3.1 Behavioral characterization:

3.1.1 Introduction:

Isolation of *Drosophila* olfactory mutants is important in identifying the genes mediating the sense of smell. First single-gene olfactory mutations were isolated by Rodrigues and Siddiqi (1978, 1981). The P-element has been the workhorse of *Drosophila* genetics since it was developed as a tool for transgenesis in 1982; the subsequent development of a variety of systems based on the transposon have provided a range of powerful and flexible tools for genetics and genomics applications. P-element insertions are frequently used as starting-points for generating chromosomal deletions to remove flanking genes, either by screening for imprecise excision events or by selecting for male recombination events. One of the early uses for P-elements was in large-scale mutagenesis screens, the major advantage over traditional chemical or radiation methods being that mutants were molecularly tagged by virtue of the P-element sequence (Russell et al., 2003). In addition to phenotypic screening, P-elements also can be used to study the pattern and timing of gene expression by enhancer trapping (Fig. 3.1) (O'Kane et al., 1987 and Wilson et al., 1989).

![Fig. 3.1 Enhancer trapping. A P-element construct containing a transformation marker, in this case a functional copy of the white gene (mini-w), and a LacZ reporter gene driven by a weak basal promoter inserts near a gene. An endogenous enhancer (grey circle) may then control the expression of the LacZ reporter in a similar pattern to the endogenous gene (black arrows). P-element ends are shown as triangles (5 and 3), and gene products are shown as squares.](image)

One widely-used variant of the enhancer trap strategy is the GAL4-UAS system developed by Brand and Perrimon (1993). This binary system utilizes enhancer trapping with a construct carrying the *Saccharomyces*
*cerevisiae* transcriptional activator, GAL4, as a reporter gene and the activity of the GAL4 protein as a transcription factor can be detected by monitoring the expression of a second reporter gene under the control of a GAL4 responsive promoter, or upstream activation sequence (UAS) (Fig. 3.2). On the one hand, reporter genes such as LacZ or GFP can be used to visualize the expression pattern of the enhancer. On the other hand, and far more importantly, any gene placed downstream of the UAS sequences in a construct can be activated by the GAL4 protein.

![GAL4-activated gene expression](image)

**Fig. 3.2** GAL4-activated gene expression. In the GAL4-UAS system, a construct containing the GAL4 gene is inserted randomly in the genome. As with the enhancer trap strategy shown in Figure 2, it may come under the influence of a genomic enhancer and express GAL4 in a pattern dictated by the enhancer. The GAL4 protein can then act at any UAS sites in the genome to activate expression of a gene of interest. Two scenarios are possible; in the first, a gene of interest is introduced into the genome in a P-element construct carrying UAS sites. In the second, a set of UAS sites in a P-element (an EP-element) are mobilized at random in the genome; if they insert in the vicinity of an endogenous gene, GAL4 can be used to activate the expression of that gene.

A set of P-element insertions containing P[1ArB] (Fig. 3.3) and P[GawB] (Fig. 3.4) were screened in our lab on the basis of their LacZ and GFP reporter expression patterns in olfactory organs, their adult and larval behavior phenotypes were characterized by me, Nixon (2001), Sukant (2003), Bilal (2004), Shamsudeen (2005), Hisham (2005), Satyajit (2006), Amulya (2007), Deepitha (2007), Shobhana (2007), Shwetha (2007) and others.
Fig. 3.3 Schematic of the molecular structure of P[ArB] It consists of enhancer trap lacZ and selectable markers rosy and Adh

Fig. 3.4 Schematic of the molecular structure of P[GawB] It consists of enhancer trap GAL4 (binary system) and selectable marker white

3.1.2 List of fly strains:

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Fly strain</th>
<th>Source of the strain</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Canton Special Benzer (CSBz)</td>
<td>Wild type stock, TIFR Stock Centre, Mumbai</td>
</tr>
<tr>
<td>2</td>
<td>003</td>
<td>Cahir O’Kane, University of Cambridge, Cambridge, UK</td>
</tr>
<tr>
<td>3</td>
<td>030</td>
<td>Cahir O’Kane, University of Cambridge, Cambridge, UK</td>
</tr>
</tbody>
</table>
3.1.3 Adult olfactory responses of P[GawB] insertion lines:

Adult flies of eight P[GawB] insertion lines were characterized behaviorally (after four days of conditioning) using the T-trap assay as described in Chapter. There are different classes of insertion lines which show varying degree of conditioned and unconditioned responses for the three chemicals tested (Ethyl acetate, Isoamyl acetate and 1-Hexanol) when compared to the wild type response (Table 3.1). In the line 238Y, the unconditioned response is increased for both Ethyl acetate and Isoamyl acetate when compared to the wild type (the conditioned response is normal).
Table 3.1 Adult behavioral phenotype of \( P[GawB] \) insertion lines compared to wild type

<table>
<thead>
<tr>
<th>Strains</th>
<th>Ethyl acetate</th>
<th>ISOamyl acetate</th>
<th>1-Hexanol</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CR</td>
<td>UCR</td>
<td>CR</td>
</tr>
<tr>
<td>003</td>
<td>↓</td>
<td>↓</td>
<td>↓</td>
</tr>
<tr>
<td>030</td>
<td>↓</td>
<td>N</td>
<td>↓</td>
</tr>
<tr>
<td>191</td>
<td>N</td>
<td>↓</td>
<td>↑</td>
</tr>
<tr>
<td>OK66</td>
<td>N</td>
<td>↑</td>
<td>↑</td>
</tr>
<tr>
<td>OK284</td>
<td>N</td>
<td>N</td>
<td>↓</td>
</tr>
<tr>
<td>OK301</td>
<td>↓</td>
<td>↓</td>
<td>↑</td>
</tr>
<tr>
<td>OK309</td>
<td>↓</td>
<td>N</td>
<td>↓</td>
</tr>
<tr>
<td>238Y</td>
<td>N</td>
<td>↑</td>
<td>N</td>
</tr>
</tbody>
</table>

CR: Conditioned Response  
UCR: Unconditioned Response  
N: Normal  
↑: Increased  
↓: Decreased

3.1.4 Larval olfactory responses of \( P[GawB] \) insertion lines:

The early third instar larval behavioral phenotype for the same eight insertion lines was measured using the larval plate test as described in Chapter. The lines were tested for a range of log dilution (10^{-1} to 10^{9}) of three chemicals (Ethyl acetate, ISOamyl acetate and 1-Hexanol). There are again different classes of insertion lines showing varying responses across different chemicals tested. Interestingly, when compared to wild type, the responses of few insertion lines are different at certain log dilutions but normal at other log dilutions of the same chemical (Tables 3.2, 3.3 and 3.4) (Figs. 3.5 to 3.12). The line 238Y show increased response to all the three chemicals tested at lower dilutions.
Table 3.2 Larval behavioral phenotype of wild type and P[GawB] insertion lines for Ethyl acetate

<table>
<thead>
<tr>
<th>Genotype</th>
<th>CsBz 003</th>
<th>030</th>
<th>191</th>
<th>OK66</th>
<th>OK284</th>
<th>OK101</th>
<th>OK209</th>
<th>238Y</th>
</tr>
</thead>
<tbody>
<tr>
<td>10⁻¹</td>
<td>43.15±2.12</td>
<td>47.32±2.36</td>
<td>63.54±5.38</td>
<td>63.19±3.59</td>
<td>46.65±3.19</td>
<td>46.88±3.12</td>
<td>41.57±3.31</td>
<td>46.17±2.56</td>
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<tr>
<td>10⁻²</td>
<td>85.53±4.53</td>
<td>80.26±4.54</td>
<td>86.89±2.76</td>
<td>82.45±4.23</td>
<td>88.74±4.38</td>
<td>82.49±4.23</td>
<td>80.92±4.28</td>
<td>82.76±3.42</td>
</tr>
<tr>
<td>10⁻³</td>
<td>86.23±3.17</td>
<td>86.72±3.20</td>
<td>90.73±3.01</td>
<td>90.87±6.52</td>
<td>85.31±3.82</td>
<td>85.37±3.54</td>
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<td>83.6±4.64</td>
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<td>91.83±4.45</td>
<td>90.53±4.27</td>
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<td>92.5±3.29</td>
<td>91.95±3.29</td>
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<td>90.53±4.52</td>
<td>92.1±4.21</td>
</tr>
<tr>
<td>10⁻⁵</td>
<td>61.38±2.27</td>
<td>55.54±4.21</td>
<td>52.5±3.21</td>
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<td>57.5±2.69</td>
<td>65.3±2.38</td>
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<td>55.2±4.21</td>
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<td>10⁻⁶</td>
<td>41.56±2.18</td>
<td>38.32±3.15</td>
<td>45.0±2.76</td>
<td>50.56±3.15</td>
<td>44.67±3.23</td>
<td>50.92±2.73</td>
<td>32.47±2.57</td>
<td>35.19±2.53</td>
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<td>10⁻⁷</td>
<td>31.11±2.71</td>
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<td>32.8±4.2</td>
<td>49.42±2.45</td>
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<td>20.3±2.42</td>
<td>19.2±2.1</td>
<td>49.1±3.85</td>
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</table>

Table 3.3 Larval behavioral phenotype of wild type and P[GawB] insertion lines for Isoamyl acetate

<table>
<thead>
<tr>
<th>Genotype</th>
<th>CsBz 003</th>
<th>030</th>
<th>191</th>
<th>OK66</th>
<th>OK284</th>
<th>OK101</th>
<th>OK209</th>
<th>238Y</th>
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</thead>
<tbody>
<tr>
<td>10⁻¹</td>
<td>65.16±3.21</td>
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<td>10⁻²</td>
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<td>66.7±2.09</td>
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<td>41.82±3.67</td>
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<td>50.18±3.72</td>
<td>32.93±2.73</td>
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<tr>
<td>10⁻⁷</td>
<td>28.51±2.34</td>
<td>24.92±2.83</td>
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<td>38.14±2.15</td>
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<td>10⁻⁹</td>
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<td>34.5±2.01</td>
<td>26.5±2.14</td>
<td>17.3±1.2</td>
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</table>
Table 3.4 Larval behavioral phenotype of wild type and P[GawB] insertion lines for 1-Hexanol

<table>
<thead>
<tr>
<th>Log dilution</th>
<th>CsBz 003</th>
<th>030</th>
<th>191</th>
<th>OK66</th>
<th>OK284</th>
<th>OK301</th>
<th>OK309</th>
<th>238Y</th>
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<td>10^4</td>
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<td>10^3</td>
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<td>78.43±3.52</td>
<td>76.29±3.61</td>
<td>81.19±2.76</td>
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<td>44.29±2.38</td>
<td>37.19±2.16</td>
<td>35.62±2.91</td>
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<td>10^-2</td>
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<td>14.21±1.01</td>
<td>7.06±1.41</td>
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</table>
Fig. 3.5 Olfactory response of third instar larvae of wild type and 003 line tested for varying log dilutions of Ethyl acetate, Isoamyl acetate and 1-Hexanol. Each response index (RI) represents the mean of six experiments, each experiment consisting six petri-dishes. The standard error was calculated as the standard error of mean.
Fig. 3.6 Olfactory response of third instar larvae of wild type and 030 line tested for varying log dilutions of Ethyl acetate, Isoamyl acetate and 1-Hexanol. Each response index (RI) represents the mean of six experiments, each experiment consisting six petri-dishes. The standard error was calculated as the standard error of mean.
Fig. 3.7 Olfactory response of third instar larvae of wild type and 191 line tested for varying log dilutions of Ethyl acetate, Isoamyl acetate and 1-Hexanol. Each response index (RI) represents the mean of six experiments, each experiment consisting six petri-dishes. The standard error was calculated as the standard error of mean.
Fig. 3.8 Olfactory response of third instar larvae of wild type and OK66 line tested for varying log dilutions of Ethyl acetate, Isoamyl acetate and 1-Hexanol. Each response index (RI) represents the mean of six experiments, each experiment consisting six petri-dishes. The standard error was calculated as the standard error of mean.
Fig. 3.9 Olfactory response of third instar larvae of wild type and OK284 line tested for varying log dilutions of Ethyl acetate, Isoamyl acetate and 1-Hexanol. Each response index (RI) represents the mean of six experiments, each experiment consisting six petri-dishes. The standard error was calculated as the standard error of mean.
Fig. 3.10 Olfactory response of third instar larvae of wild type and OK301 line tested for varying log dilutions of Ethyl acetate, Isoamyl acetate and 1-Hexanol. Each response index (RI) represents the mean of six experiments, each experiment consisting six petri-dishes. The standard error was calculated as the standard error of mean.
Fig. 3.11 Olfactory response of third instar larvae of wild type and OK309 line tested for varying log dilutions of Ethyl acetate, Isoamyl acetate and 1-Hexanol. Each response index (RI) represents the mean of six experiments, each experiment consisting six petri-dishes. The standard error was calculated as the standard error of mean.
Fig. 3.12 Olfactory response of third instar larvae of wild type and 238Y line tested for varying log dilutions of Ethyl acetate, Isoamyl acetate and 1-Hexanol. Each response index (RI) represents the mean of six experiments, each experiment consisting six petri-dishes. The standard error was calculated as the standard error of mean.
3.2 Molecular localization of the P element insertion lines:

3.2.1 Introduction:

The P-element used in mutagenesis, P[lArB] and P[GawB], allow for the rescue of genomic DNA, immediately downstream of its site of insertion, in a plasmid (Plasmid Rescue). This rescued DNA then serves as a handle in the molecular characterization and the subsequent cloning of the gene. Localization of the P-element was carried out using plasmid rescue methodology (Steller et al., 1985) in one P[lArB] line (1110) and four P[GawB] lines (191, OK294, OK309 and 238Y). Furthermore, point of insertions in these four P[GawB] lines were confirmed by inverse PCR (Ochman et al., 1988). Also, P-elements in four new P[GawB] lines (003, OK140, OK284 and OK301) were localized using the same inverse PCR methodology. Inverse PCR (iPCR) is a method for the rapid in vitro amplification of DNA sequences that flank a region of known sequence. The method uses the polymerase chain reaction (PCR), but it has the primers oriented in the reverse direction of the usual orientation. A limitation of standard PCR is that 5' and 3' flanking regions of your DNA fragment of interest must be known. Inverse PCR allows you to conduct PCR when you only have the information of one internal sequence. Thus, in a total of nine lines, P-element insertion point was localized by either plasmid rescue or inverse PCR or both.

Plasmid Rescue:

In plasmid rescue, genomic DNA from the P element insertion lines was extracted, restriction digested with XhoI, a restriction enzyme of the polylinker 3 (PL3) site of the P element, ligated under conditions to favor self-ligations, the ligated DNA was transformed into E. coli (XL-10 Gold strain) by electroporation and the transformants were selected for Ampicillin, Tetracyclin and Chloramphenicol resistance (Fig. 3.13).
Genomic DNA extracted from \( P[GawB] \) and \( P[IArB] \) insertion lines, digested with Xhol (cutting at the polylinker PL3 site of the P element and at the next restriction site in the downstream genomic region). The DNA was then self-ligated and the ligated DNA was used for transformation and the cells were plated on the selection medium containing Ampicillin, Tetracyclin and Chloramphenicol.

Plasmid DNA was extracted from the transformants, restriction digested using Xhol and NotI (PL4 restriction enzyme) to know the size of insert. This plasmid DNA with insert have T3 and T7 promoter/priming sites and DNA primers specific to these two sites were used to obtain the 3' and 5' end sequences of the rescued DNA. The T3 primed 3' end sequence includes a part of the pBluescript vector, followed by the seven base pair P element inverted repeat (p3'), CATCATG and the downstream genomic region. The first base, following the inverted repeat therefore corresponds to the exact site of P element insertion (Wilson et al., 1989).

In order to determine the site of P element insertion on the genome, the 5' and 3' end sequences were matched with the \textit{Drosophila} genome sequence available online at the National Center for Biotechnology Information (NCBI) and the FlyBase websites. This was done by using
BLAST (Basic Local Alignment Search Tool) sequence similarity search program (Altschul et al., 1990).

**Inverse PCR:**

In inverse PCR, there are initial common steps as that of plasmid rescue. The genomic DNA was digested with DpnII restriction enzyme and then it was ligated in a condition favoring self-ligation. The ligated DNA was directly taken for PCR, using PGaw2 and PGaw3 primers (Fig. 3.14). The PCR product was gel extracted and given for DNA sequencing using the SP1 (for 5' end) and SPEP1 primer (for 3' end). Then the sequence was matched with the *Drosophila* genome sequence databases as in case of plasmid rescue.

Fig. 3.14 Schematic of the Inverse PCR
The core region (the *P* element *P*(GawB)) is depicted as a jagged line. Filled and open boxes represent the upstream and downstream flanking regions, respectively, and restriction enzyme (DpnII) recognition sites are denoted by triangles. Oligonucleotide primers (PGaw2 and PGaw3) constructed to anneal to the core region and the direction of DNA synthesis are shown by arrows.
Fig. 3.15 Inverse PCR of P[GawB] insertion lines
The presence of single band in each of the lines indicate that there is only one copy of the P element inserted in them. And the varying sizes of the bands are indicative of different sites of P element insertions and thus recognition sites for DpnII in the genome.

In a total of nine P element insertion lines, the exact insertion point of the P element was molecularly localized by plasmid rescue or inverse PCR or both.

3.2.2 P-element insertion lines localized:

1) 003 line:

P element insertion site: X: 8788241 bp

Genes in vicinity of the insertion site:

i) Moesin (Moe): It has 10 annotated transcripts. The insertion is in the intron region of the transcripts Moe-RF, Moe-RG and Moe-RH.

ii) CG1885: The gene is at 4824 bp from the insertion site towards 3' side.

i) Moesin (moe):

Symbol: Moe
Name: Moesin
Annotation symbol : CG10701  
FlyBase ID : FBgn0011661  
Cytogenetic map : 8B4 - 8B6  
Sequence location : X : 8,767,636 .. 8,792,343 [-]

Gene Information:

Its molecular function is described as: protein binding; cytoskeletal protein binding; actin binding. It is involved in the biological processes described with 17 unique terms, many of which group under: anatomical structure development; anterior/posterior axis specification; organelle organization and biogenesis; oocyte axis determination; actin filament-based process; sensory organ development; cytoskeleton organization and biogenesis; organ morphogenesis; compound eye photoreceptor development; oocyte anterior/posterior axis determination. 45 alleles are reported. The phenotypes of these alleles are annotated with 15 unique terms, many of which group under: adult segment; organ system; adult mesothoracic segment; thoracic segment; peripheral nervous system; nervous system; adult; imaginal precursor; embryonic abdomen; blastoderm embryo; embryonic hindgut; dorsal thoracic disc. It has 10 annotated transcripts and 10 annotated polypeptides.

ii) CG1885:

Symbol : CG1885  
Name : CG1885  
Annotation symbol : CG1885  
FlyBase ID : FBgn0030066  
Cytogenetic map : 8B6 - 8B6  
Sequence location : X : 8,793,065 .. 8,794,211 [-]

Gene Information:

Its molecular function is described as: uroporphyrinogen-III synthase activity; caspase activity. It is involved in the biological processes: tetrapyrrole biosynthetic process; proteolysis. One allele is reported. No phenotypic data is available. It has one annotated transcript and one annotated polypeptide.
2) 191 line:

P element insertion site: X : 10633066 bp

Genes in vicinity of the insertion site:

i) CG32676: The insertion is in the intron region of this gene.

ii) Raspberry (Ras): The gene is at 5473 bp from the insertion site towards 3' side.

i) CG32676:

Symbol : CG32676
Name : CG32676
Annotation symbol : CG32676
FlyBase ID : FBgn0052676
Cytogenetic map : 9E1 - 9E2
Sequence location : X : 10,607,151..10,638,938 [-]

Gene Information:

Its molecular function is unknown. It is involved in the biological processes: cellular process; protein modification process. 18 alleles are reported. No phenotypic data is available. It has one annotated transcript and one annotated polypeptide.

ii) Raspberry (Ras):

Symbol : Ras
Name : Raspberry
Annotation symbol : CG1799
FlyBase ID : FBgn0003204
Cytogenetic map : 9E1 - 9E2
Sequence location : X : 10,638,539..10,642,840 [+]

Gene Information:

Its molecular function is described as IMP dehydrogenase activity. It is involved in the biological processes: axon guidance; GMP biosynthetic process; phagocytosis, engulfment; oogenesis. 71 alleles are reported. The phenotypes of these alleles are annotated with: pigment cell; Malpighian
tubule; testis; ovariole; egg; macrochaeta. It has 3 annotated transcripts and 3 annotated polypeptides.

PGawB

Gene Span: Xilueta

mRNA

Xilueta-Ra

CG32676-Ra

CDS

Xilueta-Fa

CG32676-Fa

CNS

Xilueta-Ph

CG32676-Ph

Gene Span: Xilueta

PGawB

10633066 bp

X: 5' - 3'

CG32676

10607151 bp - 10638938 bp

Ras

10638539 bp - 10642840 bp
3) 1110 line:

P element insertion site: 2R : 16470254 bp

Genes in vicinity of the insertion site:

i) l(2)05510: The gene is at 621 bp from the insertion site towards 5' side.

ii) Nnfla: The gene is at 3470 bp from the insertion site towards 3' side.

iii) Bancal (bl): The gene is at 4756 bp from the insertion site towards 3' side.

i) l(2)05510:

Symbol : l(2)05510
Name : lethal (2) 05510
Annotation symbol : CG13432
FlyBase ID: FBgn0028622
Cytogenetic map : 57A5 • 57A6
Sequence location : 2R : 16,450,540..16,469,633 [■]

Gene Information:

Its molecular function is unknown. The biological processes in which it is involved are not known. 9 alleles are reported. The phenotypes of these alleles are annotated with: dorsocentral bristle; scutellar bristle; trichogen cell; tormogen cell; anterior fascicle; ommatidium; eye. It has one annotated transcript and one annotated polypeptide.

ii) Nnfla:

Symbol : Nnfla
Name : Nnfla
Annotation symbol : CG13434
FlyBase ID: FBgn0034523
Cytogenetic map : 57A6 • 57A6
Sequence location : 2R : 16,473,724..16,474,712 [-]

Gene Information:

Its molecular function is unknown. It is involved in the biological processes: mitotic metaphase plate congress; chromosome segregation. 5 alleles are reported. The phenotype of these alleles is annotated with metaphase. It has one annotated transcript and one annotated polypeptide.
iii) *Bancal (bl)*:

Symbol: bl  
Name: bancal  
Annotation symbol: CG13425  
FlyBase ID: FBgn0015907  
Cytogenetic map: 57A6 - 57A7  
Sequence location: 2R: 16,475,010..16,494,359

**Gene Information:**

Its molecular function is described as: mRNA binding; transcription factor binding; RNA binding. It is involved in the biological processes: appendage morphogenesis; cell fate commitment; cell proliferation; imaginal disc growth; regulation of alternative nuclear mRNA splicing, via spliceosome. 22 alleles are reported. The phenotypes of these alleles are annotated with 13 unique terms, many of which group under: adult segment; peripheral nervous system; thoracic segment; nervous system; adult mesothoracic segment; adult; organ system; appendage segment; dorsal thoracic disc; metathoracic tarsal segment. It has 4 annotated transcripts and 4 annotated polypeptides.
**Gene Span**

**h2Q**

**cDNA**

**Non coding RNA**

**Natural transposon**

**BLAST**

---

**PGawB**

16470254 bp

**PGawB**

16450540 bp - 16469633 bp

**Nafta**

16473724 bp - 16474712 bp

**Bancal** (bl)

16475910 bp - 16494359 bp
4) OK140 line:

P element insertion site: **2R : 15614715 bp**

**Genes in vicinity of the insertion site:**

i) **snoRNA:185:** The gene is at 112 bp from the insertion site towards 5' side.

ii) **tRNA:E4:56Fb:** The gene is at 250 bp from the insertion site towards 3' side.

i) **snoRNA:185:**

Symbol: snoRNA:185 (small nucleolar RNA)
Name: snoRNA:185
Annotation symbol: CR33930
FlyBase ID: FBgn0065076
Cytogenetic map: 56E2 - 56E2
Sequence location: 2R : 15,614,603..15,614,657 [-]

**Gene Information:**

Its molecular function is unknown. The biological processes in which it is involved are not known. One allele is reported. No phenotypic data is available. It has one annotated transcript.

ii) **tRNA:E4:56Fb:**

Symbol: tRNA:E4:56Fb
Name: transfer RNA:glu4:56Fb
Annotation symbol: CR30455
FlyBase ID: FBgn0011849
Cytogenetic map: 56E2 - 56E2
Sequence location: 2R : 15,614,965..15,615,036 [-]

**Gene Information:**

Its molecular function is described as triplet codon-amino acid adaptor activity. It is involved in the biological process translation. No alleles are reported. It has one annotated transcript.
PGawB

Gene Span

Non-coding RNA

Natural transposon

BLAST

snoRNA:185
15614603 bp - 15614657 bp

tRNA:E4;56Fb
15614965 bp - 15615036 bp

15614715 bp
PGawB

2R 5' 3'
5) OK284 line:

P element insertion site: 3L: 18703561 bp

Genes in vicinity of the insertion site:

i) CG12477: The gene is at 11869 bp from the insertion site towards 3' side.

ii) CG18363: The gene is at 12049 bp from the insertion site towards 5' side.

i) CG12477:

Symbol: CG12477
Name: CG12477
Annotation symbol: CG12477
FlyBase ID: FBgn0036809
Cytogenetic map: 75D7 - 75D7
Sequence location: 3L: 18,715,430..18,716,227

Gene Information:

Its molecular function is described as: protein binding; zinc ion binding; nucleic acid binding. The biological processes in which it is involved are not known. 3 alleles are reported. No phenotypic data is available. It has one annotated transcript and one annotated polypeptide.

ii) CG18363:

Symbol: CG18363
Name: CG18363
Annotation symbol: CG18363
FlyBase ID: FBgn0036808
Cytogenetic map: 75D6 - 75D6
Sequence location: 3L: 18,690,168..18,691,512

Gene Information:

Its molecular function is described as: transmembrane transporter activity; binding. It is involved in transport. One allele is reported. No phenotypic data is available. It has one annotated transcript and one annotated polypeptide.
PGawB

mRNA

CDS

tRNA

Non-coding RNA

Natural transposon

BLAST

15614715 bp

PGawB

2R 5' 3'

snoRNA:185
15614603 bp - 15614657 bp

tRNA:E4:56Fb
15614965 bp - 15615036 bp
6) OK294 line:

P element insertion site: X : 2685776 bp

Genes in vicinity of the insertion site:

i) *white (w)*: The insertion is in the intron region of the gene.

ii) CG32795: The gene is at 1800 bp from the insertion site towards 5' side.

i) *white (w)*:

Symbol: w
Name: white
Annotation symbol: CG2759
FlyBase ID: FBgn0003996
Cytogenetic map: 3B6 - 3B6
Sequence location: X : 2,684,632..2,690,499 [-]

Gene Information:

Its molecular function is described as: ATPase activity, coupled to transmembrane movement of substances; eye pigment precursor transporter activity; transmembrane receptor activity; ATP binding. It is involved in the biological processes: eye pigment precursor transport; ommochrome biosynthetic process; eye pigment biosynthetic process; eye pigment metabolic process; transport. 1655 alleles are reported. The phenotypes of these alleles are annotated with: pigment cell; ocellus pigment granule; testis pigment cell; Malpighian tubule; eye; ocellus; testis; ommatidium; larval Malpighian tubule; main segment of Malpighian tubule. It has one annotated transcript and one annotated polypeptide.

ii) CG32795:

Symbol: CG32795
Name: CG32795
Annotation symbol: CG32795
FlyBase ID: FBgn0040384
Cytogenetic map: 3B6 - 3B6
Sequence location: X : 2,676,939..2,683,975 [-]
Its molecular function is unknown. The biological processes in which it is involved are not known. 8 alleles are reported. The phenotype of these alleles is annotated with pigment cell. It has 3 annotated transcripts and 3 annotated polypeptides.
7) OK301 line:

P element insertion site: X : 22232347 bp

Genes in vicinity of the insertion site:

i) **fog** : The insertion is in intron region of this gene.

ii) **CG41475**: The gene is at 8529 bp from the insertion site towards 3' side.

i) **fog**:

Symbol : fog
Name : folded gastrulation
Annotation symbol : CG9559
FlyBase ID : FBgn0000719
Cytogenetic map : 20D2 - 20E1
Sequence location : X : 22,227,998..22,257,020 [-]

Gene Information:

Its molecular function is unknown. It is involved in the biological processes: terminal region determination; torso signaling pathway; posterior midgut invagination; regulation of development; heterochronic; ventral furrow formation; morphogenesis of embryonic epithelium; regulation of cell shape; gastrulation; salivary gland morphogenesis; mesoderm development. 32 alleles are reported. The phenotypes of these alleles are annotated with 14 unique terms, many of which group under: anatomical structure; organ system; primordium; gastrula embryo; germ layer; organism; extended germ band embryo; portion of tissue; embryonic head; posterior midgut inclusive primordium; ventral furrow; salivary gland; plasma membrane part. It has 2 annotated transcripts and 2 annotated polypeptides.

ii) **CG41475**:

Symbol : CG41475
Name : CG41475
Annotation symbol : CG41475
FlyBase ID : FBgn0084035
Sequence location : X : 22,240,876..22,264,867 [+]

92
Gene Information:

Its molecular function is unknown. The biological processes in which it is involved are not known.
8) OK309 line:

P element insertion site: 3L: 20396454 bp

Genes in vicinity of the insertion site:

i) trbl: The gene is at 1746 bp from the insertion site towards 5' side.

ii) CG13248: The gene is at 3746 bp from the insertion site towards 3' side.

Symbol: trbl
Name: tribbles
Annotation symbol: CG5408
FlyBase ID: FBgn0028978
Cytogenetic map: 77C1 - 77C1
Sequence location: 3L: 20,389,388..20,394,708 [-]

Gene Information:

Its molecular function is described as: ATP binding; protein kinase activity; protein serine/threonine kinase activity. It is involved in the biological processes: protein amino acid phosphorylation; negative regulation of mitosis; ventral furrow formation; gastrulation; regulation of cell cycle. 24 alleles are reported. The phenotypes of these alleles are annotated with 25 unique terms, many of which group under: organ system; anatomical structure; gastrula embryo; peripheral nervous system; nervous system; germarium; adult segment; thoracic segment; adult mesothoracic segment; stage 8 embryo. It has one annotated transcript and one annotated polypeptide.

ii) CG13248:

Symbol: CG13248
Name: CG13248
Annotation symbol: CG13248
FlyBase ID: FBgn0036984
Cytogenetic map: 77C1 - 77C2
Sequence location: 3L: 20,400,200..20,403,669 [-]
Gene Information:

Its molecular function is described as: cationic amino acid transmembrane transporter activity; amino acid transmembrane transporter activity. It is involved in the biological process amino acid transport. One allele is reported. No phenotypic data is available. It has one annotated transcript and one annotated polypeptide.
P element insertion site: 3L : 14267346 bp

Genes in vicinity of the insertion site:

i) frizzled (fz):

The gene is at 101 bp from the insertion site towards 3' side.

Symbol: fz
Name: frizzled
Annotation symbol: CG17697
FlyBase ID: FBgn0001086
Cytogenetic map: 70D4 - 70D5
Sequence location: 3L : 14,267,447..14,361,748 [+]

Gene Information:

Its molecular function is described as: Wnt receptor activity; Wnt-protein binding; transmembrane receptor activity; non-G-protein coupled 7TM receptor activity; G-protein coupled receptor activity. It is involved in the biological processes described with 22 unique terms, many of which group under: anatomical structure development; establishment of planar polarity; cell communication; sensory organ development; signal transduction; macromolecule localization; protein localization; imaginal disc-derived wing hair organization and biogenesis; organ morphogenesis; homophilic cell adhesion; cell division; regulation of cellular component organization and biogenesis; Wnt receptor signaling pathway; epithelial cell differentiation; ommatidial rotation. 170 alleles are reported. The phenotypes of these alleles are annotated with 34 unique terms, many of which group under: adult segment; peripheral nervous system; nervous system; adult mesothoracic segment; organ system; embryonic nervous system; metatarsus; appendage segment; thoracic segment; adult cuticle. It has 2 annotated transcripts and 2 annotated polypeptides.
3.2.3 Summary:

The adult behavioral phenotype of flies was tested using the T-trap paradigm. The line 238Y, when tested with Ethyl acetate, shows increased unconditioned response and normal conditioned response compared to the wild type.

The third instar larvae of eight GAL4 lines (008, 030, 191, OK66, OK284, OK301, OK309 and 238Y) with P[GawB] insertions were tested using the larval plate test paradigm, for Ethyl acetate, Isoamyl acetate and 1-Hexanol across a range of dilutions from $10^{-3}$ to $10^{6}$. There are different classes of insertion lines showing varying responses for the three chemicals. The responses of few insertion lines, when compared to the wild type, are different at certain log dilutions but normal at other dilutions of the same chemical. The line 238Y show increased response to all the three chemicals tested at lower dilutions.

The molecular localization of the P element was carried out for nine insertion lines (008, 191, 1110, OK140, OK284, OK294, OK301, OK309 and 238Y) at the base pair level. The summary of the site of insertions and the candidate genes in the vicinity of the insertion point are given in the Table 3.5 below.
Table 3.5  Summary of the P-element insertions localization

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Strain</th>
<th>Cytological location</th>
<th>Site of insertion</th>
<th>Genes in the vicinity of insertion</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>003</td>
<td>X</td>
<td>8788241 bp</td>
<td>Moesin (Moe) CG1835</td>
</tr>
<tr>
<td>2</td>
<td>191</td>
<td>X</td>
<td>10633066 bp</td>
<td>CG32676 Raspberry (Ras)</td>
</tr>
<tr>
<td>3</td>
<td>1110</td>
<td>2R</td>
<td>16470254 bp</td>
<td>I(2)05510 Naf1a Bancal (bl)</td>
</tr>
<tr>
<td>4</td>
<td>OK140</td>
<td>2R</td>
<td>15614715 bp</td>
<td>snoRNA:186 tRNA:E4:56Fb</td>
</tr>
<tr>
<td>5</td>
<td>OK284</td>
<td>3L</td>
<td>18703561 bp</td>
<td>CG12477 CG18363</td>
</tr>
<tr>
<td>6</td>
<td>OK294</td>
<td>X</td>
<td>2685775 bp</td>
<td>white (w) CG32795</td>
</tr>
<tr>
<td>7</td>
<td>OK301</td>
<td>X</td>
<td>22232347 bp</td>
<td>log CG41475</td>
</tr>
<tr>
<td>8</td>
<td>OK309</td>
<td>3L</td>
<td>20396454 bp</td>
<td>trbl CG13248</td>
</tr>
<tr>
<td>9</td>
<td>238Y</td>
<td>3L</td>
<td>14267346 bp</td>
<td>frizzled (fz)</td>
</tr>
</tbody>
</table>