# Genotyping of Mutant Plants

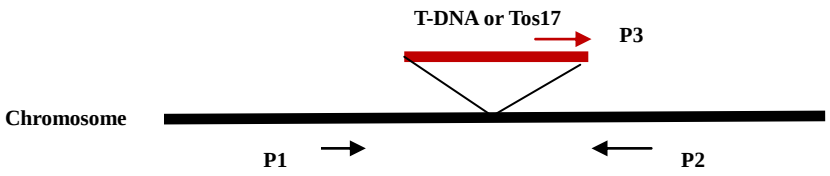
1. Dehull the mature seeds and sterilize with 70% ethanol for 1 min then 0.15% $HgCl_2$ for 15 min.
2. Rinse seeds with sterile water for 4-5 times.
3. Inoculate the seeds in 1/2MS medium for getting seedlings.
4. Transfer the seedlings to puddy field.
5. Harvest the fresh leaves from field-grown plants for DNA extraction.
6. BLAST search of the flanking sequence against the TIGR Rice Genome. Annotation database (http://rice.plantbiology.msu.edu/index.shtml) to determine the insertion site of T-DNA or Tos17.
7. Use DNA as template with gene specific primers (P1 and P2) and T-DNA or Tos17 border primer (P3) for PCR reaction.
8. Genotyping of the insertion site: If PCR fragments amplified by P1 +P2 and P2+P3, it indicates the insertion site is heterozygous of insertion; If a PCR fragment amplified by P2+P3, but no fragment amplified by P1+P2, it indicates the insertion site is homozygous of insertion; If a PCR fragment amplified by P1+P2, but no fragment amplified by P2+P3, it indicates there is no insertion in the examining site.

T-DNA left and right border primers:

**NTLB5：AATCCAGATCCCCCGAATTA** **PFRB4：TGCAGGTTCTCTCCAAATGA**

Tos17 left and right border primers:

**TosLS：CTGATACCATCTTAACTAACTTGC** **TosRS：GAAGGGGGGTGTTAAATATATATAC**




**Reference:**

1. http://rmd.ncpgr.cn/

**2.** Wu C. et al. RID1, encoding a Cys2/His2-type zinc finger transcription factor, acts as a master switch from vegetative to floral development in rice. Proc Natl Acad Sci USA, 2008, 105: 12915-12920