This site provides online material for the **www.cosmoss.org 2nd Physcomitrella genome workshop** that was held

on

July 1st-3rd 2009

at

University of Freiburg, Germany

Here are more photos...

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Venue

Biology Campus, Hauptstr. 1, Computer Pool & Lounge

Speakers

- Stefan Rensing
- Andreas Zimmer

- Daniel Lang

Cosmoss accounts

You have to be a registered cosmoss user in order to access some of linked material.

For the workshop participants, accounts have been created (Unless you already had one) with an initial password that has been sent by e-mail. Please change it as soon you log in.

Everyone else is also invited to register! Please contact helpdesk-cosmoss@uhura.biologie.uni-freiburg.de for an account!

First day

Morning session

The P. patens genome

speaker
: Stefan

slides
: Genome.pdf

Overview of cosmoss.org resources

speaker
: Daniel

slides
: cosmoss_overview.pdf

Cosmoss sequence retrieval

speaker
: Andreas

slides
: Sequence_Retrieval_09.pdf

The cosmoss.org databases

speaker
: Daniel

slides
: cosmoss_databases.pdf

Hands-on experience I

Using cosmoss.org (help, documentation), sequence retrieval, databases

prepared by
: Andreas and Daniel

Menu, documentation, mailing list and wiki

Hint: Use STRG + left mouse click to open a link in a new window

Open http://www.cosmoss.org

Tasks

1. Explore the the menu system
2. Find the FAQ
3. Find the BLAST documentation - What are the e-value threshold defaults?
4. Explore the wiki - Find out Daniel's ICQ number
5. How many predicted genes are in Genome Annotation V1.0?
6. Continents of the World: Where can *Physcomitrella* be found?

Sequence Retrieval

documentation
: Sequence Retrieval

Familiarize yourself with the Cosmoss Retrieval system:

Tasks

Try to retrieve the following sequences:

```
BJ172647
BJ179866
PP015013150R
```

You can access the sequence retrieval via the transcriptome and genome menu.

Hint: These accession numbers above are from the pp0304 annotated virtual transcript database. You can retrieve multiple sequences by providing their accession numbers as a comma- or space-separated

For a vast number of sequences you could upload your request in a text file.

```
File format: text
Provide accession number per line.
```

Select the following accession numbers and copy them into a new text file (e.g. notepad) and save it.

```
Pp1s459_1V2.1|cosmoss|Phypa_173430
Pp1s7_181V2.1|cosmoss|Phypa_201973
Pp1s204_88V2.1|cosmoss|Phypa_221004
Pp1s204_91V2.1|cosmoss|Phypa_59935
Pp1s352_13V2.1|cosmoss|Phypa_152025
Pp1s312_46V2.1|cosmoss|Phypa_225236
Pp1s66_47V2.1|cosmoss|Phypa_106210
Pp1s545_4V2.1|cosmoss|Phypa_109367
Pp1s459_9V2.1|cosmoss|Phypa_110675
Pp1s56_169V2.1|cosmoss|Phypa_125839
Pp1s56_166V2.1|cosmoss|Phypa_125903
Pp1s188_40V2.1|cosmoss|Phypa_168764
Pp1s545_4V2.2|cosmoss|Phypa_201189
Pp1s109_143V2.1|cosmoss|Phypa_233894
Pp1s352_57V2.1|cosmoss|Phypa_61317
Pp1s66_46V2.1|cosmoss|Phypa_77574
Pp1s12_223V2.1|cosmoss|Phypa_115069
Pp1s46_41V2.1|cosmoss|Phypa_123666
Pp1s251_42V2.1|cosmoss|Phypa_146969
Pp1s161_16V2.1|cosmoss|Phypa_87740
Pp1s161_32V2.1|cosmoss|Phypa_87752
Pp1s339_14V2.1|cosmoss|Phypa_151552
Pp1s459_12V2.1|cosmoss|Phypa_8310
```

Goto the cosmoss.org sequence retrieval:

Select database: P.patens.V1.2_proteins

This database contains all P.patens released proteins. In comparison to the release V1.1 all gene models overlapping with transposons, non-protein-coding genes (e.g. tRNA genes) were removed. The *Physcomitrella patens* genome accession numbers work for both transcripts and proteins databases. Just change the database to change to your favored sequence type.

Browse for the previously created file and submit your request

Save the sequences in a new file in FASTA format

Select only a subset of the sequences and save it to a new file

Keyword search

documentation
: Keyword search

How many *geranylgeranyl pyrophosphate synth(et)ases* (GGPS) are in the virtual transcriptome?

Try to find the corresponding pp0304 virtual transcripts by keyword search!

Tasks

1. Read the documentation
2. Use the simple search menu to find the GGPS's in pp0304
3. Play around with the advanced search option.

This is the advanced query that works:

```
"geranylgeranyl"[DESC] AND "phosphate"[DESC] AND "synth"[DESC]
```

Finally, here are the two loci in the genome:

- Pp1s3_331V2.1
- Pp1s8_136V2.1

The two initial pp0304 transcripts are highlighted with a yellow box.

Afternoon session

BLAST, homology and hit filtering

speaker
: Stefan

slides
: BLAST.pdf

Hands-on experience II

(batch) BLAST & hit filtering

prepared by
: Stefan

material
: BLAST_hands_on.pdf

Reciprocal BLAST

speaker
: Andreas

presentation
: Reciprocal_BLAST_searches_09.pdf

CSV BLAST

speaker
: Stefan

presentation
: CSV-BLAST.pdf

Hands-on experience III

CSV-BLAST and reciprocal BLAST

prepared by
: Stefan and Andreas

material

- CSV-BLAST_hands_on.pdf
- Hands_on_reciprocal_blast.pdf

Second day

Morning session

The cosmoss.org genome browser

speaker
: Andreas

presentation
: Basic_GenomeBrowser_09.pdf

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other gbrowse instances for additional genomes

- *Physcomitrella* plastid genome
- *Physcomitrella* mitochondrial genome

- *Arabidopsis*genome TAIR7
- *Selaginella* genome FM2
- *Vitis genome V1.0*
- organellar genomes

Hands-on experience IV

Genome browser basics

prepared by
Andreas and Daniel
presentation
Hands_on_gbrowse_basics.pdf

Genome browser: hidden treasures

speaker
Daniel
presentation
cosmoss_gbrowse_treasures.pdf
links

- Scalable Vector Graphics (SVG)
- Inkscape An Open Source vector graphics editor, with capabilities similar to Illustrator, CorelDraw, or Xara X, using the W3C standard Scalable Vector Graphics (SVG) file format.

Hands-on experience V

Genome browser: customization and special features

prepared by
Daniel
documentation gbrowse
general gbrowse help

BLAST gbrowse integration

documentation
BLAST_gbrowse_integration
Tasks

1. BLAST with an arbitrary transcript/EST vs the scaffolds and follow the 

link.
2. Compare results when BLASTing w/o low complexity filtering!
3. Compare the BLAST to the spliced-alignment results. Are there lonely exons?

4. BLAST with the *Arabidopsis* protein `AT5G13930.1` vs the v1.2 gene models and follow the  link.
5. Compare one of the hit *Physcomitrella* loci vs the *Arabidopsis* locus AT5G13930

Advanced navigation and zooming

Tasks

1. Find out the definition of a *gene* in SO by using the *Ontology_term* cross-link in a gene feature's mouse-over window
2. Zoom into a CDS exon and back again to the region of its *mommy* mRNA or gene
3. Play around with the zoom function to inspect EST and cDNA spliced alignments!

Exporting sequence annotations and publication quality images

Tasks

1. Export the upstream 10kbp your most favorite region to FASTA format and reverse it when necessary to reflect the gene's orientation.
2. *OPTIONAL:* Get another upstream region and try to find shared putative promoter elements using e.g. AlignACE
3. Save your most favorite region as a png image.
4. Check out the exemplary PDF created with Inkscape from the SVG of a region: www.cosmoss.org.inkscape_example.pdf

Highlighting

example locus
: scaffold_29:573541..579040

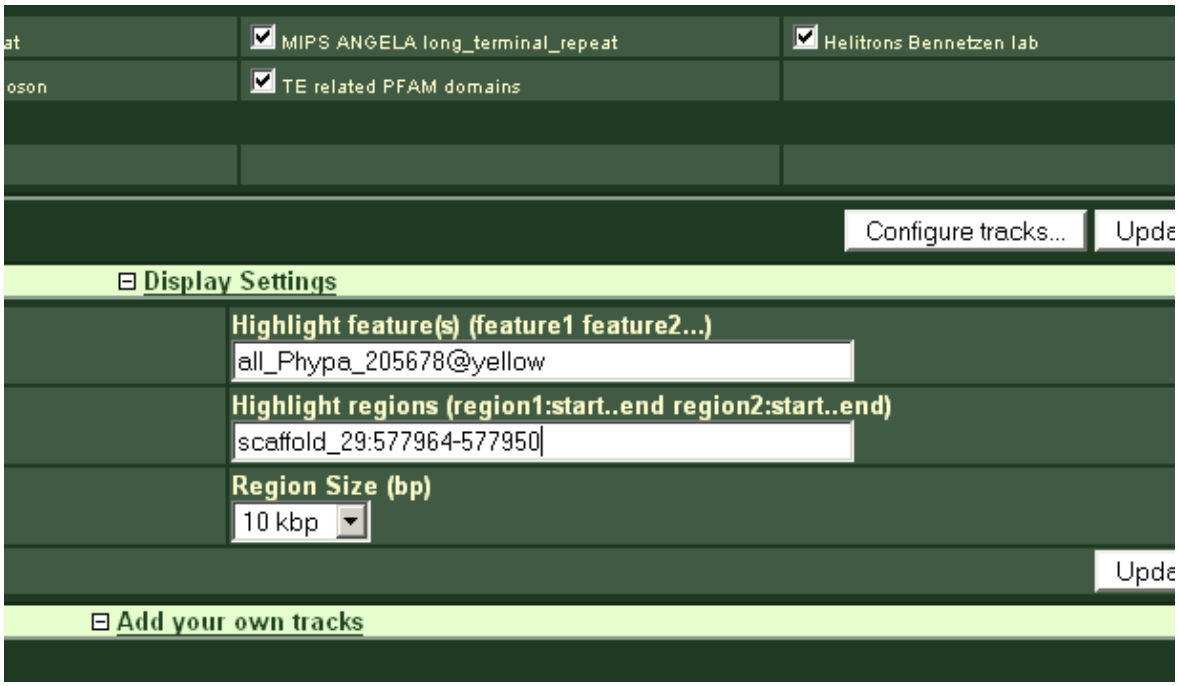
documentation
: available colors (scroll down to *Colors*)

Tasks

1. Let gbrowse highlight a feature and zoom out again, in order to see whether it overlaps with another feature in another track (*highlight and zoom to the region of this feature* link in the mouse-over)
2. Highlight your favorite gene model for the locus using the *Highlight feature* box in the *Display panel*.
3. Visualize an PCR experiment on the genomic locus! You've just amplified and sequenced an genomic PCR product for the locus using the primer coordinates below. Highlight the genomic region using the *Highlight regions* box in the *Display panel*.

forward primer mapping
: scaffold_29:575397..575417

reverse primer mapping
: scaffold_29:577964..577950



Displaying custom annotation

You can draw your own features and have your own custom track in the browser!

documentation
: gbrowse custom annotation help

example locus
: scaffold_29:573541..579040

Tasks

1. Go through the documentation
2. Visualize an RT-PCR experiment on the genomic locus! You've just amplified and sequenced an RT-PCR product for the locus below. Display it as custom annotation using the coordinates:

```
RT-exon1 scaffold_29:575397..575570
RT-exon1 scaffold_29:576849..576875
RT-exon1 scaffold_29:577042..577335
RT-exon1 scaffold_29:577629..577692
```

See the example file which combines everything. Adjust it, if you like!

Afternoon session

V1.0., 1.1, 1.2, 1.5, 2.0 ?!

speaker
: Stefan

slides
versions.pdf

Annotation interface

speaker
Daniel
slides
genonaut.pdf

Hands-on experience VI

Annotation interface

prepared by
Daniel

Hands-on experience VII

?Putting the pieces together - The Summary HowTo? & (on your own/Q&A)

The Summary HowTo

prepared by
Stefan (caution: this is using v1.2!)

slides
HowToSummary.pdf

material (in the order you might need it)

the paper

A.t. MS1 query as FASTA

P.p. MS1 homologs as FASTA

A.t. MS1 homologs as FASTA

the BLAST csv result against Swissprot

the Excel table from above - three tabs

the n.r. acc. nos. from above

the combined protein sequences in FASTA format

the alignment of above in FASTA format

the alignment in PHYLIP format

sneak preview

the two P.p. loci are

```
scaffold_314:310561..315560
scaffold_271:262092..267091
```

an ML tree:

```
(((A9TNA4_PHY:0.065768,A9TTI0_PHY:0.028371):0.304285,((((A5AL84_VIT:0.048466,A7P414_VIT:0.000000)
```

bootstrapped:

```
(((A9TNA4_PHY:0.065768,A9TTI0_PHY:0.028371)100:0.304285,((((A5AL84_VIT:0.048466,A7P414_VIT:0.0000
```