CG020 Genomika

Lesson 2

Genes Identification

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Literature

- Literature sources for Chapter 02:

  - Plant Functional Genomics, ed. Erich Grotewold, 2003, Humana Press, Totowa, New Jersey
  - Majoros, W.H., Pertea, M., Antonescu, C. and Salzberg, S.L. (2003) GlimmerM, Exonomy, and Unveil: three ab initio eukaryotic genefinders. *Nucleic Acids Research*, **31**(13).
  - Singh, G. and Lykke-Andersen, J. (2003) New insights into the formation of active nonsensemediated decay complexes. *TRENDS in Biochemical Sciences*, **28** (464).
  - Wang, L. and Wessler, S.R. (1998) Inefficient reinitiation is responsible for upstream open reading frame-mediated translational repression of the maize R gene. *Plant Cell*, **10**, (1733)
  - de Souza et al. (1998) Toward a resolution of the introns earlyylate debate: Only phase zero introns are correlated with the structure of ancient proteins *PNAS*, **95**, (5094)
  - Feuillet and Keller (2002) Comparative genomics in the grass family: molecular characterization of grass genome structure and evolution *Ann Bot,* 89 (3-10)
  - Frobius, A.C., Matus, D.Q., and Seaver, E.C. (2008). Genomic organization and expression demonstrate spatial and temporal Hox gene colinearity in the lophotrochozoan Capitella sp. I. PLoS One 3, e4004

Outline

- Forward and Reverse Genetics Approaches
  - Differences between the approaches used for identification of genes and their function
- Identification of Genes *Ab Initio*
  - Structure of genes and searching for them
  - Genomic colinearity and genomic homology
- Experimental Genes Identification
  - Constructing gene-enriched libraries using methylation filtration technology
  - EST libraries
  - Forward and reverse genetics

Outline

- Forward and Reverse Genetics Approaches
  - Differences between the approaches used for identification of genes and their function

Forward vs. Reverse Genetics

Revolution in understanding the term „gene“

„classical“ genetics approaches

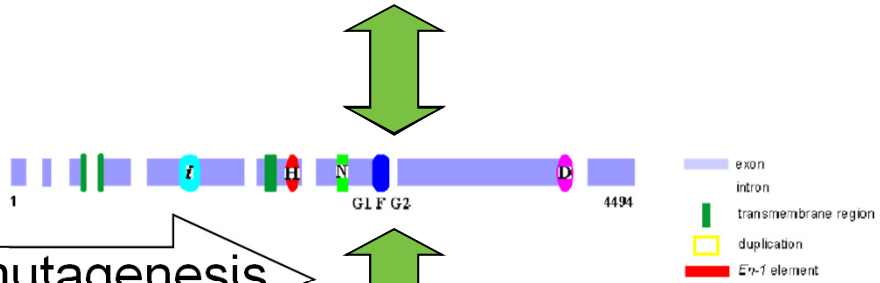
3 : 1

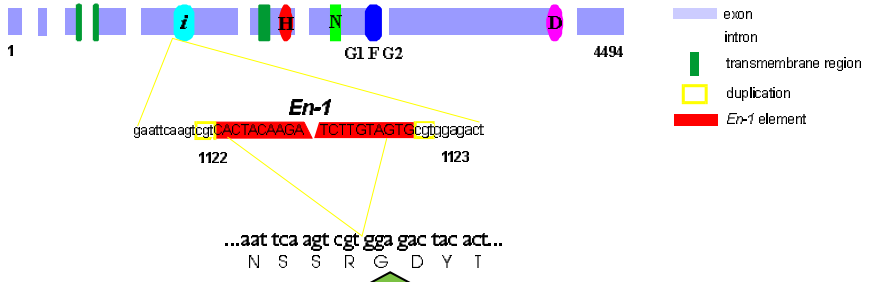
?

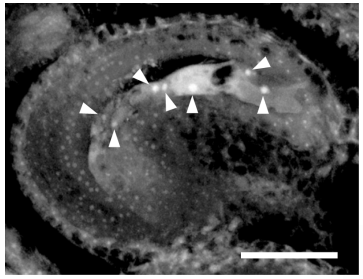
„reverse genetics“ approaches

5‘TTATATATATATATTAAAAAATAAAATAA
AAGAACAAAAAAGAAAATAAAATA….3‘

insertional mutagenesis







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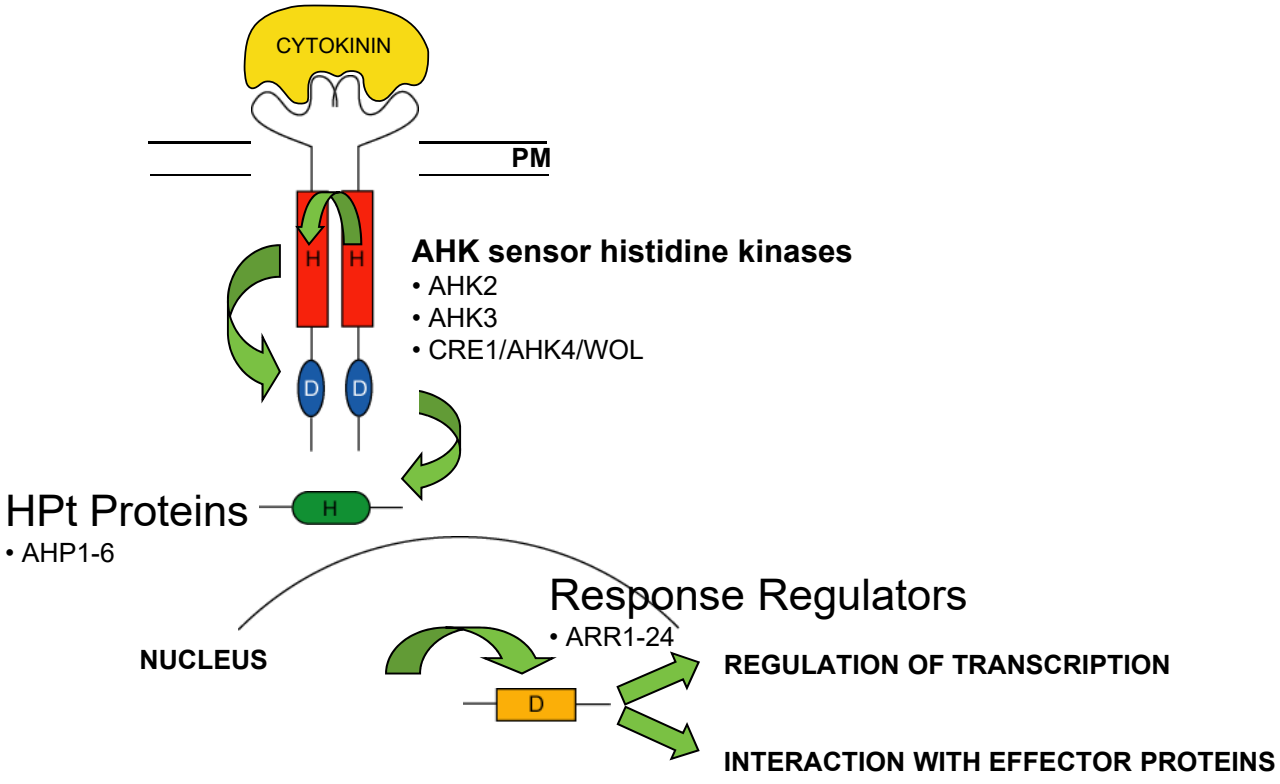
Identification of the role of *ARR21* gene

- Hypothetical signal transducer in two-component system of *Arabidopsis*

Identification of the role of *ARR21* gene

Recent Model of the CK Signaling via Multistep Phosphorelay (MSP) Pathway



Identification of the role of *ARR21* gene

- Hypothetical signal transducer in two-component system of *Arabidopsis*
- Mutant identified by searching in databases of insertional mutants (SINS-sequenced insertion site) using BLAST

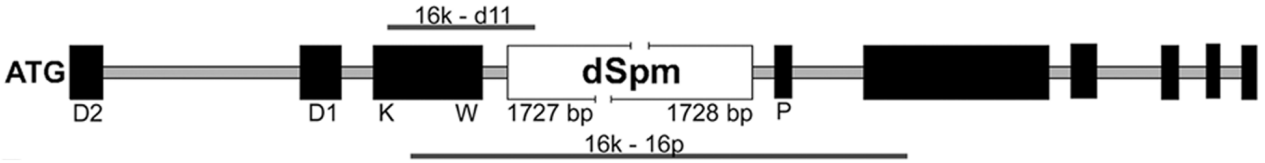
Identification of the role of *ARR21* gene – isolation of insertional mutant

- **Searching in databases of insertional mutants (SINS)**

```
Insert_SINS: 01_09_64
Query: 80    tcctagcgttcatgagcgtaccatacttgacaanagagaacgtagccagccatttacagg 139
             ||||||||||||||||||||||||||||||| |||||||||||||||||||||||||
Sbjct: 58319 tcctagcgttcatgagcgtaccatacttgacaagagagaacgtagccagccatttacagg 58378
Arr21:  1830

Insert_SINS: 01_09_64
Query: 140   tttgatatctcttgtcaaaaatgtttttggattttactgt 179
              ||||||||||||||||||||||||||||||||||||||
Sbjct: 58379 tttgatatctcttgtcaaaaatgtttttggattttactgt 58418
Arr21:  1890
```

- **Localization of *dSpm* insertion in genome sequence of *ARR21* using sequenation of PCR products**



Identification of the role of *ARR21* gene

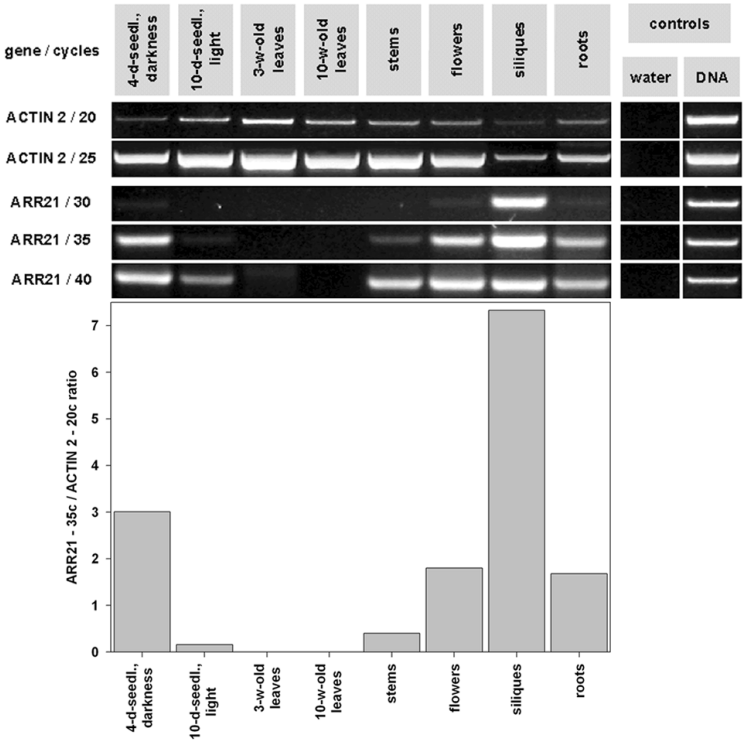
- Hypothetical signal transducer in two-component system of *Arabidopsis*

- Mutant identified by searching in databases of insertional mutants (SINS-sequenced insertion site) using BLAST

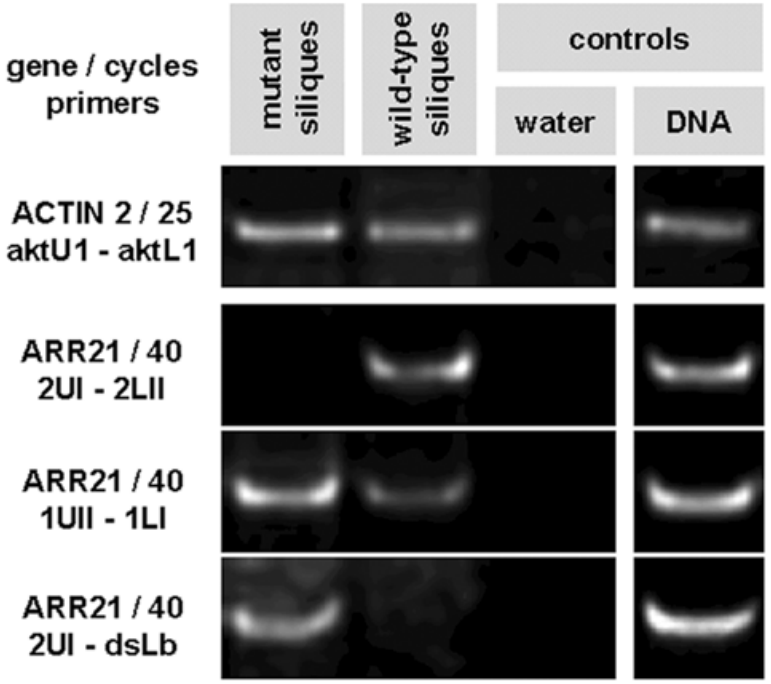
- Expression of *ARR21* in wild-type and inhibition of expression of *ARR21* in insertional mutant confirmed at the RNA level

Identification of the role of *ARR21* gene – analysis of expression

wild type expression



insertional mutant vs wild type



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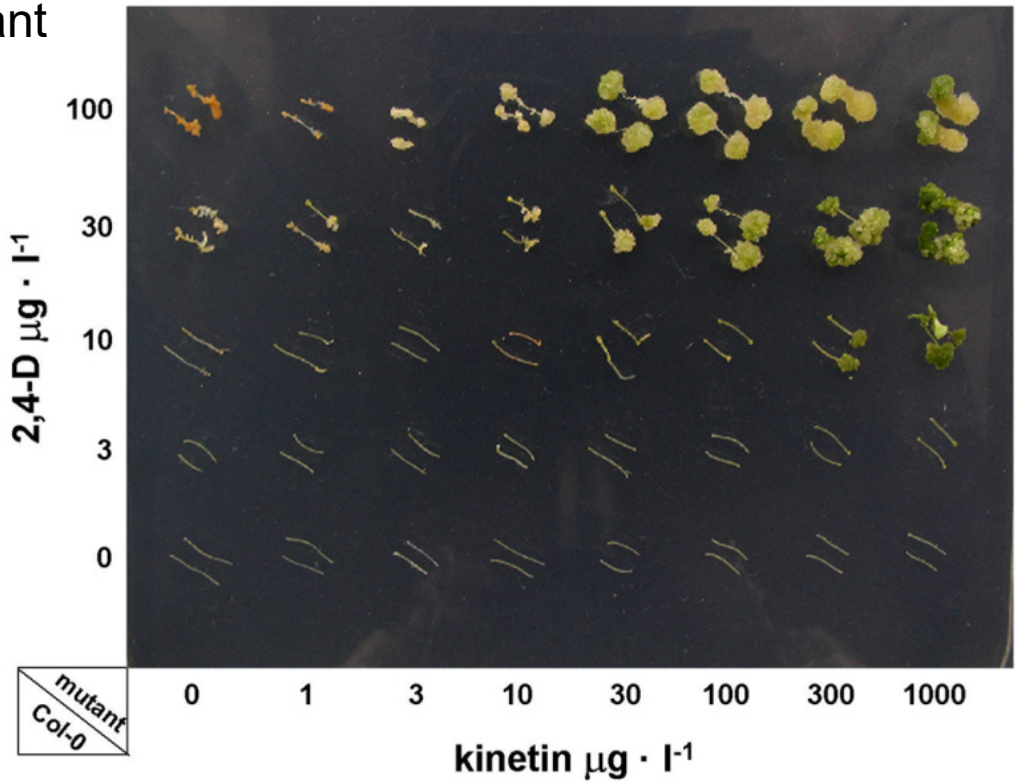
Identification of the role of *ARR21* gene

- Hypothetical signal transducer in two-component system of *Arabidopsis*
- Mutant identified by searching in databases of insertional mutants (SINS-sequenced insertion site) using BLAST
- Expression of *ARR21* in wild-type and inhibition of expression of *ARR21* in insertional mutant confirmed at the RNA level
- Phenotype analysis of insertional mutant

Identification of the role of *ARR21* gene – phenotype analysis of mutant

- Analysis of sensitivity to plant growth regulators
  - 2,4-D a kinetin
  - ethylene
  - Light of various wavelengths

- No alterations - nor in flowering, neither in the number of the seeds



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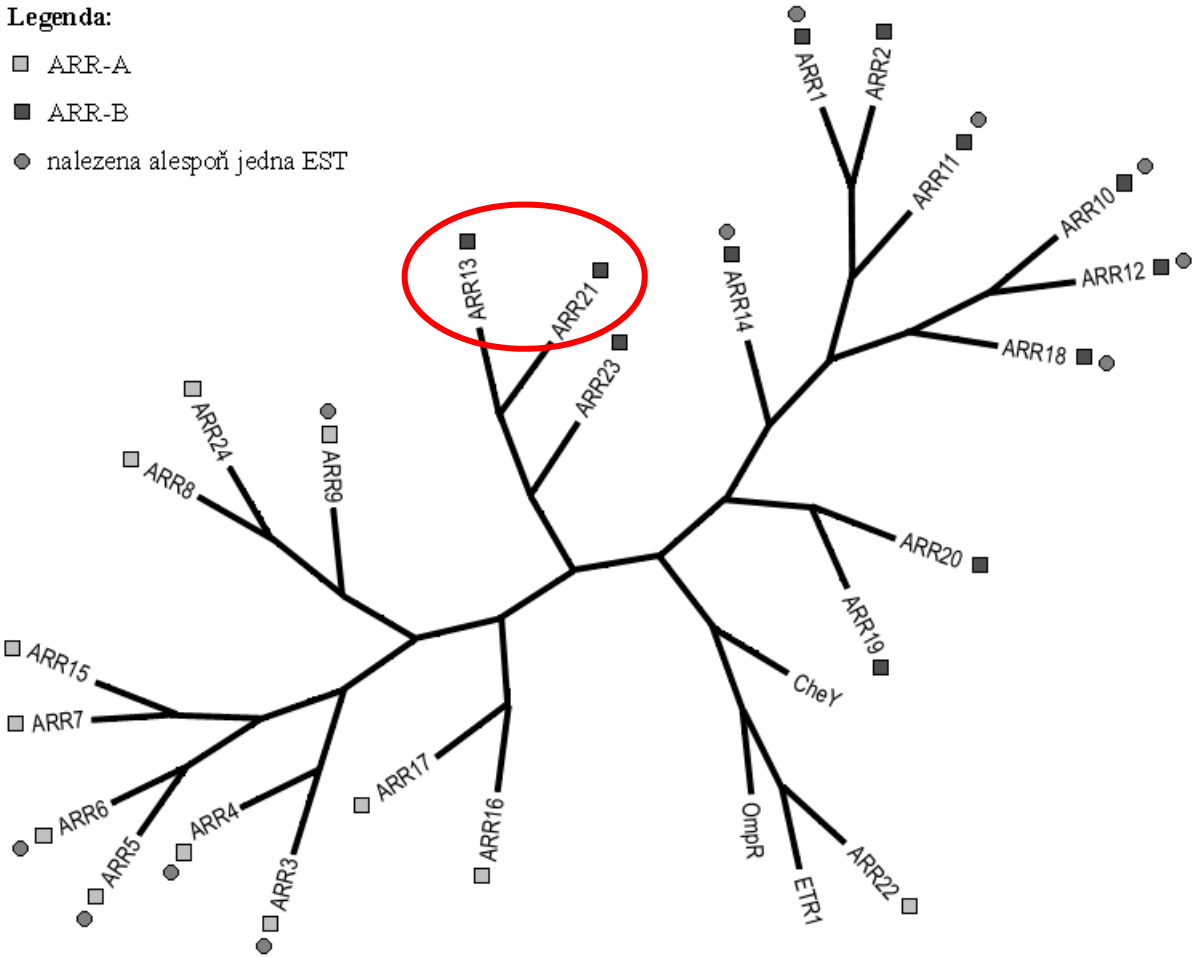
Identification of the role of *ARR21* gene – possible reasons for the absence of the phenotype

- Functional redundance within the gene family

Identification of the role of *ARR21* gene – homology of *ARR* genes



Identification of the role of *ARR21* gene – possible reasons for the absence of the phenotype

- Functional redundance within the gene family?
- Phenotype only under specific conditions

Identification of the role of *ARR21* gene – summary

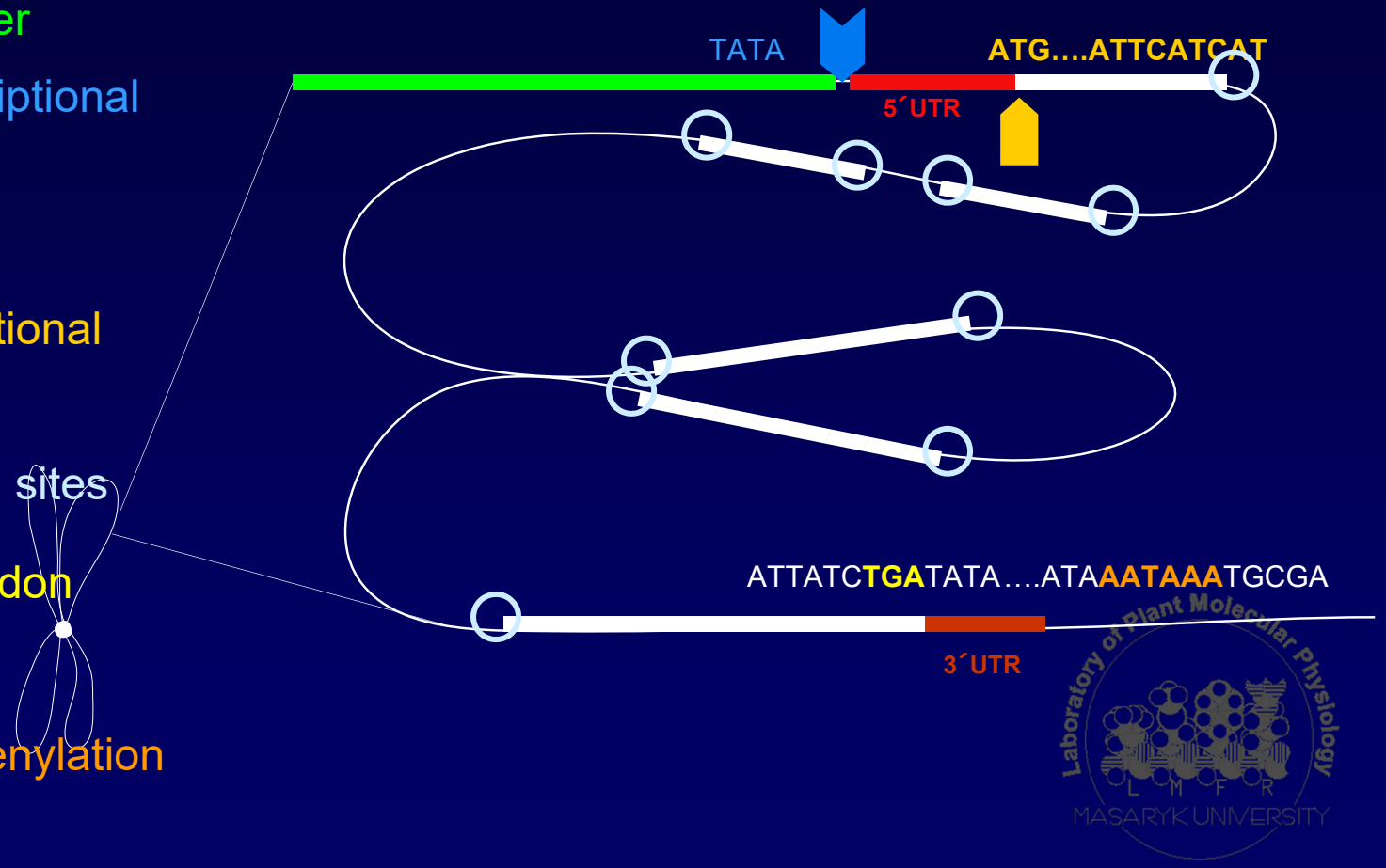
- Gene *ARR21* identified by comparative analysis of *Arabidopsis* genome
- Based on sequence analysis, its function was predicted
- Site-specific expression of *ARR21* gene was proved at the RNA-level
- Identification of gene function by insertional mutagenesis in case of *ARR21* in development of *Arabidopsis* was not successful, probably because of functional redundancy within the gene family

Outline

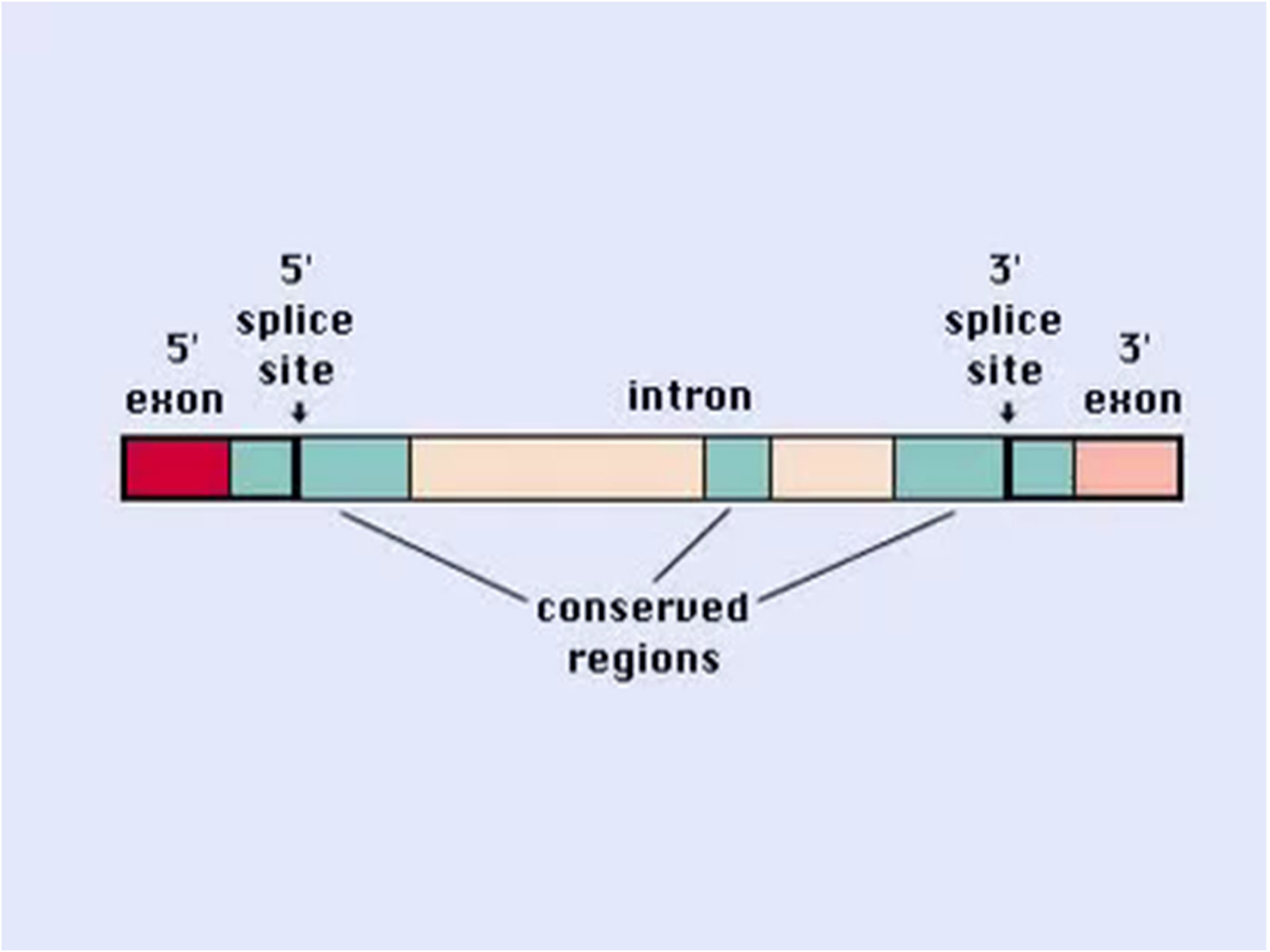
- Forward and Reverse Genetics Approaches
  - Differences between the approaches used for identification of genes and their function
- Identification of Genes *Ab Initio*
  - Structure of genes and searching for them

Genes Structure

- Promoter
- Transcriptional start
- 5´UTR
- Translational start
- Splicing sites
- Stop codon
- 3´UTR
- Polyadenylation signal



RNA Splicing



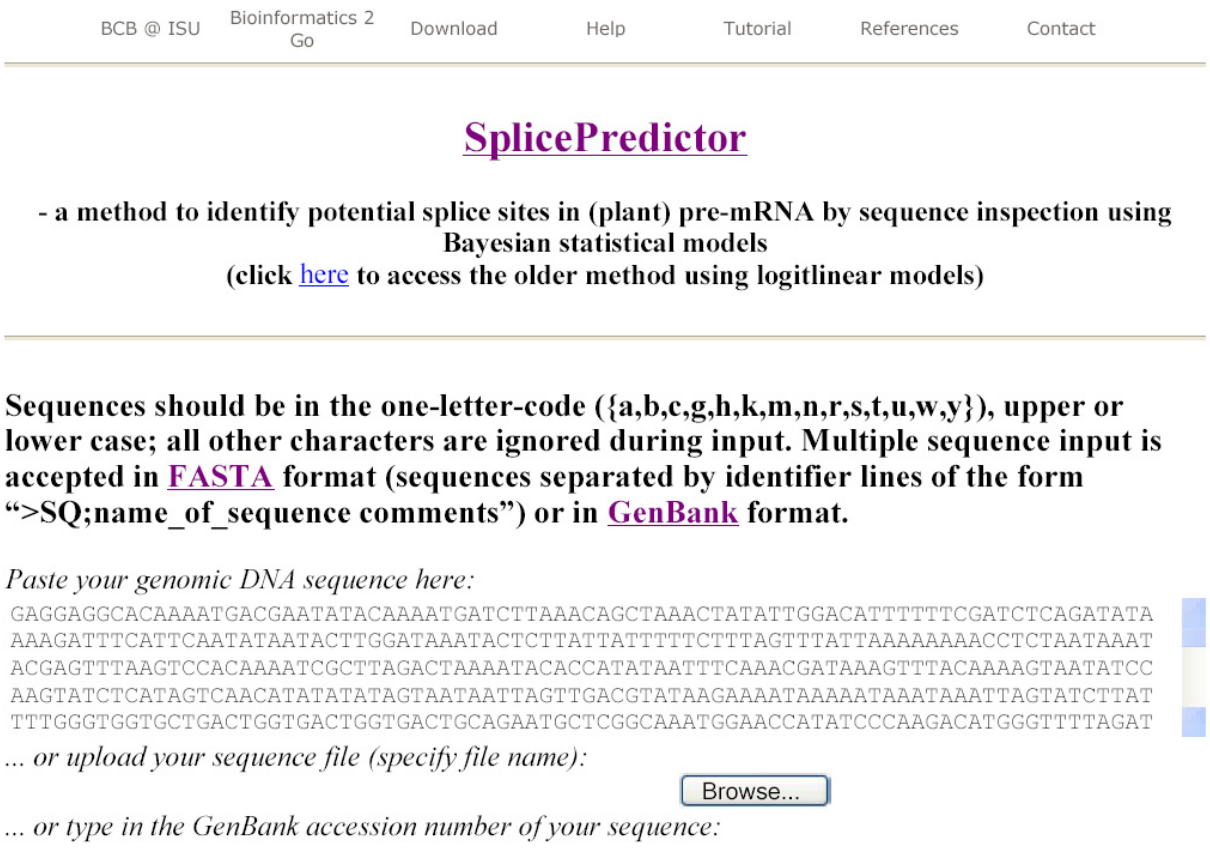
Identification of Genes *Ab Initio*

- Omitting 5‘ and 3‘ UTR
- Identification of translation start (ATG) and stop codon (TAG, TAA, TGA)
- Finding donor (typically GT) and acceptor (AG) splicing sites
- Using various statistic models (e.g. Hidden Markov Model – HMM, see recommended literature, Majoros *et al.*, 2003) to evaluate and score the weight of identified donor and acceptor sites

Splicing Site Prediction

- Programs for splice site prediction (specifity approximately 35 %)
  - GeneSplicer (http://www.tigr.org/tdb/GeneSplicer/gene_spl.html)
  - SplicePredictor (http://deepc2.psi.iastate.edu/cgi-bin/sp.cgi)

SplicePredictor



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SplicePredictor

What do the output columns mean?

```
SplicePredictor.   Version of February 13, 2005.
Date run: Wed Nov  9 11:30:14 2005

Species:                        Homo sapiens
Model:                          2-class Bayesian
Prediction cutoff (2 ln[BF]):     3.00
Local pruning:                  on
Non-canonical sites:            not scored

Sequence    1:   your-sequence, from 1 to 9490.

Potential splice sites

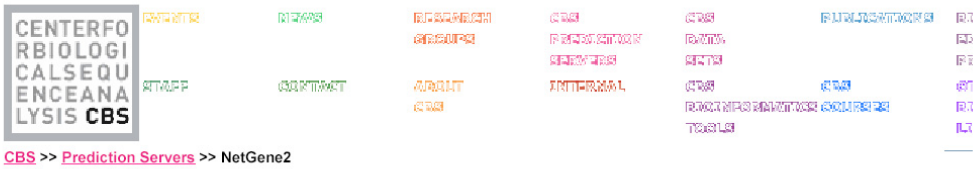
t    q      loc     sequence          P      c      rho    gamma    *  P*R*G*

A    <--     75 ttttttcgatctcAGat     0.973   7.16   0.000  0.000   7  (5 1 1)
A    <--    134 attatttttctttAGtt     0.999  14.86   0.000  0.000   7  (5 1 1)
A    <--    500 gattttgttgtttAGtc     0.977   7.48   0.000  0.000   7  (5 1 1)
A    <--    780 tctgttattgtatAGct     0.986   8.56   0.000  0.000   7  (5 1 1)
A    <--    848 tatttttttgaaatAGat     0.968   6.80   0.000  0.000   7  (5 1 1)
A    <--   1051 caatttatttttaAGaa     0.930   5.19   0.000  0.000   7  (5 1 1)
A    <--   1213 ttatttatttttttAGtt     0.998  12.14   0.000  0.000   7  (5 1 1)
A    <--   1373 tttcctctctcacAGga     0.999  13.17   0.000  0.000   7  (5 1 1)
A    <--   1487 tttatatattgatAGtg     0.883   4.04   0.000  0.000   7  (5 1 1)
A    <--   1581 atgtgttgcttgtAGga     0.982   8.03   0.000  0.000   7  (5 1 1)
A    <--   1781 ggttgtgcgaaatAGgg     0.886   4.10   0.000  0.000   7  (5 1 1)
A    <--   2440 taattaaaaatttAGat     0.939   5.46   0.000  0.000   7  (5 1 1)
A    <--   2479 catctaaaatttttAGat     0.942   5.59   0.000  0.000   7  (5 1 1)
D ----->   2546          aagGTagta     0.909   4.61   0.885  1.903  15  (5 5 5)
A    <--   2572 ttttttttttggcAGca     0.930   5.16   0.000  0.000   7  (5 1 1)
A  <----   2763 ctcaaattcacaaAGgt     0.873   3.86   0.185  0.000  11  (5 5 1)
A  <----   2782 tttcgttttcattAGcg     0.952   5.98   0.220  0.000  11  (5 5 1)
A  <----   3022 tttgtttgtactaAGct     0.956   6.16   0.221  0.000  11  (5 5 1)
A  <----   3048 ctttgcaatacatAGga     0.973   7.15   0.229  0.000  11  (5 5 1)
A    <--   3171 cgtcgtcatttatAGta     0.988   8.74   0.000  0.000   7  (5 1 1)
A  <---    3284 ctttgttatcaaAGgg     0.993  10.03   0.000  0.006   8  (5 1 2)
D ----->   3372          aatGTaagg     0.933   5.28   0.855  1.849  15  (5 5 5)
A  <----   3451 aatgcttcctcgtAGaa     0.916   4.77   0.293  0.065  12  (5 5 2)
A    <--   3581 cgatcgccgttctAGgt     0.850   3.47   0.000  0.000   7  (5 1 1)
D ---->    3649          cacGTatta     0.933   5.25   0.000  1.848  11  (5 1 5)
A    <--   3695 ttgtggttatacaAGtt     0.907   4.56   0.000  0.000   7  (5 1 1)
A  <---    4254 attattgttcttcAGat     0.998  12.82   0.000  0.002   8  (5 1 2)
A    <--   4351 tttcttacattgcAGaa     0.991   9.42   0.000  0.000   7  (5 1 1)
A    <--   4633 gtcttgtttcttttAGgg     0.879   3.97   0.000  0.000   7  (5 1 1)
A    <--   4976 cttgttgtttctcAGct     0.952   5.98   0.000  0.000   7  (5 1 1)
A    <--   5004 ttttttttttgccAGag     0.996  11.17   0.000  0.000   7  (5 1 1)
D ---->    5356          caaGTgaat     0.821   3.04   0.387  0.000  11  (5 5 1)
D ---->    5384          ttgGTaaga     0.941   5.54   0.478  0.090  13  (5 5 3)
A    <--   5403 actctgtttctttAGct     0.894   4.26   0.000  0.000   7  (5 1 1)
A  <----   5441 ctttctctctaacAGaa     0.995  10.43   0.387  0.000  11  (5 5 1)
A  <----   5472 ttgttaaaattacAGct     0.965   6.62   0.478  0.090  13  (5 5 3)
D ----->   5745          gcgGTaaga     0.991   9.48   0.990  1.956  15  (5 5 5)
A  <----   5808 catcatatcctaaAGgt     0.948   5.83   0.458  0.000  11  (5 5 1)
A  <----   6135 ggtctattattatAGgt     0.999  13.59   0.508  0.050  12  (5 5 2)
A    <--   6552 ggattttcacctcAGag     0.938   5.42   0.000  0.000   7  (5 1 1)
```

Splicing Site Prediction

- Programs for splice site prediction (specifity approximately 35 %)
  - GeneSplicer (http://www.tigr.org/tdb/GeneSplicer/gene_spl.html)
  - SplicePredictor (http://deepc2.psi.iastate.edu/cgi-bin/sp.cgi)
  - NetGene2 (http://www.cbs.dtu.dk/services/NetGene2/)

NetGene2



NetGene2 Server

The NetGene2 server is a service producing neural network predictions of splice sites in human, *C. elegans* and *A. thaliai*

Instructions Output format Abstract Performanc

SUBMISSION

Submission of a local file with a single sequence:

File in FASTA format Browse...

- (•) Human
- ( ) C. elegans
- ( ) A. thaliana

Clear fields Send file

Submission by pasting a single sequence:

Sequence name

- ( ) Human
- ( ) C. elegans
- (•) A. thaliana

Sequence

```
GAGGAGGCACAAAATGACGAATATACAAAATGATCTTAAACAGCTAAACTATATTGGACATTTTTTCGATC
TCAGATATA
AAAGATTTCATTCAATATAATACTTGGATAAATACTCTTATTATTTTTCTTTAGTTTATTAAAAAAAACCT
CTAATAAAT
ACGAGTTTAAGTCCACAAAATCGCTTAGACTAAAATACACCATATAATTTCAAACGATAAAGTTTACAAAA
```

Clear fields Send file

NOTE: The submitted sequences are kept confidential and will be erased immediately after processing.

NetGene2

Prediction done

********** NetGene2 v. 2.4 **********

The sequence: Sequence has the following composition:

Length: 9490 nucleotides.
31.8% A, 17.0% C, 19.6% G, 31.7% T, 0.0% X, 36.5% G+C

Donor splice sites, direct strand

pos 5'->3'	phase	strand	confidence	5' exon intron 3'	
1704	0	+	0.87	TTCCAAACAC^GTTAATATTT	
1906	0	+	0.99	CGGTGAACGG^GTCAGAACAT	
3582	1	+	1.00	GCCGTTCTAG^GTAATCTTGC	H
3765	1	+	1.00	TTGCGTCCTG^GTAATTCTGC	H
4134	0	+	0.74	TCAAACACAG^GTTGTTAAAA	
4619	1	+	0.74	AGCAAGAAAG^GTCTTGTTTC	
4915	0	+	0.94	CGTTCCTCTG^GTAAATACTG	
5356	0	+	0.87	TCTCAACCAA^GTGAATGTTT	
5384	1	+	1.00	GATTTGGTTG^GTAAGACTCT	H
5809	1	+	1.00	TATCCTAAAG^GTGTGTCCAA	
6057	0	+	1.00	GCAGTCTTTG^GTAAGCTACT	H
6096	1	+	0.74	CTCTTCACAA^GTAAATCTAG	
7369	0	+	1.00	GGACTGCCAA^GTAAGTTTAA	H
7886	0	+	0.74	GAACAAAATG^GTTAGATGAA	
9323	0	+	0.74	GAAGATTAGG^GTTTTTCTCT	

Donor splice sites, complement strand

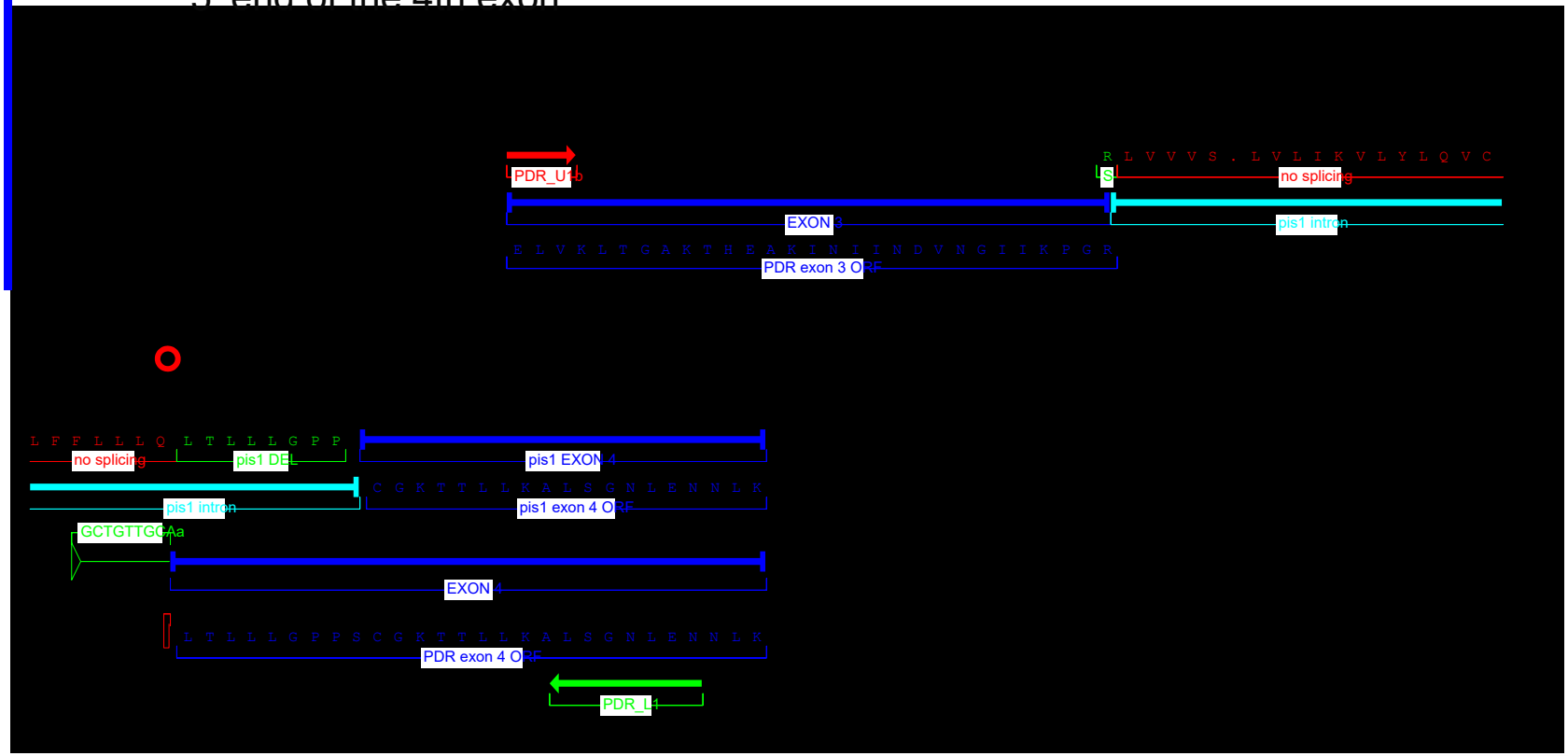
pos 3'->5'	pos 5'->3'	phase	strand	confidence	5' exon intron 3'

Acceptor splice sites, direct strand

pos 5'->3'	phase	strand	confidence	5' intron exon 3'	
1213	0	+	0.59	TATTTTTTAG^TTATGGAGAC	
1221	2	+	0.87	AGTTATGGAG^ACAAGAATCG	
1373	0	+	0.71	TCTCTCACAG^GACACAGAAT	
1487	1	+	0.81	ATATTGATAG^TGGGACATTA	
3284	0	+	0.87	GTTATCAAAG^GGTTTCGACT	
4254	0	+	1.00	TGTTCTTCAG^ATCGCACCAT	H
4832	2	+	0.54	AAAATTGCAG^TTCCAGTGGC	
5004	0	+	0.94	TTTTTGCCAG^AGATACACAC	
5472	1	+	0.96	AAAATTACAG^CTCTGCTCAA	
6135	0	+	1.00	ATTATTATAG^GTAAGATTAA	H
6490	1	+	0.90	AAAGTTACAG^TGGTGGAGAA	
6744	0	+	0.59	TGTCAAACAG^TTTCGTAGAG	
7447	0	+	0.96	TTCTGCACAG^ATGCCAGAAA	
7780	2	+	0.76	TCCATTTCAG^ATACAGAACA	
7786	2	+	0.92	TCAGATACAG^AACACATGCA	

RNA Splicing and Adaptation

- Flexibility in splicing site recognition in plants in practice – example of developmental plasticity of (not only) plants
  - Identification of mutant with point mutation (transition G→A) exactly at the splice site at the 5‘ end of the 4th exon



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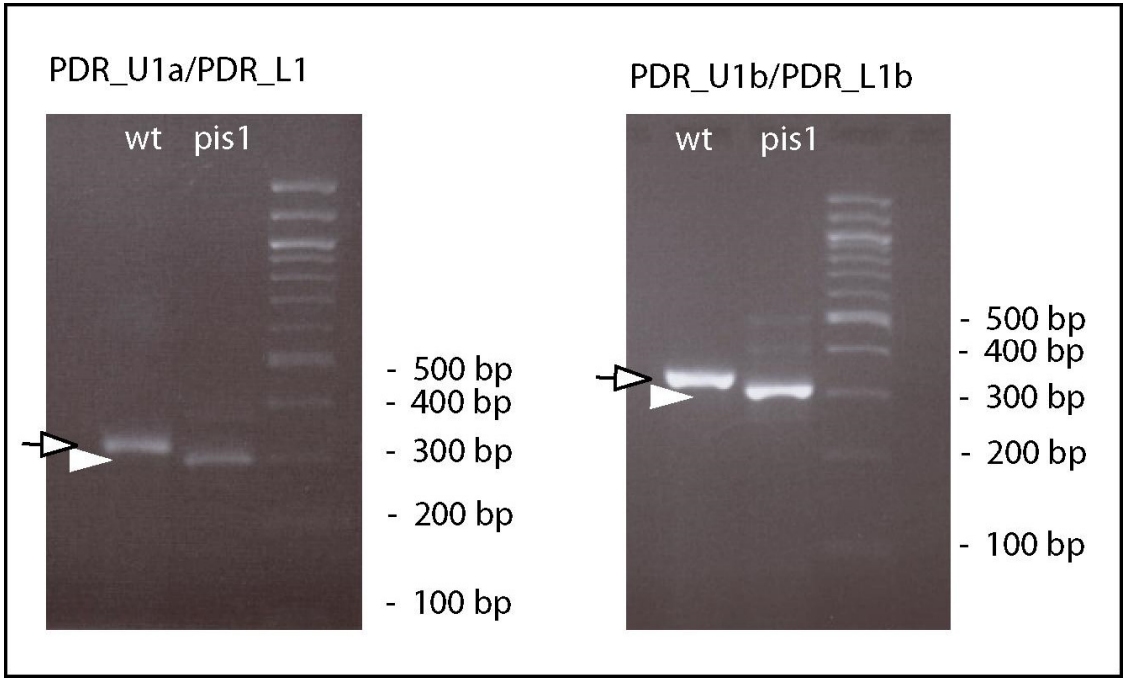
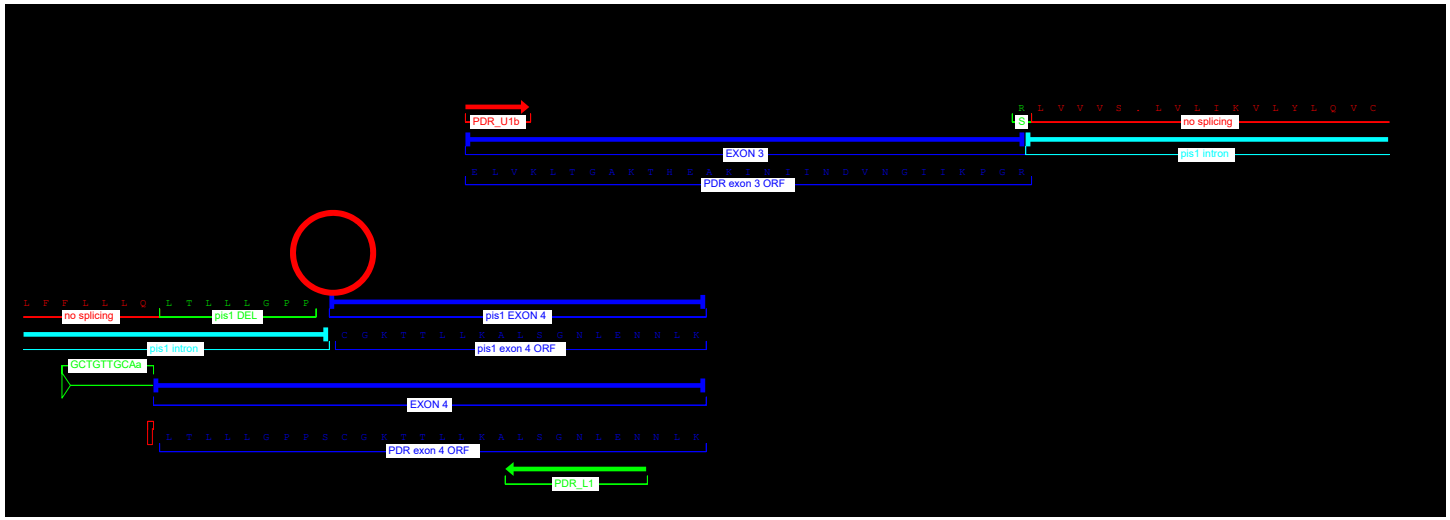
RNA Splicing and Adaptation

- Identification of mutant with point mutation (transition G→A) exactly at the splice site at the 5‘ end of the 4th exon
- Analysis by RT PCR proved the presence of a fragment shorter than cDNA should be after the typical splicing event



RNA Splicing and Adaptation

- Flexibility in splicing site recognition in plants in practice – example of developmental plasticity of (not only) plants
  - Identification of mutant with point mutation (transition G→A) exactly at the splice site at the 5‘ end of the 4th exon
  - Analysis by RT PCR proved the presence of a fragment shorter than cDNA should be after the typical splicing event
  - Sequenation of this fragment then suggested alternative splicing with the closest possible splice site in exon 4



RNA Splicing and Adaptation

- Divergencies at splice site recognition in plants in practice – example of developmental plasticity of (not only) plants

  - Identification of mutant with point mutation (transition G→A) exactly at the splice site at the 5‘ end of the 4th exon
  - Analysis by RT PCR proved the presence of a fragment shorter than cDNA should be after the typical splicing event
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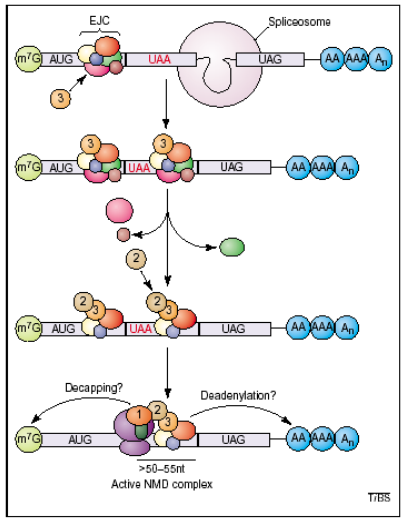


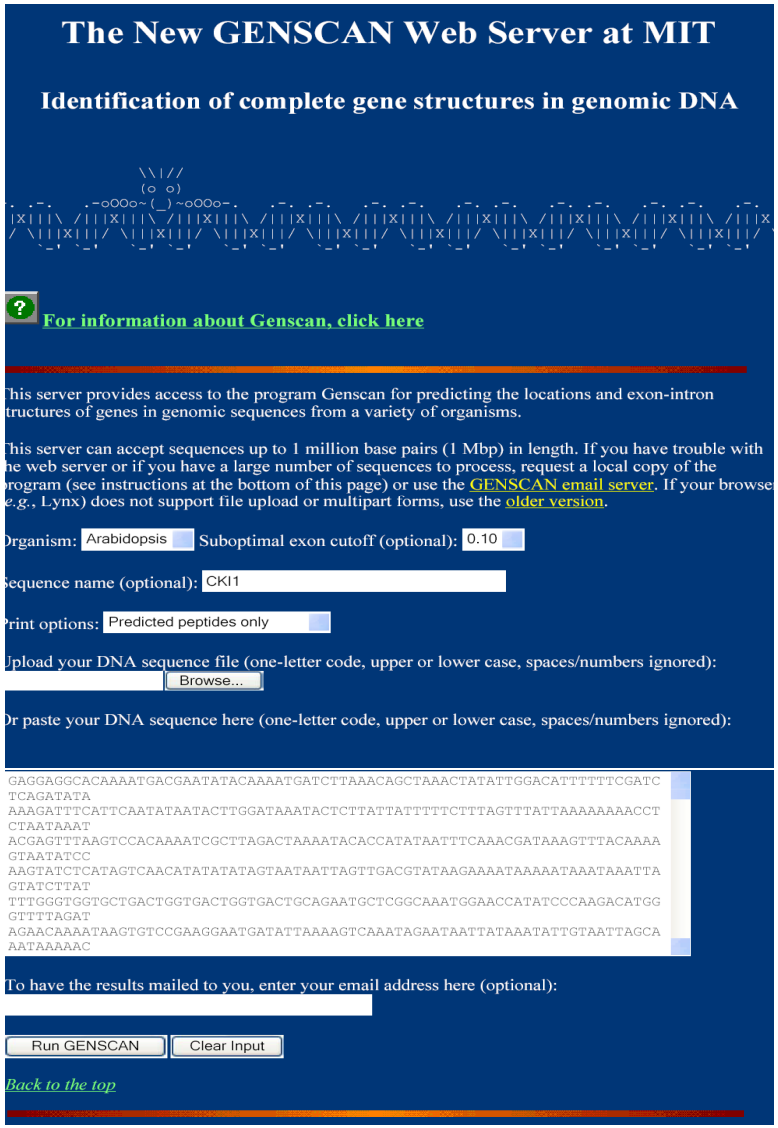
  - Existence of similar defense mechanisms was proven in different organisms as well (e.g. Instability of mutant mRNA with early stop codon formation (> 50 - 55 bp before typical stop codon) in eukaryotes, see recommended literature – Singh and Lykke-Andersen, 2003

Identification of Genes *Ab Initio*

- Programs for exon prediction
  - 4 types of exons (according to location in the gene):
    - initial
    - internal
    - terminal
    - single
  - Programs predict splice sites and they take into account the structure of the type of exon as well

- initial:
  - Genescan (http://hollywood.mit.edu/GENSCAN.html)
  - GeneMark.hmm (http://opal.biology.gatech.edu/GeneMark/)
- internal:
  - MZEF (http://rulai.cshl.org/tools/genefinder/)

GENSCAN


The New GENSCAN Web Server at MIT
Identification of complete gene structures in genomic DNA
For information about Genscan, click here
This server provides access to the program Genscan for predicting the locations and exon-intron
tructures of genes in genomic sequences from a variety of organisms.
This server can accept sequences up to 1 million base pairs (1 Mbp) in length. If you have trouble with
he web server or if you have a large number of sequences to process, request a local copy of the
rogram (see instructions at the bottom of this page) or use the GENSCAN email server. If your browse
e.g., Lynx) does not support file upload or multipart forms, use the older version.
Organism: Arabidopsis
Suboptimal exon cutoff (optional): 0.10
Sequence name (optional): CKI1
Print options: Predicted peptides only
Upload your DNA sequence file (one-letter code, upper or lower case, spaces/numbers ignored):
Browse...
Or paste your DNA sequence here (one-letter code, upper or lower case, spaces/numbers ignored):
GAGGAGGCACAAAATGACGAATATACAAAATGATCTTAAACAGCTAAACTATATTGGACATTTTTTCGATC
TCAGATATA
AAAGATTTCATTCAATATAATACTTGGATAAATACTCTTATTATTTTTCTTTAGTTTATTAAAAAAAACCT
CTAATAAAT
ACGAGTTTAAGTCCACAAAATCGCTTAGACTAAAATACACCATATAATTTCAAACGATAAAGTTTACAAAA
GTAATATCC
AAGTATCTCATAGTCAACATATATATAGTAATAATTAGTTGACGTATAAGAAAATAAAAATAAATAAATTA
GTATCTTAT
TTTGGGTGGTGCTGACTGGTGACTGGTGACTGCAGAATGCTCGGCAAATGGAACCATATCCCAAGACATGG
GTTTTAGAT
AGAACAAAATAAGTGTCCGAAGGAATGATATTAAAAGTCAAATAGAATAATTATAAATATTGTAATTAGCA
AATAAAAAC
To have the results mailed to you, enter your email address here (optional):
Run GENSCAN
Clear Input
Back to the top

GENSCAN

GENSCANW output for sequence CKI1

GENSCAN 1.0 Date run: 10-Nov-105 Time: 02:24:26

Sequence CKI1 : 9490 bp : 36.53% C+G : Isochore 1 (0 - 43 C+G%)

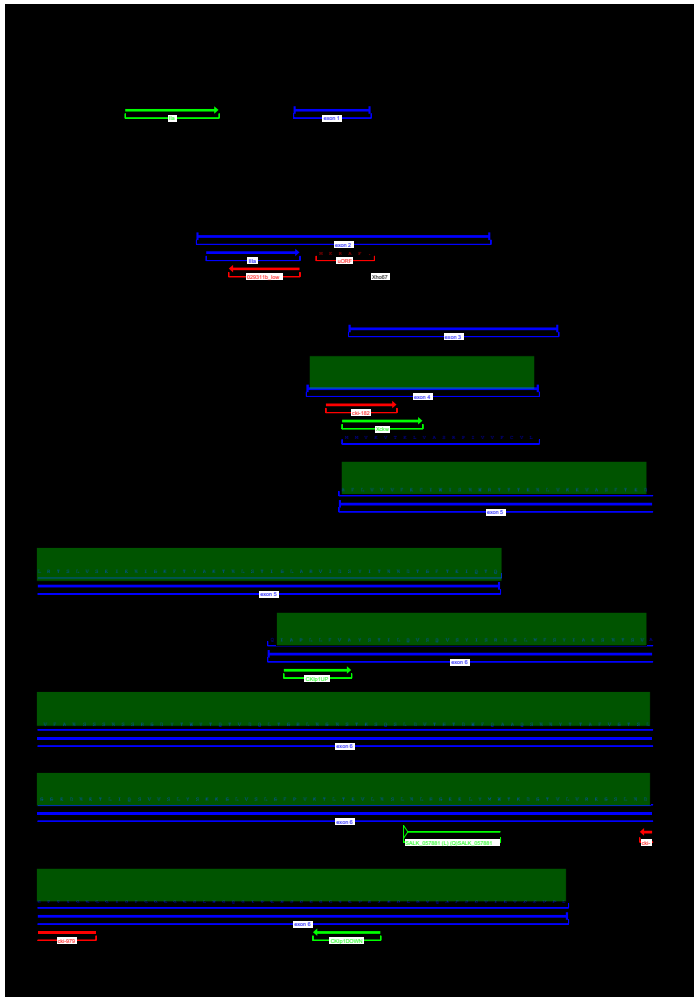
Parameter matrix: Arabidopsis.smat

Predicted genes/exons:

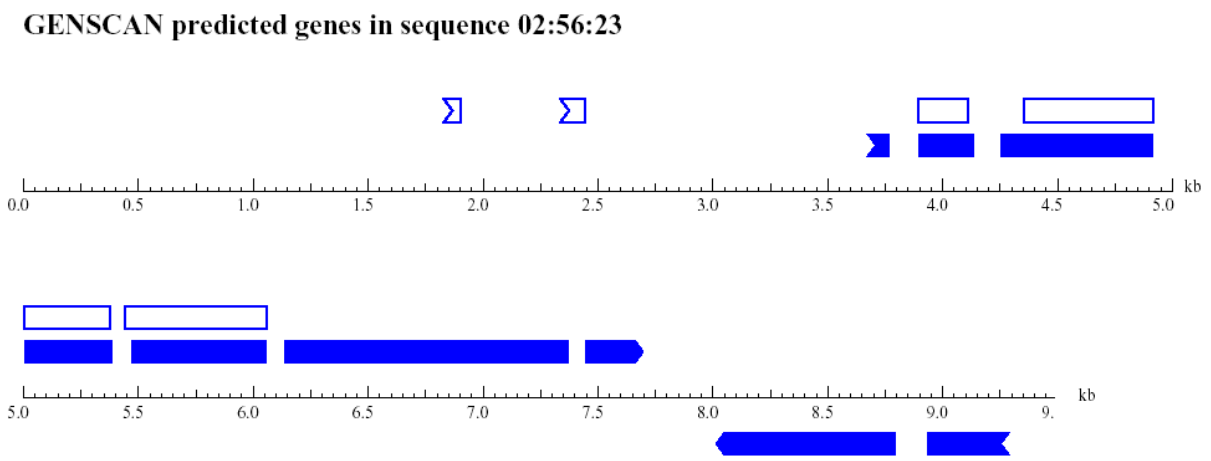
Gn.Ex	Type	S	.Begin	...End	.Len	Fr	Ph	I/Ac	Do/T	CodRg	P....	Tscr..
1.00	Prom	+	1497	1536	40							-3.85
1.01	Init	+	3708	3764	57	2	0	63	51	37	0.499	4.03
1.02	Intr	+	3894	4133	240	2	0	-3	7	327	0.713	17.32
1.03	Intr	+	4255	4914	660	0	0	86	59	296	0.771	22.57
1.04	Intr	+	5005	5383	379	0	1	70	91	343	0.772	31.41
1.05	Intr	+	5473	6056	584	2	2	38	99	582	0.722	50.76
1.06	Intr	+	6136	7368	1233	0	0	68	108	655	0.977	56.86
1.07	Term	+	7448	7660	213	1	0	43	35	212	0.999	12.65
1.08	PlyA	+	7910	7915	6							-0.45
2.03	PlyA	-	7976	7971	6							-4.83
2.02	Term	-	8793	8050	744	0	0	107	37	542	0.997	48.46
2.01	Init	-	9253	8936	318	1	0	105	73	386	0.999	41.18

Suboptimal exons with probability > 0.100

Exnum	Type	S	.Begin	...End	.Len	Fr	Ph	B/Ac	Do/T	CodRg	P....	Tscr..
S.001	Init	+	1867	1905	39	0	0	64	40	57	0.298	3.74
S.002	Init	+	2374	2442	69	0	0	55	95	-11	0.132	2.40
S.003	Intr	+	3894	4110	217	2	1	-3	-34	307	0.177	11.55
S.004	Intr	+	4352	4914	563	0	2	75	59	338	0.187	26.20
S.005	Intr	+	5005	5379	375	0	0	70	8	335	0.212	22.99
S.006	Intr	+	5442	6056	615	2	0	95	99	589	0.208	57.32



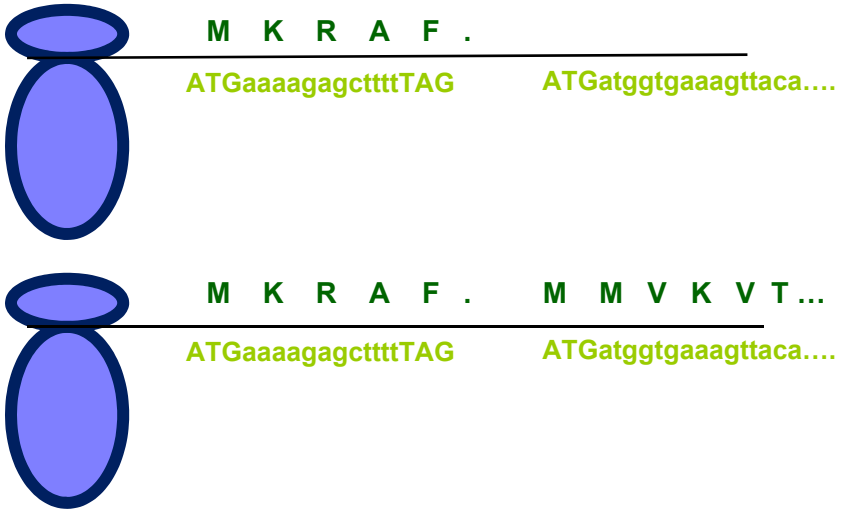
GENSCAN


GENSCAN predicted genes in sequence 02:56:23
0.0
0.5
1.0
1.5
2.0
2.5
3.0
3.5
4.0
4.5
5.0
kb
5.0
5.5
6.0
6.5
7.0
7.5
8.0
8.5
9.0
9.
kb
Key:
Initial exon
Internal exon
Terminal exon
Single-exon gene
Optimal exon
Suboptimal exon

Regulation of Translation

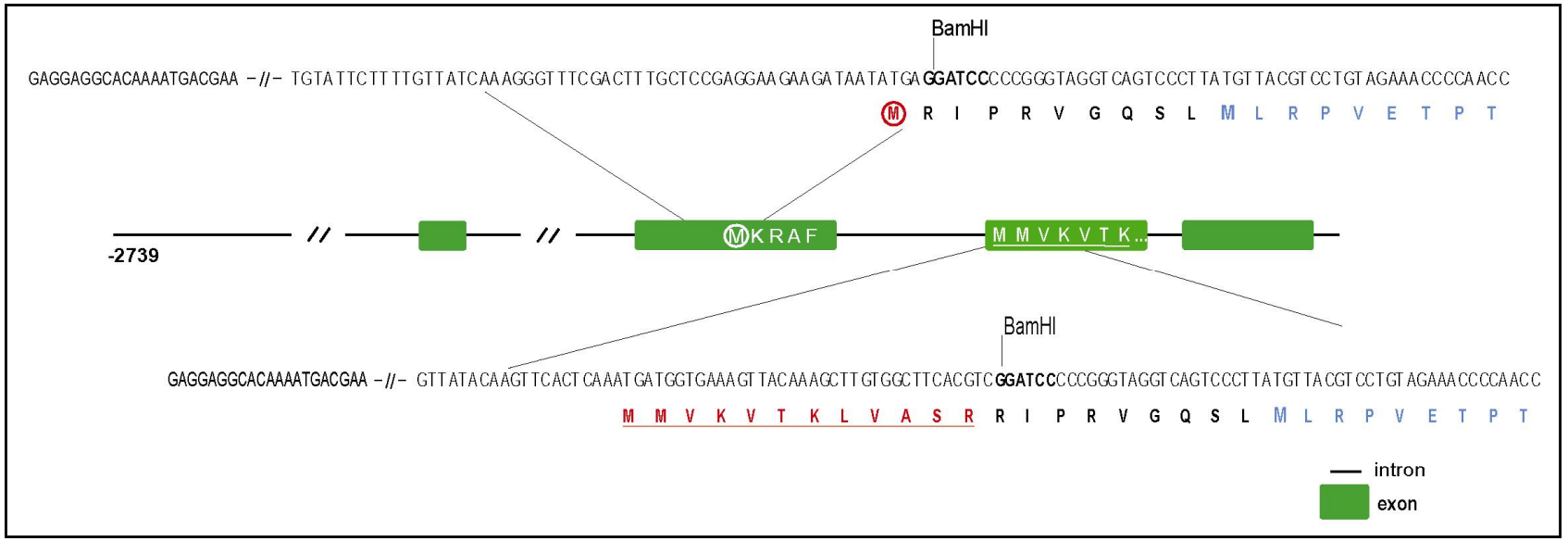
- Splicing in Untranslated Regions – important regulation part of genes

- Translational repression by short ORFs in 5‘ UTR
- Identified e.g. in maize (Wang and Wessler, 1998, see recommended literature for additional info.)
- In case of CKI1 there was an attempt to prove this mechanism of regulation using transgenic lines carrying *uidA* under control of two versions of promoter (unconfirmed so far)



Regulation of translation

- Functional purpose of splicing in untranslated regions – important regulation part of genes
  - In case of CKI1 there was an attempt to prove this mechanism of regulation using transgenic lines carrying *uidA* under control of two versions of promoter (unconfirmed so far)



CEITEC

Gene Modelling

- Programs for gene modelling
  - Those that take into account other parameters as well, e.g.continuity of ORFs
    - Genescan (http://hollywood.mit.edu/GENSCAN.html) – very good foor prediction of exons in coding regions (tested for gene *PDR9*, Genescan identified all of the 23 (!) exons)
    - GeneMark.hmm (http://opal.biology.gatech.edu/GeneMark/)
    - GlimmerHMM (https://ccb.jhu.edu/software/glimmerhmm/)

GeneMark

GeneMark™
A family of gene prediction programs provided by Mark Borodovsky's Bioinformatics Group at the Georgia Institute of Technology, Atlanta, Georgia.

What's New: - *November, 2005*
Prokaryotes: predicted gene **database**.
Prokaryotes: models for **GeneMark** *and* **GeneMark.hmm**.

Supported by NIH

Gene Prediction in Bacteria and Archaea

For bacterial and archaeal gene prediction, you can use the parallel combination of the GeneMark and GeneMark.hmm programs here.

If the DNA sequence of interest belongs to a species whose name is not in the list of available models, you should use either the Heuristic models option or, if the sequence is longer than 1 Mb, generate models with the self-training program GeneMarkS. Both options will allow you to generate models and then to use GeneMark.hmm and GeneMark in parallel.

Gene Prediction in Eukaryotes

For eukaryotic gene prediction, you can use the parallel combination of the GeneMark and GeneMark.hmm programs here.

Gene Prediction in EST and cDNA

To analyze ESTs and cDNAs, please follow this link.

Gene Prediction in Viruses

For viral gene prediction, or to access our virus database VIOLIN, please follow this link.

What the programs do:

Borodovsky Group

Gene Prediction Programs
- GeneMark
- GeneMark.hmm
- Frame-by-Frame
- GeneMarkS
- Heuristic models

Statistics
- Documented GeneMark.* usage

Help
- References
- Papers
- FAQ
- Contact

Databases of predicted genes
- Prokaryotes New!
- Viruses/Phages (VIOLIN)

Bioinformatics Resources
- Links

Bioinformatics Studies at Georgia Tech
- MS Degree Program
- PhD Program
- Lectures
- Seminars
- Center for Bioinformatics and

Eukaryotic GeneMark.hmm[1,2] (Reload this page)

References:

[1]Borodovsky M. and Lukashin A. (unpublished)
[2]Lomsadze A., Ter-Hovhannisyan V., Chernoff Y. and Borodovsky M., "Gene identification in novel eukaryotic genomes by self-training algorithm", **Nucleic Acids Research**, 2005, Vol. 33, No. 20, 6494-6506

Accuracy comparison

UPDATE October 2005. Added pre-built models of eukaryotic GeneMark.hmm ES-3.0 (E - eukaryotic; S - self-training; 3.0 - the version)

Listing of previous updates

Input Sequence

Title (optional):
CK1

Sequence:

Sequence File upload:
Procházet...

Species: Athaliana ES-3.0 Model description

Output Options

Email Address: (required for graphical output or sequences longer than 400000 bp)

- [x] Generate PDF graphics (screen)
- [ ] Generate PostScript graphics (email)
- [ ] Print GeneMark 2.4 predictions in addition to GeneMark.hmm predictions
- [ ] Translate predicted genes into protein

Run Default Start GeneMark.hmm

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GeneMark

Result of last submission:

View PDF Graphical Output

GeneMark.hmm Listing

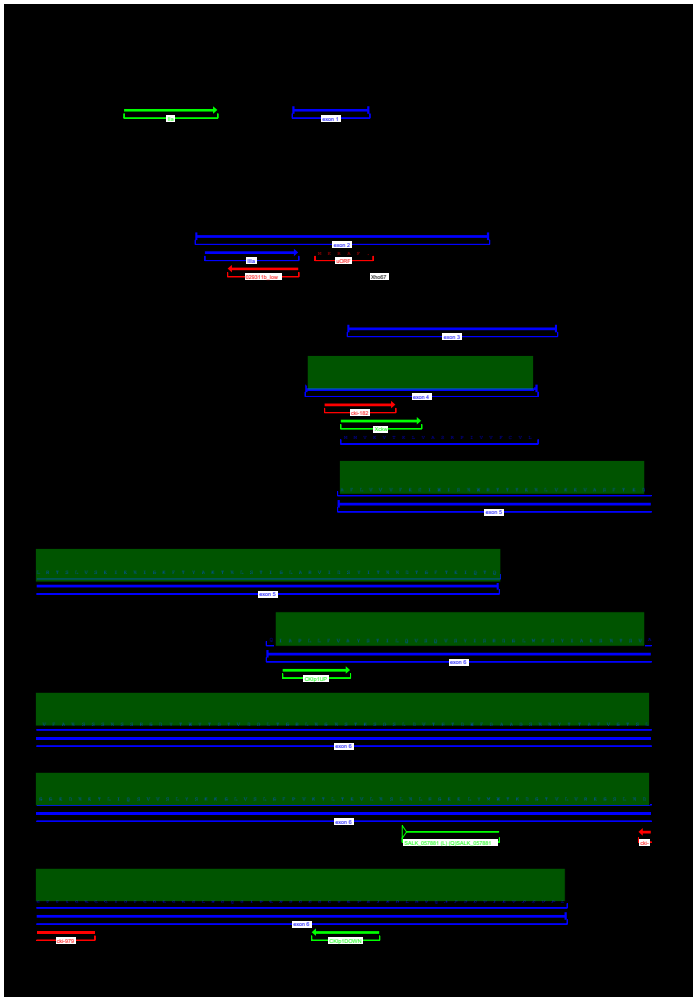
Go to: GeneMark.hmm Protein Translations

Go to: Job Submission

```
Eukariotyc GeneMark.hmm version bp 3.9 April 25, 2008
Sequence name: CKI1
Sequence length: 5043 bp
G+C content: 38.79%
Matrices file: /home/genmark/euk_ghm.matrices/athaliana_hmm3.0mod
Thu Oct  1 11:09:24 2009
```

Predicted genes/exons

Gene #	Exon #	Strand	Exon Type	Exon Range		Exon Length	Start/End Frame
1	1	+	Initial	969	1025	57	1 3 - -
1	2	+	Internal	1155	1394	240	1 3 - -
1	3	+	Internal	1516	2175	660	1 3 - -
1	4	+	Internal	2266	2644	379	1 1 - -
1	5	+	Internal	2734	3317	584	2 3 - -
1	6	+	Internal	3397	4629	1233	1 3 - -
1	7	+	Terminal	4709	4921	213	1 3 - -



GeneMark

Result of last submission:

View PDF Graphical Output

GeneMark.hmm Listing

Go to: GeneMark.hmm Protein Translations

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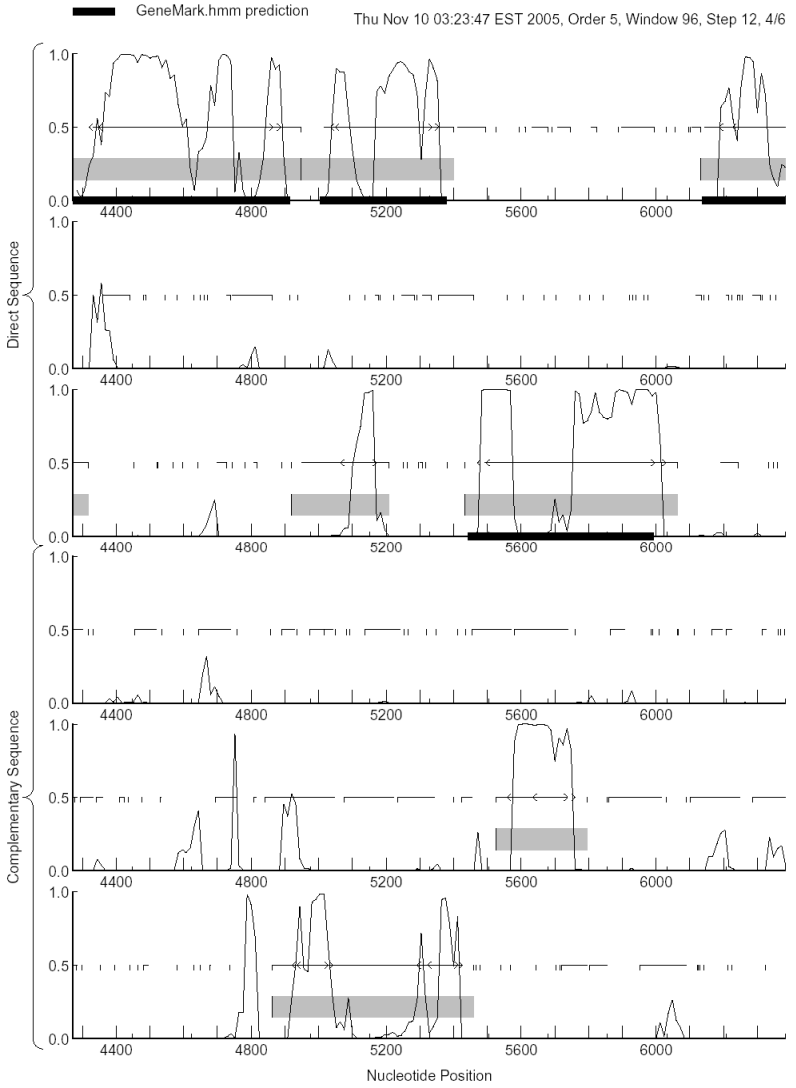
Predicted genes/exons

Gene  Exon  Strand  Exon          Exon Range          Exon      Start/End
  #     #         Type                              Length      Frame

   1    1    +   Initial          969 1025 57 1 3 - -
   1    2    +   Internal        1155       1394       240        1 3 - -
   1    3    +   Internal        1515       2175       660        1 3 - -
   1    4    +   Internal        2255       2644       379        1 1 - -
   1    5    +   Internal        2734       3317       584        2 3 - -
   1    6    +   Internal        3397       4629      1233        1 3 - -
   1    7    +   Terminal        4709       4921       213        1 3 - -
```



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Genomic Homologies

- Searching for genes according to homologies with known sequences
  - Comparison with EST databases
    - BLASTN (http://www.ncbi.nlm.nih.gov/BLAST/, http://workbench.sdsc.edu/)
  - Comparison with protein databases
    - BLASTX (http://www.ncbi.nlm.nih.gov/BLAST/, http://workbench.sdsc.edu/)
    - Genewise (http://www.ebi.ac.uk/Wise2/)

    They compare protein sequence with genomic DNA (after reverse transcription), therefore the aminoacid sequence is needed
  - Comparison with homologous genome sequences from related species
    - VISTA/AVID (http://www.lbl.gov/Tech-Transfer/techs/lbnl1690.html)

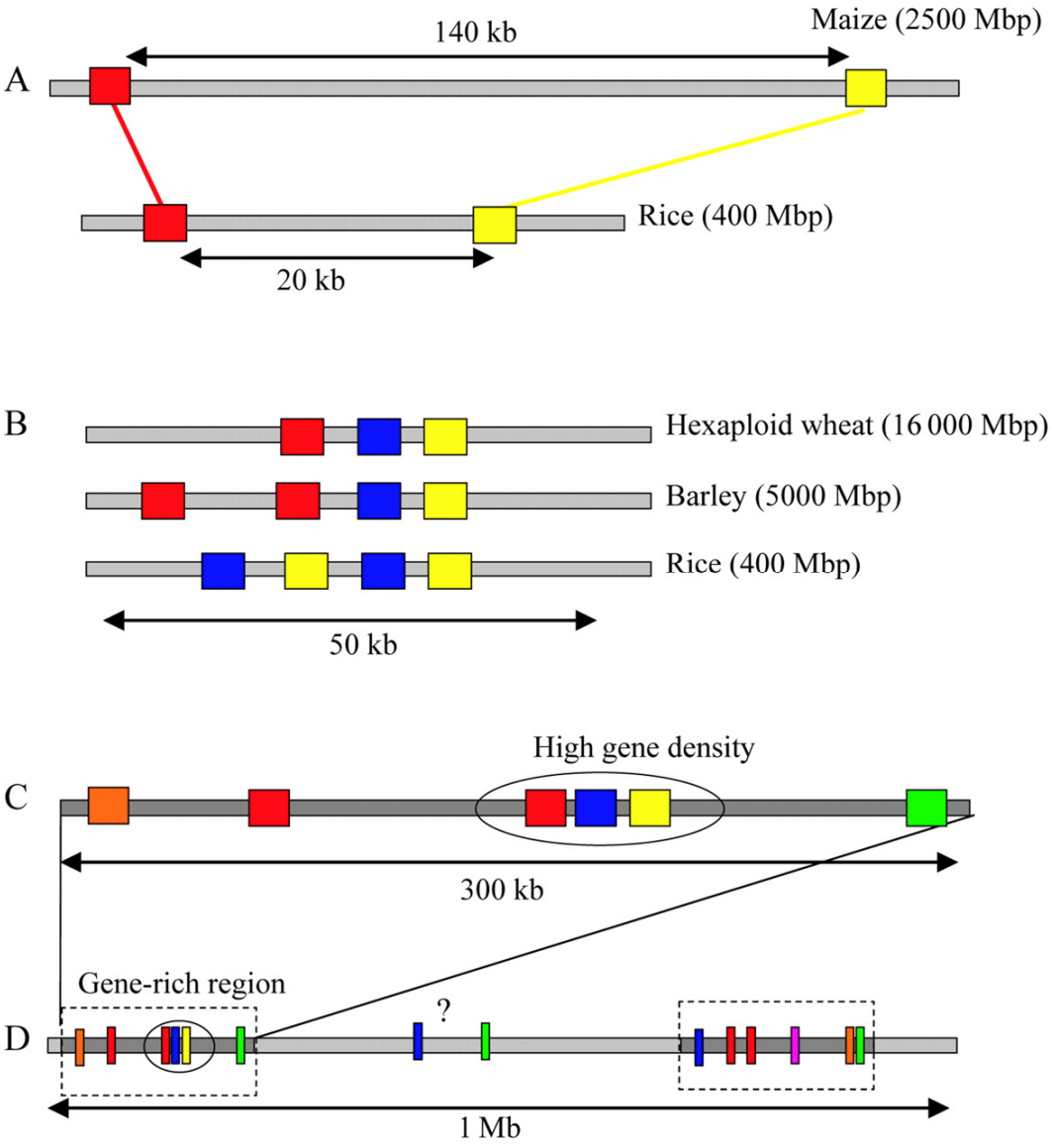
Outline

- Forward and Reverse Genetics Approaches
  - Differences between the approaches used for identification of genes and their function
- Identification of Genes *Ab Initio*
  - Structure of genes and searching for them
  - Genomic colinearity and genomic homology

Genomic Colinearity

- Genomes of related species (despite large differencies) are characterized by similarities in sequence organization -> possibility to use this information for identification of genes in related species when searching in databases
- General scheme of work while applying genomic colinearity (also called „comparative genomics“) for experimental identification of genes in related species:
  - Mapping small genomes using low-copy DNA markers (e.g. RFLP)
  - Using these markers for identification of orthologous genes (genes with the same or similar function) of related species
  - Small genome (e.g. rice, 466 Mbp) can be used as a guide: molecular low-copy markers (e.g. RFLP) bound to gene of interest are identified and these regions are then used as a probe for searching in BAC libraries during identification of orthologous regions of large genomes (e.g. barley: 5 Gbp, or wheat: 16 Gbp)

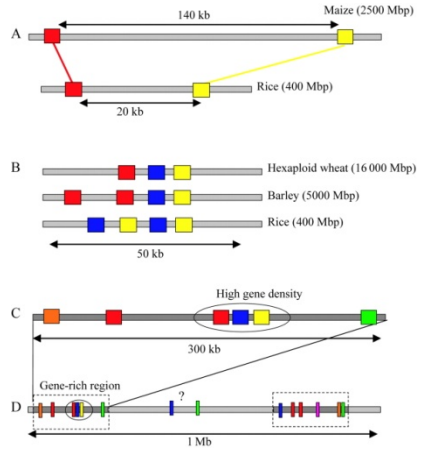
Genomic Colinearity



Feuillet and Keller, 2002

Genomic Colinearity

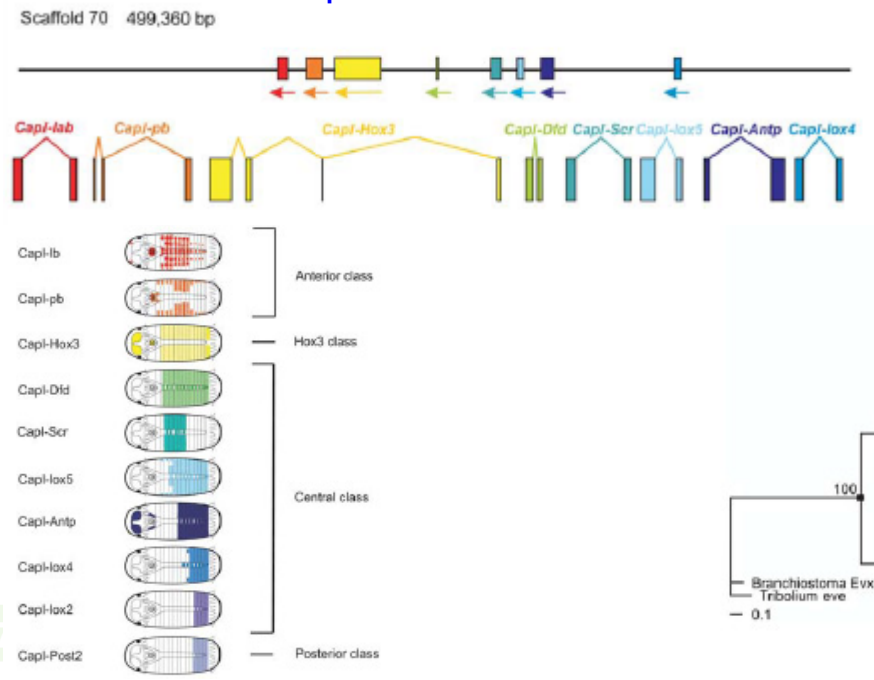
- Can be mostly used for the species of grass (e.g. using related genes of species of barely, wheat, rice, maize)
- Small genome reorganizations (deletions, duplications, inversions, translocations smaller than a few cM) are then detected by detailed sequentional comparative analysis
- During evolution there‘s occured some divergencies in related species, mostly in non-coding regions
(invasion of retrotransposons etc.)

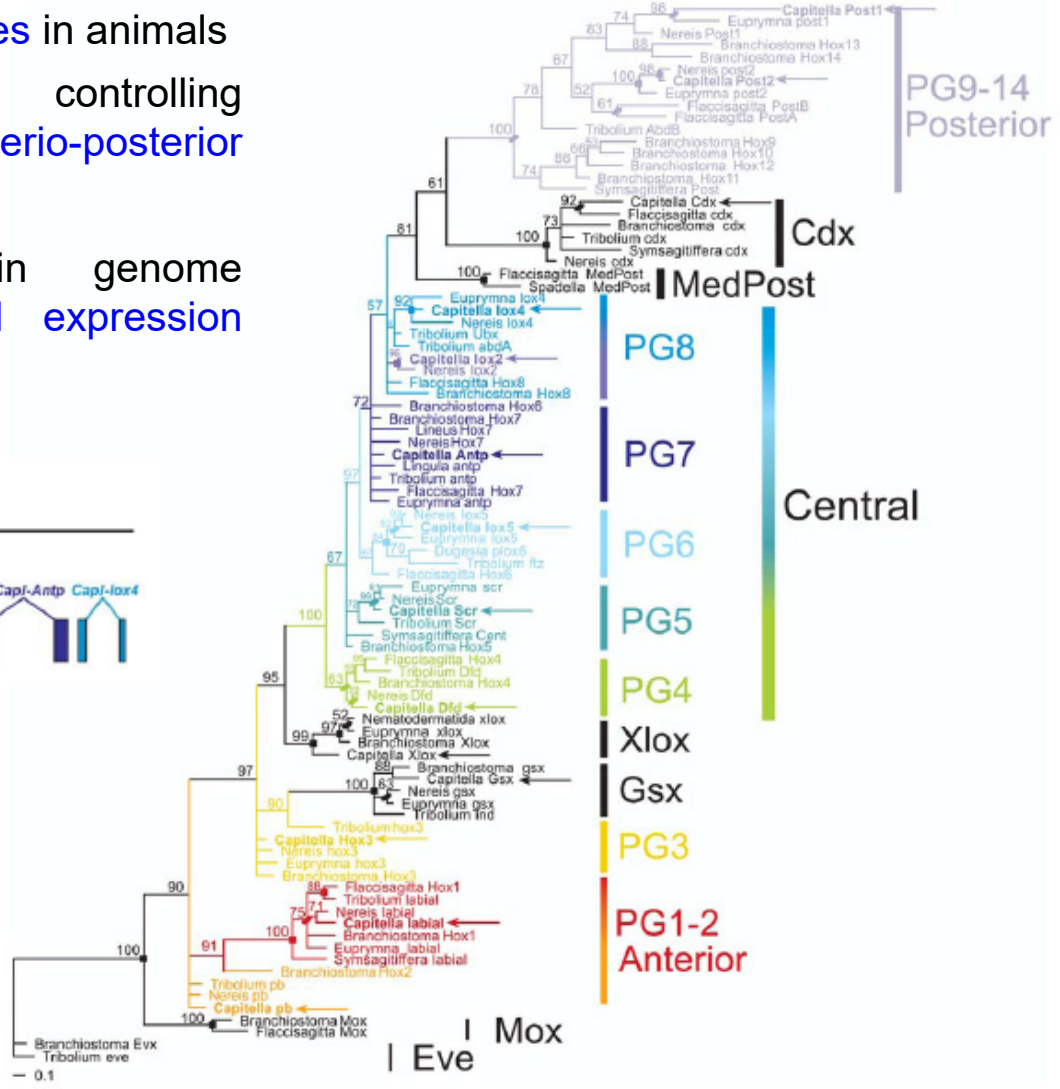


CEITEC

Genomic Colinearity

- Genomic colinearity of HOX genes in animals
  - Transcription factors controlling organisation of body in anterio-posterior axis
  - Position of genes in genome corresponds with spatial expression during development
  - Interspecies conservation





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  - Constructing gene-enriched libraries using methylation filtration technology

Methylation Filtration

- Preparation of gene-enriched libraries by technology of methylation filtration
  - genes are (mostly!) hypomethylated, noncoding regions are methylated
  - using bacterial restriction-modification system, which recognizes methylated DNA with restriction enzymes McrA a McrBC
    - McrBC recognizes methylated cytosin (in DNA), which comes after purine (G or A)
    - For cleavage the distance of these sites 40-2000 bp is necessary

Methylation Filtration

- Preparation of gene-enriched libraries by technology of methylation filtration
  - Scheme of work during preparation of BAC genome libraries using methylation filtration:
    - preparation of genomic DNA without addition of organelle DNA (chloroplasts and mitochondria)
    - fragmentation of DNA (1-4 kbp) and ligation of adaptors
    - preparation of BAC libraries in *mcrBC+* strain of *E. coli*
    - selection of positive clones
  - Limitied usage: enrichment of coding DNA only approx. 5 -10 %

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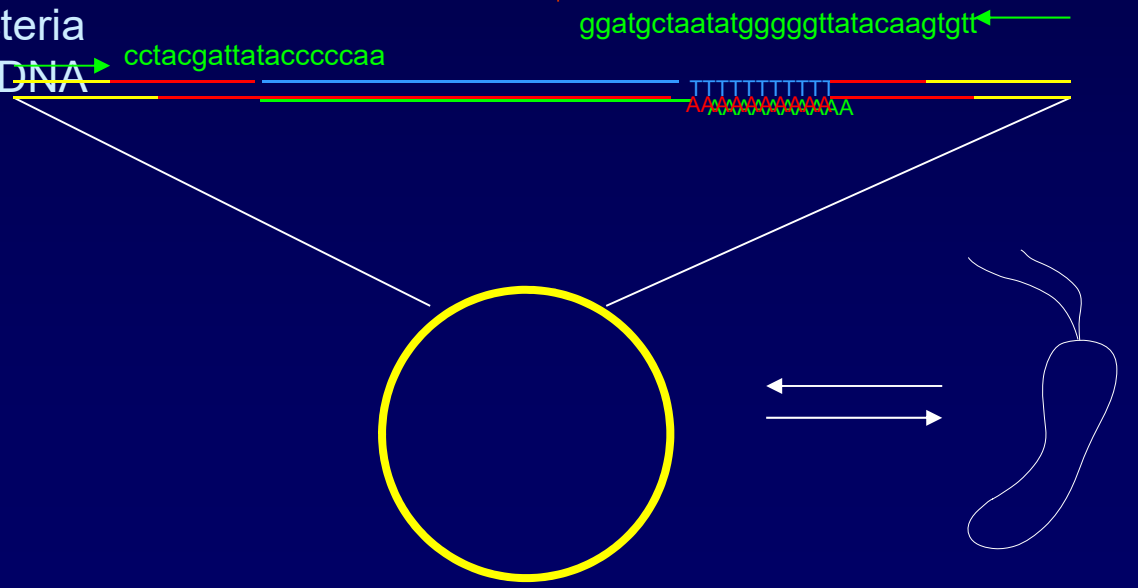
  - EST libraries

EST Libraries

- Preparation of EST libraries
  - Isolation of mRNA
  - Reverse transcription
  - Ligation of linkers and synthesis of second cDNA strand
  - Cloning into suitable bacterial vector
  - Transformation into bacteria and isolation of DNA (amplification of DNA)
  - Sequencing using primers specific for used plasmid
  - Saving the results of sequencing into public database



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  - Forward and reverse genetics

Discussion