**STANDARD OPERATING PROCEDURE (SOP) FOR DNA SEQUENCE ANALYSIS FROM ILLUMINA MISEQ PLATFORM**

**Software:**

**USEARCH v10.0.240\_i86osx32 (2017)**

**Example Data provided by Gallo & Brian Leydet**

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**This protocol closely follows the video posted by Robert Edgar:**

[**https://www.youtube.com/watch?v=pOV\_tuZqzso&t=292s**](https://www.youtube.com/watch?v=pOV_tuZqzso&t=292s)

**Part 1: Download Programs**

USEARCH: <https://www.drive5.com/usearch/download.html> (Reference Edgar 2013)

Unzip USEARCH. Activate Unix executable for USEARCH using the following command (on Mac OSX terminal):

**Apples-MacBook-Pro-2:~ ben$ chmod +x (FILENAME)**

Create a new folder directory on desktop for USEARCH – create subdirectory folders labeled ‘fq’, ‘out’, and ‘script’ respectively.

**Part 2: Merge Paired Reads using USEARCH**

Open USEARCH in the terminal:

**Apples-MacBook-Pro-2:~ ben$ cd desktop**

**Apples-MacBook-Pro-2:desktop ben$ ./usearch10.0.240\_i86osx32**

**Apples-MacBook-Pro-2:desktop ben$ cd usearch**

**Apples-MacBook-Pro-2:usearch ben$ export usearch=~/Desktop/usearch10.0.240\_i86osx32 ###Depending on where file is saved**

Upload all FASTQ files to the fq directory. **BE SURE TO MAKE IT SO THAT ALL SAMPLE NAMES HAVE THEIR ID ATTACHED (E.G. RG-025 CHANGE TO RG025) USEARCH DENOTES ‘-‘ AS THE END OF THE NAME OF THE SAMPLE.** This will be important later on when making the OTU table.

Go to the scripts folder.

**Apples-MacBook-Pro-2:usearch ben$ cd scripts**

Scripts should be run in the following order:

**Apples-MacBook-Pro-2:scripts ben$ ./merge**

**Apples-MacBook-Pro-2:scripts ben$ ./stripped.sh**

**Apples-MacBook-Pro-2:scripts ben$ ./filter**

**Apples-MacBook-Pro-2:scripts ben$ ./uniques**

**Apples-MacBook-Pro-2:scripts ben$ ./precluster**

**Apples-MacBook-Pro-2:scripts ben$ ./sortby**

**Apples-MacBook-Pro-2:scripts ben$ ./otus**

**Apples-MacBook-Pro-2:scripts ben$ ./otu\_table**

This will produce final OUT table that you can export as a .txt file and convert to excel, upload to R, etc.

**References:**

Edgar, R., C., 2013. UPARSE: highly accurate OTU sequences from microbial amplicon reads. *National Methods* **10**: 996-998. doi:10.1038/nmeth.2604.