

551-1119-00L Microbial Community Genomics

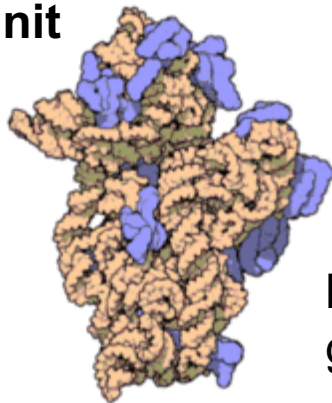
16S rRNA amplicon data analysis using DADA2

The 16S rRNA gene

- Part of prokaryotic ribosomes

50S large subunit (33 proteins)	<u>5S</u> : 120 nt <u>23S</u> : 2906 nt
30S small subunit (22 proteins)	<u>16S</u>: 1542 nt

- 16S rRNA present in all prokaryotes
- 30S small subunit

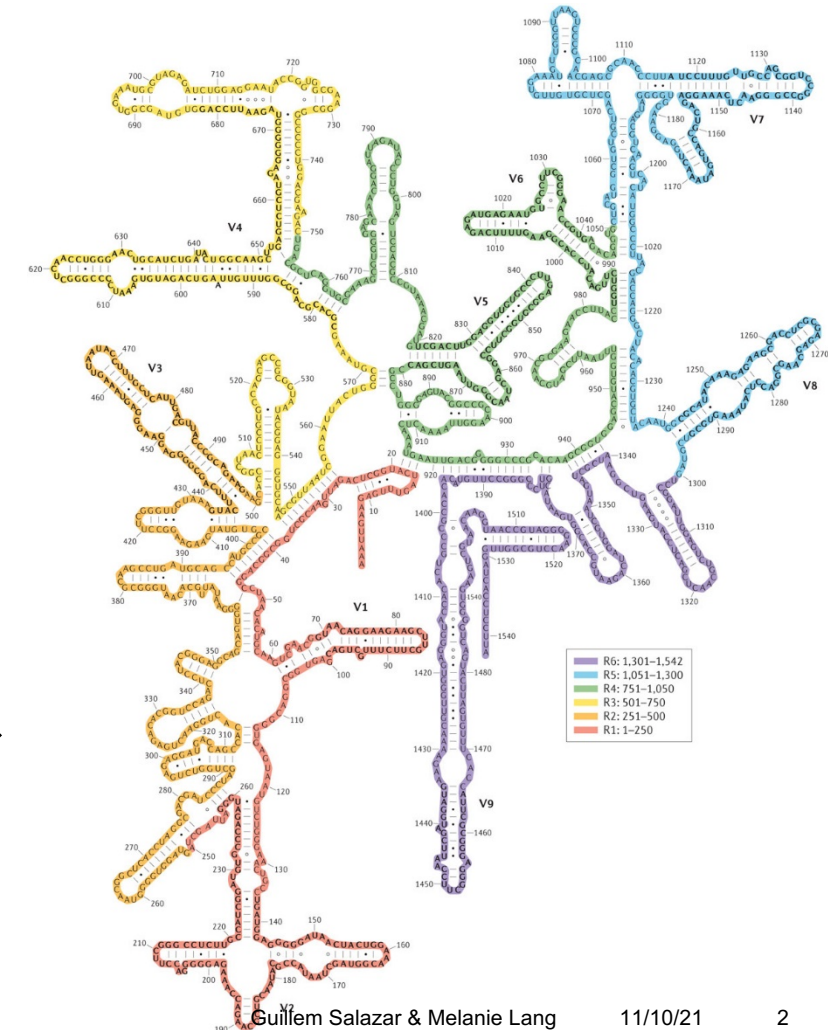


blue: ribosomal proteins
gold: 16S rRNA

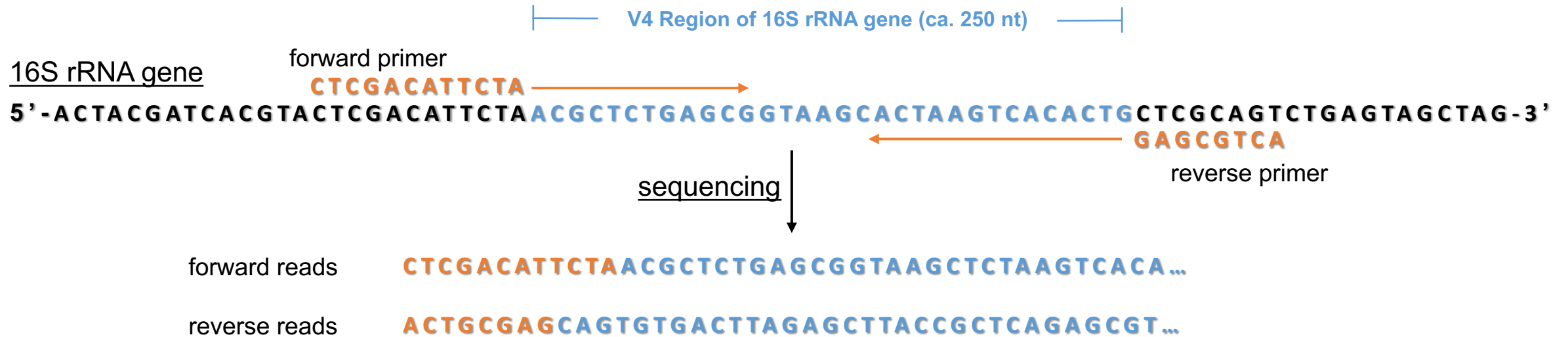
- conserved regions and variable regions



Secondary structure of 16S rRNA



Generation of 16S rRNA gene PCR amplicons



- PCR products (amplicons) are used for sequencing
- The sequencing output are forward reads and reverse reads
- Sequencing reads are saved as plain (or compressed) text files in the fastq format
 - see: https://en.wikipedia.org/wiki/FASTQ_format

Quality control reads: primer match filtering

primer match

CTCGACATTCTA

CTCGACATTCTAACGCTCTGAGCGGTAAGCACTAAGTCACACTG

primer mismatch

GATCGTCG

GAGCGTCA

discard read

primer match

CTCGACATTCTA

CTCGACATTCTAACGCTCTGAGCGGTAAGCACTAAGTCACACTG

primer match

GAGCGTCA

GAGCGTCA

ACGCTCTGAGCGGTAAGCACTAAGTCACACTG 16s rRNA V4 region

- Forward and reverse primer sequences are aligned to the read.
- If both primers perfectly match, the read is used for further steps, otherwise the read is discarded.
- This assures that all reads start at the same 16S position, which is mandatory for the pipeline to work.

Quality control of amplicon reads: error filtering

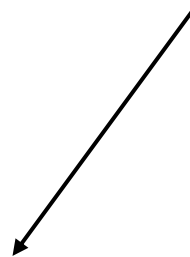
- Quality filtering by maximum expected errors (maxEE) should be performed as a first processing step

EE = expected errors = sum of error probability (sum of P)

- small EE = high quality; large EE = low quality
- By setting a maximum expected errors (maxEE) threshold, we can discard reads with $EE > \text{maxEE}$

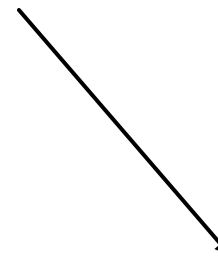
AGCTGTAAGATACGCTCTGAGCGGTAAGCACTAAGTCACACTGGAGCGTCA

If $EE < \text{maxEE}$



keep high quality read

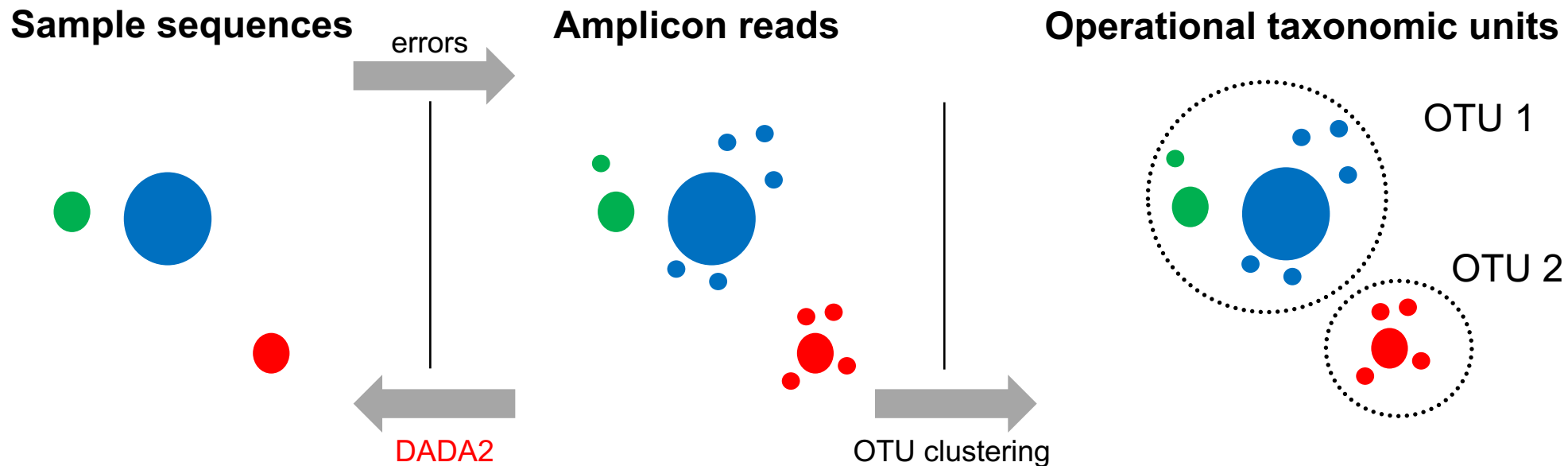
If $EE > \text{maxEE}$



discard low quality read

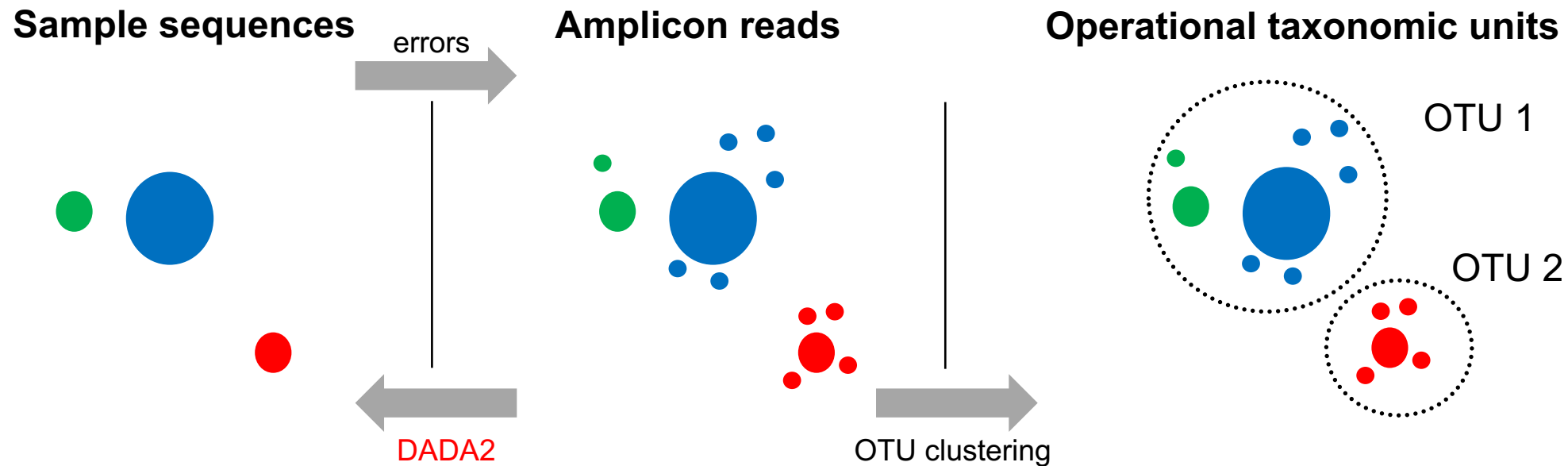
The Divisive Amplicon Denoising Algorithm (DADA)

- The core denoising algorithm in the DADA2 R package is built on a model of the errors in Illumina-sequenced amplicon reads.
- This error model quantifies the rate λ_{ji} at which an amplicon read with sequence i is produced from sample sequence j as a function of sequence composition and quality.



The Divisive Amplicon Denoising Algorithm (DADA)

- *DADA2 tries to decipher whether each amplicon is a true biological sequence or is the result of an error because of the sequencing process*



DADA2: the error model

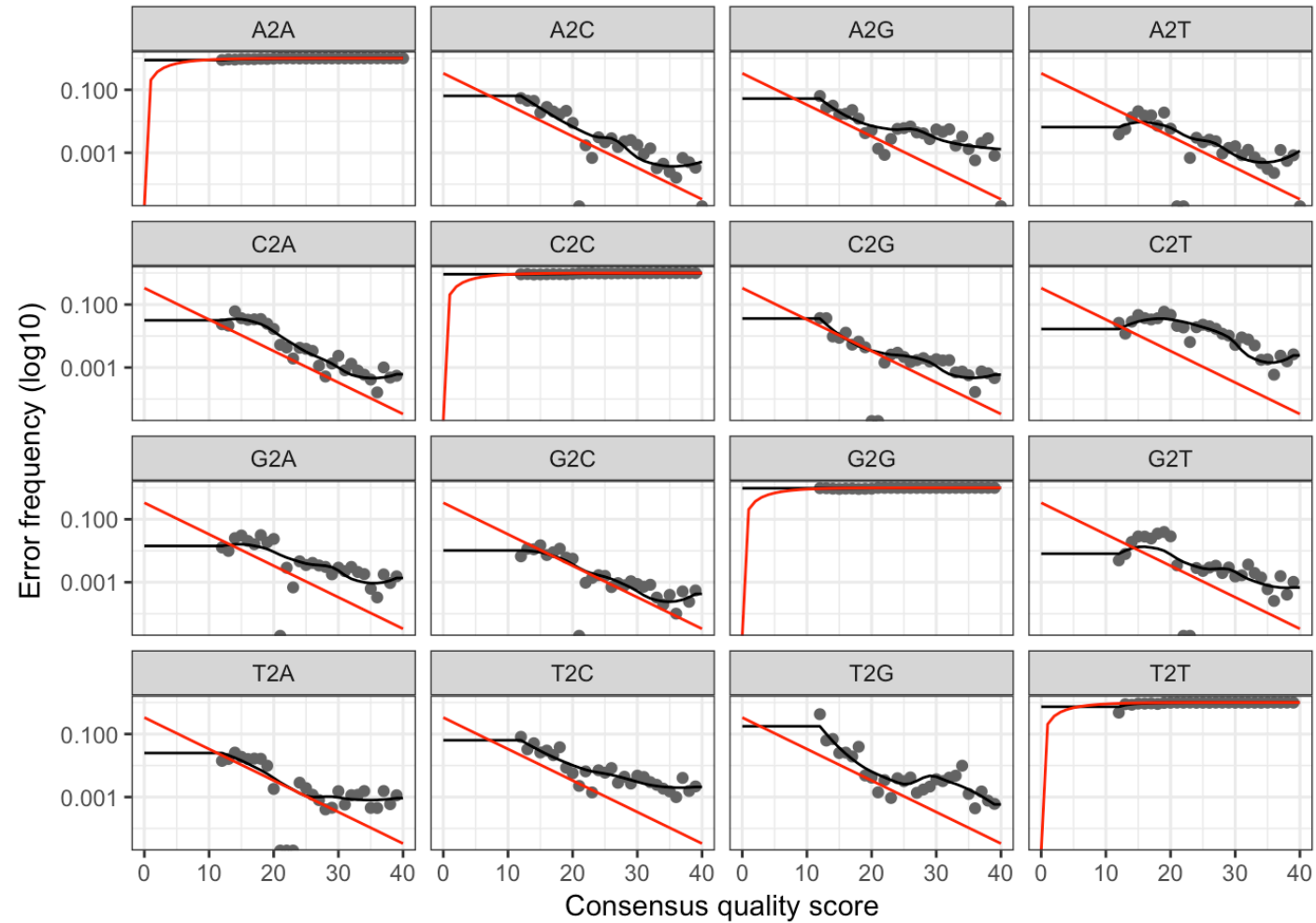
- The rate at which an amplicon read with sequence i is produced from sample sequence j is reduced to the product over the transition probabilities between the L aligned nucleotides.

$$\lambda_{ji} = \prod_{l=0}^L p(j(l) \rightarrow i(l), q_i(l))$$

- The transition probability between aligned nucleotides is allowed to depend on the original nucleotide, substituting nucleotide, and associated quality score, for example, $p(A \rightarrow T, 9)$

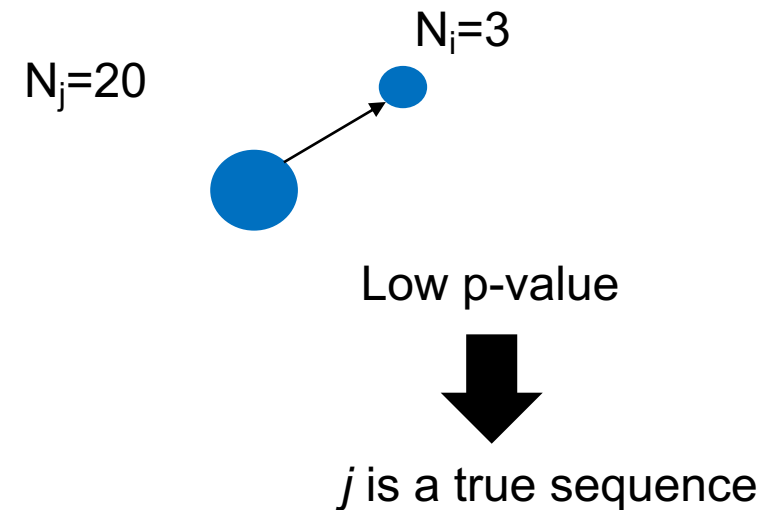
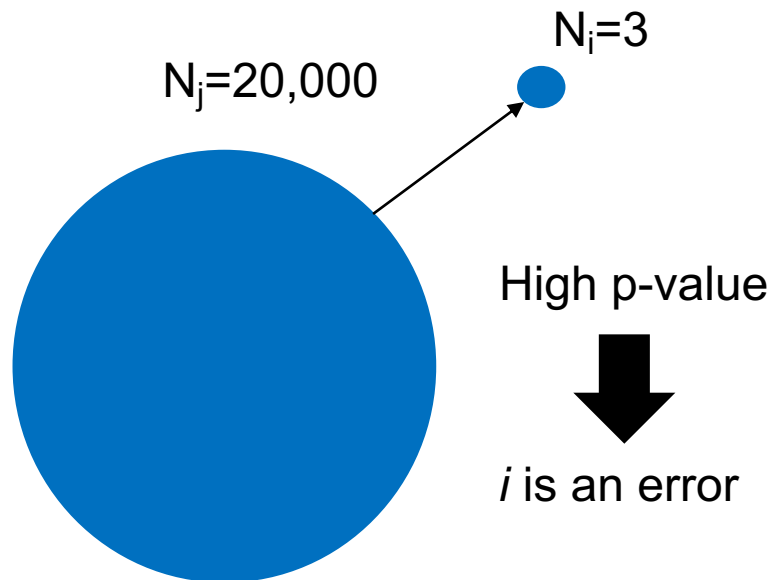
A	C	G	C	T	C	T	G	A	G	C	G	G	T	A	A	G	C	A	C	T	A	A	G	T	C	A	C	A	C	T	G
40	40	40	37	32	31	35	22	30	35	37	25	34	27	25	23	25	27	9	25	25	27	24	23	25	26	27	25	26	27	24	27
12	14	15	27	25	27	12	28	27	26	23	28	26	25	27	28	27	26	9	25	27	33	34	35	32	35	36	34	36	37	34	36
A	C	G	C	T	C	A	G	A	G	C	G	G	T	A	A	G	C	T	C	T	A	A	G	T	C	A	C	A	C	T	G

DADA2: the error model



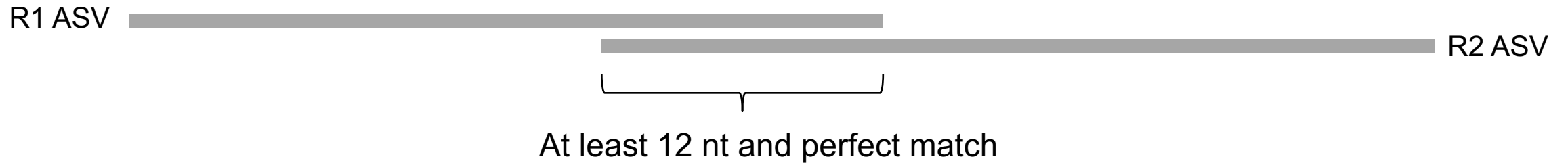
DADA2: the abundance p-value

- The abundance p -value quantifies the notion that sequence i is too abundant to be explained by errors in amplicon sequencing.
- It measures the probability of a given amplicon abundance given the model. That is, the likelihood that an amplicon is produced n times because errors.



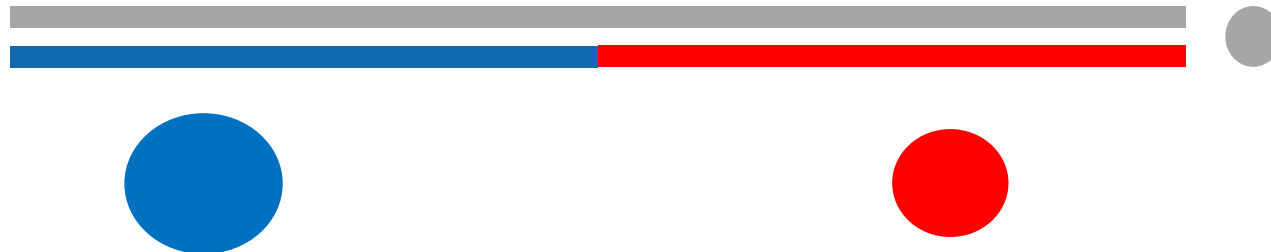
Merging and chimera removal

- Merging:



- Chimera detection and removal:

Chimeric sequences are identified if they can be exactly reconstructed by combining a left-segment and a right-segment from two more abundant “parent” sequences



Taxonomic annotation of ASVs



- Prediction of ASV taxonomy
- Each is compared to a database of annotated 16S rRNA gene sequences
- Sequences are classified to a phylum, class, family etc.