CG920 Genomics

Lesson 6

Gene Expression and Chemical Genetics

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Literature

- Literature resources for Lesson 06

- Brady, S. M. et al. A high-resolution root spatiotemporal map reveals dominant expression patterns. *Science.* **318** (5851), 801-806 (2007).
- Karaiskos N, Wahle P, Alles J, Boltengagen A, Ayoub S, Kipar C, Kocks C, Rajewsky N, Zinzen RP (2017) The Drosophila embryo at single-cell transcriptome resolution. *Science* **358**: 194-199
- Lecuyer, E., Yoshida, H., Parthasarathy, N., Alm, C., Babak, T., Cerovina, T., Hughes, T.R., Tomancak, P., and Krause, H.M. (2007). Global analysis of mRNA localization reveals a prominent role in organizing cellular architecture and function. *Cell* **131**, 174-187.
- Nevo-Dinur, K., Nussbaum-Shochat, A., Ben-Yehuda, S., and Amster-Choder, O. (2011). Translation-independent localization of mRNA in E. coli. *Science* **331**, 1081-1084
- Schonberger, J., Hammes, U.Z., and Dresselhaus, T. (2012). In vivo visualization of RNA in plants cells using the lambdaN(22) system and a GATEWAY-compatible vector series for candidate RNAs. *The Plant Journal* **71**, 173-181.
- Stahl, P. L. et al. Visualization and analysis of gene expression in tissue sections by spatial transcriptomics. *Science.* **353** (6294), 78-82 (2016).
- Xia, K. et al. The single-cell stereo-seq reveals region-specific cell subtypes and transcriptome profiling in arabidopsis leaves. *Dev Cell.* **57** (10), 1299-1310 e1294 (2022)

Outline

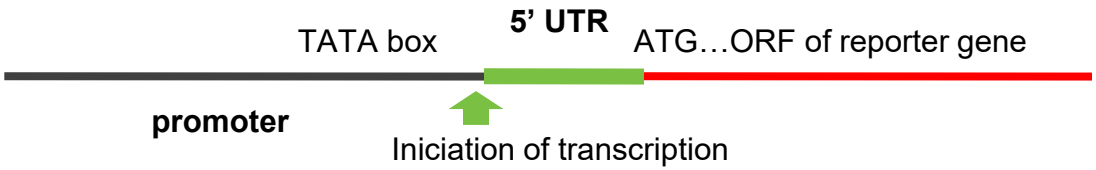
- Methods of gene expression analysis
  - Qualitative analysis of gene expression
    - Preparation of transcriptional fusion of promoter of analysed gene with a reporter gene
    - Preparation of translational fusion of the coding region of the analysed gene with reporter gene
    - Use of the data available in public databases
    - Tissue- and cell-specific gene expression analysis
    - Spatial trascriptomics
  - Quantitative analysis of gene expression
    - DNA and protein chips
    - Next generation transcriptional profiling
- Regulation of gene expression in the identification of gene function by gain-of-function approaches
  - T-DNA activation mutagenesis
  - Ectopic expression and regulated gene expression systems

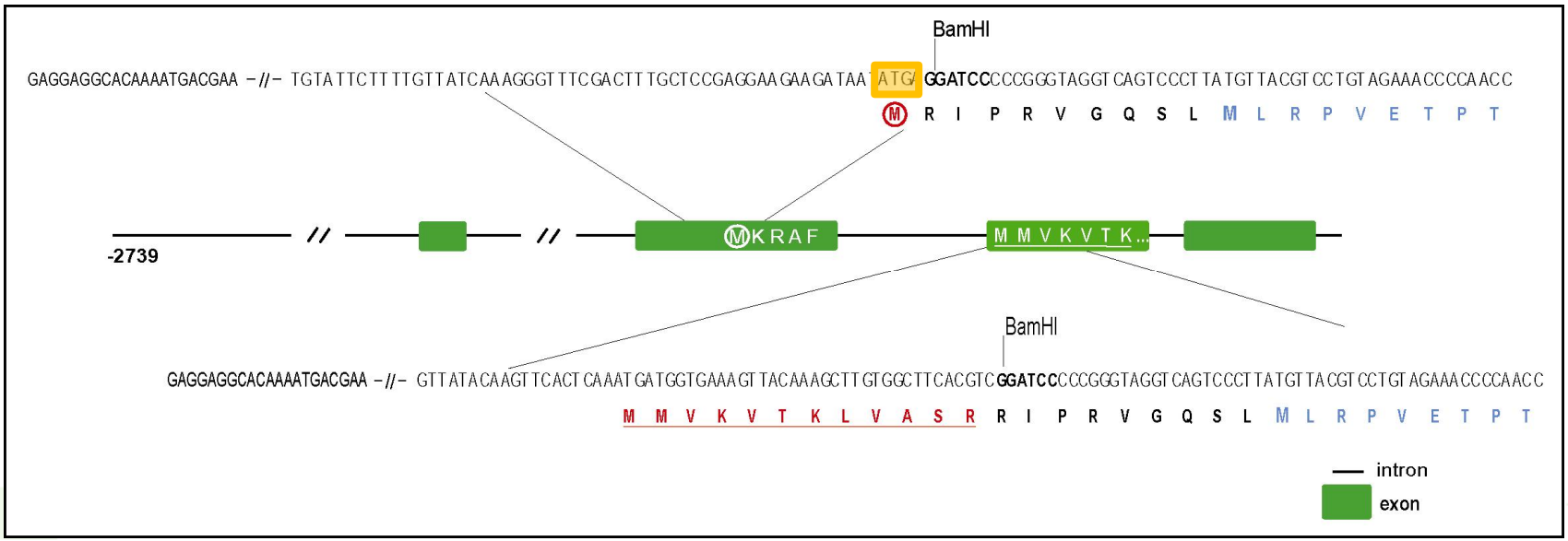
Outline

- Methods of gene expression analysis
  - Qualitative analysis of gene expression
    - Preparation of transcriptional fusion of promoter of analysed gene with a reporter gene

Transcriptional Fusion

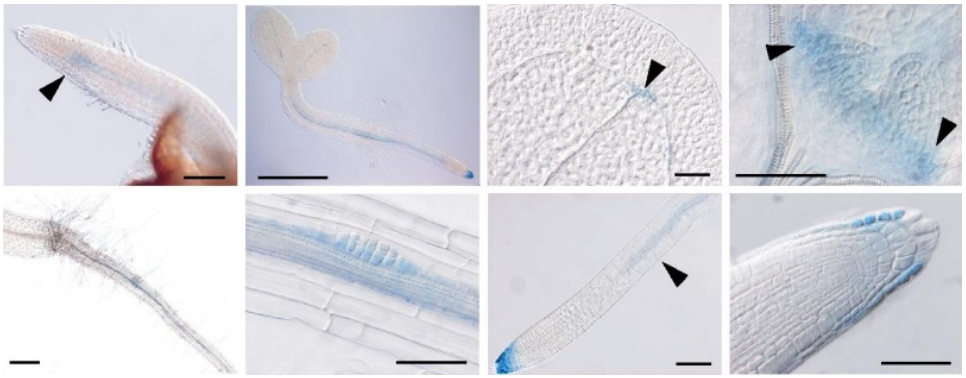
- Identification and cloning of the promoter region of the gene
- Preparation of recombinant DNA carrying the promoter and the reporter gene (uidA, GFP)

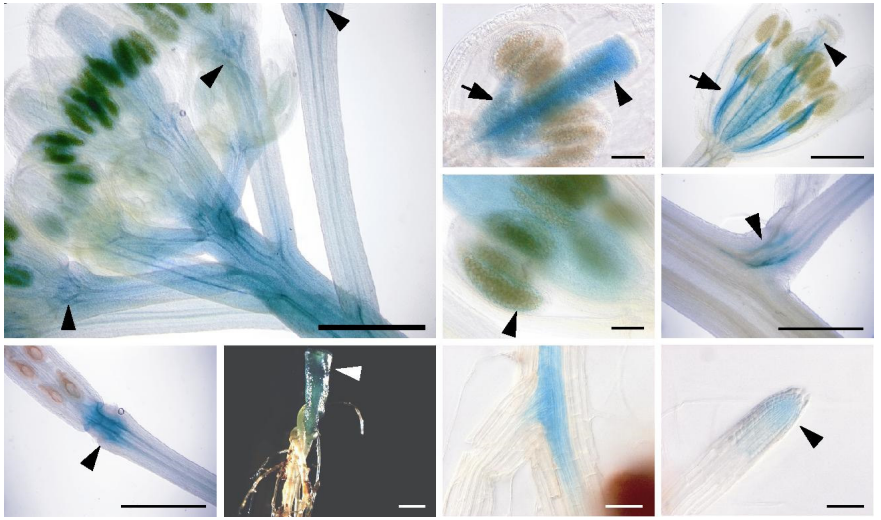




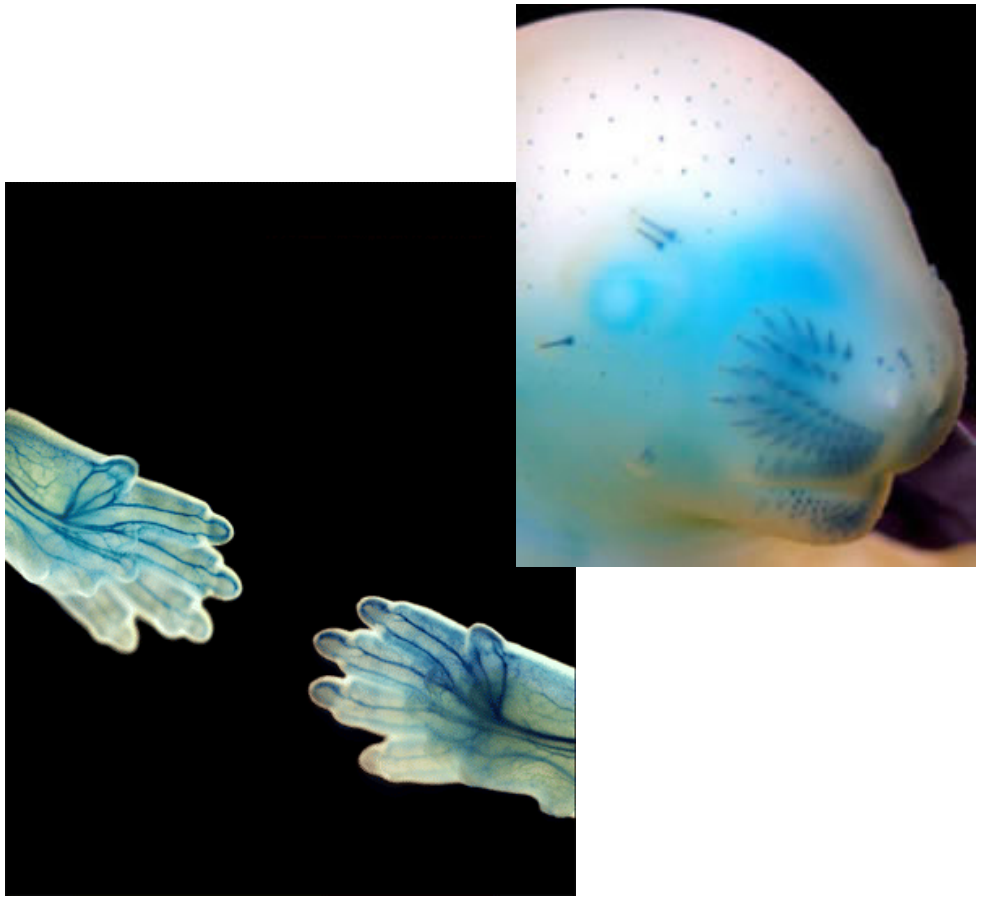
Transcriptional Fusion

- Identification and cloning of the promoter region of the gene
- Preparation of recombinant DNA carrying the promoter and the reporter gene (uidA, GFP)
- Preparation of transgenic organisms carrying this recombinant DNA and their histological analysis





GUS Reporter in Mouse Embryos



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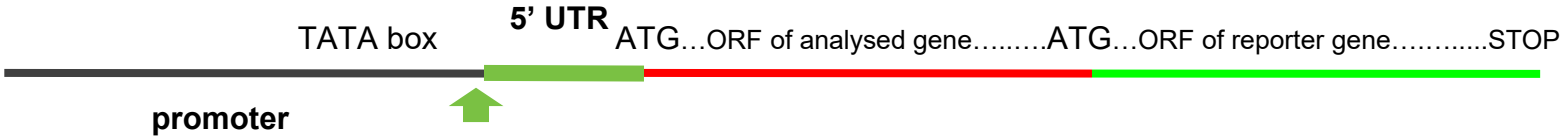
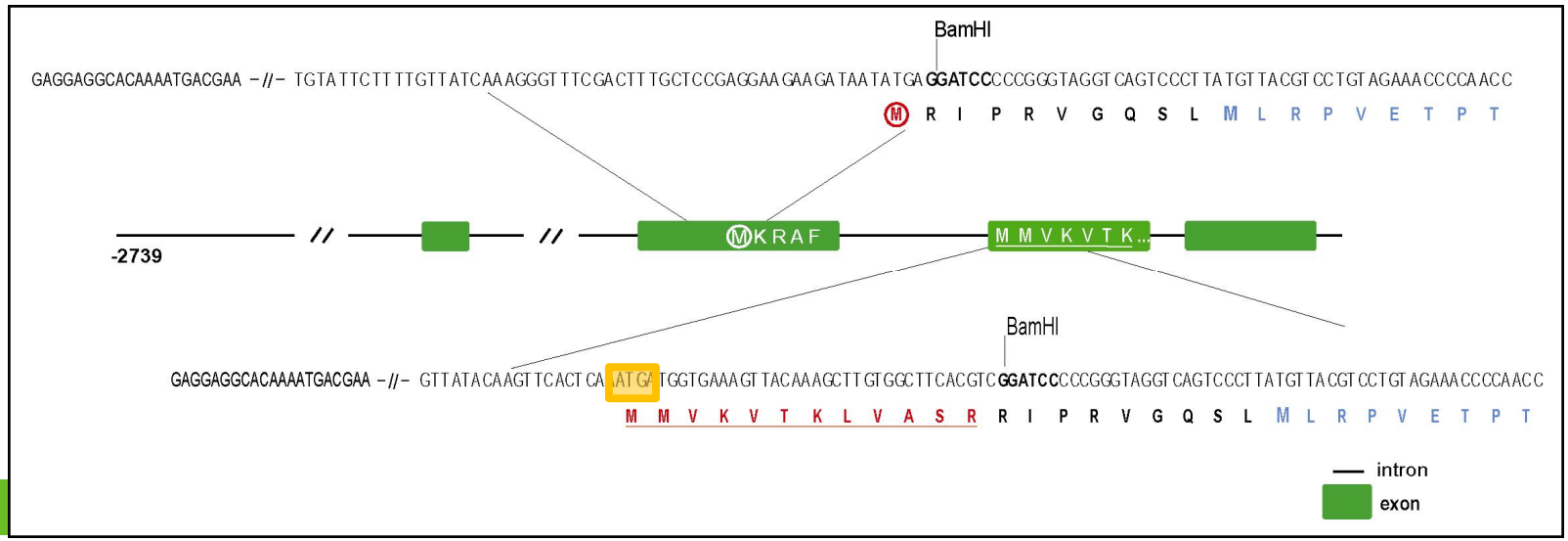
- Methods of gene expression analysis
  - Qualitative analysis of gene expression
    - Preparation of transcriptional fusion of promoter of analysed gene with a reporter gene
    - Preparation of translational fusion of the coding region of the analysed gene with reporter gene

Translational Fusion

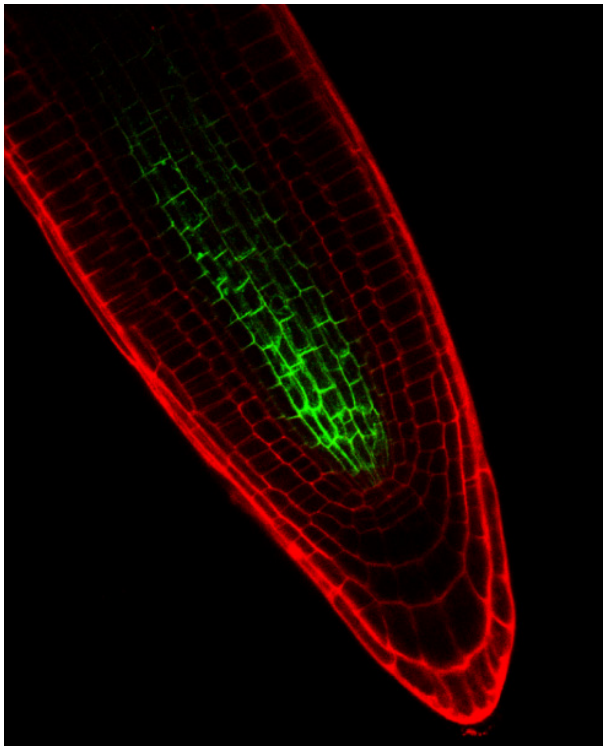
- Identification and cloning of the promoter and coding region of the analyzed gene
- Preparation of a recombinant DNA carrying the promoter and the coding sequence of the studied gene in a fusion with the reporter gene (uidA, GFP)



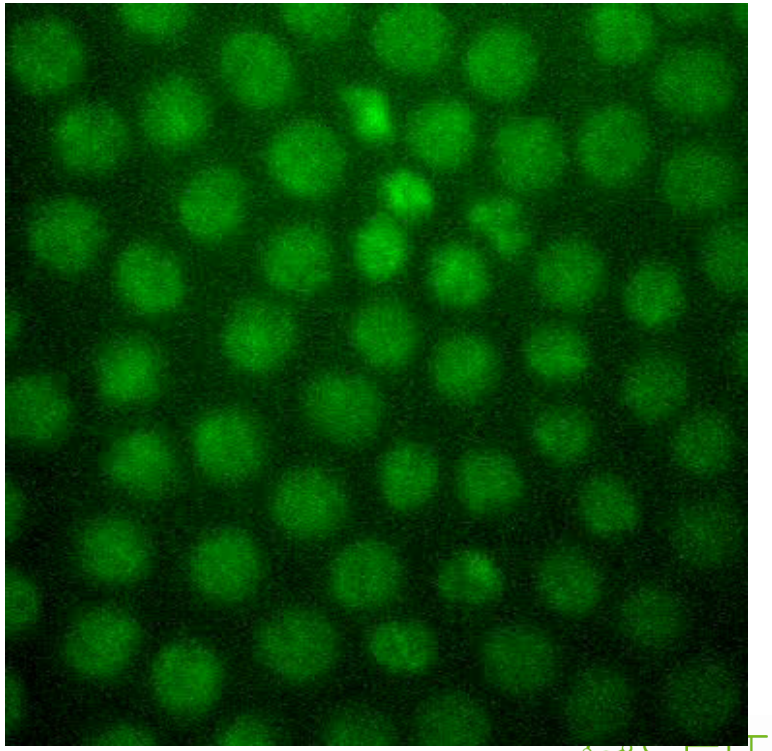


Translational Fusion

- Preparation of transgenic organisms carrying the recombinant DNA and their histological analysis
- Compared to transcriptional fusion, translation fusion allows analysis of intercellular localization of gene product (protein) or its dynamics

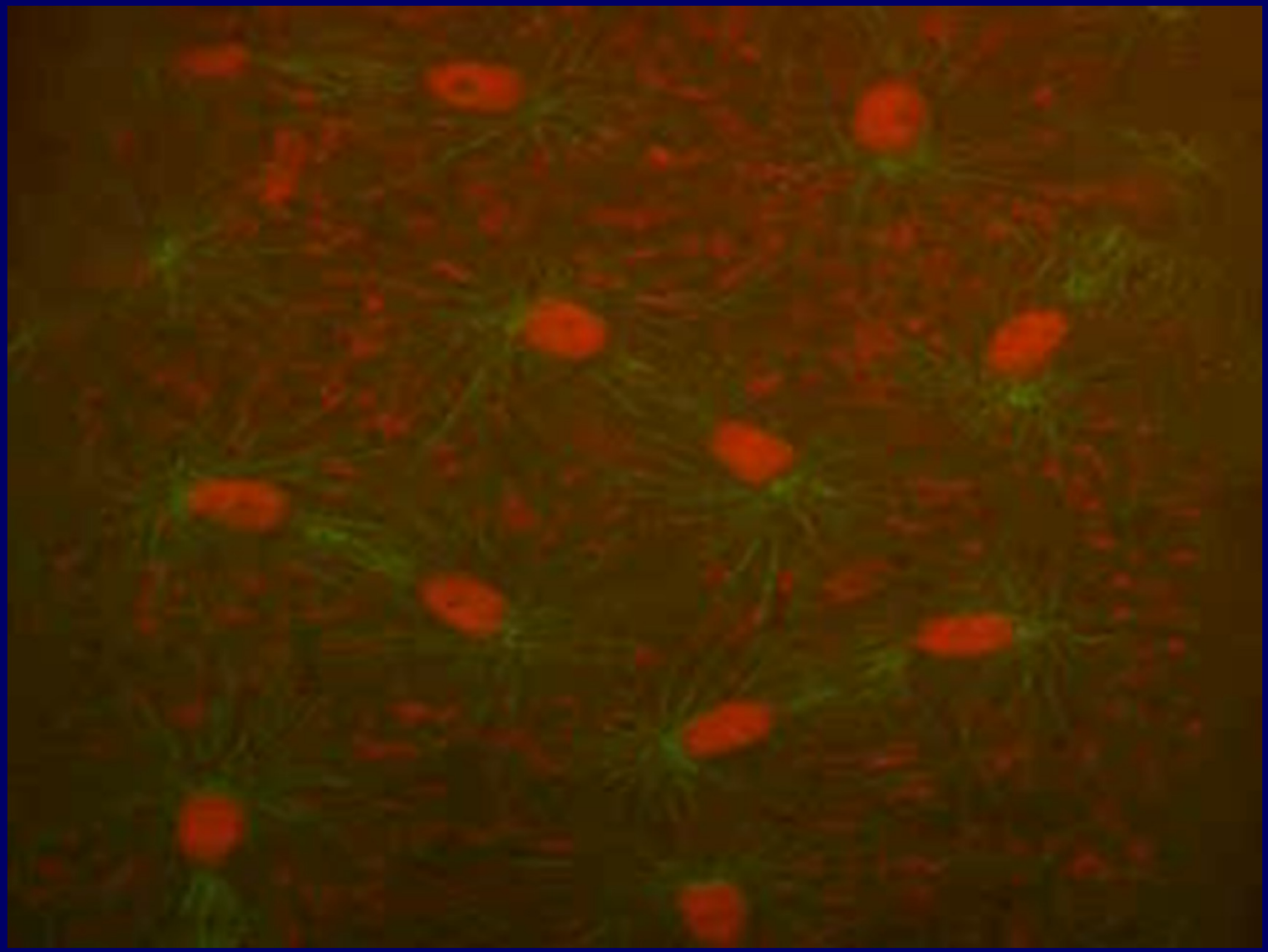


PIN1-GFP in *Arabidopsis*



Histone 2A-GFP in *Drosophila* embryo by PAM

Translational Fusion



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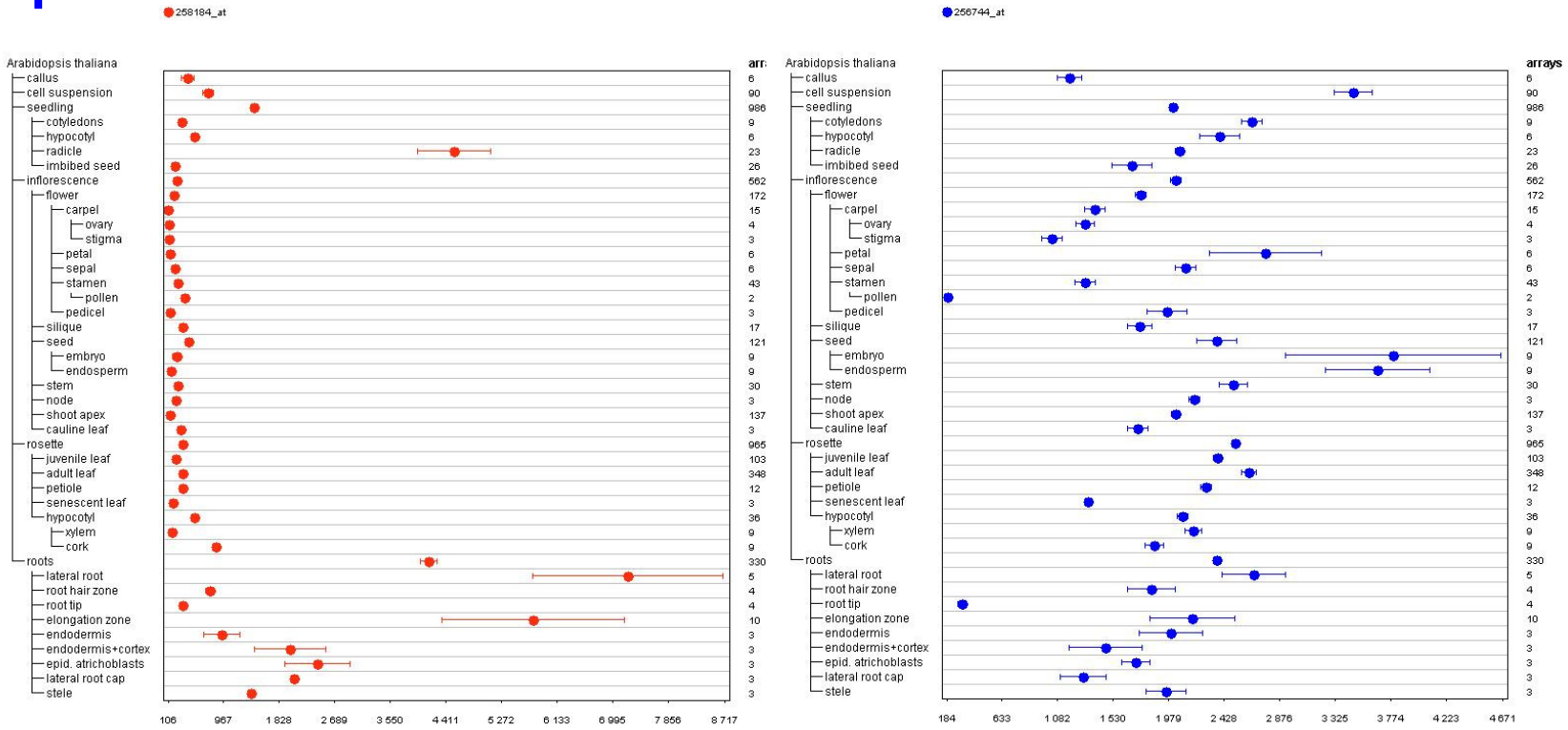
Databases

- **Analysis of expression using Genevestigator** (***AHP1*** and ***AHP2***, *Arabidopsis*, Affymetrix ATH 22K Array)



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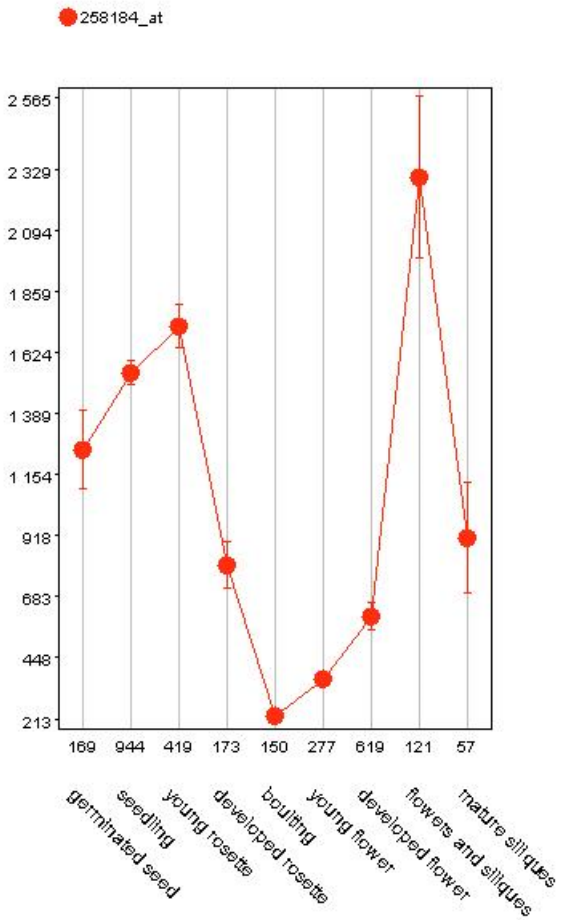

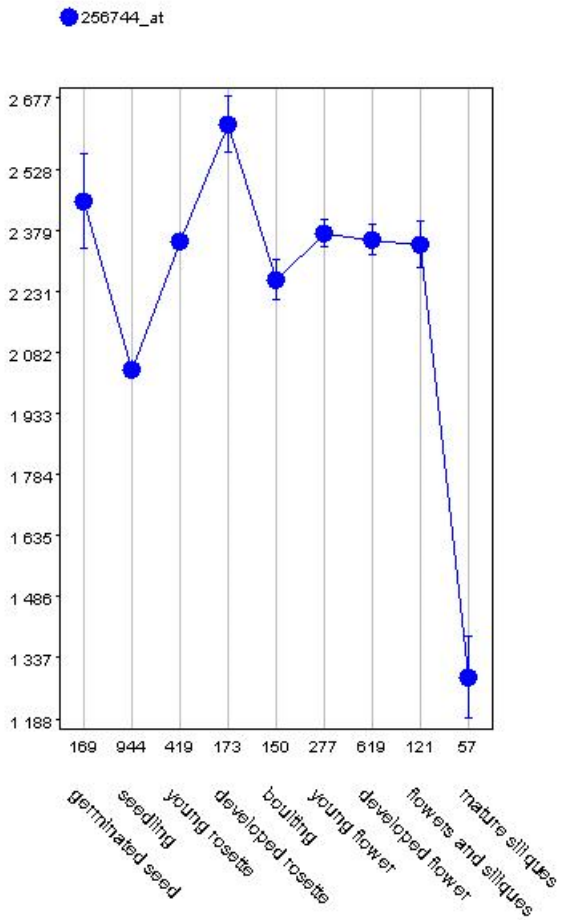

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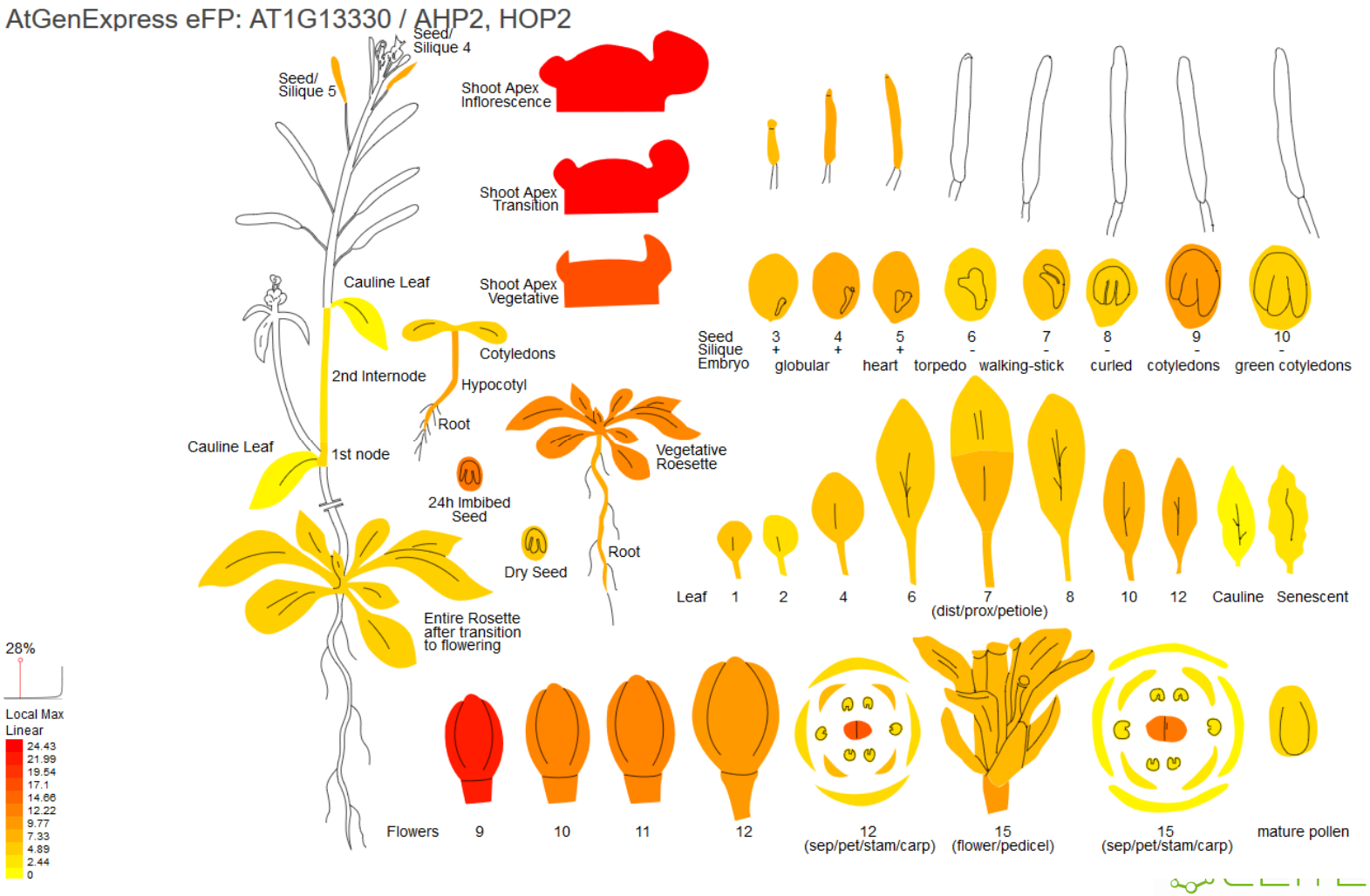
Databases

- **Analysis of expression using [ePlant]**



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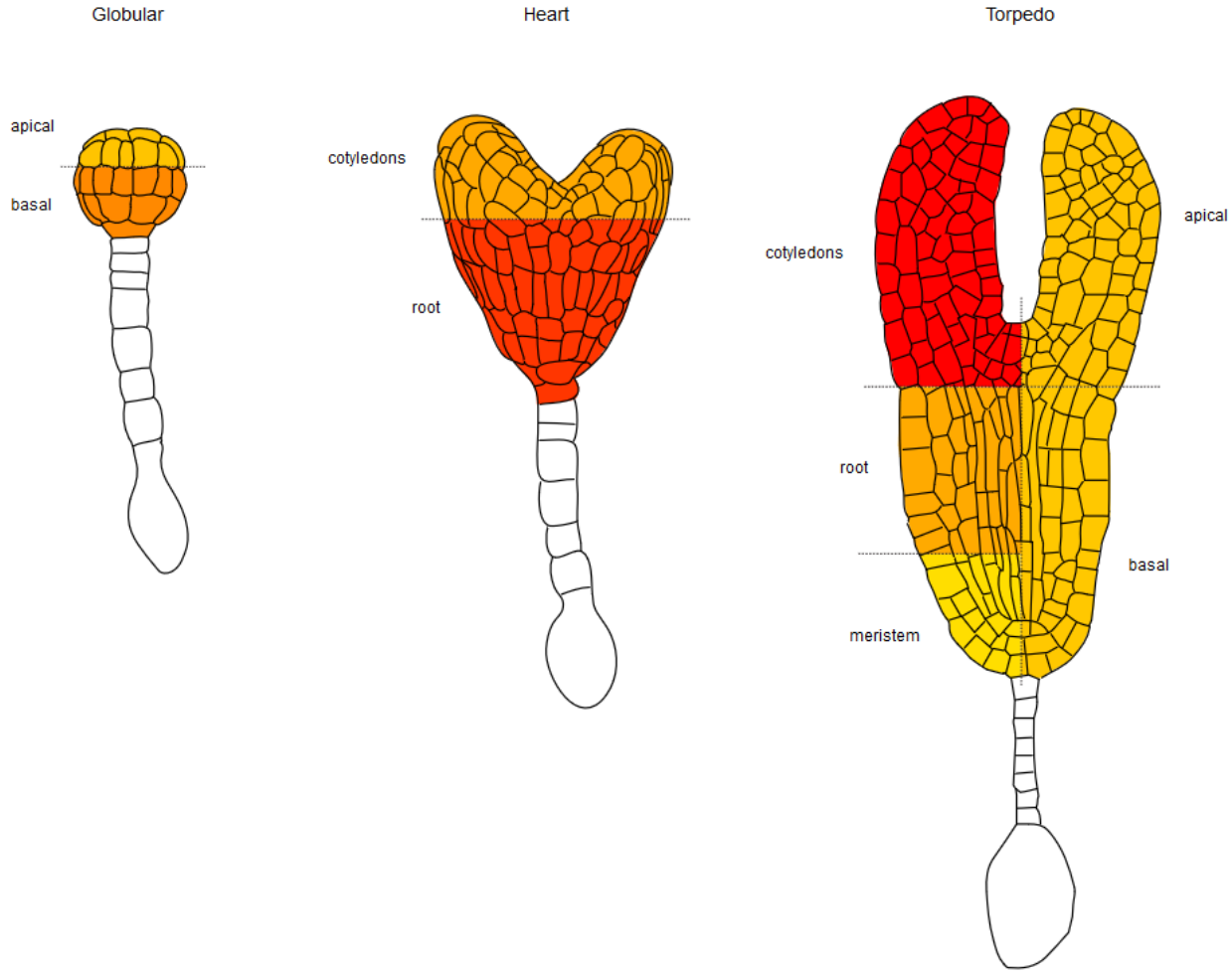
- **Analysis of expression using ePlant**



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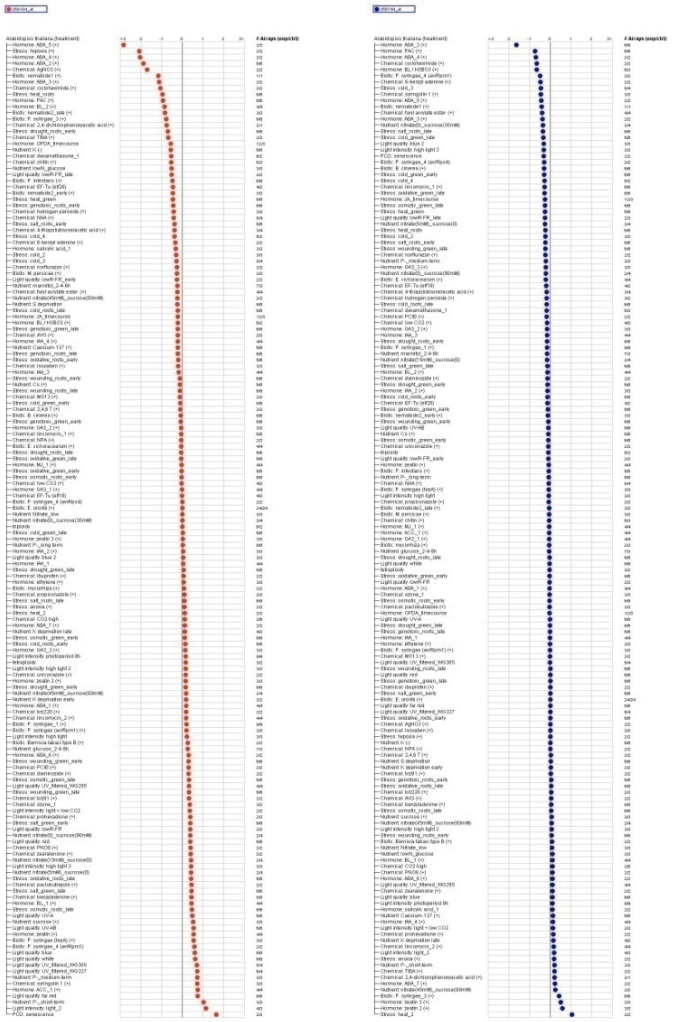

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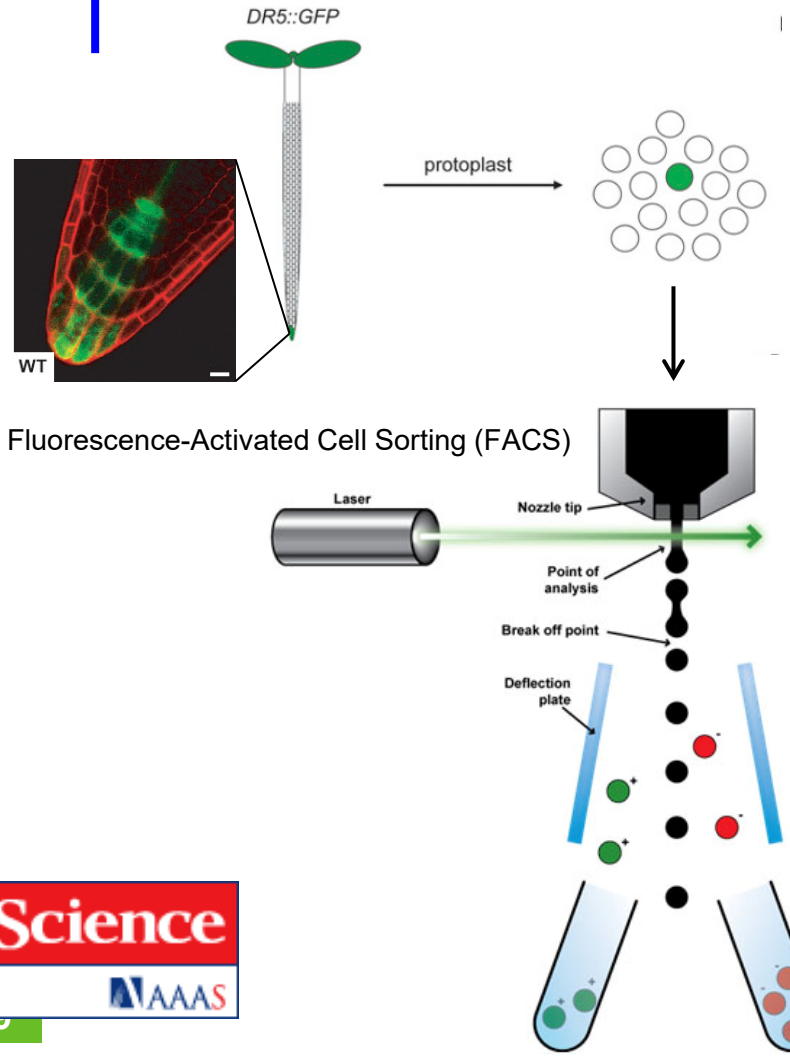
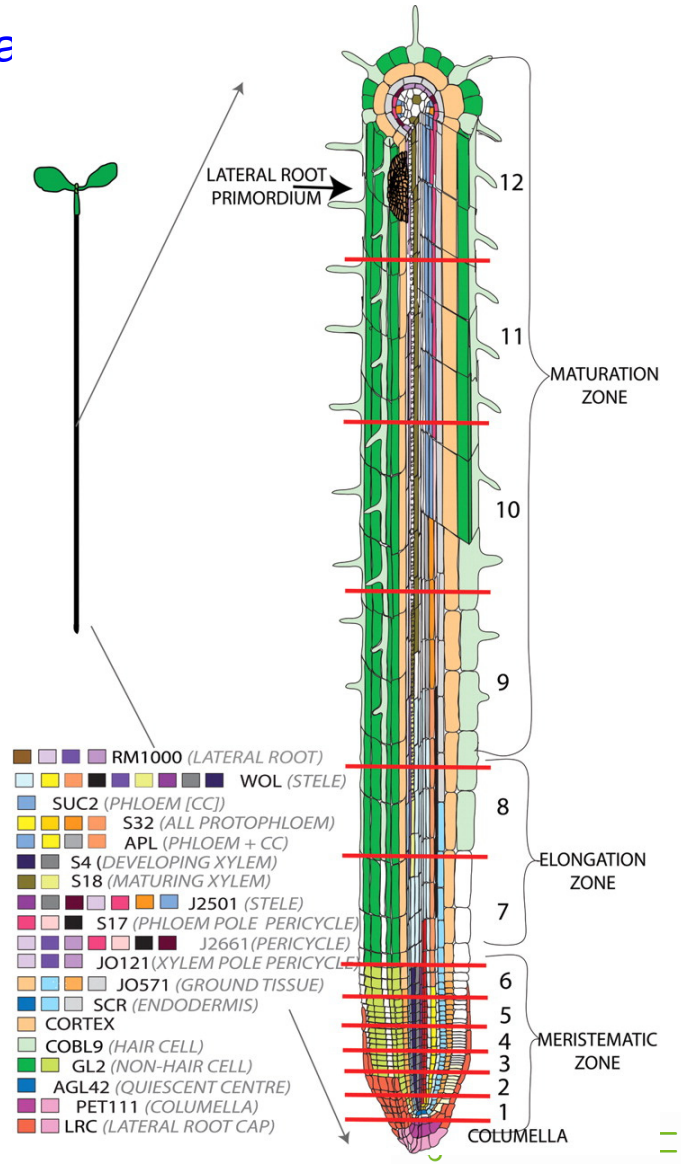
    - Tissue- and cell-specific gene expression analysis

Expression Maps - RNA

- High-Resolution Expression Map in Ara



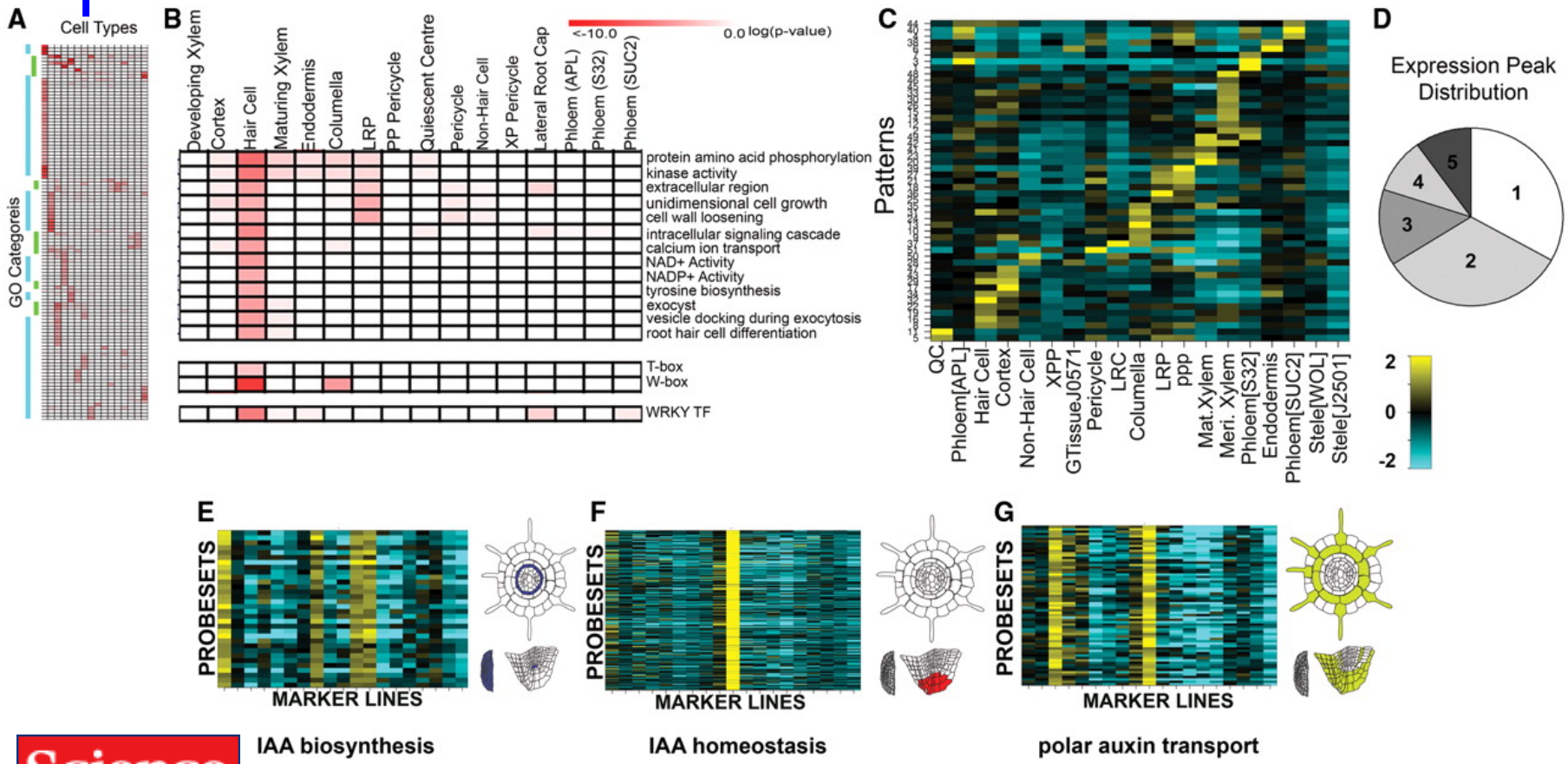


Brady et al., *Science*, 2007

Science AAAS

Expression Maps - RNA

- High-Resolution Expression Map in Arabidopsis Root



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Brady et al., *Science*, 2007

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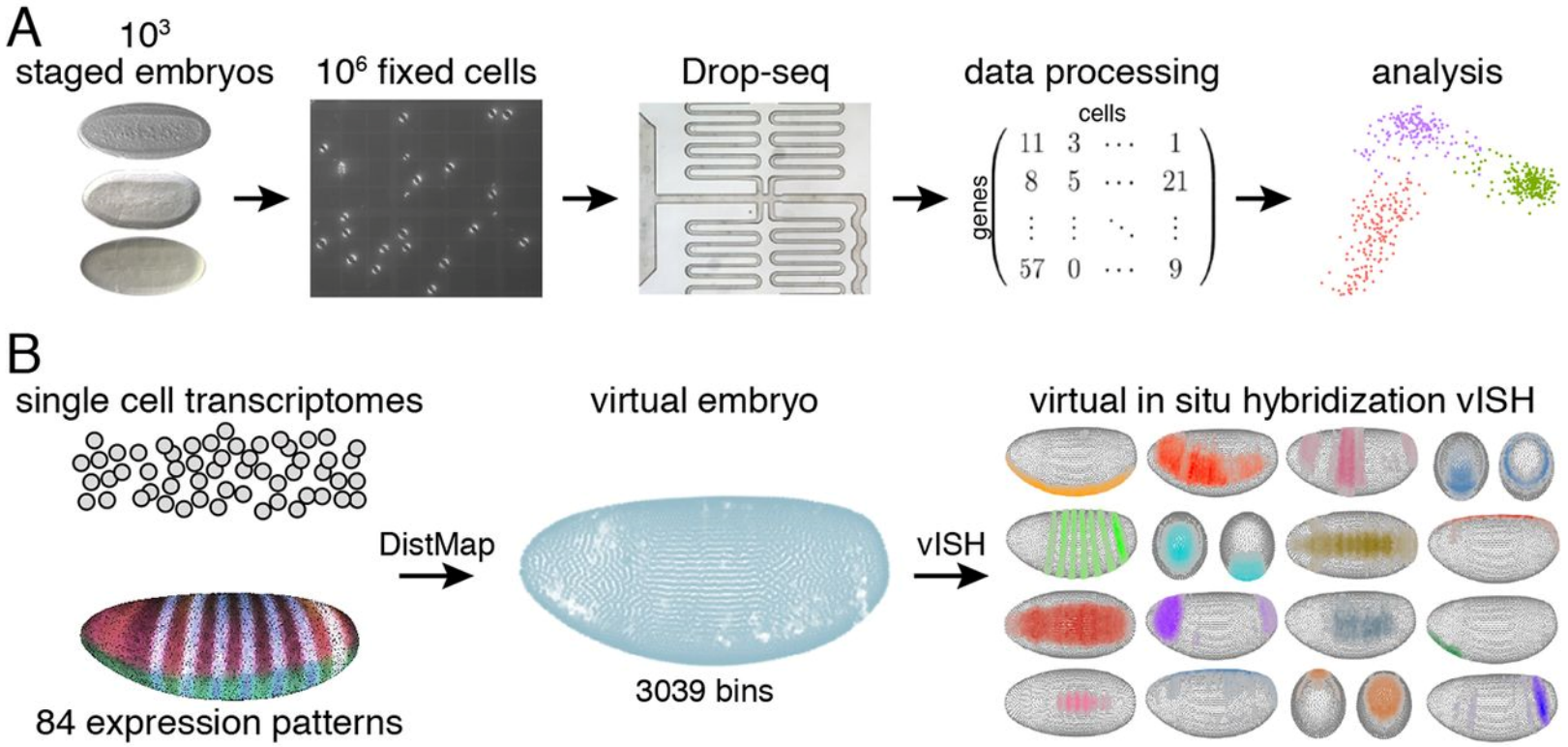
Expression Maps - RNA

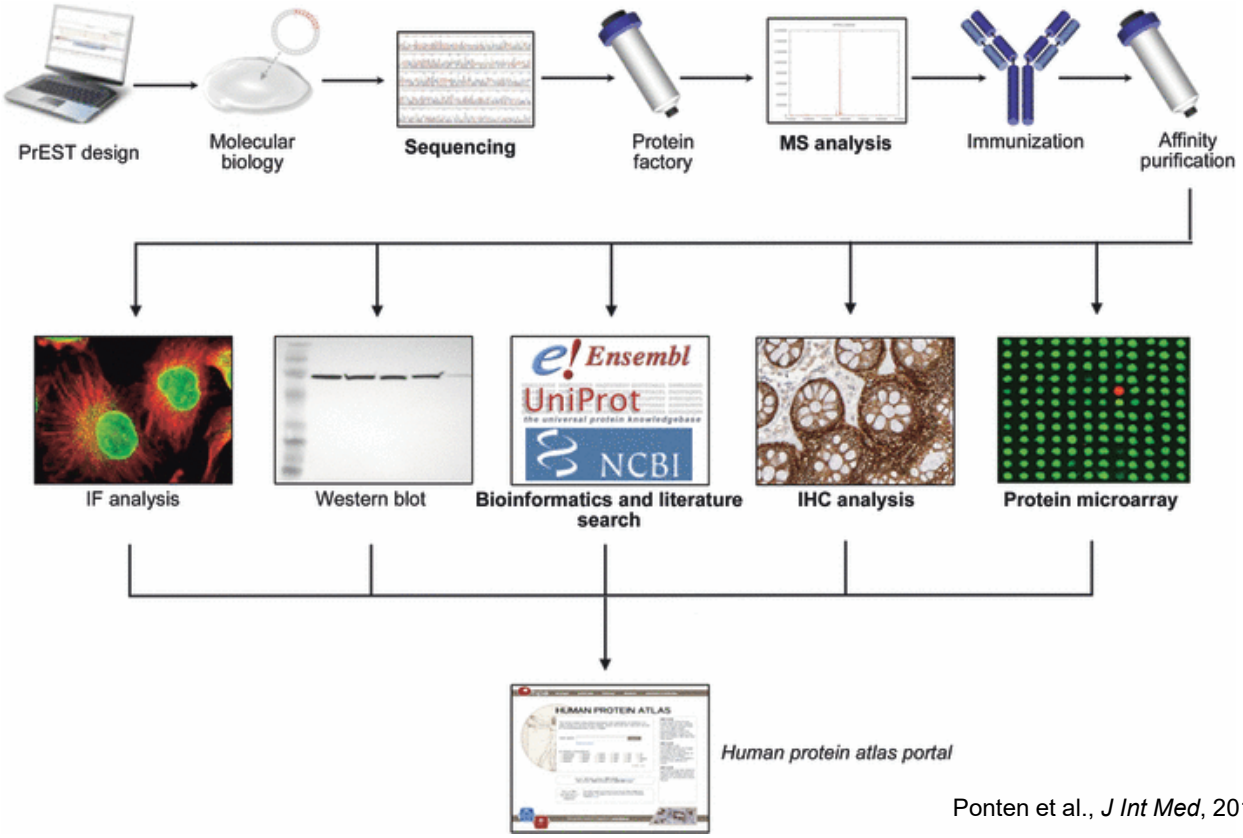
- High-Resolution Expression Map in Drosophilla



Nikos Karaiskos et al. Science 2017;science.aan3235

Expression Maps - Proteins

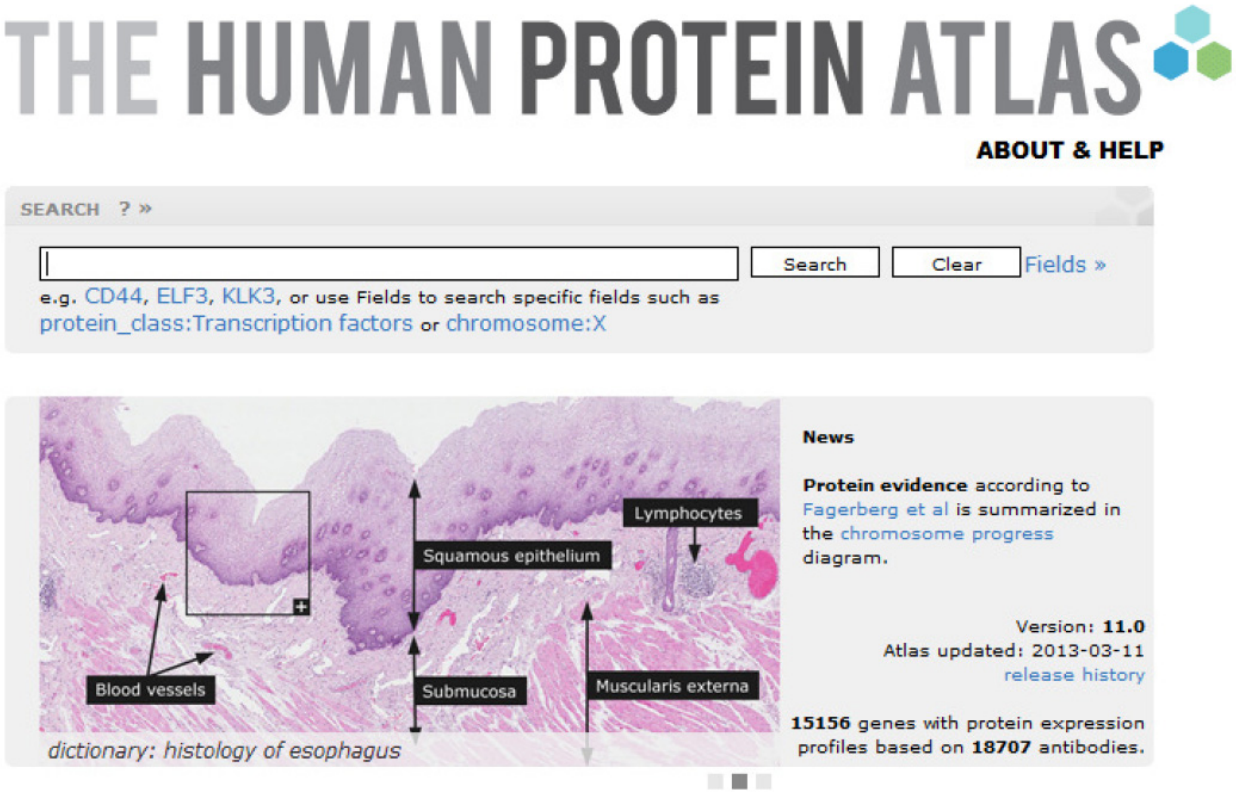
- Human Protein Atlas



Ponten et al., *J Int Med*, 2011

Expression Maps - Proteins

- Human Protein Atlas (http://www.proteinatlas.org/)



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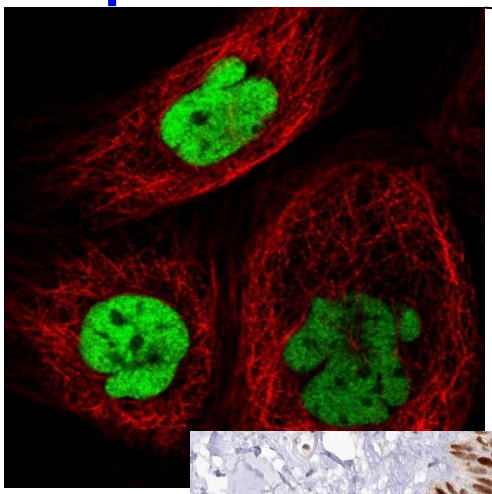

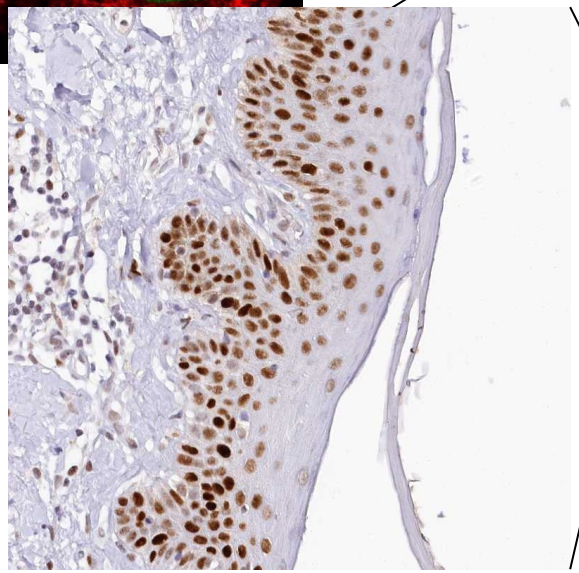

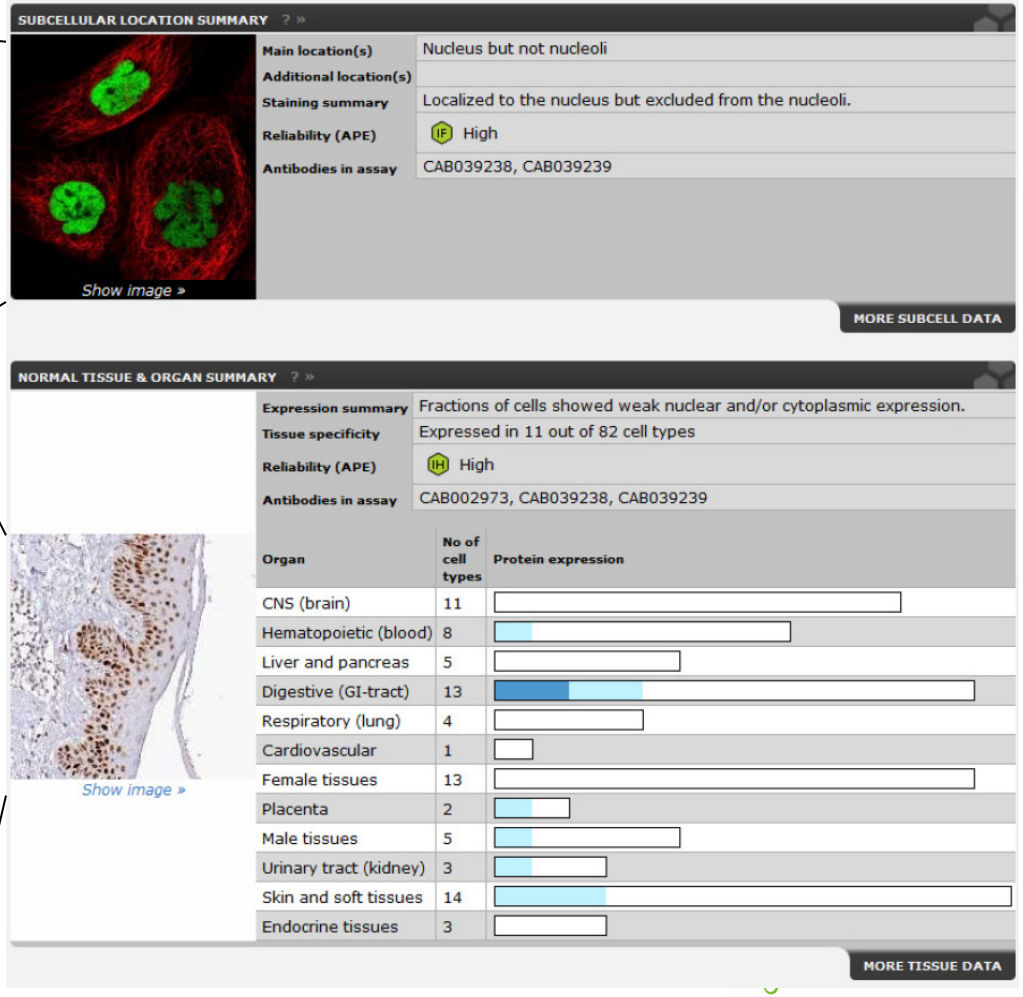

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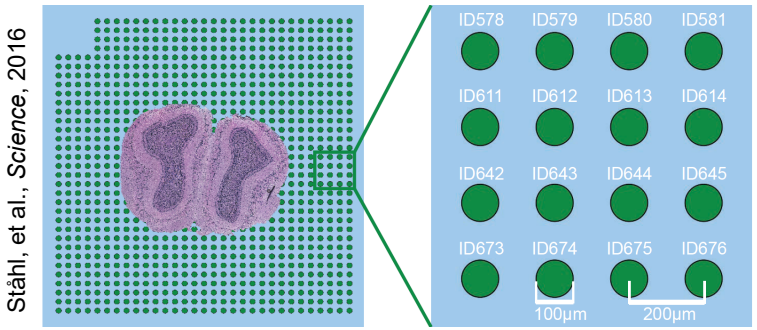
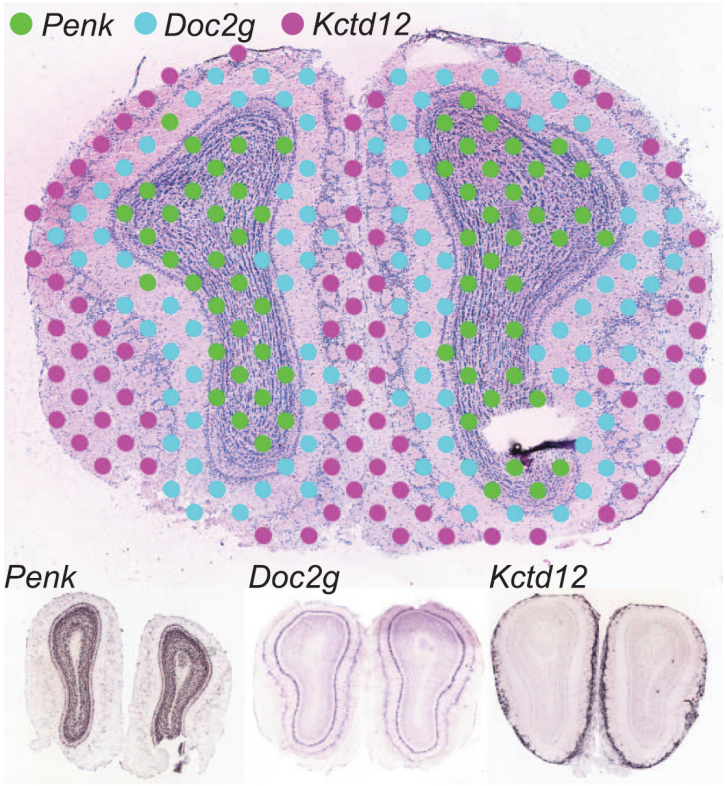
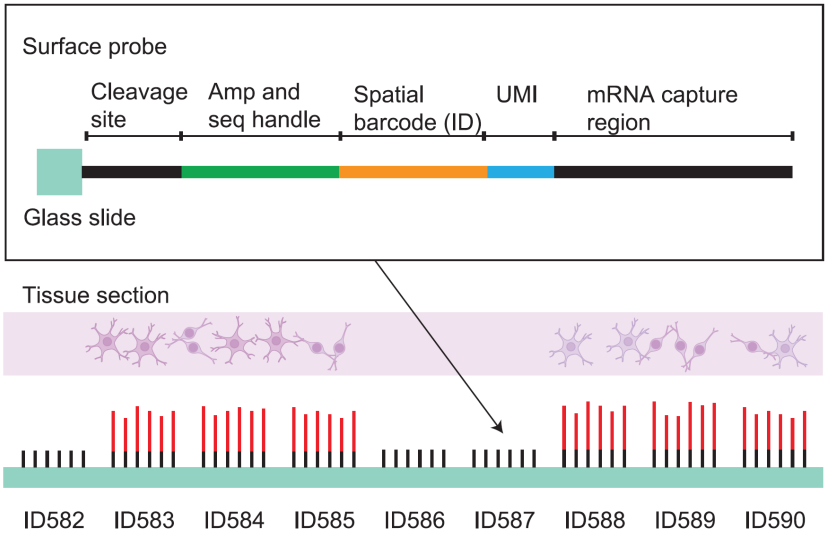
    - Tissue- and cell-specific gene expression analysis
    - Spatial trascriptomics

Spatial Transcriptomics







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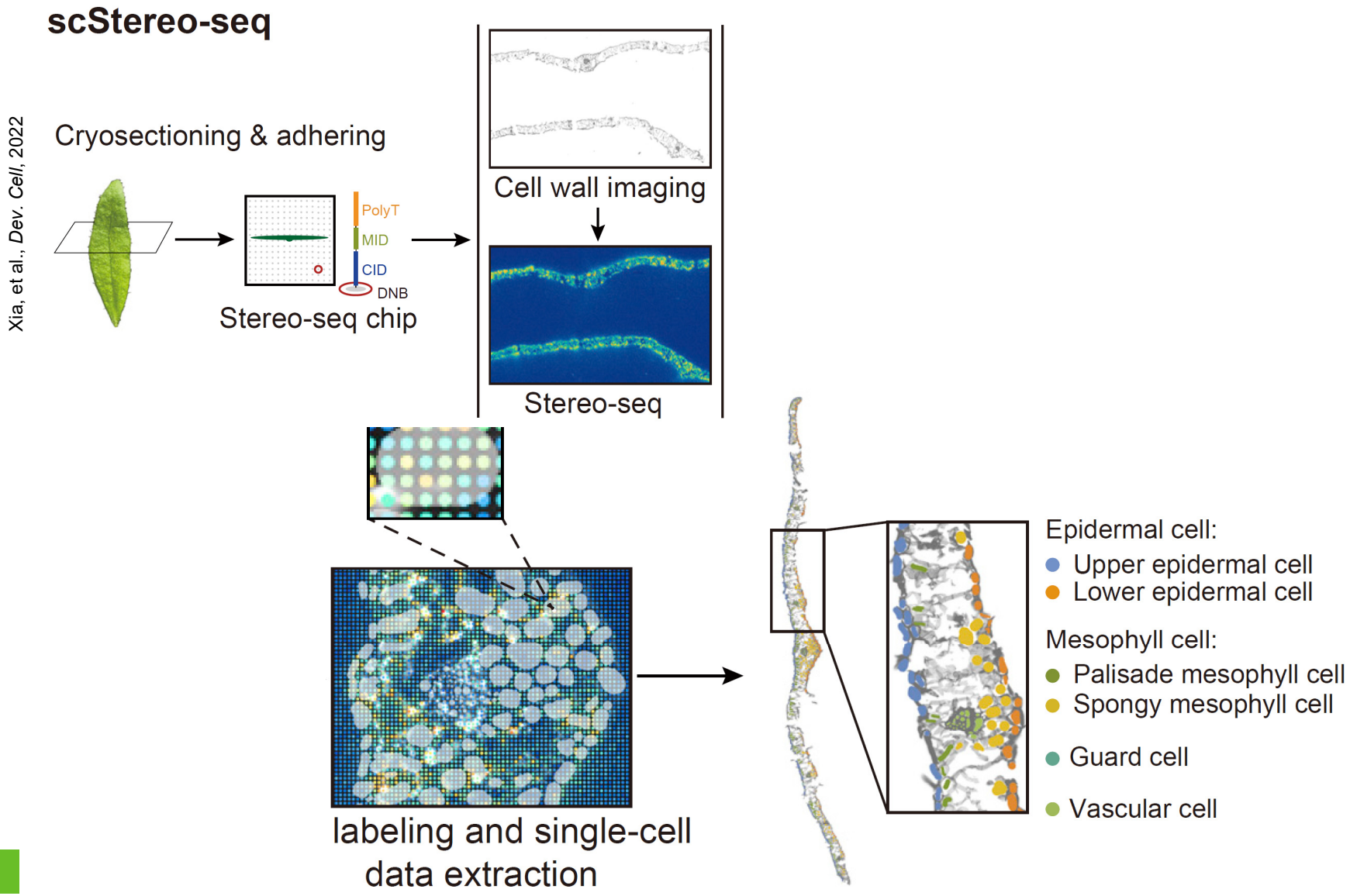
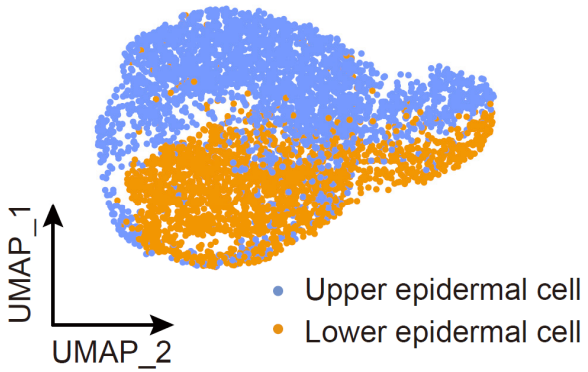
Spatial Transcriptomics



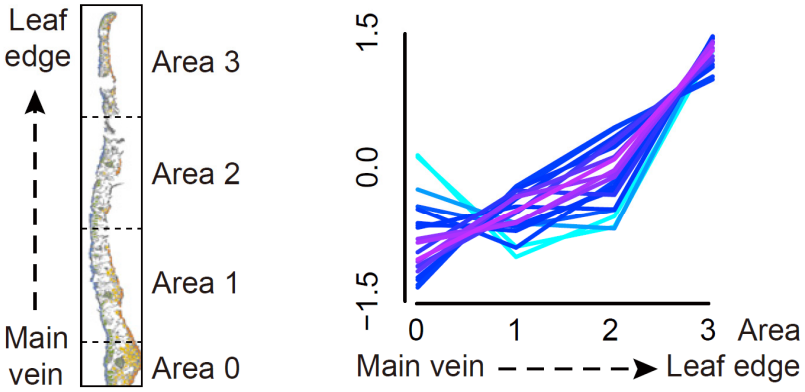
Spatial Transcriptomics

Spatial single-cell transcriptome analysis

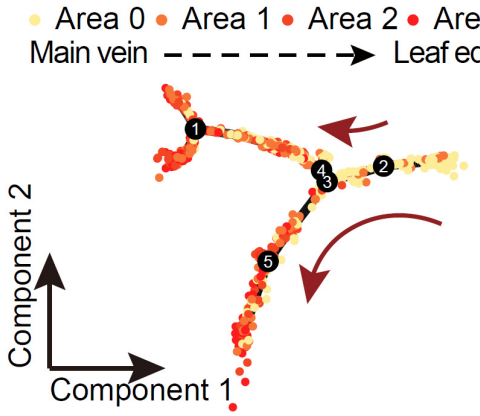
(i) Cell sub-type identification



(ii) Spatial gene expression pattern



(iii) Spatial developmental trajectory



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    - Tissue- and cell-specific gene expression analysis
    - Spatial trascriptomics
  - Quantitative analysis of gene expression
    - DNA and protein chips

DNA Chips

- Method, which provides quick comparison of a large number of genes/proteins between the test sample and control
- Oligo DNA chips are used the most

- There are commercialy available kits for the whole genome
  - company Operon (Qiagen), 29.110 of 70-mer oligonucleotides representing 26.173 genes coding proteins, 28.964 transcripts and 87 microRNA genes of *Arabidopsis thaliana*
  - Possibility of use for the preparation of photolithography chips – facilitation of oligonucletide synthesis e.g. for the whole human genome (about 3,1 x 10^9 bp) jit is possible to prepare 25-mers in only 100 steps, by this technique

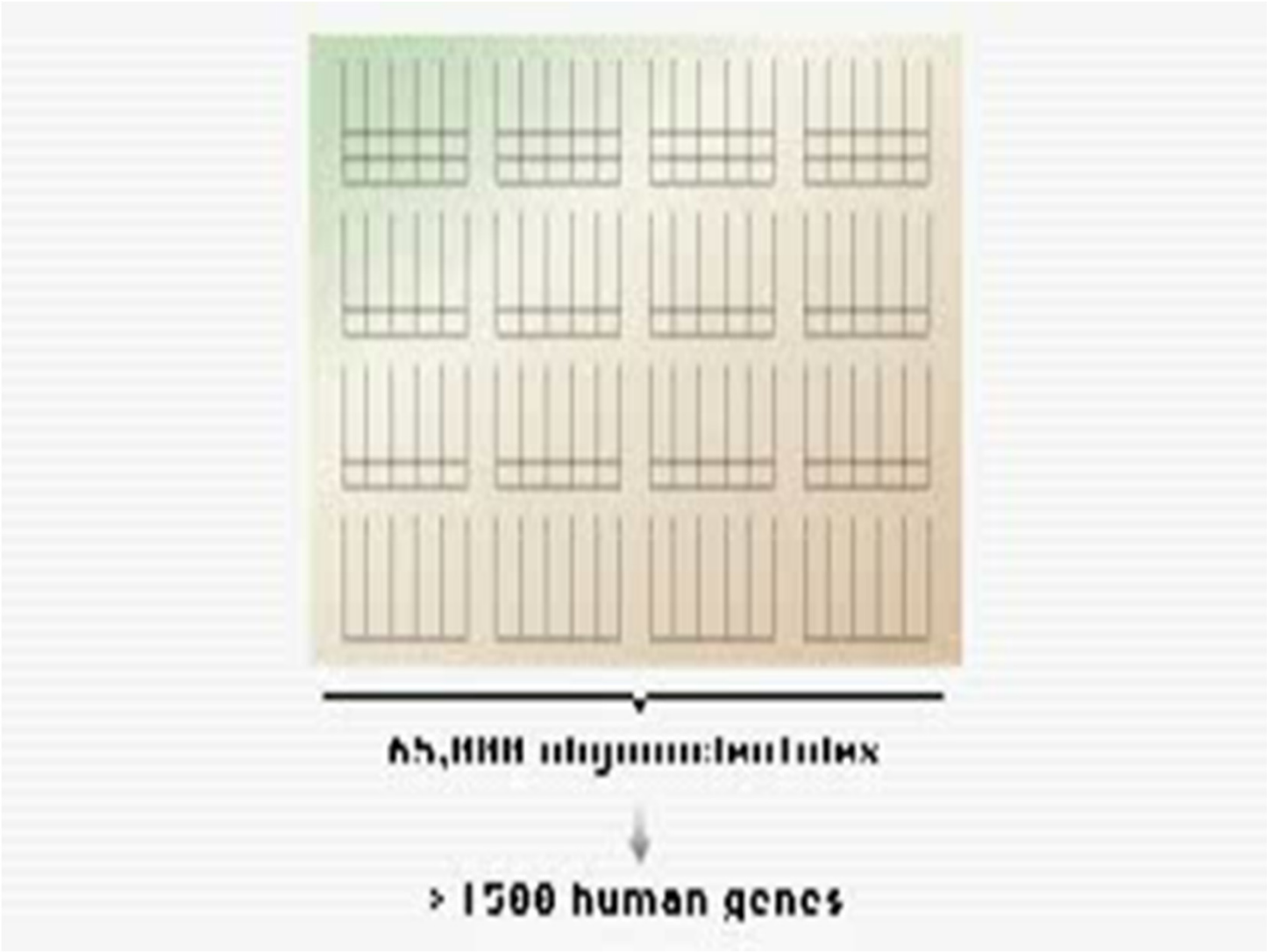
- Chips not only for the analysis of gene expression, but also for e.g. Genotyping (SNPs, sequencing with chips, …)

Affymetrix ATH1 *Arabidopsis* genome array

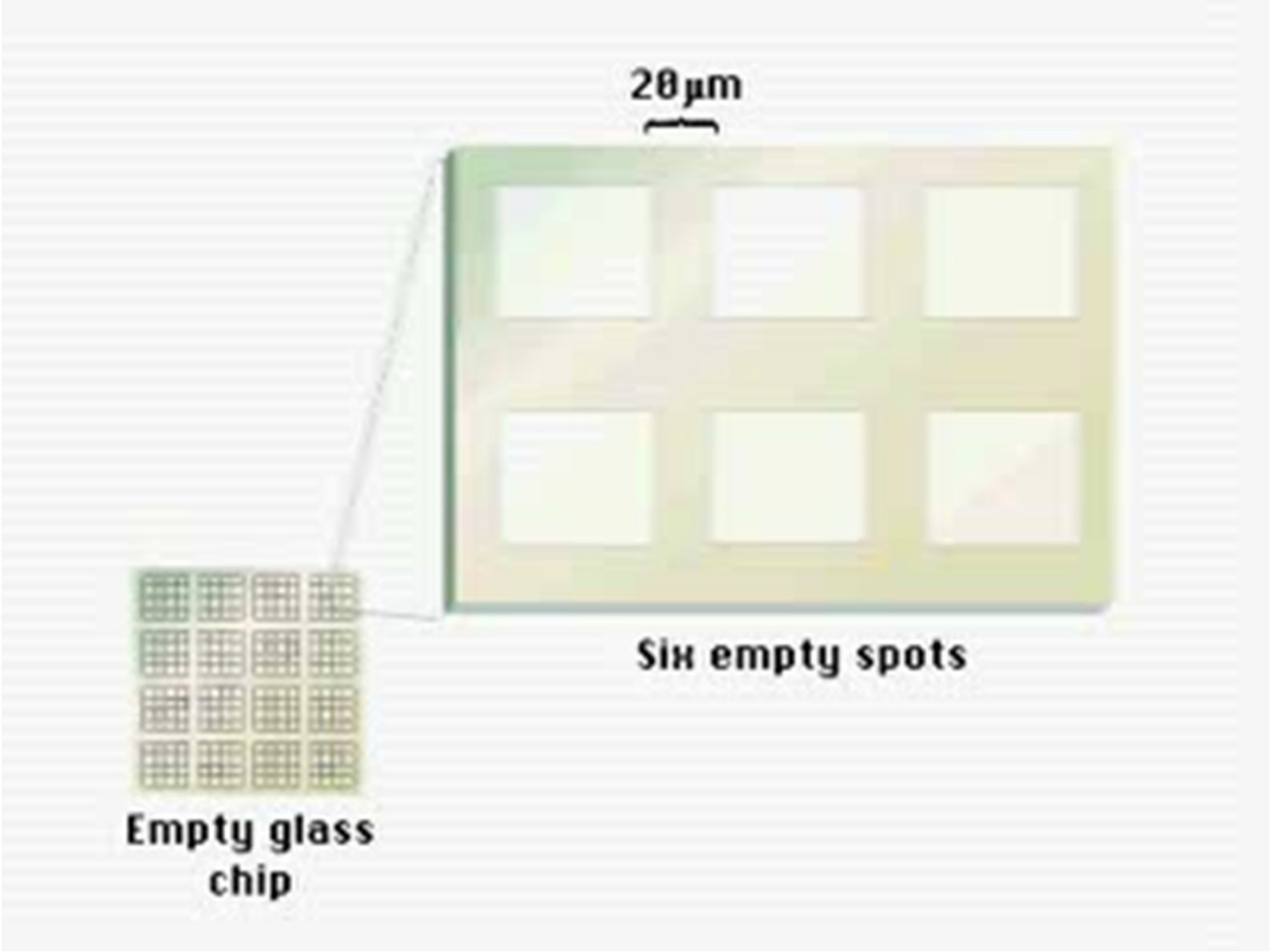
Critical Specifications	
Number of arrays	One
Number of sequence represented	>24,000 gene sequences
Feature size	18 µm
Oligonucleotide probe length	25-mer
Probe pairs/sequence	11
Control sequences	*E. coli* genes *bioB, bioC, bioD*. *B. subtilis* gene *lysA*. Phage P1 *cre* gene. Arabidopsis maintenance genes GAPDH, Ubiquitin, and Actin
Detection sensitivity	1:100,000*

*As measured by detection in comparative analysis between a complex target containing spiked control transcriptions and a complex target with no spikes.

DNA Chips



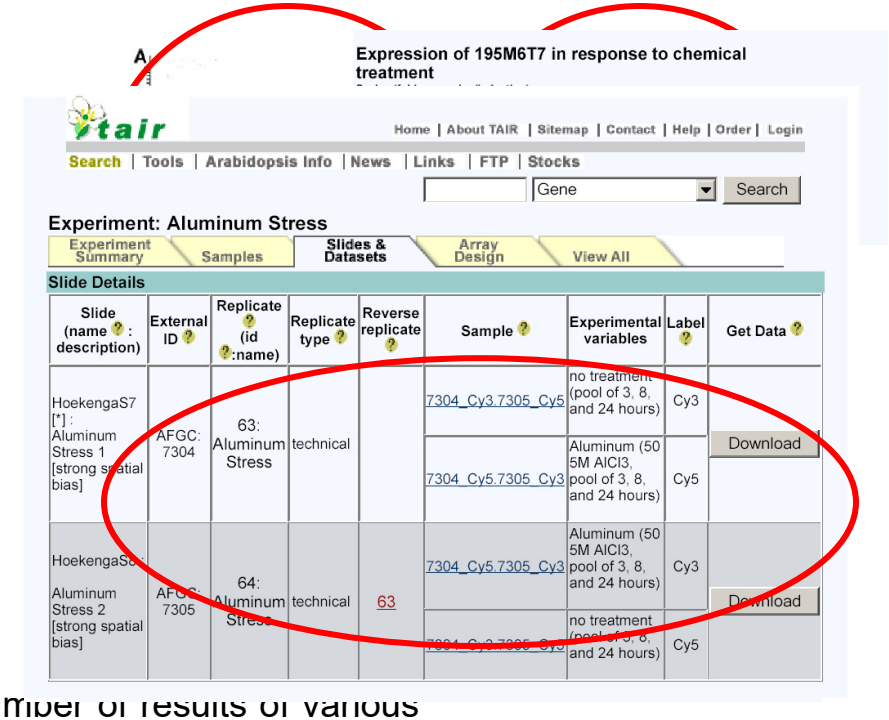
Photolitography



DNA Chips

- For the correct interpretation of the results, good knowledge of advanced statistical methods is required
- It is necessary to include a sufficient number of controls and repeats

- Control of accuracy of the measurement (repeated measurements on several chips with the same sample, comparing the same samples analysed on different chips with each other)
- Control of reproducibility of measurements (repeated measurements with different samples isolated under the same conditions on the same chip – comparing with each other)
- Identification of reliable measurement treshold
- Finally comparing the experiment with the control or comparing different conditions with each other -> the result



- Currently there's been a great number of results of various experiments in publicly accessible databases

Che et al., 2002

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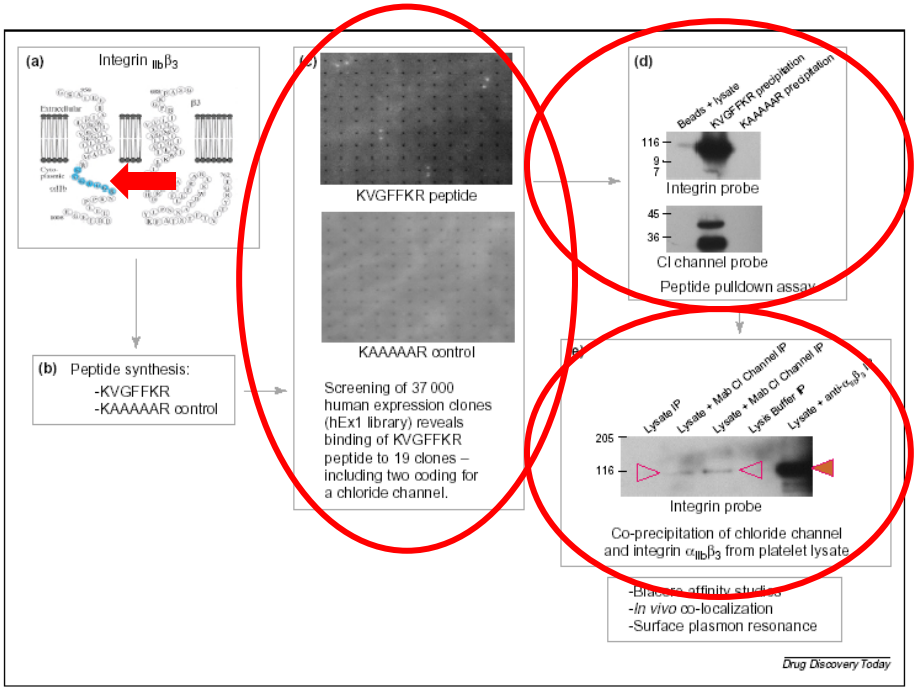
Protein Chips

- Protein chips
  - Chips with high density containing 10^4 proteins
  - Analysis of protein-protein interactions, kinase substrates and interactions with small molecules
  - Possibility of using antibodies – more stable than proteins

Protein Chips

- Identification of proteins interacting with integrin $\alpha_{IIb}\beta_3$ cytoplasmic domain of platelets
  - Expression of cytoplasmic part as a fusion peptide biotin-KVGFFKR
  - Analysis of binding to the protein chip containing 37.000 clones of *E. coli* expressing human recombinant proteins
  - Confirmation of interaction by pull-down analysis of peptides and by coprecipitation of whole proteins as well (e.g. chloride channel Icln)
  - Other use: e.g. in the identification of kinase substrates, when substrates are bound to the chip and exposed to kinases in the presense of radiolabeled ATP (786 purified proteins of barely, of which 21 were identified as CK2α kinase substrates; Kramer et al., 2004)



Lueking et al., 2005

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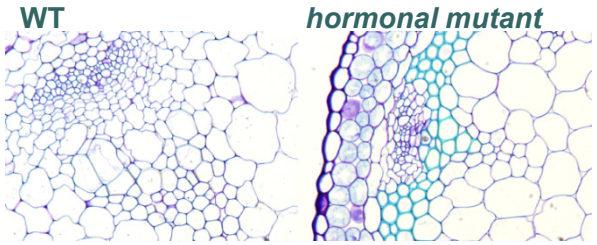
Next Gen Transcriptional Profiling

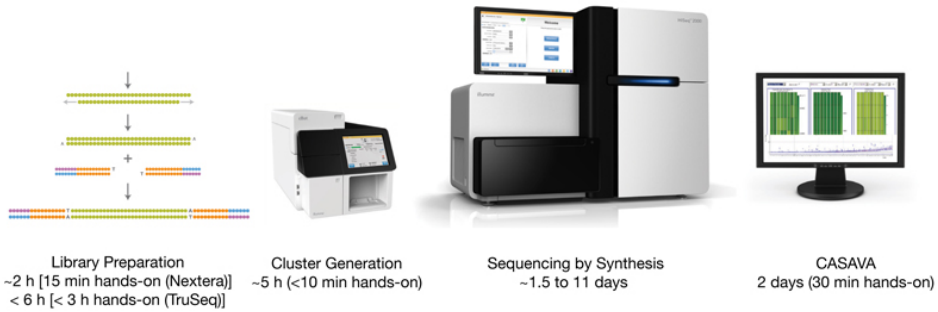
- ***Transcriptional profiling*** via ***RNA sequencing***



mRNA mRNA

cDNA cDNA

Sequencing by Illumina and
number of transcripts determination



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Results of –omics Studies vs Biologically Relevant Conclusions

- Transcriptional profiling yielded more then **7K differentially regulated genes**…

Ddii et al., *unpublished*

gene	locus	sample_1	sample_2	status	value_1	value_2	log2(fold_change)	test_stat	p_value	q_value	significant
AT1G07795	1:2414285-2414967	WT	MT	OK	0	1,1804	1.79769e+308	1.79769e+308	6.88885e-05	0,000391801	yes
HRS1	1:4556891-4558708	WT	MT	OK	0	0,696583	1.79769e+308	1.79769e+308	6.61994e-06	4.67708e-05	yes
ATMLO14	1:9227472-9232296	WT	MT	OK	0	0,514609	1.79769e+308	1.79769e+308	9.74219e-05	0,000535055	yes
NRT1.6	1:9400663-9403789	WT	MT	OK	0	0,877865	1.79769e+308	1.79769e+308	3.2692e-08	3.50131e-07	yes
AT1G27570	1:9575425-9582376	WT	MT	OK	0	2,0829	1.79769e+308	1.79769e+308	9.76039e-06	6.647e-05	yes
AT1G60095	1:22159735-22162419	WT	MT	OK	0	0,688588	1.79769e+308	1.79769e+308	9.95901e-08	9.84992e-07	yes
AT1G03020	1:698206-698515	WT	MT	OK	0	1,78859	1.79769e+308	1.79769e+308	0,00913915	0,0277958	yes
AT1G13609	1:4662720-4663471	WT	MT	OK	0	3,55814	1.79769e+308	1.79769e+308	0,00021683	0,00108079	yes
AT1G21550	1:7553100-7553876	WT	MT	OK	0	0,562868	1.79769e+308	1.79769e+308	0,00115582	0,00471497	yes
AT1G22120	1:7806308-7809632	WT	MT	OK	0	0,617354	1.79769e+308	1.79769e+308	2.48392e-06	1.91089e-05	yes
AT1G31370	1:11238297-11239363	WT	MT	OK	0	1,46254	1.79769e+308	1.79769e+308	4.83523e-05	0,000285143	yes
APUM10	1:13253397-13255570	WT	MT	OK	0	0,581031	1.79769e+308	1.79769e+308	7.87855e-06	5.46603e-05	yes
AT1G48700	1:18010728-18012871	WT	MT	OK	0	0,556525	1.79769e+308	1.79769e+308	6.53917e-05	0,000374736	yes
AT1G59077	1:21746209-21833195	WT	MT	OK	0	138,886	1.79769e+308	1.79769e+308	0,00122789	0,00496816	yes
AT1G60050	1:22121549-22123702	WT	MT	OK	0	0,370087	1.79769e+308	1.79769e+308	0,00117953	0,0048001	yes
AT4G15242	4:8705786-8706997	WT	MT	OK	0,00930712	17,9056	10,9098	-4,40523	1.05673e-05	7.13983e-05	yes
AT5G33251	5:12499071-12500433	WT	MT	OK	0,0498375	52,2837	10,0349	-9,8119	0	0	yes
AT4G12520	4:7421055-7421738	WT	MT	OK	0,0195111	15,8516	9,66612	-3,90043	9.60217e-05	0,000528904	yes
AT1G60020	1:22100651-22105276	WT	MT	OK	0,0118377	7,18823	9,24611	-7,50382	6.19504e-14	1.4988e-12	yes
AT5G15360	5:4987235-4989182	WT	MT	OK	0,0988273	56,4834	9,1587	-10,4392	0	0	yes

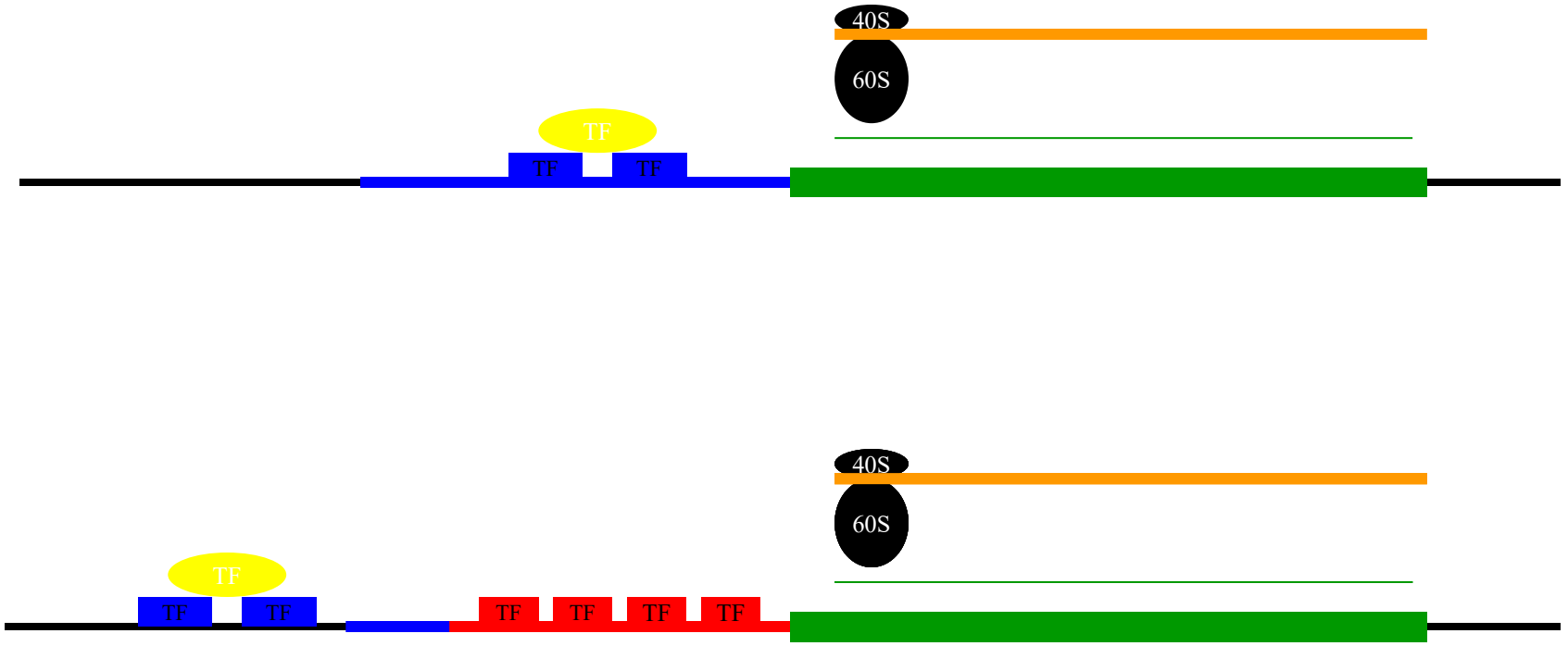
Outline

- Methods of gene expression analysis
  - Qualitative analysis of gene expression
    - Preparation of transcriptional fusion of promoter of analysed gene with a reporter gene
    - Preparation of translational fusion of the coding region of the analysed gene with reporter gene
    - Use of the data available in public databases
    - Tissue- and cell-specific gene expression analysis
    - Spatial trascriptomics
  - Quantitative analysis of gene expression
    - DNA and protein chips
    - Next generation transcriptional profiling
- Regulation of gene expression in the identification of gene function by gain-of-function approaches
  - T-DNA activation mutagenesis

Gain-of-Function Approaches

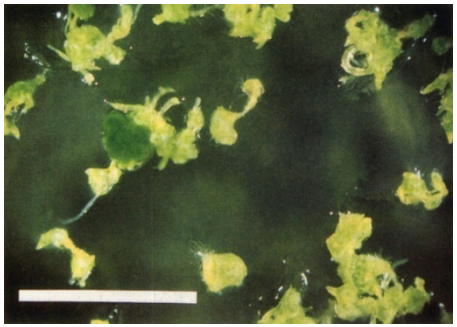
- Methods for identification of gene function using gain-of-function approaches
  - T-DNA activation mutagenesis
    - Method enabling isolation of dominant mutants by random insertion of constitutive promoter, resulting in overexpression of the gene and therefore in corresponding phenotypic changes
    - First step: preparation of mutant library prepared by tansformation of a strong constitutive promoter or enhancer
    - Next step: search of interesting phenotypes
    - Identification of the affected gene, e.g. by plasmid-rescue

Activation Mutagenesis



Isolation of *CKI1* Gene

- Tatsuo Kakimoto, *Science* 274 (1996), 982-985 *
- Isolation of the gene using activation mutagenesis



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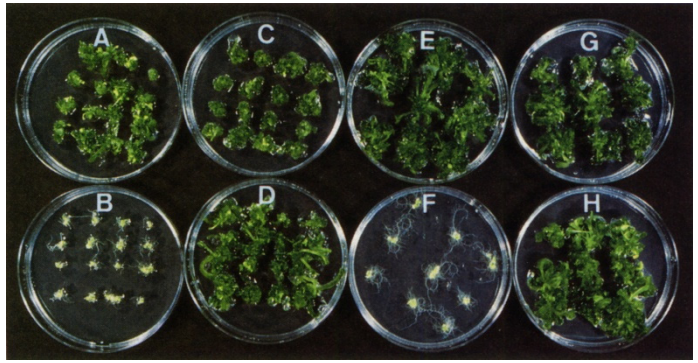
- Mutant phenotype is a phenocopy of exogenous application of cytokinins (*CKI1*, ***C****YTO****K****ININ* ***I****NDEPENDENT 1*)



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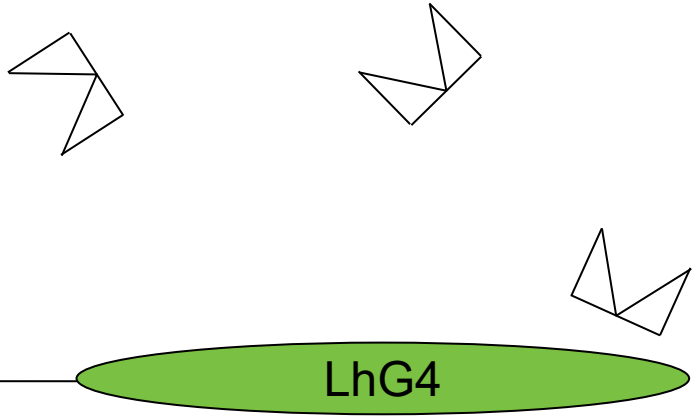
  - T-DNA activation mutagenesis
  - Ectopic expression and regulated gene expression systems

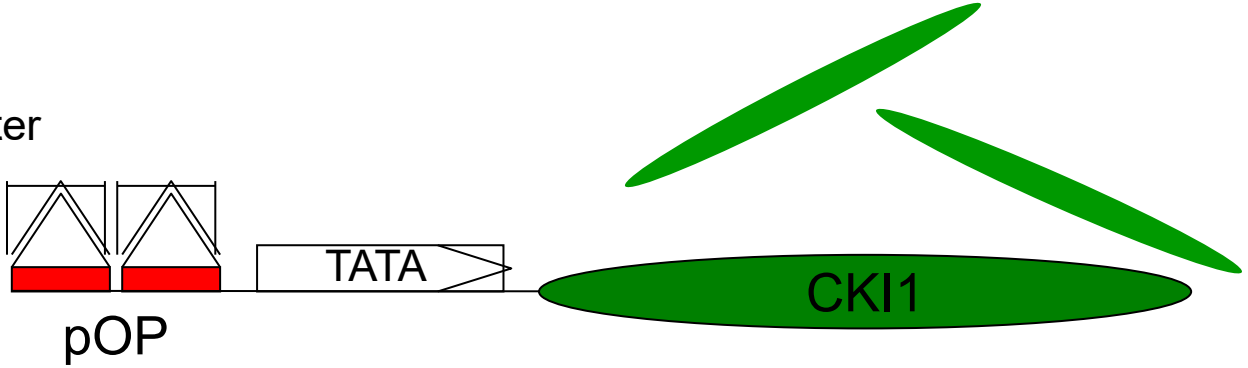
Regulated Expression Systems

activator
x

reporter

activator x reporter





pOP

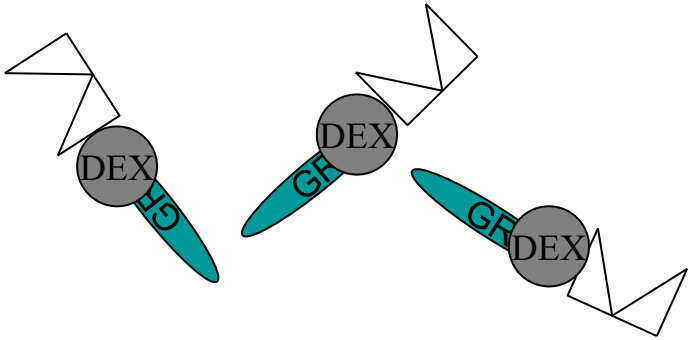
Regulated Expression Systems

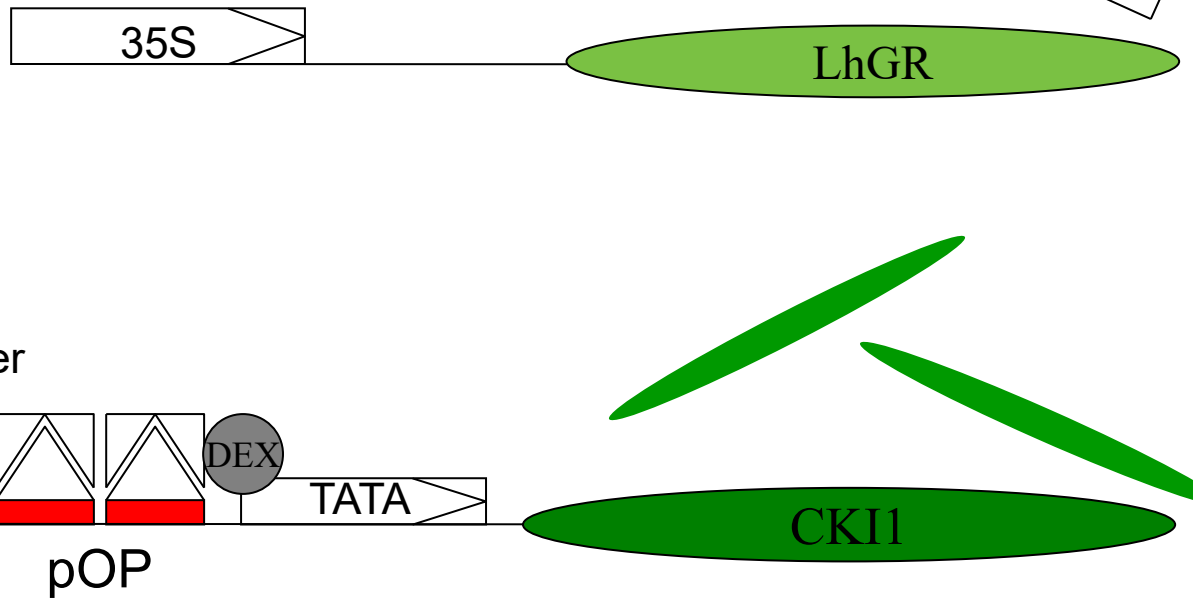
activator
x

activator x reporter

reporter

+DEX





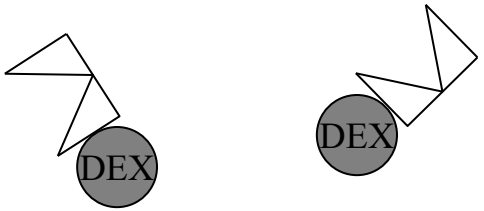
Regulated Expression Systems

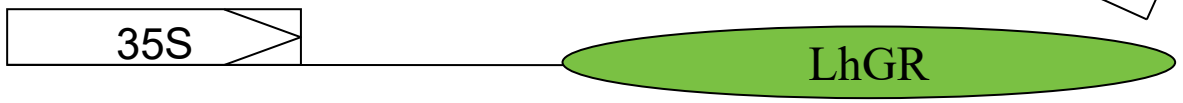
activator
X

activator x reporter

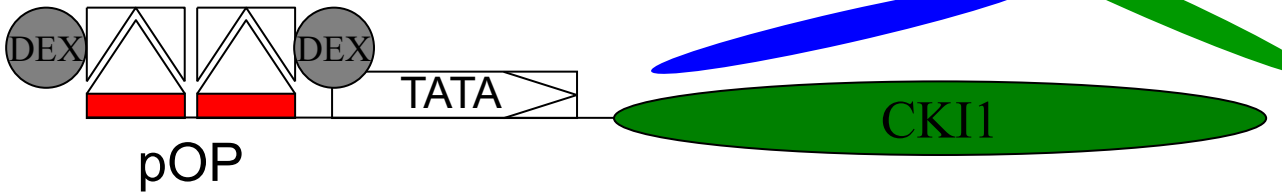
reporter

+DEX

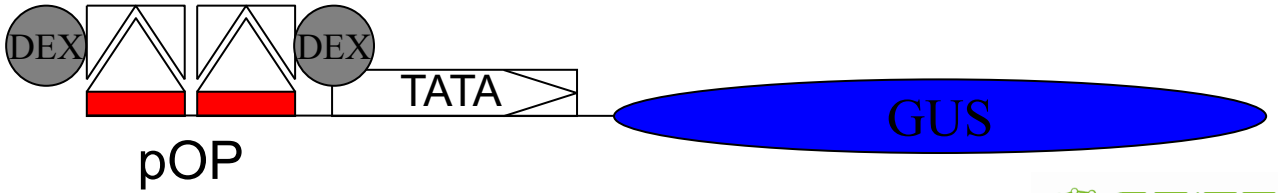




wt Col-0



4C



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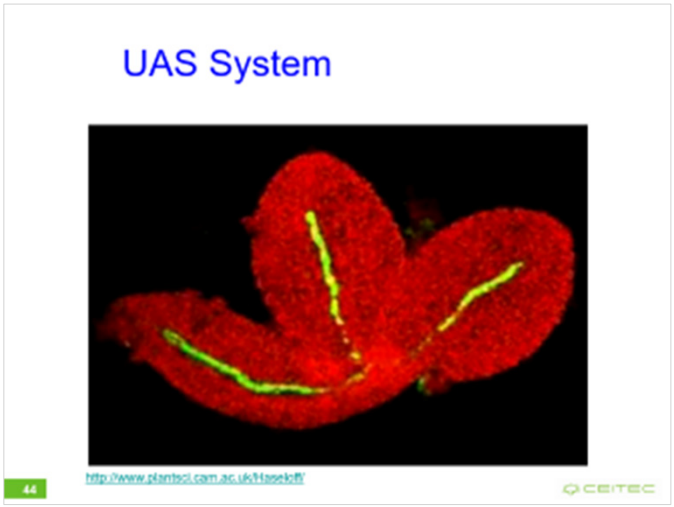
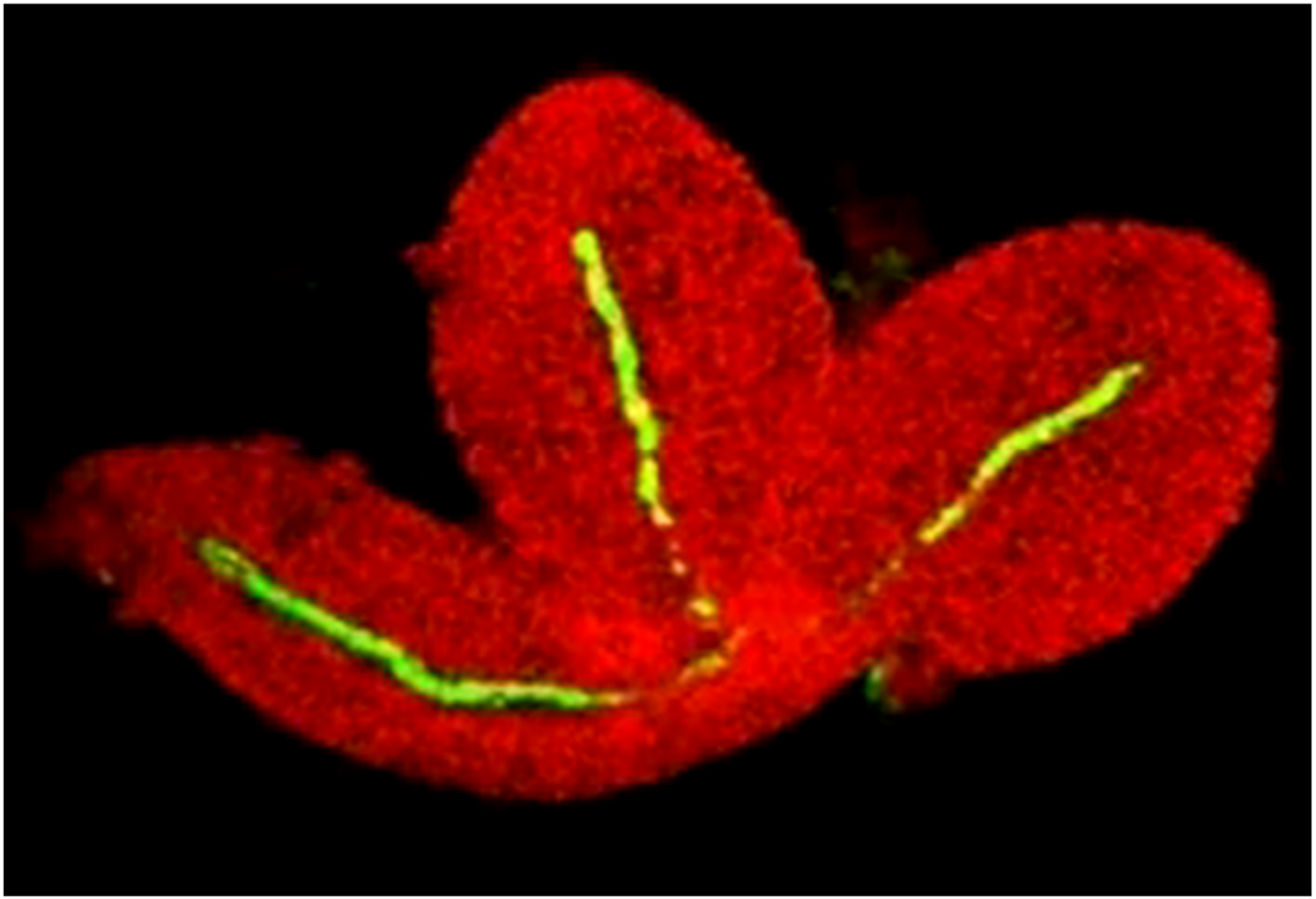
Regulated Expression Systems

- Regulated transgene expression systems
  - Allow time- or site-specific regulation of gene expression, leading to a change in phenotype and thereby identification of the natural function of the gene
    - pOP system
    - UAS system



UAS System



http://www.plantsci.cam.ac.uk/Haseloff/

Key Concepts

- Gene expression has spatiotemporal specificity
  - Analysis of spatiotemporal specificity of gene expression using
    - Transcriptional fusion of the promoter of analyzed gene with reporter gene
    - Translational fusion of coding region of teh assayed gene with reporter gene
    - Publicly accessible databases frequently with s cellular resolution
  - Quantitative analysis of gene expression
    - DNA and proteinové chips
    - Next gen transcriptional profiling
- Via regulating gene expression it is possible to identify gene function – gain of function approaches

Discussion