

CG020 Genomika

Přednáška 12

Nástroje systémové biologie
Modelové organismy, PCR a zásady navrhování primerů

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Mendelovo centrum genomiky a proteomiky rostlin,
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INVESTICE DO ROZVOJE VZDĚLÁVÁNÍ

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

Genomika 12

▪ Zdrojová literatura

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- Roscoe B. Jackson Memorial Laboratory., and Green, E.L. (1966). *Biology of the laboratory mouse*. (New York: Blakiston Division) <http://www.informatics.iax.org/greenbook/index.shtml>
- Eden, E., Navon, R., Steinfeld, I., Lipson, D., and Yakhini, Z. (2009). GOzilla: a tool for discovery and visualization of enriched GO terms in ranked gene lists. *BMC Bioinformatics* 10, 48.
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- 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)



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pro konkurenceschopnost



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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)



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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**?

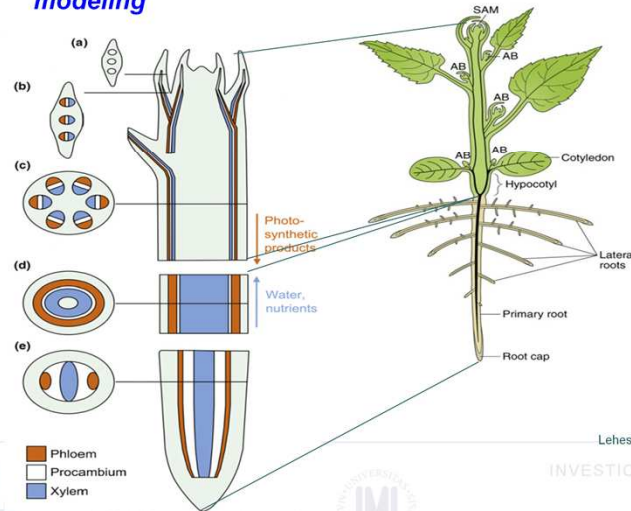
Ddii et al., unpublished

gene	locus	sample_1	sample_2	status	value_1	value_2	log2(fold_change)	test_stat	p_value	q_value	significant
AT1G07795	1:2414285-2414867	WT	MT	OK	0	1.1804179769e+308		1.73769e+308	6.88885e-05	0.00039180	1 yes
HRS1	1:4556891-4558708	WT	MT	OK	0	0.6965831.79769e+308		1.79769e+308	6.61994e-06	0.00053505	yes
ATMLC14	1:8227472-8232296	WT	MT	OK	0	0.5146091.79769e+308		1.79769e+308	9.74219e-05	0.00053505	2 yes
NRT1.6	1:9400663-9403789	WT	MT	OK	0	0.8776651.79769e+308		1.79769e+308	3.2692e-08	3.50131e-07	yes
AT1G27570	1:9575425-9582376	WT	MT	OK	0	2.08291.79769e+308		1.79769e+308	9.76039e-06	6.647e-05	yes
AT1G60095	1:22159735-22162419	WT	MT	OK	0	0.6885881.79769e+308		1.79769e+308	9.95901e-08	9.84992e-07	yes
AT1G03020	1:698206-698515	WT	MT	OK	0	1.788591.79769e+308		1.79769e+308	0.00913915	0.0277558	yes
AT1G13609	1:4662720-4663471	WT	MT	OK	0	3.558141.79769e+308		1.79769e+308	0.00021683	0.00108079	yes
AT1G21550	1:7553100-7553876	WT	MT	OK	0	0.5628681.79769e+308		1.79769e+308	0.00115582	0.00471497	yes
AT1G22120	1:7806308-7806632	WT	MT	OK	0	0.6173541.79769e+308		1.79769e+308	2.48392e-06	1.91089e-05	yes
AT1G31370	1:11236297-11239363	WT	MT	OK	0	1.462541.79769e+308		1.79769e+308	4.83523e-05	0.00028514	3 yes
APUM10	1:13253397-13255570	WT	MT	OK	0	0.5810311.79769e+308		1.79769e+308	7.87855e-06	5.46803e-05	yes
AT1G48700	1:18010728-18012671	WT	MT	OK	0	0.5565251.79769e+308		1.79769e+308	6.53917e-05	0.00037473	6 yes
AT1G59077	1:21746209-21833195	WT	MT	OK	0	138.8861.79769e+308		1.79769e+308	0.00122789	0.00496816	yes
AT1G60050	1:22121549-22123702	WT	MT	OK	0	0.3700871.79769e+308		1.79769e+308	0.00117953	0.0048001	yes
AT4G15242	4:8705786-8706997	WT	MT	OK	0.00930712	17.9056	10.9098	-4.405231.05673e-05	7.13983e-05	0 yes	
AT5G33251	5:12496071-12500433	WT	MT	OK	0.0466375	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	

Example of an output of transcriptional profiling study using Illumina sequencing performed in our lab. Shown is just a tiny fragment of the complete list, comprising about 7K genes revealing differential expression in the studied mutant.

Molecular Regulatory Networks Modeling

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



Lehesranta et al., *Trends in Plant Sci* (2010)



MINISTERSTVO AGRÁRNÍ VÝROBY A ROZVOJE
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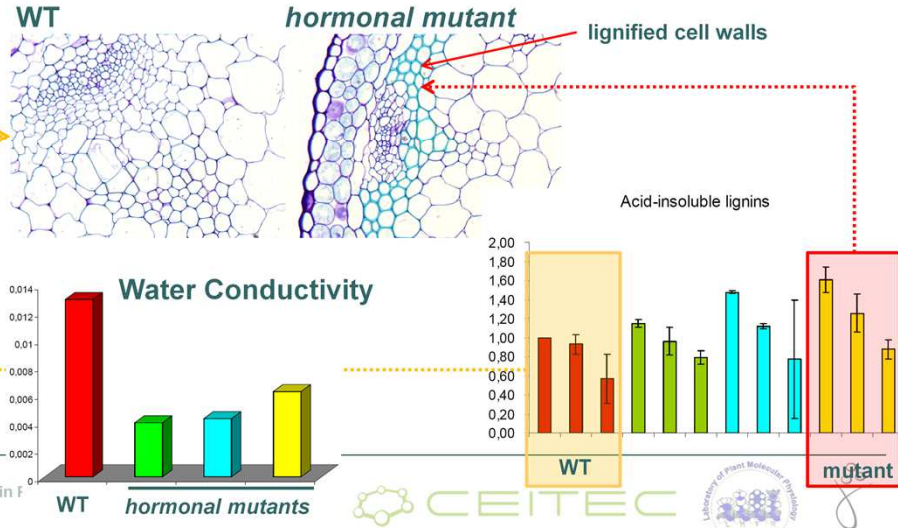
INVESTICE DO ROZVOJE VZDĚLÁVÁNÍ

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Hormonal Control Over Vascular Tissue Development

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



Hormonal Regulation in F

WT hormonal mutants

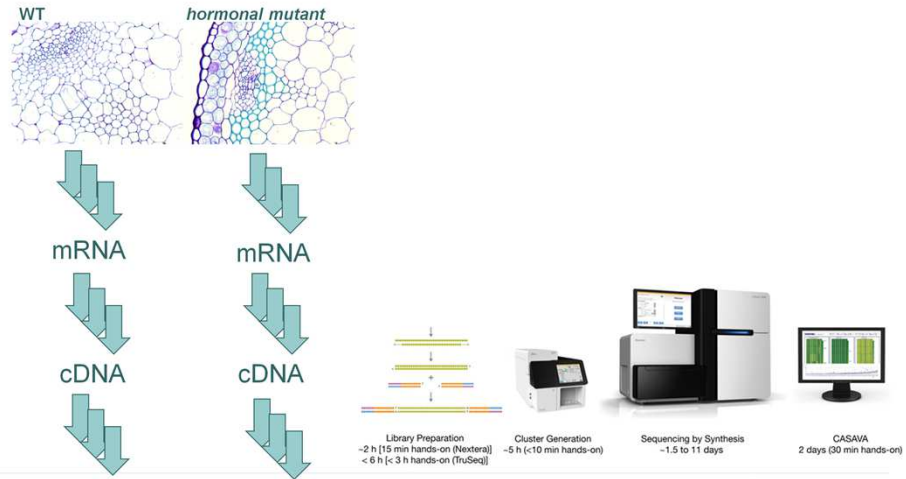
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Hormonal Control Over Vascular Tissue Development

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



Sequencing by Illumina and **number of transcripts** determination

Hormonal Regulation of Vascular Development

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Results of -omics Studies vs Biologically Relevant Conclusions

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

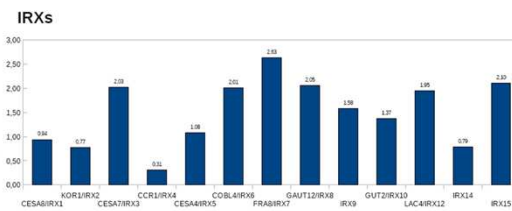
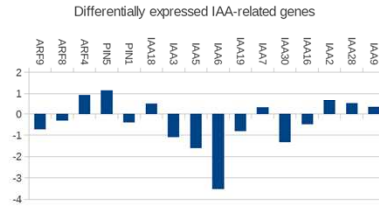
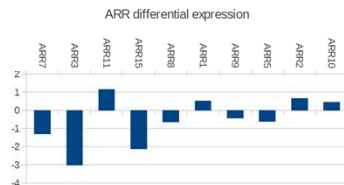
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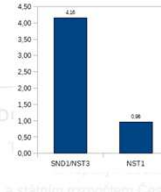
Example of an output of transcriptional profiling study using Illumina sequencing performed in our lab. Shown is just a tiny fragment of the complete list, comprising about 7K genes revealing differential expression in the studied mutant.

Gene Ontology Analysis

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



XYLEM MARKERS



Ddii et al., unpublished

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INVESTICE D

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Gene Ontology Analysis

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

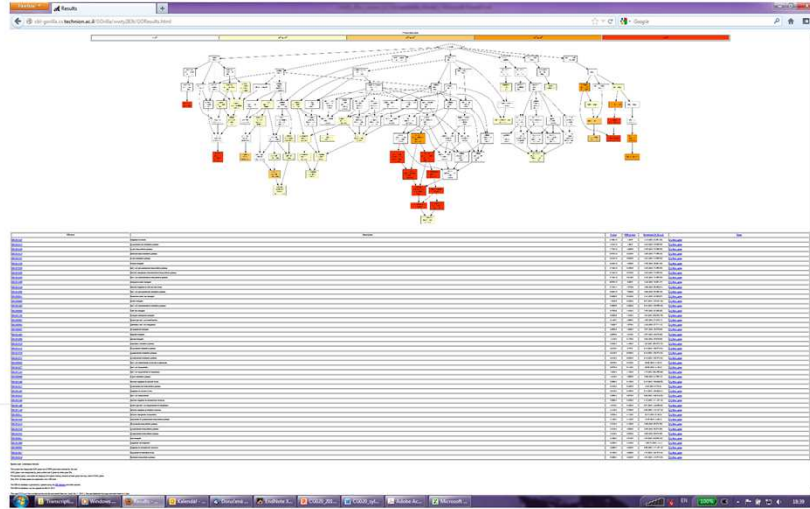
Eden et al., *BMC Bioinformatics* (2009)

The screenshot shows the GOrilla web application interface. At the top, the title "GOrilla" is displayed with the subtitle "Gene Ontology enrichment analysis and visualization tool". Below the title is a small graphic of a gorilla. The main content area contains instructions for using the tool, including a list of steps: 1. Searching for enriched GO terms that appear directly 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. The interface includes a "Step 1: Choose organism" dropdown menu, a "Step 2: Choose ranking mode" section with radio buttons for "Single ranked list of genes" and "Two ranked lists of genes (target and background lists)", and a "Step 3: Paste a ranked list of gene symbols names" section with a text input field and a "Choose an ontology" dropdown menu. The bottom of the interface features a "Search Enriched GO terms" button and a "Reset form" button. The browser's address bar shows the URL "http://cbl-gorilla.cs.technion.ac.il/".

One of such recent and very useful tools is Gorilla software, freely available at <http://cbl-gorilla.cs.technion.ac.il/>.

Gene Ontology Analysis

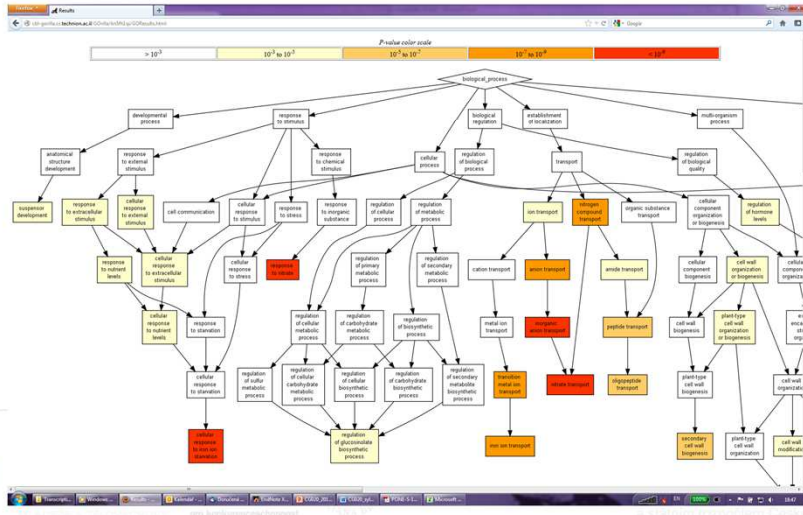
- Several tools allow **statistical evaluation** of **enrichment** for genes **associated with specific processes**



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Gene Ontology Analysis

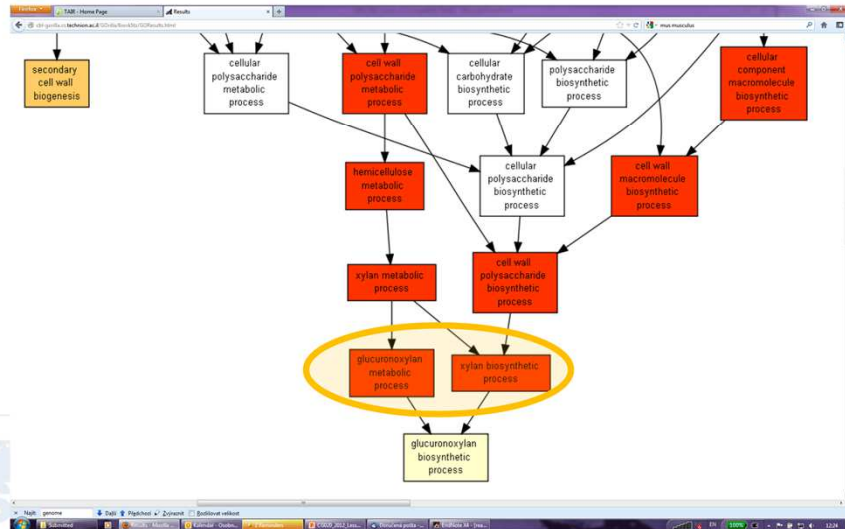
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Gene Ontology Analysis

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



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fondom
equality

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



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random
epublika

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
				[+] Hide genes
				GUT2 - putative glycosyltransferase
				PGSP1 - plant glycoprotein-like starch maturation protein 3
				PKA8 - exochitinase-like protein
				GAUT11 - alpha-1,4-galacturonosyltransferase
				AT1G021400 - bifunctional inhibitor lipid-transfer protein/seed storage 2a albumin-like protein
				AT1G042180 - peroxidase 64
				AT1G019910 - ring-h2 finger protein at172
				LACT1 - lactase 17
				KNAT1 - homeobox protein knat6d-1-like 7
				NAC013 - nac-domain-containing protein 12
				IREX9 - nucleotide-diphosphate-sugar transferase-like protein
				AT1G03500 - protein tyrosin-like protein
				CESA4 - cellulose synthase 4 catalytic subunit 4 [wlp-forming]
				AT1G008340 - rhu gpiase activating protein with pak-box/p11-aha-binding domain
				CTL3 - chitinase-like protein 2
				IRX6 - xylanase-like protein 4
				MYB61 - myb-domain protein 63
				PGSP1 - plant glycoprotein-like starch maturation protein 1
				AT1G046340 - putative alpha-acetyltransferase
				AT1G021710 - hypothetical protein
				AT1G003200 - aspartyl protease-like protein
				AT1G009440 - protein kinase family protein
				AT1G040200 - pathogenesis-related thionin-like protein
				AT1G021090 - targeting protein for skp2-like protein
				AT1G067110 - hypothetical protein
				AT1G062310 - mb-pou domain-containing protein
				AT1G019190 - hypothetical protein
				IRP8 - putative polyglucanase non-catalytic subunit ip810
				MAP16.5 - microtubule-associated protein 16.5
				AT1G032200 - hypothetical protein
				ACL44 - protein agmatase-like 44
				IRE11 - lactase 4
				NAC073 - nac-domain containing protein 73
				IRE3 - cellulose synthase 4 catalytic subunit 7 [wlp-forming]
				AT1G021415 - hypothetical protein
				MYB46 - transcription factor myb46
				AT1G032200 - ring-h2 finger protein at174
				FRD3 - maize rffla family protein
				AT1G033800 - hypothetical protein



Osnova

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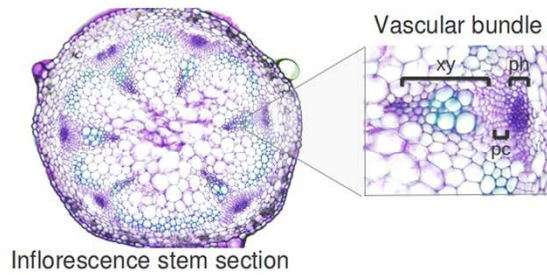


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Molecular Regulatory Networks Modeling

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



Benitez and Hejatko, *submitted*



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země České republiky



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]

Signaling and Homeostasis

Benitez and Hejatko, submitted

Signaling and Homeostasis





Molecular Regulatory Networks Modeling

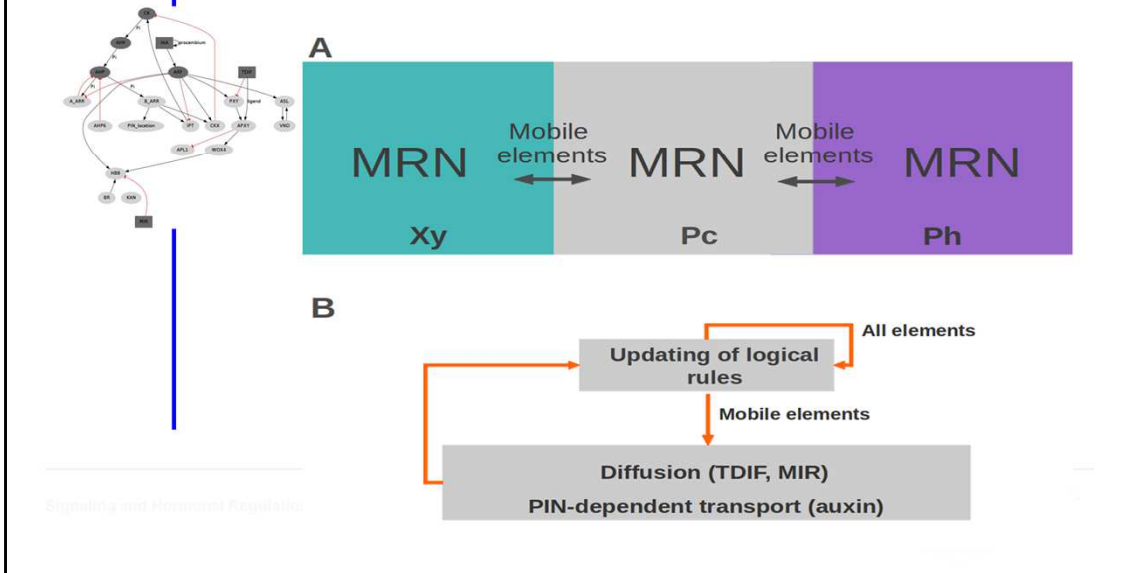
- Formulating *logical rules* defining the *model dynamics*

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



According to experimental evidence for the system under study, the hormone IAA, the peptide TDIF, and the microRNA MIR165/6 are able to move among the cells. In the case of TDIF and MIR165/6, the mobility is defined as diffusion and is given by the following equation:

$$g(t+1)T[i] = H(g(t)[i] + D(g(t)[i+1] + g(t)[i-1] - N(g(t)[i])) - b) \quad (2),$$

where $g(t)T[i]$ is the total amount of TDIF or MIR165 in cell (i). D is a parameter that determines the proportion of g that can move from any cell to neighboring ones and is correlated to the diffusion rate of g . b is a constant corresponding to a degradation term. H is a step function that converts the continuous values of g into a discrete variable that may attain values of 0, 1 or 2. N stands for the number of neighbors in each cell. Boundary conditions are zero-flux. In the case of IAA, the mobility is defined as active transport dependent on the radial localization of the PIN efflux transporters and is defined by the equation:

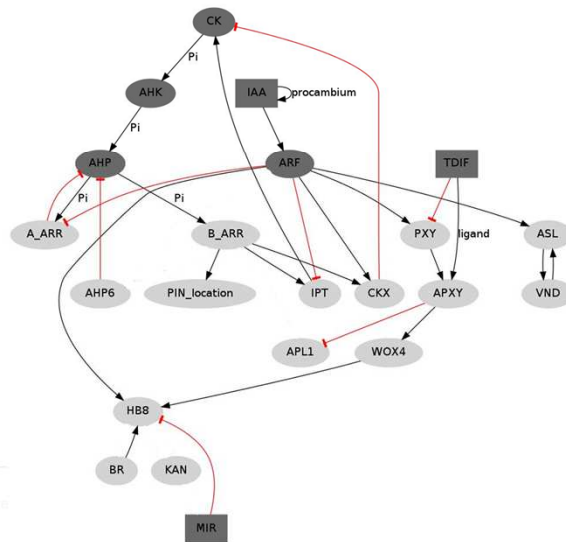
$$iaa(t+1)T[i] = H(iaa(t)[i] + Diaa(pin(t)[i+1])(iaa(t)[i+1]) + Diaa(pin(t)[i-1])(iaa(t)[i-1]) - N(Diaa)(pin(t)[i])(iaa(t)[i]) - b) \quad (3),$$

where $Diaa$ is a parameter that determines the proportion of IAA that can be transported among cells. The transport depends on the presence of IAA and PIN in the cells and b corresponds to a degradation term. As in equation 2, H is a step function

that converts the continuous values to discrete ones and N stands for the number of neighbors in each cell. Boundary conditions for IAA motion are also zero-flux.

Molecular Regulatory Networks Modeling

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



The proposed model considers data that we identified and evaluated through an extensive search (up to January 2012). It takes into account molecular interactions, hormonal and expression patterns, and cell-to-cell communication processes that have been reported to affect vascular patterning in the bundles of Arabidopsis. The model components and interactions are graphically presented in the figure above. In the network model, nodes stand for molecular elements regulating one another's activities. Most of the nodes can take only 1 or 0 values (light gray nodes in the figure), corresponding to "present" or "not present," respectively. Since the formation of gradients of hormones and diffusible elements may have important consequences in pattern formation, mobile elements TDIF and MIR, as well as members of the CK and IAA signaling systems, can take 0, 1 or 2 values (dark gray nodes in the figure above) Benitez and Hejatko, submitted.



Molecular Regulatory Networks Modeling

□ Specifying of missing interactions via *informed predictions*

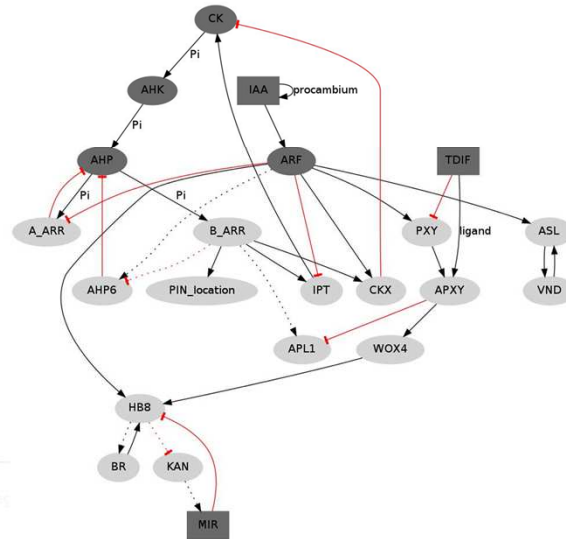
Interaction	Evidence	References
CK → PIN7 radial localization	Predicted interaction (could be direct or indirect)	
	Informed by the following data:	
	During the specification of root vascular cells in <i>Arabidopsis thaliana</i> , CK regulates the radial localization of PIN7.	[18]
CK → APL	Expression of PIN7::GFP and PIN7::GUS is upregulated by CK with no significant influence of ethylene.	[18,20]
	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.	[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.	[21]
	Partially supported by microarray data and phloem-specific expression patterns of CK response factors.	(TAIR, ExpressionSet:1005823559, [22])

Signaling and Hormon...

PLoS ONE | DOI:10.1371/journal.pone.0152107

Molecular Regulatory Networks Modeling

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

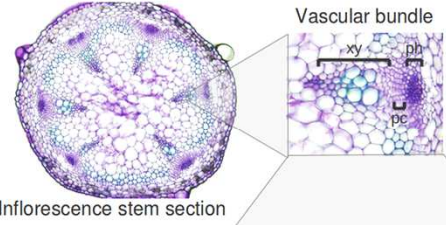
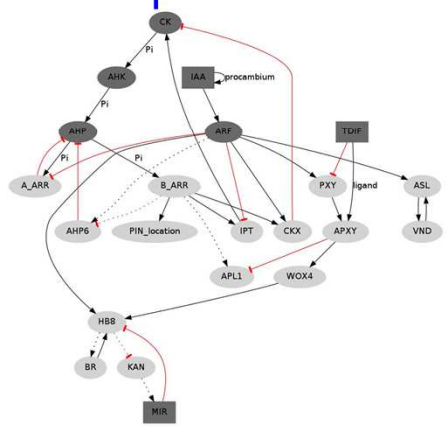


In comparison to the model shown on slide 21, the final version of the model contains the predicted interactions (dashed lines).



Molecular Regulatory Networks Modeling

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



Inflorescence stem section

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

Benitez and Hejatko, *PlosONE*, 2013

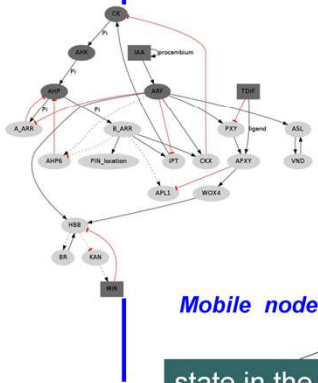
Signaling and Hormonal Regulation of Plant Development





Molecular Regulatory Networks Modeling

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



logical rule function state in the time t

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

state in the time $t+1$

Amount of 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



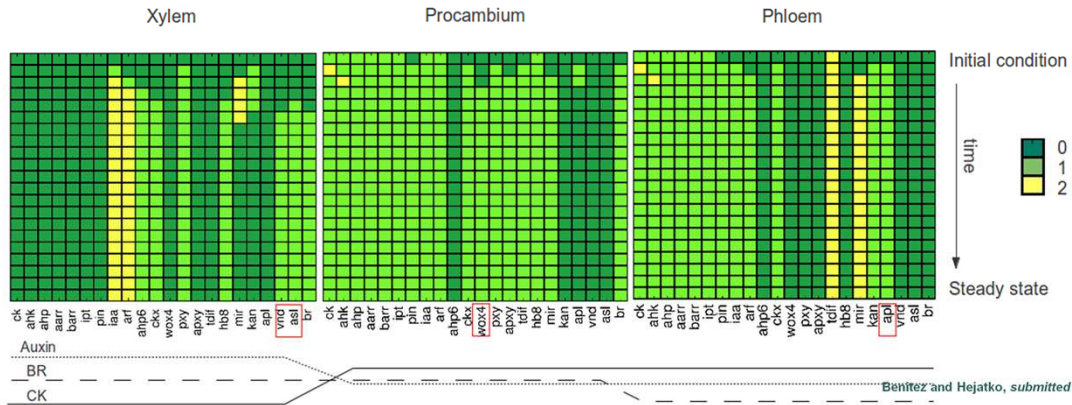


Molecular Regulatory Networks Modeling

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

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

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



The initial conditions specify the initial state of some of the network elements (figure above) and are the following :

I) In the procambial position (central compartment), CK is initially available and there is an initial and sustained IAA input or self-upregulation. This condition is supported by several lines of evidence. Also *HB8*, a marker of early vascular development that has been found in preprocambial cells, is assumed to be initially present at this position. These conditions are not fixed, however. After the initial configuration, all the members of the CK and IAA signaling pathways, as well as *HB8*, can change their states according to the logical rules.

II) In the xylem and phloem positions, it is assumed that no element is initially active except for the CK signaling pathway and TDIF, both in the phloem position. The level of expression for a given node is represented by a discrete variable g and its value at a time $t+1$ depends on the state of other components of the network (g_1, g_2, \dots, g_N) at a previous time unit. The state of every gene g therefore changes according to:

$$g_n(t+1) = F_n(g_{n1}(t), g_{n2}(t), \dots, g_{nk}(t)) \quad (1).$$

In this equation, $g_{n1}, g_{n2}, \dots, g_{nk}$ are the regulators of gene g_n and F_n is a discrete function known as a logical rule (logical rules are grounded in available

experimental data, for example see slide 20). Given the logical rules, it is possible to follow the dynamics of the network for any given initial configuration of the nodes expression state. One of the most important traits of dynamic models is the existence of steady states in which the entire network enters into a self-sustained configuration of the nodes state. It is thought that in developmental systems such self-sustained states correspond to particular cell types.

According to experimental evidence for the system under study, the hormone IAA, the peptide TDIF, and the microRNA MIR165/6 are able to move among the cells. In the case of TDIF and MIR165/6, the mobility is defined as diffusion and is given by the following equation:

$$g(t+1)T[i] = H(g(t)[i] + D(g(t)[i+1] + g(t)[i-1] - N(g(t)[i])) - b) \quad (2),$$

where $g(t)T[i]$ is the total amount of TDIF or MIR165 in cell (i). D is a parameter that determines the proportion of g that can move from any cell to neighboring ones and is correlated to the diffusion rate of g . b is a constant corresponding to a degradation term. H is a step function that converts the continuous values of g into a discrete variable that may attain values of 0, 1 or 2. N stands for the number of neighbors in each cell. Boundary conditions are zero-flux. In the case of IAA, the mobility is defined as active transport dependent on the radial localization of the PIN efflux transporters and is defined by the equation:

$$iaa(t+1)T[i] = H(iaa(t)[i] + Diaa(pin(t)[i+1])(iaa(t)[i+1]) + Diaa(pin(t)[i-1])(iaa(t)[i-1]) - N(Diaa)(pin(t)[i])(iaa(t)[i]) - b) \quad (3),$$

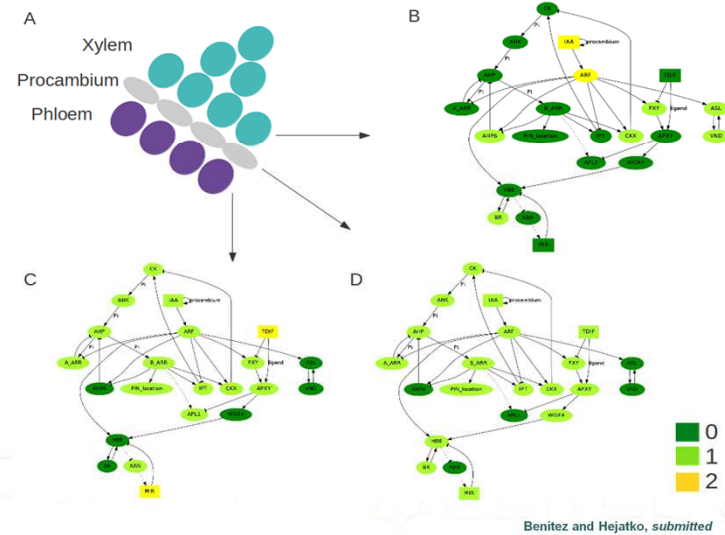
where $Diaa$ is a parameter that determines the proportion of IAA that can be transported among cells. The transport depends on the presence of IAA and PIN in the cells and b corresponds to a degradation term. As in equation 2, H is a step function that converts the continuous values to discrete ones and N stands for the number of neighbors in each cell. Boundary conditions for IAA motion are also zero-flux.

Using the logical rules, equations 1–3, and a broad range of parameter values (not shown here), it is possible fully to reproduce the results and analyses reported in the following sections (see the figure above for the simulation time course).



Molecular Regulatory Networks Modeling

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

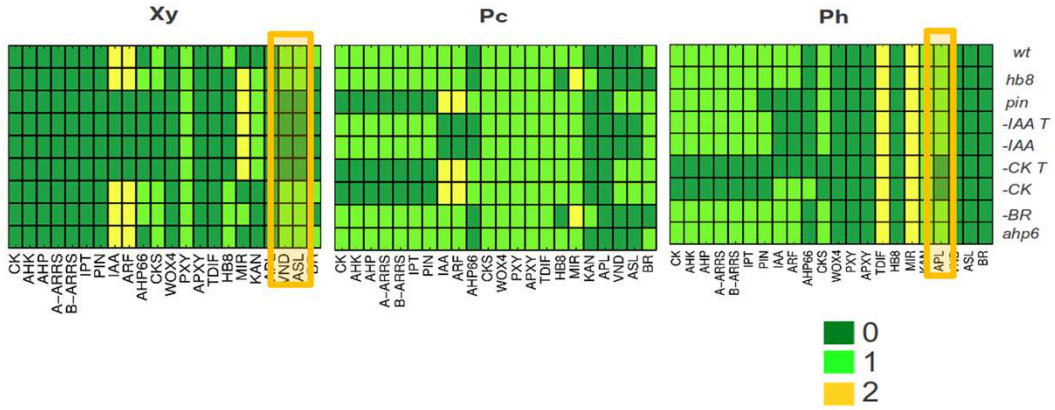
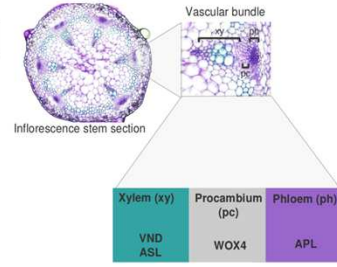


Another representation of the distinct expression profiles in the individual vascular bundle compartments (phloem, procambium and xylem).



Molecular Regulatory Networks Modeling

□ Simulation of *mutants*





Osnova

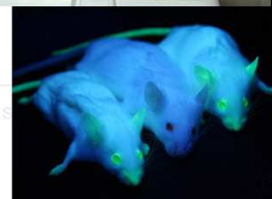
- Nástroje **systemové biologie**
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Mus musculus

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



INVE

NI

More info about mouse at

<http://www.informatics.jax.org/greenbook/index.shtml>.

Mus musculus

myš domácí, house mouse

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

The screenshot displays the 'Mouse Genome Overview' page from the Genome Reference Consortium. The page features a navigation menu at the top with options like 'GRC Home', 'Data', 'Help', 'Report an Issue', 'Contact Us', 'Credits', and 'Curators Only'. Below the navigation, there are tabs for 'Mouse Overview', 'Mouse Issues under Review', 'Mouse Assembly Data', and 'Report a Problem'. The main content area is titled 'Mouse Genome Overview' and includes an ideogram of the mouse genome with chromosomes 1-19, X, and Y. A legend indicates regions with alternate loci (red triangles) and fix patches (orange squares). Text on the page explains the GRC's goal to provide the best possible reference assembly and mentions the next assembly update (GRCm38.p1) scheduled for March 2013. A sidebar on the right contains a 'GRC Blog' with recent posts and a 'References' section.



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BP Vzdělávání pro konkurenceschopnost
EVROPSKÝ SOCIÁLNÍ FOND
Operační program Vzdělávání pro konkurenceschopnost

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spoluřadíme s vámi
Evropským sociálním fondem
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Osnova

- Nástroje **systemové biologie**
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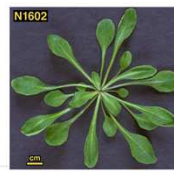
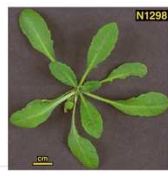
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Arabidopsis thaliana

huseníček polní, mouse-ear cress

- malé nároky na kultivační plochu
- velké množství semen (20.000/roslinu 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/>



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Tato výzkumná činnost byla financována
z státního rozpočtu České republiky

Arabidopsis thaliana

huseníček polní, mouse-ear cress

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

The Arabidopsis Information Resource (TAIR) maintains a database of genetic and molecular biology data for the model higher plant *Arabidopsis thaliana*. Data available from TAIR includes the complete genome sequence along with gene structure, gene product information, metabolism, gene expression, DNA and seed stocks, genome maps, genetic and physical markers, publications, and information about the Arabidopsis research community. Gene product function data is updated every two weeks from the latest published research literature and community data submissions. Gene structures are updated 1-2 times per year using computational and manual methods as well as community submissions of new and updated genes. TAIR also provides extensive links from our data pages to other Arabidopsis resources.

The Arabidopsis Biological Resource Center at The Ohio State University collects, reproduces, preserves and distributes seed and DNA resources of *Arabidopsis thaliana* and related species. Stock information and ordering for the ABRC are fully integrated into TAIR.

TAIR is located at the Carnegie Institution for Science Department of Plant Biology and funded by the National Science Foundation with additional support from TAIR sponsors.

Updates on TAIR funding are available [here](#)

Click here to try our new online submission form
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

Breaking News
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New Set of Confirmed T-DNA Lines Available
November 28, 2012
The fourth one-allele set of confirmed T-DNA lines representing 3,263 new loci is now available for ordering as CS27944.

New from ABRC Education and Outreach
October 31, 2012
ABRC is pleased to announce a re-designed Education and Outreach website at <http://abrcoutreach.osu.edu>. The website allows quick and easy donation of education modules, direct ordering and online evaluation of education kits.

2012 MASCC Report Now Available
July 11, 2012
Please check out the latest report from the Multinational Arabidopsis Steering Committee.

New Protein Chip and Cell Cultures at ABRC
May 9, 2012
A new protein chip (AProteinChip 2) developed by M. Snyder and S.P. Dinash-Kumar is now available. Cell

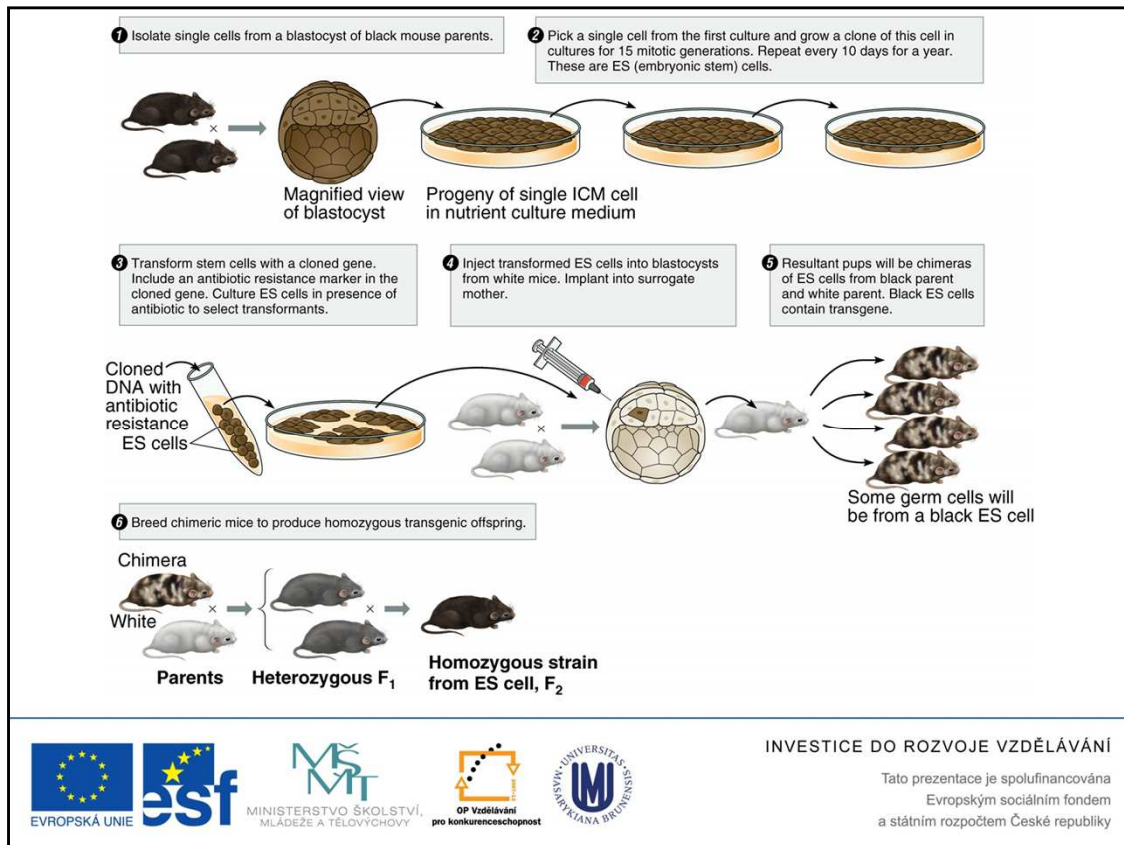
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Individuals ICM cells of the embryo could be isolated and later re-introduced into the new embryo. These ICM cells are called **embryonic stem (ES) cells**. It is a very important technique that allows production of transgenic mice.

The isolated ES cells are transformed via foreign DNA construct and it is injected within the embryo. The transformed cell becomes a part of the embryo and might result in the formation of different tissue types, among them the spermatogonia or oogonia, i.e. the tissue that provides progenitor for sperm or egg cells in the resulting chimera. Thus, the progeny of those chimeras will inherit the modified cell with certain probability and these individuals will carry the transgene in every cell of their body. Thus, the transgenic mice will be produced.

This is very important mainly with regard to the knockout mutant (K.O.) production. In the modified ES, the genes might be specifically eliminated via DNA recombination. In that way, the function of many of the mouse genes was identified.

E.g. the gene *NODAL* is expressed in the anterior portion of the primitive streak that is equivalent to the Hensen's node. *nodal/nodal* embryos are lethal, they do

not undergo gastrulation and form almost no mesoderm.

Transformace *Arabidopsis* prostřednictvím *Agrobacterium tumefaciens*



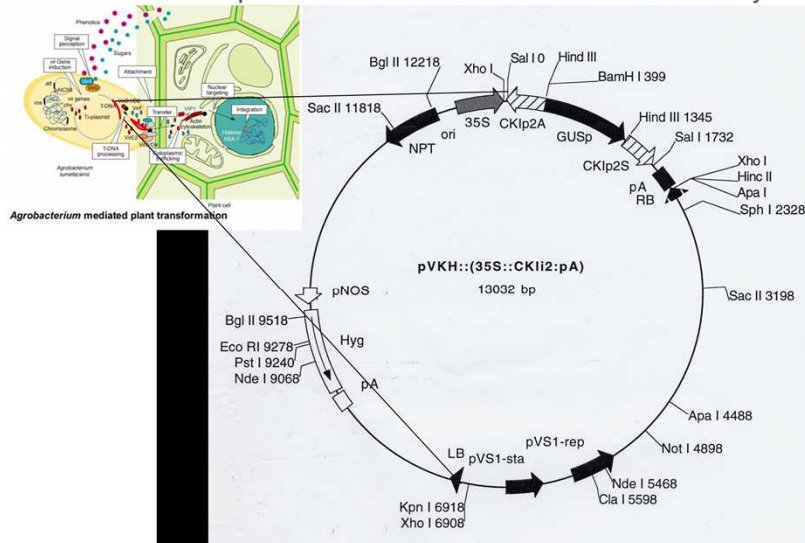
Crown gall of raspberry caused by *Agrobacterium tumefaciens*.



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Transformace *Arabidopsis* prostřednictvím *Agrobacterium tumefaciens* přenos bakteriální DNA do rostlinné buňky



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Transformace kokultivací listových disků



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MINISTERSTVO ŠKOLSTVÍ,
MLÁDEŽE A TĚLOVÝCHOVY

pro konkurenceschopnost

“ZNA BK”

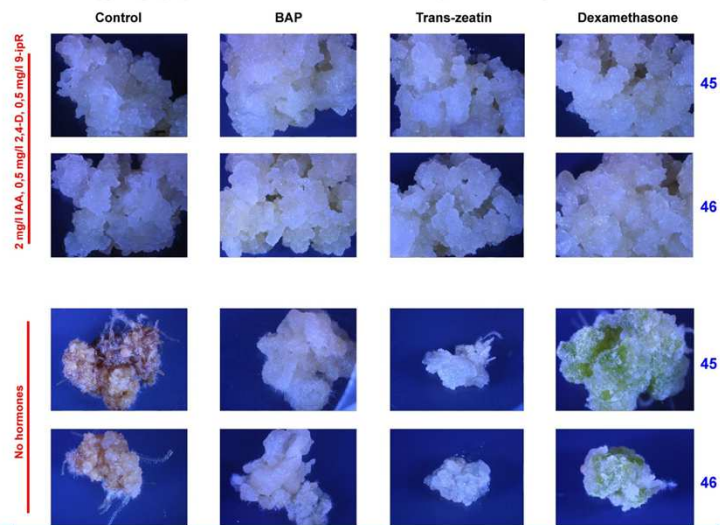
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a státním rozpočtem České republiky

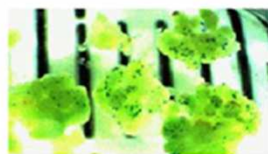
Transformace kokultivací kalusů



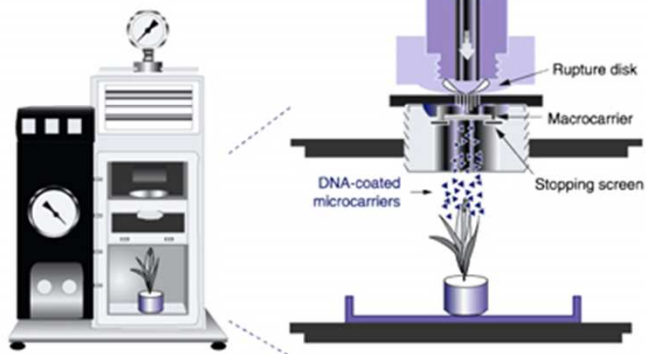
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Transformace „nastřelováním“ DNA



Biolistic delivery of DNA



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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.



EVRO

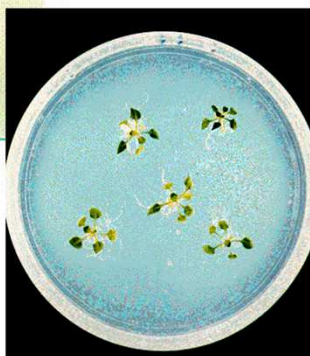
<http://www.bch.msu.edu/pamgreen/green.htm>



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Transformace květenství



<http://www.bch.msu.edu/pamgreen/green.htm>
medium (a 40mg/l kanamycin plate is shown)



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Osnova

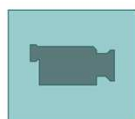
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PCR



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 - Design a příprava primerů (Dr. Hana Konečná)



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Shrnutí

- Nástroje **systemové biologie**
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Diskuse



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