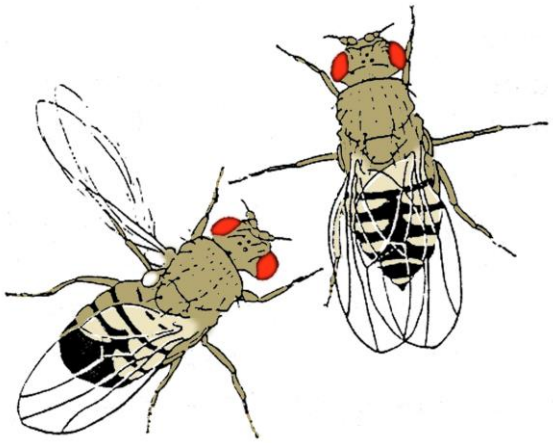
This document is one part of a *Drosophila* genetics training package, the entire strategy of which is described in detail elsewhere (see link).



General Tips

Tip 1: When solving these tasks, revisit the **manual** *(Suppl Mat. 1)* and **PowerPoint presentation** *(Suppl. Mat. 3)* for help which can be found **here**. 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: Note that elements on the same chromosome are separated by comma (*separable only upon recombination*), that sister chromosomes are separated by a horizontal line, and that different chromosomes are separated by semicolon: 1^{st} /Y (or 1^{st}) ; 2^{nd} / 2^{nd} ; 3^{rd} / 3^{rd}

Tip X: Remember that the 1^{st} chromosome always requires a male (X/Y) and a female (X/X) in each cross. The Y does not carry genes, the X is represented either by "+" or a mutation (e.g. "*w*").

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 tend to be lethal in homozygosis (*but look up B/+, B/B, B/Y in the manual*), whereas alleles starting with small letter are recessive and show a phenotype only in homozygosis.

Tip 6: The presence of a balancer chromosome in any given stock indicates that the balanced chromosome harbours at least one homozygous lethal mutant allele.

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.

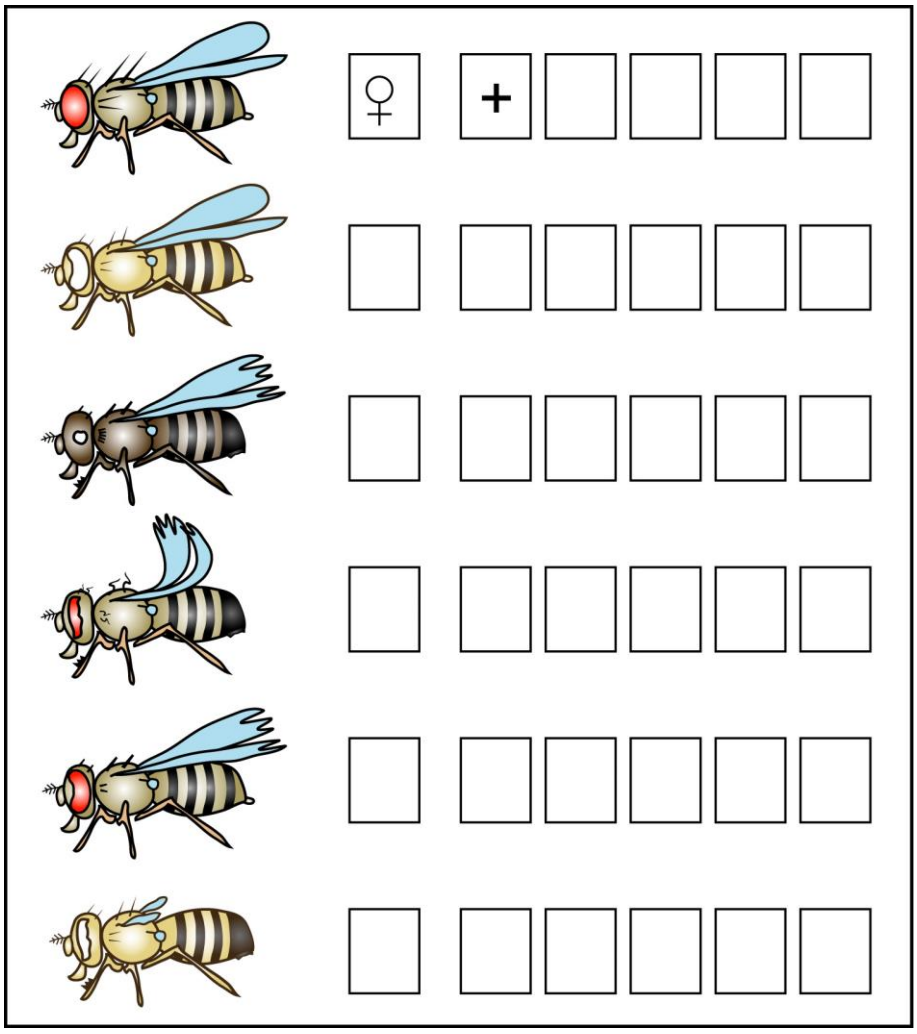
Tip 9: Many marker mutations may occur in the available stocks. Carefully consider which of these markers are relevant for the task. For complex chromosomes, use shorthand.

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

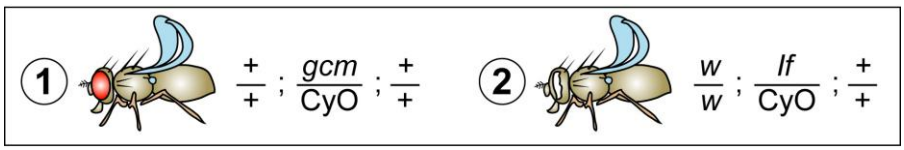
Tip 11: Recombination occurs randomly in the germline of non-balanced, transheterozygous female flies. Remember to balance potential recombination events and to use single crosses at the right step of the scheme (*see PowerPoint presentation which can be found **here***). Selecting the individuals which 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.

Tip 1: The first fly is a wildtype female.



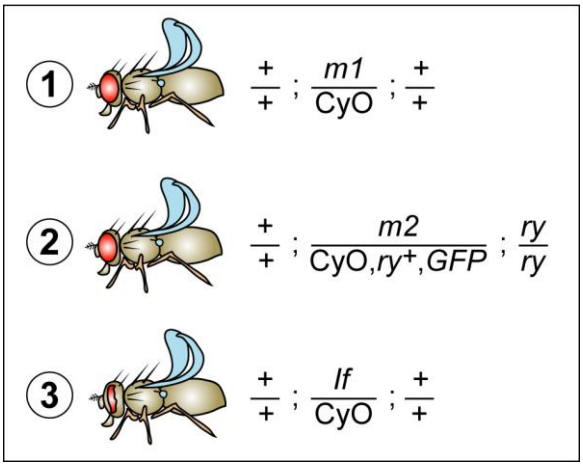
Task 2: You keep a fly stock that carries a homozygous lethal, recessive *gcm* mutant allele and is wildtype for the *white* locus on its first chromosome (stock 1). However, for a recombination experiment with a P-element line you need *gcm* in a *white* mutant background.



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

b) Using stocks 1 and 2, 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 which you keep in a fly stock over a recessive, homozygous lethal mutation *m2* (stock 2). You have currently no microscope to distinguish that CyO balancer by its GFP-expression and it carries no further markers that would distinguish it from normal CyO.



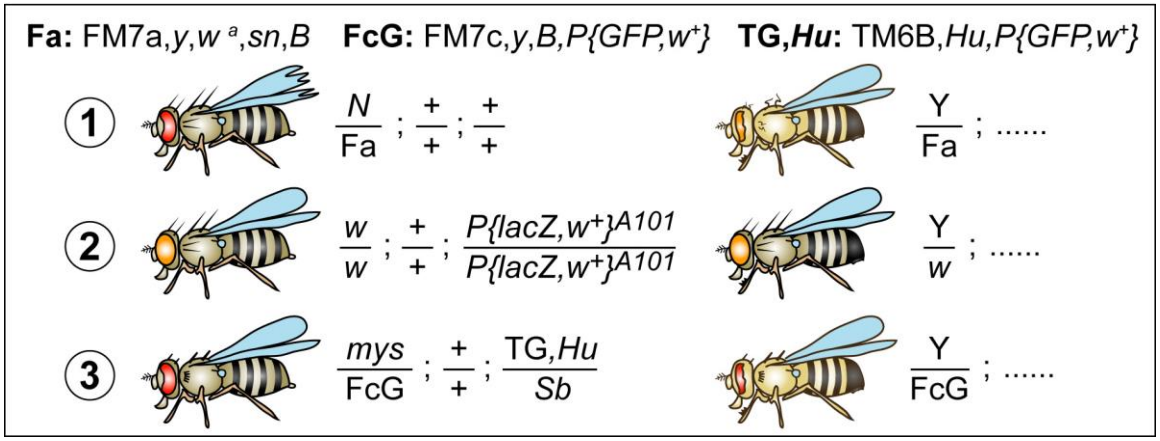
Tip 1: Be aware that *m1* and *m2* are recessive mutations. Make sure that you can follow these chromosomes safely throughout the mating scheme.

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) Making potential use of the three stocks provided, design a safe strategy by which you can bring the *m1* mutation over the GFP-expressing CyO balancer.

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.

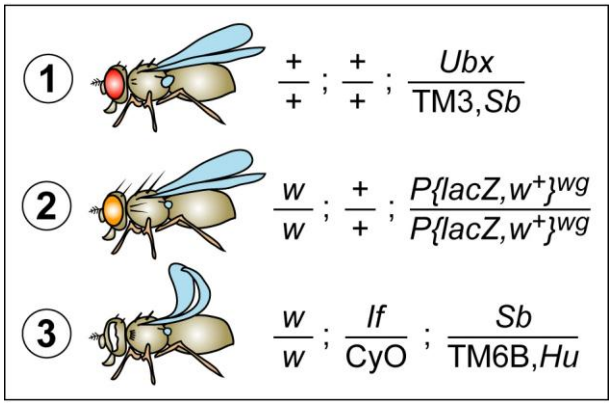
a) Why do females of stock 1 have red eyes, considering that Fa carries the w^a marker?

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

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

d) Potentially using the three stocks provided, 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; stock 1) in heterozygosis, but needs to be kept above balancer because it 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+)wg* enhancer trap insertion (stock 2). You maintain *Ubx* and *P(lacZ,w+)wg* as separate stocks in the laboratory, hence need to recombine them before you can perform the experiment.



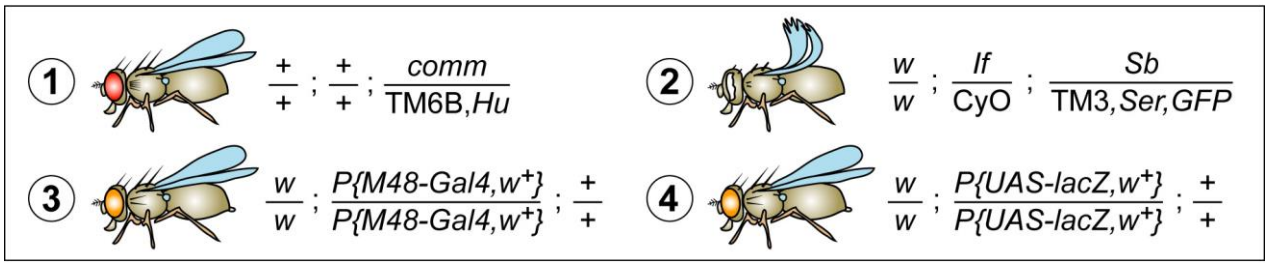
a) How important are *If* and CyO in stock 3 for your cross?

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

c) Write down the genotype of the stock you want to build that gives rise to the embryos in (b).

d) Making potential use of the three stocks provided, design a crossing strategy to generate the fly stock in (c).

Task 6: The *M48-Gal4* P-element insertion (stock 3) drives Gal4 expression in a subset of neurons in the CNS. Stock 4 carries the *UAS-lacZ* P-element insertion. If stocks 3 and 4 are crossed together, cell bodies and axons of the *M48-Gal4*-positive neurons will express the *lacZ* gene (*see the manual for explanations about the Gal4 / UAS expression system*) which can be visualised with X-Gal or anti-β-Gal staining[1]. You would like to test whether the axonal pattern of *M48-Gal4*-positive neurons is altered in *comm* homozygous mutant embryos (see stock 1). 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.



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) Write down the genotype of the embryos you would want to analyse.

b) Write down the genotypes of the two stable parental stocks that need to be crossed to give rise to the embryos in (a).

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

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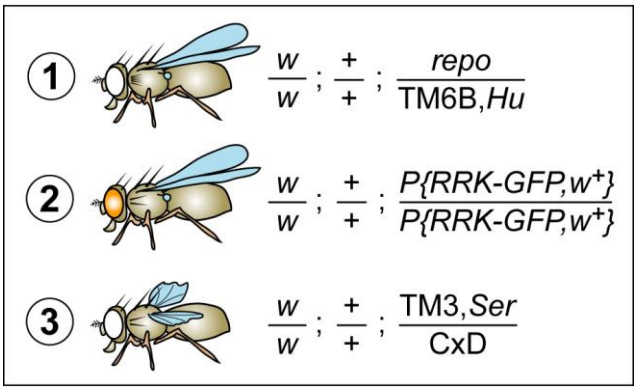
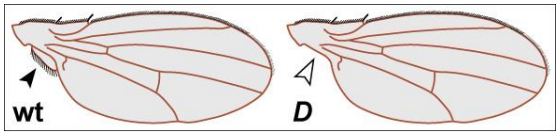
[1] Note that the *lacZ* gene from *E. coli* gives rise to the β-galactosidase enzyme, the presence of which can be detected via the lacZ colour reaction or using antibodies against the protein.

Task 7: The *P{RRK-GFP,w⁺}* insertion (stock 2) drives GFP expression in a subset of neurons. Visualising GFP, you want to study whether the morphology of *P{RRK-GFP,w⁺}*-positive neurons is altered in *repo* homozygous mutant embryos. For this you need to recombine the homozygous viable *P{RRK-GFP,w⁺}* insertion 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 identified by loss of the alula (arrow heads); note that flies usually hold out their wings but this is not a reliable indicator.



a) Write down the genotype of the embryos you want to analyse.

b) Making potential use of the fly stocks above, design a mating scheme to generate a stable stock carrying a recombination of *repo* and *P{RRK-lacZ,w⁺}* on the third chromosome. Make sure that both the mutation and the P-element are present, for example by performing suitable back-crosses.

Task 8: You use a mutant stock from the "olden days" carrying the embryonic lethal mutant allele m^1 which has originally been genetically mapped using the homozygous viable "rucuca" multi-marker chromosome: ***ru*** ^{1} h^1 th^1 ***cu*** ^{1} sr^1 e^s ***ca*** ^{1} (see a whole collection here). The recessive marker mutations on rucuca are as follows:

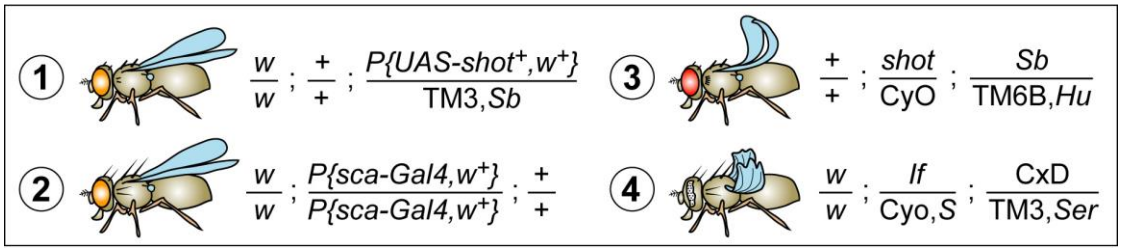
- *ru*^{1} (61F): homozygotes have a week rough eye phenotype
- *h*^{1} (*hairy*; 66D): extra micro-chaetae are found along wing veins (predominantly L2) and on the wing membrane
- *th*^{1} (*threat/Diap1;* 72D): the arista lacks all lateral branches
- *cu*^{1} (*curled*; 86D): wings are curved upward, the body color is dark, postscutellar bristles are erected and crossed
- *sr*^{1} (*stripe*; 90E-F): the trident colour pattern on the notum is replaced by a broad light grey stripe
- *e*^{s} (*ebony*^{sooty}; 93C-D): dark body colour
- *ca*^{1} (*claret*; 99C): mutant flies have reduced red pigment in the eye

Your m^1 mutant stock is described as m^1 *th, cu, sr, e*, *ca* / TM6B*, Hu*. You are concerned that the marker mutations have modifying effects on the m^1 mutant phenotype and decide to use recombination to get rid of the markers. Describe your strategy and use the following stocks to design a suitable mating scheme:

- stock 1: ***m*** ^{1} *th, cu, sr, e*, *ca* / TM6B,*Hu*
- stock 2: wildtype
- stock 3: m^2/TM3,*Sb*
- stock 4: *ru h th cu sr e ca* / *ru h th cu sr e ca* (the rucuca stock)

Task 9: You have identified the novel 2nd chromosomal *shot* mutation (stock 3) which, when homozygous, is lethal and displays 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 transgenic stock carrying a *P{UAS-shot⁺,w⁺}* insertion on the 3rd chromosome; unfortunately the insertion turns out to be lethal in homozygosis (stock 1).
- 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 (stock 2).



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 the *Gal4*-, one with the *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 making use of 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.

Now you are ready to take the test exam:

- go to: www.coursesites.com
- log on: username "flyfacility", password "prokop"
- click "Fly Facility" in the "My courses" box
- read the texts carefully, then click on "*Drosophila* Genetics Assessment" and start