

Assign taxonomy to gene calls using Centrifuge Version 2

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Abstract

Uses a custom Centrifuge pipeline to assign taxonomy to gene calls.

Citation: James Thornton Jr Assign taxonomy to gene calls using Centrifuge. **protocols.io**

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Protocol

Step 1.

Navigate to the directory on your local machine that contains the contigs.db generated during the [Anvi'o protocol](#).

Step 2.

Extract gene calls from the contigs database.

cmd **COMMAND**

```
$ anvi-get-dna-sequences-for-gene-calls -c CONTIGS.db -o nucleotides.faa
```

 **NOTES**

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Important: nucleotides.fna was generated in the prodigal protocol. HOWEVER, we will be using this version from Anvi'o for taxonomy assignment.

 **ANNOTATIONS**

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Remember windows users you must launch Anvio using docker.

```
docker run --rm -v /path/to/files:/my_data -p 8080:8080 -it meren/anvio:latest
```

Step 3.

Log into the HPC

cmd **COMMAND**

```
$ ssh hpc  
$ ocelote
```

Step 4.

Move into your class directory.

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

Step 5.

Make an anvio-genes directory.

```
cmd COMMAND
$ mkdir anvio-genes
```

Step 6.

On your local machine, scp the nucleotides.fna file generated from step 2 into the newly created anvio-genes directory.

```
cmd COMMAND
$ scp nucleotides.fna username@sftp.hpc.arizona.edu:/rsgroups/bh_class/username/anvio-genes
```

Step 7.

Clone the Centrifuge github repository into your class directory on the HPC.

```
cmd COMMAND
$ pwd
/rsgroups/bh_class/username
$ git clone git@github.com:jetjr/Centrifuge.git
```

Step 8.

Move into the Centrifuge directory.

```
cmd COMMAND
$ cd Centrifuge
```

Dependencies

Step 9.

This program uses R packages that must be installed prior to launching the job. Load the R module.

```
cmd COMMAND
$ module load unsupported
$ module load markb/R/3.1.1
```

Dependencies

Step 10.

Launch R.

```
cmd COMMAND
$ R
```

Dependencies

Step 11.

Get the "optparse" package.

cmd **COMMAND**

```
> install.packages("optparse", repos="http://R-Forge.R-project.org")
```

■ **ANNOTATIONS**

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Choose yes if prompted to use a personal library.

Dependencies

Step 12.

Get ggplot2 and plyr packages. You may be prompted to select a mirror. Any US server will work.

cmd **COMMAND**

```
> install.packages("ggplot2")
> install.packages("plyr")
```

🔗 **NOTES**

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If you receive an error when installing the dependencies, continue with the protocol.

Dependencies

Step 13.

Quit the R session. Do not save workspace image.

cmd **COMMAND**

```
> q()
> Save workspace image? [y/n/c]: n
```

Step 14.

Edit the config.sh file to include the correct variable declarations. The following steps will detail how the config.sh file should be edited.

cmd **COMMAND**

```
$ nano config.sh
```

CENT_DB

Step 15.

```
export CENT_DB="/rsgrps/bh_class/b_compressed+h+v/b_compressed+h+v"
```

FASTA_DIR

Step 16.

```
export FASTA_DIR="/rsgrps/bh_class/username/anvio-genes"
```

🔗 **NOTES**

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FASTA_DIR should point to the directory containing your nucleotides.fna file generated from step 2

and transferred to the anvio-genes directory.

TYPE

Step 17.

```
export TYPE="single"
```

FILE_EXT

Step 18.

```
export FILE_EXT="fna"
```

REPORT_DIR

Step 19.

```
export REPORT_DIR='/rsgrps/bh_class/username/anvio-genes/taxonomy/'
```

📌 NOTES

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The program will create this directory for you. Make sure to replace username.

PLOT_OUT

Step 20.

```
export PLOT_OUT='/rsgrps/bh_class/username/anvio-genes/taxonomy/'
```

📌 NOTES

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Same as REPORT_DIR but make sure to include the trailing / as stated in the config.sh file.

PLOT_FILE and PLOT_TITLE

Step 21.

These should be named according to what sample your working with. For example, ocean data may name these:

```
export PLOT_FILE='ocean_depth'
```

```
export PLOT_TITLE='ocean_depth'
```

📌 NOTES

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PLOT FILE will be the file name of the bubble plot that is generated.

PLOT TITLE will be the title found on the actual plot.

FILE_TYPE

Step 22.

```
export FILE_TYPE="f"
```

📌 NOTES

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The nucleotides.fna file is in FASTA format.

EXCLUDE

Step 23.

The exclude parameter can be left blank.

```
export EXCLUDE=""
```

Step 24.

Save and quit config.sh

Step 25.

Submit the job using the submit script found in the Centrifuge directory.

```
cmd COMMAND
$ ./submit.sh
```

Step 26.

Status of the job can be determined by the following command:

```
cmd COMMAND
$ stat -u username
```

Step 27.

A successful job will generate a centrifuge_report.tsv file in anvio-genes/taxonomy.