H3ABioNet

Pan African Bioinformatics Network for H3Africa

16SrRNA Intermediate Bioinformatics Online Course: Int_BT_2019

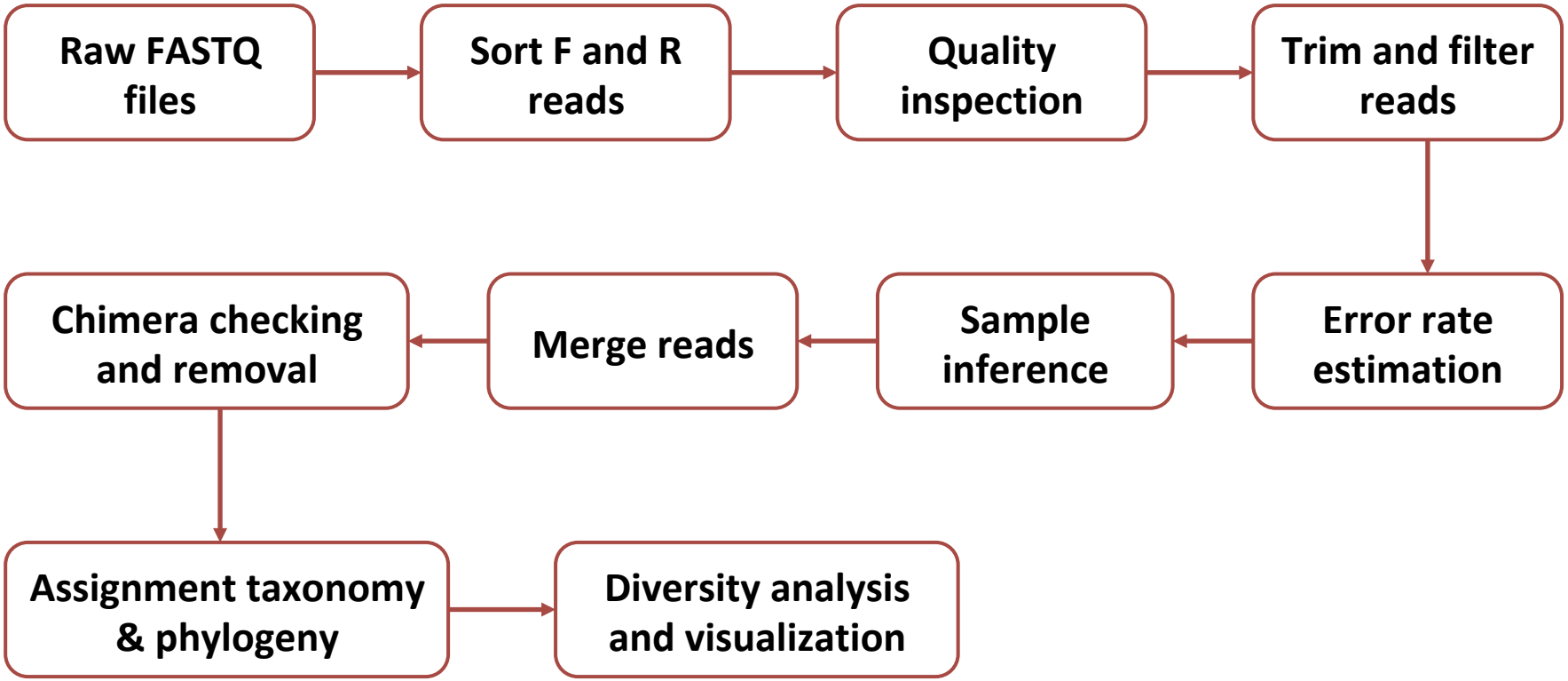
16S analysis pipeline

QC and ASV picking using the dada2 pipeline

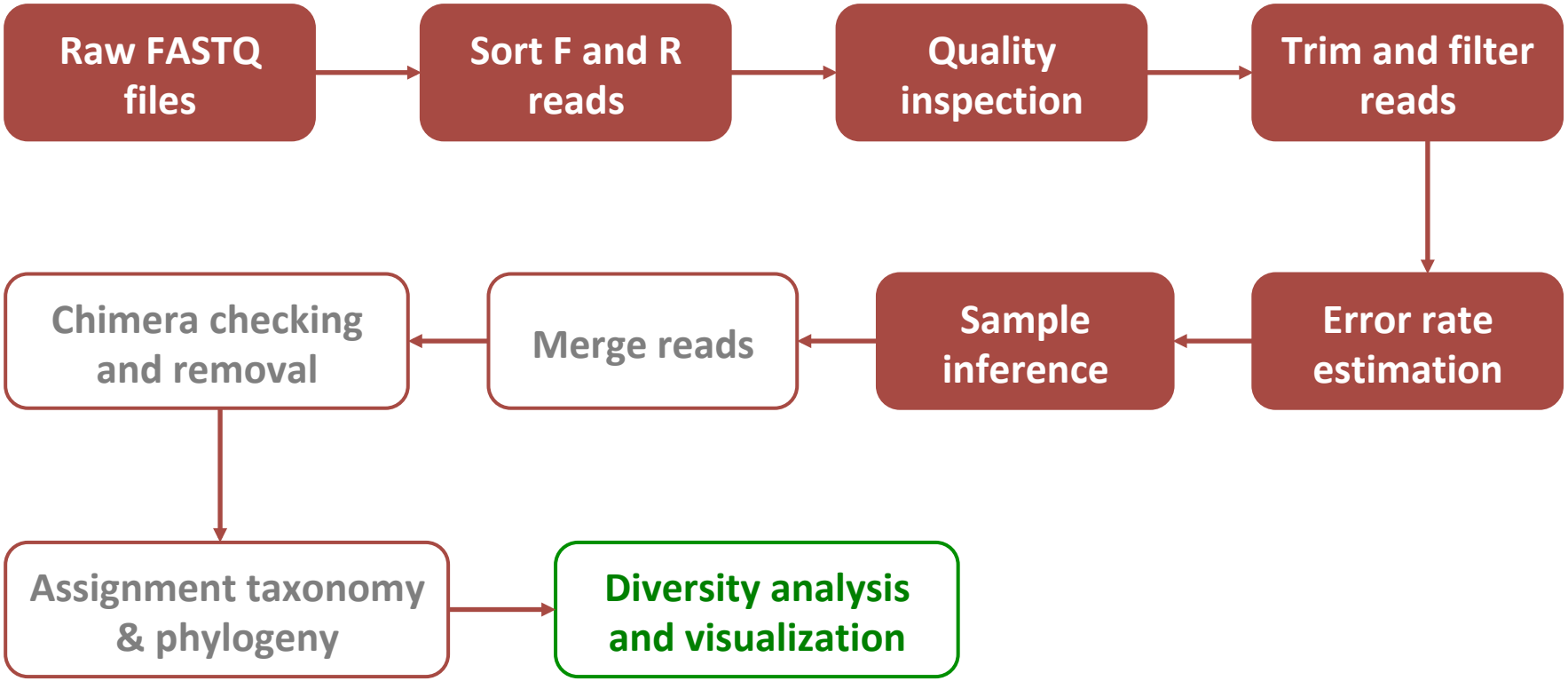
Outline

- Quality Control
- DADA2 background
- **DADA2 workflow**

DADA2 workflow



DADA2 workflow



DADA2 workflow

Before running the pipeline:

- Barcodes, adapters should be removed
  - Cutadapt, Trimmomatic, …
- Samples should be demultiplexed
  - FASTX-Toolkit, idemp, …
- For paired-end data, forward and reverse reads must be in the same order.

DADA2 workflow

The data:

- The data can be accessed here.
- Stool samples.
- Paired-end 300 bp reads.
- Barcodes/Adapters have been removed.

Sample	Dog	Treatment
Dog1	B	2
Dog2	G	3
Dog3	K	3
Dog8	B	4
Dog9	G	0
Dog10	K	4
Dog15	B	1
Dog16	G	4
Dog17	K	0
Dog22	B	3
Dog23	G	1
Dog24	K	2
Dog29	B	0
Dog30	G	2
Dog31	K	1

DADA2 workflow

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Getting Ready

Load the dada2 package in your R/RStudio

```
library(dada2); packageVersion("dada2")
```

If you do not already have it, see the dada2 installation instructions

DADA2 workflow

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Getting Ready

Set the path, it points to the **dog samples** directory:

```
MY_HOME <- Sys.getenv("HOME")
data <- paste(MY_HOME, "/dada2_tutorial_dog/dog_samples", sep='')  # change the path
list.files(data)
```

```
##  [1] "Dog1_R1.fastq"  "Dog1_R2.fastq"  "Dog10_R1.fastq" "Dog10_R2.fastq"
##  [5] "Dog15_R1.fastq" "Dog15_R2.fastq" "Dog16_R1.fastq" "Dog16_R2.fastq"
##  [9] "Dog17_R1.fastq" "Dog17_R2.fastq" "Dog2_R1.fastq"  "Dog2_R2.fastq"
## [13] "Dog22_R1.fastq" "Dog22_R2.fastq" "Dog23_R1.fastq" "Dog23_R2.fastq"
## [17] "Dog24_R1.fastq" "Dog24_R2.fastq" "Dog29_R1.fastq" "Dog29_R2.fastq"
## [21] "Dog3_R1.fastq"  "Dog3_R2.fastq"  "Dog30_R1.fastq" "Dog30_R2.fastq"
## [25] "Dog31_R1.fastq" "Dog31_R2.fastq" "Dog8_R1.fastq"  "Dog8_R2.fastq"
## [29] "Dog9_R1.fastq"  "Dog9_R2.fastq"
```

DADA2 workflow

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Getting Ready

Sort the forward and reverse reads

```
# Forward and reverse fastq filenames have format: SAMPLENAME_R1.fastq and SAMPLENAME_R2.fastq
dataF <- sort(list.files(data, pattern="_R1.fastq", full.names = TRUE))
dataR <- sort(list.files(data, pattern="_R2.fastq", full.names = TRUE))
```

Extract sample names

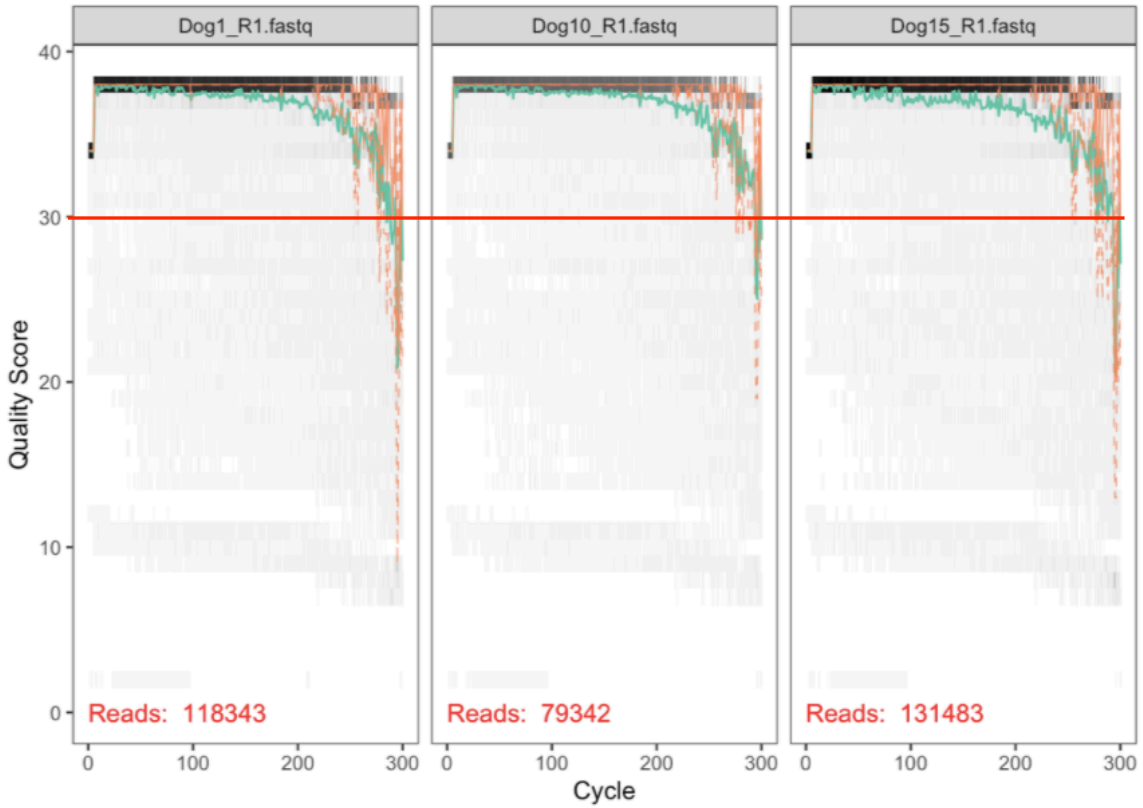
```
# Extract sample names, assuming filenames have format: SAMPLENAME_XXX.fastq
list.sample.names <- sapply(strsplit(basename(dataF), "_"), `[`, 1)
list.sample.names
```

```
##  [1] "Dog1"  "Dog10" "Dog15" "Dog16" "Dog17" "Dog2"  "Dog22" "Dog23"
##  [9] "Dog24" "Dog29" "Dog3"  "Dog30" "Dog31" "Dog8"  "Dog9"
```

DADA2 workflow

Quality Control

```
plotQualityProfile(dataF[1:3])
```



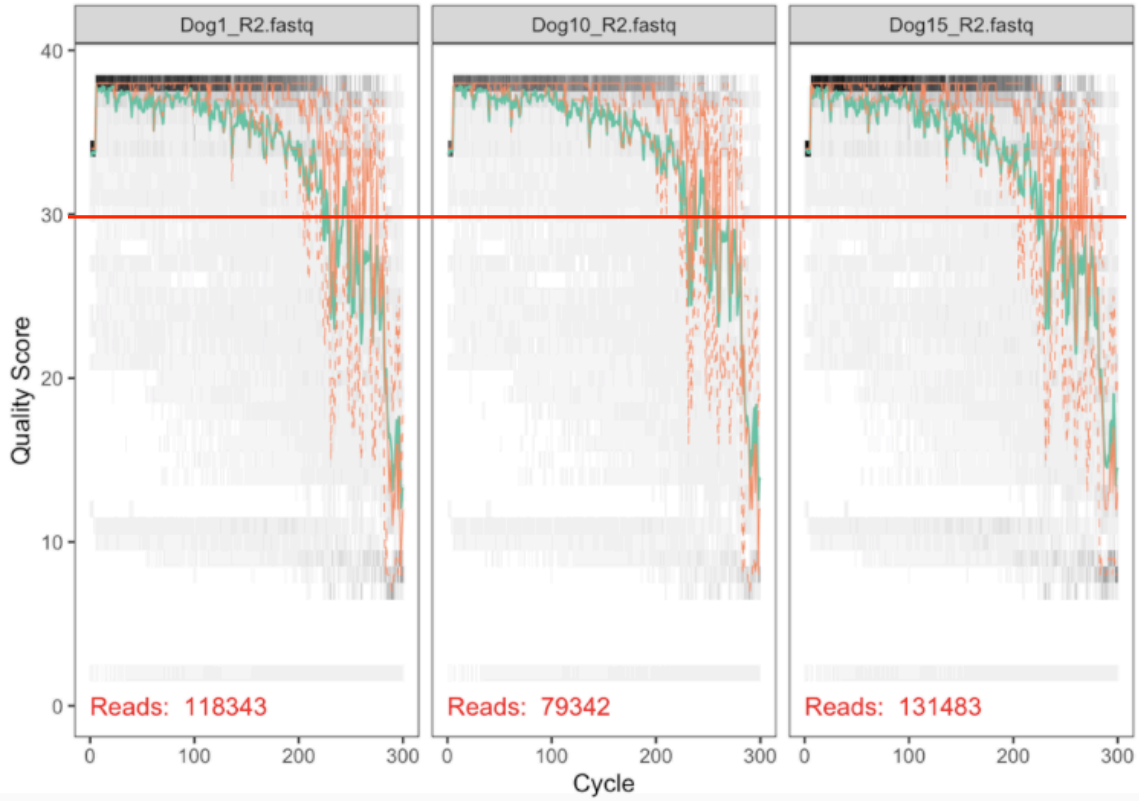
- The quality plot of three forward samples.

- Scores never really go below 30.

DADA2 workflow

Quality Control

```
plotQualityProfile(dataR[1:3])
```



- The reverse reads are slightly different.

- The scores are good but they drop off right around 275 bp.

DADA2 workflow

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Filter and Trim

Set filtered subdirectory and rename files

```
# Place filtered files in filtered/ subdirectory
filt.dataF <- file.path(data, "filtered", paste0(list.sample.names, "_F_filt.fastq.gz"))
filt.dataR <- file.path(data, "filtered", paste0(list.sample.names, "_R_filt.fastq.gz"))
names(filt.dataF) <- list.sample.names
names(filt.dataR) <- list.sample.names
```

DADA2 workflow

Filter and Trim

```
out <- filterAndTrim(dataF, filt.dataF, dataR, filt.dataR, truncLen=c(290,275),
              maxN=0, maxEE=c(2,2), truncQ=2, rm.phix=TRUE,
              compress=TRUE, multithread=TRUE) # On Windows set multithread=FALSE
```

truncLen truncates your reads at specific base.

truncLen=c(290,275)

The amplicon length.

The length of your overlap, by default is 20 for DADA2.

DADA2 workflow

Filter and Trim

```
out <- filterAndTrim(dataF, filt.dataF, dataR, filt.dataR, truncLen=c(290,275),
              maxN=0, maxEE=c(2,2), truncQ=2, rm.phix=TRUE,
              compress=TRUE, multithread=TRUE) # On Windows set multithread=FALSE
```

maxN maximum number of ambiguous nucleotides.

maxN=0

DADA2 requires no Ns.

DADA2 workflow

Filter and Trim

```
out <- filterAndTrim(dataF, filt.dataF, dataR, filt.dataR, truncLen=c(290,275),
              maxN=0, maxEE=c(2,2), truncQ=2, rm.phix=TRUE,
              compress=TRUE, multithread=TRUE) # On Windows set multithread=FALSE
```

maxEE maximum number of estimated errors allowed in your reads.

maxEE=c(2,2)

The quality of your sequences.

DADA2 workflow

Filter and Trim

```
out <- filterAndTrim(dataF, filt.dataF, dataR, filt.dataR, truncLen=c(290,275),
              maxN=0, maxEE=c(2,2), truncQ=2, rm.phix=TRUE,
              compress=TRUE, multithread=TRUE) # On Windows set multithread=FALSE
```

truncQ truncates the read at the first nucleotide with a specific quality score.

truncQ=2

Score of 2 means that the probability of the base being incorrect is 63%.

DADA2 workflow

Filter and Trim

```
out <- filterAndTrim(dataF, filt.dataF, dataR, filt.dataR, truncLen=c(290,275),
              maxN=0, maxEE=c(2,2), truncQ=2, rm.phix=TRUE,
              compress=TRUE, multithread=TRUE) # On Windows set multithread=FALSE
```

rm.phix removes reads that match against the phiX genome.

rm.phix=TRUE

DADA2 workflow

Filter and Trim

```
out <- filterAndTrim(dataF, filt.dataF, dataR, filt.dataR, truncLen=c(290,275),
              maxN=0, maxEE=c(2,2), truncQ=2, rm.phix=TRUE,
              compress=TRUE, multithread=TRUE) # On Windows set multithread=FALSE
```

Compress if you want to fastq files to be gzipped.

Multithread if you want your files to run in parallel.

DADA2 workflow

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Learn the Error Rates

It will create an error model that will be used by the DADA2 algorithm.

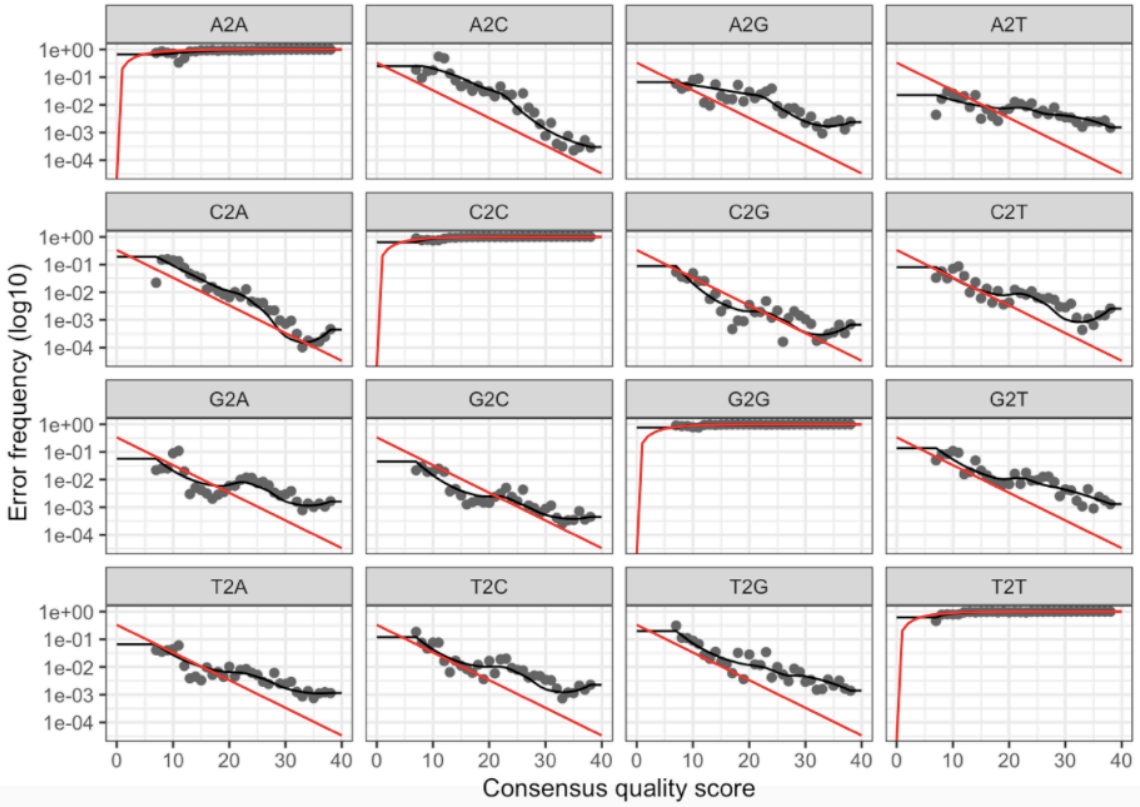
```
errF <- learnErrors(filt.dataF, multithread=TRUE)
```

```
errR <- learnErrors(filt.dataR, multithread=TRUE)
```

DADA2 workflow

Learn the Error Rates

```
plotErrors(errF, nominalQ=TRUE)
```



- The error rates for each possible transition (A -> C).

- As quality score increases, the expected error rate decreases.

DADA2 workflow

Sample Inference

Set filtered subdirectory and rename files

```
# Place filtered files in filtered/ subdirectory
filt.dataF <- file.path(data, "filtered", paste0(list.sample.names, "_F_filt.fastq.gz"))
filt.dataR <- file.path(data, "filtered", paste0(list.sample.names, "_R_filt.fastq.gz"))
names(filt.dataF) <- list.sample.names
names(filt.dataR) <- list.sample.names
```

It uses the error model that was created earlier.

p-value high -> sequence likely caused by errors.
p-value low -> sequence is real.