

# MG\_HW6: Gene Calls with Prodigal

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## Abstract

This protocol provides the procedure to generate gene calls on your contigs using Prodigal.

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## Protocol

### Step 1.

Login to the HPC and move into Cluster(ICE).

cmd **COMMAND**

```
$ ssh hpc  
$ ice
```

 **NOTES**

**James Thornton Jr** 10 Oct 2016

Option 3 if you have menu enabled.

### Step 2.

Move into your class directory.

cmd **COMMAND**

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

Use YOUR username to be in the right directory

### Step 3.

Copy the following into a new script called run-interactive.sh :

cmd **COMMAND**

```
#!/bin/bash  
  
#PBS -W group_list=bh_class  
#PBS -q windfall  
#PBS -l jobtype=cluster_only  
#PBS -l select=1:ncpus=2:mem=4gb  
#PBS -l walltime=24:00:00  
#PBS -l cput=24:00:00  
#PBS -l place=pack:shared  
#PBS -M netid@email.arizona.edu
```

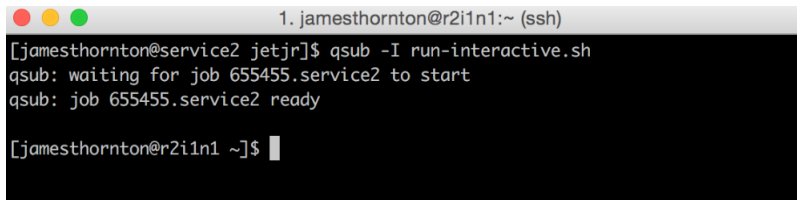
```
#PBS -m bea
Replace netid
```

#### Step 4.

Submit run-interactive.sh interactively using qsub:

```
cmd COMMAND
qsub -I run-interactive.sh
The capital "I" indicates this will be an interactive job
```

#### EXPECTED RESULTS

A terminal window titled '1. jamesthornton@r2i1n1:~ (ssh)' shows the execution of 'qsub -I run-interactive.sh'. The output indicates the job is waiting to start and then ready. The prompt returns to the user's shell.

```
1. jamesthornton@r2i1n1:~ (ssh)
[jamesthornton@service2 jetjr]$ qsub -I run-interactive.sh
qsub: waiting for job 655455.service2 to start
qsub: job 655455.service2 ready

[jamesthornton@r2i1n1 ~]$
```

#### Step 5.

Once the job is ready move back into your class directory.

```
cmd COMMAND
$ cd /rsgroups/bh_class/username
Use YOUR username
```

#### Step 6.

Make a directory named prodigal:

```
cmd COMMAND
$ mkdir prodigal
```

#### Step 7.

Move into your assembly directory which contains your contigs:

```
cmd COMMAND
$ cd /rsgroups/bh_class/username/assembly
```

#### Step 8.

Load prodigal and run it on your fixed\_contigs.fa to generate gene calls.

```
cmd COMMAND
$ module load prodigal/2.6.2
$ prodigal -i fixed_contigs.fa -o ../prodigal/gene_calls -a ../prodigal/proteins.faa -
d ../prodigal/nucleotides.fna
IMPORTANT: make sure to run prodigal on the fixed_contigs.fa file which was generated when
simplifying the fasta header lines. The output will be placed in the prodigal directory that was
created and the file name will be gene_calls in addition to a file containing the protein
(proteins.faa) and nucleotide (nucleotide.faa) sequences for the genes.
```

#### Step 9.

Move into your prodigal directory and make sure the gene calls were generated.

## cmd COMMAND

```
$ cd /rsgrps/bh_class/username/prodigal
$ head gene_calls
```

## EXPECTED RESULTS

```
1. james Thornton@r2i1n1:/rsgrps/bh_class/jetjr/prodigal (ssh)
[jamesthorton@r2i1n1 prodigal]$ head gene_calls
DEFINITION seqnum=1;seqlen=3384;seqhdr="1";version=Prodigal.v2.6.2;run_type=Single;model="Ab initio";gc_cont=59.13;transl_table=11;uses_sd=1
FEATURES             Location/Qualifiers
     CDS             <1..585
                     /note="ID=1_1;partial=10;start_type=Edge;rbs_motif=None;rbs_spacer=None;gc_cont=0.694;conf=100.00;score=64.40;cscore=61.18;sscore=3.22;rscore=0.00;uscore=0.00;tscore=3.22;"
     CDS             complement(611..1393)
                     /note="ID=1_2;partial=00;start_type=ATG;rbs_motif=GGA/GAG/AGG;rbs_spacer=5-10bp;gc_cont=0.674;conf=100.00;score=127.23;cscore=121.91;sscore=5.32;rscore=0.27;uscore=0.84;tscore=3.13;"
     CDS             complement(1412..1933)
                     /note="ID=1_3;partial=00;start_type=ATG;rbs_motif=GGAG/GAGG;rbs_spacer=5-10bp;gc_cont=0.600;conf=100.00;score=73.38;cscore=66.09;sscore=7.29;rscore=2.87;uscore=-0.31;tscore=3.13;"
     CDS             complement(1930..>3384)
                     /note="ID=1_4;partial=01;start_type=Edge;rbs_motif=None;rbs_spacer=None;gc_cont=0.635;conf=99.99;score=218.15;cscore=214.93;sscore=3.22;rscore=0.00;uscore=0.00;tscore=3.22;"
[jamesthorton@r2i1n1 prodigal]$
```

## ANNOTATIONS

**Emma Skidmore** 17 Oct 2016

I'm only getting 3 ID numbers, is this a problem?

```
[service2@/rsgrps/bh_class/emmanoelskidmore/prodigal]$ head gene_calls
DEFINITION
seqnum=1;seqlen=1466;seqhdr="1";version=Prodigal.v2.6.2;run_type=Single;model="Ab initio";gc_cont=56.96;transl_table=11;uses_sd=1
FEATURES Location/Qualifiers
CDS <1..141
/note="ID=1_1;partial=10;start_type=Edge;rbs_motif=None;rbs_spacer=None;gc_cont=0.539;conf=99.24;score=21.22;cscore=18.00;sscore=3.22;rscore=0.00;uscore=0.00;tscore=3.22;"
CDS 153..788
/note="ID=1_2;partial=00;start_type=ATG;rbs_motif=AGxAGG/AGGxGG;rbs_spacer=5-10bp;gc_cont=0.596;conf=100.00;score=66.07;cscore=57.00;sscore=9.07;rscore=4.47;uscore=0.94;tscore=2.51;"
CDS complement(785..>1465)
/note="ID=1_3;partial=01;start_type=Edge;rbs_motif=None;rbs_spacer=None;gc_cont=0.596;conf=100.00;score=80.77;cscore=77.55;sscore=3.22;rscore=0.00;uscore=0.00;tscore=3.22;"
//
DEFINITION
seqnum=2;seqlen=1053;seqhdr="2";version=Prodigal.v2.6.2;run_type=Single;model="Ab initio";gc_cont=56.96;transl_table=11;uses_sd=1
```

## Step 10.

Use your scripting or unix skills to detect how many genes were detected on each of the assemblies. Create a table in google documents that shows the number of "ORFs" or open reading frames detected on each assembly. We will add additional data to this table later on # of genes with annotation, so the table should have a single column for #ORFs and the rows should be the name of each sample "SRR..."

## ■ ANNOTATIONS

**Emma Skidmore** 18 Oct 2016

When I try and count my ORF's I'm only getting one number (22744). I doesn't distinguish between my different SRR's. Is anyone else having this problem?

My prodigal directory only has 3 items inside which are gene\_calls, Nucleotides.fna, and proteins.faa. From what I can tell, they do not have the different SRR's clearly labeled with in them.