

CG020 Genomika

Přednáška 12

Nástroje systémové biologie

Modelové organismy, PCR a zásady navrhování primerů

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INVESTICE DO ROZVOJE VZDĚLÁVÁNÍ

Tato prezentace je spolufinancována
Evropským sociálním fondem
a státním rozpočtem České republiky

Genomika 12

■ Zdrojová literatura

- Wilt, F.H., and Hake, S. (2004). [Principles of Developmental Biology](#). (New York ; London: W. W. Norton)
- Roscoe B. Jackson Memorial Laboratory., and Green, E.L. (1966). [Biology of the laboratory mouse](#). (New York: Blakiston Division) <http://www.informatics.jax.org/greenbook/index.shtml>
- Eden, E., Navon, R., Steinfeld, I., Lipson, D., and Yakhini, Z. (2009). GOrilla: a tool for discovery and visualization of enriched GO terms in ranked gene lists. *BMC Bioinformatics* 10, 48.
- The Arabidopsis Genome Initiative. (2000). Analysis of the genome sequence of the flowering plant *Arabidopsis thaliana*. *Nature* 408, 796-815.
- Gregory, S.G., Sekhon, M., Schein, J., Zhao, S., Osoegawa, K., Scott, C.E., Evans, R.S., BurrIDGE, P.W., Cox, T.V., Fox, C.A., Hutton, R.D., Mullenger, I.R., Phillips, K.J., Smith, J., Stalker, J., Threadgold, G.J., Birney, E., Wylie, K., Chinwalla, A., Wallis, J., Hillier, L., Carter, J., Gaige, T., Jaeger, S., Kremitzki, C., Layman, D., Maas, J., McGrane, R., Mead, K., Walker, R., Jones, S., Smith, M., Asano, J., Bosdet, I., Chan, S., Chittaranjan, S., Chiu, R., Fjell, C., Fuhrmann, D., Girn, N., Gray, C., Guin, R., Hsiao, L., Krzywinski, M., Kutsche, R., Lee, S.S., Mathewson, C., McLeavy, C., Messervier, S., Ness, S., Pandoh, P., Prabhu, A.L., Saeedi, P., Smailus, D., Spence, L., Stott, J., Taylor, S., Terpstra, W., Tsai, M., Vardy, J., Wye, N., Yang, G., Shatsman, S., Ayodeji, B., Geer, K., Tsegaye, G., Shvartsbeyn, A., Gebregeorgis, E., Krol, M., Russell, D., Overton, L., Malek, J.A., Holmes, M., Heaney, M., Shetty, J., Feldblyum, T., Nierman, W.C., Catanese, J.J., Hubbard, T., Waterston, R.H., Rogers, J., de Jong, P.J., Fraser, C.M., Marra, M., McPherson, J.D., and Bentley, D.R. (2002). A physical map of the mouse genome. *Nature* 418, 743-750.
- Benitez, M. and Hejatko, J. Dynamics of cell-fate determination and patterning in the vascular bundles of *Arabidopsis thaliana* (submitted)

Osnova

- Nástroje **systemové biologie**
 - Analýza **genové ontologie**
 - **Modelování molekulárních regulačních sítí**
- Modelové organismy
 - *Mus musculus*
 - *Arabidopsis thaliana*
- Vybrané **metody molekulární biologie**
 - Příprava transgenních organismů
 - PCR
 - Design a příprava primerů (Dr. Hana Konečná)

Osnova

- Nástroje **systemové biologie**
 - Analýza **genové ontologie**
- 

Results of –omics Studies vs Biologically Relevant Conclusions

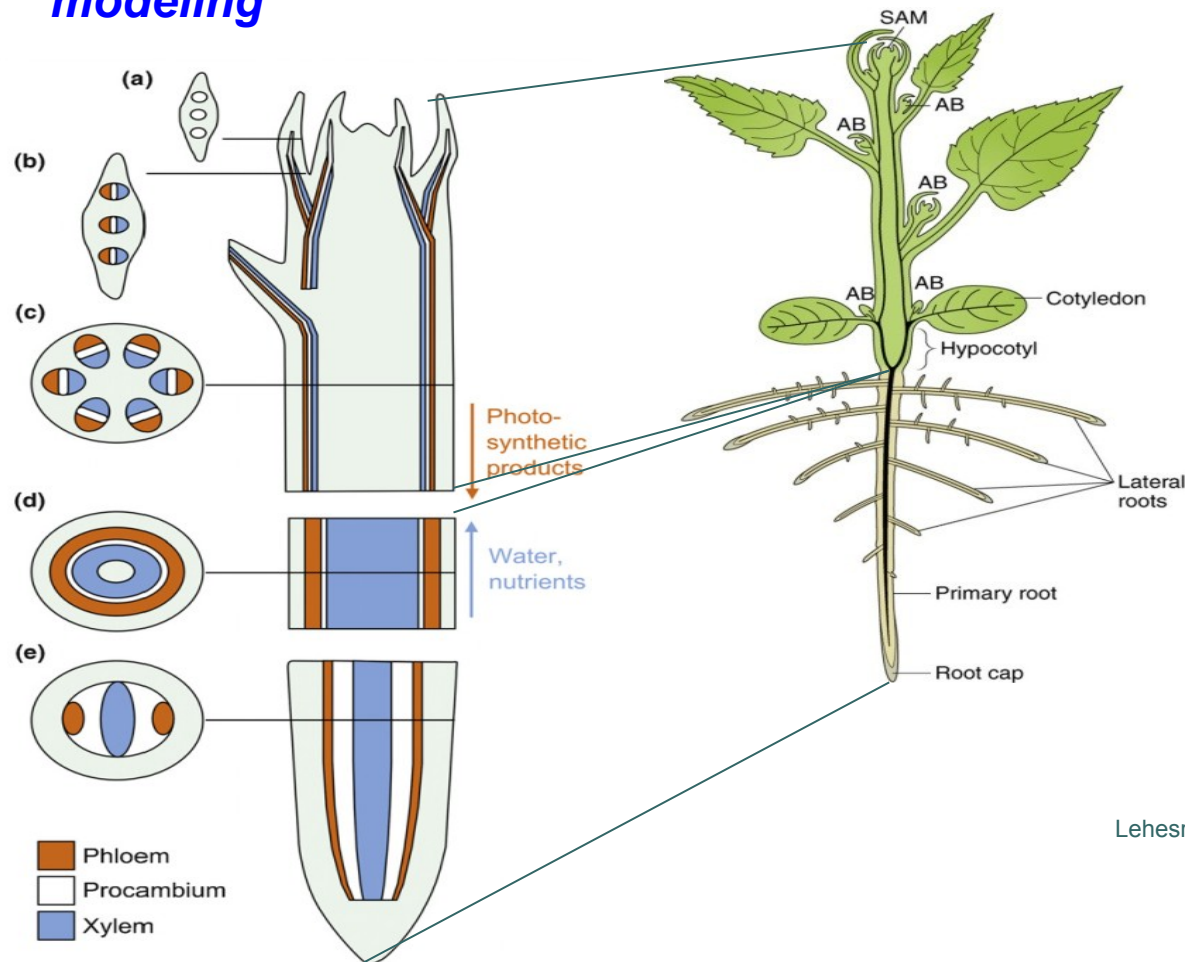
- Results of **–omics studies** are represented by **huge amount of data**, e.g. differential gene expression. But how to get any **biologically relevant conclusions**?

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	0,00039180	1 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	0,00053505	5 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	0,00028514	3 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	0,00037473	6 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

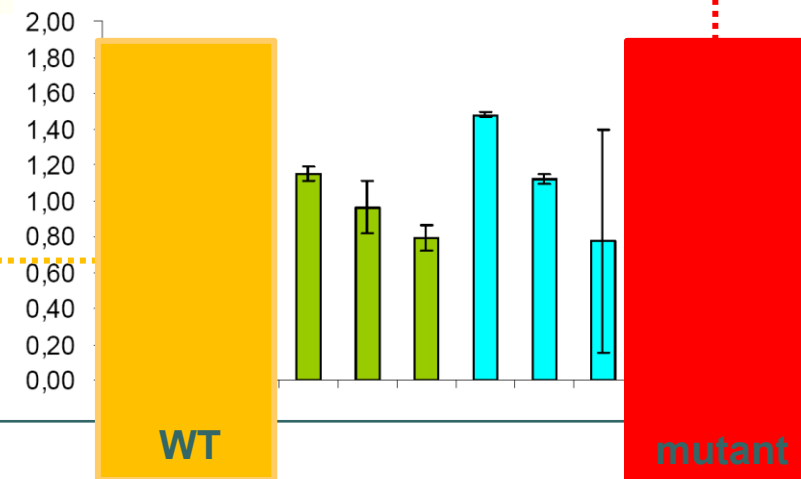
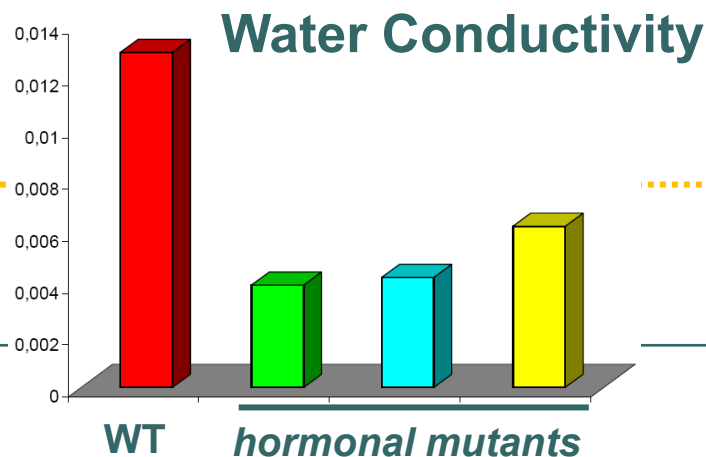
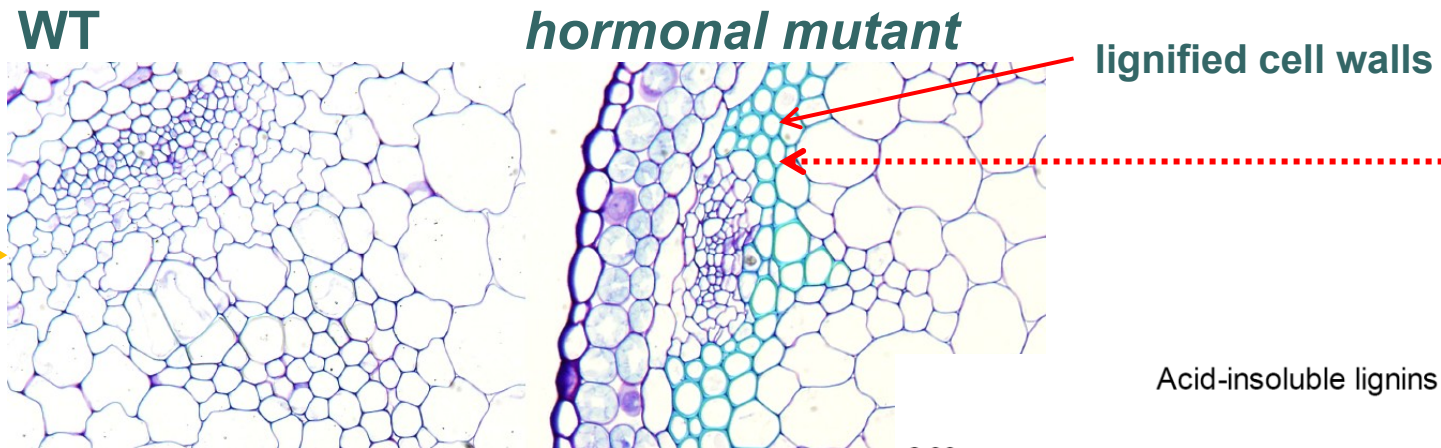
Molecular Regulatory Networks Modeling

- **Vascular tissue** as a developmental model for **GO analysis** and **MRN modeling**



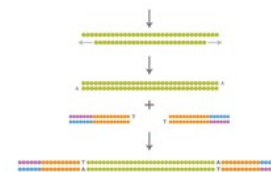
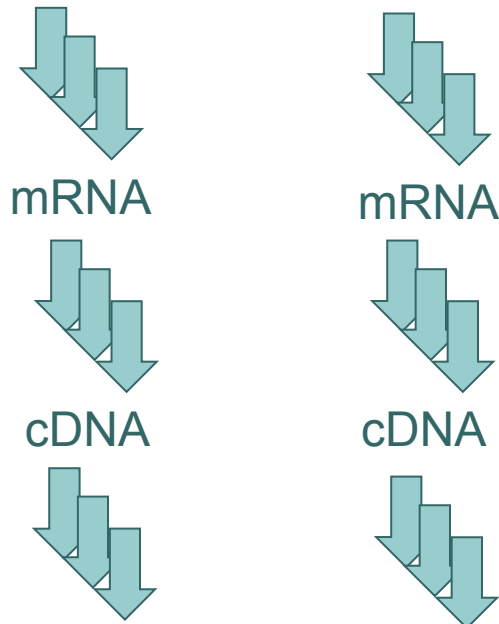
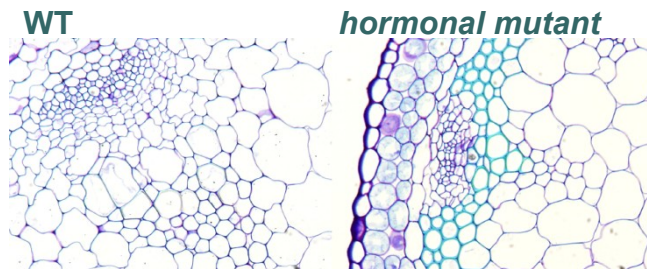
Hormonal Control Over Vascular Tissue Development

- Plant **Hormones Regulate Lignin Deposition** in Plant Cell Walls and **Xylem Water Conductivity**



Hormonal Control Over Vascular Tissue Development

- *Transcriptional profiling* via *RNA sequencing*



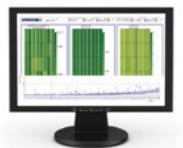
Library Preparation
~2 h [15 min hands-on (Nextera)]
< 6 h [< 3 h hands-on (TruSeq)]



Cluster Generation
~5 h (<10 min hands-on)



Sequencing by Synthesis
~1.5 to 11 days



CASAVA
2 days (30 min hands-on)

Sequencing by Illumina and
number of transcripts determination

Results of -omics Studies vs Biologically Relevant Conclusions

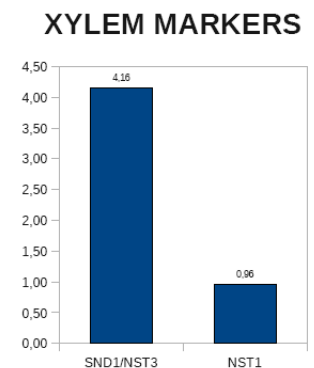
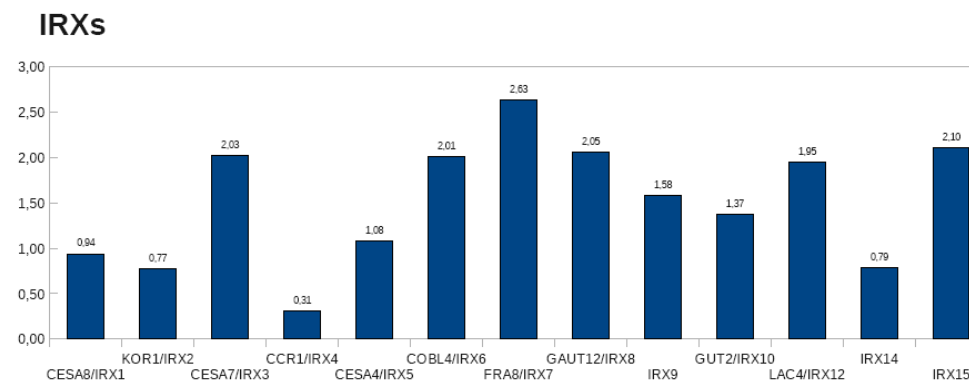
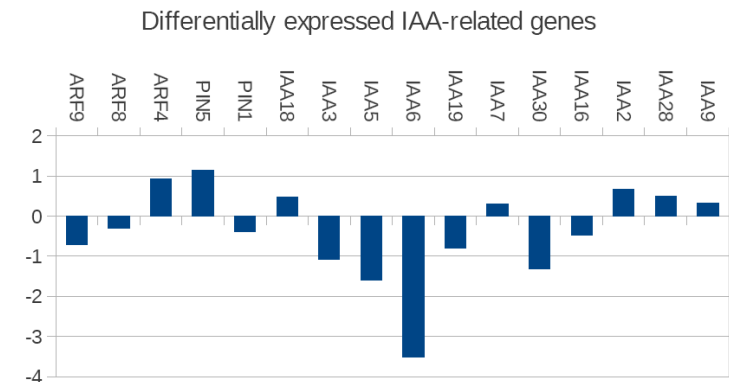
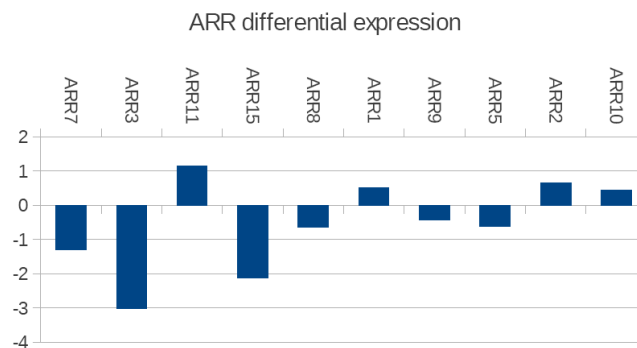
- Transcriptional profiling yielded more than **7K differentially regulated genes...**

Dii et al., unpublished

gene	locus	sample_1	sample_2	status	value_1	value_2	log2(fold_change)	test_stat	p_value	q_value	significant
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AT5G15360	5:4987235-4989182	WT	MT	OK	0,0988273	56,4834	9,1587	-10,4392	0	0	yes

Gene Ontology Analysis

- One of the possible approaches is to study **gene ontology**, i.e. previously demonstrated **association** of genes to **biological processes**



Gene Ontology Analysis

- Several tools allow **statistical evaluation** of **enrichment** for genes **associated with specific processes**

Eden et al., *BMC Bioinformatics* (2009)

GORILLA
Gene Ontology enRICHment anaLysis and visualiZation tool

GORILLA is a tool for identifying and visualizing enriched GO terms in ranked lists of genes. It can be run in one of two modes:

1. Searching for enriched GO terms that appear densely at the top of a ranked list of genes or
2. Searching for enriched GO terms in a target list of genes compared to a background list of genes.

For further details see [References](#).

[Running example](#) [Usage instructions](#) [GORilla News\(Updated December 3rd 2012\)](#) [References](#)

Step 1: Choose organism
Arabidopsis thaliana

Step 2: Choose running mode
 Single ranked list of genes Two unranked lists of genes (target and background lists)

Step 3: Paste a ranked list of gene/protein names
Names should be separated by an <ENTER>. The preferred format is gene symbol. Other supported formats are: gene and protein RefSeq, Uniprot, Unigene and Ensembl. Use [WebGestalt](#) for conversion from other identifier formats.

Or upload a file: D:\Results\2012\Mariane [Prochizet]

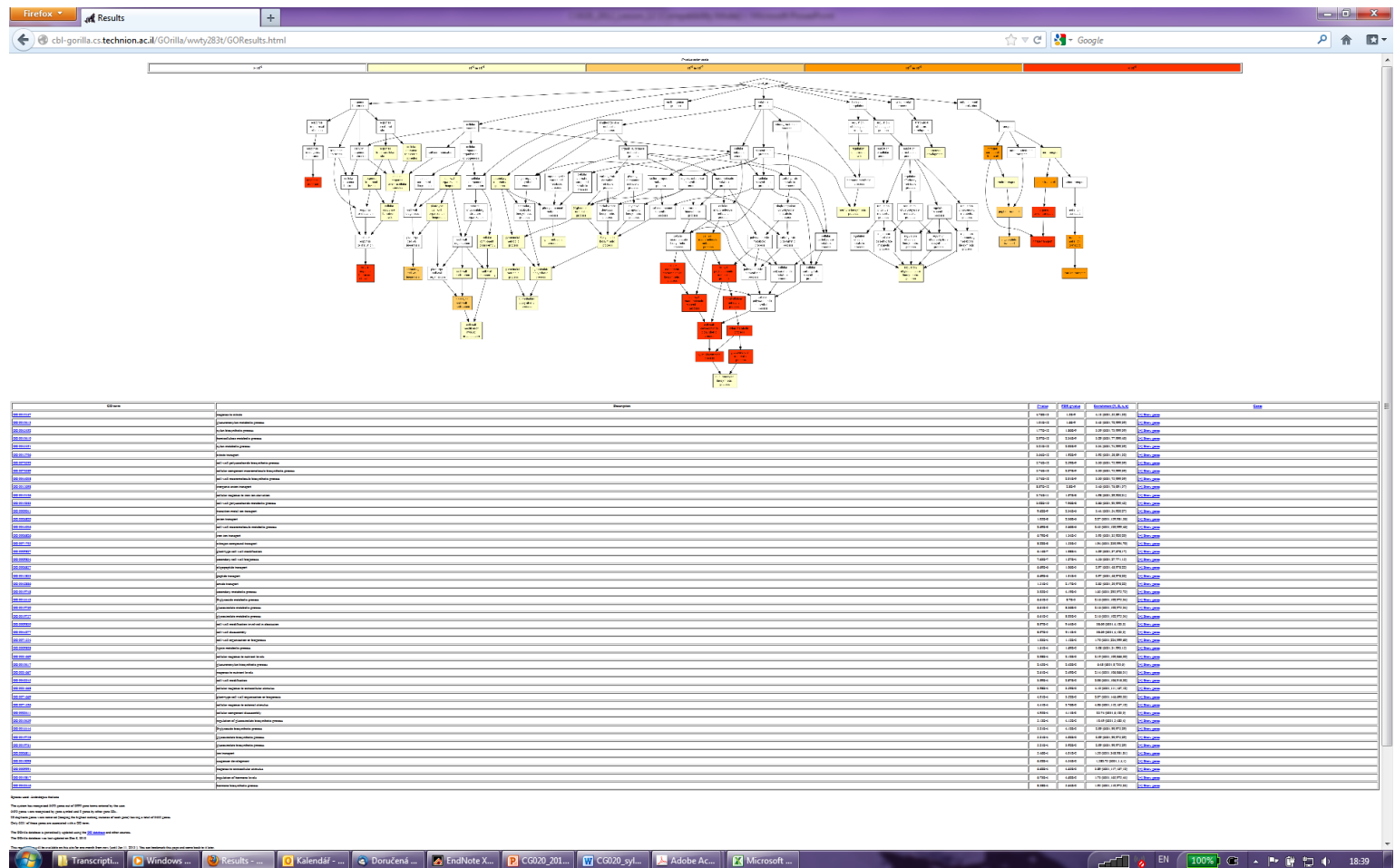
Step 4: Choose an ontology
 Process Function Component All

Search Enriched GO terms

Reset form

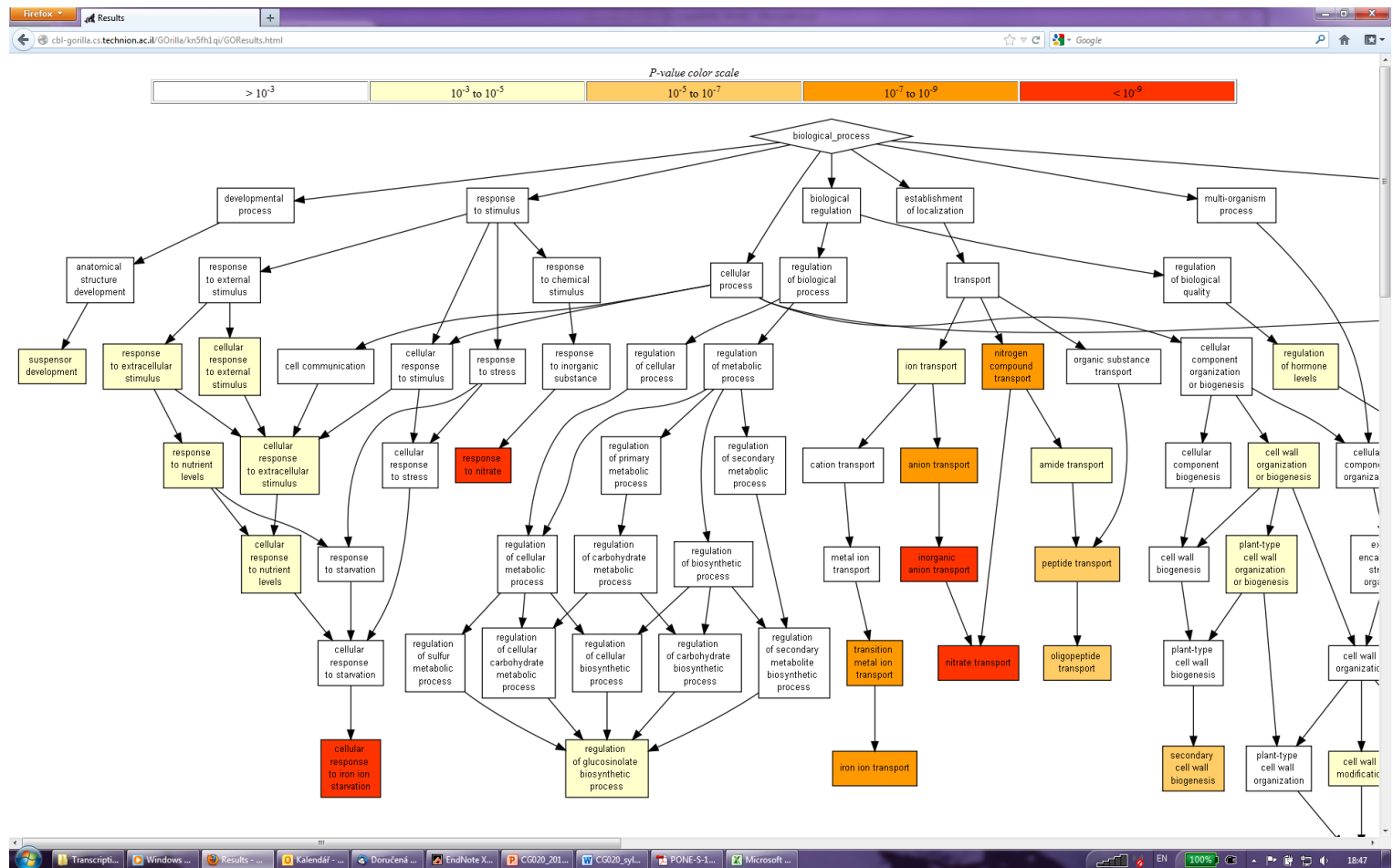
Gene Ontology Analysis

- Several tools allow **statistical evaluation** of **enrichment** for genes **associated with specific processes**



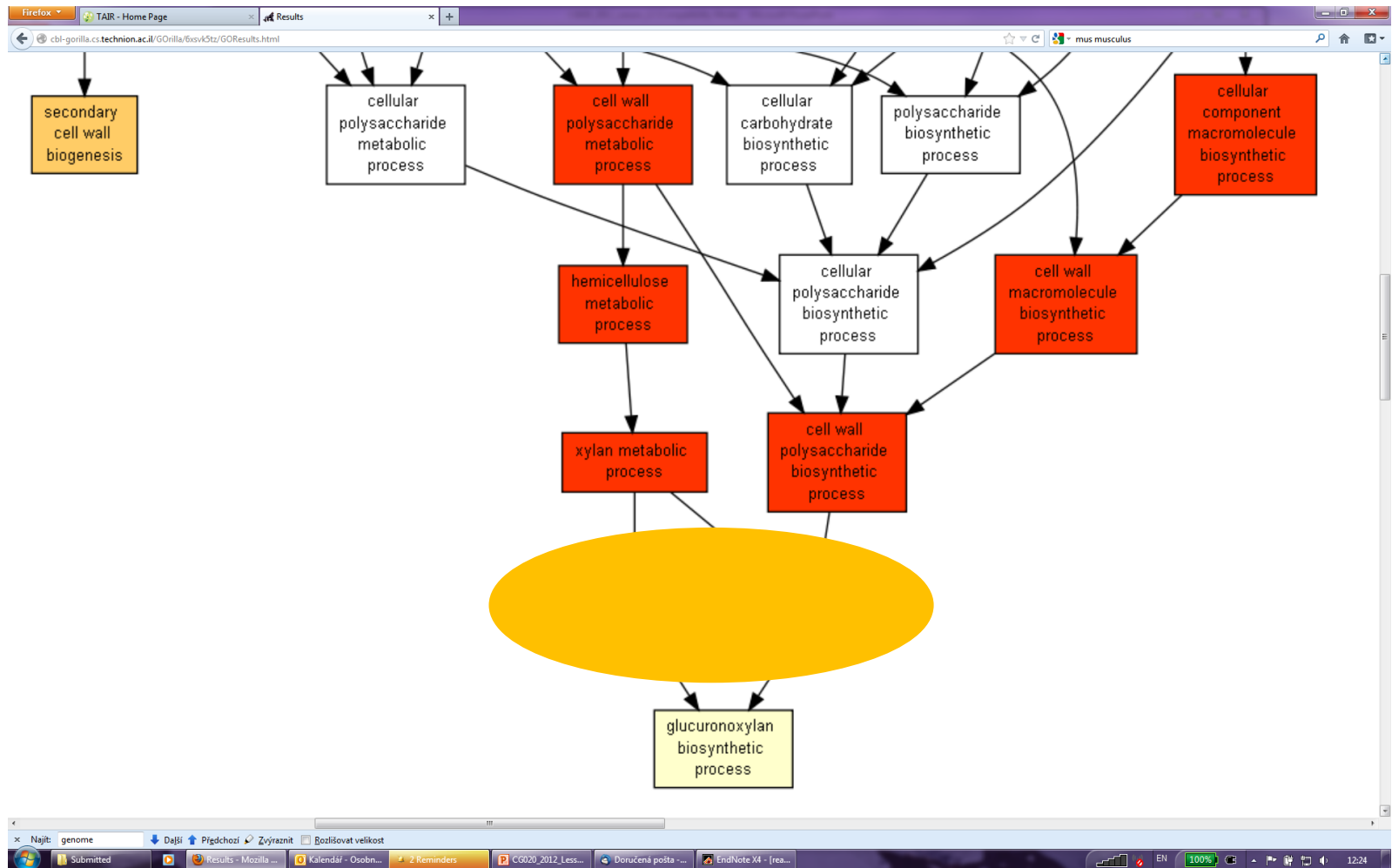
Gene Ontology Analysis

- Several tools allow **statistical evaluation** of **enrichment** for genes **associated with specific processes**



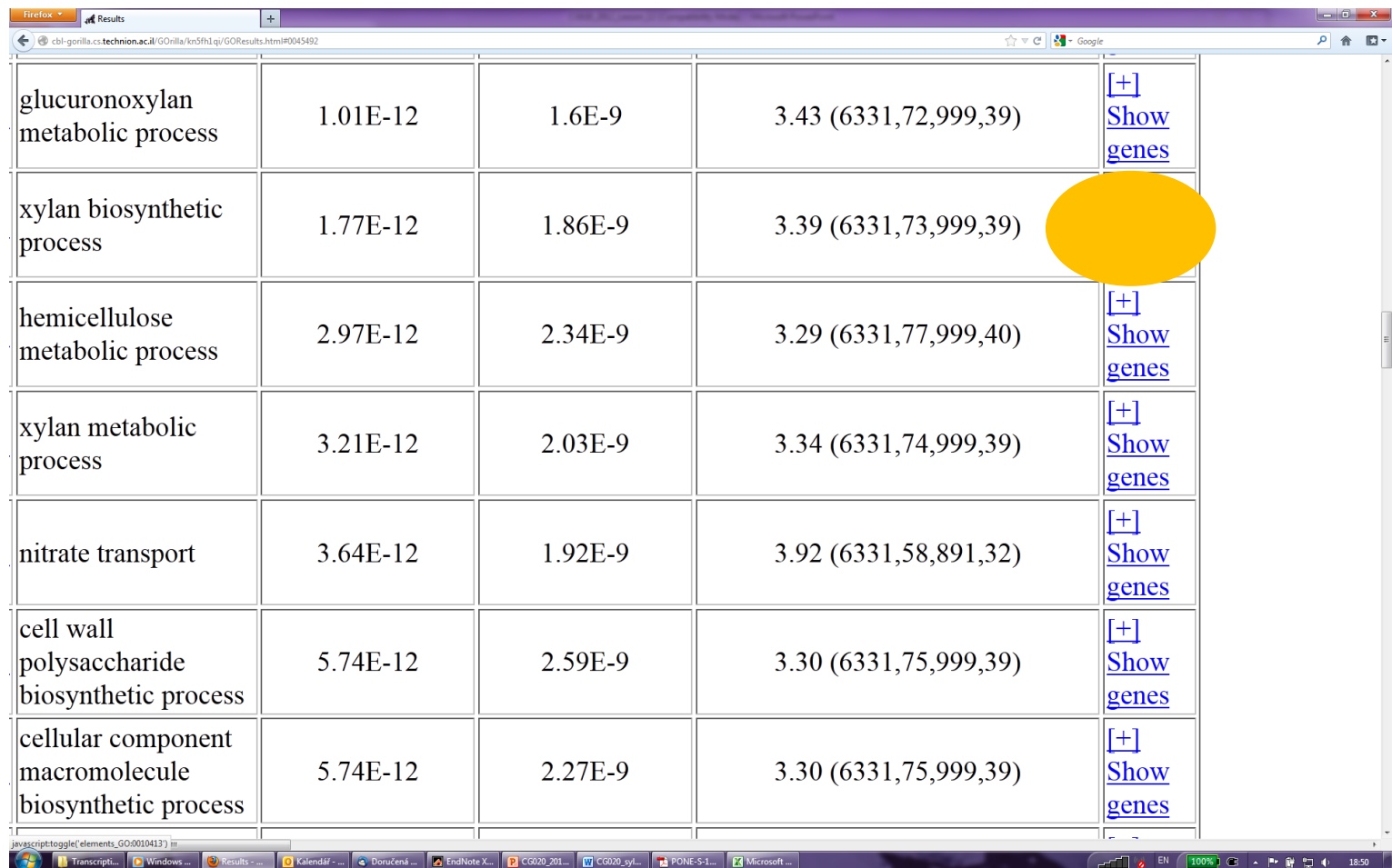
Gene Ontology Analysis

- Several tools allow **statistical evaluation** of **enrichment** for genes **associated with specific processes**



Gene Ontology Analysis

- Several tools allow **statistical evaluation** of **enrichment** for genes **associated with specific processes**



glucuronoxylan metabolic process	1.01E-12	1.6E-9	3.43 (6331,72,999,39)	[+] Show genes
xylan biosynthetic process	1.77E-12	1.86E-9	3.39 (6331,73,999,39)	[+] Show genes
hemicellulose metabolic process	2.97E-12	2.34E-9	3.29 (6331,77,999,40)	[+] Show genes
xylan metabolic process	3.21E-12	2.03E-9	3.34 (6331,74,999,39)	[+] Show genes
nitrate transport	3.64E-12	1.92E-9	3.92 (6331,58,891,32)	[+] Show genes
cell wall polysaccharide biosynthetic process	5.74E-12	2.59E-9	3.30 (6331,75,999,39)	[+] Show genes
cellular component macromolecule biosynthetic process	5.74E-12	2.27E-9	3.30 (6331,75,999,39)	[+] Show genes

Gene Ontology Analysis

- Several tools allow **statistical evaluation** of **enrichment** for genes **associated with specific processes**

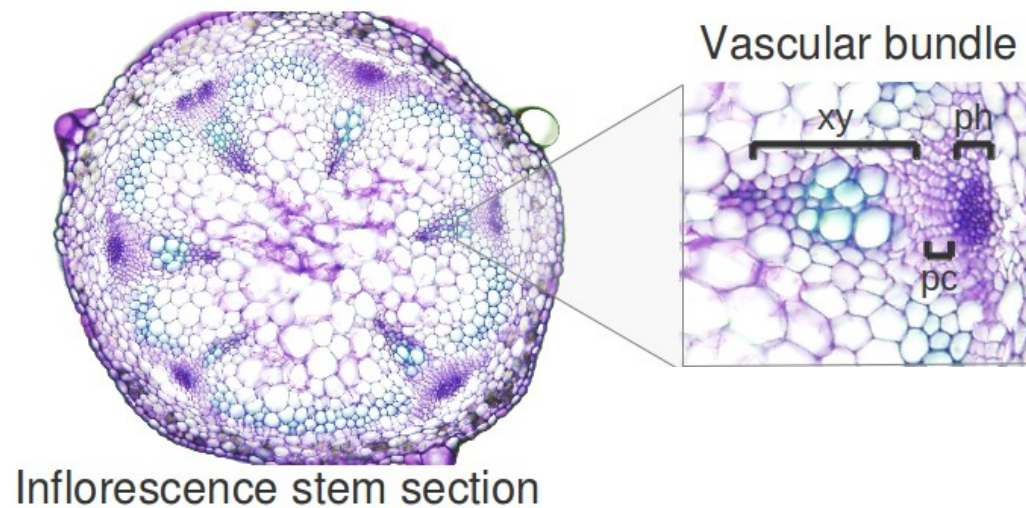
Description	P-value	FDR q-value	Enrichment (N, B, n, b)	Genes
response to nitrate	4.76E-13	1.5E-9	4.13 (6331,55,891,32)	[+] Show genes
glucuronoxytan metabolic process	1.01E-12	1.6E-9	3.43 (6331,72,999,39)	[+] Show genes
xylan biosynthetic process	1.77E-12	1.86E-9	3.39 (6331,73,999,39)	[-] Hide genes GUT2 - putative glycosyltransferase PG5IP3 - plant glycogenin-like starch initiation protein 3 FRA8 - exostosin-like protein GAUT12 - alpha-1,4-galacturonyltransferase AT4G22460 - bifunctional inhibitor/lipid-transfer protein/seed storage 2s albumin-like protein AT5G42180 - peroxidase 64 AT3G10910 - ring-h2 finger protein at172 LAC17 - laccase 17 KNAT7 - homeobox protein knotted-1-like 7 NAC012 - nac domain-containing protein 12 IRX9 - nucleotide-diphospho-sugar transferases-like protein AT1G70500 - pectin lyase-like protein CESA4 - cellulose synthase a catalytic subunit 4 [udp-forming] AT1G08340 - rho gtpase activating protein with pak-box/p21-rho-binding domain CTL2 - chitinase-like protein 2 IRX6 - cobra-like protein 4 MYB63 - myb domain protein 63 PG5IP1 - plant glycogenin-like starch initiation protein 1 AT5G46340 - putative o-acetyltransferase AT3G21710 - hypothetical protein AT2G03200 - aspartyl protease-like protein AT1G09440 - protein kinase family protein AT5G40020 - pathogenesis-related thaumatin-like protein AT3G23090 - targeting protein for xk1p2-like protein AT5G67210 - hypothetical protein AT3G56230 - btb/poz domain-containing protein AT2G31930 - hypothetical protein JP630 - putative polygalacturonase non-catalytic subunit jp630 MAP70-5 - microtubule-associated proteins 70-5 AT3G50220 - hypothetical protein AGL44 - protein agamous-like 44 IRX12 - laccase-4 NAC073 - nac domain containing protein 73 IRX3 - cellulose synthase a catalytic subunit 7 [udp-forming] AT4G27435 - hypothetical protein MYB46 - transcription factor myb46 AT1G72220 - ring-h2 finger protein at154 FRD3 - mate efflux family protein AT1G33800 - hypothetical protein
hemiacellulose metabolic process	2.07E-12	2.24E-9	3.20 (6331,77,999,40)	[+] Show genes

Osnova

- **Modelování molekulárních regulačních sítí**

Molecular Regulatory Networks Modeling

- **Vascular tissue** as a developmental model for **MRN modeling**





Molecular Regulatory Networks Modeling

- **Literature search** for published data and creating small database

Interaction	Evidence	References
A-ARRs \neg CK signaling	Double and higher order type-A ARR mutants show increased sensitivity to CK.	[27]
	Spatial patterns of A-type ARR gene expression and CK response are consistent with partially redundant function of these genes in CK signaling.	[27]
	A-type ARR decreases B-type ARR6-LUC.	[13]
	Note: In certain contexts, however, some A-ARRs appear to have effects antagonistic to other A-ARRs.	[27]
AHP6 \neg AHP	ahp6 partially recovers the mutant phenotype of the CK receptor WOL.	[9]
	Using an in vitro phosphotransfer system, it was shown that, unlike the AHPs, native AHP6 was unable to accept a phosphoryl group. Nevertheless, AHP6 is able to inhibit phosphotransfer from other AHPs to ARRs.	[9]



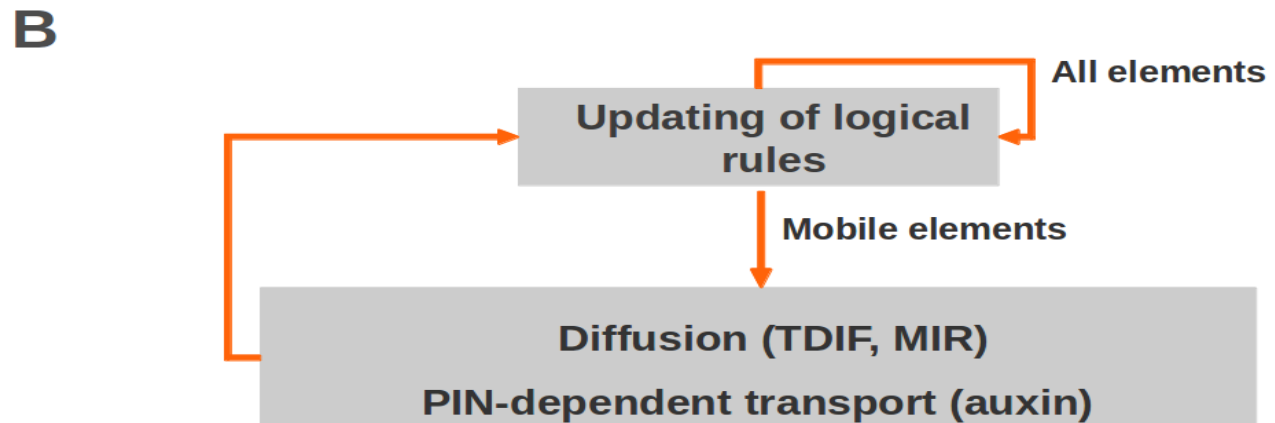
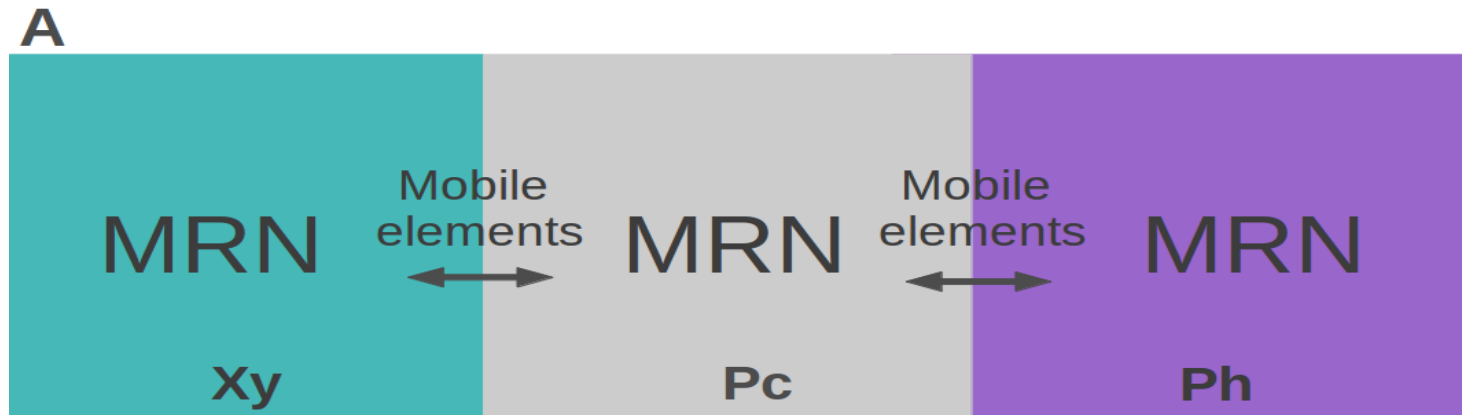
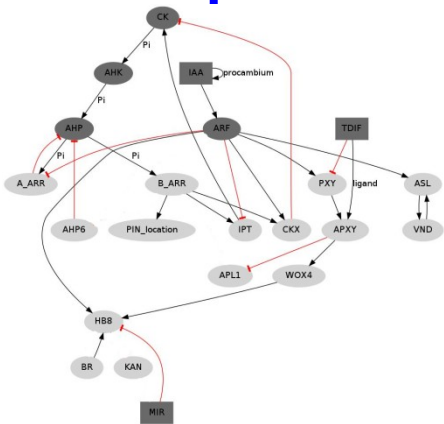
Molecular Regulatory Networks Modeling

- Formulating *logical rules* defining the *model dynamics*

Network node	Dynamical rule
CK	2 If ipt=1 and ckx=0 1 If ipt=1 and ckx=1 0 else
CKX	1 If barr>0 or arf=2 0 else
AHKs	ahk=ck
AHPs	2 If ahk=2 and ahp6=0 and aarr=0 1 If ahk=2 and (ahp6+aarr<2) 1 If ahk=1 and ahp6<1 0 else
B-Type ARRs	1 If ahp>0 0 else
A-Type ARRs	1 If arf<2 and ahp>0 0 else

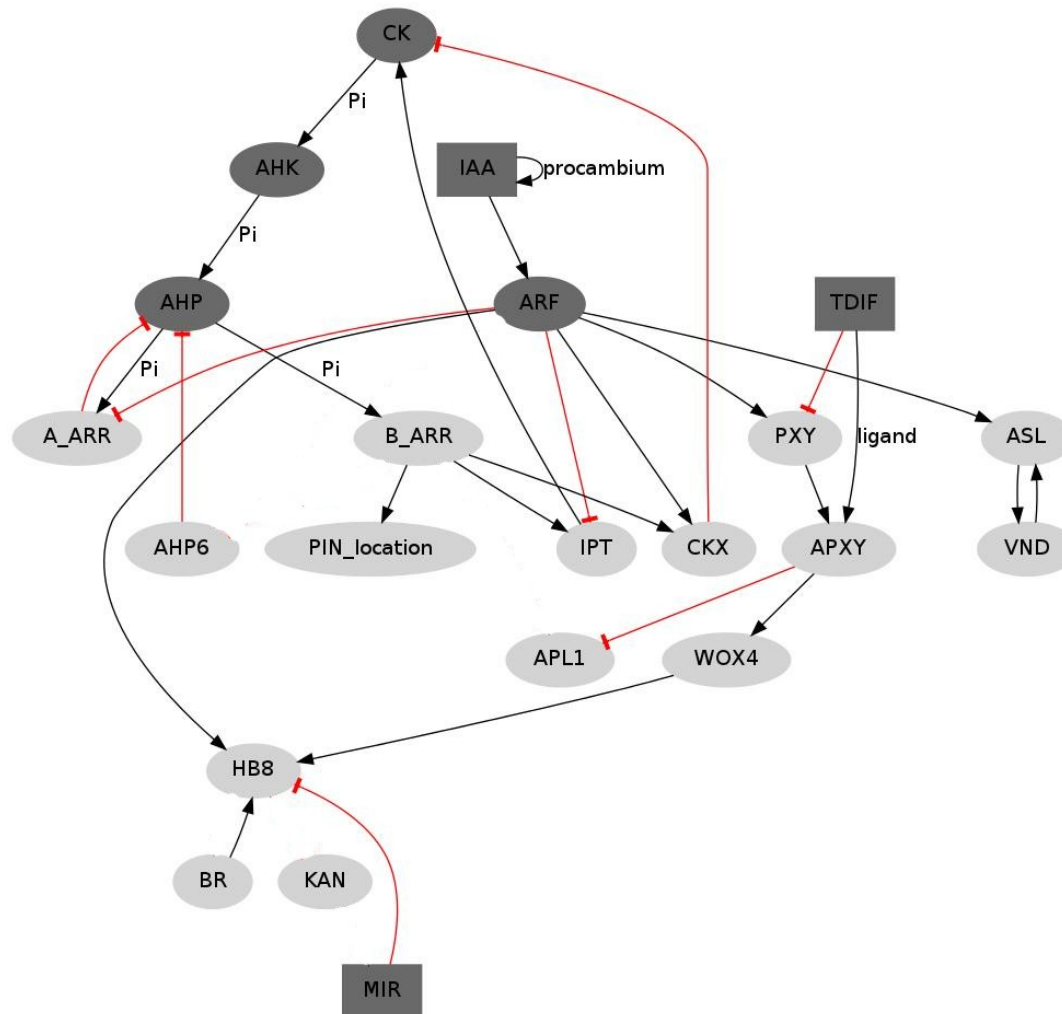
Molecular Regulatory Networks Modeling

- Specifying *mobile elements* and their model behaviour



Molecular Regulatory Networks Modeling

- Preparing the *first version* of the model and its *testing*





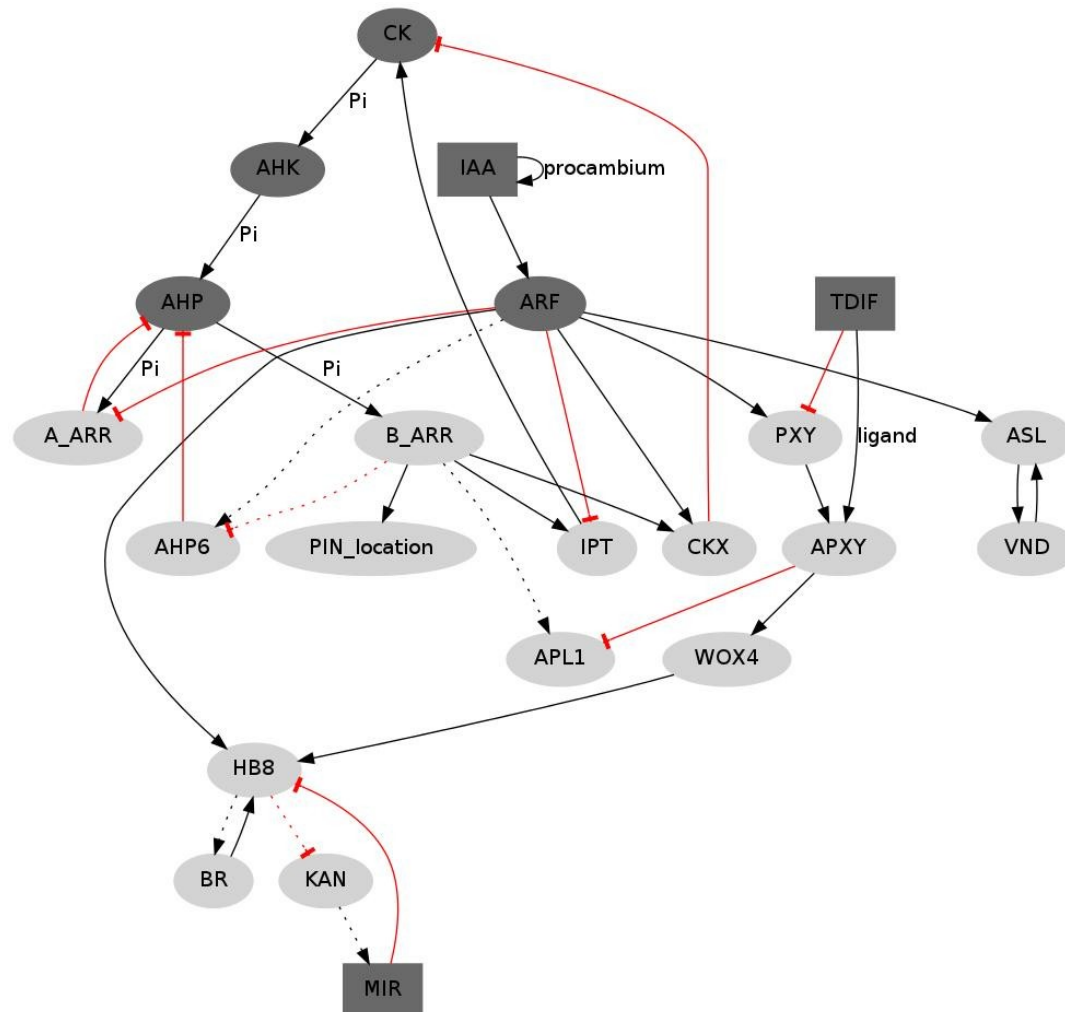
Molecular Regulatory Networks Modeling

- Specifying of missing interactions via *informed predictions*

Interaction	Evidence	References
CK → PIN7 radial localization	<p>Predicted interaction (could be direct or indirect)</p> <p>Informed by the following data:</p> <p>During the specification of root vascular cells in <i>Arabidopsis thaliana</i>, CK regulates the radial localization of PIN7.</p> <p>Expression of PIN7:GFP and PIN7::GUS is upregulated by CK with no significant influence of ethylene.</p> <p>In the root, CK signaling is required for the CK regulation of PIN1, PIN3, and PIN7. Their expression is altered in <i>wol</i>, <i>cre1</i>, <i>ahk3</i> and <i>ahp6</i> mutants.</p>	<p>[18]</p> <p>[18,20]</p> <p>[19]</p>
CK→ APL	<p>Predicted interaction (could be direct or indirect)</p> <p>Consistent with the fact that APL overexpression prevents or delays xylem cell differentiation, as does CKs.</p> <p>Partially supported by microarray data and phloem-specific expression patterns of CK response factors.</p>	<p>[21]</p> <p>(TAIR, ExpressionSet:1005823559, [22])</p>

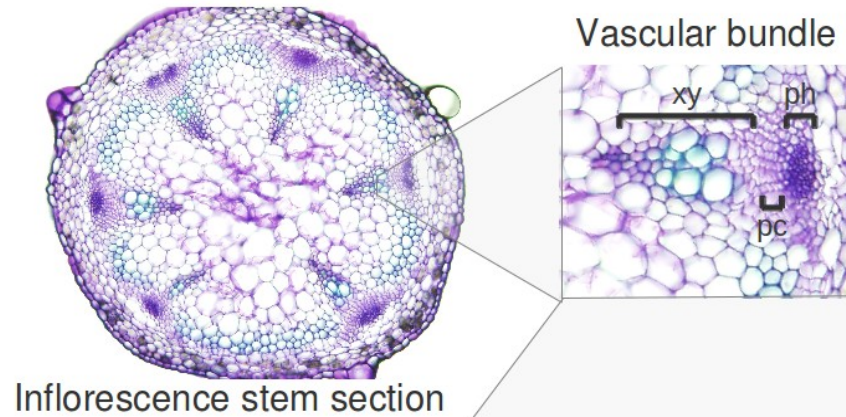
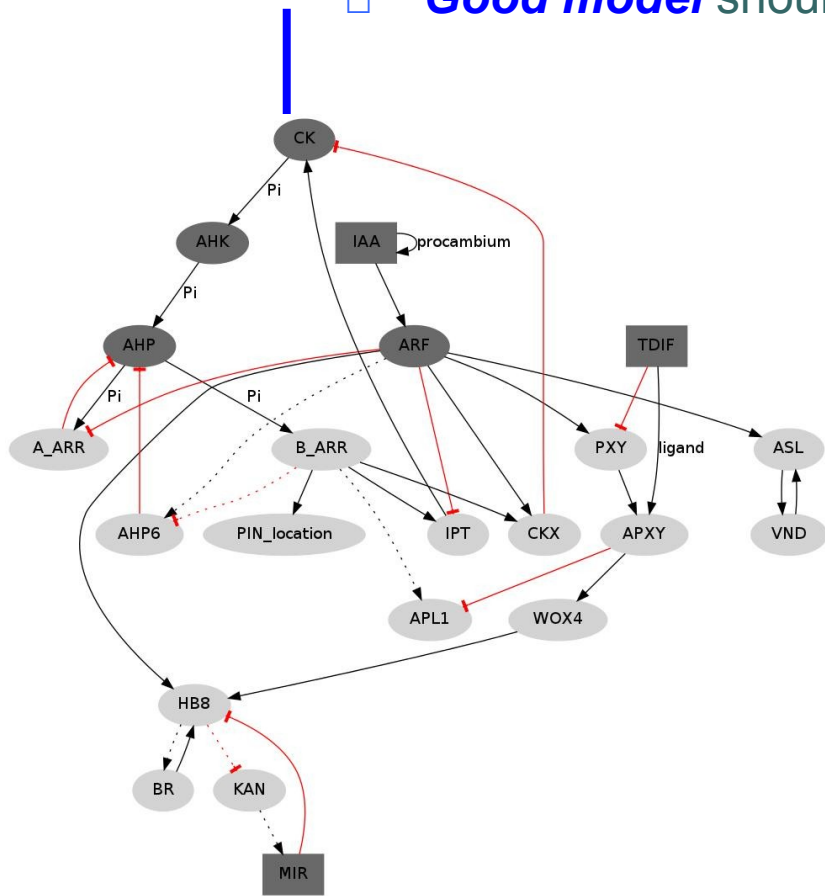
Molecular Regulatory Networks Modeling

- Preparing the *next version* of the model and its *testing*



Molecular Regulatory Networks Modeling

- **Good model** should be able to **simulate reality**

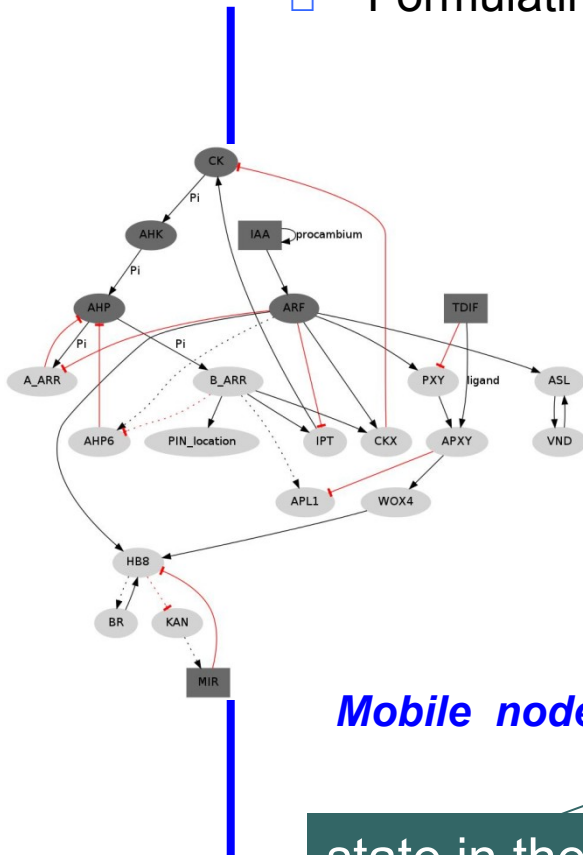


Xylem (xy)	Procambium (pc)	Phloem (ph)
VND ASL	WOX4	APL

Benitez and Hejatko, *submitted*

Molecular Regulatory Networks Modeling

- Formulating **equations** describing the **relationships** in the model



logical rule function

state in the time t

$$\text{Static nodes: } g_n(t+1) = F_n(g_{n1}(t), g_{n2}(t), \dots, g_{nk}(t))$$

state in the time $t+1$

Amount of TDIF or MIR165 in cell i

$$\text{Mobile nodes: } g_{(t+1)T[i]} = H(g_{(t)[i]} + D(g_{(t)[i+1]} + g_{(t)[i-1]} - N(g_{(t)[i]})) - b)$$

state in the time $t+1$

constant corresponding to a degradation term

proportion of movable element

Molecular Regulatory Networks Modeling

- **Good model** should be able to **simulate reality**

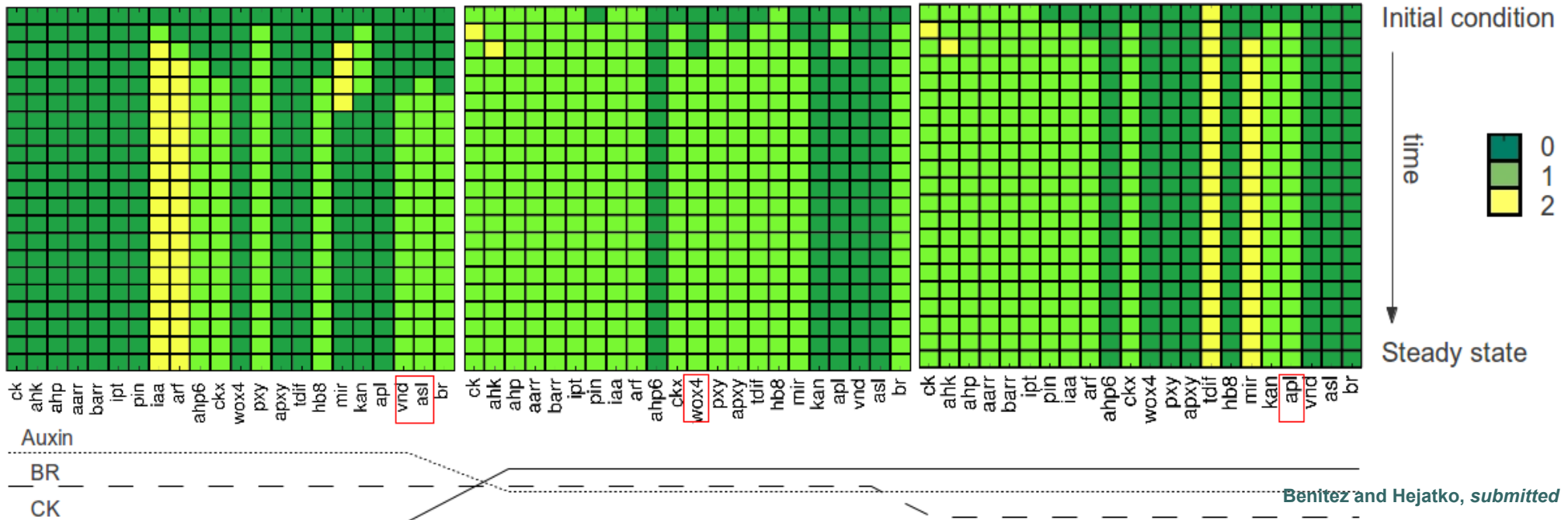
Static nodes: $g_n(t+1) = F_n(g_{n1}(t), g_{n2}(t), \dots, g_{nk}(t))$

Mobile nodes: $g_{(t+1)T [i]} = H(g_{(t) [i]} + D(g_{(t) [i+1]} + g_{(t) [i-1]} - N(g_{(t) [i]})) - b$

Xylem

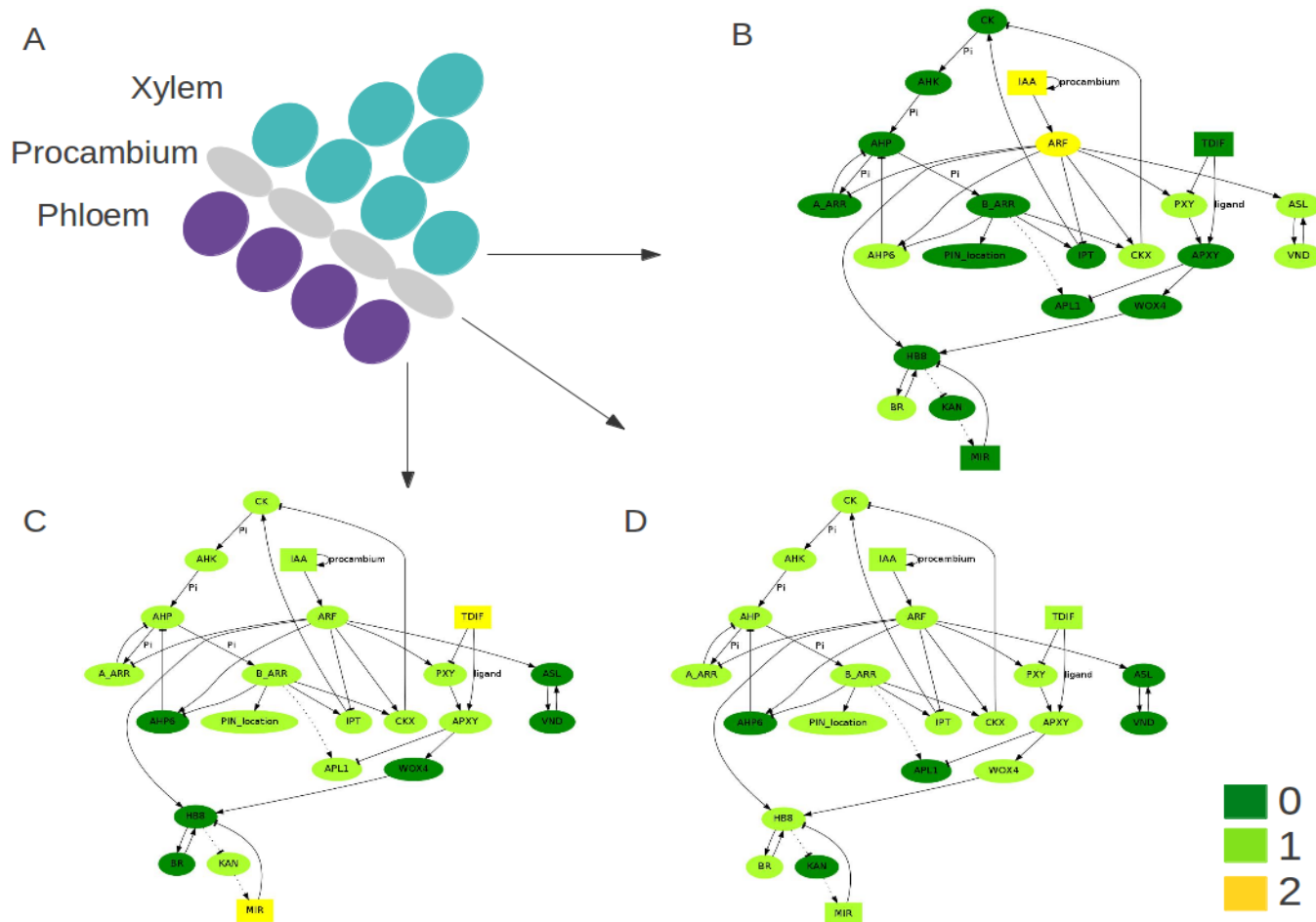
Procambium

Phloem



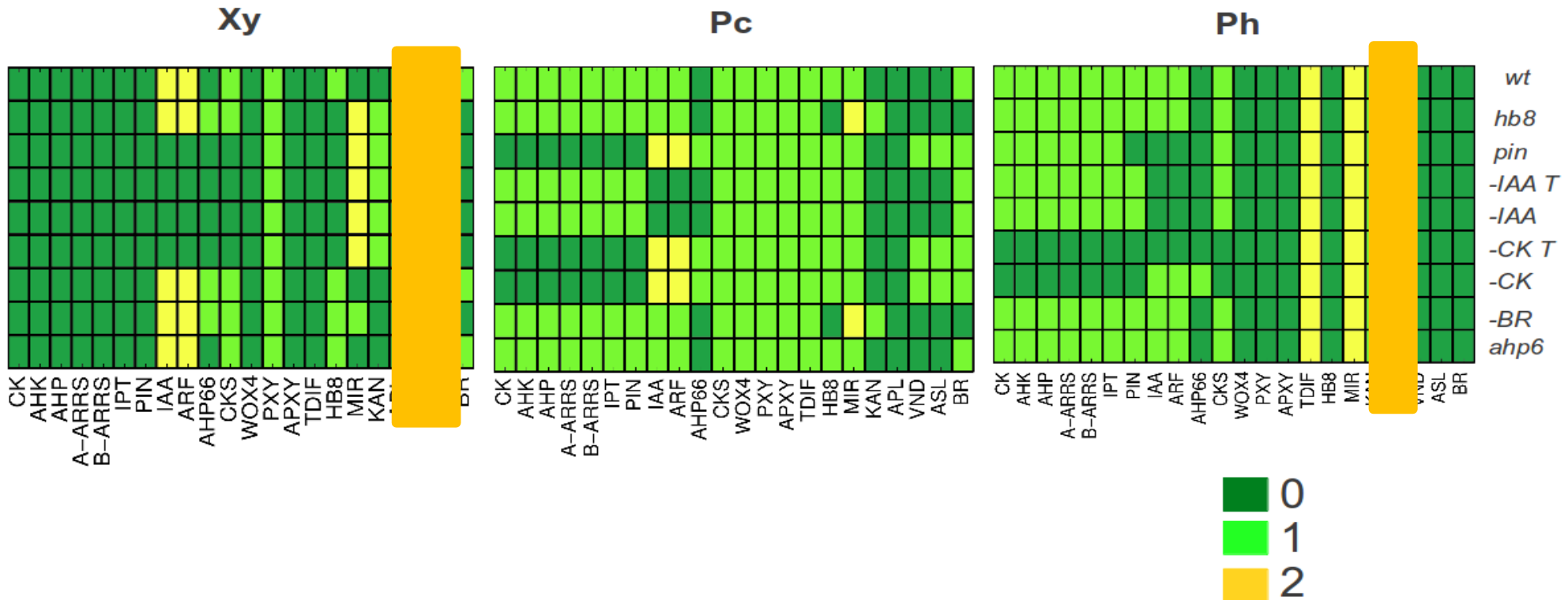
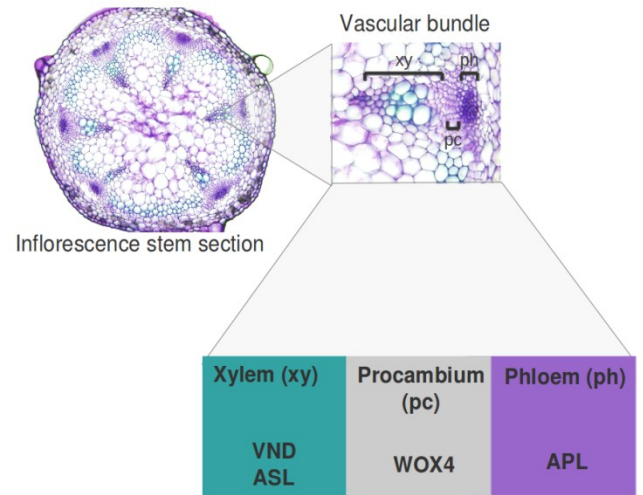
Molecular Regulatory Networks Modeling

- The **good model** should be able to **simulate reality**



Molecular Regulatory Networks Modeling

□ Simulation of *mutants*





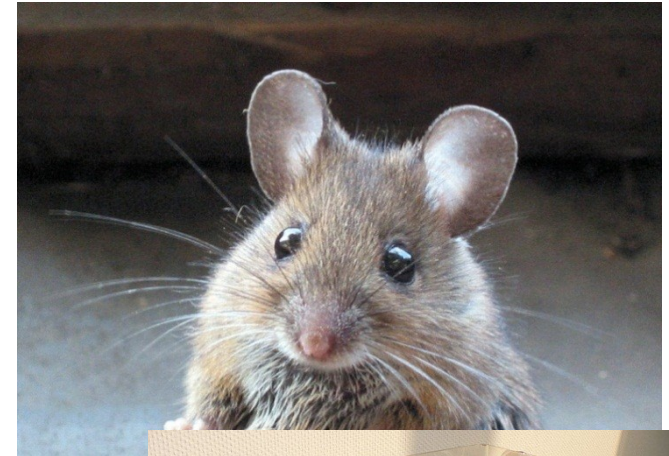
Osnova

- 
- Modelové organismy
 - *Mus musculus*

Mus musculus

myš domácí, house mouse

- malé nároky na chovnou plochu
- relativně velké množství mláďat (3-14, v průměru 6-8)
- velikost genomu se blíží velikosti genomu člověka (cca 3000 Mbp), podobně jako počet genů (cca 24K)
- 20 chromozomů (19+1)
- vhodná pro široké spektrum fyziologických experimentů (anatomicky i fyziologicky podobná člověku)
- možno poměrně snadno získávat K.O. mutanty i transgenní linie



Mus musculus

myš domácí, house mouse

- Genom známý od roku 2002 (<http://www.ncbi.nlm.nih.gov/projects/genome/assembly/grc/mouse/>)

Genome Reference Consortium

Mouse Genome Overview

Information concerning the continuing improvement of the mouse genome.

The GRC is working hard to provide the best possible reference assembly for mouse. We do this by both generating multiple representations ([alternate loci](#)) for regions that are too complex to be represented by a single path. Additionally, we are releasing regional fixes known as [patches](#). This allows users who are interested in a specific locus to get an improved representation without affecting users who need chromosome coordinate stability.

Getting Data
GRCm38.p1 (Latest minor release from the GRC): [FTP](#)
GRCm38 (Latest Major release from the GRC): [FTP](#)
Alignments of MGSCv37 to GRCm38: [GRC FTP](#)
Information on regions under review: [FTP](#)
Annotated clone assembly problems: [FTP](#)

Next assembly update
The next assembly update (patch release 2) will be a minor update (only patches) and will happen in March 2013.

GRC Blog

[The GRC and the 10th International Zebrafish Genetics and Development Meeting \(June 20-24, 2012 - Madison, Wisconsin\)](#) 26 Jul 2012

[Hidden assembly problems exposed](#) 06 Jul 2012

The human reference genome GRCh37 represents th... [see all](#)

Recently Resolved Mouse Issues

Mouse (MG-4136) Nov16, 2012
Inversion found in the assembly has been corrected in component AL611930.25.

Mouse (MG-4212) Nov16, 2012
There is an assembly gap between AC132090.3 and AC239617.4, originally described in MG-3584, and then updated in MG-4143. This duplicate Jira issue is being used to manage an update at this gap that is being considered for patch release. [see all](#)

References

Whole Genome Papers
[The Mouse Genome WGS Assembly](#)
[The Mouse Genome: Clone based assembly](#)

GRCm38.p1 GRCm38 MGSCv37

GRCm38.p1
Release date: 23 Aug 2012
Release type: minor

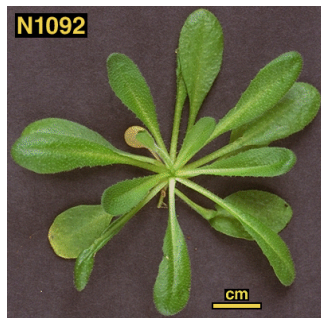
Osnova

- Nástroje **systemové biologie**
 - Analýza **genové ontologie**
 - **Modelování molekulárních regulačních sítí**
- Modelové organismy
 - *Mus musculus*
 - *Arabidopsis thaliana*

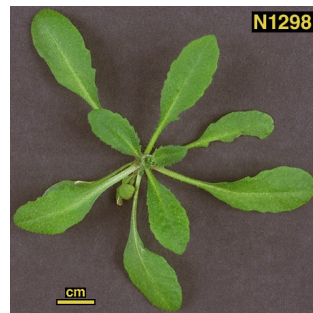
Arabidopsis thaliana

huseníček polní, mouse-ear cress

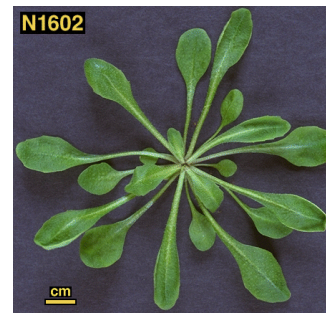
- malé nároky na kultivační plochu
- velké množství semen (20.000/rostlinu a více)
- malý a kompaktní genom, (125 MBp, cca 25.000 genů, prům. velikost 3 kb)
- 5 chromozomů
- vhodná pro široké spektrum fyziologických experimentů
- velká přirozená variabilita (cca 750 ekotypů (Nottingham Arabidopsis Seed Stock Centre))



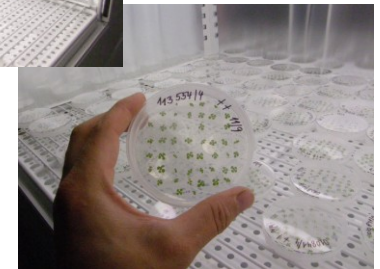
Columbia 0



Landsberg 0



Wassilewskija 0



Arabidopsis thaliana

huseníček polní, mouse-ear cress

- Genom známý od roku 2000 (<http://www.arabidopsis.org/>)

The screenshot shows the TAIR website homepage. The browser window title is "TAIR - Home Page" and the address bar shows "www.arabidopsis.org". The website features a navigation menu with links for Home, Help, Contact, About Us, and Login/Register. Below the navigation is a search bar and a menu with options: Search, Browse, Tools, Portals, Download, Submit, News, and ABRC Stocks. The main content area is titled "The Arabidopsis Information Resource" and contains several sections:

- Breaking News**: Includes links to "Subscribe to news feed", "Follow our Twitter feed", and "Join our Facebook group".
- New Set of Confirmed T-DNA Lines Available [November 28, 2012]**: Announces the fourth one-allele set of confirmed T-DNA lines representing 3,263 new loci now available for ordering as CS27944.
- New from ABRC Education and Outreach! [October 31, 2012]**: Announces a re-designed Education and Outreach website at <http://abrcoutreach.osu.edu>, highlighting the "donation of education modules" and "online evaluation of education kits".
- 2012 MASC Report Now Available [July 11, 2012]**: Promotes the latest report from the Multinational Arabidopsis Steering Committee.
- New Protein Chip and Cell Cultures at ABRC [May 9, 2012]**: Announces a new protein chip (AtProteinChip 2) developed by M. Snyder and S.P. Dinesh-Kumar.

A central banner features a laptop with a "TAIR SUBMISSION" form, a pipette, and a flower. The form includes fields for "Article #", "Gene", "Locus", "GeneFunction", and "Method". Arrows labeled "SUBMIT PAPER" and "SUBMIT DATA" point to the form. Below the banner, it says "Click here to try our new online submission form" and lists submission types: "and submit the molecular function (e.g. protein kinase), biological process (e.g. seed development), localization (e.g. plasma membrane) or interacting partner of your favorite gene".

The browser's status bar at the bottom shows the search term "genome" and various system icons, including a 100% battery level and the time 12:18.

Osnova

- Vybrané **metody molekulární biologie**
 - Příprava transgenních organismů

1 Isolate single cells from a blastocyst of black mouse parents.

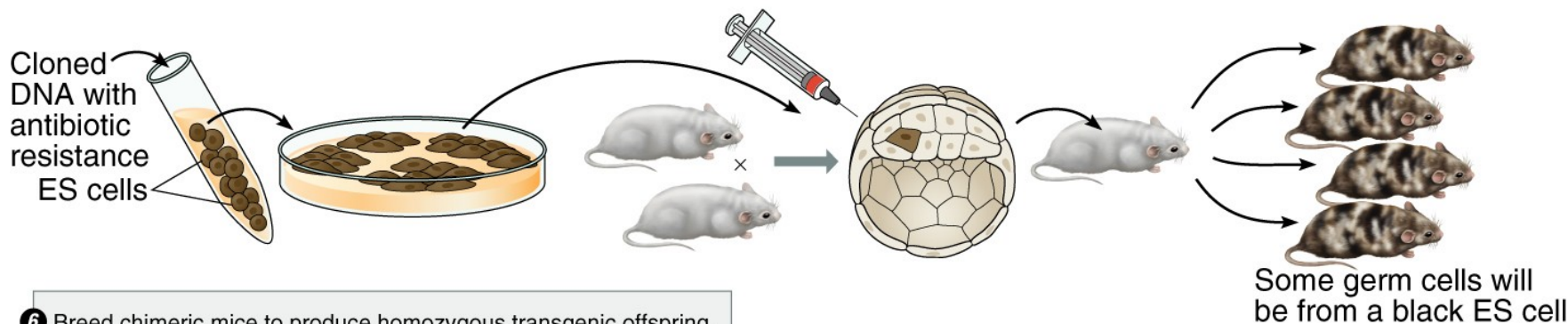
2 Pick a single cell from the first culture and grow a clone of this cell in cultures for 15 mitotic generations. Repeat every 10 days for a year. These are ES (embryonic stem) cells.



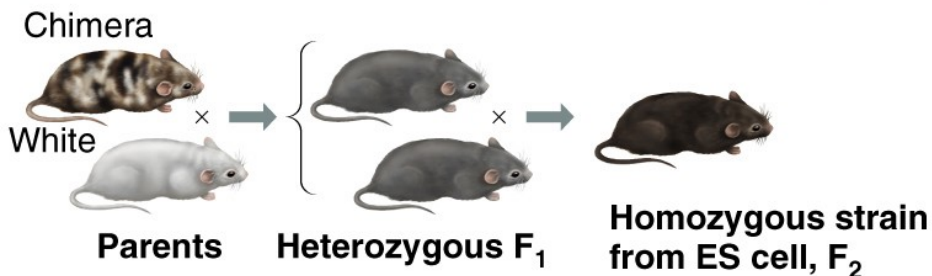
3 Transform stem cells with a cloned gene. Include an antibiotic resistance marker in the cloned gene. Culture ES cells in presence of antibiotic to select transformants.

4 Inject transformed ES cells into blastocysts from white mice. Implant into surrogate mother.

5 Resultant pups will be chimeras of ES cells from black parent and white parent. Black ES cells contain transgene.



6 Breed chimeric mice to produce homozygous transgenic offspring.



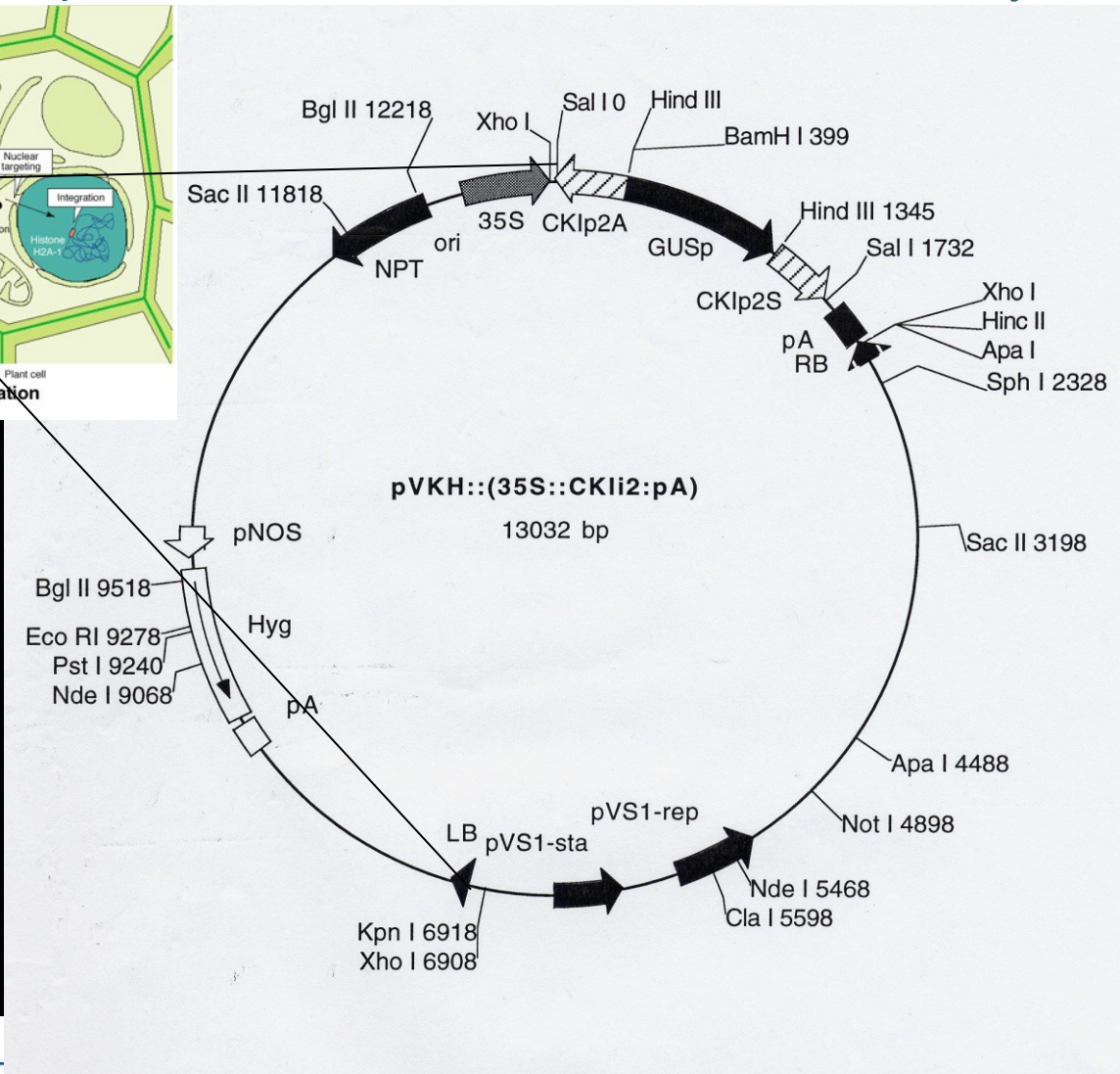
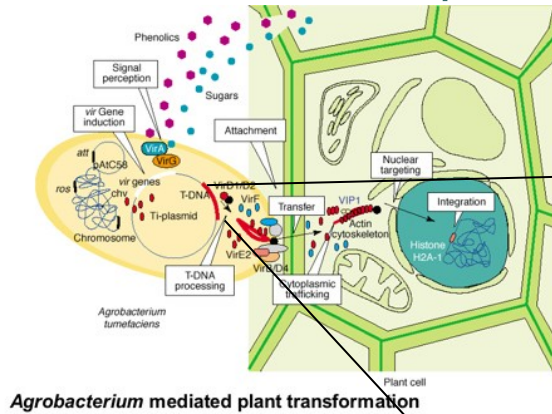
Transformace *Arabidopsis* prostřednictvím *Agrobacteria tumefaciens*



Crown gall of raspberry caused by *Agrobacterium tumefaciens*.

Transformace *Arabidopsis* prostřednictvím *Agrobacterium tumefaciens*

přenos bakteriální DNA do rostlinné buňky



Transformace kokultivací listových disků



a

Non-transformed plant in a Magenta box. Source of explants for co-cultivation.

b

Excising a leaf from an *in vitro*-grown plant. Done in a laminar flow hood.

c

Punching leaf discs. Done with sterile implements in a laminar flow hood.

d

Overnight (stationary-phase) culture of *Agrobacterium tumefaciens*.

e

Leaf discs floated on *Agrobacterium tumefaciens* suspension.

f

Leaf discs dipped in callus-induction media (CIM) after co-cultivation. Photo taken 2 days after plating. Dark bacterial growth (white) on surface of discs.

g

Callus production on leaf disc explants. Green spots represent cells that survived exposure to the selection agent.

h

Discs infected on leaf disc explants. Done in the presence of a selection agent.

i

Excised shoots inserted into root-induction media (RIM).

j

Roots beginning to form on excised shoots. Done in the presence of a selection agent.

k

Extensive root production on excised shoots.

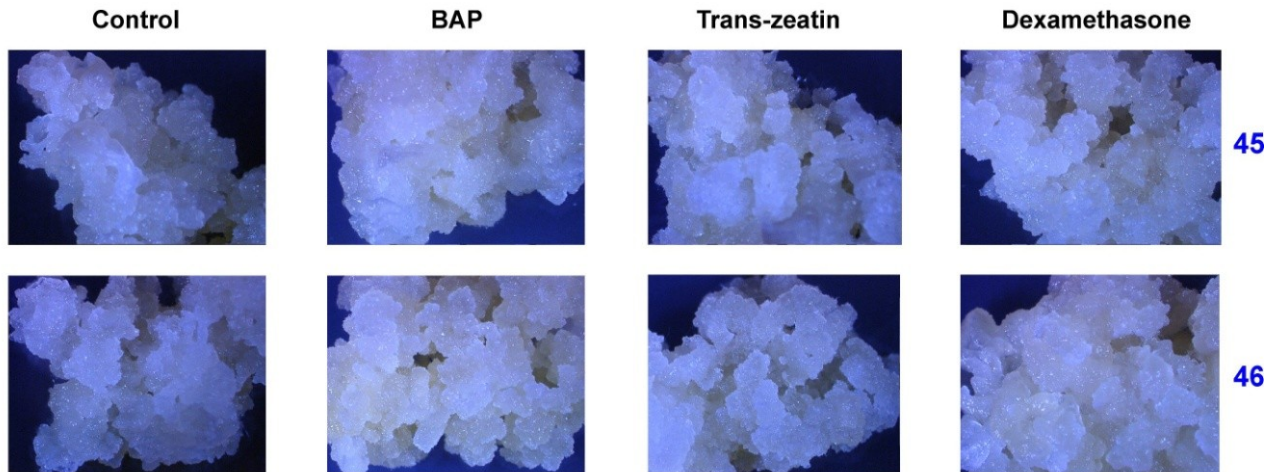
l

Fully regenerated transformed plants growing in a Magenta box.



Transformace kokultivací kalusů

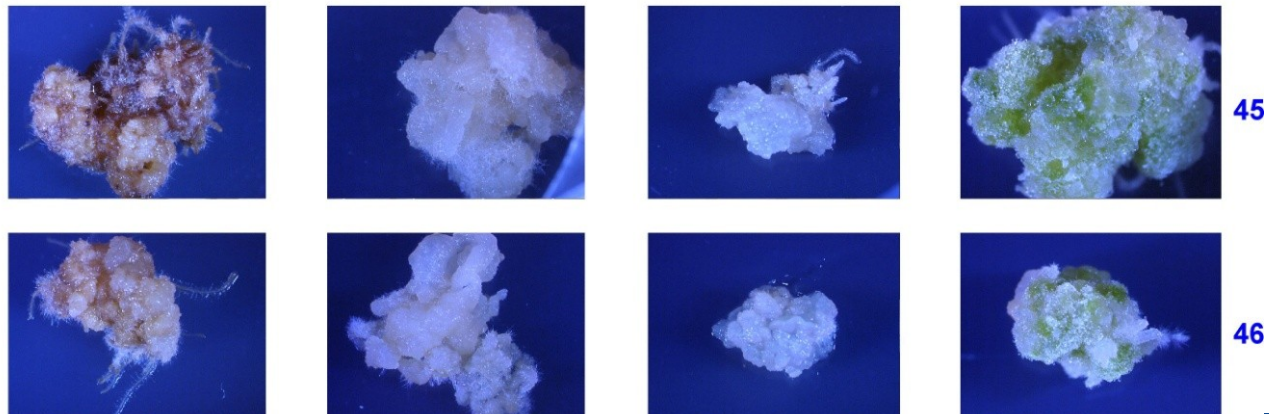
2 mg/l IAA, 0,5 mg/l 2,4-D, 0,5 mg/l 9-ipR



45

46

No hormones



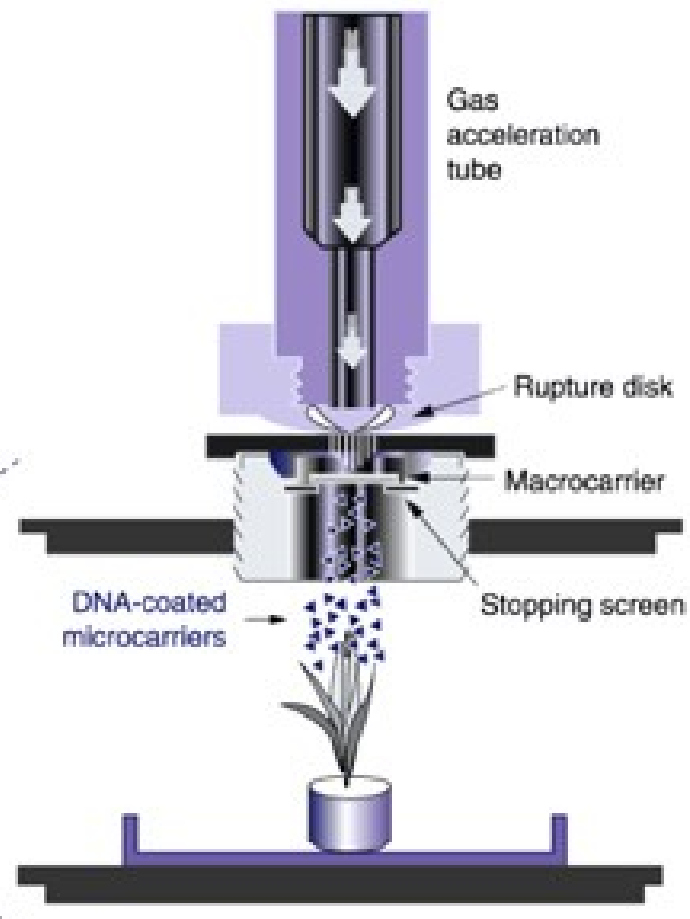
45

46

Transformace „nastřelováním“ DNA



Biolistic delivery of DNA



Transformace květenství



When plants have primary bolus 5-15 cm they are ready to infiltrate. Clipping of primary bolus is not necessary.



After infiltration, pots are placed on their sides to allow for drainage and are covered with plastic wrap. Plants are returned, in this state, to the growth chamber for 24 hours. After 24 hours, they are turned upright into a fresh flat.



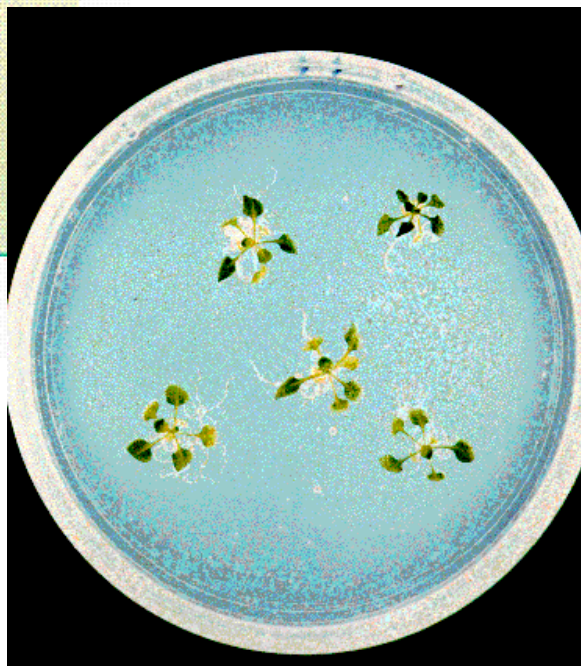
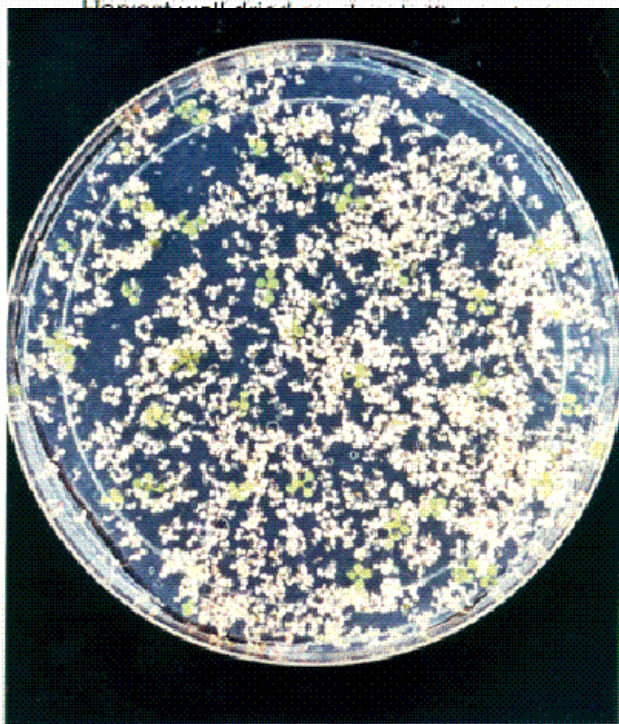
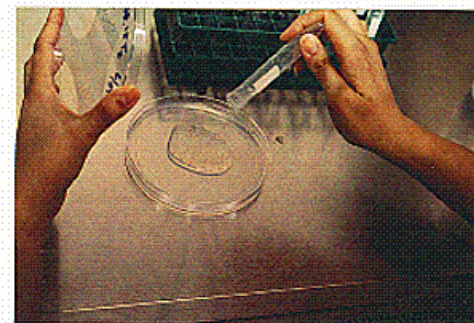
Plants are allowed to grow to maturity. They are staked to avoid seed loss and facilitate plant harvesting.

NOTE: Leaves degenerate within 2 weeks of infiltration. This is normal and does not affect seed set.

Transformace květenství



Sterilize seed in bleach solution.



Plant transformed seedlings in soil.

<http://www.bch.msu.edu/pamgreen/green.htm>
Transformed seedlings are grown and have true leaves on selective medium (a 40mg/l kanamycin plate is shown).

Osnova

- Vybrané **metody molekulární biologie**
 - Příprava transgenních organismů
 - PCR

PCR

**Polymerase Chain
Reaction**

Osnova

- Vybrané **metody molekulární biologie**
 - Příprava transgenních organismů
 - PCR
 - Design a příprava primerů (Dr. Hana Konečná)

Shrnutí

- Nástroje **systemové biologie**
 - Analýza **genové ontologie**
 - **Modelování molekulárních regulačních sítí**
- Modelové organismy
 - *Mus musculus*
 - *Arabidopsis thaliana*
- Vybrané **metody molekulární biologie**
 - Příprava transgenních organismů
 - PCR
 - Design a příprava primerů (Dr. Hana Konečná)

Diskuse



INVESTICE DO ROZVOJE VZDĚLÁVÁNÍ

Tato prezentace je spolufinancována
Evropským sociálním fondem
a státním rozpočtem České republiky