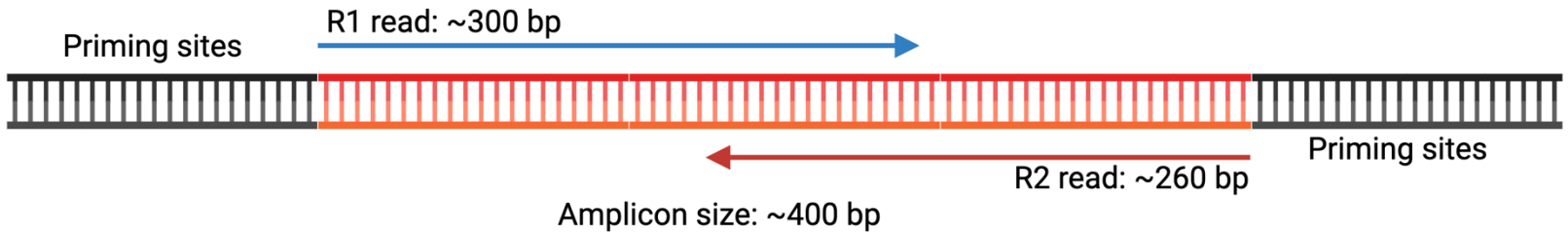
MMB-117

Amplicon sequence data analysis

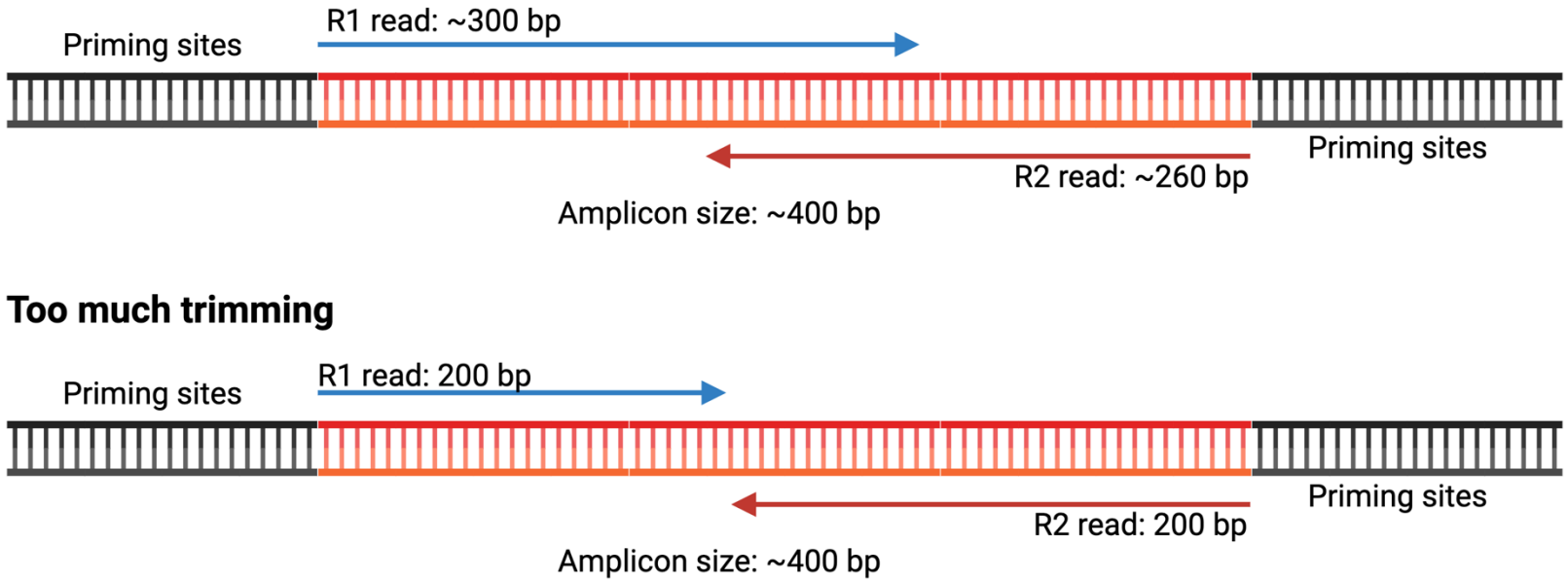
DADA2 pipeline

- Quality trimming
- Denoising
- Chimera removal
- Taxonomic annotation

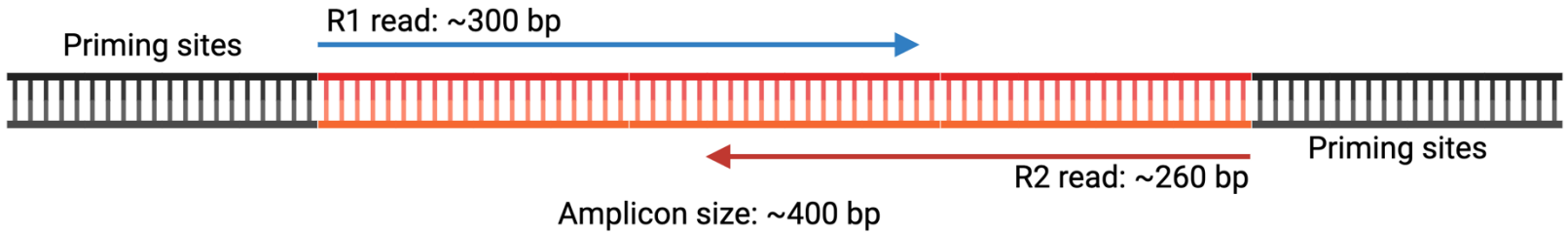
Length trimming



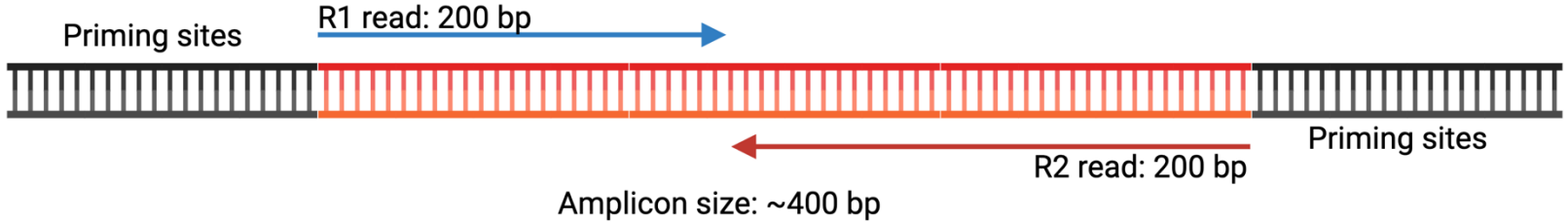
Length trimming



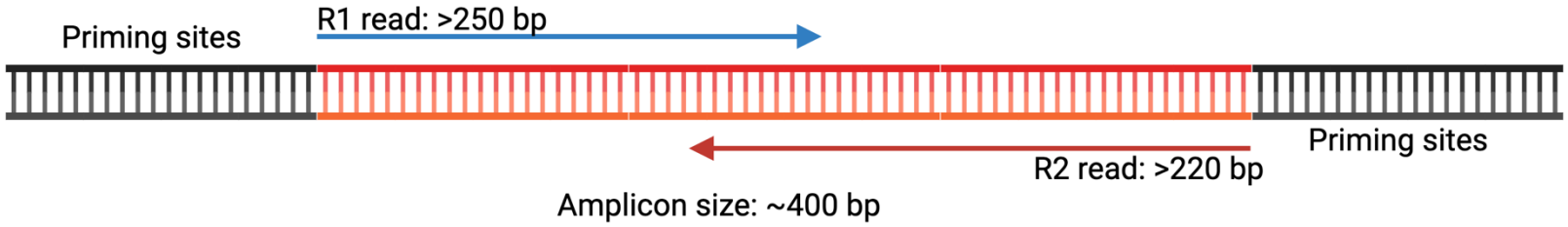
Length trimming



Too much trimming



Optimal trimming



Quality trimming

- PHRED quality scores (Q)
- Probability (P) that the base is called right

$$Q = -10\, log_{10} P$$

$$P = 10^{\frac{-Q}{10}}$$

- Expected errors (EE)

$$\mathrm{EE} = \mathrm{sum}(10^{\frac{-Q}{10}})$$

PHRED (Q)	Probability of incorrect base	Accuracy (%)
10	1 in 10	90
20	1 in 100	99
30	1 in 1 000	99.9
40	1 in 10 000	99.99

Calculate expected errors

```
@sequence1
GTCAGGTAGC
+
++++++++++
@sequence2
ATGCGGCTTATTG… (100 nt)
+
5555555555555…
```

ASCII_BASE=33 Illumina, Ion Torrent, PacBio and Sanger

Q	P_error	ASCII	Q	P_error	ASCII	Q	P_error	ASCII	Q	P_error	ASCII
0	1.00000	33 !	11	0.07943	44 ,	22	0.00631	55 7	33	0.00050	66 B
1	0.79433	34 "	12	0.06310	45 -	23	0.00501	56 8	34	0.00040	67 C
2	0.63096	35 #	13	0.05012	46 .	24	0.00398	57 9	35	0.00032	68 D
3	0.50119	36 $	14	0.03981	47 /	25	0.00316	58 :	36	0.00025	69 E
4	0.39811	37 %	15	0.03162	48 0	26	0.00251	59 ;	37	0.00020	70 F
5	0.31623	38 &	16	0.02512	49 1	27	0.00200	60 <	38	0.00016	71 G
6	0.25119	39 '	17	0.01995	50 2	28	0.00158	61 =	39	0.00013	72 H
7	0.19953	40 (	18	0.01585	51 3	29	0.00126	62 >	40	0.00010	73 I
8	0.15849	41)	19	0.01259	52 4	30	0.00100	63 ?	41	0.00008	74 J
9	0.12589	42 *	20	0.01000	53 5	31	0.00079	64 @	42	0.00006	75 K
10	0.10000	43 +	21	0.00794	54 6	32	0.00063	65 A			

- What's the expected error on average in our data (first 100nt)