



IIT ROORKEE



NPTEL ONLINE
CERTIFICATION COURSE

Molecular Genetics of Plant Development-cont..II

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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)
 - *Identifying & 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)*



Functional characterization of the gene

- **Loss-of-function (necessity of a gene)**
 - Knock-out
 - Knock-down
- **Gain-of-function (sufficiency of a gene)**
 - Ectopic expression
 - Activation tagging



Loss-of-function (Knock-out)

- **T-DNA insertion inactivation:** Screening T-DNA insertion lines
- **Transposon insertion inactivation :** Screening lines
- **Genome editing:** Targeting a gene for genome editing



Loss-of-function (Knock-out)

- **T-DNA insertion inactivation:** Screening T-DNA insertion lines
- **Transposon insertion inactivation :** Screening lines

Table 1. Gene disruption resources in *Arabidopsis*

Laboratory	Insertion element	Number	Source	Sequenced insertions
Salk Institute	T-DNA	70 000	http://signal.salk.edu/cgi-bin/tdnaexpress	~94 947
TMRI	T-DNA	100 000	http://www.tmri.org/pages/collaborations/garlic_files/	~100 000
CSHL	Ds GT and ET	27 000	http://genetrap.cshl.org/	~26 800
GABI-KAT	T-DA	23 000	http://www.mpiz-koeln.mpg.de/GABI-Kat/	~20 764
FLAG	T-DNA	50 000	http://flagdb-genoplante-info.infobiogen.fr/	~11 500
John Innes Centre	DSpm and Ds GT	50 000	http://atidb.cshl.org/	~13 000

Source: Pan et al. (2003)
Nucleic Acids Research, 31, 1245-51.

T-DNA Express - SIGnAL :
Salk - Salk Institute
 [\(signal.salk.edu/cgi-bin/tdnaexpress\)](http://signal.salk.edu/cgi-bin/tdnaexpress)

- [Download data](#)
[T-DNA FAQs](#)
[RNAi FAQs](#)

T-DNA Primer Design

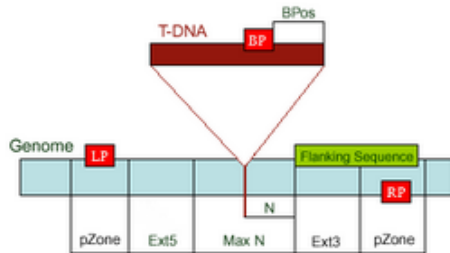
(Powered by GEED)

Please use [the backup page](#) served by AtTA, if the tdnaexpress server is down.

The new T-DNA Primer Design Tool is now powered by Genome Express Browser Server (GEED). The new tool can return the primers faster, and also give the insertion location information, the estimated T-DNA confirmation product size, as well as primer3-like format output. (July 28, 2005)

Important Change: Now the RP is always on the side of the flanking sequence, that is, RP is always on the 3' end of the insertion. Therefore, the PCR reaction should always be set up as LB+RP for HM and LP+RP for WT. (Feb. 04, 2005)

1. Protocol for SALK T-DNA primer design



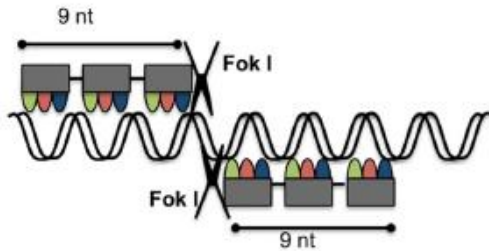
	WT	HZ	HM
900			
410+N			
* N = 0 ~ 300			

T-DNA Express - SIGnAL :
Salk - Salk Institute
(signal.salk.edu/cgi-bin/tdnaexpress)

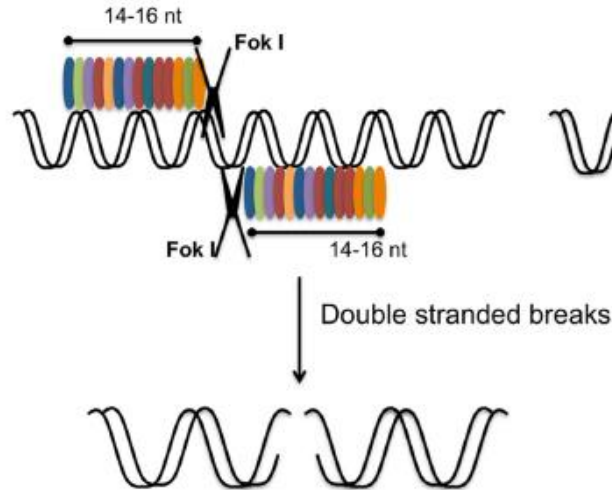
Loss-of-function (Knock-out)

Genome editing: Targeting a gene for genome editing

A Zinc Finger Nucleases (ZFNs)



B TAL Effector Nucleases (TALENs)



C CRISPR/Cas9

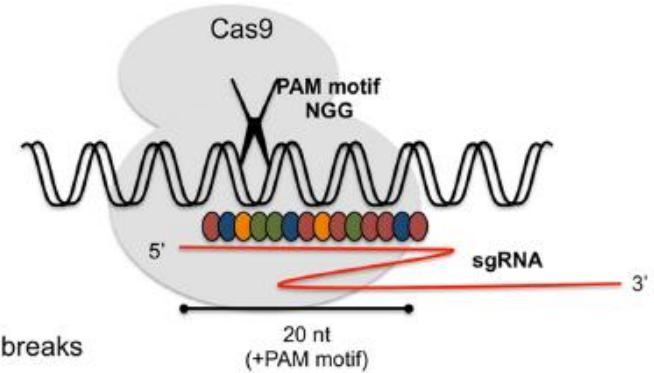


Image source: Arora & Narula (2017)
Front. Plant Sci. 8:1932.

Loss-of-function (Knock-out)

CRISPR/Cas9 based genome-editing

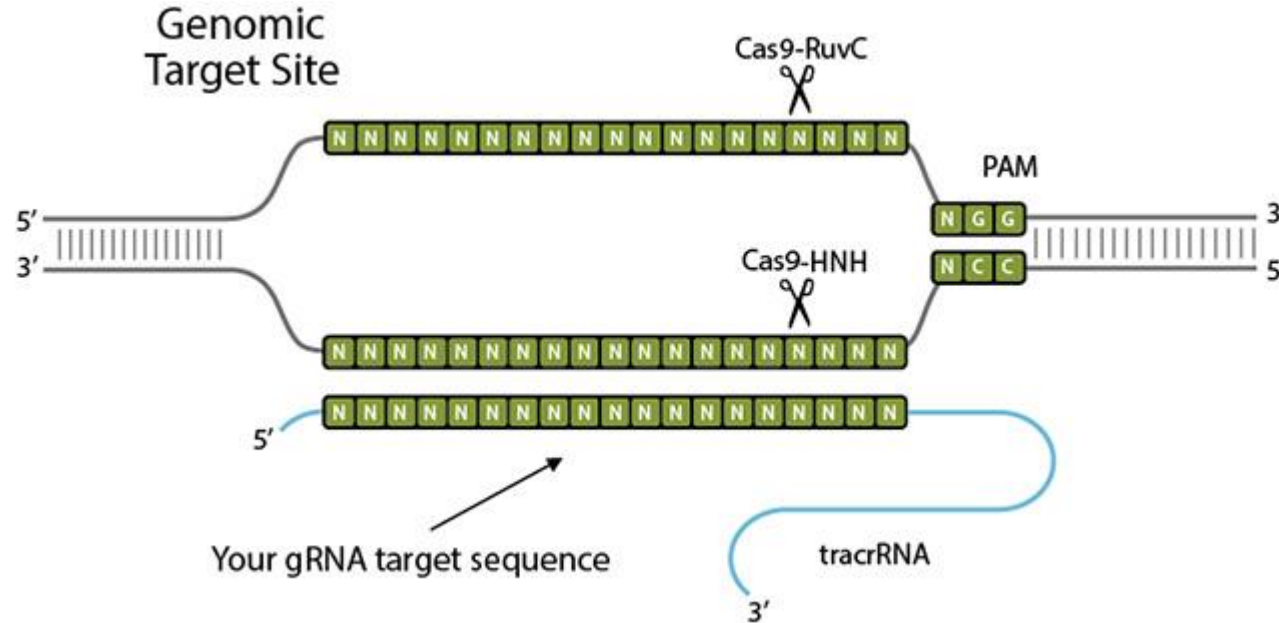


Image source:
<https://www.sigmaaldrich.com/>

CRISPR/Cas9 based genome-editing

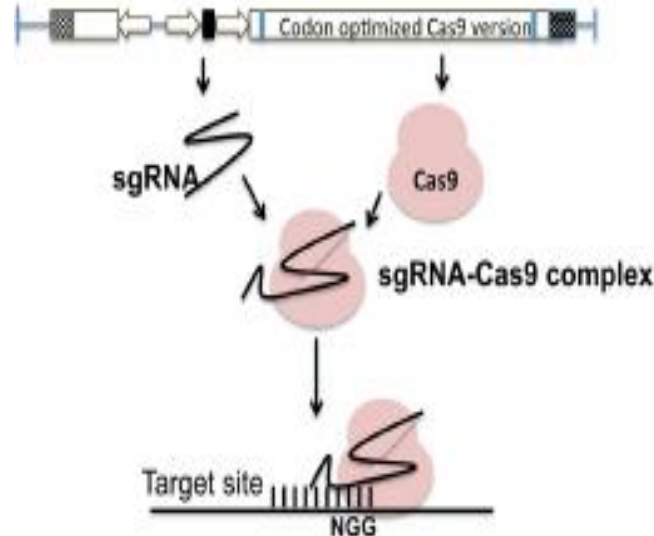
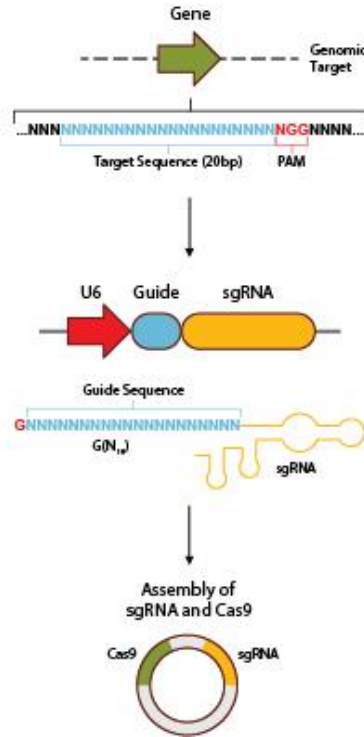
1. Select Genomic Target

- 20 bp sequence followed by the PAM (NGG)
- Use online tools to minimize off-targeting

2. Design sgRNA

- sgRNA is expressed using a small RNA promoter, such as U6p or U3p
- First nucleotide in the guide sequence is a "G", when U6p is used
- Guide sequence should match the target except for the first nucleotide (5' G or A) which can be different

3. Assemble Cas9/sqRNA Construct



A Non-homologous end joining

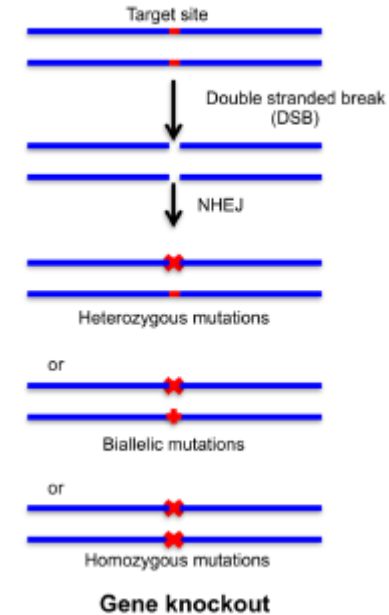


**Image source: Arora & Narula (2017)
Front. Plant Sci. 8:1932.**

Image source: <https://www.sigmaaldrich.com/>

Functional characterization of the gene

- **Loss-of-function** (Knock-down/down-regulation)
 - Anti-sense RNA Technology
 - Double-stranded RNA interference (dsRNAi)
 - Artificial miRNAs



Anti-sense RNA Technology

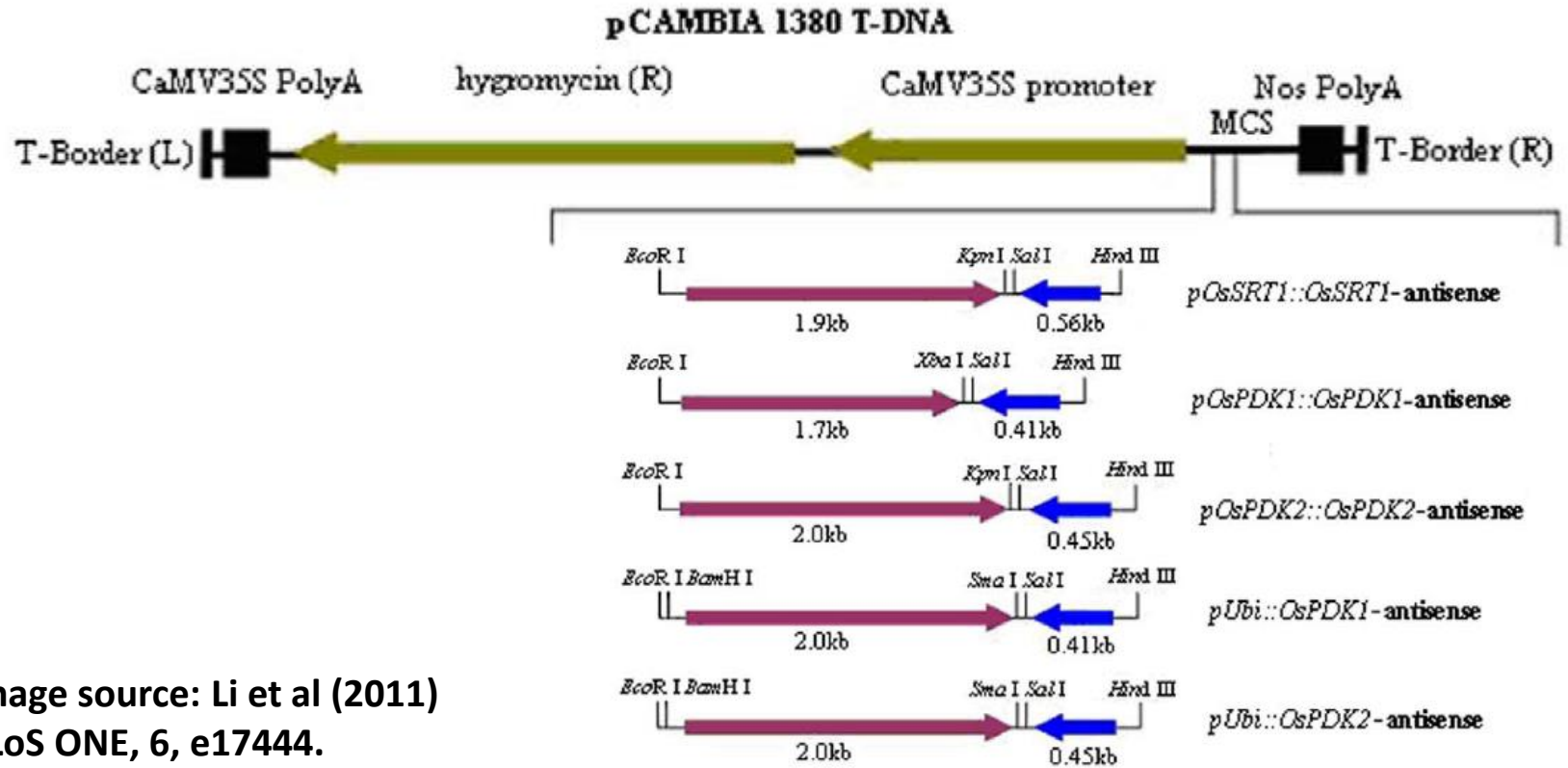


Image source: Li et al (2011)
PLOS ONE, 6, e17444.

Anti-sense RNA Technology

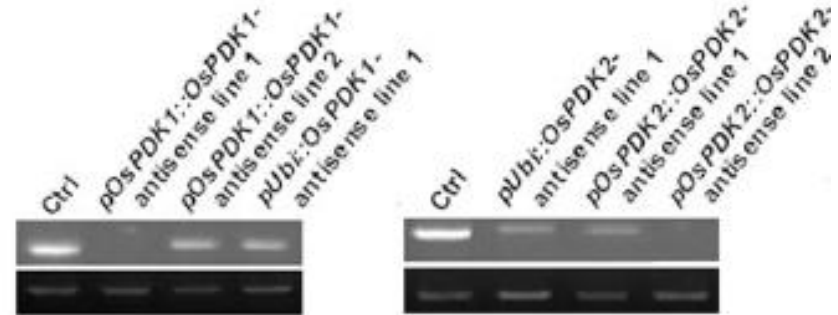
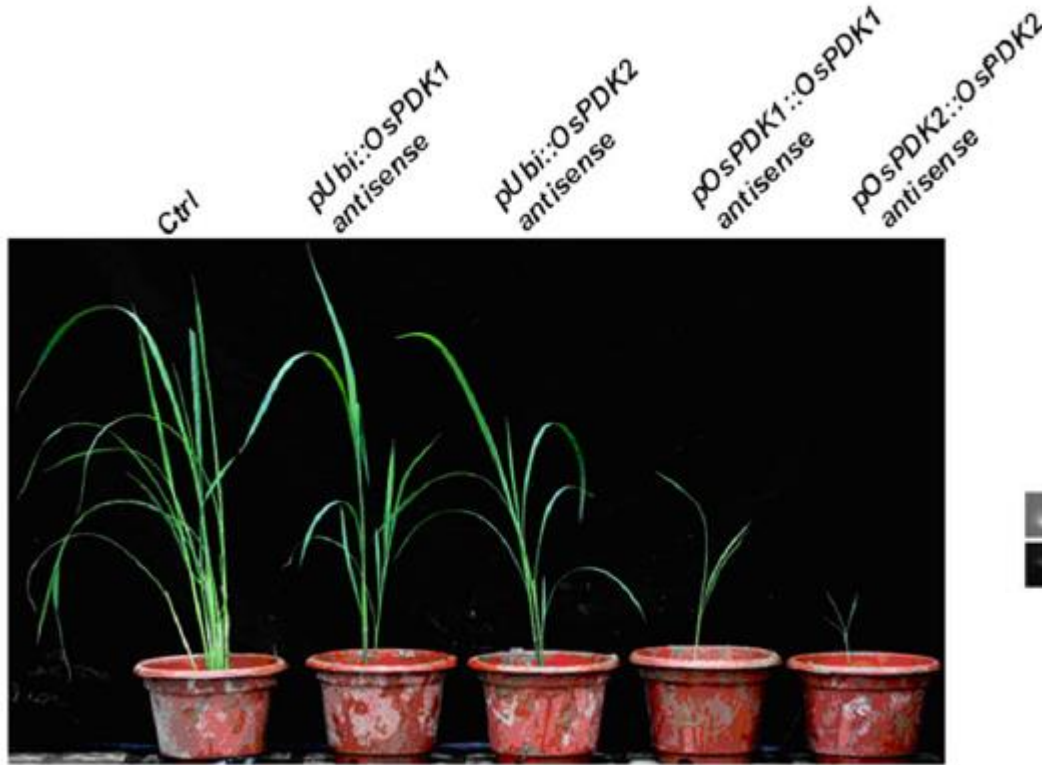


Image source: Li et al (2011)
PLoS ONE, 6, e17444.

Double-stranded RNA Interference Technology

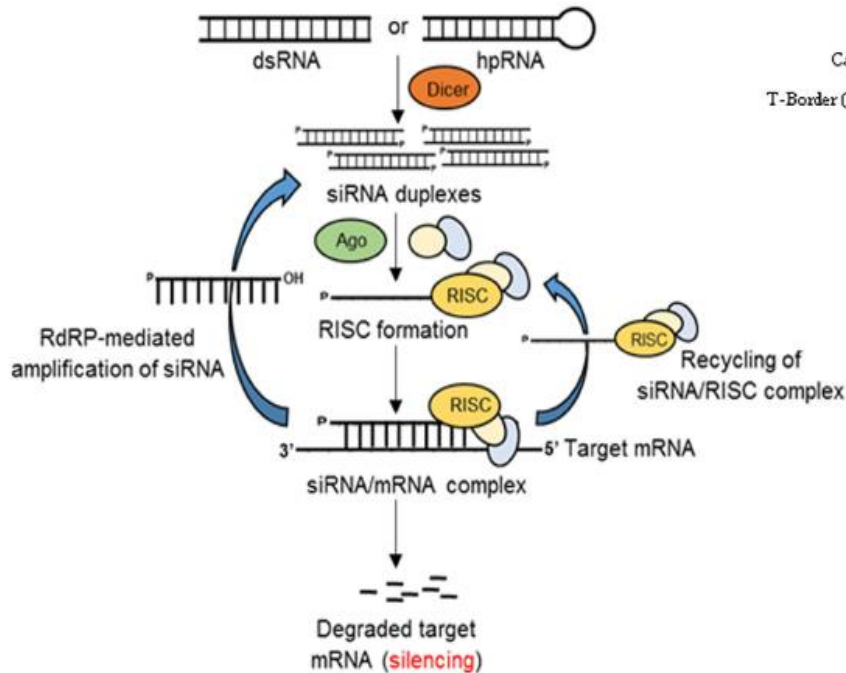


Image source: Majumdar et al (2017)
Front. Plant Sci. 8:200.

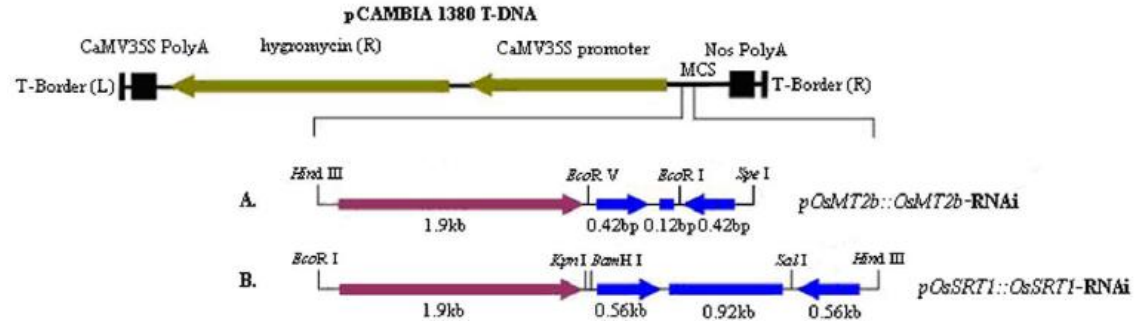


Image source: Li et al (2011)
PLoS ONE, 6, e17444.

Double-stranded RNA Interference Technology

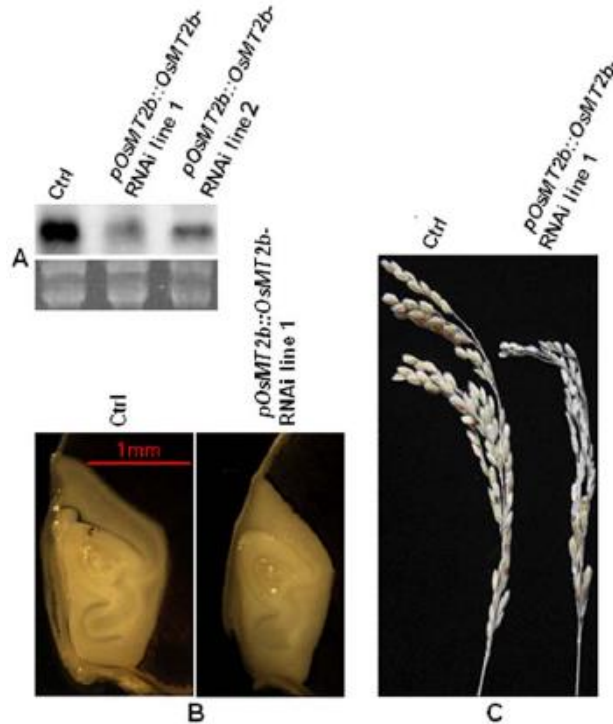


Image source: Li et al (2011)
PLOS ONE, 6, e17444.

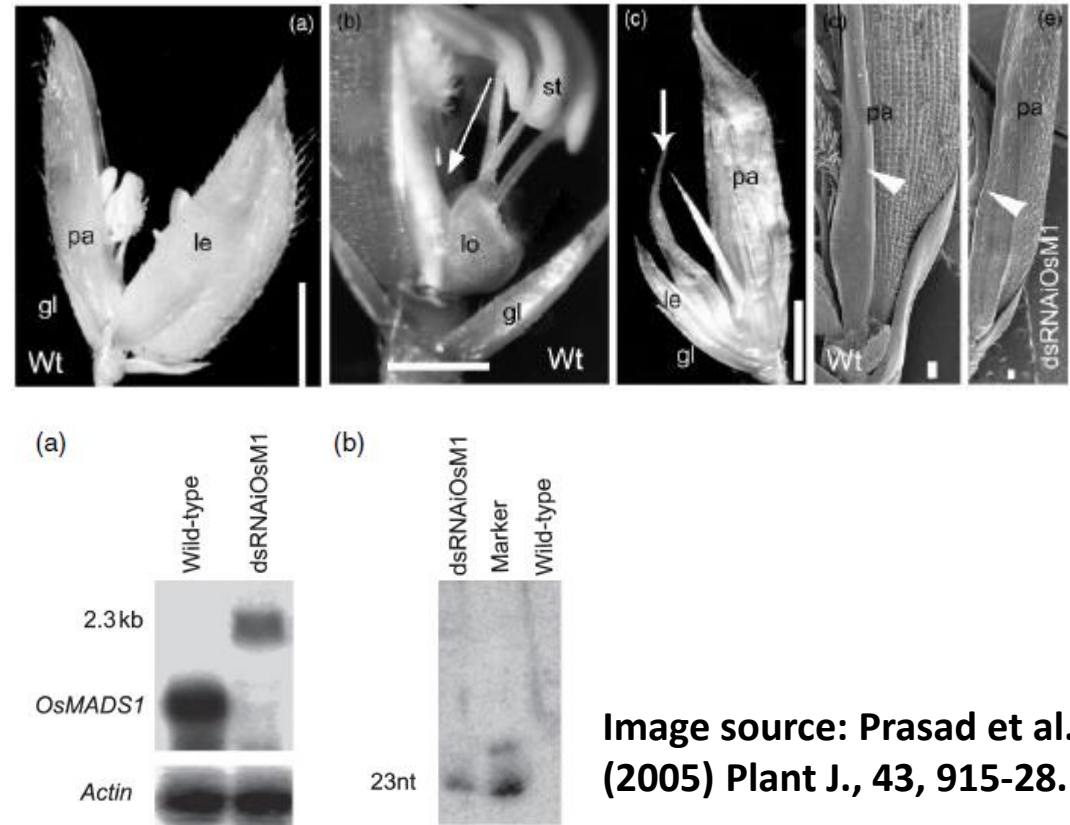
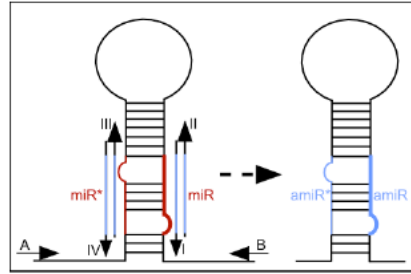
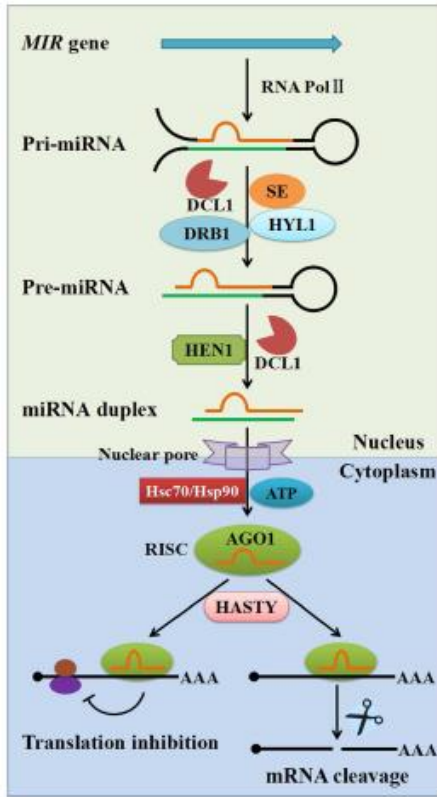


Image source: Prasad et al.
(2005) Plant J., 43, 915-28.

Artificial miRNA Technology



I: microRNA forward
II: microRNA reverse
III: microRNA* forward
IV: microRNA* reverse

	forward oligo	reverse oligo	template
(a)	A	IV	pRS300
(b)	III	II	pRS300
(c)	I	B	pRS300
(d)	A	B	(a)+(b)+(c)

PCR scheme to produce artificial miRNA constructs from pNW55

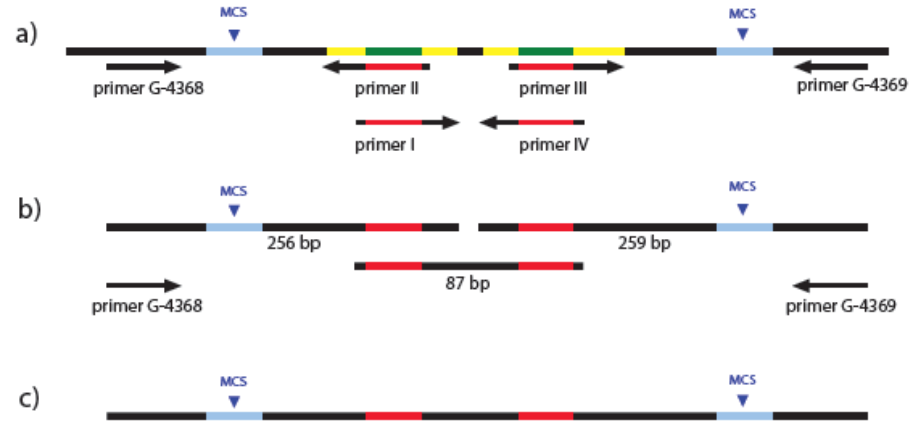
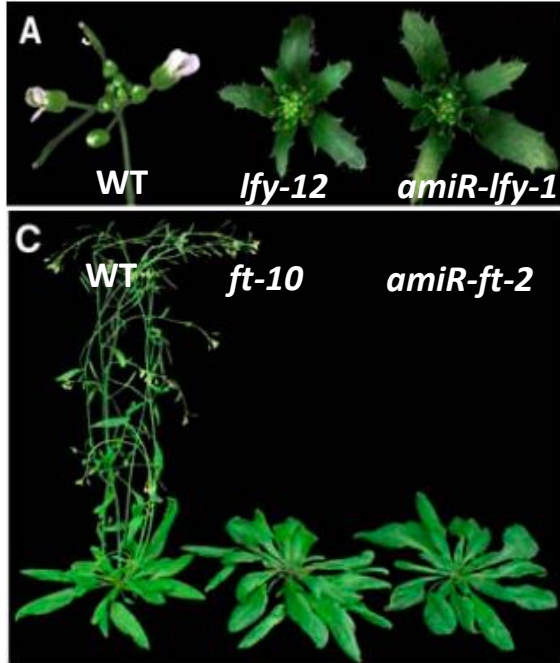


Image source: Liu et al (2017)
Front. Microbiol. 8:1801.

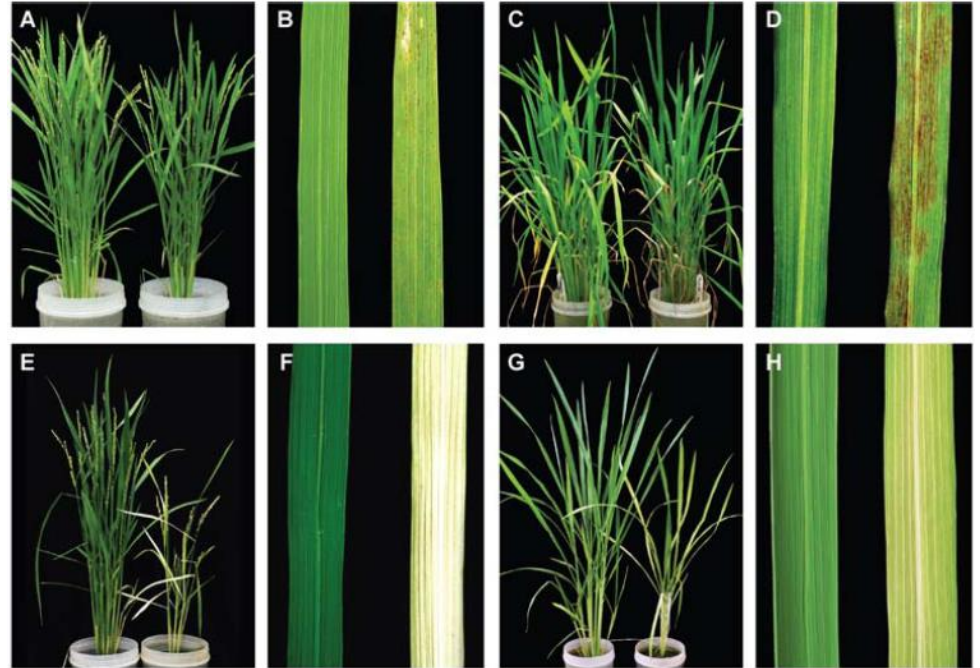
Image source: Schwab et al.
(2006) Plant Cell 18: 1121-33.

Image source: Warthmann et al.
(2008) PLoS ONE 3: e1829.

Artificial miRNA Technology



amiR-Spl11



amiR-Pds

Image source: Schwab et al.
(2006) Plant Cell 18: 1121-33.

Image source: Warthmann et al.
(2008) PLoS ONE 3: e1829.

Gain-of-function (Ectopic expression)

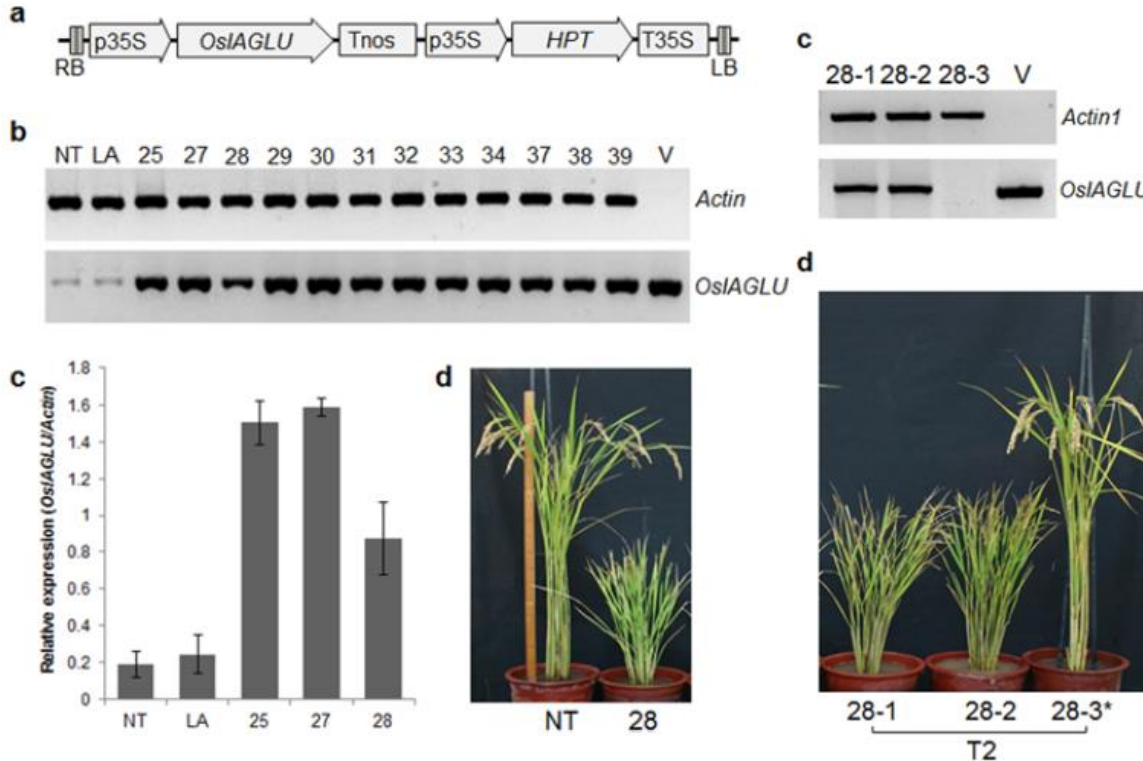


Image source: Choi et al. (2012) J. Plant Biol. 55:429-35.

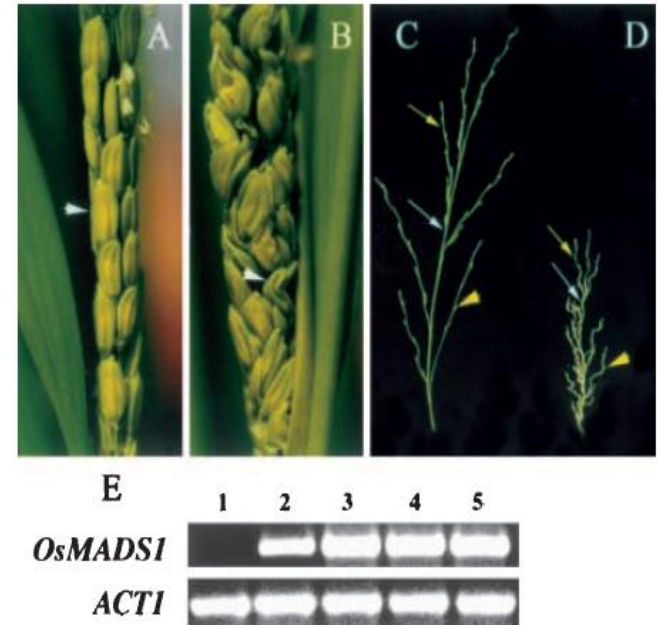


Image source: Prasad et al. (2001) Dev. Genes Evol., 211:281–90.

Gain-of-function: Activation tagging

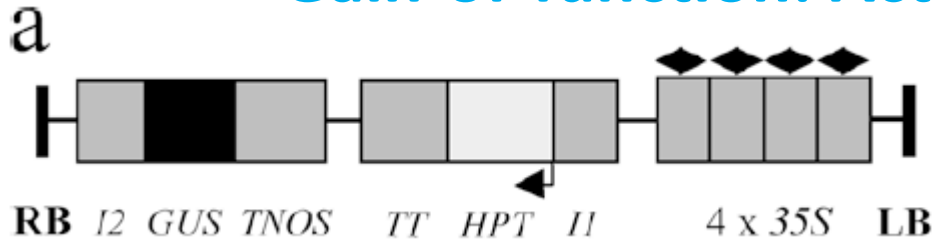


Image source: Tani et al. (2004)

Funct Integr Genomics. 4:258-66.

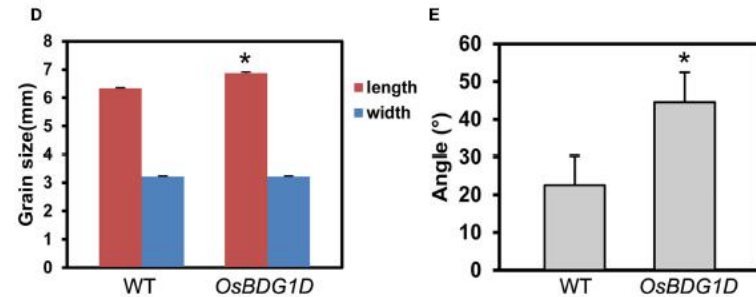
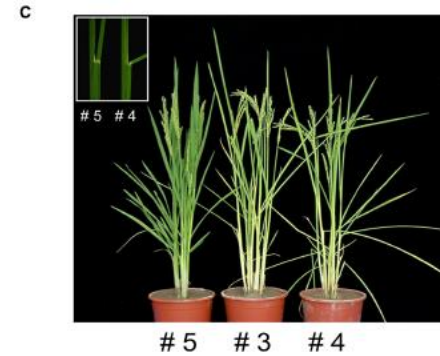
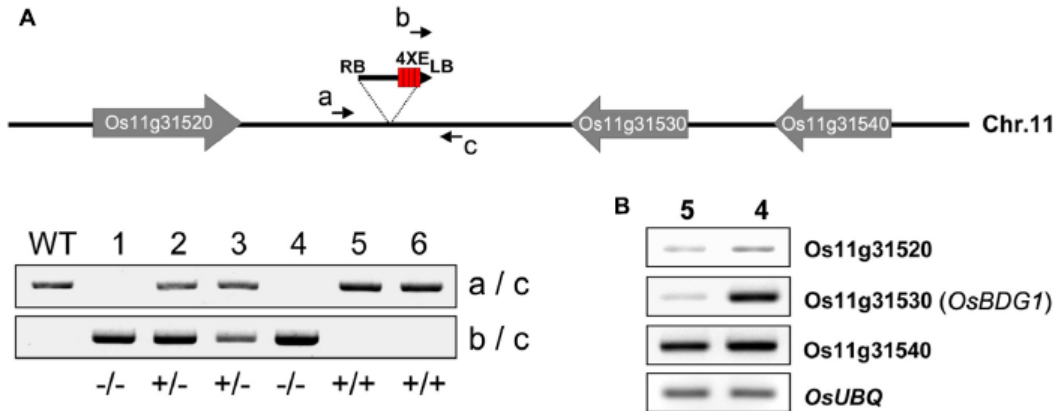


Image source: Jang & Li (2017)

Front. Plant Sci. 8:1253.

Regulated gene expression of constructs in plants

- **Spatial regulated construct:** Use of tissue-specific Promoter
- **Temporal regulated construct:** Stage-specific promoter or chemical inducible system
- **Spatial and Temporal regulated:** Combination of both

Regulated gene expression of constructs in plants

Glucocorticoid receptor-based system

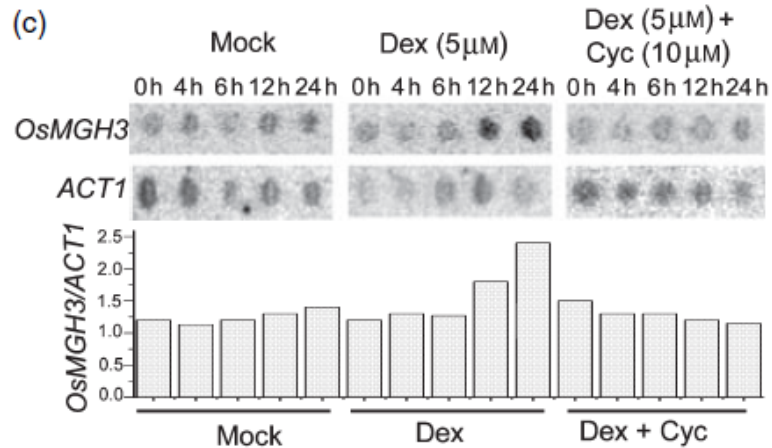


Image source: Prasad et al. (2005) *Plant J.*, 43, 915-28.

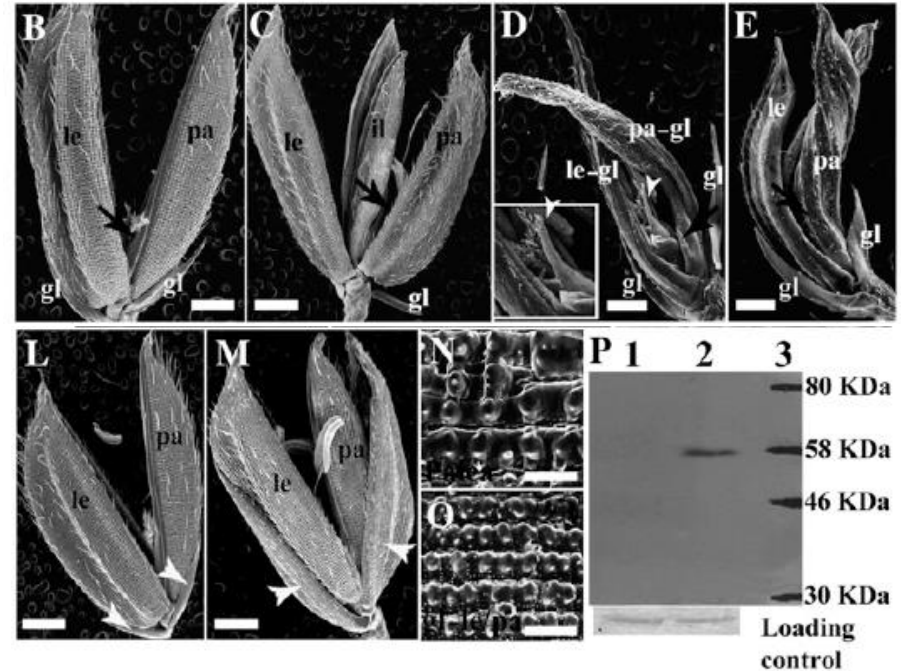


Image source: Khanday, Yadav (2013) *Plant Physiology*, 161:1970-83.

Regulated gene expression of constructs in plants

Estrogen receptor-based system

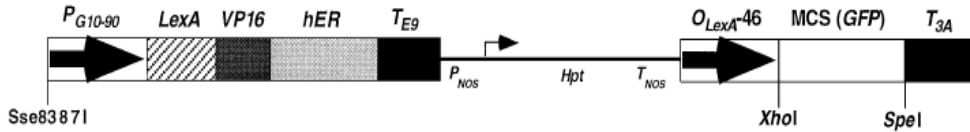


Image source: Zuo et al. (2000)
Plant J. 24:265-73.

XVE: chimeric transcription factor

X- DNA-binding domain of LexA

V- trans-activation domain of VP16

E- partial human estrogen receptor

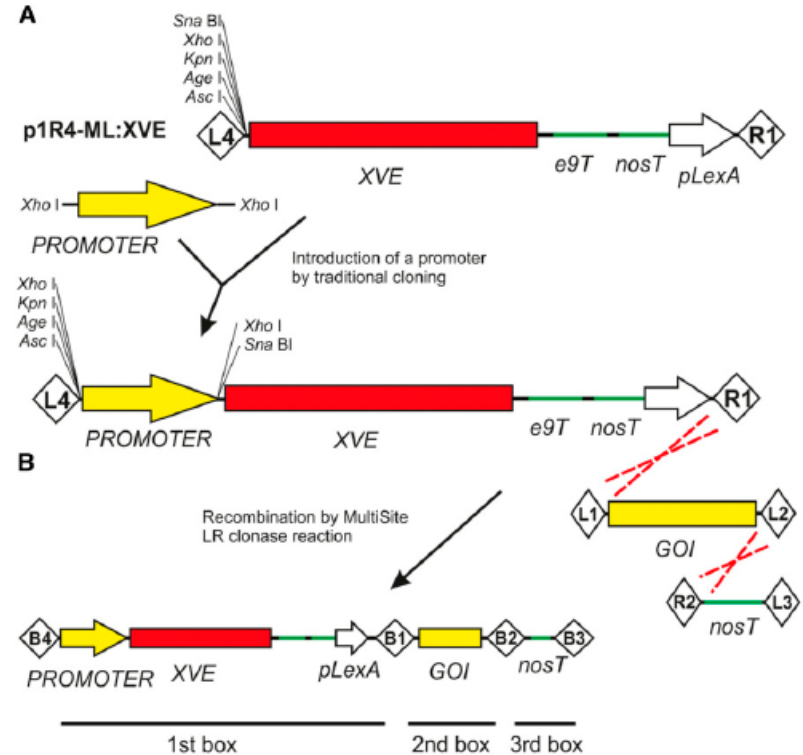
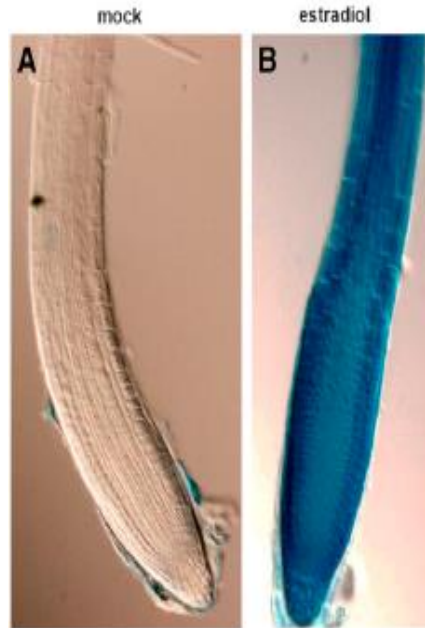
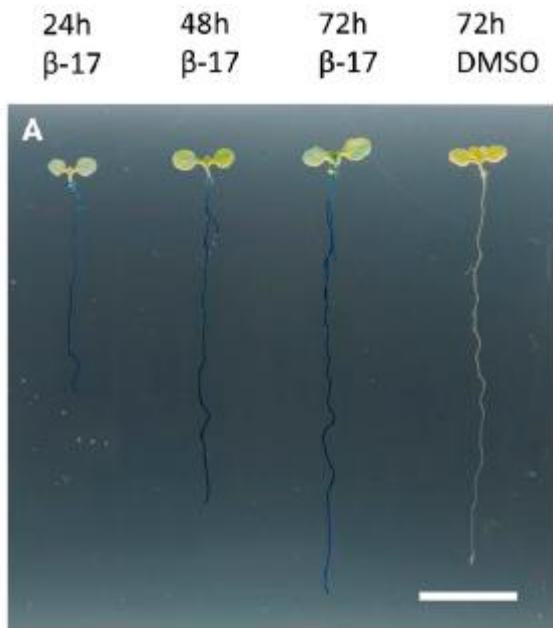


Image source: Siligato et al. (2016) Plant Physiology , 170:627-41.

Regulated gene expression of constructs in plants

Estrogen receptor-based system

pG10-90:XVE>>GUS



pG10-90:XVE>>PLT5-YFP

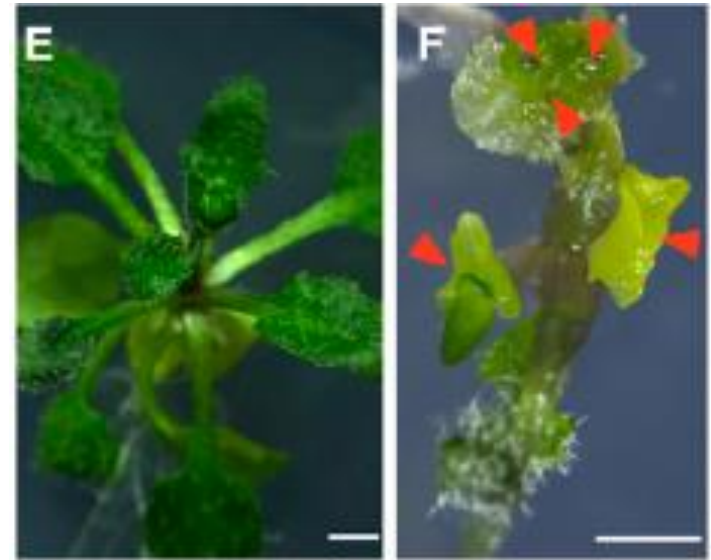


Image source: Siligato et al. (2016) Plant Physiology , 170:627-41.

Approaches to the study of plant development

- **Reverse Genetics:** An approach to study function(s) of gene(s)
(Genotype to Phenotype)
 - *Identifying & isolation of gene for functional study (gene of interest)*
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