average percentage of misqualifications of the females was calculated to be 1.68% ± 0.26. This experimental error is in the same order as misqualifying male flies (Table 1 B), and mainly due to large sample sizes and time pressure.

The most important difference between *D. simulans* and *D. melanogaster* is the black pigmentation which runs to the ventral margin in *D. melanogaster* in various patterns (Figure 1 b,c,e-i), whereas the pigmentation border line in *D. simulans* makes an angle with the tergite margin. It forms a continuous line with the pigmentation border line in the seventh tergite (Figure 1 a). The abdominal pigmentation pattern with respect to the sixth tergite was found to be monomorphic in *D. simulans* in our population, and in at least two other world-wide different populations (Brasil: Gallo, 1973; USA: Thompson *et al.*, 1979) in contrast to *D. melanogaster* (David *et al.*, 1990; Robertson *et al.*, 1977; this paper). The seventh tergite in *D. simulans* was almost completely pigmented but for a small area adjacent to the sixth tergite and its ventral margin (Figure 1 a). However, like in *D. melanogaster*, some variation existed in the pigmentation pattern of the seventh tergite of *D. simulans* with only small spots like those of *D. melanogaster* in Figure 1 g. Robertson *et al.* (1977) described the locus *fap* (female abdomen pattern) to be residing on the extreme tip of the 3L chromosome, with some effects related to chromosome four. The *D. melanogaster* Groningen-FFF strain, used as a reference in electrophoresis, showed a pigmentation pattern in the sixth tergite like *D. simulans* (Figure 1 d), but in contrast the seventh tergite was pigmented differently. This strain was homozygous for Adh^F and $\alpha Gpdh^F$ on the second chromosome and Odh^F on the third chromosome. This might be the reason why the Groningen-FFF strain with some homozygosity on the third chromosome was different from the other *D. melanogaster* strains.

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Szakmary, A. Laboratory of Molecular Genetics, NIEHS, NIH, P.O.Box 12233 Research Triangle Park NC 27709. A short guide to scoring mosaic cell clones in the *Drosophila* eye.

Somatic Mutation and Recombination tests are quick, simple and easy to perform. Because of their versatility more and more researchers in different fields use them for different purposes. Several different systems have been developed. Some use bristles and color on the body (*e.g.*, markers *y* and *sn*) or trichomes on the wings (markers *mwh* and *flr*). Mosaicism in the eye allows a lot of variation due to the large number of possible genetic markers. Still the best way to learn to identify mosaic clones correctly is to visit a laboratory where it is done routinely. This short guide is intended to help when eye mosaicism should be used and a visit is not possible. Photographic examples will be made at the image section of FlyBase.

We use a regular dissecting microscope (binocular) at a magnification of 35-75×. The lights are common light boxes with swan neck point lights. Position the lights on the same side giving you light and shade but minimum reflection. Adjust brightness to whatever feels comfortable over a long time period.

Scoring mosaic clones is certainly subjective; therefore, consistency is more important than absolute accuracy. One rule of thumb is, if you are in doubt whether you have a mosaic clone exclude it. When scoring do record spot sizes, *i.e.*, number of mutant ommatidia in a clone. This can give you additional information, *e.g.*, age of larvae at time of treatment, and serve as an additional control, because the distribution over size classes (2, 3-4, 5-8, 9-16, 17-32 etc.) should fit a Poisson-distribution (often only one-sided). However, factors such as metabolic activation or stability of the mutagen can distort it.

The flies are scored initially in a solution of 90% EtOH, 9% water, 1% Tween20. Once the flies are dead, this solution can be replaced by 2% aqueous Tween20. I prefer dark, matte tiles because they are easier on the eyes. Focus on the highest points of the eyes and bring other parts into focus by lifting the plate on one side. Position and move the flies with a thin but stiff brush. Turning the flies from light into the shadow and back will reveal mosaic clones that would not be visible in direct bright light. The shape of ommatidia and spots is important to distinguish them from artifacts like chemical burns or developmental disturbances. Because ommatidia are hexagonal in shape small spots have typical shapes of multiple hexagons. The color of spots depends not only on genotype but also on the size of the spots in the case of the white gene as marker in a wild-type background only large mosaic clones appear white. The clones are actually

pigmentless and transparent. Small clones, therefore, appear dark like a tunnel entrance. Other kinds of spots can be clones of other genotypes like roughest or chocolate or artifacts as mentioned before and should be excluded. Insect eyes do contain hairs that in the solution sometime stick together and appear like small spots, changing the angle will help to detect this. Two mosaic clones should be scored as single event if they are separated by 4 normal ommatidia or less and as two independent events if separated by more. Spots larger than 64 ommatidia are rare and can be categorized rather than their size accurately determined (as A = 64-128, B = 128-256, C = half eye, or D = full eye). They should be excluded from mean spot size calculations. Over time more dust and other debris accumulates on the eyes which can be mistaken for a mosaic clone. Brushing over the spots, therefore, becomes important. The age of the flies scored can be important too. 3-4 day old flies are usually best.

Mosaic clone frequencies are generally given as mosaic clones per 100 eyes scored. Other frequencies, *e.g.*, mosaic clones per eye or eyes with clones per eyes scored, are generally less useful. Ideal sample sizes depend very much on the purpose of the experiments and the mosaic clone frequency. To classify a chemical as non-mutagenic, a minimum of 2000 scored eyes will be necessary, while clone frequencies clearly above control level can be significant with much less. I often score till reaching 100 mosaic clones in experiments comparing different genotypes with a standard mutagen. Historic controls are useful to observe stocks over a longer period of time but cannot replace concurrent controls (equal in size to treated groups). However, clone frequencies that do not exceed the highest values in the historic controls should not be considered positive no matter how low the concurrent control frequency is (for statistics see Frei and Würgler, 1995). The variations between repeats can be fairly large compared to other types of experiments due to slight variations in age of cultures at time of treatment or feeding behavior.

References: Frei, H., and F.E. Würgler 1995, Mutat. Res. 334 : 247-258.

Crisp, Jonathan, and John Merriam. Department of Molecular, Cell and Developmental Biology, University of California, Los Angeles, CA 90095-1606. Efficiency of an F1 selection screen in a pilot two-component mutagenesis involving *Drosophila melanogaster* misexpression phenotypes .

Introduction: One of the main goals of genetic research concerns the identification of genes and their role in development. The most common method for determining the significance of a gene involves the loss of function approach in which the result of gene inactivation (deletion, mutation, etc.) is observed. In contrast, the gain of function approach utilizes misexpression of a genetic region or putative gene in order to recognize new genes. The idea used here is to hop around a P element with the promoter and Gal4-UAS of pUAST (Brand and Perrimon, 1993) and expose new insert sites to Gal4 regulation. In this way, a Gal4-dependent phenotype will result if the P element lies close enough to an endogenous gene and if the endogenous gene protein has an effect on the cells expressing Gal4.

For this purpose a P element construct was made with the promoter and UAS of pUAST located at the 5' end of the construct oriented to transcribe leftwards away from the P element (Merriam, Harrington, Merrill, Phillips, Warden, Martin-Blanco, Nygren and Poole, in prep.). This new construct carries a genomic y[+] sequence as its marker; it was transformed and an X linked strain (1-37) selected for further studies. This strain, 6.11 #4, is available from the Bloomington stock center.

Initial experiments with this (responder) strain recovered 730 exceptional y[+] sons of independent origin from 2540 fathers cultured separately. When crossed to females from a strain carrying the sca-Gal4 driver (y w; {w[+], sca::Gal4}/CyO virgins), 102 (13.8%) of the cultures showed a Gal4-dependent phenotype in the y+ w+ progeny.

In this paper a pilot two-component mutagenesis involving misexpression phenotypes was performed in which the steps traditionally used to produce the F1 generation were combined with a F2 screen. This screen has been termed an F1 screen; it enabled selection of phenotypes to be applied directly to the F1 generation instead of the F2 generation. The questions asked here are whether the F1 screen will find the same number of "keeper" inserts as the F2 screen and how much savings in time are realized.

Materials and Methods: The pilot two-component mutagenesis performed in the F2 screen involved females with the responder P element located on the X chromosome (Figure 1), which have grey bodies due to the y+ gene insert on the P element and white eyes (yw[UAS, y+]/yw[UAS, y+]) (ref Merriam, *et al.*, unpublished). The males have wild type red eyes and kinked bristles linked to the 2-3 transposase gene (Ki 2-3/Ki 2-3). Once this cross is performed, the offspring will have both the responder P element and transposase, creating a situation in which the P element can mobilize ("hop") to new, random locations. The males of this offspring (yw[UAS, y+]/Y; Ki 2-3/+) are crossed to tester females with white eyes and yellow bodies (yw). Any male offspring from this cross with grey bodies are indicative of the P element "hopping" from the X chromosome to an autosome, since the original y+ insert was on the X chromosome.