Genome assembly strategies

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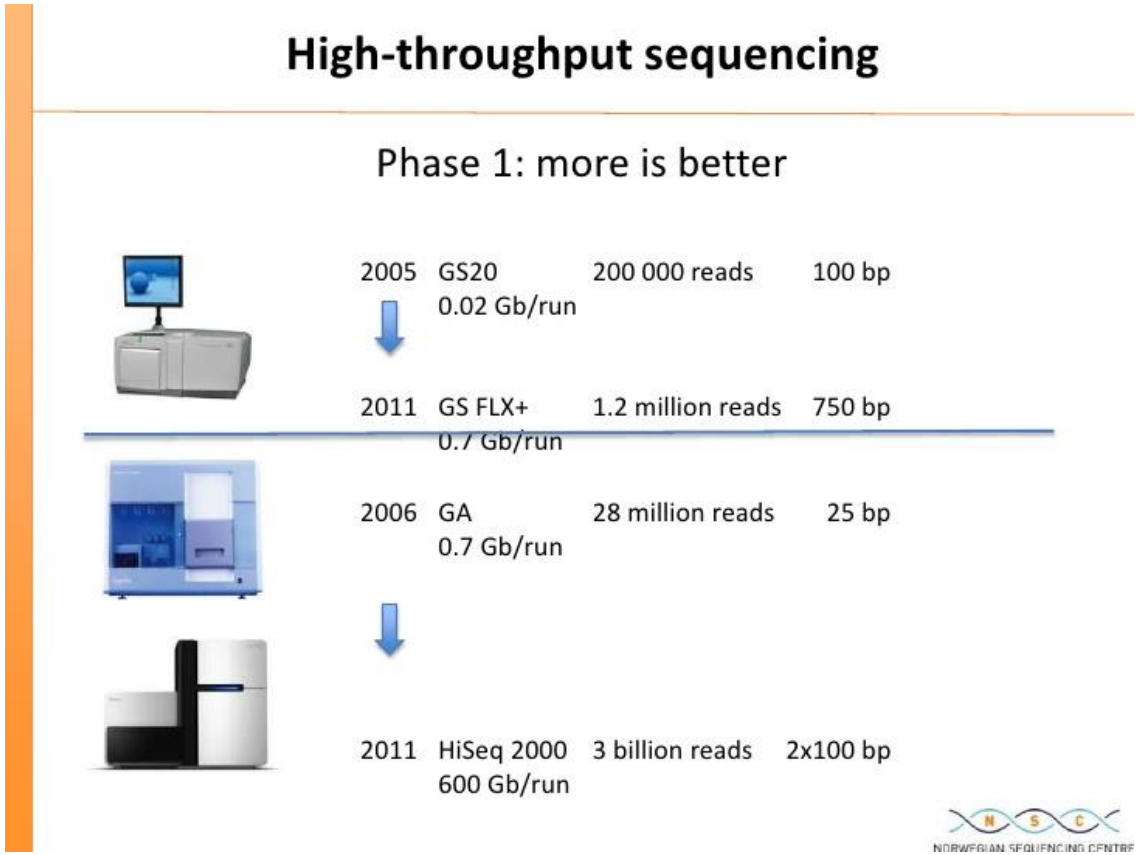
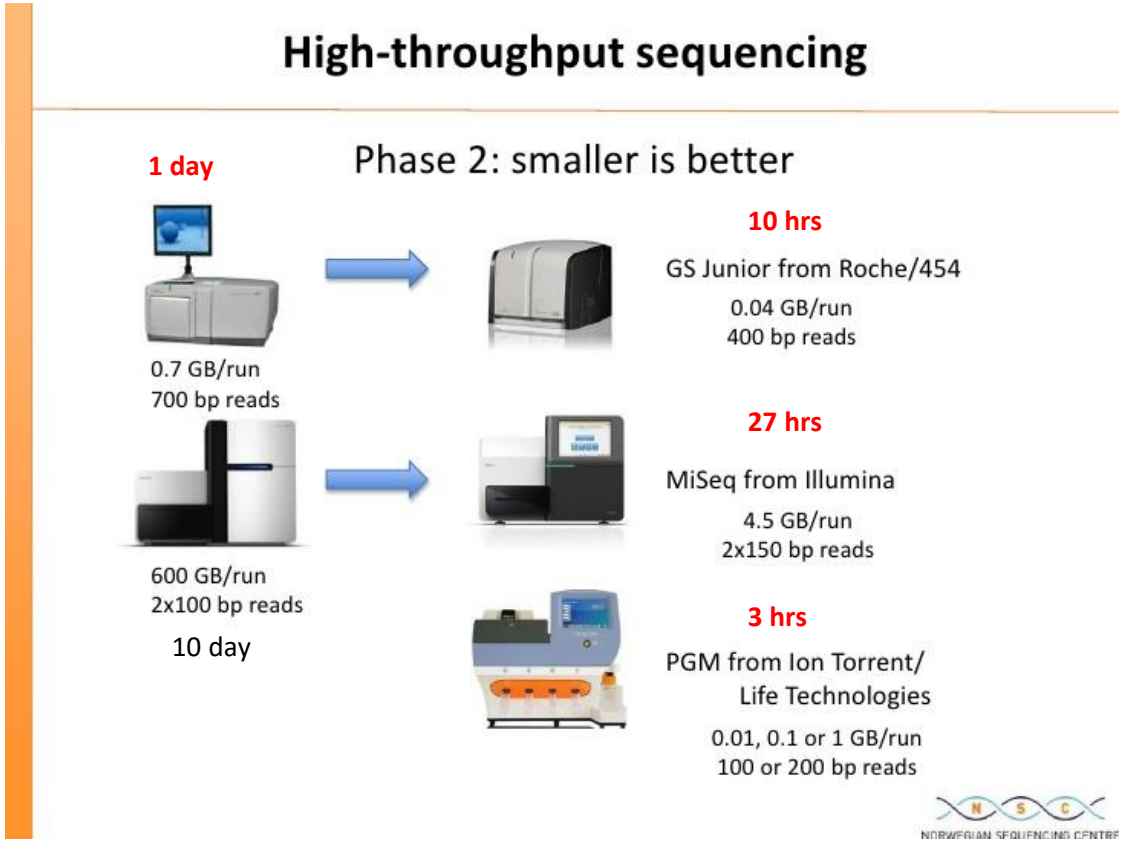
History of NGS and Quality control

High throughput sequencing or NGS

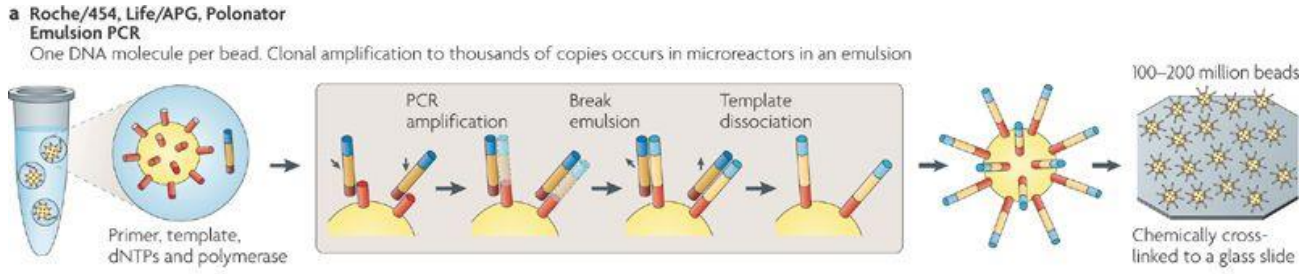




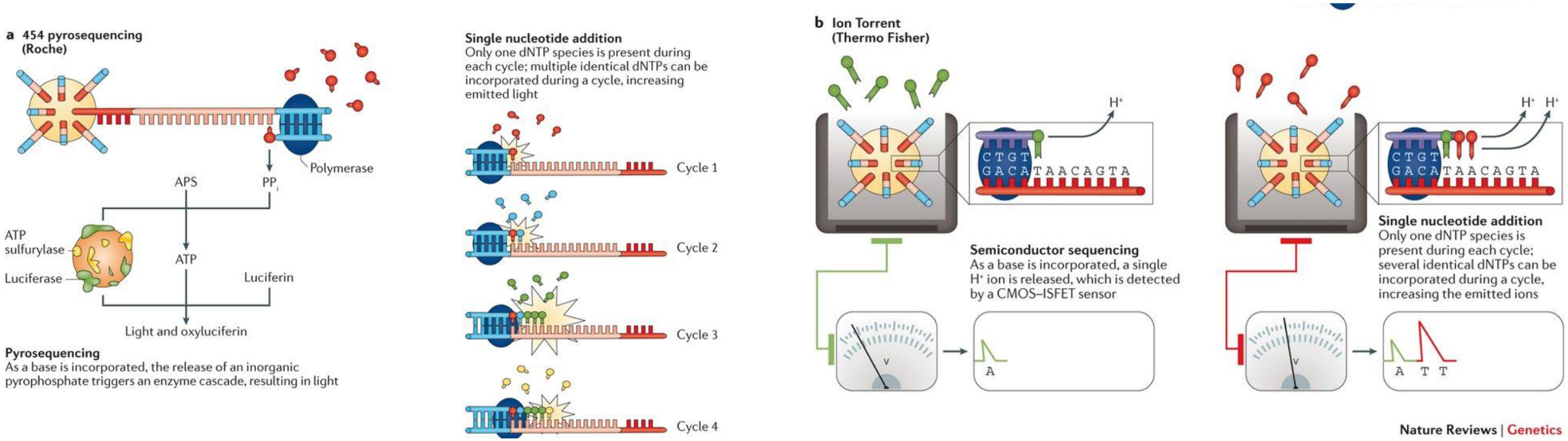
Sequencing-by-synthesis categories. SBS is a term used to describe numerous DNA-polymerase-dependent methods

454 and IonTorrent sequencing

Template immobilization strategies.



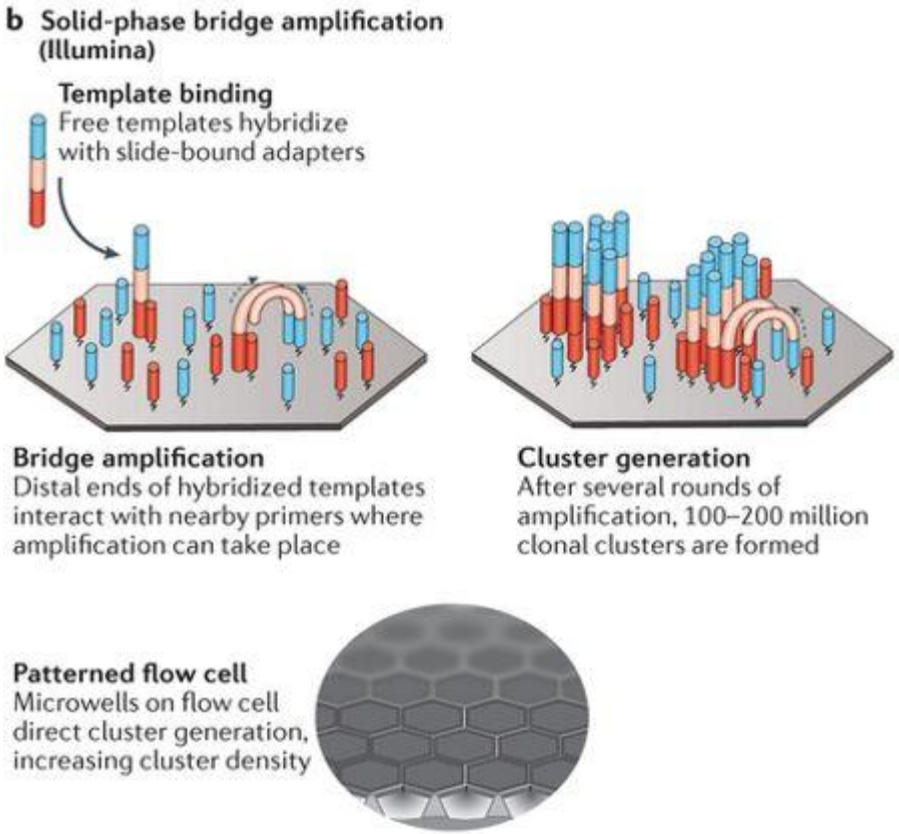
Sequencing by synthesis: single-nucleotide addition approaches.



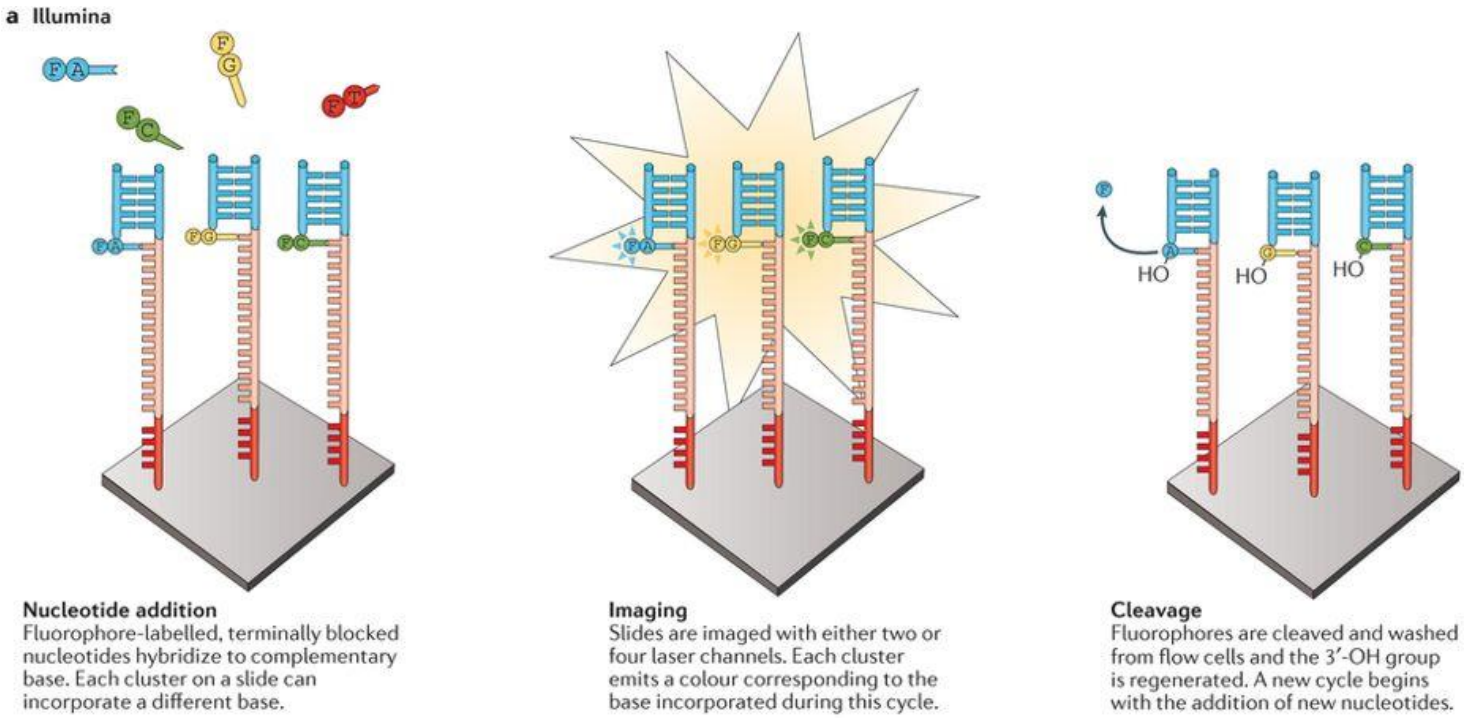
Nature Reviews Genetics **volume17**, 333–351 (2016)

Illumina technology

Template immobilization strategies.



Sequencing by synthesis: cyclic reversible termination approaches.



Nature Reviews Genetics **volume17**, 333–351 (2016)

High-throughput sequencing

Phase 3: single-molecule

PACIFIC BIOSCIENCES™

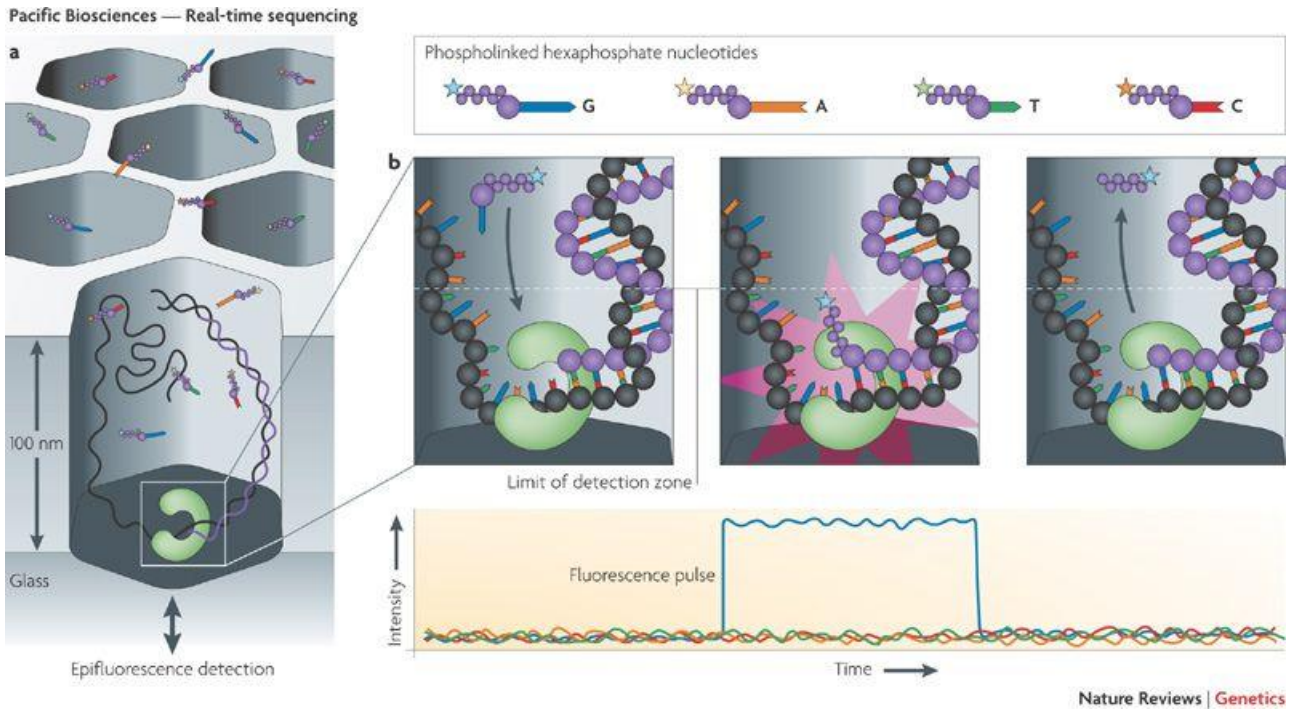
C2 (current) chemistry:
Average read length 2500 bp
36 000 reads
90 MB per 'run'

NORWEGIAN SEQUENCING CENTRE

Oxford NANOPORE Technologies

Real-time sequencing.

Library preparation comprises the ligation of hairpin adapters (yellow) to double-stranded DNA molecules (blue), thereby creating circular molecules called 'SMRTbells'.



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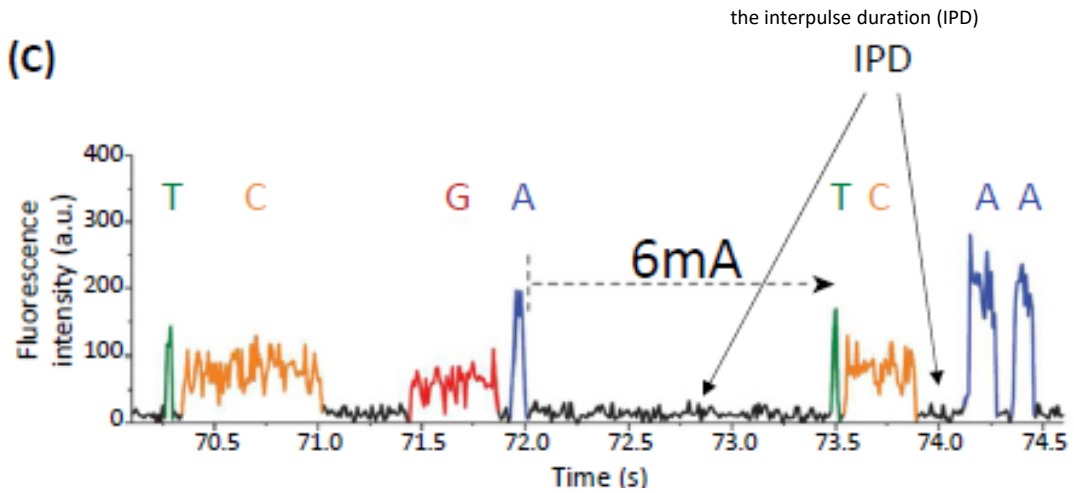


The presence of an epigenetic modification, such as 6-methyladenosine (6 mA), results in a delayed IPD

Trends in Genetics (2018) Vol. 34, No. 9 666-681

Platform Features







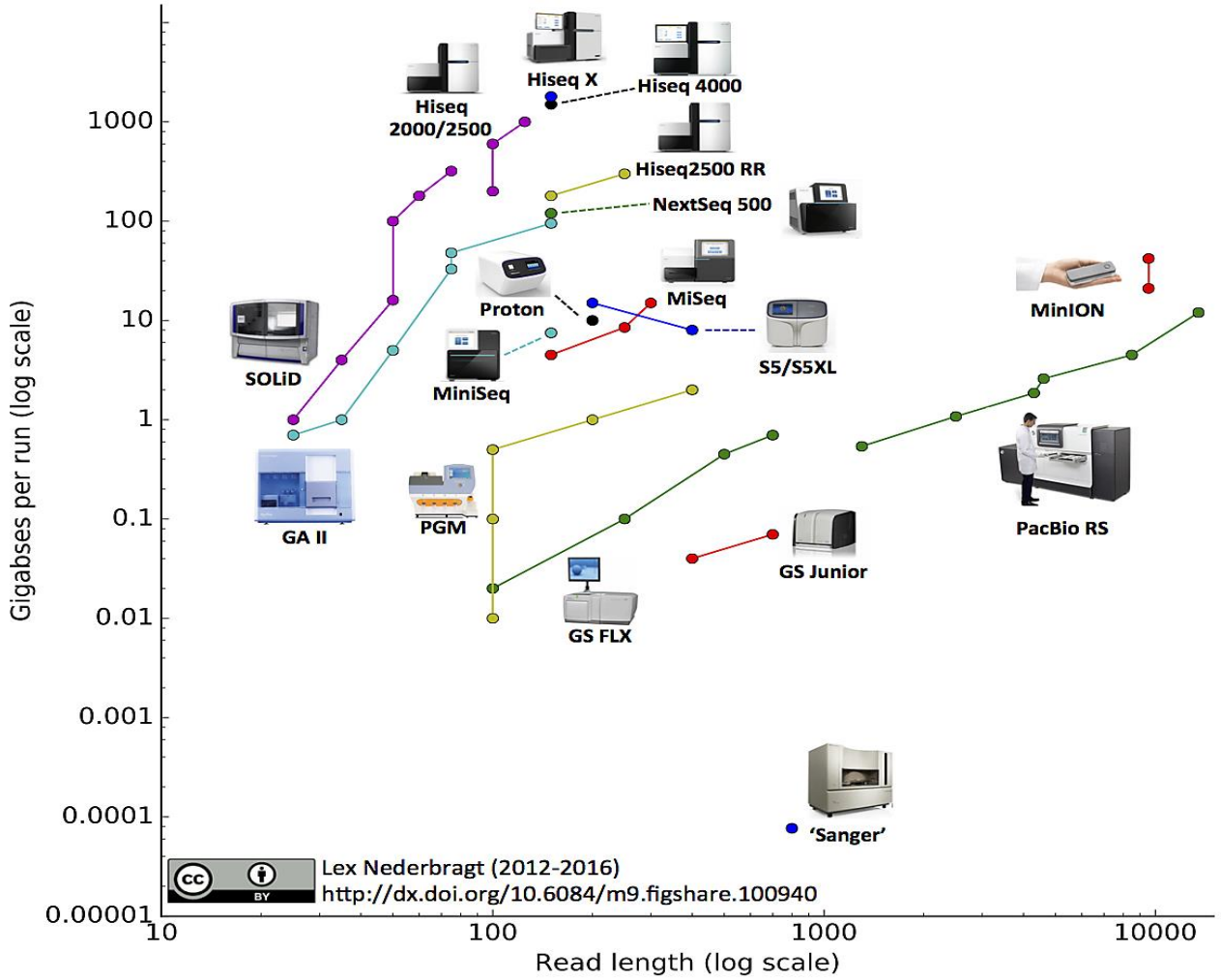
Feature	HiSeq2500 - Highoutput	HiSeq2500 – Rapid mode	MiSeq	PacBio RSII
Number of reads	150-180M/lane	100-150M/lane	12-15M (v2) 20-25M (v3)	50-80K/SMRT cell
Read length	2 x 100 bp	2 x 150 bp	2 x 300 bp (v3)	~ 10-20 kb
Yield per lane (PF data)	up to 35 Gb	up to 45Gb	up to 15 Gb	up to 0.4 Gb
Instrument Time	~12-14 days	~2 days	~2 days	~2 hours
Pricing per Gb	$59 (PE100)	$53 (PE150)	$108 (PE300)	$697

Applications

Platform	454	Illumina HiSeq	Illumina MiSeq*	Ion Torrent	PacBio
resequencing	-	+++	++	-	+
de novo	+++	+	+	+++	+++
metagenomics	+++	++	+	+++	+/-
mRNA	++	+++	++	++	++
miRNA	-	+++	+++	-	-
ChIP	-	+++	++	-	-
DNA meth	-	+++	+	-	!!!
SNP validation	+	-	-	-	++

NORWEGIAN SEQUENCING CENTRE

Multiple technologies diverse features



Which one is the good one?

Yields

A Genome of 1Mb (1 x 10^6 bases):

- By Sanger:
  C = nl/L
  10 = n(500)/1,000,000
  n = 1,000,000*10/500
  20,000 reads
  Cost per read~ 1-2 USD
  20,000 USD (~360,000 MX pesos)
- A 454 run ~700Mb (700X)
  - Cost arpox de 20,000 USD
- Un SMRT cell de PacBio (P6-C4) ~150,000 reads (1Gb)
  - Cost 800 USD (~14,400 pesos)
- An Illumina lane ~300 millions of reads (HiSeq2000)
  - An average length of 100 bp = 30 Gb = 30,000 X
    - Cost per lane 2,000 USD (~36,000 pesos)

Coverage:
C = nl/L

C=Coverage

N=Number of reads

l=Read length

L=Genome size (length) in bases

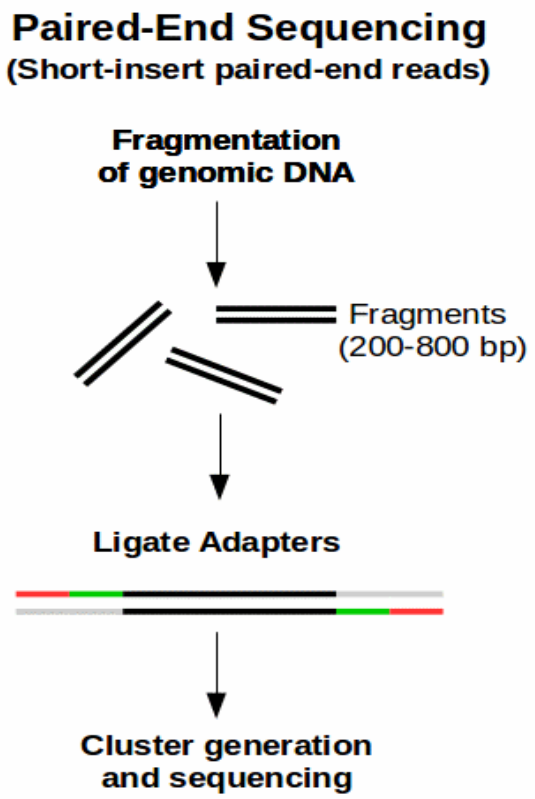
30, 000 X coverage ~ $ 36, 000 Mxpesos Illumina

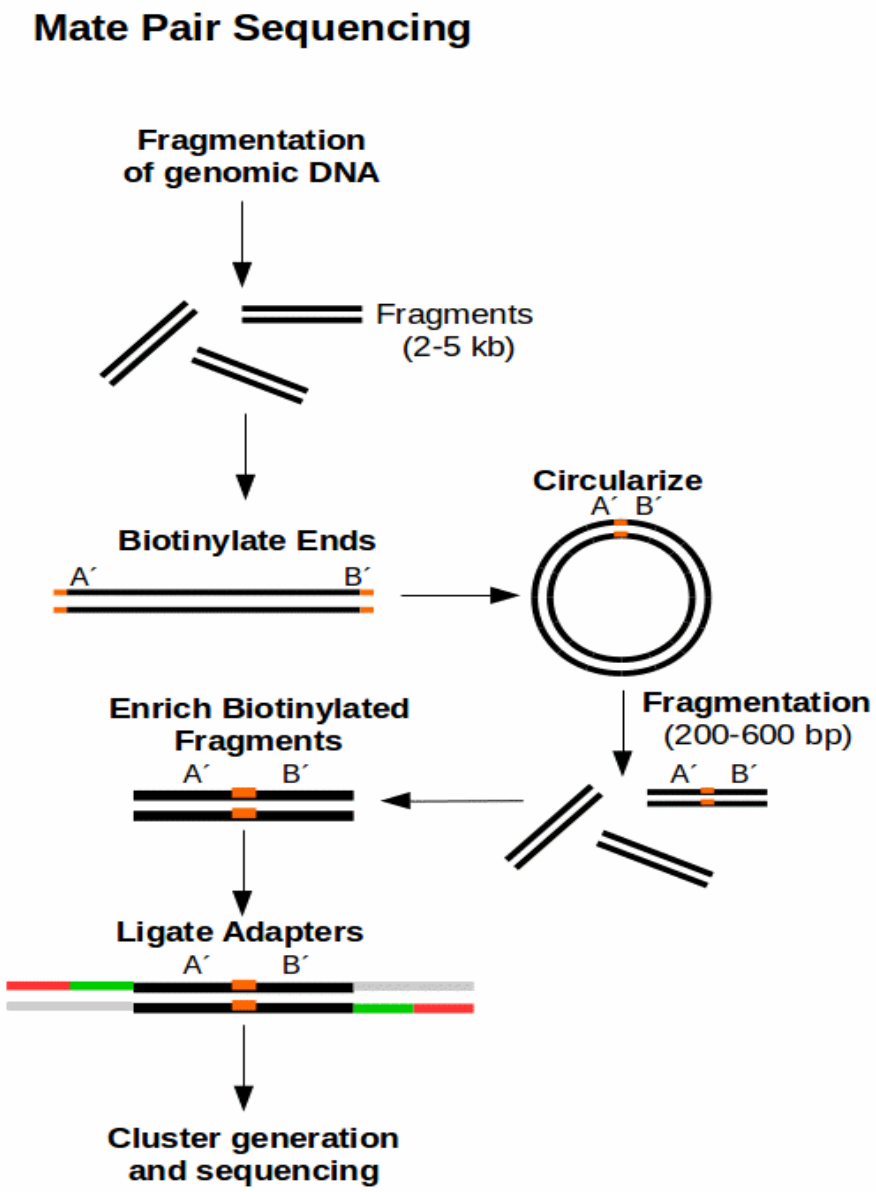
10x coverage ~ $ 360, 000 MX pesos Sanger

Minimal coverage for SNPs, annotation and completeness assessments

> 50 x

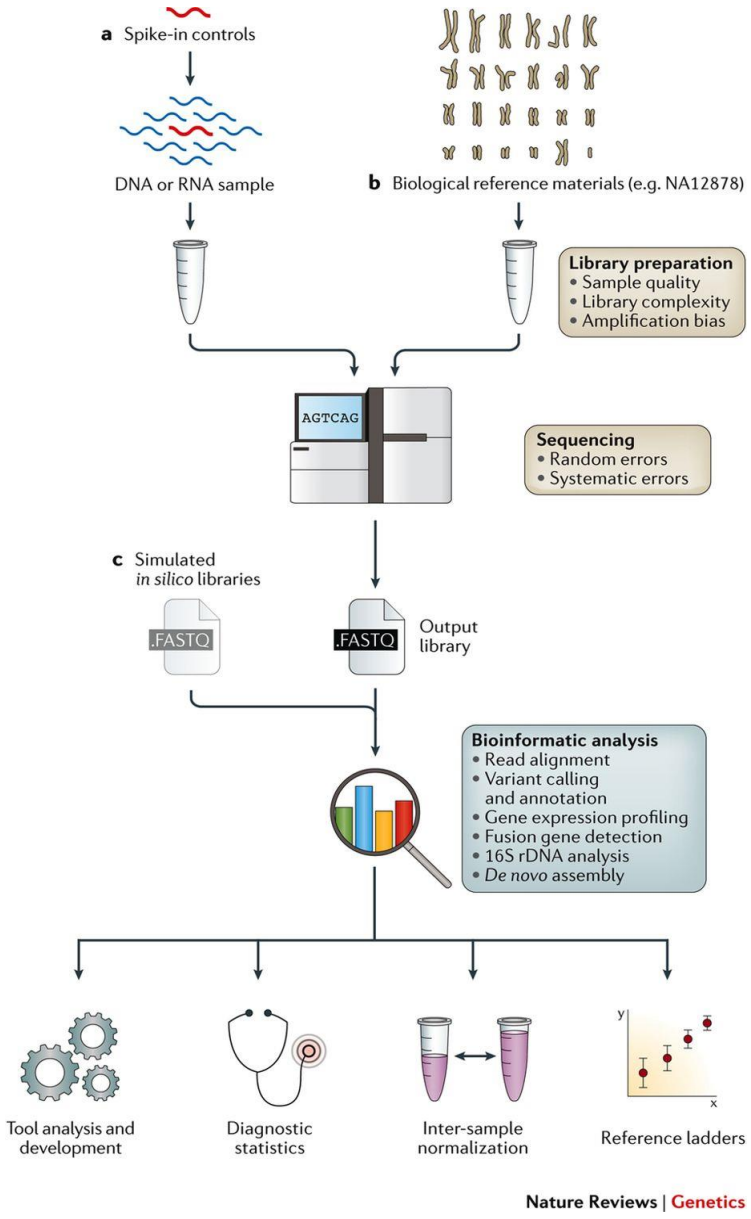
Pair end vs Mate Pair





[Lets watch a very useful video]

HTS general analysis flow chart



'Wet-lab' experimental design

Bioinformatics hard work

Nature Reviews Genetics volume 18, pages 473–484 (2017)

HTS general analysis time flow chart

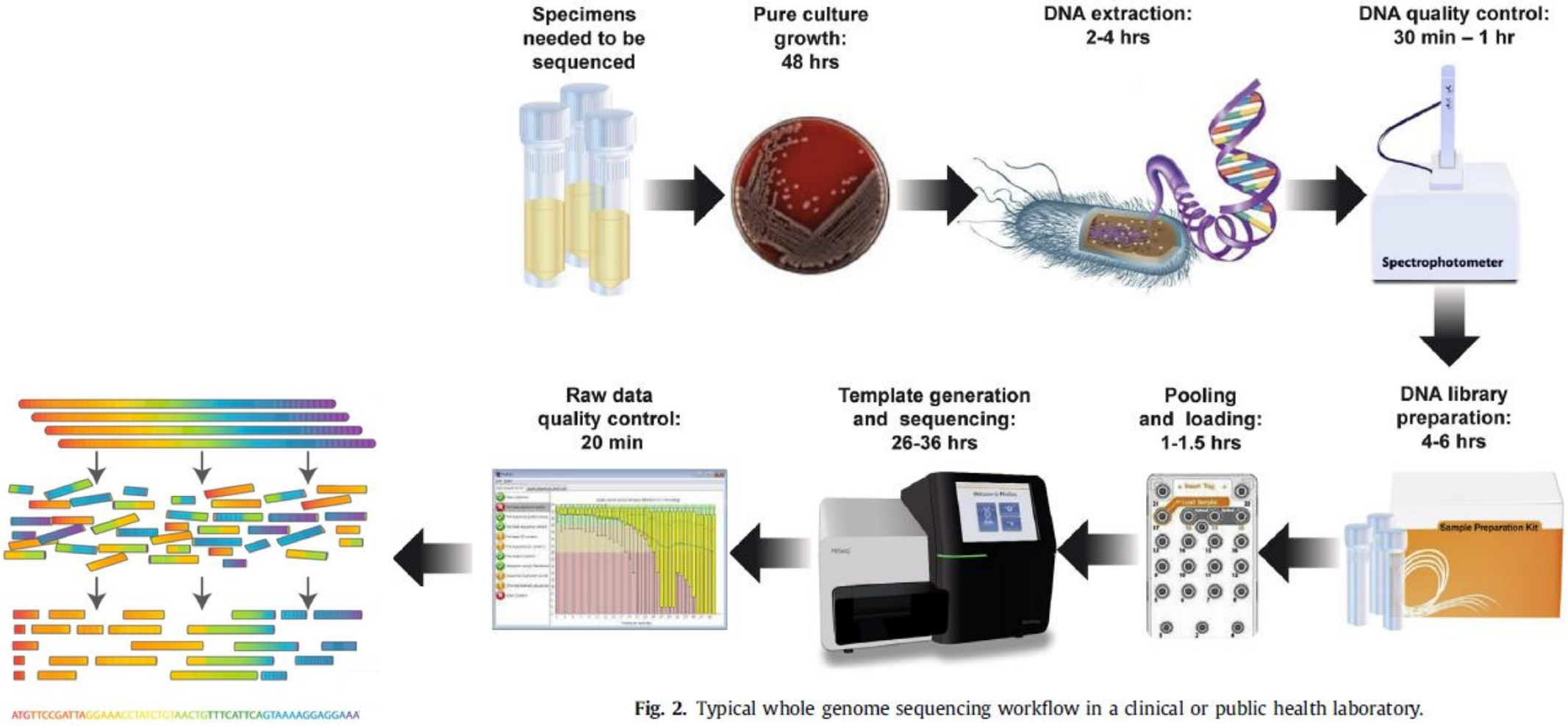


Fig. 2. Typical whole genome sequencing workflow in a clinical or public health laboratory.

Genome assembly

Sequence file formats

- Next gen sequence file formats are based on the commonly used

  FASTA format

  >sequence_ID and optional comments
  ATTCCGGTGCGGTGCGGTGCTGCCGTGCCGGTGC
  TTCGAAATTGGCGTCAGT

- The Phred quality scores per base were added to form the FASTQ format

Sequence file formats

- Illumina Fastq format (fasta format with **Q**uality values for each base)

Space to separate Read

Read ID

```
@EAS139:136:FC706VJ:2:5:1000:12850 1:Y:18:ATCACG
AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA      - base calls
+
BBBBCCCC?<A?BC?7@@??????DBBA@@@@A@@       - Base quality+33
```

Full read header description

@ <instrument-name>:<run ID>:<flowcell ID>:<lane-number>:<tile-number>: <x-pos>: <y-pos>
<read number>:<is filtered>:<control number>:<barcode sequence>

The phred quality score

Quality score interpretation

$$Q = -10 \log_{10} P \quad \Longrightarrow \quad P = 10^{\frac{-Q}{10}}$$

Phred Quality Score	Probability of incorrect base call	Base call accuracy
10	1 in 10	90%
20	1 in 100	99%
30	1 in 1000	99.9%
40	1 in 10000	99.99%
50	1 in 100000	99.999%

If base quality = 35

$P=10^{-35/10} = 0.00032$

or 1/3200 incorrect

```
@EAS139:136:FC706VJ:2:5:1000:12850 1:Y:18:ATCACG
AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA      - base calls
+
BBBBCCCC?<A?BC?7@@??????DBBA@@@@A@@       - Base quality+33
```

ASCII Table

Dec	Hex	Oct	Char	Dec	Hex	Oct	Char	Dec	Hex	Oct	Char	Dec	Hex	Oct	Char
0	0	0		32	20	40	[space]	64	40	100	@	96	60	140	`
1	1	1		33	21	41	!	65	41	101	A	97	61	141	a
2	2	2		34	22	42	"	66	42	102	B	98	62	142	b
3	3	3		35	23	43	#	67	43	103	C	99	63	143	c
4	4	4		36	24	44	$	68	44	104	D	100	64	144	d
5	5	5		37	25	45	%	69	45	105	E	101	65	145	e
6	6	6		38	26	46	&	70	46	106	F	102	66	146	f
7	7	7		39	27	47	'	71	47	107	G	103	67	147	g
8	8	10		40	28	50	(	72	48	110	H	104	68	150	h
9	9	11		41	29	51	)	73	49	111	I	105	69	151	i
10	A	12		42	2A	52	*	74	4A	112	J	106	6A	152	j
11	B	13		43	2B	53	+	75	4B	113	K	107	6B	153	k
12	C	14		44	2C	54	,	76	4C	114	L	108	6C	154	l
13	D	15		45	2D	55	-	77	4D	115	M	109	6D	155	m
14	E	16		46	2E	56	.	78	4E	116	N	110	6E	156	n
15	F	17		47	2F	57	/	79	4F	117	O	111	6F	157	o
16	10	20		48	30	60	0	80	50	120	P	112	70	160	p
17	11	21		49	31	61	1	81	51	121	Q	113	71	161	q
18	12	22		50	32	62	2	82	52	122	R	114	72	162	r
19	13	23		51	33	63	3	83	53	123	S	115	73	163	s
20	14	24		52	34	64	4	84	54	124	T	116	74	164	t
21	15	25		53	35	65	5	85	55	125	U	117	75	165	u
22	16	26		54	36	66	6	86	56	126	V	118	76	166	v
23	17	27		55	37	67	7	87	57	127	W	119	77	167	w
24	18	30		56	38	70	8	88	58	130	X	120	78	170	x
25	19	31		57	39	71	9	89	59	131	Y	121	79	171	y
26	1A	32		58	3A	72	:	90	5A	132	Z	122	7A	172	z
27	1B	33		59	3B	73	;	91	5B	133	[	123	7B	173	{
28	1C	34		60	3C	74	<	92	5C	134	\	124	7C	174	|
29	1D	35		61	3D	75	=	93	5D	135	]	125	7D	175	}
30	1E	36		62	3E	76	>	94	5E	136	^	126	7E	176	~
31	1F	37		63	3F	77	?	95	5F	137	_	127	7F	177	

AAAAA

+

BBBBC

ASCII val	66	67
	-33	-33
Q value	33	34

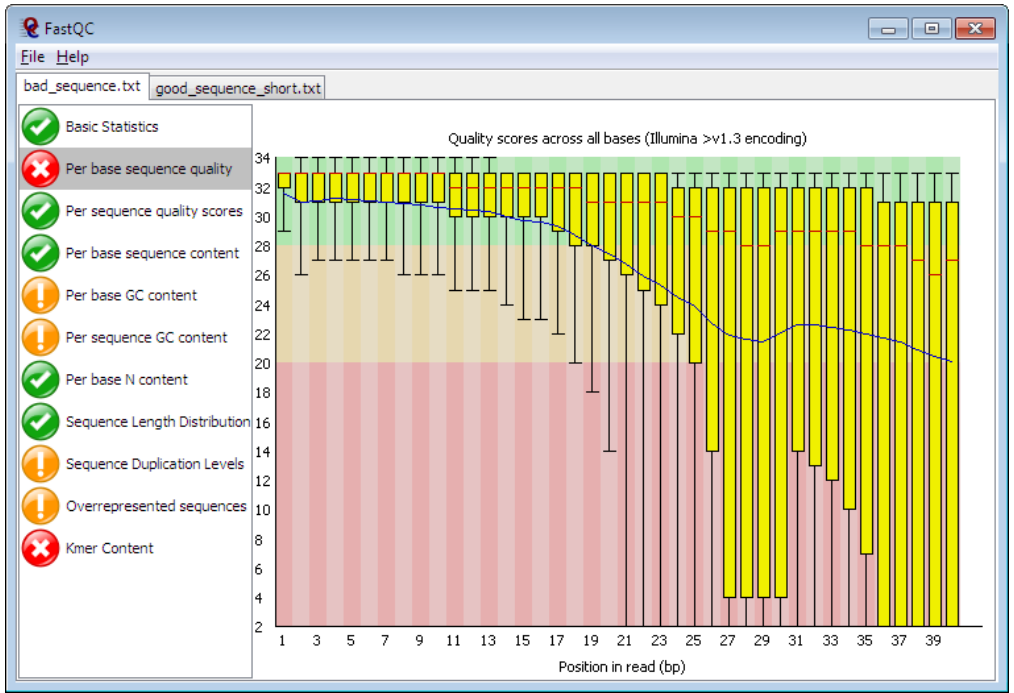
$$Q = -10 \log_{10} P \quad \Rightarrow \quad P = 10^{\frac{-Q}{10}}$$

> 10^(-33/10)
[1] 0.0005011872 = 1/5000
> 10^(-34/10)
[1] 0.0003981072=1/39000

Let’s play with FastQC to quality control visualization

Open FastQC program

Open in browser:
fastqc_report.html

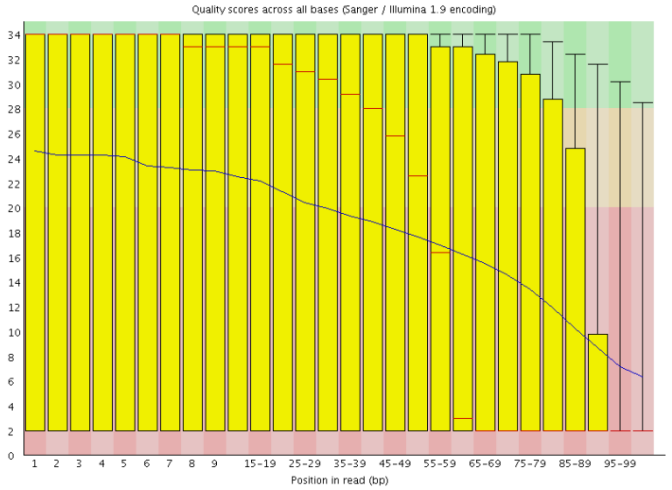


https://www.bioinformatics.babraham.ac.uk/projects/fastqc/

Quality filter trim galore

Babraham Bioinformatics

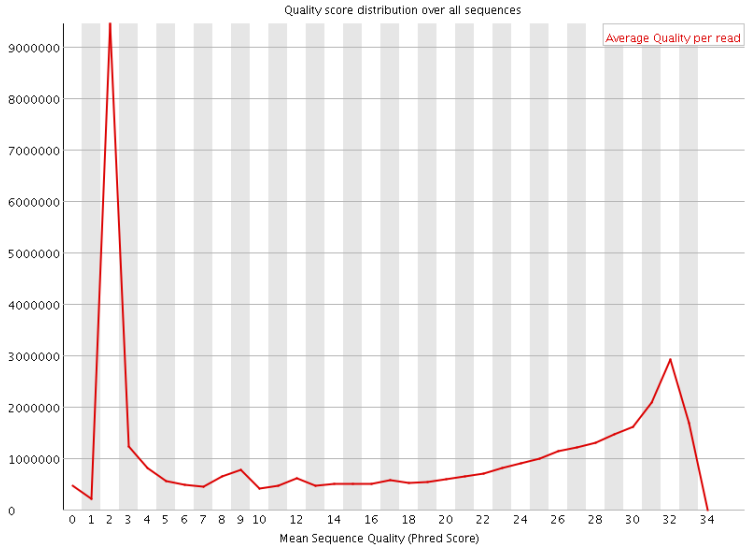
Before trimGalore



Good

Reasonable

bad



After trimGalore