CG020 Genomika

Přednáška 12

Nástroje systémové biologie

Modelové organismy, PCR a zásady navrhování primerů

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EVROPSKÁ UNIE
MINISTERSTVO ŠKOLSTVÍ, MLÁDEŽE A TĚLOVÝCHOVY
OP Vzdělávání pro konkurenceschopnost
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 **systémové 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 **systémové biologie**
  - Analýza **genové ontologie**

Results of –omics Studies vs Biologically Relevant Conclusions

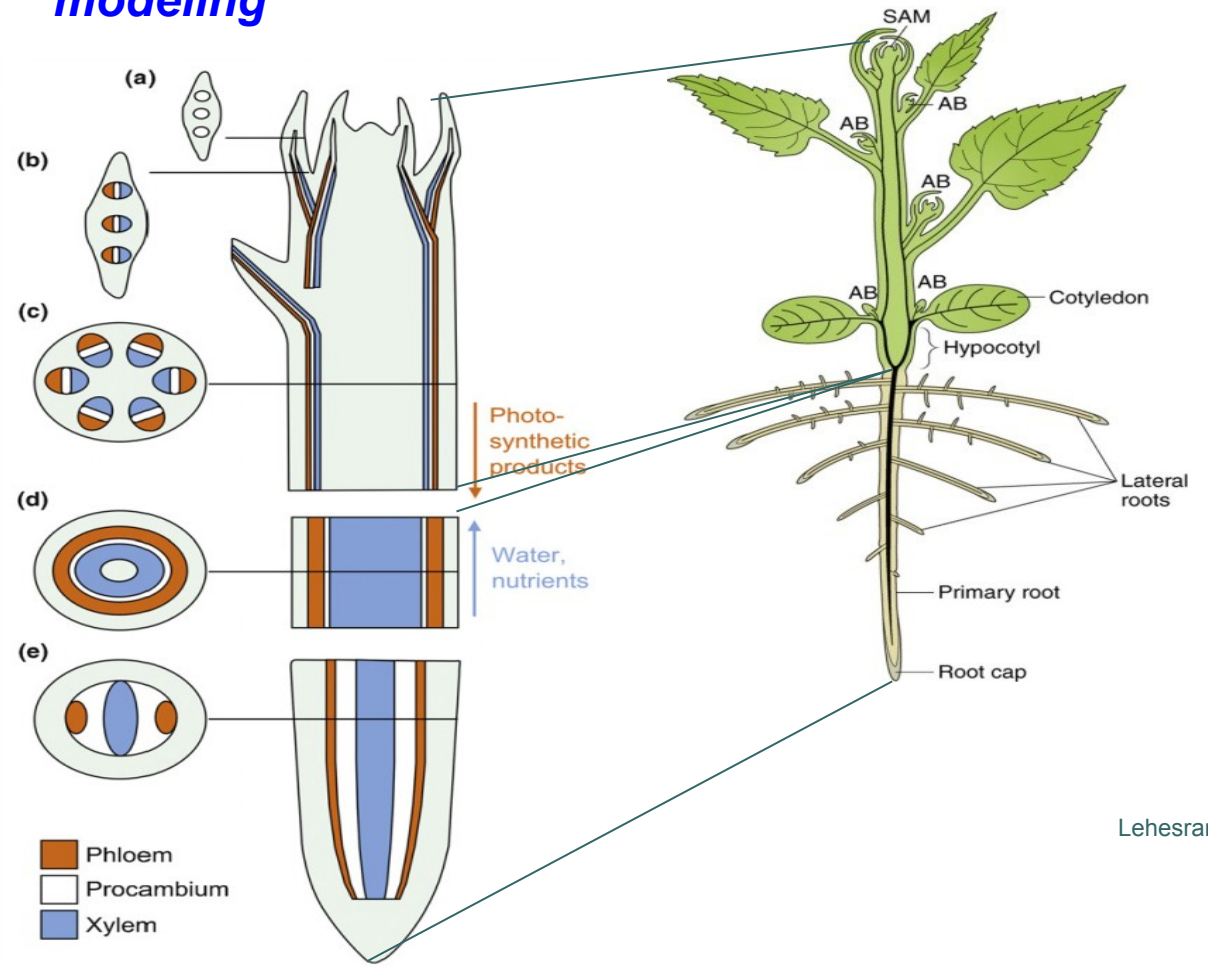
- Results of **–omics studies** are representred by **huge amount of data**, e.g. differential gene expression. But how to get any **biologically relevant conclusions**?

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	0,000391801	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

Molecular Regulatory Networks Modeling

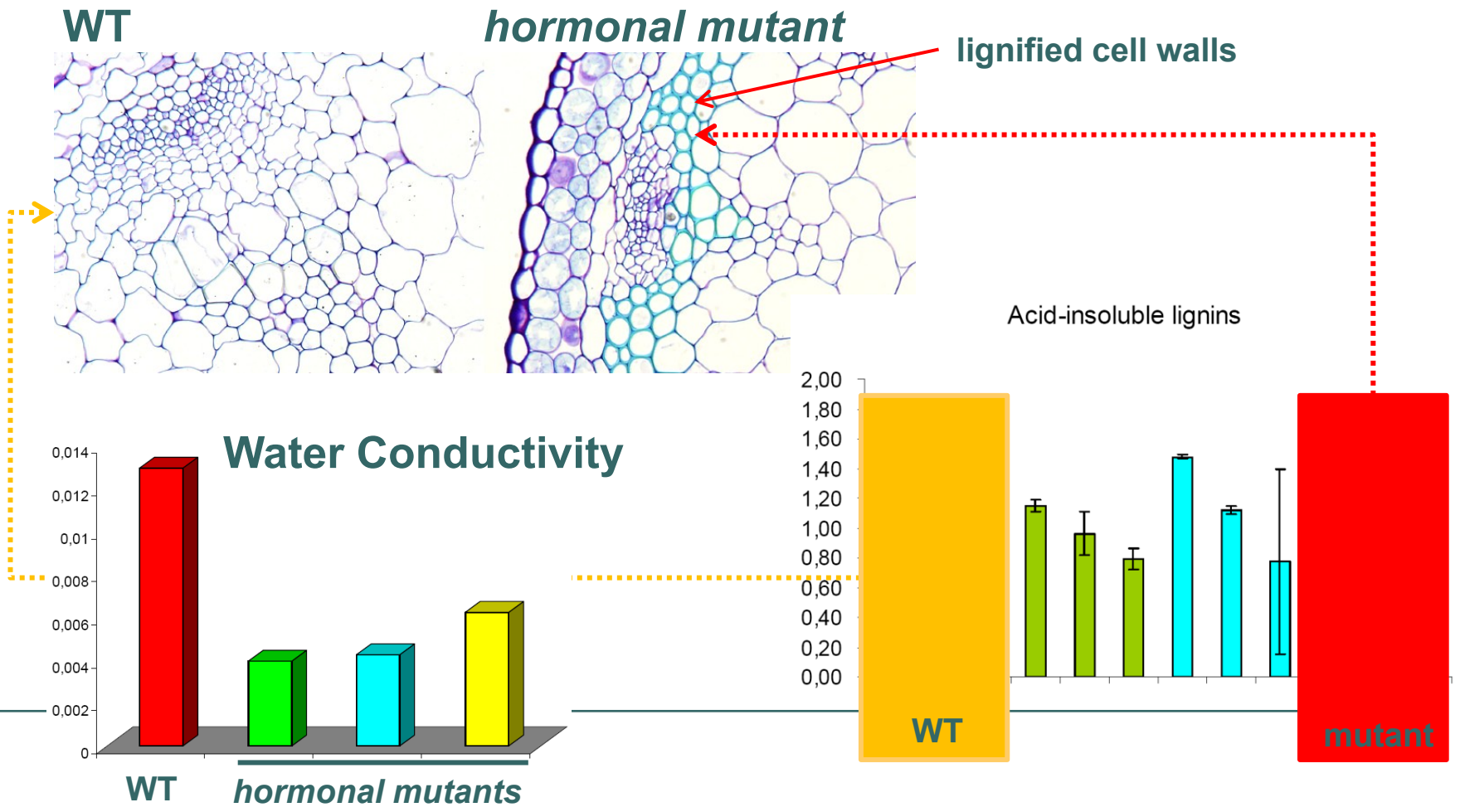
- ***Vascular tissue*** as a developmental model for ***GO analysis*** and ***MRN modeling***



Lehesranta etal., *Trends in Plant Sci* (2010)

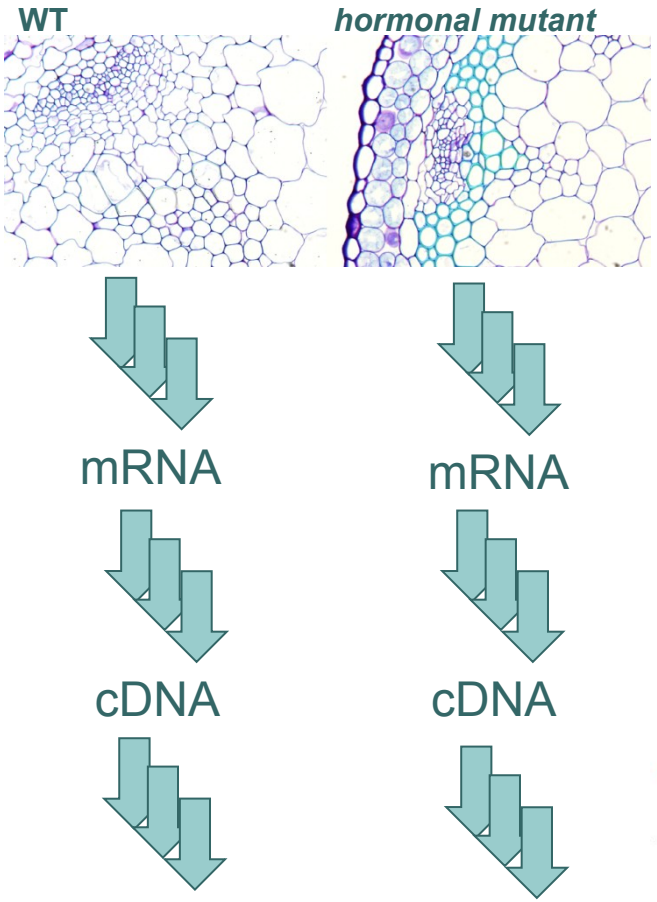
Hormonal Control Over Vascular Tissue Development

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

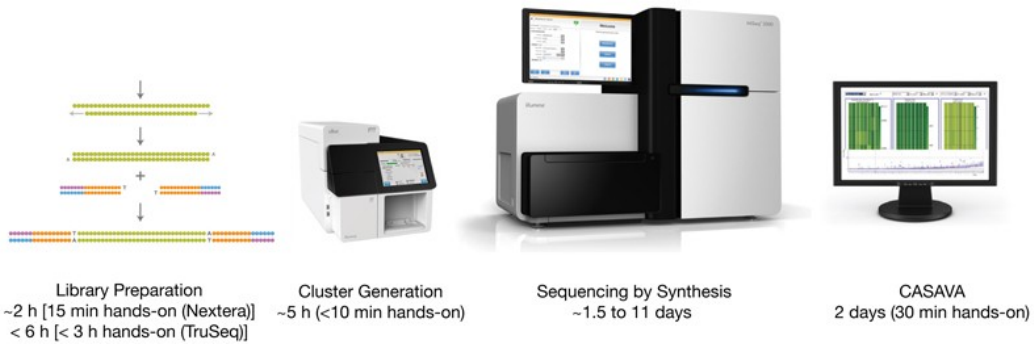


Hormonal Control Over Vascular Tissue Development

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



Sequencing by Illumina and
number of transcripts determination



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
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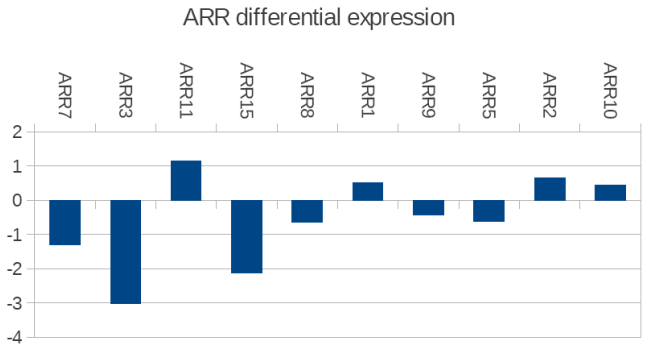
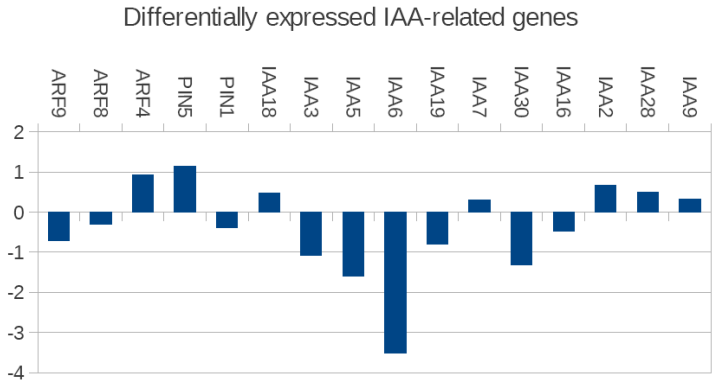
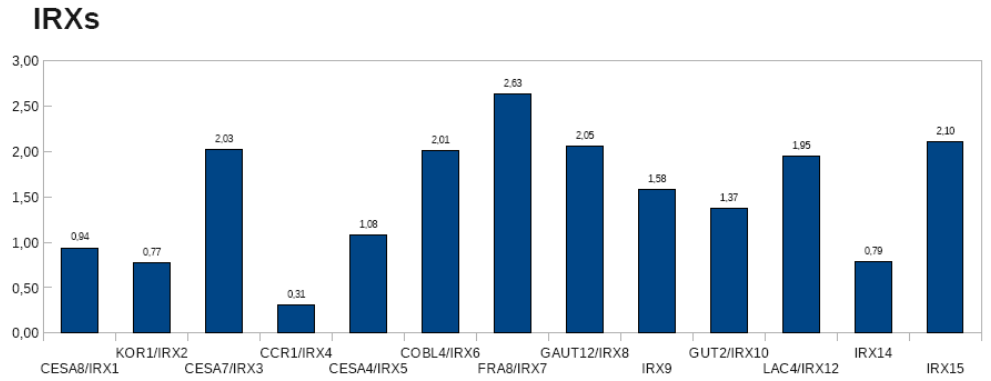
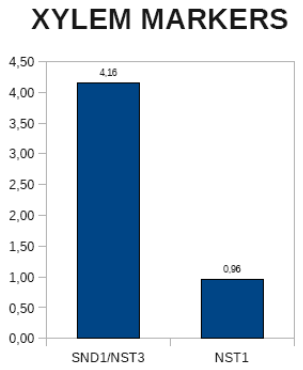
Gene Ontology Analysis

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







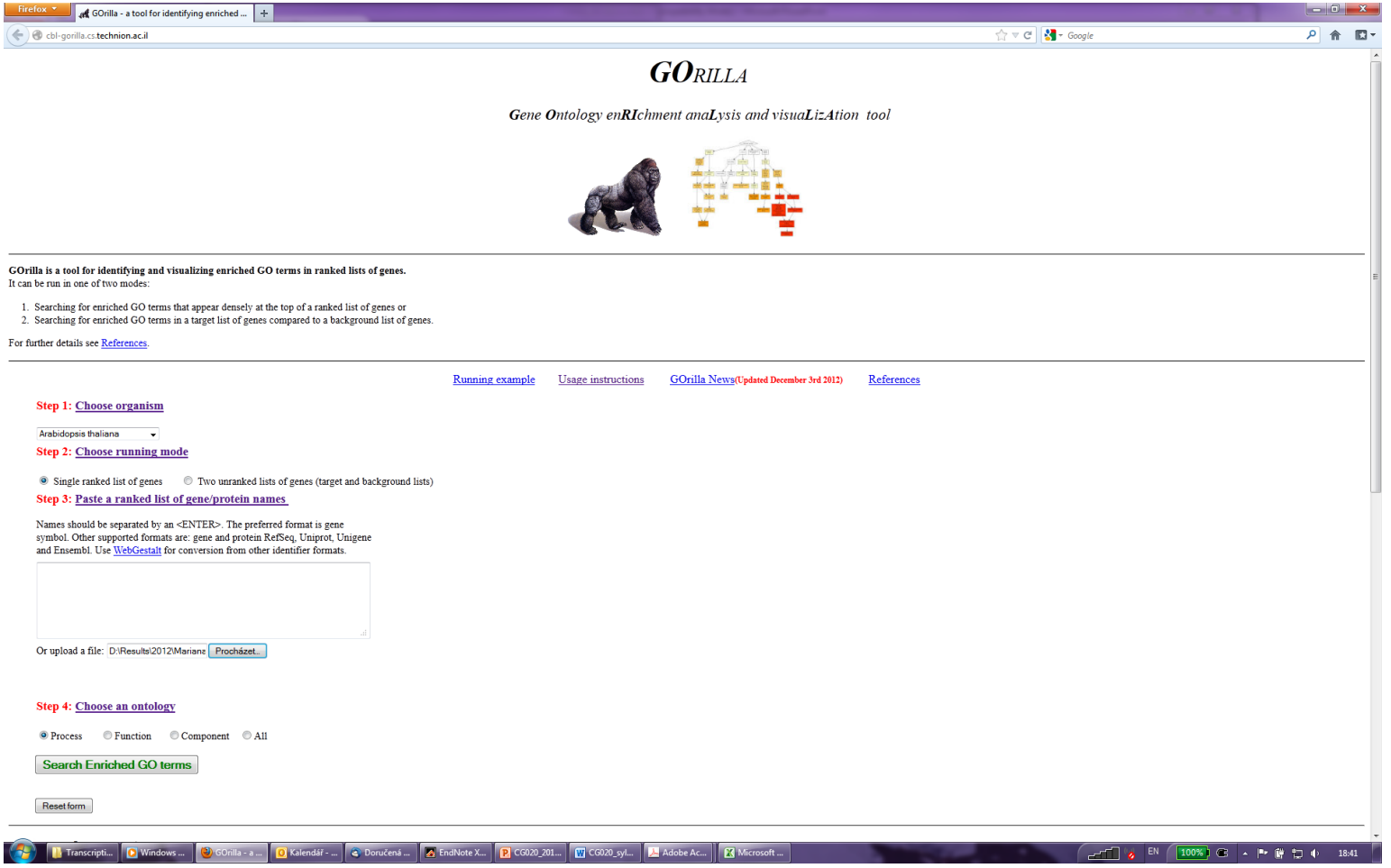


Ddii et al., *unpublished*

Gene Ontology Analysis

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

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



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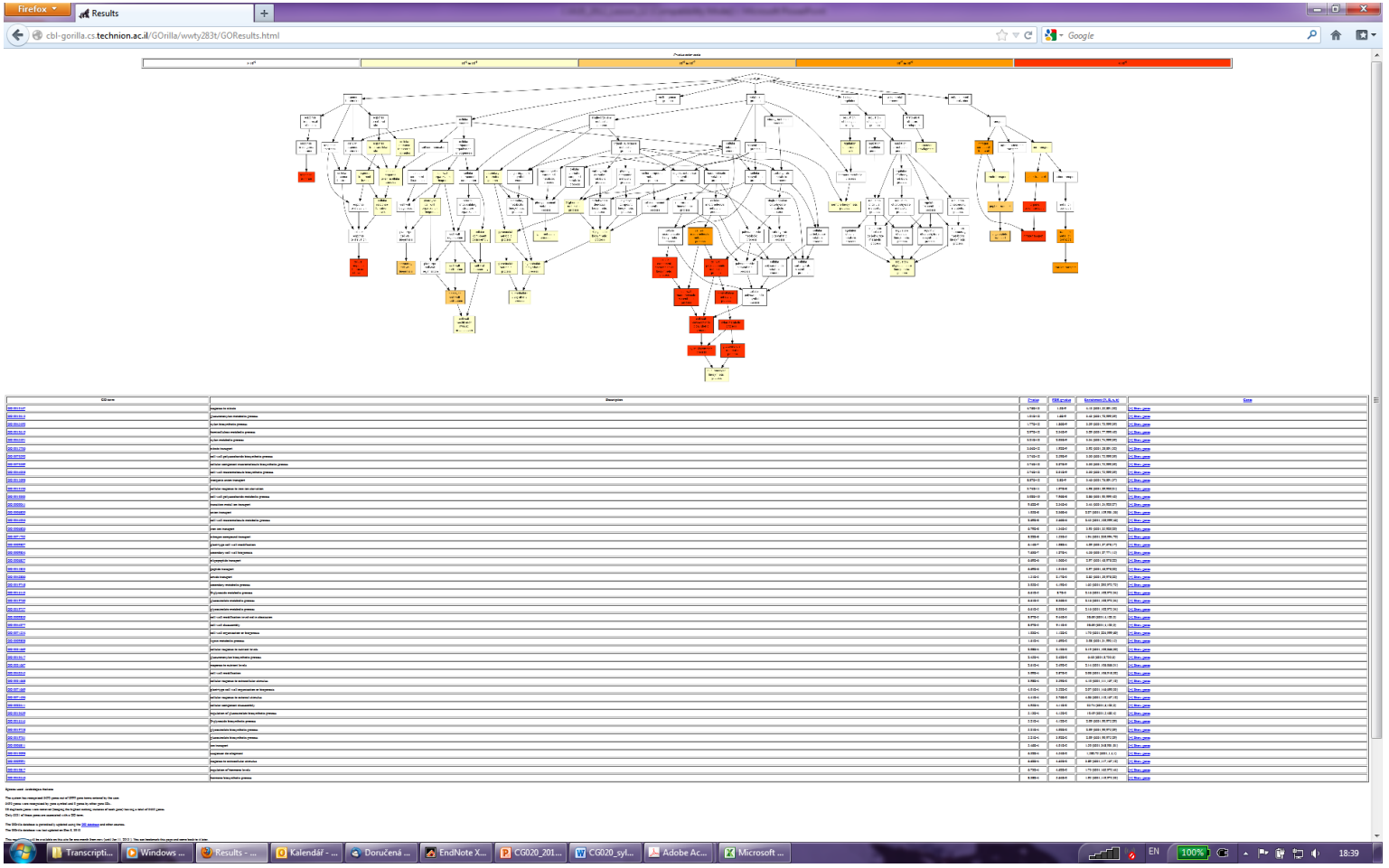

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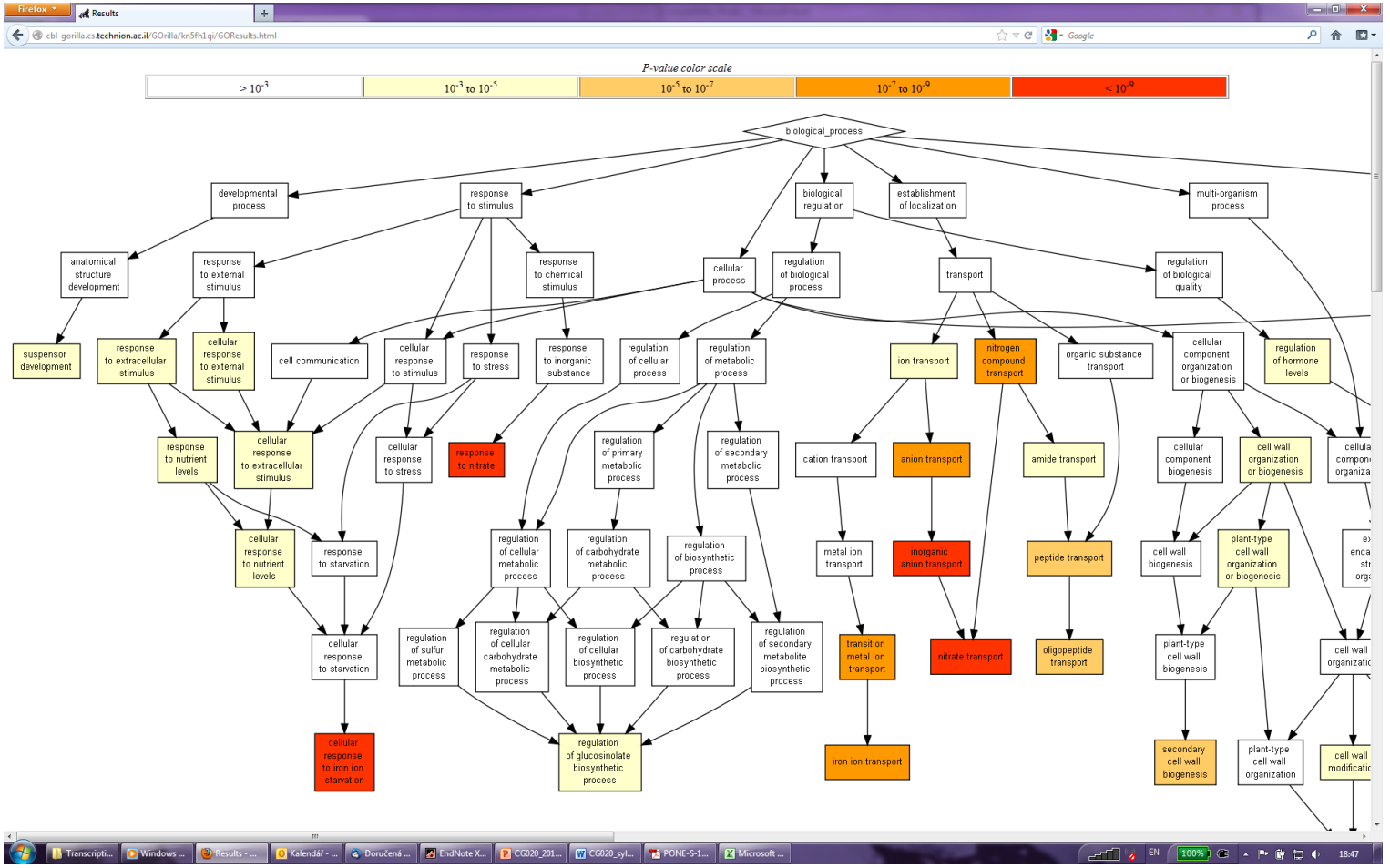
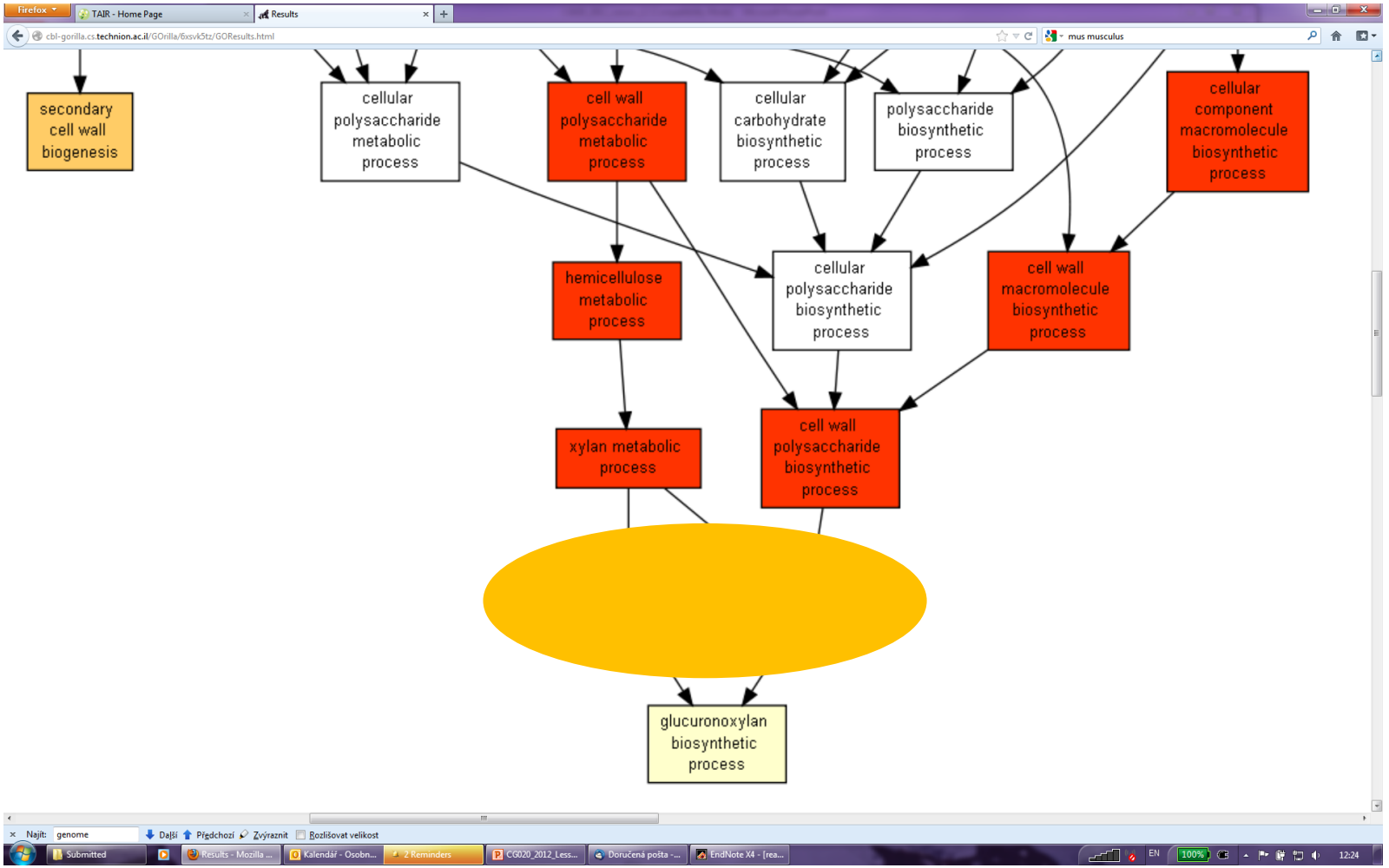


Gene Ontology Analysis

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



Gene Ontology Analysis

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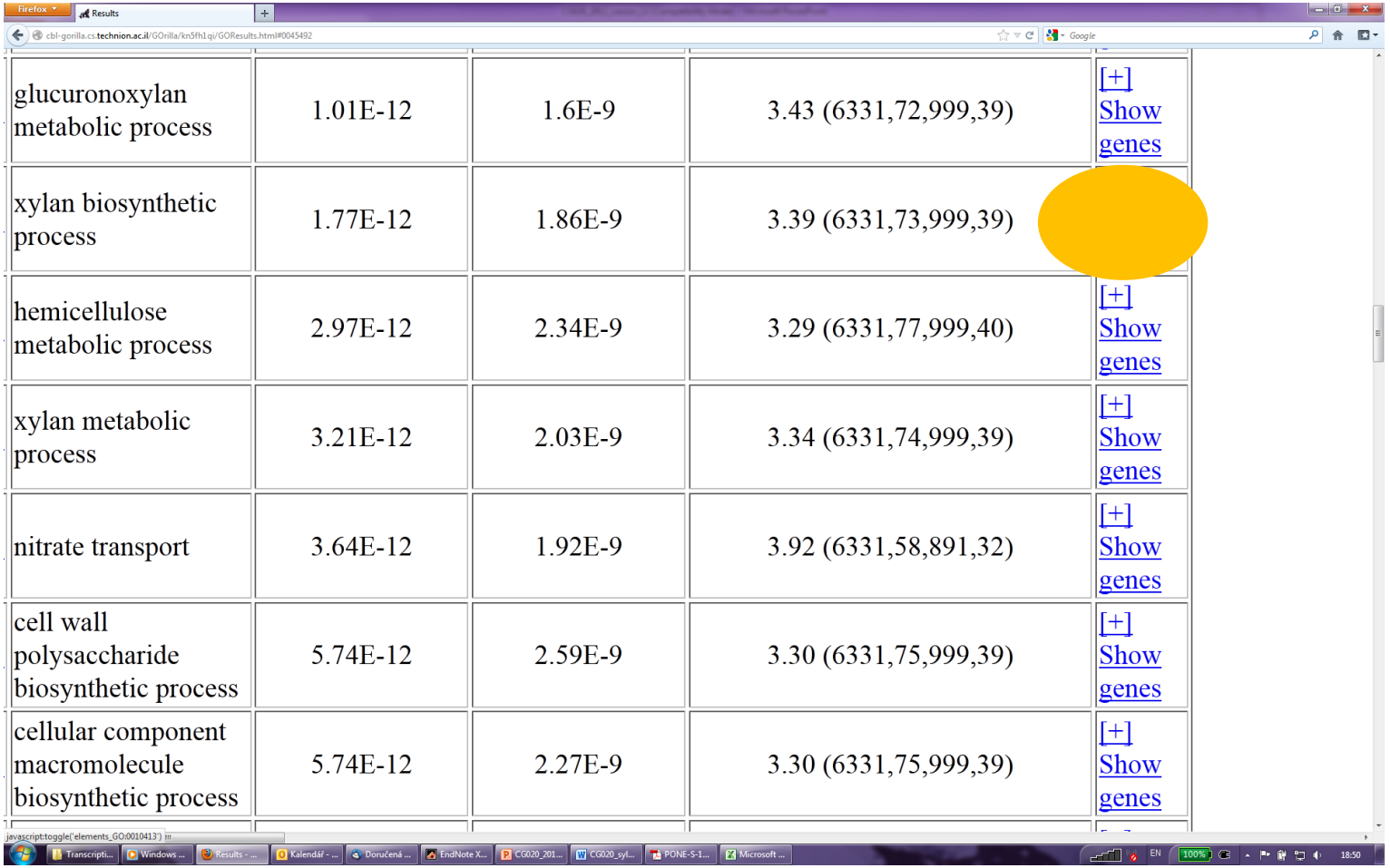
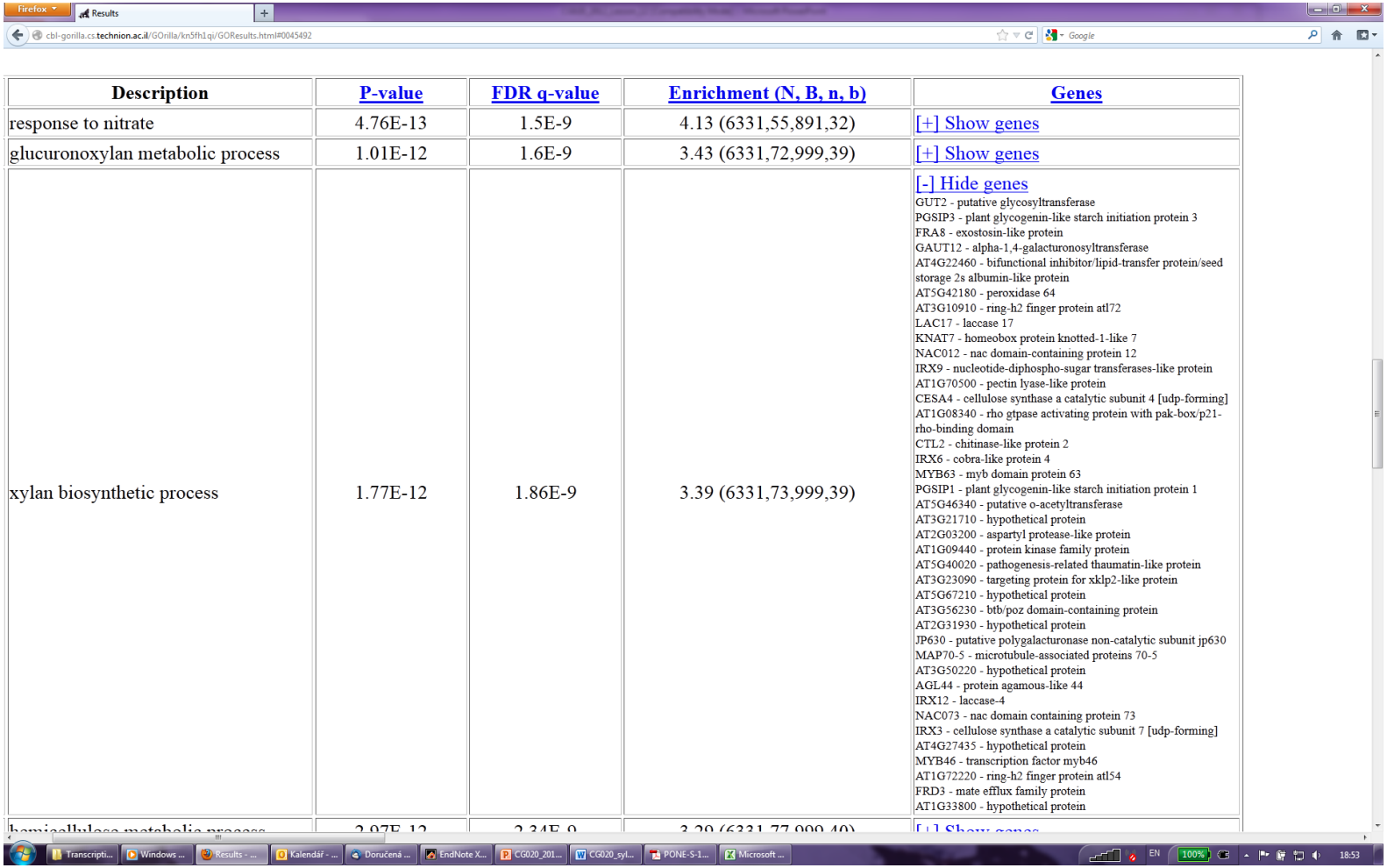


glucuronoxylan metabolic process	1.01E-12	1.6E-9	3.43 (6331,72,999,39)	[+] Show genes
xylan biosynthetic process	1.77E-12	1.86E-9	3.39 (6331,73,999,39)	
hemicellulose metabolic process	2.97E-12	2.34E-9	3.29 (6331,77,999,40)	[+] Show genes
xylan metabolic process	3.21E-12	2.03E-9	3.34 (6331,74,999,39)	[+] 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**



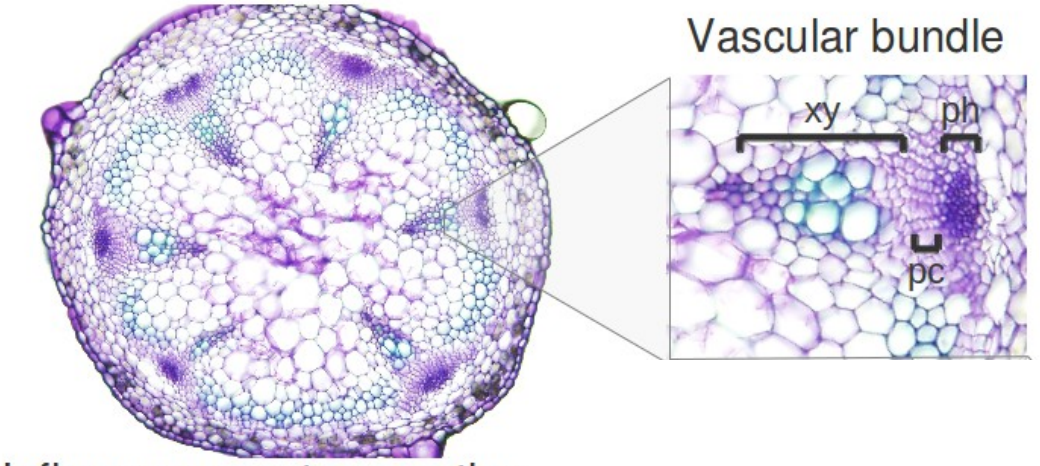
Description	P-value	FDR q-value	Enrichment (N, B, n, b)	Genes
response to nitrate	4.76E-13	1.5E-9	4.13 (6331,55,891,32)	[+] Show genes
glucuronoxylan metabolic process	1.01E-12	1.6E-9	3.43 (6331,72,999,39)	[+] Show genes
xylan biosynthetic process	1.77E-12	1.86E-9	3.39 (6331,73,999,39)	[-] Hide genes GUT2 - putative glycosyltransferase PGSIP3 - plant glycogenin-like starch initiation protein 3 FRA8 - exostosin-like protein GAUT12 - alpha-1,4-galacturonosyltransferase AT4G22460 - bifunctional inhibitor/lipid-transfer protein/seed storage 2s albumin-like protein AT5G42180 - peroxidase 64 AT3G10910 - ring-h2 finger protein atl72 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 PGSIP1 - 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 xklp2-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 atl54 FRD3 - mate efflux family protein AT1G33800 - hypothetical protein
hemicellulose metabolic process	2.97E-12	2.34E-9	3.29 (6331,77,999,40)	[+] Show genes

Osnova

- **Modelování molekulárních regulačních sítí**

Molecular Regulatory Networks Modeling

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



Benitez and Hejatko, ***submitted***

Molecular Regulatory Networks Modeling

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

Interaction	Evidence	References
A-ARRs –| 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 ARRs 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 –| 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]

Benitez and Hejatko, *submitted*

Molecular Regulatory Networks Modeling

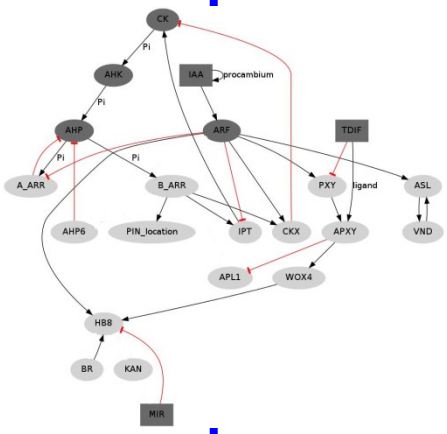
- Formulating ***logical rules*** defining the ***model dynamics***

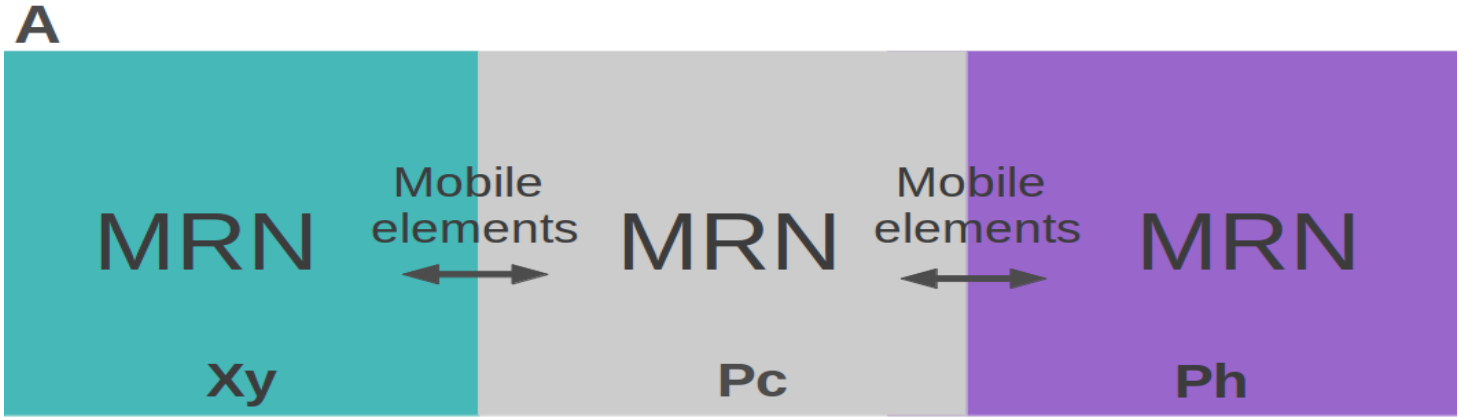
Network node	Dynamical rule
CK	2 If ipt=1 and ckx=0 1 If ipt=1 and ckx=1 0 else
CKX	1 If barr>0 or arf=2 0 else
AHKs	ahk=ck
AHPs	2 If ahk=2 and ahp6=0 and aarr=0 1 If ahk=2 and (ahp6+aarr<2) 1 If ahk=1 and ahp6<1 0 else
B-Type ARRs	1 If ahp>0 0 else
A-Type ARRs	1 If arf<2 and ahp>0 0 else

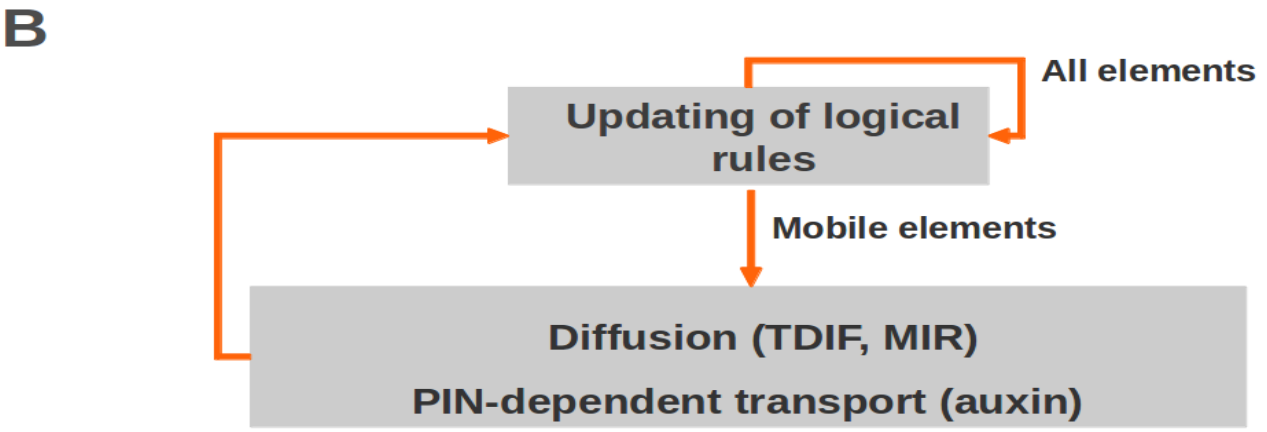
Benitez and Hejatko, *submitted*

Molecular Regulatory Networks Modeling

- Specifying ***mobile elements*** and their model behaviour

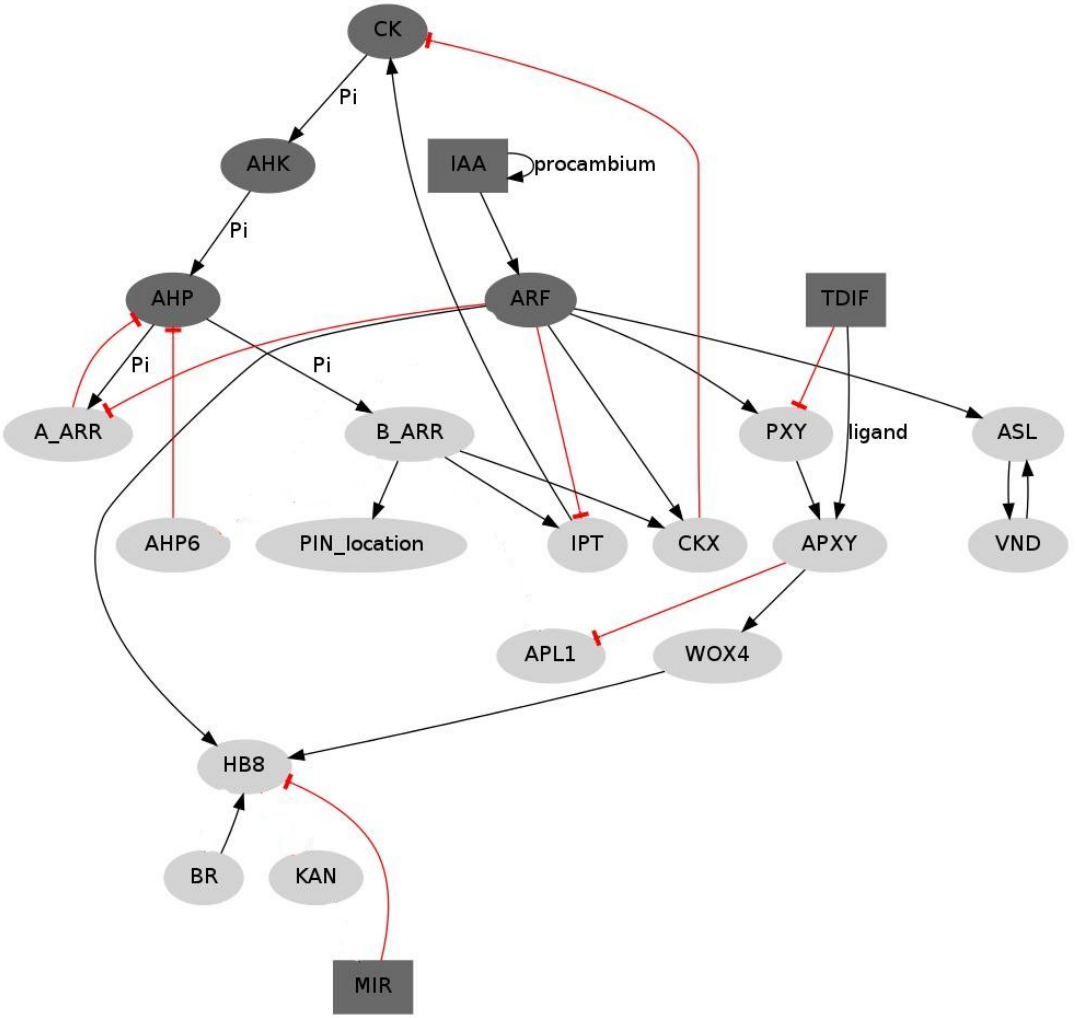






Molecular Regulatory Networks Modeling

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



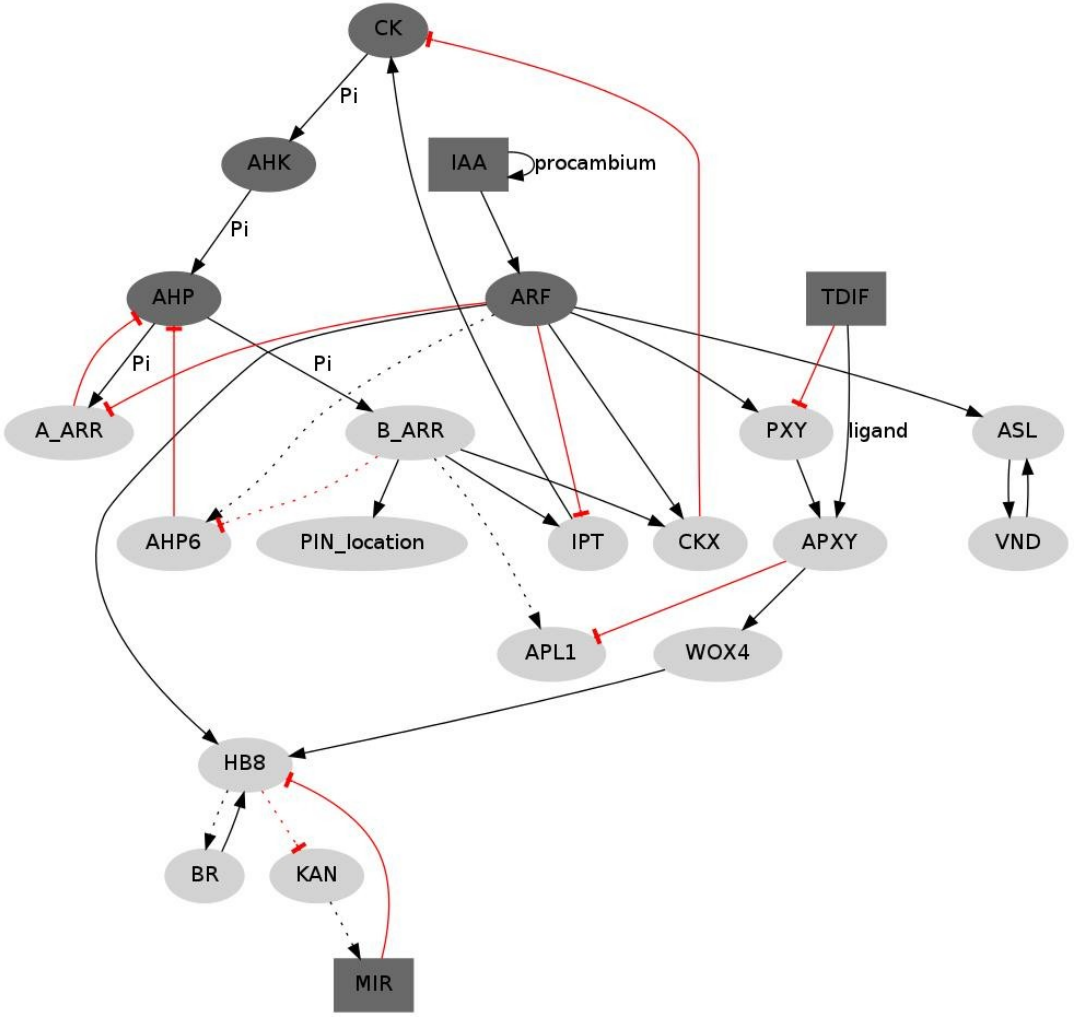
Molecular Regulatory Networks Modeling

- Specifying of missing interactions via ***informed predictions***

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

Molecular Regulatory Networks Modeling

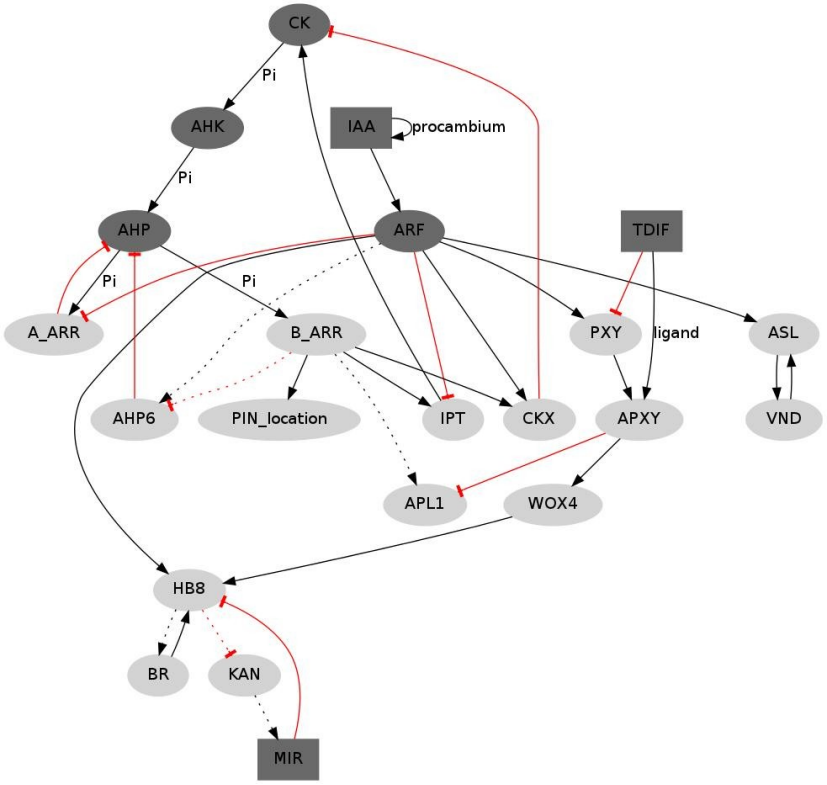
- Preparing the ***next version*** of the model and its ***testing***

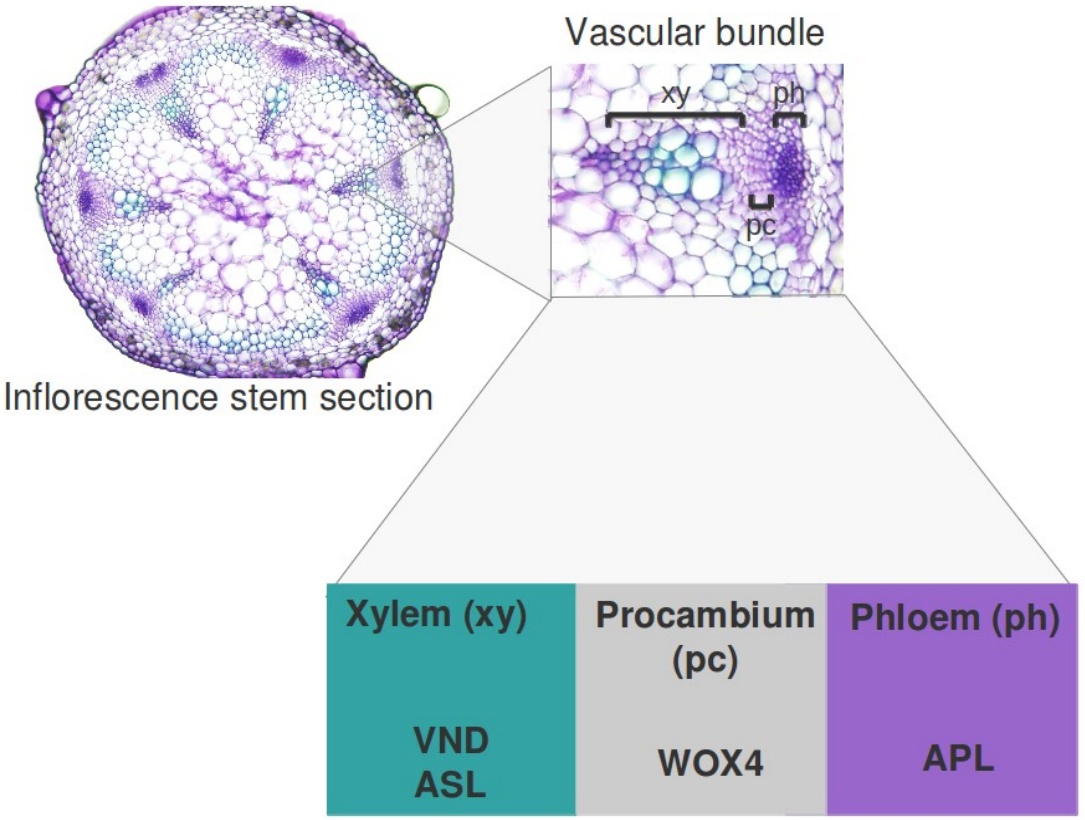


Benitez and Hejatko, *submitted*

Molecular Regulatory Networks Modeling

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

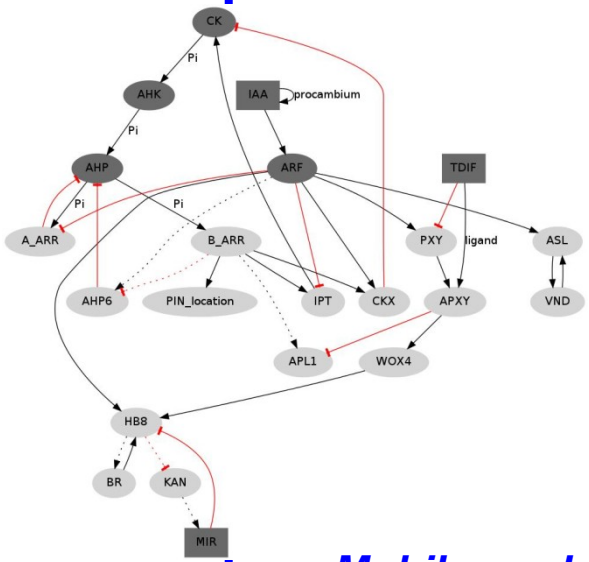




Benitez and Hejatko, *submitted*

Molecular Regulatory Networks Modeling

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



logical rule function

state in the time *t*

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

state in the time *t+1*

Amount if TDIF or MIR165 in cell *i*

***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

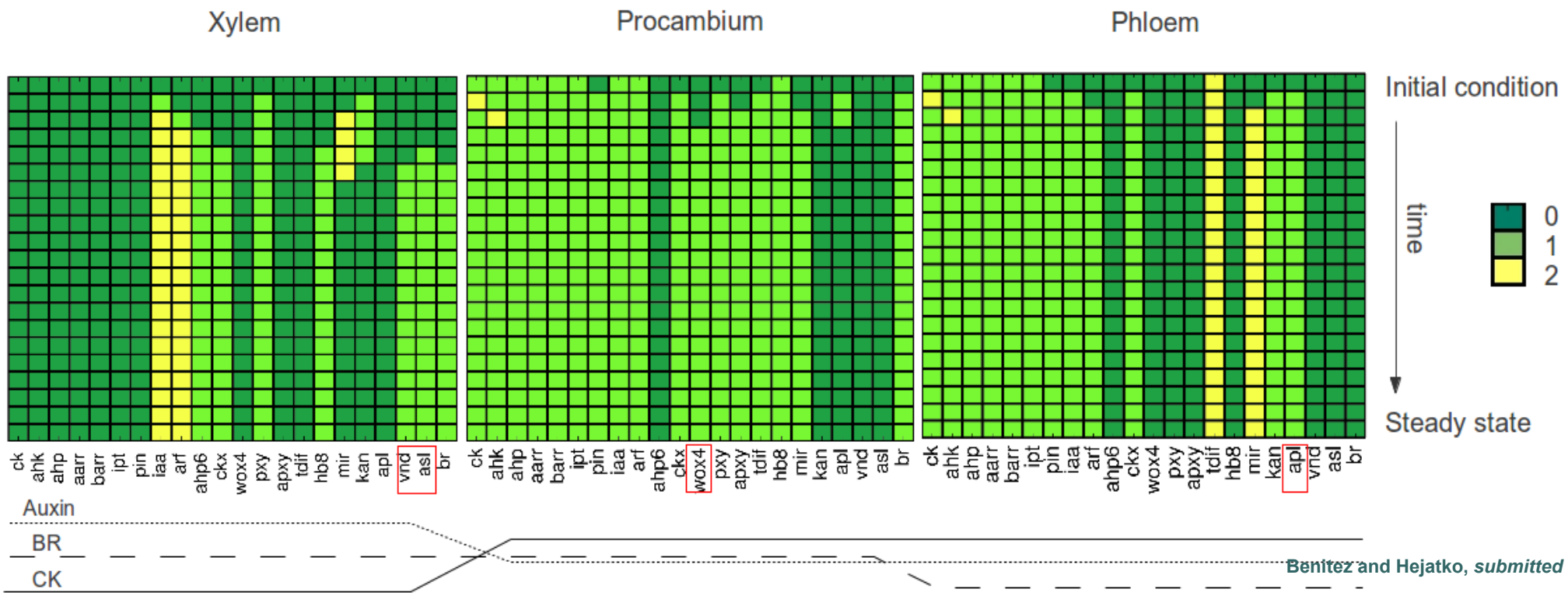
proportion of movable element

Molecular Regulatory Networks Modeling

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

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

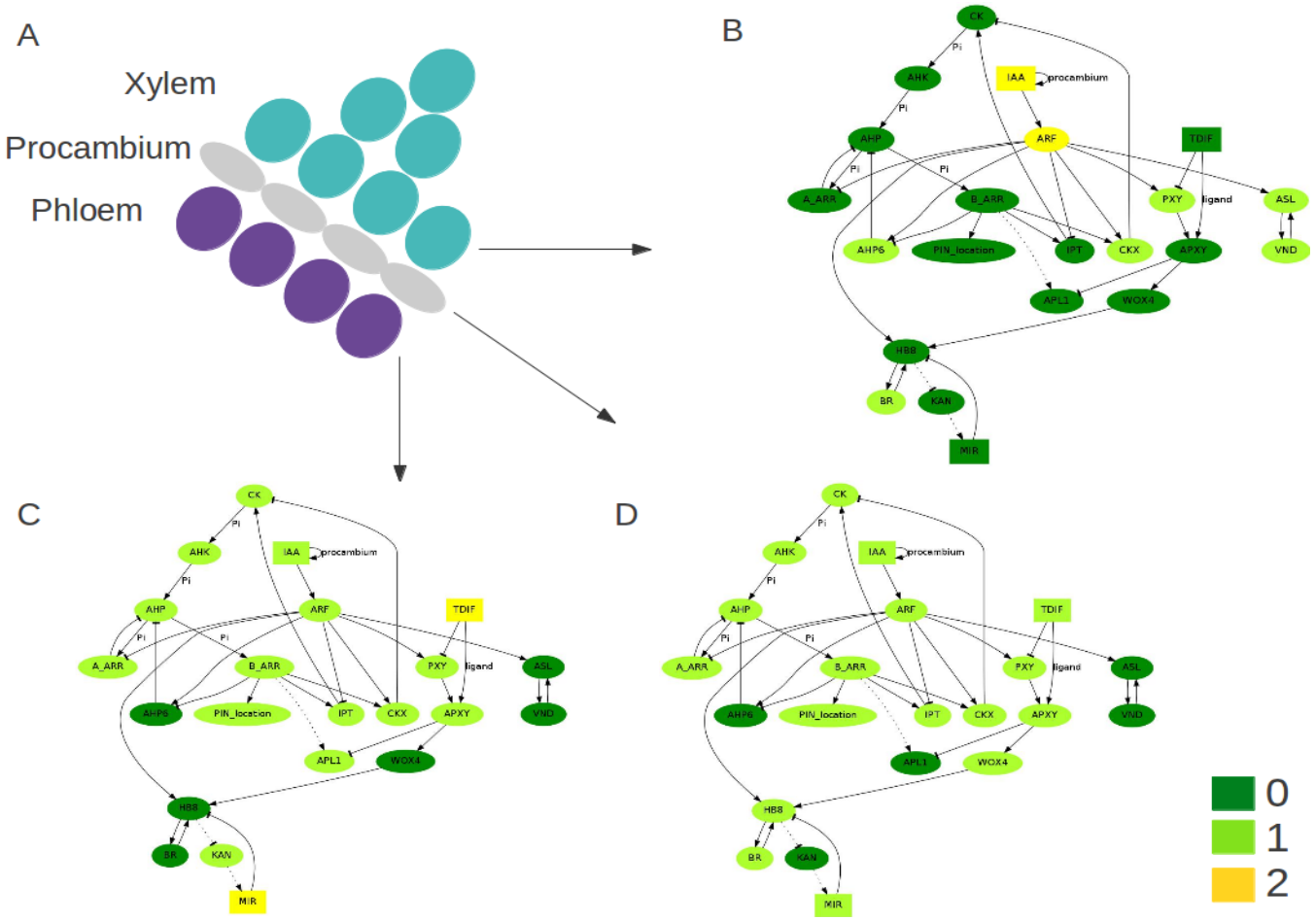
Mobile nodes: $g_{(t+1)T\,[i]}= H(g_{(t)\,[i]}+ D\,(g_{(t)\,[i+1]}+g_{(t)\,[i-1]} - N(g_{(t)\,[i]}))-b)$



Benitez and Hejatko, *submitted*

Molecular Regulatory Networks Modeling

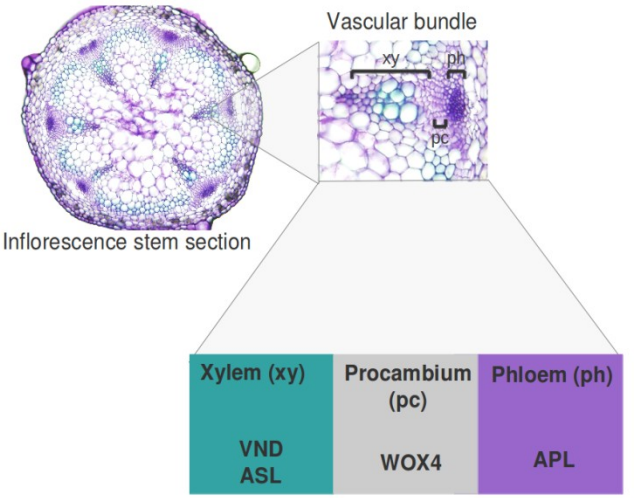
- The **good model** should be able to **simulate reality**

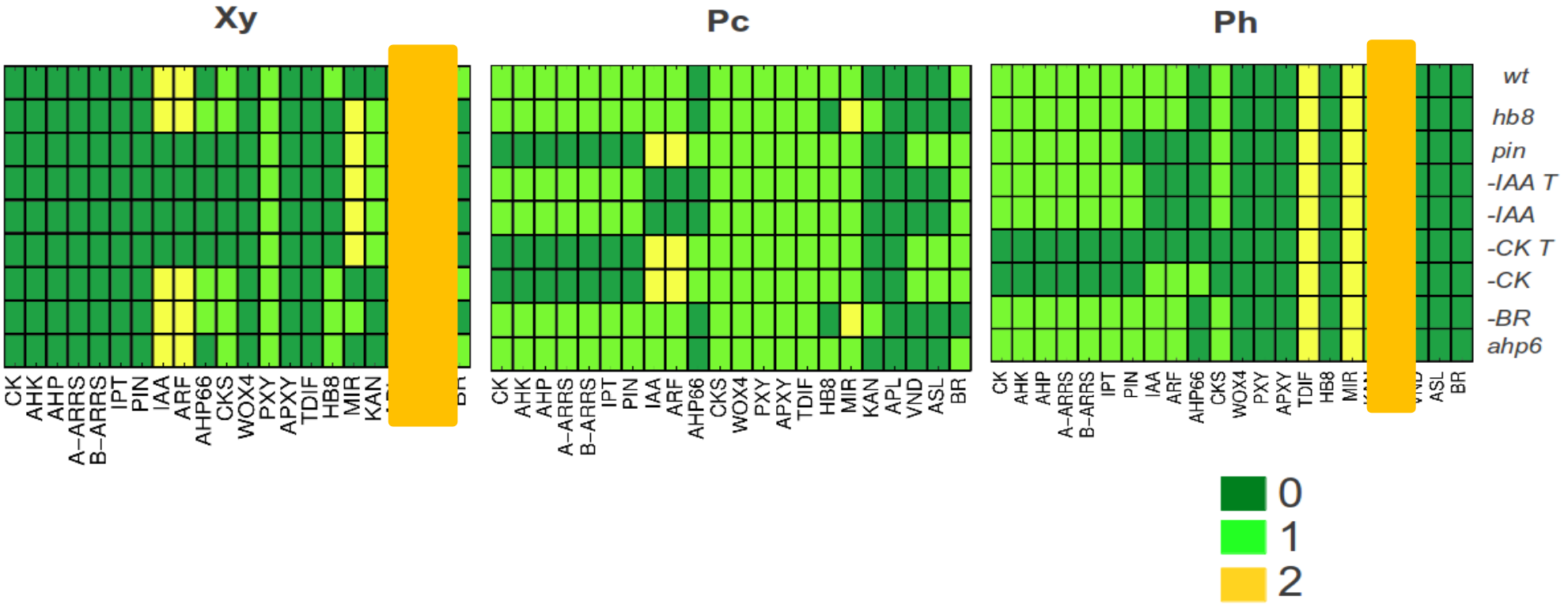


Benitez and Hejatko, *submitted*

Molecular Regulatory Networks Modeling

- Simulation of ***mutants***





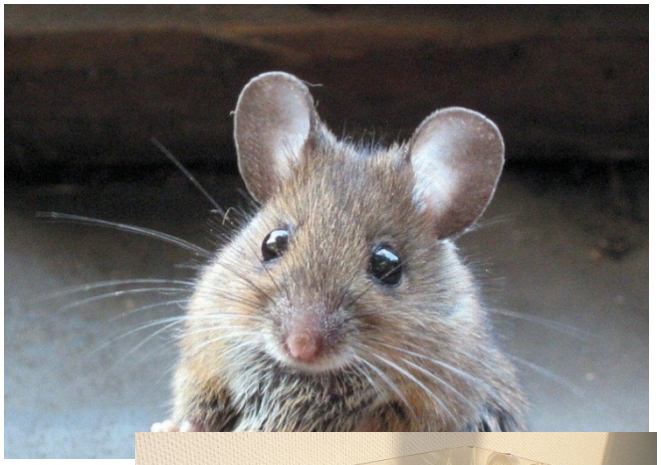
Osnova

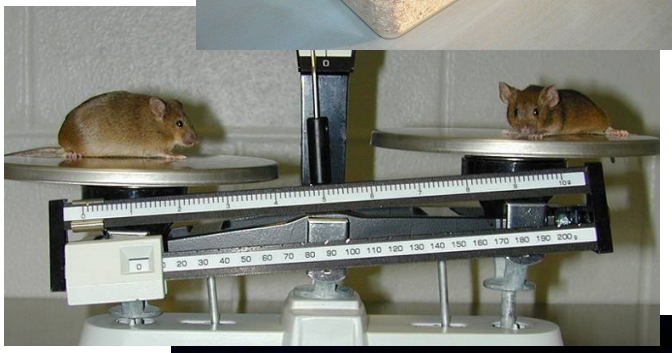
- Modelové organismy
  - *Mus musculus*

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

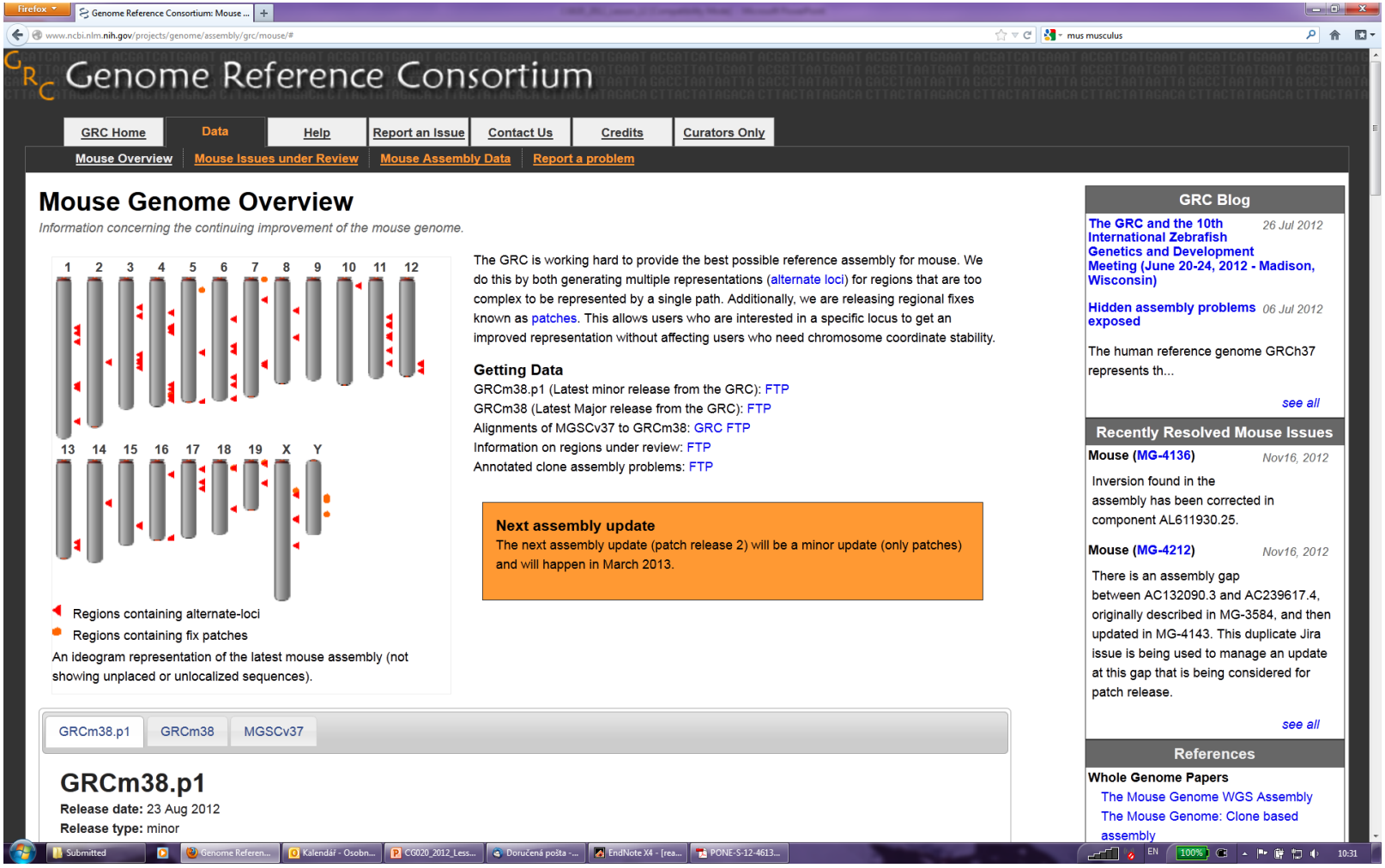




Mus musculus

myš domácí, house mouse

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



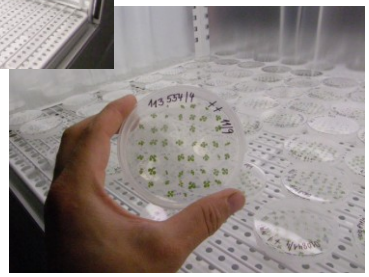
Osnova

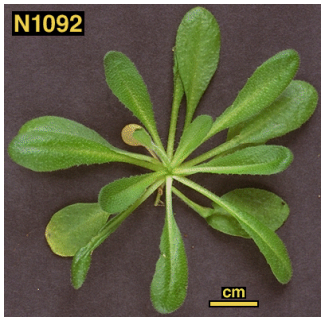
- Nástroje **systémové biologie**
  - Analýza **genové ontologie**
  - **Modelování molekulárních regulačních sítí**
- Modelové organismy
  - *Mus musculus*
  - *Arabidopsis thaliana*

Arabidopsis thaliana

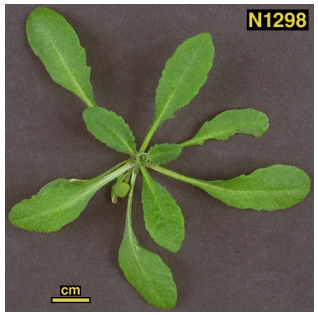
huseníček polní, mouse-ear cress

- 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))

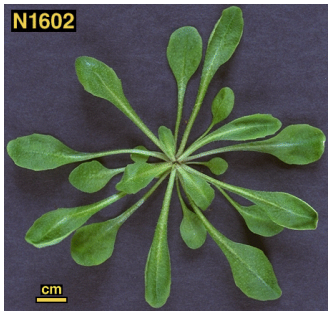




Columbia 0



Landsberg 0



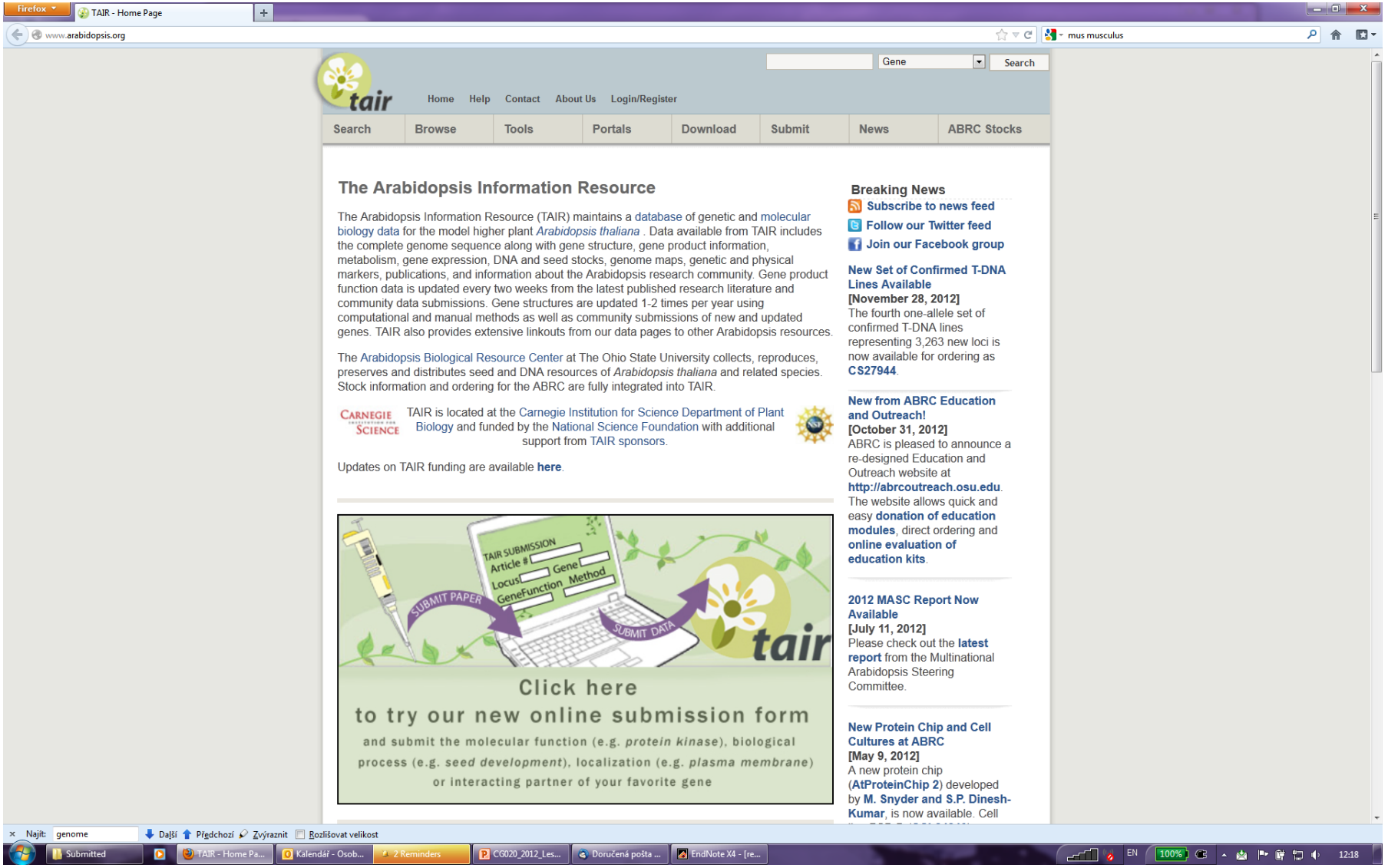
Wassilewskija 0

http://seeds.nottingham.ac.uk/

Arabidopsis thaliana

huseníček polní, mouse-ear cress

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



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.

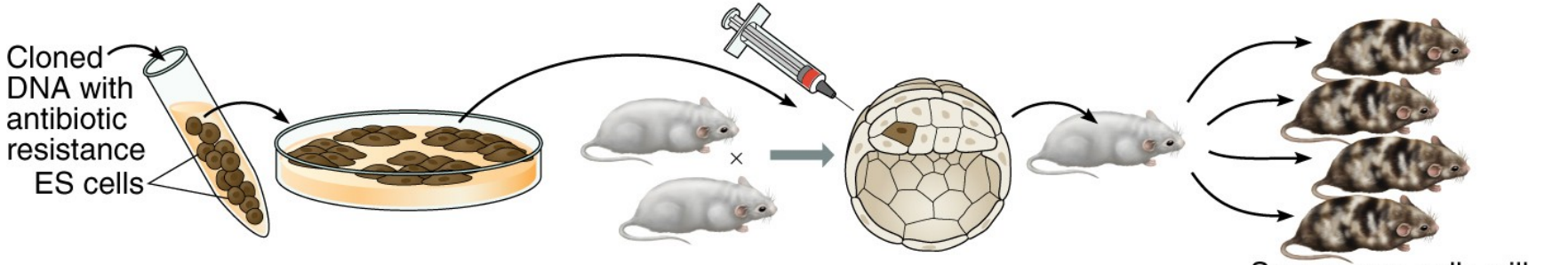
Magnified view of blastocyst

Progeny of single ICM cell in nutrient culture medium

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.



Some germ cells will be from a black ES cell

6. Breed chimeric mice to produce homozygous transgenic offspring.

Parents **Heterozygous F_1** **Homozygous strain from ES cell, F_2**

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

Transformace *Arabidopsis* prostřednictvím *Agrobacteria tumefaciens*

Crown gall of raspberry caused by *Agrobacterium tumefaciens*.

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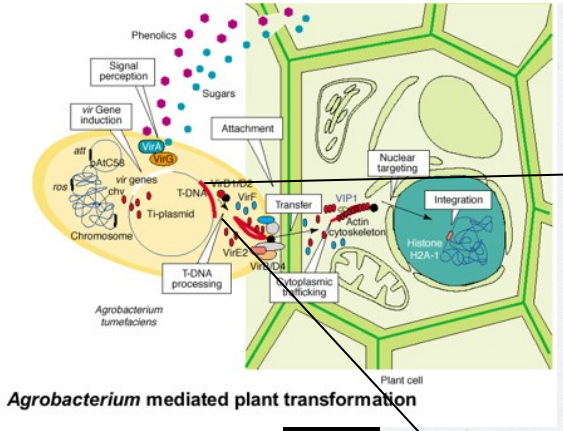
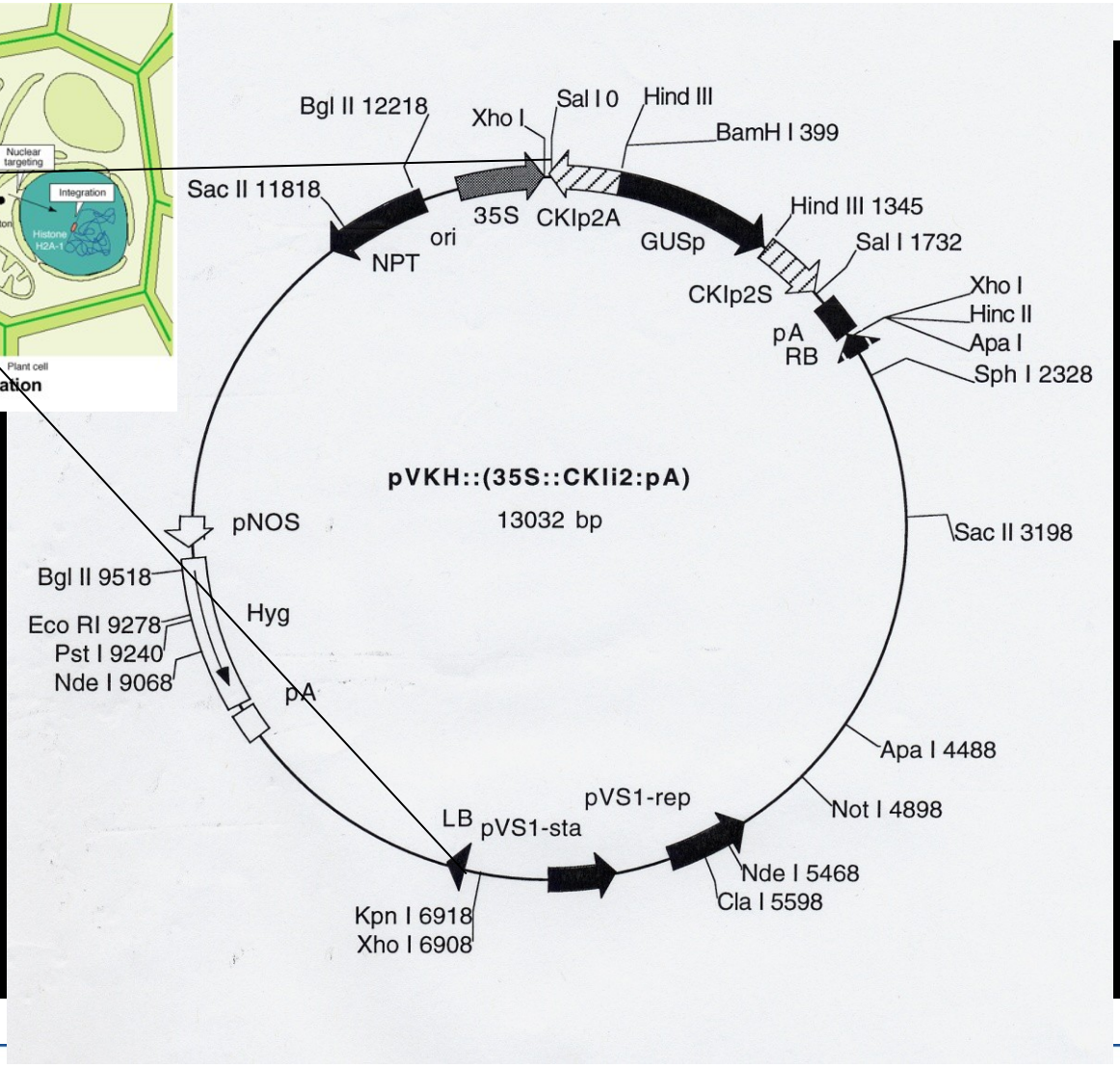
Transformace *Arabidopsis* prostřednictvím *Agrobacteria tumefaciens*

přenos bakteriální DNA do rostlinné buňky



***Agrobacterium* mediated plant transformation**



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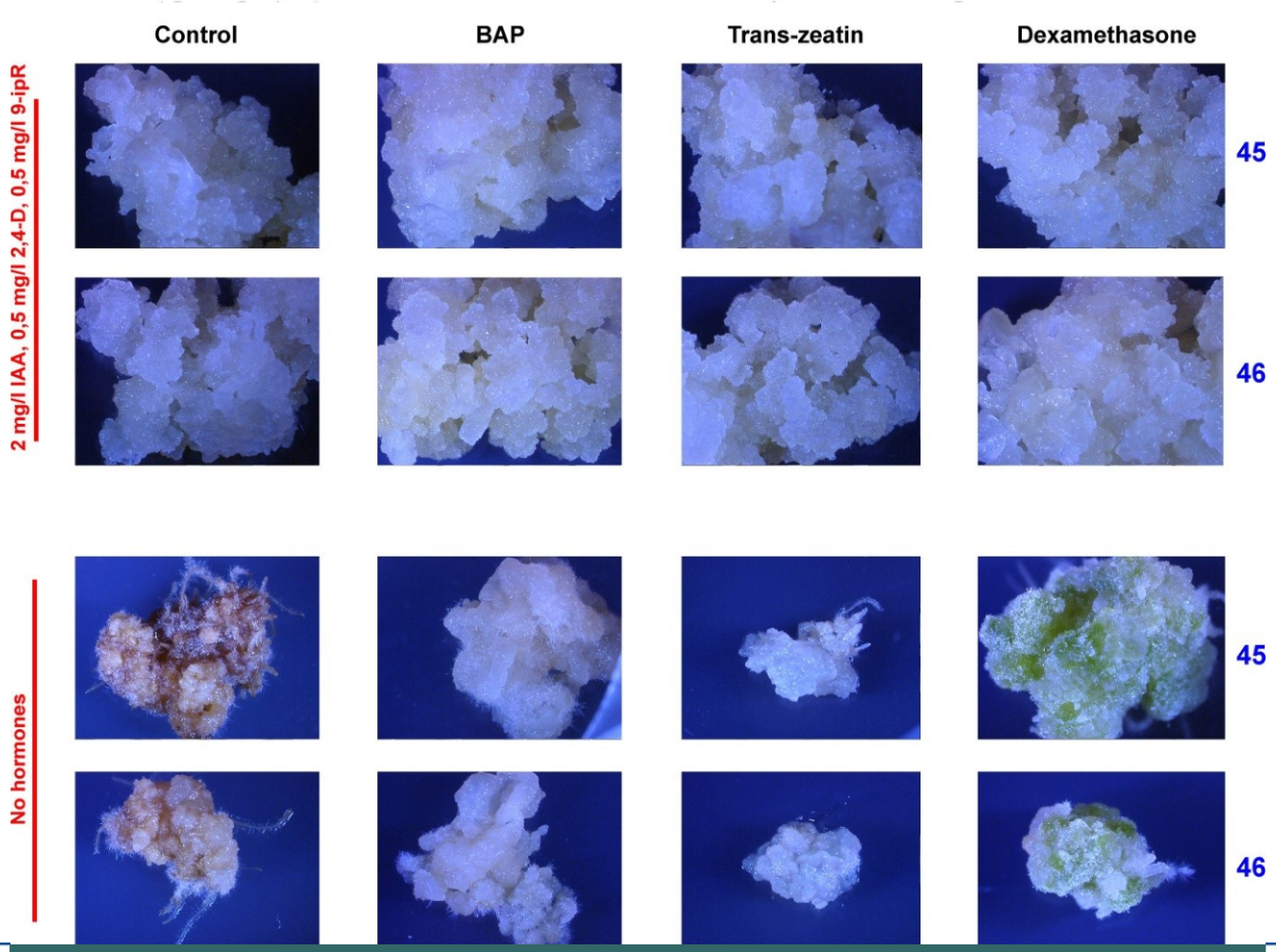
Transformace kokultivací listových disků

Transformace kokultivací kalusů



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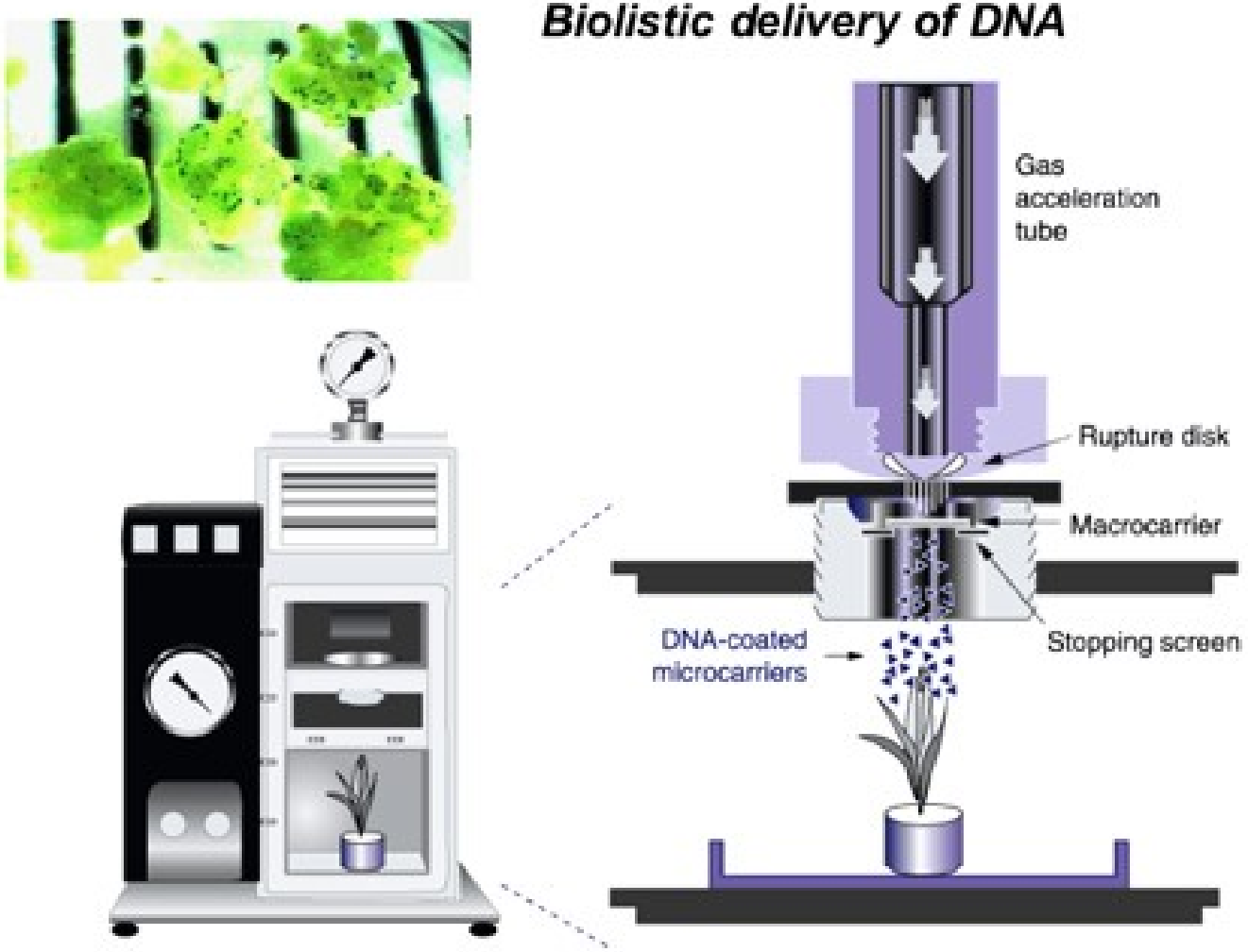
Transformace „nastřelováním“ DNA



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Transformace květenství

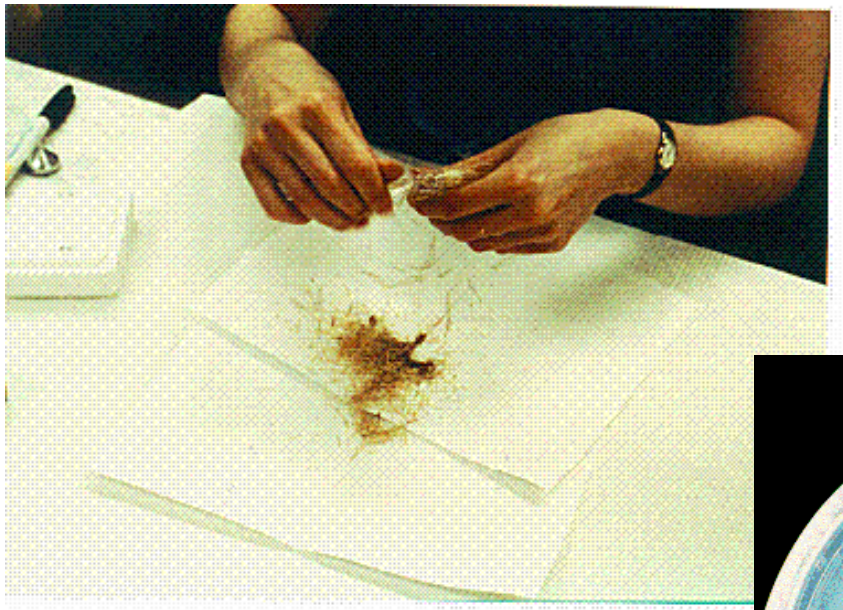
When plants have primary bolts 5-15 cm they are ready to infiltrate. Clipping of primary bolts is not necessary.

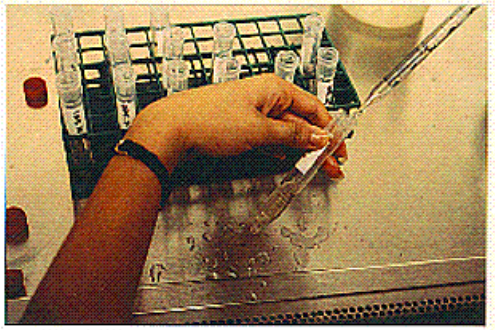
After infiltration, pots are placed on their sides to allow for drainage and are covered with plastic wrap. Plants are returned, in this state, to the growth chamber for 24 hours. After 24 hours, they are turned upright into a fresh flat.

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.

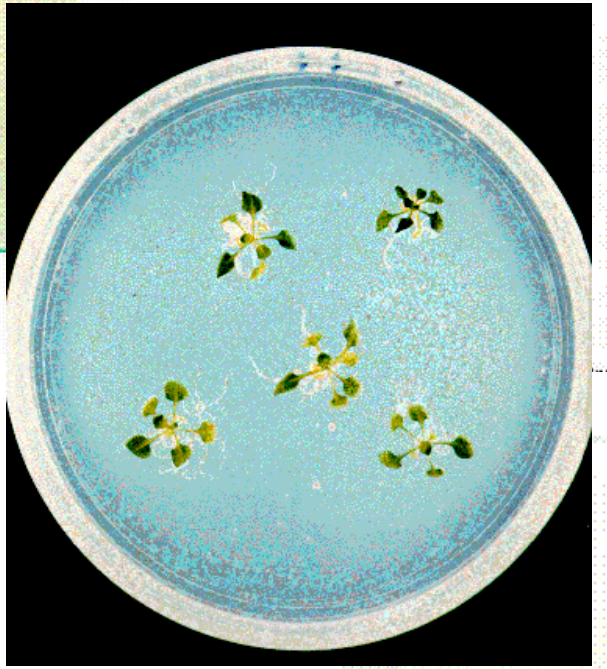
http://www.bch.msu.edu/pamgreen/green.htm

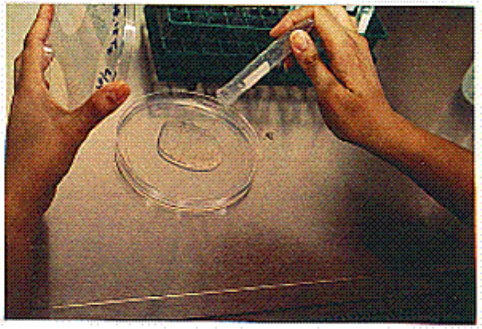
Transformace květenství

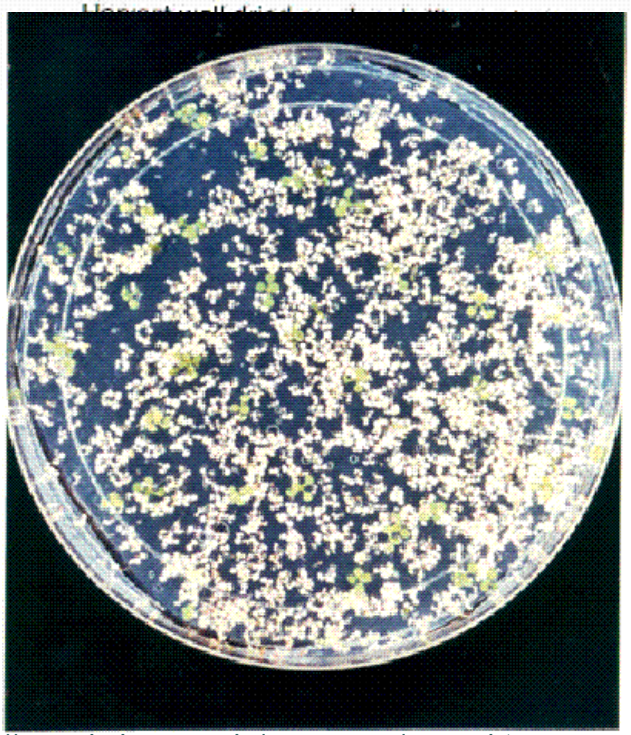




Sterilize seed in bleach solution.







Transformed seedlings are green and have true leaves on selective medium (a 40mg/l kanamycin plate is shown).

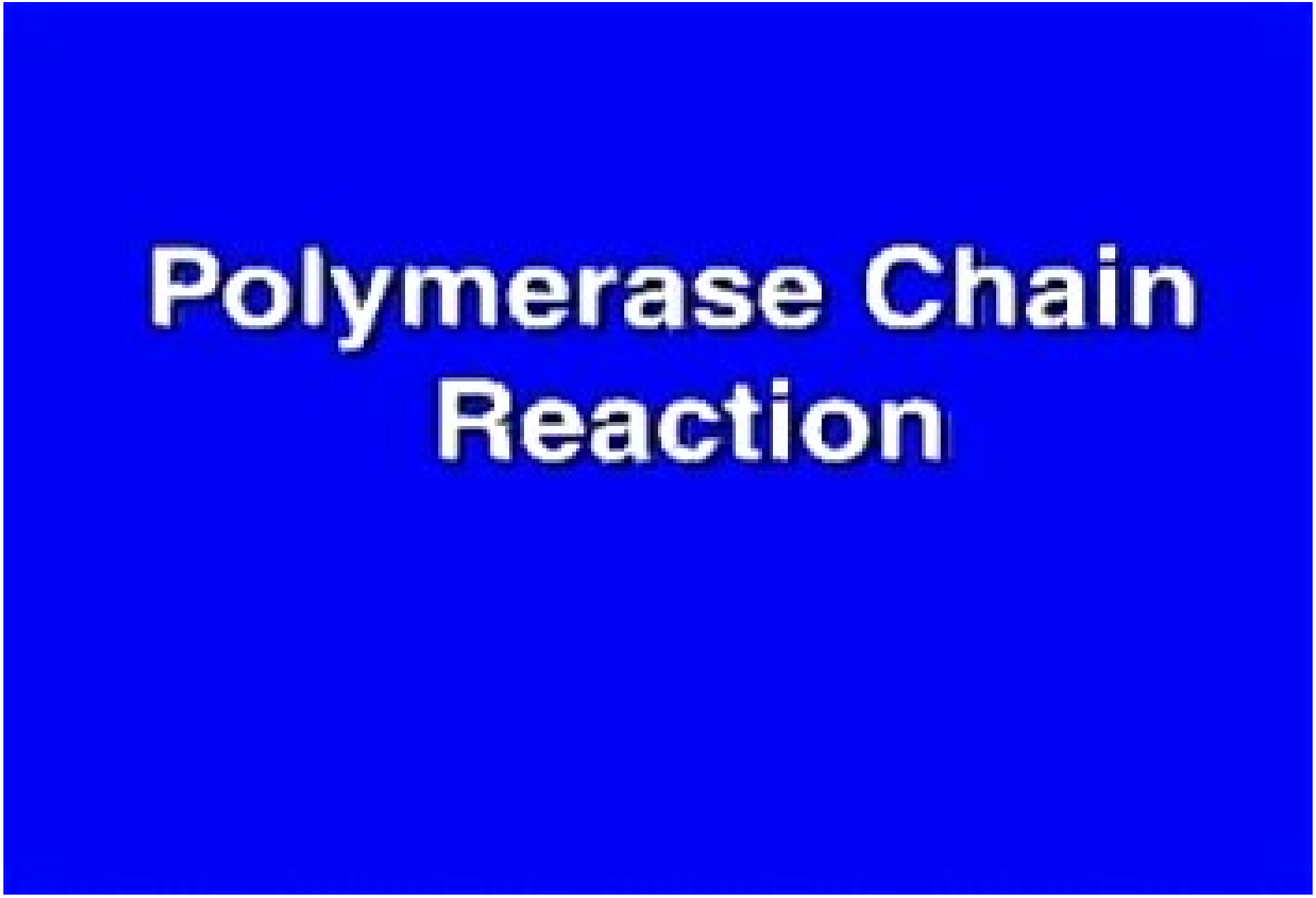
Plant transformed seedlings in soil.

http://www.bch.msu.edu/pamgreen/green.htm

Osnova

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

PCR



Osnova

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

Shrnutí

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

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