CG020 Genomika

Lesson 2

Genes Identification

Jan Hejátko

Functional Genomics and Proteomics of Plants,
Mendel Centre for Plant Genomics and Proteomics,
CEITEC - Central European Institute of Technology
and
National Centre for Biomolecular Research,
Faculty of Science,

Masaryk University, Brno
hejatko@sci.muni.cz, www.ceitec.eu

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

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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

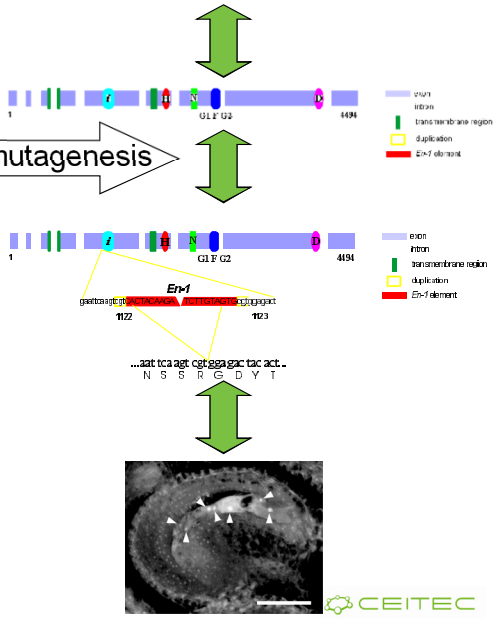
3 : 1

?

„reverse genetics“ approaches

5‘TTATATATATATATTAAAAAATAAAATAA
AAGAACAAAAAAGAAAATAAAATA….3‘

insertional mutagenesis



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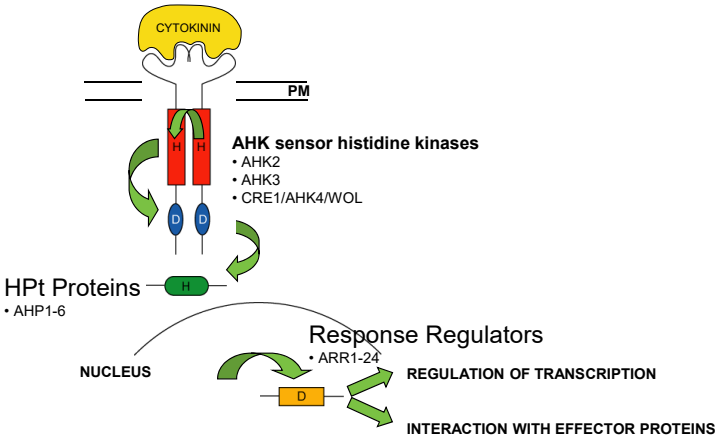
Identification of the role of *ARR21* gene

- 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

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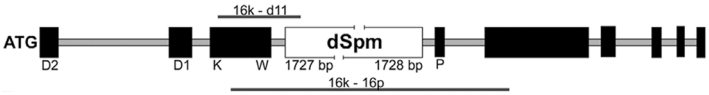
Identification of the role of *ARR21* gene – isolation of insertional mutant

- **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

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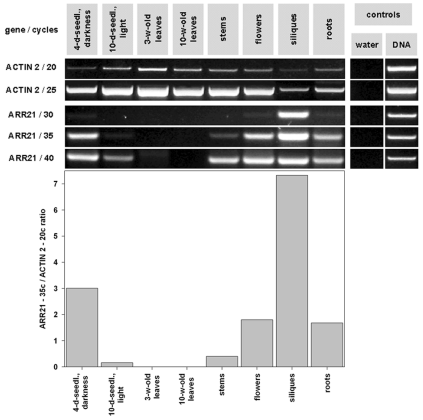
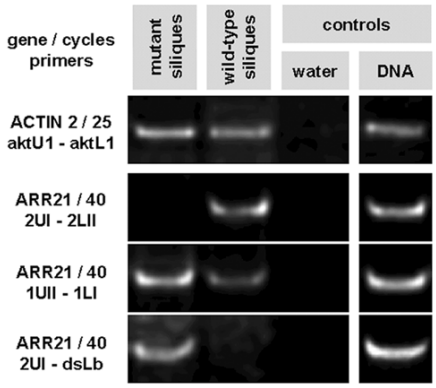
Identification of the role of *ARR21* gene – analysis of expression

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

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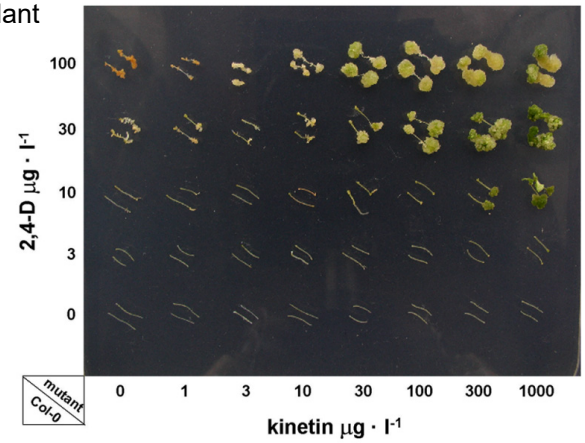
Identification of the role of *ARR21* gene – phenotype analysis of mutant

- 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

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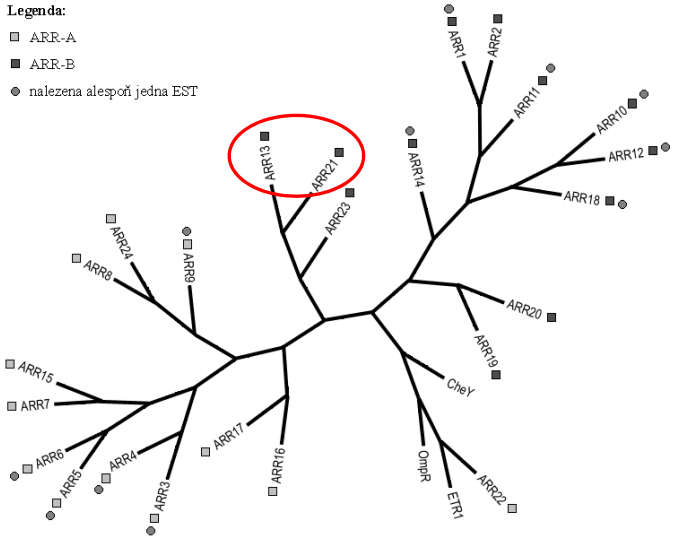
Identification of the role of *ARR21* gene – homology of *ARR* genes



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

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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

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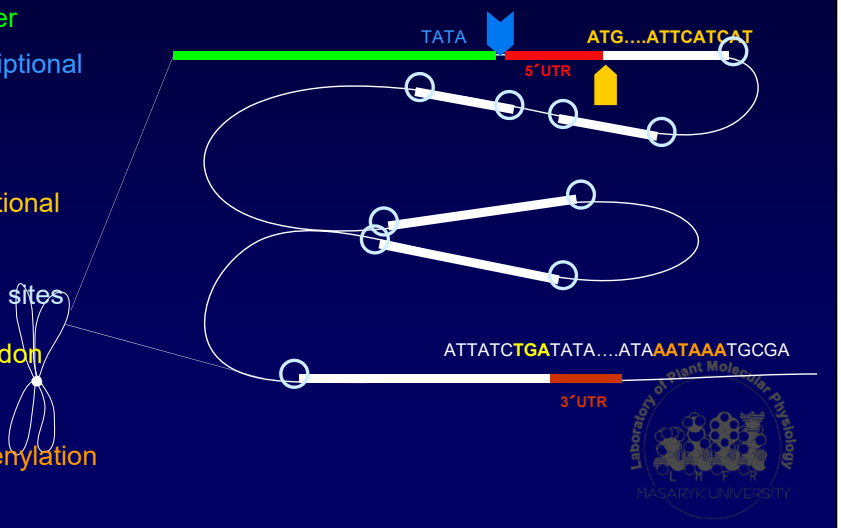
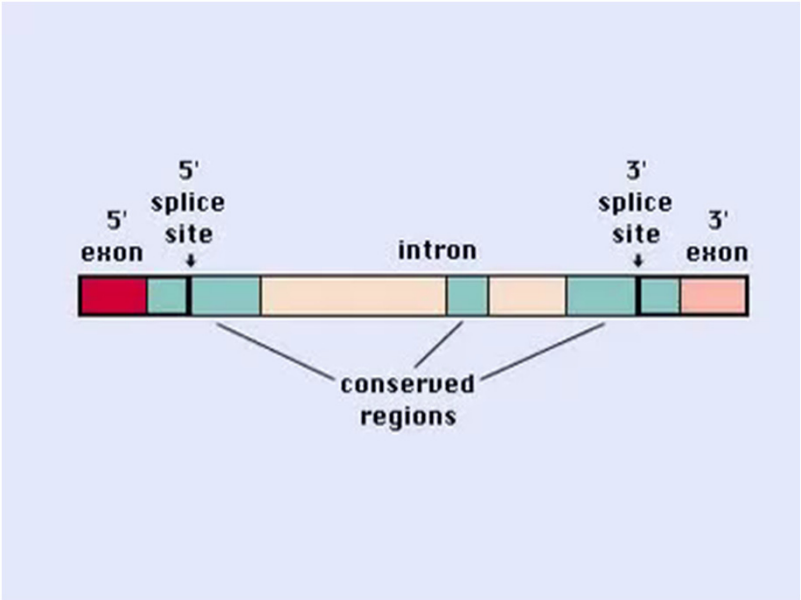
Genes Structure

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



RNA Splicing



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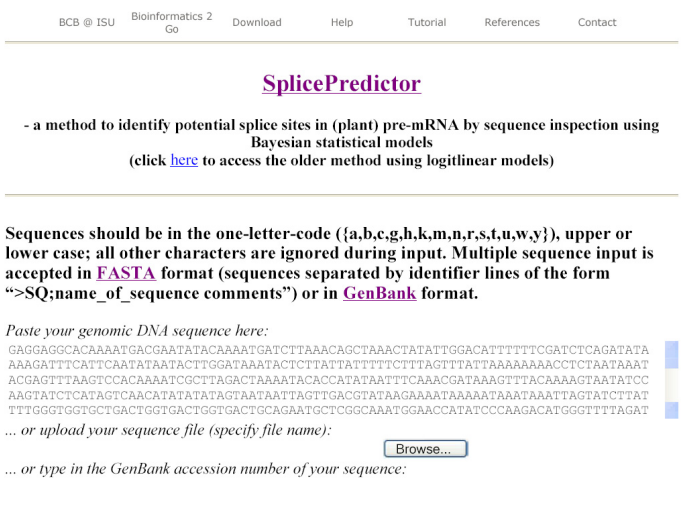
Identification of Genes *Ab Initio*

- 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.

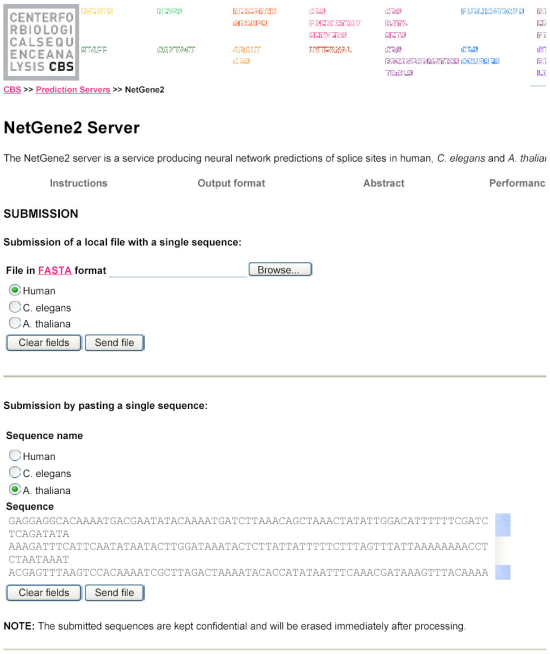
Potential splice sites

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 gatttgttgttgttAGtc   0.977   7.48   0.000  0.000    7 (5 1 1)
A   <--     780 totgttattgtatAGgt   0.986   8.56   0.000  0.000    7 (5 1 1)
A   <--     848 tatttttgaaatAGat   0.968   6.80   0.000  0.000    7 (5 1 1)
A   <--    1051 caatttattttttaAGaa   0.930   5.19   0.000  0.000    7 (5 1 1)
A   <--    1213 ttatttattttttAGtt   0.998  12.14   0.000  0.000    7 (5 1 1)
A   <--    1373 tttcctctctcaacAGga   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 catctaaaattttAGat   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 tttgttctgtactaAGct   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  <---    3286 ctttgttatcaaAGag   0.993  10.03   0.000  0.086    8 (5 1 2)
D =====>    3372           aatGTaagg   0.933   5.28   0.855  1.849   15 (5 5 5)
A  <----    3453 aatgcttcccgtatAGaa   0.916   4.77   0.293  0.065   32 (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 ttgtggttatacAGtt   0.907   4.56   0.000  0.000    7 (5 1 1)
A  <---    4234 atcatcgtcctcAGgat   0.990  12.82   0.000  0.000    8 (5 1 2)
A   <--    4351 tttcttacattgcAGaa   0.991   9.42   0.000  0.000    7 (5 1 1)
A   <--    4633 gtcttgtttctttAGgg   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 ttttttttgccAGag   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   <--    6352 ggatttttcacctcAGag   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



CENTERFO RBIOLOGI CALSEQU ENCEANA LYSIS CBS

CBS >> Prediction Servers >> NetGene2

NetGene2 Server

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

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

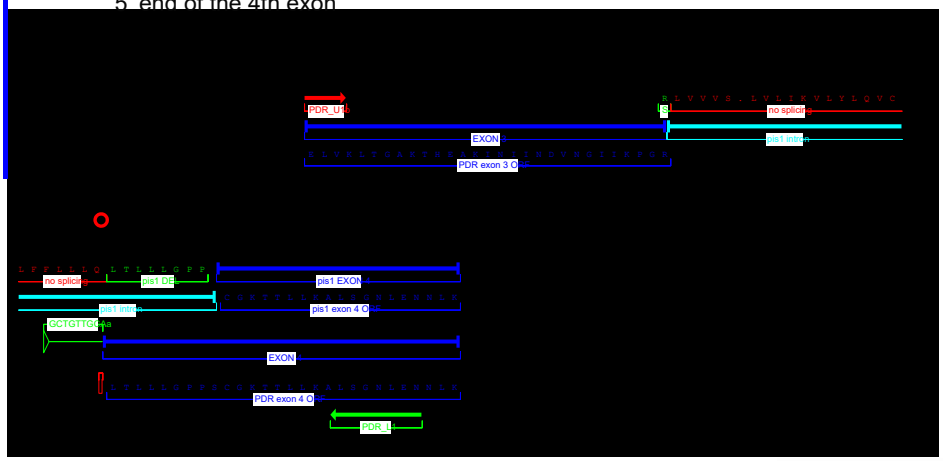
Donor splice sites, direct strand
---------------------------------
                pos 5'->3'  phase strand  confidence  5'     exon intron    3'
                      1704      0      +        0.87       TTCCAAACAC'GTTAATATTT
                      1906      0      +        0.99       CGGTGAACGG'GTCAGAACAT
                      3521      1      +        1.00       GGCGTTCTAG'GTAATGTTGA H
                      3765      1      +        1.00       TTCCGTCCTG'GTAATTCTGG H
                      4134      0      +        0.74       TCAAACACAG'GTTGTTAAAA
                      4619      1      +        0.74       AGCAAGAAAG'GTCTTGTTTC
                      4915      0      +        0.94       CGTTCCTCTG'GTAAATACTG
                      5356      0      +        0.87       TCTCAACCAA'GTCGATGTTT
                      5384      1      +        1.00       GATTTGGTTG'GTAAGCTCT 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       GAACAAAAAG'GTTAGATGAA
                      9323      0      +        0.74       GAAGATTAGG'GTTTTCTCT

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'GGTTCGACCT
                      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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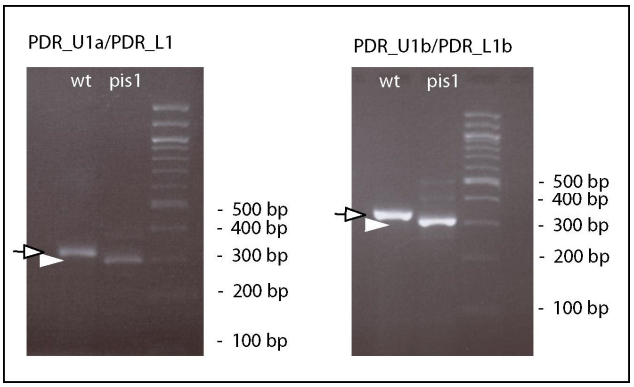
RNA Splicing and Adaptation

- 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

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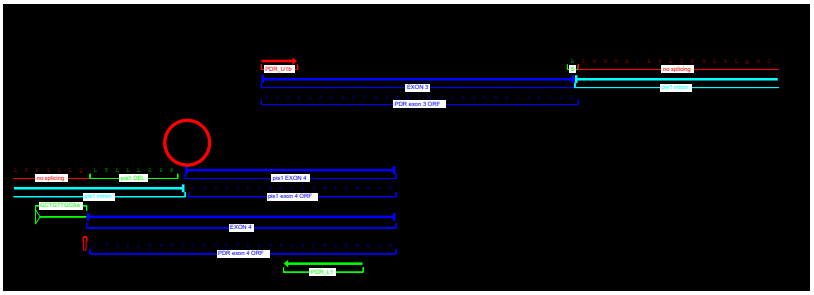
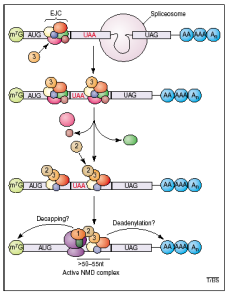
  - Identification of mutant with point mutation (transition G→A) exactly at the splice site at the 5‘ end of the 4th exon
  - Analysis by RT PCR proved the presence of a fragment shorter than cDNA should be after the typical splicing event
  - Sequenation of this fragment then suggested alternative splicing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

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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/)

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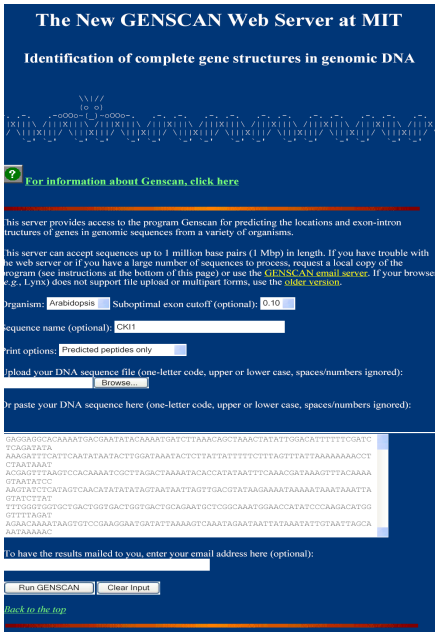
- programy kromě rozpoznávání míst sestřihu zohledňují i strukturu jednotlivých typů exonů

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):
GAGGAGGCACAAAATGACGAATATACAAAATGATCTTAAACAGCTAAACTATATTGGACATTTTTCGATC
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..
----- ---- - ------ ------ ---- -- -- ---- ---- ----- ----- ------

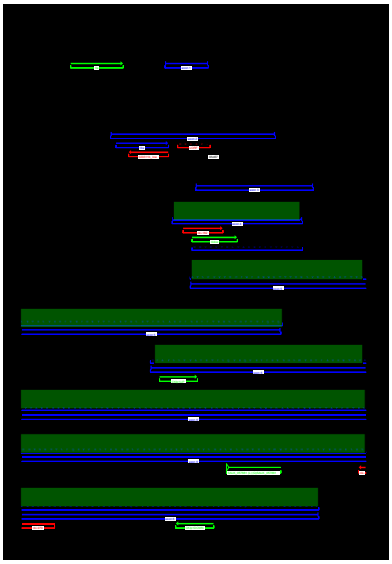
 1.00 Prom +   1497   1536   40                                -3.85
 1.01 Init +   3708   3764   57  2  0   63   51    37 0.499   4.03
 1.02 Intr +   3894   4133  240  2  0   -3    7   327 0.713  17.32
 1.03 Intr +   4255   4914  660  0  0   86   59   296 0.771  22.57
 1.04 Intr +   5005   5383  379  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
```



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Explanation Gn.Ex : gene number, exon number (for reference) **Type** : Init = Initial exon (ATG to 5' splice site) Intr = Internal exon (3' splice site to 5' splice site) Term = Terminal exon (3' splice site to stop codon) Sngl = Single-exon gene (ATG to stop) Prom = Promoter (TATA box / initation site) PlyA = poly-A signal (consensus: AATAAA) **S** : DNA strand (+ = input strand; - = opposite strand) **Begin** : beginning of exon or signal (numbered on input strand) **End** : end point of exon or signal (numbered on input strand) **Len** : length of exon or signal (bp) **Fr** : reading frame (a forward strand codon ending at x has frame x mod 3). For example, if nucleotides 1,2,3 of the sequence are read as a codon, that's called reading frame 0. If 2,3,4 are read as a codon, that's reading frame 1. If 3,4,5 are read as a codon, that's reading frame 2, and so on. This information, together with the starting and ending positions of the exon, is sufficient to give the amino acid sequence encoded by the exon. Another use of the reading frame is that if you see two adjacent predicted exons separated by a relatively short intron which share the same reading frame, it may be worth looking at the possibility that the intervening intron is not correct, i.e. that the two exons plus the intervening intron might form one long exon (assuming there are no inframe stops in the intron, of course). **Ph** : net phase of exon (exon length modulo 3). For example, an exon of length 15 bp has net phase 0 since 15 is divisible by 3, an exon of length 16 bp has net phase 1 because 16 divided by 3 leaves a remainder of 1, an exon of length 17 bp has net phase 2, and an exon of length 18 bp has net phase 0 again. The point of this is that exons whose net phase is 0 can be omitted from the gene without disrupting the reading frame: such exons are candidates for being either 1) incorrect, or 2) alternatively spliced. **I/Ac** : initiation signal or 3' splice site score (tenth bit units; x 10). If below zero, probably not a real acceptor site. **Do/T** : 5' splice site or termination signal score (tenth bit units; x 10) If below zero, probably not a real donor site. **CodRg** : coding region score (tenth bit units) **P** : probability of exon (sum over all parses containing exon). This quantity is close to the actual probability that the predicted exon is correct. **Tscr** : exon score (depends on length, I/Ac, Do/T and CodRg scores).

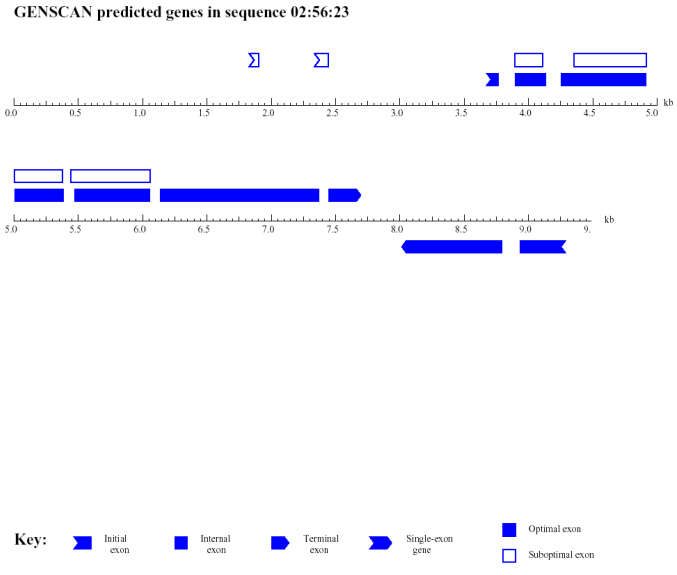
Comments The SCORE of a predicted feature (e.g., exon or splice site) is a log-odds measure of the quality of the feature based on local sequence properties. For example, a predicted 5' splice site with score > 100 is strong; 50-100 is moderate; 0-50 is weak; and below 0 is poor (more than likely not a real donor site). The PROBABILITY of a predicted exon is the estimated probability under GENSCAN's model of genomic sequence structure that the exon is correct. This probability depends in general on global as well as local sequence properties, e.g., it depends on how well the exon fits with neighboring exons. It has been shown that predicted exons with higher probabilities are more likely to be correct than those with lower probabilities.

What are the suboptimal exons?

Under the probabilistic model of gene structural and compositional properties used by GENSCAN, each possible "parse" (gene structure description) which is compatible with the sequence is assigned a probability. The default output of the program is simply the "optimal" (highest probability) parse of the sequence. The exons in this optimal parse are referred to as "optimal exons" and the translation products of the corresponding "optimal genes" are printed as GENSCAN predicted peptides. (All the data in our J Mol Biol paper and on the other GENSCAN web pages refer exclusively to the optimal parse/optimal exons.) Of course, the optimal parse does not always correspond to the actual (biological) parse of the sequence, that is, the actual set of exons/genes present. In addition, there may be more than one parse which can be considered "correct", for example, in the case of a gene which is alternatively transcribed, translated or spliced. For both of these reasons, it may be of interest to consider "suboptimal" ("near-optimal") exons as well, i.e. exons which have reasonably high probability but are not present in the optimal parse. Specifically, for every potential exon E in the sequence, the probability P(E) is defined as the sum of the probabilities under the model of all possible "parses" (gene structures) which contain the exact exon E in the correct reading frame. (This quantity is calculated as described on the GENSCAN exon probability page.) Given a probability cutoff C, suboptimal exons are those potential exons with P(E) > C which are not present in the optimal parse.

Suboptimal exons have a variety of potential uses. First, suboptimal exons sometimes correspond to real exons which were missed for whatever reason by the optimal parse of the sequence. Second, regions of a prediction which contain multiple overlapping and/or incompatible optimal and suboptimal exons may in some cases indicate alternatively spliced regions of a gene (Burge & Karlin, in preparation). The probability cutoff C used to determine which potential exons qualify as suboptimal exons can be set to any of a range of values between 0.01 and 1.00. The default value on the web page is 1.00, meaning that no suboptimal exons are printed. For most applications, a cutoff value of about 0.10 is recommended. Setting the value much lower than 0.10 will often lead to an explosion in the number of suboptimal exons, most of which will probably not be useful. On the other hand, if the value is set much higher than 0.10, then potentially interesting suboptimal exons may be missed.

GENSCAN



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)

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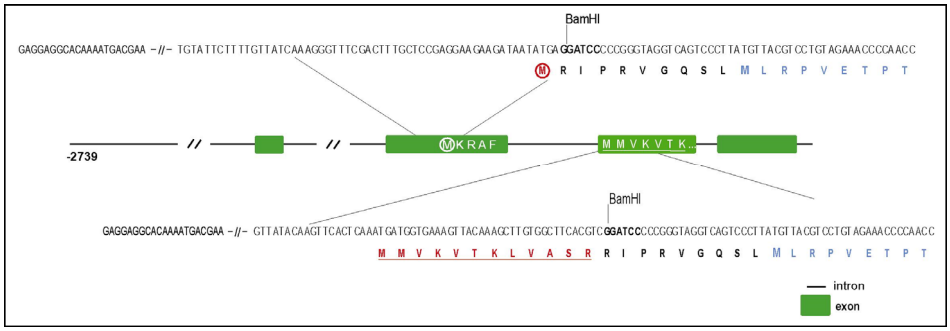
Regulation of translation

- 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)



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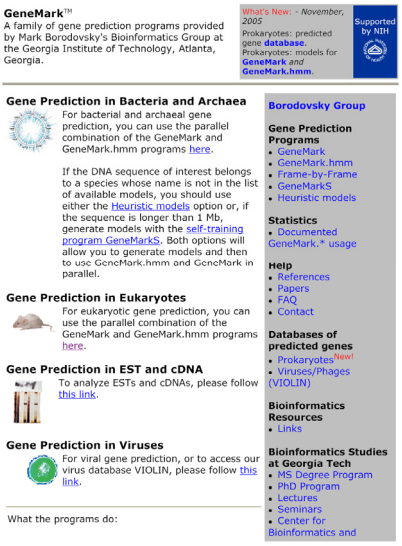
Gene Modelling

- 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



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GeneMark

Result of last submission:

View PDF Graphical Output

GeneMark.hmm Listing

Go to: GeneMark.hmm Protein Translations

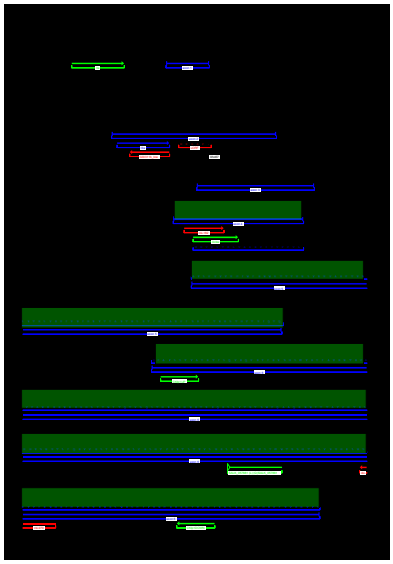
Go to: Job Submission

```
Eukariotyc GeneMark.hmm version bp 3.9 April 25, 2008
Sequence name: CK11
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           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        1516  2175         660          1  3  -  -
  1    4    +   Internal        2266  2644         379          1  1  -  -
  1    5    +   Internal        2704  3317         584          2  3  -  -
  1    6    +   Internal        3297  4629        1233          1  3  -  -
  1    7    +   Terminal        4709  4921         213          1  3  -  -
```



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Matrices file: /home/genmark/euk_ghm.matrices/athaliana_hmm3.0mod
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Predicted genes/exons

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


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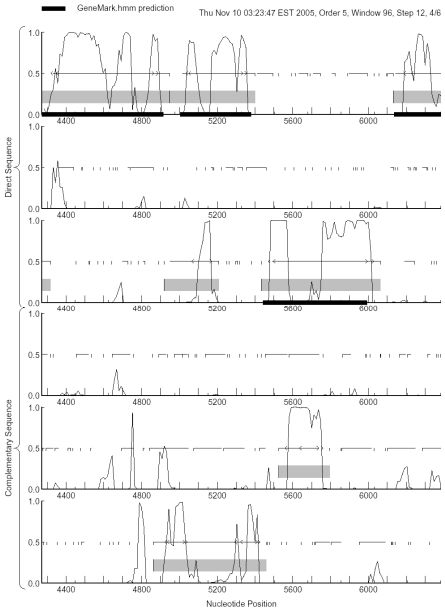
  1    3    +   Internal        1515       2175       660          1 3 - -
  1    4    +   Internal        2266       2644       379          1 1 - -
  1    5    +   Internal        2704       3317       584          2 3 - -
  1    6    +   Internal        3397       4629      1233          1 3 - -
  1    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)

CEITEC

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

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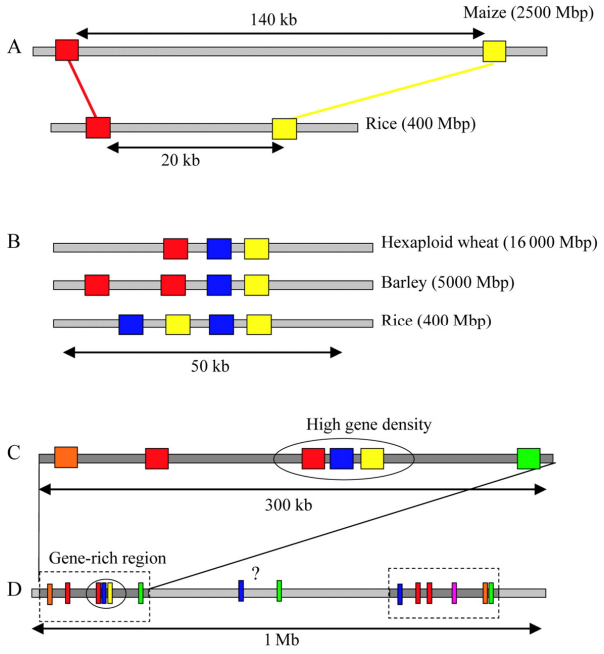
Genomic Colinearity

- 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)

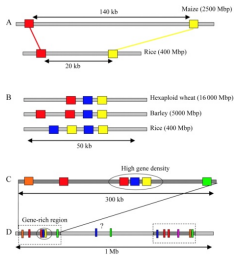
Genomic Colinearity



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.)



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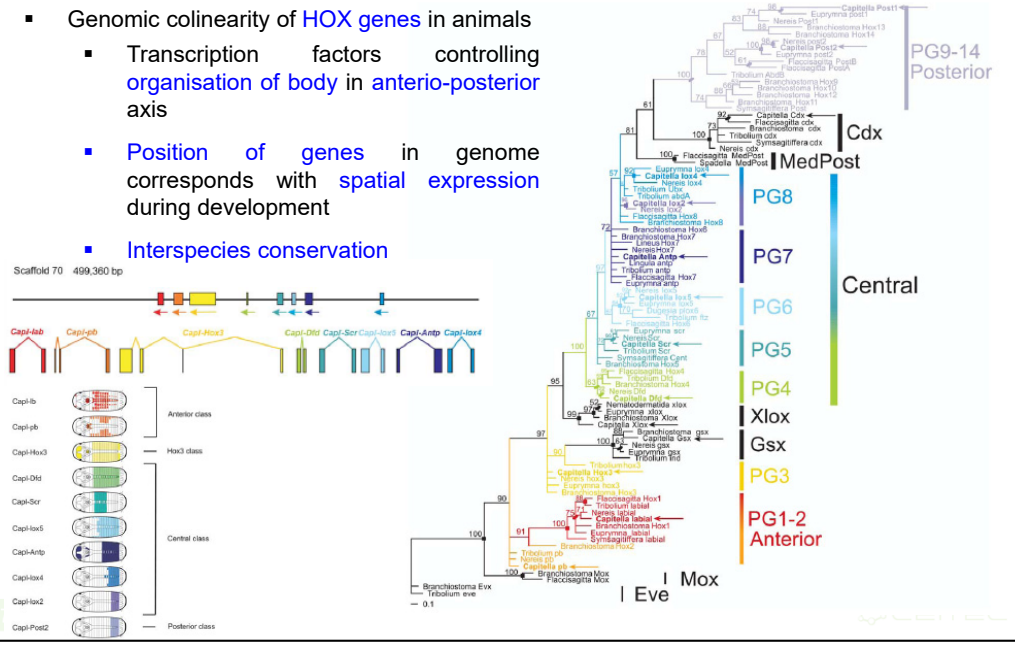
Genomic Colinearity

- 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



Genomic organization of the Capitella sp. I Hox cluster. A total of 11 Capitella sp. I Hox genes are distributed among three scaffolds. Black lines depict two scaffolds, which contain 10 of the Capitella sp. I Hox genes. The eleventh gene, CapI-Post1, is located on a separate scaffold surrounded by ORFs of non-Hox genes (unpublished data). No predicted ORFs were identified between adjacent linked Hox genes. Transcription units are shown as boxes denoting exons, connected by lines that denote introns. Transcription orientation is denoted by arrows beneath each box. Color coding is the same as that used in on the right-hand side for each ortholog.

The phylogenic tree on the right-hand side shows that the order of the genes on the chromozome is retained in several species (genome colinearity).

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  - Constructing gene-enriched libraries using methylation filtration technology

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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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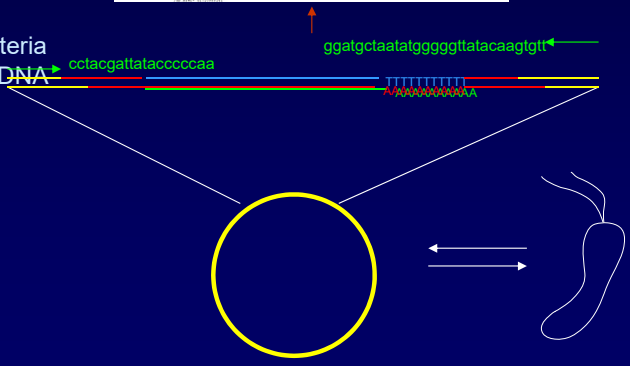
  - EST libraries

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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Discussion