Craymer, L. California Institute of Technology, Pasadena, California USNA. Automating saturation screens with crisscross lethals.

Large scale identification and isolation of chromosomes which carry lethals in a region specified by a deficiency can be a tedious operation. Muller's idea of using crisscross lethals in mutation studies (1953 DIS 27:104-106) can be adapted for saturation screening to simplify the scoring and balancing steps. The idea is to recover a mutagen-treated chromosome over a chromosome which carries two lethals, ℓ_1 ℓ_2, and mate to Df, ℓ_1/Bal, ℓ_2 where Df is a deficiency for the region of interest and Bal is a balancer chromosome. The lethals ensure that all of the F_2 progeny will carry the treated chromosome and that there will usually be two classes of progeny: */Df, ℓ_1 and */Bal, ℓ_2. If, however, the treated chromosome carries a lethal in the region of interest, then only the */Bal, ℓ_2 progeny will survive. Balanced stocks of induced lethals are thus automatically recovered by this procedure, and the scoring process can be simplified by having either the Df or Bal chromosome marked with a dominant visible mutation which can be recognized without etherization. The following screen has been used by M. Crosby to recover lethals in the 68A to 69A region:

P_1 In(3R)C, Sb ℓ(3)a/Ser Dr^{Mio} females are crossed to treated males

F_1 In(3R)C, Df(3L)vin^5, Tb ℓ(3)a/TM3, Sb Ser females are crossed to single */In(3R)C, Sb ℓ(3)a males

F_2 Look for cultures with no Tb adults; these cultures carry lethals in the deficiency region.

As originally envisioned, the */Ser Dr^{Mio} males were also to be tested, using Df(3L)vin^5, Dr^{Mio}/TM3 Sb Ser females for the screening cross. Unfortunately, Ser/TM3, Sb Ser is not lethal (M. Crosby, personal communication) and this half of the screen had to be abandoned. Tb and Dr^{Mio} were chosen for the screen since they could be easily scored through the sides of a vial: Tb, because it could be scored in pupal cases, and Dr^{Mio}, because it is easily scored in adults. At the time this screen was designed, no third chromosome balancer existed which carried an adequate dominant visible; TM6B with either D^3 or Tb (with a lethal) should be useful for other such screens, and Cy can be used for the dominant visible in second chromosome screens.

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Isogenic stocks are useful in many experiments where it is critical that there be little or no background genetic variation. Muller's triple balancer scheme (1936 DIS 6:7) is commonly used, but this method has drawbacks. Females which are simultaneously heterozygous for efficient first-, second-, and third-chromosomal balancers have seriously reduced fertility, and the individual balancer chromosomes have reduced efficiencies because of the interchromosomal effect of heterologous rearrangements on recombination. The following scheme circumvents these problems, although it does require two generations more than the triple-balancer scheme.

P_0 C(1)M4, y^2; If; Sb/TM6B, h D^3 e females x + male.

P_1 C(1)M4, y^2; If; Sb/TM6B, h D^3 e and
FM3, v^{0f}/y sc $l.z^9$ v f; Sp bw^D/SM5 females
x +; +/If; +/Sb male [1 male to 3 or 4 of each type of female]

P_{2a} C(1)M4, y^2; If; Sb/TM6B, h D^3 e females
x +; +/If; +/TM6B, h D^3 e males

P_{3a} Like P_{2a}

P_{3b} +/+; +/+; Sb/TM6B, h D^3 e females x +; +/If; +/TM6B, h D^3 e males

P_4 +; +; TM6B, h D^3 e/+ females x males

P_5 +; +; + isogenic stock.

Some comments are in order concerning the balancers listed. FM3, SM5, and TM6B are the most efficient balancers available for their respective chromosomes. FM3/+; SM5/+; TM6B/+ females could be used in a triple-balancer isolation--perhaps 2-5% of the offspring would carry a recombinant chromosome, with an average of about 2% of the genome substituted per recombinant (these figures are educated guesses based on some observations of multi-chromosomal balancer combinations)--although some problems might arise from the reduced fertility and viability of individual females. In the suggested scheme, with double-balancer heterozygotes used in one generation and single-balancer heterozygotes used in a later generation, it is unlikely that as many as 0.5% of the offpspring of the double-balancer cross would be recombinant for the balanced chromosomes and very unlikely that recombinant third chromosomes be recovered from the crosses involving TM6B/+ females.

Chromosome substitutions can also be carried out without going through a triple-balancer intermediate. For X-substitutions, the scheme is

P_0 $+_a$; $+_a$; $+_a$ females x SM6/In(2LR)bw^{V1}; TM6B, h D^3 e/In(3R)Mo, Sb sr males

P_{1a} $+_a$; $+_a$; $+_a$ females x $+_a$; SM6/+; TM6B, h D^3 e/+ males

P_{1b} $+_a$; $+_a$; $+_a$ females x $+_a$; In(2LR)bw^{V1}/+; In(3R)Mo, Sb sr/+ males

P_2 $+_a$; In(2LR)bw^{V1}/+; In(3R)Mo, Sb sr/+ females [from P_{1b}] x $+_b$; $+_b$; $+_b$ males

P_3 $+_a$; SM6/+; TM6B, h D^3 e/+ females [from P_{1a}]
x $+_a$; In(2LR)bw^{V1}/$+_b$; In(3R)Mo, Sb sr/$+_b$ males

P_4 $+_a$; SM6/$+_b$; TM6B, h D^3 e/$+_b$ females x males

P_5 $+_a$; $+_b$; $+_b$ stock

This series of crosses thus substitutes the $+_a$ X-chromosome into the $+_b$ stock. SM5 would be preferable to SM6 in this series of crosses, since SM5 has the better balancing properties; however, SM6 is listed because the SM6/In(2LR)bw^{V1}; TM6B, h D^3 e/In(3R)Mo, Sb sr stock currently exists.

To substitute a $+_b$ third chromosome in to a $+_a$ background:

P_0 $+_a$; $+_a$; $+_a$ females x SM6/In(2LR)bw^{V1}; TM6B, h D^3 e/In(3R)Mo, Sb sr males

P_{1a} $+_a$; $+_a$; $+_a$ females x $+_a$; In(2LR)bw^{V1}/$+_a$; TM6B, h D^3 e/+

P_{1b} $+_a$; $+_a$; $+_a$ females x $+_a$; SM6/+; In(3R)Mo, Sb sr/+ males

P_2 $+_a$; SM6/+; + females [from P_{1b}] x $+_b$; $+_b$; $+_b$ males

P_3 $+_a$; $+_a$; TM6B, h D^3 e/+ females [from P_{1a}] x $+_a$; SM6/+; In(3R)Mo, Sb sr/$+_b$ males

P_4 $+_a$; SM6B/$+_a$; TM6B, h D^3 e/$+_b$ females x males

P_5 $+_a$; $+_a$; $+_b$ stock

The series of crosses for substituting a second chromosomes is rather similar to the third chromosome substitution crosses.