



IIT ROORKEE



NPTEL ONLINE
CERTIFICATION COURSE

Molecular Genetics of Plant Development-II

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Approaches to the study of plant development

- **Forward Genetics:** An approach to determine genetic basis of a phenotype (Phenotype to Genotype)
 - *Identifying desired developmental phenotype (mutant)*
 - *Analyzing inheritance pattern of the phenotype (dominant/recessive)*
 - *Mapping genetic locus associated with the phenotype (gene mapping)*
 - *Validation of the genotype-phenotype association (complementation)*



Approaches to the study of plant development

➤ *Analyzing inheritance pattern of the phenotype:*

- **Heritable:** Mendelian vs. non-Mendelian pattern
- **Pattern of segregation:** dominant, semi-dominant or recessive
- **Single site vs. multisite mutations:** single or double mutants
- **Co-segregation:** if double, independent vs. co-segregation



Approaches to the study of plant development

➤ *Analyzing inheritance pattern of the phenotype:*

- Heritable or non-heritable
- Mendelian vs. non-Mendelian inheritance
 - Incomplete dominance
 - Sex-linked traits
 - Epigenetic traits



Approaches to the study of plant development

➤ *Analyzing inheritance pattern of the phenotype:*

- Pattern of segregation:

- Dominant mutants
- Semi-dominant mutants
- Recessive mutants



Approaches to the study of plant development

➤ *Analyzing inheritance pattern of the phenotype:*

- **Pattern of segregation:** if mutant is homozygous (dominant or recessive)
- Back cross

Analysis of semi-dominant mutant

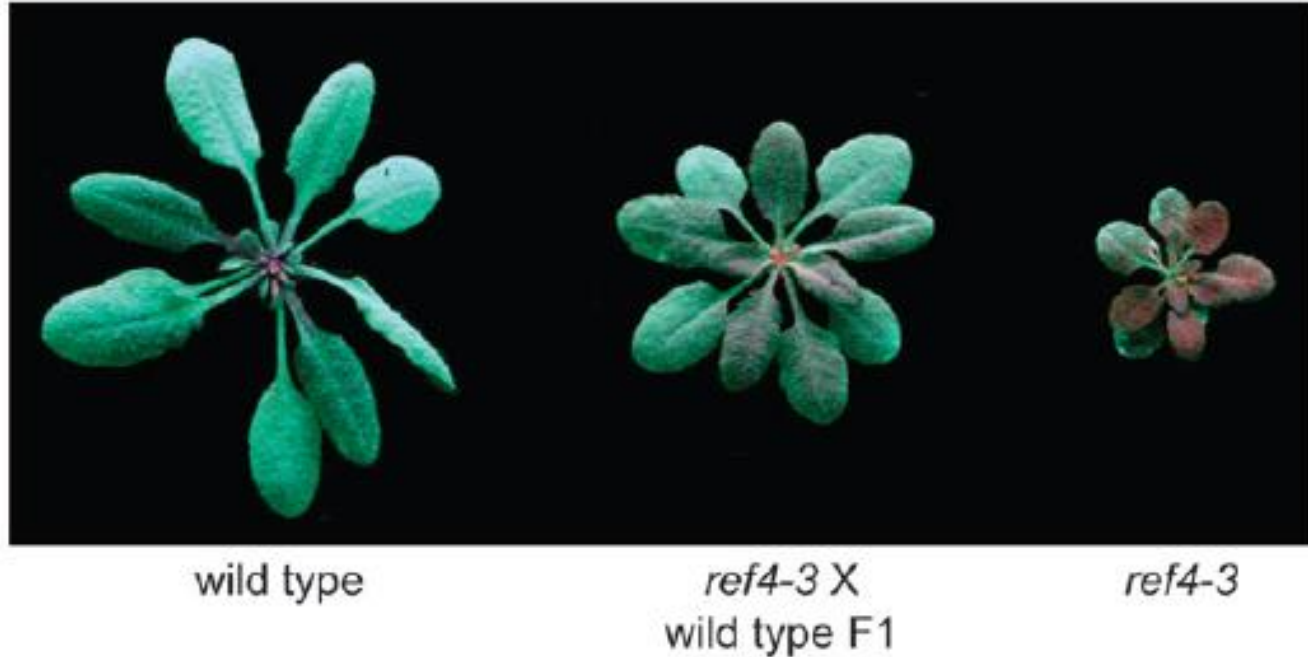


Image source: Stout et al. (2008) Genetics, 178, 2237-51.

Approaches to the study of plant development

➤ *Analyzing inheritance pattern of the phenotype:*

- **Single site vs. multisite mutations:** single or double mutants
- **Segregation pattern:** if double, independent vs. co-segregation



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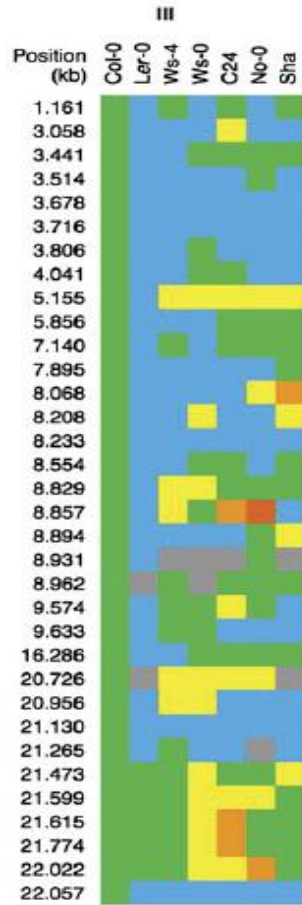
Approaches to the study of plant development

- ***Mapping genetic locus associated with the phenotype:*** gene mapping
 - **Map-based cloning:** Mapping point mutations by chromosome walking
 - **Gene tagging:** Mapping T-DNA or transposon inserted site

Approaches to the study of plant development

- ***Mapping genetic locus associated with the phenotype:*** gene mapping
 - **Map-based cloning:** Mapping point mutations by chromosome walking
 - **Molecular markers:** PCR or Restriction digestion based
 - **Linkage and co-segregation pattern analysis**
 - **INDEL markers:** Insertion-deletion based markers

INDEL markers: Insertion-deletion based markers



Marker	Chromosome	Col-0	Ws-4	Ws-0
NGA59	1	111	141	111
CIW12	1	128	120	115
NGA111	1	128	146	180
NGA280	1	105	85	85
NGA168	2	151	135	135
NGA6	3	143	147	154
NGA162	3	107	85	97
NGA172	3	162	138	180
NGA8	4	154	166	188
NGA1107	4	150	140	~145
NGA1139	4	114	~145	~100
NGA151	5	150	102	110
CA72 ^a	5	~225	~210	~205
NGA249	5	125	115	115
CIW9	5	165	145	140

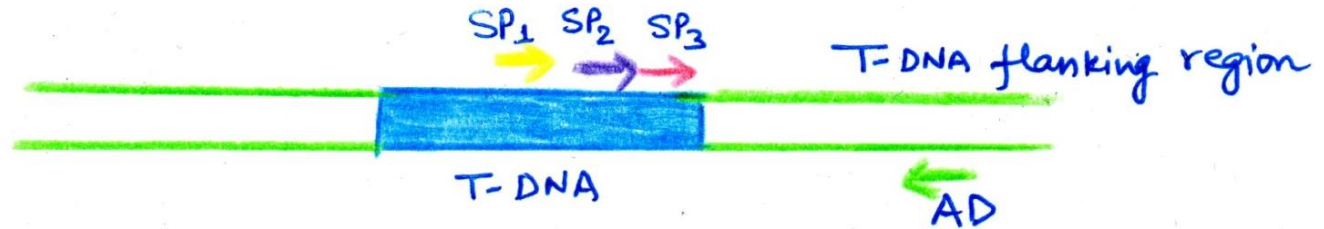
Image & data source: Pacurar et al. (2012) J. Exp. Bot., 63, 2491-2501.

Approaches to the study of plant development



➤ **Gene tagging: Mapping T-DNA inserted site**

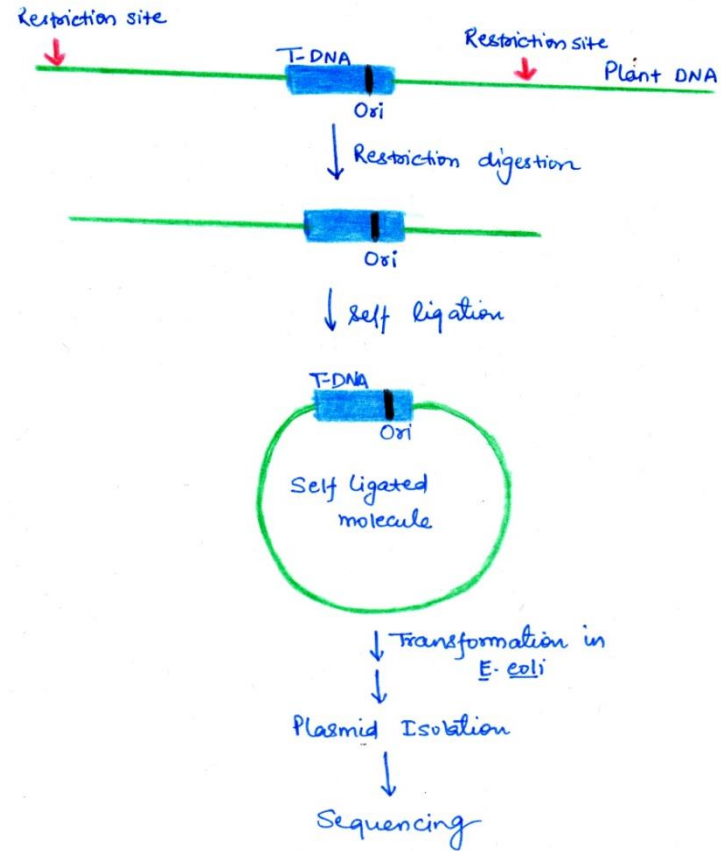
- TAIL PCR



Primary PCR : SP₁ and AD,
Secondary PCR : SP₂ and AD,
Tertiary PCR : SP₃ and AD,
↓
PCR amplified product
↓
cloning and sequencing

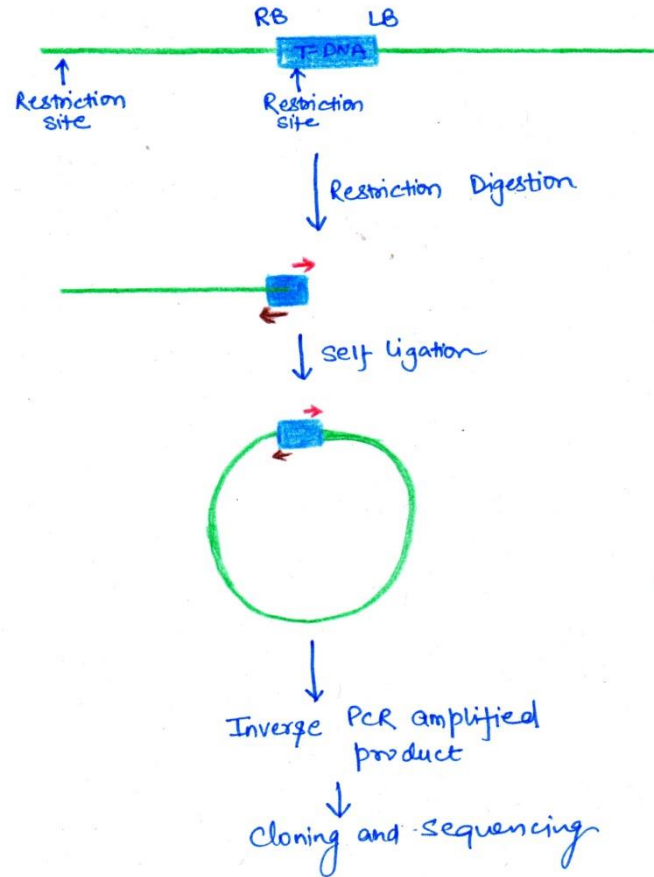
➤ Gene tagging: Mapping T-DNA inserted site

- Plasmid Rescue



➤ **Gene tagging:**
Mapping T-DNA inserted site

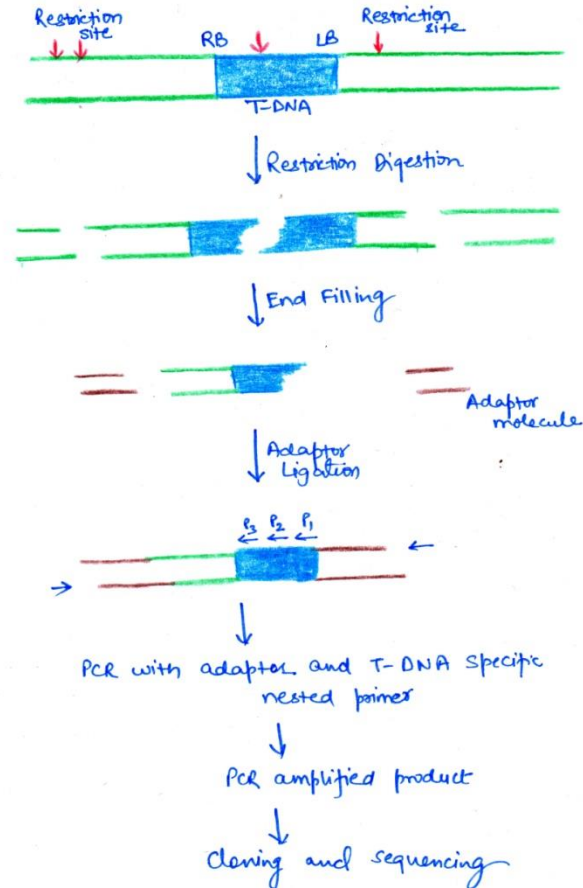
- Inverse PCR



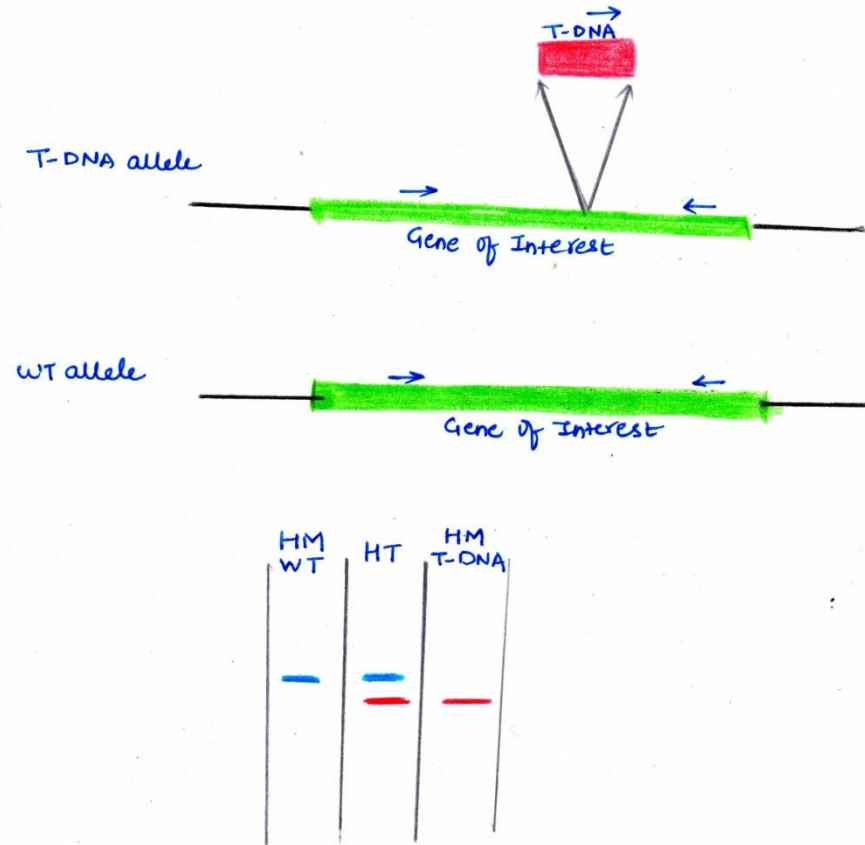
➤ Gene tagging:

Mapping T-DNA inserted site

- Adaptor PCR



➤ Genotyping: T-DNA insertion lines



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Approaches to the study of plant development

➤ *Validation of the genotype-phenotype association: complementation*

- Complementation with genomic copy
- Complementation with cDNA copy
- Translation fusion & complementation



➤ **Validation of the genotype-phenotype association:** complementation

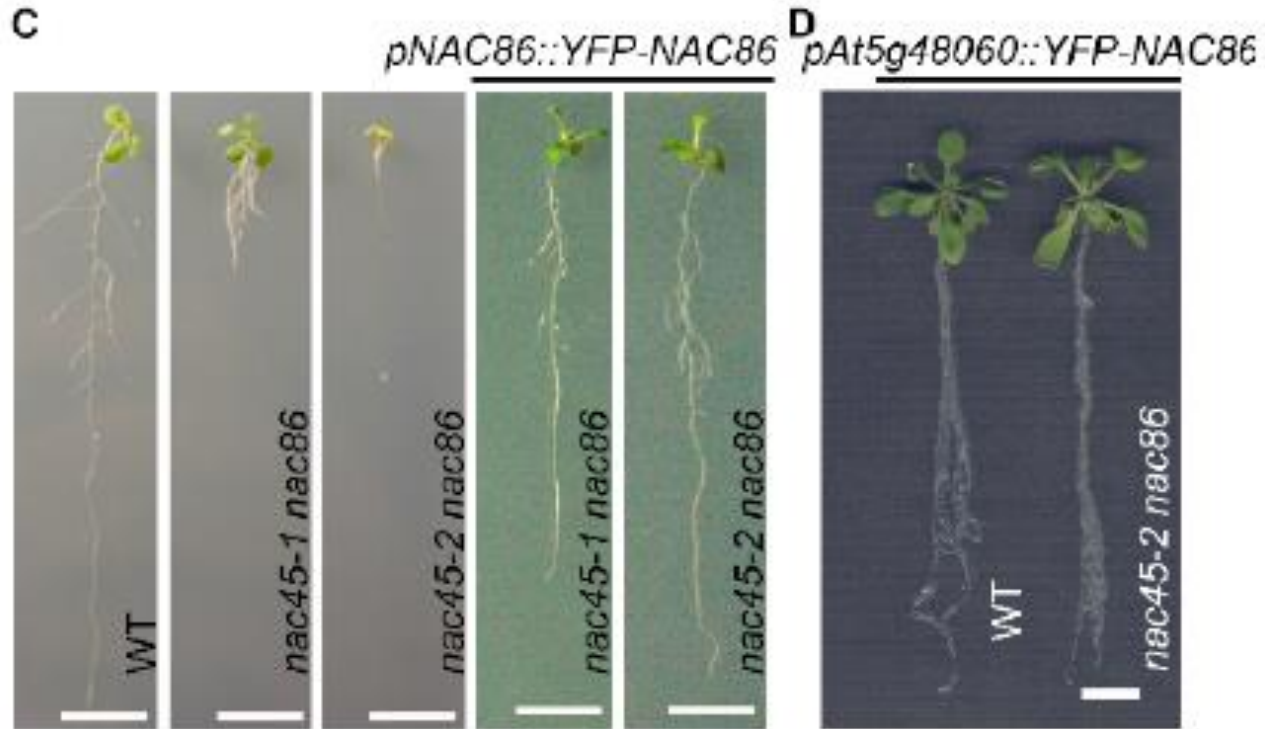


Image source: Furuta, Yadav et al. (2014) Science, 345, 933-37.