|  |  |  |  |
| --- | --- | --- | --- |
| **Step** | **QIIME/UPARSE** | **Script/Command Name** | **Parameters** |
| **Demultiplexing and quality filtering** | QIIME | split\_libraries.py | -s, --min\_qual\_score (25); -b, --barcode\_type (variable\_length); -l, --min\_seq\_length (200); L, --max\_seq\_length (400); -d, --record\_qual\_scores; -j, --added\_demultiplex\_field (run\_prefix); w, --qual\_score\_window (50); --z, reverse\_primers (truncate\_only) |
| **Quality filtering using UPARSE** | UPARSE | usearch --fastq\_filter | E, --fastq\_maxee (0.5); L, --fastq\_minlen 270 |
| **Dereplicating sequences** | UPARSE | usearch --derep\_fulllength | - |
| **Abundance sorting and discarding singletons** | UPARSE | usearch --sortbysize | --minsize (2) |
| **UPARSE-OTU method for OTU clustering with the greedy clustering algorithm and representative sequence picking with abundance algorithm** | UPARSE | usearch --cluster\_otus | --otu\_radius\_pct (3.0) |
| **Reference chimera checking and removal using uchime** | UPARSE | usearch --uchime\_ref | --mindiv (0.8); mindiffs (3); --db Greengenes, gg13\_8 |
| **Mapping original quality-filtered reads back to OTUs** | UPARSE | usearch --usearch\_global | --id (0.97) |
| **Assigning taxonomy to OTUs using RDP Classifier** | QIIME | assign\_taxonomy.py | -t Greengenes gg13\_8 |
| **Sequence alignment** | QIIME | align\_seqs.py | -e, --min\_length (75); -p, --minimum\_percent\_id (0.75) |
| **Filtering sequence alignment** | QIIME | filter\_alignment.py | - g, --allowed\_gap\_frac (0.999999) |
| **Inferring phylogeny** | QIIME | make\_phylogeny.py | - |
| **Generating a dense/sparse representation of an OTU table (observation matrix)** | QIIME | make\_otu\_table.py | -e, --exclude\_otus\_fp |
| **Diversity analyses** | QIIME | core\_diversity\_analyses.py | -e, --sampling\_depth (5000); -c, --categories; -p, --parameter\_fp (metrics: chao1, observed\_species, Shannon, simpson, PD\_whole\_tree, unweighted\_unifrac,weighted\_unifrac,bray\_curtis; single rarefaction depth: 5000) |