Assembly with Megahit Version 3

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Abstract

Co-assembly using Megahit.

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Protocol

Step 1.

Log into the HPC.

cmd COMMAND
```
$ ssh hpc
$ ocelote
```

Step 2.

From your home directory, open .bashrc file for editing.

cmd COMMAND
```
$ nano .bashrc
```

NOTES
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Remember, you are already in your home directory after logging into ocelote.

Step 3.

Input the following line into your .bashrc file:

cmd COMMAND
```
export PATH=/rsgrps/bh_class/bin:$PATH
```
This will allow us to execute tools found in /rsgrps/bh_class/bin without specifying the path name.

Step 4.

Save and close the .bashrc file

Step 5.

Move into your project directory.

cmd COMMAND

```
$ cd /rsgrps/bh_class/username
```

Step 6.

Create a directory for assembly output. Then move into that directory.

cmd COMMAND

```
$ mkdir assembly
$ cd !$
```

Step 7.

Make a directory for fasta files. This is the format of your files after doing quality control. You will also need to move all fasta files into this directory.

cmd COMMAND

```
mkdir fasta
mv fastq/*.fasta fasta
```

NOTES

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This step assumes that the fasta files are in the fastq directory. Alter the command accordingly if this is not the case.

Step 8.

Make directories for standard out and standard error.

cmd COMMAND

```
mkdir std-out std-err
```

Step 9.

Before we continue, determine if you have single end or paired end files. If you have two files per SRR number, you have paired end reads. Otherwise, you have single end reads.

1. If you have single end reads proceed to step 10.

2. If you have paired end reads, skip to step 11.

Step 10.

Assembly script for SINGLE END FILES

Create a script called run-assembly.sh

cmd COMMAND

```
#!/bin/bash
```

```
#PBS -W group_list=bh_class
#PBS -q windfall
#PBS -l select=1:ncpus=20:mem=40gb
#PBS -l pvmem=38gb
#PBS -l walltime=24:00:00
#PBS -l cput=48:00:00
#PBS -M netid@email.arizona.edu
#PBS -m bea

FASTA_DIR='/rsgrps/bh_class/username/fasta'
ASSEM_DIR='/rsgrps/bh_class/username/assembly'
MIN_CONTIG_LEN=500
OUT_DIR='/rsgrps/bh_class/username/assembly/megahit-out'

cd $ASSEM_DIR

SINGLEs=`ls $FASTA_DIR/*.fasta | python -
c 'import sys; print ",".join([x.strip() for x in sys.stdin.readlines()])'`

megahit -r $SINGLEs --preset meta-sensitive --min-contig-len $MIN_CONTIG_LEN -o $OUT_DIR -
t 12
```

NOTES

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OUT_DIR does NOT need to be created prior to running this script. Megahit will make the directory on its own.

Step 11.

Assembly script for PAIRED END FILES

Create a script called run-assembly.sh

cmd COMMAND

```
#!/bin/bash

#PBS -W group_list=bh_class
#PBS -q windfall
#PBS -l select=1:ncpus=20:mem=40gb
#PBS -l pvmem=38gb
#PBS -l walltime=24:00:00
#PBS -l cput=48:00:00
#PBS -M netid@email.arizona.edu
#PBS -m bea

FASTA_DIR='/rsgrps/bh_class/username/fasta'
ASSEM_DIR='/rsgrps/bh_class/username/assembly'
MIN_CONTIG_LEN=500
OUT_DIR='/rsgrps/bh_class/username/assembly/megahit-out'

cd $ASSEM_DIR

R1s=`ls $FASTA_DIR/*_1.fasta | python -
c 'import sys; print ",".join([x.strip() for x in sys.stdin.readlines()])'`
R2s=`ls $FASTA_DIR/*_2.fasta | python -
```

```
c 'import sys; print ",".join([x.strip() for x in sys.stdin.readlines()])'`

megahit -1 $R1s -2 $R2s --preset meta-sensitive --min-contig-len $MIN_CONTIG_LEN -
o $OUT_DIR -t 12
```

NOTES

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OUT_DIR does NOT need to be created prior to running this script. Megahit will make the directory on its own.

Step 12.

Run the assembly:

cmd COMMAND

```
$ chmod +x run-assembly.sh
$ qsub -e std-err/ -o std-out/ run-assembly.sh
```

Step 13.

You can check the status of your job with the following command:

cmd COMMAND

```
$ qstat -u username
```

NOTES

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Job runtime will vary depending on the size of your dataset.

Step 14.

Upon job completion, get assembly statistics using MetaQuast on CyVerse.

PROTOCOL

. Assembly Stats with MetaQuast

CONTACT: James Thornton Jr

Step 14.1.

Go to https://user.cyverse.org/

LINK:
https://user.cyverse.org/

Step 14.2.

Click "Sign Up" to create an account.

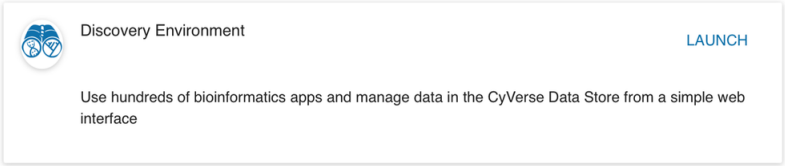
Step 14.3.

After account creation go back to https://user.cyverse.org/ and login with your account.

LINK:
https://user.cyverse.org/

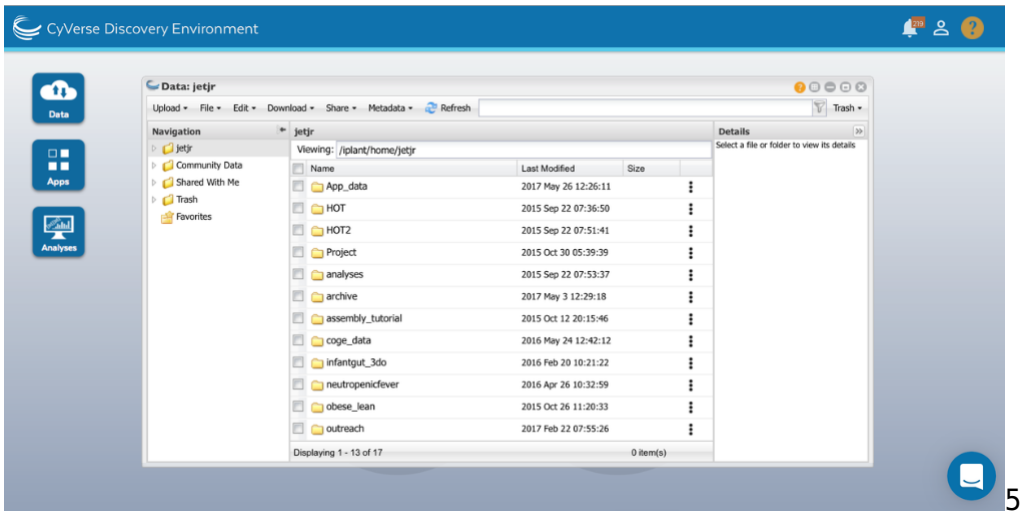
Step 14.4.

Launch the discovery environment.



Step 14.5.

Click the "Data" button found on the left. Navigate to your user folder.



5

Step 14.6.

Click "Upload" > "Simple Upload From Desktop"

Step 14.7.

Upload your final.contigs.fa file generated from Megahit.

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Important: You must scp your contigs to your local machine before you can upload.

$ scp username@sftp.hpc.arizona.edu:/rsgrps/bh_class/username/assembly/megahit-

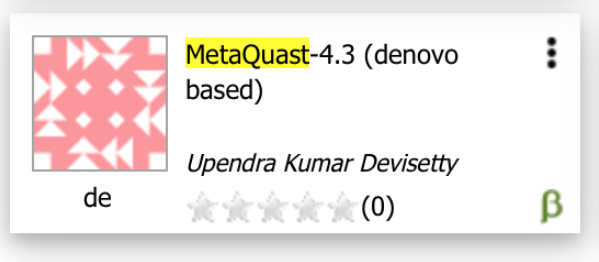
out/final.contigs.fa .

Step 14.8.

Once your upload is complete, click on the "Apps" button found on the left.

Step 14.9.

Search for "MetaQuast". Click on MetaQuast-4.3 (denovo based)



Step 14.10.

Under the "Fasta file(s)" tab, select the newly uploaded final.contigs.fa file. This is the only parameter that needs to change. Click "Launch Analysis".

Step 14.11.

Once MetaQuast is complete (email notification), navigate to the output found in the "analyses" folder in your data storage.

Step 14.12.

Download the "report.html" file found in the MegaQuast output folder.

Step 14.13.

Open the report.html file to see a summary of assembly statistics.