
```
# Open terminal
```

```
# Log into your account
user<no.>@hpc01.icipe.org
password<no.>
```

```
# Determine whether you are in the home directory
```

```
pwd
```

```
cd
```

```
# Make a new directory
```

```
mkdir Assembly
```

```
# copy the fastq files you have into the new directory
```

```
mv ../Assembly.Lab/fastq_file_*.fastq ./Assembly
```

```
# Go into the new directory
```

```
cd Assembly
```

```
# Let's have a quick look at the files...
```

```
head fastq_file_1.fastq
```

```
head fastq_file_2.fastq
```

```
less fastq_file_1.fastq
```

```
less fastq_file_2.fastq
```

```
#lets count the lines in each file
```

```
wc -l head fastq_file_1.fastq
```

```
wc -l head fastq_file_2.fastq
```

```
# Now load all the software you require for this exercise
```

```
# If you don't know what's available you can first have a peek
```

```
module avail
```

```
# Now load Velvet
```

```
module load velvet/1.2.10
```

```
# Once velvet is loaded call velvet scripts to start work on the fastq
sequences
#First have a look at how to call Velvet

velveth -help

# now let's do the first step - running the hash (this creates a hash of
reads)

velveth my_assembly_directory 21 -shortPaired -fastq -separate
fastq_file_1.fastq fastq_file_2.fastq

# what files or directories were created

# Now that the first step is completed, lets do the next step - building the
graph

velvetg --help

velvetg my_assembly_directory -exp_cov auto -cov_cutoff auto

# The assembled sequence should be in the file contigs.fa, have a look at
this! What does it look like?

# At the moment you contigs are assembled but we don't know the order
# Now the next step would be to order you contigs

# We shall use abacas for that, so lets load it

module load abacas/1.3.1

# Abacas requires Mummer to run so let's also load Mummer

module load mummer/3.23

# Now let's run abacas, we shall require a reference sequence to order
against
# So let's use the provided sequence "reference_sequence.fa"

# Let's first see how to run abacas

abacas -h

# Let's now run abacas
```

```
abacas -abcm -p nucmer -r reference_sequence.fa -q
./my_assembly_directory/contigs.fa
```

what files are produced?

```
ls -lrt
```

Your ordered contig file is now called
"contigs.fa_reference_sequence.fa.MULTIFASTA.fa".

You can convert this to a single fasta file using emboss

```
module load emboss/6.6.0
```

```
grep -v ">" contigs.fa_reference_sequence.fa.MULTIFASTA.fa | seqret -filter >
joined_file.dna
```

Now that we have a single file we can do:

1. Ab-initio gene prediction - i.e. predict genes in our assembled sequence from scratch
2. Annotation transfers using well annotated genomes as references

Ab-initio gene prediction

```
module load glimmer/3.02
```

Now use glimmer

```
g3-iterated.csh joined_file.dna joined_file
glimmer3totab.pl joined_file.predict > joined_file.predict.tab
```

Annotation transfers

```
mkdir Ref_emb1
mv FM211187.embl ./Ref_emb1
```

```
~/RATT/start.ratt.sh Ref_emb1 ~/my_assembly_director/single_fasta_file.dna
result Strain.Repetitive
```

