

# Demultiplexing Internal Indexes Using Mr. Demuxy

Author: BadDNA Lab, UGA

Lates version of Mr. Demuxy is available at: [https://pypi.org/project/Mr\\_Demuxy/](https://pypi.org/project/Mr_Demuxy/)

Mr. Demuxy was developed by Daniel E. Lefever

## Goal

If TaggiMatrix from *Adapterama II* system with indexed fusion primers was used to construct libraries, Mr. Demuxy can be used to quickly and efficiently demultiplex samples by internal indexes from Read 1 and Read 2 fastq files.

## Files to Run Mr. Demuxy

1. Mr. Demuxy uncompressed folder
2. Read 1 and Read 2 fastq Files, uncompressed
3. Indexes txt Files (forward and reverse)

Each set of indexes (Read 1 and Read 2) can be saved as .txt file

### Read 1 Indexes (See Supplemental File S1)

A GGTAC  
B CAACAC  
C ATCGGTT  
D TCGGTCAA  
E AAGCG  
F GCCACA  
G CTGGATG  
H TGATTGAC

### Read 2 Indexes (See Supplemental File S1)

1 AGGAA  
2 GAGTGG  
3 CCACGTC  
4 TTCTCAGC  
5 CTAGG  
6 TGCTTA  
7 GCGAAGT  
8 AATCCTAT  
9 ATCTG  
10 GAGACT  
11 CGATTCC  
12 TCTCAATC

## How to Run Mr. Demuxy

```
python pe_demuxer.py -r1 PATH-TO-READ-1-FASTQ-FILE.fastq \  
-r2 PATH-TO-READ-2-FASTQ-FILE.fastq -r1_bc Read_1_indexes.txt \  
-r2_bc Read_2_indexes.txt -o PATH-TO-OUTPUT-FOLDER/
```

## Output of Mr. Demuxy

You will have in the output folder Read 1 and Read 2 fastq files, which names are going to correspond to the combination of internal indexes demultiplexed. For example:

```
A1_R1.fastq A1_R2.fastq (For indexes A and 1)  
A2_R1.fastq A2_R2.fastq (For indexes A and 2)  
A3_R1.fastq A3_R2.fastq (For indexes A and 3)  
... ..  
... ..  
H10_R1.fastq H10_R2.fastq (For indexes H and 10)  
H11_R1.fastq H11_R2.fastq (For indexes H and 11)  
H12_R1.fastq H12_R2.fastq (For indexes H and 12)
```

## If changing the names of files is desired:

### *How to rename output with meaningful names*

Similarly to the last part of the demultiplexing tutorial of external indexes from Brant Faircloth Lab (<https://faircloth-lab-documentation.readthedocs.io/en/latest/protocols-computer/sequencing/sequencing-demultiplex-a-run.html>). You will need to create a tab-delimited file that has the index combination name (i.e A1, A2, ..., H11, H12) in column 1 and the names you want for the file in column 2.

For example (temp-names.txt):

```
A1 Sample_W  
A2 Sample_X  
...  
...  
H11 Sample_Y  
H12 Sample_Z
```

Then, you can rename files by using:

```
while IFS=$'\t' read -r column1 column2; do  
mv ${column1}_R1.fastq ${column2}_${column1}_R1.fastq;  
mv ${column1}_R2.fastq ${column2}_${column1}_R2.fastq;  
done < "temp-names.txt"
```