Software Tools (BINF*6210) Assignment 5

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GitHub link: https://github.com/ErikaMyler/Assignment5

Introduction

Modern microbiome research relies on software tools for the analysis of molecular data to infer microbial community compositions. DADA2 is a denoising and deduplication pipeline for microbiome analysis which generates amplicon sequence variants (ASVs; also exact sequence variants, ESVs) from Illumina-sequenced paired-end reads (Callahan et al. 2016). The DADA2 denoising algorithm has flexible parameters, allowing the user to set the number of nucleotides to trim from the 5' end and the length to truncate forward and reverse reads independently. In this project, I manipulate these parameters to assess and compare the performance of the DADA2 pipeline under two sets of conditions. Performance is defined as the success of recovering reads and identifying taxa present in a mock community.

Description of Data Set

Paired-end reads (2x300bp) were generated using the Illumina MiSeq instrument by Winand et al. (2020). The raw 16S10_V8_V9 and 16S10_V8_V9_NTC fastq.gz files were downloaded from the NCBI Sequence Read Archive (SRA) BioProject PRJNA587452. The files can be accessed here:

#https://www.ncbi.nlm.nih.gov/Traces/study/?uids=9323659%2C9323657%2C9323658%2C9323656%2C9323655%2C9323654%2C9323653%2C9323652%2C9323651%2C9323650%2C9323649%2C9323648%2C9323647%2C9323646%2C9323645%2C9323644%2C9323643%2C9323642%2C9323641%2C9323639%2C9323638%2C9323637&o=acc_s%3Aa

16S10_V8_V9 = sequenced amplicon (370 bp) spanning the V8 and V9 16S rRNA regions using the primer pair 1522F/1189R1

16S10_V8_V9_NTC = no template control for 16S10_V8_V9

Code Section 1 - Data Acquisition, Exploration, Filtering, and Quality Control

```
# Paired-end reads (2x300bp) were generated using the Illumina MiSeq instrument
# by Winand et al. (2020). The raw 16S10_V8_V9 and 16S10_V8_V9_NTC fastq.gz
# files were downloaded from the NCBI Sequence Read Archive (SRA) BioProject
# PRJNA587452.

# 16S10_V8_V9 = sequenced amplicon (370 bp) spanning the V8 and V9 16S rRNA
# regions using the primer pair 1522F/1189R1 16S10_V8_V9_NTC = no template
# control for 16S10_V8_V9
```

```
# All fastq.gz files were demultiplexed (i.e., split into two separate files
# for forward and reverse reads) to faciliate analysis using dada2.
# Demultiplexing was conducted individually for each file using the split2
# function in SeqKit (Shen et al. 2016) by running the following line in the
# command line:

# C:\>seqkit split2 \Users\Erika\Downloads\sra_data1.fastq.gz -p 2 -O out -f
# [INFO] split seqs from \Users\Erika\Downloads\sra_data1.fastq.gz [INFO] split
# into 2 parts [INFO] write 20841 sequences to file:
# out\sra_data1.part_001.fastq.gz [INFO] write 20841 sequences to file:
# out\sra_data1.part_002.fastq.gz

# sra_data1.part_001.fastq.gz = forward reads for 16S10_V8_V9
# sra_data1.part_002.fastq.gz = reverse reads for 16S10_V8_V9

# setting file path to the folder containing fastq.gz files

path <- "./testData4"
list.files(path)
```

```
## [1] "filtered"                    "sra_data1.part_001.fastq.gz"
## [3] "sra_data1.part_002.fastq.gz" "sra_data9.part_001.fastq.gz"
## [5] "sra_data9.part_002.fastq.gz"
```

```
R1s <- sort(list.files(path, pattern = ".part_001.fastq", full.names = TRUE))
R2s <- sort(list.files(path, pattern = ".part_002.fastq", full.names = TRUE))

R1s
```

```
## [1] "./testData4/sra_data1.part_001.fastq.gz"
## [2] "./testData4/sra_data9.part_001.fastq.gz"
```

```
R2s
```

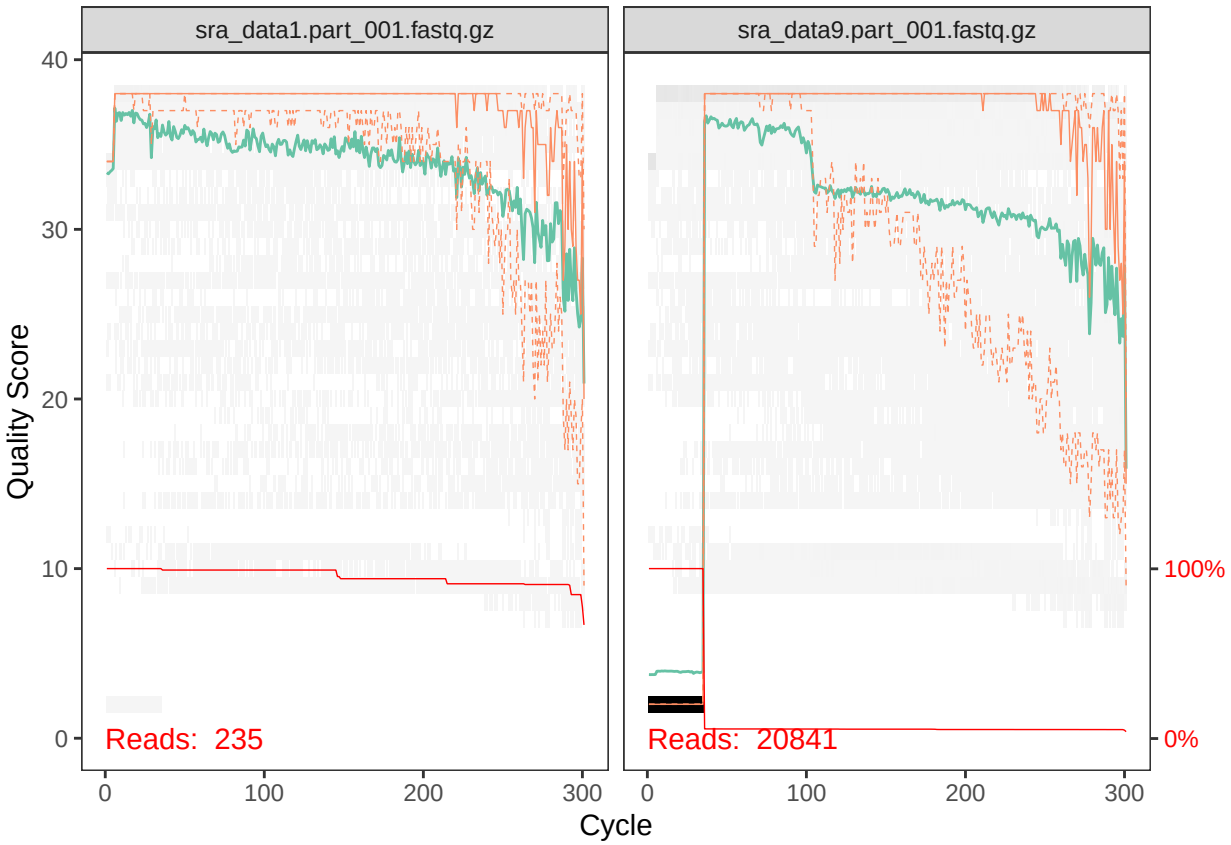
```
## [1] "./testData4/sra_data1.part_002.fastq.gz"
## [2] "./testData4/sra_data9.part_002.fastq.gz"
```

```
# extracting sample names

sample.names <- sapply(strsplit(basename(R1s), ".part"), `[`, 1)
sample.names
```

```
## [1] "sra_data1" "sra_data9"
```

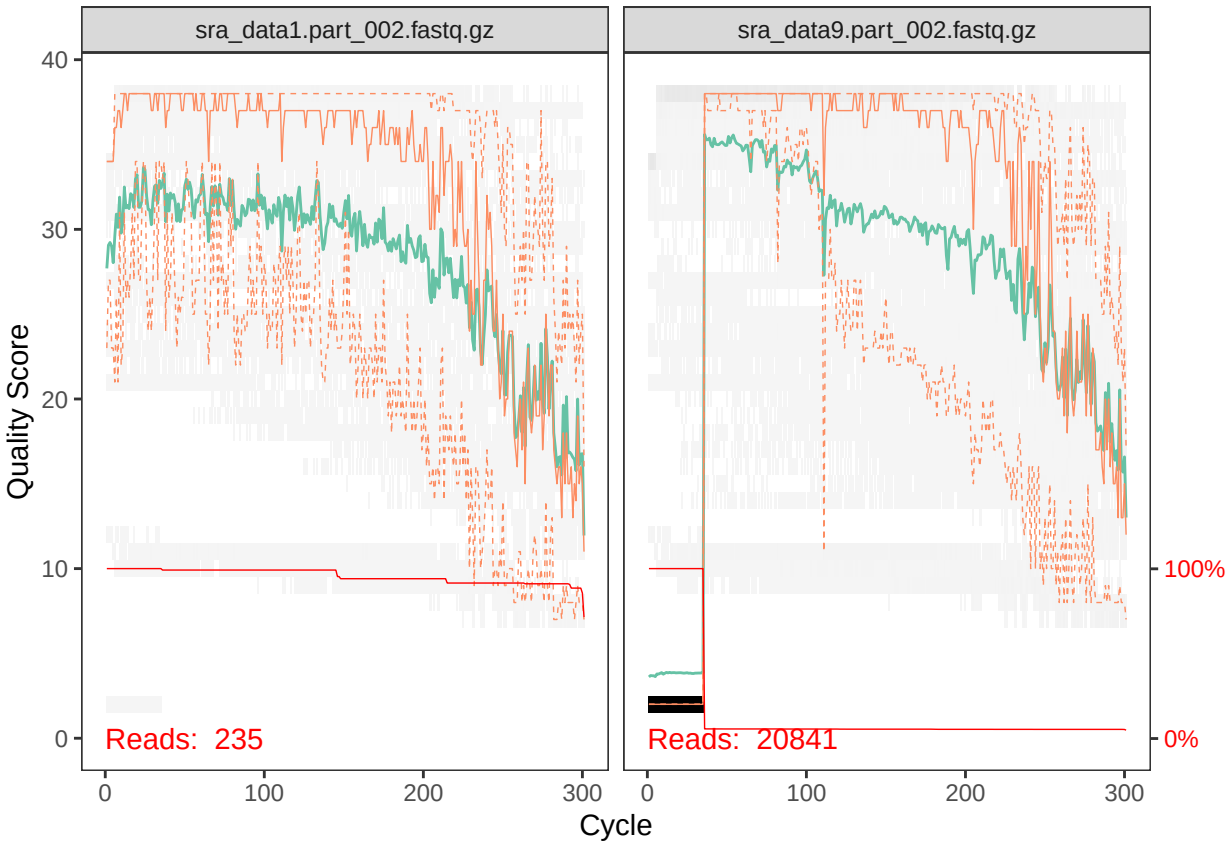
```
plotQualityProfile(R1s[1:2])
```

```
## Warning: ‘guides(<scale> = FALSE)‘ is deprecated. Please use ‘guides(<scale> =
## "none")‘ instead.
```




```
plotQualityProfile(R2s[1:2])
```

```
## Warning: `guides(<scale> = FALSE)` is deprecated. Please use `guides(<scale> =
## "none")` instead.
```




```
# pre-setting file names for filtered sequences

filtR1s <- file.path(path, "filtered", paste0(sample.names, "_R1_filt.fastq.gz"))
filtR2s <- file.path(path, "filtered", paste0(sample.names, "_R2_filt.fastq.gz"))

names(filtR1s) <- sample.names
names(filtR2s) <- sample.names

# filtering all sequences

# out <- filterAndTrim(R1s, filtR1s, R2s, filtR2s, trimLeft=10,
# truncLen=c(290,220), maxN=0, maxEE=c(2,2), truncQ=2, rm.phix=TRUE,
# compress=TRUE, verbose=TRUE, multithread=FALSE)

out <- filterAndTrim(R1s, filtR1s, R2s, filtR2s, maxN = 0, maxEE = c(2, 2), truncQ = 2,
    rm.phix = TRUE, compress = TRUE, verbose = TRUE, multithread = FALSE)
```

```
## Overwriting file:C:\Users\Erika\Documents\MSc IBIO Guelph 2021-2023_SAFE\Courses\BINF 6210_F21\Assig

## Overwriting file:C:\Users\Erika\Documents\MSc IBIO Guelph 2021-2023_SAFE\Courses\BINF 6210_F21\Assig

## Read in 235 paired-sequences, output 88 (37.4%) filtered paired-sequences.

## Overwriting file:C:\Users\Erika\Documents\MSc IBIO Guelph 2021-2023_SAFE\Courses\BINF 6210_F21\Assig
```

```
## Overwriting file:C:\Users\Erika\Documents\MSc IBIO Guelph 2021-2023_SAFE\Courses\BINF 6210_F21\Assig
```

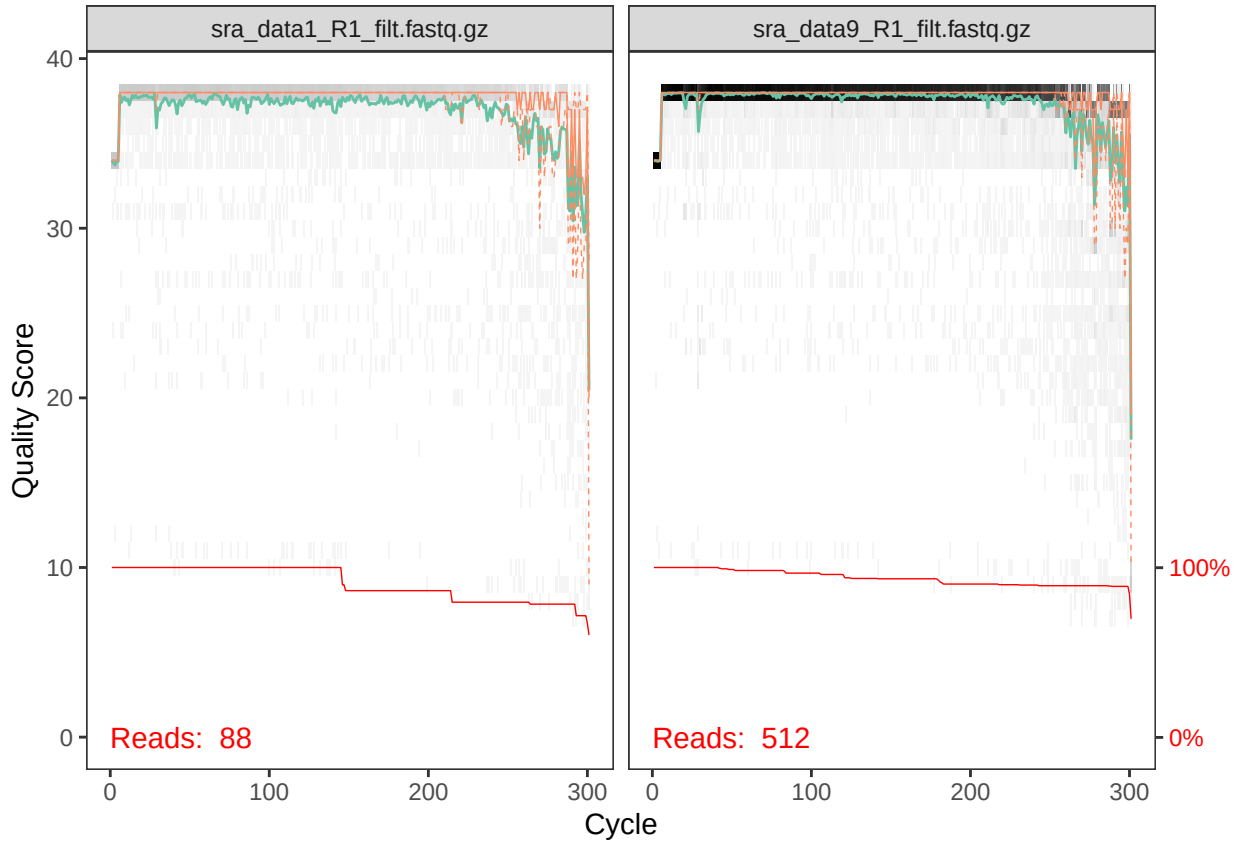
```
## Read in 20841 paired-sequences, output 512 (2.5%) filtered paired-sequences.
```

```
head(out)
```

```
##                               reads.in reads.out
## sra_data1.part_001.fastq.gz      235        88
## sra_data9.part_001.fastq.gz    20841       512
```

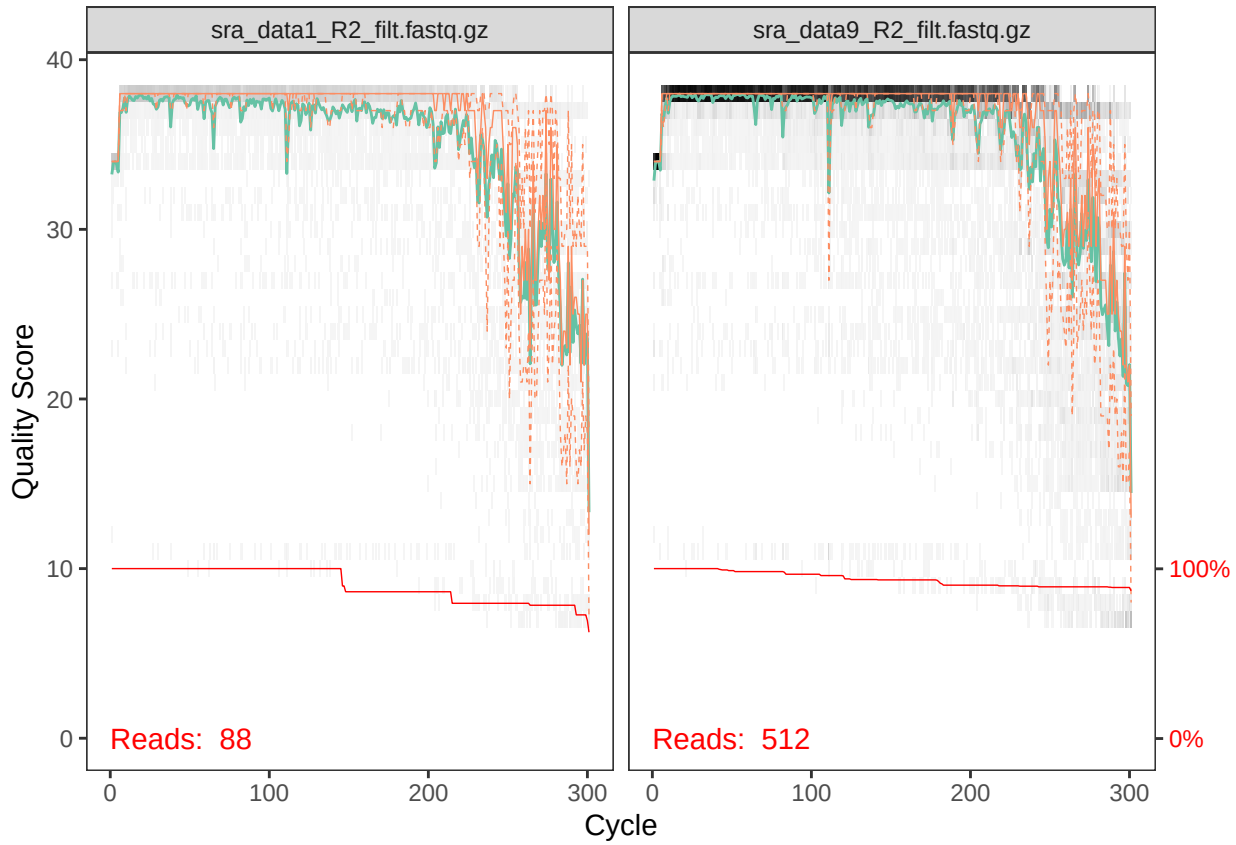
```
# re-checking quality profiles

plotQualityProfile(filtR1s[1:2])
```

```
## Warning: `guides(<scale> = FALSE)` is deprecated. Please use `guides(<scale> =
## "none")` instead.
```




```
plotQualityProfile(filtR2s[1:2])
```

```
## Warning: `guides(<scale> = FALSE)` is deprecated. Please use `guides(<scale> =
## "none")` instead.
```




```
# dereplicating

derepR1s <- derepFastq(filtR1s, verbose = TRUE)
```

```
## Dereplicating sequence entries in Fastq file: ./testData4/filtered/sra_data1_R1_filt.fastq.gz

## Encountered 61 unique sequences from 88 total sequences read.

## Dereplicating sequence entries in Fastq file: ./testData4/filtered/sra_data9_R1_filt.fastq.gz

## Encountered 151 unique sequences from 512 total sequences read.
```

```
derepR2s <- derepFastq(filtR2s, verbose = TRUE)
```

```
## Dereplicating sequence entries in Fastq file: ./testData4/filtered/sra_data1_R2_filt.fastq.gz

## Encountered 72 unique sequences from 88 total sequences read.

## Dereplicating sequence entries in Fastq file: ./testData4/filtered/sra_data9_R2_filt.fastq.gz

## Encountered 256 unique sequences from 512 total sequences read.
```

```
class(derepR1s)
```

```
## [1] "list"
```

```
class(derepR2s)
```

```
## [1] "list"
```

```
# investigating dereplicated data outputs from these investigations have been
# omitted to reduce the length of the pdf
```

```
# unique sequences and number of reads for each

for (i in sample.names) {
    print(i)
    print(derepR1s[[i]][1])
}

# sequences and read quality (Phred scores)

for (i in sample.names) {
    # print(i) print(derepR1s[[i]][2])
}

# sequence reads mapped to ESV (where each number represents which ESV the read
# is mapped to)

for (i in sample.names) {
    print(i)
    print(derepR1s[[i]][3])
}

# summary of mapped reads to display min, mean, max, etc. no. reads mapped to
# each ESV

for (i in sample.names) {
    print(i)
    print(lapply(derepR1s[[i]][3], summary))
}
```

```
# estimating error rates in forward and reverse reads across all samples

errorR1s <- learnErrors(derepR1s, multithread = FALSE)
```

```
## 168517 total bases in 600 reads from 2 samples will be used for learning the error rates.
```

```
errorR2s <- learnErrors(derepR2s, multithread = FALSE)
```

```
## 168640 total bases in 600 reads from 2 samples will be used for learning the error rates.
```

```
errorR1s
```

```
## $err_out
##             0         1         2         3         4         5         6
## A2A 0.2500000 0.2500000 0.2500000 0.2500000 0.2500000 0.2500000 0.2500000
## A2C 0.2500000 0.2500000 0.2500000 0.2500000 0.2500000 0.2500000 0.2500000
## A2G 0.2500000 0.2500000 0.2500000 0.2500000 0.2500000 0.2500000 0.2500000
## A2T 0.2500000 0.2500000 0.2500000 0.2500000 0.2500000 0.2500000 0.2500000
## C2A 0.2500000 0.2500000 0.2500000 0.2500000 0.2500000 0.2500000 0.2500000
## C2C 0.2561050 0.2561050 0.2561050 0.2561050 0.2561050 0.2561050 0.2561050
## C2G 0.2500000 0.2500000 0.2500000 0.2500000 0.2500000 0.2500000 0.2500000
## C2T 0.2438950 0.2438950 0.2438950 0.2438950 0.2438950 0.2438950 0.2438950
## G2A 0.2083099 0.2083099 0.2083099 0.2083099 0.2083099 0.2083099 0.2083099
## G2C 0.2500000 0.2500000 0.2500000 0.2500000 0.2500000 0.2500000 0.2500000
## G2G 0.2916901 0.2916901 0.2916901 0.2916901 0.2916901 0.2916901 0.2916901
## G2T 0.2500000 0.2500000 0.2500000 0.2500000 0.2500000 0.2500000 0.2500000
## T2A 0.1589106 0.1589106 0.1589106 0.1589106 0.1589106 0.1589106 0.1589106
## T2C 0.2500000 0.2500000 0.2500000 0.2500000 0.2500000 0.2500000 0.2500000
## T2G 0.1529134 0.1529134 0.1529134 0.1529134 0.1529134 0.1529134 0.1529134
## T2T 0.4381760 0.4381760 0.4381760 0.4381760 0.4381760 0.4381760 0.4381760
##             7         8         9         10         11         12         13
## A2A 0.2500000 0.4266208 0.5759671 0.73251553 0.81886018 0.86965916 0.90066062
## A2C 0.2500000 0.2500000 0.2146027 0.12476231 0.07871678 0.05322343 0.03847414
## A2G 0.2500000 0.1624597 0.1042350 0.07030786 0.04990539 0.03725415 0.02922765
## A2T 0.2500000 0.1609195 0.1051951 0.07241430 0.05251765 0.03986326 0.03163758
## C2A 0.2500000 0.2500000 0.2500000 0.25000000 0.25000000 0.25000000 0.22688092
## C2C 0.2561050 0.2500000 0.2612100 0.27888420 0.30117066 0.32832119 0.38109956
## C2G 0.2500000 0.2500000 0.2387900 0.22111580 0.20376836 0.18703919 0.17102377
## C2T 0.2438950 0.2500000 0.2500000 0.25000000 0.24506097 0.23463962 0.22099575
## G2A 0.2083099 0.2048671 0.1996446 0.19214977 0.18205064 0.16974479 0.15634338
## G2C 0.2500000 0.2500000 0.2500000 0.22322279 0.19009098 0.16088506 0.13597296
## G2G 0.2916901 0.3106020 0.3423715 0.40050325 0.46573076 0.52727799 0.58315593
## G2T 0.2500000 0.2345309 0.2079838 0.18412418 0.16212762 0.14209216 0.12452773
## T2A 0.1589106 0.1712689 0.1813114 0.18812288 0.19135918 0.19120442 0.18772763
## T2C 0.2500000 0.2500000 0.2500000 0.24690303 0.22695003 0.20665743 0.18657491
## T2G 0.1529134 0.1636254 0.1720911 0.17768365 0.18015538 0.17957727 0.17598838
## T2T 0.4381760 0.4151058 0.3965974 0.38729044 0.40153541 0.42256087 0.44970909
##             14         15         16         17         18         19
## A2A 0.92002333 0.93165619 0.93802896 0.94095249 0.94143238 0.93377498
## A2C 0.02966501 0.02459404 0.02195746 0.02085519 0.02081805 0.02444585
## A2G 0.02408316 0.02089996 0.01909535 0.01826865 0.01820236 0.02087197
## A2T 0.02622851 0.02284982 0.02091824 0.01992367 0.01954720 0.02090720
## C2A 0.20280127 0.18047832 0.15805452 0.13536561 0.11472039 0.09734529
## C2C 0.43642350 0.49066961 0.54639114 0.60317079 0.65652158 0.70392994
## C2G 0.15579643 0.14141155 0.12841114 0.11665531 0.10538613 0.09410932
## C2T 0.20497880 0.18744052 0.16714319 0.14480829 0.12337190 0.10461545
## G2A 0.14263706 0.12925463 0.11665725 0.10489339 0.09332363 0.08150975
## G2C 0.11520500 0.09823616 0.08463540 0.07450846 0.06669912 0.05949614
## G2G 0.63278028 0.67600608 0.71298616 0.74269519 0.76757825 0.79172419
## G2T 0.10937766 0.09650313 0.08572118 0.07790296 0.07239900 0.06726992
## T2A 0.18116770 0.17190810 0.16029955 0.14668383 0.13159550 0.11563880
## T2C 0.16714806 0.14871672 0.13087361 0.11366921 0.09780433 0.08368043
## T2G 0.16957788 0.16066866 0.14876573 0.13430244 0.11890441 0.10384099
## T2T 0.48210636 0.51870652 0.56006110 0.60534452 0.65169577 0.69683978
```

```
##             20         21         22         23         24         25
## A2A 0.91349477 0.88543123 0.86606935 0.87162839 0.88967260 0.90683757
## A2C 0.03406419 0.04760965 0.05641212 0.05017163 0.03674352 0.02583253
## A2G 0.02786300 0.03772523 0.04513218 0.04494667 0.04033366 0.03499054
## A2T 0.02457804 0.02923389 0.03238635 0.03325331 0.03325023 0.03233936
## C2A 0.08674501 0.08192450 0.07831438 0.07236791 0.06321011 0.05343806
## C2C 0.73976508 0.76569145 0.78873356 0.81381852 0.84552466 0.87799885
## C2G 0.08353292 0.07418297 0.06561840 0.05755398 0.04812681 0.03807928
## C2T 0.08995700 0.07820107 0.06733366 0.05625959 0.04313842 0.03048381
## G2A 0.06933768 0.06123129 0.05782448 0.05504687 0.05140467 0.04718371
## G2C 0.05182087 0.04840237 0.04980223 0.05003891 0.04605346 0.03998214
## G2G 0.81774502 0.83346099 0.83678831 0.84133961 0.85344687 0.86959135
## G2T 0.06109642 0.05690534 0.05558498 0.05357461 0.04909500 0.04324281
## T2A 0.09956080 0.08450710 0.07128230 0.06023067 0.04934815 0.03871577
## T2C 0.07209719 0.06282229 0.05488173 0.04765166 0.04035531 0.03338432
## T2G 0.09049863 0.07897615 0.06856523 0.05883548 0.04747978 0.03575178
## T2T 0.73784337 0.77369446 0.80527073 0.83328219 0.86281676 0.89214813
##             26         27         28         29          30          31
## A2A 0.91763087 0.92900625 0.94357702 0.95548907 0.962525825 0.966487583
## A2C 0.02032564 0.01707391 0.01364559 0.01092210 0.009216339 0.008196302
## A2G 0.03148477 0.02778688 0.02269764 0.01835322 0.015711723 0.014180588
## A2T 0.03055872 0.02613295 0.02007975 0.01523561 0.012546114 0.011135528
## C2A 0.04475074 0.03799241 0.03262683 0.02797332 0.024109023 0.021030837
## C2C 0.90420021 0.92226777 0.93384861 0.94199746 0.948217613 0.953563198
## C2G 0.02976630 0.02400158 0.02046199 0.01814844 0.016446480 0.014955722
## C2T 0.02128275 0.01573824 0.01306257 0.01188078 0.011226884 0.010450244
## G2A 0.04192811 0.03552601 0.02709288 0.01892421 0.013347733 0.010480893
## G2C 0.03338712 0.02734394 0.01976990 0.01237851 0.007786880 0.005709526
## G2G 0.88766935 0.90599589 0.92913291 0.95192907 0.967161530 0.974808076
## G2T 0.03701541 0.03113417 0.02400431 0.01676821 0.011703856 0.009001505
## T2A 0.03020977 0.02435163 0.02086441 0.01872793 0.017149742 0.015602246
## T2C 0.02757002 0.02322831 0.02015679 0.01781533 0.015888395 0.014165296
## T2G 0.02657347 0.02062541 0.01728993 0.01530612 0.013914823 0.012632529
## T2T 0.91564674 0.93179465 0.94168887 0.94815062 0.953047040 0.957599929
##             32          33          34          35          36          37
## A2A 0.969732382 0.972303143 0.974239224 0.975649261 0.976623922 0.977158027
## A2C 0.007426716 0.006863488 0.006476077 0.006239554 0.006139630 0.006176664
## A2G 0.012884018 0.011833337 0.011032504 0.010438519 0.010008356 0.009746963
## A2T 0.009956884 0.009000032 0.008252195 0.007672666 0.007228092 0.006918346
## C2A 0.018571108 0.016490185 0.014674167 0.013042337 0.011581952 0.010297517
## C2C 0.958183281 0.961892826 0.964978906 0.967653443 0.969946748 0.971847578
## C2G 0.013652139 0.012656320 0.011902302 0.011341607 0.010952661 0.010721663
## C2T 0.009593472 0.008960669 0.008444624 0.007962614 0.007518639 0.007133241
## G2A 0.009155287 0.008313093 0.007822078 0.007603304 0.007640370 0.007967798
## G2C 0.004875833 0.004402926 0.004224050 0.004325770 0.004730791 0.005529619
## G2G 0.978340633 0.980603010 0.981895407 0.982371956 0.982066327 0.980863033
## G2T 0.007628246 0.006680971 0.006058465 0.005698970 0.005562512 0.005639549
## T2A 0.014116304 0.012919348 0.011929771 0.011086175 0.010371572 0.009776829
## T2C 0.012628911 0.011331305 0.010230074 0.009291239 0.008489685 0.007803632
## T2G 0.011455399 0.010577199 0.009948783 0.009536883 0.009317926 0.009271648
## T2T 0.961799385 0.965172148 0.967891372 0.970085703 0.971820818 0.973147892
##             38          39          40
## A2A 0.977228756 0.977228756 0.977228756
## A2C 0.006360115 0.006360115 0.006360115
```

```
## A2G 0.009664560 0.009664560 0.009664560
## A2T 0.006746568 0.006746568 0.006746568
## C2A 0.009159375 0.009159375 0.009159375
## C2C 0.973444858 0.973444858 0.973444858
## C2G 0.010627408 0.010627408 0.010627408
## C2T 0.006768359 0.006768359 0.006768359
## G2A 0.008637328 0.008637328 0.008637328
## G2C 0.006944613 0.006944613 0.006944613
## G2G 0.978461291 0.978461291 0.978461291
## G2T 0.005956768 0.005956768 0.005956768
## T2A 0.009269609 0.009269609 0.009269609
## T2C 0.007212840 0.007212840 0.007212840
## T2G 0.009387113 0.009387113 0.009387113
## T2T 0.974130438 0.974130438 0.974130438
##
## $err_in
## $err_in[[1]]
##             0         1         2         3         4         5         6
## A2A 1.0000000 1.0000000 1.0000000 1.0000000 1.0000000 1.0000000 1.0000000
## A2C 0.2500000 0.2500000 0.2500000 0.2500000 0.2500000 0.2500000 0.2500000
## A2G 0.2458808 0.2458808 0.2458808 0.2458808 0.2458808 0.2458808 0.2458808
## A2T 0.2500000 0.2500000 0.2500000 0.2500000 0.2500000 0.2500000 0.2500000
## C2A 0.2500000 0.2500000 0.2500000 0.2500000 0.2500000 0.2500000 0.2500000
## C2C 1.0000000 1.0000000 1.0000000 1.0000000 1.0000000 1.0000000 1.0000000
## C2G 0.2500000 0.2500000 0.2500000 0.2500000 0.2500000 0.2500000 0.2500000
## C2T 0.2500000 0.2500000 0.2500000 0.2500000 0.2500000 0.2500000 0.2500000
## G2A 0.2048950 0.2048950 0.2048950 0.2048950 0.2048950 0.2048950 0.2048950
## G2C 0.2500000 0.2500000 0.2500000 0.2500000 0.2500000 0.2500000 0.2500000
## G2G 1.0000000 1.0000000 1.0000000 1.0000000 1.0000000 1.0000000 1.0000000
## G2T 0.2432204 0.2432204 0.2432204 0.2432204 0.2432204 0.2432204 0.2432204
## T2A 0.1841570 0.1841570 0.1841570 0.1841570 0.1841570 0.1841570 0.1841570
## T2C 0.2500000 0.2500000 0.2500000 0.2500000 0.2500000 0.2500000 0.2500000
## T2G 0.1626867 0.1626867 0.1626867 0.1626867 0.1626867 0.1626867 0.1626867
## T2T 1.0000000 1.0000000 1.0000000 1.0000000 1.0000000 1.0000000 1.0000000
##             7         8          9         10         11          12         13
## A2A 1.0000000 1.0000000 1.00000000 1.00000000 1.00000000 1.00000000 1.00000000
## A2C 0.2500000 0.2500000 0.19521182 0.11382217 0.07227287 0.04933574 0.03612297
## A2G 0.2458808 0.1557127 0.10342386 0.07204049 0.05267433 0.04045580 0.03262221
## A2T 0.2500000 0.1556097 0.09998101 0.06795158 0.04886996 0.03696854 0.02939337
## C2A 0.2500000 0.2500000 0.25000000 0.25000000 0.25000000 0.25000000 0.25000000
## C2C 1.0000000 1.0000000 1.00000000 1.00000000 1.00000000 1.00000000 1.00000000
## C2G 0.2500000 0.2500000 0.25000000 0.24124461 0.22907199 0.21651284 0.20366468
## C2T 0.2500000 0.2500000 0.25000000 0.25000000 0.25000000 0.23307765 0.21458465
## G2A 0.2048950 0.1925278 0.18112969 0.17047877 0.16039300 0.15122435 0.14323988
## G2C 0.2500000 0.2500000 0.25000000 0.25000000 0.23134390 0.19306945 0.16065917
## G2G 1.0000000 1.0000000 1.00000000 1.00000000 1.00000000 1.00000000 1.00000000
## G2T 0.2432204 0.2150525 0.19063372 0.16888436 0.14905178 0.13098825 0.11505199
## T2A 0.1841570 0.1877283 0.18996072 0.18993098 0.18765615 0.18389333 0.17887785
## T2C 0.2500000 0.2500000 0.25000000 0.25000000 0.24645557 0.23029838 0.21413676
## T2G 0.1626867 0.1725771 0.18119966 0.18708915 0.18976165 0.18993923 0.18796662
## T2T 1.0000000 1.0000000 1.00000000 1.00000000 1.00000000 1.00000000 1.00000000
##            14         15         16         17         18         19
## A2A 1.00000000 1.00000000 1.00000000 1.00000000 1.00000000 1.00000000
## A2C 0.02830338 0.02393859 0.02188680 0.02134795 0.02192233 0.02615277
```

```
## A2G 0.02760503 0.02455605 0.02295268 0.02245887 0.02291909 0.02647431
## A2T 0.02454551 0.02166868 0.02021917 0.01972854 0.01991397 0.02212725
## C2A 0.24918805 0.22129422 0.19286770 0.16431569 0.13892905 0.11834938
## C2C 1.00000000 1.00000000 1.00000000 1.00000000 1.00000000 1.00000000
## C2G 0.19063002 0.17751435 0.16463930 0.15205735 0.13954540 0.12697497
## C2T 0.19613895 0.17824116 0.15956407 0.14023781 0.12223167 0.10672759
## G2A 0.13619431 0.12988339 0.12413517 0.11880355 0.11437849 0.11114403
## G2C 0.13391782 0.11233541 0.09526753 0.08205926 0.07225463 0.06477336
## G2G 1.00000000 1.00000000 1.00000000 1.00000000 1.00000000 1.00000000
## G2T 0.10131899 0.08974099 0.08019778 0.07253937 0.06785956 0.06591125
## T2A 0.17285656 0.16607533 0.15975660 0.15382696 0.14677057 0.13736982
## T2C 0.19762077 0.18055431 0.16197537 0.14278378 0.12503368 0.10995699
## T2G 0.18425533 0.17924460 0.17086373 0.15866652 0.14528135 0.13276425
## T2T 1.00000000 1.00000000 1.00000000 1.00000000 1.00000000 1.00000000
##             20         21         22         23         24         25
## A2A 1.00000000 1.00000000 1.00000000 1.00000000 1.00000000 1.00000000
## A2C 0.03647787 0.05113801 0.06194159 0.05727044 0.04383383 0.03247091
## A2G 0.03486935 0.04664949 0.05647105 0.06039202 0.06141356 0.06123629
## A2T 0.02728527 0.03406544 0.03928605 0.04123172 0.04151864 0.04050947
## C2A 0.10430754 0.09552590 0.08926641 0.08358383 0.07932947 0.07714966
## C2C 1.00000000 1.00000000 1.00000000 1.00000000 1.00000000 1.00000000
## C2G 0.11187556 0.09506529 0.08006477 0.06868421 0.06073889 0.05472142
## C2T 0.09556771 0.08768366 0.08029366 0.07147942 0.05933458 0.04639404
## G2A 0.10871540 0.10675678 0.10748035 0.11139503 0.11556026 0.11165476
## G2C 0.05846273 0.05253790 0.05312264 0.06154204 0.06909771 0.06815855
## G2G 1.00000000 1.00000000 1.00000000 1.00000000 1.00000000 1.00000000
## G2T 0.06492864 0.06336632 0.06274670 0.06378927 0.06386600 0.05902352
## T2A 0.12762586 0.11939752 0.11175625 0.10398693 0.09268543 0.07858908
## T2C 0.09477100 0.07917571 0.06613002 0.05695371 0.05012033 0.04396322
## T2G 0.12461562 0.12062553 0.11745240 0.11220729 0.10710233 0.10485613
## T2T 1.00000000 1.00000000 1.00000000 1.00000000 1.00000000 1.00000000
##             26         27         28         29         30          31
## A2A 1.00000000 1.00000000 1.00000000 1.00000000 1.00000000 1.000000000
## A2C 0.02721829 0.02412252 0.02008101 0.01702008 0.01592073 0.016423649
## A2G 0.06173650 0.05750919 0.04762063 0.03904221 0.03529992 0.034728386
## A2T 0.03867721 0.03096313 0.02023294 0.01319842 0.01051133 0.010005816
## C2A 0.07541778 0.07269537 0.06699629 0.05919277 0.05171678 0.046090390
## C2C 1.00000000 1.00000000 1.00000000 1.00000000 1.00000000 1.000000000
## C2G 0.04981065 0.04543133 0.04221948 0.04047194 0.03943012 0.038466655
## C2T 0.03597426 0.02912359 0.02627527 0.02617383 0.02710304 0.027467019
## G2A 0.09811747 0.08196903 0.06804896 0.05549505 0.04510400 0.039705386
## G2C 0.06145543 0.05268381 0.04466473 0.03086040 0.01783003 0.012447679
## G2G 1.00000000 1.00000000 1.00000000 1.00000000 1.00000000 1.000000000
## G2T 0.04981460 0.03994089 0.03164911 0.02003888 0.01057955 0.007104587
## T2A 0.06590020 0.05681210 0.05307988 0.05321647 0.05417830 0.053003406
## T2C 0.03871864 0.03448871 0.03145331 0.02950849 0.02836739 0.027834529
## T2G 0.10395864 0.10305141 0.09971973 0.09336842 0.08542636 0.077132593
## T2T 1.00000000 1.00000000 1.00000000 1.00000000 1.00000000 1.000000000
##             32          33          34          35         36         37
## A2A 1.000000000 1.000000000 1.000000000 1.000000000 1.00000000 1.00000000
## A2C 0.017650548 0.019689613 0.022715122 0.027119487 0.03363081 0.04335691
## A2G 0.034472193 0.035034145 0.036992802 0.040518629 0.04567395 0.05326073
## A2T 0.009848846 0.010184047 0.011238850 0.013214146 0.01643595 0.02179530
## C2A 0.041867813 0.037967306 0.034404114 0.031181252 0.02826172 0.02562302
```

```
## C2C 1.000000000 1.000000000 1.000000000 1.000000000 1.00000000 1.00000000
## C2G 0.037615200 0.037121246 0.036744037 0.036256116 0.03568085 0.03512563
## C2T 0.027296947 0.027482939 0.027629866 0.027338888 0.02666183 0.02579762
## G2A 0.038341181 0.038568178 0.039881568 0.041833741 0.04454793 0.04853926
## G2C 0.011130164 0.010639856 0.010981027 0.012355925 0.01517366 0.02034350
## G2G 1.000000000 1.000000000 1.000000000 1.000000000 1.00000000 1.00000000
## G2T 0.006492139 0.006551975 0.007361658 0.009282854 0.01315093 0.02096178
## T2A 0.049905303 0.046821632 0.043539153 0.039913852 0.03609777 0.03226780
## T2C 0.027861080 0.028511918 0.029838760 0.031942757 0.03497874 0.03917461
## T2G 0.068695626 0.060256285 0.052487735 0.045782087 0.03994864 0.03470779
## T2T 1.000000000 1.000000000 1.000000000 1.000000000 1.00000000 1.00000000
##             38         39         40
## A2A 1.00000000 1.00000000 1.00000000
## A2C 0.05815939 0.05815939 0.05815939
## A2G 0.06458309 0.06458309 0.06458309
## A2T 0.03105399 0.03105399 0.03105399
## C2A 0.02327277 0.02327277 0.02327277
## C2C 1.00000000 1.00000000 1.00000000
## C2G 0.03450181 0.03450181 0.03450181
## C2T 0.02460082 0.02460082 0.02460082
## G2A 0.05397351 0.05397351 0.05397351
## G2C 0.03003667 0.03003667 0.03003667
## G2G 1.00000000 1.00000000 1.00000000
## G2T 0.03791607 0.03791607 0.03791607
## T2A 0.02840807 0.02840807 0.02840807
## T2C 0.04487186 0.04487186 0.04487186
## T2G 0.03010342 0.03010342 0.03010342
## T2T 1.00000000 1.00000000 1.00000000
##
## $err_in[[2]]
##             0         1         2         3         4         5         6
## A2A 0.2500000 0.2500000 0.2500000 0.2500000 0.2500000 0.2500000 0.2500000
## A2C 0.2500000 0.2500000 0.2500000 0.2500000 0.2500000 0.2500000 0.2500000
## A2G 0.2500000 0.2500000 0.2500000 0.2500000 0.2500000 0.2500000 0.2500000
## A2T 0.2500000 0.2500000 0.2500000 0.2500000 0.2500000 0.2500000 0.2500000
## C2A 0.2500000 0.2500000 0.2500000 0.2500000 0.2500000 0.2500000 0.2500000
## C2C 0.2500000 0.2500000 0.2500000 0.2500000 0.2500000 0.2500000 0.2500000
## C2G 0.2500000 0.2500000 0.2500000 0.2500000 0.2500000 0.2500000 0.2500000
## C2T 0.2500000 0.2500000 0.2500000 0.2500000 0.2500000 0.2500000 0.2500000
## G2A 0.2172217 0.2172217 0.2172217 0.2172217 0.2172217 0.2172217 0.2172217
## G2C 0.2500000 0.2500000 0.2500000 0.2500000 0.2500000 0.2500000 0.2500000
## G2G 0.2827783 0.2827783 0.2827783 0.2827783 0.2827783 0.2827783 0.2827783
## G2T 0.2500000 0.2500000 0.2500000 0.2500000 0.2500000 0.2500000 0.2500000
## T2A 0.1597072 0.1597072 0.1597072 0.1597072 0.1597072 0.1597072 0.1597072
## T2C 0.2500000 0.2500000 0.2500000 0.2500000 0.2500000 0.2500000 0.2500000
## T2G 0.1534459 0.1534459 0.1534459 0.1534459 0.1534459 0.1534459 0.1534459
## T2T 0.4368469 0.4368469 0.4368469 0.4368469 0.4368469 0.4368469 0.4368469
##             7         8         9         10         11         12         13
## A2A 0.2500000 0.4238171 0.5771115 0.73307086 0.81914411 0.86981488 0.90073845
## A2C 0.2500000 0.2500000 0.2126781 0.12424296 0.07876815 0.05347281 0.03877567
## A2G 0.2500000 0.1646717 0.1047849 0.07021144 0.04958966 0.03690101 0.02891041
## A2T 0.2500000 0.1615112 0.1054256 0.07247474 0.05249808 0.03981130 0.03157547
## C2A 0.2500000 0.2500000 0.2500000 0.25000000 0.25000000 0.25000000 0.23388666
## C2C 0.2500000 0.2500000 0.2613678 0.27867345 0.30220294 0.33466442 0.38444183
```

```
## C2G 0.2500000 0.2500000 0.2386322 0.22132655 0.20420476 0.18757115 0.17154303
## C2T 0.2500000 0.2500000 0.2500000 0.25000000 0.24359230 0.22776443 0.21012848
## G2A 0.2172217 0.2090823 0.2000710 0.18971455 0.17768999 0.16439293 0.15080484
## G2C 0.2500000 0.2500000 0.2500000 0.23187435 0.19753331 0.16708125 0.14103252
## G2G 0.2827783 0.3067289 0.3421243 0.39431898 0.46255253 0.52622466 0.58333069
## G2T 0.2500000 0.2341888 0.2078046 0.18409212 0.16222417 0.14230117 0.12483196
## T2A 0.1597072 0.1719132 0.1817951 0.18841468 0.19142475 0.19104401 0.18737306
## T2C 0.2500000 0.2500000 0.2500000 0.24718388 0.22708195 0.20665345 0.18646562
## T2G 0.1534459 0.1640485 0.1724013 0.17786287 0.18018388 0.17945754 0.17574274
## T2T 0.4368469 0.4140383 0.3958036 0.38653856 0.40130942 0.42284501 0.45041857
##             14         15         16         17         18         19
## A2A 0.92004032 0.93159225 0.93785839 0.94066630 0.94103306 0.93326091
## A2C 0.02996195 0.02489019 0.02226573 0.02116393 0.02109146 0.02446946
## A2G 0.02383108 0.02072508 0.01900824 0.01828722 0.01835605 0.02136448
## A2T 0.02616665 0.02279248 0.02086763 0.01988255 0.01951943 0.02090515
## C2A 0.21108434 0.18932654 0.16636331 0.14231300 0.12032544 0.10208952
## C2C 0.44096695 0.49556625 0.55156693 0.60869506 0.66183956 0.70823524
## C2G 0.15621621 0.14166550 0.12839434 0.11628798 0.10468316 0.09315753
## C2T 0.19173250 0.17344171 0.15367541 0.13270397 0.11315184 0.09651771
## G2A 0.13750522 0.12492655 0.11336571 0.10281111 0.09265322 0.08240224
## G2C 0.11926467 0.10143835 0.08711384 0.07631001 0.06786496 0.06015818
## G2G 0.63347452 0.67670242 0.71333863 0.74252473 0.76665536 0.78972565
## G2T 0.10975559 0.09693269 0.08618182 0.07835415 0.07282646 0.06771393
## T2A 0.18067817 0.17136237 0.15979284 0.14627534 0.13128701 0.11539157
## T2C 0.16697423 0.14852282 0.13070408 0.11354264 0.09771135 0.08359674
## T2G 0.16924579 0.16030162 0.14842534 0.13402541 0.11868960 0.10366259
## T2T 0.48310181 0.51981319 0.56107775 0.60615660 0.65231204 0.69734910
##             20         21         22         23         24         25
## A2A 0.91287511 0.88475578 0.86524354 0.87068471 0.88875669 0.90593384
## A2C 0.03331817 0.04545571 0.05304667 0.04667444 0.03371770 0.02344405
## A2G 0.02918325 0.04044439 0.04916104 0.04923403 0.04418103 0.03825505
## A2T 0.02462347 0.02934411 0.03254874 0.03340682 0.03334458 0.03236705
## C2A 0.09098694 0.08576965 0.08190470 0.07588717 0.06687636 0.05730153
## C2C 0.74092600 0.76179820 0.77969607 0.80127387 0.83154245 0.86412624
## C2G 0.08223177 0.07242063 0.06351713 0.05537709 0.04635862 0.03696313
## C2T 0.08585530 0.08001152 0.07488210 0.06746187 0.05522258 0.04160909
## G2A 0.07182432 0.06561720 0.06440194 0.06327819 0.05978106 0.05448790
## G2C 0.05218481 0.04799805 0.04804823 0.04739066 0.04432385 0.04028973
## G2G 0.81435848 0.82889803 0.83144195 0.83531346 0.84672598 0.86254247
## G2T 0.06163239 0.05748672 0.05610788 0.05401769 0.04916910 0.04267990
## T2A 0.09953912 0.08486748 0.07194875 0.06101639 0.05005374 0.03926253
## T2C 0.07223923 0.06335254 0.05565245 0.04833438 0.04044215 0.03270484
## T2G 0.09042688 0.07909232 0.06884768 0.05919969 0.04769987 0.03574492
## T2T 0.73779477 0.77268767 0.80355112 0.83144955 0.86180424 0.89228770
##             26         27         28         29          30          31
## A2A 0.91663588 0.92844990 0.94382886 0.95614463 0.963078047 0.966688866
## A2C 0.01839254 0.01561885 0.01275729 0.01047407 0.009033705 0.008187272
## A2G 0.03441762 0.03010689 0.02405980 0.01903302 0.016149578 0.014616279
## A2T 0.03055396 0.02582436 0.01935406 0.01434829 0.011738669 0.010507584
## C2A 0.04868435 0.04182945 0.03603724 0.03072458 0.026254120 0.022771835
## C2C 0.89140098 0.91076238 0.92383989 0.93359411 0.941126912 0.947294563
## C2G 0.02915681 0.02363408 0.01993782 0.01724575 0.015242831 0.013719625
## C2T 0.03075787 0.02377409 0.02018504 0.01843556 0.017376137 0.016213977
## G2A 0.04767485 0.03984351 0.02949322 0.01952314 0.013064370 0.009990515
```

```
## G2C 0.03540974 0.02993517 0.02157713 0.01318350 0.008069114 0.005846732
## G2G 0.88096522 0.90031949 0.92591942 0.95112612 0.967457383 0.975267059
## G2T 0.03595020 0.02990183 0.02301022 0.01616724 0.011409133 0.008895694
## T2A 0.03062088 0.02468882 0.02110347 0.01882223 0.017125609 0.015540777
## T2C 0.02635543 0.02182170 0.01868111 0.01626894 0.014366660 0.012822997
## T2G 0.02646226 0.02055753 0.01749282 0.01592051 0.014895447 0.013770210
## T2T 0.91656144 0.93293195 0.94272260 0.94898832 0.953612285 0.957866016
##             32          33          34          35          36          37
## A2A 0.969557329 0.971701136 0.973131499 0.973950053 0.974240586 0.973952916
## A2C 0.007579487 0.007175910 0.006956008 0.006904626 0.007017717 0.007310177
## A2G 0.013347530 0.012363034 0.011675646 0.011238466 0.011004168 0.010991977
## A2T 0.009515655 0.008759920 0.008236847 0.007906855 0.007737529 0.007744930
## C2A 0.020048243 0.017735615 0.015727433 0.013946370 0.012369855 0.010992780
## C2C 0.952376909 0.956460690 0.959918596 0.963021584 0.965754774 0.968028716
## C2G 0.012577198 0.011772077 0.011249914 0.010976696 0.010935812 0.011123969
## C2T 0.014997650 0.014031618 0.013104057 0.012055351 0.010939560 0.009854536
## G2A 0.008724614 0.008004144 0.007702336 0.007762427 0.008199060 0.009109310
## G2C 0.005008829 0.004548378 0.004400322 0.004558586 0.005059223 0.006021178
## G2G 0.978600831 0.980591609 0.981530159 0.981535037 0.980579668 0.978435255
## G2T 0.007665726 0.006855869 0.006367183 0.006143950 0.006162049 0.006434256
## T2A 0.014078046 0.012919034 0.011986859 0.011223754 0.010608466 0.010127458
## T2C 0.011569898 0.010583935 0.009825249 0.009264456 0.008872686 0.008625536
## T2G 0.012587391 0.011684281 0.011005217 0.010509564 0.010177984 0.009992499
## T2T 0.961764665 0.964812750 0.967182675 0.969002226 0.970340864 0.971254507
##             38          39          40
## A2A 0.972998873 0.972998873 0.972998873
## A2C 0.007811546 0.007811546 0.007811546
## A2G 0.011233297 0.011233297 0.011233297
## A2T 0.007956283 0.007956283 0.007956283
## C2A 0.009785578 0.009785578 0.009785578
## C2C 0.969900695 0.969900695 0.969900695
## C2G 0.011550738 0.011550738 0.011550738
## C2T 0.008762990 0.008762990 0.008762990
## G2A 0.010674557 0.010674557 0.010674557
## G2C 0.007730409 0.007730409 0.007730409
## G2G 0.974583032 0.974583032 0.974583032
## G2T 0.007012002 0.007012002 0.007012002
## T2A 0.009750853 0.009750853 0.009750853
## T2C 0.008512376 0.008512376 0.008512376
## T2G 0.009928765 0.009928765 0.009928765
## T2T 0.971808006 0.971808006 0.971808006
##
## $err_in[[3]]
##             0         1         2         3         4         5         6
## A2A 0.2500000 0.2500000 0.2500000 0.2500000 0.2500000 0.2500000 0.2500000
## A2C 0.2500000 0.2500000 0.2500000 0.2500000 0.2500000 0.2500000 0.2500000
## A2G 0.2500000 0.2500000 0.2500000 0.2500000 0.2500000 0.2500000 0.2500000
## A2T 0.2500000 0.2500000 0.2500000 0.2500000 0.2500000 0.2500000 0.2500000
## C2A 0.2500000 0.2500000 0.2500000 0.2500000 0.2500000 0.2500000 0.2500000
## C2C 0.2561050 0.2561050 0.2561050 0.2561050 0.2561050 0.2561050 0.2561050
## C2G 0.2500000 0.2500000 0.2500000 0.2500000 0.2500000 0.2500000 0.2500000
## C2T 0.2438950 0.2438950 0.2438950 0.2438950 0.2438950 0.2438950 0.2438950
## G2A 0.2083099 0.2083099 0.2083099 0.2083099 0.2083099 0.2083099 0.2083099
## G2C 0.2500000 0.2500000 0.2500000 0.2500000 0.2500000 0.2500000 0.2500000
```

```
## G2G 0.2916901 0.2916901 0.2916901 0.2916901 0.2916901 0.2916901 0.2916901
## G2T 0.2500000 0.2500000 0.2500000 0.2500000 0.2500000 0.2500000 0.2500000
## T2A 0.1589106 0.1589106 0.1589106 0.1589106 0.1589106 0.1589106 0.1589106
## T2C 0.2500000 0.2500000 0.2500000 0.2500000 0.2500000 0.2500000 0.2500000
## T2G 0.1529134 0.1529134 0.1529134 0.1529134 0.1529134 0.1529134 0.1529134
## T2T 0.4381760 0.4381760 0.4381760 0.4381760 0.4381760 0.4381760 0.4381760
##             7         8         9         10         11         12         13
## A2A 0.2500000 0.4266208 0.5759671 0.73251553 0.81886018 0.86965916 0.90066062
## A2C 0.2500000 0.2500000 0.2146027 0.12476231 0.07871678 0.05322343 0.03847414
## A2G 0.2500000 0.1624597 0.1042350 0.07030786 0.04990539 0.03725415 0.02922765
## A2T 0.2500000 0.1609195 0.1051951 0.07241430 0.05251765 0.03986326 0.03163758
## C2A 0.2500000 0.2500000 0.2500000 0.25000000 0.25000000 0.25000000 0.22688092
## C2C 0.2561050 0.2500000 0.2612100 0.27888420 0.30117066 0.32832119 0.38109956
## C2G 0.2500000 0.2500000 0.2387900 0.22111580 0.20376836 0.18703919 0.17102377
## C2T 0.2438950 0.2500000 0.2500000 0.25000000 0.24506097 0.23463962 0.22099575
## G2A 0.2083099 0.2048671 0.1996446 0.19214977 0.18205064 0.16974479 0.15634338
## G2C 0.2500000 0.2500000 0.2500000 0.22322279 0.19009098 0.16088506 0.13597296
## G2G 0.2916901 0.3106020 0.3423715 0.40050325 0.46573076 0.52727799 0.58315593
## G2T 0.2500000 0.2345309 0.2079838 0.18412418 0.16212762 0.14209216 0.12452773
## T2A 0.1589106 0.1712689 0.1813114 0.18812288 0.19135918 0.19120442 0.18772763
## T2C 0.2500000 0.2500000 0.2500000 0.24690303 0.22695003 0.20665743 0.18657491
## T2G 0.1529134 0.1636254 0.1720911 0.17768365 0.18015538 0.17957727 0.17598838
## T2T 0.4381760 0.4151058 0.3965974 0.38729044 0.40153541 0.42256087 0.44970909
##             14         15         16         17         18         19
## A2A 0.92002333 0.93165619 0.93802896 0.94095249 0.94143238 0.93377498
## A2C 0.02966501 0.02459404 0.02195746 0.02085519 0.02081805 0.02444585
## A2G 0.02408316 0.02089996 0.01909535 0.01826865 0.01820236 0.02087197
## A2T 0.02622851 0.02284982 0.02091824 0.01992367 0.01954720 0.02090720
## C2A 0.20280127 0.18047832 0.15805452 0.13536561 0.11472039 0.09734529
## C2C 0.43642350 0.49066961 0.54639114 0.60317079 0.65652158 0.70392994
## C2G 0.15579643 0.14141155 0.12841114 0.11665531 0.10538613 0.09410932
## C2T 0.20497880 0.18744052 0.16714319 0.14480829 0.12337190 0.10461545
## G2A 0.14263706 0.12925463 0.11665725 0.10489339 0.09332363 0.08150975
## G2C 0.11520500 0.09823616 0.08463540 0.07450846 0.06669912 0.05949614
## G2G 0.63278028 0.67600608 0.71298616 0.74269519 0.76757825 0.79172419
## G2T 0.10937766 0.09650313 0.08572118 0.07790296 0.07239900 0.06726992
## T2A 0.18116770 0.17190810 0.16029955 0.14668383 0.13159550 0.11563880
## T2C 0.16714806 0.14871672 0.13087361 0.11366921 0.09780433 0.08368043
## T2G 0.16957788 0.16066866 0.14876573 0.13430244 0.11890441 0.10384099
## T2T 0.48210636 0.51870652 0.56006110 0.60534452 0.65169577 0.69683978
##             20         21         22         23         24         25
## A2A 0.91349477 0.88543123 0.86606935 0.87162839 0.88967260 0.90683757
## A2C 0.03406419 0.04760965 0.05641212 0.05017163 0.03674352 0.02583253
## A2G 0.02786300 0.03772523 0.04513218 0.04494667 0.04033366 0.03499054
## A2T 0.02457804 0.02923389 0.03238635 0.03325331 0.03325023 0.03233936
## C2A 0.08674501 0.08192450 0.07831438 0.07236791 0.06321011 0.05343806
## C2C 0.73976508 0.76569145 0.78873356 0.81381852 0.84552466 0.87799885
## C2G 0.08353292 0.07418297 0.06561840 0.05755398 0.04812681 0.03807928
## C2T 0.08995700 0.07820107 0.06733366 0.05625959 0.04313842 0.03048381
## G2A 0.06933768 0.06123129 0.05782448 0.05504687 0.05140467 0.04718371
## G2C 0.05182087 0.04840237 0.04980223 0.05003891 0.04605346 0.03998214
## G2G 0.81774502 0.83346099 0.83678831 0.84133961 0.85344687 0.86959135
## G2T 0.06109642 0.05690534 0.05558498 0.05357461 0.04909500 0.04324281
## T2A 0.09956080 0.08450710 0.07128230 0.06023067 0.04934815 0.03871577
```

```
## T2C 0.07209719 0.06282229 0.05488173 0.04765166 0.04035531 0.03338432
## T2G 0.09049863 0.07897615 0.06856523 0.05883548 0.04747978 0.03575178
## T2T 0.73784337 0.77369446 0.80527073 0.83328219 0.86281676 0.89214813
##             26         27         28         29          30          31
## A2A 0.91763087 0.92900625 0.94357702 0.95548907 0.962525825 0.966487583
## A2C 0.02032564 0.01707391 0.01364559 0.01092210 0.009216339 0.008196302
## A2G 0.03148477 0.02778688 0.02269764 0.01835322 0.015711723 0.014180588
## A2T 0.03055872 0.02613295 0.02007975 0.01523561 0.012546114 0.011135528
## C2A 0.04475074 0.03799241 0.03262683 0.02797332 0.024109023 0.021030837
## C2C 0.90420021 0.92226777 0.93384861 0.94199746 0.948217613 0.953563198
## C2G 0.02976630 0.02400158 0.02046199 0.01814844 0.016446480 0.014955722
## C2T 0.02128275 0.01573824 0.01306257 0.01188078 0.011226884 0.010450244
## G2A 0.04192811 0.03552601 0.02709288 0.01892421 0.013347733 0.010480893
## G2C 0.03338712 0.02734394 0.01976990 0.01237851 0.007786880 0.005709526
## G2G 0.88766935 0.90599589 0.92913291 0.95192907 0.967161530 0.974808076
## G2T 0.03701541 0.03113417 0.02400431 0.01676821 0.011703856 0.009001505
## T2A 0.03020977 0.02435163 0.02086441 0.01872793 0.017149742 0.015602246
## T2C 0.02757002 0.02322831 0.02015679 0.01781533 0.015888395 0.014165296
## T2G 0.02657347 0.02062541 0.01728993 0.01530612 0.013914823 0.012632529
## T2T 0.91564674 0.93179465 0.94168887 0.94815062 0.953047040 0.957599929
##             32          33          34          35          36          37
## A2A 0.969732382 0.972303143 0.974239224 0.975649261 0.976623922 0.977158027
## A2C 0.007426716 0.006863488 0.006476077 0.006239554 0.006139630 0.006176664
## A2G 0.012884018 0.011833337 0.011032504 0.010438519 0.010008356 0.009746963
## A2T 0.009956884 0.009000032 0.008252195 0.007672666 0.007228092 0.006918346
## C2A 0.018571108 0.016490185 0.014674167 0.013042337 0.011581952 0.010297517
## C2C 0.958183281 0.961892826 0.964978906 0.967653443 0.969946748 0.971847578
## C2G 0.013652139 0.012656320 0.011902302 0.011341607 0.010952661 0.010721663
## C2T 0.009593472 0.008960669 0.008444624 0.007962614 0.007518639 0.007133241
## G2A 0.009155287 0.008313093 0.007822078 0.007603304 0.007640370 0.007967798
## G2C 0.004875833 0.004402926 0.004224050 0.004325770 0.004730791 0.005529619
## G2G 0.978340633 0.980603010 0.981895407 0.982371956 0.982066327 0.980863033
## G2T 0.007628246 0.006680971 0.006058465 0.005698970 0.005562512 0.005639549
## T2A 0.014116304 0.012919348 0.011929771 0.011086175 0.010371572 0.009776829
## T2C 0.012628911 0.011331305 0.010230074 0.009291239 0.008489685 0.007803632
## T2G 0.011455399 0.010577199 0.009948783 0.009536883 0.009317926 0.009271648
## T2T 0.961799385 0.965172148 0.967891372 0.970085703 0.971820818 0.973147892
##             38          39          40
## A2A 0.977228756 0.977228756 0.977228756
## A2C 0.006360115 0.006360115 0.006360115
## A2G 0.009664560 0.009664560 0.009664560
## A2T 0.006746568 0.006746568 0.006746568
## C2A 0.009159375 0.009159375 0.009159375
## C2C 0.973444858 0.973444858 0.973444858
## C2G 0.010627408 0.010627408 0.010627408
## C2T 0.006768359 0.006768359 0.006768359
## G2A 0.008637328 0.008637328 0.008637328
## G2C 0.006944613 0.006944613 0.006944613
## G2G 0.978461291 0.978461291 0.978461291
## G2T 0.005956768 0.005956768 0.005956768
## T2A 0.009269609 0.009269609 0.009269609
## T2C 0.007212840 0.007212840 0.007212840
## T2G 0.009387113 0.009387113 0.009387113
## T2T 0.974130438 0.974130438 0.974130438
```

```
##
##
## $trans
##     0 1 2 3 4 5 6 7 8  9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26  27
## A2A 0 0 0 0 0 0 0 1 6 21 16 15  1 11 17 91  1 93 96 14 15 44 16 16 34 34  6  63
## A2C 0 0 0 0 0 0 0 1 2  2  3  3  1  0  0  0  0  1  0  2  0  1  1  1  3  0  1   4
## A2G 0 0 0 0 0 0 0 0 0  0  1  1  0  0  0  1  0  0  0  1  0  0  0  3  1  3  0   2
## A2T 0 0 0 0 0 0 0 0 0  1  0  1  0  0  0  1  0  0  0  0  0  2  1  0  0  0  0   1
## C2A 0 0 0 0 0 0 0 0 1  1  0  3  0  1  0  0  0  3  1  0  2  0  0  1  3  0  0   8
## C2C 0 0 0 0 0 0 0 3 1  9  3  5  0  0  2  6  2  8  5 22  7 14 17 37 27 57 18  70
## C2G 0 0 0 0 0 0 0 0 0  1  0  1  0  0  0  0  0  0  0  0  1  0  2  0  1  3  1   0
## C2T 0 0 0 0 0 0 0 0 0  1  1  1  0  0  0  0  0  0  1  1  2  0  0  1  1  0  1   1
## G2A 0 0 0 0 0 0 0 0 0  0  1  1  0  0  0  0  0  1  0  1  0  1  2  0  0  1  0   2
## G2C 0 0 0 0 0 0 0 0 0  1  1  0  0  0  0  0  0  0  0  0  0  0  0  0  0  1  0   3
## G2G 0 0 0 0 0 0 0 3 0 10  7  2  2  0  2  4  1  3 13  9 35 18 23 28 25 39 32  67
## G2T 0 0 0 0 0 0 0 0 0  0  1  1  0  0  0  0  0  0  0  0  0  0  0  0  2  2  0   3
## T2A 0 0 0 0 0 0 0 0 0  0  1  2  0  0  0  0  1  0  1  1  0  1  0  2  0  5  1   1
## T2C 0 0 0 0 0 0 0 1 0  3  0  1  0  0  0  0  0  0  1  0  1  0  1  1  0  0  0   3
## T2G 0 0 0 0 0 0 0 0 0  0  0  2  0  0  0  0  0  0  0  0  3  1  1  1  0  1  0   2
## T2T 0 0 0 0 0 0 0 2 9  7  1  4  0  2  3  9  3  7  2 24 10 20 18 13 40 44 22 137
##     28 29  30  31  32  33   34   35   36   37    38 39 40
## A2A 50 51 271 169  98 249 1543  571 1139 3184 24976  0  0
## A2C  0  0   0   1   1   0    9    1    9   22   159  0  0
## A2G  1  1   4   1   0   1   13    9   13   28   247  0  0
## A2T  0  1   3   3   2   1    9    2    7   26   170  0  0
## C2A  2  4   3   3   6   4   16    8   16   62   371  0  0
## C2C 46 61 238 216 294 532  766 1151 1448 3983 40535  0  0
## C2G  1  2   2   3   2   2   16   14   14   46   439  0  0
## C2T  0  3   1   0   0   2   13    5   10   50   270  0  0
## G2A  2  2   8   2   1   1   10    9   12   49   268  0  0
## G2C  3  0   1   2   0   0    8    5    2   29   222  0  0
## G2G 34 77  89 104 145 289 2494  885 1474 3801 31722  0  0
## G2T  0  0   1   1   2   1   13    2    7   26   190  0  0
## T2A  0  3   1   3   1   1   22    6   10   45   288  0  0
## T2C  1  0   0   6   1   1   16    6    7   33   226  0  0
## T2G  2  1   0   4   0   1   15    6   17   29   298  0  0
## T2T 33 66 118 259 287 407  720  665 1327 3879 30767  0  0
```

```
errorR2s
```

```
## $err_out
##              0          1          2          3          4          5
## A2A 0.64180327 0.64180327 0.64180327 0.64180327 0.64180327 0.64180327
## A2C 0.25000000 0.25000000 0.25000000 0.25000000 0.25000000 0.25000000
## A2G 0.08219653 0.08219653 0.08219653 0.08219653 0.08219653 0.08219653
## A2T 0.02600020 0.02600020 0.02600020 0.02600020 0.02600020 0.02600020
## C2A 0.15027672 0.15027672 0.15027672 0.15027672 0.15027672 0.15027672
## C2C 0.68166968 0.68166968 0.68166968 0.68166968 0.68166968 0.68166968
## C2G 0.11358232 0.11358232 0.11358232 0.11358232 0.11358232 0.11358232
## C2T 0.05447128 0.05447128 0.05447128 0.05447128 0.05447128 0.05447128
## G2A 0.07690688 0.07690688 0.07690688 0.07690688 0.07690688 0.07690688
## G2C 0.04071330 0.04071330 0.04071330 0.04071330 0.04071330 0.04071330
## G2G 0.80482377 0.80482377 0.80482377 0.80482377 0.80482377 0.80482377
## G2T 0.07755605 0.07755605 0.07755605 0.07755605 0.07755605 0.07755605
```

```
## T2A 0.06414959 0.06414959 0.06414959 0.06414959 0.06414959 0.06414959
## T2C 0.10671283 0.10671283 0.10671283 0.10671283 0.10671283 0.10671283
## T2G 0.18340660 0.18340660 0.18340660 0.18340660 0.18340660 0.18340660
## T2T 0.64573098 0.64573098 0.64573098 0.64573098 0.64573098 0.64573098
##             6          7          8          9         10         11
## A2A 0.64180327 0.64180327 0.66750244 0.72996434 0.77886087 0.81791683
## A2C 0.25000000 0.25000000 0.23441715 0.18145360 0.14178758 0.11182694
## A2G 0.08219653 0.08219653 0.07434897 0.06695254 0.05979220 0.05277653
## A2T 0.02600020 0.02600020 0.02373144 0.02162952 0.01955935 0.01747969
## C2A 0.15027672 0.15027672 0.11575431 0.09019336 0.07095050 0.05637775
## C2C 0.68166968 0.68166968 0.74873957 0.79898836 0.83719414 0.86648100
## C2G 0.11358232 0.11358232 0.08664796 0.06699114 0.05262238 0.04206099
## C2T 0.05447128 0.05447128 0.04885815 0.04382714 0.03923297 0.03508027
## G2A 0.07690688 0.07690688 0.06553211 0.05645596 0.04944222 0.04411532
## G2C 0.04071330 0.04071330 0.03890016 0.03706713 0.03519819 0.03330858
## G2G 0.80482377 0.80482377 0.82822334 0.84773850 0.86388212 0.87719637
## G2T 0.07755605 0.07755605 0.06734439 0.05873841 0.05147747 0.04537973
## T2A 0.06414959 0.06414959 0.05590681 0.04886084 0.04281444 0.03763702
## T2C 0.10671283 0.10671283 0.09298317 0.08116291 0.07086072 0.06176681
## T2G 0.18340660 0.18340660 0.15225204 0.12631950 0.10459708 0.08639270
## T2T 0.64573098 0.64573098 0.69885799 0.74365675 0.78172776 0.81420347
##            12         13         14         15          16          17
## A2A 0.84920867 0.87418784 0.89400335 0.91015790 0.923700791 0.934932255
## A2C 0.08909890 0.07176374 0.05847007 0.04805007 0.039774997 0.033266199
## A2G 0.04617620 0.04027026 0.03520040 0.03080337 0.026841678 0.023273603
## A2T 0.01551623 0.01377816 0.01232618 0.01098866 0.009682534 0.008527943
## C2A 0.04533802 0.03690427 0.03040932 0.02537772 0.021427043 0.018264809
## C2C 0.88911058 0.90686587 0.92100153 0.93228843 0.941371161 0.948846128
## C2G 0.03415373 0.02811112 0.02340080 0.01974959 0.016941160 0.014738320
## C2T 0.03139767 0.02811874 0.02518835 0.02258426 0.020260637 0.018150743
## G2A 0.03994277 0.03655402 0.03367993 0.03150491 0.030083264 0.029080846
## G2C 0.03143268 0.02958546 0.02777980 0.02599819 0.024240471 0.022538362
## G2G 0.88838533 0.89801823 0.90651326 0.91376693 0.919764217 0.924888761
## G2T 0.04023922 0.03584230 0.03202702 0.02872998 0.025912048 0.023492030
## T2A 0.03321470 0.02942328 0.02616091 0.02341784 0.021101831 0.019048079
## T2C 0.05380886 0.04697761 0.04121507 0.03642434 0.032364486 0.028805050
## T2G 0.07127917 0.05883546 0.04865969 0.04040102 0.033612938 0.027907021
## T2T 0.84169726 0.86476365 0.88396434 0.89975680 0.912920746 0.924239850
##             18          19          20          21          22          23
## A2A 0.944145792 0.951436664 0.957017907 0.961299698 0.964699212 0.967002752
## A2C 0.028196442 0.024603331 0.022158733 0.020261300 0.018500393 0.016779395
## A2G 0.020065129 0.016945521 0.014061785 0.011771231 0.010205225 0.009525393
## A2T 0.007592637 0.007014484 0.006761574 0.006667771 0.006595170 0.006692460
## C2A 0.015685226 0.013996293 0.013132240 0.012564555 0.011888224 0.010924692
## C2C 0.955132683 0.959973098 0.963434432 0.966128344 0.968539051 0.971078522
## C2G 0.012975820 0.011433346 0.010046362 0.008884165 0.007979432 0.007232224
## C2T 0.016206271 0.014597263 0.013386966 0.012422937 0.011593293 0.010764562
## G2A 0.028224110 0.027180455 0.025856438 0.024360842 0.022791009 0.020528552
## G2C 0.020916374 0.019292982 0.017701974 0.016294617 0.015176006 0.014353786
## G2G 0.929455450 0.933750673 0.937886179 0.941866631 0.945695591 0.950384955
## G2T 0.021404065 0.019775890 0.018555409 0.017477911 0.016337394 0.014732707
## T2A 0.017140312 0.015608718 0.014480105 0.013446413 0.012281341 0.010643178
## T2C 0.025584271 0.022698955 0.020124424 0.017746235 0.015492965 0.013095304
## T2G 0.023025812 0.018780282 0.015246272 0.012495327 0.010485808 0.009044098
```

```
## T2T 0.934249605 0.942912045 0.950149198 0.956312025 0.961739886 0.967217420
##              24          25          26          27          28          29
## A2A 0.968206658 0.968916190 0.969760914 0.971295130 0.973051051 0.974402522
## A2C 0.015207124 0.013777432 0.012482883 0.010952100 0.009296071 0.008003055
## A2G 0.009504613 0.009717111 0.009755536 0.009532797 0.009291456 0.009102142
## A2T 0.007081605 0.007589266 0.008000668 0.008219973 0.008361422 0.008492282
## C2A 0.009904337 0.009036618 0.008464300 0.008252228 0.008261431 0.008314975
## C2C 0.973557834 0.975601324 0.976944377 0.977421628 0.977304792 0.976996319
## C2G 0.006569840 0.006033352 0.005649647 0.005439563 0.005353662 0.005318441
## C2T 0.009967989 0.009328706 0.008941675 0.008886581 0.009080115 0.009370265
## G2A 0.017659211 0.015052785 0.013191963 0.012033477 0.011222879 0.010612403
## G2C 0.013706750 0.013138335 0.012567904 0.011869435 0.011104568 0.010450912
## G2G 0.955802046 0.960656517 0.964248186 0.966765425 0.968741969 0.970262713
## G2T 0.012831993 0.011152362 0.009991947 0.009331664 0.008930584 0.008673972
## T2A 0.008828476 0.007365379 0.006493895 0.006117192 0.005948520 0.005862567
## T2C 0.010806438 0.009074125 0.008080748 0.007839260 0.008057732 0.008426785
## T2G 0.007959823 0.007131891 0.006490192 0.006065480 0.005833470 0.005682972
## T2T 0.972405263 0.976428606 0.978935166 0.979978068 0.980160278 0.980027675
##              30          31          32          33          34          35
## A2A 0.974958082 0.974937699 0.974751491 0.974349871 0.973669274 0.972699488
## A2C 0.007326579 0.007088531 0.006992571 0.007052148 0.007291017 0.007721496
## A2G 0.009030296 0.009052117 0.009113926 0.009236535 0.009442830 0.009733812
## A2T 0.008685043 0.008921652 0.009142012 0.009361446 0.009596879 0.009845204
## C2A 0.008237704 0.008056700 0.007902255 0.007758214 0.007609626 0.007461243
## C2C 0.976902927 0.976968125 0.976877993 0.976660010 0.976341350 0.975903423
## C2G 0.005265825 0.005196695 0.005163289 0.005176795 0.005249620 0.005383287
## C2T 0.009593545 0.009778480 0.010056464 0.010404981 0.010799404 0.011252048
## G2A 0.010089837 0.009676148 0.009419430 0.009274032 0.009201371 0.009206130
## G2C 0.010047641 0.009856850 0.009758794 0.009756284 0.009854829 0.010055450
## G2G 0.971394554 0.972131135 0.972487877 0.972538872 0.972345511 0.971891547
## G2T 0.008467968 0.008335867 0.008333900 0.008430812 0.008598289 0.008846873
## T2A 0.005749114 0.005615559 0.005546322 0.005553843 0.005653465 0.005849381
## T2C 0.008610327 0.008636101 0.008766544 0.008991030 0.009300765 0.009710870
## T2G 0.005520055 0.005352102 0.005241814 0.005187424 0.005188856 0.005246706
## T2T 0.980120505 0.980396238 0.980445320 0.980267703 0.979856915 0.979193043
##              36          37          38          39          40
## A2A 0.971436994 0.969793725 0.967644100 0.967644100 0.967644100
## A2C 0.008365502 0.009289385 0.010592870 0.010592870 0.010592870
## A2G 0.010101448 0.010559574 0.011125345 0.011125345 0.011125345
## A2T 0.010096056 0.010357315 0.010637685 0.010637685 0.010637685
## C2A 0.007320387 0.007177374 0.007023274 0.007023274 0.007023274
## C2C 0.975319698 0.974604909 0.973773680 0.973773680 0.973773680
## C2G 0.005570974 0.005815160 0.006119586 0.006119586 0.006119586
## C2T 0.011788941 0.012402557 0.013083460 0.013083460 0.013083460
## G2A 0.009311273 0.009510777 0.009800887 0.009800887 0.009800887
## G2C 0.010362629 0.010792603 0.011366903 0.011366903 0.011366903
## G2G 0.971119674 0.970016623 0.968559096 0.968559096 0.968559096
## G2T 0.009206424 0.009679997 0.010273114 0.010273114 0.010273114
## T2A 0.006136401 0.006521877 0.007016697 0.007016697 0.007016697
## T2C 0.010238692 0.010879446 0.011627179 0.011627179 0.011627179
## T2G 0.005360142 0.005529388 0.005756072 0.005756072 0.005756072
## T2T 0.978264765 0.977069288 0.975600053 0.975600053 0.975600053
##
## $err_in
```

```
## $err_in[[1]]
##              0          1          2          3          4          5
## A2A 1.00000000 1.00000000 1.00000000 1.00000000 1.00000000 1.00000000
## A2C 0.25000000 0.25000000 0.25000000 0.25000000 0.25000000 0.25000000
## A2G 0.13586498 0.13586498 0.13586498 0.13586498 0.13586498 0.13586498
## A2T 0.03004597 0.03004597 0.03004597 0.03004597 0.03004597 0.03004597
## C2A 0.20396659 0.20396659 0.20396659 0.20396659 0.20396659 0.20396659
## C2C 1.00000000 1.00000000 1.00000000 1.00000000 1.00000000 1.00000000
## C2G 0.10318139 0.10318139 0.10318139 0.10318139 0.10318139 0.10318139
## C2T 0.09076914 0.09076914 0.09076914 0.09076914 0.09076914 0.09076914
## G2A 0.10658505 0.10658505 0.10658505 0.10658505 0.10658505 0.10658505
## G2C 0.06119095 0.06119095 0.06119095 0.06119095 0.06119095 0.06119095
## G2G 1.00000000 1.00000000 1.00000000 1.00000000 1.00000000 1.00000000
## G2T 0.16511202 0.16511202 0.16511202 0.16511202 0.16511202 0.16511202
## T2A 0.08370374 0.08370374 0.08370374 0.08370374 0.08370374 0.08370374
## T2C 0.19495397 0.19495397 0.19495397 0.19495397 0.19495397 0.19495397
## T2G 0.17510818 0.17510818 0.17510818 0.17510818 0.17510818 0.17510818
## T2T 1.00000000 1.00000000 1.00000000 1.00000000 1.00000000 1.00000000
##              6          7          8          9         10         11
## A2A 1.00000000 1.00000000 1.00000000 1.00000000 1.00000000 1.00000000
## A2C 0.25000000 0.25000000 0.21753218 0.18109099 0.15238730 0.12979385
## A2G 0.13586498 0.13586498 0.11578703 0.09956305 0.08609525 0.07467360
## A2T 0.03004597 0.03004597 0.02958434 0.02915734 0.02873404 0.02829320
## C2A 0.20396659 0.20396659 0.16581262 0.13696644 0.11482376 0.09774731
## C2C 1.00000000 1.00000000 1.00000000 1.00000000 1.00000000 1.00000000
## C2G 0.10318139 0.10318139 0.08912447 0.07785127 0.06868527 0.06116554
## C2T 0.09076914 0.09076914 0.09194498 0.09257891 0.09274678 0.09258122
## G2A 0.10658505 0.10658505 0.09466784 0.08466954 0.07658156 0.07024025
## G2C 0.06119095 0.06119095 0.06590848 0.07014161 0.07375390 0.07661330
## G2G 1.00000000 1.00000000 1.00000000 1.00000000 1.00000000 1.00000000
## G2T 0.16511202 0.16511202 0.14128422 0.12165969 0.10549482 0.09223800
## T2A 0.08370374 0.08370374 0.10099490 0.11904613 0.13740195 0.15541290
## T2C 0.19495397 0.19495397 0.16927052 0.14824327 0.13083327 0.11624730
## T2G 0.17510818 0.17510818 0.14818286 0.12643433 0.10858025 0.09374588
## T2T 1.00000000 1.00000000 1.00000000 1.00000000 1.00000000 1.00000000
##             12         13         14         15         16         17
## A2A 1.00000000 1.00000000 1.00000000 1.00000000 1.00000000 1.00000000
## A2C 0.11185487 0.09737386 0.08548851 0.07575587 0.06784655 0.06135149
## A2G 0.06513622 0.05740473 0.05135047 0.04652941 0.04250152 0.03915309
## A2T 0.02785519 0.02745133 0.02711117 0.02668598 0.02612956 0.02561552
## C2A 0.08461159 0.07447884 0.06667147 0.06082343 0.05652192 0.05329806
## C2C 1.00000000 1.00000000 1.00000000 1.00000000 1.00000000 1.00000000
## C2G 0.05503429 0.05009360 0.04618408 0.04311666 0.04068743 0.03876892
## C2T 0.09204853 0.09098847 0.08925594 0.08708461 0.08469430 0.08195117
## G2A 0.06514714 0.06085428 0.05701803 0.05372903 0.05115082 0.04913749
## G2C 0.07861398 0.07969182 0.07981563 0.07886045 0.07683136 0.07393986
## G2G 1.00000000 1.00000000 1.00000000 1.00000000 1.00000000 1.00000000
## G2T 0.08130066 0.07212728 0.06430461 0.05765281 0.05205072 0.04729515
## T2A 0.17196423 0.18585534 0.19589658 0.20201321 0.20426102 0.20189293
## T2C 0.10407939 0.09408160 0.08602877 0.07994016 0.07537861 0.07161900
## T2G 0.08149089 0.07147913 0.06340459 0.05694483 0.05165218 0.04715885
## T2T 1.00000000 1.00000000 1.00000000 1.00000000 1.00000000 1.00000000
##             18         19         20         21         22         23
## A2A 1.00000000 1.00000000 1.00000000 1.00000000 1.00000000 1.00000000
```

```
## A2C 0.05596176 0.05138240 0.04751937 0.04440442 0.04205784 0.04109893
## A2G 0.03639177 0.03472019 0.03424782 0.03442242 0.03474511 0.03627292
## A2T 0.02530485 0.02573997 0.02698202 0.02843724 0.02939895 0.02948065
## C2A 0.05080282 0.05132229 0.05567338 0.06136619 0.06503798 0.06487030
## C2C 1.00000000 1.00000000 1.00000000 1.00000000 1.00000000 1.00000000
## C2G 0.03726157 0.03716417 0.03878332 0.04104200 0.04268552 0.04390963
## C2T 0.07874515 0.07421541 0.06846897 0.06268211 0.05772505 0.05247027
## G2A 0.04757295 0.04572833 0.04332618 0.04088657 0.03883305 0.03653787
## G2C 0.07040971 0.06433804 0.05610691 0.04858228 0.04345626 0.04163465
## G2G 1.00000000 1.00000000 1.00000000 1.00000000 1.00000000 1.00000000
## G2T 0.04322610 0.04011415 0.03788983 0.03602722 0.03410603 0.03130890
## T2A 0.19447496 0.17589945 0.14791012 0.12008139 0.09774843 0.07742250
## T2C 0.06808331 0.06528198 0.06350947 0.06204086 0.06023002 0.05859748
## T2G 0.04319339 0.03961076 0.03657759 0.03428591 0.03288571 0.03345360
## T2T 1.00000000 1.00000000 1.00000000 1.00000000 1.00000000 1.00000000
##             24         25         26         27         28         29
## A2A 1.00000000 1.00000000 1.00000000 1.00000000 1.00000000 1.00000000
## A2C 0.04130550 0.04150926 0.04055162 0.03678657 0.03145540 0.02700314
## A2G 0.03965838 0.04363024 0.04640630 0.04621412 0.04400709 0.04169298
## A2T 0.02901719 0.02831427 0.02766341 0.02636615 0.02431030 0.02248862
## C2A 0.06269402 0.05993606 0.05786451 0.05636584 0.05452934 0.05230041
## C2C 1.00000000 1.00000000 1.00000000 1.00000000 1.00000000 1.00000000
## C2G 0.04549844 0.04685485 0.04731484 0.04480773 0.03984016 0.03514574
## C2T 0.04667591 0.04184476 0.03893107 0.03867440 0.04035062 0.04256061
## G2A 0.03373765 0.03118956 0.02945233 0.02912753 0.02990650 0.03091858
## G2C 0.04209646 0.04329038 0.04363745 0.04235275 0.04028399 0.03817181
## G2G 1.00000000 1.00000000 1.00000000 1.00000000 1.00000000 1.00000000
## G2T 0.02786873 0.02474786 0.02255734 0.02160712 0.02150083 0.02169518
## T2A 0.05833320 0.04390045 0.03465291 0.02829290 0.02313349 0.01934897
## T2C 0.05770006 0.05704341 0.05616514 0.05422212 0.05140438 0.04870939
## T2G 0.03619699 0.03996996 0.04321798 0.04743676 0.05443074 0.06202290
## T2T 1.00000000 1.00000000 1.00000000 1.00000000 1.00000000 1.00000000
##             30         31         32         33         34         35
## A2A 1.00000000 1.00000000 1.00000000 1.00000000 1.00000000 1.00000000
## A2C 0.02478762 0.02408801 0.02364509 0.02362764 0.02422167 0.02543005
## A2G 0.04089198 0.04130290 0.04168162 0.04211679 0.04270095 0.04341157
## A2T 0.02164664 0.02154342 0.02157183 0.02184236 0.02247730 0.02348283
## C2A 0.04964675 0.04669824 0.04360298 0.04038231 0.03706629 0.03372733
## C2C 1.00000000 1.00000000 1.00000000 1.00000000 1.00000000 1.00000000
## C2G 0.03250717 0.03118136 0.02982383 0.02880636 0.02845596 0.02867514
## C2T 0.04368457 0.04392808 0.04451399 0.04518501 0.04567085 0.04603169
## G2A 0.03121617 0.03094903 0.03080485 0.03060578 0.03017921 0.02956826
## G2C 0.03663050 0.03540484 0.03407960 0.03292033 0.03215860 0.03172518
## G2G 1.00000000 1.00000000 1.00000000 1.00000000 1.00000000 1.00000000
## G2T 0.02166810 0.02156570 0.02175202 0.02203376 0.02221213 0.02232041
## T2A 0.01691043 0.01538163 0.01435524 0.01382658 0.01382456 0.01433486
## T2C 0.04695575 0.04593192 0.04502016 0.04432889 0.04396175 0.04388859
## T2G 0.06667121 0.06830526 0.06921644 0.06861066 0.06579446 0.06116774
## T2T 1.00000000 1.00000000 1.00000000 1.00000000 1.00000000 1.00000000
##             36         37         38         39         40
## A2A 1.00000000 1.00000000 1.00000000 1.00000000 1.00000000
## A2C 0.02720640 0.02974346 0.03332147 0.03332147 0.03332147
## A2G 0.04421075 0.04517641 0.04639420 0.04639420 0.04639420
## A2T 0.02482806 0.02661685 0.02898858 0.02898858 0.02898858
```

```
## C2A 0.03043833 0.02723506 0.02415109 0.02415109 0.02415109
## C2C 1.00000000 1.00000000 1.00000000 1.00000000 1.00000000
## C2G 0.02922996 0.03025723 0.03192977 0.03192977 0.03192977
## C2T 0.04643021 0.04673732 0.04682090 0.04682090 0.04682090
## G2A 0.02888505 0.02807316 0.02708452 0.02708452 0.02708452
## G2C 0.03143482 0.03133040 0.03145703 0.03145703 0.03145703
## G2G 1.00000000 1.00000000 1.00000000 1.00000000 1.00000000
## G2T 0.02249719 0.02269539 0.02286649 0.02286649 0.02286649
## T2A 0.01535130 0.01699764 0.01948078 0.01948078 0.01948078
## T2C 0.04403289 0.04443162 0.04512710 0.04512710 0.04512710
## T2G 0.05553220 0.04906768 0.04205457 0.04205457 0.04205457
## T2T 1.00000000 1.00000000 1.00000000 1.00000000 1.00000000
##
## $err_in[[2]]
##              0          1          2          3          4          5
## A2A 0.63898174 0.63898174 0.63898174 0.63898174 0.63898174 0.63898174
## A2C 0.25000000 0.25000000 0.25000000 0.25000000 0.25000000 0.25000000
## A2G 0.08473162 0.08473162 0.08473162 0.08473162 0.08473162 0.08473162
## A2T 0.02628664 0.02628664 0.02628664 0.02628664 0.02628664 0.02628664
## C2A 0.13151420 0.13151420 0.13151420 0.13151420 0.13151420 0.13151420
## C2C 0.70009584 0.70009584 0.70009584 0.70009584 0.70009584 0.70009584
## C2G 0.11548325 0.11548325 0.11548325 0.11548325 0.11548325 0.11548325
## C2T 0.05290671 0.05290671 0.05290671 0.05290671 0.05290671 0.05290671
## G2A 0.07667658 0.07667658 0.07667658 0.07667658 0.07667658 0.07667658
## G2C 0.04485045 0.04485045 0.04485045 0.04485045 0.04485045 0.04485045
## G2G 0.80070561 0.80070561 0.80070561 0.80070561 0.80070561 0.80070561
## G2T 0.07776735 0.07776735 0.07776735 0.07776735 0.07776735 0.07776735
## T2A 0.06443980 0.06443980 0.06443980 0.06443980 0.06443980 0.06443980
## T2C 0.10862583 0.10862583 0.10862583 0.10862583 0.10862583 0.10862583
## T2G 0.18272652 0.18272652 0.18272652 0.18272652 0.18272652 0.18272652
## T2T 0.64420785 0.64420785 0.64420785 0.64420785 0.64420785 0.64420785
##              6          7          8          9         10         11
## A2A 0.63898174 0.63898174 0.66824591 0.73136380 0.78057957 0.81970316
## A2C 0.25000000 0.25000000 0.23225900 0.17964225 0.14033147 0.11070875
## A2G 0.08473162 0.08473162 0.07570823 0.06746944 0.05973958 0.05237814
## A2T 0.02628664 0.02628664 0.02378687 0.02152451 0.01934938 0.01720995
## C2A 0.13151420 0.13151420 0.10672676 0.08720776 0.07167508 0.05930471
## C2C 0.70009584 0.70009584 0.75746894 0.80123949 0.83514425 0.86166107
## C2G 0.11548325 0.11548325 0.08679518 0.06628483 0.05158485 0.04098325
## C2T 0.05290671 0.05290671 0.04900912 0.04526791 0.04159583 0.03805097
## G2A 0.07667658 0.07667658 0.06475277 0.05545457 0.04844141 0.04327641
## G2C 0.04485045 0.04485045 0.04209219 0.03948662 0.03704550 0.03477867
## G2G 0.80070561 0.80070561 0.82615875 0.84704338 0.86400976 0.87770236
## G2T 0.07776735 0.07776735 0.06699629 0.05801542 0.05050333 0.04424257
## T2A 0.06443980 0.06443980 0.05590810 0.04867463 0.04250186 0.03723409
## T2C 0.10862583 0.10862583 0.09337478 0.08055668 0.06960984 0.06011798
## T2G 0.18272652 0.18272652 0.15154630 0.12563803 0.10396211 0.08581022
## T2T 0.64420785 0.64420785 0.69917082 0.74513066 0.78392620 0.81683770
##             12         13         14         15          16          17
## A2A 0.85088580 0.87564644 0.89518394 0.91099677 0.924161230 0.935034045
## A2C 0.08828250 0.07120251 0.05811739 0.04788439 0.039777065 0.033401424
## A2G 0.04560752 0.03965811 0.03462958 0.03034233 0.026536101 0.023135156
## A2T 0.01522418 0.01349295 0.01206909 0.01077650 0.009525604 0.008429375
## C2A 0.04945495 0.04153865 0.03511876 0.02986812 0.025547233 0.021973134
```

```
## C2C 0.88263622 0.89953673 0.91338318 0.92474710 0.934114931 0.941995584
## C2G 0.03318009 0.02730354 0.02277792 0.01933591 0.016753573 0.014764817
## C2T 0.03472873 0.03162108 0.02872014 0.02604887 0.023584263 0.021266465
## G2A 0.03937534 0.03632432 0.03382452 0.03212181 0.031308278 0.030995299
## G2C 0.03266457 0.03066974 0.02876704 0.02699157 0.025355369 0.023809465
## G2G 0.88894946 0.89842634 0.90663166 0.91336222 0.918568959 0.922779099
## G2T 0.03901063 0.03457960 0.03077679 0.02752440 0.024767394 0.022416137
## T2A 0.03275570 0.02894471 0.02569867 0.02298395 0.020694063 0.018673654
## T2C 0.05196506 0.04510502 0.03944370 0.03483519 0.030994411 0.027671893
## T2G 0.07075479 0.05837652 0.04827238 0.04007842 0.033343336 0.027685325
## T2T 0.84452444 0.86757375 0.88658525 0.90210244 0.914968190 0.925969128
##             18          19          20          21          22          23
## A2A 0.94393842 0.950817380 0.955903194 0.959798176 0.963059377 0.965560644
## A2C 0.02842715 0.024968724 0.022696577 0.020921350 0.019162348 0.017236272
## A2G 0.02008240 0.017138503 0.014427782 0.012256887 0.010749769 0.010105649
## A2T 0.00755203 0.007075393 0.006972447 0.007023587 0.007028506 0.007097435
## C2A 0.01900189 0.016859125 0.015458107 0.014363664 0.013263751 0.011919233
## C2C 0.94875377 0.954218494 0.958450342 0.961927320 0.965002313 0.968179301
## C2G 0.01318900 0.011869143 0.010731849 0.009764686 0.008954707 0.008170768
## C2T 0.01905534 0.017053237 0.015359702 0.013944331 0.012779230 0.011730699
## G2A 0.03084584 0.030632922 0.030237197 0.029505809 0.028309882 0.025733889
## G2C 0.02231497 0.020698346 0.019007285 0.017483115 0.016296749 0.015412122
## G2G 0.92644017 0.929786003 0.932935372 0.936109888 0.939513350 0.944504998
## G2T 0.02039902 0.018882728 0.017820146 0.016901188 0.015880018 0.014348991
## T2A 0.01681243 0.015378307 0.014401765 0.013534654 0.012511166 0.011015097
## T2C 0.02469167 0.022198482 0.020148556 0.018224285 0.016213658 0.013833286
## T2G 0.02285009 0.018612525 0.015069975 0.012342647 0.010406185 0.009133422
## T2T 0.93564581 0.943810686 0.950379704 0.955898414 0.960868991 0.966018195
##             24          25          26          27          28          29
## A2A 0.967132698 0.968179682 0.969179412 0.970756741 0.972583453 0.973992964
## A2C 0.015346930 0.013650877 0.012241593 0.010729943 0.009130837 0.007894900
## A2G 0.010131079 0.010393625 0.010471082 0.010262503 0.010020047 0.009829348
## A2T 0.007389293 0.007775816 0.008107913 0.008250814 0.008265663 0.008282788
## C2A 0.010514613 0.009335686 0.008553662 0.008211306 0.008131119 0.008131007
## C2C 0.971313460 0.973889230 0.975573737 0.976253271 0.976272031 0.976025567
## C2G 0.007390776 0.006729705 0.006263886 0.005940874 0.005675531 0.005476926
## C2T 0.010781151 0.010045379 0.009608715 0.009594548 0.009921319 0.010366501
## G2A 0.022076017 0.018685027 0.016313017 0.014862972 0.013826994 0.013058558
## G2C 0.014658750 0.013977212 0.013318317 0.012555478 0.011731875 0.011025182
## G2G 0.950758009 0.956449119 0.960565560 0.963346288 0.965510040 0.967152958
## G2T 0.012507224 0.010888642 0.009803107 0.009235262 0.008931090 0.008763302
## T2A 0.009330735 0.007942187 0.007094485 0.006713357 0.006528203 0.006418230
## T2C 0.011467135 0.009656730 0.008637960 0.008446617 0.008765508 0.009247524
## T2G 0.008269495 0.007624068 0.007065056 0.006596843 0.006258022 0.006005573
## T2T 0.970932635 0.974777015 0.977202500 0.978243183 0.978448267 0.978328674
##             30          31          32          33          34          35
## A2A 0.974535233 0.974431383 0.974126783 0.973548922 0.972605326 0.971272611
## A2C 0.007258963 0.007054558 0.007001643 0.007118239 0.007435244 0.007973087
## A2G 0.009770166 0.009818236 0.009909508 0.010064511 0.010305998 0.010635585
## A2T 0.008435638 0.008695824 0.008962066 0.009268328 0.009653433 0.010118717
## C2A 0.008038451 0.007877797 0.007775771 0.007719044 0.007695558 0.007708938
## C2C 0.975928346 0.975941899 0.975748813 0.975365133 0.974804887 0.974044708
## C2G 0.005353847 0.005286790 0.005269792 0.005333531 0.005513160 0.005815336
## C2T 0.010679357 0.010893514 0.011205624 0.011582292 0.011986395 0.012431018
```

```
## G2A 0.012448087 0.012034711 0.011853683 0.011821157 0.011862062 0.011990167
## G2C 0.010573581 0.010334688 0.010189767 0.010147390 0.010218783 0.010403399
## G2G 0.968354955 0.969094160 0.969380920 0.969316452 0.968988086 0.968370827
## G2T 0.008623377 0.008536442 0.008575631 0.008715001 0.008931070 0.009235608
## T2A 0.006276782 0.006110866 0.006001891 0.005961753 0.006004006 0.006129544
## T2C 0.009500785 0.009557157 0.009721663 0.009972708 0.010288797 0.010685050
## T2G 0.005805293 0.005645937 0.005543230 0.005508267 0.005553960 0.005680282
## T2T 0.978417140 0.978686039 0.978733216 0.978557272 0.978153236 0.977505124
##              36          37          38          39          40
## A2A 0.969541355 0.967291108 0.964348983 0.964348983 0.964348983
## A2C 0.008763640 0.009891588 0.011485972 0.011485972 0.011485972
## A2G 0.011046547 0.011553781 0.012175668 0.012175668 0.012175668
## A2T 0.010648458 0.011263523 0.011989378 0.011989378 0.011989378
## C2A 0.007764321 0.007852818 0.007965611 0.007965611 0.007965611
## C2C 0.973057435 0.971843615 0.970396932 0.970396932 0.970396932
## C2G 0.006231911 0.006788498 0.007520834 0.007520834 0.007520834
## C2T 0.012946332 0.013515070 0.014116623 0.014116623 0.014116623
## G2A 0.012261183 0.012667612 0.013204610 0.013204610 0.013204610
## G2C 0.010700088 0.011125244 0.011700811 0.011700811 0.011700811
## G2G 0.967381417 0.966006775 0.964223212 0.964223212 0.964223212
## G2T 0.009657313 0.010200369 0.010871367 0.010871367 0.010871367
## T2A 0.006329295 0.006605811 0.006963766 0.006963766 0.006963766
## T2C 0.011183675 0.011771660 0.012433266 0.012433266 0.012433266
## T2G 0.005881334 0.006166016 0.006546968 0.006546968 0.006546968
## T2T 0.976605696 0.975456513 0.974056000 0.974056000 0.974056000
##
## $err_in[[3]]
##              0          1          2          3          4          5
## A2A 0.64180327 0.64180327 0.64180327 0.64180327 0.64180327 0.64180327
## A2C 0.25000000 0.25000000 0.25000000 0.25000000 0.25000000 0.25000000
## A2G 0.08219653 0.08219653 0.08219653 0.08219653 0.08219653 0.08219653
## A2T 0.02600020 0.02600020 0.02600020 0.02600020 0.02600020 0.02600020
## C2A 0.15027672 0.15027672 0.15027672 0.15027672 0.15027672 0.15027672
## C2C 0.68166968 0.68166968 0.68166968 0.68166968 0.68166968 0.68166968
## C2G 0.11358232 0.11358232 0.11358232 0.11358232 0.11358232 0.11358232
## C2T 0.05447128 0.05447128 0.05447128 0.05447128 0.05447128 0.05447128
## G2A 0.07690688 0.07690688 0.07690688 0.07690688 0.07690688 0.07690688
## G2C 0.04071330 0.04071330 0.04071330 0.04071330 0.04071330 0.04071330
## G2G 0.80482377 0.80482377 0.80482377 0.80482377 0.80482377 0.80482377
## G2T 0.07755605 0.07755605 0.07755605 0.07755605 0.07755605 0.07755605
## T2A 0.06414959 0.06414959 0.06414959 0.06414959 0.06414959 0.06414959
## T2C 0.10671283 0.10671283 0.10671283 0.10671283 0.10671283 0.10671283
## T2G 0.18340660 0.18340660 0.18340660 0.18340660 0.18340660 0.18340660
## T2T 0.64573098 0.64573098 0.64573098 0.64573098 0.64573098 0.64573098
##              6          7          8          9         10         11
## A2A 0.64180327 0.64180327 0.66750244 0.72996434 0.77886087 0.81791683
## A2C 0.25000000 0.25000000 0.23441715 0.18145360 0.14178758 0.11182694
## A2G 0.08219653 0.08219653 0.07434897 0.06695254 0.05979220 0.05277653
## A2T 0.02600020 0.02600020 0.02373144 0.02162952 0.01955935 0.01747969
## C2A 0.15027672 0.15027672 0.11575431 0.09019336 0.07095050 0.05637775
## C2C 0.68166968 0.68166968 0.74873957 0.79898836 0.83719414 0.86648100
## C2G 0.11358232 0.11358232 0.08664796 0.06699114 0.05262238 0.04206099
## C2T 0.05447128 0.05447128 0.04885815 0.04382714 0.03923297 0.03508027
## G2A 0.07690688 0.07690688 0.06553211 0.05645596 0.04944222 0.04411532
```

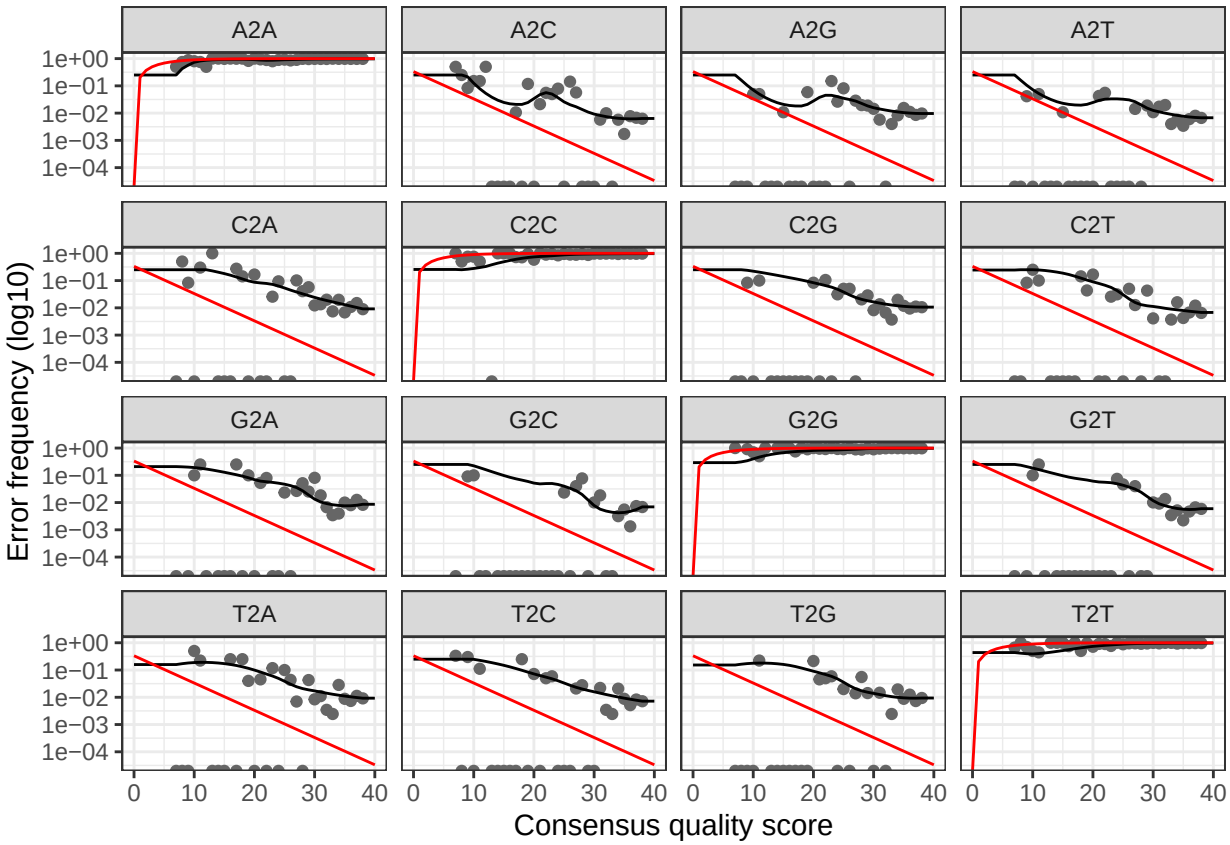
```
## G2C 0.04071330 0.04071330 0.03890016 0.03706713 0.03519819 0.03330858
## G2G 0.80482377 0.80482377 0.82822334 0.84773850 0.86388212 0.87719637
## G2T 0.07755605 0.07755605 0.06734439 0.05873841 0.05147747 0.04537973
## T2A 0.06414959 0.06414959 0.05590681 0.04886084 0.04281444 0.03763702
## T2C 0.10671283 0.10671283 0.09298317 0.08116291 0.07086072 0.06176681
## T2G 0.18340660 0.18340660 0.15225204 0.12631950 0.10459708 0.08639270
## T2T 0.64573098 0.64573098 0.69885799 0.74365675 0.78172776 0.81420347
##             12         13         14         15          16          17
## A2A 0.84920867 0.87418784 0.89400335 0.91015790 0.923700791 0.934932255
## A2C 0.08909890 0.07176374 0.05847007 0.04805007 0.039774997 0.033266199
## A2G 0.04617620 0.04027026 0.03520040 0.03080337 0.026841678 0.023273603
## A2T 0.01551623 0.01377816 0.01232618 0.01098866 0.009682534 0.008527943
## C2A 0.04533802 0.03690427 0.03040932 0.02537772 0.021427043 0.018264809
## C2C 0.88911058 0.90686587 0.92100153 0.93228843 0.941371161 0.948846128
## C2G 0.03415373 0.02811112 0.02340080 0.01974959 0.016941160 0.014738320
## C2T 0.03139767 0.02811874 0.02518835 0.02258426 0.020260637 0.018150743
## G2A 0.03994277 0.03655402 0.03367993 0.03150491 0.030083264 0.029080846
## G2C 0.03143268 0.02958546 0.02777980 0.02599819 0.024240471 0.022538362
## G2G 0.88838533 0.89801823 0.90651326 0.91376693 0.919764217 0.924888761
## G2T 0.04023922 0.03584230 0.03202702 0.02872998 0.025912048 0.023492030
## T2A 0.03321470 0.02942328 0.02616091 0.02341784 0.021101831 0.019048079
## T2C 0.05380886 0.04697761 0.04121507 0.03642434 0.032364486 0.028805050
## T2G 0.07127917 0.05883546 0.04865969 0.04040102 0.033612938 0.027907021
## T2T 0.84169726 0.86476365 0.88396434 0.89975680 0.912920746 0.924239850
##             18          19          20          21          22          23
## A2A 0.944145792 0.951436664 0.957017907 0.961299698 0.964699212 0.967002752
## A2C 0.028196442 0.024603331 0.022158733 0.020261300 0.018500393 0.016779395
## A2G 0.020065129 0.016945521 0.014061785 0.011771231 0.010205225 0.009525393
## A2T 0.007592637 0.007014484 0.006761574 0.006667771 0.006595170 0.006692460
## C2A 0.015685226 0.013996293 0.013132240 0.012564555 0.011888224 0.010924692
## C2C 0.955132683 0.959973098 0.963434432 0.966128344 0.968539051 0.971078522
## C2G 0.012975820 0.011433346 0.010046362 0.008884165 0.007979432 0.007232224
## C2T 0.016206271 0.014597263 0.013386966 0.012422937 0.011593293 0.010764562
## G2A 0.028224110 0.027180455 0.025856438 0.024360842 0.022791009 0.020528552
## G2C 0.020916374 0.019292982 0.017701974 0.016294617 0.015176006 0.014353786
## G2G 0.929455450 0.933750673 0.937886179 0.941866631 0.945695591 0.950384955
## G2T 0.021404065 0.019775890 0.018555409 0.017477911 0.016337394 0.014732707
## T2A 0.017140312 0.015608718 0.014480105 0.013446413 0.012281341 0.010643178
## T2C 0.025584271 0.022698955 0.020124424 0.017746235 0.015492965 0.013095304
## T2G 0.023025812 0.018780282 0.015246272 0.012495327 0.010485808 0.009044098
## T2T 0.934249605 0.942912045 0.950149198 0.956312025 0.961739886 0.967217420
##             24          25          26          27          28          29
## A2A 0.968206658 0.968916190 0.969760914 0.971295130 0.973051051 0.974402522
## A2C 0.015207124 0.013777432 0.012482883 0.010952100 0.009296071 0.008003055
## A2G 0.009504613 0.009717111 0.009755536 0.009532797 0.009291456 0.009102142
## A2T 0.007081605 0.007589266 0.008000668 0.008219973 0.008361422 0.008492282
## C2A 0.009904337 0.009036618 0.008464300 0.008252228 0.008261431 0.008314975
## C2C 0.973557834 0.975601324 0.976944377 0.977421628 0.977304792 0.976996319
## C2G 0.006569840 0.006033352 0.005649647 0.005439563 0.005353662 0.005318441
## C2T 0.009967989 0.009328706 0.008941675 0.008886581 0.009080115 0.009370265
## G2A 0.017659211 0.015052785 0.013191963 0.012033477 0.011222879 0.010612403
## G2C 0.013706750 0.013138335 0.012567904 0.011869435 0.011104568 0.010450912
## G2G 0.955802046 0.960656517 0.964248186 0.966765425 0.968741969 0.970262713
## G2T 0.012831993 0.011152362 0.009991947 0.009331664 0.008930584 0.008673972
```

```
## T2A 0.008828476 0.007365379 0.006493895 0.006117192 0.005948520 0.005862567
## T2C 0.010806438 0.009074125 0.008080748 0.007839260 0.008057732 0.008426785
## T2G 0.007959823 0.007131891 0.006490192 0.006065480 0.005833470 0.005682972
## T2T 0.972405263 0.976428606 0.978935166 0.979978068 0.980160278 0.980027675
##              30          31          32          33          34          35
## A2A 0.974958082 0.974937699 0.974751491 0.974349871 0.973669274 0.972699488
## A2C 0.007326579 0.007088531 0.006992571 0.007052148 0.007291017 0.007721496
## A2G 0.009030296 0.009052117 0.009113926 0.009236535 0.009442830 0.009733812
## A2T 0.008685043 0.008921652 0.009142012 0.009361446 0.009596879 0.009845204
## C2A 0.008237704 0.008056700 0.007902255 0.007758214 0.007609626 0.007461243
## C2C 0.976902927 0.976968125 0.976877993 0.976660010 0.976341350 0.975903423
## C2G 0.005265825 0.005196695 0.005163289 0.005176795 0.005249620 0.005383287
## C2T 0.009593545 0.009778480 0.010056464 0.010404981 0.010799404 0.011252048
## G2A 0.010089837 0.009676148 0.009419430 0.009274032 0.009201371 0.009206130
## G2C 0.010047641 0.009856850 0.009758794 0.009756284 0.009854829 0.010055450
## G2G 0.971394554 0.972131135 0.972487877 0.972538872 0.972345511 0.971891547
## G2T 0.008467968 0.008335867 0.008333900 0.008430812 0.008598289 0.008846873
## T2A 0.005749114 0.005615559 0.005546322 0.005553843 0.005653465 0.005849381
## T2C 0.008610327 0.008636101 0.008766544 0.008991030 0.009300765 0.009710870
## T2G 0.005520055 0.005352102 0.005241814 0.005187424 0.005188856 0.005246706
## T2T 0.980120505 0.980396238 0.980445320 0.980267703 0.979856915 0.979193043
##              36          37          38          39          40
## A2A 0.971436994 0.969793725 0.967644100 0.967644100 0.967644100
## A2C 0.008365502 0.009289385 0.010592870 0.010592870 0.010592870
## A2G 0.010101448 0.010559574 0.011125345 0.011125345 0.011125345
## A2T 0.010096056 0.010357315 0.010637685 0.010637685 0.010637685
## C2A 0.007320387 0.007177374 0.007023274 0.007023274 0.007023274
## C2C 0.975319698 0.974604909 0.973773680 0.973773680 0.973773680
## C2G 0.005570974 0.005815160 0.006119586 0.006119586 0.006119586
## C2T 0.011788941 0.012402557 0.013083460 0.013083460 0.013083460
## G2A 0.009311273 0.009510777 0.009800887 0.009800887 0.009800887
## G2C 0.010362629 0.010792603 0.011366903 0.011366903 0.011366903
## G2G 0.971119674 0.970016623 0.968559096 0.968559096 0.968559096
## G2T 0.009206424 0.009679997 0.010273114 0.010273114 0.010273114
## T2A 0.006136401 0.006521877 0.007016697 0.007016697 0.007016697
## T2C 0.010238692 0.010879446 0.011627179 0.011627179 0.011627179
## T2G 0.005360142 0.005529388 0.005756072 0.005756072 0.005756072
## T2T 0.978264765 0.977069288 0.975600053 0.975600053 0.975600053
##
##
## $trans
##     0 1 2 3 4 5 6  7   8  9 10  11 12 13 14  15 16  17  18  19  20  21  22  23
## A2A 0 0 0 0 0 0 0 87 103 75 38  92 43 43 32 124 84 161 167 229 411 262 292 299
## A2C 0 0 0 0 0 0 0 47  18 14  9  37  2  1  9   4  2   3   6  14   4   3   4   6
## A2G 0 0 0 0 0 0 0 22   5  2  0   4  1 16  0  10  4   4   6   1   3   2   2   4
## A2T 0 0 0 0 0 0 0  2   2  0  0   4  0  1  0   1  0   2   1   2   0   0   1   3
## C2A 0 0 0 0 0 0 0  8   9  4  3   7  1  1  0   2  0   2   2   0   1   1   2   3
## C2C 0 0 0 0 0 0 0 85  59 37 11  43 17 39 17  92 87 111 125 173 160 154 323 289
## C2G 0 0 0 0 0 0 0 12   6  1  0   2  0  0  0   1  0   0   0   1   1   2   3   0
## C2T 0 0 0 0 0 0 0  1   8  2  0   3  2  0  0   2  2   0   0   2   2   0   3   2
## G2A 0 0 0 0 0 0 0 12   2  2  1   1  2  1  0   0  0   1   5   2   5   4   3   4
## G2C 0 0 0 0 0 0 0  3   1  1  2   2  1  0  1   0  1   4   0   4   0   7   1   4
## G2G 0 0 0 0 0 0 0 97  46 33 14 108 34 29 25  65 89  76 108 145 142 155 247 163
## G2T 0 0 0 0 0 0 0  5   3  7  2   4  0  1  0   1  0   2   2   3   0   1   4   5
```

```
## T2A 0 0 0 0 0 0 0  4   1  1  0   3  0  0  0   0  0   0   1   2   0   1   3   0
## T2C 0 0 0 0 0 0 0 11   4  1  0   0  0  0  0   2  3   1   2   4   2   0   4   3
## T2G 0 0 0 0 0 0 0 13   6  2  8   3  2  0  1   1  2   1   2   1   1   2   1   0
## T2T 0 0 0 0 0 0 0 55  46 37 11  32 21 11 12  47 48  77  62 188 123 233 139 111
##      24  25  26  27  28  29  30  31  32   33   34   35   36   37    38 39 40
## A2A 403 450 228 498 210 587 704 496 561 1121 2155 1110 1786 5252 22697  0  0
## A2C   8   4   2   6   1   8   3   9   3    1   15    6   25   54   242  0  0
## A2G   2   5   2   1   2   4  11   4   5   13   15    9   18   50   267  0  0
## A2T   2   1   2   7   2   3   5   3   5    7   21   10   18   55   249  0  0
## C2A   4   3   1   8   2   2   6   2   7    2   22    9   10   47   176  0  0
## C2C 275 417 284 641 494 780 724 605 527  555 1592 1123 2211 5886 24859  0  0
## C2G   1   3   1   2   0   3   4   3   2    2   17    4    8   27   163  0  0
## C2T   5   6   0   6   1   5   6   6   4    9   23    9   22   83   327  0  0
## G2A   8   7   4   6   1   9   7   4   5    4   26   10   22   73   263  0  0
## G2C   4   5   1   7   1  11  13   4   4    6   26    7   17   86   311  0  0
## G2G 300 496 193 708 269 852 735 707 676  989 1743 1447 1950 6502 27095  0  0
## G2T   5   4   1   8   0   6   8   3   2    3   34   11   14   83   273  0  0
## T2A   3   2   1   2   0   1   0   3   5    4   11    6    4   28   133  0  0
## T2C   2   3   1   3   1   1   4   2   2    9   24    8   12   46   217  0  0
## T2G   2   1   0   1   0   3   3   3   0    1   13    3   11   18   110  0  0
## T2T 207 286 157 391 337 741 352 297 466  665 1390  741 1670 4668 18053  0  0
```

```
# Plotting error rates to investigate success of error estimation. The black
# points should adhere to the black line and trend downwards with increasing
# quality score.

plotErrors(errorR1s, nominalQ = TRUE)
```

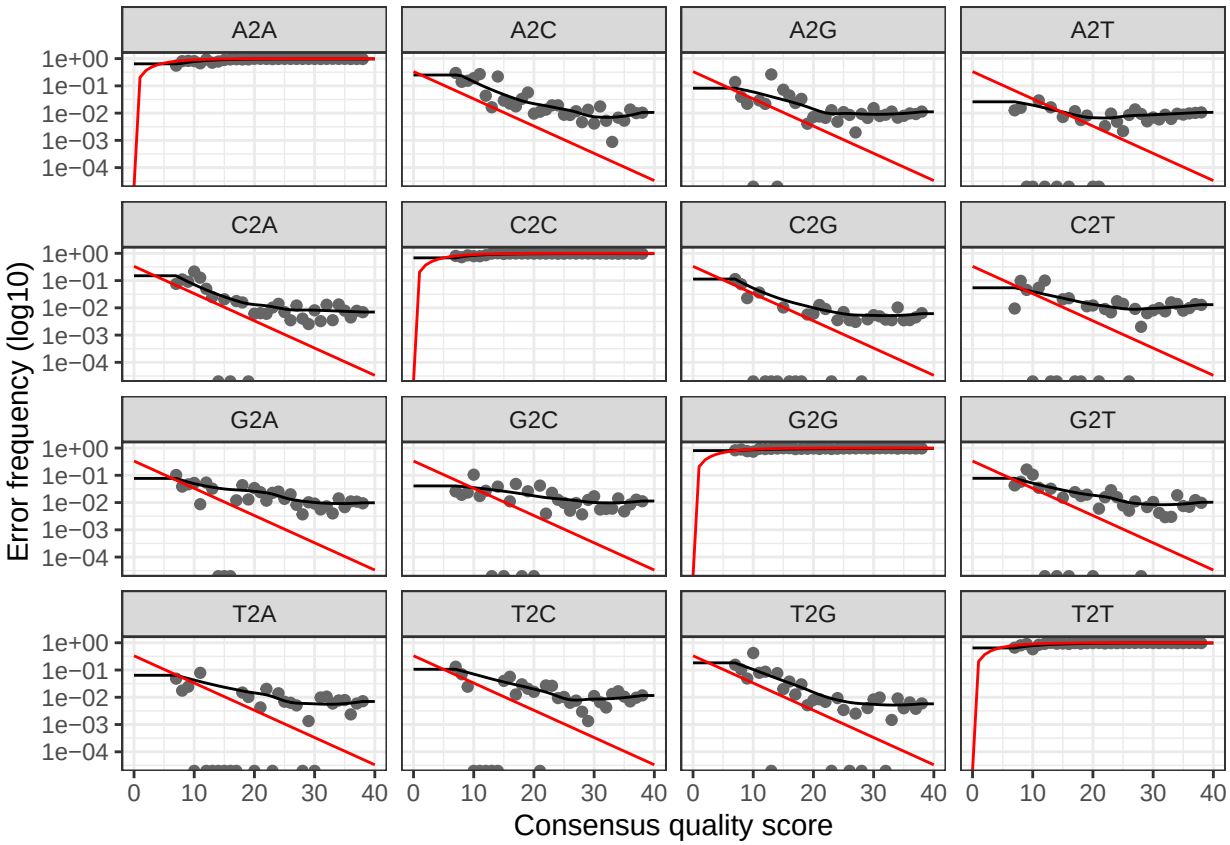
```
## Warning: Transformation introduced infinite values in continuous y-axis

## Warning: Transformation introduced infinite values in continuous y-axis
```




```
plotErrors(errorR2s, nominalQ = TRUE)
```

```
## Warning: Transformation introduced infinite values in continuous y-axis
```

```
## Warning: Transformation introduced infinite values in continuous y-axis
```



Main Software Tools Description

DADA2 is a pipeline for denoising and deduplicating paired-end reads sequenced on an Illumina system (Callahan et al. 2016). This pipeline is highly regarded in the field of microbiome research and is capable of fine-grain resolution of taxa versus pipelines which rely on clustering methods Clustering pipelines generate operational taxonomic units (OTUs) often based on 97% sequence similarity, rather than ASVs, which retain the complexity of the sample by requiring 100% sequence similarity. I chose to investigate this pipeline using online tutorials for taxonomic assignment and assessment of performance with reference to a mock community to go beyond the DADA2 vignette.

Code Section 2 - Main Analysis

```
# running DADA2

dadaR1s <- dada(derepR1s, err = errorR1s, multithread = FALSE)
```

```
## Sample 1 - 88 reads in 61 unique sequences.
## Sample 2 - 512 reads in 151 unique sequences.
```

```
dadaR2s <- dada(derepR2s, err = errorR2s, multithread = FALSE)
```

```
## Sample 1 - 88 reads in 72 unique sequences.
## Sample 2 - 512 reads in 256 unique sequences.
```

```
print(dadaR1s)
```

```
## $sra_data1
## dada-class: object describing DADA2 denoising results
## 3 sequence variants were inferred from 61 input unique sequences.
## Key parameters: OMEGA_A = 1e-40, OMEGA_C = 1e-40, BAND_SIZE = 16
##
## $sra_data9
## dada-class: object describing DADA2 denoising results
## 24 sequence variants were inferred from 151 input unique sequences.
## Key parameters: OMEGA_A = 1e-40, OMEGA_C = 1e-40, BAND_SIZE = 16
```

```
print(dadaR2s)
```

```
## $sra_data1
## dada-class: object describing DADA2 denoising results
## 3 sequence variants were inferred from 72 input unique sequences.
## Key parameters: OMEGA_A = 1e-40, OMEGA_C = 1e-40, BAND_SIZE = 16
##
## $sra_data9
## dada-class: object describing DADA2 denoising results
## 20 sequence variants were inferred from 256 input unique sequences.
## Key parameters: OMEGA_A = 1e-40, OMEGA_C = 1e-40, BAND_SIZE = 16
```

```
# merging paired reads

merged <- mergePairs(dadaR1s, derepR1s, dadaR2s, derepR2s, verbose = TRUE)
```

```
## 51 paired-reads (in 3 unique pairings) successfully merged out of 51 (in 3 pairings) input.
```

```
## 206 paired-reads (in 13 unique pairings) successfully merged out of 489 (in 25 pairings) input.
```

```
# creating sequence table to compare ESV counts across samples

seqtab <- makeSequenceTable(merged)

seqtab.nochim <- removeBimeraDenovo(seqtab, verbose = TRUE)
```

```
## Identified 0 bimeras out of 16 input sequences.
```

```
class(seqtab.nochim)
```

```
## [1] "matrix" "array"
```

```
dim(seqtab.nochim)
```

```
## [1]  2 16
```

```
# View(seqtab.nochim)

# track number of reads at each step of the pipeline

getN <- function(x) sum(getUniques(x))
track <- cbind(out, sapply(dadaR1s, getN), sapply(dadaR2s, getN), sapply(merged,
    getN), rowSums(seqtab.nochim))
colnames(track) <- c("input", "filtered", "denoisedR1", "denoisedR2", "merged", "nonchim")
rownames(track) <- sample.names
track
```

```
##           input filtered denoisedR1 denoisedR2 merged nonchim
## sra_data1   235       88         51         53     51      51
## sra_data9 20841      512        501        496    206     206
```

```
# evaluating accuracy of DADA2 for mock community

mock <- seqtab.nochim["sra_data1", ]

mock <- sort(mock[mock > 0], decreasing = TRUE)

cat("DADA2 inferred", length(mock), "sample sequences present in the V8-V9 Mock community.\n")
```

```
## DADA2 inferred 3 sample sequences present in the V8-V9 Mock community.
```

```
# comparing ESVs to fastas provided by Zymo

path <- "./ZymoFASTAs"
mock.ref <- getSequences(file.path(path, "ZymoMockCommunity.fasta"))
match.ref <- sum(sapply(names(mock), function(x) any(grepl(x, mock.ref))))
cat("Of those,", sum(match.ref), "were exact matches to the expected reference sequences.\n")
```

```
## Of those, 0 were exact matches to the expected reference sequences.
```

```
# assigning taxonomy

# convert ESVs to DNAStringSet

dna <- DNAStringSet(getSequences(mock))
dna
```

```
## DNAStringSet object of length 3:
##     width seq
## [1]   371 AGGAGGTGATCCAACCGCAGGTTCCCCTACGGCT...AGGACTTGACGTCATCCCCACCTTCCTCCAGTT
## [2]   145 GCACAAGCAGTGGAGCATGTGGTTTAATTCGAAG...CAGGTGGTGCATGGTCGTCGTCAGCTCGTGTTG
## [3]   371 AGGAGGTGATCCAACCGCAGGTTCCCCTACGGTT...ATGACTTGACGTCATCCCCACCTTCCTCCAGTT
```

```
names(dna) <- 1:length(dna)
dna
```

```
## DNAStringSet object of length 3:
##     width seq                                                 names
## [1]   371 AGGAGGTGATCCAACCGCAGGTT...GTCATCCCCACCTTCCTCCAGTT 1
## [2]   145 GCACAAGCAGTGGAGCATGTGGT...ATGGTCGTCGTCAGCTCGTGTTG 2
## [3]   371 AGGAGGTGATCCAACCGCAGGTT...GTCATCCCCACCTTCCTCCAGTT 3
```

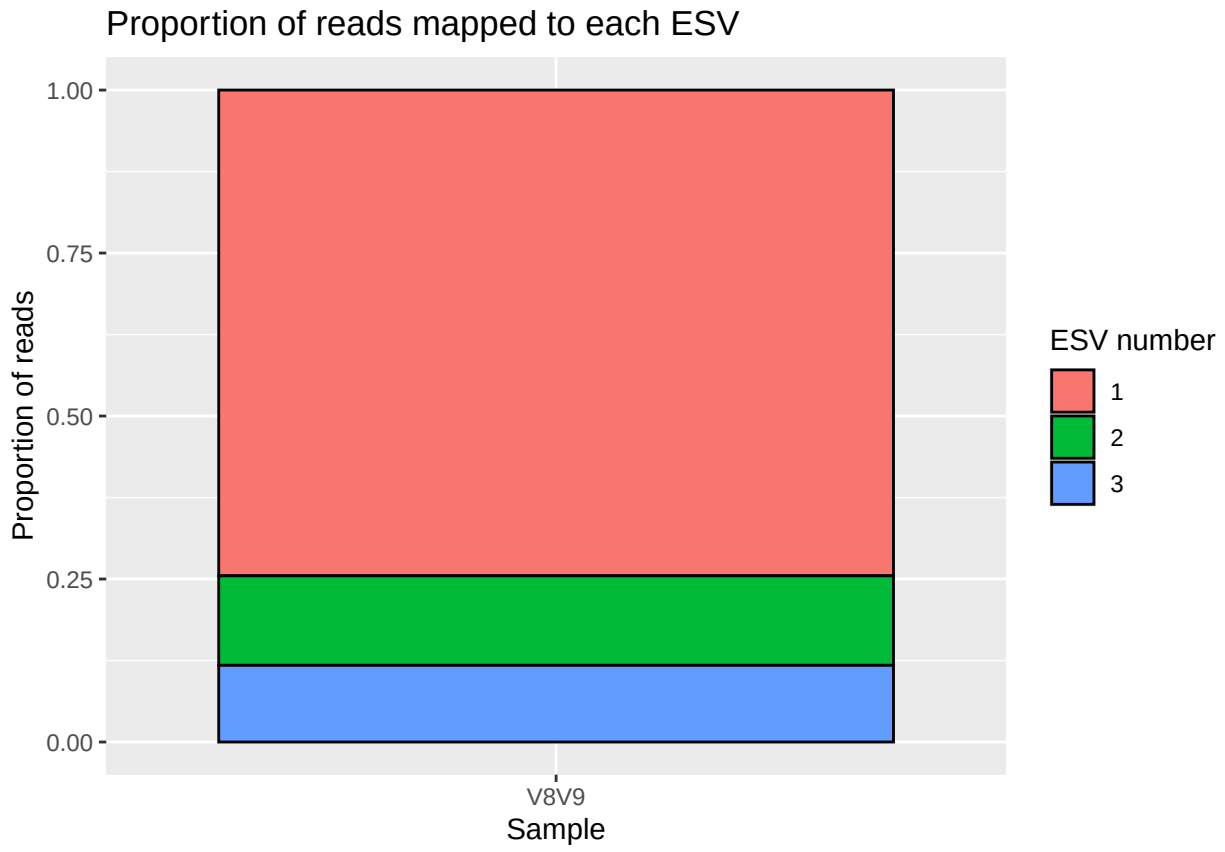
```
# loading training dataset

load("./SILVA_SSU_r138_2019.RData")

# assigning taxonomic ids to each ESV

ids <- IdTaxa(dna, trainingSet, strand = "both", processors = NULL, verbose = FALSE)

# specifying which taxonomic ranks to display

ranks <- c("domain", "phylum", "class", "order", "family", "genus", "species")  # ranks of interest

# converting the output to matrix which displays the id at each of the
# specified ranks (set as column names) for each ESV (set as row names)

taxid <- t(sapply(ids, function(x) {
    m <- match(ranks, x$rank)
    taxa <- x$taxon[m]
    taxa[startsWith(taxa, "unclassified_")] <- NA
    taxa
}))

colnames(taxid) <- ranks
rownames(taxid) <- getSequences(mock)

View(taxid)

taxid <- cbind(names(dna), taxid)

View(taxid)

# viewing the proportion of recovered reads for each member of the mock
# community

# calculating proportion of reads mapped to each ESV (i.e. member of the mock
# community)

no.reads <- unname(mock)
no.reads
```

```
## [1] 38  7  6
```

```
prop.reads <- no.reads/(sum(no.reads))
prop.reads
```

```
## [1] 0.7450980 0.1372549 0.1176471
```

```
# creating proportion table

table <- as.data.frame(taxid[, 1])
View(table)

prop.table <- cbind(table, prop.reads)
names(prop.table) <- c("ESV_number", "Proportion_of_reads")
View(prop.table)

# plotting proportion table as stacked bar plot

# creating a dummy column for sample id

Sample_ID <- "V8V9"

prop.table.plot <- cbind(prop.table, Sample_ID)
View(prop.table.plot)

ggplot(data = prop.table.plot, aes(x = Sample_ID, y = Proportion_of_reads, fill = ESV_number)) +
    geom_col() + geom_bar(position = "stack", stat = "identity", colour = "black") +
    xlab("Sample") + ylab("Proportion of reads") + guides(fill = guide_legend(title = "ESV number")) +
    ggtitle("Proportion of reads mapped to each ESV")
```



Results and Discussion

In this investigation, DADA2 was not able to recover all members of the ZymoBiomics Microbial Community Standard. A maximum of four ASVs were recovered under both sets of parameters tested here. This outcome does not align with the known mock community (see https://files.zymoresearch.com/protocols/_d6300_zymobiomics_microbial_community_standard.pdf).

This result may be expected given that Winand et al. (2020) recovered the least number of ASVs from the V8-V9 regions. The next step may involve an investigation of the V4-V6 regions which were reportedly more successful in recovering a higher number of ASVs (Winand et al. 2020).

References

https://bioconductor.org/packages/devel/bioc/vignettes/dada2/inst/doc/dada2-intro.html

https://benjjneb.github.io/dada2/tutorial.html

https://www.hadriengourle.com/tutorials/16S/

https://web.stanford.edu/class/bios221/Pune/Lectures/Lecture_Day1_dada2_workflow.pdf

Callahan, B., McMurdie, P. & Holmes, S. Exact sequence variants should replace operational taxonomic units in marker-gene data analysis. ISME J 11, 2639–2643 (2017). https://doi.org/10.1038/ismej.2017.119

Callahan, B., McMurdie, P., Rosen, M. et al. DADA2: High-resolution sample inference from Illumina amplicon data. Nat Methods 13, 581–583 (2016). https://doi.org/10.1038/nmeth.3869

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Winand R, Bogaerts B, Hoffman S, Lefevre L, Delvoye M, Van Braekel J, Fu Q, Roosens NH, De Keersmaecker SC, Vanneste K. Targeting the 16S rRNA Gene for Bacterial Identification in Complex Mixed Samples: Comparative Evaluation of Second (Illumina) and Third (Oxford Nanopore Technologies) Generation Sequencing Technologies. International Journal of Molecular Sciences. 2020; 21(1):298. https://doi.org/10.3390/ijms21010298