

Reverse genetics

Methods

- TILLING - usually not nulls
- insertional mutagenesis – can get nulls

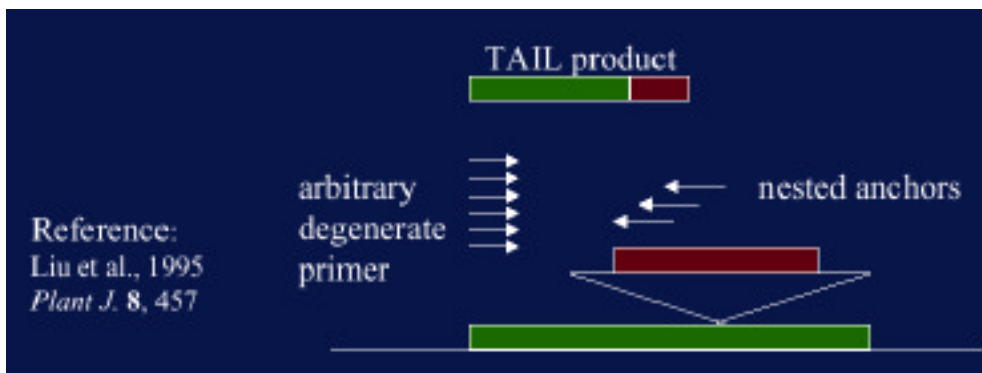
INSERTIONAL MUTAGENESIS:

“I am sure now that we can get many more newly arising mutable *al* loci. The method of detection is simple. **In fact, I think that we can go into business. If any one wants a locus to be mutable, just put in the order and one will be sent the following year.** This is not as facetious as it may sound, for that is the way it is turning out and now I know how to spot them rapidly.”

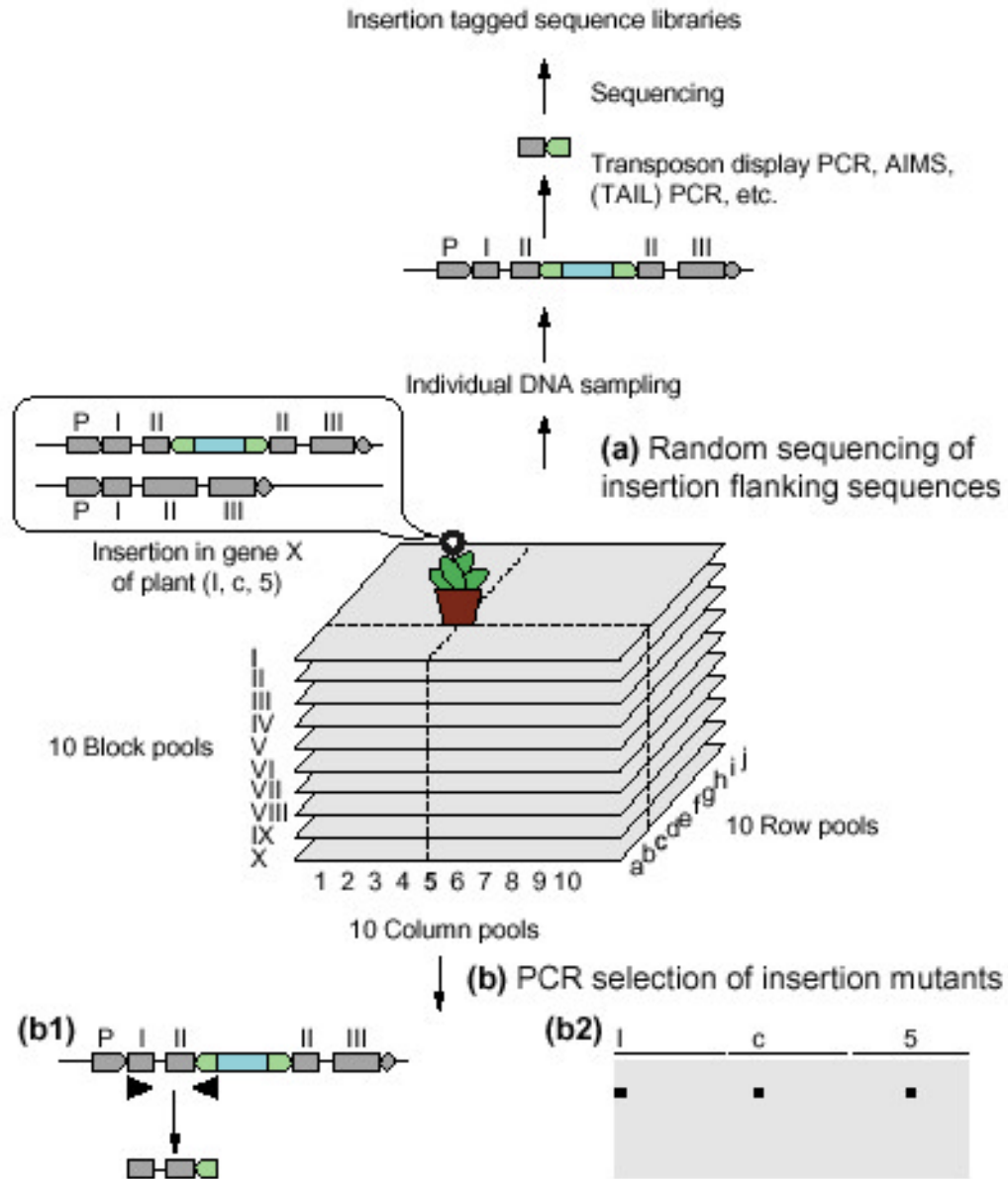
Barbara McClintock, 2 Sept. 1950, in a letter to Marcus Rhoades

The General strategy is shown on the next page

Random sequencing of insertion flanking sequences (a): Use TAIL PCR to randomly sequence DNA next to insertions and create a database that can be queried. TAIL PCR is described below. At least two groups are doing this and making the results public, the major one being the SIGnAL expression database (<http://signal.salk.edu/mabout.html>).



PCR selection of insertion mutants(b): use pooling strategy, a gene-specific and TDNA/transposon-specific primer to identify plants with a mutation in a known gene. Graphic of how TDNA or transposons can be used in reverse genetics. In maize, Robertson's mutator (Mu) is used as the mutagen. Robertson's Mu is a very active transposable element family with conserved inverted repeats that serve as site for primers.



Problem: GENETIC REDUNDANCY!!!! See linked review by Bouche and Bouchez, 2001, on the syllabus.