16S community sequencing pipeline using dada2 in R

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16S community sequencing analysis

- 16S rRNA variable regions
- Most methods cluster sequences that occur with 97% similarity and assign these to “OTUs” from references trees
- Do not incorporate quality scores of sequences

16S community sequencing analysis

- 16S rRNA variable regions
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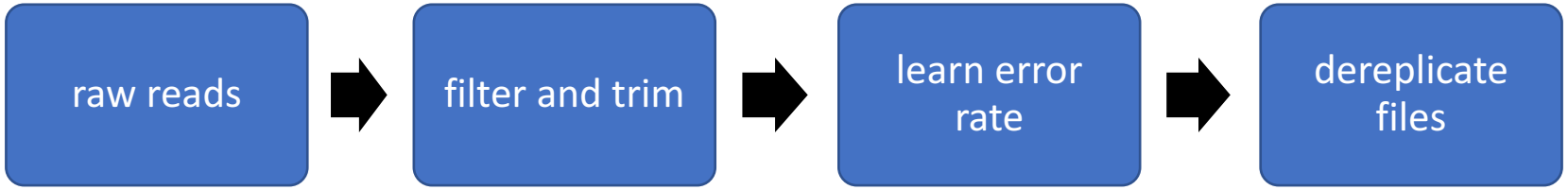
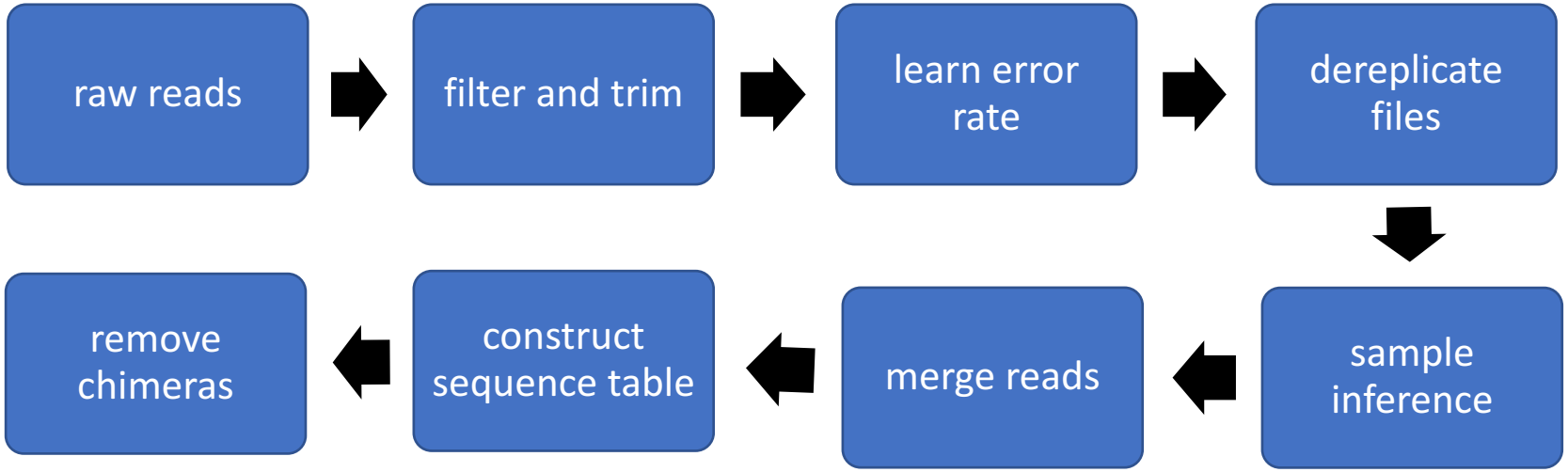
- Do not incorporate quality scores of sequences
- **Enter dada2**
- Incorporates quality score information
- Distinguishes sequencing errors from real biological variation
- Finds Amplicon Sequence Variants (ASVs)

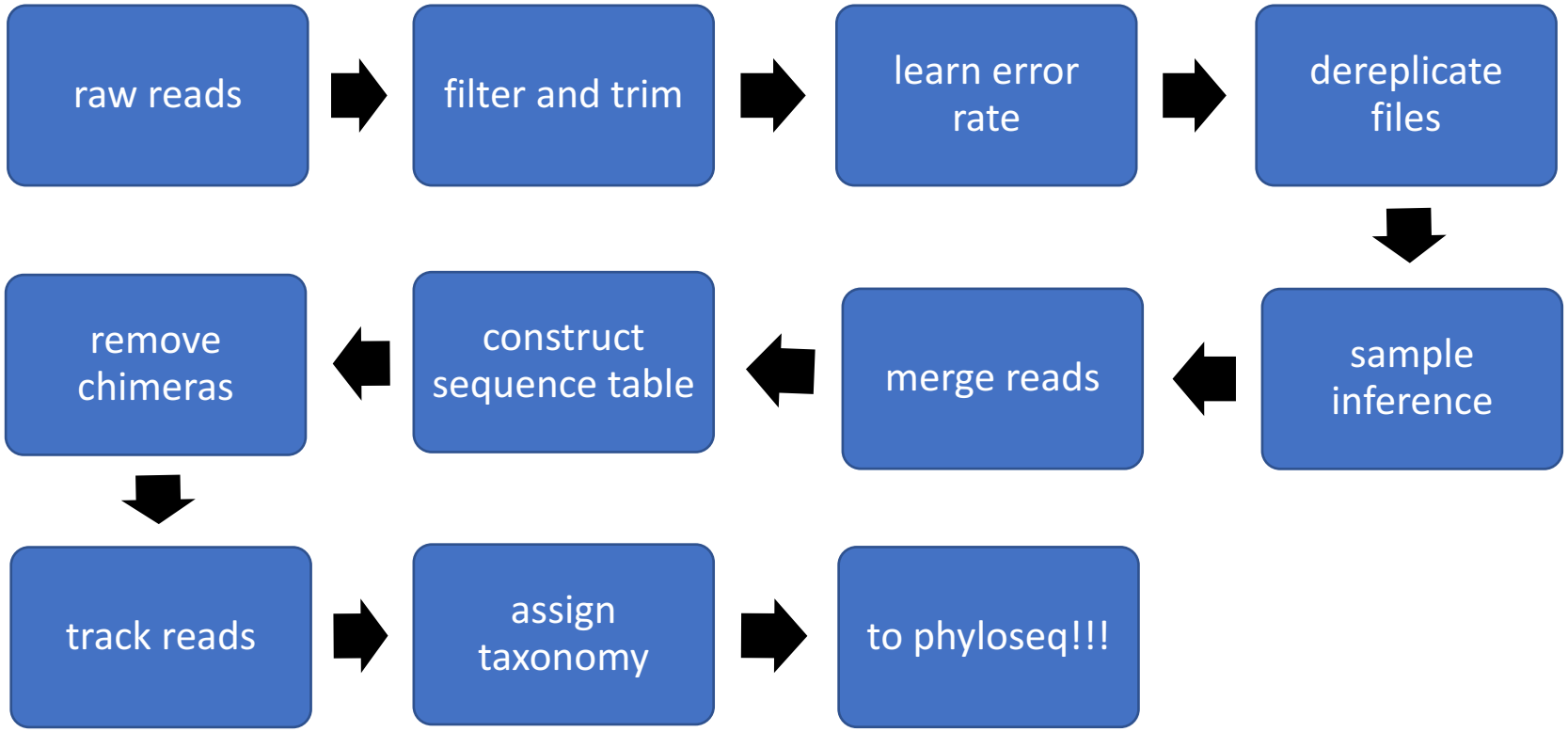
dada2 pipeline



dada2 pipeline



dada2 pipeline



The AB sequencing pipeline

padpadpadpad / AB_dada2_pipeline_R

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Pipeline for running sequencing analyses using dada2

Edit

Add topics

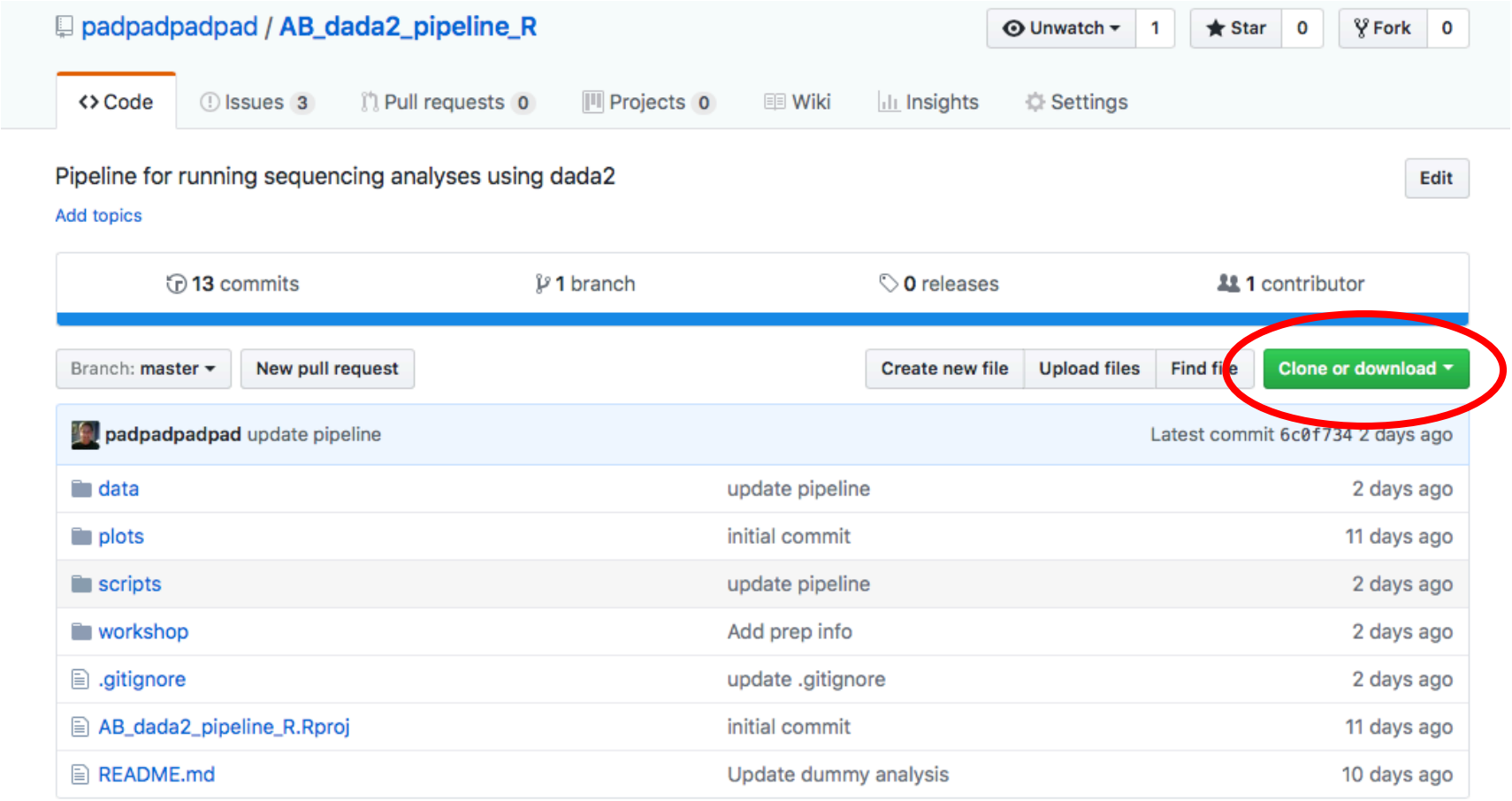
13 commits | 1 branch | 0 releases | 1 contributor

Branch: master | New pull request | Create new file | Upload files | Find file | Clone or download

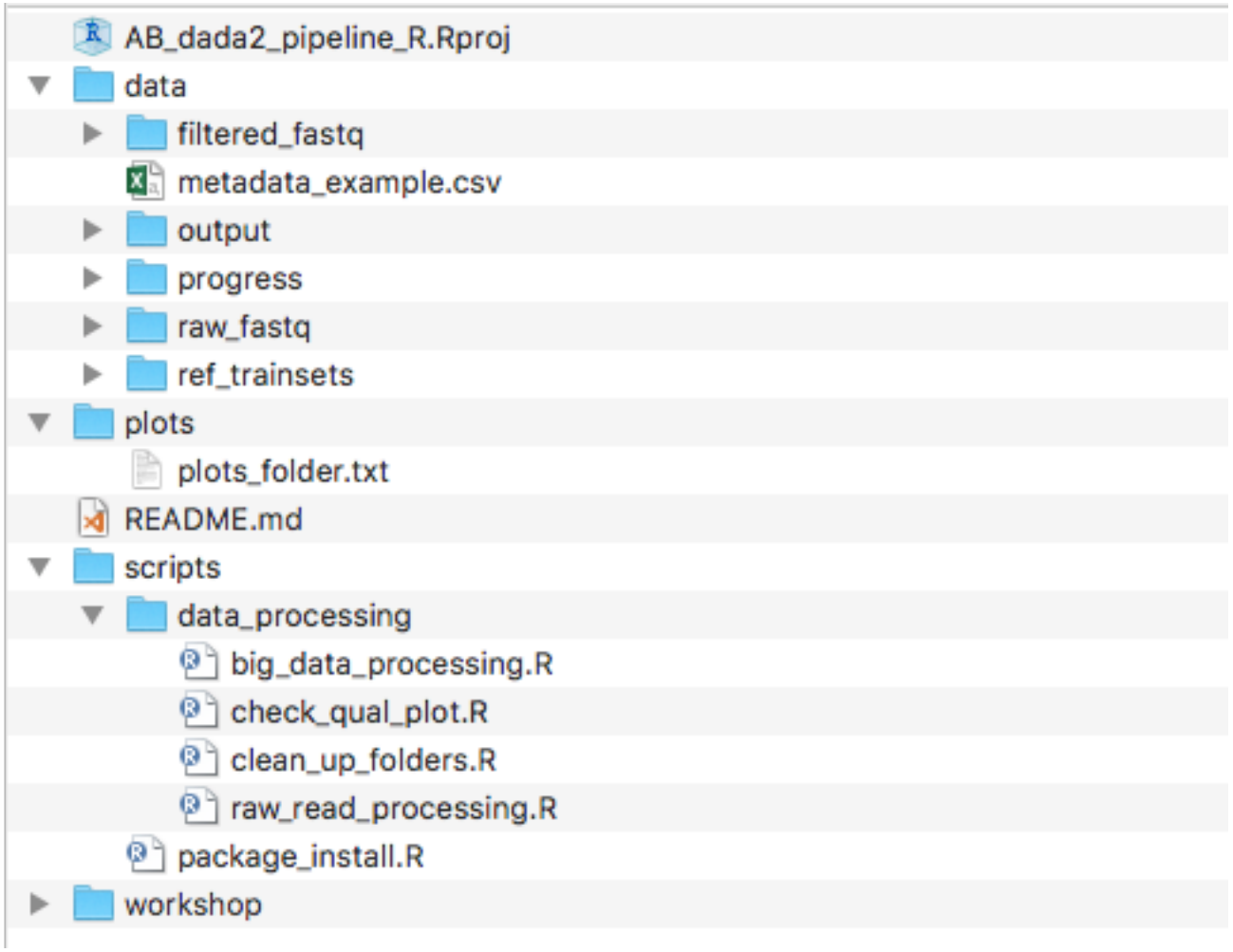
padpadpadpad update pipeline — Latest commit 6c0f734 2 days ago

data	update pipeline	2 days ago
plots	initial commit	11 days ago
scripts	update pipeline	2 days ago
workshop	Add prep info	2 days ago
.gitignore	update .gitignore	2 days ago
AB_dada2_pipeline_R.Rproj	initial commit	11 days ago
README.md	Update dummy analysis	10 days ago

The AB sequencing pipeline



The AB sequencing pipeline



raw_read_processing vs big_data_processing

- Pooling data allows information to be shared across samples. However is much much slower for big data sets
- dada2 resolves sequence variants exactly, and as such DNA sequences are consistent across samples and can be recombined separately
- big_data_processing speeds things up if needs be!

Bits to run supervised

- Quality profiles
- Setting trimming parameters
- Check sample names = metadata$SampleID
- Make sure there is a column called SampleID!

Correctly format metadata

SampleID == sample_names !!!

	A	B	C
1	SampleID	treatment	ancestral
2	sample_1-1	A	warm
3	sample_10-10	A	amb
4	sample_11-11	A	amb
5	sample_12-12	A	warm
6	sample_14-14	A	warm
7	sample_15-15	A	warm
8	sample_16-16	A	amb
9	sample_17-17	A	warm
10	sample_18-18	A	amb
11	sample_19-19	A	warm
12	sample_2-2	A	amb
13	sample_20-20	A	amb
14	sample_21-C1	A	comb
15	sample_22-C2	A	comb
16	sample_23-C3	A	comb
17	sample_24-C4	A	comb
18	sample_25-C5	A	comb
19	sample_26-C6	A	comb
20	sample_27-C7	A	comb

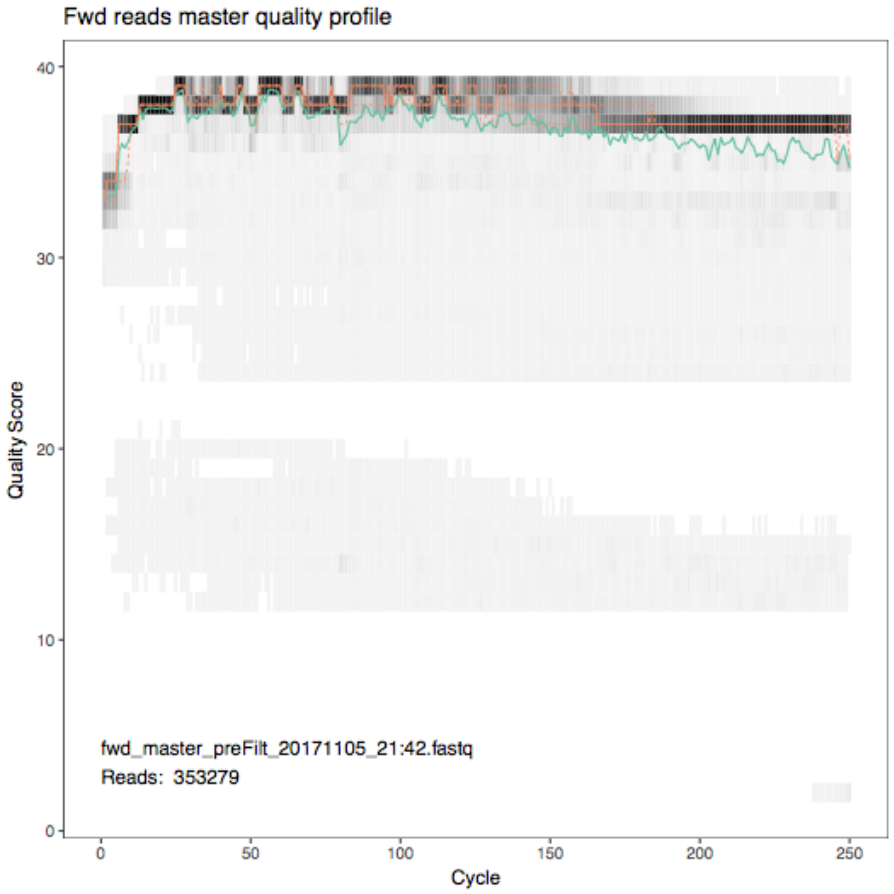
Progress file

```
Run started at  2017-11-03 23:22:55

This run is done using raw_read_processing.R
Filtering completed at  2017-11-03 23:11
Forward error rates completed at 2017-11-03 23:42:09
Forward error rate: 68.6218264955491
Forward error rate: 1.4268482212032
Forward error rate: 0.0806254374037106
Forward error rate: 0.0045528251682451
Forward error rate: 0.000171459734376565
Forward error rate: 0
Reverse error rates completed at 2017-11-04 00:01:03
Reverse error rates: 65.4204274041772
Reverse error rates: 1.28380362213372
Reverse error rates: 0.0450086135629872
Reverse error rates: 0.00562287641467286
Reverse error rates: 0.00151911546430804
Reverse error rates: 0
Dereplicated forward and reverse files 2017-11-04 00:01:19
Forward sequences inferred 2017-11-04 00:05:25
Reverse sequences inferred 2017-11-04 00:09:43
Inferred forward and reverse sequences merged 2017-11-04 00:10:00
Sequence table constructed 2017-11-04 00:10:00
Chimeric sequences removed 2017-11-04 00:10:56
Taxonomy assigned 2017-11-04 00:17:09
Assigning species at 2017-11-04 00:17:09
80 out of 1546 were assigned to the species level. Of which 74 had genera consistent with the input table.
Species assigned 2017-11-04 00:18:48
End of raw read processing without construction of phylogeny 2017-11-04 00:18:55
This run (without phylogeny estimation) took: 0.933248837457763 hours
Sequences aligned 2017-11-04 00:20:39
Constructed phylogenetic tree 2017-11-04 01:35:28
End of raw read processing 2017-11-04 01:35:29
This run took: 2.20936129470666 hours
```

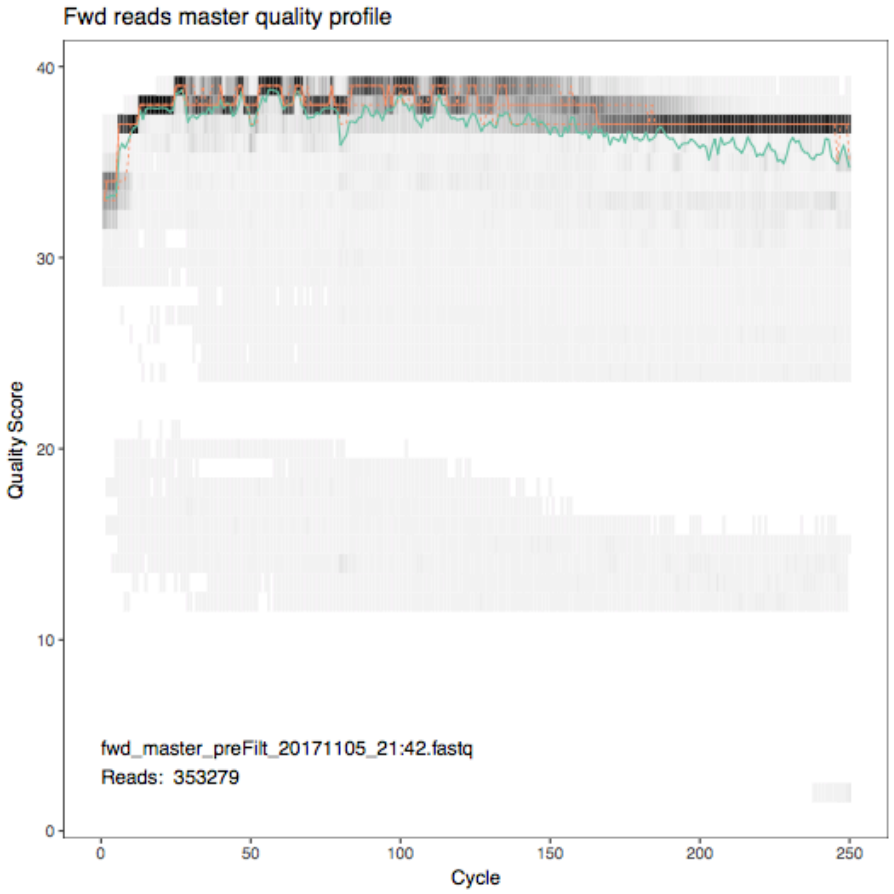
Plots

Before trimming and fltering

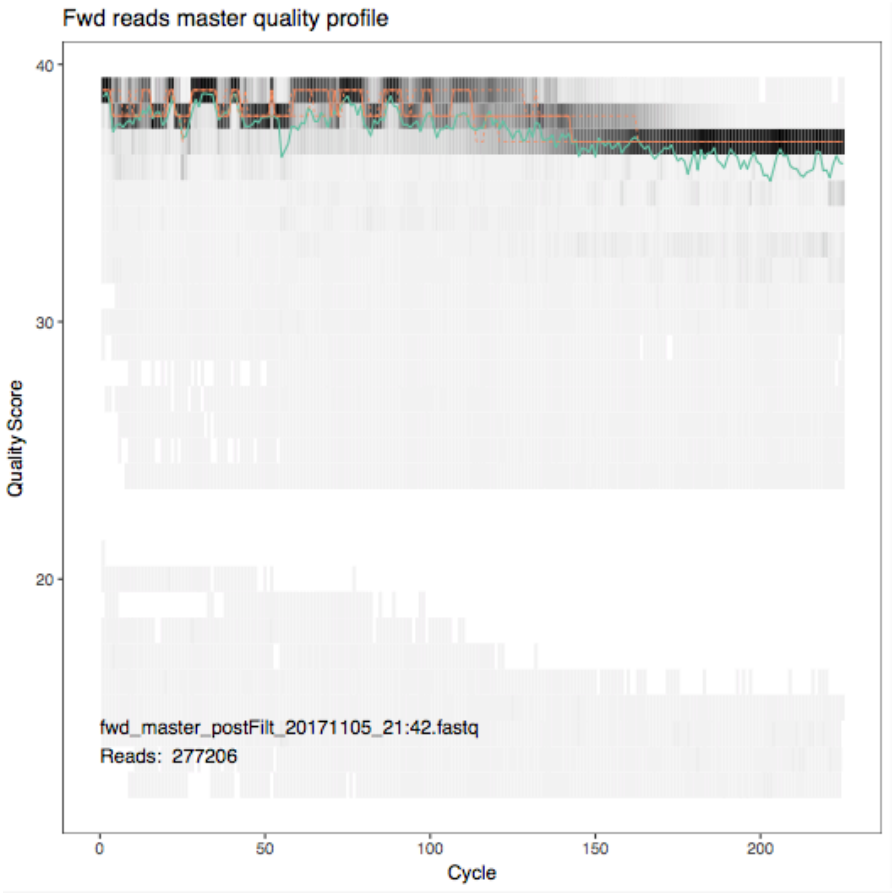


Plots

Before trimming and fltering

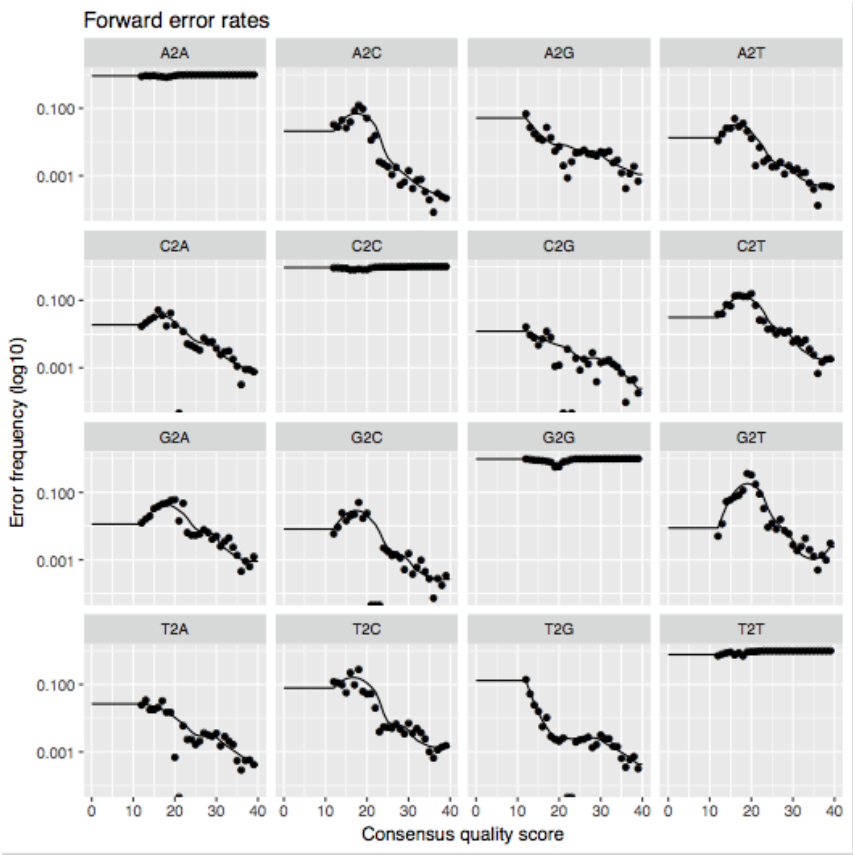


After trimming and fltering



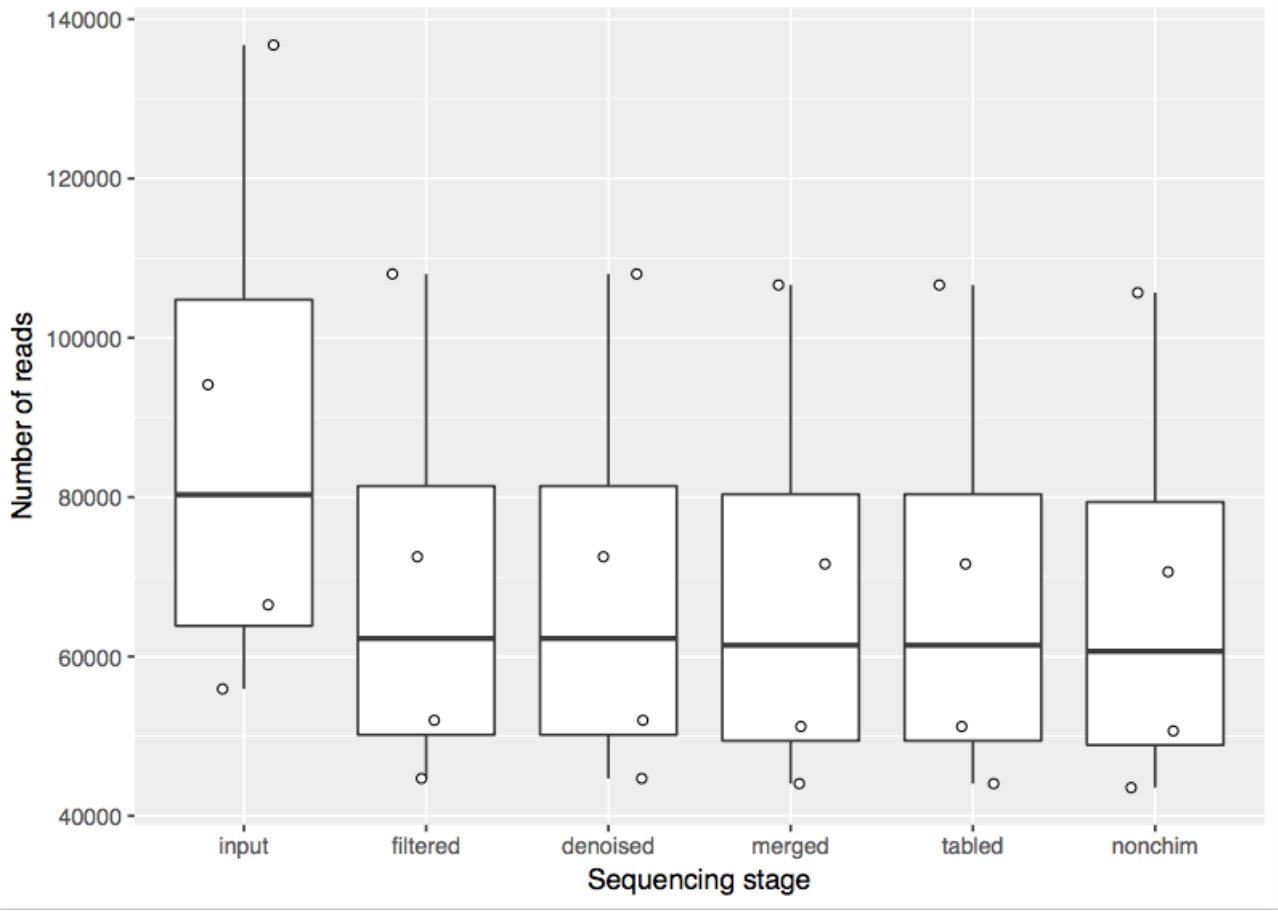
Plots

Plot of error rates



Plots

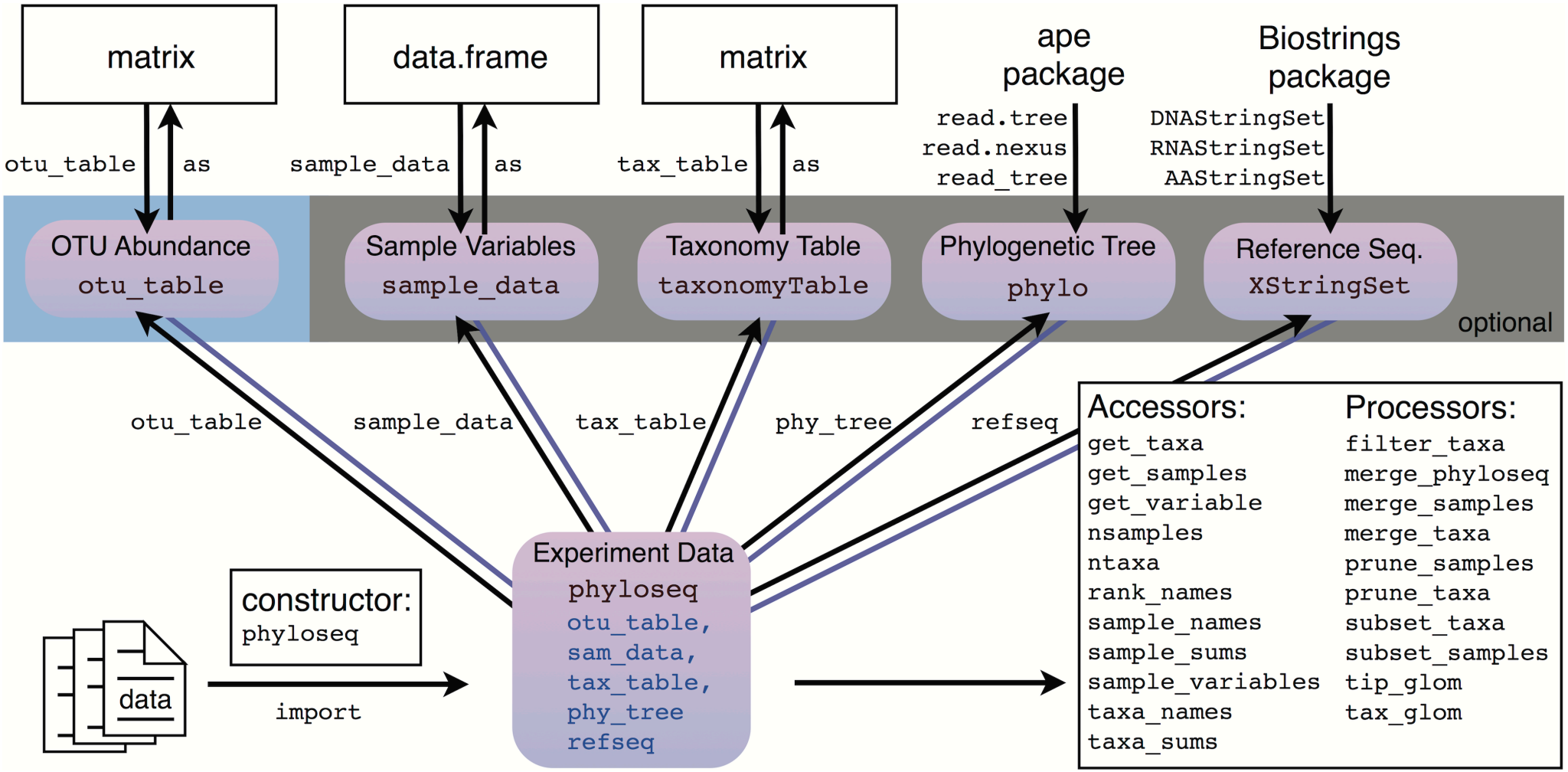
Track the number of reads through each stage



Output

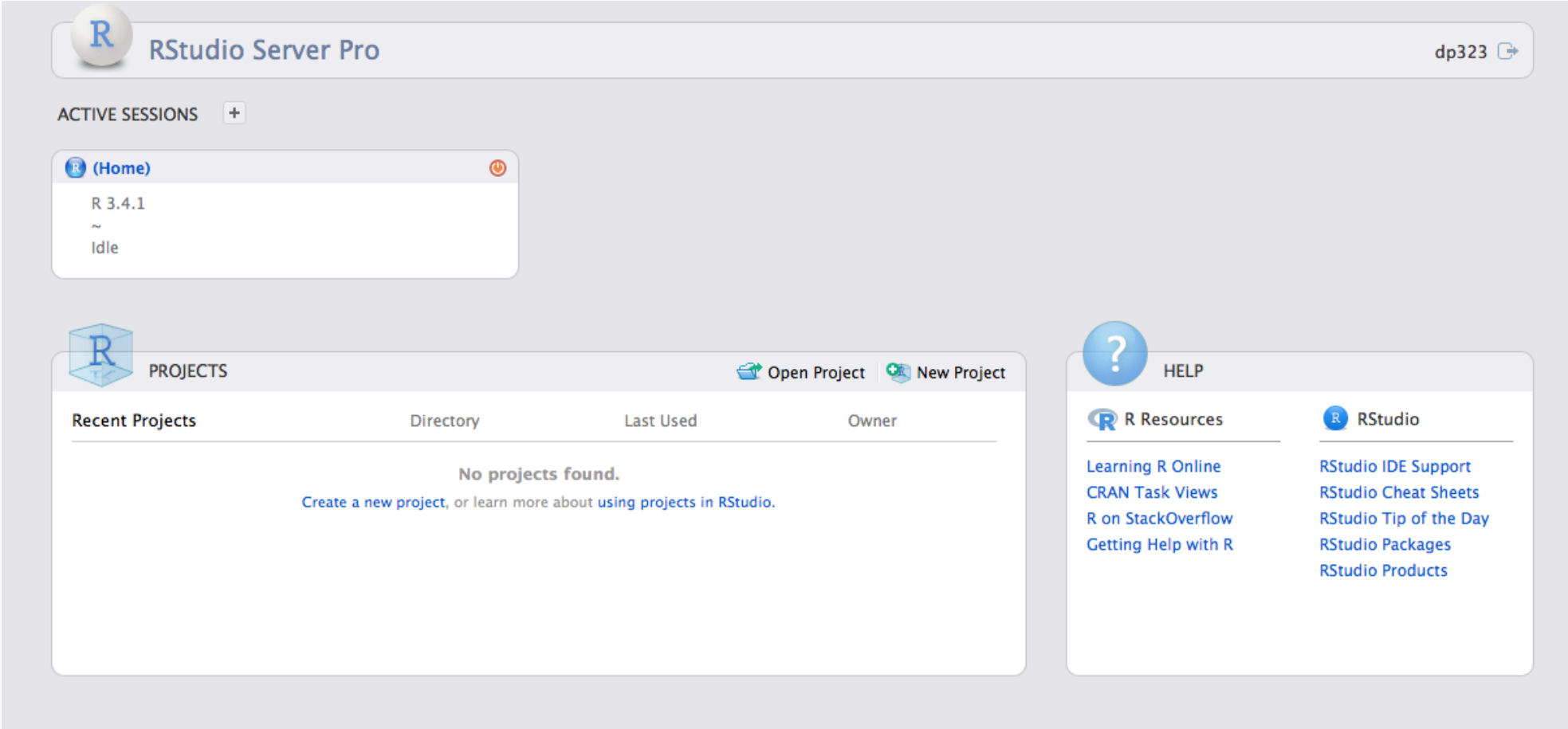
20171107_13h43m	Today, 15:11	--	Folder
20171107_13h43m_dadaFs.rds	Today, 14:02	6.6 MB	R Data File
20171107_13h43m_dadaRs.rds	Today, 14:02	6.6 MB	R Data File
20171107_13h43m_fwd_error.rds	Today, 13:58	22 KB	R Data File
20171107_13h43m_phytree.rds	Today, 15:11	216 KB	R Data File
20171107_13h43m_ps.Rdata	Today, 15:11	254 KB	R Data File
20171107_13h43m_ps.rds	Today, 15:11	254 KB	R Data File
20171107_13h43m_rev_error.rds	Today, 13:58	24 KB	R Data File
20171107_13h43m_seqtab.rds	Today, 15:11	72 KB	R Data File
20171107_13h43m_taxtab.rds	Today, 15:11	78 KB	R Data File
20171107_13h43m_track_reads_through_stages.rds	Today, 14:03	230 bytes	R Data File
fwd_master_postFilt_20171107_13h43m.fastq	Today, 13:44	24.2 MB	Document
fwd_master_preFilt_20171107_13h43m.fastq	Today, 13:43	33.9 MB	Document
rev_master_postFilt_20171107_13h43m.fastq	Today, 13:44	27.7 MB	Document
rev_master_preFilt_20171107_13h43m.fastq	Today, 13:43	38.1 MB	Document

Bonus – integration with phyloseq



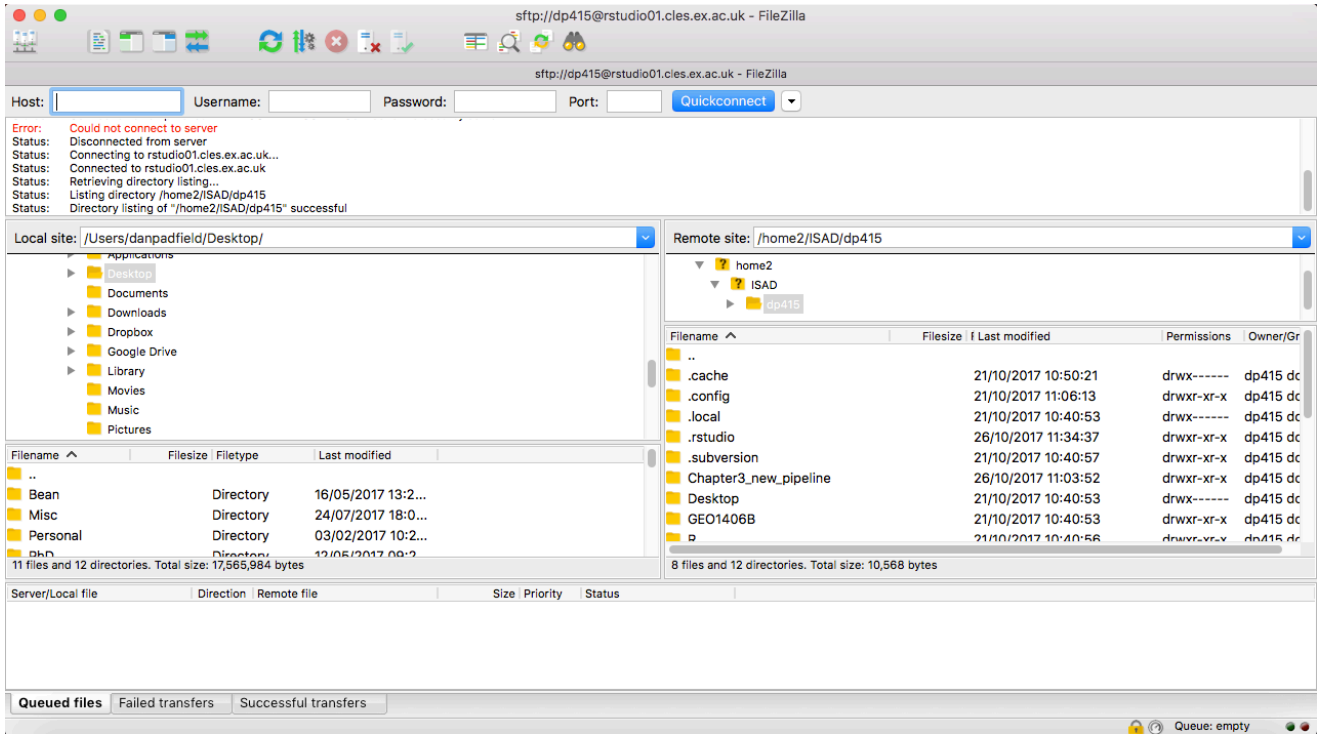
RStudio server

https://rstudio01.cles.ex.ac.uk (can be 01, 02, 03 or 04)

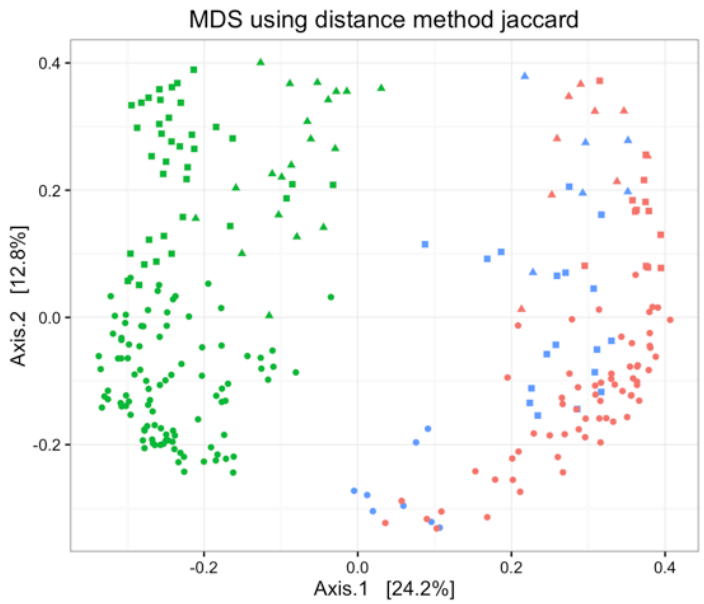


Filezilla

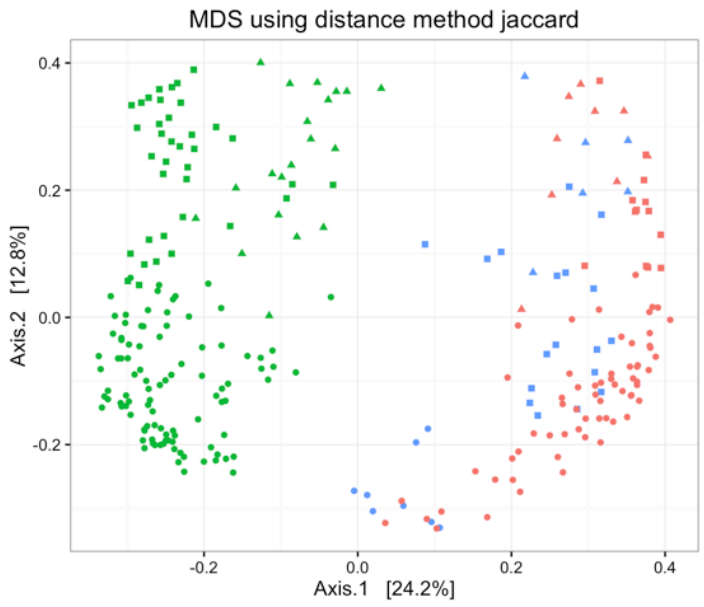
https://filezilla-project.org
sftp://https://<your username>@rstudio01.cles.ex.ac.uk

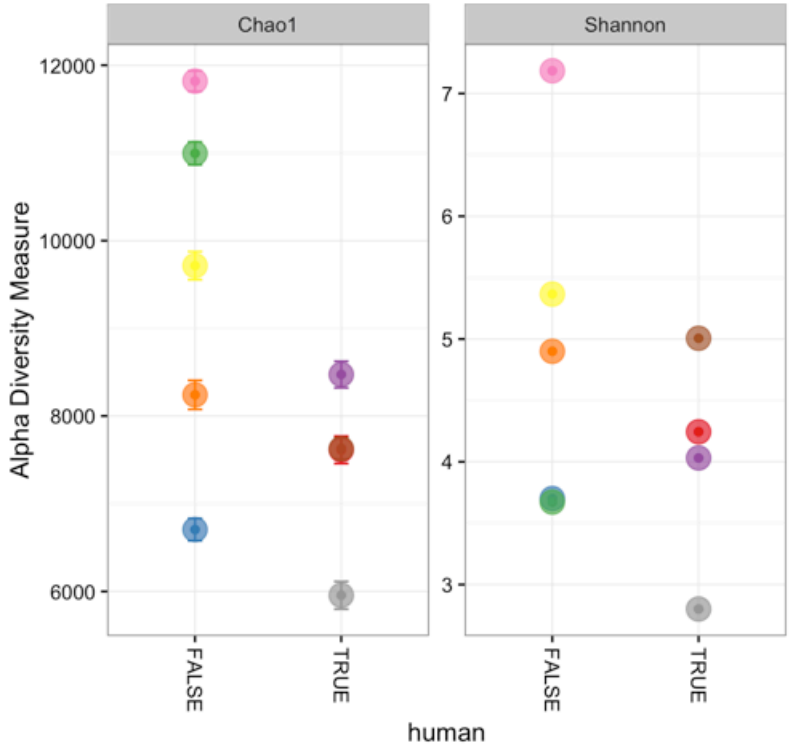


Phyloseq possibilities

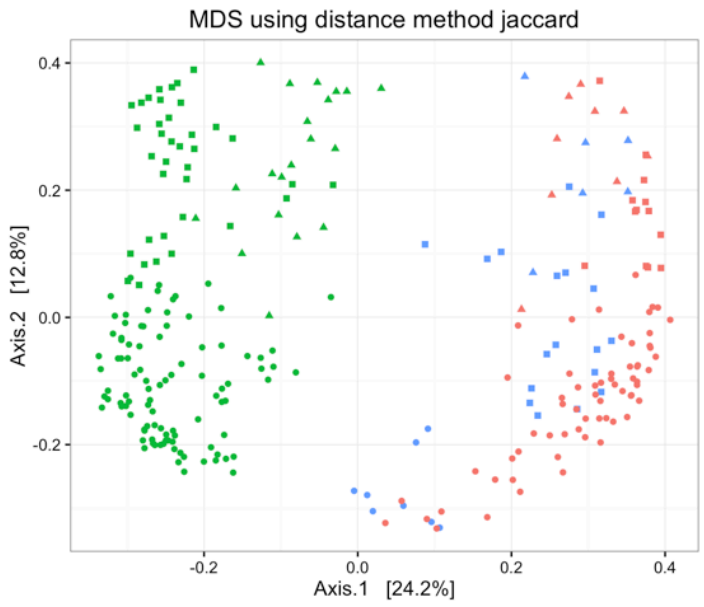


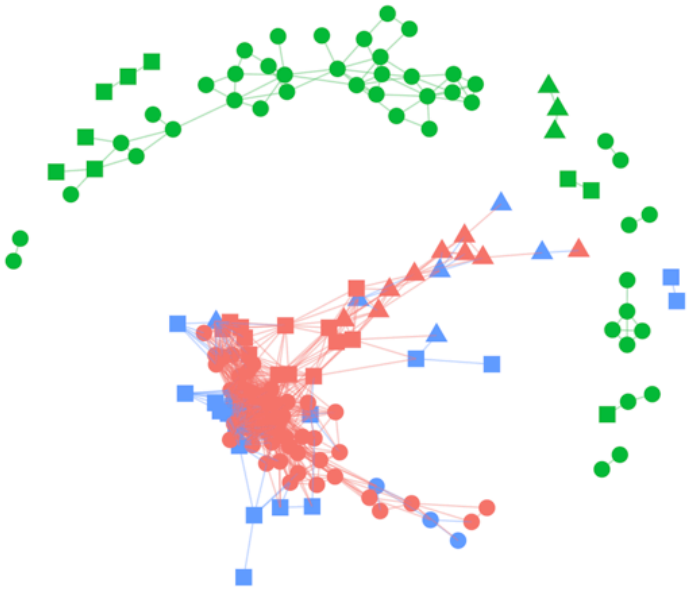
Phyloseq possibilities

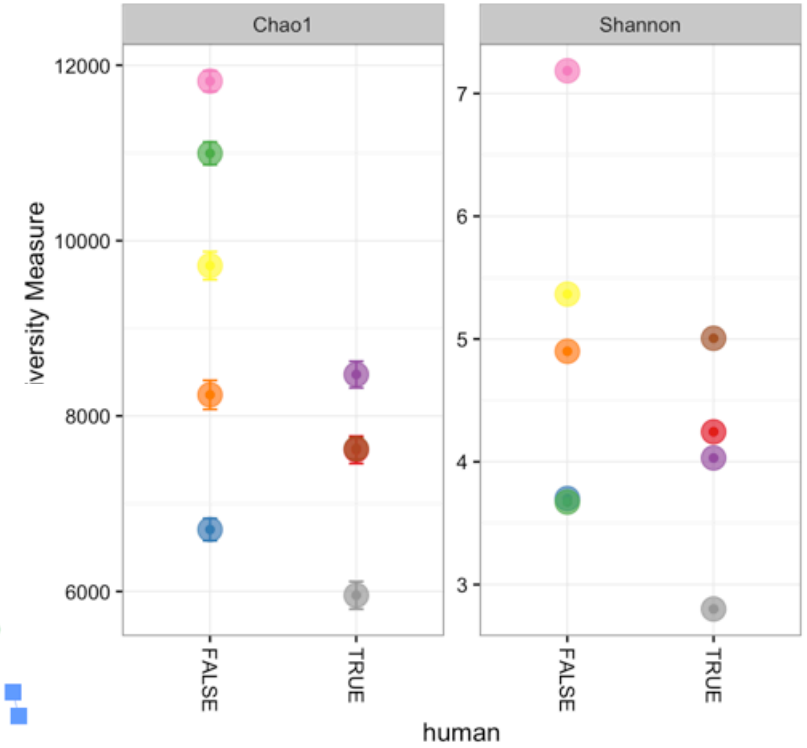




Phyloseq possibilities







Resources

- dada2
- Phyloseq
- Complete AB sequencing workflow

Lets get our hands diRty!!!