

# Bioinformatics pipeline for demultiplexed MiSeq Illumina sequences

## Sample: A

Method w/o ChimeraSlayer

### 1. Multiple join paired ends ~1 min

```
MacQIIME HamdanLabMacPro:~ $ multiple_join_paired_ends.py -i  
/Users/HamdanLab/Desktop/QuickVsFull/RawSequences -o  
/Users/HamdanLab/Desktop/QuickVsFull/multiple_join_paired_ends
```

### 2. Multiple split libraries ~ 1 min

```
MacQIIME HamdanLabMacPro:~ $ multiple_split_libraries_fastq.py -i  
/Users/HamdanLab/Desktop/QuickVsFull/multiple_join_paired_ends -o  
/Users/HamdanLab/Desktop/QuickVsFull/multiple_split_libraries --include_input_dir_path
```

### 3. Count raw sequences ~1 min

```
MacQIIME HamdanLabMacPro:~ $ count_seqs.py -i  
/Users/HamdanLab/Desktop/QuickVsFull/multiple_split_libraries/seqs.fna
```

(mean +/- std): 343.3134 +/- 63.6514)

107225 : Total

### 4. Pick open reference OTUS (default method UCLUST) ~5 min

```
MacQIIME HamdanLabMacPro:~ $ pick_open_reference_otus.py -a -O 6 -i  
/Users/HamdanLab/Desktop/QuickVsFull/Quick/SingleSample/multiple_split_libraries/seqs.fna  
-p  
/Users/HamdanLab/Desktop/QuickVsFull/Quick/SingleSample/Variations/Variation2/pick_open  
_references_otus_parallel_parameters.txt -o  
/Users/HamdanLab/Desktop/QuickVsFull/Quick/SingleSample/Variations/Variation2/pick_otus  
_open_reference
```

Parameters file:

assign\_taxonomy:assignment\_method uclust

parallel\_assign\_taxonomy\_uclust:reference\_seqs\_fp  
/macqiime/greengenes/gg\_13\_8\_otus/rep\_set/97\_otus.fasta

parallel\_assign\_taxonomy\_uclust:id\_to\_taxonomy\_fp  
/macqiime/greengenes/gg\_13\_8\_otus/taxonomy/97\_otu\_taxonomy.txt

#### 5. Summary of OTU table (sequence counts) ~1 min

```
MacQIIME HamdanLabMacPro:~ $ biom summarize-table -i  
/Users/HamdanLab/Desktop/QuickVsFull/Quick/SingleSample/Variations/Variation2/pick_otus  
_open_reference/otu_table_mc2_w_tax_no_pynast_failures.biom
```

Num samples: 1  
Num observations: 3545  
Total count: 44183  
Table density (fraction of non-zero values): 1.000

#### Counts/sample summary:

Min: 44183.0  
Max: 44183.0  
Median: 44183.000  
Mean: 44183.000  
Std. dev.: 0.000  
Sample Metadata Categories: None provided  
Observation Metadata Categories: taxonomy

#### Counts/sample detail:

SampleA: 44183.0

#### 6. Summary of OTU table (observed otu counts) ~1 min

```
MacQIIME HamdanLabMacPro:~ $ biom summarize-table -i  
/Users/HamdanLab/Desktop/QuickVsFull/Quick/SingleSample/Variations/Variation2/pick_otus  
_open_reference/otu_table_mc2_w_tax_no_pynast_failures.biom --qualitative
```

Num samples: 1  
Num observations: 3545

#### Observations/sample summary:

Min: 3545  
Max: 3545  
Median: 3545.000  
Mean: 3545.000  
Std. dev.: 0.000  
Sample Metadata Categories: None provided  
Observation Metadata Categories: taxonomy

#### Observations/sample detail:

SampleA: 3545

## Method w/ ChimeraSlayer

### 1. Multiple join paired ends ~1 min

```
MacQIIME HamdanLabMacPro:~ $ multiple_join_paired_ends.py -i  
/Users/HamdanLab/Desktop/QuickVsFull/RawSequences -o  
/Users/HamdanLab/Desktop/QuickVsFull/multiple_join_paired_ends
```

### 2. Multiple split libraries ~ 1 min

```
MacQIIME HamdanLabMacPro:~ $ multiple_split_libraries_fastq.py -i  
/Users/HamdanLab/Desktop/QuickVsFull/multiple_join_paired_ends -o  
/Users/HamdanLab/Desktop/QuickVsFull/multiple_split_libraries --include_input_dir_path
```

### 3. Count raw sequences ~1 min

```
MacQIIME HamdanLabMacPro:~ $ count_seqs.py -i  
/Users/HamdanLab/Desktop/QuickVsFull/multiple_split_libraries/seqs.fna
```

(mean +/- std): 343.3134 +/- 63.6514)

107225 : Total

### 4. Pick open reference OTUS (default method UCLUST) ~5 min

```
MacQIIME HamdanLabMacPro:~ $ pick_open_reference_otus.py -a -O 6 -i  
/Users/HamdanLab/Desktop/QuickVsFull/Quick/SingleSample/multiple_split_libraries/seqs.fna  
-p  
/Users/HamdanLab/Desktop/QuickVsFull/Quick/SingleSample/Variations/Variation2/pick_open  
_references_otus_parallel_parameters.txt -o  
/Users/HamdanLab/Desktop/QuickVsFull/Quick/SingleSample/Variations/Variation2/pick_otus  
_open_reference
```

Parameters file:

assign\_taxonomy:assignment\_method uclust

parallel\_assign\_taxonomy\_uclust:reference\_seqs\_fp  
/macqiime/greengenes/gg\_13\_8\_otus/rep\_set/97\_otus.fasta

parallel\_assign\_taxonomy\_uclust:id\_to\_taxonomy\_fp  
/macqiime/greengenes/gg\_13\_8\_otus/taxonomy/97\_otu\_taxonomy.txt

### 5. Identify chimeras (ChimeraSlayer) ~1 hours

```
MacQIIME HamdanLabMacPro:~ $ parallel_identify_chimeric_seqs.py -O 6 -m ChimeraSlayer
-i
/Users/HamdanLab/Desktop/QuickVsFull/Full/SingleSample/ChimeraSlayer/Variation33/Variati
on2/pick_otus_open_reference/pynast_aligned_seqs/rep_set_aligned.fasta -o
/Users/HamdanLab/Desktop/QuickVsFull/Full/SingleSample/ChimeraSlayer/Variation33/identif
y_chimeric_seqs_ChimeraSlayer.txt -a
/Users/HamdanLab/Desktop/QuickVsFull/Full/SingleSample/97_otus.aligned.fasta
```

### 6. Remove chimeras from alignment file ~1 min

```
MacQIIME HamdanLabMacPro:~ $ filter_fasta.py -f
/Users/HamdanLab/Desktop/QuickVsFull/Full/SingleSample/ChimeraSlayer/Variation33/Variati
on2/pick_otus_open_reference/pynast_aligned_seqs/rep_set_aligned.fasta -o
/Users/HamdanLab/Desktop/QuickVsFull/Full/SingleSample/ChimeraSlayer/Variation33/filter_f
asta_non_chimeric_rep_set_aligned.fasta -s
/Users/HamdanLab/Desktop/QuickVsFull/Full/SingleSample/ChimeraSlayer/Variation33/identif
y_chimeric_seqs_ChimeraSlayer.txt
```

### 7. Filter alignment ~1 min

```
MacQIIME HamdanLabMacPro:~ $ filter_alignment.py -o
/Users/HamdanLab/Desktop/QuickVsFull/Full/SingleSample/ChimeraSlayer/Variation33/pynast
_aligned_seqs_chimeras_removed -i
/Users/HamdanLab/Desktop/QuickVsFull/Full/SingleSample/ChimeraSlayer/Variation33/filter_f
asta_non_chimeric_rep_set_aligned.fasta
```

### 8. Build phylogenetic tree ~1 min

```
MacQIIME HamdanLabMacPro:~ $ make_phylogeny.py -i
/Users/HamdanLab/Desktop/QuickVsFull/Full/SingleSample/ChimeraSlayer/Variation33/pynast
_aligned_seqs_chimeras_removed/filter_fasta_non_chimeric_rep_set_aligned_pfiltered.fasta -o
/Users/HamdanLab/Desktop/QuickVsFull/Full/SingleSample/ChimeraSlayer/Variation33/rep_se
t_chimeras_removed.tre
```

#### 9. Exclude chimera sequences while making OTU table ~1 min

```
MacQIIME HamdanLabMacPro:~ $ make_otu_table.py -i  
/Users/HamdanLab/Desktop/QuickVsFull/Full/SingleSample/ChimeraSlayer/Variation33/Variati  
on2/pick_otus_open_reference/final_otu_map_mc2.txt -o  
/Users/HamdanLab/Desktop/QuickVsFull/Full/SingleSample/ChimeraSlayer/Variation33/otu_ta  
ble_ChimeraSlayer.biom -e  
/Users/HamdanLab/Desktop/QuickVsFull/Full/SingleSample/ChimeraSlayer/Variation33/identif  
y_chimeric_seqs_ChimeraSlayer.txt -t  
/Users/HamdanLab/Desktop/QuickVsFull/Full/SingleSample/ChimeraSlayer/Variation33/Variati  
on2/pick_otus_open_reference/uclust_assigned_taxonomy/rep_set_tax_assignments.txt
```

#### 10. Summary of OTU table (sequence counts) ~1 min

```
MacQIIME HamdanLabMacPro:~ $ biom summarize-table -i  
/Users/HamdanLab/Desktop/QuickVsFull/Full/SingleSample/ChimeraSlayer/Variation33/otu_ta  
ble_ChimeraSlayer.biom
```

Num samples: 1  
Num observations: 4934  
Total count: 48591  
Table density (fraction of non-zero values): 1.000

Counts/sample summary:  
Min: 48591.0  
Max: 48591.0  
Median: 48591.000  
Mean: 48591.000  
Std. dev.: 0.000  
Sample Metadata Categories: None provided  
Observation Metadata Categories: taxonomy

Counts/sample detail:  
SampleA: 48591.0

## 11. Summary of OTU table (observed otu counts) ~1 min

```
MacQIIME HamdanLabMacPro:~ $ biom summarize-table -i  
/Users/HamdanLab/Desktop/QuickVsFull/Full/SingleSample/ChimeraSlayer/Variation33/otu_table_ChimeraSlayer.biom --qualitative
```

Num samples: 1

Num observations: 4934

Observations/sample summary:

Min: 4934

Max: 4934

Median: 4934.000

Mean: 4934.000

Std. dev.: 0.000

Sample Metadata Categories: None provided

Observation Metadata Categories: taxonomy

Observations/sample detail:

SampleA: 4934