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Home

This page last changed on Jan 07, 2014 by jeltje.

This is the DOE Micromonas project wiki.

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Feel free to add or edit pages

For powerpoint presentations of our January meeting, see under Children(below)

Protocols and Procedures

Chemostat
Configuring nextgen to run sequenceserver and genome-browser

Adding blast databases to sequenceserver

This page last changed on Jan 07, 2014 by jeltje.

Blast databases are hosted on nextgen.shore.mbari.org. The username is geneadm, for the password see Sebastian Sudek, Mike McCann, Brian Schlining, or Rich Schramm.

To create new blast databases:

1. Get the protein or nucleotide sequences in FASTA format
2. Remove or replace all pipe symbols (|) from the sequence ID (which is anything before the first space in the fasta header)
3. Create sequence IDs that do not occur in any of the other databases. For example, you should not have two separate blast databases with IDs like >CCMP2099_1. Sequence IDs can be rather long, so for instance add the sample ID to them: >MMETSP0022C_CCMP2099_1
4. Copy the sequences to the /data/meta (for metatranscriptome and metagenome files) or /data/dbs (for everything else) on nextgen.shore.mbari.org. This can be done using ssh from another computer with username geneadm and password

   ```
   scp path/to/fasta geneadm@nextgen.shore.mbari.org:/data/meta
   ```
5. Login to nextgen and go to the directory with the sequences

   ```
   ssh geneadm@nextgen.shore.mbari.org (same password)
   cd /data/dbs (or meta)
   ```
6. Run

   ```
   makeblastdb -dbtype nucl -in <new nucleotide fasta> -title <blast db name you want> -
   parse_seqids
   ```

   For protein sequences, dbtype is prot
7. Restart the sequence server with

   ```
   sudo /sbin/service httpd restart
   ```

   This will ask you for the password again
8. Log out with

   ```
   exit
   ```

To remove databases, simply remove the files created by makeblastdb and restart the server.

Adding intensity tracks to genome browser

This page last changed on Jan 07, 2014 by jeltje.

Intensity tracks must be in the bigwig (.bw) format. Previously, those were created for us by Chee-Hong at JGI from BAM files.

genomebrowser.shore.mbari.org is hosted on nextgen.shore.mbari.org. The username is geneadm, for the password see Sebastian Sudek, Mike McCann, Brian Schlining, or Rich Schramm.

Copy the bigwig file to nextgen with

```
scp /path/to/bigwig geneadm@nextgen.shore.mbari.org:/data/genome_browser_data/bigwigs
password:<fill in password>
```

Login to nextgen

```
ssh geneadm@nextgen.shore.mbari.org
password:<fill in password>
```

Add the new track to the database (CCMP1545_v3 or RCC299_v3)

```
hgBbiDbLink CCMP1545_v3 <your_trackname> /data/genome_browser_data/bigwigs/<new_bigwig.bg>
```

Tell the browser about the new track

```
formatTrack
(then follow prompts)
```

And restart the browser

```
cd /data/genome_browser_data/genomes/trackDb && ./loadTracks.sh
```

Your new tracks should now show up under the blue RNASeq bar in the browser. They are hidden by default.

Tip for pros: you can access the mysql database as -ubrowser -pgenome. This might be useful if you want to remove tracks.

To remove tracks from the browser page, edit the pages under

```
/data/genome_browser_data/genomes/trackDb/CCMP1545/CCMP1545_v3/trackDb.ra
and
/data/genome_browser_data/genomes/trackDb/RCC299/RCC299_v3/trackDb.ra
```

Chemostat

This page last changed on Apr 05, 2011 by yansc.

Jeremy and Emily should be responsible for maintaining this category.

Procedures and Protocols

Complete workflow to assemble chemostat and start experiment

Days before assembly; start of experiments

Autoclaving media

Reconditioning and calibration of the dO probe

dO probe can be reconditioned a few days before the start of the experiment.

Recondition:

1. Open the pre-membrane cap counter clockwise. Discard the membrane. The membrane may be reusable if the probe has not been immersed in water for a long time.
2. Wash the probe under running DI/RO water. Inject DI/RO water into the probe by syringe if necessary, see Note below.
3. Dry the probe, get the DI/RO water out of the probe, such as by swinging.
4. Refill with recondition electrolyte. Manufacturer manual says about 5ml. I found electrolyte start leaking after 2ml for all four probes tested.
5. Recap with new membrane if replacing.

Calibration:

1. Before calibration, calibrate PC software to allow the hardware when connected to calibration plug, show about 0mV.

Note
If the probe has been left dry for a long time, i.e. there is not much dried salt after opening the cap, the washing process with water should not require too much work. Otherwise, one can use a syringe with needle to inject RO (DI) water through one to four of the holes. If white "powdery" substances are washed out, it is likely the probe has been left dry for a while.

Reconditioning and calibration of the pH probe

pH probe can be reconditioned and checked for performance before the start of the experiment.

1. Before calibration, calibrate PC software to allow the hardware when connected to calibration plug, show about 0mV.

Autoclaving of parts

List of parts that need to be autoclaved separately

Parts that need to be assembled before autoclaving

On the day of assembly

Sterilization of dO and pH probe

Photos and videos

Photos of assembly and diagram

Videos of chemostat

Configuring nextgen to run sequenceserver and genome-browser

This page last changed on Jan 16, 2019 by mccann.

Initial Virtual Machine setup

1. Requested VM from with Help Ticket 18084:

```
Request_Submitted_By: mccann@mbari.org
VM_Name: nextgen
VM_Purpose: Run genomics processing and server software
VM_Expiration: Permanent
VM_Support: IS_Supported
Alt_Administrator:
Project_Number:
VM_OS: CentOS 6 - Recommended for Linux
RAM: 2
Addl_RAM_Reason:
CPU: 2
Addl_CPU_Reason:
VM_disk: Data (as needed) may be mounted from atlas.  The default 75 GB should be fine to
start with.
VM_Comments: We envision this server to eventually perform the function that the wlab-hm
"cluster" currently
performs, but in a more robust manner. Processors and RAM may need to be added as we monitor
it.
The system needs to be configured by 16 December.
Submit: Submit
```

2. Followed up with Help Tickets 18119, 18120, 18124, 18125, 18127, 18143, 18219, 18222, 18223, 18253, and 18255
3. CNAMEs of **sequenceserver.shore.mbari.org** and **genomebrowser.shore.mbari.org** configured to point to nextgen's IP address
4. sudo privileges: mccann, rich, and brian; sudo /sbin/service granted to user geneadm

SequenceServer - a Ruby on Rails Web application

1. See http://www.sequenceserver.com/
2. Built Ruby 1.9.3p484 from source following instructions at http://techblogthing.blogspot.com/2012/11/complete-guide-to-install-ruby-193-on.html
3. Installed sequenceserver with:

```
sudo /usr/local/bin/gem install sequenceserver
sudo /usr/local/bin/gem install passenger
sudo /usr/local/bin/gem install rspec
sudo /usr/local/bin/gem install watir-webdriver
sudo /usr/local/bin/gem install rack-test
sudo /usr/local/bin/gem install headless
sudo /sbin/chkconfig httpd on
sudo /sbin/service httpd start
```

4. Followed the "Optional: SequenceServer on Apache or Nginx with Phusion Passenger" instructions from http://www.sequenceserver.com/ as user mccann:

```
/usr/local/lib/ruby/gems/1.9.1/gems/passenger-4.0.29/bin/passenger-install-apache2-module
```

   and followed instrcitions, it required several more 'sudo yum install's
5. Created file /etc/httpd/conf.d/sequenceserver.conf:

```
LoadModule passenger_module /usr/local/lib/ruby/gems/1.9.1/gems/passenger-4.0.29/buildout/
apache2/mod_passenger.so
PassengerRoot /usr/local/lib/ruby/gems/1.9.1/gems/passenger-4.0.29
PassengerDefaultRuby /usr/local/bin/ruby

# Sub URL
<VirtualHost *:80>
ServerName sequenceserver.shore.mbari.org
DocumentRoot /usr/local/apache/htdocs
```

```
<Directory /usr/local/apache/htdocs>
AllowOverride All
Options FollowSymLinks Indexes MultiViews
Order allow,deny
Allow from all
</Directory>
RailsBaseURI /sequenceserver-dbs
RailsBaseURI /sequenceserver-meta
RailsBaseURI /dbs
RailsBaseURI /meta
RailsEnv development
</VirtualHost>
```

6. Copied sequenceserver gem to additional directories to support multiple databases and symlinks for short names on sequenceserver.shore.mbari.org:

```
cd /usr/local/lib/ruby/gems/1.9.1/gems
sudo chown mccann .
cp -r sequenceserver-0.8.6 sequenceserver-dbs
cp -r sequenceserver-0.8.6 sequenceserver-meta
ln -s sequenceserver-dbs dbs
ln -s sequenceserver-meta meta
sudo chown root .
sudo chown -R root sequenceserver-dbs sequenceserver-meta
```

7. Create symlinks in /usr/local/apache/htdocs for web pages to work:

```
cd /usr/local/apache/htdocs
ln -s /usr/local/lib/ruby/gems/1.9.1/gems/sequenceserver-dbs/public sequenceserver-dbs
ln -s /usr/local/lib/ruby/gems/1.9.1/gems/sequenceserver-dbs/public dbs
ln -s /usr/local/lib/ruby/gems/1.9.1/gems/sequenceserver-meta/public sequenceserver-meta
ln -s /usr/local/lib/ruby/gems/1.9.1/gems/sequenceserver-meta/public meta
```

8. Modified /usr/local/lib/ruby/gems/1.9.1/gems/sequenceserver-dbs/config.ru, adding SequenceServer::App.config_file = ...

```
require 'rubygems'
require 'bundler/setup'
require 'sequenceserver'

SequenceServer::App.config_file = '/home/geneadm/conf/sequenceserver-dbs.conf'
SequenceServer::App.init
run SequenceServer::App
```

9. Modified /usr/local/lib/ruby/gems/1.9.1/gems/sequenceserver-meta/config.ru, adding SequenceServer::App.config_file = ...

```
require 'rubygems'
require 'bundler/setup'
require 'sequenceserver'

SequenceServer::App.config_file = '/home/geneadm/conf/sequenceserver-meta.conf'
SequenceServer::App.init
run SequenceServer::App
```

   See the FAQ on http://www.sequenceserver.com/. We found that the config.ru files need to be owned by root for the servers to work.
10. Restart httpd and see the sequenceservers at:

- http://sequenceserver.shore.mbari.org/dbs
- http://sequenceserver.shore.mbari.org/meta

UCSC genome-browser - a collection of C cgi-bin programs

1. As user mccann on nextgen

```
sudo yum install mysql
sudo yum install mysql-server
sudo yum install mysql-devel
sudo yum install png-devel
```

```
sudo /sbin/chkconfig mysql on
sudo /sbin/service mysql start
```

2. Set the mysql root password

```
/usr/bin/mysql -u root -p
update mysql.user set Password=PASSWORD('xxxx') where User='root';
# See Mike or Rich for the root password
```

3. As user geneadm on nextgen

```
mkdir ~/dev
cd ~/dev
git clone git://genome-source.cse.ucsc.edu/kent.git
cd kent
```

4. Follow instructions in the README and src/product/README.building.source, briefly:

```
export MACHTYPE=x86_64
mkdir -p ~/bin/${MACHTYPE}
cd src
make libs
cd gfServer
make
cd ../gfClient
make
cd ../blat
make
cd ../utils/faToNib
make
cd ../../hg
make
cd htdocs
make install
# Creates web site files in /usr/local/apache {cgi-bin-mccann, htdocs}
```

5. Setup apache server by creating a /etc/httpd/conf.d/genome-browser.conf file:

```
<VirtualHost *:80>
ScriptAlias /cgi-bin/ "/usr/local/apache/cgi-bin-mccann/"
ServerName genomebrowser.shore.mbari.org
DocumentRoot "/usr/local/apache/htdocs/"
<Directory "/usr/local/apache/htdocs">
Options Includes Indexes FollowSymLinks
AllowOverride None
Order allow,deny
Allow from all
XBitHack on
</Directory>

<Directory "/usr/local/apache/cgi-bin-mccann">
AllowOverride None
Options None
Order allow,deny
Allow from all
</Directory>

<Directory "/usr/local/apache/htdocs/trash">
Options MultiViews Indexes
AllowOverride None
Order allow,deny
Allow from all
</Directory>
</VirtualHost>
```

6. Restart httpd:

```
sudo /sbin/service httpd restart
```

7. and see the server at:

- http://genomebrowser.shore.mbari.org/

This page last changed on Mar 15, 2011 by jeltje.

For Powerpoint presentations, click the Attachments tab

Proteomics papers

This page last changed on Apr 26, 2011 by jeltje.

See Attachment tab for papers selected by Stephen

Shuangchun "Jeremy" Yan

This page last changed on Apr 05, 2011 by yansc.

Who am I

I was born and grew up in China. I earned my Bachelor of Science degree at Sichuan University in Chengdu, China and then moved to the United States for graduate school. I got my PhD in Plant Physiology at Virginia Tech, Blacksburg, Virginia in December 2010.

Projects

Click through projects to also see links to detailed updates on specific sub-projects.

The projects I am working on:

- Get chemostat (PRIMaS) running
- Determining a suitable co-habitat conditions for RCC299 and CCMP1545
- MOORE transcriptome project (not part of DOE, no details would be listed here)

Key Updates

Presentations