



IIT ROORKEE



NPTEL ONLINE
CERTIFICATION COURSE

Molecular Genetics of Plant Development-III

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Approaches to the study of plant development

- **Reverse Genetics:** An approach to study function(s) of gene(s)
(Genotype to Phenotype)
 - *Identification & isolation of gene for functional study (gene of interest)*
 - *Analyzing expression pattern of the gene (differential, spatial & temporal)*
 - *Functional characterization of the gene (gain or loss-of-function)*
 - *Gene interaction study (genetic, physical or regulatory interaction)*



Identifying gene of interest for functional study

- **Selecting developmental process:** Meristem function, organogenesis, determinacy or developmental transitions
- **Types of Genes:**
 - **Functional Genes:** Genes directly involved in the developmental process such as enzymes, cell division genes etc.
 - **Regulatory Genes:** Genes regulating the activities of the developmental genes
 - Transcriptional Regulation (e.g. transcription factors)
 - Post-transcriptional Regulation (e.g. small RNAs)
 - Translational Regulation (e.g. miRNAs)
 - Post-translational Regulation (e.g. kinases)



Identifying gene of interest for functional study

- *Candidate gene: Identifying single gene with putative developmental function*
- *Global genes (Genomics): Identifying genome-wide genes for a developmental process*



Identifying gene of interest for functional study

➤ *Identifying single gene with putative developmental function: (Candidate gene)*

- Homology-based analysis

- Gene family
- Sequence similarity
- Functional domains
- Phylogenetic analysis

- Expression pattern analysis-

- Organ-specific expression pattern (Differential expression pattern)
- Tissue-specific gene expression (Spatial expression pattern)
- Developmental stage-specific gene expression (Temporal expression pattern)



Identifying gene of interest for functional study

➤ Identifying single gene with putative developmental function: (Candidate gene)

- Homology-based analysis:

- Gene family
- Sequence similarity

Isolation and Characterization of a Rice MADS Box Gene Belonging to the AGL2 Gene Family

Hong-Gyu Kang and Gynheung An*

Department of Life Science and School of Environmental Engineering, Pohang University of Science and Technology, Pohang 790-784, Korea

Image source: Kang & An (1997)
Mol. Cells; 7: 45-51.

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                                MADS-box
OsMADS5 : GRGRVELKRIENKINRQVTFAKRRNGLLKKAYELSVLCDAEVALIIFSSRGKLYEF
OsMADS1 : GRGRVELKRIENKINRQVTFAKRRNGLLKKAYELSVLCDAEVALIIFSSRGKLYEF
FBP2 : GRGRVELKRIENKINRQVTFAKRRNGLLKKAYELSVLCDAEVALIIFSSRGKLYEF
TM5 : GRGRVELKRIENKINRQVTFAKRRNGLLKKAYELSVLCDAEVALIIFSSRGKLYEF
AGL4 : GRGRVELKRIENKINRQVTFAKRRNGLLKKAYELSVLCDAEVALIIFSSRGKLYEF
AGL2 : GRGRVELKRIENKINRQVTFAKRRNGLLKKAYELSVLCDAEVALIIFSSRGKLYEF
OM1 : GRGRVELKRIENKINRQVTFAKRRNGLLKKAYELSVLCDAEVALIIFSSRGKLYEF
ZAG3 : GRGRVELKRIENKINRQVTFAKRRNGLLKKAYELSVLCDAEVALIIFSSRGKLYEF
ZAG5 : GRGRVELKRIENKINRQVTFAKRRNGLLKKAYELSVLCDAEVALIIFSSRGKLYEF
AGL6 : GRGRVELKRIENKINRQVTFAKRRNGLLKKAYELSVLCDAEVALIIFSSRGKLYEF
AP1 : GRGRVELKRIENKINRQVTFAKRRNGLLKKAYELSVLCDAEVALIIFSSRGKLYEF
AP3 : GRGRVELKRIENKINRQVTFAKRRNGLLKKAYELSVLCDAEVALIIFSSRGKLYEF
PI : GRGRVELKRIENKINRQVTFAKRRNGLLKKAYELSVLCDAEVALIIFSSRGKLYEF
AG : GRGRVELKRIENKINRQVTFAKRRNGLLKKAYELSVLCDAEVALIIFSSRGKLYEF

                                I - region
OsMADS5 : CSGSSMLKTLERYKQCHYNAPESNNNSAAELESSYQWS
OsMADS1 : CSGSSMLKTLERYKQCHYNAPESNNNSAAELESSYQWS
FBP2 : CSGSSMLKTLERYKQCHYNAPESNNNSAAELESSYQWS
TM5 : CSGSSMLKTLERYKQCHYNAPESNNNSAAELESSYQWS
AGL4 : CSGSSMLKTLERYKQCHYNAPESNNNSAAELESSYQWS
AGL2 : CSGSSMLKTLERYKQCHYNAPESNNNSAAELESSYQWS
OM1 : CSGSSMLKTLERYKQCHYNAPESNNNSAAELESSYQWS
ZAG3 : CSGSSMLKTLERYKQCHYNAPESNNNSAAELESSYQWS
ZAG5 : CSGSSMLKTLERYKQCHYNAPESNNNSAAELESSYQWS
AGL6 : CSGSSMLKTLERYKQCHYNAPESNNNSAAELESSYQWS
AP1 : CSGSSMLKTLERYKQCHYNAPESNNNSAAELESSYQWS
AP3 : CSGSSMLKTLERYKQCHYNAPESNNNSAAELESSYQWS
PI : CSGSSMLKTLERYKQCHYNAPESNNNSAAELESSYQWS
AG : CSGSSMLKTLERYKQCHYNAPESNNNSAAELESSYQWS

                                K - box
OsMADS5 : QETYLKLRVYALORTORNLGDELGLPLSSKELEQLEROLEASLKQIRSRKKTOLMDQLEDLQRR
OsMADS1 : QETYLKLRVYALORTORNLGDELGLPLSSKELEQLEROLEASLKQIRSRKKTOLMDQLEDLQRR
FBP2 : QETYLKLRVYALORTORNLGDELGLPLSSKELEQLEROLEASLKQIRSRKKTOLMDQLEDLQRR
TM5 : QETYLKLRVYALORTORNLGDELGLPLSSKELEQLEROLEASLKQIRSRKKTOLMDQLEDLQRR
AGL4 : QETYLKLRVYALORTORNLGDELGLPLSSKELEQLEROLEASLKQIRSRKKTOLMDQLEDLQRR
AGL2 : QETYLKLRVYALORTORNLGDELGLPLSSKELEQLEROLEASLKQIRSRKKTOLMDQLEDLQRR
OM1 : QETYLKLRVYALORTORNLGDELGLPLSSKELEQLEROLEASLKQIRSRKKTOLMDQLEDLQRR
ZAG3 : QETYLKLRVYALORTORNLGDELGLPLSSKELEQLEROLEASLKQIRSRKKTOLMDQLEDLQRR
ZAG5 : QETYLKLRVYALORTORNLGDELGLPLSSKELEQLEROLEASLKQIRSRKKTOLMDQLEDLQRR
AGL6 : QETYLKLRVYALORTORNLGDELGLPLSSKELEQLEROLEASLKQIRSRKKTOLMDQLEDLQRR
AP1 : QETYLKLRVYALORTORNLGDELGLPLSSKELEQLEROLEASLKQIRSRKKTOLMDQLEDLQRR
AP3 : QETYLKLRVYALORTORNLGDELGLPLSSKELEQLEROLEASLKQIRSRKKTOLMDQLEDLQRR
PI : QETYLKLRVYALORTORNLGDELGLPLSSKELEQLEROLEASLKQIRSRKKTOLMDQLEDLQRR
AG : QETYLKLRVYALORTORNLGDELGLPLSSKELEQLEROLEASLKQIRSRKKTOLMDQLEDLQRR
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Identifying gene of interest for functional study

-Homology-based analysis:

- Functional domains
- Phylogenetic analysis

	MADS Domain
E20969	MGRGKIEIKRIENATNRQVTYSKRRTGIMGKARELTVLCDQAVALIMFSSTGKYHEF
E31864	MGRGKVQLKRIENKINRQVTFSKRRNGLJKAHEISVLCDAEVAIAIVFSPKGKLYEY
E31254	MGRGKVVLRRIENKISRQVTFAKRRNGLLKKAYELSLCDAEVALVLFSSHAGRLYQF
R3786	MARGKVQLRRIENPVIHQVTFCRRRAGLLKKARELSLCEADIGIIFSAHGKLYDL
S11905	MVRGKTQNKRIENPTSRQVTFSKRRNGLLKKAFELSVLCDAEVALIVFSPRGKLYEF
C50086	MARERRKRIKIESAAARQVTFSKRRRGLFKKAEELSVLCDAEVALIVFSTGKLSHF
S10304	MARRGRVQLRRIEDKASRQVRFKRRRAGLFFKAFELALLCDVEVALIVFSPVGKLYEY
OsMADS1	MGRGKVELKRIENKISRQVTFAKRRNGLLKKAYELSLCDAEVALIIFSGRGRLLFEF
OsMADS2	MGRGKIEIKRIENSTNRQVTFSKRRSGILKKAREISVLCDAEVGUVVIFSSAGKLYDY
OsMADS4	MGRGKIEIKRIENSTNRQVTFSKRRSGILKKAREISVLCDAEVGUVVIFSSAGKLSDY
OsMADS5	MGRGKVELKRIENKISRQVTFAKRRNGLLKKAYELSVLCDAEVALIIFSTRGRLLFEF
consensus	MGRGKVELKRIEN**RQVTFSKRR*GLLAKA*ELSVLCDAEVALI*FS**GKLYEF

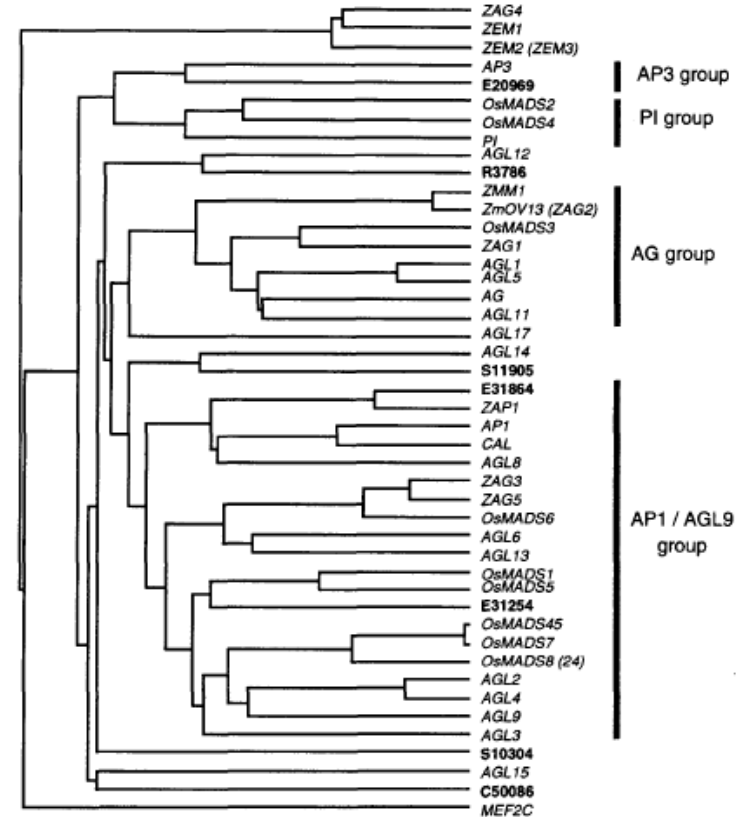


Image source:
Shinozuka et al. (1999);
DNA Res. 6:123–9.

Identifying gene of interest for functional study

➤ *Identifying single gene with putative developmental function: (Candidate gene)*

- Homology-based analysis

- Gene family
- Sequence similarity
- Functional domains
- Phylogenetic analysis

- Expression pattern analysis-

- Organ-specific expression pattern (Differential expression pattern)
- Tissue-specific gene expression (Spatial expression pattern)
- Developmental stage-specific gene expression (Temporal expression pattern)

Identifying gene of interest for functional study

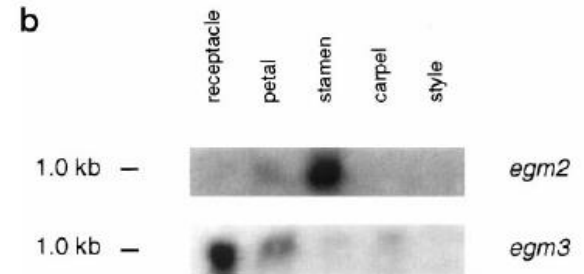
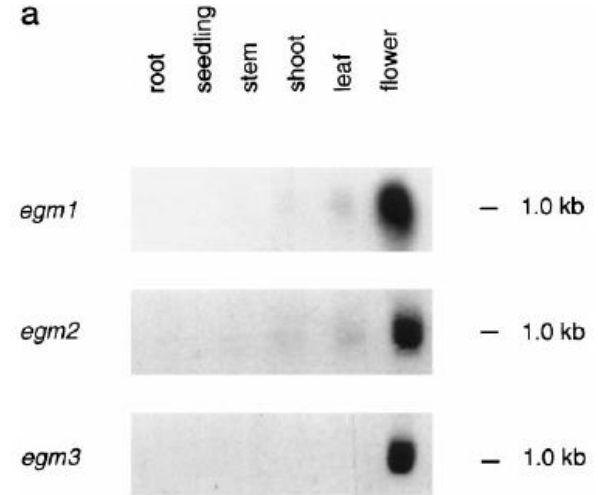
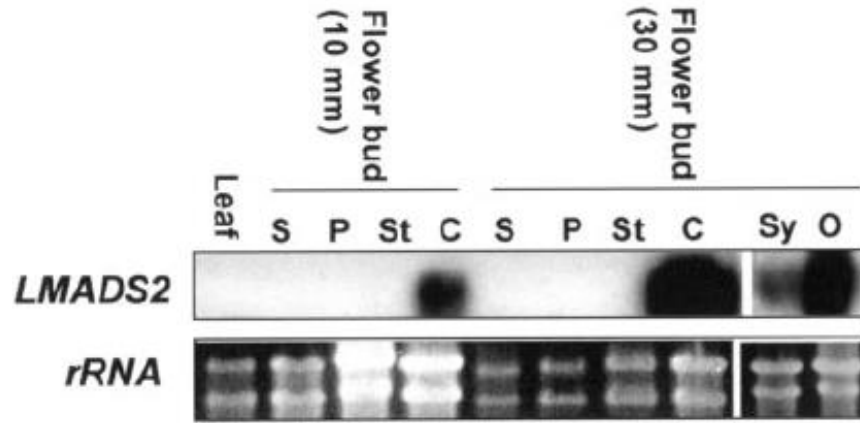


Image source:
Tzeng et al. (2002);
Plant Physiol. 130 :1827–36.

Image source:
Southerton et al. (1998);
Plant Physiol. 118:365-72.

Isolation of gene of interest for functional study

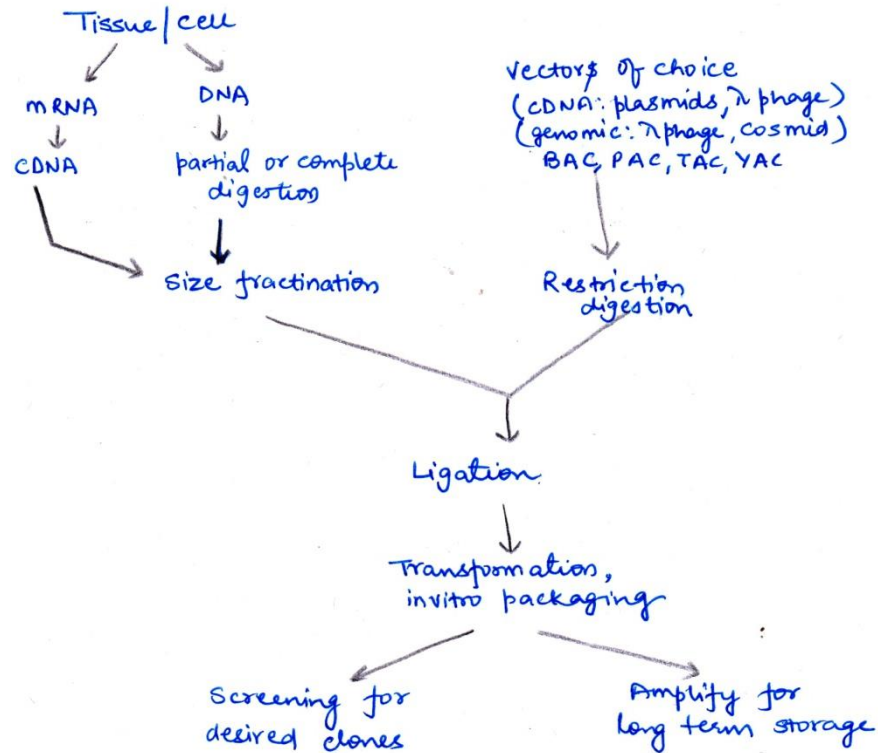
➤ *Genome not sequenced:*

- *Screening cDNA library*
- *Screening genomic library*

➤ *Genome sequenced:*

- *PCR-based gene amplification*

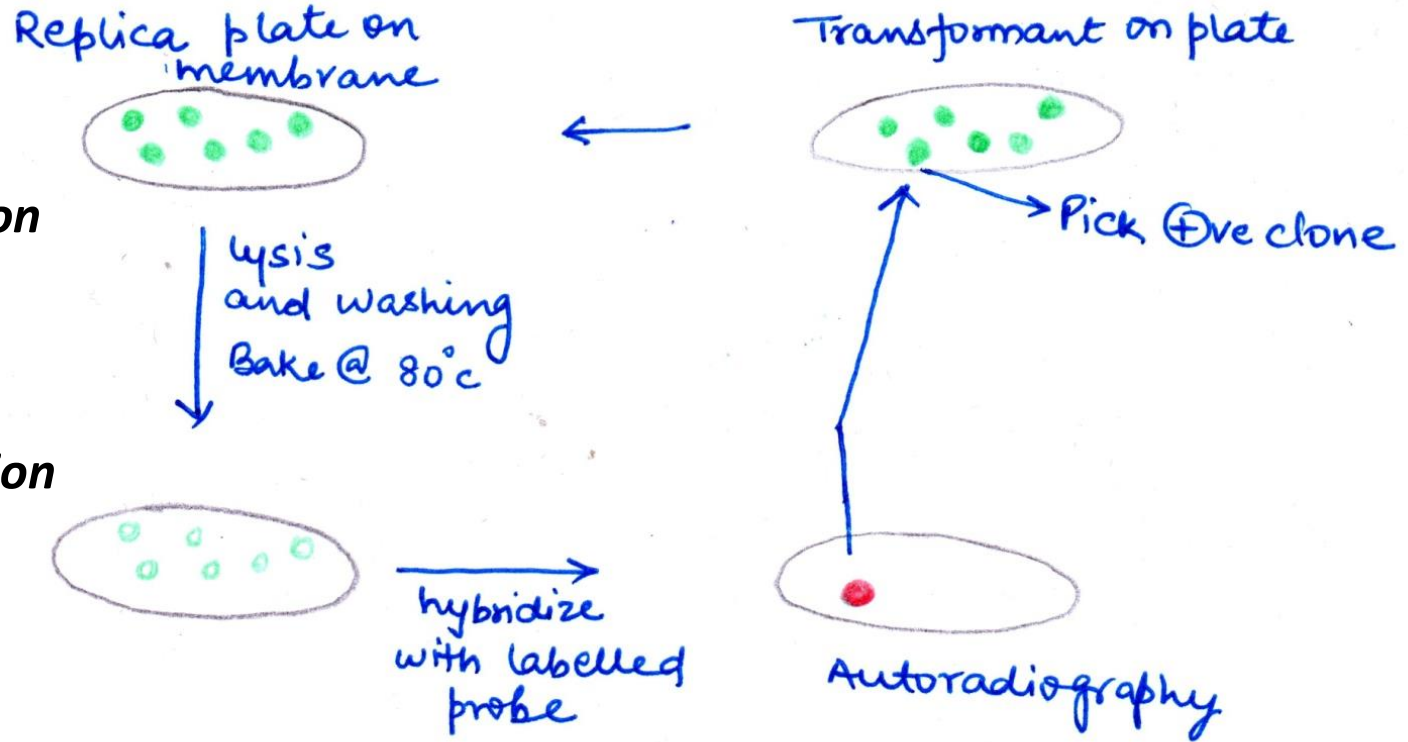
Constructing cDNA/genomic library



Screening cDNA library

➤ Colony hybridization

➤ Plaque hybridization



Isolation of gene of interest for functional study

➤ ***Genome sequenced:***

- PCR-based gene amplification

- Genomic copy (with introns)

- cDNA copy (without introns)



Identifying gene of interest for functional study

➤ *Global genes (Genomics): Identifying genome-wide genes for a developmental process*

- Structural Genomics:

- Genome sequencing: sequencing whole genome
- Genome organization: organization of genome components
- Genome annotation: predicting putative function
- EST sequencing: determining partial sequences of cDNA



Identifying gene of interest for functional study

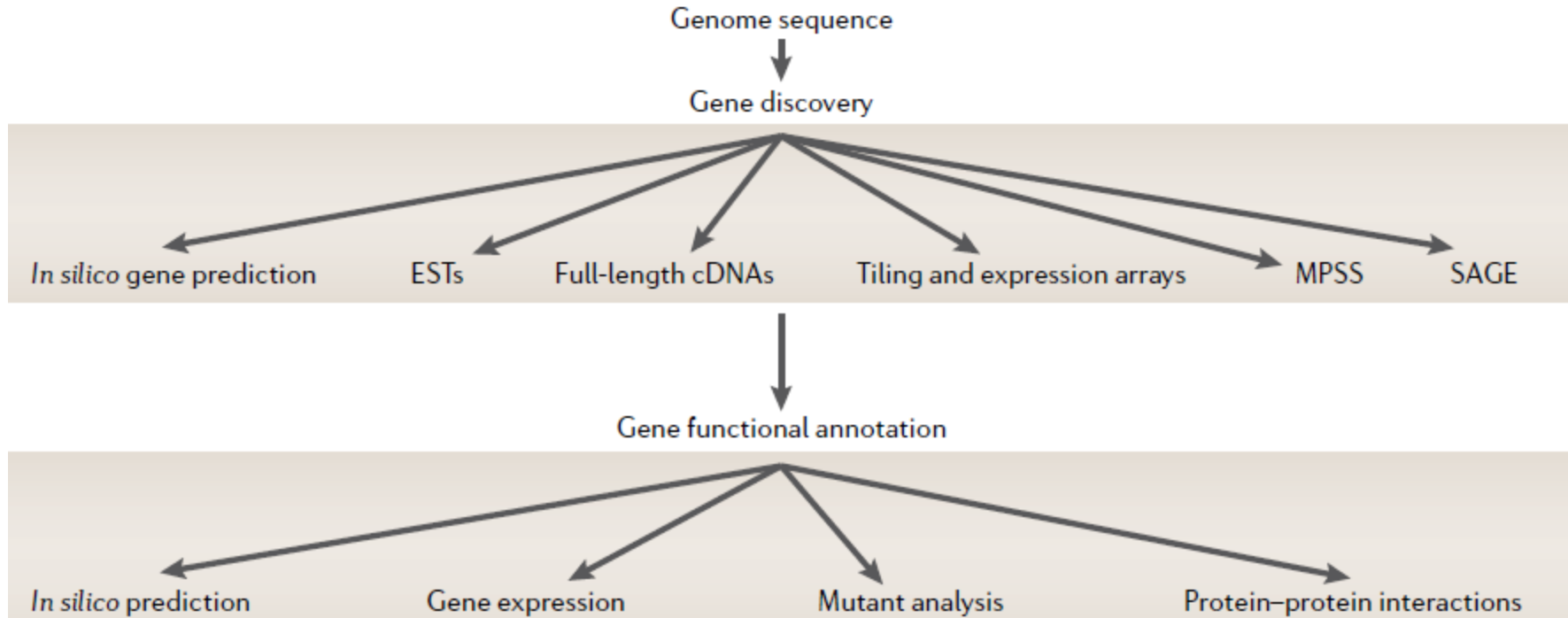


Image source: Alonso and Ecker (2006); Nat. Gent. 7:524–36.

Identifying gene of interest for functional study

➤ ***Global genes (Genomics): Identifying genome-wide genes for a developmental process***

- Functional Genomics:

- Monitoring gene expression
- Functional characterization
- Molecular mechanism

Identifying gene of interest for functional study

➤ **Monitoring gene expression:**

- Organ-specific expression pattern (Differential expression pattern)
- Tissue-specific gene expression (Spatial expression pattern)
- Developmental stage-specific gene expression (Temporal expression pattern)
- Promoter trapping (expression pattern)



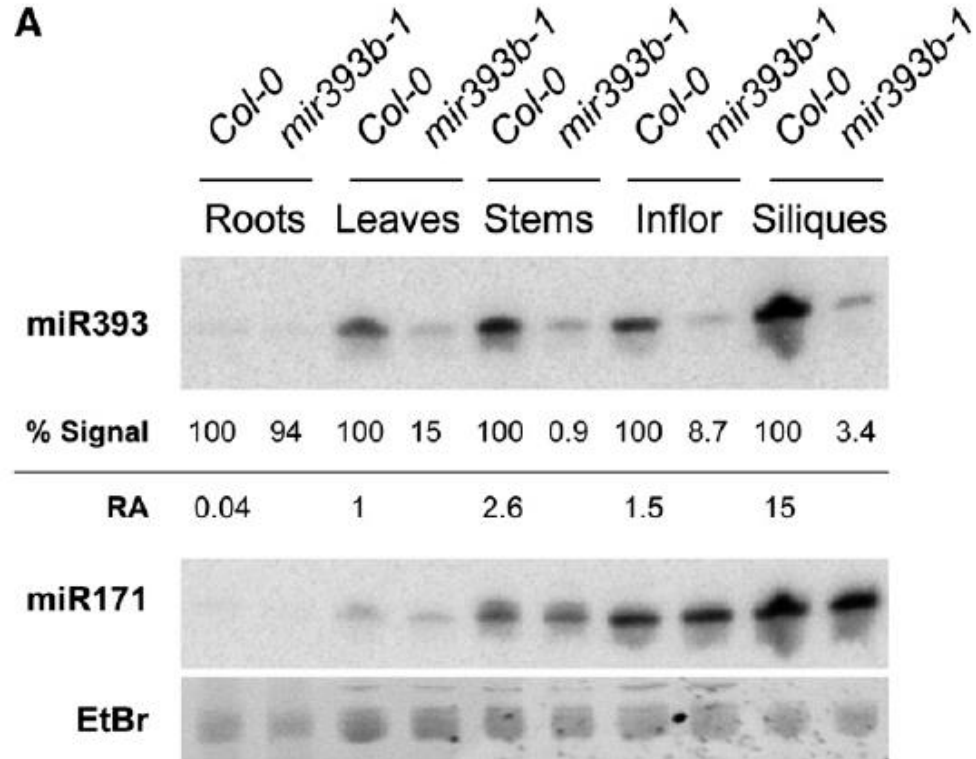
Identifying gene of interest for functional study

➤ Monitoring gene expression:

- Organ-specific expression pattern
(Differential expression pattern)

- Northern Blot analysis

Image source:
Si-Ammour et al. (2011)
Plant Physiology, 157, 683-91.



Identifying gene of interest for functional study

➤ Monitoring gene expression:

- Organ-specific expression pattern
(Differential expression pattern)

-RT-PCR analysis

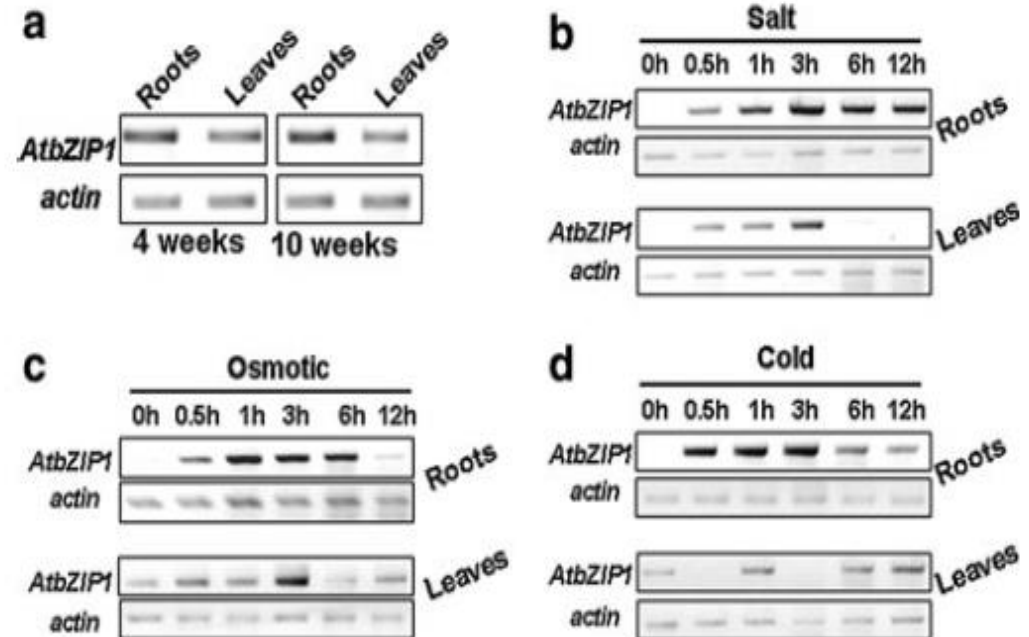


Image source:
Sun et al. (2011)
Journal of Plant Research 125:429-38.

Identifying gene of interest for functional study

➤ Monitoring gene expression:

- Organ-specific expression pattern
(Differential expression pattern)

-qRT-PCR analysis

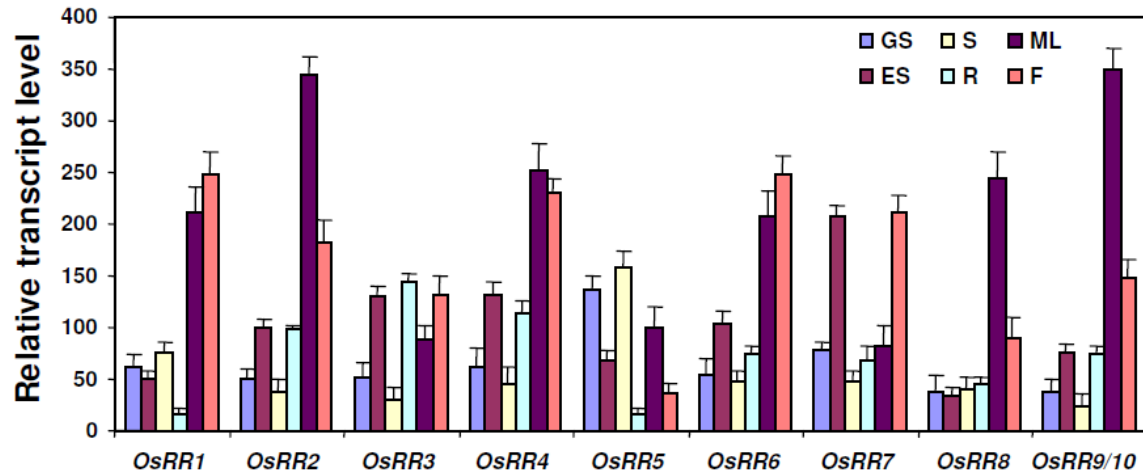


Image source:

Jain et al. (2006)

BMC Plant Biology, 6:1.

Real-time PCR analysis showing the organ-specific expression profiles of individual *OsRR* genes. The relative mRNA levels of individual *OsRR* genes normalized with respect to housekeeping gene, *UBQ5*, in different tissues (GS, green seedlings; ES, etiolated seedlings; S, green shoots; R, roots; ML, mature leaves; F, flowers) are presented.