Workflow Paired End reads:

1. Preprocessing using Trimmomatic.
   1. You can use trimmomatic\_ITS.pl but you have to talk to me first.
2. Create the treatment.file file exactly the way you do for mothur
3. Use the ITS-processing.pl script, usage:
   1. perl ITS-processing.pl treatment.file number\_of\_processors (a number between 1 and 64)
   2. You will have multiple output files the two you will want to make sure that stay around are: treatment.contigs.good.groups and treatment.trim.contigs.good.fasta
4. Use vsearch-pipleine.pl, usage:
   1. Perl vsearch-pipeline.pl treatment.trim.contigs.good.fasta directory\_name (you chose this)
   2. Output is vsearch.cent0.03.fa and vsearch.cent0.03.uchime.fa
5. Change directory to directory\_name and:
   1. Type: ls > otu.file
   2. This will absorb all the OTU names from that directory and put them in a file called otu.file.
6. Make sure that otu.file includes only out names and no other file names before you go to the next step.
7. Use cluster.pl to create you csv file that you will use with R, usage:
   1. perl custer.pl treatment.file otu.file treatment.contigs.good.groups directory\_name file.csv
   2. directory\_name is same as the one you chose above and file.csv is a file name you choose.
8. Use chimera\_cleanup.pl to filter out the chimera free OTUs, usage:
   1. perl chimera\_cleanup.pl vsearch.cent0.03.fa otu.file vsearch.cent0.03.uchime.fa new\_otu.file
   2. new\_otu.file is a name you choose to filter out the nonchimeric OTUs

Workflow Single End reads:

1. Preprocessing using Trimmomatic.
2. Create the treatment.file file exactly the way you do for mothur
3. Use fasta.create.pl to make the fasta and groups files needed as in the previous pipeline, usage:
   1. perl fasta.create.pl treatment.file somename.fasta somename.groups
   2. The files have to be those processed using trimommatic
4. Use vsearch-pipleine.pl, usage:
   1. Perl vsearch-pipeline.pl treatment.trim.contigs.good.fasta directory\_name (you chose this)
   2. Output is vsearch.cent0.03.fa and vsearch.cent0.03.uchime.fa
5. Change directory to directory\_name and:
   1. Type: ls > otu.file
   2. This will absorb all the OTU names from that directory and put them in a file called otu.file.
6. Make sure that otu.file includes only out names and no other file names before you go to the next step.
7. Use cluster.pl to create you csv file that you will use with R, usage:
   1. perl custer.pl treatment.file otu.file treatment.contigs.good.groups directory\_name file.csv
   2. directory\_name is same as the one you chose above and file.csv is a file name you choose.
8. Use chimera\_cleanup.pl to filter out the chimera free OTUs, usage:
   1. perl chimera\_cleanup.pl vsearch.cent0.03.fa otu.file vsearch.cent0.03.uchime.fa new\_otu.file
   2. new\_otu.file is a name you choose to filter out the nonchimeric OTUs

Blast:

blastn –task blastn –query in\_file.fasta –db nt –out outfile –num\_threads 4