FUNCTION AND REGULATION OF WISHFUL THINKING, A BMP TYPE II RECEPTOR, IN EGGSHELL PATTERNING

by

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ABSTRACT OF THE THESIS

Function and Regulation of Wishful thinking, a BMP Type II Receptor

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Development is controlled by a surprisingly small number of genetic pathways. One such pathway is called the bone morphogenetic protein (BMP) pathway, similar from flies to humans. We used the common fruit fly, *Drosophila melanogaster*, to study the BMP pathway during *Drosophila* oogenesis, the formation of the egg. While the pathway is relatively simple, there exist combinations between the three different ligands, and four different receptors.

My work focused largely on the two type II receptor, specifically on Wishful thinking (WIT). Much is known about the dynamic expression of the type I receptor during oogenesis, Thickveins. However, the pathway requires action of both type I and type II receptors. We found that WIT performs a necessary role during oogenesis and is regulated, indirectly, by BMP signaling. WIT is required for proper patterning of pathway target genes and necessary for proper formation of the eggshell. We also used a new technology, CRISPR/Cas9, to specifically remove the WIT locus from its endogenous location in the genome. This allowed for introduction of new alleles, including a tagged variant and conditional null, for future study of this gene. In the future, this may be applied to other genes in *Drosophila* oogenesis.

Since gene patterning is crucial to development, we also worked in a collaborative effort to describe other complex gene patterns during *Drosophila* oogenesis. The Chorion proteins (CPs) are constituents of the eggshell and are expressed in a highly complex and dynamic fashion. We developed a system to computationally analyze gene pattering, dynamics, and conservation by utilizing a simple binary matrix. This matrix was then able to be used to predict the origin of a new domain in a related species of *Drosophilid*, the dorsal ridge of *D. nebulosa* and *D. willistoni*. This work served as a jumping point for multiple lab projects to study the patterning of this domain by another signaling pathway, the epidermal growth factor receptor (EGFR).

DEDICATION

To my family

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TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

LIST OF SUPPLEMENTAL MATERIAL

CHAPTER 1: INTRODUCTION

1.1 Bone Morphogenetic Protein

The bone morphogenetic protein (BMP) signaling pathway belongs to the super family of transforming growth factors beta of growth regulators (Miyazono et al., 2010). BMP signaling was discovered by its ability to induce bone growth in tissue grafts (Urist, 1965). Since then, BMP signaling has been shown to be integral to proper development and is associated with a wide range of human disease (Yanagita, 2009). Importantly, studies have associated miss-regulation of BMP signaling with various inheritable disorders, including cancer and heart defects (Alarmo and Kallioniemi, 2010; Massague et al., 2000; Wyatt et al., 2010). As consequence, a number of pathway components are targets of various disease treatments (Hong and Yu, 2009).

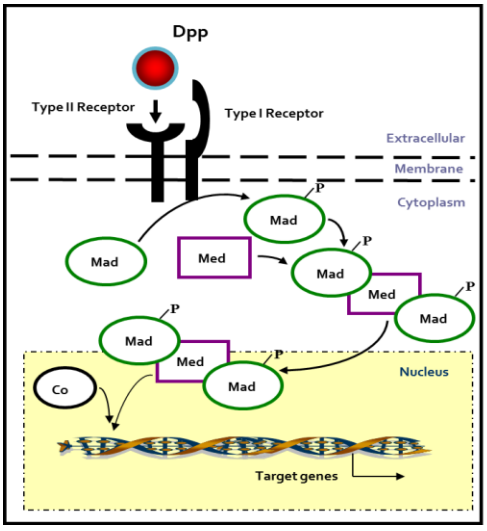

Figure 1: The BMP signaling pathway. Pathway activation begins when the ligand, Decapentaplegic (Dpp), interacts with the Type II receptor. The Type I receptor is phosphorylated, which in turn phosphorylates the intercellular signaling molecule, mothers against DPP (MAD), to generate phosphorylated MAD (P-MAD). Two P-MAD molecules, together with the coSMAD, Medea (MED), translocate to the nucleus and serve ins transcriptional regulation to act on gene expression.

On face, the pathway is relatively simple (Fig. 1). Activation of a cell occurs when the ligand, Decapentaplegic (DPP), interacts with a heterotetrameric complex of type I and type II receptors localized to the cell surface (Affolter and Basler, 2007; Padgett et al., 1987). Upon complex formation, the type I receptor is phosphorylated by the constitutively active type II, which in turn, phosphorylates a cytosolic signaling

molecule, MAD, which is referred to as Phospho-MAD (P-MAD) (Baker and Harland, 1997; Raftery and Sutherland, 1999). Throughout my dissertation, I will focus on the role of the BMP type II receptor, Wishful thinking (WIT) in eggshell patterning and morphogenesis.

In the famous example for evolutionary adaptation, Darwin observed how closely related species of finch had adjusted the size and shape of their beaks to take advantage of different food sources. It was reported that different beak morphologies are associated with the levels of the BMP ligand (Abzhanov et al., 2004). Furthermore, in a follow-up study, the same group found that the type II BMP receptor is a crucial determinator of the final shape and size of the beak in these species (Mallarino et al., 2011). In fish, using pathway component hypomorphs, precise levels of BMP signaling were shown to be required to initiate the cells of the cranial neural crest (Schumacher et al., 2011). Thus, different components of the BMP pathway control development across animals. The type II receptor has been shown to be involved in many aspects of development in animals, thus it is not surprising that malregulation of this pathway is associated with many diseases and deformations, including pulmonary hypertension and several cancers (International et al., 2000; Ma et al., 2010; Owens et al., 2012). Yet, in *Drosophila*, the utilization of WIT is exclusively described in the neuromuscular junction (Aberle et al., 2002; Marques et al., 2002; Marques et al., 2003), and we found, as a part of this dissertation, the function of WIT in oogenesis (Marmion et al., 2013).

1.2 *Drosophila* Oogenesis

Drosophila oogenesis is the process of forming an egg within the ovary of the female fly. This process occurs in an assembly line manner, consisting of fourteen

morphologically defined stages (Spradling, 1993). The entire process takes approximately three days (Bastock and St Johnston, 2008). Different cell types can be observed in an egg chamber mid-oogenesis (Fig. 2A). The nurse cells, in the anterior, are fifteen germinal derived cells, which nurse the developing oocyte by providing essential nutrients, mRNAs, and proteins. At mid-oogenesis, the oocyte nucleus is asymmetrically located in the dorso-anterior. The follicle cells (FCs) are a monolayer of epithelial tissue surrounding the oocyte. These cells will later form the eggshell. The structures of the eggshell (Fig. 2B) provide various functions. The micropile (MP) serves as conduit for sperm to fertilize the egg. The dorsal appendages (DAs) provide gas exchange for the developing embryo and the operculum (OP) is a weakened structure from which the larva emerges (James and Berg, 2003; Ward and Berg, 2005; Ward et al., 2006).

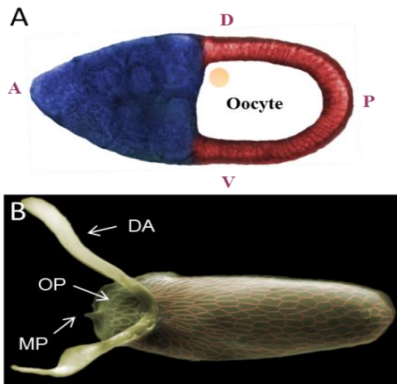

Figure 2: The egg chamber and the egg. (**A**) A, P, D, and V mark anterior, posterior, dorsal, and ventral, respectively. Located in the anterior, nurse cells (blue) nourish the developing oocyte. The oocyte nucleus (pink) is asymmetrically located, at the dorso-anterior. Surrounding the oocyte are the follicle cells (red), which form the structures of the egg. (**B**) The eggshell is oriented similarly, except this is a dorsal view, where the egg chamber is a lateral view. The eggshell consists of tractable structures including in the anterior, the micropile (MP). The dorsal appendages (DA) lift off of the dorsal side of the eggshell, and operculum (OP), in between, serves as a weakened structure for hatching.

We use *Drosophila* oogenesis as a model system to study how changes in the levels of BMP signaling affect the formation of the *Drosophila* eggshell. The eggshell is a three-dimensional structure surrounding the developing embryo, which serves as a physical protective barrier from the environment (Dapples and King, 1970; Hinton and Service, 1969; Spradling, 1993). At the same time, specialized structures on the eggshell provide a mechanism for gas exchange. These structures are highly sensitive to the

changes in the levels of BMP signaling, making the eggshell an ideal system to study how qualitative and quantitative changes in signaling affect eggshell development via proper gene patterning (Dobens and Raftery, 2000; Twombly et al., 1996; Yakoby et al., 2008b).

1.3 Morphogen Gradients

In the 50's, Alan Turing mathematically described a diffusible signal within a developing tissue that could establish cell fates based on the concentration of the chemical signal, called a morphogen (Turing, 1952). The French flag model was described in the late 60's by Lewis Wolpert. This simple model indicates that cells respond to the concentration of morphogen that they are exposed to by differential gene expression (Wolpert, 1969). In this way, one morphogen can determine several cell fates. In reality, cells within a developing tissue are actually integrating multiple morphogens in order to determine their precise location within a tissue. A hierarchical description of gene interactions to create tissue patterns was used to describe these patterning events (Meinhardt, 1986).

Axes formation in the oocyte is guided mainly by two major signaling pathways. The epidermal growth factor receptor (EGFR) ligand is localized proximal to the oocyte nucleus, establishing a gradient of high to low from dorsal to ventral (Neuman-Silberberg and Schupbach, 1993; Neuman-Silberberg and Schupbach, 1994; Queenan et al., 1997). The BMP ligand establishes a gradient of signaling of high to low from the anterior to the posterior (Deng and Bownes, 1997; Shravage et al., 2007; Twombly et al., 1996). The FCs respond to signaling gradients by differentially expressing genes and go on to form the structures of the eggshell. Research in our lab is on how two different pathways, the

epidermal growth factor receptor (EGFR) pathway and bone morphogenetic protein (BMP) pathway, get integrated to form gene expression patterns. Often, the pathway inputs are even separable by use of various enhancers of gene expression.

1.4 EGFR signaling dynamics during *Drosophila* oogenesis

During oogenesis in *D. melanogaster*, Gurken (GRK), the ligand of the EGFR pathway, is localized proximal to the oocyte nucleus. The overlying layer of follicle cells internalize the signal through ligand binding to a dimerized receptor complex and a phosphorylation cascade works through RAS, RAF, MEK, and MAPK. MAPK is then able to phosphorylate and activate/inactivate transcription factors. EGFR signaling first sets up the anterior-posterior axis and then sets up the dorsal-ventral axis (Chen and Schupbach, 2006; Cheung et al., 2011; Neuman-Silberberg and Schupbach, 1994; Queenan et al., 1997). Furthermore, this pathway interacts with other signaling pathways, including BMP (Xu et al., 2011; Yakoby et al., 2008b).

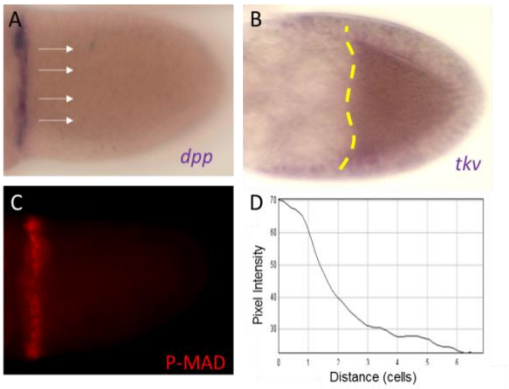

Figure 3: BMP Signaling in Oogenesis. (**A**) The ligand, *decapentaplegic* (*dpp*), is expressed in the anterior follicle cells and the DPP protein generates a gradient towards the posterior (arrows). (**B**) The initial pattern of *thickveins* (*tkv*) is uniform. Yellow line marks anterior of the developing oocyte. (**C**) BMP signaling, monitored by phospho-MAD (P-MAD), occurs where the ligand interacts with the uniform TKV (red). (**D**) The gradient of P-MAD can be measured by intensity and displays an exponential decay. All egg chambers are oriented anterior left and dorsal up.

1.5 BMP signaling dynamics during *Drosophila* oogenesis

We utilize *Drosophila* oogenesis to study BMP signaling, a model system for cell to cell communication and tissue patterning (Berg, 2005; Yakoby et al., 2008a). In *D.*

melanogaster, the ligand, Decapentalplegic (DPP) (Padgett et al., 1987; Twombly et al., 1996), emanates from an anterior source (Fig. 3A), signaling through the uniformly expressed type I receptor, Thickveins (TKV) (Fig. 3B). Internalization of the extracellular ligand, by receptors, results in the phosphorylation of an intracellular signaling molecule, phospho-Mothers against dpp (P-MAD) (Fig. 3C). This leads to an exponentially decaying gradient of signaling in the anterior (Fig. 3D) (Dobens and Raftery, 2000; Shravage et al., 2007; Twombly et al., 1996). BMP signaling requires a heterocomplex of type I and type II receptors (Parker et al., 2004; Ruberte et al., 1995). While the ligand and type I receptor are characterized in this system, the type II receptor, which is an essential component of the signaling network, has not been characterized.

1.6 Eggshell Patterning

Eggshell patterning refers to genes expression within the follicular epithelium in a particular time and space. Two genes are associated with future domains of the dorsal appendages, Broad (BR) and Rhomboid (RHO). BR is associated with cells that become the roof, or top, of the dorsal appendages while RHO is associated with the cells that become the floor, or bottom, of the dorsal appendages (Berg, 2005). EGFR signaling induces both BR and another transcriptional regulator, Pointed (PNT), which represses BR expression. Since PNT is induced only at high levels of signaling, it represses BR within the dorsal midline, leaving two dorsolateral patches of BR on either side of the midline. There exists an additional interplay between Broad and P-Mad where Broad is repressed by high levels of BMP signaling (Berg, 2008; Deng and Bownes, 1997; James and Berg, 2003; Yakoby et al., 2008b). Genes during development integrate multiple

signaling pathways to form complex patterns (Yakoby et al., 2008a). We focused on how BMP works to pattern *wishful thinking* during oogenesis.

We found *wit* to be expressed in a spatially restricted pattern in the precursor of the eggshell, the egg chamber. This pattern is evolutionarily conserved, and in *D. melanogaster* the receptor was found essential for BMP signaling. Together with powerful genetic tools (Duffy, 2002), short life cycle, different eggshell morphologies between species (Kagesawa et al., 2008), and twenty fully sequenced species (*Drosophila* 12 Genomes et al., 2007), *Drosophila* oogenesis is an excellent system to carry out an interdisciplinary study for the role of the type II receptor, Wishful thinking (WIT), in BMP signaling, tissue patterning, and morphogenesis.

CHAPTER 2: The *Drosophila* BMPRII, Wishful thinking, is required for eggshell patterning (Marmion et al., 2013)

R.A. Marmion, M. Jevtic, A. Springhorn, G. Pyrowolakis, and N. Yakoby. The Drosophila BMPRII, Wishful thinking, is required for eggshell patterning. 2013. Developmental Biology. 375:45-53.

Previously, the requirement for WIT was solely assigned to neurogenesis. Here, we established the role for WIT in oogenesis. Not only is it required for proper patterning and morphogenesis, but it is also patterned by a positive feedback loop on BMP signaling.

I designed experiments and conducted all of the immunoassays and co-wrote the paper with N. Yakoby. Reporter creation and transgenesis was conducted by M. Jevtic and G. Pyrowolakis.

CHAPTER 3: Regulation of *wishful thinking* by BMP signaling

3.1 Analysis of the *wit* reporter

cis-regulatory modules (CRMs) are regions of the DNA responsible for gene expression. They contain sequences that are recognized by transcription factors. The combination of binding sites and availability of transcription factors will determine the final pattern of a gene within different tissues (Borok et al., 2010). Since transcription factors regulate many different genes, evolution tends to select for subtle changes in gene expression via the CRM (Peter and Davidson, 2011). Since we propose *wit* to be a target of BMP signaling, we characterized the CRM responsible for *wit* expression. A P-MAD motif has been reported, called the activator element (AE), which is able to recruit the activator, P-MAD, or the suppressor, BRK, but not another pathway suppressor, Schnurri (Weiss et al., 2010). As described in Chapter 2 of this thesis, we produced a reporter for *wit* that reproduced the gene pattern (Marmion et al., 2013). However, the AE (GRCGNCNNNNNGNC) is absent from this construct, and thus we hoped to discover a novel motif.

Figure 4: Motif Analysis. MEME analysis identified a conserved fragment that contained elements similar to MAD and MED binding sites. However, the spacing is not consistent with the literature.

I utilized MEME (Bailey et al., 2006), which uses hidden markov models to find statistically overrepresented DNA sequences in attempt to computationally predict the transcription factor binding sites within the *wit locus* by comparing several different

sequenced species of *Drosophila* (Fig. 4). This conserved motif contains a P-MAD binding site as well as a conserved MED site. However, it is important to note that the binding consensus for MED is discovered frequently, since it is not complex (GNCN). For instance, in *D. melanogaster*, there are two other MED sites located within 10 bp. Furthermore, this site does not match the AE.

3.2 Development of a minimalized reporter

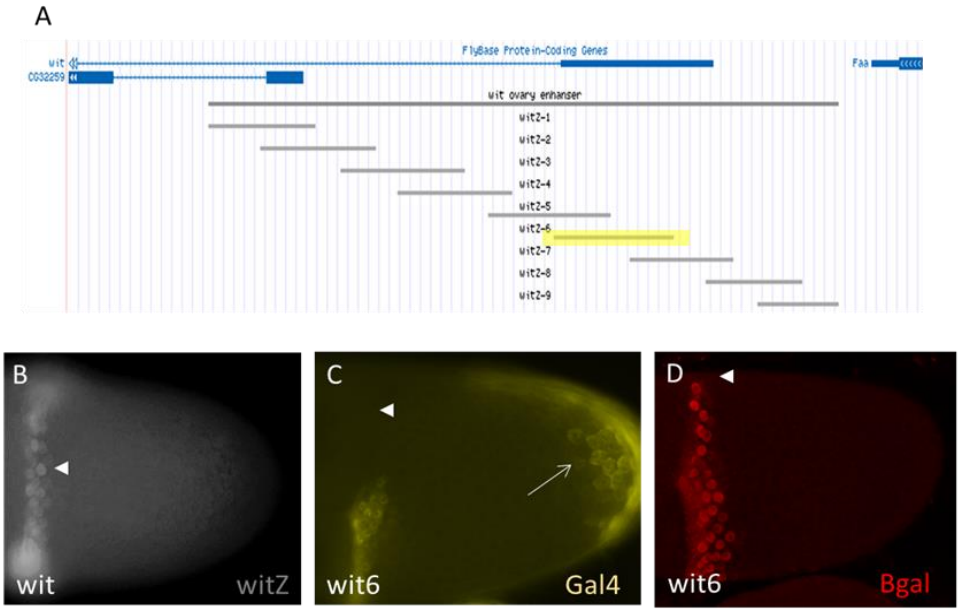

Figure 5: Minimalizing the wit enhancer. The wit CRM was fractioned into 9 overlapping fragments averaging 250 bp (A). Only one fragment, wit6, produced expression, including ectopic expression (C, arrow). When using this fragment to drive expression of Bgal, the expression pattern is in line with the original reporter (D). Arrowhead is dorsal.

The original reporter is 1.2 Kb. To narrow down the size of the enhancer, we searched, empirically, by cloning overlapping smaller fragments of the original reporter, with an average size of 250 bp (Fig. 5A). We used a Gal4 reporter with the thought of utilizing the GAL4/UAS system for follow-up studies. One fragment, wit6 (242 bp), created an anterior pattern, but this lacked the dorsal domain. Interestingly, it also produced an ectopic posterior expression (Fig. 5C, arrow). We instead went back to

using Bgal as a reporter and this same fragment (Fig. 5D), when driving Bgal, recapitulated the larger reporter dynamics (Fig. 5B). This is the fragment that contains the conserved site predicted by MEME.

3.3 The enhancer for *wit* binds BMP signaling components

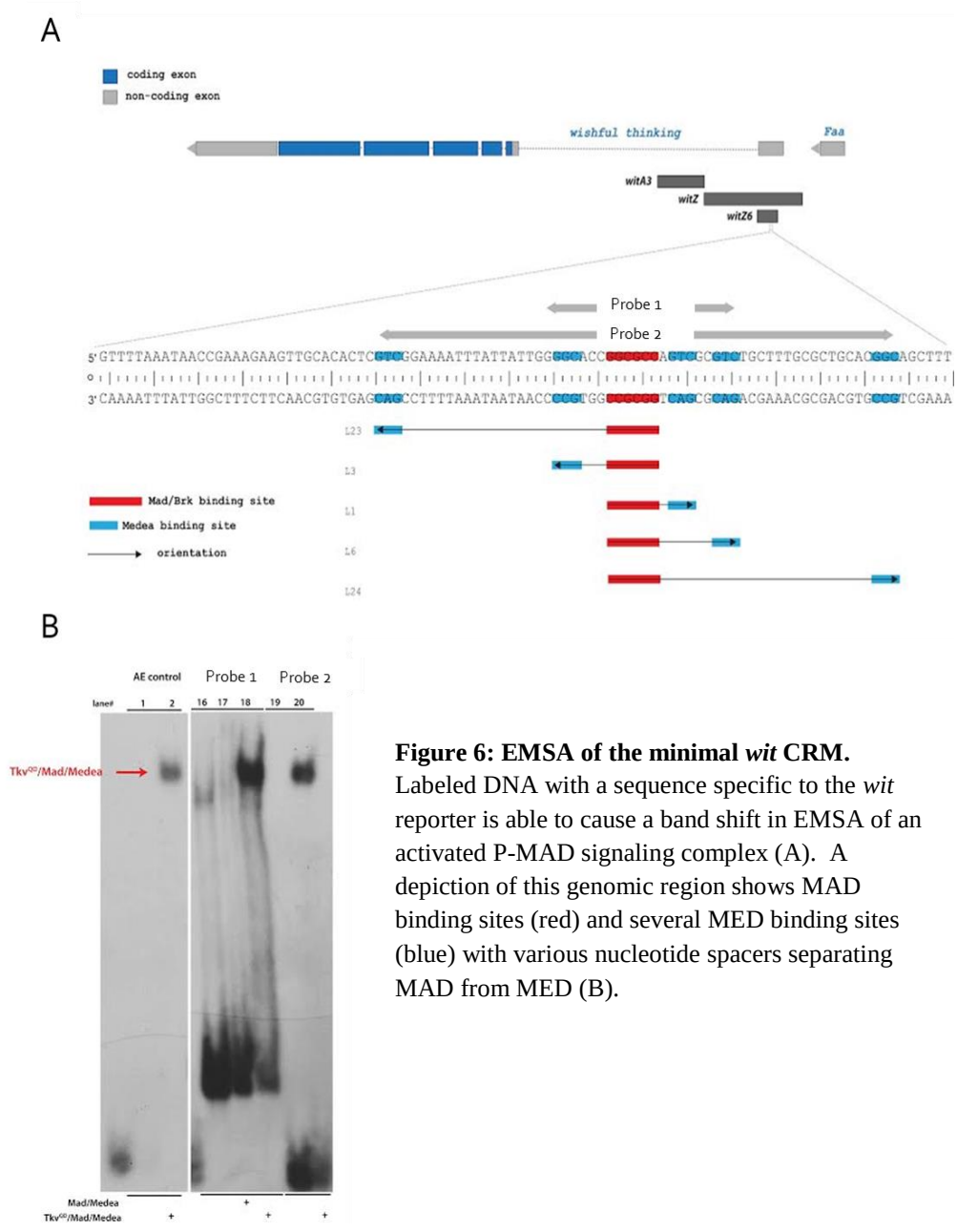

Figure 6: EMSA of the minimal *wit* CRM. Labeled DNA with a sequence specific to the *wit* reporter is able to cause a band shift in EMSA of an activated P-MAD signaling complex (A). A depiction of this genomic region shows MAD binding sites (red) and several MED binding sites (blue) with various nucleotide spacers separating MAD from MED (B).

We worked, in a collaborative effort with the Pyrowolakis Lab, to show direct binding of the BMP signaling complex to a site within the minimized CRM using gel shift assay (Fig. 6). Two different probes were utilized that bared a small sequence within wit6, a large probe that contained MAD plus 5 MED sites, and a smaller probe that only included the three most proximal MED sites. Importantly, even this small probe is able to cause a super-shift by P-MAD binding to the probe (Fig. 6B, lane 20).

3.4 The wit enhancer retains expression in absence of MED binding

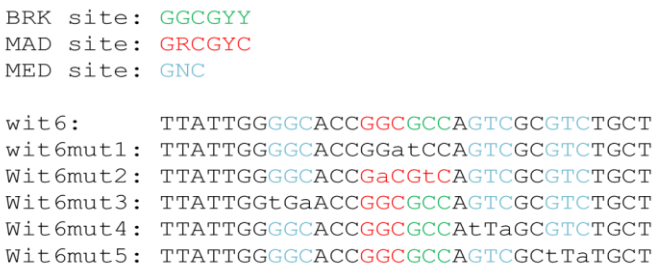

Figure 7: MED/MAD mutant sites. Nucleotide sequences depict clusters of binding sites. Brinker sites are depicted in green. Mad sites are depicted in red and Medea sites are depicted in blue. The WT wit6 can bind both Brinker as well as Mad.

There have been several published mutant forms of the MAD and MED binding sites, described by their ability to shift the P-MAD complex in EMSA (Weiss et al., 2010). We cloned these mutant forms and conducted reporter assays of the follicle cells (Fig. 7). Since SMAD binding elements are GC rich, these mutations are primarily composed of C to A conversions and G to T conversions. We used the tissue based reporter assay in order to study mutant forms of the three MED sites, local to the MAD binding site. wit6mut3, 4, and 5 correspond to MED sites with a linker size of 3, 1, and 6 respectively. MED sites were mutated using the same C to A conversions and G to T conversions to remove the MED site. Interestingly, when each of the MED sites is removed, patterning is largely unaffected (Fig. 8A-C).

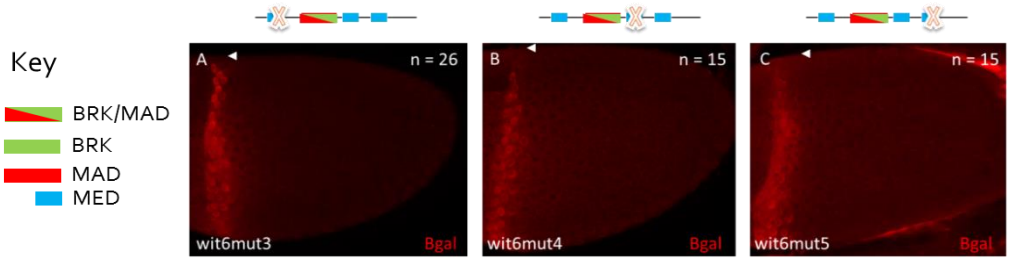

Figure 8: MED sites are not required for *wit* expression. Elimination of any of the three proximal MED sites does not considerably alter reporter expression (A-C). n is number of observations; arrowhead is dorsal.

3.5 Ectopic enhancer expression in absence of BRK binding but not MAD binding

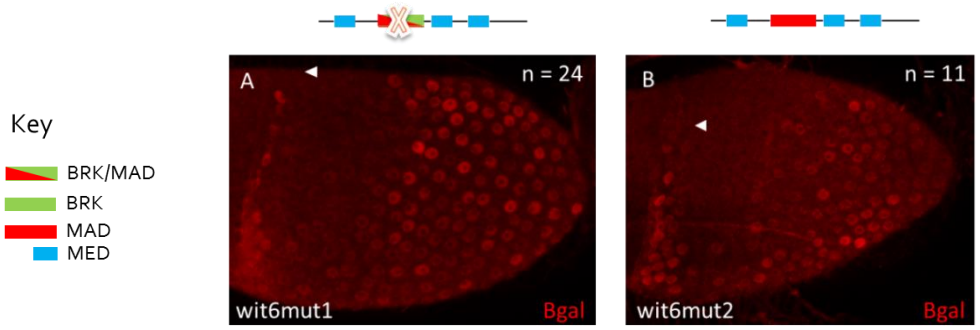

Figure 9: Removal of the BRK site leads to ectopic activation. Elimination of the SMAD binding sites leads to ectopic reporter expression (A). Binding by MAD to the reporter still leaves ectopic activation (B). n is number of observations; arrowhead is dorsal.

The MAD binding site in *wit* is composed of a palindromic GGCGCC. wit6_mut1 is a mutant of the internal CG, which is necessary for P-MAD as well as BRK binding and has been shown to not bind the P-MAD complex in EMSA. Ectopic expression is detected in this mutant (Fig. 9A). wit6-mut2 is a mutation of the second and fifth nucleotide in the palindrome and has been suggested by EMSA to not affect P-MAD binding. Interestingly, this reporter appears no different than wit6-mut1 (Fig. 9B). This suggests that *wit* regulation may, in fact, be dependent on the removal of BRK expression by P-MAD and not directly by activation on *wit*.

We confirmed this by analysis of Brinker clones. In cells null for BRK, ectopic *wit* was expressed (Fig. 10B). In the anterior, small clones of BRK have no effect on P-MAD or WIT, since BRK is not there (not shown). Outside of the anterior domain, removal of BRK has no effect on BMP signaling (Fig. 10A).

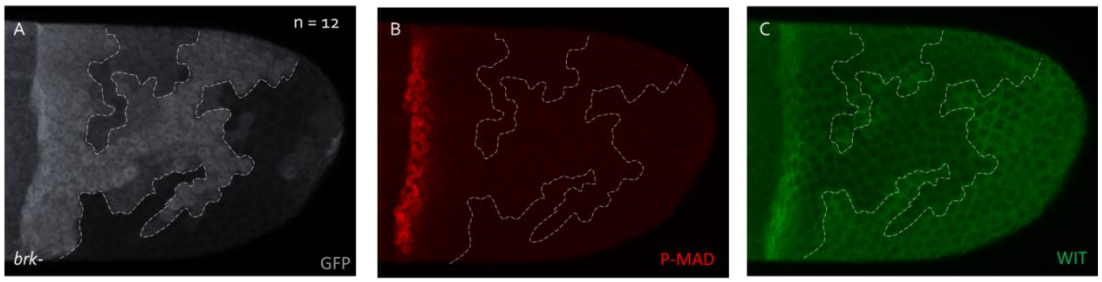

Figure 10: WIT is patterned by the loss of BRK. Clonal cells, marked by GFP (A), null for BRK exhibit normal P-MAD (B) but ectopic expression of WIT (C). n is number of observations; arrowhead is dorsal.

This is explained by the fact that the BRK expression pattern is shaped by P-MAD repression, as suggested by the mutually exclusive domains of BRK and P-MAD (Fig. 11A, B). An overlap of these two patterns indicates the repression of *brk* by P-MAD (Fig. 11C). This regulation is similar to the wing and was recently confirmed in the follicle cells (Charbonnier et al., 2015).

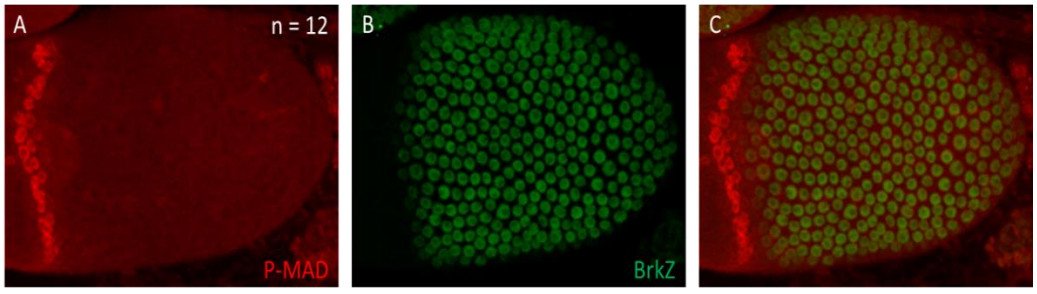

Figure 11: P-MAD and Brk are expressed in mutually exclusive domains. P-MAD is detected in an anterior to posterior gradient (A). Brinker lacZ trap is repressed in a gradient away from P-MAD (B). Merge shows the overlap of these two gradients (C). n is number of observations.

3.6 Identification of the *D. virilis wit* locus

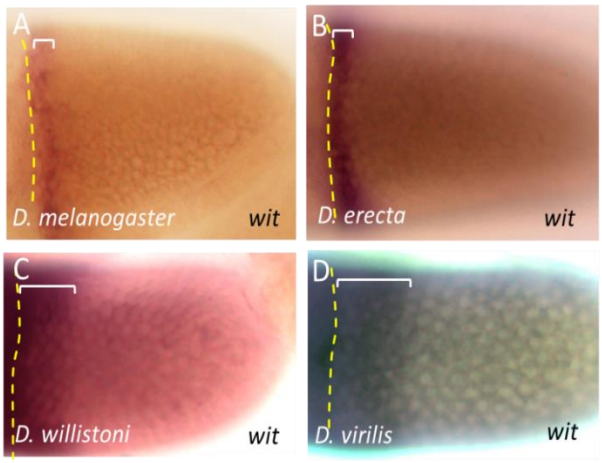

Figure 12: *wit* patterning is conserved. (**A-D**) The anterior pattern of *wit* is conserved during 45 million years of evolution. Anterior is marked by yellow dashed line. The pattern width differs between species and is either narrow (A, B), or wide (C, D). *D. erecta* (B) is closely related to *D. melanogaster* (A). *D. willistoni* (C) and *D. virilis* (D) are increasingly less related to *D. melanogaster*.

The expression pattern of *wit* is largely conserved. Throughout 45 million years of speciation, *wit* is expressed in an anterior pattern (Fig. 12).

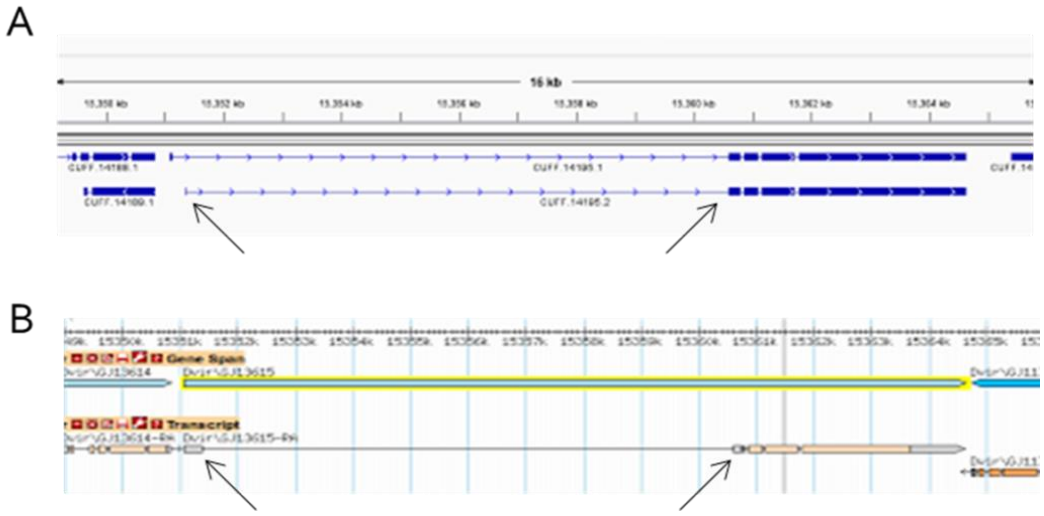

Figure 13: RNA-seq assembly. RNA-seq assembly identifies a first intron (A, between arrows). Flybase gene model has since been updated and now correctly identifies UTRs in the *D. virilis* locus (B, adjacent to marked intron).

However, *Drosophila virilis* contains no significant homology to the wit6 piece. It was previously reported that the homologous enhancers for *shavenbaby* between species could not be determined by using sequence homology. However, it is apparent

that enhancers can be discovered by searching at a similar region within the homologous locus gene model (Frankel et al., 2012). Gene homolog identity on flybase is predicted largely by sequence homology to the coding sequence. Therefore, the gene model is not complete, largely lacking UTRs.

In order to determine the homologous region within *D. virilis*, we decided to assemble sequence read archive (SRA) RNA-seq data and located the unannotated 5' UTR of *wit* in *D. virilis* (Fig. 13A). Consistent with this assembly, Gnomon gene model predictions on flybase recently became available (Fig. 13B) (Tweedie et al., 2009). As consequence, we utilized the region that flanked the predicted first exon for reporter analysis.

3.7 Analysis of *D. vir.* enhancer in *D. melanogaster*

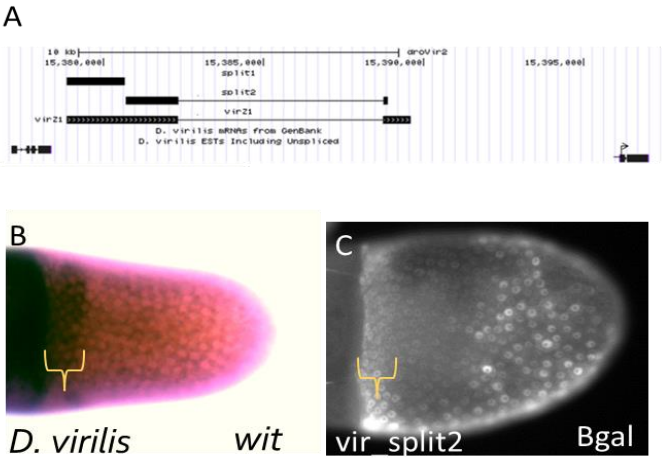

Figure 14: The *D. virilis wit* enhancer reproduces a divergent expression pattern. The *wit* locus was searched for reporter expression, including splitting one fragment into two pieces. These fragments span a repetitive element no found in the utilized strain (A, gap). A pattern similar to the endogenous gene pattern of *wit* in *D. virilis* (B) was produced in *D. melanogaster* with wider anterior expression than the *D. mel*. enhancer (C, bracket).

Using this information, we worked to identify the CRM in *Drosophila virilis*, a species 45 million years separated for *D. melanogaster*. This species exhibits patterning differences in width and dynamics. Specifically, the pattern is wider than that of *D.*

melanogaster (Fig. 14B). Since genetic tools are not widely available in *D. virilis*, we opted to screen fragments in *D. melanogaster*.

We expected that this CRM would have a pattern similar to the endogenous pattern of WIT in *D. melanogaster*. Surprisingly, this reporter is strikingly similar to the endogenous *wit* pattern in *D. virilis*, suggesting that *cis*-changes are responsible for this gene pattern evolutionary change (Fig. 14C). Later in the dissertation, we will discuss how the *D. melanogaster* SMAD/MED cluster was replaced with that of *D. virilis* within the whole *wit* locus.

3.8 A brief history of CRISPR

CRISPR allows for precise engineered editing in a wide range of organisms. This history of its discovery is a rapidly developing process and there are some legal battles still to be figured out as to its use. Repeat elements within *E. coli* were first described in 1987 (Ishino et al., 1987). Related elements were later described in 1993 and eventually predicted, correctly, to be part of an adaptive immunological response (Mojica et al., 2005; Mojica et al., 1993). Piggybacking on the discovery of many groups that characterized the action of the Cas proteins, eventually the system was greatly simplified with creation of a chimeric targeting RNA (Jinek et al., 2012). The system rapidly spread to the commonly used model organisms, including *Drosophila* (Bassett et al., 2013; Gratz et al., 2014).

3.9 Description of the generated WIT null allele

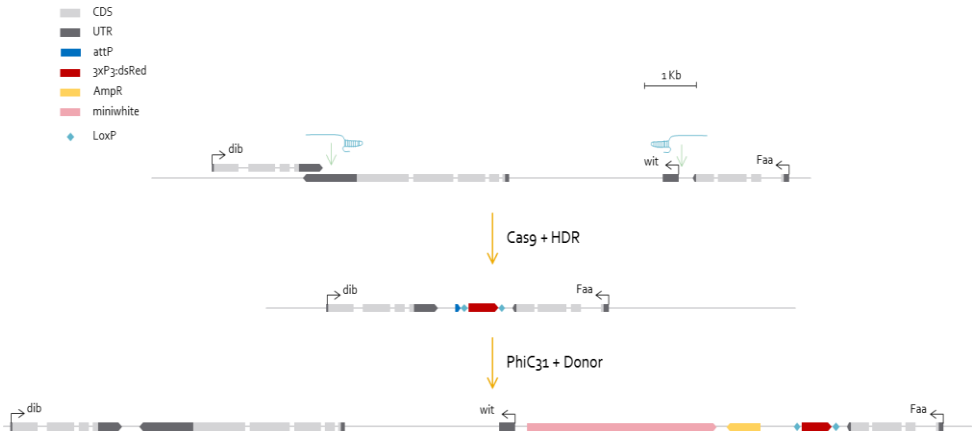

Figure 15: Creation of a CRISPR null allele of WIT. Two guide RNAs cut the wit locus, one within the 3' UTR and the other in the upstream intergenic space. Successful homology directed repair (HDR) is visualized by expression of RFP in the eye. The PhiC31 system is utilized to insert rescue fragments. In this case, a wild type wit is inserted. Insertion is confirmed by expression of red eye color.

We employed the CRISPR system coupled to homology directed repair (HDR). In short, the CRISPR/Cas9 causes a double stranded break, which is to be repaired by the cell. Either a non-templated or templated repair pathway can be utilized. By providing a plasmid with homology to the DNA surrounding the break site, we can coax the cell towards the templated repair pathway (Fig. 15). 27 surviving injected flies were test crossed and 9 lines were recovered, of which 8 were correct.

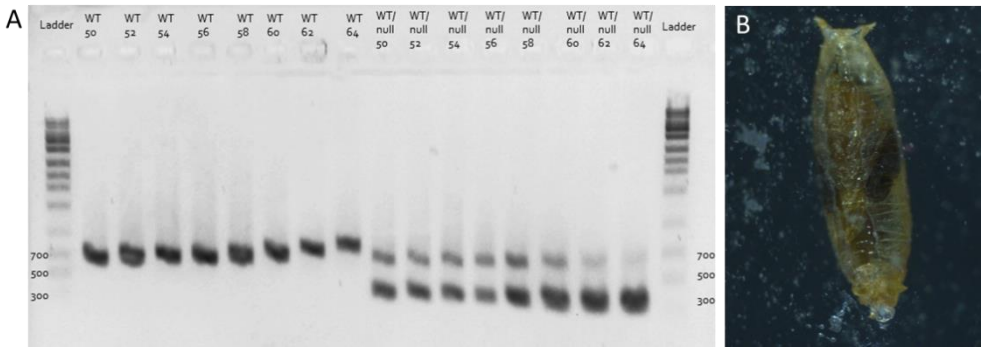

Figure 16: Confirmation of CRISPR HDR. gDNA was extracted from OreR (WT) and a heterozygote for the wit null allele. PCRs were subjected to an array of annealing temperatures and contain three primers in order to amplify either the WT allele (711bp) or null allele (370bp).

The recovered null allele was tested by PCR to confirm homology directed repair (Fig. 16A). We were able to detect both the null allele and the WT allele, since *wit* null is homozygous lethal. Importantly, this allele, like other null alleles of *wit*, when homozygous leads to pharate adults that fail to eclose from the pupal casing (Fig. 16B).

3.10 Rescue by directional insertion with PhiC31 integrase

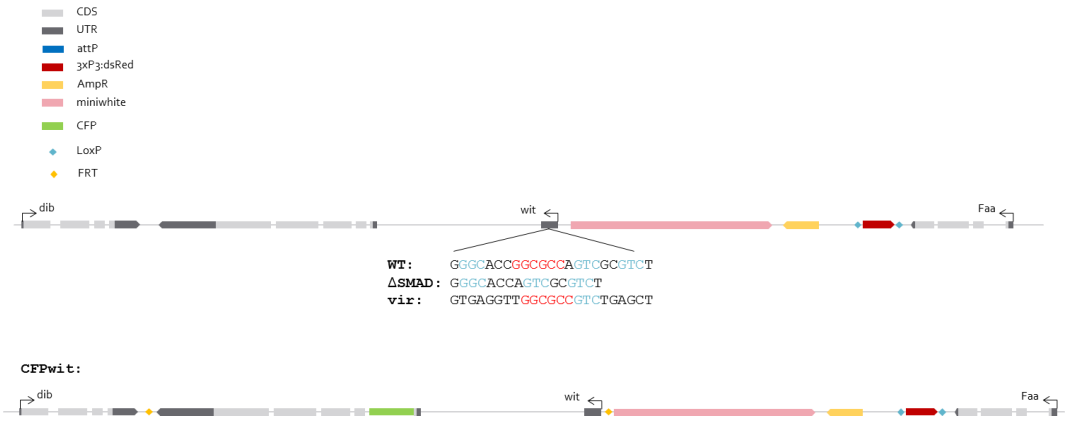

Figure 17: Creation of WIT synthetic alleles. Four separate alleles were generated for *wit* locus rescues. WT contains the WT cluster of SMAD (red) & MED (blue) sites. dSMAD is a removal of the SMAD site. virCRM contains the *D. virilis* arrangement of SMAD/MED sites replacing the WT cluster of SMAD/MED. CFPwit contains an N-terminal fusion of CFP.

The null allele contains a PhiC31 attP landing site (Groth et al., 2004). This allows for the insertion of any cloned allele into this region of the genome. We created several alleles by Gibson assembly (Fig. 17). The manufactured alleles include a wild type rescue, an N-terminal fusion of CFP with WIT, an allele of WIT where the *wit* MAD/MED binding site clusters is replaced by those from *D. virilis*, and an allele that is missing the SMAD binding site from the locus.

Importantly, the WT *wit* allele is able to fully rescue the patterning of WIT (Fig. 18A) and signaling by P-MAD (Fig. 18B). Importantly, this allele is able to be maintained homozygously.

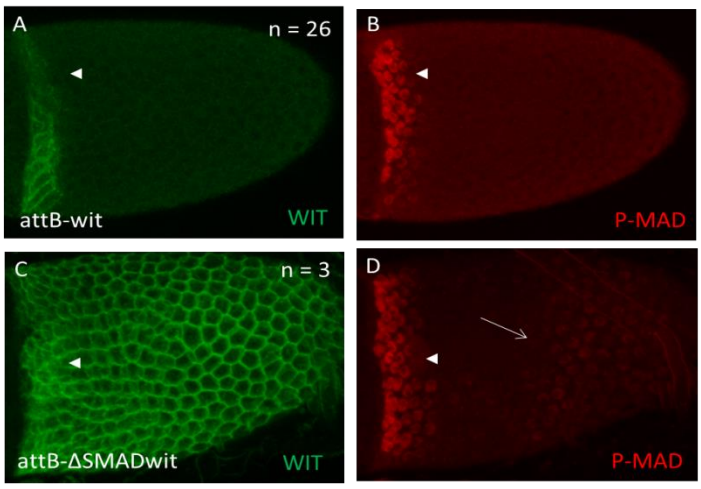

Figure 18: BRK binding site removal causes uniform expression of WIT. The wild type rescue allele is used as a control and has a normal expression pattern (A). BMP signaling is confirmed by P-MAD detection (B). Removal of the BRK binding site leads to uniform expression of WIT (C). Ectopic P-MAD is detected within the follicle cells (D) (arrow). n is number of observations; arrowhead is dorsal.

When the BRK binding site is removed from the locus, WIT is mis-expressed uniformly within the follicle cells (Fig. 18C). Of note is the ectopic P-MAD within the follicular epithelium (Fig. 18D, arrow). Since WIT is constitutively activated, this suggests that this is ligand independent signaling, although less intense than endogenous signaling in the anterior.

We wanted to utilize allele replacement to test the *D. virilis* CRM within the context of the *D. melanogaster* genome. Instead of utilizing the entire enhancer from *D. virilis*, we replaced the MAD/MED cluster in *D. melanogaster* with that of *D. virilis*. Given that the arrangement of SMAD to MED sites is different, we thought that this would contribute to differences in patterning. However, given that removal of MED sites yielded no defect in reporter expression, the *D. virilis* expression might have to do more with yet unidentified sites within the enhancer. Indeed, the *D. virilis* CRM caused no difference in WIT expression (Fig. 19A) or P-MAD differences (Fig. 19B), likely due to its ability to bind Brinker.

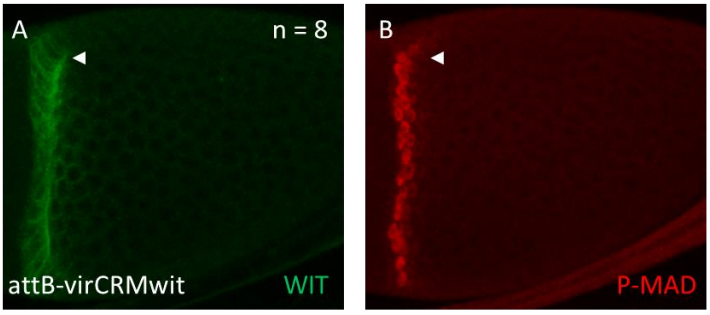

Figure 19: virCRM does not impact the expression pattern of WIT. Replacement of the *D. melanogaster* SMAD/MED arrangement does not alter the expression of WIT (A). Normal P-MAD is detected in these egg chambers (B). n is number of observations; arrowhead is dorsal.

While developing tools for the WIT locus, we wanted to see if we could tag the protein with a flurophore. Green florescent protein is perhaps the best known florescent protein, but we decided to use CFP, which can be useful for colocalization studies with YFP. This involved in silico identification of the signaling polypeptide, so that the CFP would remain in frame with the maturely shuttled protein. We were able to assemble an allele which had no defects in WIT patterning (Fig. 20A) or P-MAD (Fig. 20B). Wonderfully, this is trackable by visualization of CFP (Fig. 20C). Of importance, this

allele is conditional since it is flanked entirely by two FRT sites such that the whole locus can be flipped out using flippase, generating a true null allele.

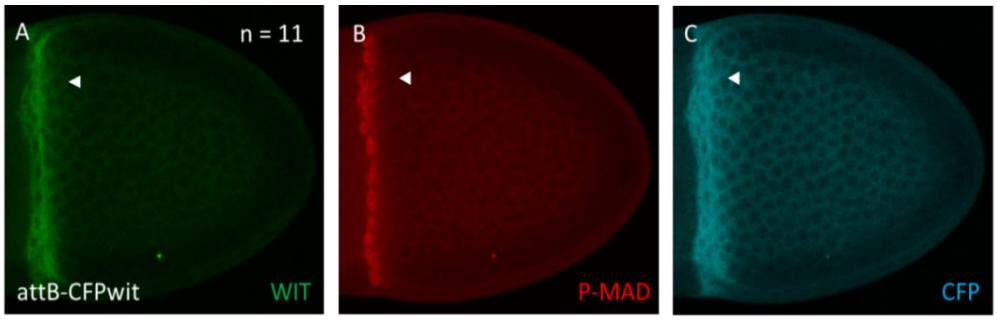

Figure 20: The CFP fusion allele of WIT is traceable and does not alter BMP signaling. WIT protein expression is unchanged by the fusion allele (A). P-MAD is unchanged in this background (B). Protein expression is able to be followed by monitoring of CFP (C). n is number of observations; arrowhead is dorsal.

Currently, we are in the process of generating alleles that allow for the removal of the positive selection markers (Supp. 8). This will allow for these alleles to be as close to WT as possible so that only the specific mutations are being tested.

CHAPTER 4: Chorion Patterning: A window into gene regulation (Niepielko et al., 2014)

M. G. Niepielko*, **R. A. Marmion***, K. Kim, D. Luor, C. Ray, N. Yakoby. Chorion Patterning: A window into gene regulation and *Drosophila* species-relatedness. 2014. Molecular Biology & Evolution. 31 (1):154-164.

Here, we describe a simple system to mathematically describe complex gene expression patterns. Not only can gene patterns estimate evolutionary distance, but we further utilized this description in order to determine the association between new and previously established expression domains.

I worked together with Matt on this project and I created the binary description and conducted the computational analysis and am a first coauthor.

CHAPTER 5: DISCUSSION & FUTURE DIRECTIONS

5.1 Description of gene patterns by binary matrices

We have applied binary matrices as a simplified way to describe complex expression patterns of genes. Amazingly, we found that gene expression patterns can be used to estimate evolutionary distance. Our results are consistent with a previous study that predicted evolutionary distance using nucleotide sequence homology of *thickveins* (*tkv*) (Niepielko et al., 2011). We reason that this approach is a way at looking at regulation absent identifying the specific binding sites. Importantly, as long as basic shapes can be identified, this can easily be applied to other tissues and even new gene expression patterns. In the case of *D. nebulosa*, this study was performed without sequencing data, which stresses that this method does not rely on binding sequences, only their output.

In the case of the chorion genes, genes are expressed in non-conserved domains. This indicates a dramatic shift in gene regulation, which is surprising since the protein sequences are highly conserved (Waring, 2000). Only one domain is conserved between species, the roof domain. The roof domain works to create the top of the dorsal appendages and is conserved among species that bare dorsal appendages (Berg, 2008; Niepielko et al., 2011). This domain is created by a specific set of CP proteins. Other domains largely require a certain number of genes but the particular gene is of little importance.

We demonstrated that gene patterns are comprised of inputs from multiple signaling pathways. In this way, genes incorporate the anterior-posterior axis as well and

the dorsal-ventral axis. In many cases, these inputs can even be separated by genetic or drug perturbation.

A potential follow-up might include trying to incorporate signaling dynamics from multiple signaling pathways. In this way, one might elucidate how the output of several pathways has an effect on a large array of genes. For the remainder of the dissertation work, we focused on a gene that is involved in AP patterning and, in addition, is further regulated by this signaling.

5.2 WIT is required for *Drosophila* oogenesis

We have shown the necessity of WIT during *Drosophila* oogenesis. This requirement is at the level of signaling, patterning, and morphogenesis. Surprisingly, *wit* is regulated by the same pathway that it works to perceive and furthermore and is regulated in a double-negative feedback loop. In neuronal cells, WIT binds the ligand Glass bottom boat (GBB) (Marques et al., 2002; McCabe et al., 2003). In oogenesis, DPP is the ligand that established the anterior to posterior gradient (Deng and Bownes, 1997; Dobens and Raftery, 2000; Peri and Roth, 2000). An *in situ* hybridization of *gbb* found it to be expressed uniformly in all FCs (not shown). In addition, using an RNAi *gbb* produced smaller eggshells with a reduced operculum (not shown). Thus, we hypothesize that DPP might function together with GBB in a heterodimer. This has been described in the embryo for DPP/SCW (Screw) (Eldar et al., 2002). Importantly, the function of the heterodimer is to signal stronger than homodimers and this confers robustness to fluctuations in ligand levels (Shimmi et al., 2005). The interplay between DPP/DPP, DPP/GBB, and GBB/GBB offers a potential follow-up study.

In addition, the most studied type II receptor, by far, is PUT (Letsou et al., 1995; Simin et al., 1998). A role for PUT in BMP signaling during oogenesis was alluded to in a study of BRK. BRK is downstream of EGFR and repressed, in the anterior, by P-MAD. In a clone null for PUT, ectopic BRK was detected. However, the ectopic BRK did not extend all the way to the anterior (Chen and Schupbach, 2006). We reason that WIT and PUT might work cooperatively to repress targets such as BRK, since in both *put* and *wit* clones, P-MAD is lost cell autonomously (not shown). Importantly, *wit* is expressed in many tissues, including the wing and the leg. At the same time, no role was found for WIT within these tissues (Aberle et al., 2002; Marques et al., 2002). This could mean that its role is masked by PUT within other developing tissues.

A similar approach to the CRISPR *wit* allele can easily be applied to *put*. In fact, we have generated the constructs for a potential follow up report and will soon be sent out for injection. PUT was fused to YFP for possible use in FRET experiments, since little is known about type II receptor cooperativity. For the first time, conditional alleles of each type II BMP receptor will be available. There is also potential for study in the role that type II receptors play in ligand selectivity.

5.3 Regulation of wit by BRK

This project began in search of a P-MAD binding site, since *wit* is expressed in the P-MAD domain, and it is regulated by BMP signaling. Along the way, we found that reporter expression can be highly dependent on properties of the reporter. This has been described previously and includes differences due to introns, promoters, and UTRs (Pfeiffer et al., 2010). Response to the BMP gradient is complex, since P-MAD and BRK bind similar motifs (Gao and Laughon, 2006; Kirkpatrick et al., 2001; Winter and

Campbell, 2004). In the case of *wit* regulation, we found negative regulation through BRK. In the embryo, the *pnr* gene response to P-MAD incorporates the gap genes *knirps*, *kruppel*, and *giant* (Liang et al., 2012). Importantly, we have described negative regulation by BRK, but there must be a uniform activator acting to pattern *wit*, since removal of BRK binding results in a uniform activation of the gene. This activator will likely be found within many other follicle cell enhancers of genes that are cared out from a uniform pattern, such as broad and tkv (Yakoby et al., 2008a).

5.4 Species differences of WIT loci

The differences in species specific enhancers for *wit* are not yet completely understood. There is a noticeable difference in width the *D. virilis* enhancer compared to *D. melanogaster*. This enhancer contains a BRK binding site that is identical to the site within the D. melanogaster enhancer, so the difference cannot be explained by weaker repression by BRK. Furthermore, we demonstrate that the differences are likely not due to different arrangement of MED sites proximal to the MAD/BRK site. We reason that perhaps the unidentified activator or activators are in some way stronger within this enhancer, since the wider pattern is produced within the context of *D. melanogaster* signaling. In fact, differences in quality of binding sites have been reported for other transcription factors for controlling different spatial domains (Papatsenko and Levine, 2005). Of note, we have already cloned the entire locus of *D. virilis* and currently we are screening transgenic flies. The observation of the correlation of width of BMP signaling and the size of the operculum of different species has been reported (Niepielko et al., 2011). Rescue by this species specific locus will allow the involvement of the type II receptor, in the process, to be tested directly. It will also allow for separation of enhancer

changes and protein sequence changes. We are also utilizing piggyback transposase in order to examine the *D. melanogaster* and *D. virilis* enhancers within *D. virilis*. These constructs have already been sent out for sequencing.

5.5 Further use for the landing site null allele of *wit*

The null allele allows for precise replacement within the endogenous *wit* locus in *D. melanogaster* utilizing the PhiC31 system (Bischof et al., 2007). Of importance, we have begun working with alleles that allow for the removal of the majority of all of the positive selection markers as well as removal of the sequences necessary for bacterial amplification (Supp. 8).

5.6 Concluding Remarks

The integrative approach for the CCIB allowed this research to take place. Specifically, all projects involved collaboration, either between graduate students or trans-atlantic labs. The *in situ* hybridization work for the CP paper was conducted primarily by undergraduate students from the Department of Biology. Insight into complex biological problems will continue to rely on integrative efforts. Throughout my dissertation, I relied on computational tools, including image analysis and bioinformatics work on genome sequencing data. Most importantly, interdisciplinary approached are essential and the CCIB provided the language for this conversation.

CHAPTER 6: MATERIALS & METHODS

6.1 Genetics

The following stocks were utilized during my dissertation: wild type *D. melanogaster* (OreR), *D. virilis* (a gift from D. Stern), *D. erecta*, *D. willistoni*, and *D. nebulosa* (*Drosophila* Species Stock Center), E4-gal4 (Queenan et al., 1997), UAS-dpp, e22c-Gal4 (Bloominggton Stock Center), and UAS-dad and DadZ (Tsuneizumi et al., 1997). The FLP/FRT mitotic recombination system (Duffy et al., 1998; Xu and Rubin, 1993) was used to generate clones of mutant follicle cells, marked by the absence of GFP. BMP input was analyzed using the Mad12 allele: FRT40A Mad12/FRT40A ubiGFP;GR1-Gal4 UAS-FLP (a gift from R. Padgett). The role of WIT in BMP signaling was conducted with the WitG15 allele: FRT79 WitG15/FRT79 ubiGFP;e22c-Gal4 UAS-FLP (a gift from M. O'Connor). Depletion of *wit* was conducted with an RNAi stock from the TRiP RNAi collection at Harvard, JF01969, and included a UAS-dicer2. Flies were grown on cornmeal agar; all crosses were completed at 22 °C.

Several flies were generated for this study including wit-attP: a knockout allele containing a PhiC31 landing site, FRT_CFPwit: a conditional null allele of an N-terminal tagged WIT, virCRMwit: an allele of *wit* with the *D. virilis* cluster of MAD/MED, and dSMADwit: an allele of *wit* with the SMAD binding site (GGCGCC) removed.

6.2 In situ Hybridization

The expression pattern for *wit* was previously described (Yakoby et al., 2008a). Additional *wit* fragments from various species were amplified from species specific oogenesis cDNA libraries generated with the Stratagene cDNA Synthesis Kit. Forward primer: CAAGTATCCCGCACCACTTT. Reverse primer: CCATCATSCGATCRTCGT

as previously described (Marmion et al., 2013). In situ hybridization was performed as previously described (Wang et al., 2006), but without the RNase digestion step (Yakoby et al., 2008a).

6.3 Immunohistochemistry and Microscopy

Dissection and fixation was conducted as reported elsewhere (Pacquelet and Rorth, 2005). Primary antibodies: mouse anti-Wit (23C7; 1:500, DSHB), rabbit anti-phosphorylated-Smad1/5/8 (1:3000, a generous gift from D. Vasiliauskas, S. Morton, T. Jessell and E. Laufer), mouse anti-Gal4 (1:1000, MP), mouse anti-betagalactosidase (1:1000, Promega), rabbit anti-betagalactosidase (1:1000, Invitrogen) and sheep anti-GFP (1:2000, Biogenesis). Secondary antibodies: Alexa Fluor (1:1000, Molecular Probes), and DAPI (1:10000). Images were captured with a Leica DM3000 Compound Microscope, a Leica SP5 or SP8 Confocal microscope (Leica) . Scanning electron microscopy was conducted with a Leo 1450EP SEM or JEOL Neoscope JCM-6000. Images were processed with ImageJ (Rasband, 1997–2009) and Gimp (GNU Image Manipulation Program, 1995–2008). To evaluate the levels of BR reduction in cells null for *wit*, a line plot of Broad was traced using ImageJ across several wild type cells as well as clones null for *wit*. The average gray value was taken for wild type cells as well as the clones in the same image. Percent reduction for each egg chamber was calculated as 1−(clone/WT).

6.4 Reporter Cloning

All reporter fragments were amplified using Phusion polymerase according to manufactures instructions using specific primers (Table 1). Mutagenic reactions were performed in two halves and a subsequent fusion PCR reaction was performed using

outside primers and templating a dilute first reaction (1:1000). PCR reactions were purified using a DNA clean and concentrate kit (Zymo Research), and subsequently incubated with Taq for A-tailing. Tailed fragments cloned into pCR8 (Invitrogen) as in manufactures instructions with approximately 2fmol in a 3ul cloning reaction. LR clonase II (Invitrogen) was utilized to facilitate movement of the test fragment into pattBGWhZn in a 5ul reaction that contained 10fmol of each vector.

6.5 Computational Analysis and Motif Conservation

MEME was utilized for prediction of a conserved enhancer within the *wit* locus by aligning four sequenced *Drosophilid* (Bailey et al., 2006). Subsequently, RNA-seq data was assembled for *D. virilis* in order to determine the location of the 5' UTR of *wit* (Kim et al., 2013; Trapnell et al., 2010) using data from the Sequence Read Archive (Duff et al., 2015).

6.6 Cloning the *D. virilis* locus and reporter

The entirety of the *D. virilis* wit locus was amplified and cloned using primers vir_locus_F & vir_locus_R and sequenced using sanger sequencing of twelve fragments from each strand. These sequences were assembled and view using phred, phrap, and consed (Gordon and Green, 2013; Machado et al., 2011). Exons were predicted, as well as the amino acid sequence that they encode (Burge and Karlin, 1997). A reporter that included all of the 5' UTR was cloned and screened for Bgal expression in *D. melanogaster* and subsequently split into two 1.7Kb fragments.

6.7 CRISPR and Gibson Assembly of WIT locus

Two CRISPR targets were chosen that cut the *wit* locus within the 3' UTR and in the upstream intergenic region using online prediction software (Housden et al., 2015).

CRISPR chiRNAs were cloned into U6-chiRNA (Gratz et al., 2014). This was conducted by annealing sense and anti-sense unphosphorylated oligos at 10uM in ligation buffer with the following thermocycler settings: 95˚C for 5 min, then ramping to 25˚C at a rate of -0.1˚C/sec. Annealed oligos were diluted (1:100) and 30fmol of each chiRNA were ligated to 10fmol of vector that had been digested with BbsI, excluding the dephosphorylation step in a 10ul reaction (The full sequence of the corresponding primers can be found in Table 1). Similarly, homology arms were amplified such that they were directly adjacent to the CRISPR cut sites. These arms were cloned into pdsRed_attP using cloning sites AarI and SapI in subsequent cloning/transformation reactions. Flies containing a nos:cas9 in the attP40 site of chromosome II were injected with a mixture of all three plasmids by Rainbow Transgenic Flies. Flies positive for repair by homology directed repair (HDR) expressed red glowing eyes. PCR confirmation of a heterozygous knockout was confirmed by mixture of primers wit_crispr_conf_left, wit_crispr_conf_right, and wit_crispr_WT_right (The full sequence of the corresponding primers can be found in Table 1). Separate PCR reactions were used to verify each side of the mutation: left arm by wit_crispr_conf_right and wit_outside_1_for and sequenced with wit_outside_1_for and right arm by wit_crispr_conf2_rev and wit_outside_2_rev and sequenced with wit_outside_2_rev (The full sequence of the corresponding primers can be found in Table 1).

The entire *wit* locus as amplified from gDNA purified by phenyl-chloroform extraction using the primers wit_locus_left & wit_locus_right and cloned into pCR8. An FRT variant of the locus was amplified using the primers wit_locus_L_FRT &

wit_locus_R_FRT (The full sequence of the corresponding primers can be found in Table 1). These flies were subsequently balanced to a fly that contained a nos:phiC31 on the X chromosome.

Gibson assemblies were conducted using New England Biolabs HiFi Assembly Master Mix containing 10fmol of each fragment in a 10ul reaction. Assemblies created a circular product with the vector backbone pSC. pSC is a linear PCR product of the primers pSC_F and pSC_R, using a pSC cloning kit template (Stratagene) (The full sequence of the corresponding primers can be found in Table 1). Assemblies were cloned and screened by restriction fragment mapping in DH10B *E. coli*. Assembly of a *D. melanogaster* wit locus with a substituted *D. virilis* SMAD binding site utilized the primers pSC2vir_wit_L_F, vir_wit_L2R_R, vir_wit_R2L_F, & vir_wit_R2pSC_R (The full sequence of the corresponding primers can be found in Table 1). Assembly of a CFP fusion of WIT utilized the primers wit_locus_left_R, wit_locus_right_F, pSC2vir_wit_L_F, vir_wit_R2pSC_R, wit_CFP_F & wit_CFP_R (The full sequence of the corresponding primers can be found in Table 1). The insertion site of CFP was after the signal polypeptide as predicted computationally (Petersen et al., 2011). A deleted SMAD locus was created utilizing the primers pSC2vir_wit_L_F, vir_wit_R2pSC_R, assembly_delSmad_for & assem_dSmad_rev_sho (The full sequence of the corresponding primers can be found in Table 1).

The donor vector, pBw, was created by modifying pBPGUw (Pfeiffer et al., 2008) to remove the promoter, *gal4*, and terminator sequences. This was accomplished by digestion with FseI and XbaI, followed by ligation of the annealed oligos CCCTAGCCCTGCAGGCT and CTAGAGCCTGGAGGGCTAGGGCCGG. Mutant

loci were subcloned with wit_locus primers including or excluding FRT sites into pCR8 and LR recombined into pBw. These destination vectors were injected into null flies containing nos:phiC31 and positive flies were selected by expression of red pigment in the eye. Maps can be found in the Supplemental Document (Supps. 4-7).

6.8 Matrices and Matrix Analysis

Gene patterns are represented as binary vectors consisting of mutually exclusive domains at four different developmental stages of Drosophila oogenesis (Spradling, 1993; Yakoby et al., 2008a). In the original combinatorial code (Yakoby et al., 2008a), the anterior, dorsal, and midline domains overlap. Here, we modified them to be mutually exclusive. The anterior domain was split into AD and AV domains, and a domain for DR and posterior (P) was added along with repression domains (for the complete details see fig. 2). Representation and manipulation of matrices were conducted with MATLAB (The MathWorks, Natick, MA) and displayed using the imagesc command.

Accumulation of domain usage was summed in excel and displayed as a bar graph for each species and color coded by stage. Pairwise comparisons between domains were calculated in MATLAB as percent co-occurrence between all domain pairs of two species. Co-occurrences is depicted as a numeral and displayed using the imagesc command. The fraction is the co-occurrence value divided by the higher of the two domain utilizations. This fraction is represented by a color scale displayed underneath the co-occurrence value. Hierarchical clustering was conducted (Eisen et al., 1998) on an averaged expression matrix of all three species to determine expression domain relatedness. Bootstrap values were calculated by assembling a an unweighted pair group method (UPGMA) tree in Mega5 (Tamura et al., 2011) with 1,000 bootstrap trees,

representing domain conservation with individual nucleotides. Distance was determined with the Euclidean distance metric and average linkage was used for tree generation. Clustergrams are generated such that genes cluster on one axis and domains cluster on the other.

Table 1. Primers

Name	Sequence	Description
witZ1_forward	AGGCTCACATTCTCCTTCG	wit1
witZ1_reverse_new	TACACCGACTGTCGCAGTTT	
witZ2_forward	GTCCCCATCCTCAGACACAT	wit2
witZ2_reverse	CCCATTTGTTTACTGTGTGGAA	
witZ3_forward	GCGAAGGGAATTTGAATGG	wit3
witZ3_reverse	TGTCTGCTCACAGTACACATGC	
witZ4_forward	AATGGCCCCTTGATCCTACT	wit4
witZ4_reverse	TCCACTCTGCGCAAGTACAC	
witZ5_forward	CCCTTCGCTAGTCAGCGTAT	wit5
witZ5_reverse	CGAGTGTGCAACTTCTTTCG	
witZ6_forward	TGCGAGCAGCACTTACACTT	wit6
witZ6_reverse	GCCTATCGGGCAAACTTGT	
witZ7_forward	GTCTGCTTTGCGCTGCAC	wit7
witZ7_reverse	CCAATGGGAGCATAGCTGAG	
witZ8_forward	CCGACTTTCGAGGAATGAAT	wit8
witZ8_reverse	TTTCGCTCTCTCGCTACGAT	
witZ9_forward	TTTACGAAGCTGCGTGGAAT	wit9
witZ9_reverse	GGCCATCCATAGTAGCGTATACG	
pBac_insert_5	TGGTTTGTCCAAACTCATCAA	
Rubin seq Forward	AAATAGGGGTTCCGCGCACAT	sequence gal4 insertions
Rubin seq Reverse	ATAATGGTGCAGGGCGCTGAC	
attB_for_seq	CGAAGTTATGCTAGCGGATCC	sequence Bgal insertions

Name	Sequence	Description
GW1	GTTGCAACAAATTGATGAGCAATGC	sequence pCR8 insertions
GW2	GTTGCAACAAATTGATGAGCAATTA	
vir_wit_big_forward	GGTCATCATTCGCGGACAC	
vir_wit_small_reverse	ACTCGTCTCGTTTCAAGTGC	
vir_wit_small_split1_reverse	TCGGGATACGATGTCAAAAACA	
vir_wit_small_split2_forward	TTTGACATCGTATCCCGACTC	
wit6_mut_Forward	TTATTGGGGCACCGGatCCAGTCGCGTCTGCT	wont bind Brk or Mad
wit6_mut_Reverse	AGCAGACGCGACTGGatCCGGTGCCCCAATAA	
wit6_mut2_Forward	TTATTGGGGCACCGaCGtCAGTCGCGTCTGCT	wont bind Brk but will mad
wit6_mut2_Reverse	AGCAGACGCGACTGaCGtCGGTGCCCCAATAA	
wit6_mut3_Forward	GGAAAATTTATTATTGGtGaACCGGCGCCAGTCGCG	wont bind Med+3
wit6_mut3_Reverse	CGCGACTGGCGCCGGTtCaCCAATAATAAATTTTCC	
wit6_mut4_Forward	GGCACCGGCGCCAtTaGCGTCTGCTTTGC	wont bind Med+1
wit6_mut4_Reverse	GCAAAGCAGACGCtAaTGGCGCCGGTGCC	
wit6_mut5_Forward	CACCGGCGCCAGTCGCtTaTGCTTTGCGCTGCAC	wont bind Med+6
wit6_mut5_Reverse	GTGCAGCGCAAAGCAtAaGCGACTGGCGCCGGTG	
wit_sense_guide1	cttcGTCTTGGACAAGAGCGAAAC	wit CRIPSR
wit_anti_guide1	aaacGTTTCGCTCTTGTCCAAGAC	
wit_sense_guide2	cttcGCGCTCAGCTATGCTCCCAT	
wit_anti_guide2	aaacATGGGAGCATAGCTGAGCGC	
wit_break1_hom_arm_for	CAAGcacctgcCAAGtcgcGTGCCAGGGATATTCAGAAGTGG	wit DONOR
wit_break1_hom_arm_rev	CAAGcacctgcCAAGctacCATTGGCGCTCTCCGCTC	
wit_break2_hom_arm_for	CAAGgctcttcCtatAACAGGTAGTAGTCTATATAGTTTGTATATGTGC	

Name	Sequence	Description
wit_break2_hom_arm_rev	CAAGgctcttcCgacGTATTATCCCTTGCCAGACCACC	
wit_crispr_conf_left	ATCCCCTTCATTCCCTACTCCCT	confirm CRISPR wit locus
wit_crispr_conf_right	GGGCCGCGACTCTAGATCATAAT	
wit_crispr_WT_right	AAGACACTGAGCTTGTACGACGA	
wit_crispr_conf2_for	GTGCTCAACTCTTTCGCTCTCTCG	
wit_crispr_conf2_rev	TAGCGACGTGTTCACTTTGCTTGT	
wit_inside_for	GGGGAAACGAGTGTGAGGTGGAG	
wit_inside_rev	ATCAATAGACGGGCCACACTCGC	
wit_outside_1_for	AGTGCCAAGGACGAGCTATCCA	
wit_outside_2_rev	CACCAATGTCGGCATTATGTTCC	
wit_locus_left	TGGGTATAAGTAAGTCGCCAGAGC	
wit_locus_right	GAAAAGAACGGACTGCGAATCGG	
wit_locus_L_FRT	GAAGTTCCTATTCtctagaaaGtATAGGAACTTcTGGGTATAAGTAAGTCGCCAGAGC	
wit_locus_R_FRT	GAAGTTCCTATaCtttctagaGAATAGGAACTTcGAAAAGAACGGACTGCGAATCGG	
wit_locus_left_R	AGTGAGTACCTCAAAGTCTTCTTATCTATG	
wit_locus_right_F	TGCTCTGCCCAGCGAGATC	
wit_CFP_F	tcgctgggcagagcaATGGTGAGCAAGGGCGAG	
wit_CFP_R	tttgaggtactcactCTTGTACAGCTCGTCCATGC	
pSC_R	ACAGTGGATATCAAGCTTATCG	
pSC2vir_wit_L_F	agcttgatatccactgtTGGGTATAAGTAAGTCGCC	
vir_wit_L2R_R	caacctcaCCAATAATAAATTTTCCGAC	
vir_wit_R2L_F	ttattattggTGAGGTTGGCGCCGTCTG	

Name	Sequence	Description
vir_wit_R2pSC_R	ctctagaactagtggatGAAAAGAACGGACTGCGAATCG	
pSC_F	ATCCACTAGTTCTAGAGCGG	
assembly_delSmad_for	CGGAAAATTTATTATTGGGGCACCAGTCGCGTCTGCTTTGCG	
assem_dSmad_rev_sho	CCCCAATAATAAATTTTCCG	
vir_locus_F	CCGGTGGAGCAATAAAAATG	
vir_locus_R	AACGTTTCCCTTGGACACAG	

CHAPTER 7: WORKS CITED

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CHAPTER 8: SUPPLEMENTAL MATERIAL

Supp. 1 *D. virilis wit* sequence

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   1 AAACTACATC CGCGGTCCTT TTAAAACTCA GCTCGCAATC AACAAGTGAT TAAAGCTCTC
  61 TGCCGCTCTC GCTTTTTTTG CTCTCTTTAC GCTGTCGCTC TCTGAGAGCG CCAGTCGGTG
 121 TCTGTGCCTT TACTCGCTGC TTCGTAAATA TGCGCATGCT ACTGCCGCCG CCGGCGTCGA
 181 CGACGCTGCT GGCGCCTAAG TCGCAGTCAG TTAGTCAGTT CTCAGCGAGC GACGACGAAG
 241 GCGGCGTCCA ACGGGCAGCA GAAATTTTTA AAATCGTCGC GCAGCAAACA AAATTGTAAT
 301 ATAAAATAAA GAGGGGAGAG TAAAATTCCT TGAAAAAAAT AGGCAAAAAA AAACAAAAGA
 361 AAACATAATA TAACAACAGA AAAGCGTCTG GCGCCGGCCG CAATCTTAAA TGACAACAGT
 421 GCTGTTTTTT GTTTAAATAA ACGCAAAGGC ATTACAAACA ACAATTTTTA AAAAGTAAAT
 481 AATTTGCATT AAAGCAGTGC AGGCATGCCC TAAAAAGCCG TTAAGAGTGT AAGAAAAACT
 541 AAAAAGTTTT GTAAATCCAT TCGCAATTTA AAAAATCGAC ACAAACGCGA CAAGTTTGGC
 601 CAGTTTTTTT TTGTTCAACT TAACGATATT ATTTTCCATA TTTTATTTGC GGCAATAATT
 661 CGCAATGACT TGGGAAAAAT CAATTTTGTA GCAGGTCTGC GCACGCACTC ACAGACACAC
 721 ACACACACGC ACGCGCACAC ATACTCGCGC ACACATGTAT GACTGCTGAG CTTGGCGCCC
 781 ACCCACCCTG GTGAACACAA AACCAGACTC AGGACCCTTT AAAATACCGA ACAATCTGCA
 841 TCTTTGTCGT TTGCTTGCGC TGTTGTGTTT GTGCTTGCAT TGATTTTCTG CATACACACA
 901 TCGTCAACGA CTCTGCCGCA GATGCACTGC AGAGAGTACA CACACACACA AACATGCACT
 961 GAGACAAAAC ACACTCTCAG CATGCAAAAA GTTAATCAGC AAACTGCACA GTCCCTCAAC
1021 AACAACAACA ACAACAACAA CGAGTAAAAA GATACGCAAC AGCAAATGGC TATAAAAAAA
1081 AAGTTTGCAA TTAACTGGCG CCGTCGGCTG TCATGCTGGA ACAGACACCC CCCCTTGCTC
1141 CTCGGCGCTC CCCCCCTATA CACCTTAGGG AACCTGTTTT GGCGCGCCAG TTGCGCTTCT
1201 CGTTTTCCTA TGCCATGCAG CGTGCGCATG TGCCAGCCAG AGCTCCTGAG TGCCATGTCT
1261 AGACTCTGGA GTGGCCGGCT CTCCCGCTCG CTCTCTTTCA CGTTCTCTTG GGGAGCACGC
1321 GTCGGCGTCC ACTCTCATTG GCGATAACGT GCGAAAACAA AGCATAATGC AAAAGGCTGC
1381 ACAATTAATT TCCATGTTTA TTTTTATAAC ATCCATAGAC CGTTGCGTTG TCAACGTGTG
1441 CATGGATAGC AAATGATGAG CAATAAATGC ATATAATTTT TTAAAAGCAA TGATAAAATA
1501 GATTTTCGTA TCACCTTGCC AAAAATTATA CATACATATG TACATATGCA TATCCCAAGA
1561 TCAAGTAAAA ATAAAACTGG ACCTAACTGA CATTTCCGGT TATTTAACCA AACAAAAATA
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1621 ATATTTCTTT GGGCTATCTA TTATAAGAGG TTTCCCATTT TTAACTTAAA GATTTGTTTT

1681 TGACATCGTA TCCCGACTCG GGCAATAGCT CGACCATCTC TAAATTTCTG CCAATTCTTA

1741 TCAACAATAA GTCATAAATT CAGAGACAAC GCTTTCTGTT TTATTCTTAA AGCTCAGACG

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2221 TACATACATA CATGATGTGT ATTCATACAG AGAGAAAAAA TAACGTACTT GATTCAATAT

2281 TAGAACTAGC TAAGAACCTC CATAAATAAA AAAAAGAAGA GTTAACTGAT AATAGCCGCC

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2581 GGCAACTTAA AAATGATATA AAATAACTGG GTAGGTTTTG TTATAGTATC TAATAATATT

2641 TCATCCCTTA GATTTCTTAC CCAATTTCTA CCTACATTGA AAAAAAAAAC GTTTCATTGA

2701 AGAAAGAGCC TTTTTTTATA GCCCAAAATA TTCATAAATA TCGGACTAGA AGCCTCGGAA

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2881 GAACGCCCCC CATTACAGCG CCAGCTCCAT TTTCAGAAAA GTGGGTGGGC GACAGACCGA

2941 CAGAAAGACA AACTGTCTGA TAAAGAAGCG CCAAGCACAA CAACAACGGC AACGACAACG

3001 ACAACTATAA CGACAATGTT GTTGGCGATG ATCAGCAACT GCGAGTGTTG CTCCTCGGCC

3061 AAGTTGATTG AGTATATTTA AGTATTTGGG TCTCGGGGTT ATCGTTGCTG TTGCTGGCGT

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3841 AGACATCGAA CGGCACCCGA TTGGTTAAGC AGGGTAGGAA AATGGATCTG AATATTTCTA

3901 CACGATGCTC ATGTCTTTCG ATTTAGGCTG CTGGAAGGAC ACGACGGATC GCAATTCCAT

3961 TTGCAGTCAA ACGGAGTGTA CCAGCTCAGC GCCCACGTCA CGCAACAATA GCCTGTACTA

4021 TTGTTGCTGC TCGGGGGAAC TGTGCAATGC TCAGGTGTCC ATTGTGGAGC CGGCGCCACT

4081 GGAGCTGGCC AACAATGTGC AATCGGCCGT GTCCAATCGG GCTGCATCCC ATCAGCAAAA

4141 CTCTGTCAGA GCCACAACGC TGCTCAGCTG TGTTGGACTC CTAACGCTCA TCATCATTGG

4201 CATGCTGTTG GCGATGCAAT ATTGTCGTGG CGTGAAGGAG AAAAGTGAGC CAGAAGAGTC

4261 GCCGCTGGCA CCGTCGGGAC CCGGCTACAG TTCCAATCTG CGCAATGTAG ATAATATGAA

4321 TTTGATCGGC ATGCTGGGCA GCGGCAAGTA TGGCACTGTG ATGAAGGGAC TGCTGCATGA

4381 ACAGGAAGTG GCTGTTAAGA TCTATCCAGA AGCCCATCAC CAGTATTATG TGAACGAAAG

4441 GAACATCTAT GCATTGCCAT TGATGGACTG TCCGGCTCTG CTCAGCTACT TTGGTTGGTC

4501 TGCTCGCGAC TTTTACTCTT ATAACAATAA TTGAATATAT ATATTCTCTT GAGCAGGCTA

4561 CGATGAACGC CGCACCATGG ATGGTCGCAT GGAGTATCAG TTGGTTTTAT CCTTGGCACC

4621 GTTGGGCTGC CTTCAGGATT GGCTAATTGC CAATAGCACA GACTTTGCCC AGTGTTGTGG

4681 CATGTTGCGC TCCATTACGC GTGGCCTATC CCATTTGCAC ACGGAACTGC GCTTGGGTGA

4741 TCTGCACAGG CCATGCGTGG CCCATCGGGA TCTCAATTCG CGCAATGTTC TGGTTCAGGC

4801 AGATCTCAGC TGTTGCATCG CAGACTTTGG ATTTGCACTG AAGGTATTCG GTTCAAAGTA

4861 TGAGTACAAG GGTGAGGTGG CCATGGCCGA GACCAAGAGC ATCAATGAGG TAGGCACGCT

4921 GCGTTACATG GCTCCGGAGC TGCTCGAGGG TGCCGTCAAT TTGCGGGACT GTGAGACATC

4981 GCTCAAACAA ATGGATGTGT ATGCACTGGG CCTGGTGCTG TGGGAGGTGG CCACACGCTG

5041 CTCGGAGTTC TATGCGCCTG GCCAGCTGAC GCCGCCCTAT AAGGCGCCCT ACGAACAGGA

5101 AGTGGGTCCG CATCCCAGTT TCGATCAGAT GCAGGCATTG GTGGTGCGTC ACAAGGCGCG

5161 CCCTCTGTTC CCAGCGGGCT GGGGAGGGGG CGCCGCCGCC AAGCTTGTGC GCGATACCTG

5221 CGAGGATTGT TGGGATCACG ATGCAGATGC CAGGCTAACT TCACTTTGTG CCGAAGAGCG

5281 CATGCAGGAG ATGTCTAGCC TGCGTCCACG GCTGCAGGCT CAGCCGGCTA GTCCGCTGCT

5341 TAATACCAAC AACCTGGCGT TGCCTGCGGC TCAGCTGCAA GTGAGCACGA TCAGCAACAC

5401 CACAACCACT GCGGCTGCAG TGCATCAGGC ACAAACGCAG CTAACGTCAG CCGACGGTGG

5461 TTTGCTGCAG CCGCCACCGA ATCAACAGTT TCCCACAGAA AAGAACCACT TAAACTATGC

5521 ACAGCCCCAG CTGCAGCCAC ATCTGCAGGG TCGCAATCCC TGTCAGGAGC GCAATCTGGC

5581 GCCGCAGCCG TTGCGTACGC CGCCTGTTCT GGTAGAGCGC AGTAAGAAGC ACAGCTTCCA

5641 GCCGCAGCAG GAGCAGAGTC TGTCCTGCCT GGAGCATGAT GTGAGCGTGG AAGAGCTCAT

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5761 CACGGATCAT ATGCTGCGCG GCTGGCATGG CGTGCGCGCC TTGATTCACA AGAAACTCTT

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5881 TCTTGTGGCT GCGCTAAAGG GCATGGACAA TGCACATTTG GCGAGCGGAC TGAGGCGGCC

5941 CAACAACCTG GACTTGAGTC CCTTGCCGCC ACTGGACAGA GCCAATCTGC TGCAGCTGCG

6001 CAGCGCGGAG CAGCGTACTG GCACCCCCGC TCACATTGTG CCCCGCTCGT TGTCCAGCAG

6061 CCTAATCAAA CACATTAACA GCAACGGCAA CAACAACAAC AACAACATCA ACGAGAACGA

6121 GCTGCACACA TTGTCTGGCT CCCGGGCTCC AAAGCGGCGT CCCGGACATC TGCGCACAAA

6181 TTCGCTGCTG GTGACCAGCA ACAGTGGCAG CAATATGCCG CCGCCCACGG AGCAGCAGCT

6241 GCGCCGCCAG CACAGTCTAG AAGTCTTTCG TGAGGTATTT AGTGGGCGTG GAAGCAGCGA

6301 ACGACTGCGC GATCCCAGCG AACGGGTGAA AACGCCCGGC GATGTGCCGG CATCGGTGCG

6361 CAAGGCGCGC GCCTCAAAGA CCCTGAGTCT GTACGATGAT CGCATGATGG ACTCATCGCT

6421 GCTGAATATA CTCTAGCATG AGGAGGTTCT GCTGCTGGAG CTATAAAGCG CGGCAGCGGA

6481 ACTGTGCAGT TAACTCTCAT ATCTCAACTT TCATCAGACA CAGAACATGC AACATATTCA

6541 ACGATAGCCA TACGAGTAGT TGATAGGACC CTCGACCTGA TACTGAAATC CGATCTTGAG

6601 AAAACGTATA TATATATATA GTATTTTTTC AAAAGCAAGA TCCGTAAGTG TTCCCGCAGC

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6721 CGTTTAGTGC GGCAAACAAC CAAAGTCCTT ATAGGTTAGA ACAGGATACA ACAGAATAAT

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6901 TTAGGTTCTC AATAGAGTTG AGCTTACAAG GCATAACTGT ACCTCGGTTA GGTTTACAGA

6961 CGGTTTAGGC TCAGAAGCAG AATGGGGCTT GTAGTGGGCC AACAGGTTAG GAACTTTTTA

7021 AAACCATATA CACACACAAC ATAATGCATA TAACTATAAA AAAAAAACAT ATAGATATTA

7081 CACAATTATA TAAATAAGCT AGCGACTGGG GCTGTCACTG CAACAGAAAT TCACGAAAAA

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7201 TGTGGCCCTT ACATACAGTG ATAGCCCTGT GGCCAGAGCG CGCACACACA CACACACATA

7261 CACATGCATA CAAACACCTA CTTTCGAGTA TTATGCGAAA CCATTCTAAA GCAATTTTGG

7321 CACCATATAA AGAATTTCAT GCGTTTGCCT TTCTATTGTA TTTTATCAAA CATGGAGATC

7381 TTTTTTGTAG TACTTGTAGA CCAAAACACC CGCAGCCTTT CAAATATAAC TAATAAAATA

7441 TATTAAAAAA AAAAAATCGC TAGTCATATG ATTTACACTA ATTATATATT TCCTAAATAA

7501 AAGTTTATTT AAACTTCGTT TTTTAAGGTA AATAAGCAAT GTACTAAACG AATAGCCACT

7561 CGGTGCTTCG GCTTTGAATT GCTAATCACG CGGAACGCGA TCTAAGCTCG ATCAGCACCG

7621 GCGCATTCGG TTTATTGATC AGCAGAGTGA CGATATCCAA GTCAACATCC GTCCGGCCGC

7681 GCCAAATGAG CTCGTAGTTG CGCAGCAGCT GTGTCCAAGG GAAACGTT

Supp. 2 *D. virilis wit* predicted cDNA sequence

```
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Supp. 3 *D. virilis wit* predicted AA sequence

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 241 GSGKYGTVMK GLLHEQEVAV KIYPEAHHQY YVNERNIYAL PLMDCPALLS YFGYDERRTM
 301 DGRMEYQLVL SLAPLGCLQD WLIANSTDFA QCCGMLRSIT RGLSHLHTEL RLGDLHRPCV
 361 AHRDLNSRNV LVQADLSCCI ADFGFALKVF GSKYEYKGEV AMAETKSINE VGTLRYMAPE
 421 LLEGAVNLRD CETSLKQMDV YALGLVLWEV ATRCSEFYAP GQLTPPYKAP YEQEVGPHPS
 481 FDQMQALVVR HKARPLFPAG WGGGAAAKLV RDTCEDCWDH DADARLTSLC AEERMQEMSS
 541 LRPRLQAQPA SPLLNTNNLA LPAAQLQVST ISNTTTTAAA VHQAQTQLTS ADGGLLQPPP
 601 NQQFPTEKNH LNYAQPQLQP HLQGRNPCQE RNLAPQPLRT PPVLVERSKK HSFQPQQEQS
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 721 ELCRQLQLGE EKSNLVAALK GMDNAHLASG LRRPNNLDLS PLPPLDRANL LQLRSAEQRT
 781 GTPAHIVPRS LSSSLIKHIN SNGNNNNNNI NENELHTLSG SRAPKRRPGH LRTNSLLVTS
 841 NSGSNMPPPT EQQLRRQHSL EVFREVFSGR GSSERLRDPS ERVKTPGDVP ASVRKARASK
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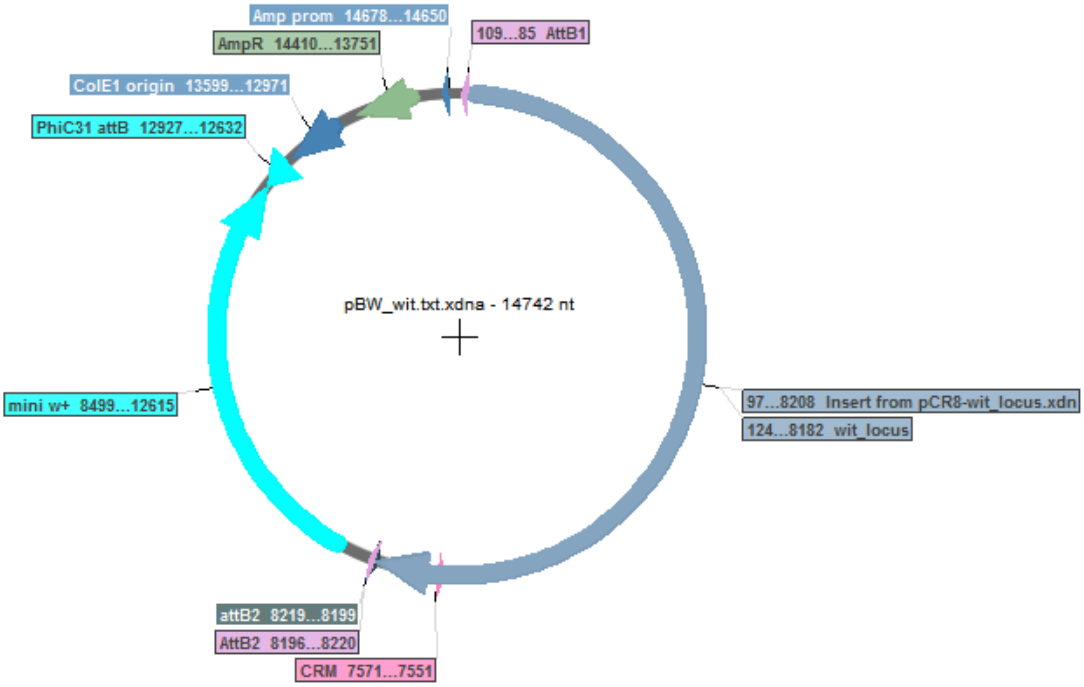
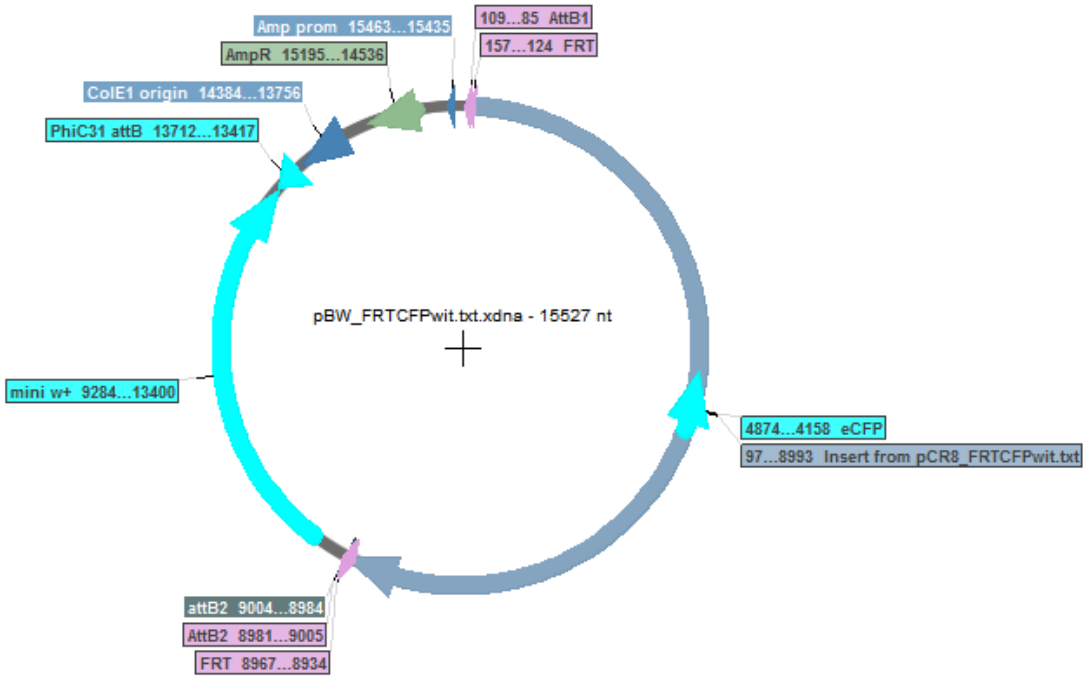
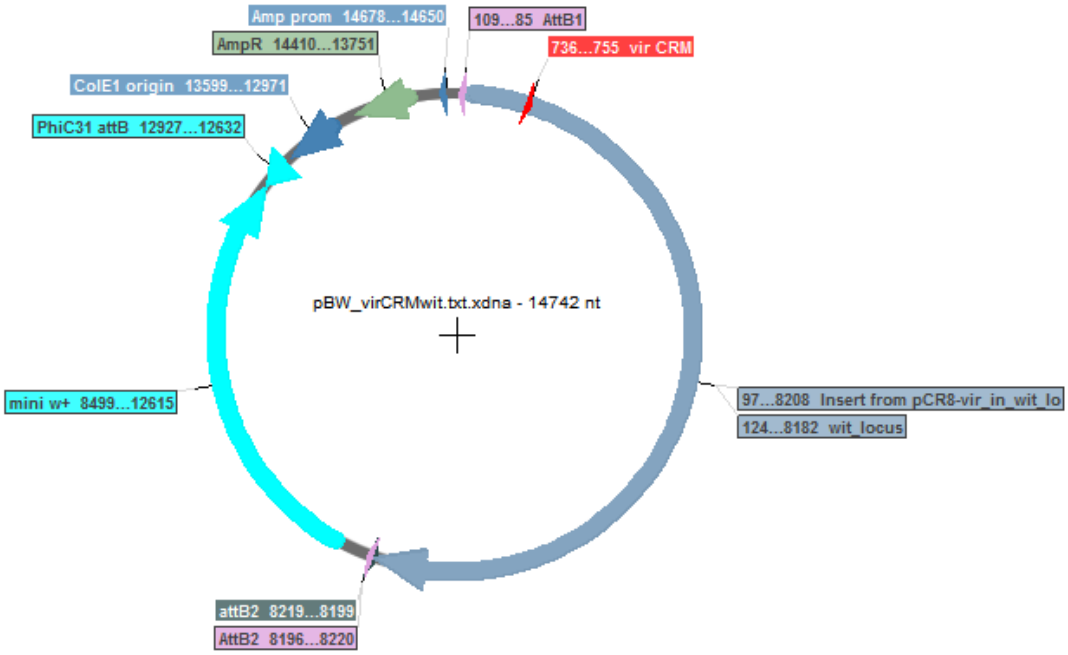
Supp. 4 pBW_wit plasmid map



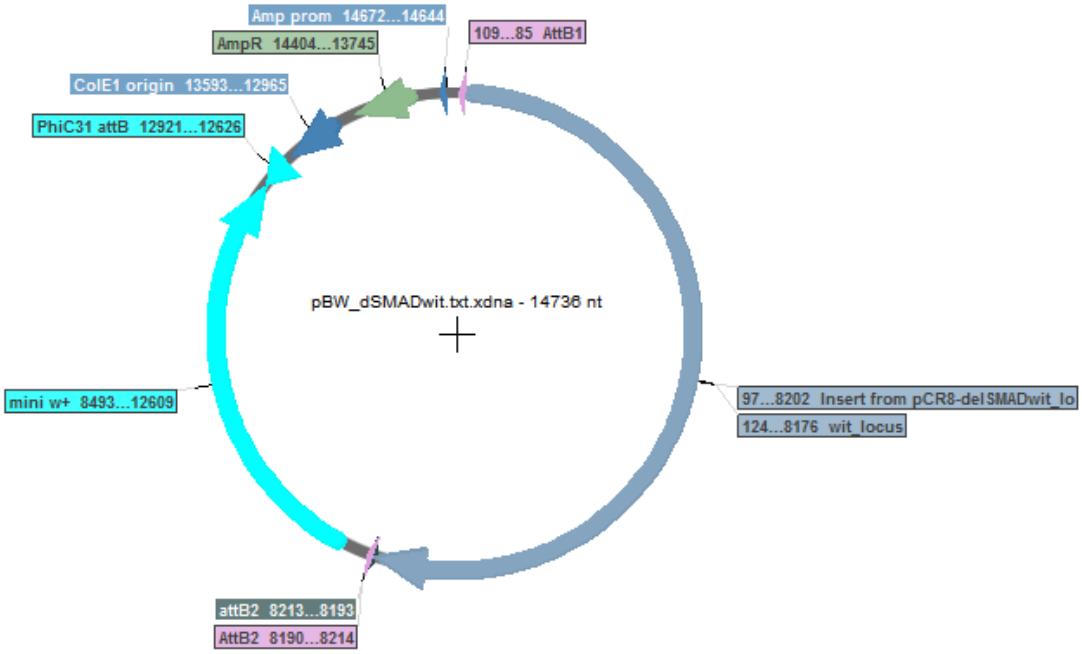
Supp. 5 pBW_FRT_CFPwit plasmid map



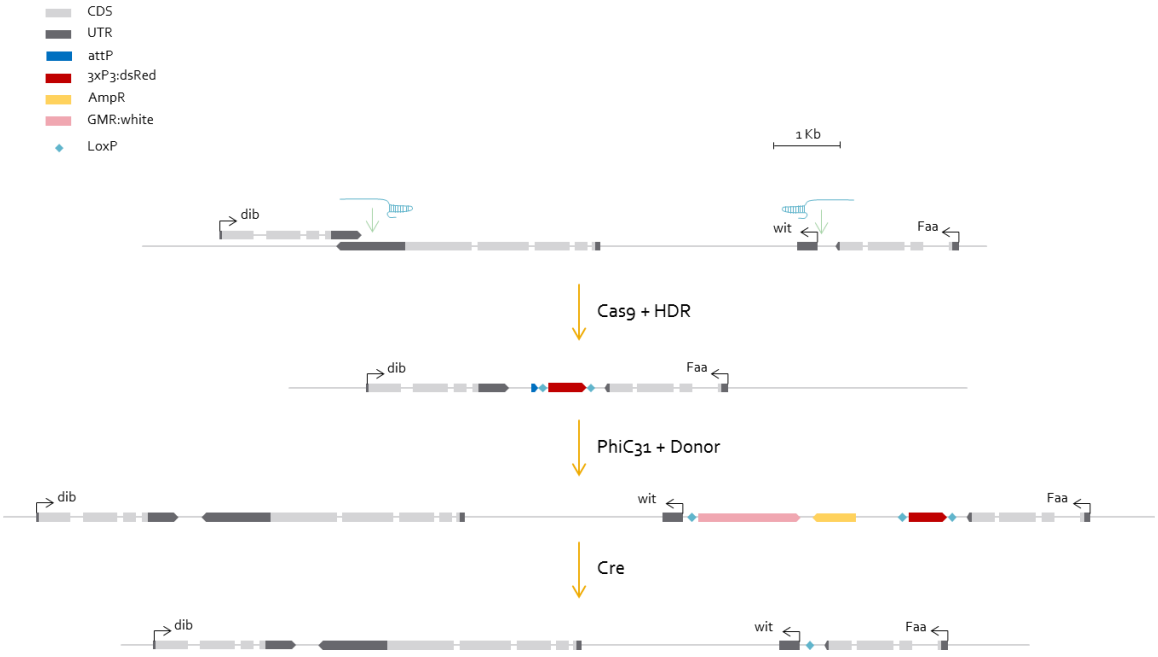
Supp. 6 virSMAD_wit plasmid map



Supp. 7 delSMAD_wit plasmid map



Supp. 8 New rescue alleles have removable markers



Supp. 9 CFP:WIT AA sequence

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  61 TLKFICTTGK LPVPWPTLVT TLTWGVQCFS RYPDHMKQHD FFKSAMPEGY VQERTIFFKD
 121 DGNYKTRAEV KFEGDTLVNR IELKGIDFKE DGNILGHKLE YNYISHNVYI TADKQKNGIK
 181 ANFKIRHNIE DGSVQLADHY QQNTPIGDGP VLLPDNHYLS TQSALSKDPN EKRDHMVLLE
 241 FVTAAGITLG MDELYKTPVP NRQYSCMSYQ EDDNSFHDDD GDQDSSGELQ EQQVESTPIP
 301 SEPHRRTCPD GYTFCFTIWN QTANGARVVK QGCWKDNTDR TSICSQSECT SSAPTSKTSS
 361 LYYCCCSGGV CNAQYSVVEP APLELGSNEG RTSITNRATE KQHQSFLAST MLGLAGGLTA
 421 LTIGIFLAVQ YCRTAKEKPE PEESPLAPSG PGYSSNLRNV DNMNLIGMLG SGKYGTVMKG
 481 LLHDQEVAVK IYPEEHHQYY VNERNIYALP LMECPALLSY FGYDERCTMD GRMEYQLVLS
 541 LAPLGCLQDW LIANTLTFSE CCGMLRSITR GISHLHTELR LGDQHKPCVA HRDINTRNVL
 601 VQADLSCCIA DFGFALKVFG SKYEYKGEVA MAETKSINEV GTLRYMAPEL LEGAVNLRDC
 661 ETSLKQMDVY ALGLVLWEVA TRCSDFYAPG QATPPYKAPY EQEVGSHPSF DQMQALVVRH
 721 KARPLFPTGW GGGAAAKVVR DTCEDCWDHD ADARLTSLCA EERMQEMSTL RPRAQAQPSS
 781 PLLNTNNLVA SPTAEQGINI IATTTTAAAV HHQMSSDTTG LIQPPPNQQI PLAALEREKN
 841 HLSYPQQQLQ PYQGRNPCQE RNLAPLTMRT PPVLVERSKK HSFQTQPQEN SLSCLEHDVS
 901 VEELIASHQH QQQKNTIVST GGNGNSCLGQ GFPKQQNTDQ KLRGWHGVRA LIHKKLFRKE
 961 HAEELCRQLQ LGEEKSNLVT ALRRPNNLDL NPRLDKPPPD QLRSAEQRMG TPAHIVPRSL
1021 SSSLIKHING TTNNNSIQSH GSELQTLTRP ASKRRPGHLR TNSLMATTGQ GPPTEQQMRR
1081 QHSLEVFREV FSGRGSSERL RDPSERVKTP GDVPPSVRKA RASKTLSLYD DRMMDSSLLN
1141 IL
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RED - Signal polypeptide

CYAN - CFP

CHAPTER 9: CURRICULUM VITAE

ROBERT MARMION
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Education

- 2016 Ph.D., Rutgers University, New Jersey
- 2010 M.S., Rutgers University, New Jersey
- 2008 B.A., Rutgers University, New Jersey

Professional Experience

- 2010-present: Research Assistant, Center for Computational and Integrative Biology, Biology Department, Rutgers University
- 2008-2009: Teacher's Assistant, Biology Department, Rutgers University
- 2010-present: Graduate Research Assistant, CCIB, Rutgers University

Publications

1) **R.A. Marmion**, M. Jevtic, A. Springhorn, G. Pyrowolakis, and N. Yakoby. The *Drosophila* BMPRII, Wishful thinking, is required for eggshell patterning. 2013. Developmental Biology. 375:45-53.

2) M. G. Niepielko*, **R. A. Marmion***, K. Kim, D. Luor, C. Ray, N. Yakoby. Chorion Patterning: A window into gene regulation and *Drosophila* species-relatedness. 2014. Molecular Biology & Evolution. 31 (1):154-164.

* Contributed equally

Presentations

Oral presentation:

56th Annual Drosophila Research Conference. March 4 - 8, 2015. Chicago, IL. **Changes in a P-MAD binding site underlie species diversity of *wishful thinking* patterning.** (selected talk)

Poster presentation:

51st Annual Drosophila Research Conference. April 7-11, 2010. Washington, DC. **Positive feedback regulation of WIT controls BMP signaling in *Drosophila* oogenesis.** (poster)

52nd Annual Drosophila Research Conference. March 30-April 3, 2011. San Diego, CA. **Wishful thinking regulates BMP signaling in the *Drosophila* follicular epithelium.** (poster)

2011 Mid-Atlantic SDB Meeting. June 3-5, 2011. Philadelphia, PA. **BMPR2, Wishful thinking: regulation of signaling through positive feedback.** (poster)

53rd Annual Drosophila Research Conference. March 7-11, 2012. Chicago, IL. **Eggshell patterning by Wishful thinking: signaling with positive feedback.** (poster)

54th Annual Drosophila Research Conference. April 3-7, 2013. Washington, DC. **The Drosophila BMPRII, Wishful thinking, is required for eggshell patterning.** (poster)

2015 Mid-Atlantic SDB Meeting. March 27-28, 2015. Princeton, NJ. **Interspecies diversity of wishful thinking patterning relies on changes in a P-MAD binding site.** (poster)

Awards:

2009: Appreciation for **Mentoring of Undergraduate Research**

2010-2011: **Research Assistant**, Supported by the Center for Computational and Integrative Biology (CCIB), Rutgers University

2011-2012: **Research Assistant**, Supported by the CCIB, Rutgers University

2012-2013: **Research Assistant**, Supported by the Yakoby Lab, CCIB and Biology Department, Rutgers University

2012: **Graduate Student Travel Fund**

2013-2014: **Research Assistant**, Supported by the Yakoby Lab, CCIB and Biology Department, Rutgers University

2014: **Best Student Paper Award**, 1st Place

2014-2015: **Research Assistant**, Supported by the Yakoby Lab, CCIB and Biology Department, Rutgers University

2015: **Graduate Student Travel Fund**

2015-2016: **Research Assistant**, Supported by the Yakoby Lab, CCIB and Biology Department, Rutgers University

2015: **Graduate Student Research Fund**

2015: **University Presidential Fellowship**