Agol, I.J. New Method of Detecting Lethals in the X-chromosome. Up to the present time Muller's method ClB has been used to detect lethals in the X-chromosome. At our laboratory we have adapted the method of y B sc^4 on the basis of the following considerations:

(1) y B sc^4 represents an inversion involving almost the whole length of the X-chromosome (from sc to bb) while the ClB inversion is far shorter (approximately from bi to Bx). Therefore y B sc^4 excludes the possibility of getting single cross-overs on both sides of the X-chromosomes.

(2) By the ClB method half of the chromosomes tested are lost, as only half of the progeny get the ClB chromosome in F_1; while by crossing the X-chromosomes tested (males) with homozygous y B sc^4 females nothing is lost.

(3) The y B sc^4 method facilitates the establishment of balanced cultures with lethals, because 1/y B sc^4 obtained in F_1 and inbred individually with their y B sc^4 brothers will give two categories of females: 1/y B sc^4 and y B sc^4/y B sc^4 which are easily distinguished one from the other, while crossing 1/ClB with any male gives two kinds of females, which it is impossible to differentiate phenotypically.

In case a new lethal has arisen in a cross with y B sc^4 we do not get maleless cultures in F_2, but all the males instead of being of two categories will be of one kind only, namely y B sc^4.

The method is used as follows. The chromosome tested (male) is crossed with a virgin homozygous female y B sc^4/y B sc^4. In F_1 all females are picked out and inbred individually with their brothers. If only one class of males is obtained in F_2, this indicates the presence of a lethal in the X-chromosome under investigation.

Muller, H.J. Accumulation of Mutations (Negativing of Natural Selection. 1. In chromosome 1 (possible in ♀♀ only) (C's B method). Key: Cs is inversion preventing crossing-over in right portion of 1 and containing gene making ♂♂ sterile. B = Bar eye. Dl-49 is inversion, preventing crossing-over to left of g in 1. lz^s = lozenge-spectacled eye, sterile in homozygous ♀. w = white eye.

P_1 Cs,B/ dl-49, w lz^s ♀ X dl-49, w lz^s♂♂ (many single-♀ cultures).

F_1 Cs,B/dl-49, w lz^s ♀ dl-49, w lz^s/dl-49, w lz^s ♀ (sterile)
Cs,B/Y ♂ (sterile) dl-49, w lz^s/Y ♂

Verify presence of B ♂♂ in each F_1 culture as preliminary test. Then breed F_1 ♀♀ in mass cultures X brothers, keeping those from different P_1 cultures separate.

F_2 offspring as before and so on to F_n (balanced ♂).

Final test: single ♀ from each culture of F_n is bred to her brothers. If lethal is present in Cs B chromosome, no B sons will appear in F_n + 1.

2. In chromosome 2 (using rotund as sterilizer)
rn - rotund wing; sterile in homozygous ♂ and ♀ (found by Glass)
sp - speck (a marker)
P_1 rn sp/Cy ♂ X S sp/Cy ♀ (in many single ♂ cultures)
F_1 rn sp/Cy (breed ♂♂ and ♀♀) (discard other types of F_1)
F_2 rn sp/Cy (breeds; balanced ♂) rn sp/rn sp (sterile
Cy/Cy (dies)
Make mass cultures of F_1, F_2, etc. to F_n corresponding to each P_1 culture. Therefore, $F_1 = F_2 = F_3 = \ldots\ldots\ldots\ldots F_n$
Preliminary test: Examine F_2 for presence and normality of homozygous rn to insure against lethal or visible present at start of experiment.
Final test: Test single ♂ from each F_n culture, making same cross as of P_1 ♂; breed $F_n \neq 2$ in same way as F_1 and ascertain presence and normality of homozygous rn in $F_n \neq 3$ as in F_2.

3. In chromosome 3 (using inserted piece of 2 as temporary lethal). Key: Homozygous chromosome 3 carrying duplicating piece of 2 from T(2-3) Pale is lethal in hyperploid having normal 2/2 but non-lethal in fly having one normal and one Pale-deficient 2. 2^- = chromosome 2 deficient for fragment translocated to 3. 3^{+2} = chromosome 3 bearing fragment of 2.
Balanced Stock: $Cy/2^-$; CC,Dfd/3^{+2}
P_1 $Cy/2^-$; CC,Dfd/3^{+2} ♂ X CC,D/H ♀♀ in many individual ♂ cultures.
F_1 (a) $2^-/\neq$; CC,D/3^{+2} ♂♂ and ♀♀ (one mass "test culture" from each P_1)
(b) $Cy/\neq$; CC,D/3^{+2} ♂♂ and ♀♀ (one mass "stock cultures" from each P_1)
F_2 (a) Examine offspring of test cultures for presence of non-D flies ($2^-/\neq$; $3^{+2}/3^{+2}$) indicating that no lethal was present in 3^{+2} of P_1 ♂. If lethal was present discard sister stock culture. (Possible visibles in 3 are discovered simultaneously).
(b) $Cy/\neq$; CC,D/3^{+2} - Continue breeding from cultures whose sister test cultures showed no lethal (i.e. produced non-D flies).
F_n Same as F_2 (b)
Final test: Test single ♂ from each F_n "stock culture" crossing it by ♀ from stock $Cy/2^-$; CC,Dfd/3^{+2}
F_{n+1}: $Cy/\neq$; CC,D/3^{+2} (in single-♂ cultures) X $Cy/2^-$/CC,Dfd/3^{+2} ♀
F_{n+2}: (Select non-Cy, non-D, Dfd) $2^-/\neq$; CC,Dfd/3^{+2} ♂ and ♀
F_{n+3}: Examine for absence of non-Dfd ($2^-/\neq$; $3^{+2}/3^{+2}$) to discover if lethal is present. (Also examine to discover visibles).

4. In chromosome 2, with automatic selection of required offspring. Symbols: ab^2 = abrupt^2 (" parted"); homozygous ♂ sterile; mr = morula; homozygous ♀ sterile; "n" indicates possible newly arisen mutant; L^2 = dominant marker, Lobe eye.
P_1 ab^2 mr/L^2 ♂ X Cy/$\neq$ ♀ (many single-♂ cultures)
F_1 ab^2 mr/Cy ♂♂ and ♀♀ (Breed F_1 in mass cultures, corresponding to each P_1 male)

F_2 ab^2 mr/Cy (balanced ⚥⚥; examine each to see that ab^2 mr is present)

F_n Continue mass cultures of each non-lethal line to F_n

Test ab^2 mr chromosome of one ♂ from each F_n culture by fly from tester balanced stock ab^2 L^2 mr/Cy as follows:

$F_{n\neq1}$ Cy/ab^2 "n" mr ♂ X Cy/ab^2 L^2 mr ♀

$F_{n\neq2}$ ab^2 "n" mr/Cy (breed ♂♂ and ♀♀; balanced ⚥ of "n"

Look for "ab^2 mr" appearing $F_{n\neq3}$ flies. If absent, "n" is lethal. If abnormal, "n" is a visible. If present and normal, no detectable mutation has occurred.

<u>Muller, H.J. Accumulation of Mutations in Given Sex</u>

1. In 1 of ♀: see "Cs,B" method above.

2. In 2 of ♂: by use of "Blond", a mutual translocation of 1 and 2, viable when homozygous and containing a dominant marker, the Blond bristles. (Found by Burkart with Stern). Key: 1 carrying right end of of 2 and deficient for left end of 1 = $Bld^{-1L\neq2R}$. 2 carrying left end of 1 and deficient for right end of 2 = $2^{-2R\neq1L}$. "all" = a series of 7 recessive markers scattered through 2.

P_1 $Bld^{-1L\neq2R}$; $2^{-2R\neq1L}$/Cy ♂ (many single ♂ cultures) X <u>X</u> ; Cy/"all" ♀

F_1 $Bld^{-1L\neq2R}$; $2^{-2R\neq1L}$/Cy(or "all") ♂ X <u>X</u> ; Cy/"all" ♀ (balanced ⚥). One mass culture and line from each P_1 ♂. (other combinations die or are highly infertile; if in latter crossing-over occurs, it is revealed by genes of "all").

Preliminary test for preexisting lethals and visibles in each P_1 ♂, and final test for new mutants is made as follows:

P_1 and $F_{n\neq1}$ $Bld^{-1L\neq2R}$; $2^{-2R\neq1L}$ <u>l</u>(?)/Cy ♂ (single ♂ from each F_n line) X sc v <u>lx</u> f bb/<u>Cl</u>B, sc v/Y;S/Cy ♀ (virgin ♀♀ of "Curly Pat" stock)

F_1 and $F_{n\neq2}$ $Bld^{-1L\neq2R}$; $2^{-2R\neq1L}$ <u>l</u>(?)/S (or Cy) ♂ (only ♂♂ which live) X $Bld^{-1L\neq2R}$/<u>Cl</u>B, sc v (or sc v <u>lx</u> f bb) ; $2^{-2R\neq1L}$ <u>l</u>(?)/Cy ♀.

(Choose non-sc, non-v, Cy orthoploid ♀♀, need not be virgin).

F_2 and $F_{n\neq2}$ Look for non-S, non-Cy ♀:

$Bld^{-1L\neq2R}$/$Bld^{-1L\neq2R}$; $2^{-2R\neq1L}$ <u>l</u>(?)/$2^{-2R\neq1L}$ <u>l</u>(?)

If absent, lethal is present; if they are abnormal (except Blond character) visible is present.

3. In 2 of ♂ and 2 of ♀ simultaneously; also in two 2's which alternate between ♂ and ♀. (By use of "$abrupt^2$", sterile in homozygous ♂, of "morula", sterile in homozygous ♀, and of "rotund", sterile in both; crossing-over to be prevented by use of homozygous C3G, or inversions in "ab^2 pr mr" chromosome.)

P_1 rn mr/ab^2 pr mr ♂ X ab^2 rn/ab^2 pr mr ♀ (single ♂ and ♀ in many individual cultures)

F_1 (and F_{3-5} etc.) rn mr/ab^2 pr mr (♀ sterile; ♂ breeds) rn mr/ab^2rn (♂ and ♀ sterile) ab^2 pr mr/ab^2 pr mr (♂ and ♀ sterile) ab^2 pr mr/ab^2 rn (♂ sterile; ♀ breeds)