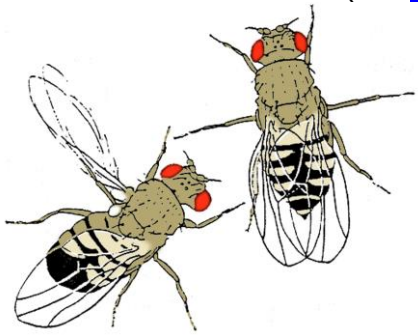
This document is one part of a *Drosophila* genetics training package, the entire strategy of which is described in detail elsewhere (see link).



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 4 Remember that crosses always require a male (X/Y) and a female (X/X). The Y does not carry genes, the X is represented either by "+" or a mutation (e.g. "*w*").

Tip 5 Always check carefully whether it will have implications for the next generation(s) if you choose male/female gender from one or the other genotype involved in your cross; this is important for first-chromosomal genetic loci and balancers (hemizygous in males but present with 2 alleles in females), or to allow/prevent recombination which takes place only in females.

Tip 6 To simplify matters in these exercises, mutant allele names starting with capital letter always cause a visible phenotype in heterozygosis and are lethal in homozygosis (*be aware of intermediate traits: see B/+, B/B, B/Y in the manual*); alleles starting with small letter are recessive and show a phenotype only in homozygosis.

Tip 7 The presence of a balancer chromosome in stocks provided for a task indicates that the sister chromosome harbours at least one homozygous lethal mutant allele; absence of a balancer indicates homozygous viability.

Tip 8 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 9 P-elements carrying expression constructs may display a difference in expression strengths between hetero- *versus* homozygous insertions; this will be irrelevant here.

Tip 10 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 11 Make sure you distinguish balancers from normal chromosomes with marker mutations.

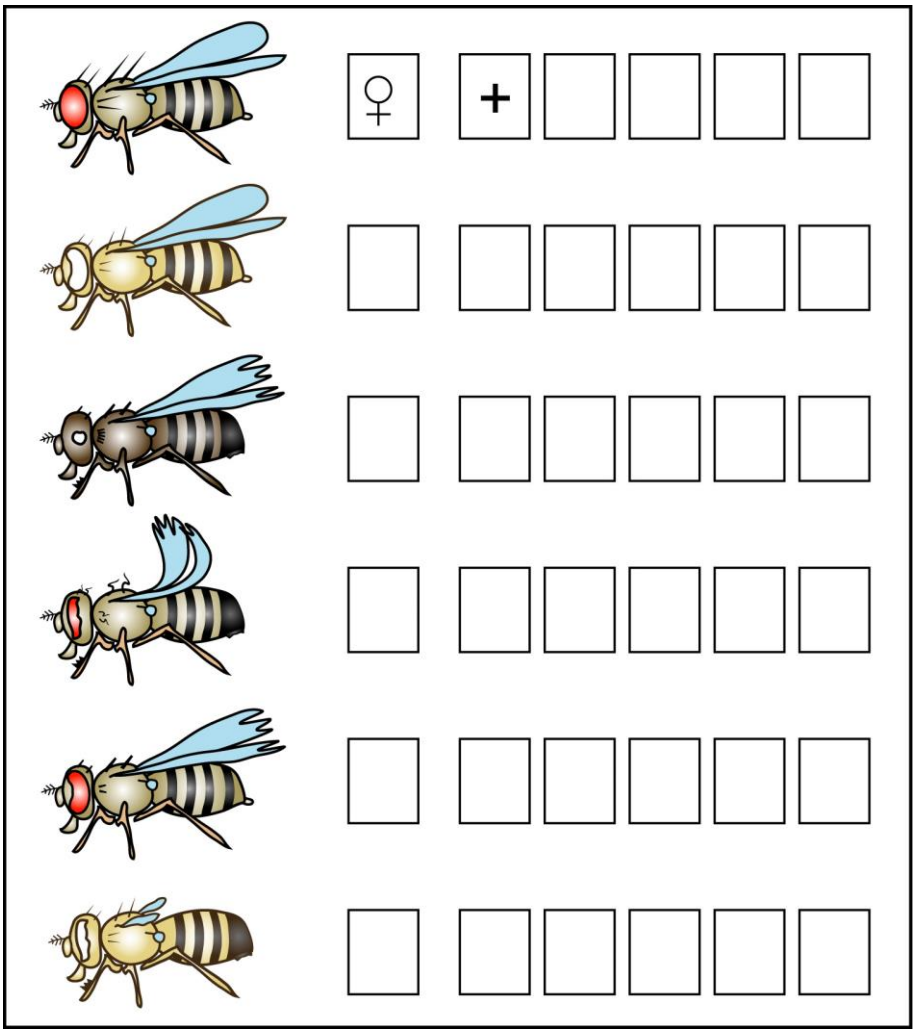
Tip 12 Recombination occurs randomly in the germline of non-balanced, trans-heterozygous female flies in F1. Always cross these females to balancer males and use single crosses for the F2 offspring (*see PowerPoint presentation which can be found **here***). Selecting the individuals carrying recombinant chromosomes is the actual challenge in these tasks.

Tip 13 Crosses involving different recessive alleles on a given chromosome require asymmetric constellations: in the offspring of "♀ *m1*/CyO X ♂ *m2*/CyO" you will not be able to distinguish *m1* from *m2*; but it works for this cross: "♀ *m1*/CyO X ♂ *m2*/*If*".

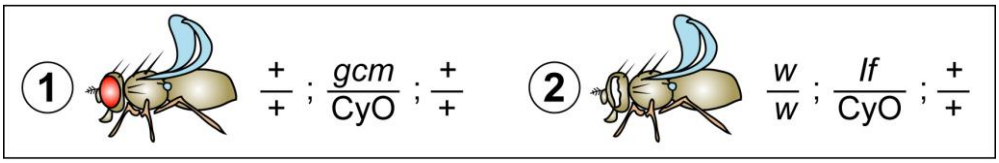
Tip 14 If your task is to bring a specific chromosome over a certain balancer, make sure the balancer is crossed in in the very last step!

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.



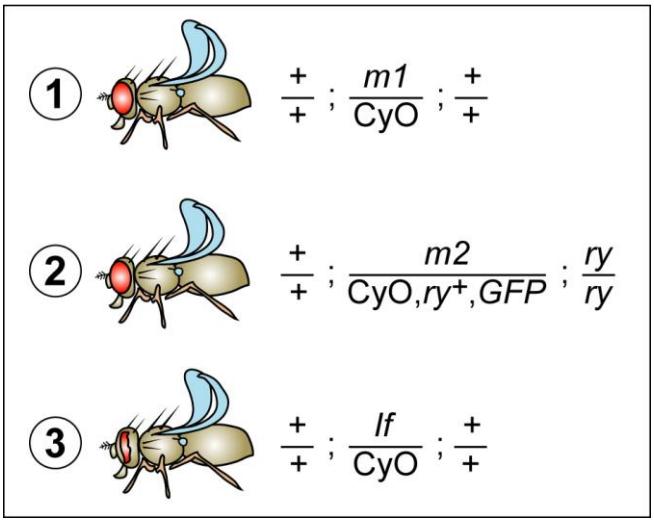
Task 2: You keep a fly stock that carries the homozygous lethal, recessive mutant allele *gcm* and is wildtype for the *white* locus on its first chromosome (stock 1). However, for a recombination experiment with a P-element line you need *gcm* in a *white* mutant background. For this, you have a suitable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



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* with the homozygous lethal *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.



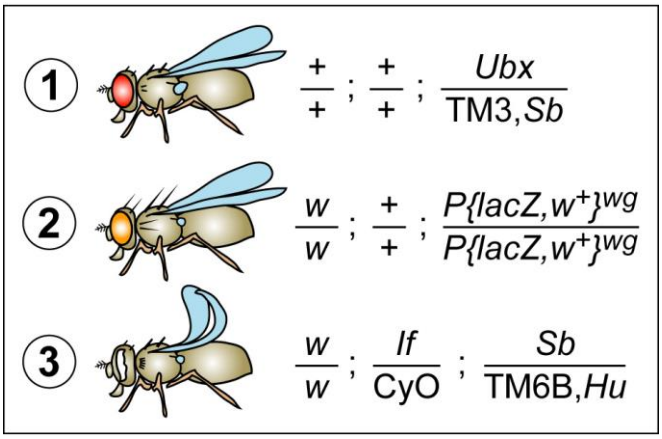
Tip 1: Be aware that *m1* and *m2* are recessive mutations. Make sure that you can follow these chromosomes safely throughout the mating scheme.

a) Is the recessive eye colour marker *ry* (stock 2) important during this cross?

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

c) 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: A specific mutant allele of the 3rd chromosomal *Ubx* gene causes a heterozygous dominant phenotype (enlarged halteres; stock 1), but is embryonic lethal in homozygosis. You want to study whether homozyous *Ubx* affects the segmental lacZ expression pattern [1] produced by 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.



Tip: Re-read Tip 12 on page 1!

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 **stable fly stock** you will use to generate the embryos in (b).

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

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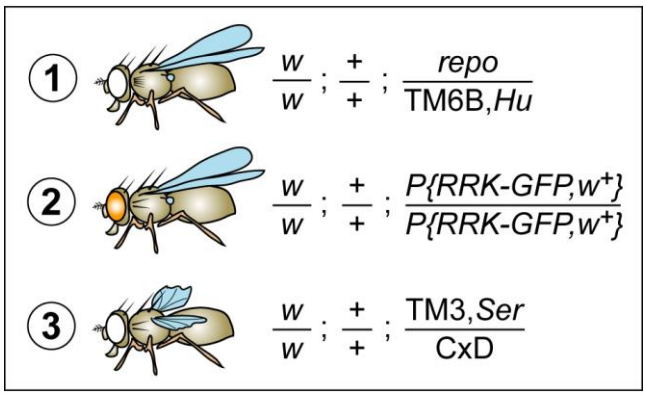
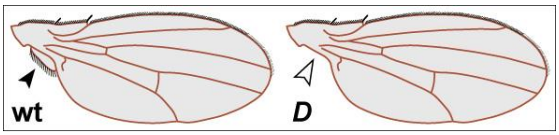
[1] Note that the *lacZ* gene from *E. coli* gives rise to the β-galactosidase enzyme, the presence of which can be detected via the lacZ colour reaction or using antibodies against the protein.

Task 5: The *P{RRK-GFP,w⁺}* insertion (stock 2) drives GFP expression in a subset of neurons. Visualising *P{RRK-GFP,w⁺}*-labelled neurons with a fluorescent microscope, you want to study whether their morphology is altered in *repo* [2] 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: Re-read Tip 12 on page 1!

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. In this task, treat the *CxD* chromosome like a dominant marker mutation.



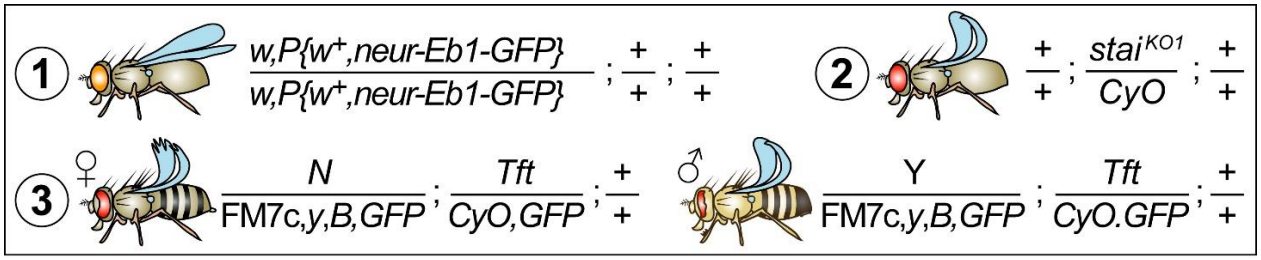
a) Write down the genotype of the embryos you want to analyse.

b) Write down the genotype of the stable stock that gives rise to embryos in (a):

c) 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.

[2] In *repo* mutant embryos, glia cells are absent, and the experiment explores to which degree the presence of glia cells is important for neurons to form their neurites appropriately.

Task 6: You hypothesise that the Stathmin (Stai) protein is required for microtubule polymerisation. You want to test this hypothesis in primary neurons that are homozygous for the *stai*^{KO1} loss-of-function mutant allele (*stock 2; shorten in your answer to 's'*). In these neurons, you want to monitor microtubule polymerisation via live imaging using the polymerisation marker Eb1::GFP driven by a neuronal promoter (*neur-Eb1-GFP*). For this, you have a first chromosomal P-element insertion carrying that construct (*stock 1*; *shorten to 'PE'*). To combine *stai*^{KO1} and the P-element into the same flies, you have a double-balanced stock (stock 3) carrying a first-chromosomal FM7c green balancer (*you may shorten in your answer to 'FG'*) kept over the homozygous lethal *Notch* (*N*) mutation, and a second-chromosomal CyO green balancer (*you may shorten in your answer to 'CG'*) kept over the homozygous lethal *Tft* marker mutation (*multiple hairs on the notum; stock 3*).



a) Write down the genotype of the embryos from which primary neurons will be generated:

b) Write down the genotype of the stable fly stock that will produce these embryos:

c) Making potential use of fly stocks 1 to 3, design a cross to generate the desired stable stock.

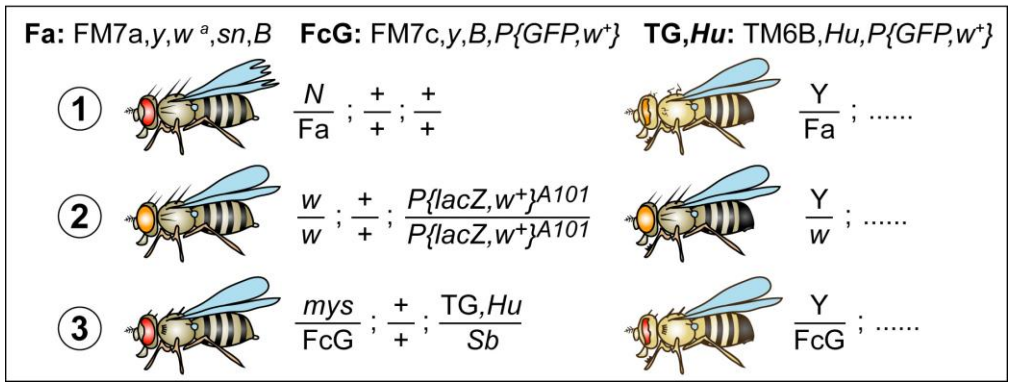
Tip 1: Note that *B*/+ has kidney-shaped eyes and *B*/*B* or *B*/Y have narrow eyes (see stock 3).

Tip 2: Note that the *Notched* mutation in heterozygosis causes *Ser*-like wing notches (stock 3).

Tip 3: Re-read Tip 5 on page 1.

Tip 4: This cross might require that you start two crossed in parallel. Label one cross with “P, F1, F2..”, the parallel one with “P’, F1’, F2’..”

Task 7: 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.



Tip1: Re-read Tip 5 on page 1! Carefully compare phenotypes of females (left) and males (right) of each stock.

Tip 2: *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 3: Note that the used balancers FM7a and FM7c carry different marker mutations, and that *mys* is a recessive lethal mutation with no heterozygous phenotype.

Tip 4: This cross might require that you start two crossed in parallel. Label one cross with "P, F1, F2..", the parallel one with "P', F1', F2'.."

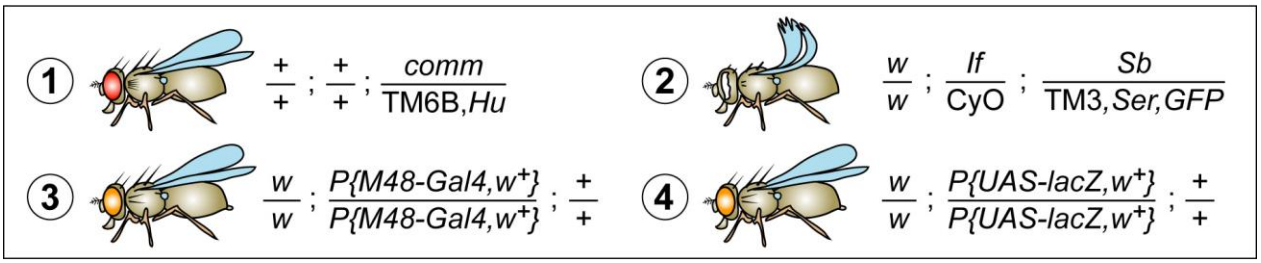
a) Why do females of stock 1 have red eyes, considering that Fa carries the w^a marker?

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 8: Stock 3 is a driver line carrying the *M48-Gal4* P-element insertion (stock 3) which expresses Gal4 expression in a subset of neurons in the CNS. Stock 4 is a reporter line carrying the *UAS-lacZ* P-element insertion[3]. 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. You would like to test whether the axonal pattern of *M48-Gal4*-positive neurons is altered in *comm* homozygous mutant embryos (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: Re-read Tip 8 on page 1!

Tip 2: Only one copy of the *Gal4*- and one copy of the *UAS*-construct are required to perform your experiment in *comm* mutant embryos.

Tip 3: If you solve the task for one P-element, the other P-element works analogously.

Tip 4: This cross might require that you start two crossed in parallel. Label one cross with “P, F1, F2..”, the parallel one with “P’, F1’, F2’..”

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

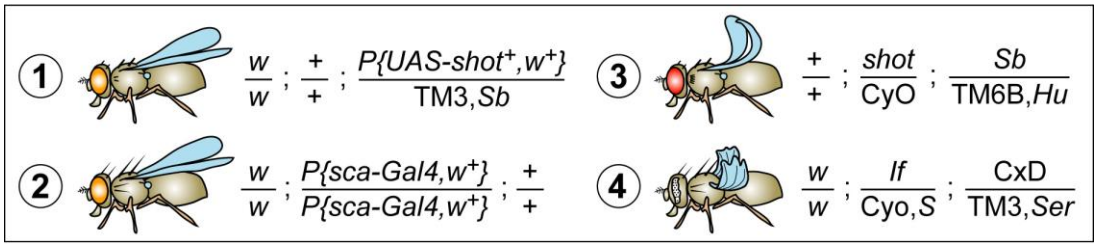
b) Write down the genotypes of the two stable parental stocks that need to be crossed to give rise to the embryos in (a).

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

[3] 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 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.

Task 10: 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 along wing veins (mainly 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 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)