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.

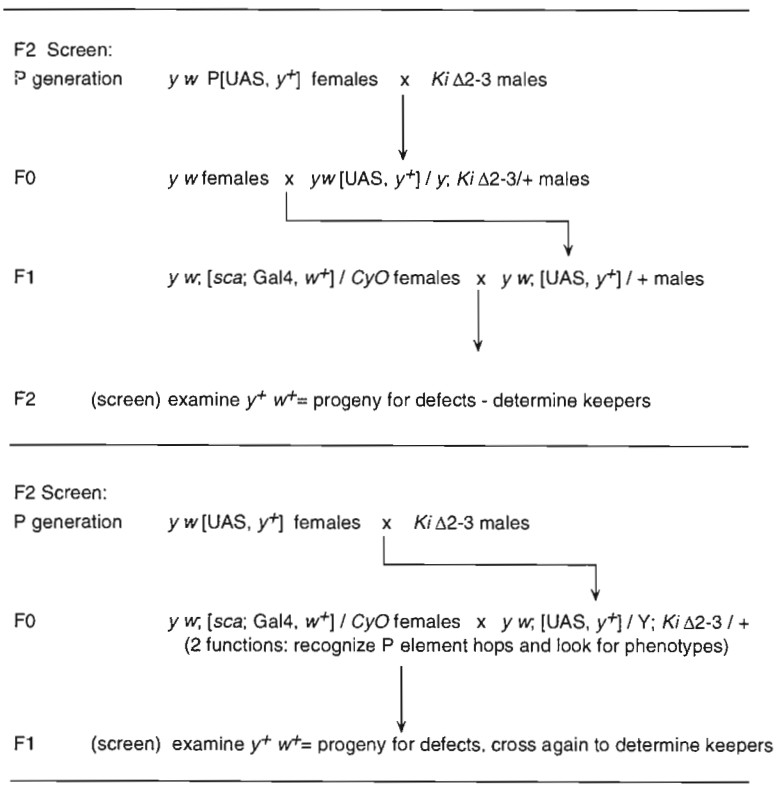

Figure 1. Comparison of F2 and F1 screens.

These grey bodied males are then crossed to females heterozygous for curly wings and a chosen driver element such as scabrous, along with the GAL4 gene (yw; [sca: GAL4, w+]/CyO) in order to recognize "keeper" phenotypes. A keeper phenotype is one in which the misexpression of the region adjacent to the P element insertion has led to an observable phenotype. A heterozygous driver with GAL4 allows lethal jumps to be preserved, for if the responder integrated next to a gene that was lethal when misexpressed, this would not produce any offspring while the dark bodied curly flies that did not receive the GAL4 gene would be viable. Due to the temperature sensitivity of GAL4, all crosses for both F1 and F2 screens were incubated at 25°C.

In the F1 screen (Figure 1), instead of crossing the F0 males to a yw female in order to isolate the P element jump and eliminate the Ki phenotype, the males were crossed to the driver element in a yw background. However, only the non-Ki males observed to contain a phenotype different from wild type were used for the next step. The rules used to screen the F1 progeny involved careful inspection of the scutum and scutellar bristles. Any deviation from the observed wild type pattern was noted as a phenotype that was different from wild type, and this made up one of the three classes of y+ flies representing a "hop" that were kept. The other two distinguishing features of flies that were selected for in the F1 generation included the y+w Cy progeny that did not have any y+w+ brothers, indicating a possible lethal phenotype, and the y+w Cy brothers of those y+w+ progeny displaying a phenotype. No Ki progeny were selected because the Ki gene affected scutum and scutellar bristle appearance, creating false phenotypes. Thus, the F1 screen combined the recognition of P element hops and the phenotype identification into one step.

The next step in the F1 screen involved test crossing selected males to a new driver (sd-Gal4) females and examining the F2 progeny for keeper phenotypes. Ths savings represented is that only a few F1 males are so screened, compared with the "F2 screen." The rules used to select keepers included a detailed inspection of the scutum and scutellar bristles along with the wings, since the sd driver is involved in the development of these areas. If four or more F2 progeny exhibited the same phenotype at the time of the initial screen, the jump was called a keeper. The number of progeny exhibiting a specific phenotype is given in Table 2. Keepers were determined in the same manner in the F2 screen.

Once the keepers were identified on the basis of their phenotype in combination with the sd driver, analysis of the progeny revealed the chromosome location of the y+ insert. If the y+ insert were on the second chromosome, the two progeny would either be y+w+ or w+ Cy, with no y+ Cy. The progeny with the insert on the third chromosome would show all possible offspring,and thus the insert location was determined for the eight keepers (Table 2).

Results and Discussion: Refer to Table 1 and Table 2 for the data of the two screens. When evaluating the effectiveness of the F1 selection imposed screen in comparison to the F2 selection imposed screen, the number of keeper phenotypes must be considered along with the effort required to generate these keepers. Selection at the next round generated 8 keepers out of 45 F1 crosses, while the F2 screen generated 102 keepers out of 730 F2 crosses, having the respective ratios of 18% to 14% keepers/successful hop crosses (Table 2). It should be noted that there were no lethal mutations observed in the F1 screen, while 24 lethal mutations were observed in the F2 screen.

Table 1.

	F1 Screen	F2 Screen
Number of initial fathers	120	2540
Number of F1 crosses	45	730
Number of F2 crosses	8	102
Ratio of keepers / F1 crosses	0.18	0.14

Table 2.

Initial Phenotype of F1(*sca* driver)	F2 Keeper Phenotype (*sd* driver)	Chromosome location
\$5, missing scutellar	small crumpled wings	2 (homozygous viable)
\$6, missing scutellars	small crumpled wings	2 (homozygous viable)
\$24, smaller wings	jagged wing edges	3 (homozygous viable)
\$26, *w Cy* heterozygote	crumpled wings	2 (homozygous viable)
\$27c, *w Cy* heterozygote	crumpled wing edges	2 (homozygous viable)
\$38, missing scutellar	wings 2/3 size	3 (homozygous viable)
\$77, *w Cy* heterozygote	small crumpled wings	2 (homozygous viable)
\$115, wrinkled wing	wrinkled wing	2 (homozygous lethal)

Table 3. Estimated time in minutes required to generate a keeper.

Mobilization cross (F0) (1 min per cross)
F1 screen total 120 +120 (transfer)= 240 mins.
F2 screen total 2540 mins.
F1 score (4 min)
F1 screen total 480 mins.
F2 screen total 10288 mins.
F1 cross (2 min)
F1 screen total 64 x 2 = 128 mins.
F2 screen total 1460 mins.
F2 score (7 min)
F1 screen total 224 mins.
F2 screen total 5110 mins.
Totals
F1 screen 1072 mins.
F2 screen 19398 mins.
Minutes per keeper
F1 screen 134
F2 screen 190

The effort required to generate each keeper was calculated using time estimates for each step in the respective screens, with the data shown in Table 3. One fact that must be mentioned is that each parent cross of the F1 screen was transferred to a second vial five days after the initial cross, to generate more progeny. This was not done for the F2 screen. Additionally, the F1 phenotypes selected to cross with the scabrous driver were also transferred five days after the cross, while the F2 screen was kept in a single vial. When this is broken down into the effort required to generate each keeper, the F1 screen is approximately one hour faster per keeper than the F2 screen.

In the F2 screen, once a y+ male was identified it was assumed that each y+ progeny in the cluster of F1 progeny was a result of the same "hop", and therefore only one was crossed to generate F2 progeny. In the F1 screen, it was assumed that there could be more that one "hop" per cluster and thus every fly with a distinct phenotype was crossed to generate F2 progeny. However, one of the keepers (\$26) from the F1 screen gave interesting results. This was the result of the cross of a brother of a Ki male, a cross that should not have been made according to the rules of F1 phenotype selection. The fact that this cross generated a keeper phenotype provided evidence that more than one "hop" could occur in the same cluster, since the crossed brothers of \$26 did not exhibit a keeper phenotype. If the results were corrected for \$26, the efficiency percentage would drop from 6.7% to 5.8%, while the minutes per keeper value increases from 134 to 151.

Conclusions: In a direct comparison of the two types of screens involved in this experiment, the F1 screen has advantages over the classic F2 screen. The proportion of keeper phenotypes obtained was greater than the F2 screen, indicating that the F1 screen did not suffer from a decrease in positive results. Considering effort, the time difference between the F1 and F2 screens was found to be large, lending additional support to the efficiency of the F1 screen. Once the keepers were identified, the next step would be to perform crosses that generate a homozygous stock of each individual keeper. This stock can then be crossed to other drivers and analyzed by molecular genetic techniques in an effort to identify new genes through gain of function phenotypes.

Castrezana, Sergio. Department of Zoology, Arizona State University, Tempe, AZ 85287. A new recipe for rearing cactophilic *Drosophila*.

The cactophilic species *Drosophila mettleri* and especially *D. nigrospiracula* have difficulty adapting to laboratory culture. Both species are associated with necrotic Saguaro (*Carnagiea gigantea*) or Cardon (*Pachycereus pringlei*) in nature, with *D. nigrospiracula* breeding in the cactus tissue and *D. mettleri* in soil soaked with fermented cactus juice (Heed, 1978).

In developing an improved method to culture these species in the laboratory, I first made a food from commercial instant mashed potatoes (Betty Crocker potato flakes) with a piece of sterilized Saguaro cactus added to the surface. Although the number of offspring was higher than with standard cornmeal or banana recipes, sometimes field-caught flies carried mold that efficiently use the food, directly affecting the development of the flies. Thus, cultures were