Using RNA-seq to identify new genes regulating a developmental process; experience form underground and above ground plant biology.

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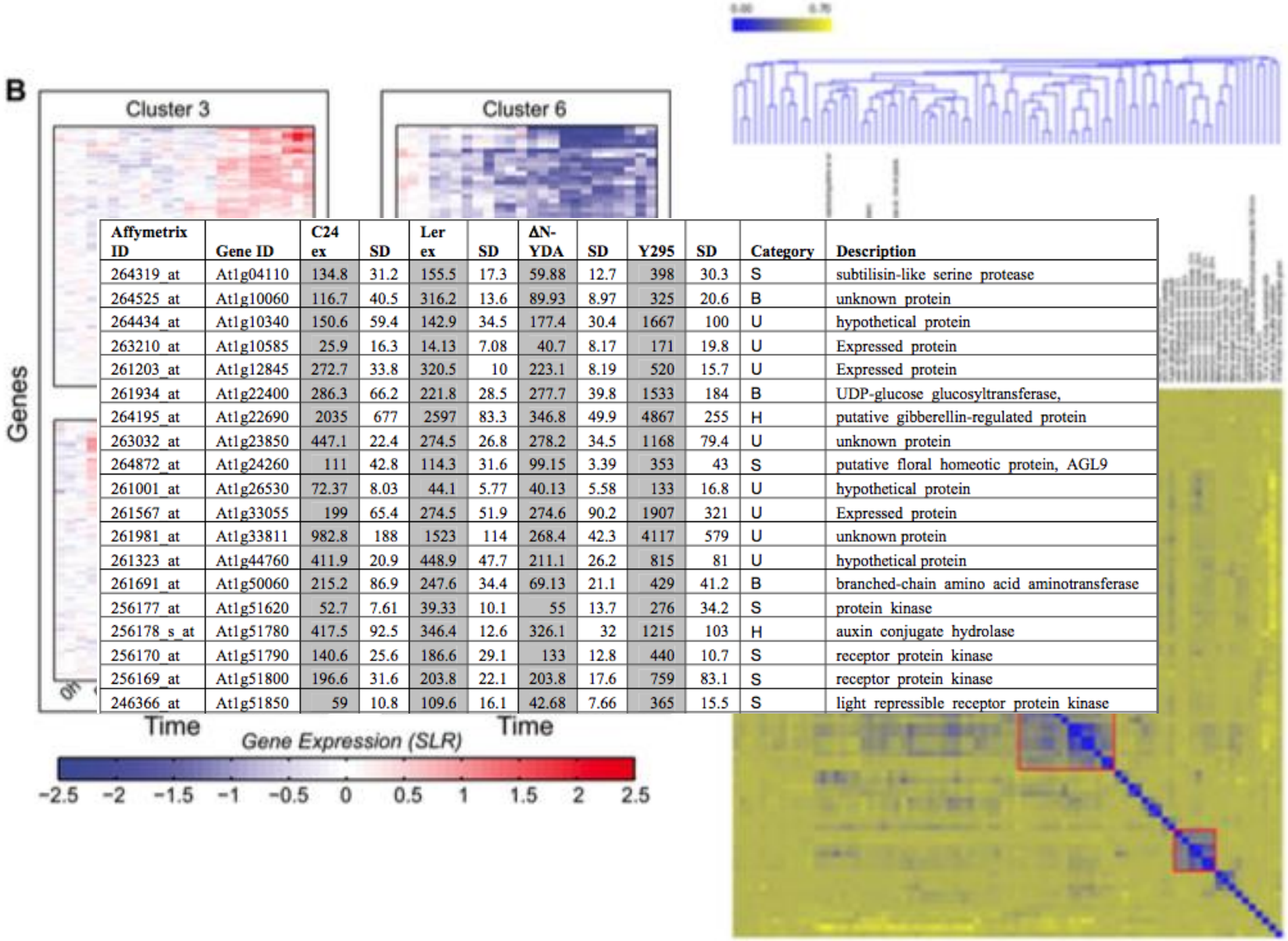
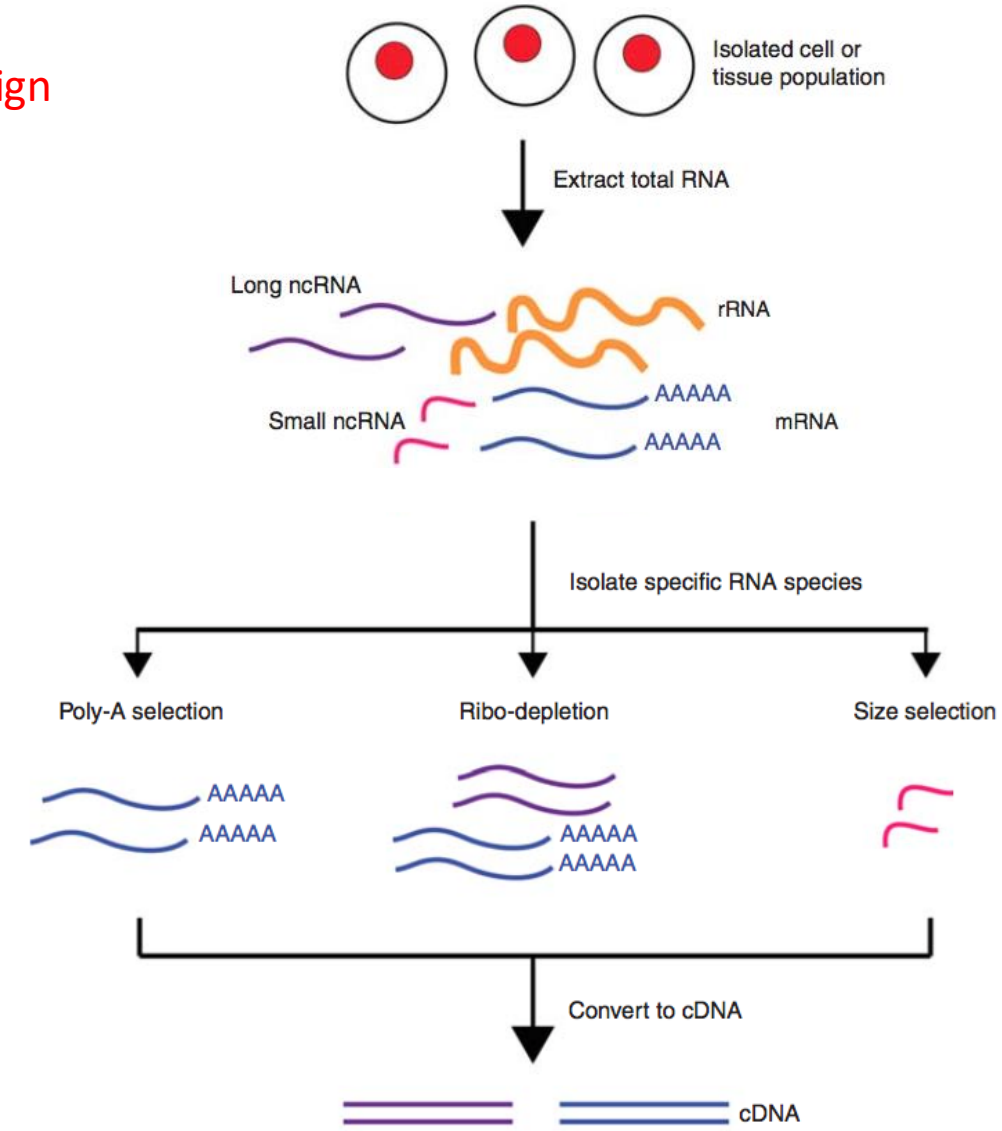


Affymetrix ID	Gene ID	C24 ex	SD	Ler ex	SD	ΔN-YDA	SD	Y295	SD	Category	Description
264319_at	At1g04110	134.8	31.2	155.5	17.3	59.88	12.7	398	30.3	S	subtilisin-like serine protease
264525_at	At1g10060	116.7	40.5	316.2	13.6	89.93	8.97	325	20.6	B	unknown protein
264434_at	At1g10340	150.6	59.4	142.9	34.5	177.4	30.4	1667	100	U	hypothetical protein
263210_at	At1g10585	25.9	16.3	14.13	7.08	40.7	8.17	171	19.8	U	Expressed protein
261203_at	At1g12845	272.7	33.8	320.5	10	223.1	8.19	520	15.7	U	Expressed protein
261934_at	At1g22400	286.3	66.2	221.8	28.5	277.7	39.8	1533	184	B	UDP-glucose glucosyltransferase,
264195_at	At1g22690	2035	677	2597	83.3	346.8	49.9	4867	255	H	putative gibberellin-regulated protein
263032_at	At1g23850	447.1	22.4	274.5	26.8	278.2	34.5	1168	79.4	U	unknown protein
264872_at	At1g24260	111	42.8	114.3	31.6	99.15	3.39	353	43	S	putative floral homeotic protein, AGL9
261001_at	At1g26530	72.37	8.03	44.1	5.77	40.13	5.58	133	16.8	U	hypothetical protein
261567_at	At1g33055	199	65.4	274.5	51.9	274.6	90.2	1907	321	U	Expressed protein
261981_at	At1g33811	982.8	188	1523	114	268.4	42.3	4117	579	U	unknown protein
261323_at	At1g44760	411.9	20.9	448.9	47.7	211.1	26.2	815	81	U	hypothetical protein
261691_at	At1g50060	215.2	86.9	247.6	34.4	69.13	21.1	429	41.2	B	branched-chain amino acid aminotransferase
256177_at	At1g51620	52.7	7.61	39.33	10.1	55	13.7	276	34.2	S	protein kinase
256178_s_at	At1g51780	417.5	92.5	346.4	12.6	326.1	32	1215	103	H	auxin conjugate hydrolase
256170_at	At1g51790	140.6	25.6	186.6	29.1	133	12.8	440	10.7	S	receptor protein kinase
256169_at	At1g51800	196.6	31.6	203.8	22.1	203.8	17.6	759	83.1	S	receptor protein kinase
246366_at	At1g51850	59	10.8	109.6	16.1	42.68	7.66	365	15.5	S	light repressible receptor protein kinase

Overview of RNA-seq transcriptional profiling

Experimental Design

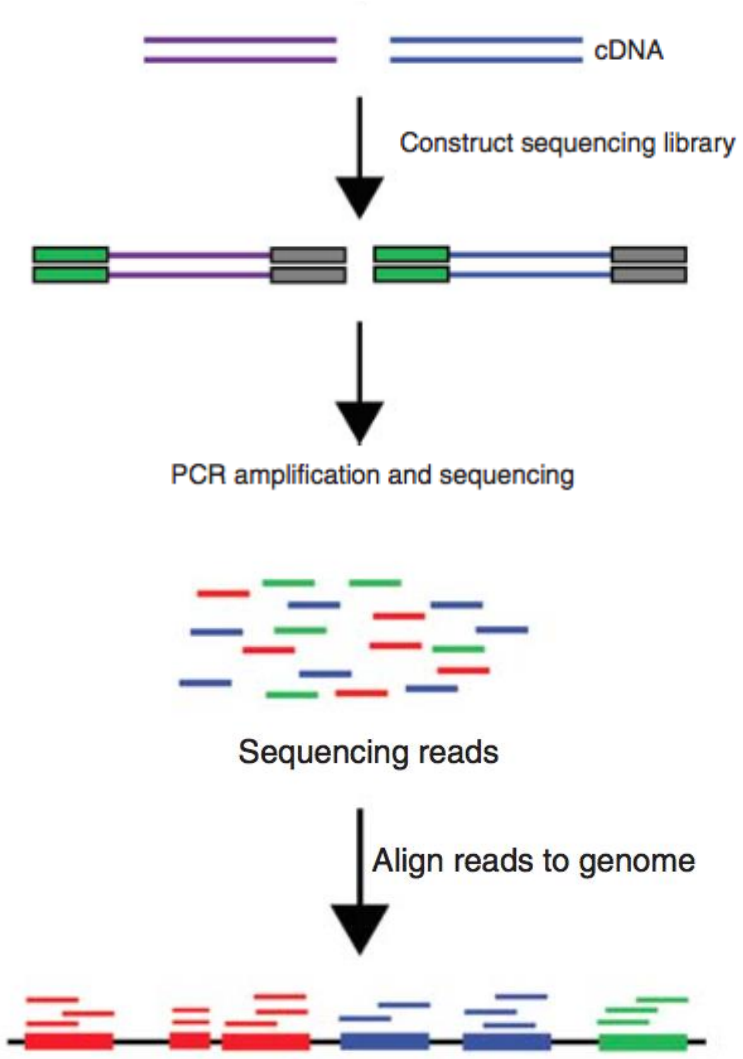


Kukurba and Montgomery. *Cold Spring Harb Protoc* 2015

Overview of RNA-seq transcriptional profiling

Sequencing

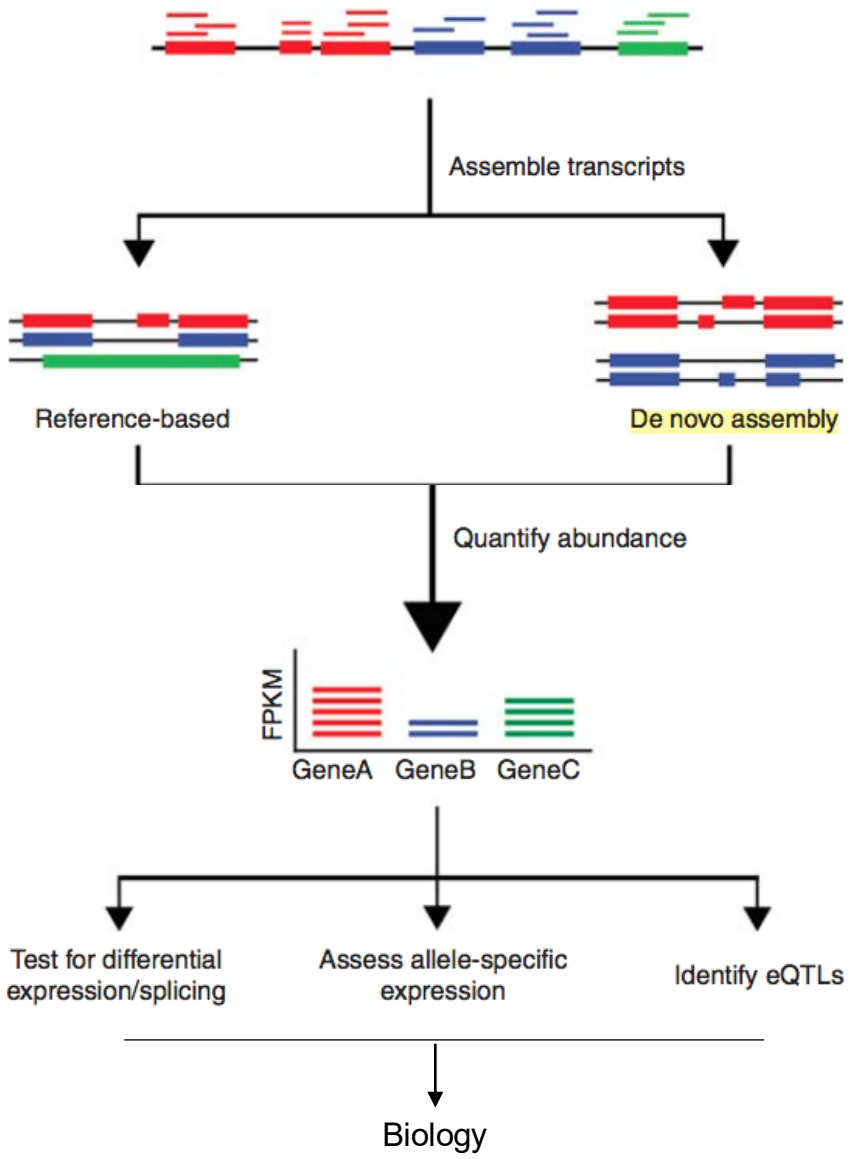
Data management



Kukurba and Montgomery. *Cold Spring Harb Protoc* 2015

Overview of RNA-seq transcriptional profiling

Data reduction

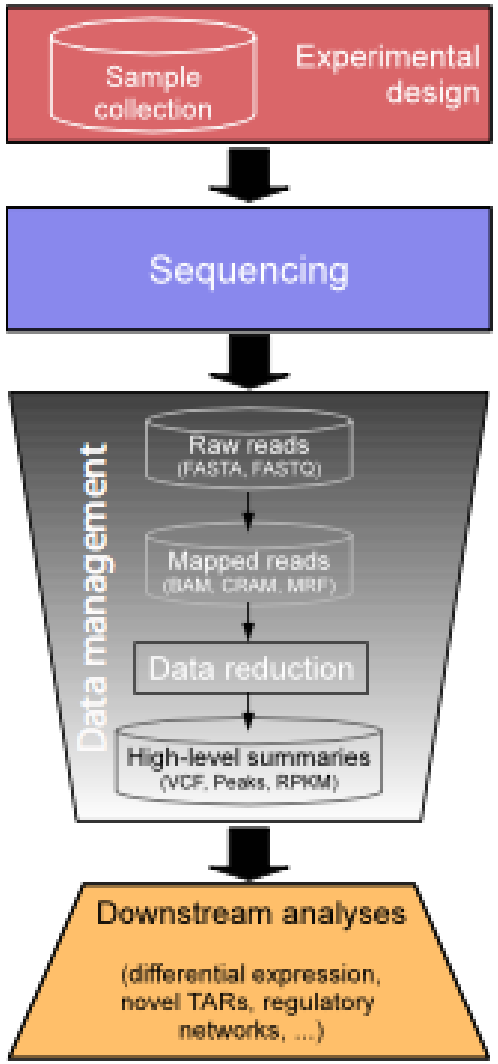


Downstream data analysis

Kukurba and Montgomery. *Cold Spring Harb Protoc* 2015

Summarized overview of \ transcriptional profiling

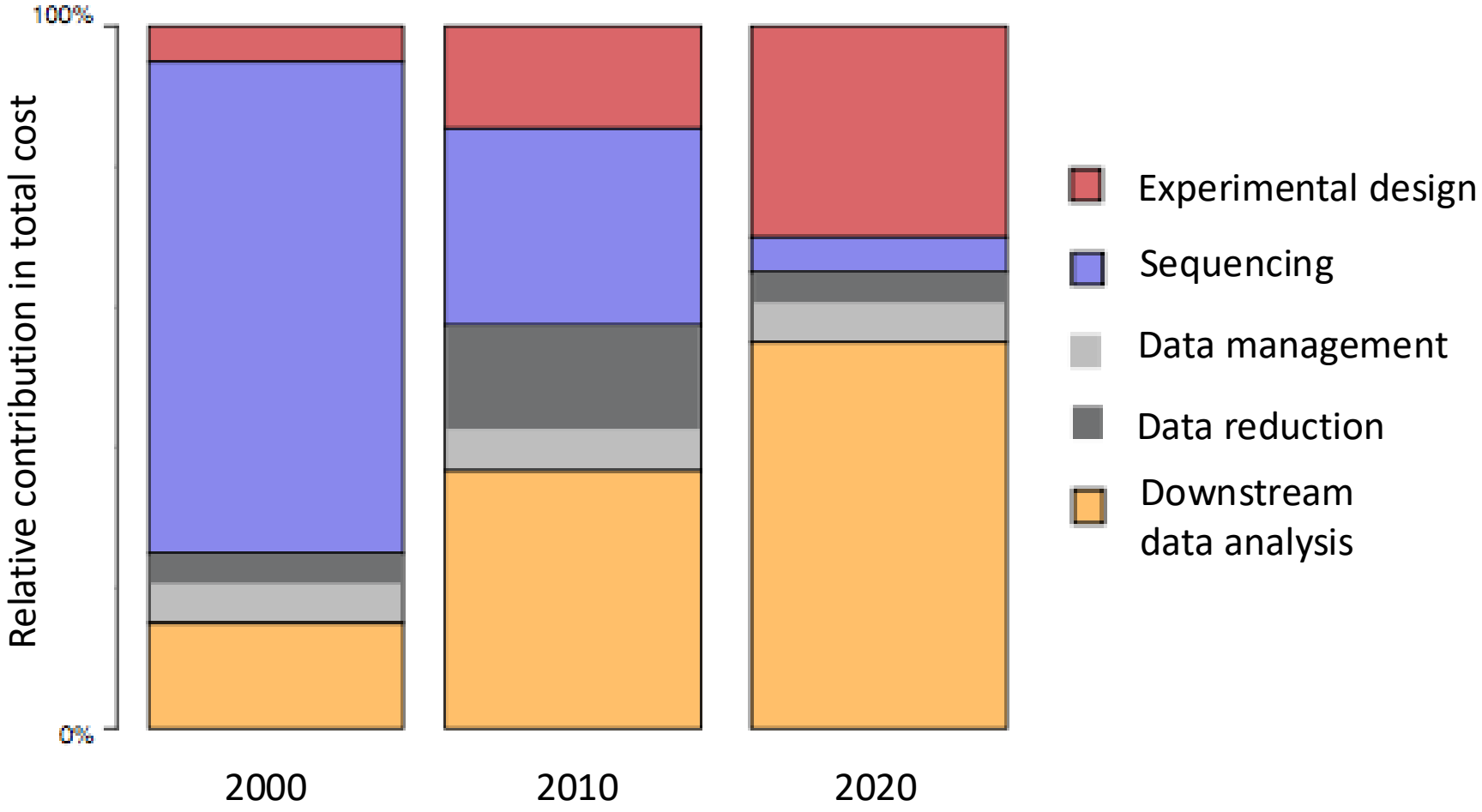
The four main steps in RNA-seq transcriptional profiling



Sboner *et al*. *Genome Biology* 2011

Key steps in RNA-seq; experimental design and data analysis

The gap between ”data generation” and ”knowledge generation”



Adapted from Sboner *et al*. *Genome Biology* 2011

To detect new candidate genes regulating specific biological phenomena

Considerations on experimental design and data analysis

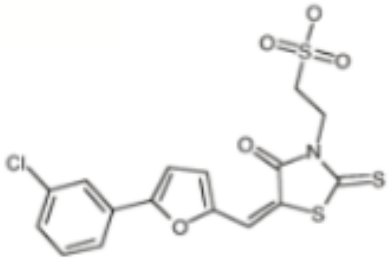
Increase successful further biological evaluation of the candidate genes

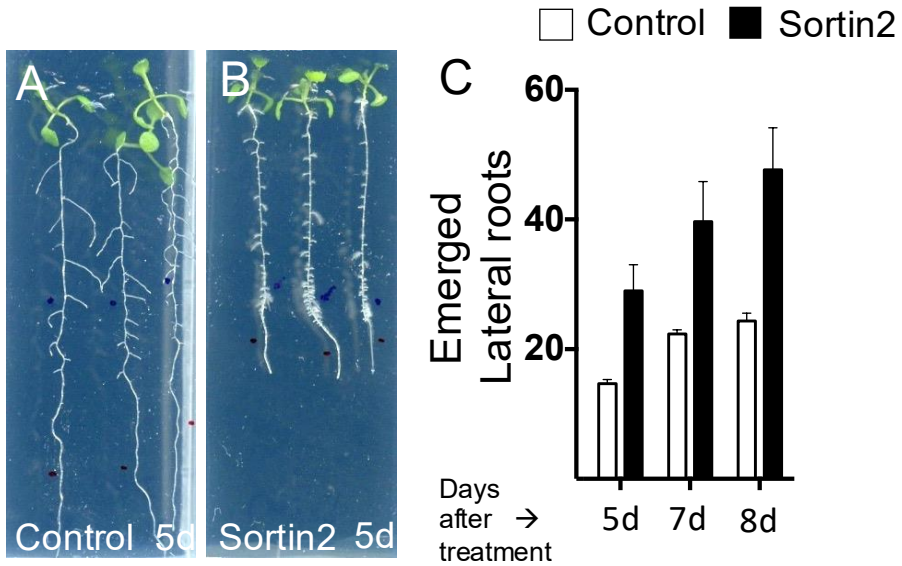
Two specific studies on cell specification

- Lateral root development
- Pavement cell formation

Experimental design and data analysis on lateral root development

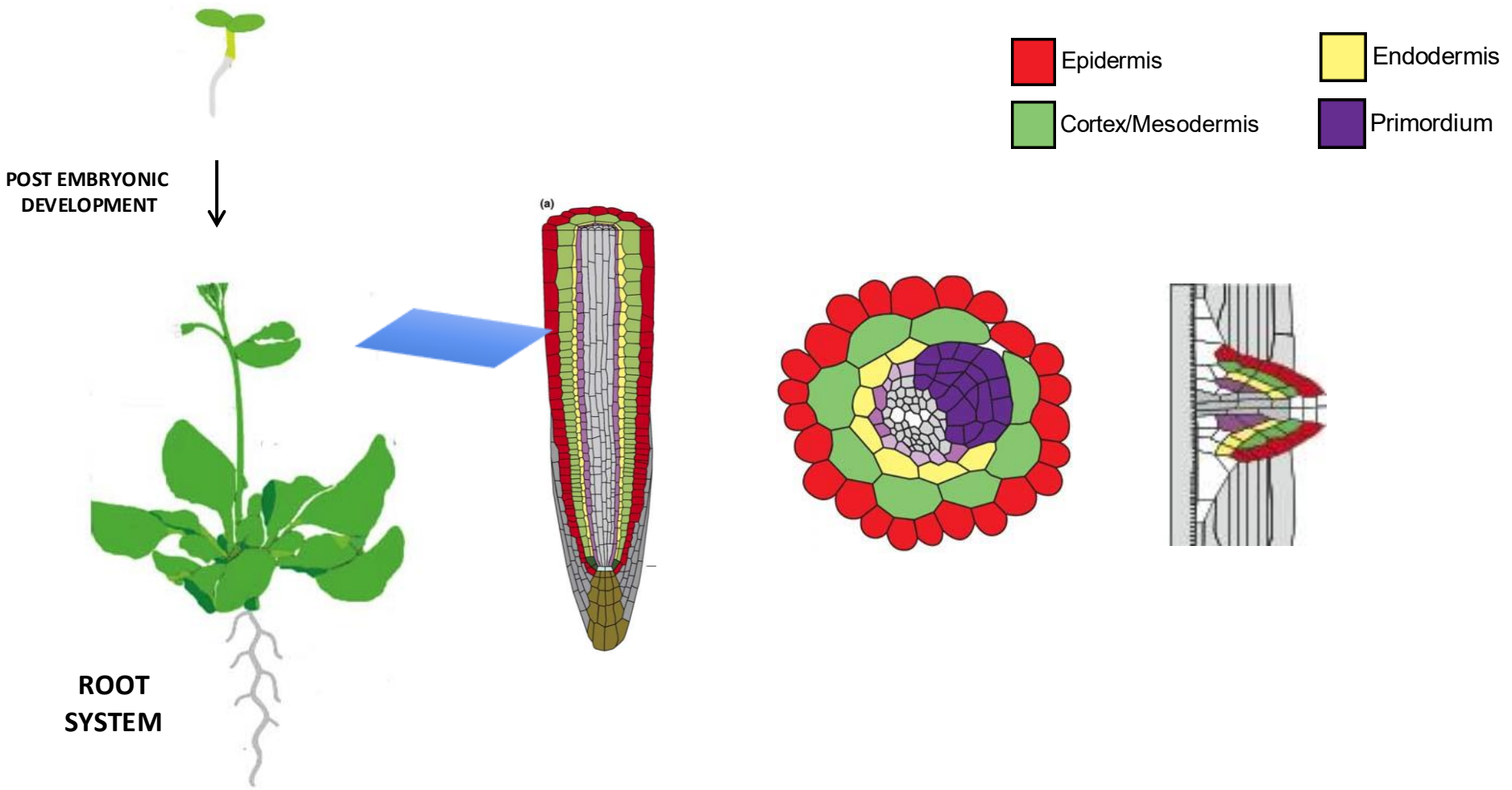
Soritn2 induces lateral roots in *A thaliana*





Increased number of emerged lateral roots is observed in prolonged Sortin2 treatments
(**A-B**) 7-day old seedlings of Arabidopsis wild type Col-0 were treated during 5 days in solid medium with (**A**) Control (DMSO 1%) and (**B**) Sortin2 (25µg/mL).
(**C**) Quantification of emerged lateral roots of seedlings treated as in A-B.

Root system architecture largely relies on the continuous process of lateral root initiation



Auxin is a key regulator of lateral root development

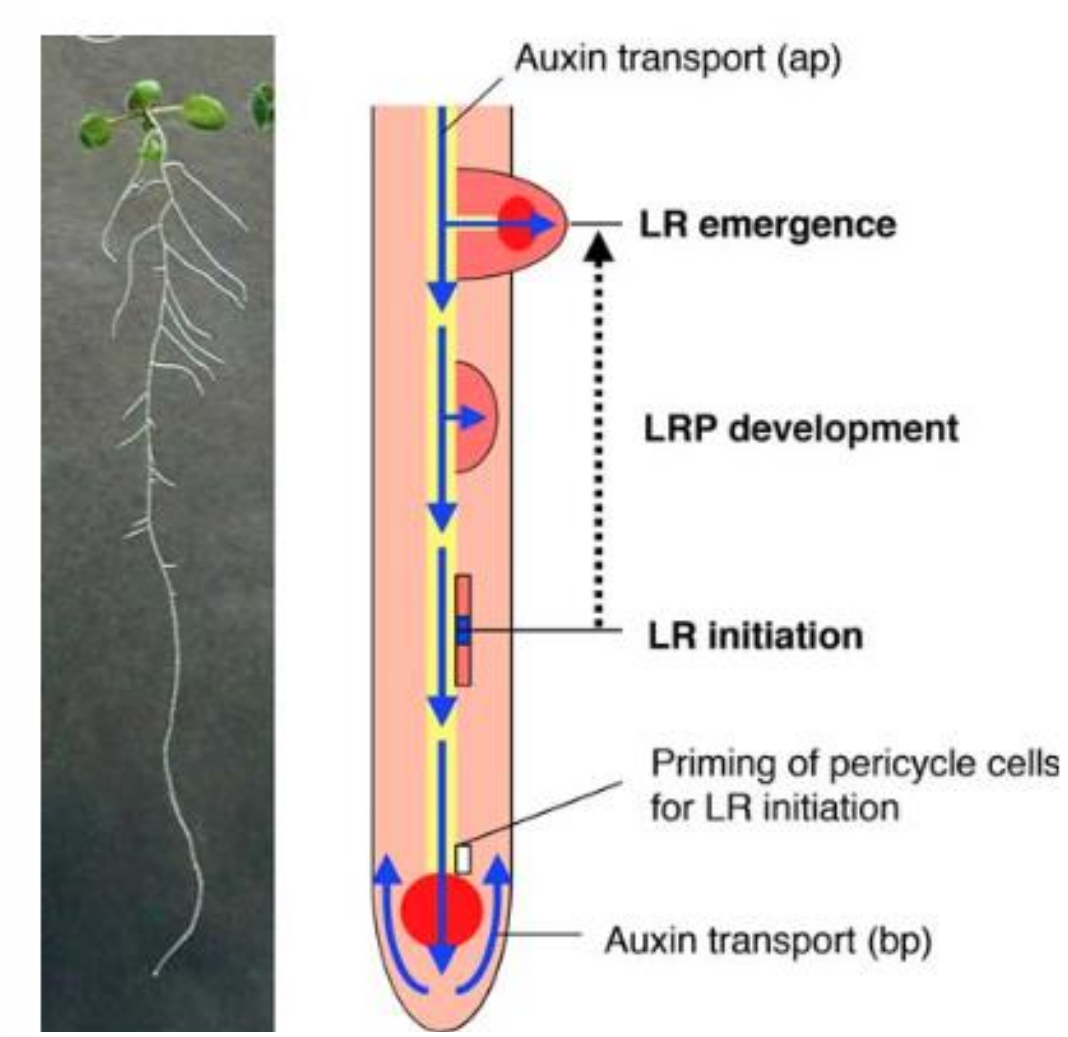
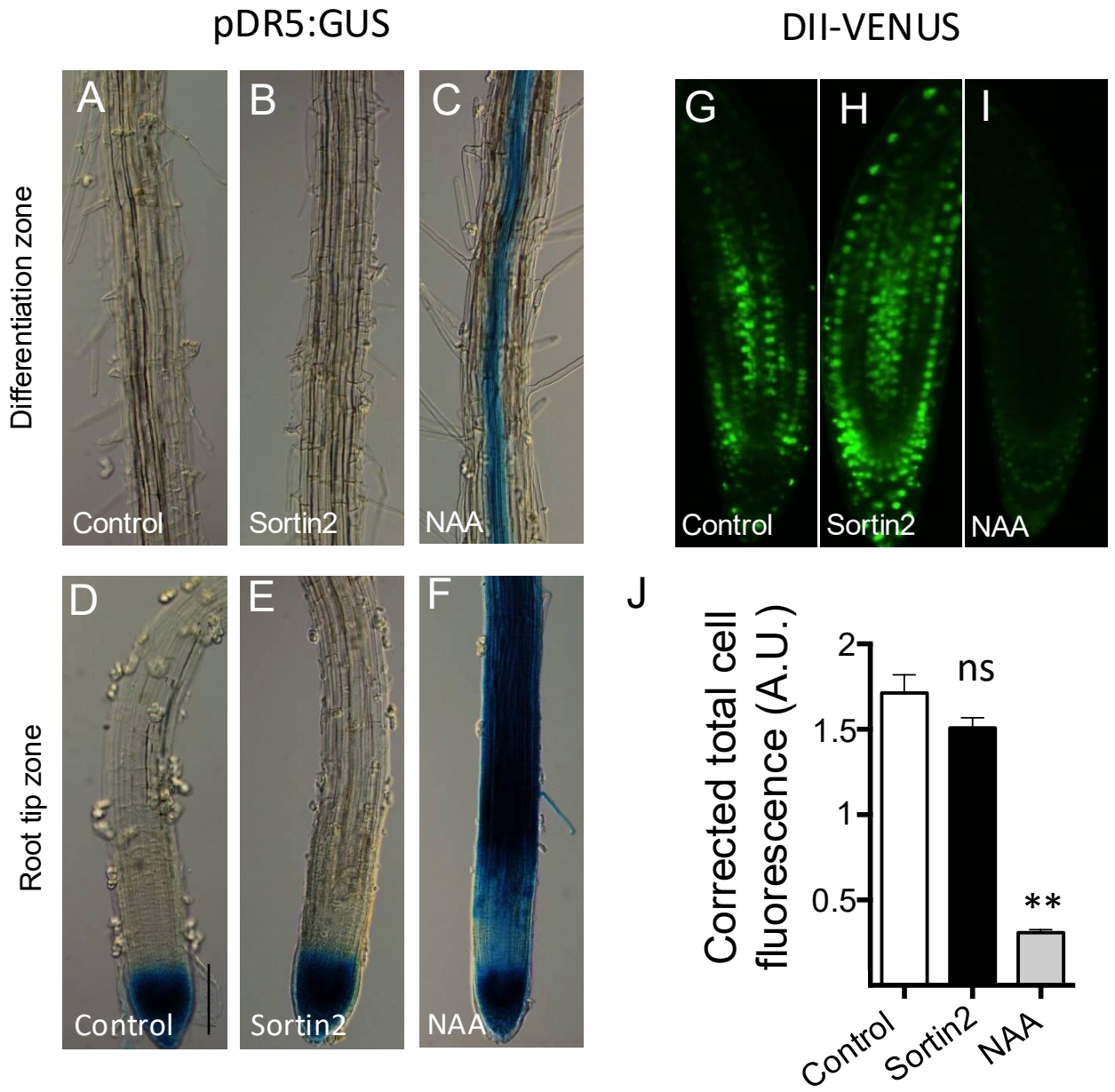


Fig. 1 Developmental events during LR formation.

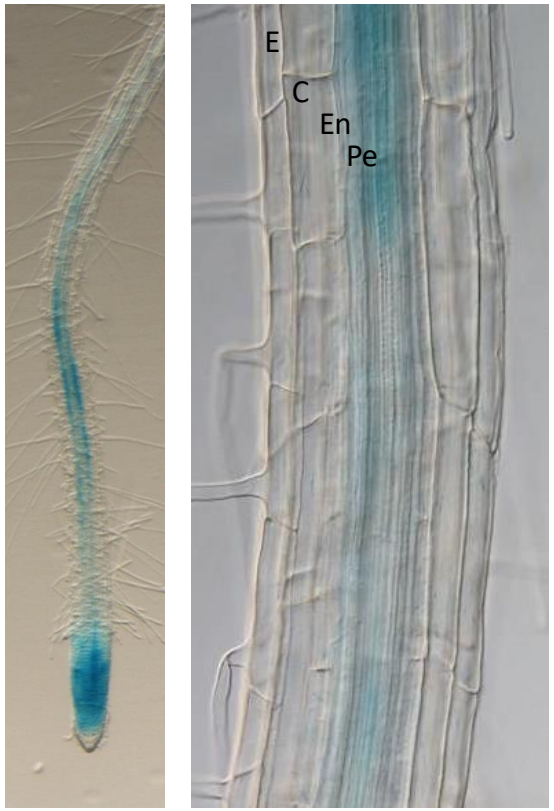
Comparative condition

Sortin2 and auxin signaling

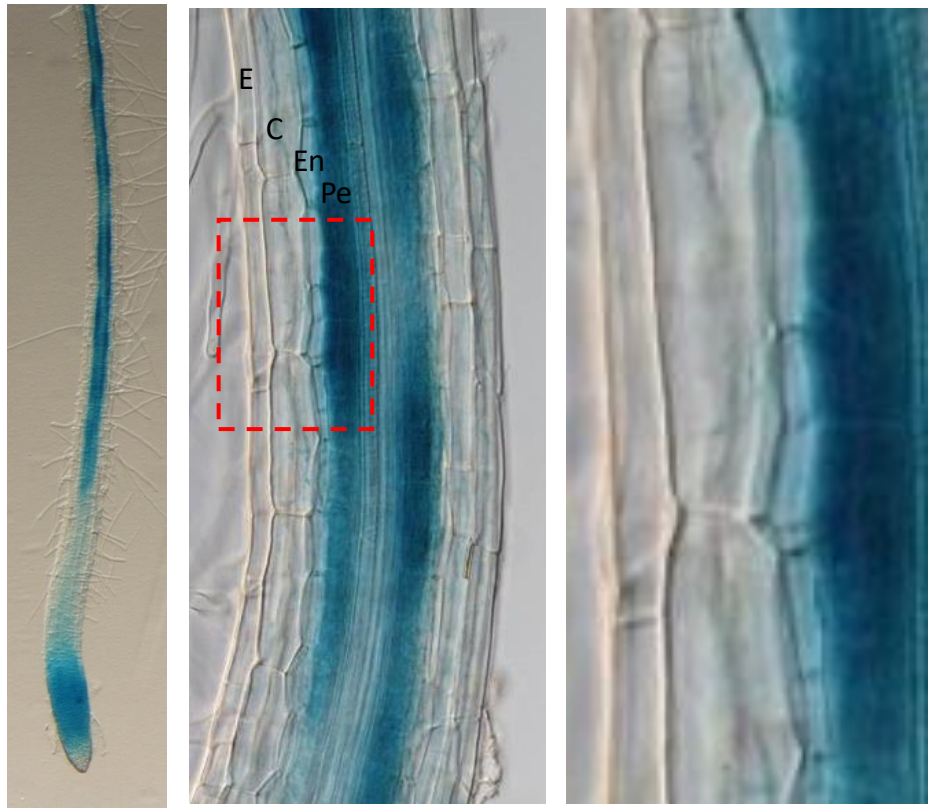


Sortin2 induces mitotic activity specifically on pericycle cells

Sortin2 -12 h

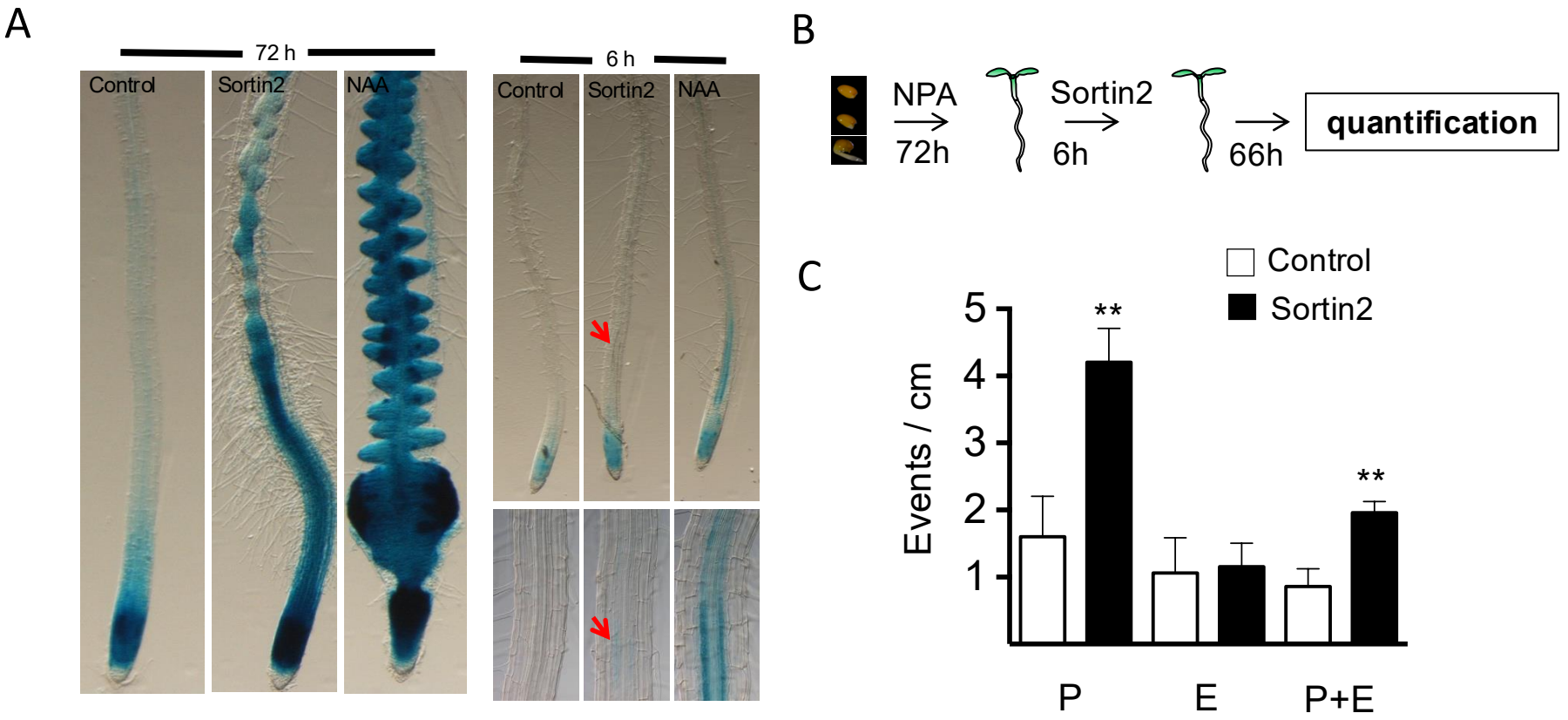


Sortin2 - 24 h



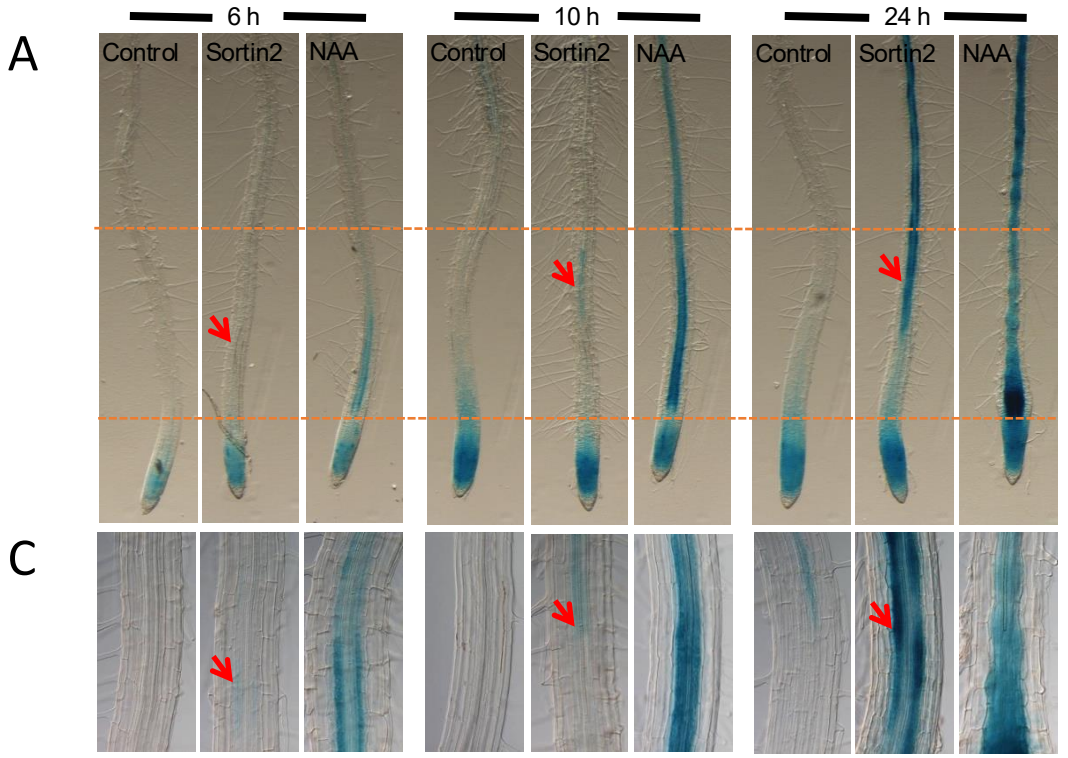
pCYCB1::GUS

Sortin2 induces mitotic activity that leads to LR formation



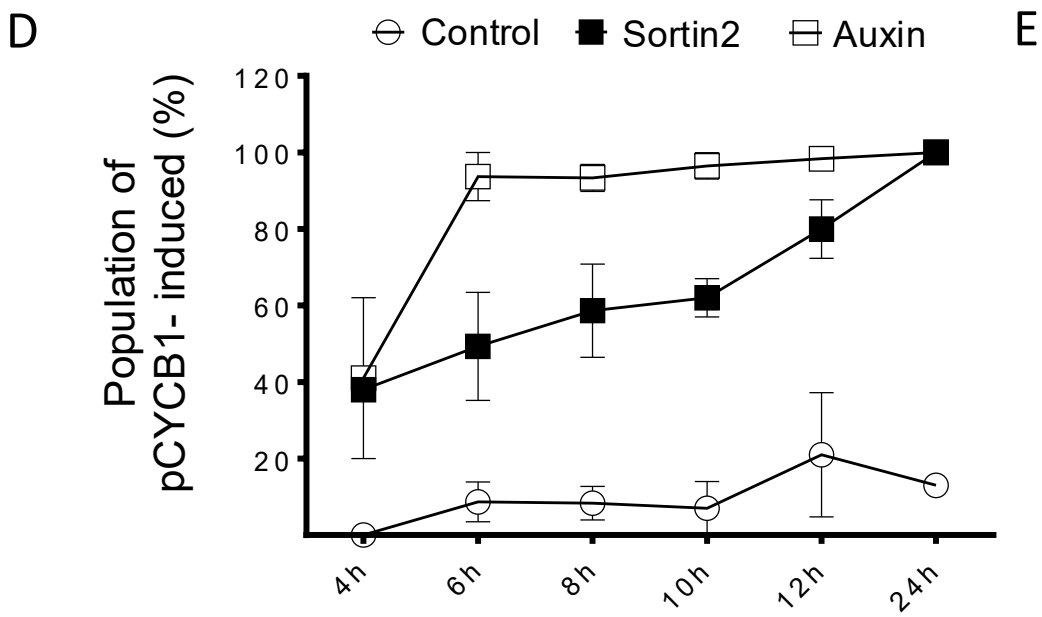
pCYCB1::GUS

Time response on Sortin2 mitotic activity induction



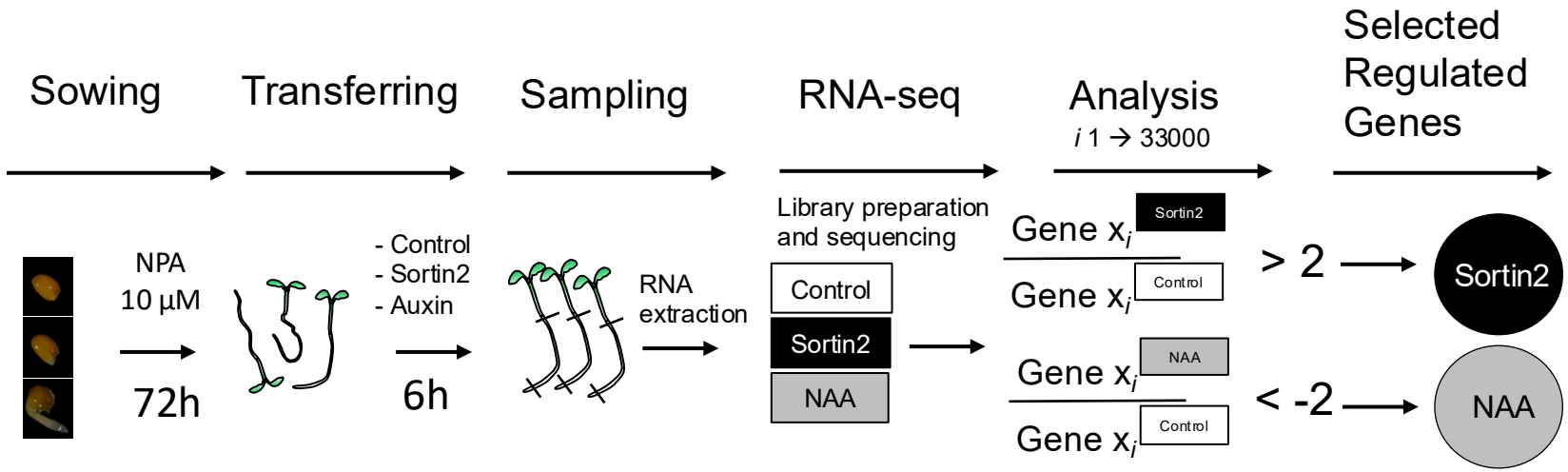
Time resolution

Time response on Sortin2 mitotic activity induction



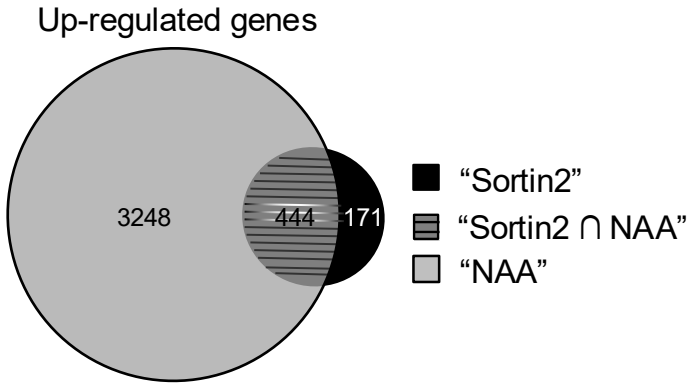
Experimental design

Workflow scheme for RNA sequencing

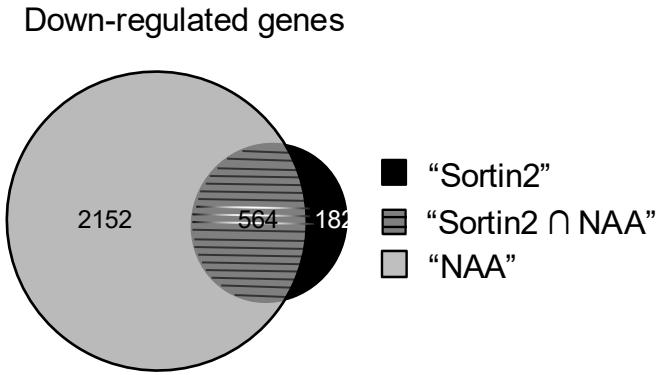


Overview of Sortin2 and NAA comparative transtripctional profile

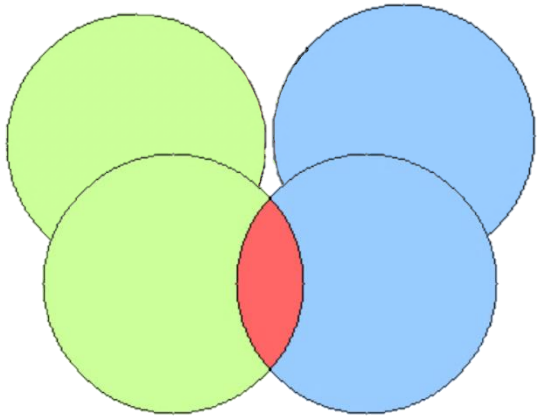
A



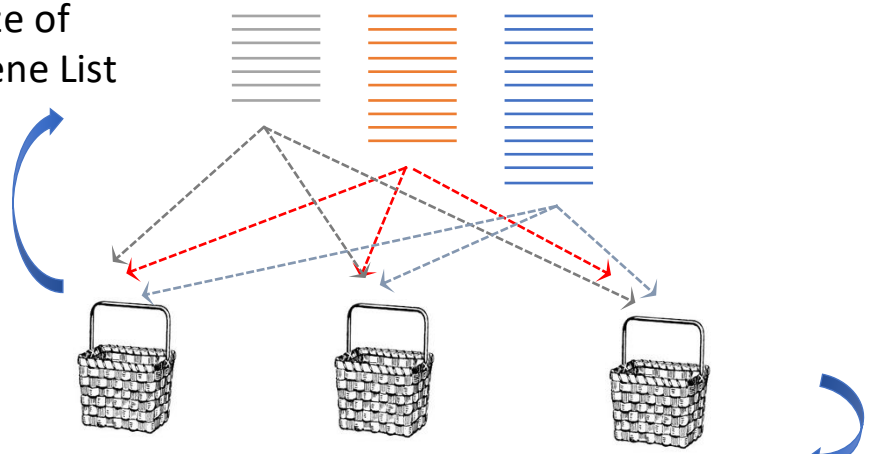
B



Biological processes enrichment



Size of Gene List



Size of Biological Process

Response to stress (GO:0006950)

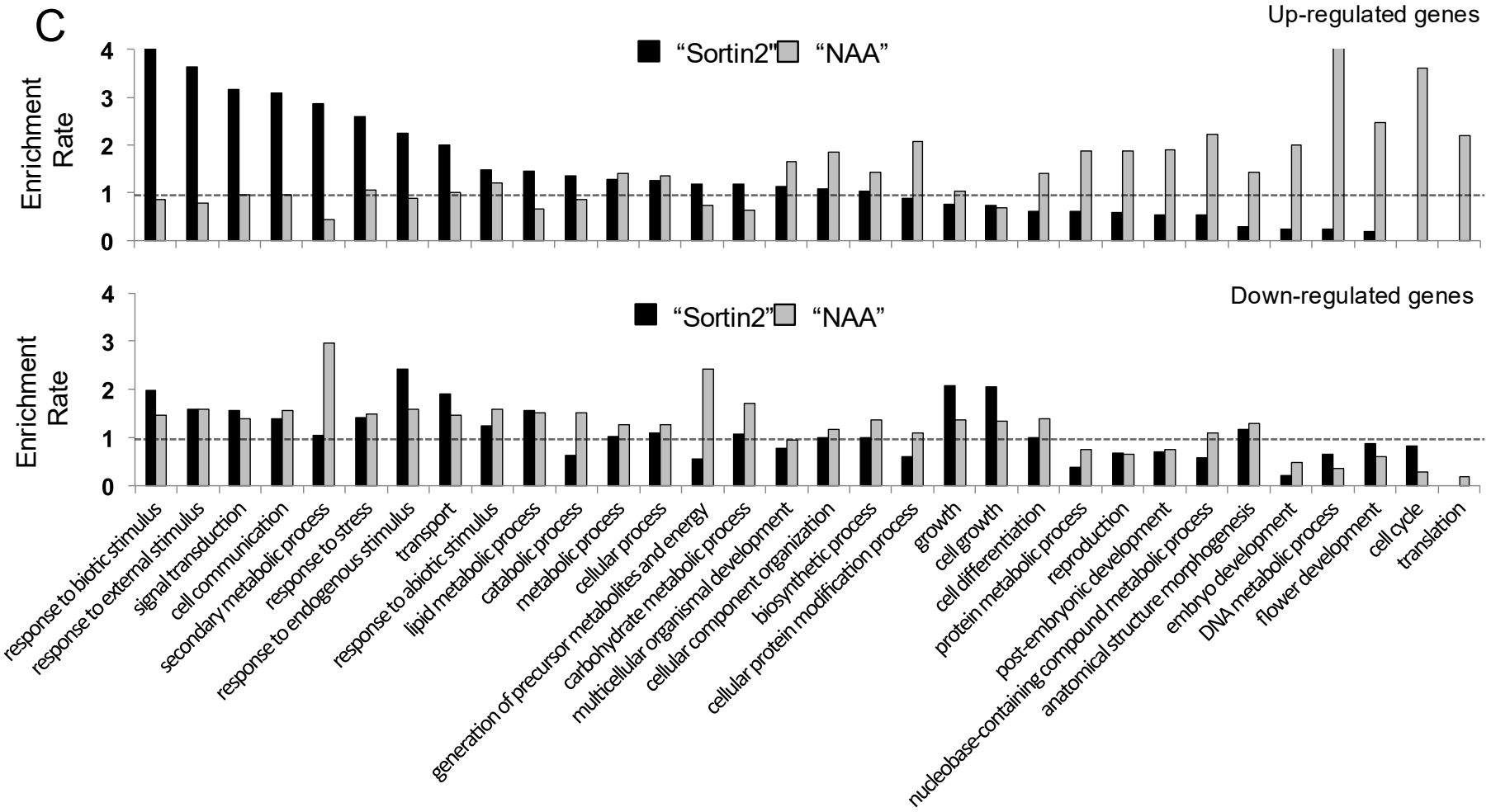
Metabolic process (GO:0008152)

Cell cycle (GO:0007049)

ENRICHEMENT OF EACH BIOLOGICAL PROCESS IN EACH GENE LIST

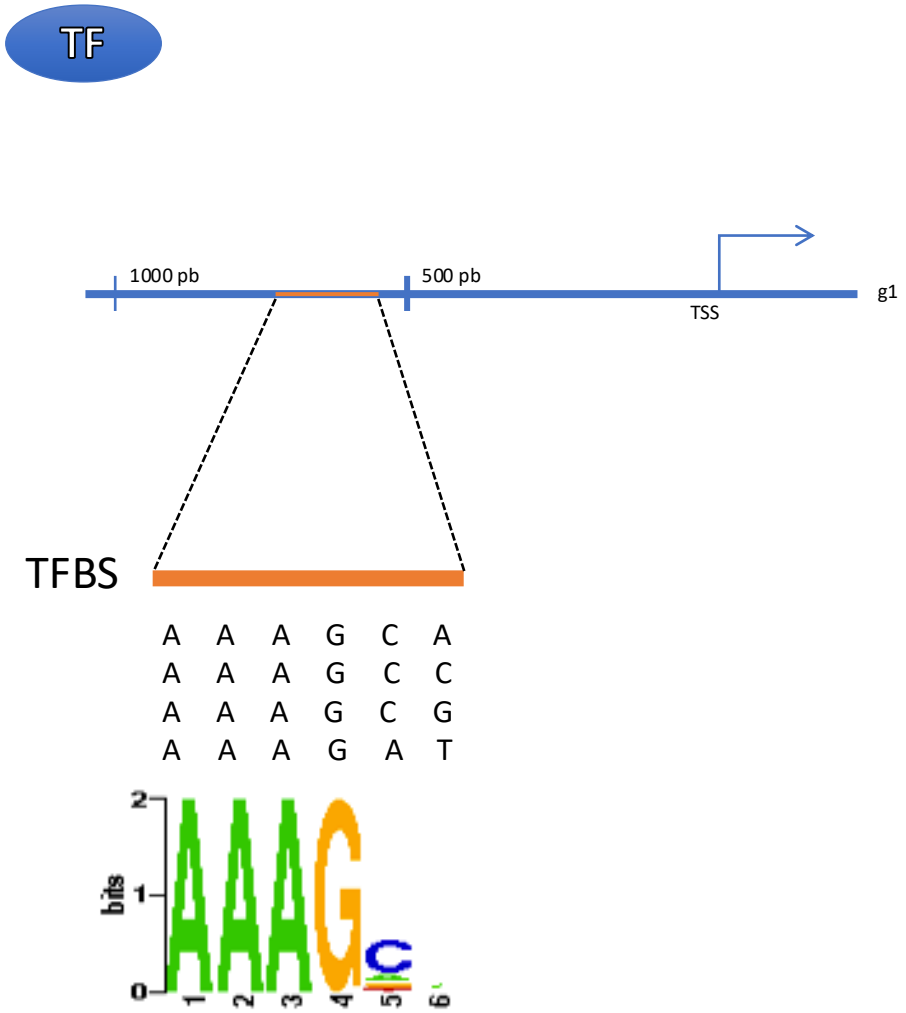
$$\text{ENRICHMENT} = \frac{\dfrac{\text{N° of genes from the list in each GO category}}{\text{N° genes in the list}}}{\dfrac{\text{N° genes from the genome annotated in each GO category}}{\text{N° genes in the genome}}}$$

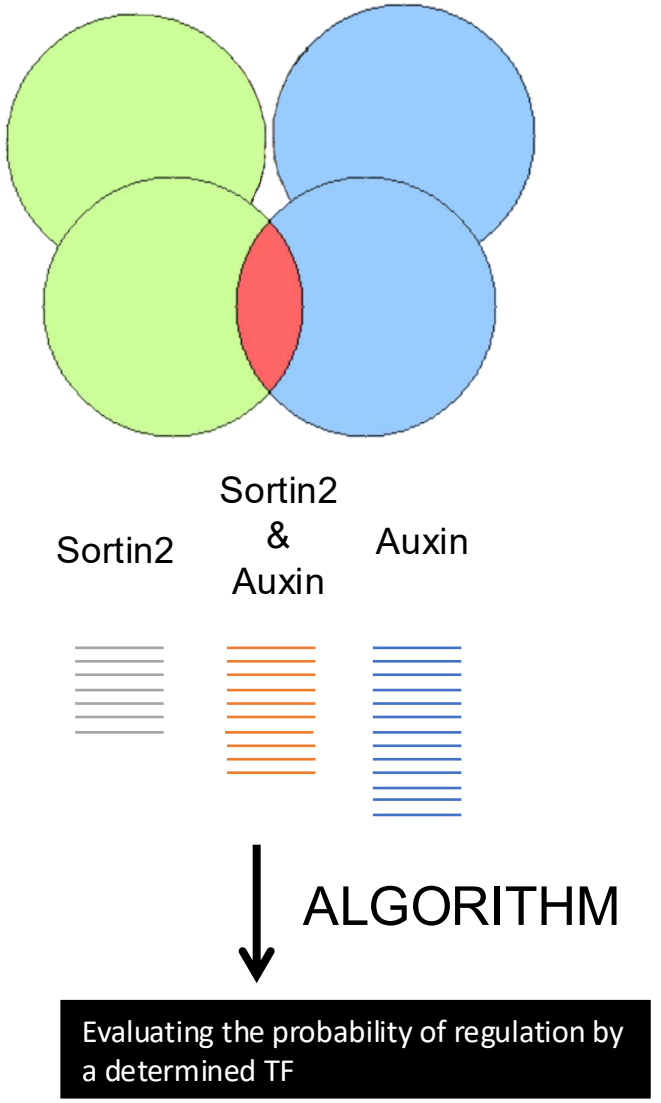
Comphenensive overview of biological processes represented among the Sortin2 and NAA transtripctional profiles



Finding overrepresented TFBS motifs in regulatory sequences from co-expressed genes

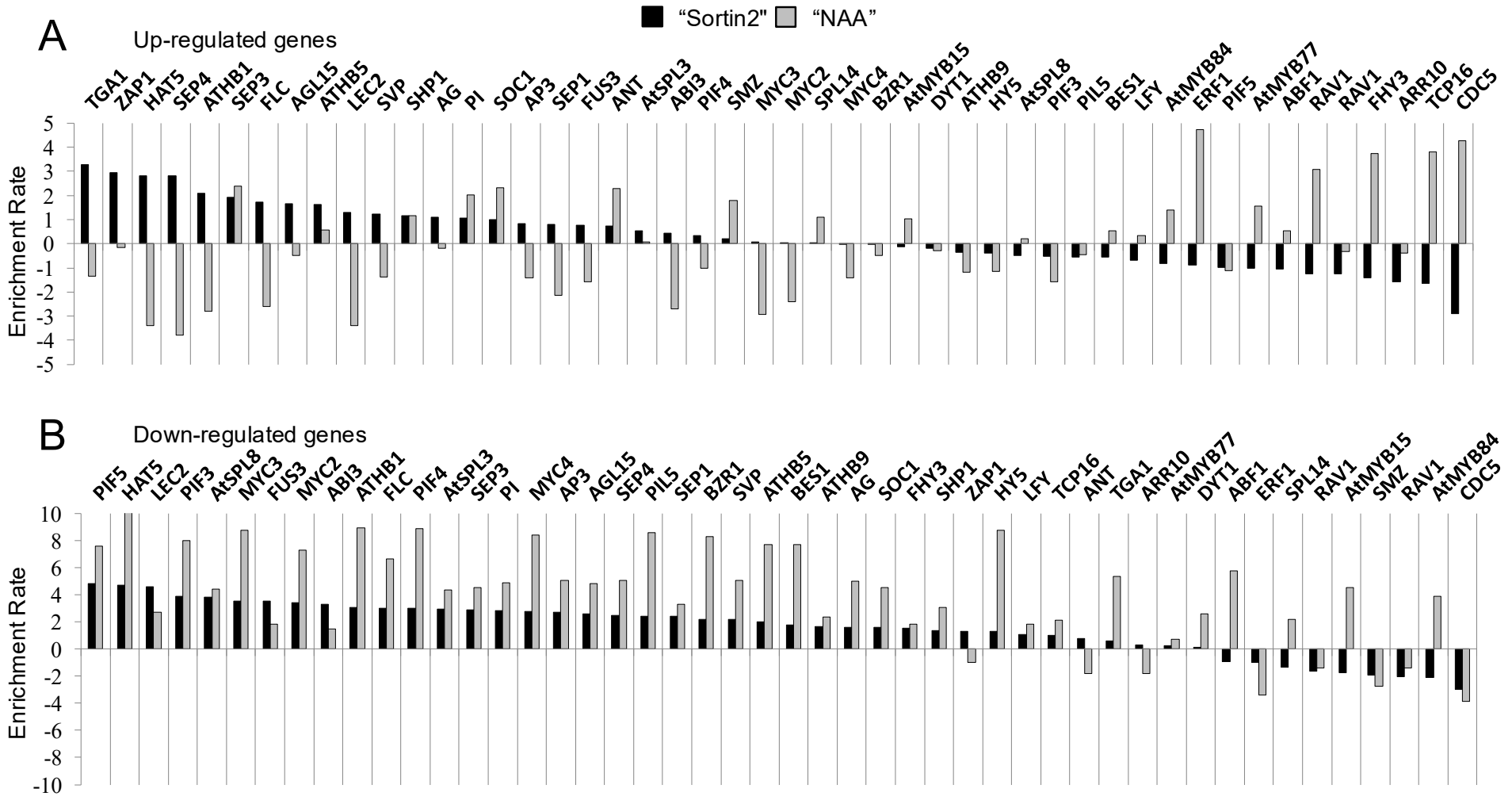
Transcription factors binding sites (TFBS)





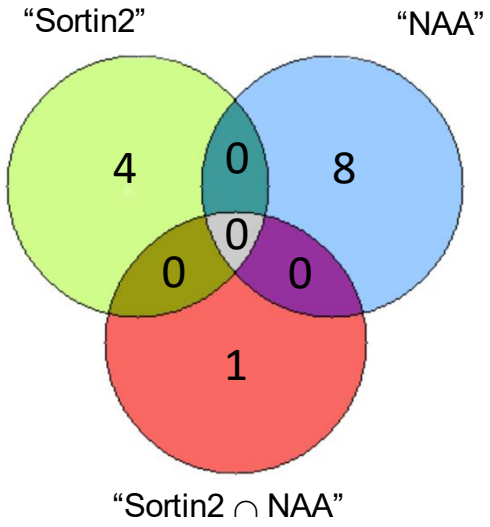
TFBS; Transcription Factor Binding Site

Compheneensive overview of TFBS represented among the Sortin2 and NAA transtripctional profiles

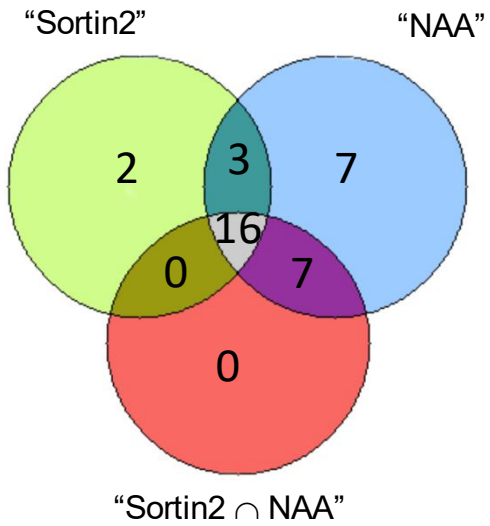


Upregulated genes showed more distinctive cis-regulatory elements than down regulated genes

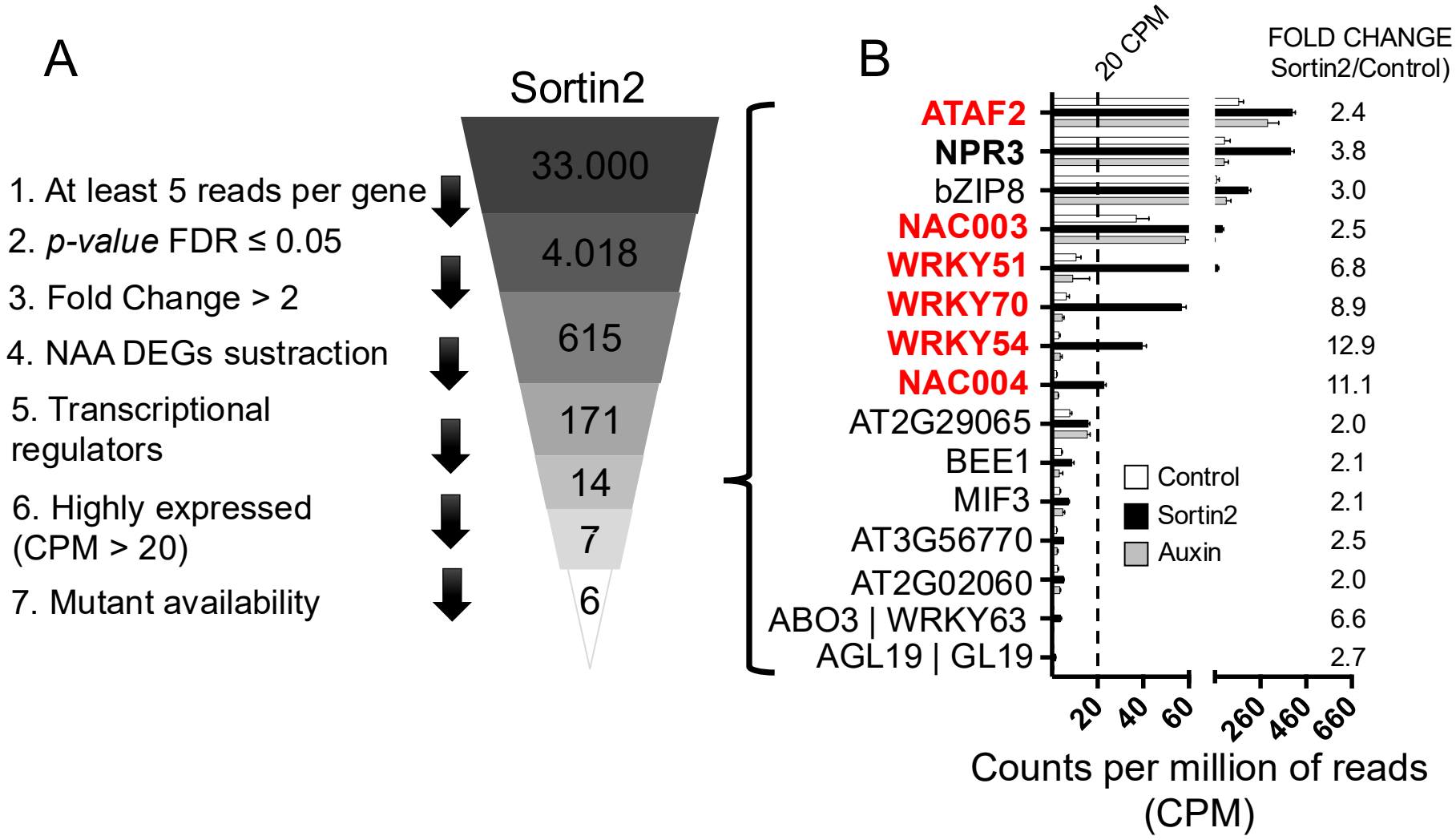
DEG+



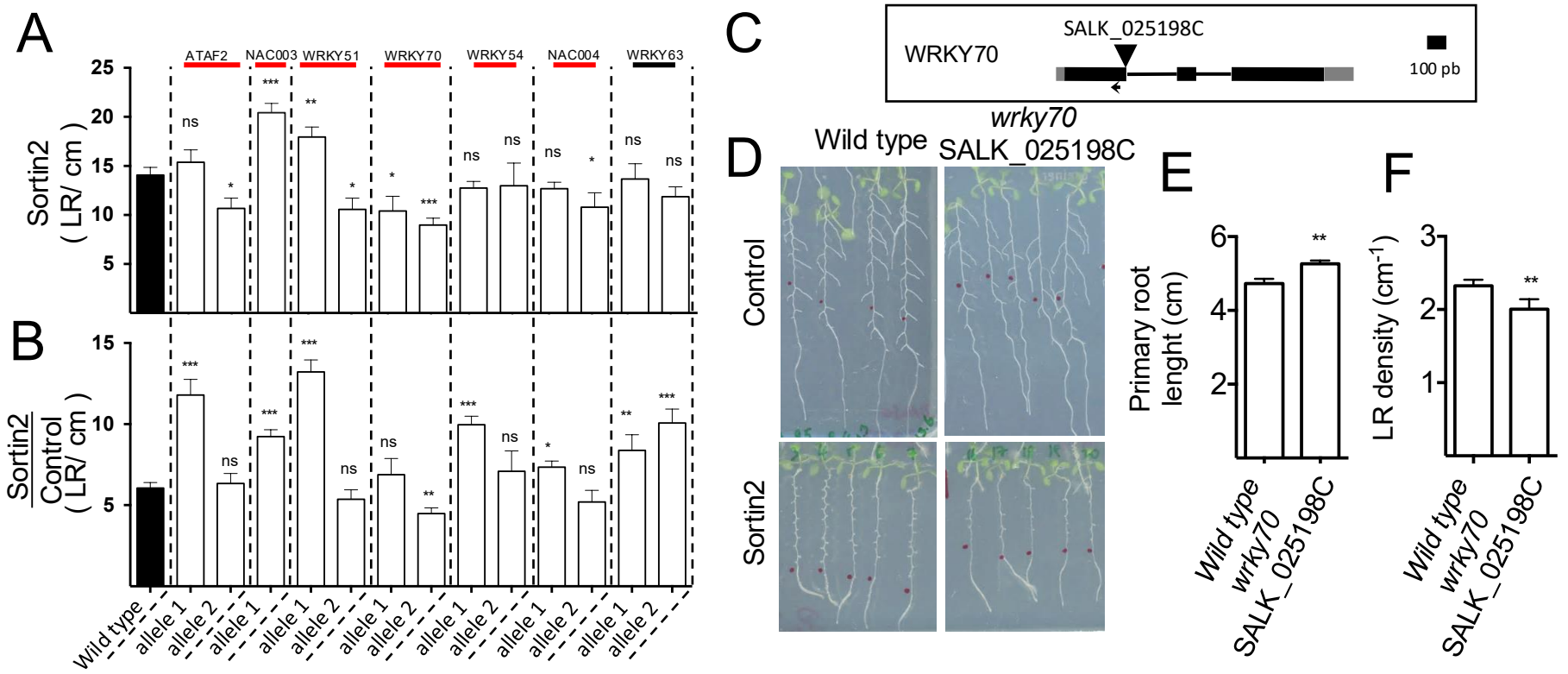
DEG-

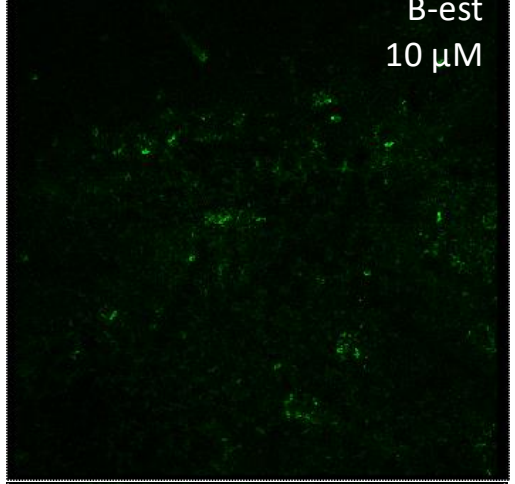


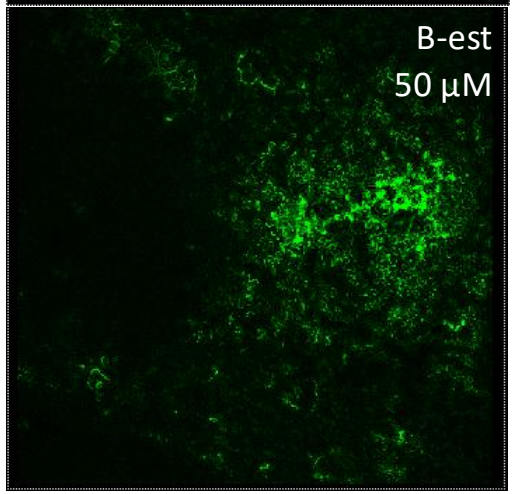
Selecting candidate genes for further biological evaluations

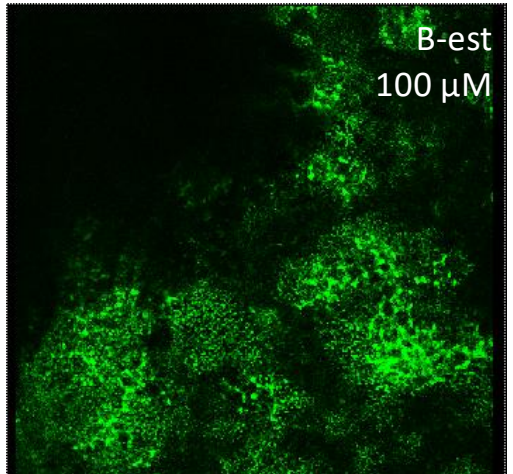


Testing the effect of insertional mutants on selected candidate genes



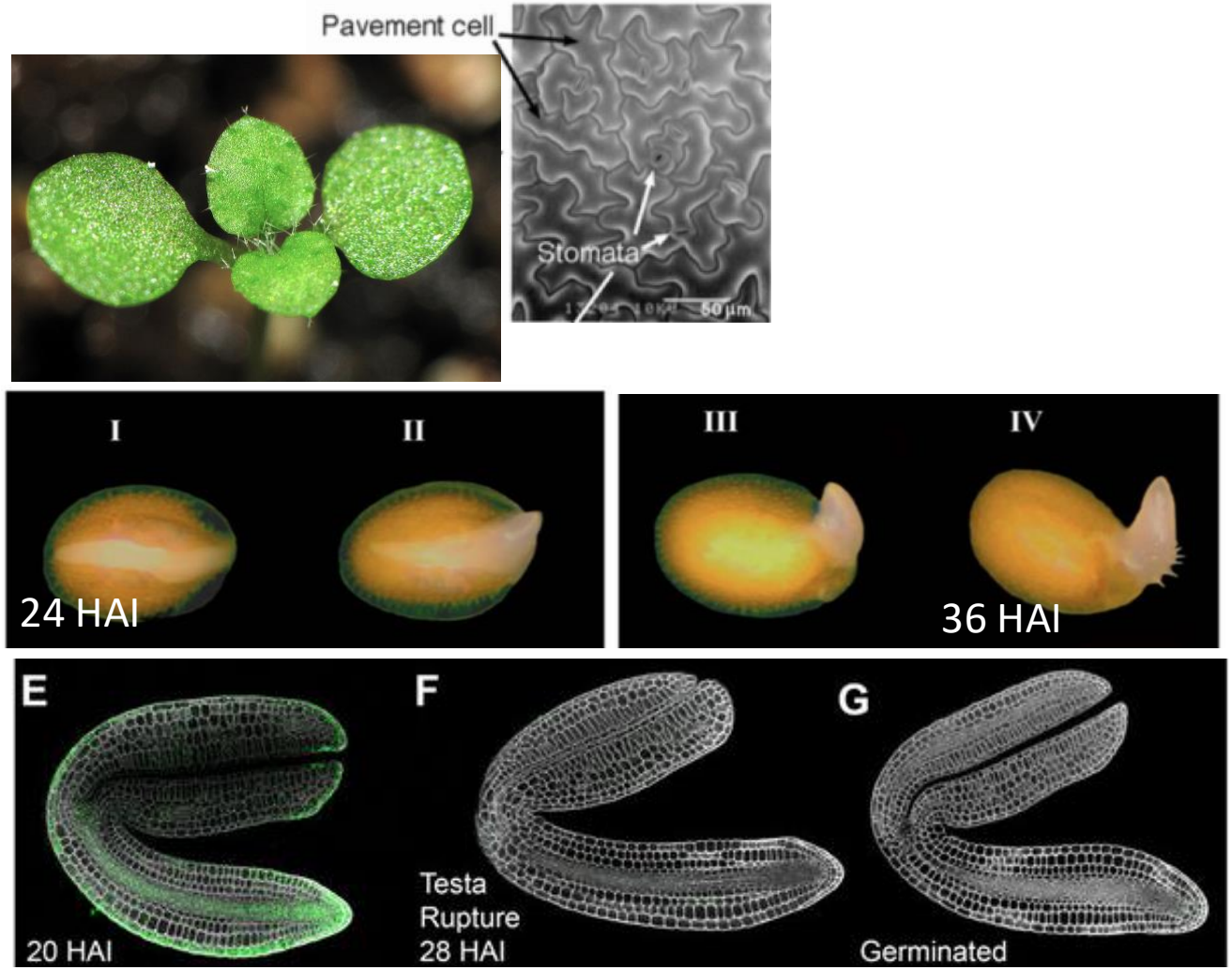






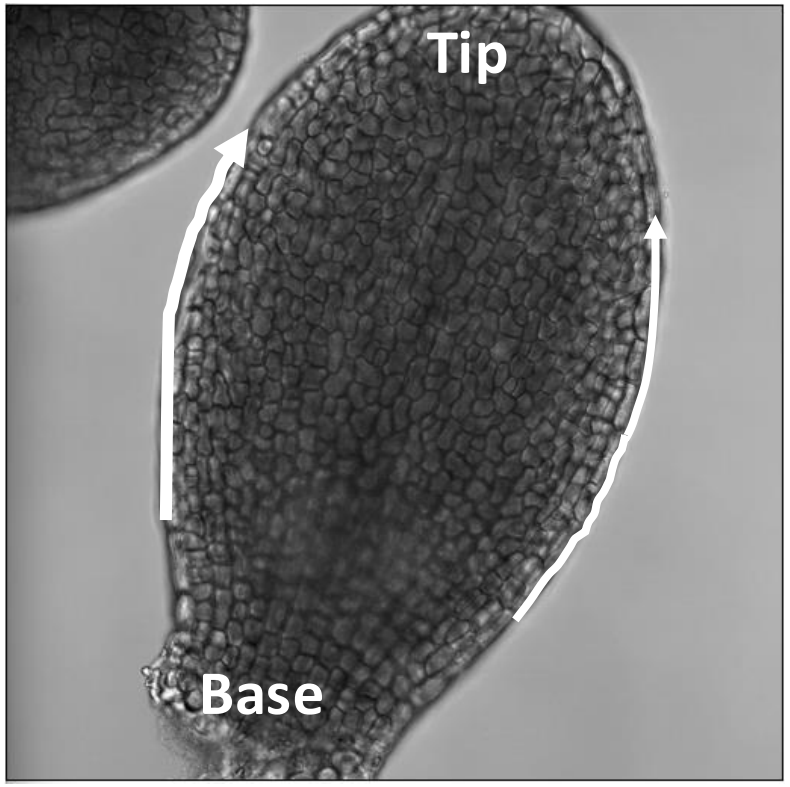
Experimental design
on pavement cell formation

Overview on pavement cell formation

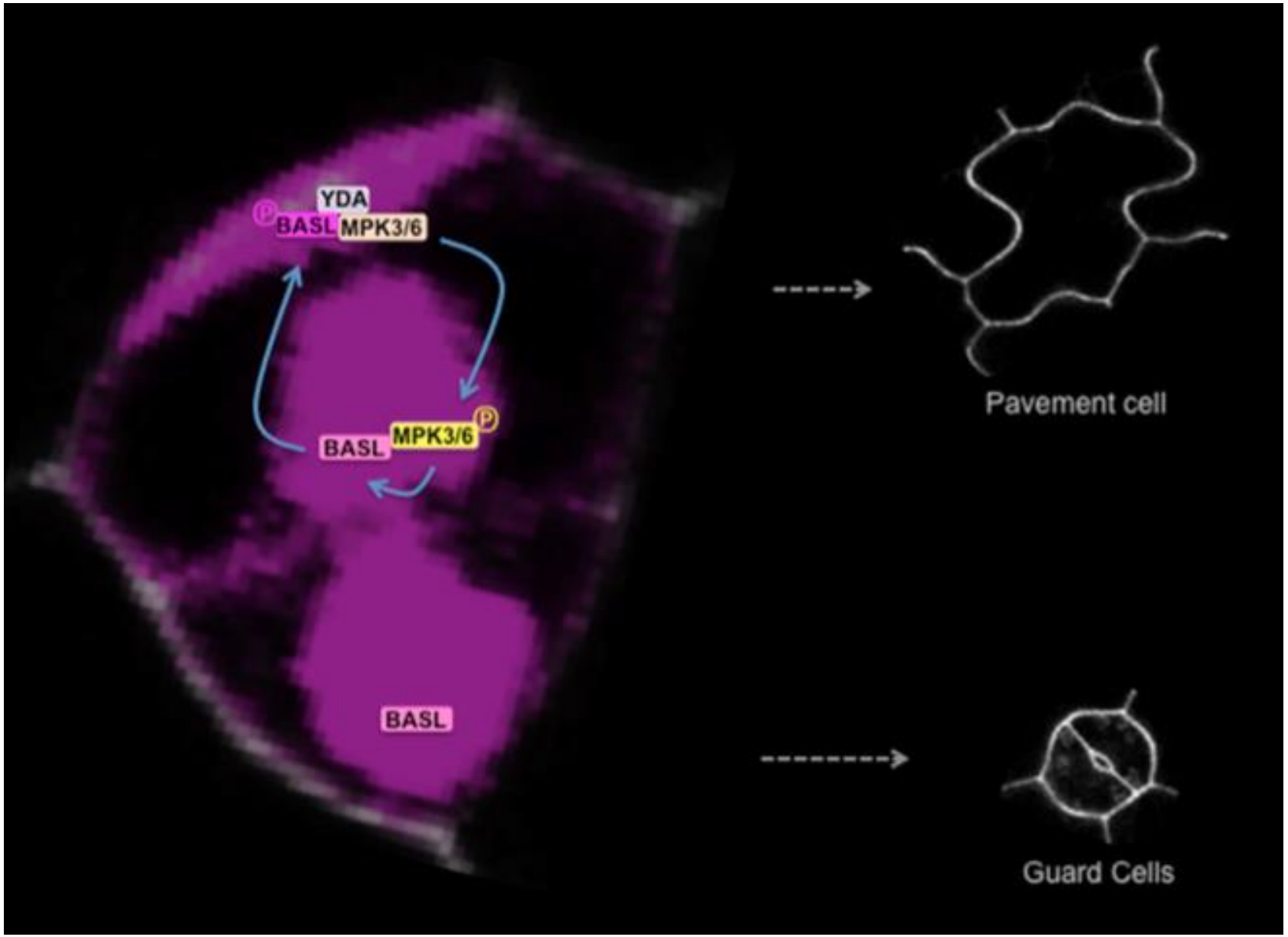


Takada y Jurgens. 2007. Development
Maia et al. 2011. PloS one
Nieuwland et al. 2016. Scientific Report

Overview on pavement cell formation



Overview on pavement cell formation

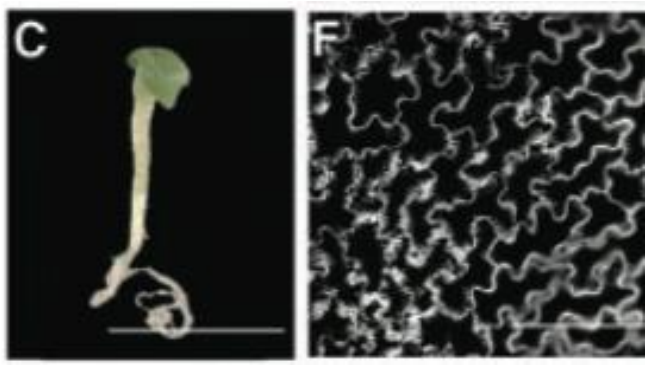


Zhang et al. 2014. Dev Cell

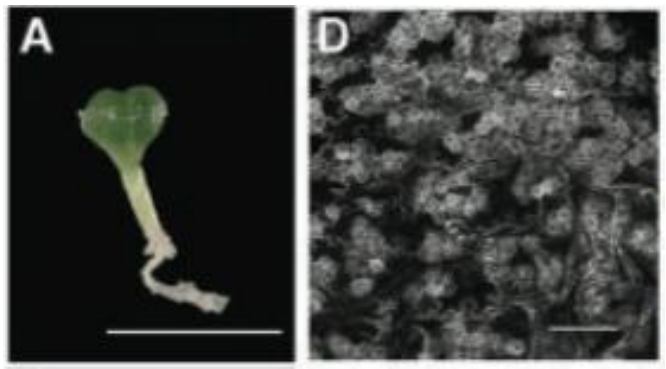
Comparative condition

CA-YDA induces al pavement cell epidermis on *A.thaliana*

ΔN-YDA (CA-YDA)

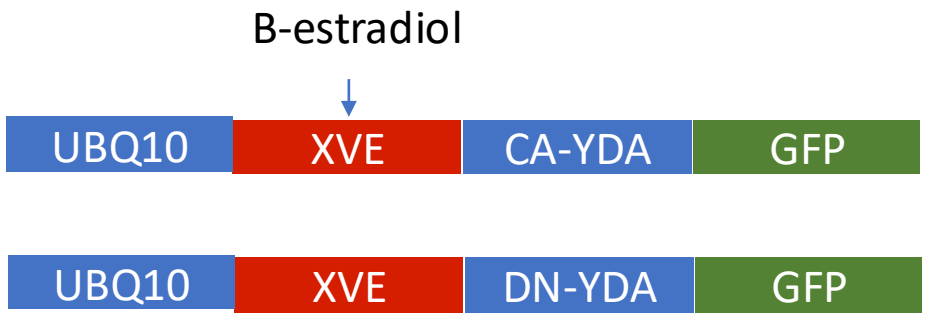


yda-1 (DN-YDA)



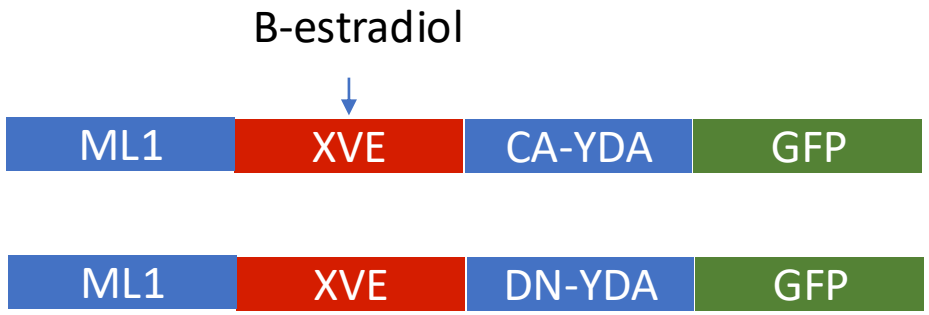
Inducible expression of CA-YDA or DN-YDA

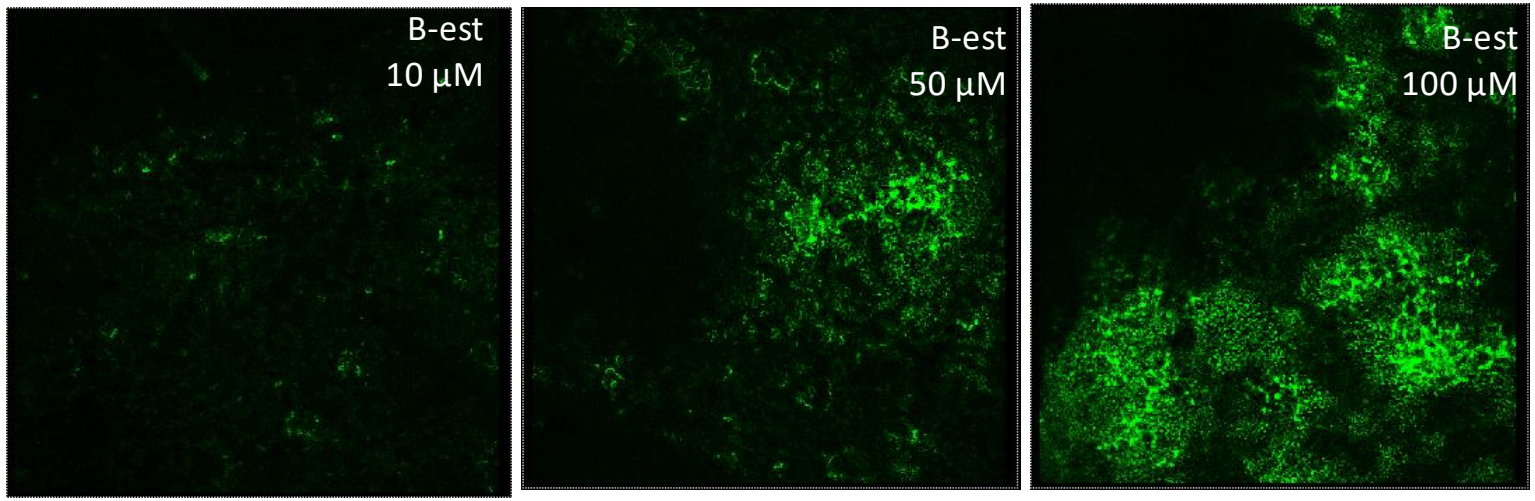
Inducible phenomena



Tissue specificity

Inducible expression of CA-YDA or DN-YDA specifically on epidermis tissue



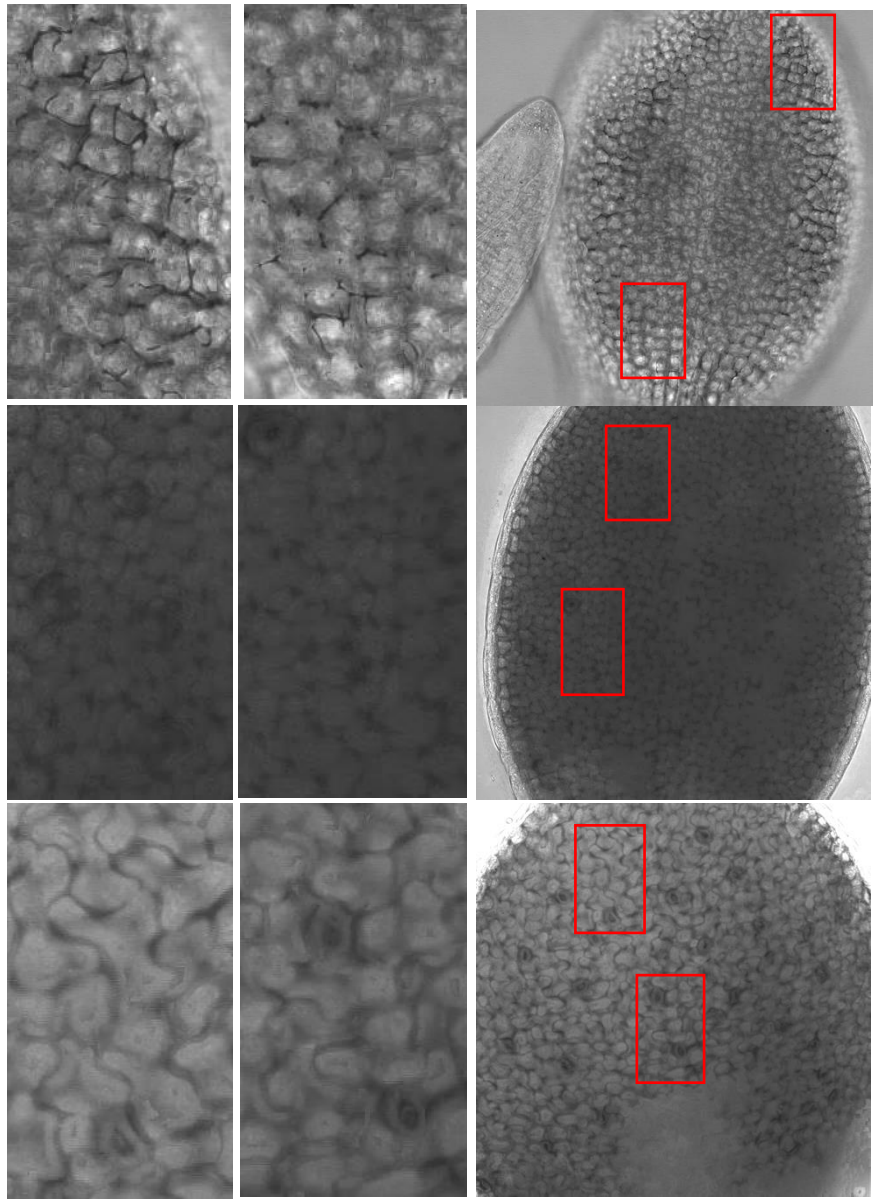


Time resolution

48 HAI
(0 DAG)

66 HAI
(18 HAG)

72 HAI
(24 HAG)



Using RNA-seq to identify new genes regulating a developmental process; experience form underground and above ground plant biology.

Considerations on experimental design
Comparative conditions
Inducible
Tissue specificity
Time resolution

Considerations on data analysis
Overview of biological process
Overview of regulatory elements (cis and trans)
Selection based on gained knowledge