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

Task 1: 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, unfortunately, harbours no further dominant genetic marker that would easily distinguish it from the normal CyO balancer chromosome. Currently, you keep the GFP-expressing CyO balancer in a fly stock carrying the recessive, homozygous lethal mutation *m2* (stock 2), and you can use this stock as a source for the desired balancer. 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.

stock 1	stock 2	stock 3
$\frac{+}{+}$; $\frac{m1}{CyO}$; $\frac{+}{+}$	$\frac{+}{+}$; $\frac{m2}{CyO,ry+,GFP}$; $\frac{ry^-}{ry^-}$	$\frac{+}{+}$; $\frac{If}{CyO}$; $\frac{+}{+}$

Tip 1: Be aware that *m1* and *m2* are recessive mutations. How do you make sure that you can follow these chromosomes safely throughout the cross without risking to mix them up?

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

Task 2: The *M48-Gal4* P-element insertion stock (stock 3) shows Gal4 expression in a subset of commissural neurons in the CNS, the axons of which can be visualised with the help of a *UAS-lacZ* insertion stock (stock 4) and X-Gal staining. You would like to test, whether the *commissureless* mutation (*comm*; stock 1) in homozygosis affects the axonal pattern of the *M48-Gal4*-positive neurons. To be able to select the *comm* mutant animals, the mutation should be kept over a GFP-expressing *TM3* balancer (stock 2), the presence of which can be easily spotted under a fluorescent microscope.

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

b) What are the genotypes of the parents of the embryos in (a)?

c) Design crosses to generate the parental strains in (b) as established maintainable fly strains, using the following stocks as source:

stock 1	stock 2
$\frac{+}{+}$; $\frac{+}{+}$; $\frac{comm}{TM6B, Hu}$	$\frac{w^-}{w^-}$; $\frac{If}{CyO}$; $\frac{TM3, Ser, GFP}{Sb}$
stock 3	**stock 4**
$\frac{w^-}{w^-}$; $\frac{M48\text{-}Gal4, w^+}{M48\text{-}Gal4, w^+}$; $\frac{+}{+}$	$\frac{w^-}{w^-}$; $\frac{UAS\text{-}lacZ, w^+}{UAS\text{-}lacZ, w^+}$; $\frac{+}{+}$

Tip 1: The w^+ on the P-elements gives orange eyes in *white* mutant background, the endogenous *white* locus on the first chromosome gives red eyes.

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

Task 3: You keep a fly stock that carries a homozygous lethal, recessive *gcm* mutant allele and is wild type for the *white* locus on its first chromosome. However, for a recombination experiment with a P-element line you need a *white* mutant background. Design a strategy by which you can combine the recessive non-lethal white mutation with the *gcm* mutation.

mutant stock 1	balancer stock
$\frac{+}{+}$; $\frac{gcm}{CyO}$; $\frac{+}{+}$	$\frac{w^-}{w^-}$; $\frac{If}{CyO}$; $\frac{+}{+}$

Task 4: You want to recombine the homozygous viable P-element insertion *P{lacZ,w}*^{RRK} with the recessive, homozygous lethal *repo* mutation. Both are on the third chromosome but kept in two separate fly stocks.

a) Design a scheme using recombination in which you bring both genes onto the same chromosome, stabilised over a balancer chromosome.
b) How do you check for the presence of mutation and P-element?

mutant stock	P-element line	balancer stock
$\frac{w^-}{w^-}$; $\frac{+}{+}$; $\frac{repo}{TM6B, Hu}$	$\frac{w^-}{w^-}$; $\frac{+}{+}$; $\frac{P\text{-}(lac\text{-}w^+)^{RRK}}{P\text{-}(lac\text{-}w^+)^{RRK}}$	$\frac{w^-}{w^-}$; $\frac{+}{+}$; $\frac{TM3, Ser}{CxD}$

Tip 1: *CxD* bears the dominant *Dichaete* marker, which is visible as loss of the alula (a part of the proximal wing); it is only a partial balancer.
Tip 2: Recombination simply occurs during meiosis in the germline of female flies. Selecting the chromosomes in which recombination has occurred is the actual challenge in this question.
Tip 3: The w^+ marker of *P-(lac-w*$^+$*)*^{RRK} causes orange eyes in *white* mutant background.

Task 5: You want to carry out experiments with a P-element insertion *P(lacZ,w*$^+$*)* on the third chromosome (stock 2) in combination with a dominant, homozygous lethal mutation, likewise on the third chromosome (stock 1). You need to recombine both onto the same chromosome. Design a suitable crossing scheme. You may make use of stock 3.

stock 1	stock 2	stock 3
$\frac{+}{+}$; $\frac{+}{+}$; $\frac{M}{TM3, Sb}$	$\frac{w^-}{w^-}$; $\frac{+}{+}$; $\frac{lacZ,w^+}{lacZ,w^+}$	$\frac{w^-}{w^-}$; $\frac{If}{CyO}$; $\frac{Sb}{TM6B, Hu}$

Tip 1: The dominant mutation *M* shows a phenotype in heterozygosis consisting in gaps in wing veins.

Tip 2: The w^+ on the P-element produces an orange eye colour in white mutant background.

Task 6: You have identified a novel 2nd chromosomal mutation called *shot* which, when in homozygosis, correlates with an exciting brain phenotype. You want to proof that the brain phenotype is indeed caused by loss of *shot* function. To this end you perform a gene rescue experiment in embryos. This experiment involves that you express the cloned *shot* gene in the nervous system of *shot* homozygous mutant embryos, with the aim of recovering normal brain morphology.

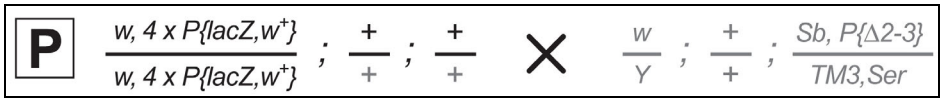
- For this you have generated a *P{UAS-shot,w+}* transgenic line (stock 1) where the P-element is inserted on the third chromosome; unfortunately the insertion turns out to be lethal in homozygosis.
- You hold a suitable transgenic fly stock carrying the *P{sca-Gal4,w+}* insertion on the second chromosome (stock 2); this Gal4 line targets expression to the nervous system.

> ➢ stock 1: ***w/w; +; P{UAS-shot+,w+}/TM3,Sb*** (orange eyes in *w* mutant flies; shorten to $\mathbf{P^{U}w^{+}}$)
> ➢ stock 2: ***w/w; P{sca-Gal4,w+}/P{sca-Gal4,w+};+/+*** (orange eyes in *w* flies; shorten to $\mathbf{P^{G}w^{+}}$)
> ➢ stock 3: ***+/+; shot/CyO; Sb/TM6B,Hu***
> stock 4: ***w/w; If/CyO,S; CxD/TM3,Ser*** (*S*: rough eyes; *D*: lack of alulae from wing hinges)

Design the genetic crosses required for this task, using the above stocks. To make this task easier, answer first the following questions:

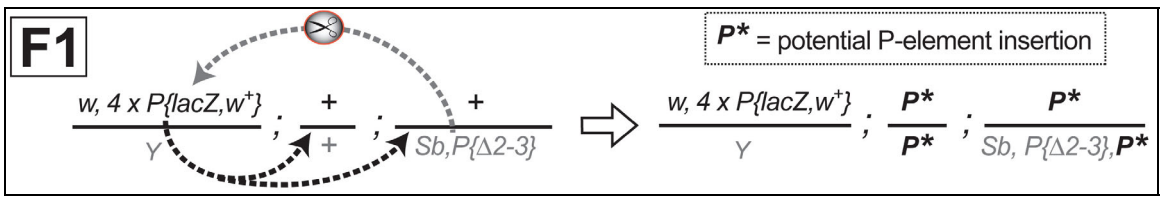
a) Write down the genotype of the embryos in which you can assess rescue of the *shot* mutant phenotype.
b) To obtain these embryos, you will have to establish two independent parental stocks that can be kept in the laboratory for future purposes. Please, write down the genotypes of these two parental stocks.
c) Design the crossing strategies to obtain these two parental fly lines using the above stocks.

Task 7: You carry out a P-element enhancer trap experiment, as mentioned in the recent presentation about transposable elements. For this, you cross females of a source stock (left in box) with males of a "jump start" strain (right in box):



- The source stock carries four P-elements "*P{lacZ,w+}*" on the first chromosome. It has orange eyes because it carries the *white* mutation, but the P-elements are marked with w^{+}.
- The jump start strain harbours a stable, non-excisable P-element insertion "P{Δ2-3}" with recombinase/transposase activity on the third chromosome, which also carries the *Sb* marker mutation. This stock has white eyes.

In F1, you select males against *Ser* and for *Sb*, i.e. you select males in which the P-element insertions are exposed to transposase activity (grey dashed arrow):



In these selected males, potential transposition events of "*P{lacZ,w+}*" into new genomic location are likely to happen (black dashed arrows), i.e. new P-element insertions MAY randomly occur on one or more locations on the 2nd and/or 3rd chromosome (P* indicates potential insertions). You want to isolate those fly lines that carry new insertions, stabilise them as stocks and determine the chromosome of insertion. This will take several steps:

Task 7.1: You hold the following triple-balancer stock in the laboratory:

$$\frac{w}{w} \; ; \; \frac{If}{CyO} \; ; \; \frac{TM3,Ser}{TM6B,Hu}$$

You cross females of this balancer stock to single males of the F1 cross (those carrying the potential P-element transposition events).

a) You carry out many parallel crosses of this kind. Why?

b) Write down all possible combinations of F2 flies and name the selection criteria for those individuals in which
   - any new P-element insertions have occurred (regardless of whether on 2nd and/or 3rd chromosome),
   - and transposition events are "stable" (balanced and no longer exposed to transposase activity)

c) Do you select males of females? Why?

Task 7.2: For the selected F2 animals carrying new stable P-element insertions you carry out a second cross with the triple-balancer stock. This cross will allow you to determine in F3 whether a P-element transposition has occurred onto the 2nd and/or 3rd chromosome. Write down all possibilities assuming that P* is either on the 2nd or the 3rd chromosome. Name the criteria which unequivocally inform you about the chromosome of insertion.