unique effector proteins were upregulated significantly when the fungus is infecting *S. latifolia*. Enhanced expression of the 19 effector proteins indicates that they could facilitate the fungal colonization during the infection (Kuppireddy et al., 2017). The current study focuses on two predicted effector proteins, MVLG_06175 and MVLG_05122, to elucidate their functions within the host tissues via identification of the host proteins potentially affected or modified by the two fungal proteins. Table 2.1 lists the amino sequences and the values from EffectorP 2.0 of the two fungal effector proteins. Fungal effector proteins with values > 0.5 are considered SSPs or effectors.
Table 2.1: Determination of MVLG_06175 and MVLG_05122 as protein effectors

<table>
<thead>
<tr>
<th>Protein</th>
<th>EffectorP 2.0</th>
<th>Amino Acid Sequence</th>
</tr>
</thead>
<tbody>
<tr>
<td>MVLG_06175</td>
<td>Effector</td>
<td>MWTSSIVQAALLFAIVLYSSSPVVAWAFCPFGKTAEHMAI</td>
</tr>
<tr>
<td></td>
<td>(0.991)</td>
<td>CSSLCRMRCYDPSNGTSTCRNACTGQYHVSRLNADQ</td>
</tr>
<tr>
<td></td>
<td></td>
<td>CMQCDRFTKDKKQGEGKLEHKRCLHKCTDWFPLNL</td>
</tr>
<tr>
<td>MVLG_05122</td>
<td>Effector</td>
<td>MLFKVSAALVLAGLSALPSMSTESRAQPSPSSNKSP</td>
</tr>
<tr>
<td></td>
<td>(0.701)</td>
<td>YGRTGYIDSPADRTYKVGDIHFVYTSAPATYFVDVS</td>
</tr>
<tr>
<td></td>
<td></td>
<td>LMLANGSQSFQLANRTGSMISNDANARAYFRMPENLKT</td>
</tr>
<tr>
<td></td>
<td></td>
<td>IATELLAASQDEHSIAMKNINCILAYLIAKETQNGQYGLV</td>
</tr>
<tr>
<td></td>
<td></td>
<td>GNLETKQAIASM</td>
</tr>
</tbody>
</table>

Verification of Secretion by Yeast Secretion Trap Test

To verify that both fungal protein effectors MVLG_06175 and MVLG_05122 were secreted from fungal cells, yeast secretion trap (YSP) test (Lee et al., 2006) was used. *Saccharomyces cerevisiae* strain SEY 6210 has a deletion of the *SUC2* locus encoding invertase. Invertase catalyzes the hydrolysis of sucrose to fructose and glucose. As a result, SEY 6210 is unable to proliferate in media where sucrose is the sole carbon source. To rescue this defect in sucrose utilization, vector pYSTO-0 was employed as a test of predicted secretion signal peptides in putatively secreted proteins. The vector contains the coding region of *SUC2*, but the gene lacks the start codon and a portion encoding the signal sequence. *SUC2* will not express invertase in the absence of the start codon and, unless a functional secretion signal is provided, invertase will remain in the cytosol and thus, not contribute to ability to utilize sucrose as a sole carbon source. This is due to the inability of *S. cerevisiae* and many fungi, to transport...
sucrose from the media. Additionally, the vector encodes a gene for production of leucine, allowing selection of successfully transformed colonies in medium lacking leucine. The signal sequence of *MVLG_06175* and *MVLG_05122* were amplified by PCR and inserted ahead of *SUC2* in-frame in the vector pYSTO-0, and the newly constructed vectors were transformed into yeast strain SEY 6210. The yeasts were cultured in medium in which leucine was absent and sucrose was the only carbon source. If the examined protein effectors are normally secreted from fungal cells, their signal peptides would lead invertase to be secreted out of the yeast cells as well; the yeast could thereby digest sucrose and absorb glucose to grow. This was indeed observed with the YSP test for both effectors (Figure 2.1). Comparing with the yeast transformed with vector only, those transformed with the fungal signal sequence grew well in the media where sucrose was the only carbon source.

![Figure 2.1: Yeast secretion trap assay. *S. cerevisiae* SEY 6210 cells transformed with pYSTO-0 encoding either the secretion signal sequence of *MVLG_06175* (Left) or *MVLG_05122* (Right) upstream of *SUC2* were able to proliferate on agar](image-url)
where leucine was absent and sucrose was the only carbon source, indicating both MVLG_06175 and MVLG_05122 are normally secreted from fungal cells. Suc0 only, the pYSTO-0 vector without the signal sequence and start codon, as a negative control. 6175SP/5122SP in Suc0, the vector with the signal sequence of MVLG_06175/MVLG_05122. 10X and 1/10, 10-fold dilution. 100X and 1/100, 100-fold dilution.

**MVLG_06175 Interacts with Two Fungal Proteins and One Plant Host Protein**

Yeast two-hybrid screening on *MVLG_06175* yielded around 2500 yeast colonies presenting different degrees of blue color on QDO/X-α-gal + 3AT (5 mM) medium. Around one thousand colonies were transferred to medium with a more stringent concentration of 3-AT (50 mM) to reduce the leaky activity of the *HIS* allele, and 220 yeast colonies with the deepest blue color were selected for further investigation. Among the 60 samples analyzed by sequencing, 39 were characterized bioinformatically (data illustrated in the supplemental material, appendix). *In silico* analysis provided insight into the possible genes encoded by the respective cDNA clones. Comparison of these DNA sequences using blastn or blastx against the database of the Broad Institute and the National Center for Biotechnology Information (NCBI) identified protein products of two *M. lychnidis-dioicae* genes and one plant gene potentially interacting with the fungal protein MVLG_06175. Of the 39 samples, four matched plant gene SOVF_158740 and/or CASP-like proteins (Table 2.2), one matched a *M. lychnidis-dioicae* fungal
gene MVLG_06379 (Table 2.3), and the remaining 34 samples matched M. lycnthis-dioicae fungal gene MVLG_07305 (Table 2.4). For the matches to plant genes, the highest matches were the SOVF_158740 of spinach (Spinacia oleracea), the CASP-like protein 2C1 (CASPL2C1) of beetroot (Beta vulgaris subsp. vulgaris), the CASP-like protein 2C1 of soybean (Glycine max), and CASP-like protein 3 of wild soybean (Glycine soja) (Table 2.2). Moreover, comparison of the cDNA clone sequences that matched plant sequences with transcriptome data from the S. latifolia identified putative S. latifolia transcripts of CASPL2C1 (S. Toh, personal communication).

Table 2.2: BLASTX outcome of yeast colony J7-2 on NCBI

<table>
<thead>
<tr>
<th>Description</th>
<th>Max score</th>
<th>Total score</th>
<th>Query cover</th>
<th>E value</th>
<th>Identi</th>
<th>Accession</th>
</tr>
</thead>
<tbody>
<tr>
<td>hypothetical protein SOVF_158740 [Spinacia oleracea]</td>
<td>204</td>
<td>204</td>
<td>96%</td>
<td>7e-53</td>
<td>59%</td>
<td>KNA06881.1</td>
</tr>
<tr>
<td>PREDICTED: CASP-like protein 2C1 [Beta vulgaris subsp. vulgaris]</td>
<td>173</td>
<td>173</td>
<td>99%</td>
<td>7e-51</td>
<td>50%</td>
<td>XP_010691480.1</td>
</tr>
<tr>
<td>CASP-like protein 2C1 [Glycine max]</td>
<td>127</td>
<td>127</td>
<td>95%</td>
<td>4e-33</td>
<td>41%</td>
<td>NP_001237350.1</td>
</tr>
<tr>
<td>CASP-like protein 3 [Glycine soja]</td>
<td>127</td>
<td>127</td>
<td>95%</td>
<td>4e-33</td>
<td>41%</td>
<td>KH277101.1</td>
</tr>
<tr>
<td>PREDICTED: CASP-like protein 2C1 [Eucalyptus grandis]</td>
<td>126</td>
<td>126</td>
<td>95%</td>
<td>1e-32</td>
<td>38%</td>
<td>XP_010046989.1</td>
</tr>
<tr>
<td>CASP POPTRDRAFT-like protein [Medicago truncatula]</td>
<td>124</td>
<td>124</td>
<td>93%</td>
<td>4e-32</td>
<td>43%</td>
<td>XP_013441625.1</td>
</tr>
<tr>
<td>CASP-like protein XL3 [Gossypium arboreum]</td>
<td>122</td>
<td>122</td>
<td>95%</td>
<td>3e-31</td>
<td>42%</td>
<td>KH981801.0</td>
</tr>
</tbody>
</table>

Table 2.3: BLASTX outcome of yeast colony J117 on NCBI

<table>
<thead>
<tr>
<th>Description</th>
<th>Max score</th>
<th>Total score</th>
<th>Query cover</th>
<th>E value</th>
<th>Identi</th>
<th>Accession</th>
</tr>
</thead>
<tbody>
<tr>
<td>hypothetical protein MVLG_06379 [Microbotryum lycnthis-dioicae 210 Latmeij]</td>
<td>140</td>
<td>265</td>
<td>69%</td>
<td>5e-81</td>
<td>100.00%</td>
<td>KDE03115.1</td>
</tr>
<tr>
<td>SOFM0001_C025o121776 [Microbotryum silene-dioicae]</td>
<td>137</td>
<td>259</td>
<td>49%</td>
<td>2e-59</td>
<td>58.55%</td>
<td>SGO279102.1</td>
</tr>
<tr>
<td>EZ3900_MvG0-128-A1_R1_Ch19-6p01313 [Microbotryum asperagel]</td>
<td>132</td>
<td>255</td>
<td>62%</td>
<td>4e-58</td>
<td>64.20%</td>
<td>SGD011677.1</td>
</tr>
<tr>
<td>EZ3801_MvG0-128-A2_R1_Ch19-3p226A1 [Microbotryum asperagel]</td>
<td>132</td>
<td>254</td>
<td>52%</td>
<td>5e-58</td>
<td>64.20%</td>
<td>SGO279104.1</td>
</tr>
<tr>
<td>Piroxetine/phosphorothionyl sultone/keto acid-containing protein [Cuscuta chinensis]</td>
<td>96.6</td>
<td>146</td>
<td>66%</td>
<td>1e-25</td>
<td>47.73%</td>
<td>DRY03001.1</td>
</tr>
<tr>
<td>SPO4146820_01730 [Rhodotorula asteroides]</td>
<td>54.7</td>
<td>143</td>
<td>62%</td>
<td>7e-19</td>
<td>36.96%</td>
<td>C82460193.1</td>
</tr>
<tr>
<td>Phosphorothionyl sultone/keto acid-containing protein [Acosyusses luteus]</td>
<td>68.8</td>
<td>105</td>
<td>63%</td>
<td>2e-13</td>
<td>38.68%</td>
<td>XP_022301287.1</td>
</tr>
<tr>
<td>citrate lyase subunit beta-like protein [Rhodotorula asteroides NF11]</td>
<td>60.8</td>
<td>90.0</td>
<td>38%</td>
<td>1e-11</td>
<td>50.00%</td>
<td>XP_016279038.1</td>
</tr>
</tbody>
</table>
Table 2.4: BLASTX outcome of yeast colony A1 on NCBI

<table>
<thead>
<tr>
<th>Description</th>
<th>Max Score</th>
<th>Total Score</th>
<th>Query Cover</th>
<th>E value</th>
<th>Per identity</th>
<th>Accession</th>
</tr>
</thead>
<tbody>
<tr>
<td>hypothetical protein MVLG_07305 [Microbotrys lichidae-diosiae A1 Lamiidae]</td>
<td>155</td>
<td>355</td>
<td>78%</td>
<td>1e-81</td>
<td>88.89%</td>
<td>MVLG_07305_1</td>
</tr>
<tr>
<td>BQ6050 C119p12302 [Microbotrys lichidae-diosiae]</td>
<td>164</td>
<td>370</td>
<td>86%</td>
<td>1e-67</td>
<td>88.89%</td>
<td>BQ233292_1</td>
</tr>
<tr>
<td>BQ6050 C001p02003 [Microbotrys lichidae-diosiae]</td>
<td>154</td>
<td>285</td>
<td>66%</td>
<td>5e-67</td>
<td>93.14%</td>
<td>BQ743457_1</td>
</tr>
<tr>
<td>BQ6050 D320p1500 [Microbotrys lichidae-diosiae]</td>
<td>149</td>
<td>280</td>
<td>58%</td>
<td>2e-65</td>
<td>92.41%</td>
<td>BQ235006_1</td>
</tr>
<tr>
<td>B25001_MvGst-1-66-A1-1_1 Chr-1p71762 [Microbotrys lichidae-diosiae]</td>
<td>155</td>
<td>328</td>
<td>86%</td>
<td>6e-56</td>
<td>89.81%</td>
<td>BCC21019_1</td>
</tr>
<tr>
<td>BQ6050 D380p12737 [Microbotrys lichidae-diosiae]</td>
<td>182</td>
<td>244</td>
<td>69%</td>
<td>5e-54</td>
<td>97.97%</td>
<td>BQ254682_1</td>
</tr>
<tr>
<td>BQ6050 D560p12697 [Microbotrys lichidae-diosiae]</td>
<td>153</td>
<td>367</td>
<td>86%</td>
<td>2e-52</td>
<td>93.14%</td>
<td>BQ220074_1</td>
</tr>
<tr>
<td>BQ6050 C001p02009 [Microbotrys lichidae-diosiae]</td>
<td>153</td>
<td>367</td>
<td>86%</td>
<td>2e-52</td>
<td>93.14%</td>
<td>BQ743459_1</td>
</tr>
</tbody>
</table>

**MVLG_05122 Interacts with One Plant Host Protein**

The current study acquired a total of 53 blue yeast colonies on QDO/X-α-gal + 3AT (25 mM) medium from Y2H of MVLG_05122, and 30 of them were analyzed by sequencing (data illustrated in the supplemental material, appendix). After comparing these DNA sequences using blastx against the database of the National Center for Biotechnology Information (NCBI), the result showed all of the identified protein products of the cDNA library matched the constitutive photomorphogenesis 9 (COP9) signalosome complex subunit 5a and/or 5b (CSN5a/5b) from plants (Table 2.5). CSN5a and CSN5b are two homologous proteins although the expression level of CSN5a is higher in plant cells. The CSN protein complex consists of 8 subunits, and CSN5a/5b is the enzymatic center for the isopeptidase activity.
Further Confirmation of Y2H Interactions: Swap of Fungal Effector Genes into the Opposite Vector

Yeast two-hybrid is notorious for generating false positive interaction results. The interactions between proteins expressed from the bait vectors and prey vectors observed in Y2H thus require further confirmation by swapping genes into the opposite vectors and repeating the Y2H. Both MVLG_06175 and MVLG_05122 continued to show interaction with their corresponding plant proteins after exchange of vectors, i.e., after the respective effector genes were cloned into the Prey vector and their respective plant host genes cloned into the Bait vector. These results indicated that the protein-protein interactions between the phytopathogenic fungal and plant host proteins initially observed were likely genuine (Figure 2.2 and 2.3).
Figure 2.2: Y2H spot test on MVLG_06175. In order to reconfirm the protein-protein interactions between the fungal and host plant proteins, Y2H spot tests with and without vector-switch were conducted for comparison. The fungal gene MVLG_06175 was first expressed from the bait vector bearing a binding domain (BD) and plant gene CASPL2C1 was expressed from the prey vector bearing an activating domain (AD) (Left panel). The bait vector and prey vector were transformed into AH109 and Y187 yeast strains, respectively. The two yeast strains were mated and spotted onto agar containing QDO medium/X-α-gal + 3AT (50 mM) (Left panel). To ensure that interactions were not due to artifacts of the particular vectors, both genes were amplified and inserted into the opposite vector, followed by transformation and mating of yeast (Right panel). The result showed MVLG_06175 and CASPL2C1 interacted although they were expressed from the opposite vector, suggesting the protein-protein interactions are genuine. BD-p53+AD-T, the positive control for Y2H interactions. BD+AD, bait and prey vectors with no inserts, as a negative control. Undil, undiluted. 10X and 100x, 10-fold and 100-fold of dilution.
Figure 2.3: Panels A and B, Y2H spot test. Similar to the trial of MVLG_06175, Y2H spot tests with and without vector-switch were conducted to reconfirm the protein-protein interactions. The result also showed MVLG_05122 interacted with
CSN5a/5b (Panel A). The protein-protein interactions remained after I switched the vector containing the fungal and plant genes (Panel B). BD-p53+AD-T, positive control for interaction. AD, AD-T, BD, and BD-p53, the negative controls. BD-5122+AD, BD+AD-CSN5a/5b, BD-CSN5a/5b+AD, and BD+AD-5122, one of the two mating yeast strains carries bait or prey vectors with no insertions, as negative controls. Undil, undiluted. 10X and 100X, 10-fold and 100-fold of dilutions.

Discussion

Fungal Protein MVLG_06175 and Plant Protein CASPL2C1

It is significant that the majority of clones found interacting with the fungal protein MVLG_06175 in Y2H are two other *M. lychnidis-dioicae* fungal proteins, MVLG_07305 and MVLG_06379. Based on the database of the Joint Genome Institute (JGI), MVLG_07305 is a fungal protein predicted to encode a mannose binding domain protein, while MVLG_06379 is predicted to encode a citrate lyase. Although a significant number of blue yeast cells showed fungal protein MVLG_07305 interacted with fungal protein MVLG_06175 in the current study, transcriptome analysis using Next-Generation Sequencing (RNA-Seq) demonstrated that MVLG_07305 was down-regulated late in the infection in *S. latifolia* (Toh et al., 2017). Since MVLG_07305 is down-regulated during infection, perhaps it functions as an inhibitor of fungal protein MVLG_06175 before
MVLG_06175 enters the tissues of the plant host. The same hypothesis could be applied to MVLG_06379 because both MVLG_07305 and MVLG_06379 were predicted via bioinformatic tools not to be secreted. Their interactions with MVLG_06175 are most likely in the fungal cytoplasm.

The comparison of sequencing results on NCBI showed that during the infection of *M. lychnidis-dioicae*, the major plant proteins interacting with the fungal protein MVLG_06175 are the hypothetical protein SOFA_158740 and/or CASP-like proteins (Table 2.2). The function of the protein SOFA_158740 is unknown, but the CASP-like proteins are homologs of the Casparian strip membrane domain proteins (CASPs) that are associated with the formation of the Casparian strip. CASPs are four-span transmembrane proteins with the carboxyl and amino ends in the cytoplasm. These proteins deposit on the surface of the endodermal cell membrane and polymerize to form a protein scaffold surrounding the endodermis as the precursor of the Casparian strip (Roppolo et al., 2011). This process is followed by the attachment of lignin. The synthesis of lignin requires the formation of hydrogen peroxide, which is catalyzed by localized NADPH oxidases, as the electron acceptor. Monolignols are oxidized to lignin when the hydrogen peroxide is reduced to water, and the oxidation-reduction reaction is catalyzed by localized peroxidases. (Lee, Rubio, Alassimone, & Geldner, 2013). Additionally, the deposition of lignin on the protein scaffold might need the guidance of the enhanced suberin 1 (ESB1), a dirigent domain-containing protein. ESB1 binds to the protein scaffold and attracts lignin to deposit at the correct locations (Hosmani et al., 2013).
The Casparian strip is a specialized cell wall structure surrounding the endodermis. It separates the cortex and stele as well as regulates the flow of water and transportation of solutes in the plant. It blocks apoplastic diffusion so that all solutes, salts and water can only enter the xylem and phloem through the cytoplasm of the endodermis. (Naseer et al., 2012; Robbins, Trontin, Duan, & Dinneny, 2014; Roppolo et al., 2011; Steudle, 2000). The Casparian strip mainly forms in the root, but it also is found in specific tissues of certain plant species, such as the stem of pea (Karahara et al., 2011), the needles of pine (Wu et al., 2003), and the leaves of quillworts (Romeo, 2000). The Casparian strip might be directly involved in plant defense against microbial invasion as well. Interestingly, it has been demonstrated that the hyphae of mycorrhizal fungi, forming a mutualistic relationship with plants, are unable to pass the Casparian strip to enter the stele of roots (Machado, Pereira, & Teixeira, 2013). In terms of the functions of Casparian strip membrane domain-like proteins (CASPLs), previous research shows CASPLs could be associated with the membrane protein barriers for the regulation of diffusion and protein scaffolds for the synthesis of the Casparian strip. Some of CASPLs are identified to be expressed at the root endodermis, peripheral root cap, root meristem zone, trichomes, lateral root primordia, young leaves, and floral organ abscission zone in Arabidopsis thaliana (Roppolo et al., 2014). Although studies specifically on CASPL2C1 are currently absent, this protein might be correlated with the formation of the Casparian strip, too.

Both CASPs and CASPLs are integral membrane proteins with four
transmembrane helices. This type of protein structure is a common feature in the myelin and lymphocyte (MAL) and is related to proteins for vesicle trafficking and membrane link (MARVEL) domain protein family in the animal kingdom. MARVEL domain proteins are associated with the functions of epithelial tight junctions (Sánchez-Pulido et al., 2002). Since CASPLs and MARVEL domain proteins are orthologous, CASPLs could as well be involved in tight junction functions in plant cells. Relating this information to the fungal infection, *M. lychnidis-dioicae* might alter the formation or structure of the Casparian strip by the interactions between MVLG_06175 and CASPL2C1; facilitated by the fungal protein effector the invasive hyphae could then be able to penetrate into the xylem and phloem of the plant host. The hyphae of *M. lychnidis-dioicae* are observed in the intercellular space in the rootstocks of *S. latifolia* (Schäfer, Kemler, Bauer, & Begerow, 2010). The researchers unearthed the rootstock of infected *S. latifolia* in early February and cut the rootstocks to thin slices. Under light microscopy they found the cells of *M. lychnidis-dioicae* in the intercellular space. The study outcome showed that the fungal hyphae penetrated into stele of the host plant and extended to the root. Alterations of the Casparian strip resulting from fungal effectors might also occur at the meristem of new tissues such as shoots.

**Fungal Protein MVLG_05122 and Plant Protein CSN5a/5b**

Sequencing results showed that the plant proteins which were identified as potential interactors for MVLG_05122 in 30 blue colonies in Y2H were constitutive photomorphogenesis 9 (COP9) signalosome complex subunit 5a and
The CSN complex is composed of eight subunits, and subunits 5a and 5b are the catalytic center with the isopeptidase activity. The two subunits are homologous proteins in *S. latifolia*, with *CSN5a* being more highly expressed and mutations in that subunit producing more prominent phenotypes (Gusmaroli, Feng, and Deng, 2004). In the model plant, *Arabidopsis thaliana*, the homozygous silencing or knockdown of *CSN5b* did not cause significant changes in the phenotype of the plant. In contrast, homozygous silencing or knockdown of *CSN5a* resulted in significant growth defects such as reduced growth at seedling and adult stages, impaired lateral root formation, impaired root hair formation, loss of apical dominance, depleted trichomes, and smaller flower size. It is noteworthy that silencing of both *CSN5a* and 5b led to lethality at the seedling stage (Dohmann, Kuhnle, & Schwechheimer, 2005; Gusmaroli, Figueroa, Serino, & Deng, 2007).

The CSN protein complex could interfere with the enzymatic activity of Cullin-RING E3 ubiquitin ligases (CRLs), a superfamily of ubiquitin ligases that transfers ubiquitin to proteins which are subjected to degradation by the 26S-proteasome (Choi, Gray, Mooney, & Hellmann, 2014). CSN5a/5b functions as an isopeptidase catalyzing the removal of the Nedd8 protein from CRLs. Without the subunit Nedd8 the CRLs are unable to transfer ubiquitin to the protein destined to be degraded (Wei, Serino, & Deng, 2008). The process of ubiquitination is mediated by the E1 enzyme for activation, E2 enzyme for conjugation, and E3 enzyme for ligation. Ubiquitin attaches to the E1 enzyme, an ATP-dependent reaction, through a thioester bond. The ubiquitin is transferred from the E1
enzyme to the E2 enzyme. The E3 enzyme recruits the E2 enzyme and catalyzes the transfer of the ubiquitin to the target protein (Dikic & Roberson, 2012).

The ubiquitination and degradation of proteins through CRLs and 26S-proteasomes are important in regulating plant development and immune responses such as the induction of genes, oxidative burst, hormone signaling, and programmed cell death (PCD) (Trujillo & Shirasu, 2010; Seo, Song, Chung, & Lee, 2013). Plants have evolved a large quantity of proteins to adjust rates of protein turnover. Taking A. thaliana as an example, up to 6% of the plant proteome is associated with protein removal, and the plant genome encodes more than 1400 different E3 ligase components (Vierstra, 2009). Due to their prominent influences, these plant ligases and proteasomes have become potential targets to be modified by various phytopathogen species. Effector proteins could directly bind to the CRLs to alter the rate of ubiquitination. AVR3a is a protein effector synthesized by Phytophthora infestans, an oomycete species infecting potato, maize, and tobacco. The U-box E3 ligase CMPG1 of the plant host constantly undergoes self-ubiquitination and the subsequent degradation by the 26S-proteasome, and the fast degradation of the CMPG1 is essential to initiate programmed cell death (PCD) of plant tissues. The binding of AVR3a to CMPG1 could stabilize the CMPG1 by preventing further self-ubiquitination. The reduction in degradation rate of the CMPG1 might in turn decrease the occurrence of PCD during the colonization of P. infestans (Bos et al, 2010). Additionally, the effector proteins may function as a ubiquitin ligase rather than bind to host E3 ligases. The type III secretion system effector AvrPtoB of
*Pseudomonas syringae* pv. *tomato*, a bacterial species infecting tomato, carries a domain mimicking the activity of plant E3 ubiquitin ligases. The bacterial effector could ubiquitinate many plant host kinases responsible for the initiation of immune responses. The higher degradation rate of these kinases leads to increased susceptibility of tomatoes to the bacterium (Rosebrock et al., 2007).

Effector proteins could also target the proteasome to alter the protein degradation rate. Syringolin A (SylA) synthesized and secreted by *P. syringae* pv *syringae* is a virulence factor covalently binding to the catalytic subunits of eukaryotic 20S proteasomes, which causes irreversible inhibition of the proteasomes (Groll et al., 2008). The *sylA* mutated bacteria are unable to reduce the accumulation of salicylic acid, leading to the activation of the PCD in host plants (Misas-Villamil et al., 2013).

With respect to MVLG_05122 of *M. lychnidis-dioicae*, perhaps it modifies the ubiquitination and degradation rate of plant proteins, as well. As phytopathogens enter plant host tissues, the plant disease resistance (R) receptor proteins recognize and bind to protein effectors released from pathogens. The perception of protein effectors initiates the effector-triggered immunity (ETI). The immune responses include elevated salicylic acid (SA) concentrations and PCD to limit further colonization by the phytopathogens (Yan & Dong, 2014). Cytoplasmic levels of R receptor proteins and the following signaling pathways are tightly regulated to avoid autoimmune responses in the plant. The Cullin 1-RING E3 ubiquitin ligase (CRL1) consisting of the CUL1 backbone is one of the ubiquitin ligases responsible for ubiquitination of R
receptor proteins for their subsequent degradation by 26S-proteasome (Figure 2.4). CSN5a/5a potentially interacting with protein effector MVLG_05122 catalyzes the cleavage of Nedd8 protein from

Figure 2.4: Ubiquitination and degradation of the R receptor protein and NPR1 transcriptional coactivator. The two proteins are labeled with ubiquitin by the Cullin 1 (CRL1) and Cullin 3-RING E3 ubiquitin ligase (CRL3), respectively. The marked proteins are subsequently degraded by the 26S-proteasome. U, ubiquitin. E2, E2 enzyme conjugating ubiquitin. RBX, RING BOX-1 protein. N, Nedd8 protein. F (F-box protein), SKP1 (S-phase kinase-associated protein 1), and BTB (bric-a-brac, tramtrack, and broad complex), adaptor and receptor proteins recognizing R receptor protein and NPR1 transcriptional coactivator.
CRLs. The removal of Nedd8 inhibits the CRLs enzymatic activity and thus hinders the CRLs-mediated ubiquitination on R receptor proteins (Furniss & Spoel, 2015). As a result, deactivation of CSN would potentially resume R receptor proteins ubiquitination and degradation by the CRLs and proteasome. It is not clear whether the effector protein MVLG_05122 would inhibit or enhance CSN activity. However, if the effector protein inhibits CSN, it would lead to a reduction in intracellular R receptor protein levels which might weaken the recognition of fungal effectors and delay the following effector triggered immunity (ETI); this would benefit the fungal colonization by *M. lycnhdis-dioicae* (Figure 2.5).

In addition, the interactions between MVLG_05122 and CSN might also affect the activity of the Cullin 3-RING E3 ubiquitin ligase (CRL3), another member of the E3 ligase family. CRL3, consisting of the CUL3 backbone, can alter cellular levels of NPR1 by ubiquitination and the following protein degradation (Figure 2.4). NPR1 is a transcriptional coactivator triggering the expression of genes associated with the systematic acquired resistance (SAR) and PCD. SAR grants plants immunity against a broad spectrum of phytopathogens for a longer period of time. NPR1 forms oligomers in the cytoplasm by disulfide bonds between two cysteine residues. As the microbial infection progresses the cellular SA concentration continues rising, leading to changes in the cellular oxidation-reduction status. The disulfide bonds within the NPR1 oligomers are broken due to the redox changes, and oligomers turn into monomers which translocate to the nucleus to stimulate defense-related gene
Figure 2.5: A hypothetical model of the inhibition of CSN complex resulting from the interactions between MVLG_05122 and CSN5a/5b. The removal of Nedd8 protein catalyzed by the CSN complex causes the dissociation of the CRL1 and CRL3, which in turn inhibits the ubiquitination of the R receptor protein and NPR1 transcriptional coactivator. When MVLG_05122 is interacting with the CSN5a/5b, which is the catalytic center of the protein complex, the enzyme activity of E3 ligase might be resumed and ubiquitination of proteins would continue. U, ubiquitin. E2, E2 enzyme conjugating ubiquitin. RBX, RING BOX-1 protein. N, nedd8 protein. F (F-box protein), SKP1 (S-phase kinase-associated protein 1), and BTB (bric-a-brac, tramtrack, and broad complex), adaptor and receptor proteins recognizing R receptor protein and NPR1 transcriptional coactivator.
expression. It is estimated that this transcriptional coactivator regulates expression of more than 2200 genes in Arabidopsis thaliana (Furniss & Spoel, 2015; Kinkema, Kan, & Dong, 2000; Wang, Amornsiripanitch, & Dong, 2006). By interacting with the plant protein complex CSN which is responsible for regulation of CRL3, the MVLG_05122 could affect the cytoplasmic concentrations of the NPR1 to delay or inhibit the occurrences of subsequent immune responses (Figure 2.5).

In summary, we hypothesize that the fungus could enhance the ubiquitination and degradation of the R receptor proteins and the transcriptional coactivator NPR1 by inhibiting CSN activity as a result of the protein-protein interactions between MVLG_05122 and CSN5a/5b. This suggests that the plant protein turnover might be an important process to target in order for M. lychnidis-dioicae to successfully colonize the host plant.

Materials and Methods

cDNA Library Construction

Total RNAs were isolated from a variety of stages of the M. lychnidis-dioicae life cycle, including in vitro stages and different stages of infection of male and female S. latifolia (Table 2.6). The samples included RNA sequences of both the fungus and the plant host. The samples from each life stage were pooled and sent to CD Genomics (Shirley, NY) for reverse transcription and establishment of the cDNA library within a prey vector, pGADT7 (Clonetech, pGADT7 AD Vector
Information, protocol No. PT3249-5, version No. 010312), for use in yeast two-hybrid analysis.

Table 2.6: Total RNAs extracted from different developmental stages of the *M. lycnhdis-dioicae*

<table>
<thead>
<tr>
<th>ID</th>
<th>Tissue type</th>
<th>Concentration (ng/μl)</th>
<th>Bioanalyser concentration (ng/μl)</th>
<th>Volume used (μl)</th>
<th>Volume left (μl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C2</td>
<td>p1A1 Nutrient free</td>
<td>132.9</td>
<td>90</td>
<td>535</td>
<td>0</td>
</tr>
<tr>
<td>D2</td>
<td>p1A2 Nutrient free</td>
<td>144.7</td>
<td>105</td>
<td>475</td>
<td>95</td>
</tr>
<tr>
<td>E1</td>
<td>Mated</td>
<td>161.5</td>
<td>131</td>
<td>385</td>
<td>410</td>
</tr>
<tr>
<td>A</td>
<td>FI Big (15-24 mm)</td>
<td>134.7</td>
<td>102</td>
<td>475</td>
<td>0</td>
</tr>
<tr>
<td>B</td>
<td>FI FS (pedicle and remaining of cluster and sepals) *</td>
<td>144.3</td>
<td>120</td>
<td>420</td>
<td>250</td>
</tr>
<tr>
<td>C</td>
<td>FI Young (7-14 mm)</td>
<td>162.4</td>
<td>135</td>
<td>370</td>
<td>210</td>
</tr>
<tr>
<td>D</td>
<td>FI Young (3-6 mm)</td>
<td>154.7</td>
<td>128</td>
<td>390</td>
<td>295</td>
</tr>
<tr>
<td>E</td>
<td>MI FS (pedicle and remaining of cluster and sepals) #</td>
<td>150.1</td>
<td>129</td>
<td>385</td>
<td>0</td>
</tr>
<tr>
<td>F</td>
<td>MI Young and Small (2-6 mm)</td>
<td>135.1</td>
<td>122</td>
<td>410</td>
<td>190</td>
</tr>
<tr>
<td>G</td>
<td>MI Big (8 mm onwards)</td>
<td>140.7</td>
<td>129</td>
<td>390</td>
<td>92</td>
</tr>
</tbody>
</table>

* FI FS is the abbreviation of female infected flora stem

# MI FS is the abbreviation of male infected flora stem

Ligation and Cloning of the Gene *MVLG_06175* and *MVLG_05122*

The fungal effector sequences are available in the JGI Fungal Genome database (Nordberg, Cantor et al. 2014). In order to acquire the gene *MVLG_06175* and *MVLG_05122*, two short DNA sequences located at the
beginning and end of the coding regions in the *M. lychnidis-dioicae* genome were used to design primers for polymerase chain reaction (PCR). The primer MVLG_6175NewF (5'-GCCGAATTCTTTTGTCCCTTTTGAAAAACGGCG-3') and MVLG_06175R (5'-GACGGATCCTTAGAGATTAGAGGAAAGAAC-3') were for the amplification of MVLG_06175, while the MVLG_05122NewF (5'-GCCGAATTCCTCCCCAGCATGAGCACGGAGTCG-3') and MVLG_05122R (5'-GACGGATCCCTACATGCTTATAGCGATCGCTTG-3') were for that of MVLG_05122. The upstream primer included a sequence recognized by the restriction enzyme EcoRI (GAATTC) at its 5' end, whereas the downstream primer contained one recognized by BamHI (GGATCC). The signal sequence of the MVLG_06175 and MVLG_05122 were identified *in silico* (SignalP 4.1 Server: Center for Biological Sequence Analysis, Technical University of Denmark DTU), and primers were designed to exclude the signal sequence in PCR to reduce the likelihood that this might interfere with the subsequent yeast two-hybrid screening. The setting of the PCR cycle was 94°C of initial denaturation temperature for 4 min, followed by 35 cycles of 94°C for 30 sec, 60°C for 30 sec, and 72°C for 45 sec. The final extension was 72°C for 5 min. Gel electrophoresis by 1.5% agarose (Agarose Unlimited USB Corp., Cleveland, OH, USA) was used to separate the amplified fungal effector sequences. After PCR, both fungal effector genes were cloned by TOPO TA Cloning into the vector pCR® 2.1 (Thermo Fisher Scientific. Inc), and transformed into *Escherichia coli* strain TOP-10 cells, according to the recommendations of the manufacturer. The addition of 250 μl of SOC broth and one-hour shaking followed by incubation at 37°C
allowed expression of its introduced DNA, after which 120 μl of aliquot was spread to Luria broth (LB) agar plates (0.5% yeast extract, 1% sodium chloride, 0.5% tryptone, and 2% agar). The media contained 200 μg/ml ampicillin and X-β-gal, as the colorimetric indicator of β-galactosidase activity; this allowed screening for plasmids with inserts, for which the colonies would be white. The transcription of β-galactosidase would be interrupted if the MVLG_06175 and MVLG_05122 were successfully inserted into the multiple cloning sites of the vector. The vector pCR® 2.1 containing the cloned fungal effector genes were propagated in E. coli and later extracted by an alkaline lysis protocol (Sambrook & Russell, 2001). Purified plasmids were then digested with restriction enzymes EcoRI and BamHI to ensure the sequence of MVLG_06175 and MVLG_05122 included the two enzyme cutting sites at two ends, and the sequences were preserved for ligation.

The vectors used in the following yeast-two hybrid screening are the bait vector pGBKT7 (Clonetech, pGBKT7 Vector Information, protocol No. PT3248-5, version No. PR8Y2643) and the prey vector pGADT7 (Clonetech, pGADT7 AD Vector Information, protocol No. PT3249-5, version No. 010312). For directed yeast two-hybrid experiments with specific genes, the bait and prey vector were digested with restriction enzymes EcoRI and BamHI (with the appropriate buffer) to become linear. The sequences of MVLG_06175 and MVLG_05122 collected from the restriction digestion were connected to the opened bait vector pGBKT7 by T4 DNA ligase, while the cDNA library prepared by CD Genomics (Shirley, NY) was constructed in the prey vector. These vectors were propagated in E. coli
strain DH5α (Bethesda research Laboratories, Bethesda, MD, USA).

Yeast Transformation

Frozen-EZ Yeast Transformation II™ (Zymo Research Corp.) was applied to transform gene constructs into competent yeast cells. The bait vector pGBKT7 carried the two fungal effector genes, while the prey vector pGADT7 cDNA library carried the cDNA library prepared by CD Genomics (Shirley, NY). These genetically modified bait and prey vectors were transformed into yeast strain AH109 and Y187, respectively, with certain modifications in the procedure. The Kit includes solution EZ 1, 2 and 3. To make competent AH109 and Y187 strains, yeast cells were grown shaking in 10 ml YPD broth (1% yeast extract, 2% peptone, 10% dextrose, and 0.1% kanamycin) at 30°C overnight. The ideal absorbance at 660 nm (OD660) to harvest the competent cells was between 0.8 and 1.0 (between $5 \times 10^6$ and $2 \times 10^7$ cells/ml). The yeast culture was centrifuged at 1000 rpm for 4 min. The pellet was resuspended in 10 ml of solution EZ 1 after discarding the supernatant, followed by repeating the centrifugation of the yeast culture and resuspending the pellet with solution EZ 2. According to the protocol, the transformation efficiency would be greatly enhanced if the cell concentration of the yeast culture was high, hence the current study resuspended the pellet in 0.5 ml of solution EZ 2 rather than 1 ml. The yeast culture was aliquoted into ten 1.5 ml microcentrifuge tubes. Each tube contained 50 μl of yeast culture and was stored at -80°C.

For transformation, one 1.5 ml microcentrifuge tube with the yeast culture
was thawed and mixed with 0.2-1 μg of pGBK7 bearing fungal effector genes or pGADT7 bearing cDNA library, 500 μl of the solution EZ 3, and 2 ml of YPD broth. The mixture was incubated at 30°C for 2 hours. It is recommended to flick the tube with fingers every 20 min during the incubation. 150 μl of the mixture was spread on plates containing selection medium (leucine drop-out medium for pGADT7 and tryptophan drop-out medium for pGBK7), and the plate was incubated at 30°C for 2-4 days. Yeast colonies were harvested and resuspended in 5 ml of YPD broth containing 10% glycerol. The suspension was aliquoted into 1.5 ml microcentrifuge tubes and frozen at -80°C for long-term storage.

**Yeast Secretion Trap Assay**

Signal peptides lead the newly synthesized protein products to reside on the cell membrane and on certain organelles such as Golgi apparatus. They also guide some proteins to be secreted from the cell to the surrounding medium or environment. As for fungal effector proteins, they need to subsequently enter tissues or the apoplast to affect host structures and/or metabolism. As a result, it was necessary to determine whether the two fungal effector proteins in the current study were truly secreted out of the fungal cell. The yeast secretion trap assay applied the SEY 6210 yeast strain which carries pYSTO-0 vector encoding an invertase gene SUC2. Invertase can hydrolyze a sucrose molecule to a fructose and a glucose molecule, and the yeast can absorb and utilize the glucose as the carbon source. However, the invertase synthesized by the SEY 6210 strain will not be secreted extracellularly because the SUC2 gene in the
pYSTO-0 vector lacks the signal sequence. Additionally, the vector also encodes LEU2 gene expressing leucine for selection. Therefore, the yeast cells will not be able to proliferate in media where sucrose is the sole carbon source and leucine is absent.

The secretion signal sequences of MVLG_06175 and MVLG_05122 were amplified by PCR and inserted upstream of the SUC2 gene in the pYSTO-0 vector by restriction enzyme digestion and ligation. Primers to amplify the signal sequences are listed in Table 2.7. The genetically constructed vector was cloned into an E. coli strain DH5α (Bethesda research Laboratories, Bethesda, MD, USA), and later was extracted from bacterial transformants and then transferred into the SEY 6210 yeast strain.

Table 2.7: Primers to acquire signal sequences of MVLG_06175 and MVLG_05122.

<table>
<thead>
<tr>
<th>Name</th>
<th>Sequence (5' to 3')</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>6175 Signal F</td>
<td>5'-GCCCATATGATACATCGTCCTCAAGCCAG-3'</td>
<td>This study</td>
</tr>
<tr>
<td>6175 signal R</td>
<td>5'-GCAGGATCCGAGATTTAGAGGAAGACCCAAT-3'</td>
<td>This study</td>
</tr>
<tr>
<td>5122 Signal F</td>
<td>5'-GCCGAATTCATGCTCTTTGAAAGTTTCCGCC-3'</td>
<td>This study</td>
</tr>
<tr>
<td>5122 Signal R</td>
<td>5'-GCCCGGGCGCCGCGCCGCGAAGCACCGAGTGA-3'</td>
<td>This study</td>
</tr>
</tbody>
</table>
Yeast Two-Hybrid Screening

Yeast-two hybrid screening (Y2H) is one of the methods to identify the interactions between biotrophic fungal proteins and host plant proteins (Fields & Song, 1989). It is designed to detect potential protein-protein interactions through yeast mating. Genes of interest and genes of potential target are cloned into “bait” and “prey” vectors, respectively. The bait vector in the current study was pGBKKT7 (Clonetech, pGBKKT7 Vector Information, protocol No. PT3248-5, version No. PR8Y2643) encoding a Gal4 DNA binding domain (BD) and a gene required for tryptophan biosynthesis, while the prey vector was pGADT7 (Clonetech, pGADT7 AD Vector Information, protocol No. PT3249-5, version No. 010312) encoding a Gal4 DNA activation domain (AD) and a gene for leucine biosynthesis. Subsequently, the bait vector was transformed into the yeast strain AH109 and the prey vector was transformed into the strain Y187. For selection of the desired yeasts in the medium, yeast strain AH109 bearing bait vectors are able to synthesize tryptophan and proliferate in tryptophan-deficient medium, in the case of Y187 bearing prey vectors, the cells are able to synthesize leuine and proliferate in leucine-deficient medium. Furthermore, the chromosomes of yeast AH109 encode reporter genes which include HIS3 gene required for histidine synthesis, ADE2 required for adenine synthesis, and MEL1 expressing α-galactosidase. α-galactosidase can cleave a chromogenic substrate 5-bromo-4-chloro-3-indolyl alpha-d-galactopyranoside (X-α-gal) in the medium. The cleavage of X-α-gal releases a blue biomarker resulting in blue yeast colonies. BD or AD alone will not trigger the expression of these reporter genes. After
genes of interest are fused with BD in the bait vector and genes of potential
target are fused with AD in the prey vector, if in the offspring yeasts the two
protein products are interacting physically with each other and thus BD and AD
are in close proximity, the reporter genes will initiate expression and the protein-
protein interactions yield proliferation of yeasts on nutrient-deficient medium.
Additionally, 5 mM 3-amino-1,2,4-triazole (3-AT) when added to the medium,
inhibits the potential leaky expression of the histidine marker. As a competitive
inhibitor, 3-AT can hinder the low-level expression of the HIS3 but not the high-
level expression caused by BD-AD interactions.

The protocol of Matchmaker™ Pretransformed Library User Manual
(Clontech Laboratories, Inc.) was applied for Y2H. In the current study the genes
of interest were fungal effectors MVLG_06175 and MVLG_05122 cloned in the
bait vector pGBKT7, while genes of potential targets were from a cDNA library
generated from RNAs extracted from both axenic fungal cultures and from flower
buds of S. latifolia infected by M. lychnidis-dioicae, this cDNA library was cloned
in the prey vector pGADT7, as indicated above. Yeast strain AH109 transformed
with the bait vector and Y187 transformed with the prey vector were mixed for
mating and incubated overnight. The yeast culture was examined to determine
whether typical three-lobed zygotes were visible under a microscope the next
day. The three-lobed zygotes indicated the presence of haploid parental yeast
cells and the budding off of a diploid offspring yeast cell. According to the
protocol, it is imperative that both AH109 and Y187 yeast strains must yield more
than one million colonies or the positive interactions may not be observed.
Therefore, 100 μl of mated yeast culture which is diluted one thousand-fold, ten thousand fold, and one hundred thousand fold, were spread onto leucine drop out (-Leu), tryptophan drop out (-Trp), and leucine and tryptophan double drop out (DDO) medium to screen the amount of colonies as determined by cfu/ml. The remaining yeast culture was spread and cultivated on quadruple drop-out media lacking adenine, histidine, leucine, and tryptophan (QDO SD/-Ade/-His/-Leu/-Trp). The offspring yeast cells would not proliferate on the QDO medium if they did not carry bait vectors to express tryptophan synthesis and prey vectors to express leucine synthesis from parental yeasts, and if the reporter genes were not triggered by protein-protein interactions. Yeast colonies showing blue color were chosen for repurification on higher stringency QDO medium in which 50 mM 3-AT was added. Such colonies were used for further DNA extraction and sequencing. All drop out media were made with sterile deionized water, glucose at 20 g/L, yeast nitrogen base at 6.7 g/L, drop out mix 2 g/L, and agar at 15 g/L.

In order to confirm the protein-protein interactions were genuine rather than false positives, after initial identification, genes of interest and genes of potential targets are cloned into the opposite vectors to proceed with the Y2H spot test. That is, genes of interest are cloned into the prey vector carried by the yeast strain Y187 and genes of potential target were cloned into the bait vector carried by the yeast strain AH109. Additionally, a positive control and certain negative controls were later used in the Y2H spot test as well. The positive control used was the interaction between the bait vector bearing a p53 protein sequence (pGBKT7-p53) and the prey vector bearing a T antigen sequence (pGADT7-T).
The negative controls include AH109 with the bait vector alone (BD), Y187 with the prey vector alone (AD), AH109 with the bait vector bearing p53 alone (BD-p53), Y187 with the prey vector bearing T antigen alone (AD-T), and two yeast strains mating without genes fused in the vectors (BD+AD).
CHAPTER III: CHARACTERIZATION OF THE FUNGAL EFFECTOR PROTEINS
IN ARABIDOPSIS THALIANA, A HETEROLOGOUS PLANT HOST MODEL

Overview

To further elucidate the influences of fungal proteins MVLG_06175 and MVLG_05122 on host plants during fungal colonization, the current study applied genetic recombination to stably express these two fungal genes in the model plant Arabidopsis thaliana. To reveal the subcellular locations of the effector proteins in the plant tissues, the fungal genes MVLG_06175 and MVLG_05122 were linked to fluorescent tag genes expressing mCherry (MVLG_06175-mCherry and MVLG_06175ΔSP-mCherry) or the cyan fluorescent protein (CFP) (MVLG_05122ΔSP-CFP), respectively, through Gibson assembly (Gibson et al., 2009). These gene constructs, whose transcription was driven by CaMV 35S promoter, were transformed into A. thaliana by Agrobacterium-mediated transformation. The test of Mendel’s law of segregation was used to confirm the genetically modified A. thaliana were homozygous for the transgenes in the third generation (T3 progenies). qRT-PCR demonstrated that the MVLG_06175, mCherry, MVLG_05122 and CFP genes were transcribed into mRNA in leaves of the two-week-old transgenic seedlings. Furthermore, DNA sequencing of cDNA reverse-transcribed from the mRNA also showed the presence of MVLG_06175-
mCherry, MVLG_06175ΔSP-mCherry, and MVLG_05122ΔSP-CFP transcripts. The tests suggest that the gene constructs were successfully transcribed into mRNA in A. thaliana. Transgenic A. thaliana expressing MVLG_06175ΔSP-mCherry and MVLG_06175-mCherry showed statistically smaller rosette diameter and leaf quantity. However, plants transformed with MVLG_05122ΔSP-CFP did not show any significant phenotype variations. The fluorescence confocal microscopic images from 4-week-old A. thaliana transformed with MVLG_06175-mCherry and MVLG_06175ΔSP-mCherry displayed clustered mCherry signals at the tip of trichomes, but only the plant transformed with MVLG_06175ΔSP-mCherry displayed clear clustered mCherry signals in the roots. The images taken from 6 and 7-week-old plants transformed with MVLG_05122ΔSP-CFP displayed band-like CFP signals gathered at the base of trichomes. The infection assay of Botrytis cinerea on transgenic A. thaliana expressing MVLG_05122ΔSP-CFP did not show any differences in comparison with the wild type Col-0 (WT).

Introduction

The yeast two-hybrid screening and sequence alignment have revealed the potential identities and functions of the plant proteins interacting with the fungal effector proteins in the current study (See Chapter II). The subsequent questions are what phenotype changes the protein-protein interactions could cause and
where the interactions would occur. The current study selected *Arabidopsis thaliana* as the model plant to investigate the pathogen-host interactions, and thus generated and cultivated transgenic *A. thaliana* lines expressing the fungal genes *MVLG_06175* and *MVLG_05122*, absent fungal infection of this species, which is not a host for *M. lychnitis-dioicae*. The lifespan of *A. thaliana* is 10-12 weeks, which is relatively short and ideal for genetic and populational research. In contrast, *S. latifolia* requires about 6 months to become mature and to flower. Like *Silene latifolia*, *A. thaliana* also synthesizes the protein CASPL2C1 and CSN5a/5b. However, the two proteins of *A. thaliana* are not identical to those of *S. latifolia*. The CSN5a of *S. latifolia* and that of *A. thaliana* share 65.1% similarity in the DNA sequence and 82.4% similarity in the amino acid sequence. The CSN5b in the two plants share 83.3% similarity in the DNA sequence and 81.1% similarity in the amino acid sequence. In contrast, the CASPL2C1 in the two plants share 33.8% similarity in the DNA sequence and only 48.7% similarity in the amino acid sequence.

The result of the yeast two-hybrid screening (Chapter II) showed the fungal effector protein *MVLG_06175* likely interacts with the plant protein CASP-like protein 2C1 (CASPL2C1) associated with the formation of the Casparian strip. The Casparian strip is a specialized cell wall surrounding the endodermis that separates the cortex and vascular bundle. The other effector protein *MVLG_05122* was found (Chapter II) to interact with the plant protein COP9 signalosome complex subunit 5a/5b (CSN5a/5b) which was associated with the inhibition of the Cullin-RING E3 ubiquitin ligases (CRLs). CRLs is an enzyme
superfamily catalyzing the protein ubiquitination to facilitate the subsequent protein degradation by proteasomes (Choi, Gray, Mooney, & Hellmann, 2014). In the discussion of Chapter II, I proposed the hypothesis that MVLG_05122 might change the R receptor level in the plant cells through the interactions between CSN complex and Cullin 1-RING E3 ubiquitin ligase (CRL1) because R receptor directly recognizes and binds to fungal effector proteins (figure 2.5). However, CRL1 has a more influential role in plant development. CRLs associated with Cullin 1 protein and F-box protein in plants are prominently involved in signal transduction pathways of phytohormones such as auxin. When the transport inhibitor response 1 (TIR1) protein acts as the adaptor and F-box protein acts as the receptor, they together recruit auxin to bind to the F-box protein. The binding of auxin to the F-box unit in the CRL1 stimulates the conformational change of F-box unit, and the transcriptional repressor auxin/indole-3-acetic acid (Aux/IAA) protein complex can bind to the F-box unit of CRL1. The transcriptional repressor subsequently undergoes ubiquitination and degradation, which will resume the auxin-induced gene expression (Choi, Gray, Mooney, & Hellmann, 2014; Woodward & Bartel, 2005). The protein-protein interaction hypothesis proposed in Chapter II (figure 2.5) also includes CRL3 associated with the Cullin 3 protein and BTB protein. By transferring ubiquitin to the non-expressor of pathogenesis related (NPR) transcriptional coactivators for the subsequent protein degradation, the E3 ligase families are primary regulators of plant immune responses stimulated and repressed by jasmonic acid (JA) and salicylic acid. Moreover, CRL3 is involved in the biosynthesis of ethylene and signaling pathway of
In summary, *M. lychnidis-dioicae* might apply the two effector proteins to penetrate into the vascular tissues and to modulate rates of protein turnover in the plant host. Due to the broad metabolic processes the CRLs can modify, different protein turnover rates might cause profound phenotypic changes. In order to examine changes *in planta* potentially resulting from the protein-protein interactions, and to identify the subcellular locations of the interactions in the plant cells, I generated *MVLG_06175* and *MVLG_05122* linked to the mCherry and cyan fluorescent protein (CFP), respectively (*MVLG_06175*Δ*SP-mCherry*, *MVLG_06175-mCherry*, and *MVLG_05122*Δ*SP-CFP*). The expression of the genes was driven by the constitutive CaMV 35S promoter. The two genetic constructs were transformed and expressed in the model plant *A. thaliana* by *Agrobacterium*-mediated transformation. Lines homozygous for the respective trans genes were identified after examination of proper segregation in crosses.

**Results**

**Protein-protein Interactions between Fungal Effector Proteins and Corresponding plant proteins of *Arabidopsis thaliana***

Since the current study chose *Arabidopsis thaliana* as the model plant to investigate the effects of expression of the fungal effector proteins, it is necessary to determine whether the protein-protein interactions occur in the model plant as
A. thaliana also synthesizes CSN5a/5b and CASPL2C1 protein, but the amino acid sequences of these proteins in Silene latifolia are not identical to those in A. thaliana; CSN5a and 5b in A. thaliana and S. latifolia share 82.4% and 81.1% identity in amino acid sequence, respectively, while CASPL2C1 in the two plants shares only 48.7% identity at the amino acid level. Primers were designed to acquire CSN5a/5b and CASPL2C1 from A. thaliana genome, and the plant genes were inserted into the prey vector to be used in Y2H spot tests. The outcomes showed the interactions between MVLG_5122 and CSN5a of A. thaliana resulted in the growth of offspring in the nutrient-deficient media, but MVLG_06175 might not interact with CASPL2C1 ortholog of A. thaliana, leading to absence of yeast growth in this assay of protein-protein interaction (Figure 3.1).
Figure 3.1: Y2H spot test on protein-protein interactions between plant proteins of *A. thaliana* and fungal protein MVLG_06175 and MVLG_05122. To confirm the protein-protein interactions in the model plant *A. thaliana*, CASPL2C1 and CSN5a of the plant were inserted into the prey vector bearing the transcriptional activation domain (AD). The modified prey vectors were transformed into the Y187 yeast strain. After the transformation, the Y187 yeast strain was mated with the AH109 yeast strain bearing the bait vector encoding either MVLG_06175 or MVLG_05122 (see Chapter II) in QDO medium/X-α-gal + 3AT (50 mM). The interaction between MVLG_05122 and CSN5a of *A. thaliana* was observed, but MVLG_06175 did not interact with CASPL2C1 of this plant. BD-6175+AD-CASPL2C1 (Silene) and BD-5122+AD-CSN5a (Silene), positive controls. BD-6175+AD, BD+AD-CASPL2C1 (Arabidopsis), BD-5122+AD, and BD+AD-CSN5a (Arabidopsis), one of the two mating yeast strains carries bait or prey vectors with no insertions, as negative controls. Undil, undiluted. 10X and 100X, 10 folds and 100 folds of dilutions.
Determination of Phenotype Changes in Transgenic *A. thaliana*

The current study examined four parameters of *A. thaliana* plants: leaf quantity, rosette diameter, days to flower opening, and silique quantity in the T3 generation of transgenic *A. thaliana*. T3 generation is the third generation of plants that were transformed with gene constructs. Based on Mendel’s law of segregation these plants are homozygous lines (see Materials and Methods). Plants were initially grown in MS media and transferred to soil at 14 days. All parameters showed no differences except the rosette diameter and leaf quantity in the 4-week-old plants transformed with MVLG_06175. Both MVLG_06175-mCherry and MVLG_06175ΔSP-mCherry transformed *A. thaliana* had statistically smaller rosette diameter and leaf quantity than the mCherry alone transformed plants and WT (Figure 3.2 & 3.3). Data were collected from 34 plants of MVLG_06175ΔSP-mCherry, 28 plants of MVLG_06175-mCherry, 35 plants of mCherry alone, and 35 plants of the wild type (*Col-0*).
Figure 3.2: Box-and-Whisker plot showing rosette diameter among transgenic *A. thaliana*. The experimental groups are plants transformed with the *MVLG_06175* (with and without the signal sequence) linking to the *mCherry* fluorescence gene. The control groups are plants transformed with *mCherry* fluorescence gene alone and wild type *Col-0* (WT). The t-test analysis demonstrated plant strains expressing *MVLG_06175*, both with and without the signal sequence, were significantly smaller in rosette diameter than the controls. *6175Δ SP-m, MVLG_06175Δ SP-mCherry*. 6175-m, *MVLG_06175-mCherry*. m alone, *mCherry* alone. *6175Δ SP-m vs m alone, p < 0.0001****. 6175-m vs m alone, p < 0.001***. *6175Δ SP-m vs WT, p < 0.0001****. 6175-m vs WT, p < 0.01**
Figure 3.3: Box-and-Whisker plot showing leaf quantity among transgenic *A. thaliana*. The experimental groups are plants transformed with the *MVLG_06175* (with and without the signal sequence) linking to the *mCherry* fluorescence gene. The control groups are plants transformed with *mCherry* fluorescence gene alone and wild type *Col-0* (WT). The t-test analysis demonstrated plant strains expressing MVLG_06175, both with and without the signal sequence, were significantly smaller in leave quantity than the controls. *6175ΔSP-m*, *MVLG_06175ΔSP-mCherry*. *6175-m*, *MVLG_06175-mCherry*. *m alone*, *mCherry* alone. *6175ΔSP-m vs m alone*, p < 0.001**. *6175-m vs m alone*, p < 0.001**. *6175ΔSP-m vs WT*, p < 0.05*. *6175-m vs WT*, p < 0.05*
Evaluation of Expression of MVLG_06175-mCherry, MVLG_06175 Δ SP-mCherry, and MVLG_05122 Δ SP-CFP in Transgenic Arabidopsis thaliana

Genomic DNA was extracted from leaves of two-week-old transgenic A. thaliana transformed with MVLG_05122 Δ SP-CFP, MVLG_06175-mCherry, and MVLG_06175 Δ SP-mCherry. The PCR showed the presence of the genes (figure 3.4). For the qRT-PCR assay, the current study used the housekeeping gene UBQ10 as the internal standard to normalize the RNA expression of two fungal effector genes, CFP, and mCherry in the transgenic and wild type (Col-0) A. thaliana. The mRNA was extracted from leaves of four-week-old transgenic A. thaliana and used as the template to synthesize cDNA. The following qRT-PCR based on the cDNA confirmed the transcription of MVLG_05122 and cyan fluorescence gene in the transgenic A. thaliana, as well as those of MVLG_06175 and mCherry (figure 3.5, supplemental table S3.1, S3.2, and S3.3, appendix) DNA sequencing of the cDNA also confirmed their corresponding identities, MVLG_05122ΔSP-CFP, MVLG_06175-mCherry, and MVLG_06175ΔSP-mCherry. In conclusion, the PCR result, DNA sequencing result and qRT-PCR based on the mRNA of the transgenic A. thaliana confirmed the gene construct was transformed into the plant.
Figure 3.4: PCR results of genomic DNA extracted from transgenic *A. thaliana* and wild type *Col-0* (WT). Left panel, transgenic plant expressing *MVLG_06175-mCherry* and *MVLG_06175ΔSP-mCherry* showed bands around 1.1K bps, while the WT plant did not. Right panel, transgenic plants expressing *MVLG_05122ΔSP-CFP* (three samples) showed bands around 1K bps. +control, an indicator showing the PCR was functioning normally.
**Figure 3.5:** Log2-fold changes of gene construct expression compared with wild type *Col-0* (WT) in qRT-PCR. Each gene construct used only one qRT-PCR biological sample in the trial.

**Localization of *M. lychnidis-dioicae* Effectors in the Transgenic Plant Tissues by Fluorescent Tags: MVLG_06175**

In the 4-week-old transgenic plants, the signals of mCherry linked to MVLG_06175ΔSP and MVLG_06175 displayed granules clustered at the tips of trichomes on leaves. In contrast, signals of mCherry alone expressed in the plant as the positive control concentrated at the center of the trichomes, and auto-fluorescent signals of WT displayed a weaker and relatively random distribution (Figure 3.6). The punctated signals indicated the potential interactions between
MVLG_06175 and CASPL2C1 or some other host protein(s), located at the tips of the trichome, and the effector protein might be affecting the functions of CASPL2C1/other proteins associated with structures and/or functions in the trichomes.

Transgenic *A. thaliana* strains expressing *MVLG_06175 ΔSP-mCherry*, *MVLG_06175-mCherry*, and *mCherry* alone exhibited clear mCherry signals in the roots of the 2-week-old seedlings in comparing with the WT plant. While plants carrying *MVLG_06175-mCherry* and *mCherry* alone did not show specific patterns, those carrying *MVLG_06175 ΔSP-mCherry* displayed concentrated granules (Figure 3.7).
**Figure 3.6**: Localization studies of MVLG_06175 in trichomes in 4-week-old *A. thaliana*. Confocal fluorescence images were taken from leaves of the 4-week-old stable transgenic *A. thaliana* expressing MVLG_06175-mCherry, MVLG_06175ΔSP-mCherry, and mCherry alone, as well as of the WT plant. Signals of MVLG_06175-mCherry and MVLG_06175ΔSP-mCherry transgenic lines formed granules clustered at the tips of trichomes on leaves. Expression was under the control of CaMV 35S promoter. In each sample, the upper panel is the fluorescence image and the lower panel is the merged image. Size bar, 20 μm.
Figure 3.7: Localization studies of MVLG_06175 in roots in 2-week-old A. thaliana. Confocal fluorescence images were taken from roots of the 2-week-old stable transgenic A. thaliana expressing MVLG_06175-mCherry, MVLG_06175ΔSP-mCherry, and mCherry alone, as well as of the WT plant. Signals of MVLG_06175ΔSP-mCherry transgenic lines formed granules in the roots, but those of MVLG_06175ΔSP-mCherry transgenic lines did not or formed granules with a weak intensity. Expression was under the control of CaMV 35S promoter. In each sample, the upper panel is the fluorescence image and the lower panel is the merged image. Size bar, 20 μm.

Localization of *M. lychnidis-dioicae* Effectors in the Transgenic Plant Tissues by Fluorescent Tags: MVLG_05122

Under confocal microscope, fluorescence images of MVLG_05122ΔSP-CFP transgenic A. thaliana showed similar overall intensity of signals to those of the CFP alone transgenic line and those of wild type Col-0 (WT). However, there were significant and clear band structures at the bases of trichomes on leaves of the MVLG_05122ΔSP-CFP transgenic plant (Figure 3.8). The band-like CFP signals were also observed in trichomes on leaves of WT and CFP alone transgenic lines, but the MVLG_05122ΔSP-CFP transgenic line displayed the strongest signal intensity. After reducing the intensity of light to the level at which the fluorescence signals of the WT was barely seen, the band structure at the base of trichomes remained clear in the MVLG_05122ΔSP-CFP transgenic line.
Figure 3.8: Localization studies of MVLG_05122 in trichomes in 6 and 7-week-old transgenic A. thaliana. Fluorescence images taken from a confocal microscope showed trichomes of plant strains expressing MVLG_05122ΔSP-CFP and CFP alone as well as of WT plant. Expression of trans genes was driven the by constitutive CaMV 35S promoter. Size bar, 20 µm.
Figure 3.9: Fluorescence images of trichomes in WT and transgenic *A. thaliana* expressing MVLG_05122ΔSP-CFP and CFP alone at the lower intensity of light. The intensity was decreased so that the signals in WT were barely seen. The band-like CFP signals remained strong in the *A. thaliana* expressing MVLG_05122ΔSP-CFP. Expression of *trans* genes was driven by constitutive CaMV 35S promoter. Size bar, 20 μm.

Susceptibility Assay of Transgenic Plant toward *Botrytis cinerea* Infection

The current study proposed a hypothesis in Chapter II that the protein-protein interactions between MVLG_05122 and CSN5a might alter the ubiquitination and degradation of R protein and NPR1 transcriptional coactivator,
two plant proteins that directly trigger immune-responses against phytopathogen infection. The hypothesis leads to the prediction that the expression of MVLG_05122 in the transgenic A. thaliana would increase the plant’s susceptibility to fungal infection. Thus, a fungal infection assay was conducted to determine if the transgenic plant had a lower resistance against fungi. The current study applied the protocol and data analysis of Liu et al. (2015) and Liu (2020) to infect A. thaliana expressing MVLG_05122 with Botrytis cinerea. The genomic DNA of plant and fungus were extracted from leaves inoculated with spores of B. cinerea three days after inoculation. As a proxy for biomass, the amount of fungal cutinase A (CutA) DNA and plant α-shaggy kinase (SKII) DNA were determined by qRT-PCR. Cutinase A is a fungal lipolytic enzyme, while SKII is a plant shikimate kinase for synthesis of certain secondary metabolites. These respective genes were used to determine relative amounts of fungal and plant biomass in the experiment. The presence of the two genes was quantified by taking the reciprocal of their cycle threshold (Ct) values. For instance, one of the Ct values of SKII in wild type Col-0 (WT) was 20, thus 1/20 was the relative value of plant DNA in the sample. Data were analyzed by comparing the fractions of the relative values of CutA over those of SKII \[ \frac{1}{Ct \text{ of CutA}} / \frac{1}{Ct \text{ of SKII}} \]. Each study group contained three plants. These experiments did not find statistical differences in fungal biomass during infection among groups of WT, WT infected with fungus, MVLG_05122ΔSP-CFP transgenic plant infected with fungus, and CFP alone transgenic plant infected with fungus (Figure 3.10).
Figure 3.10: Ratios of relative amount of CutA over those of SKII (i.e., fungal biomass) among four study groups. Each group had three trials, and there were no significant differences among the four groups. WT mock, wild type Col-0 plants inoculated with Vogel buffer only. WT Inf, WT plants inoculated with fungal (i.e., B. cinerea) spores. 5122-CFP Inf, MVLG_05122ΔSP-CFP transgenic plants inoculated with fungal spores. CFP Inf, CFP alone transgenic plants inoculated with fungal spores.
Discussion

Although the *in vitro* Y2H spot test (Figure 3.1) did not detect the interactions between MVLG_06175 and CASPL2C1 of *A. thaliana*, fluorescence-labeled MVLG_06175 showed different signal distributions in the transgenic *A. thaliana* in comparison with the WT and mCherry alone transgenic plants. The images revealed the localization of fungal effector protein MVLG_06175 at the tip of trichomes and of roots. We expected that the MVLG_06175 without signal sequence would stay in the cytoplasm, while MVLG_06175 with signal sequence would be secreted out of the cell, but our images showed their fluorescence signals exhibited a similar pattern in the trichomes; they all aggregated at tips of trichomes, like small particles. In terms of mCherry signals in the roots, MVLG_06175 without signal sequence showed aggregation at the root tips, but MVLG_06175 with signal sequence did not or the aggregation was very weak. Considering the *in vitro* Y2H spot test, the aggregation of fluorescence signals in trichomes and roots might indicate that MVLG_06175 was interacting with different plant proteins in the two tissues.

The fluorescence images only indicated where the fungal effector proteins were located in the plant. The true identities of plant proteins interacting with effector proteins, if there are, may require a co-immunoprecipitation assay to reveal. Nevertheless, CASPL2C1 still could be a candidate, and the smaller rosette diameter and leaf quantity observed in *MVLG_06175* transgenic *A. thaliana* (figure 3.2 and 3.3) might result from the modifications of plant proteins.
by the fungal effector protein. Although there were no studies directly tracing the
distribution of CASPL2C1 in plant tissues, a former study showed the
fluorescence-labeled CASPL5B1 of A. thaliana, a homologous protein of CSAP5,
was expressed in immature and differentiated trichomes (Roppolo et al., 2014).
Casparian strip membrane domain proteins (CASPs) are expressed exclusively
in the endodermal cells for the polymerization of lignin, but the homologous
CASP-like proteins (CASPLs) can be expressed in different tissues such as
abscission zone cells, peripheral root cap cells, trichomes, and xylem pole
pericycle cells, where they could function as modifiers of cell wall-related
structures (Roppolo et al., 2014). Therefore, we cannot exclude the possibility
that the MVLG_06175 is interacting with other CASP-like proteins in trichomes.
As the first defensive line of the plant, trichomes are the protruding epidermal
structures on aerial plant tissues. They protect plants from damage caused by
ultraviolet (UV) light or by insects, and excess transpiration, and phytochemicals
synthesized and secreted from trichomes are often involved in phytopathogen
resistance as well as pollinator attraction (Wagner, Wang, & Shepperd, 2004). If
the interactions between the effector protein MVLG_06175 and plant protein
CASPL2C1 do happen at the tips of trichomes, the protein-protein interaction
could be involved in the trichome development and flavonoid synthesis, and M.
lychnidis-dioicae might be neutralizing the immune responses of S. latifolia to
enhance the colonization. Since M. lychnidis-dioicae starts its infection cycle on
the flowers of the host plants or in wounded host tissues, the modifications
potentially happening in trichomes on the flower buds could provide advantages
to the fungal invasion.

It is even more intriguing that the potential interactions between MVLG_05122 and CSN5a also appeared in the trichome. The CFP signals formed an organized band at the basal cells of trichomes, suggesting that the effector protein MVLG_05122 might be affecting the trichome growth through interactions with the CSN protein complex. CSN5a has previously shown influences on trichome development and metabolism. Inactivation of CSN5a in A. thaliana results in elevated production of many carotenoids and phenylpropanoids including anthocyanins in seeds and leaves. This mutated plant strain also demonstrates significantly reduced density and abnormal morphology of trichomes. These phenotypic changes could be due to altered gene expressions in two tri-protein complexes when CSN5a is disrupted (Wei et al., 2018), although the same changes could also have resulted from altered hormone signaling pathways followed by inactivation of CSN5a. Plant hormones, including jasmonic acid, salicylic acid, and gibberellin could regulate the growth of trichomes (Traw & Bergelson, 2003). Jasmonic acid, in conjunction with gibberellin induces trichome production, while salicylic acid decreases the number and density of trichomes (Traw & Bergelson, 2003). An earlier study silencing the CSN5a in tomato, found reduced synthesis of jasmonic acid but not of salicylic acid (Hind et al., 2011). A lower jasmonic acid concentration is a potential cause of the reduced density and abnormal morphology of trichomes, too.
In terms of the infection assay of *Botrytis cinerea*, the transgenic *A. thaliana* expressing *MVLG_05122*Δ*SP-CFP* did not show evidence of greater fungal biomass compared with the two control groups, mock WT and infected WT. This result suggests that *MVLG_05122* might not affect the cellular levels of R receptor and NPR1 transcriptional coactivator in the host plant, although three plants in each study group was a very small sample size (Figure 3.10). It is worth noting that between the two control groups mock WT and infected WT, there were also no differences in fungal infection. This suggests that *A. thaliana* was naturally resistant to infection by *B. cinerea*, a finding consistent with some of the results found in Liu at al. (2016). Hence the infection assay in the future might need to select fungal species to which *A. thaliana* is more susceptible. To verify the hypothesis that the transgenic *Arabidopsis* strain expressing *MVLG_05122-CFP* is more venerable to pathogenic infection, future studies can also evaluate the cellular levels of R receptor and NPR1 transcriptional coactivator in the transgenic plant because the decreased levels might indicate that the enzyme activity of CSN protein complex is inhibited by *MVLG_05122* and the ubiquitination of the two proteins is resumed.

It is an interesting finding that, during infection, *M. lychnidis-dioicae* might modify the trichome structure and metabolism, and it is more interesting that the fungus might utilize two different effector proteins to interfere with the development of trichomes by interacting with two different plant proteins, CASPLs and CSN5a. These findings suggests that *M. lychnidis-dioicae* evolved
to apply multiple effector proteins to modulate the same plant structures and processes.

Materials and Methods

Gibson Assembly to Construct MVLG_05122 Tagged with Cyan Fluorescent Protein Gene and MVLG_06175 Tagged with mCherry Protein Gene, as Well as CASPL2C1 and CSN5a/5b of Arabidopsis thaliana in the Prey Vector pGADT7

Unlike the gene construction by restriction digestion and ligation for yeast-two hybrid screening in Chapter II, Gibson assembly was used to create plasmids bearing the desired constructs. Primers are listed in Table 3.1. The vector pRI-101AN used here encodes a kanamycin resistance gene, the cauliflower mosaic virus 35S promoter (CaMV 35S), and a NOS transcriptional terminator. CaMV 35S is a robust promoter to enhance the expression of the inserted genes of interest in plants. Vector pRI-101AN was cut by restriction enzymes BamHI and Ndel and underwent ethanol precipitation. The linear vector of pRI-101AN purified from agarose after gel electrophoresis was mixed with 1/10 volume of 3M sodium acetate (pH = 5.2) and 3 volume of ice cold 100% ethanol. The mixture was stored at -80°C for an hour, followed by microcentrifugation at top speed at 4°C for 30 minutes. The supernatant was removed by aspiration, and the pellet was washed with 3 volumes of ice cold 70% ethanol and spun again at 4°C for 30 minutes. The ethanol supernatant was
Table 3.1: Primers used to construct *MVLG_06175-mCherry*, *MVLG_06175 Δ SP -mCherry*, and *MVLG_05122 Δ SP-CFP* in pRI-101AN vector

<table>
<thead>
<tr>
<th>Name</th>
<th>Sequence (5' to 3')</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>pRI to 6175ns Forward</td>
<td>5'-TTCTTCACTGTTGATACATAATGTGGTTTGCCTTTGGAAAAAC-3'</td>
<td>This study</td>
</tr>
<tr>
<td>pRI to 6175sp Forward</td>
<td>5'-TTCTTCACTGTTGATACATAATGTCCTTGCATCGTCCA-3'</td>
<td>This study</td>
</tr>
<tr>
<td>pRI to mCherry Forward</td>
<td>5'-TTCTTCACTGTTGATACATAATGGCGCTTCAGGTGCACATGGA-3'</td>
<td>This study</td>
</tr>
<tr>
<td>pRI to Myc Reverse</td>
<td>5'-GTTGATTCAGAATTCCGGATCTTACGAGAGGTCCTCTTTCCGAGA-3'</td>
<td>This study</td>
</tr>
<tr>
<td>pRI to 5122 Forward</td>
<td>5'-TTCTTCACTGTTGATACATAATGGCGCTTCAGGTGCACATGGA-3'</td>
<td>This study</td>
</tr>
<tr>
<td>3rd mtCFP to 5122+GGS Reverse</td>
<td>5'-tcctcgcccttgctcaccatAGAGCCGCCCATGCTTATAGCGATCGCTT-3'</td>
<td>This study</td>
</tr>
<tr>
<td>Control mtCFP Forward</td>
<td>5'-TTCTTCACTGTTGATACATAatggtagcgcaagggcgagaa-3'</td>
<td>This study</td>
</tr>
<tr>
<td>CFP to pRI Forward</td>
<td>5'-TTCTTCACTGTTGATACATAATGTTGACCAAGGCGAGGAGA-3'</td>
<td>This study</td>
</tr>
<tr>
<td>3rd pRI to mtCFP Reverse</td>
<td>5'-GTTGATTCAGAATTCCGGATCttacttgtaacgtcgtc-3'</td>
<td>This study</td>
</tr>
</tbody>
</table>
removed by vacuum drying and the pellet was resuspended in 40 μl of H₂O.

For the yeast two-hybrid screening (Y2H) spot test on the interactions between fungal effector proteins and A. thaliana CASPL2C1 and CSN5a/5b, primers are designed to acquire the two plant genes (Table 3.2). The two plant genes were inserted into the prey vector pGADT7 by Gibson assembly rather than by restriction digestion and ligation.

Table 3.2: Primers to acquire CSN5a/5b and CASPL2C1 from A. thaliana genome

<table>
<thead>
<tr>
<th>Name</th>
<th>Sequence (5’ to 3’)</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>PGA to CSN5a Left</td>
<td>5'-GCCATGGAGGCGCAGTGAATTAGGTTCCTCGTCAG-3'</td>
<td>This study</td>
</tr>
<tr>
<td>PGA to CSN5a Right</td>
<td>5'-CAGCTCGAGCTCGATGGATCTCAGCTACGAAGGTTCCTCGTCAG-3'</td>
<td>This study</td>
</tr>
<tr>
<td>PGA to CSN5b Left</td>
<td>5'-GCCATGGAGGCGCAGTGAATTAGGTTCCTCGTCAG-3'</td>
<td>This study</td>
</tr>
<tr>
<td>PGA to CSN5b Right</td>
<td>5'-CAGCTCGAGCTCGATGGATCTCAGCTACGAAGGTTCCTCGTCAG-3'</td>
<td>This study</td>
</tr>
<tr>
<td>PGA to CASP Left</td>
<td>5'-GCCATGGAGGCGCAGTGAATTAGGTTCCTCGTCAG-3'</td>
<td>This study</td>
</tr>
<tr>
<td>PGA to CASP Right</td>
<td>5'-CAGCTCGAGCTCGATGGATCTCAGCTACGAAGGTTCCTCGTCAG-3'</td>
<td>This study</td>
</tr>
</tbody>
</table>
The fungal effector gene \textit{MVLG\_05122} lacking its signal sequence and a cyan fluorescent protein (CFP) gene were incorporated into the vector pRI-101AN. Additionally, nine nucleotides encoding glycine-glycine-serine served as a linker, were added in-frame between \textit{MVLG\_05122} and the CFP gene; by doing so the linker provided a certain level of flexibility between the respective proteins in the resulting fusion protein. The fungal effector \textit{MVLG\_06175} with and without its signal sequence and the mCherry protein gene were incorporated into the pRI-101AN vector, to yield additional expression constructs. In order to complete the Gibson assembly, adjacent DNA fragments are required to have 20 to 40 nucleotide overlap. I used NEBuilder® HiFi DNA Assembly Master Mix (New England BioLabs, Inc.) to accomplish Gibson assembly. The master mix contained exonuclease, DNA polymerase, and DNA ligase. The exonuclease will digest the 5’ terminus of the DNA strands, DNA polymerase starts filling in gaps with nucleotides based on the complementary strand after the alignment of two adjacent DNA fragments, and ligase links adjacent DNA fragments at the end.

Ten \( \mu l \) of NEBuilder® HiFi DNA Assembly Master Mix (New England BioLabs, Inc.) was mixed with 0.5 \( \mu l \) of the fungal effector DNA sequence, 0.5 \( \mu l \) of the fluorescent protein DNA sequence, and 9 \( \mu l \) of linear vector pRI-101AN. The mixture was incubated at 50°C for 15 minutes, followed by transferring 10 \( \mu l \) of the mixture to a 2 ml tube of pre-thawed competent \textit{E. coli} cells and keeping the bacteria/DNA on ice for 30 minutes. The cell mixture underwent heat shock at 42°C for 30 seconds and was transferred to ice for 2 minutes. After the addition of 950 \( \mu l \) of SOC media the culture was incubated at 37°C for 60 minutes and
was shaken vigorously at 250 rpm. Finally, 120 µl of the culture was spread onto a selection Petri dish of LB agar and kanamycin (50 µg/ml), and the Petri dish was incubated overnight at 37°C. The bacterial colonies acquired from the Petri dish underwent DNA extraction, PCR sequencing, and sequencing to confirm the presence of the respective fungal gene and the gene for its tag.

**Agrobacterium Transformation through Electroporation**

Competent cells of bacterium *Agrobacterium tumefaciens* were prepared based on the Pikaard’s Lab protocol (Pikaard). A 0.5 ml tube containing competent cells was thawed on ice. The bacterial culture was then aliquoted into two 0.5 ml tubes, and 10 µl of the constructed plasmid (*MVLG_05122 Δ SP-CFP*, *MVLG_06175 Δ SP-mCherry*, or *MVLG_06175-mcherry in the vector pRI-101AN*) extracted from *E. coli* was added to one of the 0.5 ml tubes, while 10 µl of water was added to the other 0.5 ml tube as the negative control sample. *Agrobacterium* cells and constructed plasmids were transferred to electroporation cuvettes which were pre-chilled on ice. The transformation was conducted in a BioRad micropulser electroporator with voltage of 2.5 kV using the 25 µF capacitor and at 400-ohm settings (Mattanovich et al. 1989). The electrical shock lasted for about 4.7 seconds, followed by addition of 1 ml of LB to the cuvette. After pipetting up and down several times to mix the bacterial culture and LB broth, the mixture was transferred to a 2 ml tube and incubated at 28 °C for 2 hours, with shaking. 150 µl of the bacterial culture was spread on each Petri dish containing LB media and kanamycin (50 µg/ml). These Petri dishes were
incubated at 28 °C for 2 days. DNA extraction from Agrobacterium colonies and PCR were conducted to determine the presence of the MVLG_05122 ΔSP-CFP, MVLG_06175 ΔSP-mCherry, and MVLG_06175-mCherry within the vector. Agrobacterium clones confirmed to carry the constructed vector would be used to infect Arabidopsis and deliver the respective constructs.

**Arabidopsis and Growth Conditions**

Arabidopsis thaliana ecotype Col-0 was used as the wild-type background in the current study (kindly provided by Dr. Mark Running, University of Louisville). The plant seeds underwent surface-sterilization, and were spread onto and cultivated on 1/2x MS solid media (Murashige & Skoog, Phytotechnology Laboratories, Cat No: M524) which contained 0.05% MES buffer (2-(N-morpholino) ethanesulfonic acid, ThermoFisher, Pittsburgh, Pennsylvania, USA) and 0.8% agar as well as kanamycin (50 μg/ml) for selection of transgenic plants. The media needed to be adjusted to a pH 5.7. The seeds were kept at 4°C for 2 to 3 days and were transferred to 20-24°C for 10 days for further germination. Seedlings were transplanted to pots with soil (Sungro Horticulture propagation mix, Premium Horticultural Supply, Louisville, KY, cat no.5232601). The environment for the growth of Arabidopsis was at 22°C, with 68% relative humidity (RH), light intensity 120 μmol m⁻² s⁻¹ and with a 16 h / 8 h day/night cycle.
Floral Dipping Transformation of *Arabidopsis thaliana* Mediated by *Agrobacterium*

The transformation was based upon the protocol of Zhang et al, (2006). After confirming, via PCR and DNA sequencing, that the *Agrobacterium tumefaciens* strains carried genes of interest, a single *Agrobacterium* colony was inoculated into 5 ml of liquid LB media containing kanamycin for selection and the bacterial culture was incubated at 28°C for 2 days. The 5 ml of bacterial culture was poured into a flask with 500 ml of liquid LB media containing kanamycin, and the culture was incubated 28°C for 24 hours until it reached the stationary phase in which absorbance at 600nm (OD600) was between 1.5 and 2.0. *Agrobacterium* pellets were collected after centrifugation at 4000 x g for 10 minutes at room temperature and were suspended in 500 ml of 5% sucrose solution. Before transferring the bacterial suspension to a 500 ml beaker, 100 µl of Silwet L-77 was added to the bacterial suspension to reduce the surface tension (Silwet L-77 concentration was 0.02%). The 500 ml of bacterial suspension was ready for floral dipping. However, based on our lab experience it was very difficult for the 500 ml of *Agrobacterium* culture to reach OD600 1.5-2.0 even after a 26-hour incubation at 28°C. We modified the protocol; we prepared two sets of tubes, each contained 5 ml of liquid LB media and was inoculated with an *Agrobacterium* colony (1 colony per 5 ml LB). After incubation at 28°C for 2 days, the two tubes containing *Agrobacterium* culture were separately poured into two flasks with 500 ml of liquid LB media (5 ml bacterial culture per 500 ml LB). Bacterial pellets collected from the two flasks were combined into 500 ml of
5% sucrose solution.

Four-week-old *A. thaliana* showing 20-30 inflorescences in pots were prepared to be transformed. Siliques were clipped off before the floral dipping process. We inverted the pots and immersed the aerial parts of plants in the 500 ml of the *Agrobacterium* cell suspension no more than 10 seconds. The dipped plants were covered with plastic bags to maintain the high moisture rate over night. The bags were removed the next day and plants were grown in the growth chamber for a month, then seeds were collected to continue crosses leading to T3 generation plants homozygous for the trans gene(s). Such plants were subsequently tested for segregation of the *trans* gene alleles.

**Test of Mendel's Law of Segregation**

The test of Mendel's law of independent segregation is a conventional approach to determine whether the transgenic *Arabidopsis* lines are homozygous for the transformed *trans* genes. *A. thaliana* that underwent *Agrobacterium* transformation was considered the T0 generation. The T0 plant yielded seeds which were the T1 progeny. These seeds of T1 progeny were spread onto agar containing MS medium and kanamycin. The antibiotic was used to select seedlings successfully transformed with the gene construct MVLG_0512 ΔSP-CFP, MVLG_06175 ΔSP-mCherry, and MVLG_06175-mCherry. Without the kanamycin resistance gene encoded in the pRI vector, the kanamycin sensitive seedlings usually do not grow well in media and become bleached. In contrast, seedlings homozygous or heterozygous for the *trans* gene and seedlings with
multiple insertions of the genetic construct are usually green and flourishing on the media.

The green T1 progeny were transplanted to soil for further growth, and they yielded seeds of T2 progeny. Around one hundred seeds of T2 progeny from a single T1 green plant were placed in MS media with kanamycin. Although homozygous and heterozygous *Arabidopsis* strains as well as strains with multiple insertion of gene constructs were all kanamycin resistant, their seedlings would show different ratios in kanamycin resistance. Since *Arabidopsis* is a species capable of self-pollination, the progeny of a heterozygous strain will have 75% kanamycin-resistant seedlings and 25% kanamycin-sensitive offspring, while a homozygous strain and a multiple-inserted strain will show 100% kanamycin-resistant seedlings. The seedlings showing 100% kanamycin-resistance were discarded, and the 75% kanamycin-resistant green seedlings were selected and transferred to soil for growth. These green seedlings are by Mendel’s law either homozygous or heterozygous. They yielded seeds of the T3 progeny. Because the T3 offspring batch was out of either heterozygous or homozygous plant strain, not out of the multiple-inserted one, the 100% kanamycin-resistant green seedlings of the T3 progeny were considered homozygous and would progress to the subsequent DNA and RNA extraction.

**Plant DNA Extraction**

Leaves of two-week-old *Arabidopsis thaliana* were collected and ground in 1.5 ml microcentrifuge tubes with small plastic pestles. 400 µl of extraction buffer
(1M Tris-HCl with pH 7.5, 5M NaCl, 0.5M EDTA with pH 8.0, 10% SDS) was added in each microcentrifuge tube. The plant tissues and extraction buffer were vortexed for 5 seconds and centrifuged at 14000 rpm for 5 minutes. 300 µl of the supernatant was transferred to a new microcentrifuge tube, followed by addition of 300 µl of isopropanol. The tubes were inverted several times to fully mix the isopropanol and supernatant, incubated at room temperature for 2 minutes, and centrifuged at 14000 rpm for 10 minutes. The supernatant was carefully removed without disturbing the pellet, then 400 µl of 70% ethanol was added in. After the pellet was dried, 50 µl of sterile distilled water was added to dissolve the pellet. Tubes carrying the dissolved pellet were heated at 95 °C for 3 minutes to denature any DNases and kill microorganisms. The concentration and quality of the extracted plant DNA was checked by a Nanodrop 2000™ UV-Vis spectrophotometer (Thermo Fisher Scientific, Waltham, MA).

**Plant mRNA Extraction**

The current study used Zymoclean kit (Zymo Research Corp., Orange, CA, USA) to conduct RNA extraction of the transgenic *A. thaliana*. Leaves of four-week-old plants were placed in frozen (-80 °C freezer) mortar, and liquid nitrogen was added to quick-freeze leaf tissues. Frozen pestle was used to grind the tissues into fine powder and the powder was transferred to a 2 ml microcentrifuge tube. 600 µl of Trizol was added into the tube, and the tube was vortexed for about 5 minutes. The tube was centrifuged at 14,000 rpm for 1 minute, and around 600 µl of supernatant was transferred to a new 2 ml
microcentrifuge tube without disturbing the cell pellets. 600 µl of ethanol was added into the new tube, and pipetting up and down was done gently to fully mix the supernatant and ethanol. Half of the mixture solution (600 µl) was transferred to a filter column in a collection tube, and then the filter column-tube set was centrifuged at 10,000 rpm for 1 minute. The flow through was discarded. The other half of mixture solution (approximately 600 µl) was transferred to the filter column. The filter column-tube set was again centrifuged at 10,000 rpm for 1 minute. The filter column, expected to contain the plant DNA, was transferred to a new collection tube. For description purpose the filter column and the new collection tube together is named the new filter column-tube set. After addition of 400 ml of wash buffer, the new filter column-tube set was centrifuged at 10,000 rpm for 1 minute, and flow through was discarded. 75 µl of digestion buffer + 5 µl of DNase enzyme was added into the filter column, and the new filter column-tube set was allowed to sit without disturbance on the table for 15 minutes, followed by centrifugation at 14,000 rpm for 1 minute. 400 µl of prewash was added into the filter column, and the new filter column-tube set was centrifuged at 10,000 rpm for 1 minute, and this process was repeated once time. 700 µl of wash buffer was added into the filter column, followed by centrifugation at 10,000 rpm for 2 minutes. After discarding the flow-through, the new filter column-tube set was centrifuged at 14,000 rpm for 1 minute. The filter column was transferred to a new 1.5 ml microcentrifuge tube, then the lid of tube was opened and stayed in hood for 10 minutes to evaporate ethanol. 50 µl of DNA-RNA free water was added into the filter column to elute the RNA, the 1.5 ml microcentrifuge tube was
allowed to sit without disturbance for 1 minute, and the tube was centrifuged at 10,000 rpm for 1 minute. This process was repeated with addition of another 50 µl of DNA-RNA free water (final total 100 µl of DNA-RNA free water).

qRT-PCR

The eluted plant mRNA was the template to synthesize the cDNA for use in qRT-PCR. For the synthesis of cDNA, oligodT primers and Superscript III cDNA synthesis kit (Invitrogen Corp.) were used. The cycle threshold values of the housekeeping gene *ubiquitin 10 (UBQ10)* was selected as the standardized index to normalize the RNA expression of the target genes in the study. 1x Power SYBR Green Mango Bio Eva-Green (Applied Biosystems) was the detector. The reaction was performed in an Applied Biosystems Step-One thermocycler. Primers to amplify sequences of *MVLG_06175, MVLG_05122, UBQ10, mCherry*, and *CFP* are listed in Table 3.3. PCR conditions is 95 °C for 10 minutes, followed by 95 °C for 15 seconds, and 60 °C for 1 minute. The total process is 40 cycles. Analysis of melting curve was performed at the end of each cycle to ensure the specificity of the reaction.
Table 3.3: Primers used for qRT-PCR to determine the expression of fungal effectors and fluorescent tags.

<table>
<thead>
<tr>
<th>Name</th>
<th>Sequence (5’ to 3’)</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>UBQ10 F (AT4G05320)</td>
<td>5’-GTTGGAGGATGGGCAGAAACTC-3’</td>
<td>Czechowski et al., 2005</td>
</tr>
<tr>
<td>UBQ10 R (AT4G05320)</td>
<td>5’-GGAGCCTGAGAACAAGATGAA-3’</td>
<td>Czechowski et al., 2005</td>
</tr>
<tr>
<td>qRT CFP F</td>
<td>5’-GCAAAGACCCCAACGAGA-3’</td>
<td>This study</td>
</tr>
<tr>
<td>qRT CFP R</td>
<td>5’-CCATGCCGAGAGGTCC-3’</td>
<td>This study</td>
</tr>
<tr>
<td>mCherry qRT F</td>
<td>5’-CACTACGACGCTGAGGTCAA-3’</td>
<td>This study</td>
</tr>
<tr>
<td>mCherry qRT R</td>
<td>5’-GTGGGAGGATGTCCAACT-3’</td>
<td>This study</td>
</tr>
<tr>
<td>MVLG6175 qRT F</td>
<td>5’-CAGGGGGAGGGGCAAACGA-3’</td>
<td>This study</td>
</tr>
<tr>
<td>MVLG6175 qRT R</td>
<td>5’-AGAGATTTAGAGAAAGAAACCAAC-3’</td>
<td>This study</td>
</tr>
<tr>
<td>MVLG5122 qRT F</td>
<td>5’-GGACCGCAAACGACGAC-3’</td>
<td>This study</td>
</tr>
<tr>
<td>MVLG5122 qRT R</td>
<td>5’-GTATGTTGCGAGGAGCCGAAG-3’</td>
<td>This study</td>
</tr>
</tbody>
</table>

**Fluorescence Images Taken by a Confocal Microscopy**

Roots of 2-week-old *Arabidopsis* line expressing *MVLG_06175 Δ SP-mCherry*, *MVLG_06175-mCherry*, and *mCherry* alone, leaves of 4 or 6-week-old *Arabidopsis* line expressing *MVLG_06175 Δ SP-mCherry*, *MVLG_06175-mCherry*, *MVLG_05122 Δ SP-CFP*, *mCherry* alone, and *CFP* alone, and their corresponding WT controls were observed by a confocal microscopy. Images were acquired by an Olympus Fluoview FV-1000 confocal coupled to an Olympus 1X81 inverted microscope, a PlanApoN 60× objective, and FV-10 ASW 2.1 software. A single channel scanning configuration was set up for the acquisition of mCherry (excitation 587nm, emission 610nm) and CFP (excitation...
458nm, emission 476 nm) using a 543 nm HeNe laser and a 458 line of argon laser, respectively. Scanning was set at a speed of 2 μs/pixel to acquire z-stacks of each visual field. Images are presented as either single plane images or stacked images.

**Botrytis cinerea Infection Assay**

The current study applied the protocol and data analysis of Liu et al. (2015) and Shouan Liu (2020) to infect *A. thaliana* expressing MVLG_05122ΔSP-CFP with *Botrytis cinerea*. The mycelium of *Botrytis cinerea* was inoculated in PDA media in 5 to 10 Petri dishes. After growth for 10 days, 10 ml of sterile water or 0.8% NaCl solution was added into each Petri dish. The spore and mycelium solution from the 5 to 10 Petri dishes was collected by pipet and was poured into a 50 ml falcon tube. The solution was vigorously mixed and filtered with three layers of sterile gauze. The solution, expected to contain mainly fungal spores, was collected in a new 50 ml falcon tube. The solution was centrifuged at 2000 rpm for 10 minutes, and the supernatant was removed. The pellet was resuspended with sterile water and diluted to the final concentration 2 x 10⁷ spores/ml, as determined by a spectrophotometer. The solution was then stored at -80°C.

Before inoculation, the fungal spore stock was thawed and diluted to 2.5 x 10⁵ spores/ml with Vogel buffer (1 L: 15 g of Sucrose, 3 g of Na-citrate, 5 g of K₂HPO₄, 0.2 g of MgSO₄·7H₂O, 0.1 g of CaCl₂·2H₂O, and 2 g of NH₄NO₃). Four-week-old transgenic *Arabidopsis thaliana* expressing MVLG_05122ΔSP-CFP or
expressing CFP alone as well as wild type Col-0 were subjected to infection. Plants were infected with two droplets of fungal spore solution (2 µl for one droplet) on the surface of a single leaf. For mock treatment, 2 droplets of Vogel buffer alone were applied on leaves of WT. Genomic DNA extraction from leaves was conducted three days after fungal spore inoculation. For qRT-PCR, the current study followed the protocol to apply the primers amplifying cutinase A of B. cinerea (CutA) and α-shaggy kinase of A. thaliana (SKII). Primers are listed in Table 3.4.

Table 3.4: Primers to amplify cutinase A and α-shaggy kinase in qRT-PCR

<table>
<thead>
<tr>
<th>Name</th>
<th>Sequence (5’ to 3’)</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>CutA Forward</td>
<td>5'-AGCCTTATGTCCCTTCCCTTG-3'</td>
<td>Gachon &amp; Saindrenan, 2004</td>
</tr>
<tr>
<td>CutA Reverse</td>
<td>5'-GAAGAGAAATGGAAAAATGGTGAG-3'</td>
<td>Gachon &amp; Saindrenan, 2004</td>
</tr>
<tr>
<td>SKII Forward</td>
<td>5'-CTTATCGGATTCTCTATGTTTGGGC-3'</td>
<td>Gachon &amp; Saindrenan, 2004</td>
</tr>
<tr>
<td>SKII Reverse</td>
<td>5'-GAGCTCCTGTTTATTTAACTTGACATACC-3'</td>
<td>Gachon &amp; Saindrenan, 2004</td>
</tr>
</tbody>
</table>
CHAPTER IV: DISCUSSION AND FUTURE STUDY DIRECTIONS

The major achievements of the study are the identification of the potential plant proteins interacting with our two fungal effector proteins in the \textit{S. latifolia}, and potential cellular locations where the protein-protein interactions may occur in the model plant \textit{A. thaliana}. These discoveries expand our understandings of fungal effector proteins’ roles in plant host during infection. By the yeast two-hybrid screening and DNA sequencing, the current study identified potential plant protein interactors CASPL2C1 and CSN5a/5b as potentially associated with formation of Casparian strip, and activities of Cullin-Ring E3 ubiquitin ligases, respectively. With respect to the potential locations of the protein-protein interactions in the plant tissues, the current study detected signals from fluorescence-tagged effector proteins at the tips of trichomes and root. Although these findings might reflect that the fungus is applying the effector proteins MVLG\_06175 and MVLG\_05122 to change plant vascular structure and immune system, additional studies such as Co-IP are needed to confirm the identities of the plant protein interactors in these tissues. To investigate whether our fungal effector proteins alter immune responses in the transgenic \textit{A. thaliana}, future studies need to select phytopathogenic species to which \textit{A. thaliana} is vulnerable. Furthermore, it is necessary to detect if MVLG\_06175 and MVLG\_05122 tagged with mCherry and CFP also demonstrated identical
fluorescent patterns in the roots and trichomes in the original plant host, *S. latifolia*.

The discussions of Chapter II and III emphasize plant structures and proteins which are related to pathogen resistance and are potentially affected by the protein-protein interactions, including Casparian strip, trichome, the non-expressor of pathogenesis related (NPR) transcriptional coactivator, and the R receptor. However, the plant protein CASPL2C1 and CSN5a/5b may have other functions which fungal effector proteins could manipulate. In the introduction of Chapter III, it was mentioned that the Cullin 1 and Cullin 3-RING E3 ubiquitin ligases could function as regulators in the synthesis of plant hormones including auxin and ABA. I will review other possibilities in the following section.

**Other Structures and Mechanisms Regulated by Plant Protein CASPL2C1**

Studies specifically on CASPL2C1 are absent, but there are studies to elucidate the roles of Casparian strip membrane domain-like proteins (CASPLs) in plants. The functions of CASPLs are mainly associated with the development and differentiation of specialized cell wall structures, and these special cell walls could be applied to isolate the pathogens in the plant tissues, to regulate water transportation in the roots, and to facilitate starch granule accumulation.

As homologues of CASPs, CASPLs might form a protein scaffold for lignin deposition to form the Casparian strip, too. However, the polymerization of lignin on the protein scaffold guided by CASPLs could also occur at the pathogenic infection sites in order to isolate microorganisms. Fluorescence-labeled
CASPL4D1 (a homolog of CASP4) and CASPL1D1 (a homolog of CASP1) were found to gather and encircled the bacterial infiltration sites of *Pseudomonas syringae*. Disruption *CASPL4D1* and *CASPL1D1* in *A. thaliana* significantly increased colony forming units of *P. syringae*. The study outcomes suggested that the deposition of lignin on the protein scaffold could be applied to isolate the pathogen at the infection sites (Lee et al., 2019). From the point of view of phytopathogens, mitigating the functions of *CASPL4D1* and *CASPL1D1* by effector proteins, if they exist, would help the spreading of pathogens in *A. thaliana*.

*CASPLs* might contribute to the regulation of water transport in the roots, and by protein-protein interaction the fungal effector proteins could change the water flow. After the deposition of lignin between adjacent endodermal cells, the second stage of Casparian strip formation occurs as suberin lamellas deposit between the cell wall and cell membrane of the endodermal cells. The hydrophobic suberin restricts the diffusion of water and solutes in the apoplastic route, and all molecules need to pass the plasma membrane of endodermal cells to enter the vascular cylinder (Barberon et al., 2016; Niko Gelder, 2013; Meyer & Peterson, 2013). Additionally, water permeability at plant roots is constantly adjusting to the environment. Aquaporins are plasma membrane intrinsic proteins (PIPs) responsible for water transport, and the *A. thaliana* genome encodes 39 homologous PIPs. Studies applying immuno-purification approach and quantification by mass spectrometry (IP-MS) identify plant proteins potentially interacting with PIPs. Of all protein interactors, four candidates belong to
CASPLs. They are CASPL1B1, CASPL1B2, CASPL1D1, and CASPL1D2 (Bellati et al., 2016; Champeyroux et al., 2019). Furthermore, CASL1B1, CASPL1B2, and CASPL1D2 are expressed only in endodermal cells which are suberized, indicating they could participate in the suberization of endodermis and transportation of water (Champeyroux et al., 2019). Although it is not clear if *M. lychnis-dioicae* utilizes effector proteins to interact with CASPL protein families associated with water flow, the modification of suberin polymerization in the second stage of Casparian strip formation might benefit the development of fungal hyphae in the plant.

Functions of CASPL2C1 might not be limited to those that are cell wall-related. Sapkota et al., 2020 conducted a bioinformatic study looking for genes correlated with starch granule content and accumulation in sorghum. The researcher identified the genomic regions by comparing the single nucleotide polymorphisms in the sorghum gene database, and one of the regions was a Casparian strip membrane protein (CASP)-like protein gene (*Sobic.008G111500*) in chromosome 8. The study result suggests the potential effects of CASPLs on plant nutrient and growth. Since phytopathogens acquire nutrients from host plants, starch accumulation could be an important metabolism to modify by effector proteins.

**Other Structures and Mechanisms Regulated Plant Protein CSN5a/5b**

As Chapter II describes, protein ubiquitination by Cullin-RING E3 ubiquitin ligases and degradation by 26S-proteosomes regulate plant development and
defensive responses including the oxidative burst, hormone signaling, and programmed cell death (PCD) (Trujillo & Shirasu, 2010; Seo, Song, Chung, & Lee, 2013). To manage the metabolism, genome of A. thaliana encodes more than 1400 different E3 ligase components, and as many as 6% of the A. thaliana proteome is associated with the protein removal (Vierstra, 2009). The significant band structure of CFP at the base of trichomes is perhaps the most interesting finding in the current study (Figure 3.8 and 3.9). It leads to the speculation that MVLG_05122 is modifying trichome development by interacting with CSN5a, and this may be relevant to fungal infection. Nonetheless, trichomes also provide plants protection against abiotic stress including UV light, water evaporation, and drastic temperature changes (Karabourniotis, Liakopoulos, Nikolopoulos, & Bresta, 2020). We cannot exclude the possibility that MVLG_05122 is involved in the abiotic responses of the plant host, and CSN5a is actually found to be associated with plant responses against environmental abiotic stimuli such as salt and heat in the laboratory studies described.

CSN5a was found to be an intermediate regulator in salt stress responses in A. thaliana. The plant carries the gene Salt-Responsive Alternatively Spliced Gene 1 (SRAS1) which encodes a RING-type E3 ligase. This gene has two splicing variants, SRAS1.1 and SRAS1.2. The study by Zhou et al., 2021 demonstrated high level of salt stress caused a higher expression in SRAS1.1 and a lower one in SRAS1.2. The study also showed that overexpression of SRAS1.1 resulted in greater tolerance to salt stress while that of SRAS1.2 resulted in reduced tolerance. As a RING-type E3 ligase, SRAS1.1 facilitated the
ubiquitination and degradation of CSN5a by the 26S proteosome. In contrast, 
SRAS1.2 decreased the degradation of CSN5a by competing with SRAS1.1.
Although the mechanism whereby cellular CSN5a concentration influences plant 
salt stress is not yet clear, it is intriguing that as a regulator of Cullin-RING E3 
ubiquitin ligases, CSN5a is also a protein target to be labeled with ubiquitin for 
further degradation with respect to plant salt tolerance. The interactions between 
MVLG_05122 and CSN5a might also influence the responses of plant salt stress 
if the protein-protein interactions alter the ubiquitination rate of CSN5a. In a 
different study on the influences of temperature on plant growth, A. thaliana 
CSN5a-1 mutant shows growth retardation and is unable to fully remove the 
Nedd8 protein from the Cullin subunit of E3 ligases. However, with the heat 
stress treatment the mutated plant strain exhibited enhanced growth caused by a 
rearrangement of auxin homeostasis, due to an increase in deneddylation of 
Cullin 1-RING E3 (CRL1) ubiquitin ligase. Because heat treatment in the CSN5a- 
1 mutated plant leads to the subsequent inhibition of CRL1 enzymatic activity 
and protein ubiquitination, a process identical to the deneddylation of CRL1 
caused by CSN5a in the WT plant, it is possible that the inhibition of CRL1 by 
CSN5a is associated with the temperature-related auxin concentration changes 
and plant development (Singh et al., 2019).

In sum, the book “The Structure of Scientific Revolutions” by Thomas Kuhn, 
an American physicist and philosopher, indicated that progress or revolution 
occurring in science was caused by “a paradigm shift”. Each scientific era has its 
dominant paradigm, and the protein-protein interactions between
phytopathogens and plant hosts could be a new candidate for such a paradigm shift.
REFERENCES


Casparian strip formation in the endodermis. *Nature*, 473(7347), 380-383. doi: 10.1038/nature10070


APPENDIX

Yeast-Two Hybrid Sequencing and BlastX Results Showing MVLG_07305

1. I162
5'—
CGGCGAAGGAGAGATTTTCGGGGGGAAGATACTACGCAACAAAGAAAAAGACC
GTGCTGACGGACCGCCTGTCTGTTCTGGGTGCGGCCTCCCGGATCGGCAGCGAC
GTTCACCACCTGTTGACCGCTGGTGATGCTGCTTCCGAGGACCAG
CGAACCCTLTTGTCTAGCGCTGTTCCTTGAAAGCGTGTGAATATACAGAAGAG
GAAAGATGATGTGTTGTGATTCCTGTTTTTTATTATTTTTTTTTTTGTATTTTTACT
TTTTTTTTTTTTTGCTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTT
2. K58

5'—
GAAAGAGGGGCCCCCTCTGGGAGATAATCTCAATGTAAAAAAAGGAGAGAAAAAGATGCGCAGCCGACAGGACCCAGCCCTGTTCGGGGGCGCCCGGCGTCCCTCGGAGGCCGGCAGCGAAGGGACAACCACGTGGTGACGCGGGGGTGGAGGCCGATGCCGCCTAGGGACCAGCGAACCCCGGCCTAGCGCTGTACCAGGAAAAGCGTGATGAAGAACATAATAGGAAAGATGATGTCTCGAGAATCTTGAAGATGATTATGTCGATGATGACTC—3'

Query Sequence

<table>
<thead>
<tr>
<th>Summary of Hits by Genome</th>
</tr>
</thead>
<tbody>
<tr>
<td>M. violaceum mito</td>
</tr>
<tr>
<td>M. violaceum nuclear</td>
</tr>
</tbody>
</table>

[Show All Alignments] [Hide All Alignments] [View Raw Output]

<table>
<thead>
<tr>
<th>Target</th>
<th>Score (Bits)</th>
<th>Expect Length</th>
<th>Alignment Length</th>
<th>Identities</th>
<th>Positives</th>
</tr>
</thead>
<tbody>
<tr>
<td>M. violaceum nuclear: MVLG_07305.1: hypothetical protein</td>
<td>45.0542</td>
<td>3.19741E-06</td>
<td>50</td>
<td>24</td>
<td>27</td>
</tr>
</tbody>
</table>

Reference


3. L20

5'—
GGGAGATAAAGGTTTATCTGGGGTTAACGAAACTCATGCTTAAAAAGGAAAGAAGGACTCGACGACCAGGACCCCGCTGTCGGGGCACCACCTCAGGAGCCCGATGCCGCCATCTACGGTACAACCACGTGGTGACGCGGGGGAGGGAGGCCGATGCCTCGAGGAACCCCGGCCTAGCGCTGTACCAGGAAAAGCGTGATGAAGAACAGAAGGAAAGATGATGTCGCGAGAATCCTGAAGATGATCAGTGTCAAAGATGACACACACGGGCAACCGCATCCTTGTGCTCAAAGACGGCTACGCTTCGGCAAGGACCGGGCCACCTCCCTGCGCTTCAGCGCTCTCAATGCAACGGAACCAATCGCCGACACGTCTCGAGGCGGGTACGCTGACTCAAGAGGGGAGGGATGCTCCGTACCTGTCGCCCGACACCTTGTTGTGTTTTAACCTGCCGACAGGAGTTTACCTC

107
CCGCCGAGCCCCAGATACGCTCCAATTCATCCATGGAAATTCGACACCAGA
ATCGATAGCCAAAGCGGTAACACCCGAATTCAAAGTTCGTGGTGC
TGCGCTGGGCAGCTGGGCGCTTTTGACCCACGCACGCCACGCGACC
AACGAAATCGAGTGGAAACGCTCCCTTGGTCAGCGTCTACA
CTTCGTTCAAACGGTCTCTAAACTGCTTTCATATTGATCTAATGGCCA
ATTCTCCTATCAGACACTTCTGGAACCAACCTGCAAGGGATTGTCCGCA
AGCCTTACCGGAGCGGACCATAAGTGGGCATTTCTCACTCA—3'

Query Sequence

Summary of Hits by Genome

M. violaceum mito 0
M. violaceum nuclear 3

[Show All Alignments] [Hide All Alignments] [View Raw Output]

<table>
<thead>
<tr>
<th>Target</th>
<th>Score (Bits)</th>
<th>Expect Length</th>
<th>Alignment Length</th>
<th>Identities</th>
<th>Positives</th>
</tr>
</thead>
<tbody>
<tr>
<td>M. violaceum nuclear: MVLG_07340.1: hypothetical protein</td>
<td>61.6178</td>
<td>1.9307E-10</td>
<td>33</td>
<td>28</td>
<td>30</td>
</tr>
</tbody>
</table>

Reference


4. B52

5'—
CCGTGGGATTCCATAACGACGTACCAGATTACGCTCATATGACAAGTTTTGTAC
AAAAAAAAAGTTTGCCACCTAAAGCGCAGAACGTAATACGTAATACATCTCAACT
GCTATACAGCTAAACGACCCGCCAGTGCCCAGGACCAGCCCGCTTGGTCGGGC
CGCGCCCTCCCGGAGCAGCGCAGCCGAGGCAAACCCACGCCGACGGTAGTGGTCGCCG
GGCGGAGGCGATGGCGGCGAGGACCAGAACCACCGGCTTAGCGCC
GTACCAGGAAAGCGTGATGAAGAAGAACGAGGAAAGATGATGTCGCGAG
AATCCTGAAGATGATCATCAGTGTGAGATGACAGACACACCAGGGCACCGCAT

108
CCTTGTGCTCAAAGACGCACTCGGCTTCGGCAAGGACCGGGCCACCTCCTCACGCTTTACAGCAGCTCTCAATGCAACGAAACCAATCGCCGACACGTCTGGTGCTCGAGGTTGCTCCGTACCCTGTCGCCCGACACCTTGTTGTGTTTTAACCTGCAGGAGTTATCCTCCCGCCCGAGCCCCAGAATACGCTCCAATCATCCATGGAGTCGCACCAGATCGATCAGCCAAAGCGGTAACACCCGATTCAAGTTCGTGCTCGCTCGCTGCAGCGCAGTGCTGCTCGCTGGAGCGCCCGGCCCTCCGGAGCCGGCAGCGACGGGACAACCACGTGGTGACCGGGGGCGGAGGCCGATGCCGCCGAGGGACCAGCGAACCCCGGCCTAGCGCTGTACCAGGAAAAGCGTGATGAAGAACAGAAGAGGAAAGATGATGTCGC

5'—B149

5'—

TCCCCCTCCTGCTAAAAACCCTCTCCGTACCTTTACGCTCATATGAAAAAGTTTG
TACAAAAAGTTTGACACCCTTTATTCCAGAAACGCTAACATCAATACATCG
ACTGCTATACCGATCGACCGAGGCCGGACCGGACCCGGCTTCCCG
GGCCCGCGCCCTCCGGAGCGCGCGAGCGACGAGGAACCCACGCTTGAGCC
CGGGCGCGGGCGGTGGGCAGCGAGCGGACCGAACCACCACGAGCACCAG
GCTGTACCAGGAAAAGCGTGAAGAAGACGAGAAAGATGATGTGGC
GAGAATCCTGAAAGATGATCGAGTGTCAAGATGACAGACACACAGGAGGCACCCCG
CATCCTGTGCTCAAAGACGCACTCGGCTTCGGCAAGGACCGGGCCACCTCCTCA

Query Sequence

Summary of Hits by Genome

<table>
<thead>
<tr>
<th>Genome</th>
<th>Score (Bits)</th>
<th>Expect Length</th>
<th>Identities</th>
<th>Positives</th>
</tr>
</thead>
<tbody>
<tr>
<td>M. violaceum mito</td>
<td>149.443</td>
<td>1.84056E-37</td>
<td>102</td>
<td>79</td>
</tr>
<tr>
<td>M. violaceum nuclear</td>
<td>98.2117</td>
<td>4.8661E-22</td>
<td>59</td>
<td>49</td>
</tr>
</tbody>
</table>

Reference

CCTGCCTTCAGCGCTCTCAATGCAACGAAACCAATCGCCGACACGTCTGGTGCCGTTCCTGAAAGTTCTCGACGCTTCTGACGAGGTGCTACTGAGGTGCTCTGACTGCCTGTAATCCTGCGCCAGACCGTCTGGTGCCGTTCTGGAAGTCACGTTCAAACACACGCACGCGAGAAGTCCGAGGTG
TACTCGAAGGTGCTCCTCGATCCCTGTCGCCCGACACCTTGGTTGTGTTTTAACCCTGCCGCAGAGTCTCCCTCCCGCCGCCAAGAGATACGCTTATCATCATGAGTCGCAGCAGAATCGATCAGCCCAAAGCGGTAACACCCGATTCAAGTTCCGTGGTGCGCTGCGCTTGGCGCAGTGGCCGCTTTGACCACGTCCGACGACGCGTCCACCAAGATCGAGTGGAAACGCTCCCTTTGCCCAAGTCCGGAAATGCGCTTTCGTTCAAAACGTCTTTCGAACTGCGTCTTTCGAAACTGCCTCCATTAT

6. E125

5’—
GGGTGGGGTCCCATACGACGTACCACATATTACGCTCATATGACAAGTTTGTACAAAGTTGGCCACGAAACGGCAAACGTCAATTTTTCGAATACATCAACTGC
TATTCCAGTAAACGACCGAGGCGACCTCGCTTCCCGGGCCGCAGCGGACCTACGCTGCTCCGCAGGAAAAGCGTGATGAAGAACAGAAGAGGAAAGATGATGTCGCGAGAA

Reference
TCCTGAAGATGATCAGTGTCGAAGATGACACACACCAGGGCACCCGCATCC
TTGTGCTCAAAGACGCACTCGGGTTTCGGCAAGGACCGGGCCACCTCCCTGC
GCTTTTTTTTTTTTTATGCAACGAAACCAATCGCCGATTTTTTTTTTGTGCC
GTTCTGGAAGGTTTTTTGTCTATCTC—3'

**Query Sequence**

**Summary of Hits by Genome**

<table>
<thead>
<tr>
<th>Target</th>
<th>Score (Bits)</th>
<th>Expect Length</th>
<th>Identities</th>
<th>Positives</th>
</tr>
</thead>
<tbody>
<tr>
<td>M. violaceum mito</td>
<td>0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>M. violaceum nuclear</td>
<td>2</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

[Show All Alignments] [Hide Alignments] [View Raw Output]

7. F161

5’—
GGGGGGGTCAAATACGAGTACCGATATTACGCTCATATGACAAGTTTGTACA
AAAAAGTTTGCCACGCATAGAAGCAAAACGTCATTCATGAAATACATCAACTG
CTATACCAGCTAACCAGCCGGCATTGCGCCGGGACCAGGACCCGGCTGTTCCCGGC
GCGGCTCCGAGGCCGGCCGAGCGGGAGGCACCGGTGACGCGGGCAGG
GGGCGAGCCGGATGCGCGCGAGGGACCAGCGAACCACCAGGGCGCTAGCGCTG
TACCAGGAAAAGCGTGATGAAAGAAGAAAGAGAAACGATGATGCACGAGAGA
ATCCTGTGATGATGTCATTTGTCAGAGATGACACACACCAGGGCACCGCTTCC
TTGTG—3’
### Query Sequence

<table>
<thead>
<tr>
<th>Summary of Hits by Genome</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>M. violaceum mito</td>
<td>0</td>
</tr>
<tr>
<td>M. violaceum nuclear</td>
<td>1</td>
</tr>
</tbody>
</table>

[Show All Alignments] [Hide All Alignments] [View Raw Output]

<table>
<thead>
<tr>
<th>Target</th>
<th>Score (Bits)</th>
<th>Expect</th>
<th>Length</th>
<th>Identities</th>
<th>Positives</th>
</tr>
</thead>
<tbody>
<tr>
<td>M. violaceum nuclear: MVLG_07305.1: hypothetical protein</td>
<td>62.003</td>
<td>2.47665E-11</td>
<td>57</td>
<td>34</td>
<td>36</td>
</tr>
</tbody>
</table>

### Reference


8. **H103**

5'—

GGGTGGGGTACATACGACGTACCATTATAGCTCATATGACAAGTTTGTTGTACAAA
AAAAGTTTGCCCAAGCAAAAGAAAACGTCTTACATCGAATACATCAACTCAACTCTGTA
TACCAGCTAACGACCAGTGCCCGACCAGGCACCACCACCCTGTTTCCGGGCCGCG
GGCCCTCCCGAGCCGGCAGCGACGGGACAACCGCTGTTGACGCGGGGG
CGGAGGCGATGCCCGAGGGAGCCAGCGACCGAGCCCGCCGCGCTAGCGCTGTAC
CAGGAAAGCGCTGATGAAGAAACGAAAGGAAAGATGATGTCGAGAATAC
CTGCTCAAGACGCATCCTGTTTCGGCAAGGACCAGCGGGCCACCCTCCTGCTTT
TTTTTTTTTTTCATATGCAACGGAAACCAATCATTTTTTTTTTTTGTTGCTTTTCTGA
CTCTCTCTGCTTTTT—3'
9. H146

5'—
GGTGGGATAACATACGACGTACCTGATTACGCTCATATGACAAGTTTGTACAA
AAAAAGTTGGCCAAAAAAACTCGAGACGTCATACATCGCATAATCAGACATCAACTGCTA
TACCAGCTAACCGGATGCCACCAGGGCACCAGCGCCTGTTCCGGGCC
GGCCCTCCGGAGCCGGACACGGAGACAGCGGCAACCGGGACCAGCGAACCCCGGCCTAGCGCTGTAC
CAGGAAAAGGTGATGTAAGAACAGAAGAGGAAAGATGATGTCGCGAGAATCTGAAGATGATCAGTGTCGAAGATGACACACACCAGGGCACCCGCATCCTG
GTGCTCAAAGACGACTCGCTTGGTGGCAAGGACCAGGGCCACCTCCCTGCG
CTTCAGCGCTCTCAATGCAACGAAACCAATTTTTTTTCGTCTGGTGCCGTT
CTGGAAAGT—3'

Query Sequence

Summary of Hits by Genome

<table>
<thead>
<tr>
<th>Genome</th>
<th>Score (Bits)</th>
<th>Expect</th>
<th>Alignment Length</th>
<th>Identities</th>
<th>Positives</th>
</tr>
</thead>
<tbody>
<tr>
<td>M. violaceum mito</td>
<td>0</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>M. violaceum nuclear</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Reference

Query Sequence

Summary of Hits by Genome

<table>
<thead>
<tr>
<th>Target</th>
<th>Score (Bits)</th>
<th>Expect</th>
<th>Alignment Length</th>
<th>Identities</th>
<th>Positives</th>
</tr>
</thead>
<tbody>
<tr>
<td>M. violaceum nuclear: MVLG_07305.1:</td>
<td>115.931</td>
<td>1.47567E-27</td>
<td>84</td>
<td>62</td>
<td>65</td>
</tr>
<tr>
<td>hypothetical protein</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>M. violaceum nuclear: MVLG_07215.1:</td>
<td>74.7146</td>
<td>3.77005E-15</td>
<td>42</td>
<td>38</td>
<td>39</td>
</tr>
<tr>
<td>hypothetical protein</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Reference


10. A166-2

5’—

GGACATGTATACATACGACGTACCAGATTACGCTCATATGACAAGTTTGTACAA
AAAAGTTGGCAATTACCTCTGCAAACGTCAATAAACATGGAATACATCAACTGC
TATAACCAGCTAACCGGCGCTGCGCTGGCGCAGTGGCGCTTGACCAGTCACGACGCGG
CGACCAAGATCGAGTGGAAACGCTCCTTTGCCCAAGTCCGCAAGCGCTTGGCGTTCGTCAAAAACGTCGTCGAACTGCGTTTCATTAT
CAGTCCTAAAGGCCGAAATACACGAACGACGACCTCTGGAACAAACTGCAAGGCATTGTCGCCCAAG

114
CTTACCGGGGACGGG—3'

Query Sequence

115

11. B139-2

5’—

GGAACGTGCGATACCATAACGACGTACCAGATTACGCTCATATGACAAGTTTGTACAAAAAGTGGCCAATGACTGTGCAAACGTCAATACATCGAATACATCAACTGCTATACCAGCTGACCCGACCAGGAAGCCCGCAGCGCGACCCCGCTAGCGCCTGTACCAGGAAAAGCGTGATGAAGAACAGAAGAGGAAAGATGATGTCGCGAGAATCCTGAAGATGATCAGTGTCGAAGATGACACACACCAGGGCACCCCGCTCTTGTGCTCAAAGACGCACTCGGCTTCGGCAAGGACCGGGCCACCTCCGCGCTTCAGCGCTCTCAATGCAACGAAACCAATCGCCGACACGTCTGGTGCGGTTCTGGAAGTCACGTTCAAACACACGCACGCGAGAAGTCCGAGGTGTACTCGAAGGTGCTCCGTACCCTGTCGCCCGACACCTTGTTGTGTTTTAACCTGCCCGCAGGAGTTATCCTCCCGCCCGAGCCCCAGAATACGCTCCAATCATC

Summary of Hits by Genome

<table>
<thead>
<tr>
<th>Genome</th>
<th>Score (Bits)</th>
<th>Expect</th>
<th>Alignment Length</th>
<th>Identities</th>
<th>Positives</th>
</tr>
</thead>
<tbody>
<tr>
<td>M. violaceum mito</td>
<td>0</td>
<td>108</td>
<td>80</td>
<td>85</td>
<td></td>
</tr>
<tr>
<td>M. violaceum nuclear</td>
<td>8</td>
<td>35</td>
<td>31</td>
<td>26</td>
<td>28</td>
</tr>
</tbody>
</table>
CATGGAGTCGCACCAGAATCGATCAGCCAAAGCGGTAACACCCGATTCAAG
TTCTGGTGTCGCTGCGCTGCGCTGCGCTGCGCTGCGCTGCGCTGCGCTGCGCTGCGCTG
GGCGACCAAGATCGAGTGAAACGCCTCTCTTGCCAAAGTGCCGCAAGCGCTT
GGCGTTCGGTCAAAACCGTGGTCGAACTCGGTTCATTTCATGCTCTTAAGGCC
GAATACACGAACGACGACCTCTGGAACAAACTGCAAGGCATTGTCGCCAG
CTTACCAGGGACGCCCACAAGTG—3’

### Query Sequence

<table>
<thead>
<tr>
<th>Summary of Hits by Genome</th>
</tr>
</thead>
<tbody>
<tr>
<td>M. violaceum mito</td>
</tr>
<tr>
<td>M. violaceum nuclear</td>
</tr>
</tbody>
</table>

[Show All Alignments] [Hide All Alignments] [View Raw Output]

<table>
<thead>
<tr>
<th>Target</th>
<th>Score</th>
<th>Expect</th>
<th>Alignment Length</th>
<th>Identities</th>
<th>Positives</th>
</tr>
</thead>
<tbody>
<tr>
<td>M. violaceum nuclear: MVLG_07305.1:</td>
<td>150.214</td>
<td>0.0</td>
<td>108</td>
<td>80</td>
<td>85</td>
</tr>
<tr>
<td>hypothetical protein</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>M. violaceum nuclear: MVLG_07305.1:</td>
<td>66.6254</td>
<td>0.0</td>
<td>35</td>
<td>31</td>
<td>33</td>
</tr>
<tr>
<td>hypothetical protein</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>M. violaceum nuclear: MVLG_07215.1:</td>
<td>56.9954</td>
<td>0.0</td>
<td>31</td>
<td>26</td>
<td>28</td>
</tr>
<tr>
<td>hypothetical protein</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>M. violaceum nuclear: MVLG_07215.1:</td>
<td>98.2117</td>
<td>8.30642E-35</td>
<td>59</td>
<td>49</td>
<td>52</td>
</tr>
<tr>
<td>hypothetical protein</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>hypothetical protein</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>M. violaceum nuclear: MVLG_07215.1:</td>
<td>36.965</td>
<td>8.30642E-35</td>
<td>32</td>
<td>18</td>
<td>21</td>
</tr>
<tr>
<td>hypothetical protein</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

12. C1
5’—
ATCAGTCCGATACCCATACGACGTACCAGATTACGCTCATATGACAAGTTTGTA
CAAAAAGTTGCTGCTACTGCAAAGTGCTCAATACATCGAATACATC
CTCGCTATACCAACGACCAGCCAGCCGAAACAGCCACCGGCTGCT
TCCGGGGCCGCCGCCCTTCGCGAAGCCGCGACGCCGACCGGAGACAA
CCAACGCTGGTGAGCAGCGGCGGGGCGGAGAGCGATGCGCCCGCGAGGG
GAACCCAGCAAGCCCGGCGCCTAGCGCTGTAACCAGGAAAAGCGTGTAG
AAGAACAGAAGATGGAAAGATGATGTCGCCGAGAATCCTGAAGATGATCAGTG

116
TCGAAGATGACACACACCAGGGACACCCCGCATCCTTGGTGCTCAAAGAACG
CACTCGGGCTTTTCGGCAAGATCAGGGCCACCTTCTCTGCGCTTCAGCGCTCT
CATGCAACGAACAAATTCCGCAACCACAGTCTGGTTGCGTTTCTGAAAGTCAC
GTTCAAAAAACCGCCAGCGAGAAGTCCGAGGTGTACTCGAAGGGGTCCGT
AACCTGTTGCGGCAACTTGTTGGATAACCGGCCGCAGGAATTATCTTTCCGC
CGAAGCCGAGAAAAGGG—3’

Query Sequence

Summary of Hits by Genome
M. violaceum mito 0
M. violaceum nuclear 2

[Show All Alignments] [Hide All Alignments] [View Raw Output]

<table>
<thead>
<tr>
<th>Target</th>
<th>Score (Bits)</th>
<th>Expect</th>
<th>Alignment Length</th>
<th>Identities</th>
<th>Positives</th>
</tr>
</thead>
<tbody>
<tr>
<td>M. violaceum nuclear: MVLG_07305.1: hypothetical protein</td>
<td>49.6766</td>
<td>2.37652E-7</td>
<td>114</td>
<td>46</td>
<td>53</td>
</tr>
<tr>
<td>M. violaceum nuclear: MVLG_07305.1: hypothetical protein</td>
<td>40.4318</td>
<td>1.44179E-4</td>
<td>122</td>
<td>44</td>
<td>52</td>
</tr>
</tbody>
</table>

Reference
Altschul, Stephen F., Thomas L. Madden, Alejandro A. Schaffer, Jinghui Zhang, Zheng Zhang, Webb Miller, and

13. D106
5’—
GATCAGCGTGATACGCGATACCAGATTACGCTCATATGACAAGTTTGCTACAA
AAAAGTTGGCCACCCTACCTCCTGCAAACGTCAATACATCGAATACATCAACTG
CTATACCAGCTAACGACCACCATGCGACGACCCGCACCCCGCTCTTCCGGGAC
GCGGCCCTTCCGGAGCAGCGAGCAGGGACAGGGACACCACGAGTGCTGAGCGCGG
GGCGGAGGCATGCGCGGAGGGACACCGGAACCCCGCCGCTACTGCTCCT
TACCCAGAAAAGCGTGATGAAGAACAGAAGAGGAAAGATGTACAGCTCAGA
ATCCTGAAGATGATCGTGAAGATGACACACACGAGGGACACGCAACCGCATC
CTTGTTGCTCAAAGACGCATCGGCTTTCCGGCAAGGACCCGACCCACGCCCTCCT
GCGCTTCACGCAGCTCTCAATGCAACGAAACCAATCGCCGCAGACGTCTGGTG
CGTTCTGAAGATGTCGGTTCAACACACGACGCGAGAGTCCGAGGGTAC
TCGAAGGTGCTCCGTACCCTGTCGCCCGACACCTTGTTGTGTTTTAACCTGC
CCGCAGGAGTTATCCTCCCCGCCCCGAGCCCACAATACGCTCCAATCATCCAT
GGAGTCGACCAGAATCGATCGACAAAAAGGTAAACACCCGATTCAAGTTC
GTGGTGCGCTCGCTGCGGTGACGGTCACGGTCCAGTCCACAGCAGCGGC
CCGACCAAGATCGAGTGGAAACGCTCTTTGCCCCAATGTCGGAAGGCTTTGG
CTTTCGTCAAAAACGTCGTCGGAACTGCGTTTCATTATCGATGTCCTAAGCCGATA
CACGAACAGACGACCTCTGGAACAAACTGCAAGGCAAGGATTTGTCGCGCAAAGCTAC—
3’

Query Sequence

<table>
<thead>
<tr>
<th>Target</th>
<th>Score (Bits)</th>
<th>Expect</th>
<th>Alignment Length</th>
<th>Identities</th>
<th>Positives</th>
</tr>
</thead>
<tbody>
<tr>
<td>M. violaceum nuclear: MVLG_07305.1: hypothetical protein</td>
<td>150.214</td>
<td>0.0</td>
<td>108</td>
<td>80</td>
<td>85</td>
</tr>
<tr>
<td>M. violaceum nuclear: MVLG_07305.1: hypothetical protein</td>
<td>66.6254</td>
<td>0.0</td>
<td>35</td>
<td>31</td>
<td>33</td>
</tr>
<tr>
<td>M. violaceum nuclear: MVLG_07305.1: hypothetical protein</td>
<td>56.9954</td>
<td>0.0</td>
<td>31</td>
<td>26</td>
<td>28</td>
</tr>
<tr>
<td>M. violaceum nuclear: MVLG_07215.1: hypothetical protein</td>
<td>98.2117</td>
<td>9.20653E-35</td>
<td>59</td>
<td>49</td>
<td>52</td>
</tr>
<tr>
<td>M. violaceum nuclear: MVLG_07215.1: hypothetical protein</td>
<td>36.965</td>
<td>9.20653E-35</td>
<td>32</td>
<td>18</td>
<td>21</td>
</tr>
</tbody>
</table>

14. E105
5’—
TCAGCCGCGTGGGTTACCATACGACGTACCAGATTACGCTCATATGACAAGTT
TGTAACAAAAAGTGGCAGGAAACTGCAAAACGTCAATACATCGAATAACATCA
ACTGCTATACAGCTAACGACCCGATGCCCGACCAGGCACCACAGCGCTTCCG
GGCCGCGGGCCTCCGAGCCCGCGACGCAGCGACGGGACAACCACGTTGTCGACG
CGGGGCGAGGGCCGATGCCGCCGAGGGACCGAACCACAGCGACCCGCGGCTAAGC

118
GCTGTACCAGGAAAAGCGTGATGAAGAACAGAAGAGGAAAGATGATGTCGC
GAGAATCCTGAAGATGATCAGTGTGAGAAGATGACACACACAGGGGACCCCG
CATCCTTGTGCTCAAAGACGCACTCGGCTTGCGCAAGGACCACCAGGCCACCTC
CCTGGCGTTTCAGCGCCTCTCAATTGCAAGAAGACCAATCGCAGACACGTCTGG
TGCCGTTCTGGAAGTCACGTTCGTTCAAACACACAGCGACGAGAAGTCGAGGTTG
TACTCGAAAGGTCTCCGTACCCTGTGTCGCCCGACACCTTTTGTGTTTGTGTTTAA
CGCCCGCAGGAGTTATCCTCCCCGCGCCCGAGGCCCCCCAGAATACGCTCTCAAATCAT
CCATGGAGTCGACCCAGAATGCTACGAGCAGCTAACCAACCGATTCAA
GTTCGTGGTGCGTCGCTCGCGTCAGTGGCCTTTGACCACGTCCACACGCG
CGGCCAGCAAGATCGAGTGGAAACGCTCCTTTGCCCAAGTCCGCAAGCGCT
GCGTTCGTCAAAAACGTCGTCGAACTGCTTTTATTATCATGCCTAAGG
CCGAATACACGAACGACGACCTCTTGGAACACTGCAAGGCAATGGTCGACAA
GCTTACCGGGGACGGCCACAAATGCGCGACTT—3'

**Query Sequence**

<table>
<thead>
<tr>
<th>Summary of Hits by Genome</th>
</tr>
</thead>
<tbody>
<tr>
<td>M. violaceum mito</td>
</tr>
<tr>
<td>M. violaceum nuclear</td>
</tr>
</tbody>
</table>

[Show All Alignments] [Hide All Alignments] [View Raw Output]

<table>
<thead>
<tr>
<th>Target</th>
<th>Score (Bits)</th>
<th>Expect</th>
<th>Alignment Length</th>
<th>Identities</th>
<th>Positives</th>
</tr>
</thead>
<tbody>
<tr>
<td>M. violaceum nuclear: MVLG_07305.1:</td>
<td>150.214</td>
<td>0.0</td>
<td>108</td>
<td>80</td>
<td>85</td>
</tr>
<tr>
<td>hypothetical protein</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>M. violaceum nuclear: MVLG_07305.1:</td>
<td>66.6254</td>
<td>0.0</td>
<td>35</td>
<td>31</td>
<td>33</td>
</tr>
<tr>
<td>hypothetical protein</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>M. violaceum nuclear: MVLG_07305.1:</td>
<td>56.9954</td>
<td>0.0</td>
<td>31</td>
<td>26</td>
<td>28</td>
</tr>
<tr>
<td>hypothetical protein</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>M. violaceum nuclear: MVLG_07215.1:</td>
<td>98.2117</td>
<td>8.30642E-35</td>
<td>59</td>
<td>49</td>
<td>52</td>
</tr>
<tr>
<td>hypothetical protein</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>hypothetical protein</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>M. violaceum nuclear: MVLG_07215.1:</td>
<td>36.965</td>
<td>8.30642E-35</td>
<td>32</td>
<td>18</td>
<td>21</td>
</tr>
<tr>
<td>hypothetical protein</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

15. E126
5′—
GATCATCGATACATACGACGTACCAGATTACGCTCATATGACAAGTTTGTACAA
AAAAGTTGGCCACTTACCTCTGCAAACGTCAATACATCGAATACATCAACTGC
TATACCAGCTAACGACCCGATGCCCCGCCGACGGCACCCTCTGTTCCGGGCCG
CGGCCCTCCGGAGCCGGAGCGACGGGACAACCACGTGGTGACGCGGGG
GCGGAGGCCGATGCCGCCGAGGGACCAGCGAACCCCGGCCTAGCGCTGT
ACCAGGAAAAGCCTGATGAAGAACAGAAGAGGAAAGATGATGTCGCGAGAA
TCCTGAAGATGATCAGTGCAGTGGAGTCACACACACCACACCAGGGCAACCCGCATCC
TTGTGCTCAAGACGCACTCGGTTCGGCAAGGACCGGGCCACCTCCTCG
CGCTTCAGCGCTCTCTCAATGCAACGAAGAAACAAATCGCGACACGTCTGGTGCC
GTTCTGGAAGTACGTCTCCTTTAAACACACGCACCGGAGAAATCGCCAGGTACT
CGAAGGTGCTCCCGTACCTGTCGCCGCCACACCTTGTTGTGTTTTAAACCTGC
CCGCGAGGTTATCTCCTCCGCAGGCCCCAGAATACGCTCTGGCAATCCCAT
GGAGTGCACCAGAATCGTGACCCAAAAAGCTGGAACCCCGATTCAAGGTT
GTGGTGCGCTGGCTGGCAGTGGCGCTTTGACCAGTCCAGCAGCGCG
CGACCAAGATCGAGTGGAAACGCTTCCCTTGCCCCAGTCCGCAAGCGCTTG
CGTTCGTCAAAAAAAAAAGTCGTCGGAACGTGGCTTTCAATACAGTCCTAGAAAGCGGA
ATACACGAACGACGCCACCTCTGGAAACAAACTGCAAGGATTGTCGCGACG
TACCAGGGACGGG—3'

Query Sequence

<table>
<thead>
<tr>
<th>Target</th>
<th>Score (Bits)</th>
<th>Expect</th>
<th>Alignment Length</th>
<th>Identities</th>
<th>Positives</th>
</tr>
</thead>
<tbody>
<tr>
<td>M. violaceum nuclear: MVLG_07305.1: hypothetical protein</td>
<td>150.214</td>
<td>0.0</td>
<td>108</td>
<td>80</td>
<td>85</td>
</tr>
<tr>
<td>M. violaceum nuclear: MVLG_07305.1: hypothetical protein</td>
<td>66.6254</td>
<td>0.0</td>
<td>35</td>
<td>31</td>
<td>33</td>
</tr>
<tr>
<td>M. violaceum nuclear: MVLG_07305.1: hypothetical protein</td>
<td>56.9954</td>
<td>0.0</td>
<td>31</td>
<td>26</td>
<td>28</td>
</tr>
<tr>
<td>M. violaceum nuclear: MVLG_07215.1: hypothetical protein</td>
<td>98.2117</td>
<td>8.30642E-35</td>
<td>59</td>
<td>49</td>
<td>52</td>
</tr>
<tr>
<td>M. violaceum nuclear: MVLG_07215.1: hypothetical protein</td>
<td>36.965</td>
<td>8.30642E-35</td>
<td>32</td>
<td>18</td>
<td>21</td>
</tr>
</tbody>
</table>

5'—
GAGCAGTCGATACCATACGACGTACGAGATTACGTACATGACTATGACAAAGTTGTA
CAAAAAAGTTGGCACGGCCTGCTGCAAAACGGCTAATACATCGGAAAACACTCAAC
TGCTATACCAGCTAAGCGACCAGCAGCCGACCGAGACGGACCTGCTGCTTCCGGG
CCGCGGCCCTCCGGAGCCAGCGACGGAGAACGCAAGCTGGGGACGCGAGCG
GGGGCGGAGGCCGATGCCCGACCAGGCGCCCTGCTGCTAGCACGT
TGACCAGGAAAAGCGTGACTGAAAAAAGAGAGAAAGATGACTGCGGGA
GAATCTGAGAGTACGTGTCGAGATGACACGACAGACCCAGCCACCCGCA
TCCTGTGCTCAAAGACGCAGCCTCAGCAGCTGACCCGGCAAAGACCAGGGGCACCTCC
CTGGCCTTCAGCGCTCTCAATGCAACGAAAACCAATCGCGCGAGACGTCTGGA
GCGTTTGGAAAGTCAGTTCAGTTGCAACACAGCACGCGAGAGTCCGAGGTGT
ACTCGAAAGGTGTCCGTACCCGACTCTGTCGCCCCGACACCTTTGTTGTTTTAACCT
GCCGCCAGGAGTTATCCTCCCGCCGCGCCGCCAGCCGAAATACGCTCAATCAG
CATGGAGTCCGCCCACAGCAGCCGAAAAGCGGTAAAAACAGCCAGTTCAAG
TTCTGTTGTCGCTGCTGCGCTGGGGCGACTGCGCCTGACCCAGCCACCGACGC
GGCGACCGAAGACGGTGGAGAGCTCCTCTGCTCTGGCAAGTCCCGAGGCGCTT
GGCGTTCTGCAAAAAACGTCGCTGACACTGCGTTTCTATTACGTCCTCAAAAGCC
GAATACACCGAAGCGCACCTCTGGAAACAAACTGCAAGGCTATTGTCGCAA
GCTTACCAGGGGACCGGGCA—3'
17. N53-1

5’—
GAACATCCATAACATAAGACGCATGCATTACGCTCATATGACAAAGTTTGTACAAAAAATTTGGCCACTCACTCTCTCTGCAAAACGTCAATACATCGAATACATCAACTGCTATACCAGCTAAGCAGCCGATGCCCCGACCAGGACCAGGCCTGTCTTCCGGGCCC
GCGGCCTTCGGAGCCGACGACGCAGGCGAACAACCAGCTGGTGACGCCG
GCCGAGGCCAGCGCCGGAGGAGCACCCAGCAACGGGCCCTGCTTCTG
AGTCCGAGGTGTAC
TCGGAGGTGCTCCGTACCCTGTCGCCCGACACCTTGTTGTGTTTACCTGC
CCGCAGGAGTTATCCTCCCGCCGGAGCCGAGTACCATCGCACCAGAATACGCTCCATCATCCATGGAGTCGCACCAGAATCGATCAGCCAAAGCGGTAACACCCGATTCAAG TTGGTGCGCTGCGCTGGCGCAGTGGCGCTTGACCACGTCCACGACGCGG

---

<table>
<thead>
<tr>
<th>Target</th>
<th>Score (Bits)</th>
<th>Expect</th>
<th>Alignment Length</th>
<th>Identities</th>
<th>Positives</th>
</tr>
</thead>
<tbody>
<tr>
<td>M. violaceum nuclear: MVLG_07305.1: hypothetical protein</td>
<td>150.214</td>
<td>0.0</td>
<td>108</td>
<td>80</td>
<td>85</td>
</tr>
<tr>
<td>M. violaceum nuclear: MVLG_07305.1: hypothetical protein</td>
<td>66.6254</td>
<td>0.0</td>
<td>35</td>
<td>31</td>
<td>33</td>
</tr>
<tr>
<td>M. violaceum nuclear: MVLG_07305.1: hypothetical protein</td>
<td>56.9954</td>
<td>0.0</td>
<td>31</td>
<td>26</td>
<td>28</td>
</tr>
<tr>
<td>M. violaceum nuclear: MVLG_07215.1: hypothetical protein</td>
<td>98.2117</td>
<td>7.9992E-35</td>
<td>59</td>
<td>49</td>
<td>52</td>
</tr>
<tr>
<td>M. violaceum nuclear: MVLG_07215.1: hypothetical protein</td>
<td>36.965</td>
<td>7.9992E-35</td>
<td>32</td>
<td>18</td>
<td>21</td>
</tr>
</tbody>
</table>

---

122
CGACCAAGATCGAGTGGAAACGCTCCTTTGCCCAAGTCGCAAGCGCTTGGCGTTCGTCAAAAACGTGCGAACTGCGTTTCATTATCGTCTAAGGCCGAATACACGAACGACGACCTCTGGAACAAACTGCAAGGGCATTGTCCGCGACGCGTTACCCGGGACGGGCCAC

**Query Sequence**

**Summary of Hits by Genome**

<table>
<thead>
<tr>
<th>Target</th>
<th>Score (Bits)</th>
<th>Expect</th>
<th>Alignment Length</th>
<th>Identities</th>
<th>Positives</th>
</tr>
</thead>
<tbody>
<tr>
<td>M. violaceum nuclear: MVLG_07305.1:</td>
<td>150.214</td>
<td>0.0</td>
<td>108</td>
<td>80</td>
<td>85</td>
</tr>
<tr>
<td>hypothetical protein</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>M. violaceum nuclear: MVLG_07305.1:</td>
<td>66.6254</td>
<td>0.0</td>
<td>35</td>
<td>31</td>
<td>33</td>
</tr>
<tr>
<td>hypothetical protein</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>M. violaceum nuclear: MVLG_07305.1:</td>
<td>56.9954</td>
<td>0.0</td>
<td>31</td>
<td>26</td>
<td>28</td>
</tr>
<tr>
<td>hypothetical protein</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>M. violaceum nuclear: MVLG_07215.1:</td>
<td>98.2117</td>
<td>8.30642E-35</td>
<td>59</td>
<td>49</td>
<td>52</td>
</tr>
<tr>
<td>hypothetical protein</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>hypothetical protein</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>M. violaceum nuclear: MVLG_07215.1:</td>
<td>36.965</td>
<td>8.30642E-35</td>
<td>32</td>
<td>18</td>
<td>21</td>
</tr>
<tr>
<td>hypothetical protein</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

18. O133-2

5’—

GCCAACAGGGGATACATACGACGTACCAGATTACGCTCATATGACAAGTTTGTGCAAAAAAGTTGGAACAAACCTCTGCAAAACGCTCAATACATCGAATACATACTCAACTGCTATACAGCTAACGGACCAGGACCAGCGCTGTTCCCGGCGCGGCCCTCCGGAGCCGGCAGCGACGGGACAACCACGTGGTGACGCGGCGGAGGCCGATGCCGCCGAGGGACCAGCGAACCCCGGCCTAGCGCTG

18. O133-2

5’—

GCCAACAGGGGATACATACGACGTACCAGATTACGCTCATATGACAAGTTTGTGCAAAAAAGTTGGAACAAACCTCTGCAAAACGCTCAATACATCGAATACATACTCAACTGCTATACAGCTAACGGACCAGGACCAGCGCTGTTCCCGGCGCGGCCCTCCGGAGCCGGCAGCGACGGGACAACCACGTGGTGACGCGGCGGAGGCCGATGCCGCCGAGGGACCAGCGAACCCCGGCCTAGCGCTG

123
CCGTTCTGGAAGTCACGTTCAAACACACGCACGCGAGAAGTCCGAGGTGTA
CTCGAAGGTTGCTCCGTACCCTGTCGCCCGACACCTTTGTTGTGGTTTAACTTG
CCGCAGAGGTATTATCTCCTCCGCGCGCGAGCCCACAATACGCTCCAAATCATCC
ATGGAGGTCGCCACCAGAATCGATCAGCGCCAAGCGTTAACCACCGGATTCAAGTT
CGTGGGTGCGCTGCGCTGCGATGGCCGAGCGTGGTTGACCCAGTCCACGACCGCG
GCGACCAAAATCGAGTGGAAACGCTCTTTGGCCAAAGTCCCGAAGCGGCTTTG
GCGTTCGTCAAAAACGTCGTCGAACTCGGTCTTTATTATCAGTCCCTAAAGGGCG
AATACACGAAACGACCGAACTTCTGGGAAACAAACTGCAAGGGCAGTGTCGCA
AGCTTTACCGGGGACCGGCAAACAAG—3'

**Query Sequence**

<table>
<thead>
<tr>
<th>Summary of Hits by Genome</th>
</tr>
</thead>
<tbody>
<tr>
<td>M. violaceum mito 0</td>
</tr>
<tr>
<td>M. violaceum nuclear 5</td>
</tr>
</tbody>
</table>

[Show All Alignments] [Hide All Alignments] [View Raw Output]

<table>
<thead>
<tr>
<th>Target</th>
<th>Score (Bits)</th>
<th>Expect</th>
<th>Alignment Length</th>
<th>Identities</th>
<th>Positives</th>
</tr>
</thead>
<tbody>
<tr>
<td>M. violaceum nuclear: MVLG_07305.1: hypothetical protein</td>
<td>150.214</td>
<td>0.0</td>
<td>108</td>
<td>80</td>
<td>85</td>
</tr>
<tr>
<td>M. violaceum nuclear: MVLG_07215.1: hypothetical protein</td>
<td>66.6254</td>
<td>0.0</td>
<td>35</td>
<td>31</td>
<td>33</td>
</tr>
<tr>
<td>M. violaceum nuclear: MVLG_07215.1: hypothetical protein</td>
<td>98.2117</td>
<td>1.07637E-26</td>
<td>59</td>
<td>49</td>
<td>52</td>
</tr>
<tr>
<td>M. violaceum nuclear: MVLG_07340.1: hypothetical protein</td>
<td>36.965</td>
<td>1.07637E-26</td>
<td>32</td>
<td>18</td>
<td>21</td>
</tr>
<tr>
<td>M. violaceum nuclear: MVLG_07340.1: hypothetical protein</td>
<td>63.1586</td>
<td>2.75119E-11</td>
<td>33</td>
<td>28</td>
<td>31</td>
</tr>
</tbody>
</table>

19. P43-5

5'—
GGGGCATGTGATACATACGACGTACCAGATTACGCTCATATGACAAGTTTGTACAAAAAGTTTGGCCGCTCCTGCAAAACGTCATACATCGCAATACCATCAACT
GCTATACGAGCTAACGACCGGATGCCGGGACCAGGCACGCCGCTGTTCCGGGC
CGCGGGCCCTCAGGAGCCGCGGACGCGAGCGAACCACAGTGGCGACCGG
GGGCGGAGGGCAGTGCCGAGGGAGCCAGCGAACCACCGGCGCCTAGGCGCT
GTACCAGGAAAAGCGTGATGAAGAACAGAAGAGGAAAGATGATGTCGCGAG
AATCCTGAAGATGATCAGTGTCGAAAGATGACACAGGGCCACCTCCC
TCGCTTCTACGCTTCGACTTGACAGGACGGAGGCCACCTCCC
TGCGCTTCTACGCTTCGACTTGACAGGACGGAGGCCACCTCCC
TGCGCTTCTACGCTTCGACTTGACAGGACGGAGGCCACCTCCC
TGCGCTTCTACGCTTCGACTTGACAGGACGGAGGCCACCTCCC
TGCGCTTCTACGCTTCGACTTGACAGGACGGAGGCCACCTCCC
TGCGCTTCTACGCTTCGACTTGACAGGACGGAGGCCACCTCCC
TGCGCTTCTACGCTTCGACTTGACAGGACGGAGGCCACCTCCC
TGCGCTTCTACGCTTCGACTTGACAGGACGGAGGCCACCTCCC

Query Sequence

<table>
<thead>
<tr>
<th>Summary of Hits by Genome</th>
</tr>
</thead>
<tbody>
<tr>
<td>M. violaceum mito</td>
</tr>
<tr>
<td>M. violaceum nuclear</td>
</tr>
</tbody>
</table>

[Show All Alignments] [Hide All Alignments] [View Raw Output]

<table>
<thead>
<tr>
<th>Target</th>
<th>Score (Bits)</th>
<th>Expect Length</th>
<th>Alignment Length</th>
<th>Identities</th>
<th>Positives</th>
</tr>
</thead>
<tbody>
<tr>
<td>M. violaceum nuclear: MVLG_07305.1: hypothetical protein</td>
<td>150.214</td>
<td>108</td>
<td>0.0</td>
<td>80</td>
<td>85</td>
</tr>
<tr>
<td>M. violaceum nuclear: MVLG_07305.1: hypothetical protein</td>
<td>66.6254</td>
<td>35</td>
<td>0.0</td>
<td>31</td>
<td>33</td>
</tr>
<tr>
<td>M. violaceum nuclear: MVLG_07305.1: hypothetical protein</td>
<td>56.9954</td>
<td>31</td>
<td>0.0</td>
<td>26</td>
<td>28</td>
</tr>
<tr>
<td>M. violaceum nuclear: MVLG_07215.1: hypothetical protein</td>
<td>98.2117</td>
<td>59</td>
<td>8.30642E-35</td>
<td>49</td>
<td>52</td>
</tr>
<tr>
<td>M. violaceum nuclear: MVLG_07215.1: hypothetical protein</td>
<td>36.965</td>
<td>32</td>
<td>8.30642E-35</td>
<td>18</td>
<td>21</td>
</tr>
</tbody>
</table>

20. Q150-2
5'—
GGAGCAGTCAAATACATACGAGCTACCAGATTACGCTCATATGACAAGTTTGTACAAAAAGTTTGCCACCCTACCTCTTGCAAAACGTCAATACATCGAATACATCAGCTGCTATACCAGCTAACGACCCGATGCCCGACCAGGCACCGCCTGTTCCGGGCCGCGGCCCTCCGGAGCCGGCAGCGACGGGACAACCACGTGGTGACCGGGGGCGGAGGCCGATGCCGCCGAGGGACCAGCGAACCCCGGCCTAGCGCTGTACCAGGAAAAGCGTGATGAAGAACAGAAGAGGAAAGATGATGTCGC
GAGAATCCTGAAGATGATCAGTGTCGAAGATGACACACACCAGGGCACCCGATCCTTGTGCGAGTCGCCGCAGGAGTTATCCTCCCGCCCGAGCCCCGAAATACGCTCCAATCATCCATGGAGTCGCACCAGAATCGATCAGCCAAAGCGGTAACACCCGATTCAA
GTTCGTGTCGCTGGCTGGCTGGCGCAGTGGCGCTTGACCACGTCCACGACGGCGACCAAGATCGAGTGGAAACGCTCCTTTGCCCAAGTCCGCAAGCGCTGGTCGTAAAAACGTCGTCGAACTGCGTTTCATTATCAGTCCTAAAGCCGAATACAGAAGGACGCCAATGGAGACACCGCCTCGCGTCCAGTCAAACACCGTCCGACACGGTCCCGTCCCGCAGGAGTTATCCTCCCGCCCGAGCCCCGAAATACGCTCCAATCATCCATGGAGTCGCACCAGAATCGATCAGCCAAAGCGGTAACACCCGATTCAA
GTTCGTGTCGCTGGCTGGCTGGCGCAGTGGCGCTTGACCACGTCCACGACGGCGACCAAGATCGAGTGGAAACGCTCCTTTGCCCAAGTCCGCAAGCGCTGGTCGTAAAAACGTCGTCGAACTGCGTTTCATTATCAGTCCTAAAGCCGAATACAGAAGGACGCCAATGGAGACACCGCCTCGCGTCCAGTCAAACACCGTCCGACACGGTCCCGTCCCGCAGGAGTTATCCTCCCGCCCGAGCCCCGAAATACGCTCCAATCATCCATGGAGTCGCACCAGAATCGATCAGCCAAAGCGGTAACACCCGATTCAA
GTTCGTGTCGCTGGCTGGCTGGCGCAGTGGCGCTTGACCACGTCCACGACGGCGACCAAGATCGAGTGGAAACGCTCCTTTGCCCAAGTCCGCAAGCGCTGGTCGTAAAAACGTCGTCGAACTGCGTTTCATTATCAGTCCTAAAGCCGAATACAGAAGGACGCCAATGGAGACACCGCCTCGCGTCCAGTCAAACACCGTCCGACACGGTCCCGTCCCGCAGGAGTTATCCTCCCGCCCGAGCCCCGAAATACGCTCCAATCATCCATGGAGTCGCACCAGAATCGATCAGCCAAAGCGGTAACACCCGATTCAA

<table>
<thead>
<tr>
<th>Target</th>
<th>Score (Bits)</th>
<th>Expect</th>
<th>Alignment Length</th>
<th>Identities</th>
<th>Positives</th>
</tr>
</thead>
<tbody>
<tr>
<td>M. violaceum nuclear: MVLG_07305.1: hypothetical protein</td>
<td>150.214</td>
<td>0.0</td>
<td>108</td>
<td>80</td>
<td>85</td>
</tr>
<tr>
<td>M. violaceum nuclear: MVLG_07305.1: hypothetical protein</td>
<td>66.6254</td>
<td>0.0</td>
<td>35</td>
<td>31</td>
<td>33</td>
</tr>
<tr>
<td>M. violaceum nuclear: MVLG_07305.1: hypothetical protein</td>
<td>54.6842</td>
<td>0.0</td>
<td>30</td>
<td>25</td>
<td>27</td>
</tr>
<tr>
<td>M. violaceum nuclear: MVLG_07215.1: hypothetical protein</td>
<td>98.2117</td>
<td>4.31179E-34</td>
<td>59</td>
<td>49</td>
<td>52</td>
</tr>
<tr>
<td>M. violaceum nuclear: MVLG_07215.1: hypothetical protein</td>
<td>45.8246</td>
<td>4.31179E-34</td>
<td>30</td>
<td>20</td>
<td>24</td>
</tr>
<tr>
<td>M. violaceum nuclear: MVLG_07215.1: hypothetical protein</td>
<td>36.965</td>
<td>4.31179E-34</td>
<td>32</td>
<td>18</td>
<td>21</td>
</tr>
</tbody>
</table>
21. A1

5’—

GGCATCATCGTACATACGACGTACCAGATTACGCTCATATGACAAGTTTGTACAAAAAGTTGGCCTTATACCTCTGCAAACGTCAATACATCGAATACATCAACTGCTATACCGCTAAACGACCCCATGCCCCGACCCGGCGACGGCAGCTCTGCTTTCCCGGGCCCGCGCCCTCCGGAGCCGGCAGCGACGGGACAACCACGTGGTGACGCGGGGGCGGAGGCCGATGCCGCGAGGGACCAGCGAACCCCGGCCTAGCGCTGTACCAGGAAAAGCGTGATGAAGAACAGAAGAGGAAAGATGATGTCGCGAGAATCCTGAAGAATGATCAGTGTGCAAGATGACACACACCAGGCCGACCATCCTTGTGCTCAAAGACGCACTCGGCTTCGGCAAGGACCGGGCCACCTCCCTGCGCTTTCAGCGCTCTCAATGCAACGAAACCAATCGCCGACACGTCTGGTGCCGTTCTGGAAGTCACGTTCAAACACACGCACGCGGAGGATCCGAGGTA
CTCGAAGGTGCTCCGATACCTGTCGACACCCCGACACCTTGTTGTGTTTTAACCTGCCCGCAGGAGTTATCCTCCCGCCCGAGCCCCAGAATACGCTCCAATCATCCATGGAGTCGCACCAGAATCGATCAGCCAAAGCGGTGACACCCGATTCAAGTT

GCGACCAAGATCGAATGGAAGGCCGTGCTTCTCTTGGCCCTTTGCCCCAAGTCCGAAGCGCTTGCGTTCGGCTACCGGGCCACCTCCC3’
### Query Sequence

#### Summary of Hits by Genome

<table>
<thead>
<tr>
<th>Genome</th>
<th>Score (Bits)</th>
<th>Expect</th>
<th>Alignment Length</th>
<th>Identities</th>
<th>Positives</th>
</tr>
</thead>
<tbody>
<tr>
<td>M. violaceum mito</td>
<td>0</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>M. violaceum nuclear</td>
<td>8</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

[Show All Alignments] [Hide All Alignments] [View Raw Output]

<table>
<thead>
<tr>
<th>Target</th>
<th>Score (Bits)</th>
<th>Expect</th>
<th>Alignment Length</th>
<th>Identities</th>
<th>Positives</th>
</tr>
</thead>
<tbody>
<tr>
<td>M. violaceum nuclear: MVLG_07305.1:</td>
<td>150.214</td>
<td>0.0</td>
<td>108</td>
<td>80</td>
<td>85</td>
</tr>
<tr>
<td>hypothetical protein</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>M. violaceum nuclear: MVLG_07305.1:</td>
<td>66.6254</td>
<td>0.0</td>
<td>35</td>
<td>31</td>
<td>33</td>
</tr>
<tr>
<td>hypothetical protein</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>M. violaceum nuclear: MVLG_07215.1:</td>
<td>56.9954</td>
<td>0.0</td>
<td>31</td>
<td>26</td>
<td>28</td>
</tr>
<tr>
<td>hypothetical protein</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>M. violaceum nuclear: MVLG_07215.1:</td>
<td>98.2117</td>
<td>8.30642E-35</td>
<td>59</td>
<td>49</td>
<td>52</td>
</tr>
<tr>
<td>hypothetical protein</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>hypothetical protein</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>M. violaceum nuclear: MVLG_07215.1:</td>
<td>36.965</td>
<td>8.30642E-35</td>
<td>32</td>
<td>18</td>
<td>21</td>
</tr>
<tr>
<td>hypothetical protein</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

22. A170

5'—

GCAACATCGTACATACGACGTACCAGATTACGCTCATATGACAAGTTTTGTACA

AAAAAGTTGGCCCCTTCTACCTCTCTGCAAACGTCAATACATCGAATACATCAACT

GCTATACCAGCTAACGACCCCGATGCCCCGACCAGGCACCCGCTTCCCGGGC

CGCGGCCCTCCGAGCGCCGCAGCGACGGCACAACCACGTGGTGACGCGG

GGGCGGAGGCAGGCAGCGCCGACACGCACCGAACCACCGCGTTCTCCGGGC

CGTACCAGAAAGCGTGATGAAACAGAAGAGAAGAAAGATGATGTCGCAG

AATCCTGAAGATGATCAGTGTCAAGATGACACACACACACAGCGACCCACCAT

CCCTTGCTCAAAGACGCACTCGGCTTCCGGCAAGGACCACGGGCACGCCACCTCC

TCGCCTTCAGCGCTCTCAATGCAACGAAACCAATCGCCGCACCGTCTTGGTG

CCGTTCGAGAATGCAGTCAACCAGCCAAAACACACGCAGCGAGAAGTCCAGAGGTGA

CTCGAAGGTGCTCCGTAACCTCTGTCGCACACCTTGGTTGTTTTAACTCTG

CCGCAGGAGATTTATCTCCGCCCAGCCGAGCAGAATACGCTCAATCCATCC

ATGGAGTCGCACCAGAATCGATCAGCCAAAAAGCGTGAACACCCAGTTCAAGTT

CGTGAGTCGCTGCGCTGCGCAGCGCTTGACCACGTCACCACGACCGG
GCGACCAAGATCGAGGGAAACGCTCCTTTGCCCA—3’

**Query Sequence**

<table>
<thead>
<tr>
<th>Summary of Hits by Genome</th>
</tr>
</thead>
<tbody>
<tr>
<td>M. violaceum mito 0</td>
</tr>
<tr>
<td>M. violaceum nuclear 2</td>
</tr>
</tbody>
</table>

[Show All Alignments] [Hide All Alignments] [View Raw Output]

<table>
<thead>
<tr>
<th>Target</th>
<th>Score (Bits)</th>
<th>Expect</th>
<th>Alignment Length</th>
<th>Identities</th>
<th>Positives</th>
</tr>
</thead>
<tbody>
<tr>
<td>M. violaceum nuclear: MVLG_07305.1: hypothetical protein</td>
<td>149.443</td>
<td>1.95895E-37</td>
<td>102</td>
<td>79</td>
<td>83</td>
</tr>
<tr>
<td>M. violaceum nuclear: MVLG_07215.1: hypothetical protein</td>
<td>98.2117</td>
<td>5.1791E-22</td>
<td>59</td>
<td>49</td>
<td>52</td>
</tr>
</tbody>
</table>

23. C148

5’—

GATCCGTGATACCATACGAGTACCAGATTACGCTCATATGACAAGTGGTGAATGGTGATGGTGAAGAAGTTGCGGCAAGTACCTACTCCTCTGCAAAACGTCAAATACATCGAATACCATCA
CTGCTATACCAGCTAAGCCGAGCCGCCGACGCAGAAGCTAAGCACCTGGTGGAAGAGAGAC
GCCGCCGCCCGCTCCGGAAGCCGGCAGACAGTGCCAGGGAAAGTCGATGCAAGACCCACACCTGCAAGAGAGATACGCTCCAATCATCATGGAGTCGCCAAATCGATCAGCCAAAGCGGTAACACCCGATTCAAGTT
CTGCGCGCTGCTCCCGTGGCGCTGACGTGCCAGCCTTGCTACCTGTCCTGGATGGCACCGGCTGGCAGTGGCGCTTGACCACGTCCACCAGGC
GGCGACCAAGATCGAGGGAAACGCTCCTTTGCCCA—3’
Query Sequence

Summary of Hits by Genome

<table>
<thead>
<tr>
<th>Target</th>
<th>Score (Bits)</th>
<th>Expect</th>
<th>Alignment Length</th>
<th>Identities</th>
<th>Positives</th>
</tr>
</thead>
<tbody>
<tr>
<td>M. violaceum nuclear: MVLG_07305.1: theoretical protein</td>
<td>150.214</td>
<td>2.297E-42</td>
<td>108</td>
<td>80</td>
<td>85</td>
</tr>
<tr>
<td>M. violaceum nuclear: MVLG_07305.1: hypothetical protein</td>
<td>37.3502</td>
<td>2.297E-42</td>
<td>18</td>
<td>17</td>
<td>18</td>
</tr>
<tr>
<td>M. violaceum nuclear: MVLG_07215.1: hypothetical protein</td>
<td>98.2117</td>
<td>6.45962E-22</td>
<td>59</td>
<td>49</td>
<td>52</td>
</tr>
</tbody>
</table>

24. D44-3

5’—
AAAAATCCAATCGATAGATACGTCGTACCAGATTACGCTCATATGACAAGTTTG
TACAAAAAAGTTGGCCATGCCCCACTCTGCAAACGTCAATACATCGAATACAT
CAACTGTCTATCCAGCTAACGACCCCGATGCCCGACCCAGGCACCGCCTGTT
CCGGGCGCGGCCCTCGAGGACGCGACCGACCGACGGACAAACCACGTTGGTG
ACGCAGGGGGGCGGAGGAGGAGGAGGACCCCGCGCCGAGGGGACCCAGGAAACCCCG
GCCCTAGGCCTGTTACCAGGGAAAAAGCGTGATGAAAGAAGAAGAAGAAGA
TGATGTTCCGAGAATTCTTGAGGATGATCAGTGGTGCAAGATGAAACACAC
ACCAGGGGCACCCCGCCTCCCTTGGTGTTCAAGAACTCTACCTCGCTTGCCG
GCAAAAGGACCAGGTCAACTTCTTGGCCTTCAGGGCTCTAATGCGACGAATCA
TTCGCGAAACGCTTGTGCGCTCTGAGTTCCGTTCCTCAACACGCACGAGAGT
TCGAGGGTAACTCGAGGARSTCGGGAACCTTGTGCGCAGAATCTTT---3’
Query Sequence

Summary of Hits by Genome

<table>
<thead>
<tr>
<th>Genome</th>
<th>Score (Bits)</th>
<th>Expect Length</th>
<th>Identities</th>
<th>Positives</th>
</tr>
</thead>
<tbody>
<tr>
<td>M. violaceum mito</td>
<td>38.5058</td>
<td>2.96593E-4</td>
<td>42</td>
<td>23</td>
</tr>
<tr>
<td>M. violaceum nuclear</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Target: M. violaceum nuclear: MVLG_07305.1: hypothetical protein

25. F127-2

5’—

GATCAGT CGATACATACGACGTACCAGATTACGCTCATATGACAAGTTTTGTAC
AAAAAAGTTGGCTAATATCTCTGCAAACGTCAATACATCGAATACATCAACTGC
TATACCAGCTAACGACCCGATGCCGCCGACCCGACCCCGCTGTTCCGGGCCG
CGGCCCTCCCGGAGCCGGCAGCGACGGGACAAACCACGTGTTGACGCGCGG
GGGCGGGAGGGCGTGCCGCCGCCAGGGACCCAGCGACGCTGCCCGCCCGCCTAGC
GCTGTACCAGGGAAAGCGTGTGAGACAGAAGAGGAAAGATGATGTCGC
GAGAAATCCTGAAGATGATCAGTTTGTCGAAGATGACACACCAACCAAGGGC
CGCCATCTTTGTTTGTCTCAAGACGCACTCCGGCTTTCGCAAGATCGCGGC
ACCCTCCCCTGGGCGGCTTCAGCGCTTCTCAATTGCACCGAATCAGCAGCG
GTCTGGTGCTTCTGGAGTCCCGTTCAACCCGCCGAGGAGAAGATCCCGAG
GTTTACTCGAAGGGGTCTCGTACCTGTGCGCGGACAACCTGTTGGGGTTAC—
3’
26. F152

5’—

GGATCATCGTACATACGACGTACCAGATTACGCTCATATGACAAGTTTGTACA
AAAAAGTTGGCTGACCCTCTTGGAAACGCATACATCGAATACATCAACTGCT
ATACCAGCTAACGACCCGATGCCCGACCACCAGGCACGGCCTGTTCCGGGCCG
CGGCCCTCCGGAGGACGGCAGCGACCCGATGCCCGACCAGGCACCGCCTG
AC

M. violaceum nuclear: MVLG_07305.1:

<table>
<thead>
<tr>
<th>Target</th>
<th>Score (Bits)</th>
<th>Expect</th>
<th>Alignment Length</th>
<th>Identities</th>
<th>Positives</th>
</tr>
</thead>
<tbody>
<tr>
<td>M. violaceum nuclear: MVLG_07305.1: hypothetical protein</td>
<td>38.5058</td>
<td>3.00971E-4</td>
<td>28</td>
<td>20</td>
<td>22</td>
</tr>
</tbody>
</table>

Reference

27. H145-2

5'—

GGATCAGGTGTACATACGACGTACCAGATTACGCTCATATGACAAGTTTGTACAAAAAAGTTGGCTGATAGTCTGCAAACGTCAATACATCGAATACATCAACTGCATATACCAGCTAACGACCCGATGCCCGACCAGGCACCGCCTGTTCCGGGCCGCGGCCCTCCGGAGCCGGCAGCGACGGGACAACCACGTGGTGACGCGGGGCGGAGGCCGATGCCGCCGAGGGACCAGCGAACCCCGGCCTAGCGCTGTCCAGGAAAAGCGTGATGAAGAACAGAAGAGGAAAGATGATGTCGCGAGAATCCTGAAGATGATCAGTGTCGAAGATGACACACACCAGGGCACCCGCATCCGTGTGCTCAAAGACGCACTCGGCTTCGGCAAGGACCGGGCCACCTCCCTCGCTTCAGCGCTCTCTCAATGCAACGAAACCAATCGCGACACGTCTGGTGCCGTTCTGGAAGTCACGTTCAAACACGCACGCGAGAAGTCCGAGGTGTACTCGAGGCTCCGTACCCTGTCGCCCGACACCTTGTTGTGTTTTAACCTGCAGCCAGGAGTTATCCTCCCGCCCGAGCCCCAGAATACGCTCCAATCATCCATGGAGTCGCACCAGAATCGATCAGCCAAAGCGGTAACACCCGATTCAAGTTCGTGGTGCGCTGCGCTGGCGCAGTGGCGCTTGACCACGTCCACGACGCGGCACCAAGATCGAGTGGAAACGCTCCTTTGCCCAAGTCCGCAAGCGCTTGGCGTTCTGTTCAAAAACGTCGTCGAACTGCGTTTCATTATCAGTCCTAAAGG---3'
Summary of Hits by Genome

M. violaceum mito  
0

M. violaceum nuclear  
8

[Show All Alignments] [Hide All Alignments] [View Raw Output]

<table>
<thead>
<tr>
<th>Target</th>
<th>Score (Bits)</th>
<th>Expect</th>
<th>Alignment Length</th>
<th>Identities</th>
<th>Positives</th>
</tr>
</thead>
<tbody>
<tr>
<td>M. violaceum nuclear: MVLG_07305.1: hypothetical protein</td>
<td>150.214</td>
<td>0.0</td>
<td>108</td>
<td>80</td>
<td>85</td>
</tr>
<tr>
<td>M. violaceum nuclear: MVLG_07305.1: hypothetical protein</td>
<td>66.6254</td>
<td>0.0</td>
<td>35</td>
<td>31</td>
<td>33</td>
</tr>
<tr>
<td>M. violaceum nuclear: MVLG_07305.1: hypothetical protein</td>
<td>56.9954</td>
<td>0.0</td>
<td>31</td>
<td>26</td>
<td>28</td>
</tr>
<tr>
<td>M. violaceum nuclear: MVLG_07215.1: hypothetical protein</td>
<td>98.2117</td>
<td>8.3064E-35</td>
<td>59</td>
<td>49</td>
<td>52</td>
</tr>
<tr>
<td>M. violaceum nuclear: MVLG_07215.1: hypothetical protein</td>
<td>36.965</td>
<td>8.3064E-35</td>
<td>32</td>
<td>18</td>
<td>21</td>
</tr>
</tbody>
</table>

28. 154

5’—

ACAAAGTGAAAGTGCCAGTATCCATACGAGTGTCGCTCATATGAC
AAGTTTGATACAAAAAAGTTGGCCATGCCCGCTCTCTGCAACACGTCAATACATCGA
ATACATCAACTGCTATACCAGCTAACCAGCGATGCCGCCGACCGGCCACCGCTC
TGTCGCGGCGGCGGCCCGCGACTCCCTCAGGAGGCGAGCAGGCAGGACACACGT
GGTGACCGGCGGCGAGCGAGCCGATGCCGCCGCAGGAGGACCACGACACCGG
GCCTAGCGCCTGTCAACCAAAAGCGTGATGGAACAGAGAGAGGAAGATAG
ATGTGCGGAATCTCGAAGATGATCGTCAAGAGATGACAACACACCGG
GCACCCGCATCTTTGCTCAAGAGACGCACTCGGCTTCGGGCAAGGACCCG
GCCACCTCCCTGCGTCTCAGCCTCTCAATGCAACGAAACCAATCGCCGAC
AGTCTCGGCTGGTTCTGGAAGTCAGACGTTCAAGCCACACGCAGCGAGAAGT
CCAGGATGTAATCGAAGGTGCTCGGTACCCCTGTGCGGCCGACACCTGTGT
GGTTTAACCTGCCCAGGTTATTCTCTCCGCCGCCGAGCCCGAGACACGAC
TCCAATCATCCATAGGAGTCCGCCAAGAATCGATCGACGAAACACCGGTTAACACC
CGATTCAAGTTGCTGGTGCTGGCTGCTGGCAGTGGCAGTGGGCGCTTGACCACGT
CCACGACCGCGCCGACAAAGATCAGTGAGAAGACGTCTCGCTTTGCCCCCGTCC
GCAAGCGCTTGGCGTTCGTCAAAAACGTCGTCGAACTGCGTTTCATTATCAG
TCCTAAAGGCCG—3’

Summary of Hits by Genome

<table>
<thead>
<tr>
<th>Genome</th>
<th>Score (Bits)</th>
<th>Expect</th>
<th>Alignment Length</th>
<th>Identities</th>
<th>Positives</th>
</tr>
</thead>
<tbody>
<tr>
<td>M. violaceum mito</td>
<td>0</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>M. violaceum nuclear</td>
<td>8</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

[Show All Alignments] [Hide All Alignments] [View Raw Output]

<table>
<thead>
<tr>
<th>Target</th>
<th>Score (Bits)</th>
<th>Expect</th>
<th>Alignment Length</th>
<th>Identities</th>
<th>Positives</th>
</tr>
</thead>
<tbody>
<tr>
<td>M. violaceum nuclear: MVLG_07305.1: hypothetical protein</td>
<td>150.214</td>
<td>0.0</td>
<td>108</td>
<td>80</td>
<td>85</td>
</tr>
<tr>
<td>M. violaceum nuclear: MVLG_07305.1: hypothetical protein</td>
<td>66.6254</td>
<td>0.0</td>
<td>35</td>
<td>31</td>
<td>33</td>
</tr>
<tr>
<td>M. violaceum nuclear: MVLG_07305.1: hypothetical protein</td>
<td>51.6026</td>
<td>0.0</td>
<td>27</td>
<td>23</td>
<td>25</td>
</tr>
<tr>
<td>M. violaceum nuclear: MVLG_07215.1: hypothetical protein</td>
<td>98.2117</td>
<td>4.99057E-34</td>
<td>59</td>
<td>49</td>
<td>52</td>
</tr>
<tr>
<td>M. violaceum nuclear: MVLG_07215.1: hypothetical protein</td>
<td>45.4394</td>
<td>4.99057E-34</td>
<td>25</td>
<td>19</td>
<td>23</td>
</tr>
<tr>
<td>M. violaceum nuclear: MVLG_07215.1: hypothetical protein</td>
<td>36.965</td>
<td>4.99057E-34</td>
<td>32</td>
<td>18</td>
<td>21</td>
</tr>
</tbody>
</table>

29. B79

5’—

AACATTTAACTCTGCGTGAATTCTCCTAATCCCTTTGAAGTTGACAAAAAAGTTGGT
ATTTCCTCTGCAACGTCATACATCGAAATACATCAACTGCTATACCAGCTAACG
ACCCGATGCACCGACCAGGCACCACGCCTGTCCGGCCGCGCCGGCCCTCCGGGA
GCCGCAGCGACGGGACAACCAACGTCGTTGACGCGGGGCGGAAGGCAGAT
GCCGCAGGAGGACCAGCGAACCCCCGGGCTAGCGCTGTACCAGGAAAAAGCG
TGATGAAGAACACAAAGAGGAAGAGATGATGTCGCGAGAATCCCTGAAAGATGATC
AGTGTGCGAAGATGACACACACACAGGGCACCACCACATCTGTGCTGCTAAAGAC
GCACTCAGCTTCGCGAACAGGCCGGGCACCTCCCTGCGCTTCAGCGCTCTC
CAATGCAAGAACAATCGCCGACACGTCTGTGTCGCCGTTCTGGAAGTGAC
GTTTAAACACACGACGCAGGACGGTGACTCGAAGGGTGCTCCGT
ACCCTGTCGCACCCAGACACCTGTGTTGTTTTAADTCTGCCGAGGAGGTATAC
TCCGCAGCCGACCCAGAATACGCTCCAAATCATCCATGGAGTGCAGGACCAAGA
Summary of Hits by Genome

<table>
<thead>
<tr>
<th>Target</th>
<th>Score (Bits)</th>
<th>Expect</th>
<th>Alignment Length</th>
<th>Identities</th>
<th>Positives</th>
</tr>
</thead>
<tbody>
<tr>
<td>M. violaeum nuclear: MVPX_07305.1:</td>
<td>150.214</td>
<td>0.0</td>
<td>108</td>
<td>80</td>
<td>85</td>
</tr>
<tr>
<td>hypothetical protein</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>M. violaeum nuclear: MVPX_07305.1:</td>
<td>66.6254</td>
<td>0.0</td>
<td>35</td>
<td>31</td>
<td>33</td>
</tr>
<tr>
<td>hypothetical protein</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>M. violaeum nuclear: MVPX_07305.1:</td>
<td>65.4698</td>
<td>0.0</td>
<td>36</td>
<td>30</td>
<td>32</td>
</tr>
<tr>
<td>hypothetical protein</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>M. violaeum nuclear: MVPX_07215.1:</td>
<td>98.2117</td>
<td>1.02256E-36</td>
<td>59</td>
<td>49</td>
<td>52</td>
</tr>
<tr>
<td>hypothetical protein</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>M. violaeum nuclear: MVPX_07215.1:</td>
<td>54.6842</td>
<td>1.02256E-36</td>
<td>36</td>
<td>24</td>
<td>29</td>
</tr>
<tr>
<td>hypothetical protein</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>M. violaeum nuclear: MVPX_07215.1:</td>
<td>36.965</td>
<td>1.02256E-36</td>
<td>32</td>
<td>18</td>
<td>21</td>
</tr>
<tr>
<td>hypothetical protein</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

30. G38
5'—
GGAATCAAGCTATAACACGACGTACCCAGATTACGCTCATATGACAAAGTTTGTACAAACGCTGCCGGGACCTACACGACTGCCGGGACGTCTATGCAACGCTACTACCTCTGCAACGGACATGCATACATCGAATACATCAACGCTCTCTTGTGCTATACACGACGTACCCAGATTACGCTCATATGACAAAGTTTGTACAAACGCTGCCGGGACCTACACGACTGCCGGGACGTCTATGCAACGCTACTACCTCTGCAACGGACATGCATACATCGAATACATCAACGCTCTCTTGTGCTATACACGACGTACCCAGATTACGCTCATATGACAAAGTTTGTACAAACGCTGCCGGGACCTACACGACTGCCGGGACGTCTATGCAACGCTACTACCTCTGCAACGGACATGCATACATCGAATACATCAACGCTCTCTTGTGCTATACACGACGTACCCAGATTACGCTCATATGACAAAGTTTGTACAAACGCTGCCGGGACCTACACGACTGCCGGGACGTCTATGCAACGCTACTACCTCTGCAACGGACATGCATACATCGAATACATCAACGCTCTCTTGTGCTATACACGACGTACCCAGATTACGCTCATATGACAAAGTTTGTACAAACGCTGCCGGGACCTACACGACTGCCGGGACGTCTATGCAACGCTACTACCTCTGCAACGGACATGCATACATCGAATACATCAACGCTCTCTTGTGCTATACACGACGTACCCAGATTACGCTCATATGACAAAGTTTGTACAAACGCTGCCGGGACCTACACGACTGCCGGGACGTCTATGCAACGCTACTACCTCTGCAACGGACATGCATACATCGAATACATCAACGCTCTCTTGTGCTATACACGACGTACCCAGATTACGCTCATATGACAAAGTTTGTACAAACGCTGCCGGGACCTACACGACTGCCGGGACGTCTATGCAACGCTACTACCTCTGCAACGGACATGCATACATCGAATACATCAACGCTCTCTTGTGCTATACACGACGTACCCAGATTACGCTCATATGACAAAGTTTGTACAAACGCTGCCGGGACCTACACGACTGCCGGGACGTCTATGCAACGCTACTACCTCTGCAACGGACATGCATACATCGAATACATCAACGCTCTCTTGTGCTATACACGACGTACCCAGATTACGCTCATATGACAAAGTTTGTACAAACGCTGCCGGGACCTACACGACTGCCGGGACGTCTATGCAACGCTACTACCTCTGCAACGGACATGCATACATCGAATACATCAACGCTCTCTTGTGCTATACACGACGTACCCAGATTACGCTCATATGACAAAGTTTGTACAAACGCTGCCGGGACCTACACGACTGCCGGGACGTCTATGCAACGCTACTACCTCTGCAACGGACATGCATACATCGAATACATCAACGCTCTCTTGTGCTATACACGACGTACCCAGATTACGCTCATATGACAAAGTTTGTACAAACGCTGCCGGGACCTACACGACTGCCGGGACGTCTATGCAACGCTACTACCTCTGCAACGGACATGCATACATCGAATACATCAACGCTCTCTTGTGCTATACACGACGTACCCAGATTACGCTCATATGACAAAGTTTGTACAAACGCTGCCGGGACCTACACGACTGCCGGGACGTCTATGCAACGCTACTACCTCTGCAACGGACATGCATACATCGAATACATCAACGCTCTCTTGTGCTATACACGACGTACCCAGATTACGCTCATATGACAAAGTTTGTACAAACGCTGCCGGGACCTACACGACTGCCGGGACGTCTATGCAACGCTACTACCTCTGCAACGGACATGCATACATCGAATACATCAACGCTCTCTTGTGCTATACACGACGTACCCAGATTACGCTCATATGACAAAGTTTGTACAAACGCTGCCGGGACCTACACGACTGCCGGGACGTCTATGCAACGCTACTACCTCTGCAACGGACATGCATACATCGAATACATCAACGCTCTCTTGTGCTATACACGACGTACCCAGATTACGCTCATATGACAAAGTTTGTACAAACGCTGCCGGGACCTACACGACTGCCGGGACGTCTATGCAACGCTACTACCTCTGCAACGGACATGCATACATCGAATACATCAACGCTCTCTTGTGCTATACACGACGTACCCAGATTACGCTCATATGACAAAGTTTGTACAAACGCTGCCGGGACCTACACGACTGCCGGGACGTCTATGCAACGCTACTACCTCTGCAACGGACATGCATACATCGAATACATCAACGCTCTCTTGTGCTATACACGACGTACCCAGATTACGCTCATATGACAAAGTTTGTACAAACGCTGCCGGGACCTACACGACTGCCGGGACGTCTATGCAACGCTACTACCTCTGCAACGGACATGCATACATCGAATACATCAACGCTCTCTTGTGCTATACACGACGTACCCAGATTACGCTCATATGACAAAGTTTGTACAAACGCTGCCGGGACCTACACGACTGCCGGGACGTCTATGCAACGCTACTACCTCTGCAACGGACATGCATACATCGAATACATCAACGCTCTCTTGTGCTATACACGACGTACCCAGATTACGCTCATATGACAAAGTTTGTACAAACGCTGCCGGGACCTACACGACTGCCGGGACGTCTATGCAACGCTACTACCTCTGCAACGGACATGCATACATCGAATACATCAACGCTCTCTTGTGCTATACACGACGTACCCAGATTACGCTCATATGACAAAGTTTGTACAAACGCTGCCGGGACCTACACGACTGCCGGGACGTCTATGCAACGCTACTACCTCTGCAACGGACATGCATACATCGAATACATCAACGCTCTCTTGTGCTATACACGACGTACCCAGATTACGCTCATATGACAAAGTTTGTACAAACGCTGCCGGGACCTACACGACTGCCGGGACGTCTATGCAACGCTACTACCTCTGCAACGGACATGCATACATCGAATACATCAACGCTCTCTTGTGCTATACACGACGTACCCAGATTACGCTCATATGACAAAGTTTGTACAAACGCTGCCGGGACCTACACGACTGCCGGGACGTCTATGCAACGCTACTACCTCTGCAACGGACATGCATACATCGAATACATCAACGCTCTCTTGTGCTATACACGACGTACCCAGATTACGCTCATATGACAAAGTTTGTACAAACGCTGCCGGGACCTACACGACTGCCGGGACGTCTATGCAACGCTACTACCTCTGCAACGGACATGCATACATCGAATACATCAACGCTCTCTTGTGCTATACACGACGTACCCAGATTACGCTCATATGACAAAGTTTGTACAAACGCTGCCGGGACCTACACGACTGCCGGGACGTCTATGCAACGCTACTACCTCTGCAACGGACATG
### Summary of Hits by Genome

<table>
<thead>
<tr>
<th>Target</th>
<th>Score (Bits)</th>
<th>Expect</th>
<th>Alignment Length</th>
<th>Identities</th>
<th>Positives</th>
</tr>
</thead>
<tbody>
<tr>
<td>M. violaceum mito</td>
<td>0</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>M. violaceum nuclear</td>
<td>8</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

[Show All Alignments] [Hide All Alignments] [View Raw Output]

<table>
<thead>
<tr>
<th>Target</th>
<th>Score (Bits)</th>
<th>Expect</th>
<th>Alignment Length</th>
<th>Identities</th>
<th>Positives</th>
</tr>
</thead>
<tbody>
<tr>
<td>M. violaceum nuclear: MVLG_07305.1: hypothetical protein</td>
<td>150.214</td>
<td>0.0</td>
<td>108</td>
<td>80</td>
<td>85</td>
</tr>
<tr>
<td>M. violaceum nuclear: MVLG_07305.1: hypothetical protein</td>
<td>66.6254</td>
<td>0.0</td>
<td>35</td>
<td>31</td>
<td>33</td>
</tr>
<tr>
<td>M. violaceum nuclear: MVLG_07305.1: hypothetical protein</td>
<td>54.6842</td>
<td>0.0</td>
<td>30</td>
<td>25</td>
<td>27</td>
</tr>
<tr>
<td>M. violaceum nuclear: MVLG_07215.1: hypothetical protein</td>
<td>98.2117</td>
<td>3.98974E-34</td>
<td>59</td>
<td>49</td>
<td>52</td>
</tr>
<tr>
<td>M. violaceum nuclear: MVLG_07215.1: hypothetical protein</td>
<td>45.8246</td>
<td>3.98974E-34</td>
<td>30</td>
<td>20</td>
<td>24</td>
</tr>
<tr>
<td>M. violaceum nuclear: MVLG_07215.1: hypothetical protein</td>
<td>36.965</td>
<td>3.98974E-34</td>
<td>32</td>
<td>18</td>
<td>21</td>
</tr>
</tbody>
</table>

31. I153-1

5'—

GGCCCGGGGATACATACGACGTACCAGATTACGCTCATATGACAAGTTTGTA
CAAAAAAGTTTGCCACCTACCTCTGCAAACGTCAATACATCGAATACATCAACT
GCTATACCAGCTAACCAGCCCGATGCCCAGACCAGGCACCGCACGTGGTTCCGGGC
CGCCGGCCCTCCGGAGCCCGCAGCGACGGGACAACCACGTGGTGACGCGG
GGGCGGAGGCCGAGGAGGAGGCACCGGACCCCGCACCCCGCCTAGCGCTC
GTACCAGGAAAGCGTGTAGAAAGAAAGAAAGAAAGATGATGATCGCAG
AATCCCTGAAGATGATCATCAGTGCGAGGAGATGACACACACACAGGGCACCAGCAT
CCTTTGTGCTCAAGAGACGACTCCGGTTGGCAAGGACCAGGGCCACCTCCC
TGCAGTGCTACAGCGCTCTCAATGCAACCAACCAATCGCGGACAGGTCTCGTG
CCGTTTCTGGAAGTCCGTTCAACACACGCCACACGCGAAGTCCGAGGTGTA
CTCAAGGAGGTGCTCCGTACCCTGTGCCCCGAGACACCTTTGTGTTTTTAACCTG
CCGCAGAGGTATTACCCCTCCGCCCAGGAGCCAGGAGACTACAGCTCCAATCTCC
ATGGGAGTCGCCACCAAGATCGATCGACGCAAGGAAAGCGTAAACACCCGATT
CGTGCAGTCGCCTCGCCGCTGAGTGGGCTGCTGACCAGTCACCGACGCACG
GGACCAAAGATCGAGTGGAAAACGCTCCTTTGCCCAAGTCCCGCAAGCG

Summary of Hits by Genome

<table>
<thead>
<tr>
<th>Target</th>
<th>Score (Bits)</th>
<th>Expect Length</th>
<th>Identities</th>
<th>Positives</th>
</tr>
</thead>
<tbody>
<tr>
<td>M. violaceum nuclear: MVLG_07305.1: hypothetical protein</td>
<td>150.214</td>
<td>108</td>
<td>80</td>
<td>85</td>
</tr>
<tr>
<td>M. violaceum nuclear: MVLG_07305.1: hypothetical protein</td>
<td>66.6254</td>
<td>35</td>
<td>31</td>
<td>33</td>
</tr>
<tr>
<td>M. violaceum nuclear: MVLG_07305.1: hypothetical protein</td>
<td>29.6462</td>
<td>15</td>
<td>12</td>
<td>14</td>
</tr>
<tr>
<td>M. violaceum nuclear: MVLG_07215.1: hypothetical protein</td>
<td>98.2117</td>
<td>59</td>
<td>49</td>
<td>52</td>
</tr>
<tr>
<td>M. violaceum nuclear: MVLG_07215.1: hypothetical protein</td>
<td>36.965</td>
<td>32</td>
<td>18</td>
<td>21</td>
</tr>
<tr>
<td>M. violaceum nuclear: MVLG_07215.1: hypothetical protein</td>
<td>25.0238</td>
<td>15</td>
<td>9</td>
<td>13</td>
</tr>
</tbody>
</table>

32. L177-1

5’—

GACCAGCGTACATACGAGTACCAGATTACGCTCATATGCAAGATTTGTACAA
AAAAGTTGGCCTCTCCTACCTCTGCAAAGTCAATAACTGACATCAACTGAC
CTATACGCTAACGGGCCAGCCGCGACGCAGACCCAGCGTGGTGAGCGCGG
GGACGAGCCGAGTCGCCAGGAGGACCGACCCGAGCGTCTGGGAGTC
TACCAGGAAAAGCGTGTGAAGAACAGAGGAAAGATGATGTGCTCGAGA
ATCCTGAAAGATGTCACTGCTGAAGATGACACACACGAGGACCCGACCTAGCGT
CTTTGCTCAAGAGACGCACCTCGCTTCCGCAAGGACCGGACACCTGCT
GCCGGCTACGCTCTCATTGAAACCAGAAACATCGCCGACACGTGCTGG
CGTTCTGGAAGTCGTTCAACACACACGCAAGGAGTCCGAGGTTGAC
TCGAAGGTGCTCCGTACCCTGTGCCCACACCTTTGTGTTTTTAACCTGC
CCGCAGGAGTTATCTCCGCCCCGAGCCCCAGAATCCTCCATCATCCCAT
GGAGTCGACCAAGAATGATCAGGCGCACAACCCGATTCAGTTTC
GTGTTGCGCTGCGCTGGCGCAGTGGCGCTTGACCAGTC—3’

Summary of Hits by Genome
M. violaceum mito 0
M. violaceum nuclear 2

<table>
<thead>
<tr>
<th>Target</th>
<th>Score (Bits)</th>
<th>Expect Length</th>
<th>Identities</th>
<th>Positives</th>
</tr>
</thead>
<tbody>
<tr>
<td>M. violaceum nuclear: MVLG_07305.1:</td>
<td>149.443</td>
<td>2.28041E-37</td>
<td>79</td>
<td>83</td>
</tr>
<tr>
<td>hypothetical protein</td>
<td></td>
<td>102</td>
<td></td>
<td></td>
</tr>
<tr>
<td>M. violaceum nuclear: MVLG_07215.1:</td>
<td>98.2117</td>
<td>6.02898E-22</td>
<td>49</td>
<td>52</td>
</tr>
<tr>
<td>hypothetical protein</td>
<td></td>
<td>59</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Reference

33. M95
5’—
GGGCGGTGTGAAACTAAGACGTATATAGATTCGCTCTATGACAAATTGTACAA
AATATTGTAAAAACTTTGCCAAACGTCATACATCGAATACATCAATGCTATAAG
CTAACGAGCGCGATGCCGCCAGGCAACCGGCTGTTCCGGGGCCGCGGCCCTC
CGAGCCGCGAGCGCCGACGGGACAAACCAGTGCGTGACGGCGGGGCGGGAGG
CGATGCGCCGAGGGGACAGCGAACCACCCGGCTTACGCTGTACCAGGA
AAGCGTGATGAAGAACAGAAGAGGAAAGTGATGTCGGCGAAATGTCGTGAAG
ATGATCAGTGTCCAGAAGATGACACACACAGGGCCGACTCCTTGTGCTC
AAAGACGACACTGCGCTTCCGGCAAGGACCCGGACCTCCCTGCGCTTCAG
CGCTCTCAATGCAAAGAGGAAATCGCCGACAGCTGCTGCCGTGC
AAAAGTCAGTCTTTGAAACACACCGCAACAGCGAGAAGTCCGTTGCTAGTGTCG
TGCTCCGTTACTGCGCCGACACCTTTGTGTTGTGTTTAAACGCTGCCGCAG
GAGTTATCCTCCCCGCCCCTGGCCGAAATACGCTCCATCCATGGGAG
TCGCACCAAGAATCGATCAGGCCAACAGGCTAAACCCGATTCAAGGTCCGTTG
GCGCTGCGCTGCGCAGTGCGGCTTTGACCACGTCACCGACGCGGCGACC
AAGATCGAGTGAAACCAGCTCCTTTG—3’
34. M178-1

5’—
TGCTCAGCATACTACGACGTACCAGATTACGCTCATATGACACAAGTTTTGTA
AAAAAGTTGTTAATTATCACTCTCTGCAAAGCTCAATACATCGAATACATCAACTG
CTATACCAGCTAACGAGCCGATGCCCCGACCGACCGGCCTGTTCGCGGAGCC
CGGACCCTCCGGAGCCGAGCGACGCAGCGACAGGACAACCACGTGAGTCGACGCGG
CGGAGGAGGCAGTGGCCCGAGGAGAAGCCGCAACCAGAATCAGGCTCCCG
TACCCAGGAAAAGCCTGTGATAAGAAGAAAGAGAAGATGATGTCGAGG
ATCCTGAAGAGATGATCATTGTGCAAGAGATGACACACACACGGGCAACCAGCATC
CTTGCTGCTCAAGACGACTCGGCTTCCGGAAGGCAGCGGCCACCTCCCT
GCGTTTCAAGGCCTCTCAATGCAAAGAAACCAATCCGCCGACAGCCTGCTG
CGTCTGAAGAGTACGTTCAAAACACACACGCAAGCGAAGTGTCGAGGTCGAC
TCGAAAGTGGCTCCTGACACCTGTGTCGCGCAGACACCTTTGTGTGTGTTTAACCTTG
CGCAGGAGGTTATCCTCCCGGCGAGCCGAGAATACGCTCCCATCATCCAT
GGAGTGCGGCGGATCAGTGCAAGCGAAGCGGTAACCCGCAGTCAA—3’
Yeast-Two Hybrid Sequencing and BlastX Results Showing *MVLG_06379*

J117-1
5’—
GGAGCCGGTGGGATCCATACGACGTACCAGATTACGCTCATATGACAAGTTTGTACAAAAAAGTTGGAAAGCGAAGGGGTTGTACGTGTGCGGTGCGCCCCCTTGCCGCACCGCAGCCTCCTCATCATCGCCGCACGTTCATTGCTGCCCCACTTGAGCGCGCGACTCGGCATCCGCATCGACGTCCAAGCTTGCCCCGGAGCCACCGCGCGCTCAGTATCGGCGAGGAGGGCGTTGCTGTATGTTCCCGGATCGAACGTCAAGATGTTGAGGAGTGCGTTGAGCAAGGGCACCAGCGACGCGTTGATCTTGGACCTCGAGGTCGGTGCCCATCCCAGTTCAGGTGCAGTCAATGTAAGCACTGCTTGTGATCTGATCGCAGCTCGGATCATCCTTGCTTGGATCGGAAAGGAGCGGAGGAGGAATGTTTGCGATGCACCGTCTCTCGTTGATAAACCTTCAAAGA

AATCGCAACCTCCTCCAACAGGATTGACGCCTTGCTCTTTGCCCGCAGAAG
ACTATTGC\ldots

Query Sequence

Summary of Hits by Genome

<table>
<thead>
<tr>
<th>Target</th>
<th>Score (Bits)</th>
<th>Expect</th>
<th>Alignment Length</th>
<th>Identities</th>
<th>Positives</th>
</tr>
</thead>
<tbody>
<tr>
<td>M. violaceum nuclear: MVLG_06379.1: hypothetical protein</td>
<td>133.265</td>
<td>2.60999E-32</td>
<td>69</td>
<td>68</td>
<td>69</td>
</tr>
<tr>
<td>M. violaceum nuclear: MVLG_06379.1: hypothetical protein</td>
<td>125.946</td>
<td>4.16691E-30</td>
<td>173</td>
<td>88</td>
<td>95</td>
</tr>
</tbody>
</table>

Reference


Yeast-Two Hybrid Sequencing and BlastX Results Showing \textit{CASP-like protein 2C1 (CASPL2C1)}

1. R162
5’—
GACCAGGCAATACATACGACGTACCAGATTACGCTCATATGACAAGTTTGTAC
AAAAAAAACTTGGGGGAAACTTTGTACCAACAAAAGTTGGGATTATTTGCGTTG
AACACTCTTTCTTGCTTGCTTTGATTGCTGCAGAAAGTAAGTGA
TCTTTGGTTATTACAAACAAACGGCTAGCTACAAGTTGGTAACCATTGCAAGG
ATGTAATGTATGTCCATTTTATTGGAGCCGGGTATAGCCTATTTCAATTCGTT
AGATGCTTTGCTTGGACGACCAGCAGCAGGAGACCATTGACCTTTTCAAA
GTCATATGCAAAAGTTGGAATGCATTTTCACTTTTCATATTGATCGTTGATGTG
TTTATATGCGAGGAGGATGAAATTATGCAACAAAGTTTTACAAAGTTTTTGCTG
CGGAAGTGTCCAGTGGATGAAATTATGCAACAAGGTTTTACAAAGTTTTTGTG

142
GATTTCGGGCATTTCTACCTTTAATTTATTTTAGGTTATTCTCCAAACGCTTT
GTGCCCTTAACCTAAGAGAAACGACGAGGCAATGTTGCTCCAGGTATTATTAGAGT
TTACTTTGATAAAAAAGTGTAACACAAAAATAACAAAAAATTTTGCTCACAAGTACGTA
GTATATATATATATATATTGAGTAAATATATATATTGAGTAGCCTATACATACATTT
ATTATCACACTTTACCTCTCCGATAAAATATATATAATGTGTAAGAAGAA
CAATGTACCAATACATTGTAATAATACCTAGGACACTTGTATTCTGTAAGTTTAGC
AAT—3’

<table>
<thead>
<tr>
<th>Description</th>
<th>Max score</th>
<th>Total score</th>
<th>Query cover</th>
<th>E value</th>
<th>Idnt</th>
<th>Accession</th>
</tr>
</thead>
<tbody>
<tr>
<td>hypothetical protein SOVF_156740 [Spinacia oleracea]</td>
<td>211</td>
<td>211</td>
<td>93%</td>
<td>2e-65</td>
<td>59%</td>
<td>KNA08881.1</td>
</tr>
<tr>
<td>PREDICTED: CASP-like protein 2C1 [Beta vulgaris subsp. vulgaris]</td>
<td>179</td>
<td>179</td>
<td>94%</td>
<td>4e-53</td>
<td>50%</td>
<td>XP_010691480.1</td>
</tr>
<tr>
<td>CASP-like protein 2C1 [Glycine max]</td>
<td>125</td>
<td>125</td>
<td>86%</td>
<td>3e-32</td>
<td>41%</td>
<td>NP_001337350.1</td>
</tr>
<tr>
<td>CASP-like protein 5 [Glycine soja]</td>
<td>125</td>
<td>125</td>
<td>86%</td>
<td>4e-32</td>
<td>41%</td>
<td>KHN22710.1</td>
</tr>
<tr>
<td>CASP PSTR DRIFT-like protein [Medicago truncatula]</td>
<td>124</td>
<td>124</td>
<td>86%</td>
<td>5e-32</td>
<td>43%</td>
<td>XP_013441625.1</td>
</tr>
<tr>
<td>PREDICTED: CASP-like protein 2C1 [Eucalyptus grandis]</td>
<td>124</td>
<td>124</td>
<td>90%</td>
<td>8e-32</td>
<td>38%</td>
<td>XP_010046898.1</td>
</tr>
<tr>
<td>CASP-like protein XL3 [Gossypium arboreum]</td>
<td>120</td>
<td>120</td>
<td>86%</td>
<td>2e-30</td>
<td>42%</td>
<td>KHSF81580.1</td>
</tr>
</tbody>
</table>

2. J7-2

5’—

GGACACCGGTACCATACGACGTACCAGATTACGCTCATATGACAAAGTTTTGTACA
AAAAATTTGTTGGGGCAACACTTTGTACAAAAGTTGGGATTATTTCGTTTG
TAACACTCTTTCTCTGCTGCTTATTGTCGGTTTTGCTTCCGGAAGATGAGTAGT
ATCTTTGTTTATTACAAAACAAAGGGCTAGCTACAAAGTTGGTAACCATTGCAAG
GATGTACGTGTAGTCCATTTTATGGAGCCGGGTATAGCCCTATTCACTTCCGT
TAGATGCTTTGGCTTTGACCGACCACGAGCCAAGGAGACATTTGACCTTTCCA
AGTCATATGCAAAAAGTGGACTCATTTCCTTACTTGCAGGATGTGATGATACCTA
ATATTTGCAAACAAATTTGCACATTCCGAAAATAGCTGATATGTTGTTAATGAGGG
TCGGAAGTGTTGCCAGTTGAAAATTATGCAAAAGTTTTACAAAGTTTTGTGTC
CCAAATGGAGGTGCTTACATTGTTGAGATTACTGCAACACTATTACTGCTG
TGATTTCGGGCATTTCTACCTTTAATTTATTTAGGTTATTCTCCAAACGCTTT
GTGCCCTTAACCTAAGAGAAACGACGAGGCAATGTTGCTCCAGGTATTATTAGAGT
TTACTTTGATAAAAAAGTGTAACACAAAAATAACAAAAAATTTTGCTCACAAGTACGTA
GTATATATATATATATATTGAGTAAATATATATATTGAGTAGCCTATACATACATTT
ATTATCACACTTTACCTCTCCGATAAAATATATATAATGTGTAAGAAGAA
CAATGTACCAATACATTGTAATAATACCTAGGACACTTGTATTCTGTAAGTTTAGC
AAT—3’
3. L21-2
5’—
GATCAGCGTACATACGACGTACCAGATTACGCTCATATGACAAGTTTGTACAA
AAAAGTTGGGGGGGATAACTTTGTACAAAAAAGTTGGGATTATTTGCGTTTGTA
ACACTCTTTTCTTGGCTCATTTATTGTCGTTGTTGATTCGGAAAGTAAGATGAT
CTTTGGTTATTTACAAAACAAACGGGCTAGCTACAAAGTTGGTAACCATTGCAGGA
TGATACGTATGTCCATTCTTTATTTGGAGCCGGGTATAGCCCTATTTCAATTTCGTA
GATGCTTGCGTTTGACGACCAGCAAGGAGACCAATTGCCACCTTTTCAAG
TCATATGCAAAGGTGACTCTTTTTCACCTTGATCGGTGATGTAACCTAAT
ATTTTCAACAAATTTGGCCTATCCGAAATAGCTGATATGGGTAATGGGGT
CGGAAAGTGGATCTCCAGTGAACATTATATGCAACAAAGTTTACAAAGTTTGTGTC
CAAATTGAGGGTGACACTTTTTTTGGAATTATGCAACACTATTACTAGCTGTT
GATTTTCGGGCATTCTTACCTTTCAATTATTAGGTTATGGTATTTCTCCAAACGCTTT
GTGCCTTAAACCTAGAGAAACGGGCGTGGTTGCTCCAGTATTTATTTAGGTTAG
TTACTTGTAATAATAAAGGTCAAACAAACAAACCTTGCGTACAAAGTACGTA
GTATATATATATATAGGAGTAATAAATTGGTAGACCCCATACAG—3’

<table>
<thead>
<tr>
<th>Description</th>
<th>Max score</th>
<th>Total score</th>
<th>Query cover</th>
<th>E value</th>
<th>Ident</th>
<th>Accession</th>
</tr>
</thead>
<tbody>
<tr>
<td>hypothetical protein SOVF_158740 [Spinacia oleracea]</td>
<td>204</td>
<td>204</td>
<td>96%</td>
<td>7e-63</td>
<td>59%</td>
<td>KNA08881.1</td>
</tr>
<tr>
<td>PREDICTED: CASP-like protein 2C1 [Beta vulgaris subsp. vulgaris]</td>
<td>173</td>
<td>173</td>
<td>99%</td>
<td>7e-51</td>
<td>50%</td>
<td>XP_016891480.1</td>
</tr>
<tr>
<td>CASP-like protein 2C1 [Glycine max]</td>
<td>127</td>
<td>127</td>
<td>95%</td>
<td>4e-33</td>
<td>41%</td>
<td>NP_001237350.1</td>
</tr>
<tr>
<td>CASP-like protein 3 [Glycine soja]</td>
<td>127</td>
<td>127</td>
<td>95%</td>
<td>4e-33</td>
<td>41%</td>
<td>KHN22710.1</td>
</tr>
<tr>
<td>PREDICTED: CASP-like protein 2C1 [Eucalyptus grandis]</td>
<td>126</td>
<td>126</td>
<td>95%</td>
<td>1e-32</td>
<td>38%</td>
<td>XP_010046989.1</td>
</tr>
<tr>
<td>CASP POPTRDRAFT-like protein [Medicago truncatula]</td>
<td>124</td>
<td>124</td>
<td>93%</td>
<td>4e-32</td>
<td>43%</td>
<td>XP_013441620.1</td>
</tr>
<tr>
<td>CASP-like protein XL3 [Gossypium arboreum]</td>
<td>122</td>
<td>122</td>
<td>95%</td>
<td>3e-31</td>
<td>42%</td>
<td>KHF98180.1</td>
</tr>
</tbody>
</table>
4. H12

5’—

CCTCTATGAAGTTTGTACAAGAAGTGGGGGAACATTTGTACAAGAAGTGGG
TTAGGGCAACACTCTTTTTGGTCTGCTCTTTATTGTCAAGATGATTCCGAAA
GTAAGATGATCTTTGGTTATTACAACAAAGGCTAGCTACAACAGTTGGTAACC
ATTGGCAAGGATGTAAGGAGATGCTGACGTTCTATTGACGACCGAGCAAGGAGAACAGCATG
CCTTTTCAAGCTCATACTGCAAGAAGTGACTCATTTTTCACTTGAGTTGATG
GTATACCTAATATTGCAACAAATTGCCCATATCCGAATAGCTGATATGGTGA
TTAATGGGGTCAAGGATGTTTCCAGTGGATGAAATTATGCAACAAAGTTTTCAAA
GTTTTTGATGTCGCAAAATGGGAAGGTGTCGATACACAGTTATGGGAACAGCAGCTATTT
ACTAGCTGATATTCCGGCCATTCTACCTCTCAATTATTAGATGTTATTCCCA
AACGCTTTTTGCTCTAAAACAAAGAGACGCGCATGGTTGTCAGTTTTTATTAG
TATGACTTGTATAAAAGTGTCAGAATAAAGGCTCGAAATCAACAGGCTGGCTCCAGAGCGTAAGTT
TTTTTTTTTTGGGGAATAATTG—3’

<table>
<thead>
<tr>
<th>Description</th>
<th>Max score</th>
<th>Total score</th>
<th>Query cover</th>
<th>E value</th>
<th>Ident</th>
<th>Accession</th>
</tr>
</thead>
<tbody>
<tr>
<td>hypothetical protein SOV_f_158740 (Spinacia oleracea)</td>
<td>147</td>
<td>186</td>
<td>76%</td>
<td>2e-40</td>
<td>57%</td>
<td>KNA08881.1</td>
</tr>
<tr>
<td>PREDICTED: CASP-like protein 2C1 (Beta vulgaris subsp. vulgaris)</td>
<td>120</td>
<td>120</td>
<td>65%</td>
<td>3e-30</td>
<td>47%</td>
<td>XP_010691460.1</td>
</tr>
<tr>
<td>PREDICTED: CASP-like protein 2C1 (Eucalyptus grandis)</td>
<td>99.0</td>
<td>99.0</td>
<td>65%</td>
<td>5e-22</td>
<td>38%</td>
<td>XP_010649089.1</td>
</tr>
<tr>
<td>PREDICTED: CASP-like protein 2C1 (Nicoliana tomentosiformis)</td>
<td>97.4</td>
<td>97.4</td>
<td>65%</td>
<td>4e-21</td>
<td>39%</td>
<td>XP_000611405.1</td>
</tr>
<tr>
<td>CASP-POPTDRAFT-like protein [Medicago truncatula]</td>
<td>96.3</td>
<td>96.3</td>
<td>64%</td>
<td>4e-21</td>
<td>43%</td>
<td>XP_013441925.1</td>
</tr>
<tr>
<td>CASP-like protein 2C1 [Glycine max]</td>
<td>93.6</td>
<td>93.6</td>
<td>65%</td>
<td>4e-20</td>
<td>40%</td>
<td>NP_001237350.1</td>
</tr>
<tr>
<td>CASP-like protein 3 (Glycine soja)</td>
<td>93.2</td>
<td>93.2</td>
<td>65%</td>
<td>5e-20</td>
<td>40%</td>
<td>KHN22770.1</td>
</tr>
</tbody>
</table>

Yeast-Two Hybrid Sequencing Results Showing **Constitutive photomorphogenesis 9 (COP9) signalosome complex subunit 5a and/or 5b (CSN5a/5b)**

1. 20-1

5’—

GGGGCGAGCGCGCCATGGAGTACCCATAGGCAGTACGATTACGCTCATAT
GACAAAGTGTACAAAAAGTTGGAAATAAGGGCAAGAAGAAGAAGAAGAAGAAGA

145
ATGGATCCGAGACAAATAGCAGCAGAAAAAACATGGGAATAGAAGACAAATATAGA
AACAGTAAACGAGCAGCGCATCAGATGCAATATTCAAGTACGACGATG
GCTATTATTATTAGGGTACGCTTCTTTGCTTTACCTGTTGAGGTTGACTGAAACTAGG
GTTAATGCCAGGGCGGTTATGAGTTATATATGTTGATTATTTCTACTACTATAA
AACAGGCTGGAAGACTGGAAAATGGTCAGCTCAACACCCAA
CAATTCAGAAGACTGGAAAATGGTCAGCTCAACACCCAA

<table>
<thead>
<tr>
<th>Description</th>
<th>Max score</th>
<th>Total score</th>
<th>Query cover</th>
<th>E value</th>
<th>Ident</th>
<th>Accession</th>
</tr>
</thead>
<tbody>
<tr>
<td>PREDICTED: COP9 signalosome complex subunit 5b-like [Cucumis melo]</td>
<td>572</td>
<td>572</td>
<td>90%</td>
<td>0.0</td>
<td>90%</td>
<td>XP_008445093.1</td>
</tr>
<tr>
<td>PREDICTED: COP9 signalosome complex subunit 5b [Cucumis sativus]</td>
<td>572</td>
<td>572</td>
<td>90%</td>
<td>0.0</td>
<td>90%</td>
<td>XP_008445093.1</td>
</tr>
<tr>
<td>JAB1/Mov34/MPN/PAD-1 [Corchorus capsularis]</td>
<td>571</td>
<td>571</td>
<td>94%</td>
<td>0.0</td>
<td>89%</td>
<td>OMO85839.1</td>
</tr>
<tr>
<td>PREDICTED: COP9 signalosome complex subunit 5b [Vitis vinifera]</td>
<td>570</td>
<td>570</td>
<td>90%</td>
<td>0.0</td>
<td>91%</td>
<td>XP_002283641.1</td>
</tr>
<tr>
<td>COP9 signalosome complex subunit 5a-like [Momordica charantia]</td>
<td>570</td>
<td>570</td>
<td>90%</td>
<td>0.0</td>
<td>90%</td>
<td>XP_02132158.1</td>
</tr>
<tr>
<td>COP9 signalosome complex subunit 5a [Jatropha curcas]</td>
<td>569</td>
<td>569</td>
<td>91%</td>
<td>0.0</td>
<td>87%</td>
<td>XP_012076350.1</td>
</tr>
</tbody>
</table>

2. 21-2
5’—
TAGGGCGAGCGCCGCCNTGGAAGTACCCATACGAGCTACGAGATTAACGCTCA
TATGACAAGTTGTTACAAGAAATGTTGGAAATAAGGGCAAGAGAAGAAAGAAGA
AGAATGGATCCGAGACAATAGCAGCAGAAAAACATGGGAATAGAAGACAAATATAGA
AAGAATGGATCCGAGACAAATAGCAGCAGAAAAACATGGGAATAGAAGACAAATATAGA
TTCAAAAGGGTAAAGTATCAGCATTAGCATTACTAAAGATGGTAGTACATGCTAGATCAGGTGGGAATATAGAAGTAATGGGTTTAAAGGCGAGCGCCGCCNTGGAGTACCCATACGACGTACCAGATTACGCTCATATGACAAGTTTGTACAAAAAAGTTGGAAATAAGGGCAAGAGAAGAAGAAGAA

<table>
<thead>
<tr>
<th>Description</th>
<th>Max score</th>
<th>Total score</th>
<th>Query cover</th>
<th>E value</th>
<th>Ident</th>
<th>Accession</th>
</tr>
</thead>
<tbody>
<tr>
<td>PREDICTED: COP9 signalosome complex subunit 5b-like [Cucumis melo]</td>
<td>572</td>
<td>572</td>
<td>90%</td>
<td>0.0</td>
<td>90%</td>
<td>XP_068445990.1</td>
</tr>
<tr>
<td>PREDICTED: COP9 signalosome complex subunit 5b [Cucumis sativus]</td>
<td>572</td>
<td>572</td>
<td>90%</td>
<td>0.0</td>
<td>90%</td>
<td>XP_064138765.1</td>
</tr>
<tr>
<td>JAB1/Mov34/MPN/PAD-1 [Corchorus capsularis]</td>
<td>571</td>
<td>571</td>
<td>94%</td>
<td>0.0</td>
<td>89%</td>
<td>OMO86129.1</td>
</tr>
<tr>
<td>PREDICTED: COP9 signalosome complex subunit 5b [Vitis vinifera]</td>
<td>570</td>
<td>570</td>
<td>90%</td>
<td>0.0</td>
<td>91%</td>
<td>XP_002283581.1</td>
</tr>
<tr>
<td>COP9 signalosome complex subunit 5a-like [Momordica charantia]</td>
<td>570</td>
<td>570</td>
<td>90%</td>
<td>0.0</td>
<td>90%</td>
<td>XP_022152138.1</td>
</tr>
<tr>
<td>COP9 signalosome complex subunit 5a [Jatropha curcas]</td>
<td>569</td>
<td>569</td>
<td>91%</td>
<td>0.0</td>
<td>87%</td>
<td>XP_012078326.1</td>
</tr>
</tbody>
</table>

3. 22-3

5’—
AGGGCGAGCGCGCGCCNTGGAGTACCCATACGACGTACCAGATTACGCTCATATGACAAGTTTGTACAAAAAAAGTTGGAAATAAGGGCAAGAGAAGAAGAAGAA
GAATGGATCCGAAGACATAAGCGCAGAAGACATGGGAATAGGGAAGAAGAAGAAGAA
GAATGGATCCGAAGACATAAGCGCAGAAGACATGGGAATAGGGAAGAAGAAGAAGAA
GAATGGATCCGAAGACATAAGCGCAGAAGACATGGGAATAGGGAAGAAGAAGAAGAA
GAATGGATCCGAAGACATAAGCGCAGAAGACATGGGAATAGGGAAGAAGAAGAAGAA

147
GATCAGGTGGGAATATAGAATATGGGTTTAATGCAGGGTAAAACTGATGGTG
GATGCTATTATGTATGGATGCTTTTGCTTTACCTGTAGATTATTCTACTACT
AGGGTTAATGCCCAGGCTGATGCTTATGAATATATGGTTGATTATTCTACTACT
AATAAACAGGCTGAAAGCTGGAAATGTGTTGGCTGGTACCCTCTCATC
CTGGTTATAGGGTTGGCTGGCTGGCTTTCTGGCATTGATGTTTCTACAAATGCTCAAC
CAACAATTCAGGAACCTCTTTCTGCTGGTGCATGTCATTGATCCAACCAGAAACTG
TTTCTGCTGAAAAGTGGAAATGGTCATTTTCAGGGACATACCCCGGAGGGGTA
TAAGCCACCGAGTACAGGCCTATCTCAGAATATCAAAACATTCCCTAATAAGAT
TGAAGACTTTGGAGTGCATTGTAAACAGATATTACTCATATGGGACATCATAATTT
CAAGTCCTCCTTTGATTGCCACCTCTTTGATGCTTTTTGGAACAAATACTGGG
TGAATACCCCTTCCCCATACCTTTTGGCTGGGAATGGAAGACTATATTGCTGG
CAAATATCTGATCTCGCTGAGAAATGGAACAGCAGGCAAAACCAGTTGGCTC
ATTTCGTTTTGGTCTCATTGTCGCTTTCTCAAAGAAAAANGAGGAAGA
GCCAGCATTCTGCTAGATAACAGC(TGATAGTACTAGTACAAAGCTAAACGT
CGAGCGTGGTTTGATGNCC—3'  

<table>
<thead>
<tr>
<th>Description</th>
<th>Max score</th>
<th>Total score</th>
<th>Query cover</th>
<th>E value</th>
<th>Identities</th>
<th>Accession</th>
</tr>
</thead>
<tbody>
<tr>
<td>PREDICTED: COP9 signalosome complex subunit 5b-like [Cucumis melo]</td>
<td>572</td>
<td>572</td>
<td>90%</td>
<td>0.0</td>
<td>90%</td>
<td>XP_088445090_1</td>
</tr>
<tr>
<td>PREDICTED: COP9 signalosome complex subunit 5b [Cucumis sativus]</td>
<td>572</td>
<td>572</td>
<td>90%</td>
<td>0.0</td>
<td>90%</td>
<td>XP_084138785_1</td>
</tr>
<tr>
<td>JAB1/ MJV34/MPN/PAD-1 [Corchorus capsularis]</td>
<td>571</td>
<td>571</td>
<td>94%</td>
<td>0.0</td>
<td>89%</td>
<td>OME66129_1</td>
</tr>
<tr>
<td>PREDICTED: COP9 signalosome complex subunit 5b [Vitis vinifera]</td>
<td>570</td>
<td>570</td>
<td>90%</td>
<td>0.0</td>
<td>91%</td>
<td>XP_062283581_1</td>
</tr>
<tr>
<td>COP9 signalosome complex subunit 5a-like [Momordica charantia]</td>
<td>570</td>
<td>570</td>
<td>90%</td>
<td>0.0</td>
<td>90%</td>
<td>XP_022132138_1</td>
</tr>
<tr>
<td>COP9 signalosome complex subunit 5a [Jatropha curcas]</td>
<td>569</td>
<td>569</td>
<td>91%</td>
<td>0.0</td>
<td>87%</td>
<td>XP_012076336_1</td>
</tr>
</tbody>
</table>

4. 23-2
5‘—
CGAGCGCGCCCATGGGANTACCCCATGACGTCCAGATTACGCTCATATGAC
AAGTTTTGTACAAAAAGTTGGGAAATAAGGGCAAGAGAAGAAGAAGAAGAAGATG
GATCCGAAGACAAATAGCGCACAACACACTGGCAAATAGAGAACAATATAAGAAA
AGTAACAGCGCAGCATCAGATGCAATTATTTCAATCGACAGGCACACAG
GTGAATTCCAACAAAGAAGAACCATTGGACGACTAGAAACCTACTTTCAAAA
GGGTAAAAGTATCAGCCATTACATATTACCAAAGATGTGTAGTGACTAGATCA
GGTGGGAATATAGAATATGGGTTTAATGCAGGGTAAAAACTGATGGTG

148
CTATTATGTATCGATTCGGTATTTAACCTCTTCTTTGAAGGTACTGAAACTAGGGTTAATGCCCAGGCTGATGCTTATGAATATATGGTTGGATTATTCTACTACTAATAAACAGAGATGAGCCTATCT
ACAGGCTGGAAGACTGGAAAATGTGGTTGGCTGGTACCACTCTCATCCTGGTATGGTTGCTGGCTTTCTGGCATTGATGTTTCTACACAAATGCTCAACCCATCCT
ACAGAATATCAAACCATTCCCTTAAATAAGATTGAAGACTTTGGAGTGCATTGTAAACAGTATTACTCATTTGGACATCATACATATTCTTCTCTTGATATCGAGAAATTGGGACAGGCAAGAACCAGTTGGCATTCTCGTTTGGGTCTTAGTGCAGCGCTCTCTCTCTCCTAANAAAAANGAGGAAGAGCCAGCATGTAAGAATCGTACTAGAATAGTAAACAGTACGACGAAGCGGCACAGGTGAAATTCCA

<table>
<thead>
<tr>
<th>Description</th>
<th>Max score</th>
<th>Total score</th>
<th>Query cover</th>
<th>E value</th>
<th>Identi</th>
<th>Accession</th>
</tr>
</thead>
<tbody>
<tr>
<td>PREDICTED: COP9 signalosome complex subunit 5b-like [Cucumis melo]</td>
<td>570</td>
<td>570</td>
<td>90%</td>
<td>0.0</td>
<td>90%</td>
<td>XP_098445090.1</td>
</tr>
<tr>
<td>PREDICTED: COP9 signalosome complex subunit 5b [Cucumis sativus]</td>
<td>569</td>
<td>569</td>
<td>90%</td>
<td>0.0</td>
<td>90%</td>
<td>XP_004138765.1</td>
</tr>
<tr>
<td>JAB1/Mov34/MPN/PAD-1 [Corchorus capsularis]</td>
<td>568</td>
<td>568</td>
<td>95%</td>
<td>0.0</td>
<td>88%</td>
<td>OMO86129.1</td>
</tr>
<tr>
<td>PREDICTED: COP9 signalosome complex subunit 5b [Vitis vinifera]</td>
<td>568</td>
<td>568</td>
<td>91%</td>
<td>0.0</td>
<td>91%</td>
<td>XP_002283561.1</td>
</tr>
<tr>
<td>COP9 signalosome complex subunit 5a-like [Momordica charantia]</td>
<td>567</td>
<td>567</td>
<td>90%</td>
<td>0.0</td>
<td>90%</td>
<td>XP_022132138.1</td>
</tr>
<tr>
<td>COP9 signalosome complex subunit 5a [Jatropha curcas]</td>
<td>566</td>
<td>566</td>
<td>92%</td>
<td>0.0</td>
<td>87%</td>
<td>XP_012076336.1</td>
</tr>
</tbody>
</table>

5. 24-1
5’—
TGGGAGTACCCAATACGACGTACCAGATTACGCTCATATGACAAAGTTTGTACAAAANGTGGAAAATAAGGGCAAGGAAGAAAGAAGAAAGAAGGATGGATCCGAAGACAATAGCGCAGAAACATGGGAAATAGAGAAACATAGAAACAGTAAACGCAGCAGCATCAGATGCAATATCTTCAAAGTACGACGAAGGGCACACAGGGTGAATTCCA
ACAAGAGAAACCATGGACGAATGAACCCTATTACTTTCAAAAAAGGTGAAAGTAT
CAGCATTAGCATTACTAAAGATGGTAGTACATGCTAGATCAGGTGGGAATATA
GAAATGTGGGAATTTTGCTTTACCTGTTGAAGGTACTGAAACTAGGGTTAATGCCCAGG
GACTGGAAAATGTGGTTGGTACTCACCCTCTCTCCTGTTATGTTGGCTG
GCTTTCTGGCATTGATGTTTCTACACAATGTGCAAACCAACATTTCCAAGAAC
CCCTCTGGGTCTGGTCATTGACACCAACCAGAAGTGTCTTTGCTGGGAAAGT
TGAAATTTGGTGGCTTTTCAGGACATACCAGGGGCTATAAAGCCACCAGATGAG
CTATCTCAAGATATCACCACATTCCCTTTAATAGATTGAAGACCTTGGAAATG
CATTGTAACAGTATTACTCATTGGGACATCACAATATTTCAAGTCTTTCTTTGAT
GCCACCTCTGGGTCTTTGGAAACAAATCTGGGTATGCTACCTTCTTCCAAC
TCACCTTTGTGGGAATTTGGAATATTATGCTGGAATAAGAATCTCAGTCGC
TGAGAAATTTGGAGCCAGCAGAACAGGAGCCATTGTCATTTCATTCTTTGGCTCT
ATAGTTGGCCCTCTTCTCAAAAAAGAAAAAGAGGAAAANCCAGCACTTCTGAAAN
TAACACGTGATAAGTACTAACGATAACAGTCGAGCGAGGTGCTGTTGGATGTCC
CAGGTAATCAA—3

<table>
<thead>
<tr>
<th>Description</th>
<th>Max score</th>
<th>Total score</th>
<th>Query cover</th>
<th>E value</th>
<th>Ident</th>
<th>Accession</th>
</tr>
</thead>
<tbody>
<tr>
<td>PREDICTED: COP9 signalosome complex subunit 5b-like [Cucumis melo]</td>
<td>571</td>
<td>571</td>
<td>89%</td>
<td>0.0</td>
<td>91%</td>
<td>XP_008445090.1</td>
</tr>
<tr>
<td>PREDICTED: COP9 signalosome complex subunit 5b [Cucumis sativus]</td>
<td>571</td>
<td>571</td>
<td>89%</td>
<td>0.0</td>
<td>91%</td>
<td>XP_004138765.1</td>
</tr>
<tr>
<td>PREDICTED: COP9 signalosome complex subunit 5b [Vitis vinifera]</td>
<td>569</td>
<td>569</td>
<td>90%</td>
<td>0.0</td>
<td>91%</td>
<td>XP_002283581.1</td>
</tr>
<tr>
<td>COP9 signalosome complex subunit 5a-like [Momordica charantia]</td>
<td>568</td>
<td>568</td>
<td>89%</td>
<td>0.0</td>
<td>90%</td>
<td>XP_022132313.1</td>
</tr>
<tr>
<td>JAB1/Mov34/MPN/PAD-1 [Corchorus capsularis]</td>
<td>568</td>
<td>568</td>
<td>94%</td>
<td>0.0</td>
<td>88%</td>
<td>OMO09129.1</td>
</tr>
<tr>
<td>COP9 signalosome complex subunit 5a [Jatropha curcas]</td>
<td>567</td>
<td>567</td>
<td>91%</td>
<td>0.0</td>
<td>88%</td>
<td>XP_012078335.1</td>
</tr>
</tbody>
</table>

6. 25-2
5'—
AGCGCCCGCCATGGAGTACCNATACGCAGCTACCAGATTACGCTCATATGACAA
GTTTGTACAAAAAAAGTTGGAAATAAGGGGAAGAGAAGAAGAAGAAGAAGAAGA
TCCGAAGACAATAGCCAGAAACATGGGAATAGAAGAAACAAATAGAAACA
GTAAGCGACGCACATGCAGTATCTATATTTCAAGTGACGAGCGGACAGG
TGAAATTTTCAAAAAGGAAACATGGAGCAATGAAACCTATTACTTCAAAAG
GGTAAAAGTATCAGCATTAGCATTACTAAAGATGGTAGTACATGCTAGATCAG
GTGGGAATATAGAAGTAATGGGTTTAATGCAGGGTAAAACTGATGGGTTGCT
ATTATTGTTATGGATGCTTTTGCTTTACCTGTTGAAGGTACTGAAACTAGGGTT
AATGCCCAGCTTCTGGCATTGATGTGTTTCTACACAAATGCTCAACCAAACAT
TGAAAAAGTTGAAATTGGTGGCTTTGCAGGACATACCCGGAGGCTATAAGGCA
CCAGATGAGGCTATCTCAGAATATCAAACCATTCTCTTAAATAAGATTTGAAAGAC
TTGGGAGTGCATTGAAACAGTATTACTCATTTGACATACATATTTCAAGTCT
TCTTGGATTGCCACCTCCTTGGATCTTTTTGTGGAACAAACTGCGGTAATAC
CTTTCCTCATCACCTTTGCTGGAATGGAAGACTATATTGCTGGGGAATATAT
CTGATCTCGTGGACGCAAGGCCAAAAACATGGTGGCTCTTCAAGTCT
GTTTGGTTCTTATAGTTGCGCCTTCTCAAAGAAAAAAGGGAAGGACGCAGC
ACTTGGCTAAGATAAACACGTGAGTACTAAGANAAACAGTGGAGCAGTTCCAT
GGTTTGATGTCGGAGTTCAAGATTTGCTCCTATTATAATGCGTTAGAAACATT
ACGTCTCAG—3'

<table>
<thead>
<tr>
<th>Description</th>
<th>Max score</th>
<th>Total score</th>
<th>Query cover</th>
<th>E value</th>
<th>Ident</th>
<th>Accession</th>
</tr>
</thead>
<tbody>
<tr>
<td>PREDICTED: COP9 signalosome complex subunit 5b-like [Cucumis melo]</td>
<td>570</td>
<td>570</td>
<td>90%</td>
<td>0.0</td>
<td>91%</td>
<td>XP_008445090.1</td>
</tr>
<tr>
<td>PREDICTED: COP9 signalosome complex subunit 5b [Cucumis sativus]</td>
<td>570</td>
<td>570</td>
<td>90%</td>
<td>0.0</td>
<td>91%</td>
<td>XP_004138765.1</td>
</tr>
<tr>
<td>JAB1/Mov34/MPN/PAD-1 [Corchorus capsularis]</td>
<td>569</td>
<td>569</td>
<td>94%</td>
<td>0.0</td>
<td>89%</td>
<td>OMO96120.1</td>
</tr>
<tr>
<td>PREDICTED: COP9 signalosome complex subunit 5b [Vitis vinifera]</td>
<td>568</td>
<td>568</td>
<td>90%</td>
<td>0.0</td>
<td>91%</td>
<td>XP_002283561.1</td>
</tr>
<tr>
<td>COP9 signalosome complex subunit 5a-like [Momordica charantia]</td>
<td>567</td>
<td>567</td>
<td>90%</td>
<td>0.0</td>
<td>90%</td>
<td>XP_022132138.1</td>
</tr>
<tr>
<td>COP9 signalosome complex subunit 5a [Jatropha curcas]</td>
<td>566</td>
<td>566</td>
<td>92%</td>
<td>0.0</td>
<td>88%</td>
<td>XP_012076336.1</td>
</tr>
</tbody>
</table>

7. 26-1
5’—
CGCCGCSCATGGANTACCNATACGCACGTACCAGATTACGCCTCATATGACAAAGT
TTTGTCACACAAAGTTGGGAATAAAGGGCAAGAGAAGAAGAAGAAGAATGGGATC
CGAAGACAATAGCGCAGAAAAACATGGGAAATAGAGAACAATAGAAGACAGTA
AAGCAGCAGCATACGATGCAAATATTTCAAGTACGACGAAGCGGCACAGGTG
AAATCCCAAAAGAGAAAACCATGGGAGAATGACCTATTACTTCAAAAAAGGG
TAAAAGTATCAGCATTAGCATTACTAAAGATGGTAGTACATGCTAGATCAGGTG
GGAATATAGAAGTAATGGGTTTAATGCAGGGTAAAACTGATGGGATGCTATT
ATTGTTATGGGATGCTTTTGGCTTTACACTGTTGAAAGGTACTGAAACTAGGGTTAAT
GCCCAGGCTAGCTTTTCTGATGTGTTTCTACTACACAAATGCTCAACCAAAATTC
CAAGAACCCTTCCTGCGCTGTCATGATACTGAAACTACAGGCTGACCCACATACCCGGAGGGCTATAAGGCCAC
AGATGAGCCTATCTCAGAATATCAAAACCATTCCCTTAAATAAGATTGGAAGACTTTGGAGTCATTGTAAACAGTATTACTCATTGCTGGACATCAGTATTTCAAGTCTTCCTCTTTGATATGGGATGCCCACTCTTTGATCTTTTGGTGAACAAATCTGGGTGAATACCC
TTTCCCTACATCAATTGGAAAGTGGAGACTATATTGTCTGGGAAATATCTGTCTCGAGAAATTGGAGCAGGCAGAAAACCAGTTGGCTCATTCTCGTTTGGGTCACTATAGTTGCCCTTTCTCTAAAAANAAAAAGAGGAAAGGACAGGACTGTGCTAAGATAACCCNNNAGATGCAACTAACAGGATCTGCAGGTCATCGAG
TGATGTCCAGGTAATCNAAGATGTCCTATTTTAATTCCGTT

<table>
<thead>
<tr>
<th>Description</th>
<th>Max score</th>
<th>Total score</th>
<th>Query cover</th>
<th>E value</th>
<th>Ident</th>
<th>Accession</th>
</tr>
</thead>
<tbody>
<tr>
<td>PREDICTED: COP9 signalosome complex subunit 5β-like</td>
<td>570</td>
<td>570</td>
<td>90%</td>
<td>0.0</td>
<td>91%</td>
<td>XP_008445090.1</td>
</tr>
<tr>
<td>[Cucurbita melo]</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PREDICTED: COP9 signalosome complex subunit 5β</td>
<td>570</td>
<td>570</td>
<td>90%</td>
<td>0.0</td>
<td>91%</td>
<td>XP_004138765.1</td>
</tr>
<tr>
<td>[Cucurbita salivax]</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>JAB1/MPV/MPN/PAD-1 [Corchorus capsularis]</td>
<td>569</td>
<td>569</td>
<td>94%</td>
<td>0.0</td>
<td>89%</td>
<td>OMO86129.1</td>
</tr>
<tr>
<td>PREDICTED: COP9 signalosome complex subunit 5β [Vitis</td>
<td>568</td>
<td>568</td>
<td>90%</td>
<td>0.0</td>
<td>91%</td>
<td>XP_002283581.1</td>
</tr>
<tr>
<td>vinifera]</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>COP9 signalosome complex subunit 5α-like [Momordica</td>
<td>567</td>
<td>567</td>
<td>90%</td>
<td>0.0</td>
<td>90%</td>
<td>XP_022132138.1</td>
</tr>
<tr>
<td>charantia]</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>COP9 signalosome complex subunit 5α [Jatropha curcas]</td>
<td>566</td>
<td>566</td>
<td>92%</td>
<td>0.0</td>
<td>88%</td>
<td>XP_012076336.1</td>
</tr>
</tbody>
</table>

8. 27-3
5‘—
GGGCAGCAGCGCGCNCNTGGAGTACCNATAGCAGTACCAATGCTATGCTCATAT
GACAAGTTTTGTCACAAAAAAGTGGGAATAAGGGCAAGAGAGAAGAGAAGAGAAGAGA
ATGGATCCAGAAGACATAGCAGAAACCATGGGAAATAGGAGAACAATATAGA
AACAGTAAACGAGCAGCATCAGATGCAATATTCAATACGACGAGCGAGCGA
CAGGTGAATTCACAAAGAGGAACCATGGGACATGAACCTCATTACTTCA
AAAGGGTAAAGTGATCAGCATTAGCATTACTAAAGATGGTAGTACATGCTAGATCGGTG
GGGAATATAGAAGTAATGGGTTTAATGCAGGGTAAAACTGATGGGATGCTATT
ATTGTTATGGGATGCTTTTGGCTTTACACTGTTGAAAGGTACTGAAACTAGGGTTAAT
GCCCAGGCTAGCTTTTCTGATGTGTTTCTACTACACAAATGCTCAACCAAAATTC
CAAGAACCCTTCCTGCGCTGTCATGATACTGAAACTACAGGCTGACCCACATACCCGGAGGGCTATAAGGCCAC
AGATGAGCCTATCTCAGAATATCAAAACCATTCCCTTAAATAAGATTGGAAGACTTTGGAGTCATTGTAAACAGTATTACTCATTGCTGGACATCAGTATTTCAAGTCTTCCTCTTTGATATGGGATGCCCACTCTTTGATCTTTTGGTGAACAAATCTGGGTGAATACCC
TTTCCCTACATCAATTGGAAAGTGGAGACTATATTGTCTGGGAAATATCTGTCTCGAGAAATTGGAGCAGGCAGAAAACCAGTTGGCTCATTCTCGTTTGGGTCACTATAGTTGCCCTTTCTCTAAAAANAAAAAGAGGAAAGGACAGGACTGTGCTAAGATAACCCNNNAGATGCAACTAACAGGATCTGCAGGTCATCGAG
TGATGTCCAGGTAATCNAAGATGTCCTATTTTAATTCCGTT

152
CAGGTGGGAATATAGAAGTAATGGGTTTAATGCAGGGTAAAACTGATGGTGAT
GCTATTATTGTATGAGTCTTTTTGCTTTACCTGTGAAGGTACTGAAACTAGG
TTAATGCCAGGGCTATGCTTTATGAATATATGGTTGATTATTCTACTACTATAA
AACAGGCTTGGAAAGACTGGAAATGTGGTTGGGCTGGTACCACTCTCAGGCAAAC
CTGCTGGAAAAGTTGAAATTGGTGCTTTTCAGGACATACCCCGGAGGGCTATAA
GCCACCAGATGAGGCCTATCTCAGAATTCAAAACCATTCCCCAAAGAAGATTG
AAGACCTTGGAGTTGACATTGAAACAGATTACTCATTGGACATCCATATTCCA
GCTTCTTCTTGTGACCGCTCTCCCTTGTGCTTTTGTGGAACAAAATACGGAAGT
AATACCCCTCCCTCATCACCCTTTGGCTGGAAAATGGAAGACTATATTGCTGGGCA
AATATCTGATTCGCTGAAATTTGGAGACGAGAGAAACCACTGGGCTCATT
TCTCTTTGGTGCTCTATAGCTTCGCTTCTCTCAAAANAAAAANGAGGAAGAGC
CAGCAGTGTGAAGATAACGTGATGACTGACTAGATCAGAAAAACGTCAGGCA
CCATGGTTTGATGTCAGATCCATTTATAGTTGACATTTCCAATTCCGTGAGAC
CAATTACGTCTCAGGGCNA—3'

<table>
<thead>
<tr>
<th>Description</th>
<th>Max score</th>
<th>Total score</th>
<th>Query cover</th>
<th>E value</th>
<th>Ident</th>
<th>Accession</th>
</tr>
</thead>
<tbody>
<tr>
<td>PREDICTED: COP9 signalosome complex subunit 5b [Cucumis sativus]</td>
<td>571</td>
<td>571</td>
<td>89%</td>
<td>0.0</td>
<td>91%</td>
<td>XP_004138765.1</td>
</tr>
<tr>
<td>PREDICTED: COP9 signalosome complex subunit 5b-like [Cucumis melo]</td>
<td>571</td>
<td>571</td>
<td>89%</td>
<td>0.0</td>
<td>91%</td>
<td>XP_008445090.1</td>
</tr>
<tr>
<td>JAB1/MP/MPFAD-1 [Corchorus capsularis]</td>
<td>570</td>
<td>570</td>
<td>94%</td>
<td>0.0</td>
<td>88%</td>
<td>OMO89129.1</td>
</tr>
<tr>
<td>PREDICTED: COP9 signalosome complex subunit 5b [Vitis vinifera]</td>
<td>570</td>
<td>570</td>
<td>90%</td>
<td>0.0</td>
<td>91%</td>
<td>XP_002283581.1</td>
</tr>
<tr>
<td>COP9 signalosome complex subunit 5a-like [Momordica charantia]</td>
<td>568</td>
<td>568</td>
<td>89%</td>
<td>0.0</td>
<td>90%</td>
<td>XP_022132138.1</td>
</tr>
<tr>
<td>COP9 signalosome complex subunit 5a [Jatropha curcas]</td>
<td>568</td>
<td>568</td>
<td>91%</td>
<td>0.0</td>
<td>88%</td>
<td>XP_012076336.1</td>
</tr>
</tbody>
</table>

9. 28-1
5’—
GGGCGAGCGCCCGCCNTGGGAGTACCNNATACGACGTACCAAGTACCTCATATT
GACAAGTTTGTACAAAAAGTTGGAATAAGGGCAAGAGAAGAAGAAGAAGAAGA
ATGGATCCGAAGACAATAGCAGCAGAAAACATGGGAATAAGAAGAAGAAGAAGA
AACAGTAAACGAGCGACGACATGCAATATCAAGTAGACGACGACGACGAC
CAGGTGAAATTCCAAAGAAGAAACCATGGGACGAAATGACCTATTACAT
AAAGGGTAAGTTATACGACATTAGCATTACTAAAGATGGTAGTCATGCTAGAT
CAGTGAGAATAGAAGTAAATTGTTTATGCAAGGATTATGTCTATTTTACCTGTGGAGAAGTACTGAAACTAGG
GTTAATGGCAGGCTAGTCTTCTATGATATATGTTGATTATTTCTACTACTATAA
AACAGGCTGGAAGACTGGGAAATGGTGGTGGCTGGCTTTCTGGCATTGATGTTTCTACACAAATGCTCAACAA
CAATTCAAGAACCTCCCGGCTGGGCTGAAACTAGG
GTTAATGCCCAGGCTGATGCTTATGAATATATGGTTGATTATTCTACTACTAATA
AACAGGCTGGAAGACTGGAAAATGTGGTTGGCTGGTACCACTCTCATCCTG
GTTATGGTTGCTGGCTTTTCTGGCATTGATGTTTCTACACAAATGCTCAACCAA
CTGCTGAAAAGTGGGAAATGGTGGTGGCTGGCTTTCTGGCATTGATGTTTCTACACAAATGCTCAACCAA
AACAGGCTGGAAGACTGGAAAATGTGGTTGGCTGGTACCACTCTCATCCTG
GTTATGGTTGCTGGCTTTTCTGGCATTGATGTTTCTACACAAATGCTCAACCAA

<table>
<thead>
<tr>
<th>Description</th>
<th>Max score</th>
<th>Total score</th>
<th>Query cover</th>
<th>E value</th>
<th>Ident</th>
<th>Accession</th>
</tr>
</thead>
<tbody>
<tr>
<td>PREDICTED: COP9 signalosome complex subunit 5b-like [Cucumis melo]</td>
<td>572</td>
<td>572</td>
<td>90%</td>
<td>0.0</td>
<td>91%</td>
<td>XP_008450900.1</td>
</tr>
<tr>
<td>PREDICTED: COP9 signalosome complex subunit 5b [Cucumis sativus]</td>
<td>572</td>
<td>572</td>
<td>90%</td>
<td>0.0</td>
<td>91%</td>
<td>XP_004130765.1</td>
</tr>
<tr>
<td>PREDICTED: COP9 signalosome complex subunit 5a-like [Vitis vinifera]</td>
<td>570</td>
<td>570</td>
<td>91%</td>
<td>0.0</td>
<td>91%</td>
<td>XP_002283581.1</td>
</tr>
<tr>
<td>COP9 signalosome complex subunit 5a [Monomorcha charantiflora]</td>
<td>569</td>
<td>569</td>
<td>90%</td>
<td>0.0</td>
<td>90%</td>
<td>XP_022132138.1</td>
</tr>
<tr>
<td>JAB1/Mov34/MFN/PAD-1 [Corchorus capsularis]</td>
<td>569</td>
<td>569</td>
<td>95%</td>
<td>0.0</td>
<td>88%</td>
<td>OMO06129.1</td>
</tr>
<tr>
<td>COP9 signalosome complex subunit 5a [Jatropha curcas]</td>
<td>568</td>
<td>568</td>
<td>92%</td>
<td>0.0</td>
<td>88%</td>
<td>XP_012076336.1</td>
</tr>
</tbody>
</table>

10.29-2
5'—
TAGGGGCAGCGCCGCCATGGAGTACCCATACGACGTACCAGATTACGCTCAT
ATGACAAGTTTGTACAACAAAAGGTTGGAAATAAGGGCAAGAGAAGAAGAAGAAGA
GAATGGATCCGAAAGACATAGCGCAGAACACATGGGAAATAGAGAACAATATA
GAAACAGTAGAACAGCGCAGCATCAGAGCATAATTCCAAGTACGACGAGAGG
CACAGGTGAATTTCCAACAAAGAGAAACCATGGAGAATGAACCTATTACTT
CAAAAAGGGTTAAAGTGACACGCTTTACATTAAAGATGGTAGTACATGCTA
GATCAGGTTGGAATATAGAATATGGTTTAATGCAGGGTAAAAACTGATGGT
GATGCTATTATTGTTATGGATGCTTTTGCTTTACCTGTGTGTTGCTGGCTC
CTGTTATGTTGCTGGTTTCTGGCATAGTGGTTTCTACACAAATGCTCAAC
CAACAATTTCAAGCCACCTTCTGCTGTGCTGTCAATGGATCCAACCAGAAACTG
TTTCGCTGAAAAAGTTGATGTTGCTGTTCAGACATACCCCGGAGGGCTA
TAAGCCACCAGATGAGCCTATACCAAAACTCCATCCTAAATAAGAT
TGAAGACTTGGAGTGGGATTTGAACAGATTACTACATATTGGGACTACATATT
TCAAGTCCTCTCTGATTGCCACCTCTTTTGCTTTTGTGGAACAAATACTGGG
TGAATACCCCTTCTCATTACATTTGCTGGGAAATGGAACATATATTGCTGGG
CAAATATCTGATCTCGACTGAGAAATTTGGAGCAGGACAGAAACCAGGTGGGCTC
TTTCGCTTTGGGCTATAGGTGGCTCTTATGGCGCTTCTATTAAAGAAAAANGAGGAAGA
GCCAGCATTGCTAAGATAAACGTTGATAGTACTAAAGATAAAACAGTGGAGCAG
GTCATATGGTTGATGTCAGCAGGAATACAGAGATGTCTATTTAATTCCGGTTANN
CCAATTACGTTCAGGCAAA—3' 

<table>
<thead>
<tr>
<th>Description</th>
<th>Max score</th>
<th>Total score</th>
<th>Query cover</th>
<th>E value</th>
<th>Identi</th>
<th>Accession</th>
</tr>
</thead>
<tbody>
<tr>
<td>PREDICTED: COP9 signalosome complex subunit 5b [Beta vulgaris subsp. vulgaris]</td>
<td>629</td>
<td>629</td>
<td>90%</td>
<td>0.0</td>
<td>86%</td>
<td>XP_01068282.1</td>
</tr>
<tr>
<td>PREDICTED: COP9 signalosome complex subunit 5b-like [Cucumis melo]</td>
<td>627</td>
<td>627</td>
<td>89%</td>
<td>0.0</td>
<td>90%</td>
<td>XP_009445090.1</td>
</tr>
<tr>
<td>PREDICTED: COP9 signalosome complex subunit 5b [Vitis vinifera]</td>
<td>627</td>
<td>627</td>
<td>89%</td>
<td>0.0</td>
<td>91%</td>
<td>XP_006223581.1</td>
</tr>
<tr>
<td>PREDICTED: COP9 signalosome complex subunit 5b [Cucumis sativus]</td>
<td>627</td>
<td>627</td>
<td>89%</td>
<td>0.0</td>
<td>90%</td>
<td>XP_006138758.1</td>
</tr>
<tr>
<td>COP9 signalosome complex subunit 5a [Jatropha curcas]</td>
<td>625</td>
<td>625</td>
<td>90%</td>
<td>0.0</td>
<td>87%</td>
<td>XP_012076336.1</td>
</tr>
<tr>
<td>COP9 signalosome complex subunit 5a-like [Momordica charantia]</td>
<td>624</td>
<td>624</td>
<td>89%</td>
<td>0.0</td>
<td>89%</td>
<td>XP_022132138.1</td>
</tr>
</tbody>
</table>

11.30-2

5’—
AGGGCGAGCGCCGCGCATGGAGTACCCCATACGACGTACCAGATTACGCTCAT
ATGACAAAGTTTGTACAAAAAAAGTTGGAATAAGGGCAAGAGAAAGAGAGAAA
GAATGGATCCGAGAAATAGCAGCAGAAAAACATGGGAAATAGGAGAAATATA
GAAACAGTAAACGCAGCAGCTACAGTGCAATATTTCAAGTACGCAGAGCGG
CACAGGTGAATTCCAAAGAGAAACCATGGGAGAATGAAACCTTACATT
CAAAAGGGTAAAAGTATCAGCATTAGCATTACTAAAGATGGTAGTACATGCTA
GATGCTATTATTGTATTGATGCTTTTGTCTTTACCTGTTGAAGGTACTGAAACT
AGGGTTAATTGCCAGGCTGATGCTTTATGAATATAGTGGTATTATTCTACTACT
AAATAACAGGCGTGGAAAGACTGGAATAATGGGTGSGTGGTACCACTCTCATC
CTGGTTATGGTTGCTGTTTCTGCGATTTGATGTTTCTACACAAATGCTCAAAC
CAACAACTCAAAGACCCCTTCTCCCCGTGGCTGGTATCAGCCAAACTCGAG
TTCTGCTGGAAAAGTTGAAATTGGTCTTTCAGGACATACCAGGAGGCTATAAGCCACAGTGGAGCCTATCTCAGAATATCAAACCATTTCCCTAAATAAGAT
GAAGACTTTTGGAGTGCATTGTAAACAGTAT
TACTCATTGGACATCACATATTT
CAAGTCTTCTTGGATGACCACCTCTTGGATCTTCTTGTTGGAACAAATACTGGGG
TGAATACCCCTTTCCTCATACCTTTTCTGGGGAATGGGAGACTTATATTGCTGGG
CAAATATCTGATCTCGCTGAGAAATTGGAGCAGGCAGAAAACCAGTTGGCTC
ATTCTCGTTTTGGGTCTATAGTTGCCTCTTCTCCTAAAGAAAAANGAGGAANA
NCCAGCAGTTCTGCTAAGATAAACCGTGAGTGACTAAGATAACAGTCGAGCAG
GTCCATAGTTTGTAGTTCCAGGATATCAAGAGATGCTCTTATTTAATTC—3'

<table>
<thead>
<tr>
<th>Description</th>
<th>Max score</th>
<th>Total score</th>
<th>Query cover</th>
<th>E value</th>
<th>Ident</th>
<th>Accession</th>
</tr>
</thead>
<tbody>
<tr>
<td>PREDICTED: COP9 signalosome complex subunit 5b [Beta vulgaris subsp. vulgaris]</td>
<td>629</td>
<td>629</td>
<td>90%</td>
<td>0.0</td>
<td>88%</td>
<td>XP_010969222.1</td>
</tr>
<tr>
<td>PREDICTED: COP9 signalosome complex subunit 5b-like [Cucumis melo]</td>
<td>627</td>
<td>627</td>
<td>89%</td>
<td>0.0</td>
<td>90%</td>
<td>XP_008445099.1</td>
</tr>
<tr>
<td>PREDICTED: COP9 signalosome complex subunit 5b [Vitis vinifera]</td>
<td>627</td>
<td>627</td>
<td>89%</td>
<td>0.0</td>
<td>91%</td>
<td>XP_002283561.1</td>
</tr>
<tr>
<td>PREDICTED: COP9 signalosome complex subunit 5b [Cucumis sativus]</td>
<td>627</td>
<td>627</td>
<td>89%</td>
<td>0.0</td>
<td>90%</td>
<td>XP_004136755.1</td>
</tr>
<tr>
<td>COP9 signalosome complex subunit 5a [Jatropha curcas]</td>
<td>625</td>
<td>625</td>
<td>90%</td>
<td>0.0</td>
<td>87%</td>
<td>XP_012076336.1</td>
</tr>
<tr>
<td>COP9 signalosome complex subunit 5a-like [Momordica charantia]</td>
<td>624</td>
<td>624</td>
<td>89%</td>
<td>0.0</td>
<td>89%</td>
<td>XP_022132138.1</td>
</tr>
</tbody>
</table>

12.32-1
5'—
AGGGCGAGCGCAGCCCTNTGGAGTATCCCATACGAGCTACAGATTAGCCTCAT
ATGACAAGTTTTGNNNNNAAAGTTGGGAATAAGGGCAAGAGAAGAAGAAGA
AGAATTTGATCCGAAGACATAGCGCAGAAACATGGGAAATAGAGAACAATA
TAGAAAAAGTAAACGACGCAGCATCAGATGCAATATTCAAAGTACGACGAAGC
GGCAGGATGAAATTTCCAAACAGAGAAACCATGGACGAATGAACCTATTAC

156
TTCAAAAGGGTAAAAGTATCAGCATTACTAAAGATGGTAGTACATGCTAGATCAGGTGGGAATATAGAAGTAATGGGTTTAATGCAGGGTAAAACTGATGGTGATGCTATTATTGTTATGGATGCTTTTGCTTTACCTGTTGAAGGTACTGAAAC

 compromised by a decrease in the number of functional copies of the gene, resulting in an increased susceptibility to infection by pathogenic microorganisms. In plants, this may lead to reduced growth and impaired development, ultimately affecting crop yields. Therefore, strategies for the maintenance and enhancement of the genetic diversity of the signalosome complex are crucial to sustain agricultural productivity.
AAAGGGTAAAAAGTATCAGCATTAGCATTACTAAAGATGGTAGTACATGCTAGAT
CAGGTGGAATATAGAAAGTAATGGGTTTAATGCAGGGTAAAAACTGATGGTGAT
GCTATTATTTATGGATGCTTTTTCCTGTATATTAGAATATATGTTGTATTATTCTACTATAA
AACAGGGCTGGGAAGACTGAAATGGTGTGCTGGTACCACCTCACCCTGG
GTTATGGTTGCCTCTTCTGGCATTTGATTTTCTACACAAATGCTCAACCAA
CAATTCCAGAACCCTTCTCCGCTGCTTCATTTGACTCAACGAGACTGTTT
CTGCTGAAAGTTGAAATTGGTGCTTTCAGGACATACCCGGAGGGCTATAA
GCCACCCAGATGAGCCTATCTCATAGAATATCAAAACCATTCCCCTTTAAAATAAGATG
AAGACTTTGGAGTGCATTGTAACACGATTATTACCTCATTGGACATCACATATTTC
AGTCTTCTCTTGATTGCCACCTCTTGGATCTTTTGTGAAAATACTCGGTTG
AATACCCCTTCTCATACCTTTTGCCTGGGAATGGAGACTATATTGGCTGGGCA
AATATCTGATTCGTGCCTAGAAAGTCGACAGGCAGAAACCAGCTTTGGGCTCAT
TCTCCTTTTGGGTCTATAGTGGCGCCTTCTCCTACAAANAAAAANGAGGAGAAGGC
AGCACTTGCTAAGATAAACACGTGATAGTACTAAGATAACAGTCGAGCAGGT
CATGGTTGTATGCTCCCAGATAAACAGATGTCTCTATTTAAATCC—3’

<table>
<thead>
<tr>
<th>Description</th>
<th>Max score</th>
<th>Total score</th>
<th>Query cover</th>
<th>E value</th>
<th>Ident</th>
<th>Accession</th>
</tr>
</thead>
<tbody>
<tr>
<td>PREDICTED: COP9 signalosome complex subunit 5b-like [Cucumis melo]</td>
<td>571</td>
<td>571</td>
<td>89%</td>
<td>0.0</td>
<td>91%</td>
<td>XP_008446090.1</td>
</tr>
<tr>
<td>PREDICTED: COP9 signalosome complex subunit 5b [Cucumis sativus]</td>
<td>571</td>
<td>571</td>
<td>89%</td>
<td>0.0</td>
<td>91%</td>
<td>XP_004138769.1</td>
</tr>
<tr>
<td>PREDICTED: COP9 signalosome complex subunit 5b [Vitis vinifera]</td>
<td>570</td>
<td>570</td>
<td>90%</td>
<td>0.0</td>
<td>91%</td>
<td>XP_002283556.1</td>
</tr>
<tr>
<td>JAB1/Mov34/MPN/PAD-1 [Corchorus capsularis]</td>
<td>569</td>
<td>569</td>
<td>94%</td>
<td>0.0</td>
<td>88%</td>
<td>OMO86129.1</td>
</tr>
<tr>
<td>COP9 signalosome complex subunit 5a-like [Monemotrix charantia]</td>
<td>569</td>
<td>569</td>
<td>89%</td>
<td>0.0</td>
<td>90%</td>
<td>XP_022132138.1</td>
</tr>
<tr>
<td>COP9 signalosome complex subunit 5a [Jatropha curcas]</td>
<td>568</td>
<td>568</td>
<td>91%</td>
<td>0.0</td>
<td>88%</td>
<td>XP_012076236.1</td>
</tr>
</tbody>
</table>

14.34-1
5’—
CGCCGCCTTGGAGTACCCTACGACGTACCAGATTGCTCATATGACAAGTTGTACAAAGTTGGAATAAGGGCAAGAGAAGAGAAGAAGAAGATGGGAC
CGAAGCAATAGCCGAAACATGAAAATAGAGAAACATATAGAAGAAGAAGAAGATGGGAC
AAGAGAACATAGGCCGAAAATAGGAAGAAAGAAGAAGAAGATGGGAC
AAATCCAAAGAGAAACCCTAGGCGAGAATGAAACCTATTACTTCAAAAGGG
TAAAAAGTATCAGCATTAGCATTACTAAAGATGGTAGTACATGCTAGATCGGGAATATAGAAAGTAATGGGTTTAATGCAGGGTAAAAACTGATGGTGAT
GCTATTATTTATGGATGCTTTTTCCTGTATATTAGAATATATGTTGTATTATTCTACTATAA
AACAGGGCTGGGAAGACTGAAATGGTGTGCTGGTACCACCTCACCCTGG
GTTATGGTTGCCTCTTCTGGCATTTGATTTTCTACACAAATGCTCAACCAA
CAATTCCAGAACCCTTCTCCGCTGCTTCATTTGACTCAACGAGACTGTTT
CTGCTGAAAGTTGAAATTGGTGCTTTCAGGACATACCCGGAGGGCTATAA
GCCACCCAGATGAGCCTATCTCATAGAATATCAAAACCATTCCCCTTTAAAATAAGATG
AAGACTTTGGAGTGCATTGTAACACGATTATTACCTCATTGGACATCACATATTTC
AGTCTTCTCTTGATTGCCACCTCTTGGATCTTTTGTGAAAATACTCGGTTG
AATACCCCTTCTCATACCTTTTGCCTGGGAATGGAGACTATATTGGCTGGGCA
AATATCTGATTCGTGCCTAGAAAGTCGACAGGCAGAAACCAGCTTTGGGCTCAT
TCTCCTTTTGGGTCTATAGTGGCGCCTTCTCCTACAAANAAAAANGAGGAGAAGGC
AGCACTTGCTAAGATAAACACGTGATAGTACTAAGATAACAGTCGAGCAGGT
CATGGTTGTATGCTCCCAGATAAACAGATGTCTCTATTTAAATCC—3’

158
GGGCGAGCGCCGCGCCTTTGGAAGTACCATGACGCTACCAGATTACGCTCATAT
GACAAGTGTGNNCNNAAAAAGTTGGGAAAATAAAGGGAAGAGAAGAAGAAGAAG
AATGGATCCGAGACATAGCGCAGAAATCAATTTCAAGTACGAGCGAAGCGGC
ACAGGTGAAAATTCAACAGAAGAAAACCATGGGACATGAACCTACTATTCTTC
AAAAGGTAAAAGTATCGACATTACATTAAAGATGGTAGTACATGCNTAG

<table>
<thead>
<tr>
<th>Description</th>
<th>Max score</th>
<th>Total score</th>
<th>Query cover</th>
<th>E value</th>
<th>Ident</th>
<th>Accession</th>
</tr>
</thead>
<tbody>
<tr>
<td>PREDICTED: COP9 signalosome complex subunit 5b-like [Cucumis melo]</td>
<td>570</td>
<td>570</td>
<td>88%</td>
<td>0.0</td>
<td>91%</td>
<td>XP_008445090.1</td>
</tr>
<tr>
<td>PREDICTED: COP9 signalosome complex subunit 5b [Cucumis sativus]</td>
<td>570</td>
<td>570</td>
<td>88%</td>
<td>0.0</td>
<td>91%</td>
<td>XP_004138765.1</td>
</tr>
<tr>
<td>JAB1/Mov34/MPP/NAP-1 [Cochlora capsularis]</td>
<td>569</td>
<td>569</td>
<td>93%</td>
<td>0.0</td>
<td>88%</td>
<td>OMO86128.1</td>
</tr>
<tr>
<td>PREDICTED: COP9 signalosome complex subunit 5b [Vitis vinifera]</td>
<td>569</td>
<td>569</td>
<td>89%</td>
<td>0.0</td>
<td>91%</td>
<td>XP_002283561.1</td>
</tr>
<tr>
<td>COP9 signalosome complex subunit 5a-like [Momordica charantia]</td>
<td>568</td>
<td>568</td>
<td>88%</td>
<td>0.0</td>
<td>90%</td>
<td>XP_022132138.1</td>
</tr>
<tr>
<td>COP9 signalosome complex subunit 5a [Jatropha curcas]</td>
<td>567</td>
<td>567</td>
<td>90%</td>
<td>0.0</td>
<td>88%</td>
<td>XP_012076336.1</td>
</tr>
</tbody>
</table>

15.35-1

5'—

GGAATATAGAAGTAATGGGTTTATGGGTTGATGCTATTATTGTGCTATGCTTATGGGTTAATGGCTTACCTGTTTACCTGATTGATTATTCTACTACTAAATAAACAGGCTGGGAGCTGAAATGGAATTGAGGGCTTCTGCTATGCTTTGCTTTACCTGTTGAAGGTACTGAAACTAGGGTTAATGCCAGGCTGATGCTTATGAATATATGGTTGATTATTCTACTACCTAATAAACAGGAGAACCCCTCCTCTGCTGCTTGTTGCTGGCTTTCTGGCATTGATGTTTCTACAAATGCTCAACCAACAATTCACAAGACCCCTCTCTGGCTGCTGTTTACCTGCAACCATACCAGGAGTGAAGACTATATTGCTGGGCAAATATCTGATCTCGCTGAAAATGGGAGACAGGACAGGAGAAAACAGTTGGGCTATTCTCGTTTTGTTGCTCTATGTTGCGCCTTCTCTCAAAAAAGAAAAAGAGGAGAGCCAGCAGCTTGCTAAGATAAACACGTGATAGTACTAAGATAAACAGTCGACAGGTCCATGTTTGATGCCCCAGGGTATCAAGATGTCCCTATTTATTCATCCGTAGACCAATTACG

159
TCAGGTGGGAATAGAATGGGTGTTAATGTGAGGGTAAAAACTGTGAGGTAG
TGCTATTATATTGTAATGGTGCTTTTGGCTTTACCTGTGTTAAGGTACTG
AGGTTAGCAGGCTGCTGATTGAAATATGAGGTTGATTATTCTACTACTAAAT
AACACAGGCTGGAAGACTGGAAAAATGTGTTGGCTGTTACACTCTCATTCT
GGTTATAGGTGTGGCTTTTGGCCATTGATGTTTCTACAAATGCTCAACCA
ACAATCAGAGACACTCTATTATCTCAGATACCACCAGAGAGGGTATA
AGCCACAGTGAAGCTATTCGAGATTATAGGTGCTTTTCAGGACATACCACC
GGTTATGGTGCTGGCTTTCTGGCATTGATGTTTCTACACAAATGCTCAACCA
ACAATTCCAAGACACTCCCTGGCTGCTGATTACTATTTTCAGTCTTCTTCTGATGCCCACCTCTTTGGATCTTTTGGGAACATACTG
GAATACCCCTTTCTCATACCTTTTGCTGGGAAATGGAAGACTATATTATGGGC
AAATATCGATCTCGTGAAGAAATTGGGAGCGAGGAGGAAAACCAGTTGGCTCA
TTCTCGTTTTGGTGTATAGTTGCAGCTTTTCTCAANNNAAAAAGAGGAAGAGGC
CAGCAGCTTTGACTAACACGCTAGTAGCTAAGATAACAGTGGACAGTCC
CC—3′

<table>
<thead>
<tr>
<th>Description</th>
<th>Max score</th>
<th>Total score</th>
<th>Query cover</th>
<th>E value</th>
<th>Ident</th>
<th>Accession</th>
</tr>
</thead>
<tbody>
<tr>
<td>JAB1/Mov34I/MPN/PAD-1 [Corchorus capsularis]</td>
<td>570</td>
<td>570</td>
<td>96%</td>
<td>0.0</td>
<td>90%</td>
<td>OMO38129.1</td>
</tr>
<tr>
<td>PREDICTED: COP9 signalosome complex subunit 5b-like [Cucumis melo]</td>
<td>566</td>
<td>566</td>
<td>94%</td>
<td>0.0</td>
<td>90%</td>
<td>XP_004845090.1</td>
</tr>
<tr>
<td>PREDICTED: COP9 signalosome complex subunit 5b [Cucumis sativus]</td>
<td>566</td>
<td>566</td>
<td>94%</td>
<td>0.0</td>
<td>90%</td>
<td>XP_004128785.1</td>
</tr>
<tr>
<td>PREDICTED: COP9 signalosome complex subunit 5b [Vitis vinifera]</td>
<td>564</td>
<td>564</td>
<td>95%</td>
<td>0.0</td>
<td>91%</td>
<td>XP_002283561.1</td>
</tr>
<tr>
<td>COP9 signalosome complex subunit 5a-like [Momordica charantia]</td>
<td>564</td>
<td>564</td>
<td>94%</td>
<td>0.0</td>
<td>90%</td>
<td>XP_022132136.1</td>
</tr>
<tr>
<td>COP9 signalosome complex subunit 5b-like [Hevea brasiliensis]</td>
<td>563</td>
<td>563</td>
<td>96%</td>
<td>0.0</td>
<td>87%</td>
<td>XP_021671864.1</td>
</tr>
</tbody>
</table>

16.36-2
5′—
TAGGGCGAGCGCCGCCNTGGGAGTACCATACGAGCAGTACCAGATTACGCTCA
TATGACAAGTTGGTACAATAAAAGTTGGAAATAAGGGCAAGAAGAGAGAAGAGAAG
AGAATGGGTACCGAAGACATAGCGCAGAAACATGGGAATAGAGGAAACATA
TAGAAAACAGTAAACGGACGTCAGCATCAGTAGCATTTCAATCGTAGAGCAGGCG
GGCAGGTGAAATCCCAAGAGAAGAAACCATGGGACAAATGGAACCTCATTA
TTCAAAAAGGGTAAAGTAGTACCATAGTAGTACATATCCTAAGGATGGTAGTACATGCT
AGATCAGGTGGGAATATAGAAGTAATGGGTTTAATGCAGGGTAA
AACTGATGG
TGATGCTATTATTGTATGAGCTTTTACCTGTGTGAAGTACTGAAAC
TAATAAACAGGCTGAAGACTGGAATATGTTGCTGTACCCTCTCAT
CCTGGTTATGGTTGTGCTTTTCTGCAATTAGTTTTTACAAATGCTC
AACAATCCAAGAACCCTCTCCTGGCTGTCAATTGATCCAACCAGAACT
GTTTCTGCTGAAAGATTGTGAATTGGCTTTTACAGGACATACCAGGAGGCT
ATAAGCCACCAGATGAGGCTATCTCACAAATACAAACATTTCCTTTAATAAGA
TTGAAGACTTTTGAGTGCATTGTAACACAGATATTACTCATTGAGACATCACATATT
TCAAGTCTTTCTTGTACCTGCAACCCTCTTTGTGATTTTGAGAACAAATACTGG
GTGAATACCCCTTTCTCATACACTTTTGTGGGAAATGGAGACTATATTGGCTGG
GCAAATATCTGATCTCGTCAAGAAATTTGAGACAGGCAAGAAACCAGTGGGCT
CATTCTCGTTTTGGGCCTATATGTTGGCGCCTTCTCAAAAGAAAAAGAGGAAG
AGCCAGCAGCTTGCTAAGATAACACGTCGATGACTAGATACATAGATACAGTCGAGCA
GGTCCATGTTTGAGTGCTCCAGGTAAATCAAAGATGGCTCTATTATATCCGTTA
GACCAATTACGTCT—3’

<table>
<thead>
<tr>
<th>Description</th>
<th>Max score</th>
<th>Total score</th>
<th>Query cover</th>
<th>E value</th>
<th>Ident</th>
<th>Accession</th>
</tr>
</thead>
<tbody>
<tr>
<td>PREDICTED: COP9 signalosome complex subunit 5b [Cucumis sativus]</td>
<td>571</td>
<td>571</td>
<td>89%</td>
<td>0.0</td>
<td>91%</td>
<td>XP_004138765.1</td>
</tr>
<tr>
<td>PREDICTED: COP9 signalosome complex subunit 5b-like [Cucumis melo]</td>
<td>571</td>
<td>571</td>
<td>89%</td>
<td>0.0</td>
<td>91%</td>
<td>XP_008445090.1</td>
</tr>
<tr>
<td>JAB1/Mov34/MPN/PAD-1 [Corchorus capsularis]</td>
<td>570</td>
<td>570</td>
<td>94%</td>
<td>0.0</td>
<td>88%</td>
<td>OMO86129.1</td>
</tr>
<tr>
<td>PREDICTED: COP9 signalosome complex subunit 5b [Vitis vinifera]</td>
<td>570</td>
<td>570</td>
<td>90%</td>
<td>0.0</td>
<td>91%</td>
<td>XP_002283561.1</td>
</tr>
<tr>
<td>COP9 signalosome complex subunit 5a-like [Momordica charantia]</td>
<td>568</td>
<td>568</td>
<td>89%</td>
<td>0.0</td>
<td>90%</td>
<td>XP_022132138.1</td>
</tr>
<tr>
<td>COP9 signalosome complex subunit 5a [Jatropha curcas]</td>
<td>568</td>
<td>568</td>
<td>91%</td>
<td>0.0</td>
<td>88%</td>
<td>XP_012070336.1</td>
</tr>
</tbody>
</table>

17.37-1
5’—
ATACGACGTACCAGATTACGCTCATATGACAAGTTGGTACAAAAAGTTGGAA
ATAAGGGCAGAGAGGAAGAAGAAGAAGATGGATCCGAAGACATAGCCGAGA
AAACATGGGAATAGAGAAACATATAGAAACAGTAAACGTCGAGCATCAT
GCAAATTCAAGTACAGCAGGAGCAGCGCAGGCTATGAAATCCCAACAAGAAGAAAC
CATGGACGAAATGAACCTTACTTCAAAAGGGTTAAAAGTATCAGCATAGCA
TTACTAAAGATGGTAGTACATCAGTCTGATCGTGGGTAATAGAAGTATGGG

161
TTTAATGCAGGGTAAAACTGATGGTGATGCTATTATTGTTATGGATGCTTTTGC
TTTACCTGTGTTGAAAGCTAAGGTTAATGCCCCAGGCTGATGCTTTATG
AATATATGGTTGATTATTCTACTACTATAAAACAGGCTGGAAGACTGAAAATG
TGTTGGGCTGACTCTCATTCTCCTGGTATTGTTTGCTGGCTTTTCTGGCAT
TGATTTTCTACACAAATGCTCAACAAACTTCCAGAGGACTCCTGCTG
TCGTCATTGATCACCACAAACTGTTTCTGCTGGAAAGTTGAAATTGGTGCT
TTTACGAGATACCCGAGGGCTATAAGCCACCAGATGAGGCTATCTCAGAA
TATCAAAACATCCCTAAATAAAGTGAAGACCTTGGAGTCTTGATGAAACAG
TATTAACCTCATTGGACATCACCATATTCAAGTCTTCTCTTGTGGCCACCTCTTG
GATCTTTTTGGAAACAAATACTGGGTGAATACCCCTTCTCCTACACCTTCTGCT
GGGAATGGAGACTATATTTGCTGGGCAATATCTGTCTCGTGAGGAAATTGG
AGCAGCGAGAAAACAGTGGGCTATTCTCCTTGGTCTATAAGGGCAGCC
TTCTCAAAAGAAAAANGAGGAAGAGGACGACTTGCCTAGATAACACGTTGAT
AGTACTAAGAATACAGTCGACAGGTCATGGTTGATGTCCAGGTAATCC
A—3' 

<table>
<thead>
<tr>
<th>Description</th>
<th>Max score</th>
<th>Total score</th>
<th>Query cover</th>
<th>E value</th>
<th>Identi</th>
<th>Accession</th>
</tr>
</thead>
<tbody>
<tr>
<td>PREDICTED: COP9 signalosome complex subunb 5a-like [Cucumis melo]</td>
<td>572</td>
<td>572</td>
<td>90%</td>
<td>0.0</td>
<td>90%</td>
<td>XP_008445090.1</td>
</tr>
<tr>
<td>PREDICTED: COP9 signalosome complex subunit 5b [Cucumis sativus]</td>
<td>572</td>
<td>572</td>
<td>90%</td>
<td>0.0</td>
<td>90%</td>
<td>XP_004138765.1</td>
</tr>
<tr>
<td>JAB1/Nov34/PMN/PAD-1 [Corchorus capsularis]</td>
<td>571</td>
<td>571</td>
<td>94%</td>
<td>0.0</td>
<td>89%</td>
<td>QMO68129.1</td>
</tr>
<tr>
<td>PREDICTED: COP9 signalosome complex subunit 5b [Vitis vinifera]</td>
<td>570</td>
<td>570</td>
<td>90%</td>
<td>0.0</td>
<td>91%</td>
<td>XP_002283561.1</td>
</tr>
<tr>
<td>COP9 signalosome complex subunb 5a-like [Momordica charantia]</td>
<td>570</td>
<td>570</td>
<td>90%</td>
<td>0.0</td>
<td>90%</td>
<td>XP_022132138.1</td>
</tr>
<tr>
<td>COP9 signalosome complex subunit 5a [Jatropha curcas]</td>
<td>569</td>
<td>569</td>
<td>91%</td>
<td>0.0</td>
<td>87%</td>
<td>XP_012076336.1</td>
</tr>
</tbody>
</table>

18. 38-2
5’—
GGCGGAGCGCCGCNTGGGAATACNNATACGACGTACCAGATTACGCTCATATG
ACAAGTTTTGTACANAAANNNGTTGGAAAATAAGGGCAAGAGAAGAAGAGAAAGA
TGGATCCGAGAACATAAGCAGCAGAAAAACTGGGAAATAGAGAACAATAGA
AACAGTAAACAGGACGAGCAGCATGACAGTCAATATCCAGTGACGACGAAGCGGCA
CAGGTGAATTCTCAAAACAGAAACCATGGGACGAGAATGACCTACTATTCG
AAAGGGAATAGTATACGATAGCATTACTAAAGATGGTAGTACGCTAGAT
CAGGTGGAATATAGAAATGGGTGTTTAATGCAGGTTAAACTGATGCTGAT
GCTATTATTGTTATGGATGCTTTTGCTTTACCTGTTGAAGGTACTGAAACTAGG
GTTAATGCCCCAGGCTGATGCTTTATGAATATATGGTTGATTATTCTACTAATA
AACAGGCTGGAAGACTGGGAAATGTGTTGGCTGGCTACCACTTCATCCTG
GTTATGGTTGGCTGTTTCTGGCATGTGGTTTCTACACAAATGCTCAACAA
GTTAATGCCCAGGCTGATGCTTATGAATATATGGTTGATTATTCTACTAATA
AACAGGCTGGAAGACTGGAAAATGTGGTTGGCTGGT
ACCACTCTCATCCTG
GTTATGGTTGCTGGCTTTCTGGCATTGATGTTTCTACACAAATGCTCAACAA
CAATTCCAAGAAACCCTTCTGGCTGTCATATTGATTCAACTCCACCTATCCTATTA
AGTCTCTTCTTGATTGCCACCTCTTTGATCTTTTGTGAACAAATATGCTGGTG
AACTACCTTTCCATCATACCTTGGCTGGAAATGGAGACTATATTGCTGGGCA
AATATCTGATCTCGCTGAGAAATGGAGCAGCAGAAACCAGGTGGCTCAT
TCTCGTTTTGGGTTATAGTTGCGCCTTCTCAAGAAGAAGAGAGAAGGC
CAGCAGTCTAGAATACGCCTGGATAGTACTAAGATACAAGCTGAGCAGGT
CCATGGTT—3'

<table>
<thead>
<tr>
<th>Description</th>
<th>Max score</th>
<th>Total score</th>
<th>Query cover</th>
<th>E value</th>
<th>Ident</th>
<th>Accession</th>
</tr>
</thead>
<tbody>
<tr>
<td>PREDICTED: COP9 signalosome complex subunit 5b-like [Cucumis melo]</td>
<td>569</td>
<td>569</td>
<td>90%</td>
<td>0.0</td>
<td>91%</td>
<td>XP_008440590.1</td>
</tr>
<tr>
<td>JAB1/Mov34/MPN/FAD1 [Corchorus capsularis]</td>
<td>569</td>
<td>569</td>
<td>94%</td>
<td>0.0</td>
<td>89%</td>
<td>OMO86129.1</td>
</tr>
<tr>
<td>PREDICTED: COP9 signalosome complex subunit 5b [Cucumis sativus]</td>
<td>569</td>
<td>569</td>
<td>90%</td>
<td>0.0</td>
<td>91%</td>
<td>XP_004138765.1</td>
</tr>
<tr>
<td>PREDICTED: COP9 signalosome complex subunit 5b [Vitis vinifera]</td>
<td>567</td>
<td>567</td>
<td>90%</td>
<td>0.0</td>
<td>91%</td>
<td>XP_002283561.1</td>
</tr>
<tr>
<td>COP9 signalosome complex subunit 5a-like [Momordica charantia]</td>
<td>566</td>
<td>566</td>
<td>90%</td>
<td>0.0</td>
<td>90%</td>
<td>XP_022132138.1</td>
</tr>
<tr>
<td>COP9 signalosome complex subunit 5a [Jatropha curcas]</td>
<td>565</td>
<td>565</td>
<td>91%</td>
<td>0.0</td>
<td>88%</td>
<td>XP_012076338.1</td>
</tr>
</tbody>
</table>

19. 39-3
5’—
GGCGGAGCGCGCCNTGGAATACCATAAGCTGACGTCATACGAATAGCGCTCATATG
ACAAGTTGTACAAAAAAGTGGGAAATAAAGGGAAGAGAAAGGAAGAAAGAA
TGGATCCGAAGACAATAGCGCAGAAGAACATGGGAAATAGAGAAACATAG
AAGCAGTAAAAGCAGCGACCATAGCATGCAATATCGAAGATGCAGCACAGC
CAGGTGAAATTTCCAAAGAAGAAGAACATTGGGACGTAATGACTACTATTCTCA
AAAGGGAATAAGTAGCGACATTACTAAAGATGTTAGACTCATGCTAGAT
CAGGTGGAATACATAGAATAGGGTTAATGCAGGGTAAAATCGATGGTGTAG
GCTATTATTGTTATGGATGCTTTTGCTTTACCTGTTGAAGGTACTGAAACTAGG
GTATGACCAGGCGTGATGCTTATGAAATATATGGTGGATTATTTCTACTACTAATA
AACAGGCTGGAAAGACTGGGAAATATGTTGGGCTGTTGATCCACTCTCATCTCTG
GTATGTTGCTGGCTTTCTTCTGCATTGATGGTTTCTACACAAATGCTCAACCAA
CAATTCCAAGAAGACCCTCTCTCGTGGTCTGCGTCTGATCCAAACCAGAATGTTT
CTGCTGAAAAAGTTGAAATTTGTTGCTTTCCAGGACATACCAGGAGGGCTATAA
GCCACCAGATGAGCCTATCTCAGAATATCAAAACCATTCCCTTAAATAAGATTG
AAGACTTTGGAGTGCATTGTAAACAGATATTACTCATTGGACATCACATATTCTCA
AGTCTTCTTCTGATTGCCNCTCTTGGATCTTTTNGGAAGAAATACNGGGTT
—3’

<table>
<thead>
<tr>
<th>Description</th>
<th>Max score</th>
<th>Total score</th>
<th>Query cover</th>
<th>E value</th>
<th>Ident</th>
<th>Accession</th>
</tr>
</thead>
<tbody>
<tr>
<td>PREDICTED: CDPK signalosome complex subunit 5b-like [Cucumis melo]</td>
<td>451</td>
<td>451</td>
<td>87%</td>
<td>1e-157</td>
<td>90%</td>
<td>XP_008445095.1</td>
</tr>
<tr>
<td>hypothetical protein B456_009G006200 [Gossypium raimondii]</td>
<td>450</td>
<td>450</td>
<td>87%</td>
<td>2e-157</td>
<td>93%</td>
<td>KJ551954.1</td>
</tr>
<tr>
<td>PREDICTED: CDPK signalosome complex subunit 5b [Cucumis sativus]</td>
<td>451</td>
<td>451</td>
<td>87%</td>
<td>2e-157</td>
<td>90%</td>
<td>XP_004138765.1</td>
</tr>
<tr>
<td>JAB domain-containing protein [Cephalotus follicularis]</td>
<td>451</td>
<td>451</td>
<td>87%</td>
<td>2e-157</td>
<td>91%</td>
<td>GAV56783.1</td>
</tr>
<tr>
<td>hypothetical protein B456_009G006200 [Gossypium raimondii]</td>
<td>450</td>
<td>450</td>
<td>87%</td>
<td>3e-157</td>
<td>93%</td>
<td>KJ551954.1</td>
</tr>
<tr>
<td>JAB1/Mv34/MPN/PAD-1 [Corchorus olitorius]</td>
<td>450</td>
<td>450</td>
<td>87%</td>
<td>3e-157</td>
<td>93%</td>
<td>OMO90826.1</td>
</tr>
</tbody>
</table>

20. 40-1

5’—
GGGCCAGCGCGGCNCNTGGAGTACCNATACGACGTACCAGATTACGCTCATAT
GACAAGTTTGTACAAAAAAGTTGGAAATAAAGGGCAAGAGAAGAGAGAGAGGA
ATGGATCCGAAAGAATAGCGCAGAAACCATGGGAAATAGAGAAGAAATAGAG
AACAGTAAACGCAGCAGACATAGCAATATTAATATGAGAGATGCTGATGCTG
GTCATTATTTGTGTGTGACTTATGCTTTGCTTTACCTTTGAAGAGTACTAAACATTG
GTTATGCTGATGCTTATGAAATATATGGTGGATTATTTCTACTACTAATA
AACAGGCTGGAAAGACTGGGAAATATGTTGGGCTGTTGATCCACTCTCATCTCTG
GTATGTTGCTGGCTTTCTTCTGCATTGATGGTTTCTACACAAATGCTCAACCAA
CAATTCCAAGAAGACCCTCTCTGCTGCTGCTGATCCAAACCAGAATGTTT
CTGCTGAAAAAGTTGAAATTTGTTGCTTTCCAGGACATACCAGGAGGGCTATAA
GCCACCAGATGAGCCCTATCTCAAGAATATCAAAACATTCCCTTAAATAAAGATTG
AAGACTTTGGAGTGCTATTGTAAACAGTATTATTACCTTCTGGGACATCAATATTTCA
AGTCCTCTCTATTGCGCCACCTCTTGGATCTTTGTGGAACAAATACTGGGTG
ATACCTTTTCTCTATCACCTTTTTGCTGGGAATGGAGACTATATTTGCTGGGCA
AATATCTGATTCGCTGAAGAATTTGGAGCAGGCAGAAACCAGTTGCTGCT
TCTCGTTTTGGGCTATAGTGGCCGCTTTCTCATAAAGAAAAGAAGAGAGC
CAGCACTTGCTAAGATAAACACGTGATAGTACTAGATAACAGTCGAGCAAT
CCATGTTTGTATGCCAGGTTATCAAAGATGTCTCTATTTAATTCGCTTA
CAATTACGTCTCAGG—3'

<table>
<thead>
<tr>
<th>Description</th>
<th>Max score</th>
<th>Total score</th>
<th>Query cover</th>
<th>E value</th>
<th>Ident</th>
<th>Accession</th>
</tr>
</thead>
<tbody>
<tr>
<td>PREDICTED: COP9 signalosome complex subunit 5b-like [Cucumis mixus]</td>
<td>572</td>
<td>572</td>
<td>90%</td>
<td>0.0</td>
<td>90%</td>
<td>XP_008445500.1</td>
</tr>
<tr>
<td>PREDICTED: COP9 signalosome complex subunit 5b [Cucumis sativus]</td>
<td>572</td>
<td>572</td>
<td>90%</td>
<td>0.0</td>
<td>90%</td>
<td>XP_00438765.1</td>
</tr>
<tr>
<td>JAB1/Nov34/MFN/PAD-1 [Corchorus capsularis]</td>
<td>571</td>
<td>571</td>
<td>94%</td>
<td>0.0</td>
<td>89%</td>
<td>OMO0129.1</td>
</tr>
<tr>
<td>PREDICTED: COP9 signalosome complex subunit 5b [Vitis vinifera]</td>
<td>570</td>
<td>570</td>
<td>90%</td>
<td>0.0</td>
<td>91%</td>
<td>XP_002283565.1</td>
</tr>
<tr>
<td>COP9 signalosome complex subunit 5a-like [Momordica charantia]</td>
<td>570</td>
<td>570</td>
<td>90%</td>
<td>0.0</td>
<td>90%</td>
<td>XP_022132158.1</td>
</tr>
<tr>
<td>COP9 signalosome complex subunit 5a [Jatropha oenca]</td>
<td>569</td>
<td>569</td>
<td>91%</td>
<td>0.0</td>
<td>87%</td>
<td>XP_012076325.1</td>
</tr>
</tbody>
</table>

21.41-2
5’—
ATACGACGTACCGAGTATTGCTCATATGCAAAAGTTGTGATCAAAAAAGTTGGAA
ATAAAGGGCAAGAGAAGAAAGAAGAAGAAATGGAACCGAAGCACATAGCCAGA
AAAACTGGGAAATAGGAAAAAAATAGAAGAAACAGTAAACGACGCAGCATCAGAT
GCAATTACGTACGACGAGCGGCAACAGGTGAAATTCCAAAAGAGAGAAGAC
CATGGGAGAATGAACTCATATTCTAAAAGGTTAAAAATGATAGCATAGTACAG
TTACTAAAGATGGTATGATCATACAGATCAGTAGTTGGAATATTAGAAGTATGGG
TTAATGCGAGGTAATTATCGATGCTATTATTTATTGTGATAGATTGTGCT
TTAATTAGTGTGGT ATTGATTATTATTCTACTACTAAATAAACAGGCTGAAGAAGCTGGA
TGTTGCTGTGCTATCCATCCTGTGGTTAGTGGTTCTGCTAGGTGCT
TGATGTTTCTACACAAATGCTCAAAGGAAACCTCTCTCCTGCTG
TCGTATTGATACCAACCAACTGGATTCTTCTGCTGAAAGTTGAAATTGGTGC
TTTCAAGGACATACCGGAGGCTATAAGCCACCATGAGCCTATCTCAGAAA
TATCAAAACCATTCCCTTTAAAATAAGATTGAAGACTTTGGAGTGCAATTGTAAACAG
TATTACTCATTTGAGACATCAACATATTCTCAAGTCTTCTTTGATGCGACCCTCTTTG
GATCTTTTGTGGAACAAATACGGGTAATACCCTTTTCCATCATACCTTTGCT
GGGAAATGGAGACTATATTGCTGGGCAAATATCTGATCTCGCTGAGAAATTGG
AGCAGGCAAAACCAGTTTGACTATCTCTGTGTTTTGGGTCTATTAGTGTGCGCC
TTCTCAAAGAAAGANGAGGAGAGCAAGCAGACTTTGCTAAAGATACACGTTGAT
AGTACTAAGATAACAGTGCAGCAGGTCCATTTGATGTCGCCAGGTAATCA
A—3’

<table>
<thead>
<tr>
<th>Description</th>
<th>Max score</th>
<th>Total score</th>
<th>Query cover</th>
<th>E value</th>
<th>Identi</th>
<th>Accession</th>
</tr>
</thead>
<tbody>
<tr>
<td>PREDICTED: COPII signalosome complex subunit 5b-like (Cucumis melo)</td>
<td>572</td>
<td>572</td>
<td>90%</td>
<td>0.0</td>
<td>90%</td>
<td>XP_008445090.1</td>
</tr>
<tr>
<td>PREDICTED: COPII signalosome complex subunit 5b (Cucumis sativus)</td>
<td>572</td>
<td>572</td>
<td>90%</td>
<td>0.0</td>
<td>90%</td>
<td>XP_004138765.1</td>
</tr>
<tr>
<td>JAB1/Mov34/MPPN/PAD-1 (Corchorus capsularis)</td>
<td>571</td>
<td>571</td>
<td>94%</td>
<td>0.0</td>
<td>89%</td>
<td>OMO86129.1</td>
</tr>
<tr>
<td>PREDICTED: COPII signalosome complex subunit 5b [Vitis vinifera]</td>
<td>570</td>
<td>570</td>
<td>90%</td>
<td>0.0</td>
<td>91%</td>
<td>XP_002283561.1</td>
</tr>
<tr>
<td>COPII signalosome complex subunit 5a-like (Momordica charantia)</td>
<td>570</td>
<td>570</td>
<td>90%</td>
<td>0.0</td>
<td>90%</td>
<td>XP_022132138.1</td>
</tr>
<tr>
<td>COPII signalosome complex subunit 5a [Jatropha curcas]</td>
<td>569</td>
<td>569</td>
<td>91%</td>
<td>0.0</td>
<td>87%</td>
<td>XP_012078336.1</td>
</tr>
</tbody>
</table>

22. 42-1

5’—
TGGAGTACCCATACGCAGTACCAGATTACGCTCATATGCAAAAGTTTTGTACAAA
AAAGTTGGAAATAAGGGCGAAGAAAGAAGAAAGAAGATGGGATCGGAAGACA
ATAGCGCAGAAACATGGGAAATAGAGAAACATAGAAACAGAAAACAGACG
CAGCATCAGATGCAATATTCAAGTACGACGAGCGGACAGGGTAAATTCCA
ACAAGAGAAAAACATGGGACGAATGAACCTTATTATGGTAAAGGGACTATAGATAT
CAGCATGATTACATATAAGATGGATGATCATGCTAGACAGTGGAATATA
GAAGTATGAGGTTAAAAATGCAGGGTAAAACACTGATGTTATGCTATTATGTTATG
GATGCTTTTGCTTTTACCTGTGGAAGGTACTGAAAATGGTTAATGCCAGG
CTGATGCTTATGAAATATGGGTAGTTATTTCTACTACTATAAAACAGGCCTGAA
GACTGGGAAAATGGTTGGCTGTCCTGCCTACACTCTCTGTGTTATGTTGCTTG
GCTTTCTGGCATTGATTTTCCTCAAAATGCTCAACAAATGATCCCCAGGACAG
CCTTCCCTGCGCTGTCATTGATCCAAACCAAGACTGGTTTTCTTCGTTGAAAAGT
TGAAATTGGGCTTTTACGAGATAACCCGCGAGGCTATAGGCAACGACGTAG
CCTATCTCAGAAATACCAACCATTCCCTTTAAATAGATTGAAGACTTTGGAGTG
CATTGTAAACAGTATTACTCATTGGACATCACATATTTCAAGTCTTTCTTGATT
GCCACCTCTTTGGATCTTTTGTGGAACAAATACTGGGTGAATACCCCTTTTCTCA
TCACCTTTTGCTGGGAAATGGAGACGATATATTGCTGCGAAATATCTGATCTCGC
TGAGAATAATGGGAGCAGGCAGAAACCAACAGTTGCTTCATTCTCGTTTTGGGTCT
ATAGTTGCAGCCTTCTCAAAAGAAAAANGAGGAAGCCAGCACTTTGCTAAGA
TAACACGTGATAAGTACTAAGATAAACAGTGCAGCAGTCCCATGGTTTGATGTC
CCAGGTAATCAAAGATGTCTCTTATATTTAATTCGTTA—3’

<table>
<thead>
<tr>
<th>Description</th>
<th>Max score</th>
<th>Total score</th>
<th>Query cover</th>
<th>E value</th>
<th>Ident</th>
<th>Accession</th>
</tr>
</thead>
<tbody>
<tr>
<td>PREDICTED: COP9 signalosome complex subunit 5b [Beta vulgaris subsp. vulgaris]</td>
<td>629</td>
<td>629</td>
<td>92%</td>
<td>0.0</td>
<td>96%</td>
<td>XP_010669282.1</td>
</tr>
<tr>
<td>PREDICTED: COP9 signalosome complex subunit 5b-like [Cucumis melo]</td>
<td>627</td>
<td>627</td>
<td>91%</td>
<td>0.0</td>
<td>90%</td>
<td>XP_008445090.1</td>
</tr>
<tr>
<td>PREDICTED: COP9 signalosome complex subunit 5b [Vitis vinifera]</td>
<td>627</td>
<td>627</td>
<td>91%</td>
<td>0.0</td>
<td>91%</td>
<td>XP_002283561.1</td>
</tr>
<tr>
<td>PREDICTED: COP9 signalosome complex subunit 5b [Cucumis sativus]</td>
<td>626</td>
<td>626</td>
<td>91%</td>
<td>0.0</td>
<td>90%</td>
<td>XP_004138765.1</td>
</tr>
<tr>
<td>COP9 signalosome complex subunit 5a [Jatropha curcas]</td>
<td>625</td>
<td>625</td>
<td>92%</td>
<td>0.0</td>
<td>87%</td>
<td>XP_012076336.1</td>
</tr>
<tr>
<td>COP9 signalosome complex subunit 5a-like [Momordica charantia]</td>
<td>624</td>
<td>624</td>
<td>91%</td>
<td>0.0</td>
<td>89%</td>
<td>XP_022132138.1</td>
</tr>
</tbody>
</table>

23. 43-3

5’—
TACGACGTACCAGATTACGCTCATATGACAAGTTGGTAGCAAAAGAAAAAGTTGGAAA
TAAGGGCAAGAGAAAGAGAGAAGAGATGGATCCGAGAAACATAACGCGCAGAAA
AACATGGGAAATAGAGAAACATAGAAGAACGAGGGACGGCCAGTGGTACCTTTATTAC
CAATATTCAAGTACGACGAAGCAGGGCACAGGTGAAATTCCAACAGAGAAACC
ATGGGACGAATGAACCTCATTACTTAAAAGGGTAAAGTATCACGACGATGAT
TACTAAAGATGTAGTACATGTACAGGTGGGAATATAGTTAAGTTGGGT
TTAATGCAGGTTAAAACTGATGGGTATGCTATTATTTGTATGGATGCTTTTGCT
TTACCTGTGAGGTACTGAAACTAGGGGTATTAGCCAGGGCTGATGCTATTAGA
ATATATGGGGATTATTTTACTAATAAAACAGGTGCGAGACGTGAAAAGTGT
GGTTGGCTGTTACACTCTCATTCTGGTTATGTTGTGCTGCTTTCCTGCCATT
GATGTGTGTCACAAATGGCTCAACCAACAAATCCACAGAACCCCTCTCTGCTGCT
CGTCATTGATCCAACCAGAACTGTGTCTGCTGGAAATGTTGGATGCT
TTACGAGACATACCCCGAGGGCTATAAGCCACCAGATGAGCCTATCTCAGAAT
ATCAAACACCTACCCTTAAATAGATTGAAGACTTTGGAGTCTGGTGCAAAACAG
TATTACTCATGGACATCACATATTTCAATTTCTCTTGATTGCCACCTCTTTG
GATCTTTTGTGGAACAAATACTGGGTGAATACCCTTTTCTCATCACCCTTTTGCT
GGGAAATGGGAAGCTATTTGCTGGGCAAATATCTGATCTCGTGAGAAATTGG
AGCAGGCAGAAACCACAGTTGGGTCAATTCTCGTGTTTTGGCTTATAATGGCGCC
TTCTCAAANAAAAANGAGGAAGAGCCAGCAGCTTGCCTAAGATAACACAGTGT
AGTAATAGATAACAGTCAGCAGGCTCATTGCTGGTTGTAGTCCAGAGTAAATCA
AAGATGTCTCTTTAATCCGGTATA—3’

<table>
<thead>
<tr>
<th>Description</th>
<th>Max score</th>
<th>Total score</th>
<th>Query cover</th>
<th>E value</th>
<th>Ident</th>
<th>Accession</th>
</tr>
</thead>
<tbody>
<tr>
<td>PREDICTED: COP9 signalosome complex subunit 5b [Cucumis sativus]</td>
<td>571</td>
<td>571</td>
<td>89%</td>
<td>0.0</td>
<td>91%</td>
<td>XP_004138765.1</td>
</tr>
<tr>
<td>PREDICTED: COP9 signalosome complex subunit 5b-like [Cucumis melo]</td>
<td>571</td>
<td>571</td>
<td>89%</td>
<td>0.0</td>
<td>91%</td>
<td>XP_008445090.1</td>
</tr>
<tr>
<td>JAB1/Nov34/MPN/PAD-1 [Corchorus capsularis]</td>
<td>570</td>
<td>570</td>
<td>94%</td>
<td>0.0</td>
<td>88%</td>
<td>OMO85129.1</td>
</tr>
<tr>
<td>PREDICTED: COP9 signalosome complex subunit 5b [Vitis vinifera]</td>
<td>570</td>
<td>570</td>
<td>90%</td>
<td>0.0</td>
<td>91%</td>
<td>XP_002283561.1</td>
</tr>
<tr>
<td>COP9 signalosome complex subunit 5a-like [Momordica charantia]</td>
<td>568</td>
<td>568</td>
<td>89%</td>
<td>0.0</td>
<td>90%</td>
<td>XP_022132138.1</td>
</tr>
<tr>
<td>COP9 signalosome complex subunit 5a [Jatropha curcas]</td>
<td>568</td>
<td>568</td>
<td>91%</td>
<td>0.0</td>
<td>88%</td>
<td>XP_012978336.1</td>
</tr>
</tbody>
</table>

24. 44-2
5’—
ATACGACGTACCAGATTACGCTCATATGACAAAGTTTTGTACAAAAAAAAAGTTGGAAG
ATAAAGGGCAAGAAGAAGAAAGAAGAAGAAGAAGAATGGATCCGAAGACATAGGCAGA
AAACATGGGAATAGAGAACAATATAGAAAAACGTAAGACGCAGGCACATCGAT
GCAATATTCAAGTACCAGAAGCAGCGCGACAGTGAAATTCCAAACAAGAGAAGAC
CATGGACGAATGAAACTCATTACTTTCAAGAGGTATATATGATATGGCTATTTATG
TTACCTGTGAAGGTACTGAAACTAGGGTTATGCATGACCTGAGTGATTTATG
AAATATTGTTGATTTATTTACTACTAATACAGAGCTGGAAGACTGGAAGAT
TGTTGGCTGGTACACTCTCTACCTCGGTATGATTGTCGGCTGTTCTCGCAT
TGATGTTTCTACCAAATGGCTCAAACCAAAATTCGAAGACCCTTTCTGTGGCTG
TCGTATTGATCCACACCAGAATTGTGTTACCAAGAAGATTGGAAATTTGGTCG
TTTCAGGACATACCAGGAGGGCTATAAGGCACCAGATGAGCTTACTGACAA
TATCAAAACCATTCCCTTAAATAAGATTGAAGACTTTGGAAGTGATTTGAAATCAG
TATTACTCATGGACATCACATATTTCAAGTTCTCTCTCGATTGCCACCTCTTTG
GATCTTTTGTTGGAAACAATACTGGGTGAATACCCCTTCTCATACCTACCTTTTGGCT
GGGAAATGGGAGCTATATTGGCTGGGCAAATATCTGATCTCGCTGAGAAATTGG
AGCAGGCCAGAAAACCAGTGGCTCATTCTCGTTTTGGGTCTATAGTTGCGCC
TTCTCAAAAGAAAAAGAGGAAGAGGCAGCAGCAGTCTAAGATACACNNGTAT
AGTACTAAGATAACAGTCGACAGCAGGTCCATGGTTTGATGTCCCAGGTAATCA
AAGATGTCCATTATTAATTCCCGTATA—3′

<table>
<thead>
<tr>
<th>Description</th>
<th>Max score</th>
<th>Total score</th>
<th>Query cover</th>
<th>E value</th>
<th>Ident</th>
<th>Accession</th>
</tr>
</thead>
<tbody>
<tr>
<td>PREDICTED: COP9 signalosome complex subunit 5b [Cucumis sativus]</td>
<td>571</td>
<td>571</td>
<td>89%</td>
<td>0.0</td>
<td>91%</td>
<td>XP_004138765.1</td>
</tr>
<tr>
<td>PREDICTED: COP9 signalosome complex subunit 5b-like [Cucumis melo]</td>
<td>571</td>
<td>571</td>
<td>89%</td>
<td>0.0</td>
<td>91%</td>
<td>XP_008446390.1</td>
</tr>
<tr>
<td>JAB1/Mov34/MPN/PRD-1 [Corchorus capsularis]</td>
<td>570</td>
<td>570</td>
<td>94%</td>
<td>0.0</td>
<td>88%</td>
<td>CMO66129.1</td>
</tr>
<tr>
<td>PREDICTED: COP9 signalosome complex subunit 5b [Vitis vinifera]</td>
<td>570</td>
<td>570</td>
<td>90%</td>
<td>0.0</td>
<td>91%</td>
<td>XP_002282561.1</td>
</tr>
<tr>
<td>COP9 signalosome complex subunit 5a-like [Momordica charantia]</td>
<td>568</td>
<td>568</td>
<td>89%</td>
<td>0.0</td>
<td>90%</td>
<td>XP_022132153.1</td>
</tr>
<tr>
<td>COP9 signalosome complex subunit 5a [Jatropha curcas]</td>
<td>568</td>
<td>568</td>
<td>91%</td>
<td>0.0</td>
<td>86%</td>
<td>XP_012076338.1</td>
</tr>
</tbody>
</table>

25. 45-1
5′—
TGGAGTACCCATACGACGTACCAGATTACGCTCATATGACAAGTTTTGATACNN
NNAGTTGGAAATAAGGGGAAAGAGAAGAAGAAGAAGAAGAATGGATCCGAAGACA
ATAGCGCAGAAAACATGGGAAATAGAGAACAATATAGAAACAGTAAACGACG
CAGCATCAGATGCAATATTCAGAGAGACCATGGAACCGGCACAGTGGAATTCCA
ACAAGAGAAACCATGGGACGATAGAAACCTACATTCTCAGAAAAAGGTAAAAAGTAT
CAGCATTAGCATTACTAAGAGATGGTAGCTACATGCTAGATCAGGTTGGGAATATA
GAAGTAATGGGGTTTAATGCAGGTTAAAAGCTGGTGGTGGATGTATTAGTTATG
GATGCTTTTGCTTTTCGTCAGGTTGGTACTGAAACTAGGGTTAATGCCCAGG
CTGATGCTTTAGATATATGTTGTGATTATTTCTACTACTAATAACAGGCTGGAA
GACTGGAATAACGTGGTGCGGCTGGAATCCACTCTCATCTGCTGTTATGGTGGCTG
GCTTTCTGGCATTGATGTTTCTACACAATGCTCAACCACCAATTTCCAAAGAAC
CCTTCCTGGCTGTCATTGATCCAAACCAGAAGCTGTTTCTGCTGGAAGATGT
GAAATTGCTGCTTTACAGACATACCGGAGGCTGATAGCCACCAGATGAG
CCTATCTCAGATATCACAAACCATTCCCCTTTAATAGGATTTGAA—3′
26. 48-2

5'—
TGGAGTACCCATACGACGTACCAGATTACGCTCATATGACAAGTTTGTACAAA
AAAGTTGGAAAATAAGGGCAAGAGAAGAAGAAGAAGAATGGATCCGAAGACA
ATAGCGCAGAAAACTGGGAAATAGAGAACAATATAGAAACAGTAAACGACG
CAGCATCAGATGCAATATTTCAAGTACGACGAAGCGGCACAGGTGAAATTCCA
ACAAGAGAAACCATGAGCAGGAATGAAACCTCATTACTTTCAAAAAGGGTAAAAGTAT
CAGCATTAGCTATTACTAAAGATGGTAGTACATGCTAGATCAGGTGGGAATATA
GAAGTAATGGGTTTATAATGCAGGGTAAAACTGATGGTGATGCTATTATTGTTATG
GATGCTTTTGCTTTACCTGTTGAAAGGTACTGAAACTAGGTTAATGCCCAAGG
CTGATGCTTTATGAAATATATGTTGATTATTCTACTACTAATAAACAGGCTGGA
GACTGAAAATGTGGTGTGCTGACACTCTCATCTCTGTTATGGTTCTG
GCTTTTGGGCAATGATTTCTCACAATAATGCTCAAACAAATATCCAAAGAACC
CCTTCCCTGGGTGCTCATTGATCCAAACCAGAACTCTGTTGCTCCGAAAGGT
TGAAATTGGGTGCTTTTCAGGACATACCCGGAGGGCTATAAGCCACCATGAGG
CCTCATGAGAATATCAAACCATTCCCTTAAATAAGATTGGAAGATTGGAGTG
CATTGTAACAGTATTACTCATTGGAACATCAATTTCAAGTCTTCTCTTGATT
GCCACCTCTTGGATCTTTTGTGGAACAAATACTGGGTGAATACCCTTTCCTCA
TCACCTTTGGGGAAATGGAGACTATATTGCTGGGCAATATCTGTATCTCGCC
TGAGAAATTGGGAGCAGCGAAGAAACCAGTTTGGCTCATTCTGTTTGGTCT
ATAGTTGGCTCTTTCTCAGGAA---3'
<table>
<thead>
<tr>
<th>Description</th>
<th>Max score</th>
<th>Total score</th>
<th>Query cover</th>
<th>E value</th>
<th>Ident</th>
<th>Accession</th>
</tr>
</thead>
<tbody>
<tr>
<td>PREDICTED: COPI signalosome complex subunit 5b-like [Cucumis melo]</td>
<td>511</td>
<td>511</td>
<td>88%</td>
<td>0.0</td>
<td>91%</td>
<td>XP_008445090.1</td>
</tr>
<tr>
<td>PREDICTED: COPI signalosome complex subunit 5b [Cucumis sativus]</td>
<td>511</td>
<td>511</td>
<td>88%</td>
<td>1e-180</td>
<td>91%</td>
<td>XP_004138765.1</td>
</tr>
<tr>
<td>JAB1/Myv34/MPN/PAD-1 [Coevolution]</td>
<td>509</td>
<td>509</td>
<td>89%</td>
<td>2e-180</td>
<td>93%</td>
<td>OMO90626.1</td>
</tr>
<tr>
<td>hypothetical protein B456.009G068200 [Gossypium raimondii]</td>
<td>509</td>
<td>509</td>
<td>88%</td>
<td>2e-180</td>
<td>93%</td>
<td>KJB55194.1</td>
</tr>
<tr>
<td>hypothetical protein GOBAR_AA21742 [Gossypium barbadense]</td>
<td>509</td>
<td>509</td>
<td>88%</td>
<td>2e-180</td>
<td>93%</td>
<td>PPR003311.1</td>
</tr>
<tr>
<td>PREDICTED: COPI signalosome complex subunit 5b-like isoform X2 [Gossypium raimondii]</td>
<td>509</td>
<td>509</td>
<td>88%</td>
<td>2e-180</td>
<td>93%</td>
<td>XP_012443255.1</td>
</tr>
</tbody>
</table>

27. 49-3

5′—

GGCGAGCGCCGCCATGGAGTACCCATACGACGTACCAGATTACGCTCATATG
ACAAGGTTCTGATACAAAAAGTTGGAATAAGGGCAAGAGAAGAGAAGAGAAA
TGGATCCGAAGACAATAGCGCAGAAAACATGGGAAATAGAGAACAATATAGA
AACAGTAAACGACAAGCATCAGATGCAATATTCAAGTACGACGAAGCGGA
CAGGTGAAATTCCAAACAGAAACCATGGACGAATGAAACCTCATTACTTCA
AAAGAGTAAAAGTATCAGCATTACGATTACTAAAGATGGTAGACATGCTAGAT
CAGGTGGAATATAGAATATGGTTTTAATGCAGGGTAAAAGCTGATGGTGAT
GCTATTATTGTATGGATGCTTTTGGCTTTTACCTGTGAAAGGTACTGAAACTAGG
GTTAATGCCCAGGCTGATGCTTATGAAATATATAGGTTGATTATTTCTACTAATA
AACAGGCTGGAAGACCTGGAATAATGTGGTTTGCGTGGTACCACCTGCTTCTG
GTTAGGTTATGCTGGCTTTTCTGGCATGATGTTTCTACACAAATGCTCAACCA
CAATTCCAAAGAACCCTTCTCCTGGCTGCTGATGATCACCAGAACACTGTTT
CTGCTGGAAGAATTGGAATGTGCTCTCAGAGACATCCCCGGAGGGGCTATAA
GCCACCAGATGAGCCTATCTCGAATATCAAACATTCCCTTAAATAGATTG
AAGACTTTGGAGTGCTTTGAAACAGATTATTCATTATCATTGGACATCAGCATATTCTAC
AGCATTCTCTGTAGGACCACCTTTGGATCTTTTGTGGGAACAAATATCTGGTGT
AATACCCCTCTCCTCATACCCCCCTTTGGCTGGGAATGGAGACTATATTGGCTTGG
AATATCTGATCTCGCTGANAATTGGGAAGCAGGCAGAAAGAACAGTGGGCTCAT
TCTCGTTTGGGTCTATAGTGGCCTTCCTCCAAGAAAA—3′
28. 50-2

5’—
ATACGACGTACCAGATTACGCTCATATGACAAAGTTTGTACAAAAAAGTTGGAAATAAGGGCAAGAGAAGAAGAAGAATGGATCCGAAGACAATAGCGCAGAACATGGGAAATAGAGAACAATATAGAAACAGTAAACGACGCAGCATCAGATGCAATATTCAAGTACGACGAAGCGGCACAGGTGAAATTCCAACAAGAGAAACATGGACGAATGAACCTCATTACTTCAAAAGGGTAAAAGTATCAGCATTAGCATTTACTAAAGATGTTAGTACATGCTAGATCGGTGGGAATATAGAAGTAATGGGTTTAATGCAGGGTAAAACGATGTTGTGCTATTATTGTTATGGATGCTTTTGCAACAAATGCTGTTGAAGGTACTGAAACTAGGGTTAATGCCCAGGCTGATGCTTATGAAATATATGGTTGATTATTCTACTACTAATAAACCAGGCTGGAAGACTGGAATATGGGTTGGCTGGTACCACTCTCATCCTGGTTATGGTTGCTGGCTTTCTGGCATGATGTTTCTACACAAATGCTCACCACACAATTCCAAGAACCCTTCTCTGGCTGGTCATTGATCCAACCAGAACTGTTTCTGCTGGAAAAGTTGAAATTGGTGCTTTCAGGACATACCAGGAGGGCTATAAAGCCACCAGATGAGCCTATCTCAAGATTCAAACCATTCCCTTTAATAAGATGGTAGACCTGCTATTGTAACAGTATTACTCATTTGAGATCAGACATATTTTCAATTCTCTTGATTGCCACCTCTTGAGCTTTTGTGGAACAAATACTGGGTGAATACCCTTTCCTCATCACCTTTGCTGGGAAATGGAGACTATATTGCTGGGCAAATATCTGATCTCGCTGAGAAATTGGAGCAGGCAGAAAACCAGTTGGCTCATTCTCGTTTTGGGTCTATAGTTGCCCTTT

TTCT—3’
<table>
<thead>
<tr>
<th>Description</th>
<th>Max score</th>
<th>Total score</th>
<th>Query cover</th>
<th>E value</th>
<th>Ident</th>
<th>Accession</th>
</tr>
</thead>
<tbody>
<tr>
<td>JAB1/Mov3/MPN/PAD-1 [Cochinhus capsulatus]</td>
<td>568</td>
<td>568</td>
<td>94%</td>
<td>0.0</td>
<td>90%</td>
<td>OMO385129.1</td>
</tr>
<tr>
<td>PREDICTED: COP9 signalosome complex subunit 5b-like [Cucumis melo]</td>
<td>565</td>
<td>565</td>
<td>90%</td>
<td>0.0</td>
<td>90%</td>
<td>XP_008446090.1</td>
</tr>
<tr>
<td>PREDICTED: COP9 signalosome complex subunit 5b [Cucumis sativus]</td>
<td>565</td>
<td>565</td>
<td>90%</td>
<td>0.0</td>
<td>90%</td>
<td>XP_001438765.1</td>
</tr>
<tr>
<td>COP9 signalosome complex subunit 5a-like [Momordica charantia]</td>
<td>564</td>
<td>564</td>
<td>90%</td>
<td>0.0</td>
<td>90%</td>
<td>XP_022132138.1</td>
</tr>
<tr>
<td>PREDICTED: COP9 signalosome complex subunit 5b [Vitis vinifera]</td>
<td>564</td>
<td>564</td>
<td>91%</td>
<td>0.0</td>
<td>91%</td>
<td>XP_002283561.1</td>
</tr>
<tr>
<td>COP9 signalosome complex subunit 5b-like [Hevea brasiliensis]</td>
<td>563</td>
<td>563</td>
<td>92%</td>
<td>0.0</td>
<td>87%</td>
<td>XP_021971864.1</td>
</tr>
</tbody>
</table>

29. 51-1

5′—

TGGAGTACCCCATACGAGCGTACCAGATTACGCTCATATGACAAGTTTGTACAAA AAATGGAAATAAGGGCAAGGAAAGAAGAAGAATGGGATCCGAAGACA ATAGCGCAGAAAACATGGGAAATAGAGAAACATATAGAAACAGTAAACGACG CAGCATTAGCATTACAAAAGATGGTATCGATCATGCTAGATCAGGTGGAATATA GAAGAATGGGTTTAAATGCAGGGTAAACCTGATGCTTTGCTTTACCTAGGGTA AATGCTTTTGGCTTTTACTTGTTGAAGGACTGAACATTAGGTTTAATGCACCAGG CTGATGCTTTATGAATATATGGTATTATTCTACTACTATAAAACAGGGCTGGAAGA GTGTTGAAATGTGGTTGCTGGTTACACTCTCTGCTGTTGTATGGTTCTGTG GCTTTCTGGCATTGATGTTTTTCTACACAAAAAGTCTACAACCAAAATTCCAAGAAC CTTCTGGCTTGCTGTCATTGATCCAACCCGAACATGTTTCTGTGCTGGAAAAAGT TGAAATTTGCGTCTTTCCAGGACATAACCCCGGGAGGGCTATAAGCCACCCAGATGAG CCTATACAGAATTATACAAACCATTCCCTAATAAGATTGGAAGACTTTGGAGTG CATTGGAAACAGTATTACCTGATGGGACATCAGATATTCTCTTCTCTTGATT GCGCACCCTTTGGATCCTTTGTTGGAACAAATACTGGGTGAATACCAACTTTCCCTCA TCACCTTTGTGGGAAATGGAGACTATATTGCTTGGGCAATATCTGATCTGCGC TGANAAATTGGGACAGGCAGAAACCCAGTTGGCTGATTCTCCTTGGGGTTCT ATAGGGT—3′
<table>
<thead>
<tr>
<th>Description</th>
<th>Max score</th>
<th>Total score</th>
<th>Query cover</th>
<th>E value</th>
<th>Ident</th>
<th>Accession</th>
</tr>
</thead>
<tbody>
<tr>
<td>PREDICTED: CCP9 signalosome complex subunit 5b-like [Cucumis melo]</td>
<td>508</td>
<td>508</td>
<td>88%</td>
<td>8e-180</td>
<td>91%</td>
<td>XP_008445050.1</td>
</tr>
<tr>
<td>PREDICTED: CCP9 signalosome complex subunit 5b [Cucumis sativus]</td>
<td>508</td>
<td>508</td>
<td>88%</td>
<td>1e-179</td>
<td>91%</td>
<td>XP_004136765.1</td>
</tr>
<tr>
<td>JAB1/Myo34/MPN/PAK-1 [Corchorus olitorius]</td>
<td>507</td>
<td>507</td>
<td>88%</td>
<td>2e-179</td>
<td>93%</td>
<td>OMO96626.1</td>
</tr>
<tr>
<td>hypothetical protein GOBAR_AA21742 [Gossypium barbadense]</td>
<td>507</td>
<td>507</td>
<td>88%</td>
<td>2e-179</td>
<td>93%</td>
<td>PPR98931.1</td>
</tr>
<tr>
<td>hypothetical protein B456_009G068200 [Gossypium raimondii]</td>
<td>508</td>
<td>508</td>
<td>88%</td>
<td>2e-179</td>
<td>93%</td>
<td>KjB5194.1</td>
</tr>
<tr>
<td>PREDICTED: CCP9 signalosome complex subunit 5b-like isoform X1 [Gossypium raimondii]</td>
<td>507</td>
<td>507</td>
<td>88%</td>
<td>2e-179</td>
<td>93%</td>
<td>XP_012443254.1</td>
</tr>
</tbody>
</table>

30. 52-1

5’—

TGGAGTACCCATACGACGTACCAGATTACGCTCATATGACAAAGTGTGTTACTAAA AAAGTTGGAAATAAGGGCAAGAAGAAGAAGAAGAAGAATGGATCCAGAGACA ATAGCGCAGAAAACATGGGAAATAGAAGAACATATAGAAGAACAGTAGAAGCAG CAGCATCGAGATGCAATATTCAGTAGCAGAGGAGCCAGGGTGAATTCCA ACAAGAAACCATGGACGAATGAACCTCATTTACTCTAAAAAGGGTAAAAGATAT CAGCATTAGCATTACTAAAGATGGTAGTACAGCATCGACTAGATCGGTGGAATATA GAAGTAATGGGTTTATGCAAGGGTAAAAACAGTAGGGTCAGATGCTTTGTTATG GATGCTTTTGCATTACTGTTGAAGGTACTGAAACTAGGGTTAATGCCAGAG CTGATGCTTTATGAAATATATGGTTGATTATTCTACTACTATAAACCAGGCTGGAA GACCTGGGAAAATGTGGTTGGCTGTGATTACCACCTCCTCATCTGGTTGATGTG GCTTTTCGGCATGATGTTCCTACACAAAATGCTCAACCACAATTTCCAAGAAC CTTTCCATGCTGCTGTCATTGATCCAAAACCAGAAGCGTTCTCGTTTCTGCGGAAAAGT TGAAAAATTGGTGGTTTCAAGACATACCCGGAGGGTCTATAAGCCACCAACGATGAG CCTATCCTCGAATATCAAAACCATTCCCCTAAAATAAGATTGGAAGCCTTGGAGTG CATTGTAACAGTGTTACTCATTGAGACATCAGATATTATTTCAAGCTCTTTCTCTGGATT GCCACCTTCTGGATCTTTGTTGGAACAAATATGGGTGAATACCCTTTCTCTCA TCACCTTTGCTGGGAAATGGAGACTATATGTCGTTGCGGAAATATCTGATCTCGG TGNAAATTGGAGGCGAGCCAGAAAACCAGTTGGCTTCTCGATTGTTGAGGCGC —3’
Table S3.1: qRT-PCR result of MVLG_05122 in the transgenic A. thaliana

<table>
<thead>
<tr>
<th>Arabidopsis Strain</th>
<th>Gene</th>
<th>Ct Value</th>
<th>Delta</th>
</tr>
</thead>
<tbody>
<tr>
<td>WT</td>
<td>UBQ10</td>
<td>24.778</td>
<td></td>
</tr>
<tr>
<td></td>
<td>5122</td>
<td>31.477</td>
<td>6.699</td>
</tr>
<tr>
<td>5122 Δ SP-CFP a2</td>
<td>UBQ10</td>
<td>25.13</td>
<td></td>
</tr>
<tr>
<td></td>
<td>5122</td>
<td>21.861</td>
<td>-3.269</td>
</tr>
<tr>
<td>5122 Δ SP-CFP a5</td>
<td>UBQ10</td>
<td>22.896</td>
<td></td>
</tr>
<tr>
<td></td>
<td>5122</td>
<td>20.985</td>
<td>-1.911</td>
</tr>
</tbody>
</table>

Table S3.2: qRT-PCR result of CFP in the transgenic A. thaliana

<table>
<thead>
<tr>
<th>Arabidopsis Strain</th>
<th>Gene</th>
<th>Ct Value</th>
<th>Delta</th>
</tr>
</thead>
<tbody>
<tr>
<td>WT</td>
<td>UBQ10</td>
<td>24.352</td>
<td></td>
</tr>
<tr>
<td></td>
<td>CFP</td>
<td>33.408</td>
<td>9.056</td>
</tr>
<tr>
<td>5122 Δ SP-CFP a2</td>
<td>UBQ10</td>
<td>24.517</td>
<td></td>
</tr>
<tr>
<td></td>
<td>CFP</td>
<td>21.066</td>
<td>-3.451</td>
</tr>
<tr>
<td>CFP only</td>
<td>UBQ10</td>
<td>24.715</td>
<td></td>
</tr>
<tr>
<td></td>
<td>CFP</td>
<td>18.149</td>
<td>-6.566</td>
</tr>
</tbody>
</table>
Table S3.3: qRT-PCR result of *MVLG_06175* and *mCherry* in the transgenic *A. thaliana*

<table>
<thead>
<tr>
<th>Arabidopsis Strain</th>
<th>Gene</th>
<th>Ct Value</th>
<th>Delta</th>
</tr>
</thead>
<tbody>
<tr>
<td>WT</td>
<td>UBQ10</td>
<td>24.762</td>
<td></td>
</tr>
<tr>
<td></td>
<td>6175</td>
<td>n/a</td>
<td></td>
</tr>
<tr>
<td></td>
<td>mCherry</td>
<td>31.757</td>
<td>6.995</td>
</tr>
<tr>
<td>6175ΔSP-mCherry</td>
<td>UBQ10</td>
<td>24.185</td>
<td></td>
</tr>
<tr>
<td></td>
<td>6175</td>
<td>24.313</td>
<td>0.129</td>
</tr>
<tr>
<td></td>
<td>mCherry</td>
<td>24.269</td>
<td>0.084</td>
</tr>
<tr>
<td>6175-mCherry</td>
<td>UBQ10</td>
<td>24.191</td>
<td></td>
</tr>
<tr>
<td></td>
<td>6175</td>
<td>26.13</td>
<td>1.939</td>
</tr>
<tr>
<td></td>
<td>mCherry</td>
<td>26.686</td>
<td>2.495</td>
</tr>
<tr>
<td>mCherry only</td>
<td>UBQ10</td>
<td>23.847</td>
<td></td>
</tr>
<tr>
<td></td>
<td>mCherry</td>
<td>24.104</td>
<td>0.256</td>
</tr>
</tbody>
</table>

Sequencing Results of cDNA from *A. thaliana* Transformed with *MVLG_05122 ΔSP-CFP*

Sequencing with a forward primer

5'—

TTAGGNACCNTGCCTGAGTAAGCTTTAAGACGTATTGCCACCAGAGCTCTTATAG
CGGCTTCGCAAGAGACAGCAGACAGCGAGCGCGATGAAAAACAACAAAATGCTCTCT
TGGCATAACCTCATTGCAAAGGAAACCCAAACCGGTCAATACGGGCTCGTGG
ATAACTTTGCAAAGCAGCGATCGCTATAAGCATGGCGGCTCTCTATGGT
GAGCAAAGGGCGAGGAGCTGTTCACCGGAGGTGTGCCCATCCTGTGTCGACG
TGGAGCCGACGTAAACGGCCAAAGTTTCAGCGCAGTCGCCGAGGGCGAG
GGCGATGCGCAGCTACGGCAAGCTGACCTGAACTTTACCTGAGCGCACCACCCGC
AAGCTCCCGCGTGCCTGCCCTGACCACCCCTGCTCACCACCCCTGACCTGGGGG
GCAAGCTGCCCCTGCCCTGCCCTGACCCCTCTGCTGACCACCCCTGACCTGGGGGG
GCAGTGCTGTTAGCGCGCTACCCGGACCCACATTGAAACGAGCAGCAGACTTCTTTCAA
GCCTCGGCGATGCCCGAAGGGCTACGTACGTCAGGAGCGACCCCACTCTTTTCAAGGA
Sequencing Result of cDNA from *A. thaliana* Transformed with **MVLG_06175ΔSP-mCherry** and **MVLG_06175-mCherry**

MVLG_06175ΔSP-mCherry (sequencing with a forward primer)

5’—

AAAAGGCGTGAGTCCATCGGGCCATACACTAGCTCAGGCGCCCTTTGTGAATGCG
CTGCTACGACCCCAACTCGGGAACCTCCAACTCGACGTGCCGCAATGCCTG
CACGGGACAATACCACGTTTCAAGATCGTTGAACGCAGCGGATCAATGTATG
CAGCAATGCGATAGATTTACCAAAGACAAAAAGAAGCAGGGGGAGGGCAAA
CTAGAACACAAGAGATGTCTACACAAGTGTACGGATTGGTTCTTTCCTA
AA
TCTCGGATCCATGGTGAGCAAGGGCCAGGGCCGAGGGCCGCCCCCTACGA
GACCGGCAAAGCTGAAGGTGACCAAGGGTGGCCCCTGCGGCTTTGCGCTTG
GG
ACATCTCTGCTCCCTCTAGTTCTAGTACGCTTCCAAAGGCTCTACGTAAGG
ACCCACATCCCGACTACTTGAAGCTGTCCTTCCCCGAGGGCAGGCTGAG
TGGAGCCTCCTCCGAGGGGATGATCCCGGAGGGCCGCTGGAAGGGGCA
GATCAAGCAGAGCTGAAGCTGAAGGACGGCGGCCACTACGACGCTGAGG
TCAAGACCACCTACAAGGCGGAGATGGCCGCTGAGCTGCCGCGGCGCCTAC
AACGTAACGATCAAGGCGACATCACCTACCTCCAGAACGACCGACAG

BlastX result of 1<sup>st</sup>-323<sup>rd</sup> nucleotides

<table>
<thead>
<tr>
<th>Description</th>
<th>Scientific Name</th>
<th>Max Score</th>
<th>Total Score</th>
<th>Query Cover</th>
<th>E Value</th>
<th>Per. Identity</th>
<th>Acc. Length</th>
<th>Accession</th>
</tr>
</thead>
<tbody>
<tr>
<td>hypothetical protein MvLG_08175 (Monobothrium hydrida-dioice βA1 Lamano)</td>
<td>Monobothrium hydrida-dioice βA1 Lamano</td>
<td>167</td>
<td>167</td>
<td>71%</td>
<td>3e-01</td>
<td>100.00%</td>
<td>118</td>
<td>KDG00332.1</td>
</tr>
<tr>
<td>ROD965_0970841 (Monobothrium silanes-dioice)</td>
<td>Monobothrium silanes-dioice</td>
<td>154</td>
<td>154</td>
<td>77%</td>
<td>8e-01</td>
<td>62.02%</td>
<td>118</td>
<td>SGY99194.1</td>
</tr>
<tr>
<td>RZ2699_MvSc1284-α1-β1_60-99 (Monobothrium silanes-dioice)</td>
<td>Monobothrium silanes-dioice</td>
<td>78.6</td>
<td>78.6</td>
<td>71%</td>
<td>7e-10</td>
<td>48.75%</td>
<td>120</td>
<td>SCZ69198.1</td>
</tr>
<tr>
<td>YP4949900_HF560-Chenry_aminase_sense_synthetic_construct</td>
<td>synthetic construct</td>
<td>45.8</td>
<td>45.8</td>
<td>19%</td>
<td>0.02</td>
<td>95.24%</td>
<td>503</td>
<td>AT647137.1</td>
</tr>
<tr>
<td>YP4949900_HF560-Chenry_aminase_antisense_synthetic_construct</td>
<td>synthetic construct</td>
<td>45.8</td>
<td>45.8</td>
<td>19%</td>
<td>0.02</td>
<td>95.24%</td>
<td>503</td>
<td>AT647138.1</td>
</tr>
</tbody>
</table>

BlastX result of 324<sup>th</sup>-899<sup>th</sup> nucleotides

<table>
<thead>
<tr>
<th>Description</th>
<th>Scientific Name</th>
<th>Max Score</th>
<th>Total Score</th>
<th>Query Cover</th>
<th>E Value</th>
<th>Per. Identity</th>
<th>Acc. Length</th>
<th>Accession</th>
</tr>
</thead>
<tbody>
<tr>
<td>mCherry-F (Expression vector pTrGtC008G250p_pono-mCherry-F)</td>
<td>Expression vector pTrGtC008G250p_pono-mCherry-F</td>
<td>368</td>
<td>368</td>
<td>99%</td>
<td>5e-127</td>
<td>99.48%</td>
<td>265</td>
<td>ADF31028.1</td>
</tr>
<tr>
<td>mCherry (Synthetic construct)</td>
<td>synthetic construct</td>
<td>368</td>
<td>368</td>
<td>99%</td>
<td>5e-127</td>
<td>99.48%</td>
<td>262</td>
<td>QSL23322.1</td>
</tr>
<tr>
<td>mCherry (Synthetic construct)</td>
<td>synthetic construct</td>
<td>367</td>
<td>367</td>
<td>99%</td>
<td>6e-127</td>
<td>99.48%</td>
<td>228</td>
<td>AID043648.1</td>
</tr>
<tr>
<td>Crystal structure of human APOBEC3H RNA complex (Drososoma sp.)</td>
<td>Drososoma sp.</td>
<td>366</td>
<td>366</td>
<td>99%</td>
<td>8e-127</td>
<td>99.48%</td>
<td>219</td>
<td>6BB0_D</td>
</tr>
<tr>
<td>mCherry (Binary vector pYBA-1138)</td>
<td>Binary vector pYBA-1138</td>
<td>367</td>
<td>367</td>
<td>99%</td>
<td>8e-127</td>
<td>99.48%</td>
<td>238</td>
<td>AHO32762.1</td>
</tr>
</tbody>
</table>
MVLG_06175-mCherry (sequencing with a forward primer)

5’—

AAAAGGCGGAGCACATGGGCCATCTGCTCGAGCCTTTGTCGAATGCGCTGCTACGACCCCAACTCGGGAACCTCCAATCGACGTGGCCGCAATGCCTGACGGACAATACCACGTTTCAAGATCGTTGAACGCAGCGGATCAATGTATGCAGCAATGCGATAGATTTACCAAAGACAAAAAGAAGCAGGGGGAGGGC

BlastX result of 1st-258th nucleotides

MVLG_06175-mCherry (sequencing with a reverse primer)

5’—

ATTGAGCTTCTGCTCGGCCGCGCTTGGAAGCAAGCGATATGGGCACCGTGCTGTACAGCTCGTCATGGCCGCGCGCTAGTGGGCGCCGCGCTCGCGTGGCCAGGCGTTGGCCGTGGGCGGCTTCGAGGAGGAGTCCTGGGTACGGTCACCACGCCGTCCTCGGAAGTTCATCA

MVLG_06175-mCherry (sequencing with a reverse primer)

5’—

GACAGGATGTTGGGTACCTGCAGCTTCAGCTTGAGTTGAGGAAGGAGGAGTCCTGGGTACGGTCACCACGCCGTCCTCGGAAGTTCATCA

CCTGGAGATGTTGGGTACCTGCAGCTTCAGCTTGAGTTGAGGAAGGAGGAGTCCTGGGTACGGTCACCACGCCGTCCTCGGAAGTTCATCA

CCTGGAGATGTTGGGTACCTGCAGCTTCAGCTTGAGTTGAGGAAGGAGGAGTCCTGGGTACGGTCACCACGCCGTCCTCGGAAGTTCATCA

CCTGGAGATGTTGGGTACCTGCAGCTTCAGCTTGAGTTGAGGAAGGAGGAGTCCTGGGTACGGTCACCACGCCGTCCTCGGAAGTTCATCA

CCTGGAGATGTTGGGTACCTGCAGCTTCAGCTTGAGTTGAGGAAGGAGGAGTCCTGGGTACGGTCACCACGCCGTCCTCGGAAGTTCATCA

CCTGGAGATGTTGGGTACCTGCAGCTTCAGCTTGAGTTGAGGAAGGAGGAGTCCTGGGTACGGTCACCACGCCGTCCTCGGAAGTTCATCA

CCTGGAGATGTTGGGTACCTGCAGCTTCAGCTTGAGTTGAGGAAGGAGGAGTCCTGGGTACGGTCACCACGCCGTCCTCGGAAGTTCATCA

CCTGGAGATGTTGGGTACCTGCAGCTTCAGCTTGAGTTGAGGAAGGAGGAGTCCTGGGTACGGTCACCACGCCGTCCTCGGAAGTTCATCA

CCTGGAGATGTTGGGTACCTGCAGCTTCAGCTTGAGTTGAGGAAGGAGGAGTCCTGGGTACGGTCACCACGCCGTCCTCGGAAGTTCATCA

CCTGGAGATGTTGGGTACCTGCAGCTTCAGCTTGAGTTGAGGAAGGAGGAGTCCTGGGTACGGTCACCACGCCGTCCTCGGAAGTTCATCA
BlastX result of 1\textsuperscript{st}-628\textsuperscript{th} nucleotides

<table>
<thead>
<tr>
<th>Description</th>
<th>Scientific Name</th>
<th>Max Score</th>
<th>Total Score</th>
<th>Query Cover</th>
<th>E value</th>
<th>Per. Ident</th>
<th>Acc. Len</th>
<th>Accession</th>
</tr>
</thead>
<tbody>
<tr>
<td>mCherry (Cloning vector pCSWH006)</td>
<td>Cloning vector pCSWH006</td>
<td>405</td>
<td>405</td>
<td>94%</td>
<td>4e-141</td>
<td>95.97%</td>
<td>267</td>
<td>ACD01422.1</td>
</tr>
<tr>
<td>EYOS1-mCherry (Cloning vector pLM006-EYOS1-mCherry)</td>
<td>Cloning vector pLM006-EYOS1-mCherry</td>
<td>415</td>
<td>415</td>
<td>93%</td>
<td>1e-140</td>
<td>97.64%</td>
<td>576</td>
<td>ANF29637.1</td>
</tr>
<tr>
<td>mCherry {synthetic construct}</td>
<td>synthetic construct</td>
<td>403</td>
<td>403</td>
<td>99%</td>
<td>1e-140</td>
<td>93.76%</td>
<td>262</td>
<td>Q5V222.1</td>
</tr>
<tr>
<td>mCherry-linker (Cloning vector pG6001)</td>
<td>Cloning vector pG6001</td>
<td>402</td>
<td>402</td>
<td>93%</td>
<td>5e-140</td>
<td>97.46%</td>
<td>269</td>
<td>AHG5497.1</td>
</tr>
<tr>
<td>linker-mCherry (Cloning vector pGGX003)</td>
<td>Cloning vector pGGX003</td>
<td>401</td>
<td>401</td>
<td>92%</td>
<td>1e-139</td>
<td>98.46%</td>
<td>269</td>
<td>AHG5497.1</td>
</tr>
</tbody>
</table>
CURRICULUM VITAE

NAME: Ming-Chang Tsai

ADDRESS: 333 East Market Street Room 604, Louisville, KY 40202, USA

DOB: Taichung, Taiwan — July 1, 1978

PHONE: (510)225-5572

EMAIL: mingchangtsai@hotmail.com; M0TSAI02@louisville.edu

EDUCATION:

Ph.D. Biology-MCDB Program, University of Louisville, KY, USA.
Aug 2017 – August 2011

M.Sc. Biology (GPA 3.61), University of Louisville, KY, USA.
Aug 2013 – Aug 2017

M.Sc. Nutrition, Food Science & Packaging (GPA 3.271), San Jose State University, CA, USA.
Sep 2005 – Aug 2011

B.Sc. Biology (GPA 3.1), Tunghai University, Taichung, Taiwan
Sep 1996 – Jun 2000

PUBLICATIONS:
(*Contributed equally)

PRESENTATIONS:

1. Biology Department Awards Day Graduate Student Research Presentations
   Feb 19, 2017
   “Functional Characterization of Microbotryum lychnidis-dioicae Effectors” (Oral)

2. Biology Department Awards Day Graduate Student Research Presentations
   Apr 23, 2019
   “Identification of Protein-Protein Interactions during Infection of Silene latifolia by the Fungal Phytopathogen Microbotryum lychnidis-dioicae” (Oral)

3. Kentucky Academy of Science Annual Meeting
   Nov 1-2, 2019
   “Identification of Protein-Protein Interactions during Infection of Silene latifolia by the Fungal Phytopathogen Microbotryum lychnidis-dioicae”

4. GNAS GRAD talks Brown Bag Series
   Nov 12, 2019
   “Identification of Protein-Protein Interactions during Infection of Silene latifolia by the Fungal Phytopathogen Microbotryum lychnidis-dioicae”

5. Kentucky Academy of Science Annual Meeting (Oral and virtual)
   Nov 6-7, 2020
   “Characterization of Microbotryum lychnidis-dioicae secreted effector proteins that manipulate its host plant, Silene latifolia”

HONOURS/AWARDS/SCHOLARSHIP:

1. Grant by Graduate Student Research and Creative Activities by College of Arts and Sciences, University of Louisville
   Mar 2017

2. GNAS (Graduate Network in Arts and Sciences) Graduate Student Research Grant, University of Louisville
   Feb 2017 and Sep 2019

MEMBERSHIP:

- KAS (Kentucky Academy of Science). https://kyscience.org/