I. Specific Aim

Through the use of reverse genetics, “methods of disrupting gene function when only the sequence and position in the genome are known,” it is possible to decipher the function of newly discovered genes (Adams, 189). This model, when applied to several stocks of flies carrying P-element mutations in minimally scrutinized genes will accomplish four goals: 1) creating small and large P-element mutated *Drosophila melanogaster* clones capable of mitotic recombination localized in the eye 2) observing and recording phenotypes produced by such mitotic recombination and 3) linking these observable phenotypes to the function of the mutated gene within the eye.

Depending on the function of the disrupted gene, homozygous lethal fly ommatidia will display different phenotypes. A crucial piece of information about these mutations has previously been determined; all mutations covered in this study are homozygous recessive organismic lethal. Flies carrying two mutated alleles for the particular gene of interest ultimately die and cannot be studied, necessitating the use of the FRT flip mitotic recombination system localized in a non-essential area of the fly (the eye). However, the nature of this lethality has heretofore escaped exploration. This study will determine whether each mutation can be characterized as cell lethal, having a role in eye development, or having no role in eye development. Mutations having a role in eye development will be examined in great detail to determine precisely the mutant gene’s normal function in this process. The results of this assessment will then lend a hand in decoding the role of the gene in which the mutation occurred.

The phenotypic information gleaned from observing mutant stocks will be compared to rudimentary gene function information available from Fly Base and BLAST searches to clarify the precise role of the gene. Some of these genes may have functions linked to diseases and disorders found in humans. BLAST searches may be performed to search for homologous sequences in the human genome for later experimentation.

Additionally, creating stocks of P-element mutated flies is crucial to the development of further experiments involving the mutated gene. Once the genes have been linked to a specific function, further studies may proceed using these balanced stocks.

II. Background

Simplicity is the gateway to understanding. As far as functional genomic research goes, *Drosophila melanogaster* seems to fit this statement like a key in a lock. Labeled a premiere model invertebrate system by the National Institutes of Health (NIH), fruit flies fit the bill with their simple genome, easy of care, and spectacular reproductive capabilities. With only four pairs of chromosomes, the drosophila genome constitutes a tangible, manipulatable entity. The flies are small; approximately 100 individuals can fit in a vial while 600 flies can survive in a stock bottle. Typical *Drosophila* life cycles last approximately 10 days when progeny are incubated at 25°C. Each female can produce thousands of eggs with a single mating, allowing propagation of a desired trait to several flies in a brief time period. Distinct traits and a lack of meiotic crossing over in male gametes further facilitate genetic research.

Gene regulation is responsible for great complexity of life without onerous maintenance of multitudinous genes. By manipulating this regulation, deviations from
natural expressivity patterns may arise which are often useful in the laboratory. Determining the function of a gene can be carried out through employing reverse genetics, which involves disrupting gene function and observing the effect of its absence to derive function. Introducing mutations can be accomplished through exposure to chemical mutagens, such as ethyl methanesulphonate (EMS), or exposure to x-ray radiation. However, these methods are not only dangerous, but also low in efficiency and difficult to track in the genome.

An inordinately safer and efficient way of introducing mutations involves the P-element, a transposable sequence, or “jumping gene,” existing naturally in the genome. The P-element consists of two P ends flanking a transposase gene. This gene codes for an enzyme that both cleaves the gene from the P ends and aids in inserting the sequence into another set of P ends. Mutations arise when a P-element exists within a sequence necessary for the expression of a gene. When the transposase gene is supplied outside of the P ends, the enzyme may insert alternative sequences, such as P{PZ} (rosy) and P{LacW} (mini-white), between the P ends (Adams, 191). Removal of the transposase gene by performing a cross permanently abandons the new gene within the P ends and normal transcription of the flanking gene is disrupted.

Even after creating a mutation in a drosophila gene, if the mutant allele is recessive it cannot be studied until a homozygous mutant is created. Unfortunately, if heterozygous flies are crossed and the mutant allele is recessive for lethality, all homozygous mutants would perish. However, if homozygous mutant cells arose in a non-essential structure, such as the eye, a phenotype could be observed in viable adults. Mitotic recombination, a process involving the yeast flip gene (flp) and flippase responsive target sequences (FRTs), can be employed to create patches of homozygous mutant cells during mitosis rather than just during meiosis. When flp presides in the genome, its enzymatic product, flippase, binds to FRT sites catalyzing a recombination event between the two locations. Each homologous chromosome must contain an FRT site; pieces of the chromosome can only move to the extent that they have a place to go. An additional problem concerns generating mitotic recombination only within the eye. When coupled with the eyeless (ey) enhancer, flp will only be transcribed during early eye development, localizing mitotic recombination within the organ (Tapon, 345).

Mitotic recombination results in three types of daughter cells: parental heterozygous, recombinant wild type, and recombinant mutants (see fig. 1). Depending on the nature of the mutation, the eye will display multifarious phenotypes. Color markers such as ry or w+, allow particular phenotypes to visibly reflect genotype. For example, if the mini-white marker is inserted in the P-element to create a lethal mutation (l) in a white-eyed fly background, recombinant mutant cells will display a deep orange-red hue. Parental cells, those with one copy of the mutant allele and thus one copy of w+ will appear orange. Recombinants receiving two normal alleles will show the white background color. Color combinations of this sort give rise to “mosaic eyes.”

The arrangement of these blocks of color depends on fly eye development. Ideally, most of the eye cells will be mutant recombinants. Although all cells in the eye are capable of undergoing mitotic recombination, they do not. Flippase production and function is attached to a certain frequency of success. Additionally, for every mitotic recombination that produces a homozygous mutant, there will be a homozygous wild-type cell (see figure 1). This results in the formation of “twin spots,” adjacent areas of differing color useful for detecting areas of recombination. To minimize the number of homozygous wild-type and heterozygous cells, the minute gene (M) along with a “uni-white” copy for wild-type eye color is crossed into a stock. The M gene is homozygous lethal and slows growth in the heterozygote. Only cells homozygous for the lethal allele...
of interest (with no copies of the M gene) will exhibit normal growth. The eye will display primarily homozygous lethal cells, which facilitates phenotype analysis. With the minute gene screening out unwanted genotypes, one can readily ascertain the nature of the mutation. Eyes with cell lethal mutations will show no cells of the correct recombination color. Eyes will mutations of genes involved in eye development will possess cells of the correct color with an atypical phenotype. Eyes with mutations that do not affect eye development will be the correct color but normal in all other respects.

Occasionally, an interesting phenotype will arise in a fly that is prime for further study. Unlike bacteria, flies cannot be frozen for future use. Instead, stocks requiring continuous (and inevitably tedious) maintenance act as substitutes. However, when a stock possesses a homozygous recessive lethal, simply tossing flies together and transferring them over time will result in a diminished allele frequency of the lethal allele. Balancer chromosomes eliminate this hurdle. By coupling the lethal allele with the wild-type of another lethal allele on one chromosome and pairing the wild-type of the lethal of interest with a lethal allele of the marker, any animal homozygous for either the lethal of interest or the lethal marker will perish. A dominant marker, usually the same extra lethal allele, allows one to visibly ensure that this process occurs. This process can easily be disrupted during meiotic recombination, so as a preventative measure, an inversion is created in the region of interest. Crossing over with inversions produces chromosomes with either two telomeres or two centromeres, both lethal situations. Essentially, homozygous recessive lethal mutant fly stocks can be maintained when another lethal marker with some dominant phenotype is combined with an inversion.

III. Significance

As with most research, this project seeks to add to the collective knowledge of the drosophila genome as a means of unraveling the genetic mystery behind human diseases and disorders. Although the approximately 14,000 genes of the Drosophila genome have been sequenced, many of these genes have not been linked to specific functions. A tool, no matter how useful, cannot be exploited without knowing its purpose. By screening the Drosophila genome using the successful FRT/flp mitotic recombination technique, the scientific community will gain insight to the role these genes play in fly eye formation. Determination of gene function is difficult to accomplish in humans due to ethical and practical constraints. Drosophila serves as an adequate substitute organism. Like humans, fruit flies are eukaryotes with transcription and translational methods homologous to mammals. Essential genes may show great conservation among all species, making their study in flies even more valid. Studying Drosophila through reverse genetics will aid in fleshing out databases devoted to fly genetics. These databases can in turn be used to identify homologies in human or other organismal DNA sequences to reveal the molecular mechanisms behind diseases and disorders. Several genes linked to neurological disease, cardiovascular malformation, renal, hematological, and metabolic syndromes were discovered in Drosophila and C. elegans. Also, multiple genes involved in cancers have been isolated from invertebrate studies. Two genes, BRCA 1 and BRCA 2, were first identified in Drosophila. Additional cancer genes may be unearthed as such research continues.

This research will not only generate quality information for incorporation into databases, such as Flybase, but will also generate stocks of mutant flies for additional experiments relating to our own research or the research of others.
IV. Research Methods

The proposed procedures are as follows: 1) generate homozygous mutant flies for each line of mutations, 2) examine eye phenotype of small and large clones and take pictures of mosaic phenotypes, 3) calculate the recombination distance between the mini-white insertion and FRT, and 4) create a balanced stock for each line.

In order to create homozygous mutants, a series of crosses must be performed. The particular chromosomal segment of interest in this case is the left arm of the second chromosome. Stocks from Bloomington, Indiana with pre-made P-element insertions of mini-white containing a curly oster (CyO) dominant marker will be obtained (+/Y; P{w+}/CyO). In order to combine the x-linked yellow-bodied, eyeless-FLP (yw ey-FLP) traits with the P-element, males from the original Bloomington stock will be crossed with females homozygous for yw ey-FLP and containing scutoid (Sco) and CyO, y+ markers on the 2L chromosome.

Cross 1: yw ey-FLP/yw ey-FLP; Sco/CyO, y+ X +/Y; P[w+]/CyO

Male progeny possessing yw ey-FLP on the x-chromosome and the P-element with CyO, y+ on the 2L chromosome will have red eyes, gray bodies, and curly wings. These males will be collected and mated to females homozygous for both yw ey-FLP and FRT40A.

Cross 2: yw ey-FLP/ yw ey-FLP; FRT40A/FRT40A  x  yw ey-FLP/Y; P[w+]/ CyO, y+

The goal of this cross is to create flies containing both the P-element and an FRT site. All progeny from this cross will possess the yw-ey-FLP and half will contain the P-element. However, we will only collect females possessing the P-element and FRT40A, which are color-eyed (color depends on mini-white expression), yellow-bodied, and straight-winged. Only the female genome recombines during meiosis, a step which is crucial to generating flies with FRT sites on both 2L chromosomes. This is only possible through meiotic recombination in the female to produce gametes with mini-white and FRT40A on the same arm.

When these females are crossed with yw ey-FLP, homozygous FRT40A males, some of the resulting progeny will have two FRT sites and possibly a recombination event.

Cross 3: yw ey-FLP/ yw ey-FLP; P[w+], FRT40A  X  yw ey-FLP/Y; FRT40A/FRT40A

Once flies with mosaic eyes have been isolated, scanning electron and light microscope pictures of mutant phenotypes will be obtained. The number of progeny obtained with mutant phenotypes will be counted along with the total number of progeny produced in order to calculate the recombination frequency between the P-element and FRT40A.

Mosaic males from the last cross will be mated to red-eyed, gray-bodied, curly-winged females carrying the uniwhite, minute (w+ M) marker and a curly balancer:

Cross 4: yw ey-FLP/ yw ey-FLP; w+ M, FRT40A/ CyO, y+  X  yw ey-FLP/Y; P[w+], FRT40A/FRT40A
The resulting progeny will be used for two purposes: setting up a balanced stock for future use and examining eye phenotype once again. The balanced stock will consist of male and female yw ey-FLP; P-element, FRT40A/CyO, y+ flies. CyO, y+ serves as a balancer chromosome, killing off flies homozygous wild type for the lethal mutation of interest. As for the second phenotype examination, both male and female flies with yw ey-FLP, the P-element, w+ M, and FRT sites on each chromosome will be examined. The addition of the minute allele ensures that cells with only one P-element will experience slow growth and cells without a P-element will die. A “large clone” results and more light and SEM pictures will be taken. Additional recombination data will be used to correct or corroborate data from the small clones.

V. Timeline

The experiment will occupy 10 weeks time. An instructor cross of gray-bodied, white-eyed, scutoid, curly-winged females carrying ey-FLP with gray-bodied, red-eyed, curly-winged males will be performed during the week prior to the commencement of the experiment.

During week 1, the gray-bodied, red-eyed, curly-winged male progeny from week -1 carrying the P-element will be mated to yellow-bodied, white-eyed females carrying FRT.

In week 3, yellow-bodied, red-eyed female progeny carrying both a P-element and an FRT will be mated to yellow-bodied, white-eyed males carrying two FRT sites.

During weeks 5 and 6, mosaic eyed males from the week 3 cross will be examined under a light and scanning electron microscope (SEM). Pictures will be taken of particularly interesting mosaics and recombination frequencies will be determined between the P-element and FRT40A using progeny genotype numbers. These mosaic males will be crossed with gray-bodied, red-eyed, curly winged females carrying the minute gene.

In weeks 7 and 8, balanced stocks will be constructed using gray-bodied, color-eyed, curly-winged males and females containing the P-element and one FRT site. Also, mosaic eye progeny from week 5 and 6 will be examined under the light and scanning electron microscope. Again, recombination frequencies will be calculated from progeny genotype numbers.

Weeks 9 and 10 will consist of analyzing data obtained throughout the experiment to make a conclusion as to the function of each gene examined.
VI. References