Increasing Active Mutator Stocks
(as practiced by the Schnable Lab, Iowa State University)

Information on Lines Commonly Used to Increase Active Mutator Lines

- In the past at ISU, active Mu lines were typically maintained by crossing onto the hybrid lines Standard Q60, made by crossing inbred lines Q67 & Q66, & B70, made by crossing inbred lines B77 & B79.

- More recently at ISU, active Mu stocks have been maintained by using Standard Q60 and not B70. Note: Q67 often produces smaller amounts of pollen, so Std Q60 is often made using Q67 as the female and Q66 as male.

- Robertson used the nomenclature "Std Q60". Std Q60 should not be confused with nor should it be abbreviated to Q60, because Arnel Hallauer, who developed the Q series of inbreds at ISU, developed the inbred line called Q60.

- Robertson chose to use hybrid lines Std Q60 & B70 partially because of the opportunity to cross both primary and secondary ears. One can use other vigorous lines that are inclined to develop better in one's growing environment. An inbred line may work better than the hybrid lines such that any subsequent molecular and phenotypic analysis will be simplified.

- Both primary and secondary ears on the hybrid lines Std Q60 & B70 can be crossed to produce full ears. If crossing both primary and secondary ears on the hybrid lines, one should shootbag both ears; cross the secondary ear first; then a day or two later cross the primary ear. Shoots are fairly high on the plant and a step ladder can prove useful. Standard Q60 also produces tillers, which can be crossed as well.

- When planting Std Q60 for the first time, staggered plantings are suggested in order to cross by Mu *per se*.
    - Std Q60 is similar in maturity to B73, maybe 2-5 days later
    - The parent inbred lines of the hybrids are a bit later in maturity than the hybrid, parent inbred lines maybe 5-8 days later than B73

Increasing of Active Mu Stocks

- There are two options for the increasing of Mu Stocks, either by creating Mu x Mu lines a.k.a. Mu *per se* lines or by creating Mu outcross (OC) lines. One can produce more Mu OC easier than Mu *per se* because a single Mu pollen source can be crossed onto multiple ears. What we usually do is to maintain active Mu stock as Mu *per se* lines and create Mu OC lines for Mu-tagging experiments when a large quantity of Mu seeds are needed.

- When creating active Mu stocks, Robertson concluded that there is a limit to the new Mu activity level when Mu lines are crossed. See below for nomenclature for crosses between Mu plants. Mu activity peaks at the level of 4, so it is best to not create Mu plants with levels higher than 4.

Symbol

Mu^1 - An Mu line that has only been propagated by outcrossing.

Mu^2 per se - The F_1 generation of a $Mu^1 \times Mu^1$ cross.

Mu^3 per se - The F_1 generation of a $Mu^1 \times Mu^2$ cross.

Mu^4 per se (2 x2) - The F_1 generation of a $Mu^2 \times Mu^2$ cross.

Mu^4 per se (1 x 3) - The F_1 generation of a $Mu^1 \times Mu^3$ cross.

Mu^5 per se (1 x 4) - The F_1 generation of a $Mu^1 \times Mu^4$ cross.

Mu^5 per se (2 x 3) - The F_1 generation of a $Mu^2 \times Mu^3$ cross.

Mu^6 per se (3 x 3) - The F_1 generation of a $Mu^3 \times Mu^3$ cross.

Mu^6 per se (2 x 4) - The F_1 generation of a $Mu^2 \times Mu^4$ cross. The Mu^4 was a (2 x 2).

Mu^7 per se (3 x 4) - The F_1 generation of a $Mu^3 \times Mu^4$ cross. The Mu^4 was a (2 x 2).

Mu^7 per se (1/3 x 3) - The F_1 generation of a $Mu^1 \times Mu^6$. The Mu^6 was a (3 x 3).

Mu^7 per se (2/2 x 3) - The F_1 generation of a $Mu^2 \times Mu^5$ cross. The Mu^5 was a (2 x 3).

Mu^8 per se (4 x 4) - The F_1 generation of a $Mu^4 \times Mu^4$ each Mu^4 was a (2 x 2).

Mu^8 per se (3/4 x 1) - The F_1 generation of a $Mu^3 \times Mu^5$. The Mu^5 was a (4 x 1) in which the 4 was a (2 x 2).

Mu^{10} per se (2 x 2/2 x 4) - The F_1 generation of a $Mu^4 \times Mu^6$. The Mu^4 was a (2 x 2) and the Mu^6 a (2 x 4).

Mu^{10} per se (5 x 5) - The F_1 generation of a $Mu^5 \times Mu^5$. Each Mu^5 was a (2 x 3).

Mu^{12} per se $(3 \times 3)^2$ - The F_1 generation of a $Mu^6 \times Mu^6$. Each Mu^6 was a (3 x 3).

Mu^{12} per se $(2 \times 4)^2$ - The F_1 generation of a $Mu^6 \times Mu^6$. Each Mu^6 was a (2 x 4) in which each 4 was a (2 x 2).

Mu^{16} per se $(4 \times 4)^2$ - The F_1 generation of a $Mu^8 \times Mu^8$. Each Mu^8 was a (4 x 4) and each Mu4 was a (2 x 2).

Mu^{16} per se $(3/4 \times 1)^2$ - The F_1 generation of a $Mu^8 \times Mu^8$. Each Mu^8 was a Mu^3 x Mu^5 and each Mu^5 was a (4 x 1) and the Mu^4 was a (2 x 2).

Mu^{16} per se $(2 \times 3/3)^2$ - The F_1 generation of a $Mu^8 \times Mu^8$. Each Mu^8 was a (5 x 3) and each Mu^5 was a (2 x 3).

Mu^2 o.c., Mu^3 o.c. Mu^5 (1 x 4) o.c., Mu^8 (3/4 x 1) o.c., etc. = an outcross of the respective per se line.

Mu^2 parents, Mu^3 parents, Mu^5 (1 x 4) parents Mu^8 (3/4 x 1) parents, etc. = The results of a mutator test of the parent lines that produced the indicated Mu per se line.

Creating Mu per se lines

Mu *per se* lines are created by crossing between Mu lines. Because the Mu stocks were crossed with hybrid lines, which are vigorous, one can often cross both primary and secondary ears of Mu lines as well. Mu'x' *per se* lines should be planted; the secondary ear should be self pollinated (for Mu activity sand bench test purpose); and a day or two after the secondary ear was self pollinated, the primary ear should be crossed by another Mu'y' plant from a different source thereby creating a Mu'x+y' *per se* ear of corn (which will be the Mu stock seeds). For example, Mu1 x Mu3 => Mu4.

Creating Mu OC lines:

Mu OC lines are created outcrossing Mu *per se* lines to vigorous hybrid lines (Std Q60, B70 at ISU or your regional vigorous lines). Mu *per se* should be self pollinated (for sand bench test purposes of determining Mu activity level of your newly created Mu stocks) and outcrossed to multiple hybrid line females (creating F1s). Crosses should be made to create multiple Mu OC ears from one pollen parent so that when one later identifies a parent with high new mutation rate, multiple OC ears of this parent will be available as Mu stock.

Testing New Mutation Rate of Newly Created Stocks

- After creating a set of Mu stock, one needs to test the new Mu activity level of the developed seed. 10% of Mu cross progeny loses Mu activity so it is strongly recommended to test the seeds from a Mu cross for Mu activity and select those with high Mu activity as your stock.

- Plant 70 kernels of your F1 (hybrid x Mu plant) and self all to create F2s. You should plant 70 kernels because you need to obtain 50 selfs (F2s) to do seedling tests to check the new mutation rate of your newly created stocks. Plant more as contingency. Sandbenches work well to do seedling tests for Mu activity.

- After your F2s have been created, plant 50 kernels from each of your 50 F2s as well as 50 kernels from your original Mutator self (i.e. the self done when outcrossing your Mutator line to mutator hybrid lines) to do a seedling comparison test. Compare mutations present in your Mutator self-pollinated plant to mutations present in your F2 i.e. determine how many NEW mutations are present in your F2 seed compared to your Mutator self-pollinated plant.

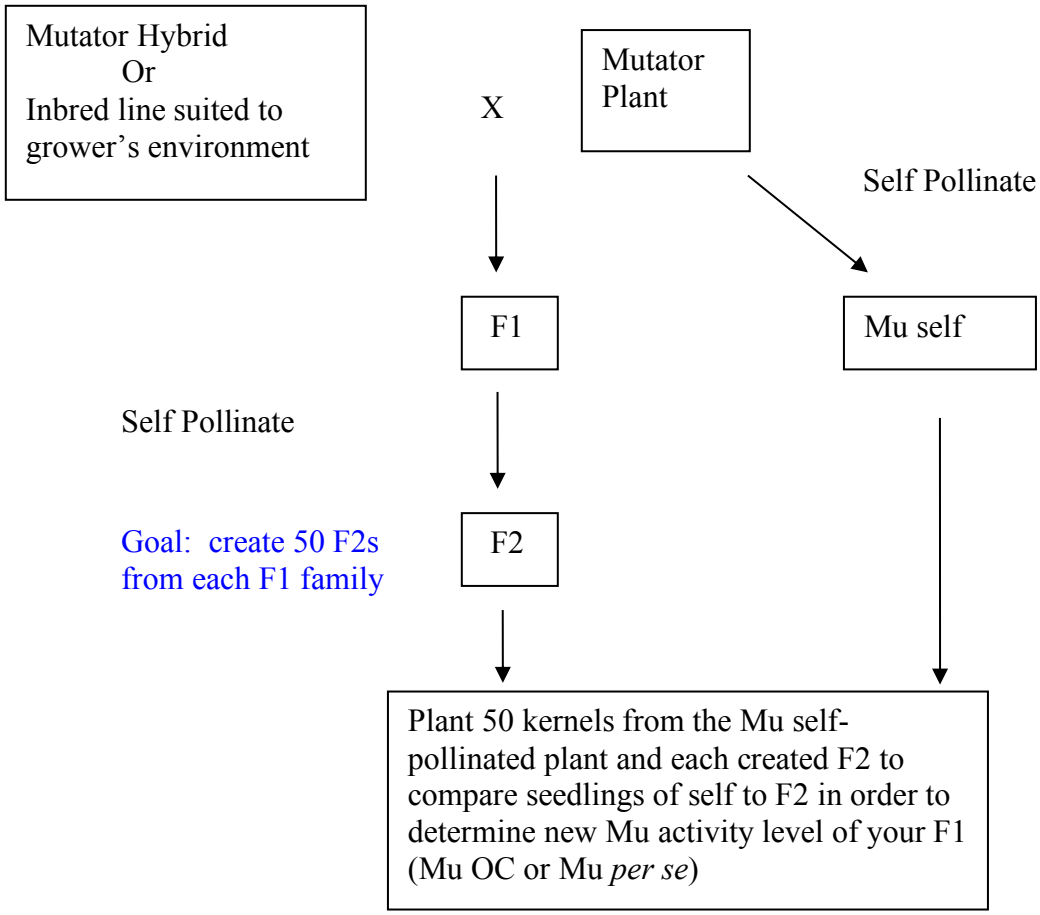
- Seedlings should be scored at 7-10 days after planting. The person scoring seedlings needs to be consistent in scoring. Phenotypes to look for include: glossy, shades of pigmentation (albino, pale green, yellow, pale yellow, etc), dwarfs, twisted plants, narrow leaf mutants, plants with lesions, etc. Each different phenotype is counted as a separate mutation. Combinations of pigmentation differences & other mutations should be treated as a separate mutation. Note: you should look for any differences that look like 'real mutations' and not a sick plant. 'Real mutations' will often be a mutation that is present in a few seedlings within a family (i.e. segregating in a set of 50 F2s), whereas a sick plant phenotype might only be present once in a family.

- A mutant phenotype is only counted towards the new mutation rate once per F1 parent. If a mutation is occurring within many F2 families from the same F1 parent, the mutation is most likely a fixed mutation that was inherited and is therefore <u>not</u> a <u>new</u> mutant and is not counted in the new mutation rate. If a specific mutant phenotype is present in the original Mu self plant <u>and</u> any F2 family, the phenotype is <u>not</u> a new phenotype and is therefore <u>not</u> counted towards the new mutation rate.

- The new mutation rate is expressed as "new mutants / total # of families" such as 3/43, 1/45, etc. and the higher the rate the better.

- Newly created Mu lines (the F1 generation) are deemed "Active Mu Lines" if they have at least 10% new mutations occurring in seedling plants of the F2 generation when compared to the original Mu self-pollinated plant.

- For lines that are determined to be Mu active (based on the F2 seedling test), about 90% of the F1 seed exhibit Mu activity when tested by further outcrossing.


Mutator Hybrid
Or
Inbred line suited to grower's environment
X
Mutator Plant
Self Pollinate
F1
Mu self
Self Pollinate
Goal: create 50 F2s from each F1 family
F2
Plant 50 kernels from the Mu self-pollinated plant and each created F2 to compare seedlings of self to F2 in order to determine new Mu activity level of your F1 (Mu OC or Mu *per se*)