This document is one part of a *Drosophila* genetics training package, the entire strategy of which is described in detail elsewhere (see link).
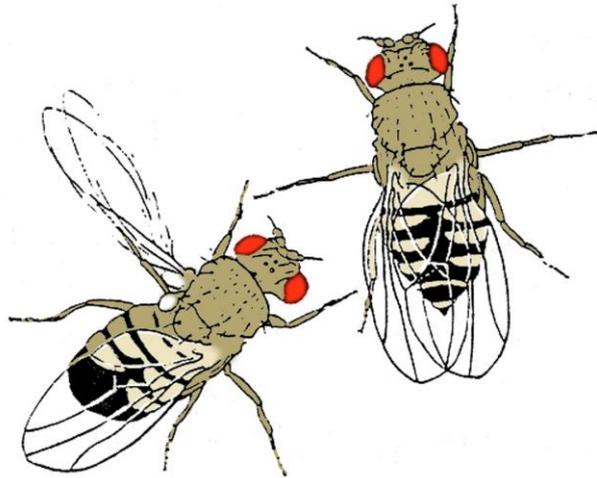
General Tips

Tip 1: **When solving these tasks, revisit the manual and PowerPoint presentation for help. If this does not solve the problem, please, come forward with specific questions.**

Tip 2: Always start by writing down the final stock you want to generate.

Tip 3: Always make sure you include a male (X/Y) and a female (X/X) in each cross.

Tip 4: Always check carefully whether specific gender choice from the two genotypes of a cross will have implications for the next generation(s), in particular when dealing with mutant alleles such as *white* or FM7 balancers on the first chromosome, or when recombination is intended (only in females) or needs to be prevented (using males or balancers).

Tip 5: To simplify matters, capitalised mutant alleles in these tasks always cause a visible phenotype in heterozygosis and are lethal in homozygosis, whereas alleles starting with small letter are recessive and show a phenotype only in homozygosis.

Tip 6: The presence of a balancer chromosome in a given stock indicates that the balanced chromosome harbours at least one homozygous lethal mutant allele. Therefore, following from Tip 5, dominant alleles will always be balanced, whereas recessive alleles or P-element insertions may be homozygous viable in some tasks.

Tip 7: The w^+ on P-elements always gives orange eyes in these tasks. But be aware that this phenotype is visible only if the first chromosome is w/Y or w/w.

Tip 8 Expression of any gene constructs cloned into P-elements occurs both in heterozygosis and homozygosis - whereby a difference in expression strengths between hetero- *versus* homozygous insertions will be irrelevant during these tasks.

Tip 9: Many marker mutations may occur in the available stocks. Carefully consider which of these markers are relevant for the task. For complex chromosomes, use shorthand.

Tip 10: Make sure you distinguish balancers from normal chromosomes with marker mutations.

Tip 11: Recombination occurs randomly in the germline of non-balanced, transheterozygous female flies. Remember to balance potential recombination events and to use single crosses at the right step of the scheme (see PowerPoint presentation). Selecting the individuals which carry recombinant chromosomes is the actual challenge in these tasks.

Task 1: For the following flies, write down the gender and the marker mutations they display.

    <u>Tip 1:</u> The first fly is a wildtype female.

♀	+				
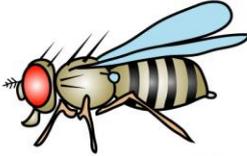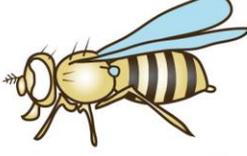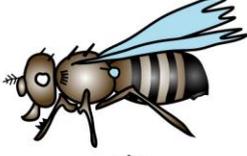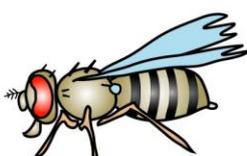
Task 2: You keep a fly stock that carries a homozygous lethal, recessive *gcm* mutant allele and is wildtype for the *white* locus on its first chromosome (stock 1). However, for a recombination experiment with a P-element line you need *gcm* in a *white* mutant background.

(1) $\dfrac{+}{+}$; $\dfrac{gcm}{CyO}$; $\dfrac{+}{+}$ (2) $\dfrac{w}{w}$; $\dfrac{If}{CyO}$; $\dfrac{+}{+}$

    a) Write down the genotype of the stock you want to generate:

    b) Using both stocks, design a strategy by which you can combine the recessive non-lethal *white* mutation (stock 2) with the *gcm* mutation.

Task 3: You have a stock carrying the recessive, homozygous lethal mutation *m1* over a standard CyO balancer (stock 1). For experimental reasons you want to bring *m1* over a GFP-expressing CyO balancer. The GFP-expressing CyO balancer carries no further markers that would distinguish it from normal CyO, and you have it available in a fly stock over a recessive, homozygous lethal mutation *m2* (stock 2).
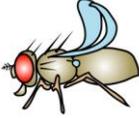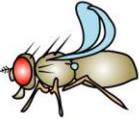
Tip 1: Be aware that *m1* and *m2* are recessive mutations. Make sure that you can follow these chromosomes safely throughout the mating scheme.

Tip 2: Does the *ry* marker of stock 2 have to be considered during this cross?

a) Write down the genotype of the stock you want to generate:

b) Design a safe strategy by which you can bring the *m1* mutation over this GFP-expressing CyO balancer. You may use stock 3 as a further aid.

Task 4: You want to use the *A101* (*neuralized-lacZ*) enhancer trap line to analyse *Notch* (*N*) mutant embryos. For this, you decide to combine the *A101* P-element insertion and *N* mutation into one fly stock which can thereafter be maintained in the laboratory. Furthermore, you want to use a "GFP balancer" (FcG) which will enable you to select the hemizygous *N* mutant embryos directly under the fluorescent microscope.

Tip 1: *N*/Y (hemizygous) and *N*/*N* (homozygous) individuals are embryonic lethal, whereas heterozygous flies are viable and have notched wing tips similar to *Ser* (see stock 1).

Tip 2: Note that the used balancers FM7a and FM7c carry different marker mutations, and that *mys* is a recessive lethal mutation with no heterozygous phenotype.

a) Write down the genotype of the hemizygous mutant embryos you will analyse:

b) Write down the genotype of the stock you keep:

c) Design a mating scheme to generate this stock.

Task 5: A specific mutant allele of the 3rd chromosomal *Ubx* gene causes a dominant phenotype (enlarged halteres) in heterozygosis, and is embryonic lethal in homozygosis. You want to study the impact of homozygous mutant *Ubx* on the lacZ expression pattern of the 3rd chromosomal *P(lacZ,w$^+$)^{hb}* enhancer trap insertion. You maintain *Ubx* and *P(lacZ,w$^+$)^{hb}* as separate stocks in the laboratory, hence need to recombine them before you can perform the experiment.
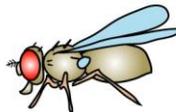
① $\dfrac{+}{+}$; $\dfrac{+}{+}$; $\dfrac{Ubx}{TM3,Sb}$
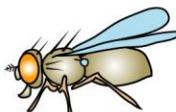
② $\dfrac{w}{w}$; $\dfrac{+}{+}$; $\dfrac{P\{lacZ,w^+\}^{wg}}{P\{lacZ,w^+\}^{wg}}$

③ $\dfrac{w}{w}$; $\dfrac{If}{CyO}$; $\dfrac{Sb}{TM6B,Hu}$

a) How important are *If* and CyO in stock 3 for your cross?

b) Write down the genotype of the embryos you want to study.

c) What is the genotype of the stock you want to build and which gives rise to those embryos?

d) Design a crossing strategy using stocks 1 to 3.

Task 6: The *M48-Gal4* P-element insertion stock (stock 3) expresses Gal4 in a subset of neurons in the CNS. The axons of *M48-Gal4*-positive neurons can be visualised with X-Gal staining if the *UAS-lacZ* insertion (stock 4) is present in the same animals. You would like to test whether the axonal pattern of *M48-Gal4*-positive neurons is altered in *comm* homozygous mutant embryos. To be able to select *comm* mutant embryos in your experiment, you decide to keep the *comm* mutant chromosome over a "green balancer" (TM3,*Ser,GFP*; stock 2). You realise that the experiment is best performed by establishing two different fly stocks that can thereafter be maintained in the laboratory and will allow you to repeat the experiment at a later stage if required.

① $\dfrac{+}{+}$; $\dfrac{+}{+}$; $\dfrac{comm}{TM6B,Hu}$

② $\dfrac{w}{w}$; $\dfrac{If}{CyO}$; $\dfrac{Sb}{TM3,Ser,GFP}$
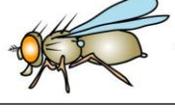
③ $\dfrac{w}{w}$; $\dfrac{P\{M48\text{-}Gal4,w^+\}}{P\{M48\text{-}Gal4,w^+\}}$; $\dfrac{+}{+}$
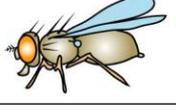
④ $\dfrac{w}{w}$; $\dfrac{P\{UAS\text{-}lacZ,w^+\}}{P\{UAS\text{-}lacZ,w^+\}}$; $\dfrac{+}{+}$

Tip 1: Only one copy of the *Gal4-* and one copy of the *UAS*-construct are required to perform your experiment in *comm* mutant embryos.

a) Which is the genotype of the embryos you would want to analyse?

b) What are the genotypes of the parental stocks that will give rise to the embryos in (a)?

d) Using the above strains, design crosses to generate the stable parental stocks in (b).

Task 7: You want to study whether the morphology of *P{RRK-lacZ,w⁺}*-positive neurons is altered in *repo* homozygous mutant embryos. For this you need to recombine the homozygous viable P-element insertion *P{RRK-lacZ,w⁺}* with the recessive, homozygous lethal *repo* mutation. Both are on the third chromosome but kept in two separate fly stocks.

Tip 1: *CxD* is a partial balancer chromosome which bears the dominant *Dichaete* (*D*) marker which causes loss of the alula (arrow heads) and held out wings.
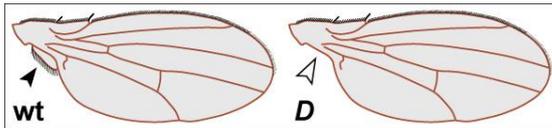
$$① \quad \frac{w}{w} \; ; \; \frac{+}{+} \; ; \; \frac{repo}{TM6B,Hu}$$

$$② \quad \frac{w}{w} \; ; \; \frac{+}{+} \; ; \; \frac{P\{RRK\text{-}lacZ,w^+\}}{P\{RRK\text{-}lacZ,w^+\}}$$

$$③ \quad \frac{w}{w} \; ; \; \frac{+}{+} \; ; \; \frac{TM3,Ser}{CxD}$$

wt   *D*

a) Which is the genotype of the embryos you want to analyse?

b) Design a mating scheme which recombines *repo* and *P{RRK-lacZ,w⁺}* on one chromosome which can be kept in a stable stock thereafter.

c) How do you know that both mutation and P-element are present?

Task 8: You have identified the novel 2ⁿᵈ chromosomal *shot* mutation which, in homozygosis, correlates with embryonic lethality and an exciting brain phenotype. You need to proof that *shot* causes the brain phenotype. For this, you want to perform a gene rescue experiment in which you express the cloned *shot* gene in the nervous system of *shot* homozygous mutant embryos and then assess whether normal brain morphology has been reinstated.

• You have generated a *P{UAS-shot⁺,w⁺}* transgenic line where the P-element is inserted on the third chromosome; unfortunately the insertion turns out to be lethal in homozygosis.

• The expression of *UAS*-constructs in the brain can be driven with the *P{sca-Gal4,w⁺}* enhancer trap line which, like *shot*, maps to the second chromosome.

$$① \quad \frac{w}{w} \; ; \; \frac{+}{+} \; ; \; \frac{P\{UAS\text{-}shot^+,w^+\}}{TM3,Sb} \qquad ③ \quad \frac{+}{+} \; ; \; \frac{shot}{CyO} \; ; \; \frac{Sb}{TM6B,Hu}$$

$$② \quad \frac{w}{w} \; ; \; \frac{P\{sca\text{-}Gal4,w^+\}}{P\{sca\text{-}Gal4,w^+\}} \; ; \; \frac{+}{+} \qquad ④ \quad \frac{w}{w} \; ; \; \frac{If}{Cyo,S} \; ; \; \frac{CxD}{TM3,Ser}$$

a) Write down the genotype of the embryos in which you can assess rescue of *shot*.

b) To obtain these embryos, you establish two parental stocks (one with *Gal4*-, one with *UAS*-construct) that can be maintained in the laboratory. Write down their genotypes:

c) Design the crossing strategies to obtain these two parental fly lines using the above stocks. Note that one CyO balancer carries the dominant Star (S) marker which generates rough eyes in heterozygous flies; for the *Dichaete* marker on CxD see task 7.