This site provides online material for the **www.cosmoss.org Physcomitrella Genome Workshop** that is held in Freiburg.

date

16.-18.3.2008

Contents

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. The password is on the sheet with the wifi credentials at the bottom. Please change it as soon you log in.

But everyone is 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
presentation
    Genome.pdf

Overview of the cosmoss.org resources

speaker
    Daniel
presentation
    cosmoss_overview.pdf

Cosmoss sequence retrieval

speaker
    Andreas
presentation
    SequenceRetrieval.pdf

BLAST, homology and hit filtering

speaker
    Stefan
presentation
    BLAST.pdf

Hands-on experience I

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

prepared by
    Andreas and Daniel

Menu, documentation, mailing list and wiki

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. Register to both of the mailing lists

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.

```
Phypa_173430
Phypa_201973
Phypa_221004
Phypa_59935
Phypa_152025
Phypa_225236
Phypa_106210
Phypa_109367
Phypa_110675
Phypa_125839
Phypa_125903
Phypa_168764
Phypa_201189
Phypa_233894
Phypa_61317
Phypa_77574
Phypa_115069
Phypa_123666
Phypa_146969
Phypa_87740
Phypa_87752
Phypa_151552
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:

- Phypa_201614
- Phypa_65868

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

Sequence Viewer

documentation
: Sequence Viewer

The accession numbers (sequence identifiers) in the search results from the above keywords search link out to the (transcriptome) sequence viewer!

Tasks

1. Check it out and play around with the (sometimes hidden, I know) links to inspect all features that we annotated for these sequences.
2. PP_9795_C1 is an assembled (contig) transcript. How many ESTs are included in this contig?
3. Retrieve the contig members in Genbank format!

In the databases talk you'll hear more on the process, to help you interpret the things you see in the Sequence Viewer...

The cosmoss.org databases

speaker
: Daniel

presentation
cosmoss_databases.pdf
further information

- The DDBJ/EMBL/GenBank Feature Table Definition
- List of features and qualifiers, with examples
- EMBL (format) manual
- EMBL annotation examples
- Sequence Ontology Project (SO)
- GENERIC FEATURE FORMAT VERSION 3 (GFF)
- Gene Ontology

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mossDB

speaker
Stefan
presentation
mossDB.pdf

Afternoon session

Hands-on experience II

(batch) BLAST & hit filtering

prepared by
Stefan
material
BLAST_hands_on.pdf

Reciprocal BLAST

speaker
Andreas
presentation
Reciprocal_BLAST_searches.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
GenomeBrowser.pdf

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.

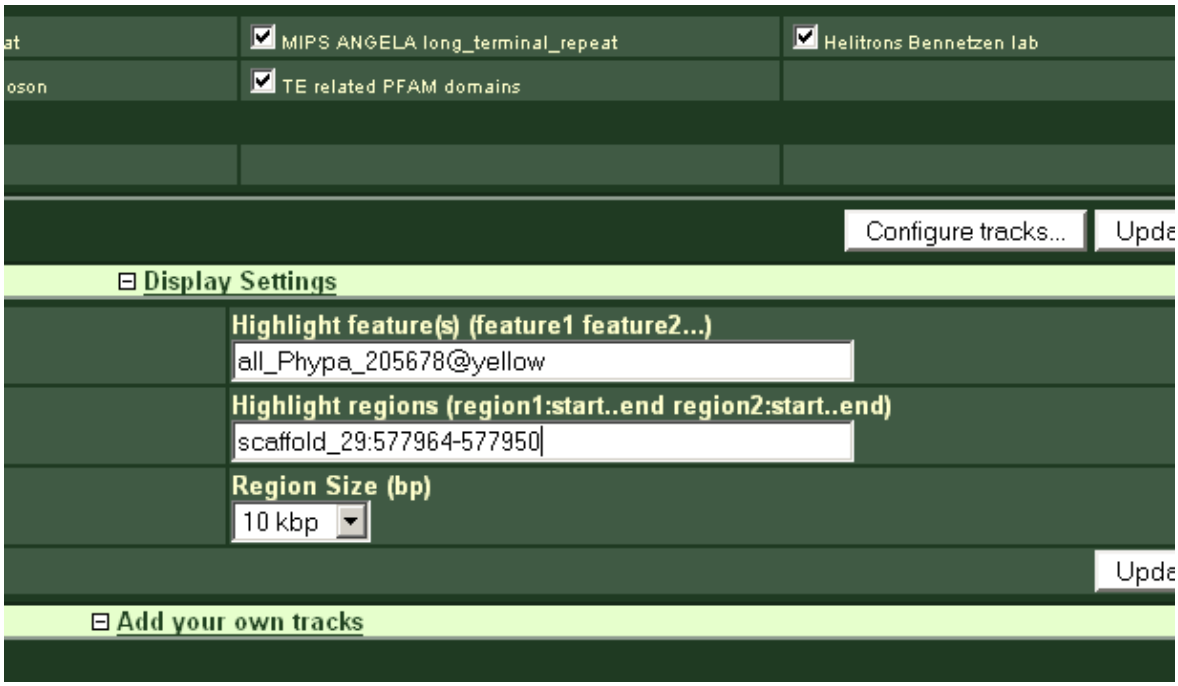
4. Check out the exemplary PDF created with Inkscape from the SVG of a region:
   www.cosmoss.org.inkscape_example.pdf

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

PlanTAPDB as a knowledge base

speaker
: Daniel

presentation
: cosmoss_plantapdb.pdf

related resource
: phylogenies from the genome paper

Hands-on experience VI

PlanTAPDB: keyword search, filtering, phylogenies, taxonomic profiles

showed by
: Daniel

Hands-on experience VII

?The Summary HowTo I.?

Stefan

material
: HowToSummaryI.pdf and see this folder

Hands-on experience VIII

?The Summary HowTo II.?

Depending on what you'd like to work upon...