Quality trimming

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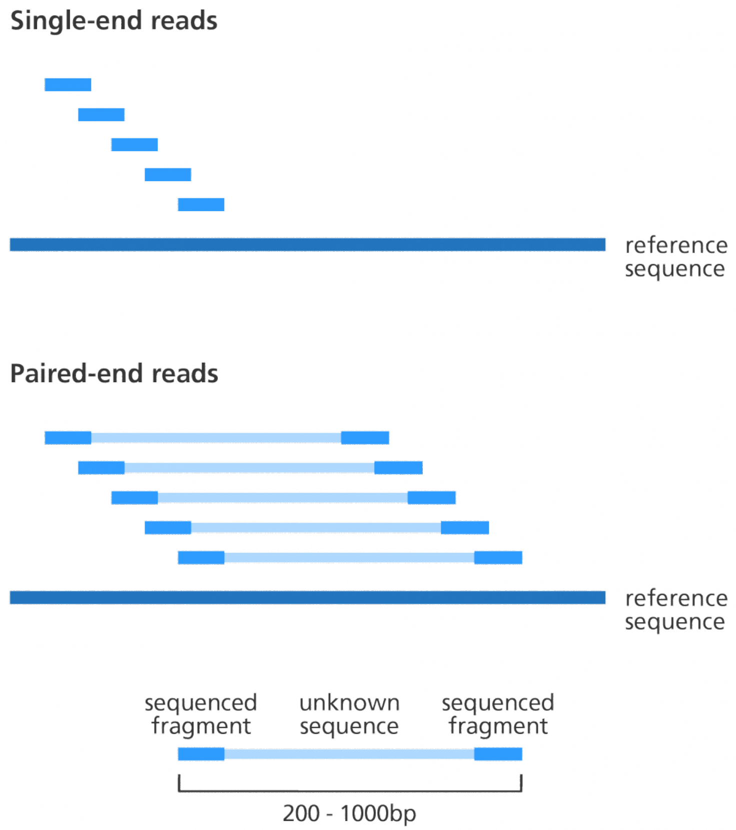
Raw data Illumina

- We have paired end data on the sequenced genomes

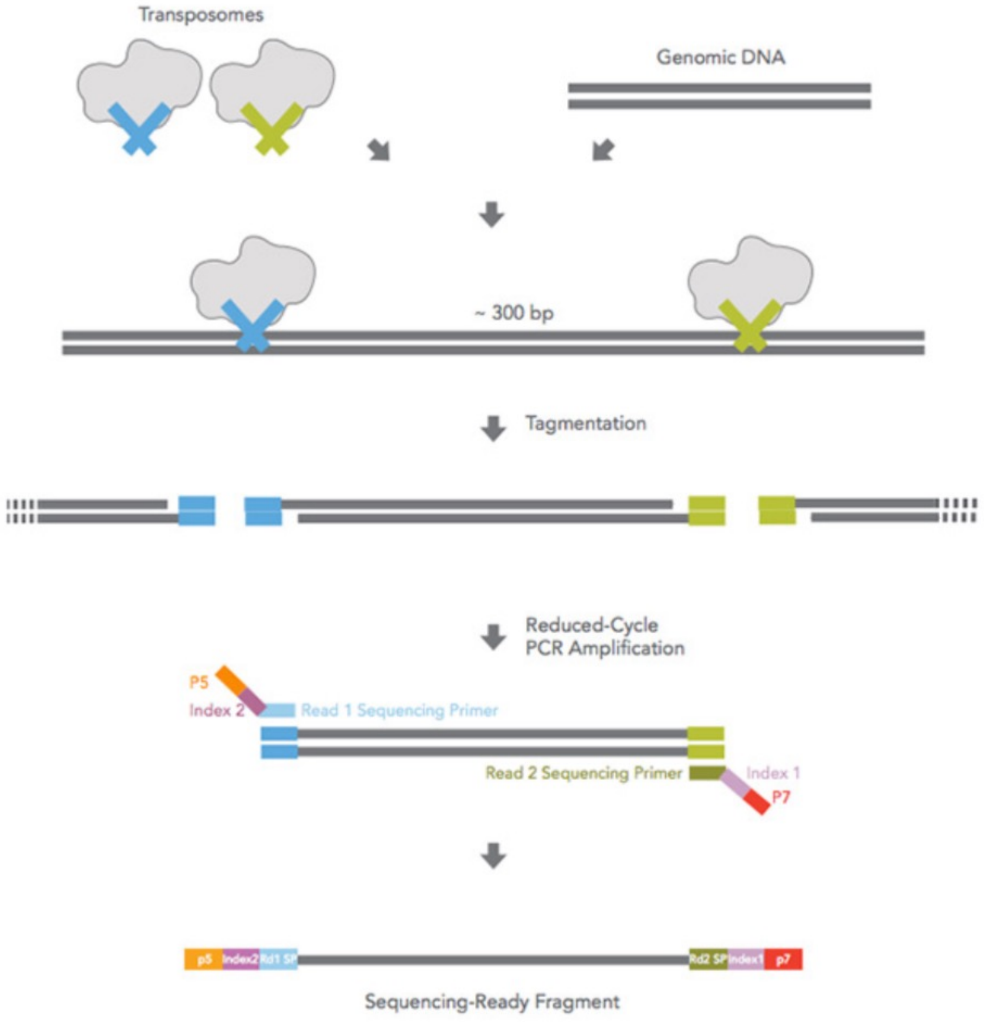
  A004_07004-B_TTGCATGT_GACTCGCA_run20171107N_S4_R1_001.fastq

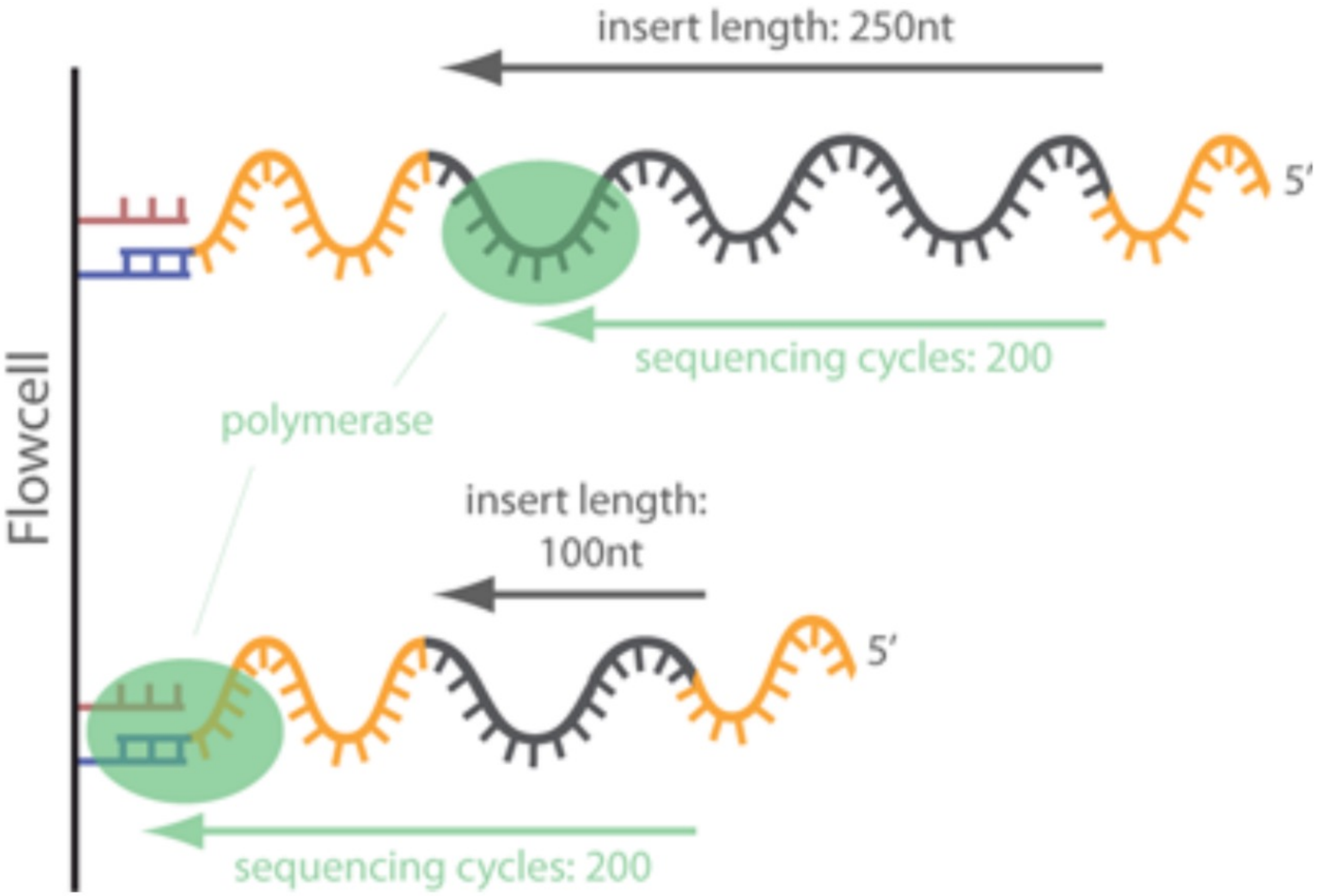
  A004_07004-B_TTGCATGT_GACTCGCA_run20171107N_S4_R2_001.fastq

- 150 + 150 bp
- Can contain
  - Sequence adapters
  - low quality sequence (usually in the end)
    - Occurrence: substitutions > indels
    - Quality scores: substitutions < indels
    - Overall quality: R1 > R2; beginning > end
- Need to check quality and trim the reads



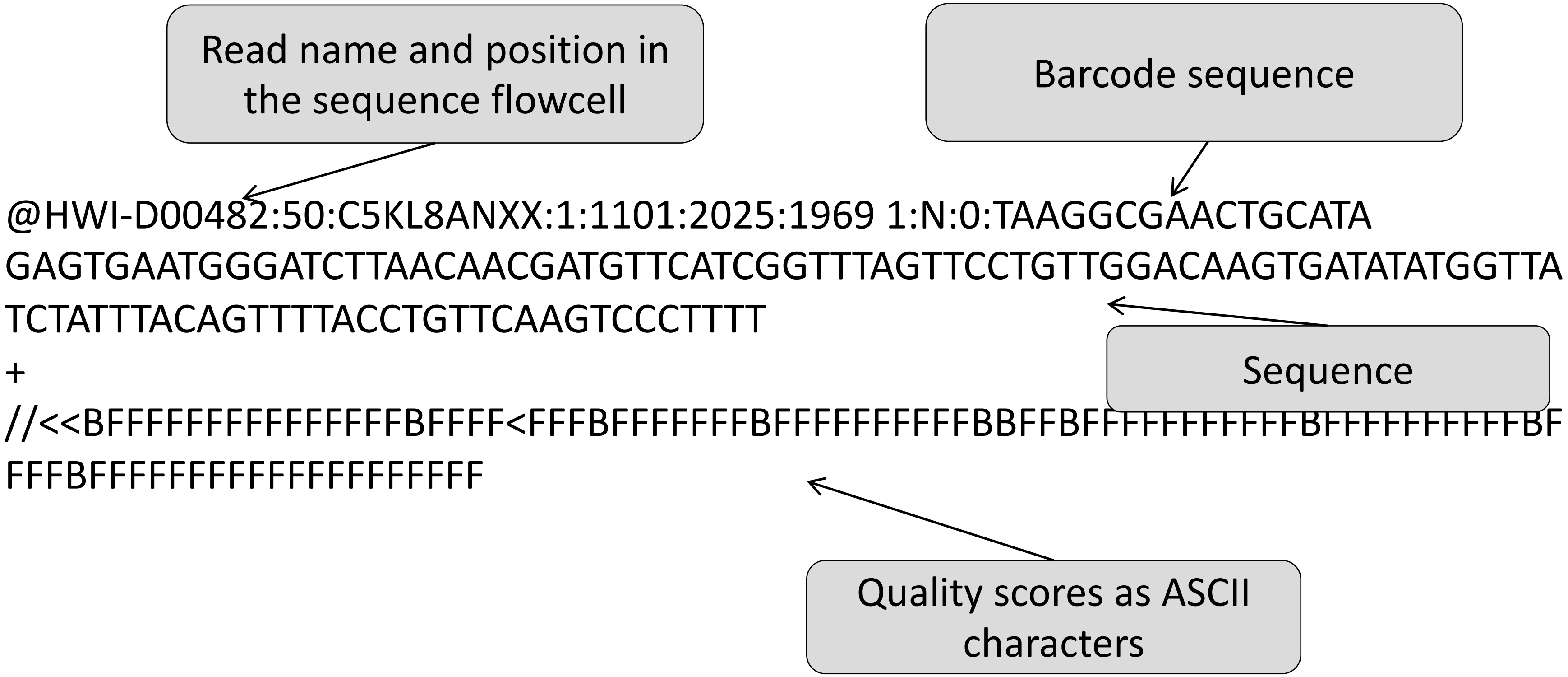
Adapter contamination





Fastq

- Sequence data is commonly delivered in FASTQ format. No chromatograms!



Read name and position in the sequence flowcell

Barcode sequence

```
@HWI-D00482:50:C5KL8ANXX:1:1101:2025:1969 1:N:0:TAAGGCGAACTGCATA
GAGTGAATGGGATCTTAACAACGATGTTCATCGGTTTAGTTCCTGTTGGACAAGTGATATATGGTTA
TCTATTTACAGTTTTACCTGTTCAAGTCCCTTTT
+
//<<BFFFFFFFFFFFFFFFBFFFF<FFFBFFFFFFFBFFFFFFFFFFBBFFBFFFFFFFFFFFBFFFFFFFFFFBF
FFFBFFFFFFFFFFFFFFFFFFFF
```

Sequence

Quality scores as ASCII characters

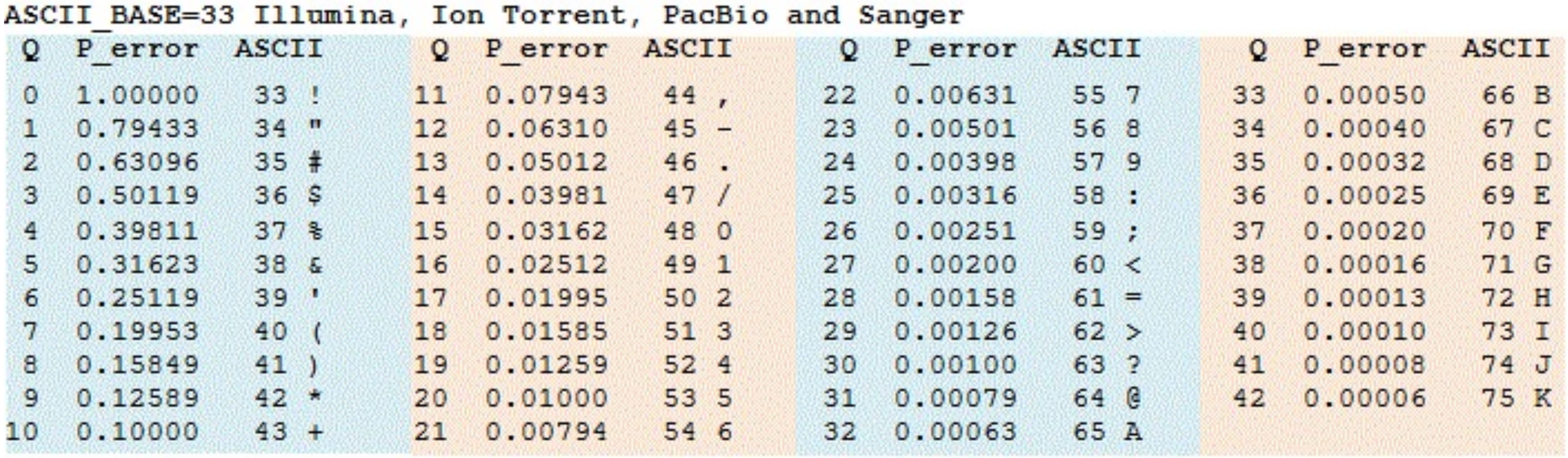
Quality scores

- measure of the quality of the identification of the bases generated by sequencer
- Phred-score

Phred Quality Score	Probability of incorrect base call	Base call accuracy	ASCHII
10	1 in 10	90%	+
20	1 in 100	99%	5
30	1 in 1000	99.9%	?
40	1 in 10000	99.99%	I

- Phred score above 20-25 considered as acceptable
  - 1 mistake in 100

```
@HWI-D00482:50:C5KL8ANXX:1:1101:2025:1969 1:N:0:TAAGGCGAACTGCATA
GAGTGAATGGGATCTTAACAACGATGTTCATCGGTTTAGTTCCTGTTGGACAAGTGATATATGGTTATCT
ATTTACAGTTTTACCTGTTCAAGTCCCTTTT
+
//<<BFFFFFFFFFFFFFFFBFFFF<FFFBFFFFFFFBFFFFFFFFFFBBFFBFFFFFFFFFFFBFFFFFFFFFFBFFFF
BFFFFFFFFFFFFFFFFFFFF
```



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			

FASTQC

- Quality assessment program
  - How the data looks like. No trimming.
  - http://www.bioinformatics.babraham.ac.uk/projects/fastqc/

- Output of FASTQC is a zip archive and an HTML document
- Combine files with **multiqc**

- View the HTML in web browser

How does the data look like?

- Where is the best quality sequence?
  - Begin, middle, end?
- Are there adapters?
  - What are adapters? Why to remove?
- Differences in R1 and R2?
  - Forward and reserve reads

What kind of trimming do you think should be done?

Quality filtering

- Removal of low-quality regions and adapters

- Several programs available, we will use **cutadapt**
  http://cutadapt.readthedocs.io/en/stable/

Cutadapt

- When looking at the cutadapt manual, which flags (=“-letter”) are for
    - Length trimming ____
    - 3’ adapter ____
    - Paired end 3’adapter ____
    - Quality score ____
    - Output name ____
    - Paired end output ____

Cutadapt

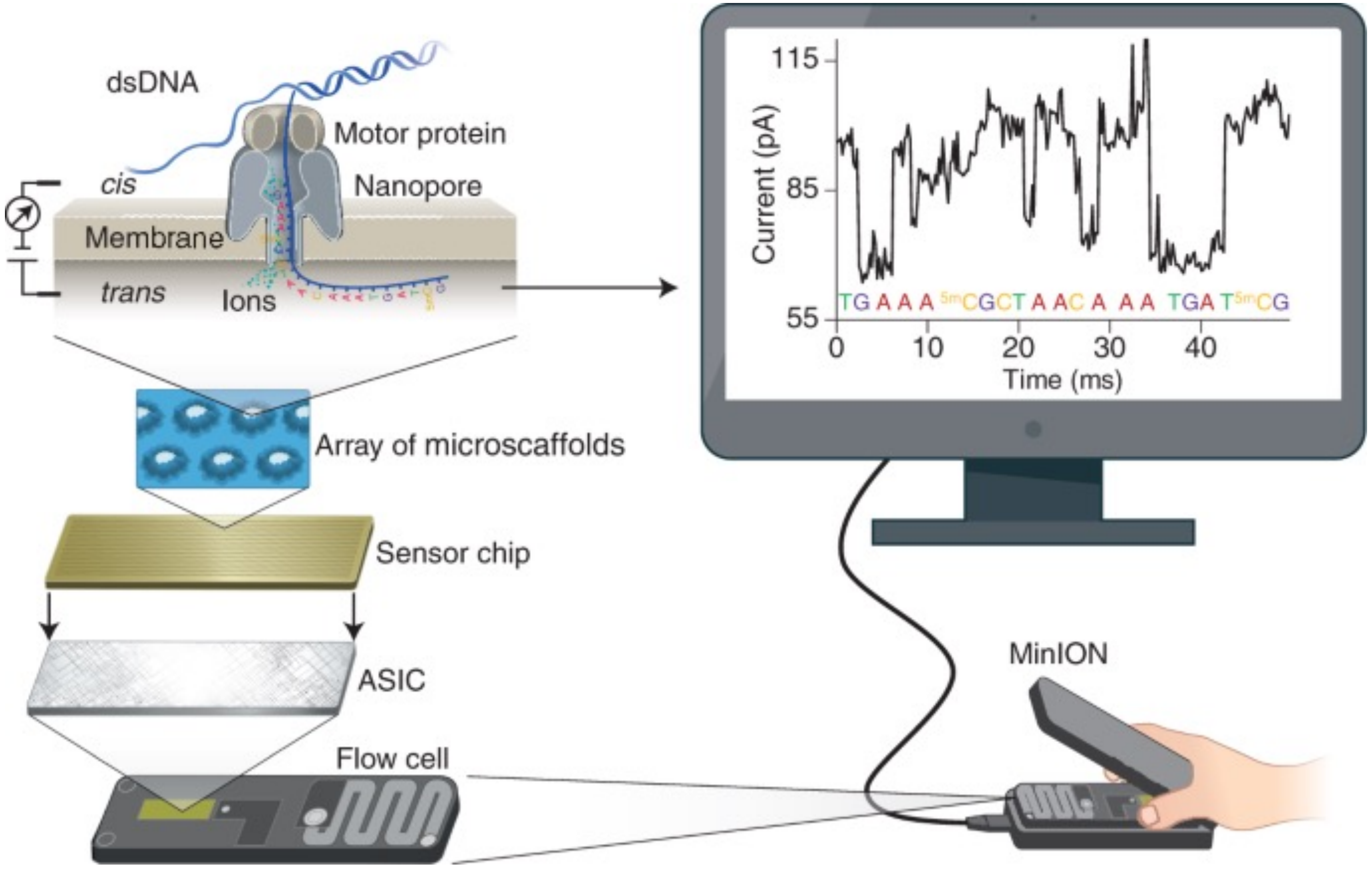
- When looking at the cutadapt manual, which flags (=“-letter”) are for

Length trimming	-m
3’ adapter	-a
Paired end 3’adapter	-A
Quality score	-q
Output name	-o
Paired end output	-p

Nanopore data

- One nanopore MinION Flow Cell /sample
- Quality issues of Nanopore: substitutions

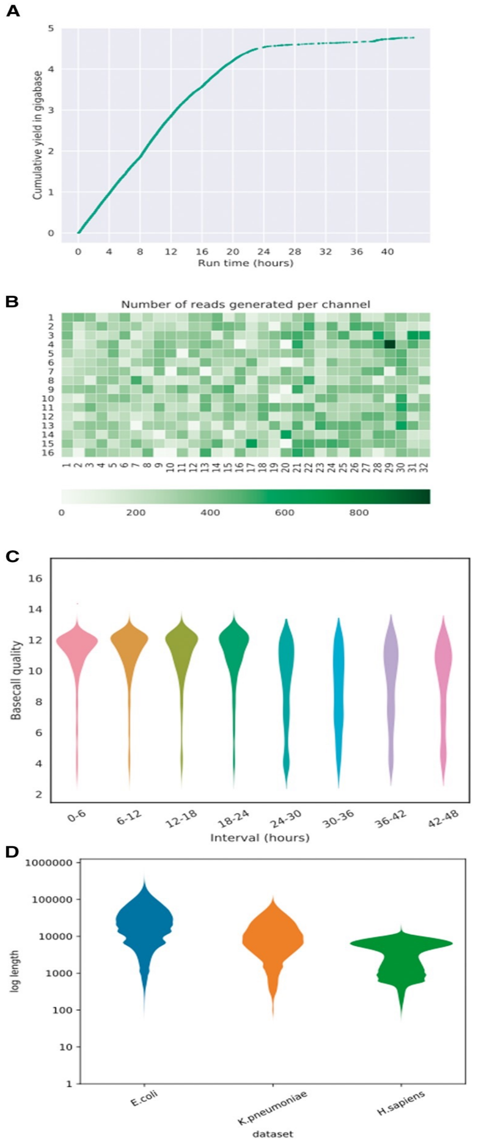
- A MinION flow cell contains 512 channels with 4 nanopores in each channel, for a total of 2,048 nanopores used to sequence DNA or RNA.
  - As nucleotides pass through the nanopore, a characteristic current change is measured and is used to determine the corresponding nucleotide type at ~450 bases per s



https://www.nature.com/articles/s41587-021-01108-x

QC & filtering: NanoPlot, nanoQC, Nanofilt

- Nanoplot: (**A**) Cumulative yield plot (**B**) Flow cell activity heatmap showing number of reads per channel. (**C**) Violin plots comparing base call quality over time. (**D**) NanoComp plot comparing log transformed read lengths of the *E.coli* dataset with a *K.pneumoniae* and human dataset.
- NanoOQ
- Nanofilt: Filtering and trimming of long read sequencing data.



https://doi.org/10.1093/bioinformatics/bty149

Garbage in – garbage out