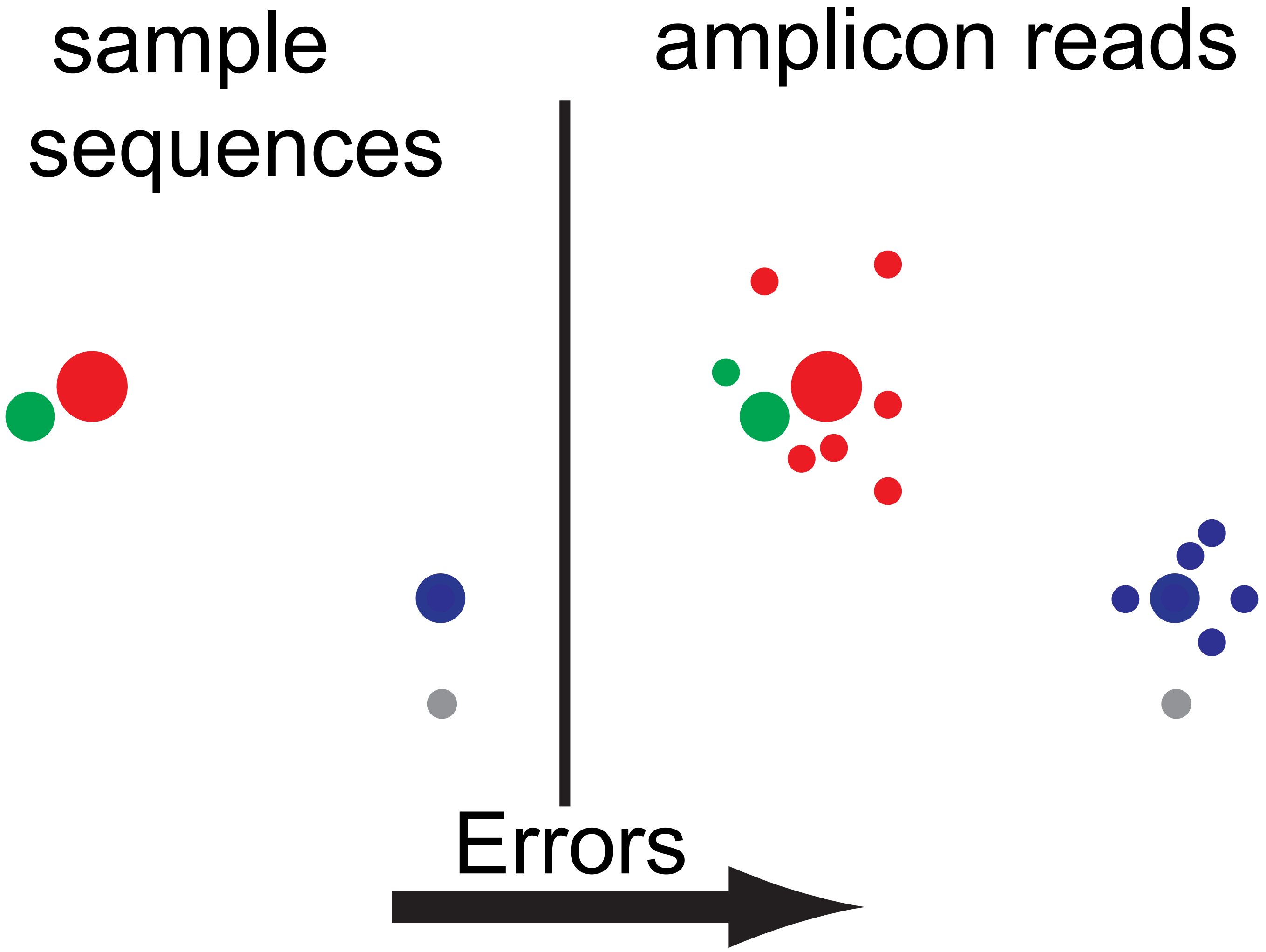
The DADA2 Method

The amplicon inference problem

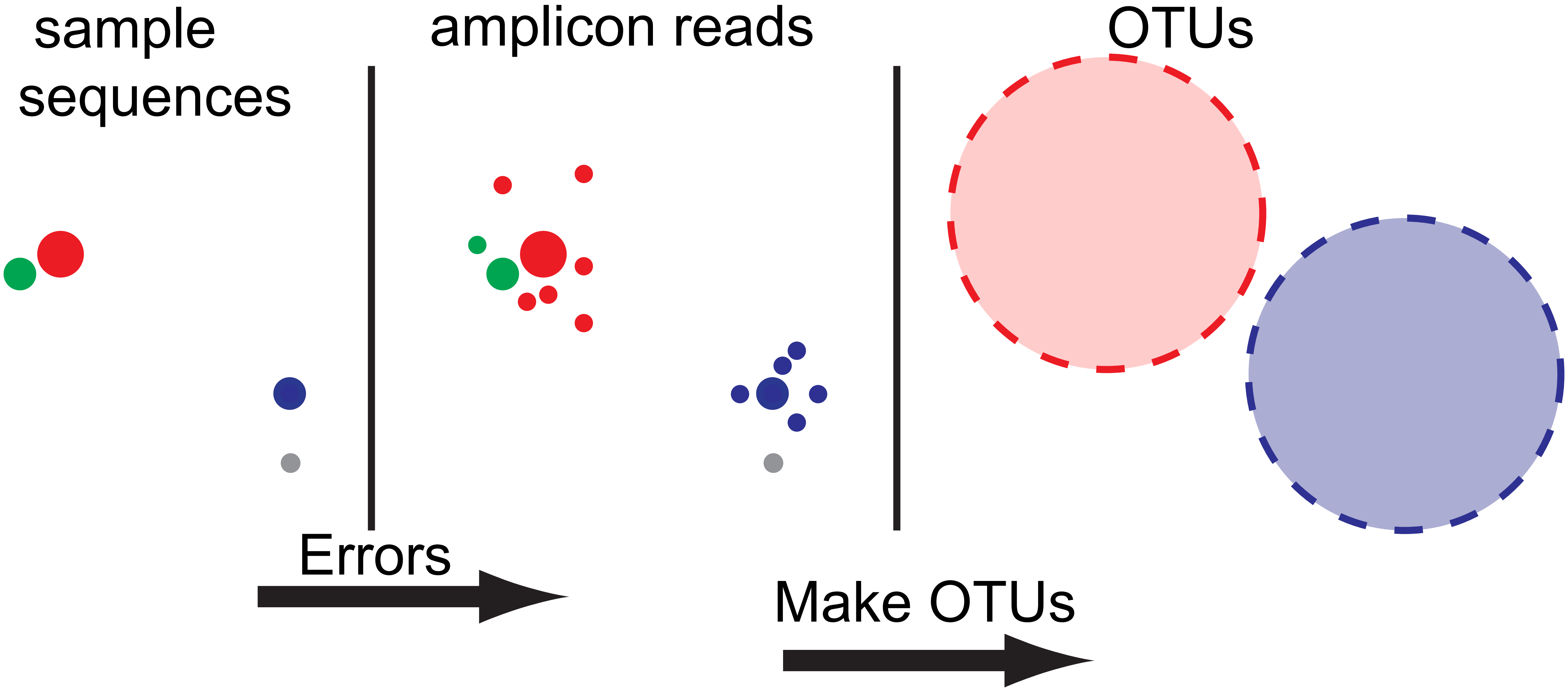
Infer the sample types and abundances $\{(s, a)\}$ from error-ful amplicon reads $\{r\}$.



sample sequences

amplicon reads

Errors



sample sequences

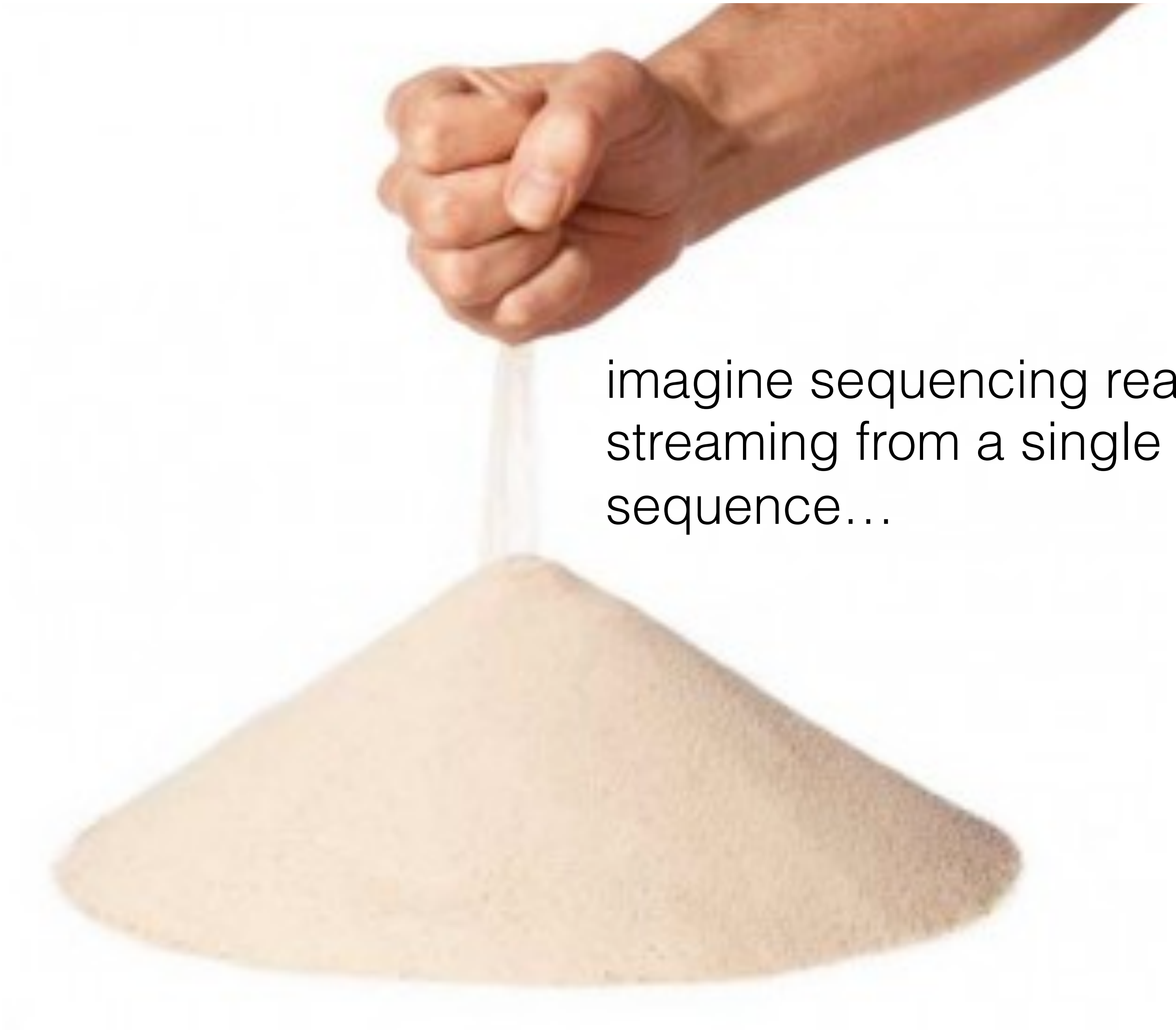
amplicon reads

OTUs

Errors

Make OTUs

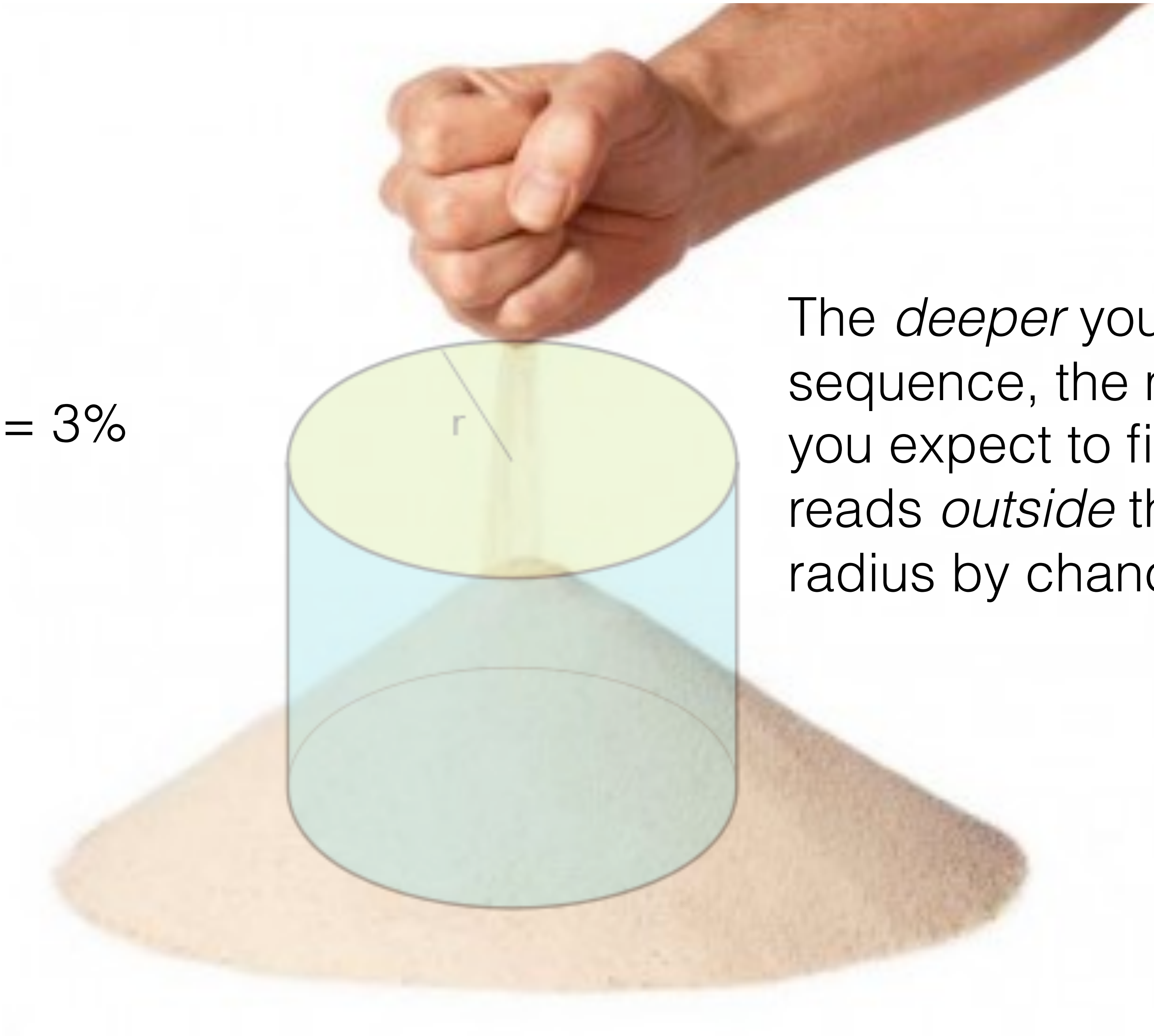
Motivation: Lingering problems with “OTU”



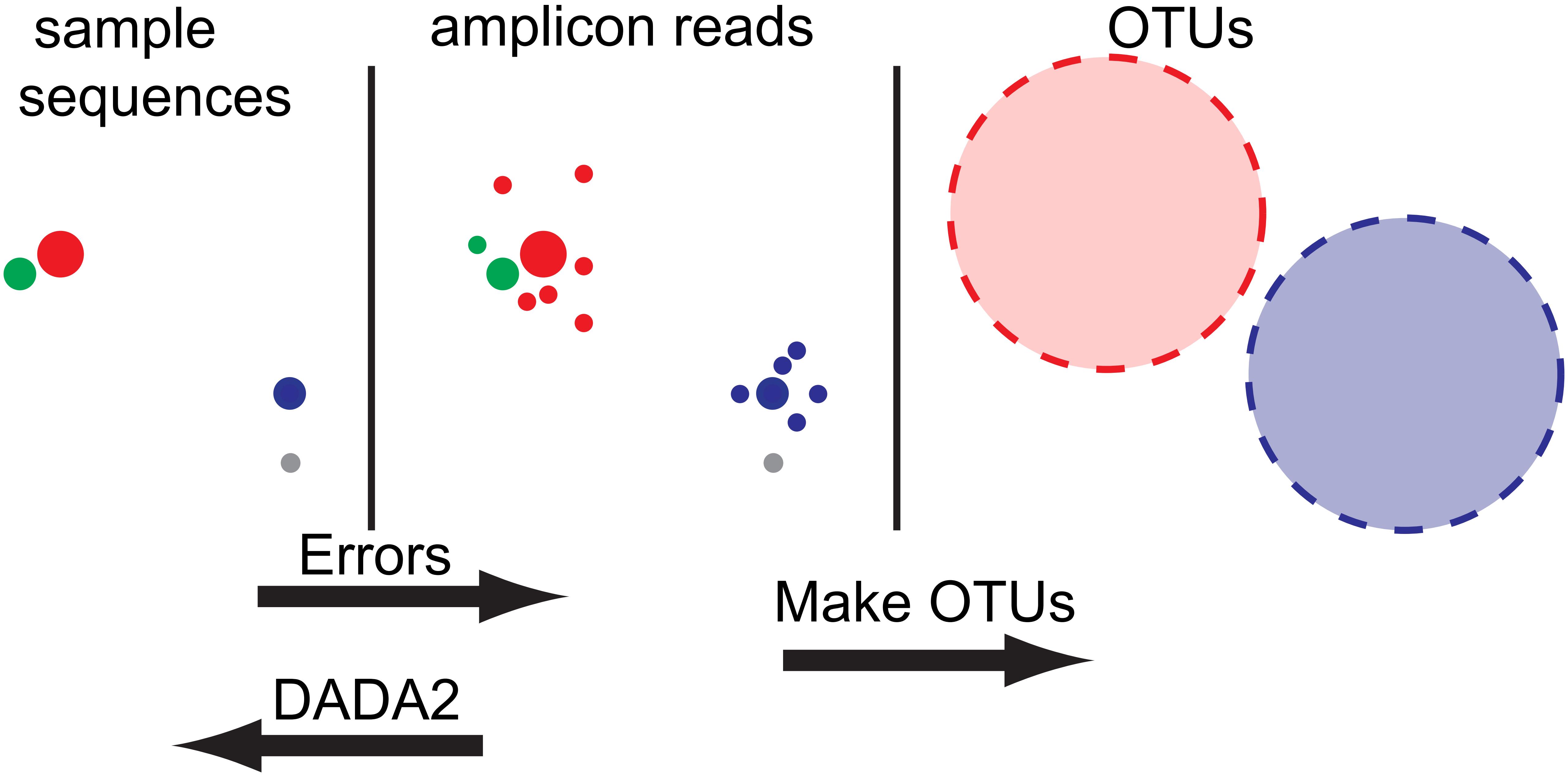
imagine sequencing reads streaming from a single true sequence…

Motivation: Lingering problems with "OTU"

r = 3%



The *deeper* you sequence, the more you expect to find reads *outside* the radius by chance.



sample sequences

amplicon reads

OTUs

Errors

DADA2

Make OTUs

Callahan, et al. Nature Methods, 2016.

DADA^2

Amplicon Sequencing. **Exactly.** ***Version 1.10***

Error Model

An Error Model

```
s: ATTAACGAGATTATAACCAGAGTACGAATA...
     |              |
r: ATCAACGAGATTATAACAAGAGTACGAATA...
```

An Error Model

```
s:  ATTAACGAGATTATAACCAGAGTACGAATA...
      |              |
r:  ATCAACGAGATTATAACAAGAGTACGAATA...
```

$$\boxed{p(r|s) = \prod_{i=1}^{L} p(r(i)|s(i), q_r(i), Z)}$$

An Error Model

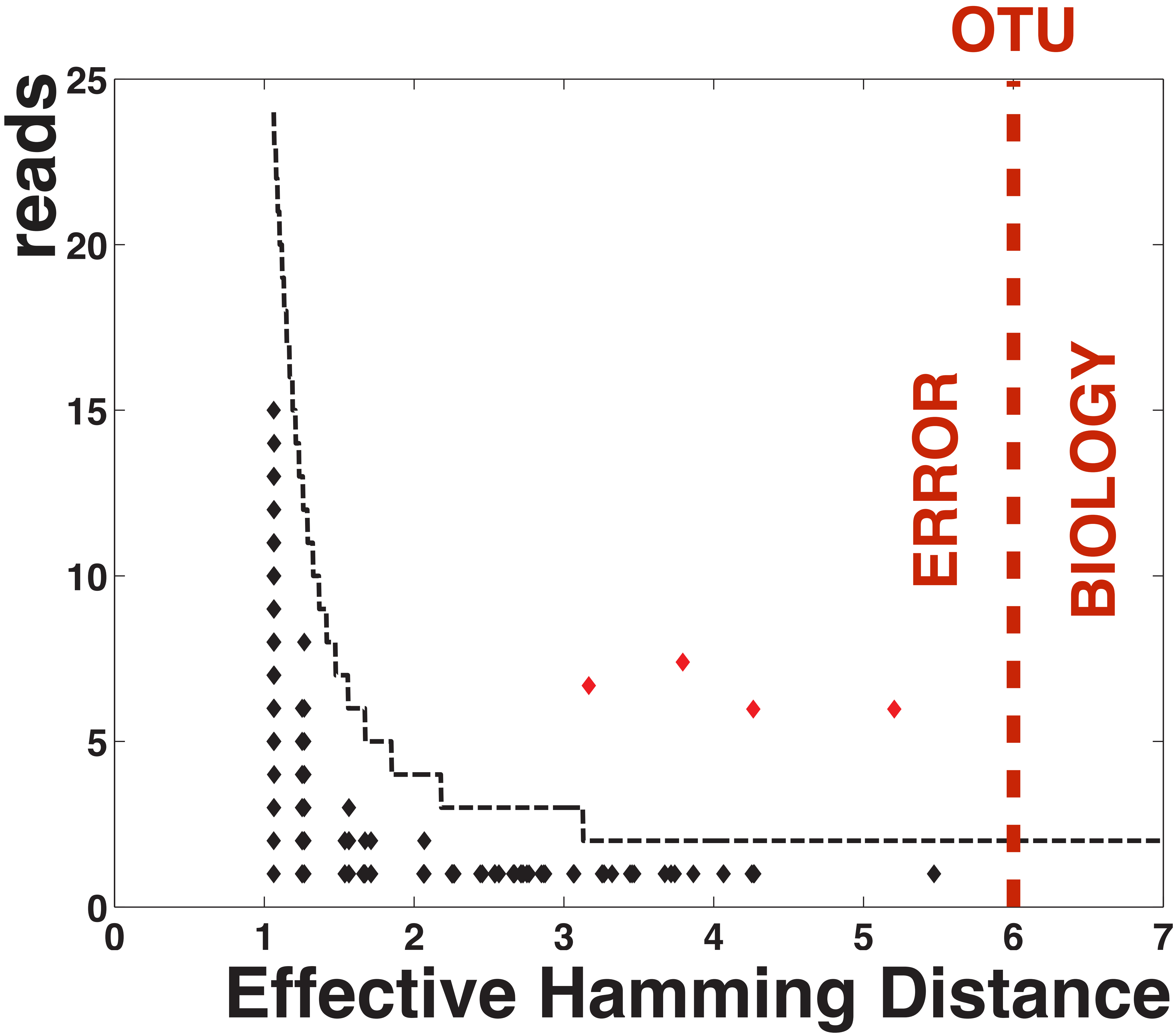
```
s:  ATTAACGAGATTATAACCAGAGTACGAATA...
      |              |
r:  ATCAACGAGATTATAACAAGAGTACGAATA...
```

$$\boxed{p(r|s) = \prod_{i=1}^{L} p(r(i)|s(i), q_r(i), Z)}$$

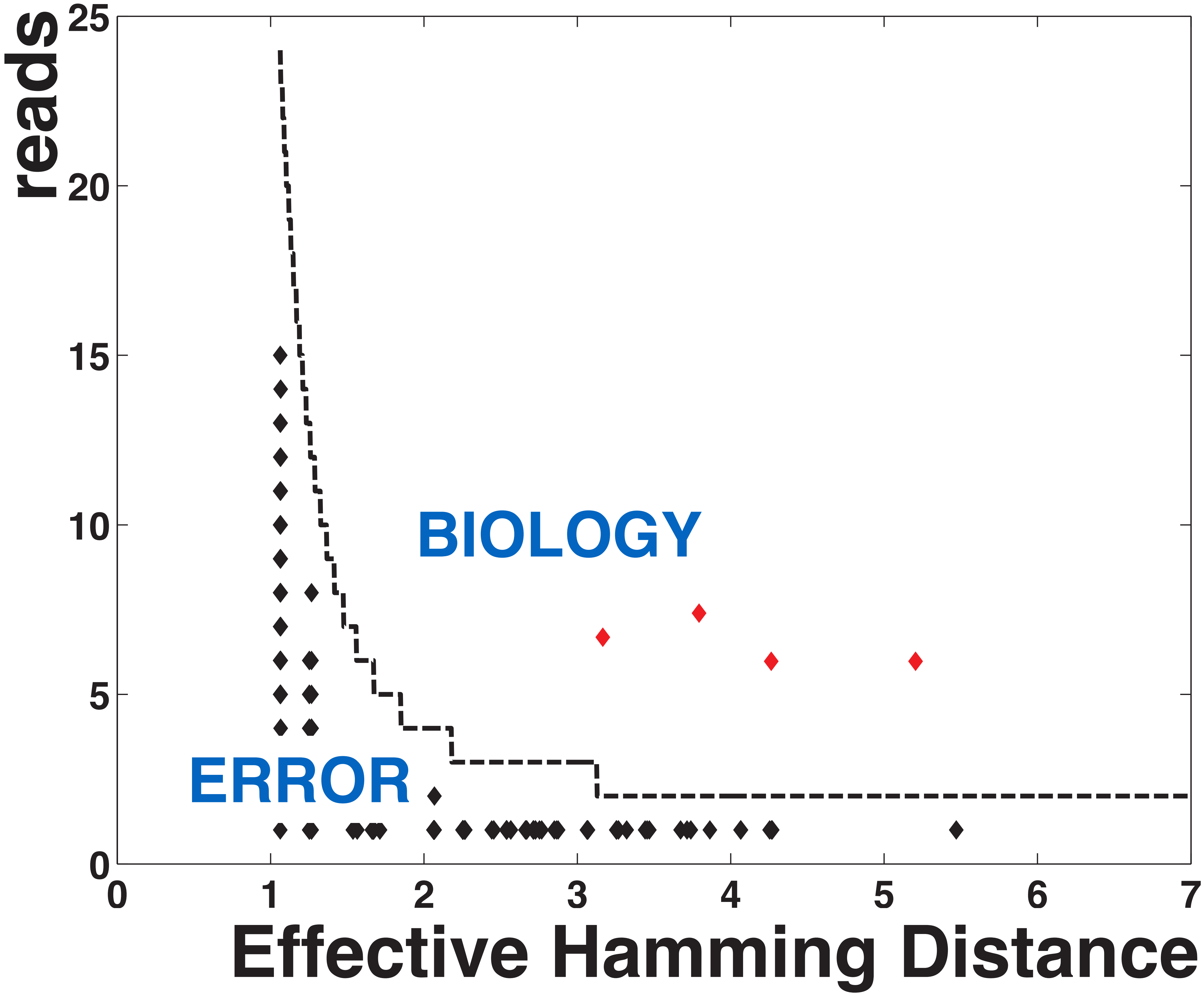
Error process is independent across nucleotides.

Per-nucleotide transition rate depends on:

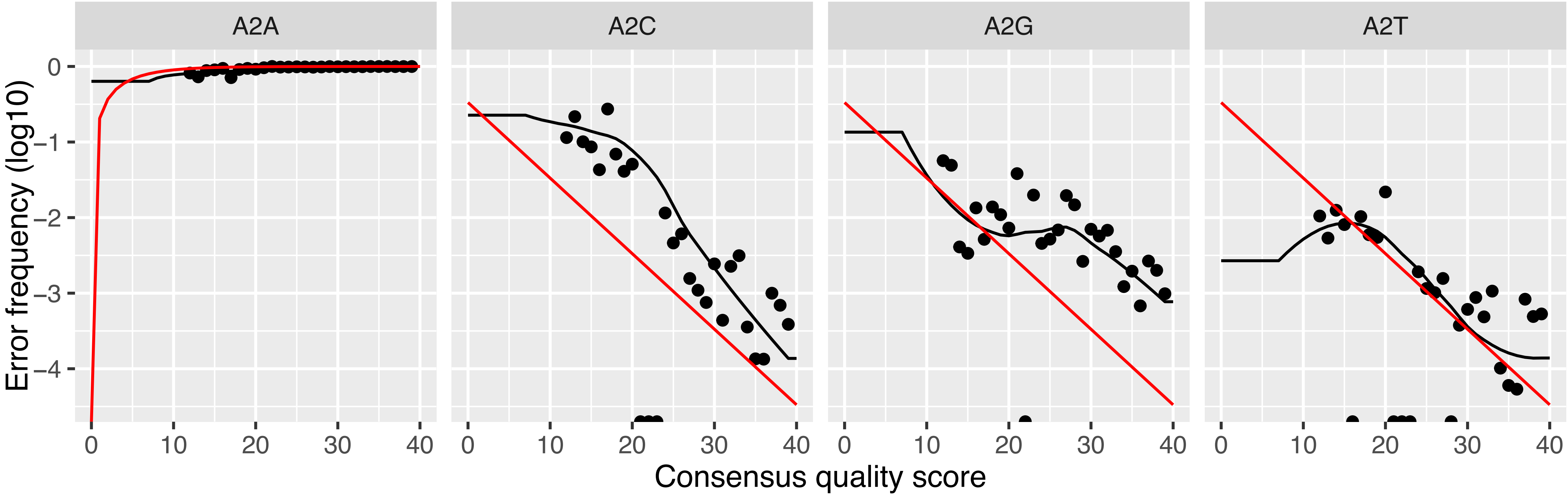
- Sample nucleotide
- Read nucleotide
- Read quality at that position
- Batch effect (eg. run)


OTU
reads
25
20
15
10
5
0
ERROR
BIOLOGY
0
1
2
3
4
5
6
7
Effective Hamming Distance

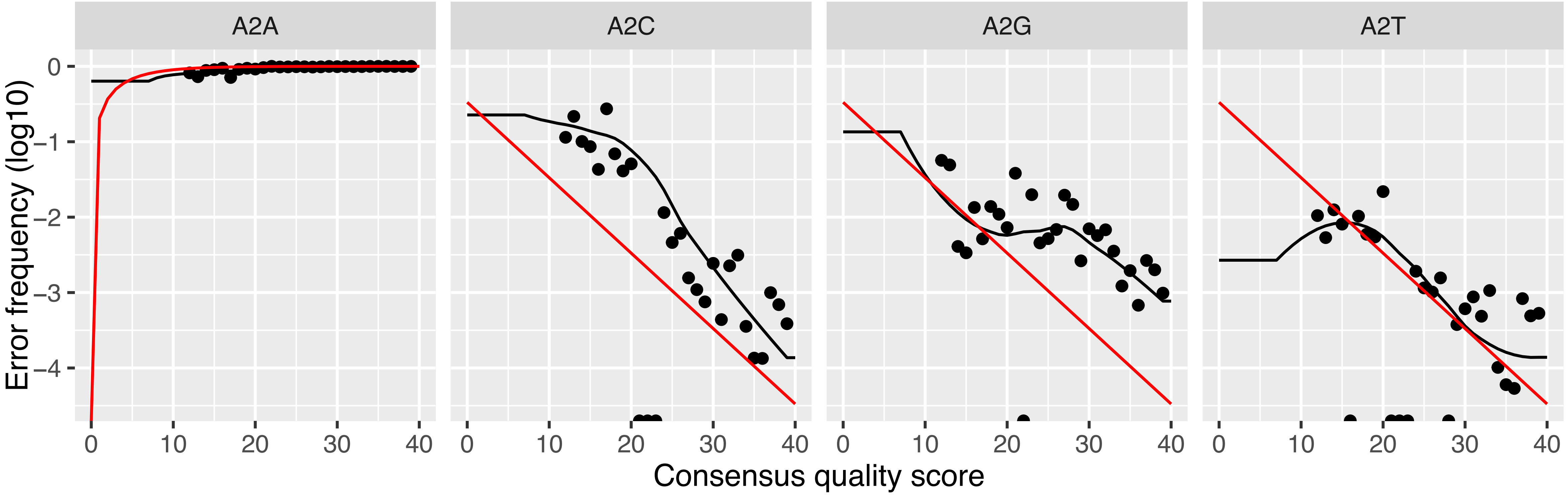
DADA2


reads
25
20
15
10
5
0
BIOLOGY
ERROR
0
1
2
3
4
5
6
7
Effective Hamming Distance

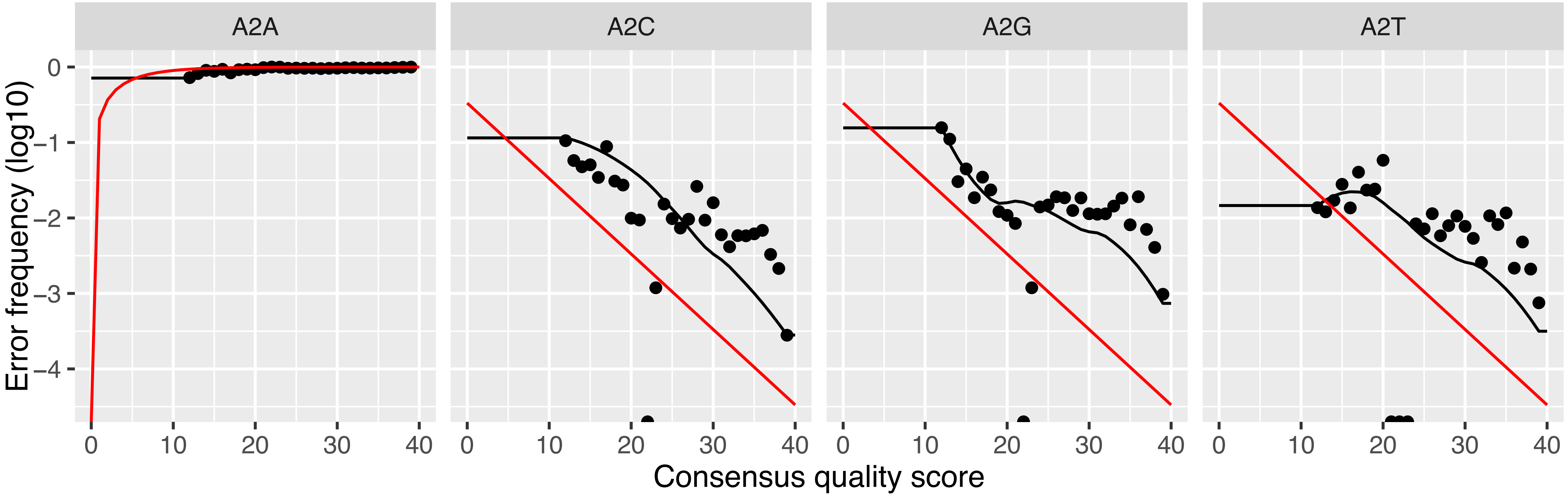
Learning Errors


A2A
A2C
A2G
A2T
0
−1
−2
−3
−4
Error frequency (log10)
0
10
20
30
40
Consensus quality score

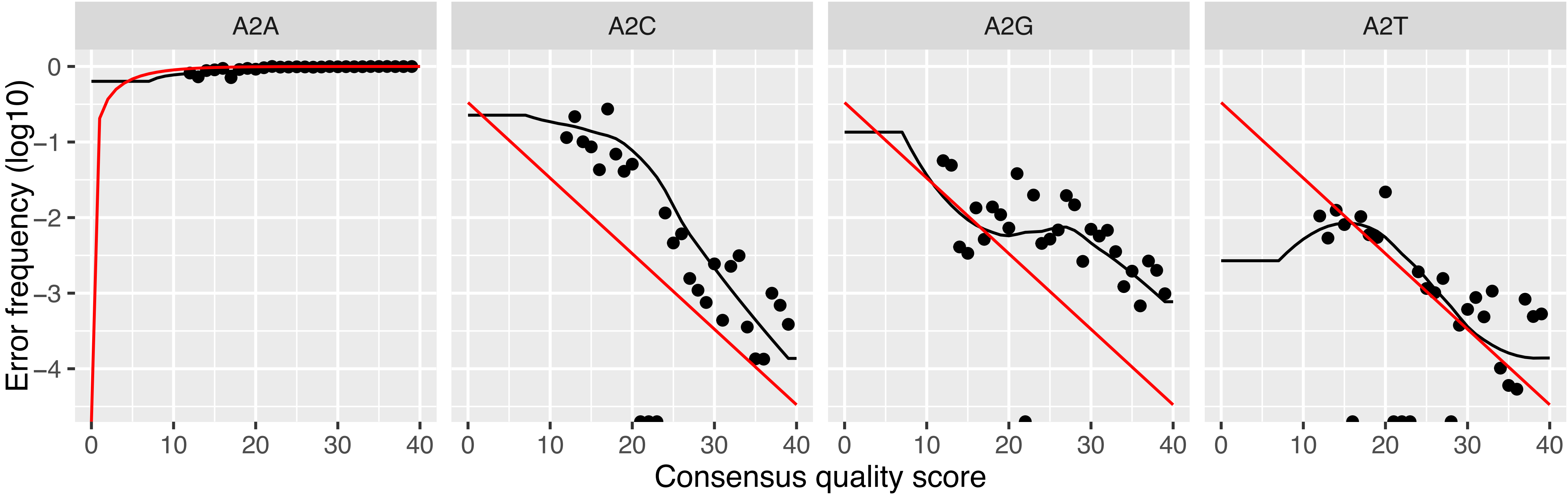
Study A



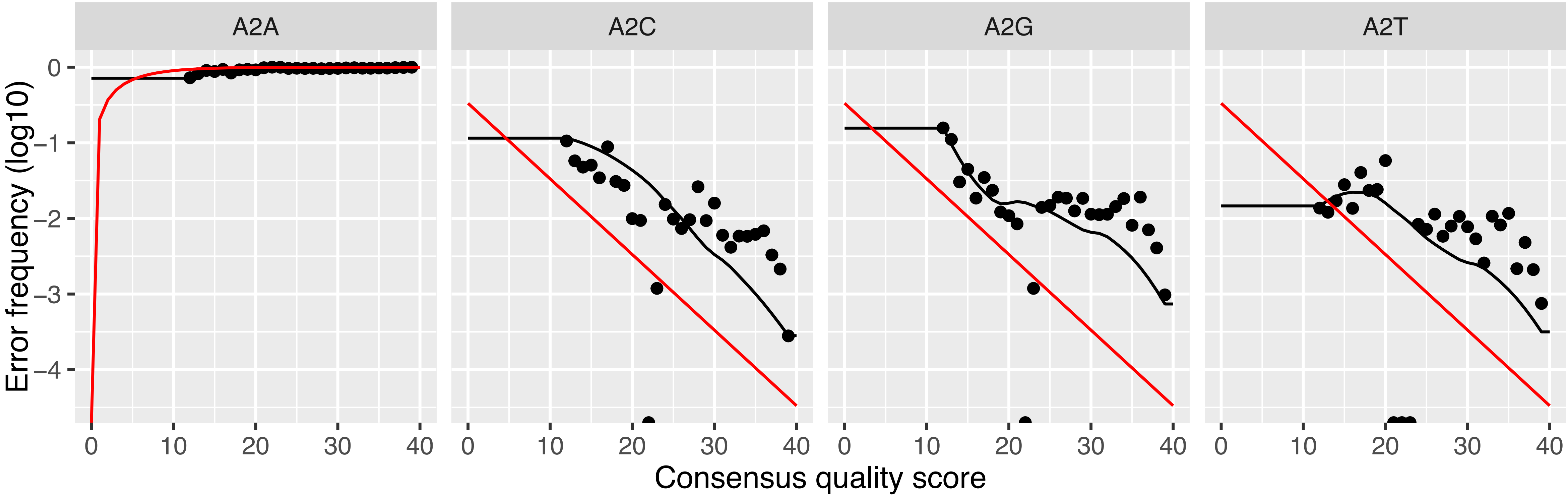
Study B



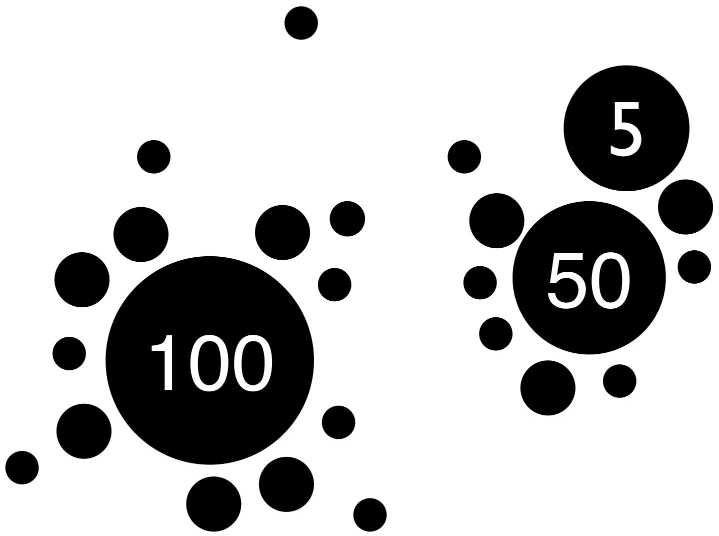
Study A



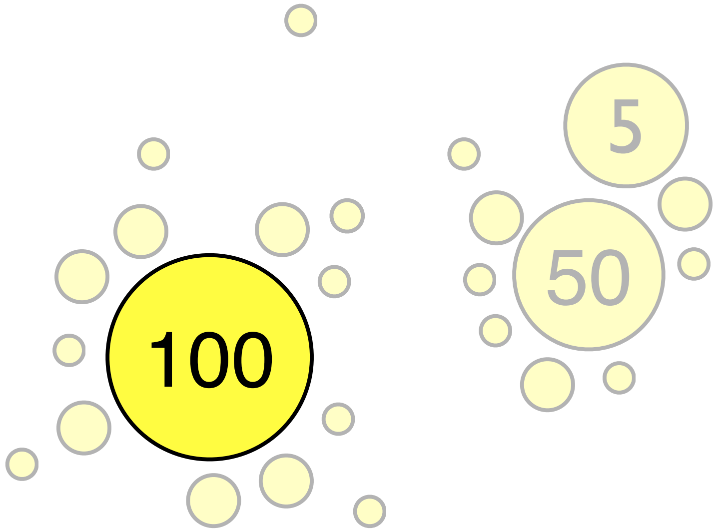
Study B



But How?

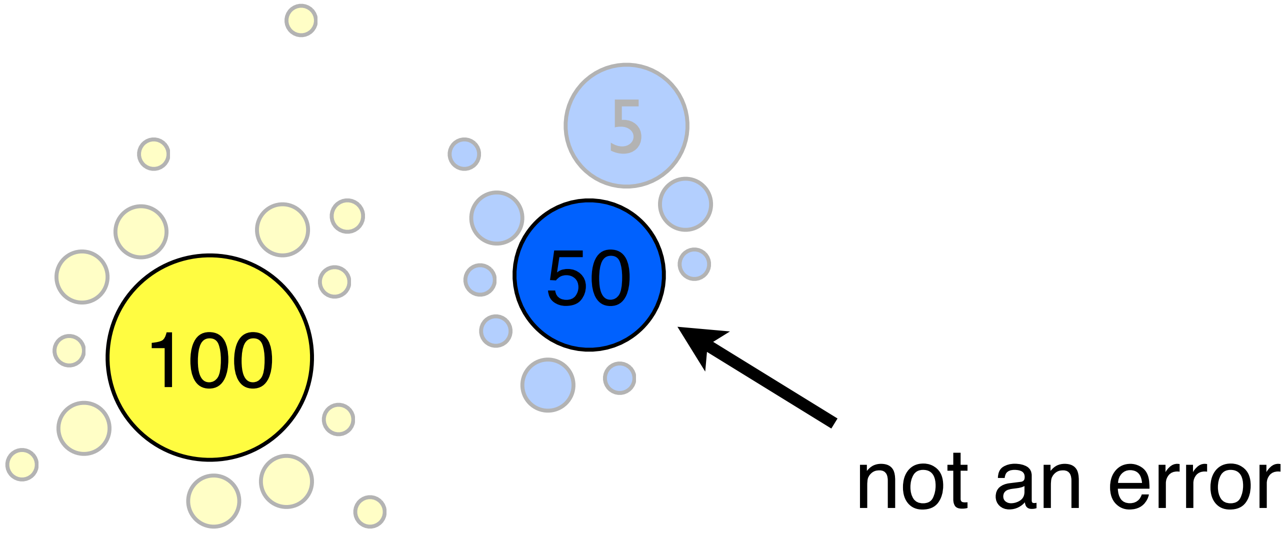


Initial guess: one real sequence + errors



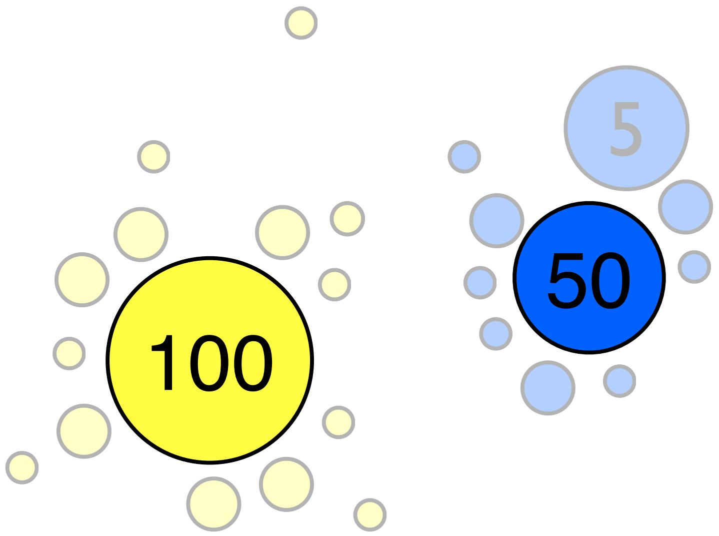
Infer initial *error model* under this assumption.

$$\Pr(i \rightarrow j) = \begin{array}{c|c|c|c|c} & \mathbf{A} & \mathbf{C} & \mathbf{G} & \mathbf{T} \\ \hline \mathbf{A} & 0.97 & 10^{-2} & 10^{-2} & 10^{-2} \\ \hline \mathbf{C} & 10^{-2} & 0.97 & 10^{-2} & 10^{-2} \\ \hline \mathbf{G} & 10^{-2} & 10^{-2} & 0.97 & 10^{-2} \\ \hline \mathbf{T} & 10^{-2} & 10^{-2} & 10^{-2} & 0.97 \end{array}$$



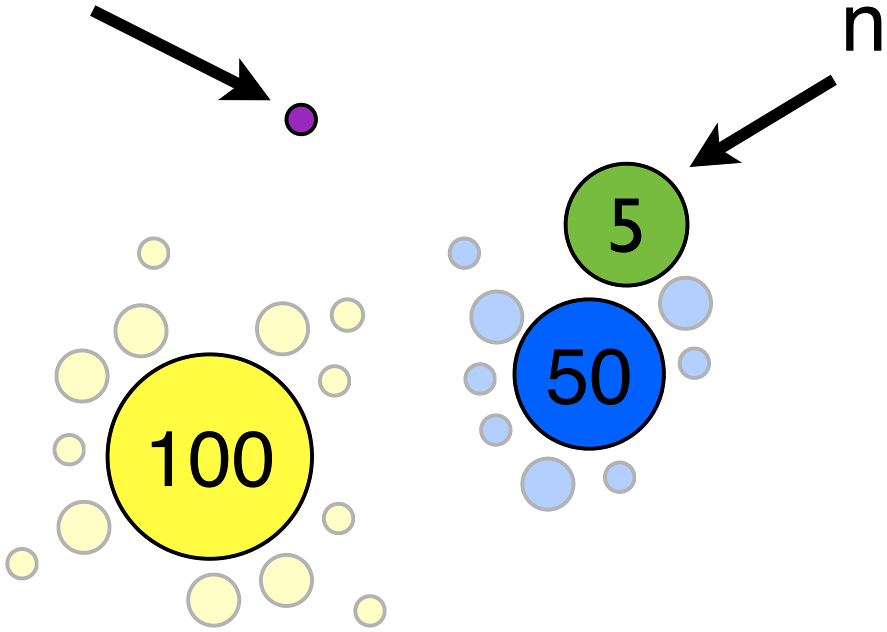
Reject unlikely error under model. **Recruit** errors.

	A	C	G	T
A	0.97	10^{-2}	10^{-2}	10^{-2}
C	10^{-2}	0.97	10^{-2}	10^{-2}
G	10^{-2}	10^{-2}	0.97	10^{-2}
T	10^{-2}	10^{-2}	10^{-2}	0.97



Update the model.

	A	C	G	T
A	0.997	10^{-3}	10^{-3}	10^{-3}
C	10^{-3}	0.997	10^{-3}	10^{-3}
G	10^{-3}	10^{-3}	0.997	10^{-3}
T	10^{-3}	10^{-3}	10^{-3}	0.997

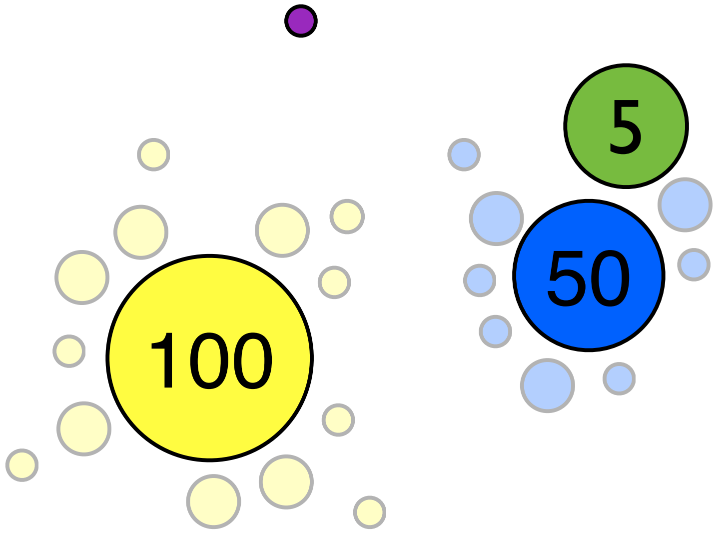


not an error

not an error

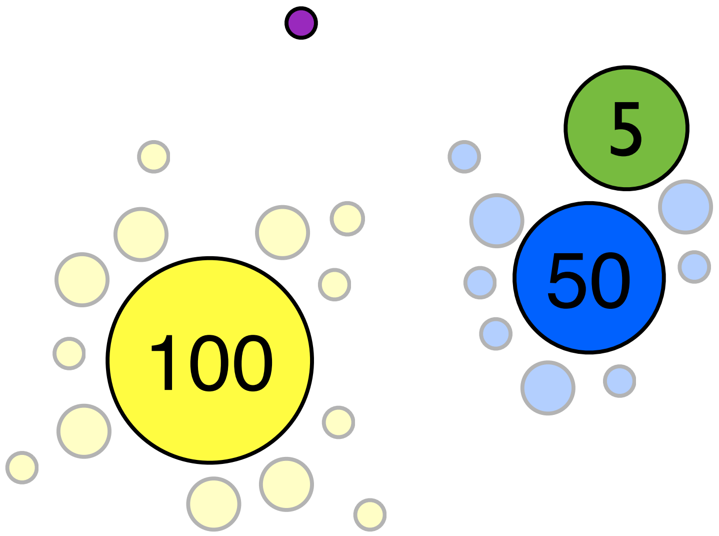
Reject more sequences under ***new*** model

	A	C	G	T
A	0.997	10^{-3}	10^{-3}	10^{-3}
C	10^{-3}	0.997	10^{-3}	10^{-3}
G	10^{-3}	10^{-3}	0.997	10^{-3}
T	10^{-3}	10^{-3}	10^{-3}	0.997



Update model again

	A	C	G	T
A	0.998	1x10^{-4}	2x10^{-3}	2x10^{-4}
C	6x10^{-5}	0.999	3x10^{-6}	1x10^{-3}
G	1x10^{-3}	3x10^{-6}	0.999	6x10^{-5}
T	2x10^{-4}	2x10^{-3}	1x10^{-4}	0.998



Convergence: all errors are plausible

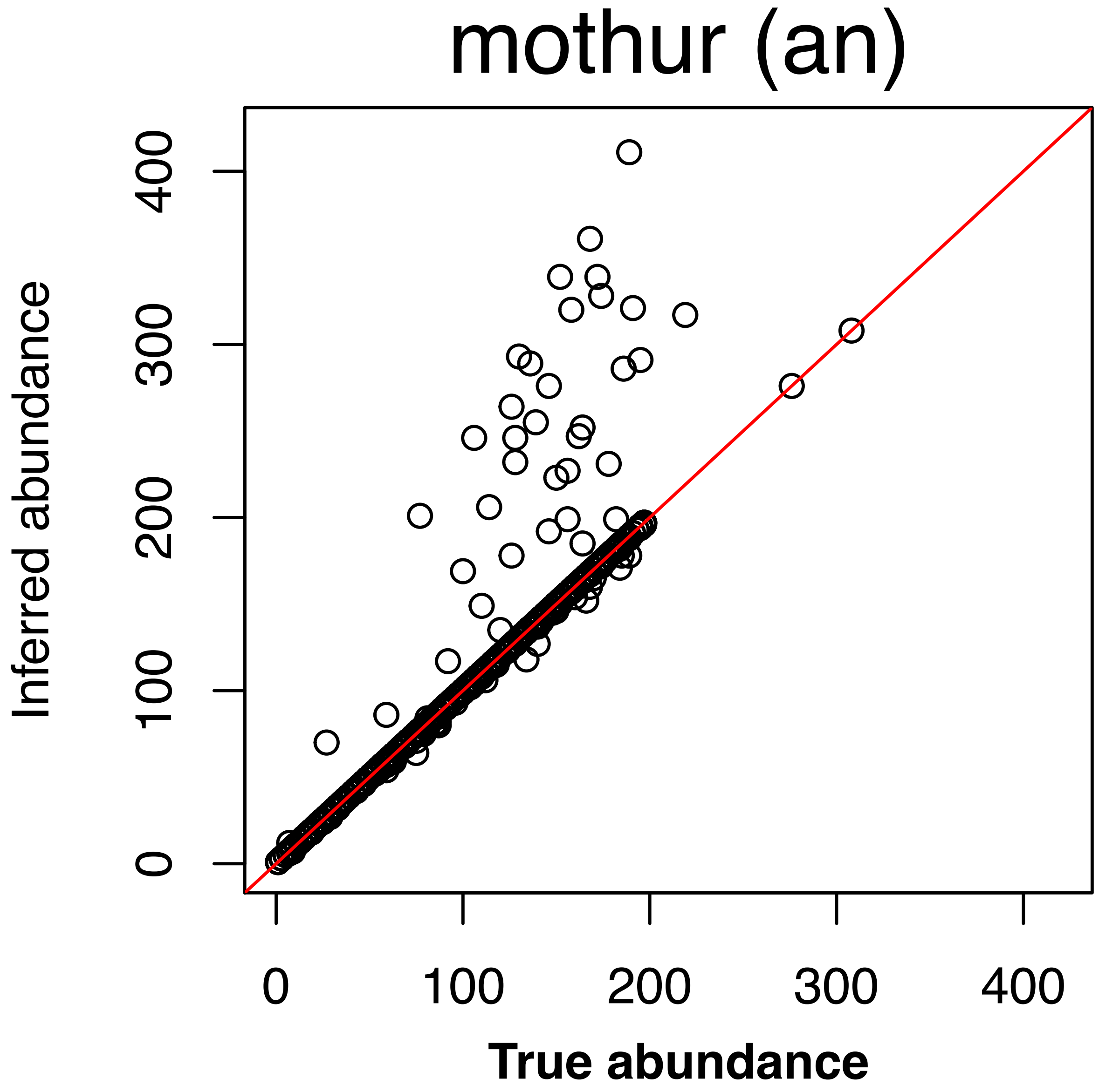
	A	C	G	T
A	0.998	$1x10^{-4}$	$2x10^{-3}$	$2x10^{-4}$
C	$6x10^{-5}$	0.999	$3x10^{-6}$	$1x10^{-3}$
G	$1x10^{-3}$	$3x10^{-6}$	0.999	$6x10^{-5}$
T	$2x10^{-4}$	$2x10^{-3}$	$1x10^{-4}$	0.998

Accuracy

and

Resolution

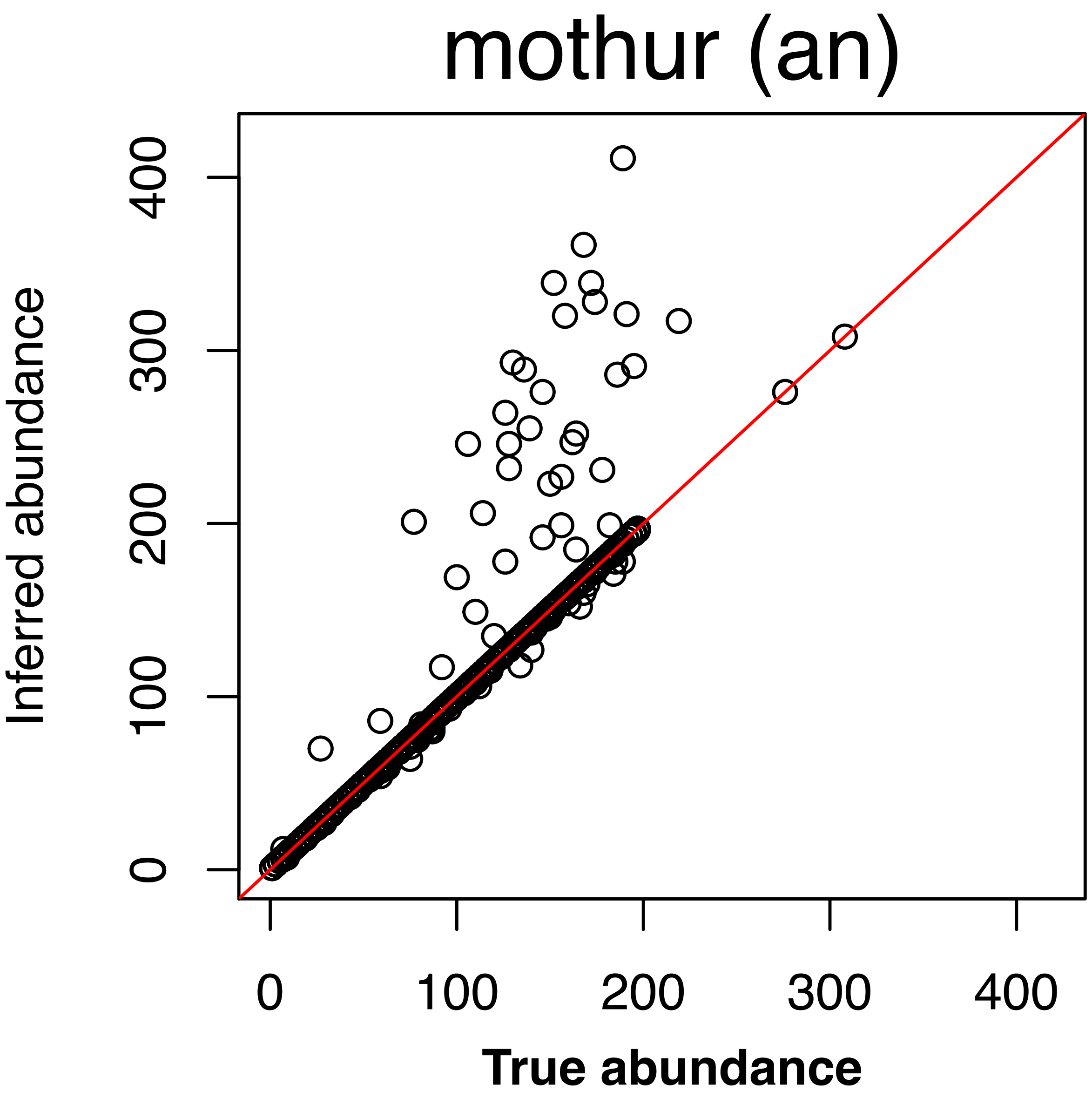
Accuracy: Simulated data



TP: 978
FP: 272
FN: 77
cor: 0.935

Data: Kopylova, et al. mSystems, 2016.

Accuracy: Simulated data



TP:	978
FP:	272
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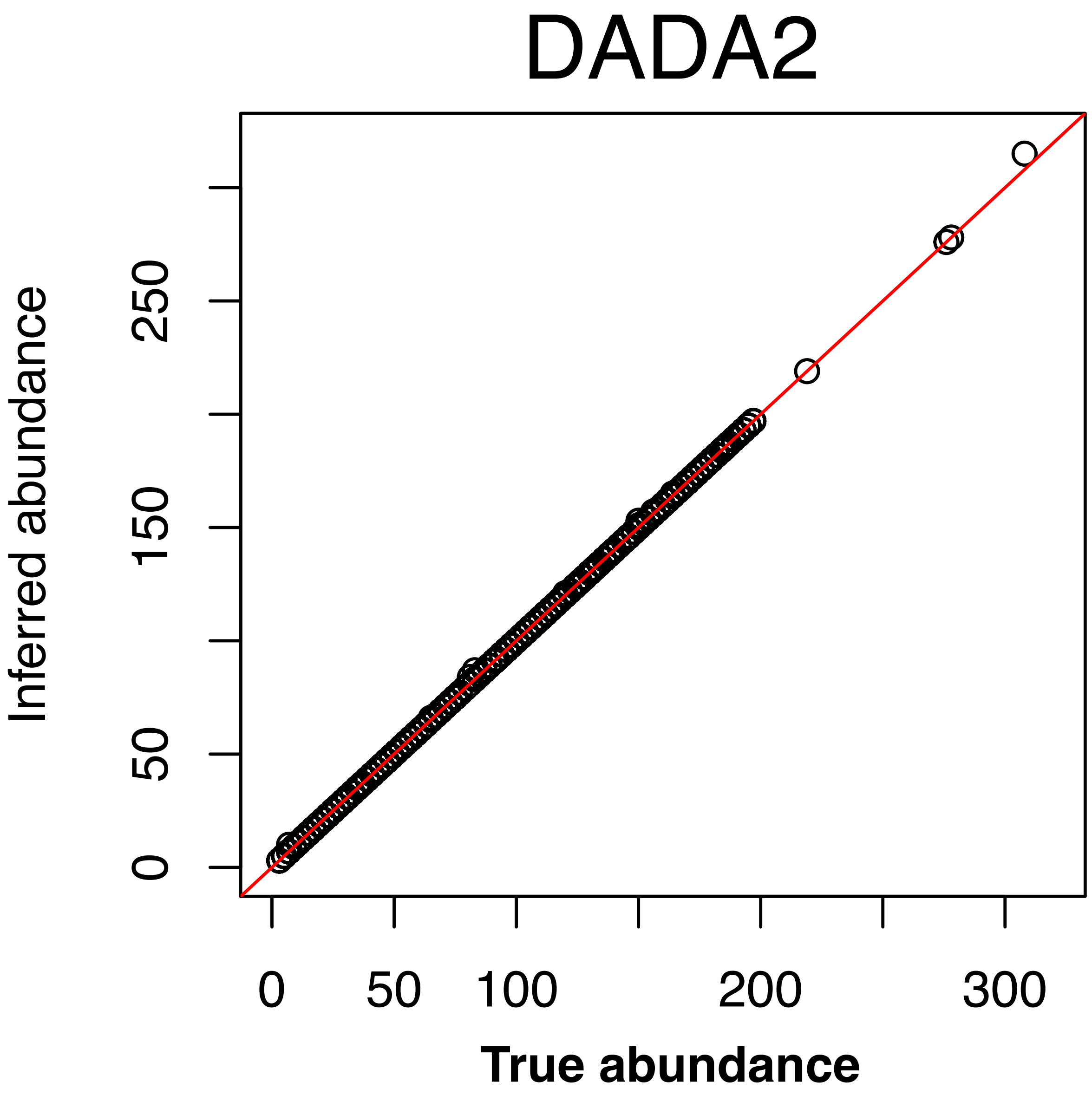
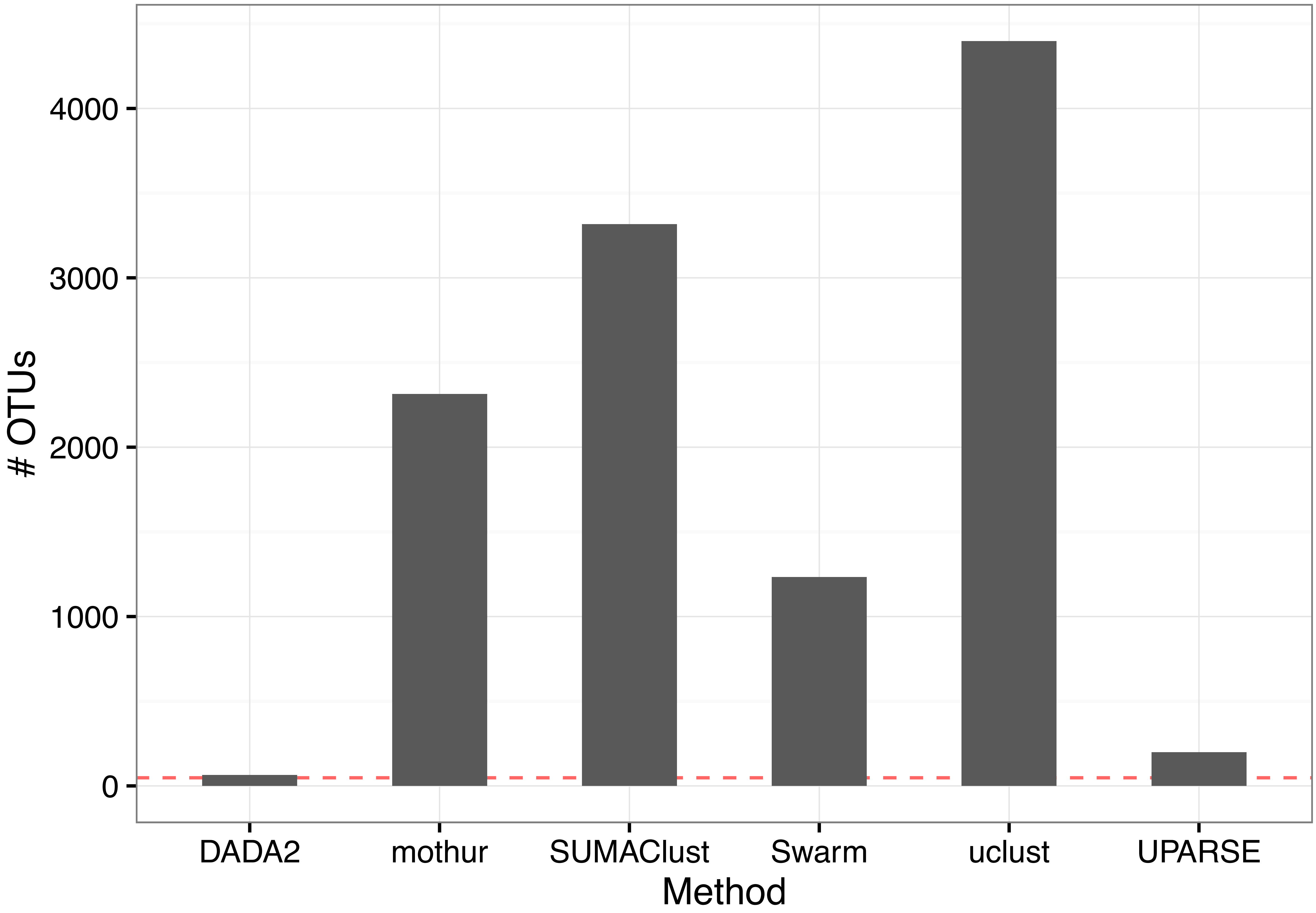


TP:	1042
FP:	0
FN:	13
cor:	0.999

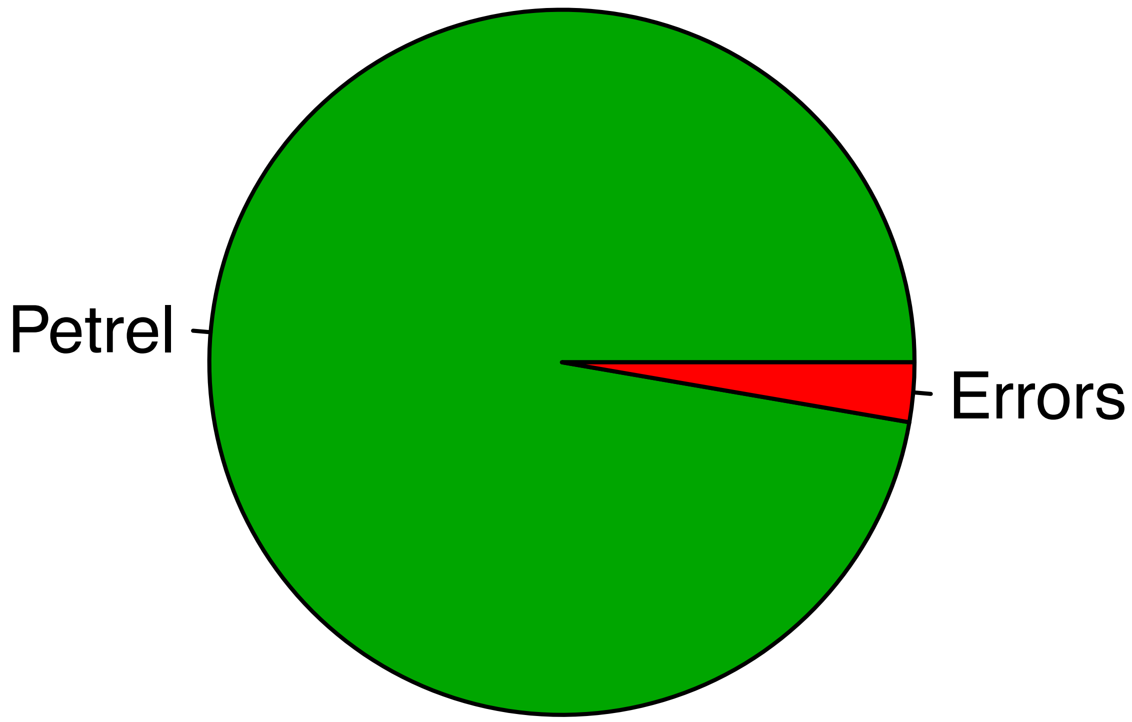
Data: Kopylova, et al. mSystems, 2016.

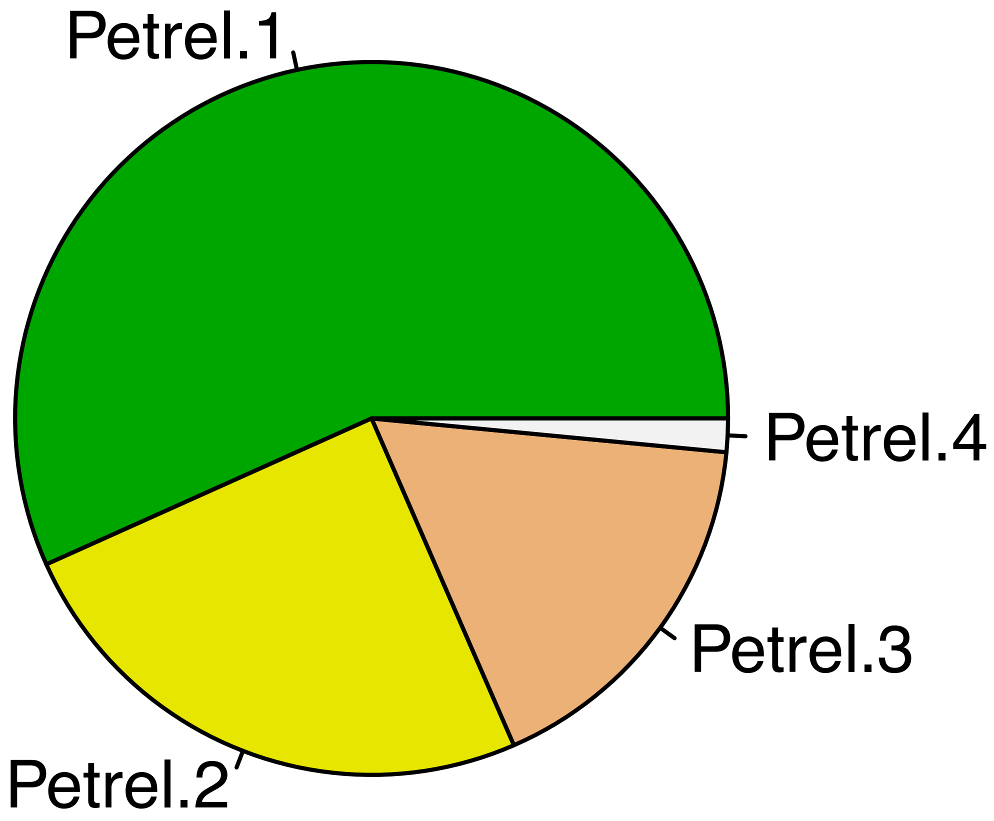
Accuracy: Mock community



Credit: Kopylova, et al. mSystems, 2016.

Resolution: Petrel aDNA


QIIME: De novo


DADA2

Credit: Kealoha Kinney, Michael Bunce, Andreanna Welch

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NSF

BIO-X

NIH National Institutes of Health

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