

DTU



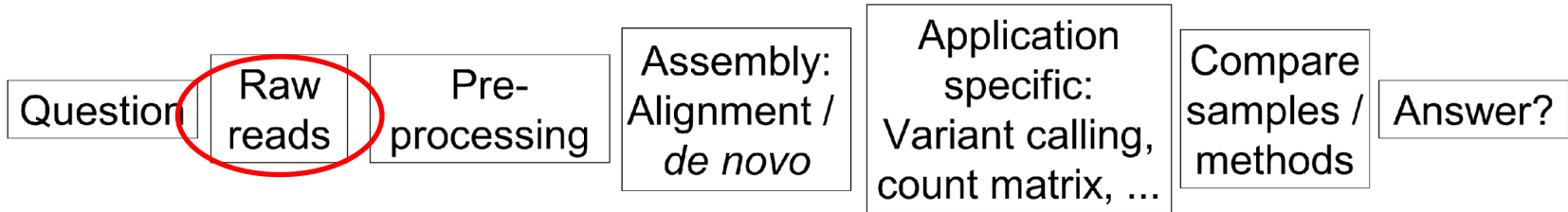
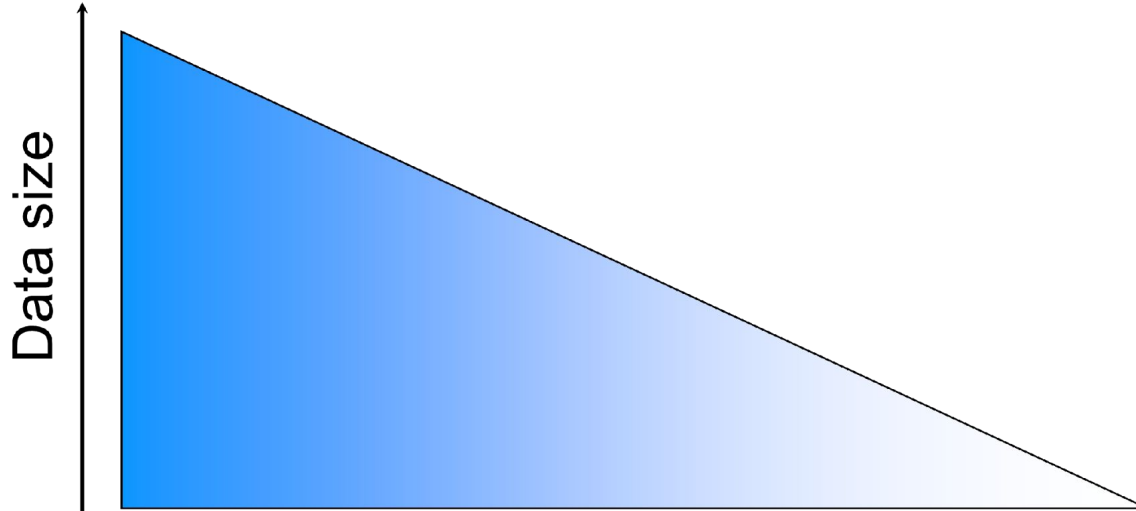


**DTU Health Technology
Bioinformatics**

Data basics

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Section of Bioinformatics
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Generalized NGS analysis



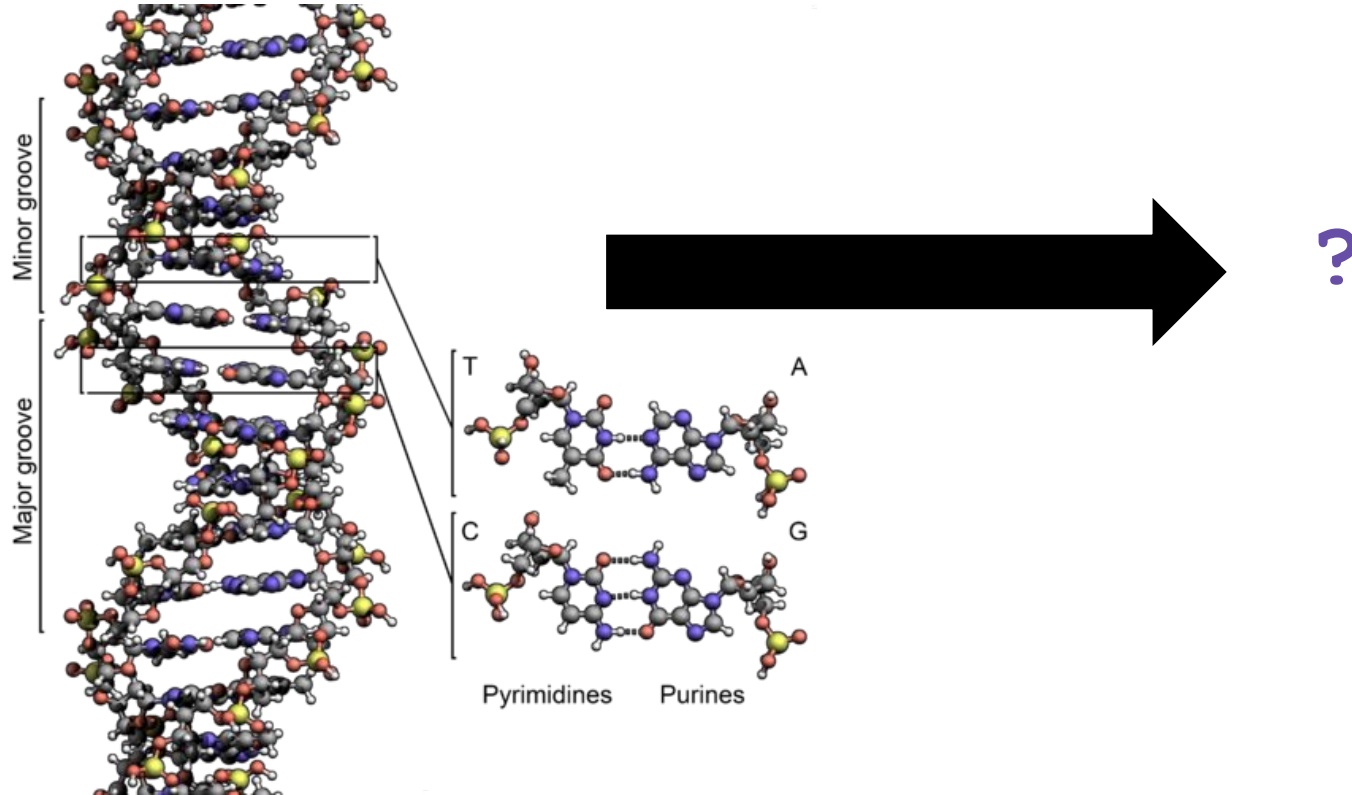
Outline

- Starting point – what we learned yesterday
- Sequence data storage
 - Fasta format
 - Fastq format
- Quality scores
- Multiplexing/Demultiplexing (briefly mentioned)
- Sequencing read types
 - Single end
 - Paired end

What we learned yesterday

- Sequencing technologies
- Key concepts:
 - Read length
 - Error types
 - Throughput
- 2nd generation vs 3rd generation sequencing

The problem at hand, storing DNA information in a computer



What is sequence data?

Sequences are stored in fasta-files

Header →

```
>gi|218693476|ref|NC_011748.1| Escherichia coli 55989 chromosome, complete genome
```

```
GTAAGTATTTTTCAGCTTTTCATTCTGACTGCAACGGGCAATATGTCTCTGTGTGGATTAAAAAAGAGT
```

```
GTCTGATAGCAGCTTCTGAACTGGTTACCTGCCGTGAGTAAATTTAAATTTTATTGACTTAGGTCACTAA
```

Sequence →

```
ATACTTTAACCAATATAGGCATAGCGCACAGACAGATAAAAATTACAGAGTACACAACATCCATGAAACG
```

```
CATTAGCACCACCATTACCACCACCATCACCATTACCACAGGTAACGGTGCGGGCTGACGCGTACAGGAA
```

```
ACACAGAAAAAAGCCCGCACCTGACAGTGC GGCTTTTTTTTTCGACCAAAGGTAACGAGGTAACAACCAT
```

```
GCGAGTGTGAAAGTTTCGGCGGTACATCAGTGGCAAATGCAGAACGTTTTCTGCGTGTGCCGATATCTG
```

```
GAAAGCAATGCCAGGCAGGGGCAGGTGGCCACCGTCCTCTCTGCCCCCGCCAAAATCACCAACCACCTGG
```

```
TGGCGATGATTGAAAAAACCATTAGCGGCCAGGATGCTTTACCCAATATCAGCGATGCCGAACGTATTTT
```

```
TGCCGAACTTTTGACGGGACTCGCCGCCGCCAGCCGGGGTTCCCGCTGGCGCAATTGAAAACTTTTCGTC
```

```
GATCAGGAATTTGCCCAAATAAAACATGTCCTGCATGGCATTAGTTTGTGGGGCAGTGCCCGGATAGCA
```

E.coli ~ 4.5 - 6 Mbases

Human ~ 3.2 Gbases

Reminder: things ~~will~~ can go wrong

template
read

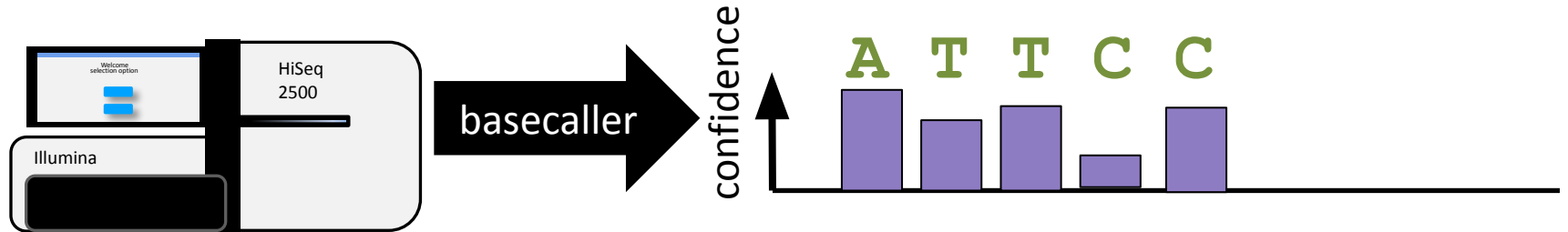
mismatch

AGCAATCTCAATTACAAATATACACCAACAAA
AGCAATCTCAATTACAGATATACACCAACAAA

AGCAATCTCAATTACA-AATATACACCAACAA
AGCAATCTCAATTACACGATATACACCAACAA

AGCAATCTCAATTACAAATATACACCAACAAA
AGCAATCTCAATTACA-ATATACACCAACAAA

Basecalling

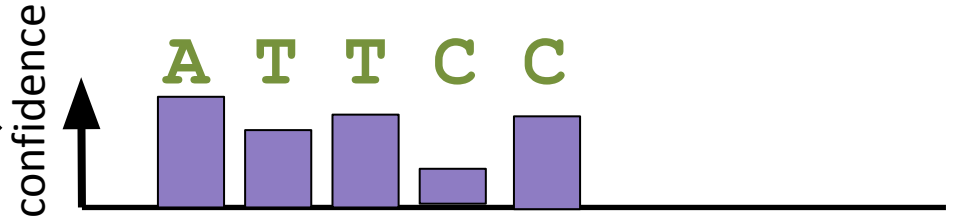
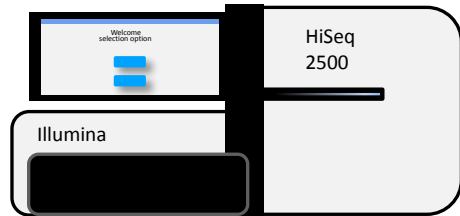


Quality predictor values of individuals bases:

- Intensity profiles
- phasing
- signal to noise ratio

Depends on machine, chemistry, software

Basecalling



@ILLUMINA-C90280_0030_FC:5:1:2675:1090#NNNNNN/1

A T T C C C G ...

0.6837722 0.9369043 0.9920567 0.9996019 0.9997488 0.9993690 0.9999000 ...



How to store the probability that a base is wrong?

Idea#1:

Store a floating-point number for each base:

```
@ILLUMINA-C90280_0030_FC:5:1:2675:1090#NNNNNN/1
```

```
ATTCCCGGCCTTTTCCAGGCCTGCCTGCTCGAGC
```

```
+ . . . .
```

```
0.6838 0.9369 0.9921 0.9996 0.9997 0.9994 0.9999
```

Not great
(4 bytes/qual score)

'6'
'8'
'3'
'8'

How to store the probability that a base is wrong?

Idea#2:

Store $10 \times \log_{10}$ of a floating-point number for each base:

@ILLUMINA-C90280_0030_FC:5:1:2675:1090#NNNNNN/1

ATTCCCGGCCTTTTCCAGGCCTGCCTGCTCGAGC



5 12 20 34 36 32 40

better...

(2 bytes/qual score)

'0'
'5'

Phred-scale

$$Quality = -10 \times \log_{10}(P_{error})$$

$$P_{error} = 10^{\frac{-Quality}{10}}$$

Q ~ Prob

10 ~ 0.1

20 ~ 0.01

30 ~ 0.001

40 ~ 0.0001

How to store the probability that a base is wrong?

Idea#3:

Store the ASCII character corresponding to $10 \times \log_{10}$ of a floating-point number for each base:

@ILLUMINA-C90280_0030_FC:5:1:2675:1090#NNNNNN/1
 ATTCCCGGCCTTTTTCCAGGCCTGCCTGCTCGAGC
 ↓ ↓ . . .
 &-5CEAI

much better...
 (1 bytes/qual score)

'&'

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			

Then what is NGS data?

Fastq

Header → @ILLUMINA-C90280_0030_FC:5:1:2675:1090#NNNNNN/1

Sequence → ATTCCCGGCCTTTTTCCAGGCCTGCCTGCTCGAGC

+
Qualities → BAAAGECEE<EEDFEDF3DBDBB=A+==>9>>88?
(probability that basecall is wrong)

Millions to billions of these...

Phred scale (offset 33 vs 64)

@ILLUMINA-C90280_0030_FC:5:1:2675:1090#NNNNNN/1

ATTCCCGGCCTTTTCCAGGCCTGCCTGCTCGAGC

+

BAAAGECEE<EEDFEDF3DBDBB=A+==>9>>88?

Q ~ Prob

10 ~ 0.1

20 ~ 0.01

30 ~ 0.001

40 ~ 0.0001

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
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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			

ASCII_BASE=64 Old Illumina											
Q	P_error	ASCII	Q	P_error	ASCII	Q	P_error	ASCII	Q	P_error	ASCII
0	1.00000	64 @	11	0.07943	75 K	22	0.00631	86 V	33	0.00050	97 a
1	0.79433	65 A	12	0.06310	76 L	23	0.00501	87 W	34	0.00040	98 b
2	0.63096	66 B	13	0.05012	77 M	24	0.00398	88 X	35	0.00032	99 c
3	0.50119	67 C	14	0.03981	78 N	25	0.00316	89 Y	36	0.00025	100 d
4	0.39811	68 D	15	0.03162	79 O	26	0.00251	90 Z	37	0.00020	101 e
5	0.31623	69 E	16	0.02512	80 P	27	0.00200	91 [38	0.00016	102 f
6	0.25119	70 F	17	0.01995	81 Q	28	0.00158	92 \	39	0.00013	103 g
7	0.19953	71 G	18	0.01585	82 R	29	0.00126	93]	40	0.00010	104 h
8	0.15849	72 H	19	0.01259	83 S	30	0.00100	94 ^	41	0.00008	105 i
9	0.12589	73 I	20	0.01000	84 T	31	0.00079	95 _	42	0.00006	106 j
10	0.10000	74 J	21	0.00794	85 U	32	0.00063	96 `			

Read types

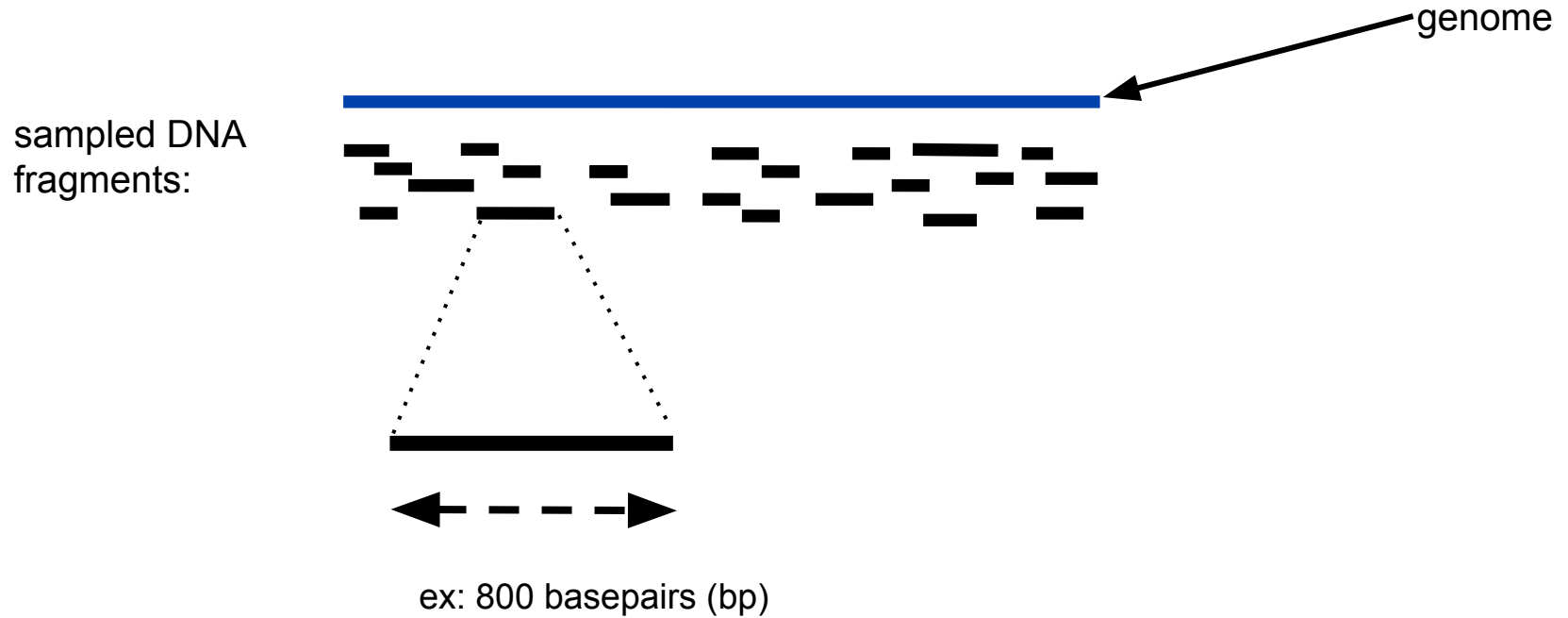
genome

sampled DNA
fragments:

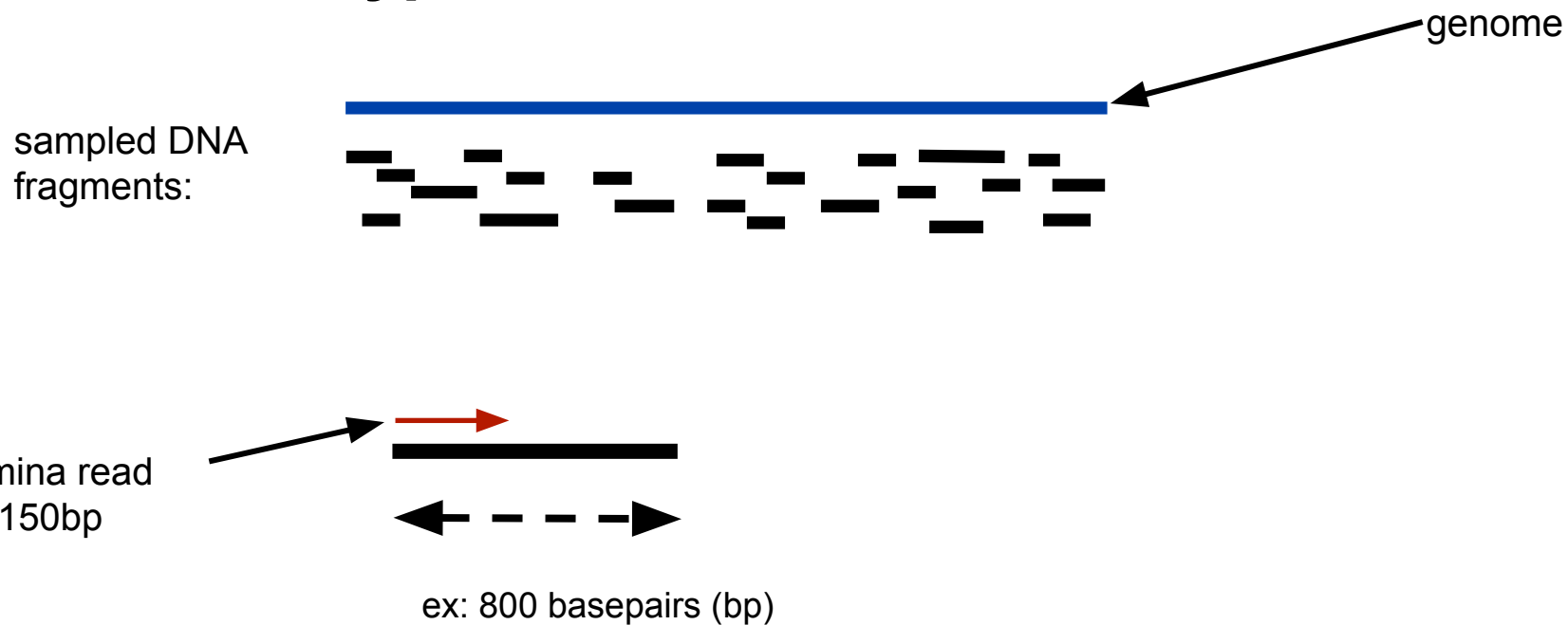


aka "inserts"

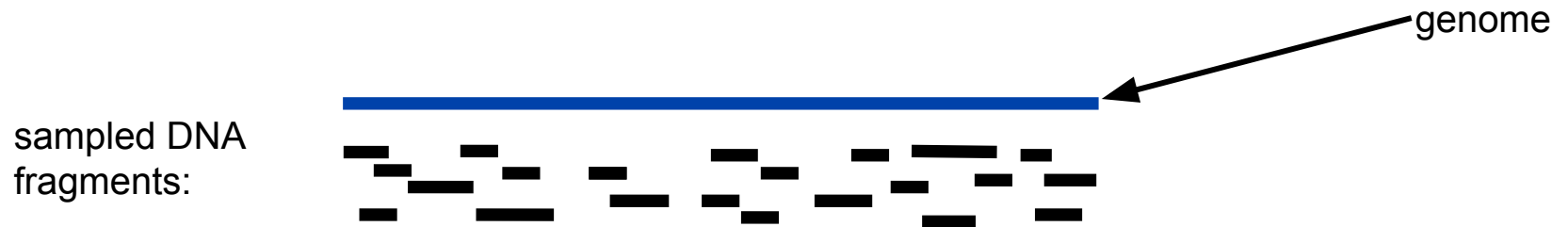
Read types



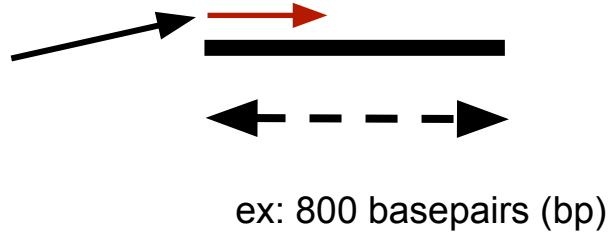
Read types



Read types

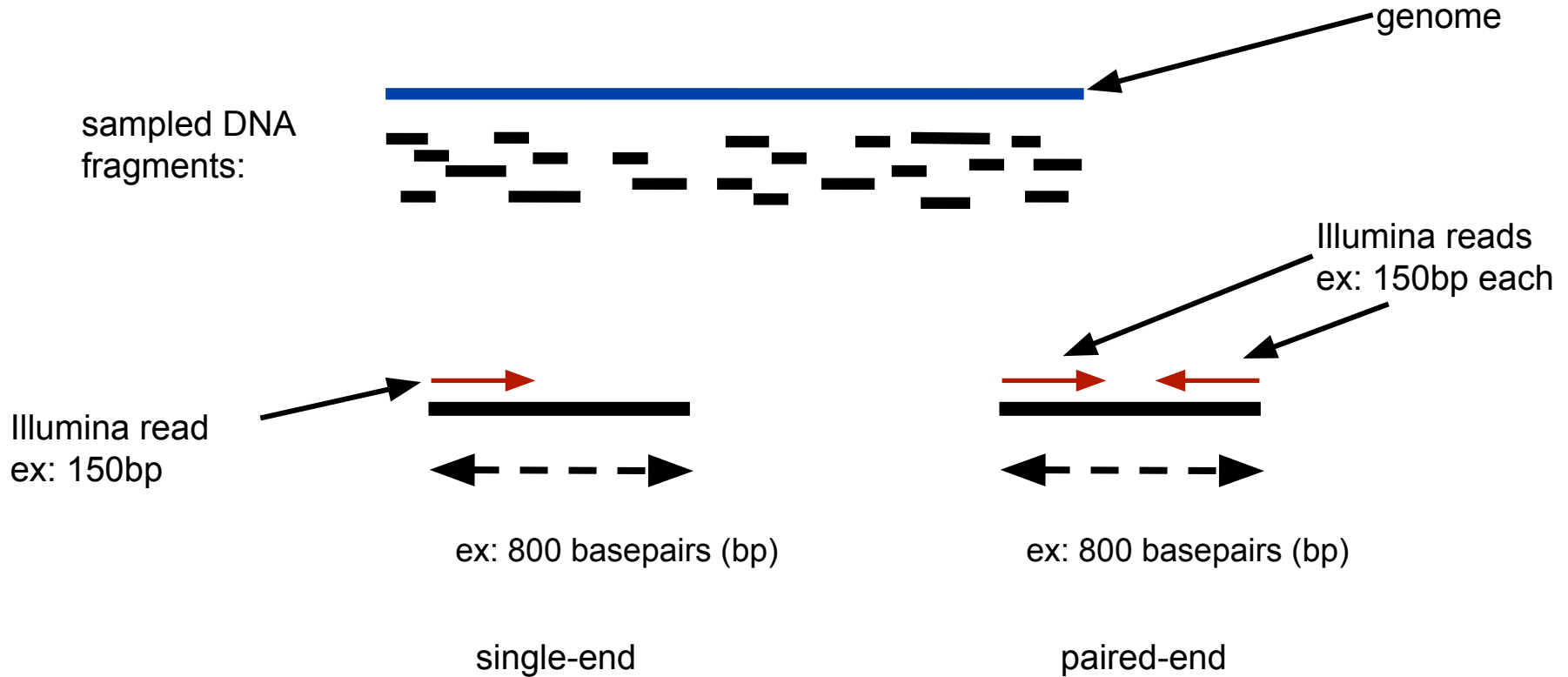


Illumina read
ex: 150bp

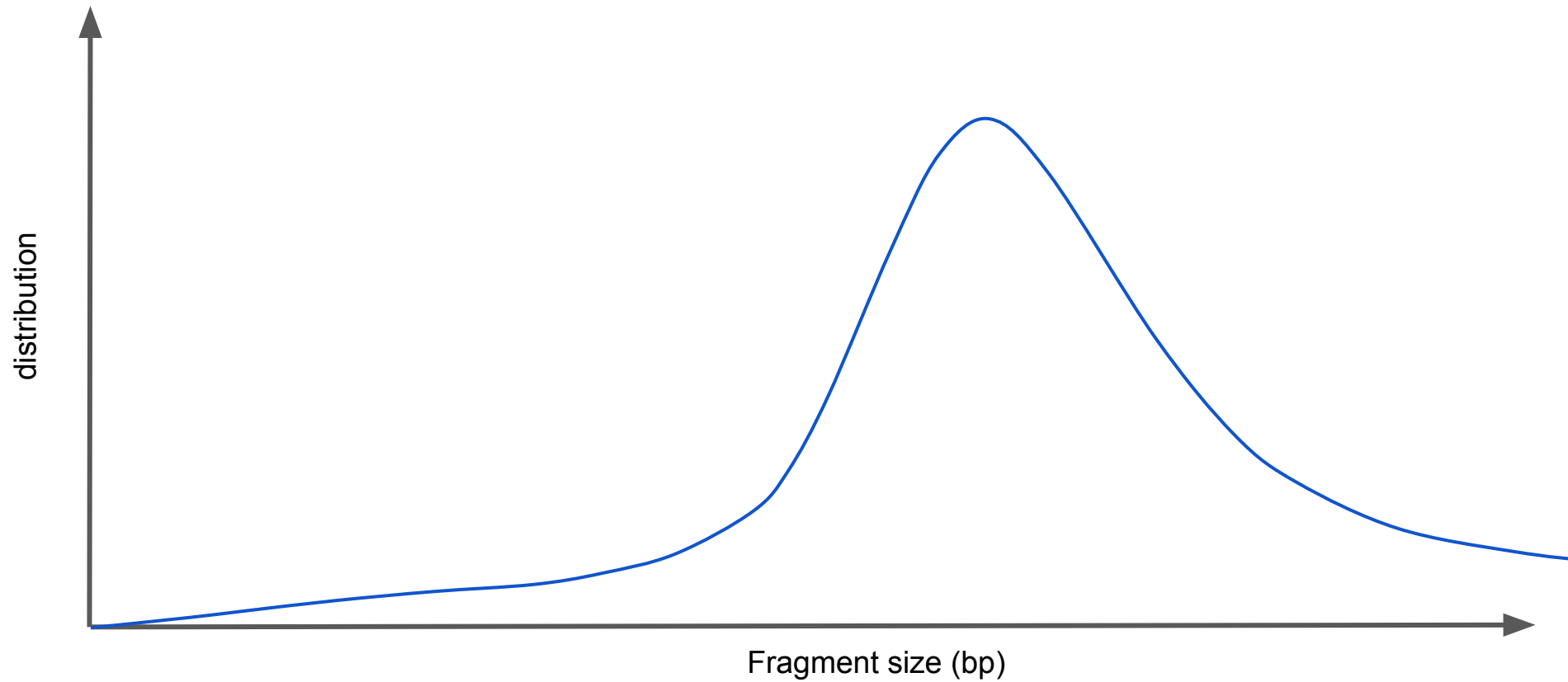


single-end

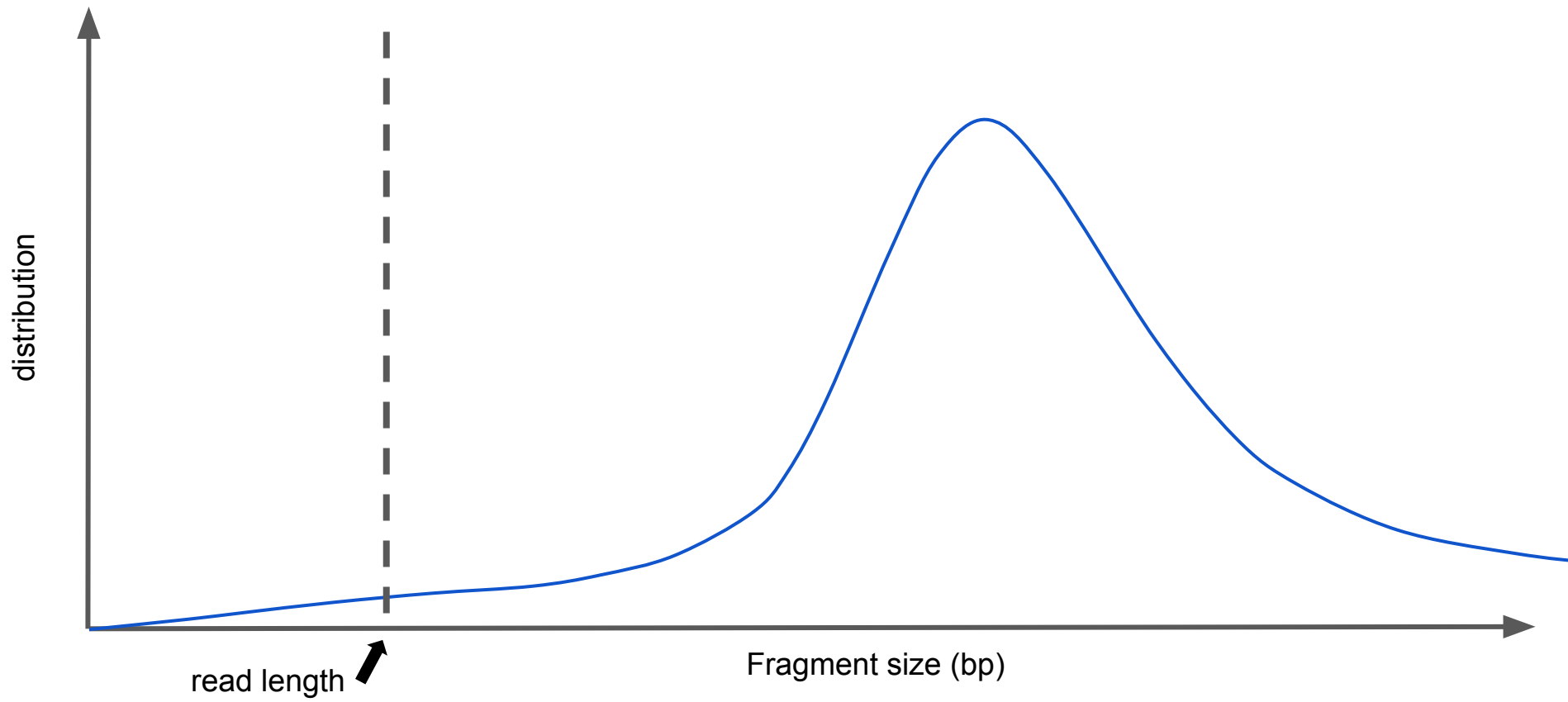
Read types

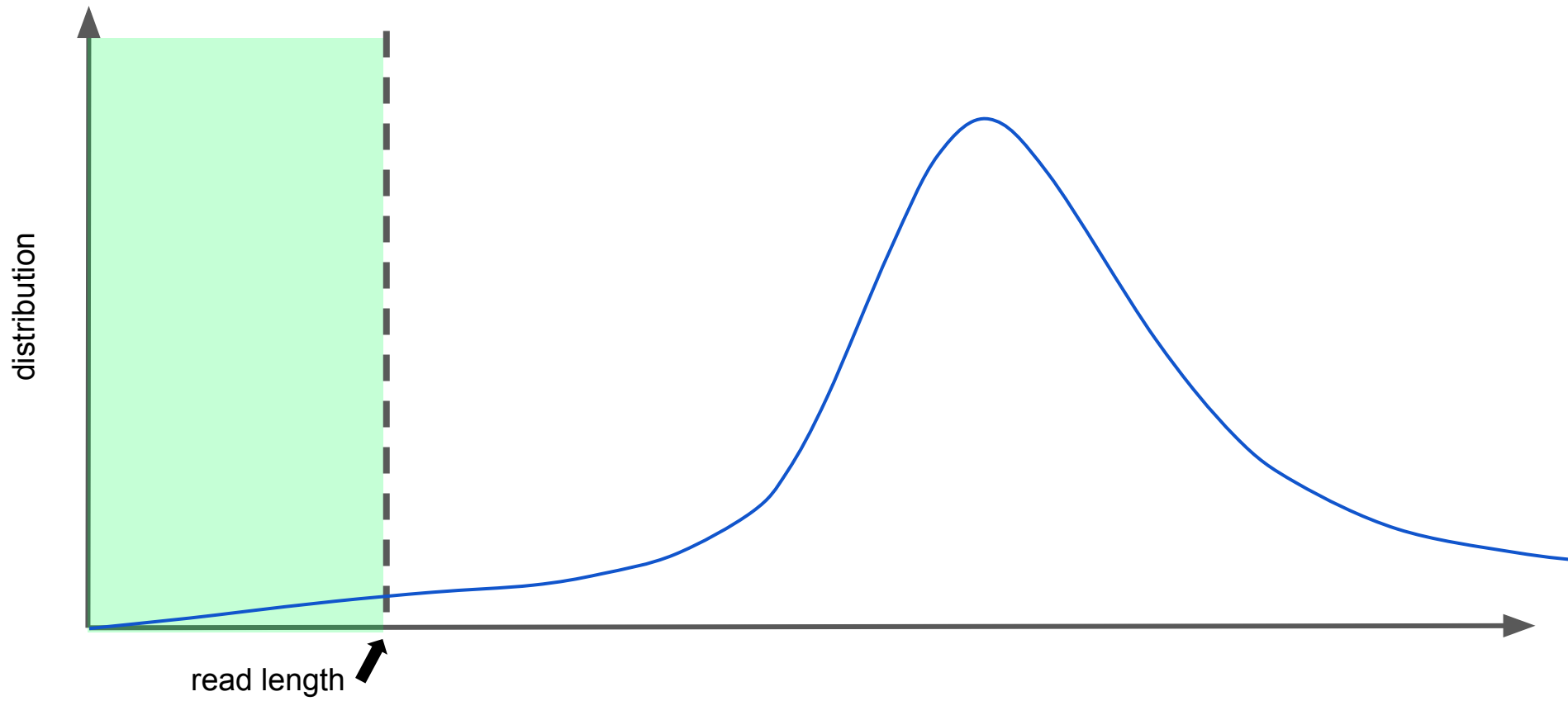
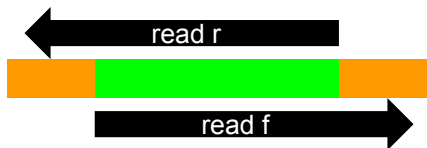


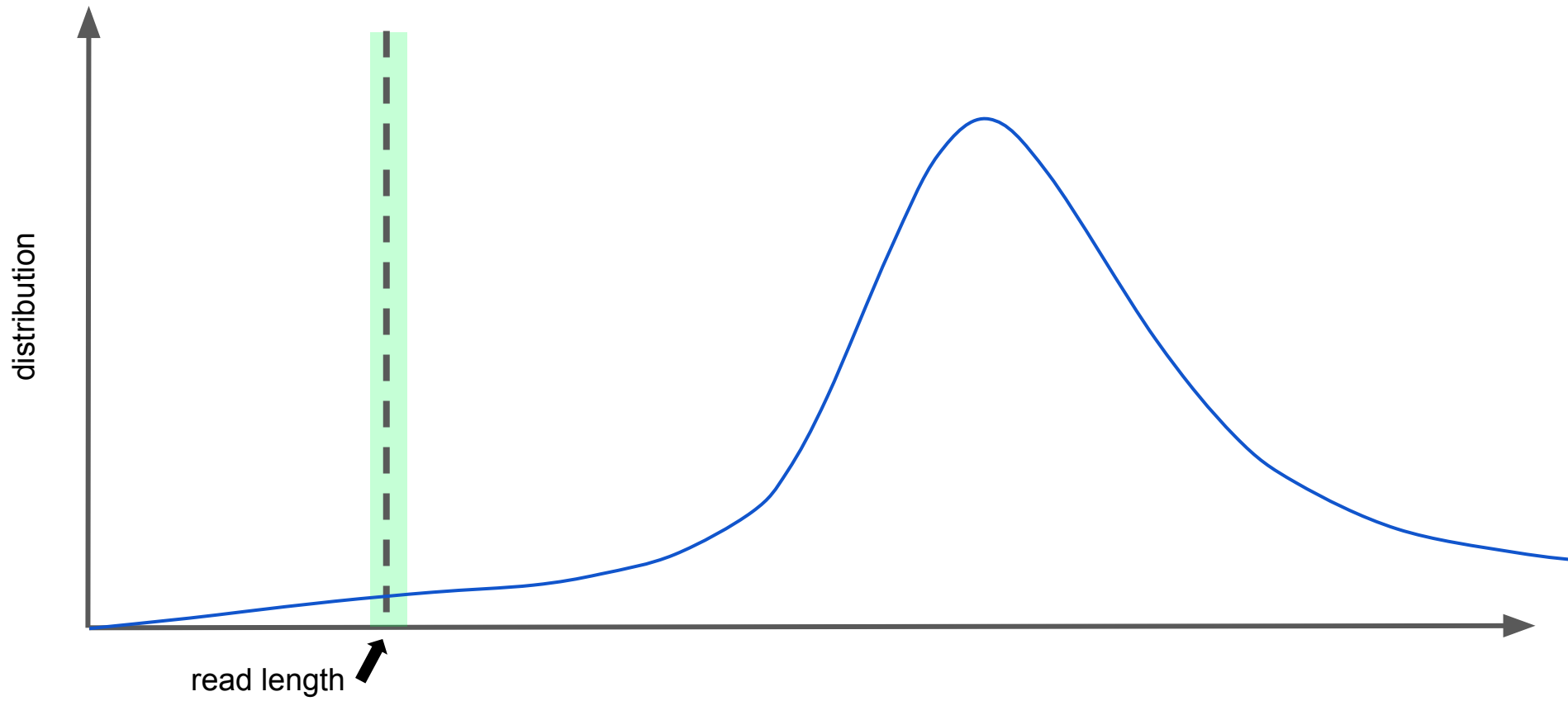
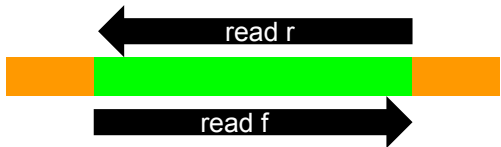
Insert size distribution

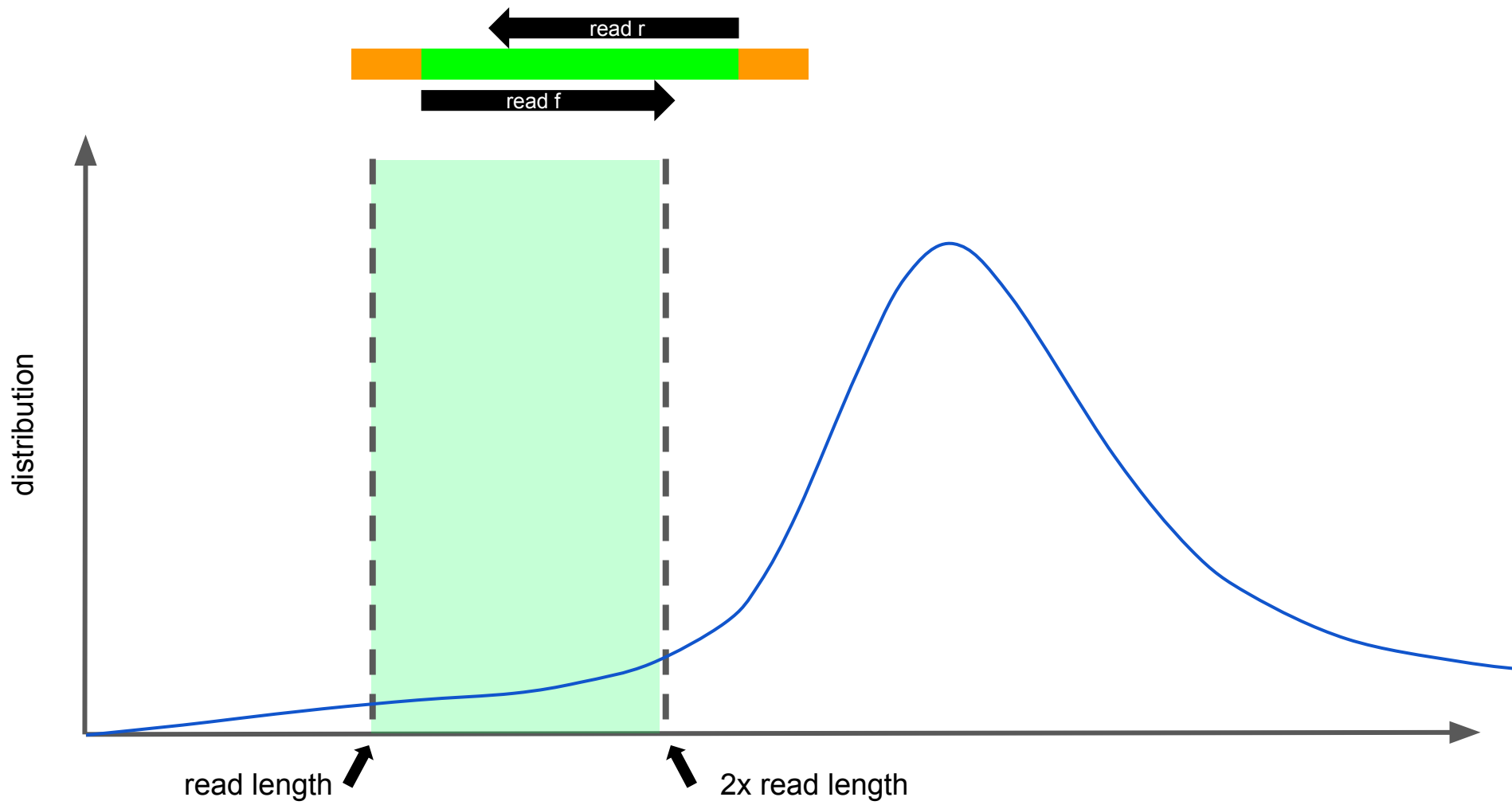


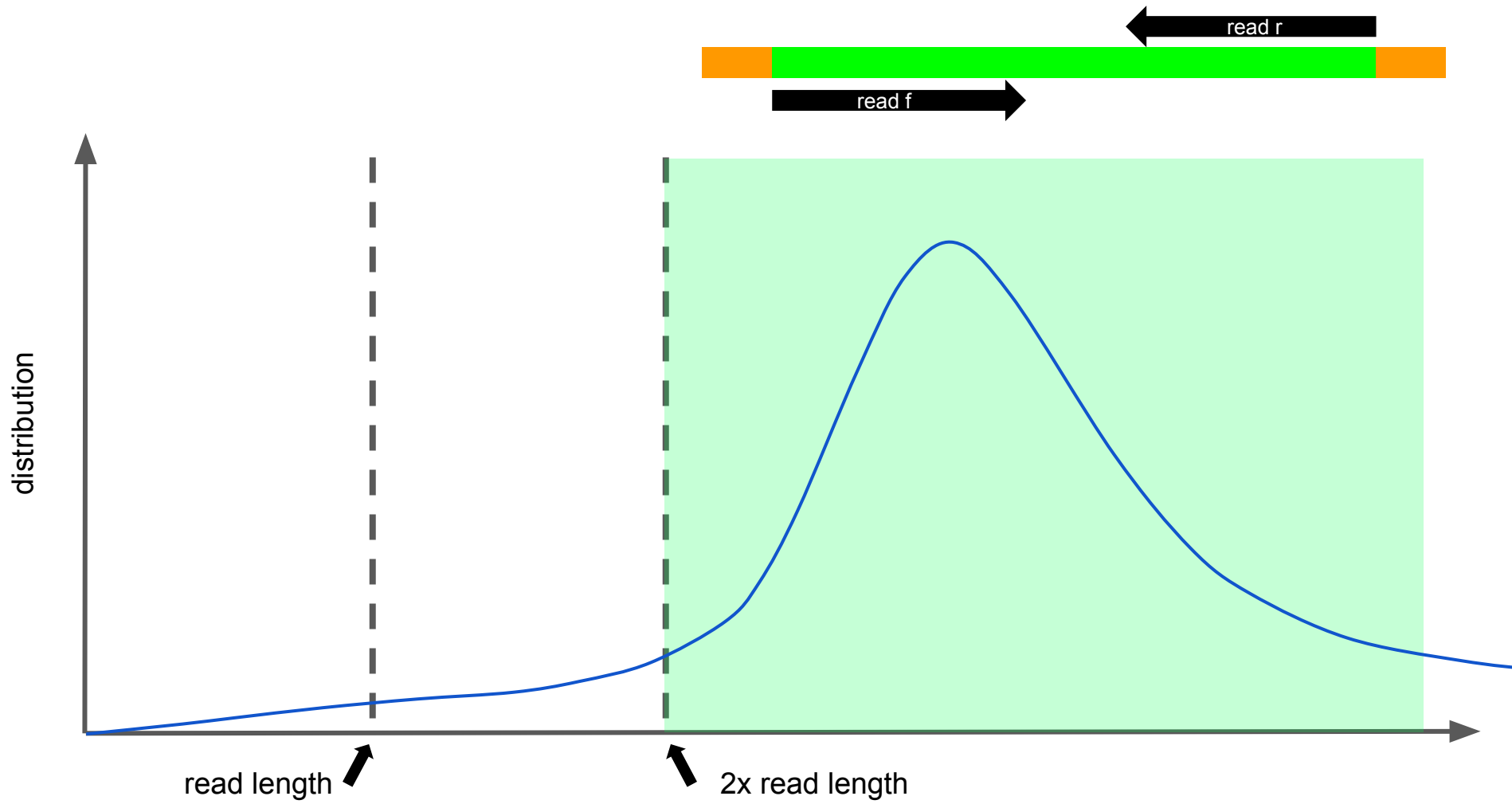
Insert size distribution

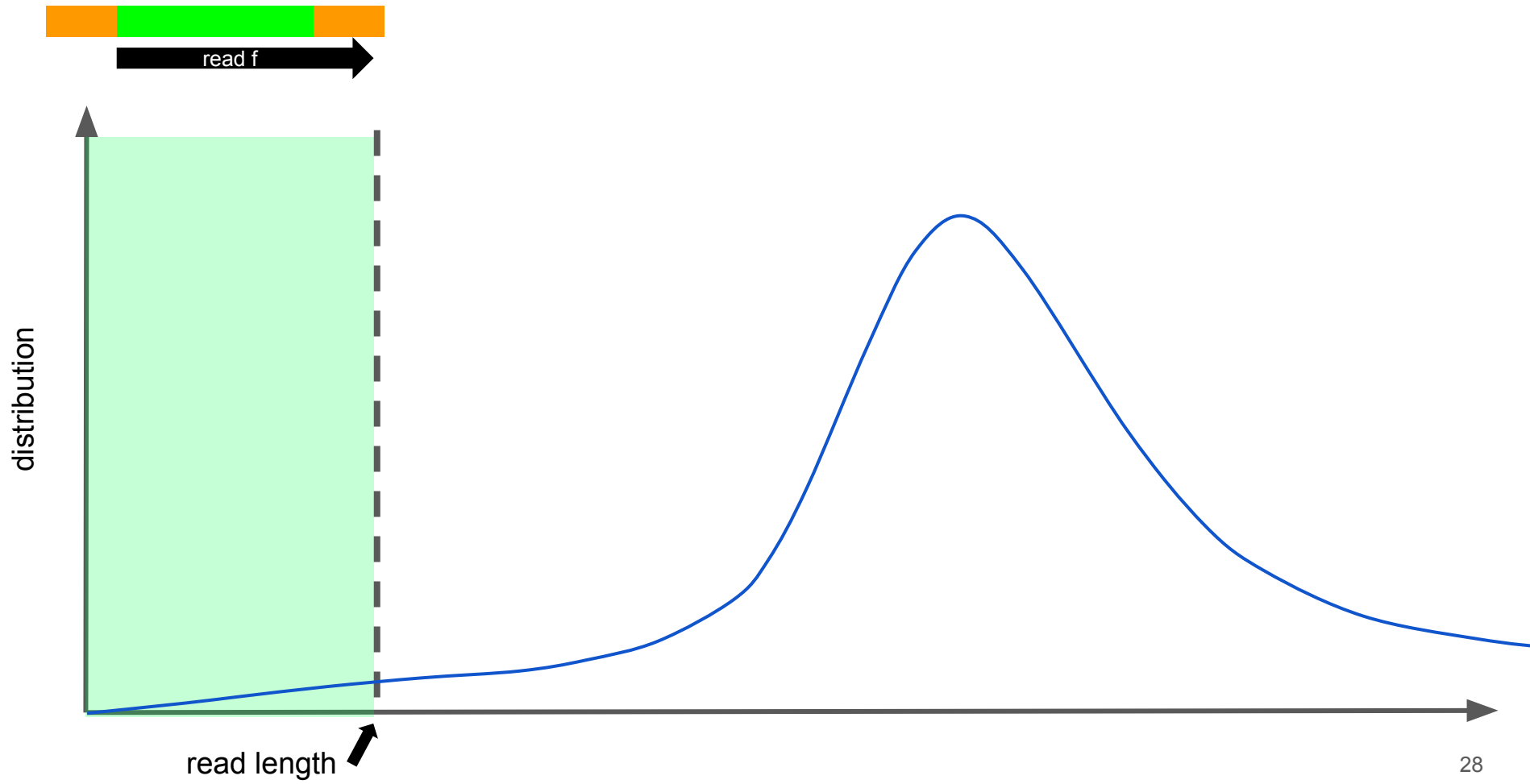






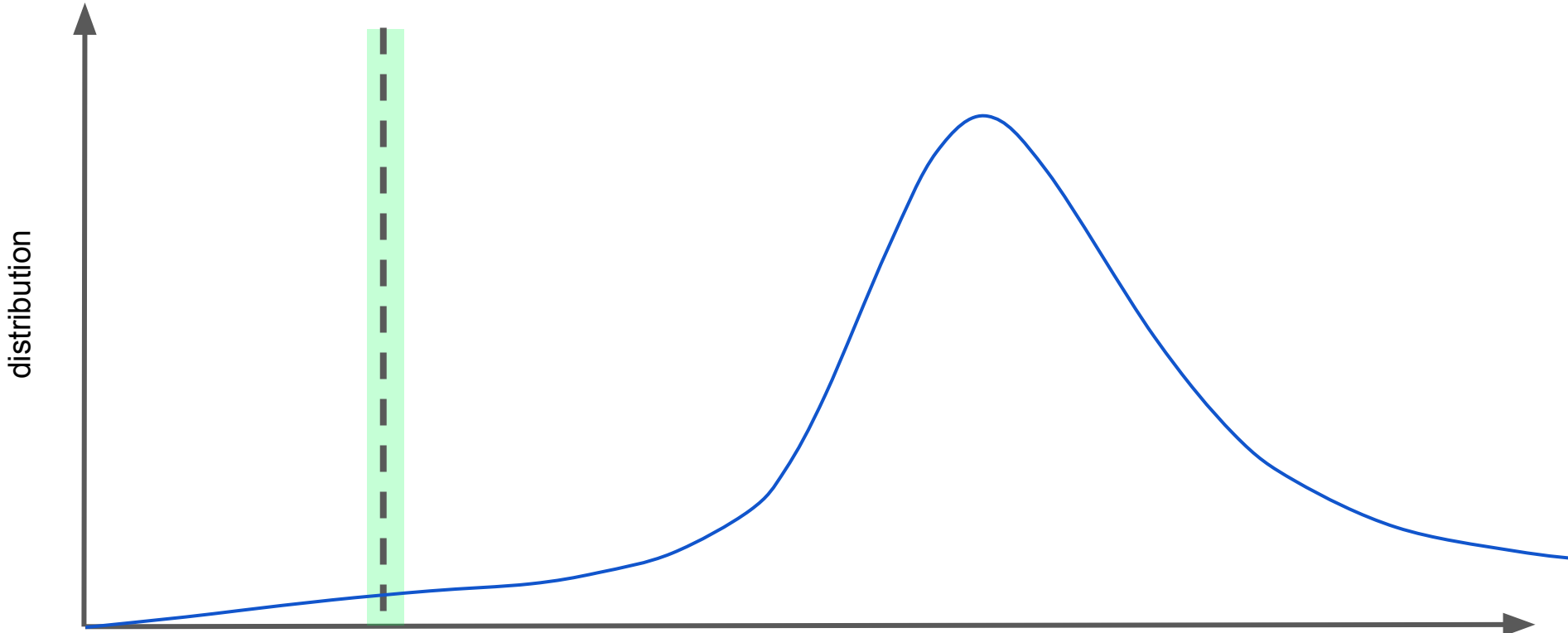




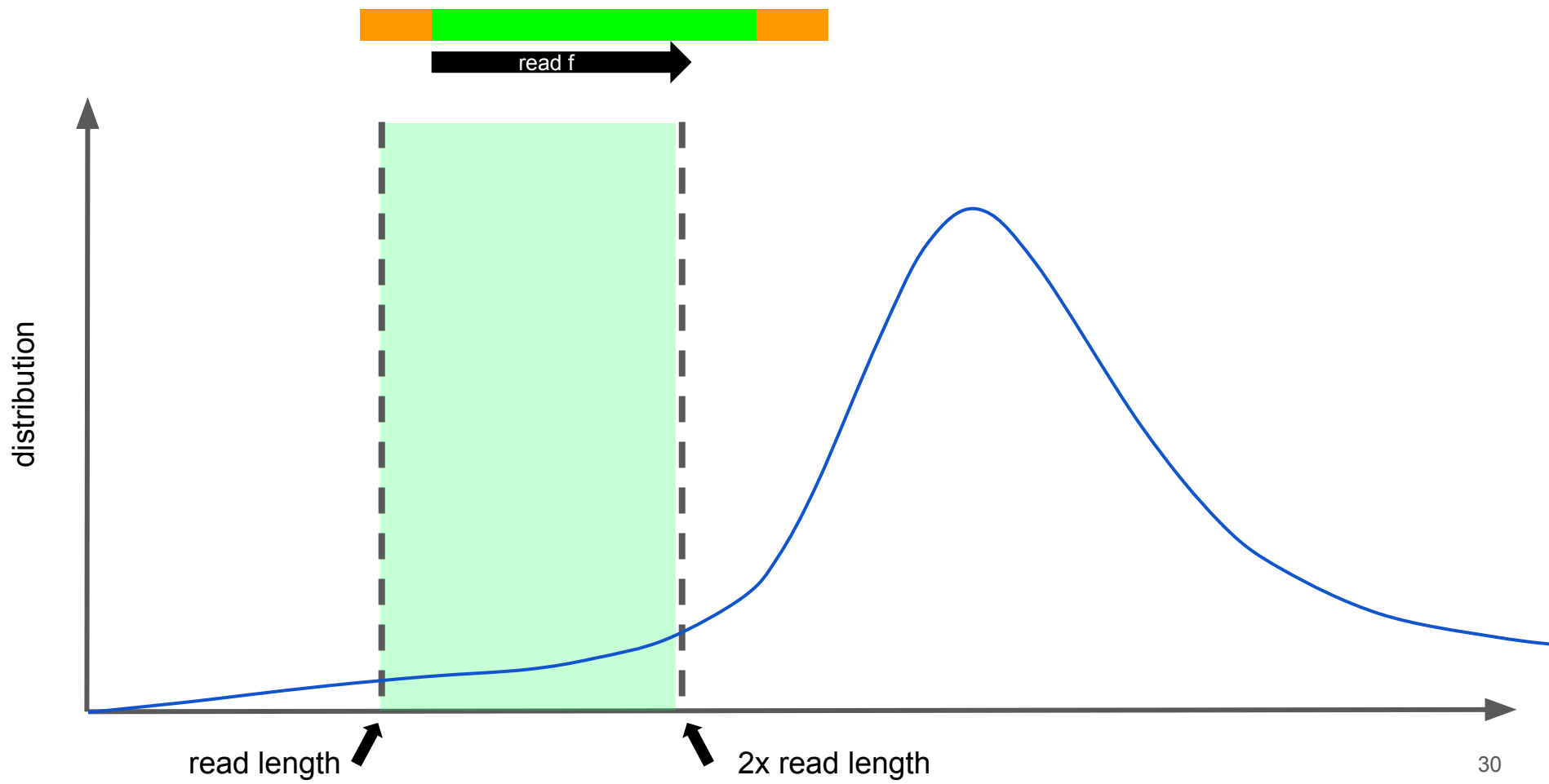


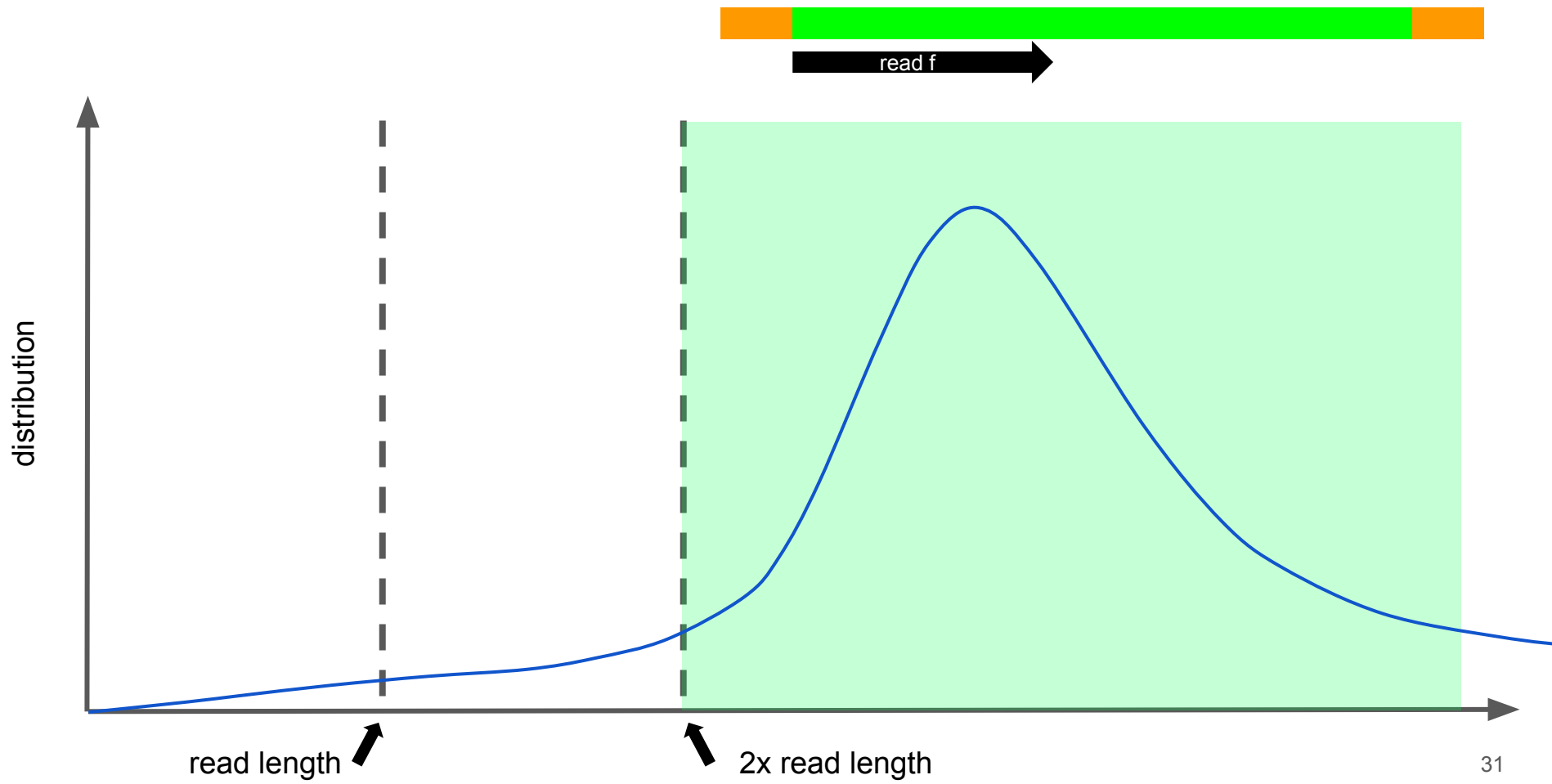


read f

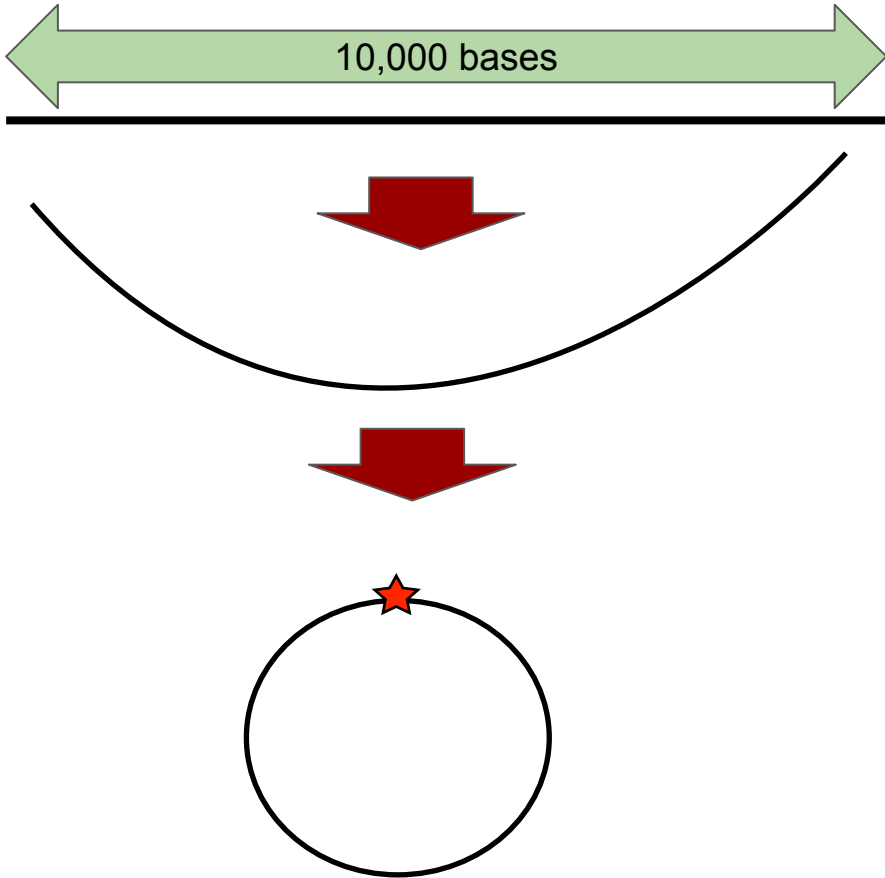


read length

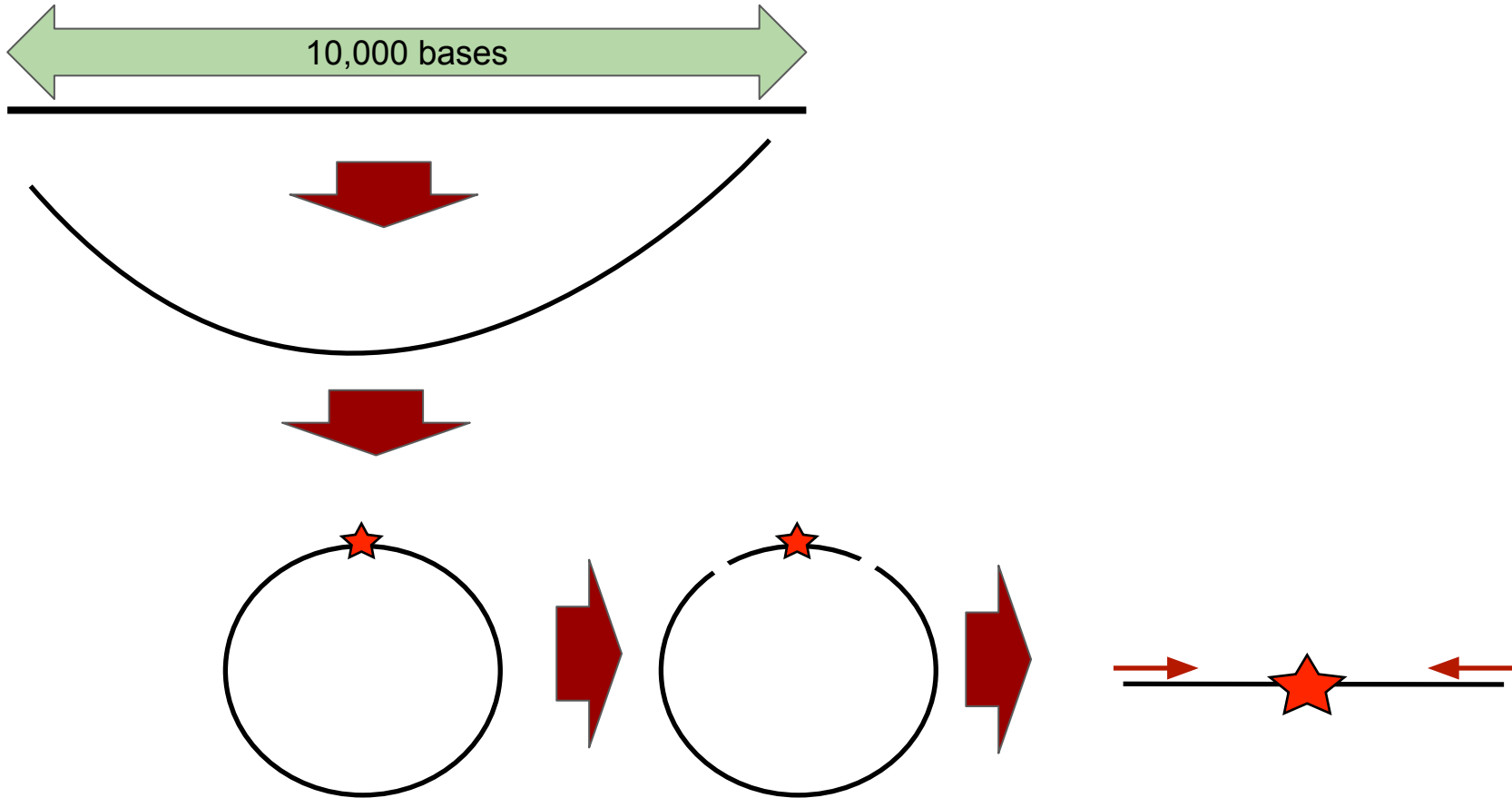




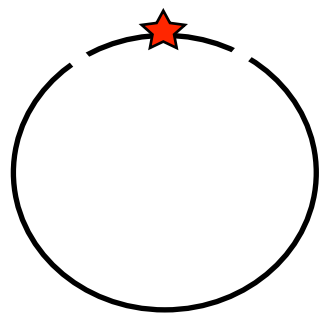
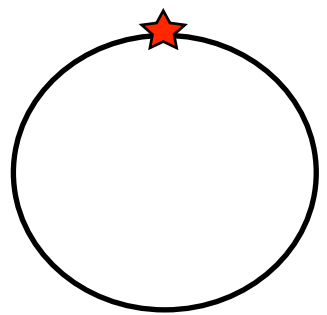
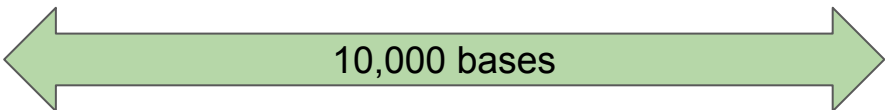
Mate pair reads (for assembly)



Mate pair reads (for assembly)



Mate pair reads



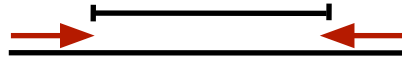
Read orientation

Single end



Forward

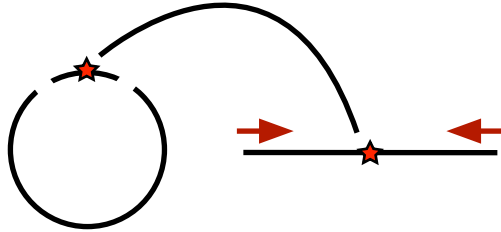
Paired end



Illumina: Forward - Reverse



Mate pair



Illumina: Reverse - Forward

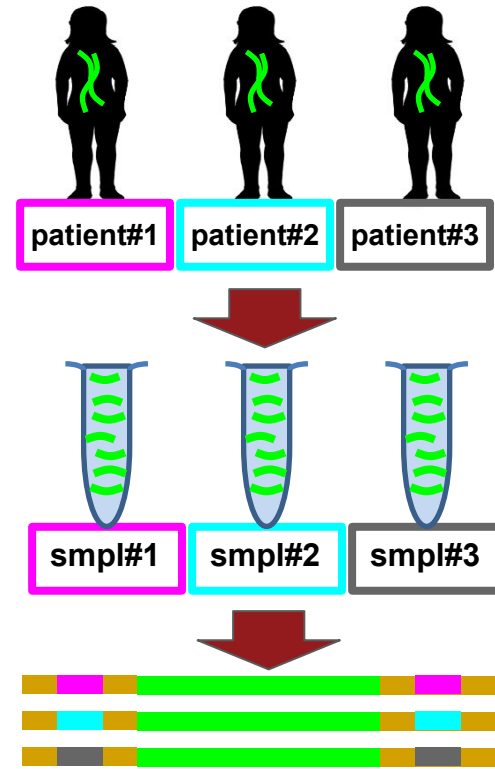


Different for other technologies!

Multiplexing/Demultiplexing

example: 100M reads per patient is sufficient

idea: pool samples together to save costs

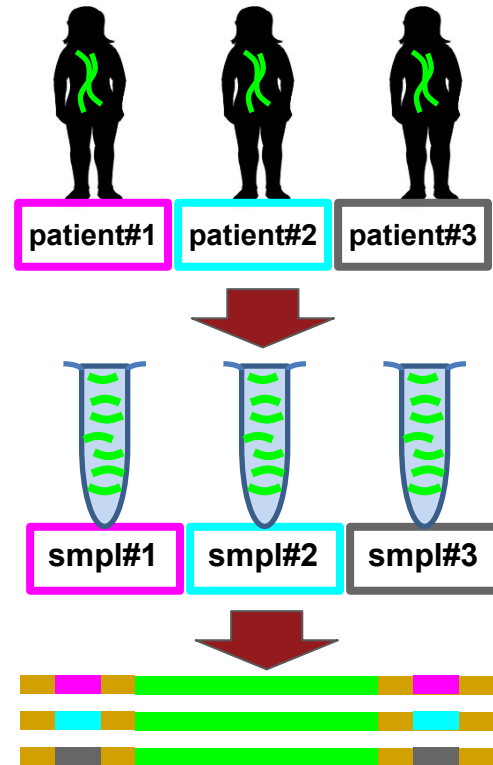


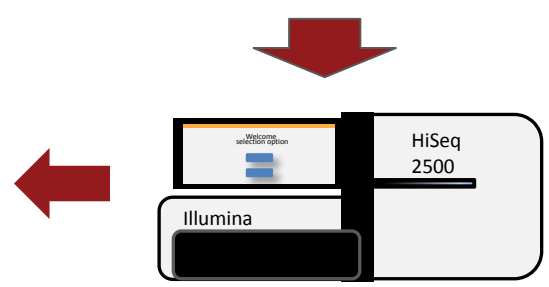
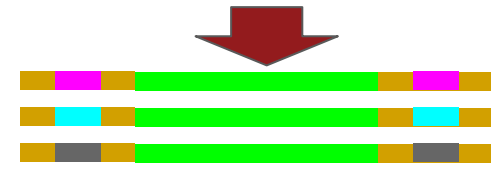
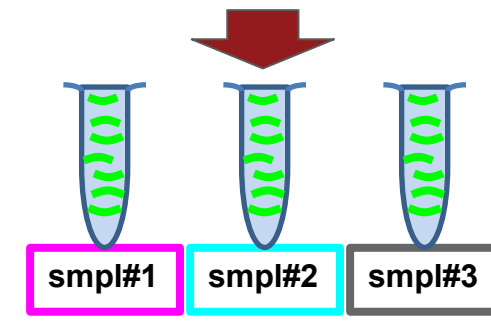
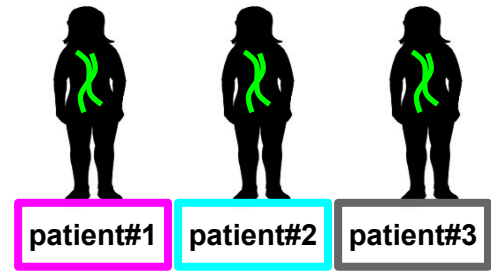
indices



TTCAGACTAGTTGGAAGTTTGTCT AGATCTCAGATTTTGTCACTAGAGGACGCACGCT
C
TTCAGACTAGTTGGAAGTTTGTCT TTCACTAAGATTTTGTCACTAGAGGACGCACGCT
C
TTCAGACTAGTTGGAAGTTTGTCT GACTAGTAGATTTTGTCACTAGAGGACGCACGCT
C

Multiplexing: in the wet lab

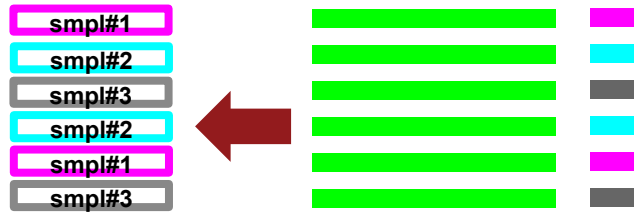
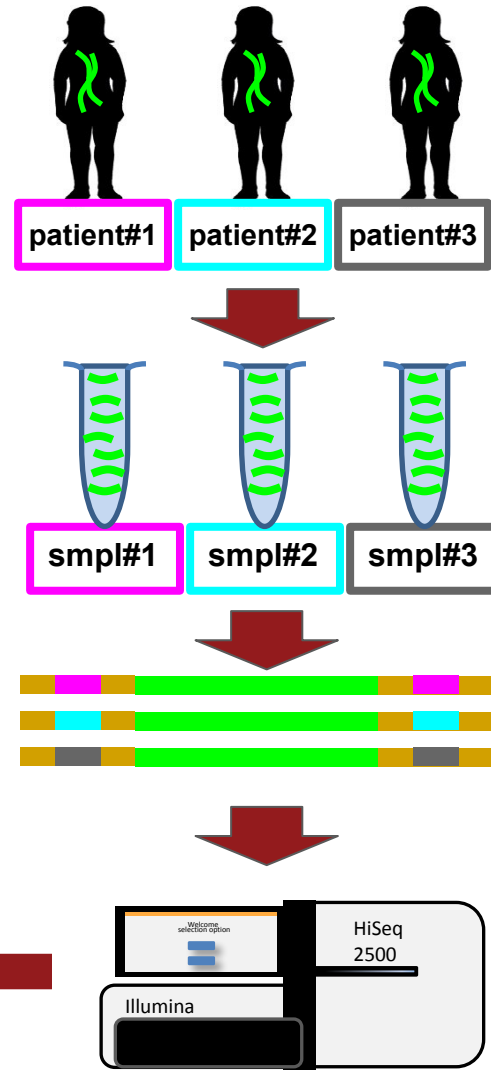




Basecalling



Demultiplexing: computational



Takeaways

- Sequence data storage
 - Fasta format
 - Fastq format + quality scores
- Learn when to use which
- Multiplexing/Demultiplexing
- Sequencing read types (single, paired, mate-pair)

Exercise time!