Genome assembly strategies

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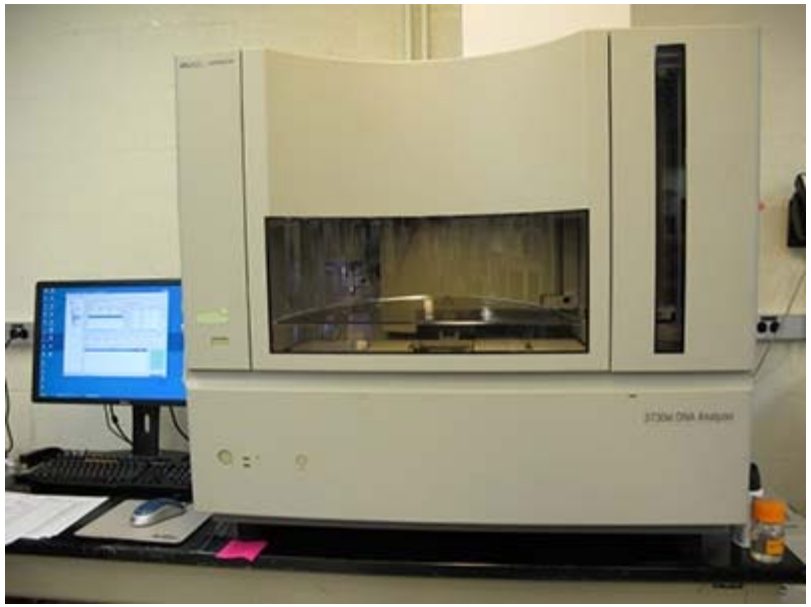
History of NGS and Quality control

1st Generation sequencing (Sanger Sequencing)



1970's-90's

- Max 96 reactions per run
- Single "read" per reaction (600-800bp)
- >5 $USD per read
- Chemistry = Chain termination reaction
- High Quality data
- Complex and demanding sample preparation (cloning)

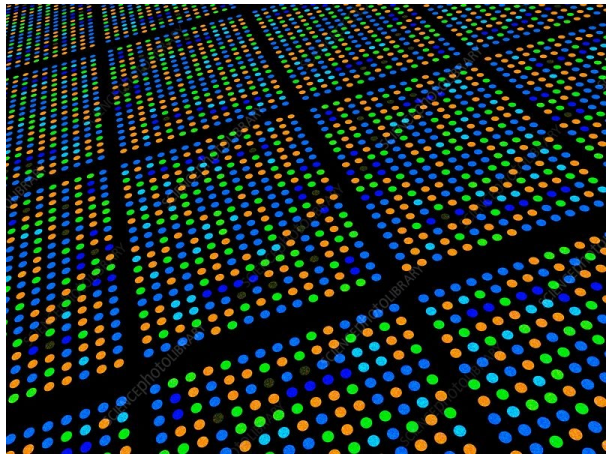
Theoretical yield from 1 full run : 96 x 700 bp = 67,200bp

Cost per full run : 96 x 50 = 480 $USD

Cost per million bp (Mb) : (1,000,000 / 67,200) * 480 =

7,142.8 USD per Mb

DNA microarrays – not sequencing



From Mid 90's

- Used to analyse DNA or RNA
- Most commonly for gene expression
- ssDNA probe targets "printed" into a slide (thousands)
- DNA or RNA chemically labelled and allowed to hybridize to the probes
- After washing, bound labels can be detected and indicate the presence and relative abundance of targets in sample.

2nt Generation sequencing (Next Generation Seq; NGS)

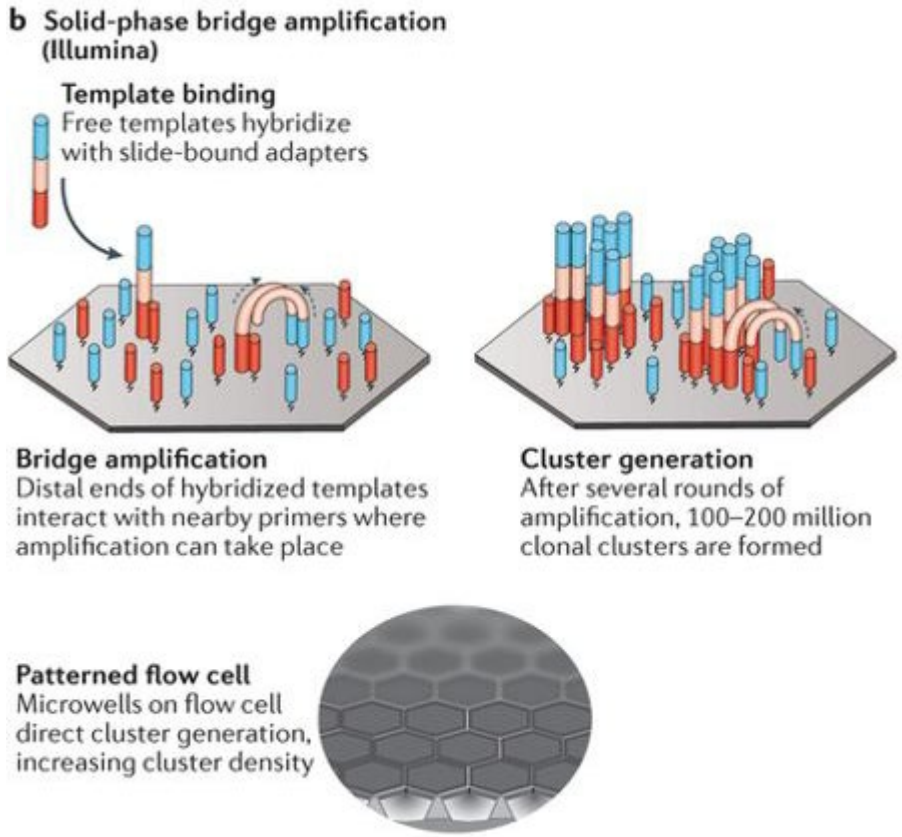
From 2005

- Multiple manufacturers (competition ☺)
- Expensive instrumentation (millions of $$$)
- Able to sequence millions of fragments simultaneously
- Single reads from 35bp to 450bp (depends on tech)
- Chemistry, target amplification and =
  - Sequencing by synthesis (illumina, 454)
  - Sequencing by ligation (solid)
- High read quality

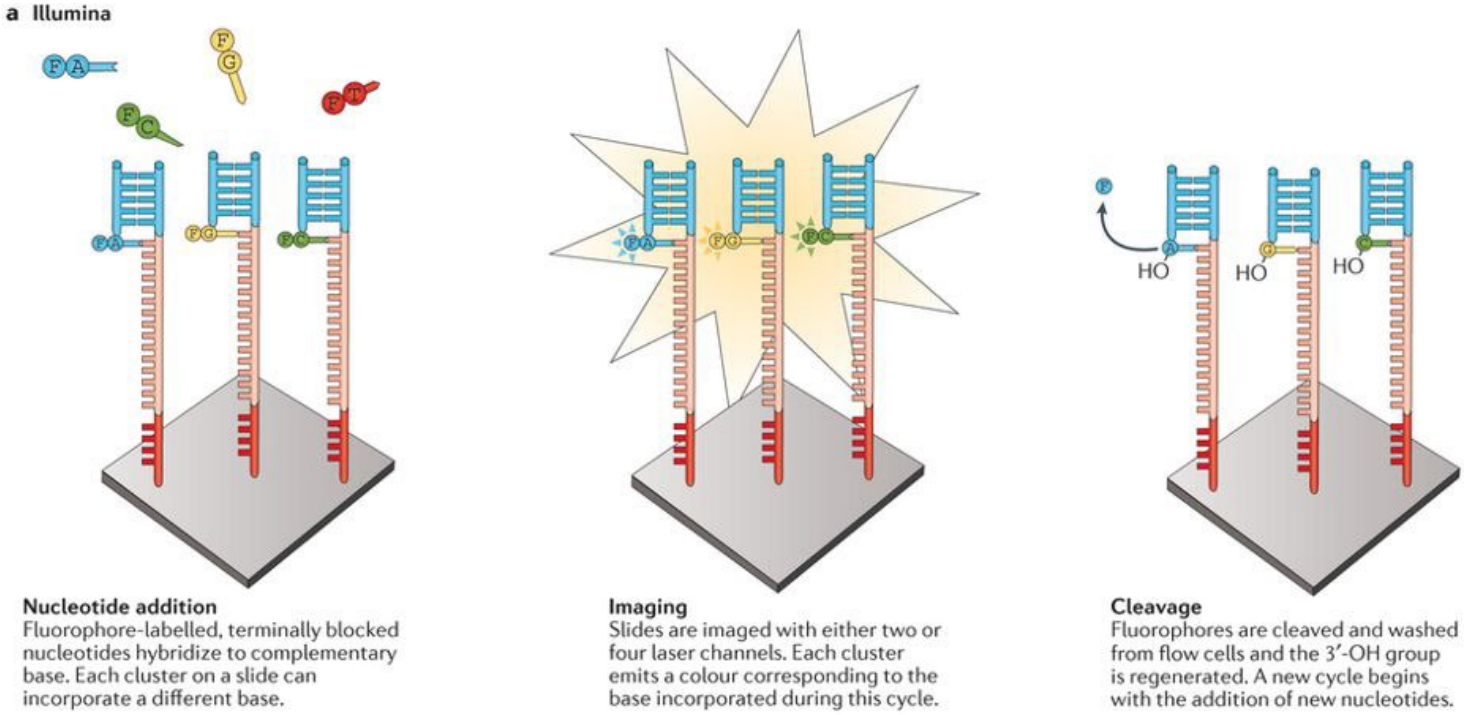
Extremely high outputs means cost per Mb is relatively low.

Illumina technology

Template immobilization strategies.

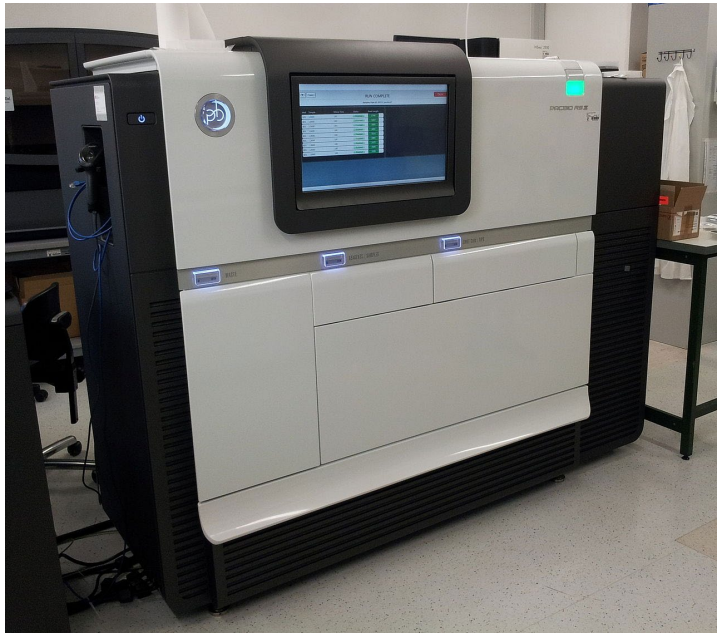


Sequencing by synthesis: cyclic reversible termination approaches.



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

3rt Generation sequencing



From 2011

From 2015

High-throughput sequencing

Phase 3: single-molecule

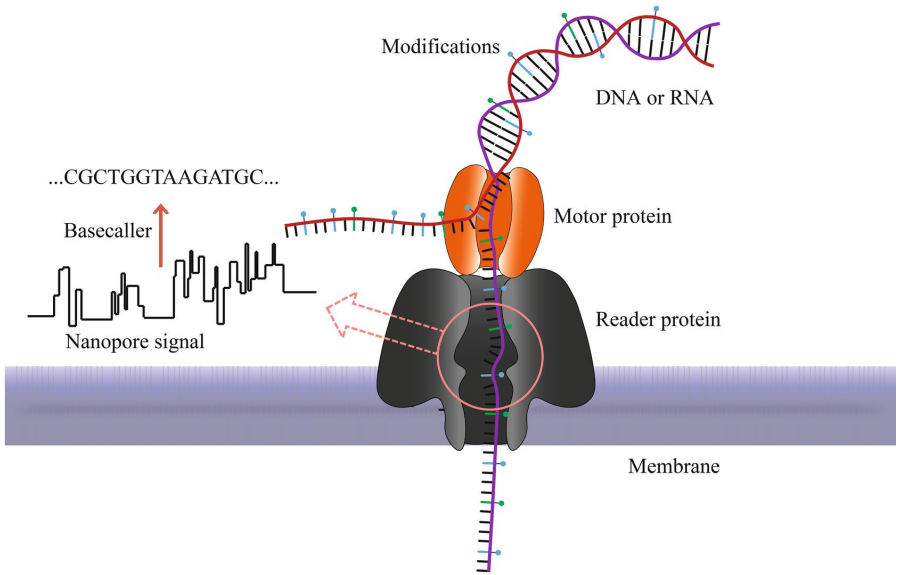
PACIFIC BIOSCIENCES™

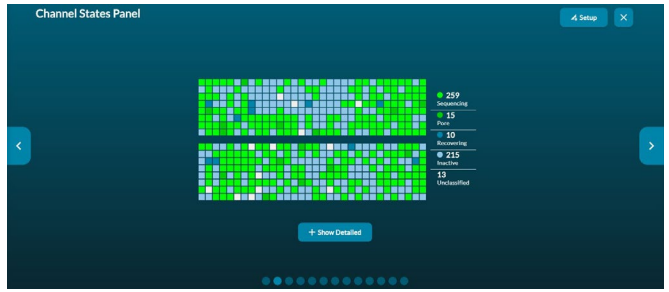
C2 (current) chemistry:
Average read length 2500 bp
36 000 reads
90 MB per 'run'

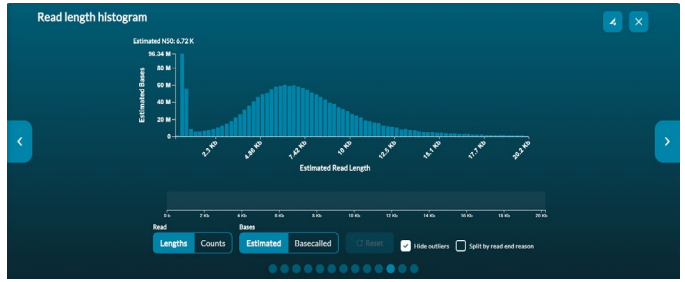
NORWEGIAN SEQUENCING CENTRE

Oxford NANOPORE Technologies

Nanopore sequencing

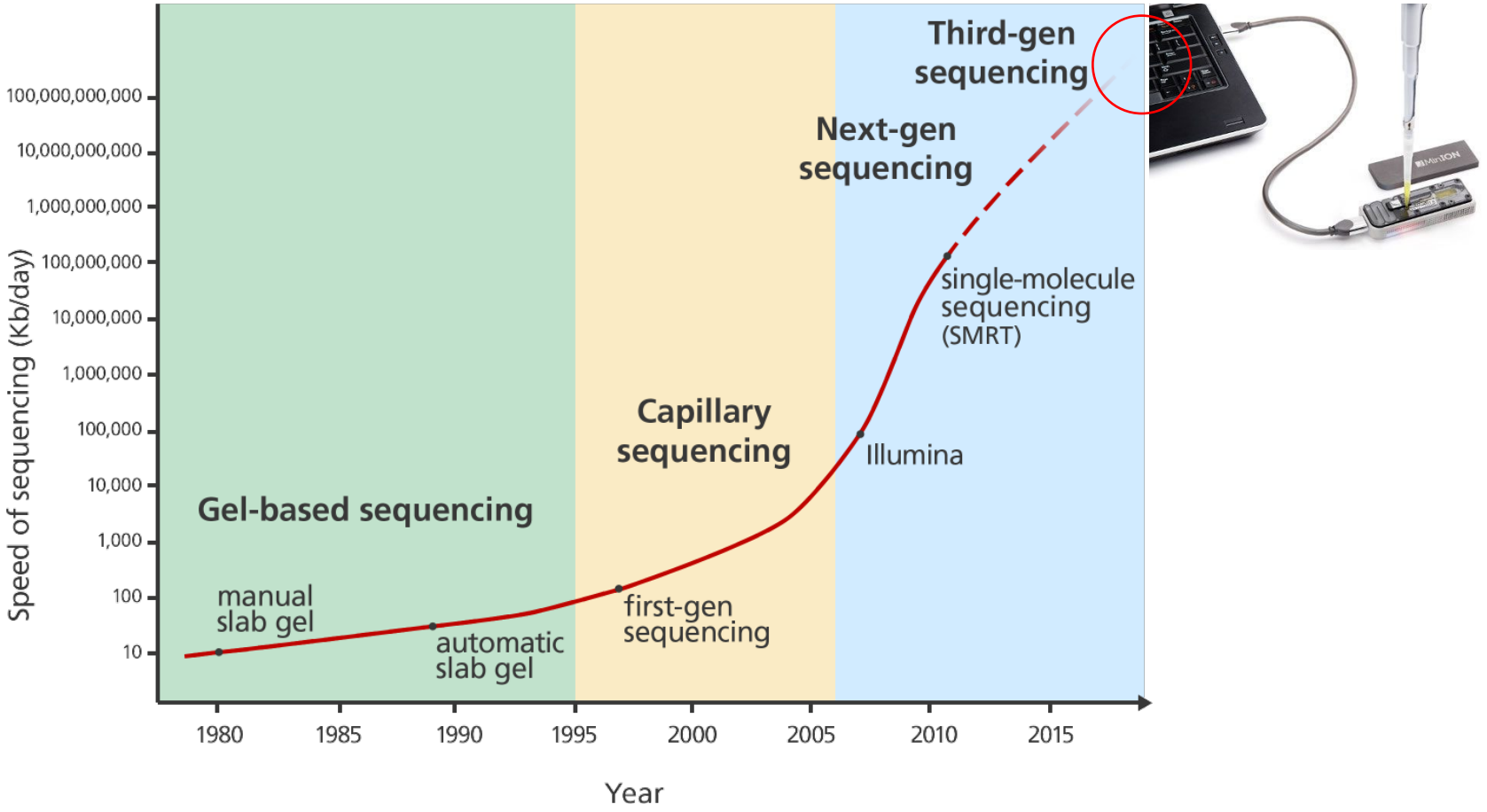






Three generations of sequencing

We are here

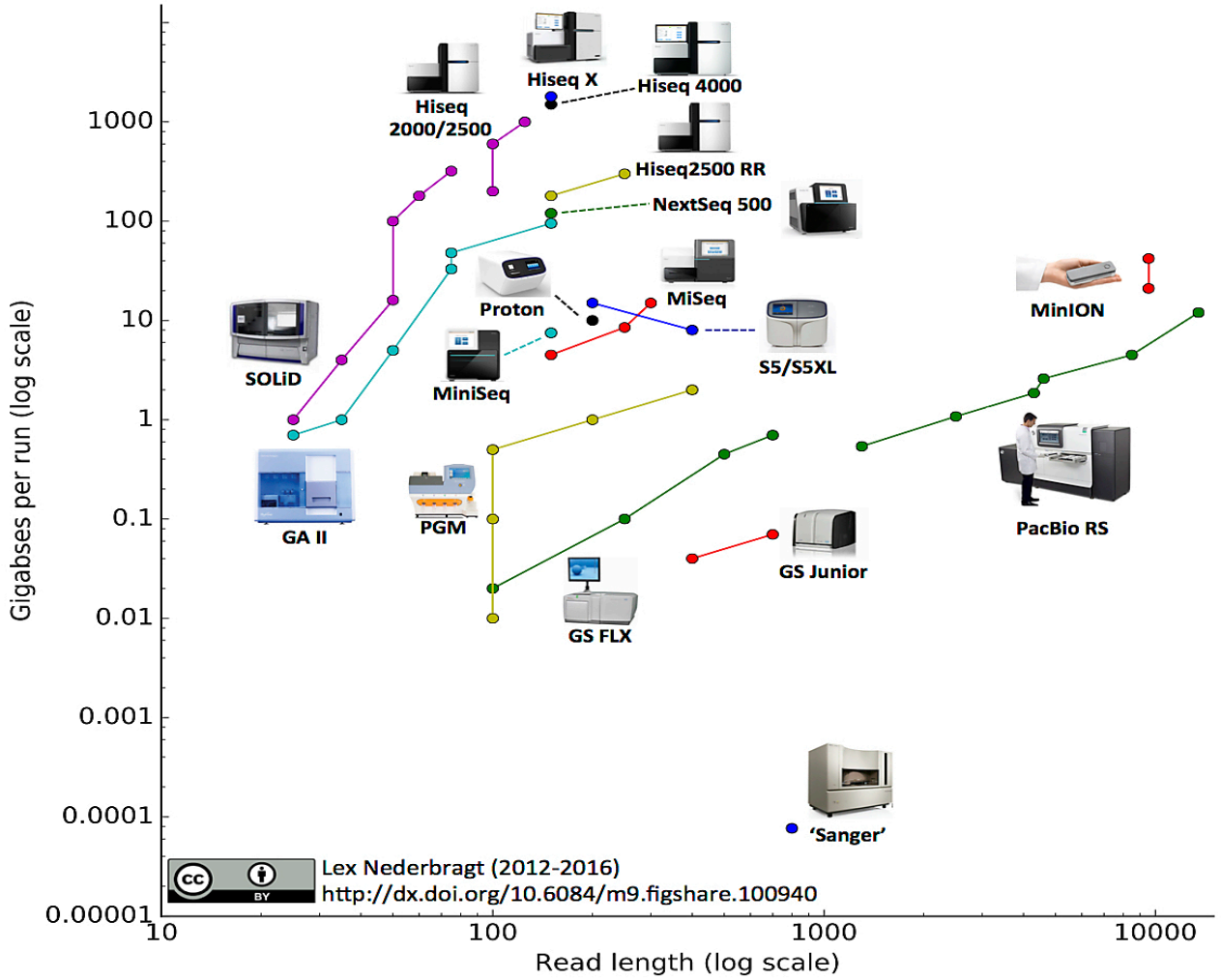


Sequencing machines

Most common… **Next in line!**

	illumina	ion torrent by life technologies	PACIFIC BIOSCIENCES	Oxford NANOPORE Technologies
Read length, bp	100-300	200-400	1000-70000	5000-900000
Error rate	0.1-1%	1-2%	10-20%	10-20%
Error type	Mismatches only	Indels & Mismatches	Indels & Mismatches	Indels & Mismatches
Comments	Quality drops towards end of the read. Sequence specific biases	Problems with homopolymers	Random errors	Problems with homopolymers
$ per 1 Mbp	0.05	0.5 - 20	2+	0.1-0.5
Sequencer cost	100-500 K	80K	700 K	1 K

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

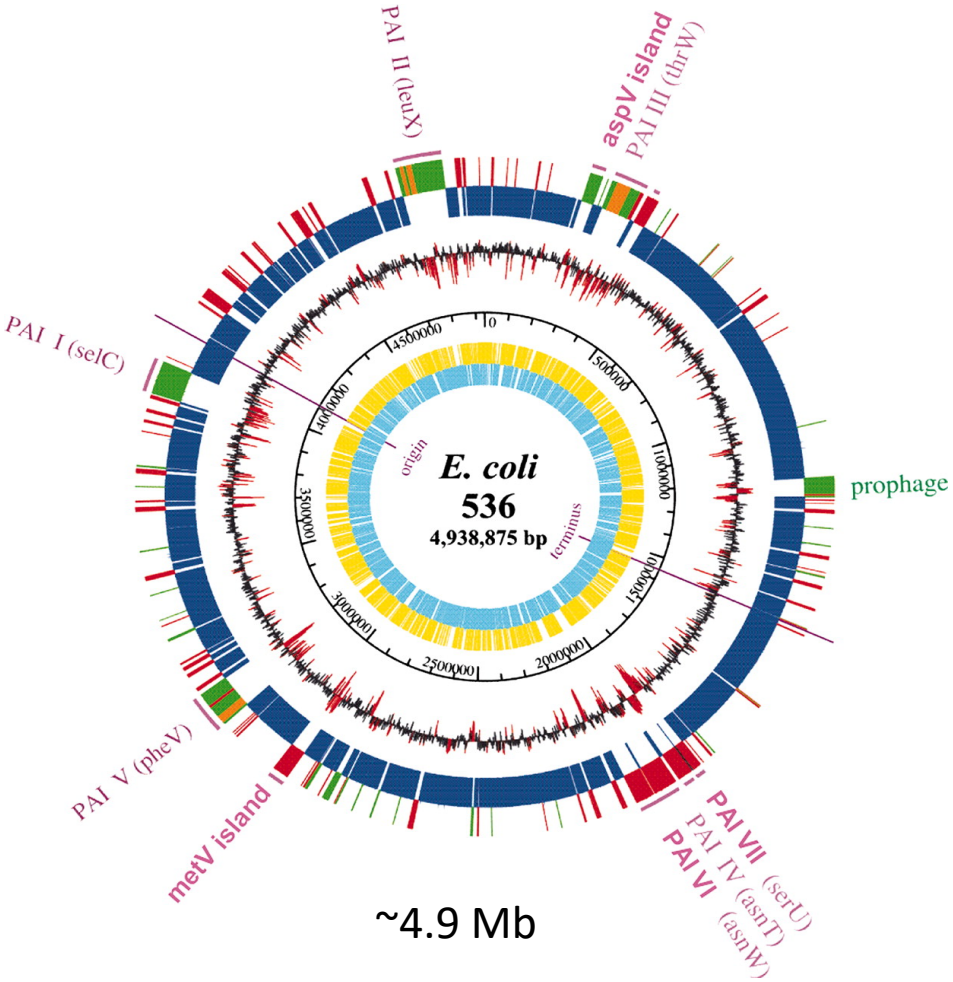
Minimal coverage for SNPs, annotation and completeness assessments

> 50 x

Bacterial Genomes

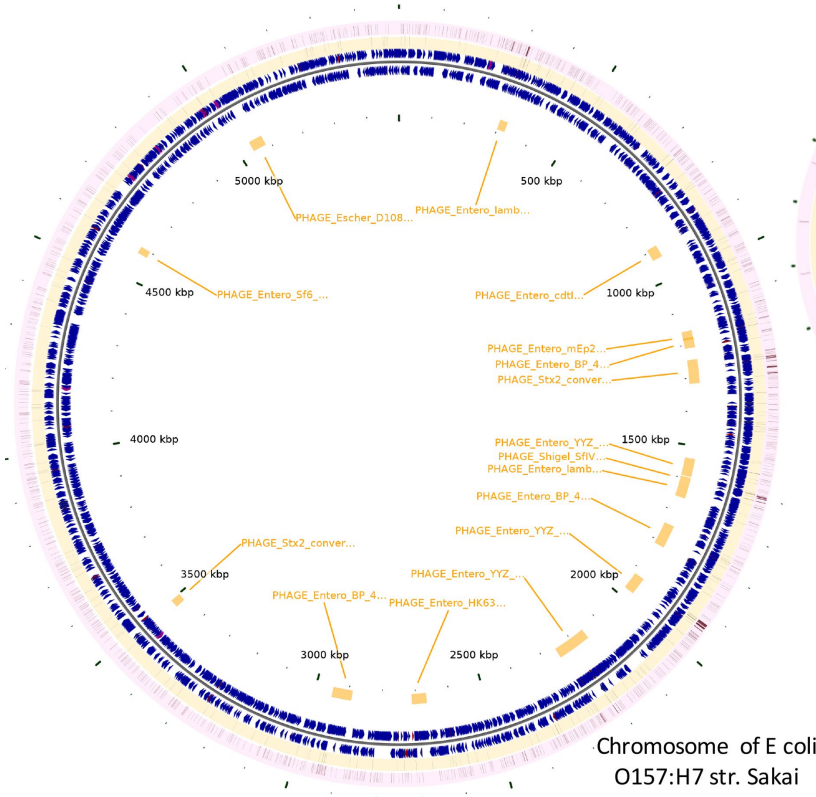
Escherichia coli

Circular chromosome



~4.9 Mb

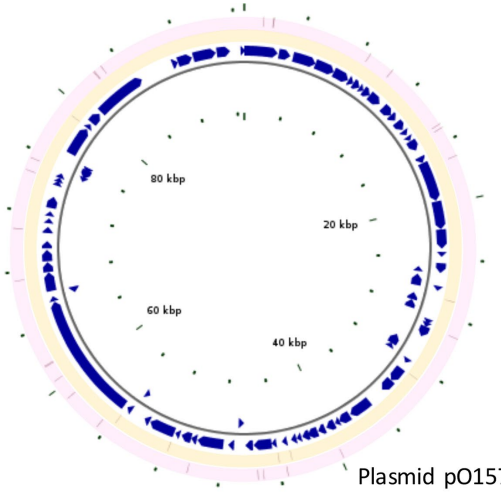
Circular chromosome



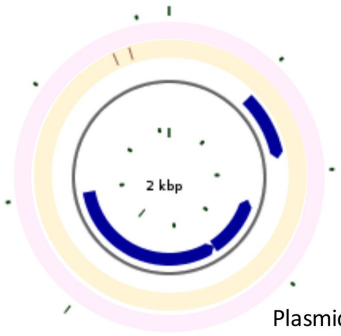
Chromosome of E coli O157:H7 str. Sakai 5.4M

~5.5 Mb

Plasmids



Plasmid pO157 92.7K



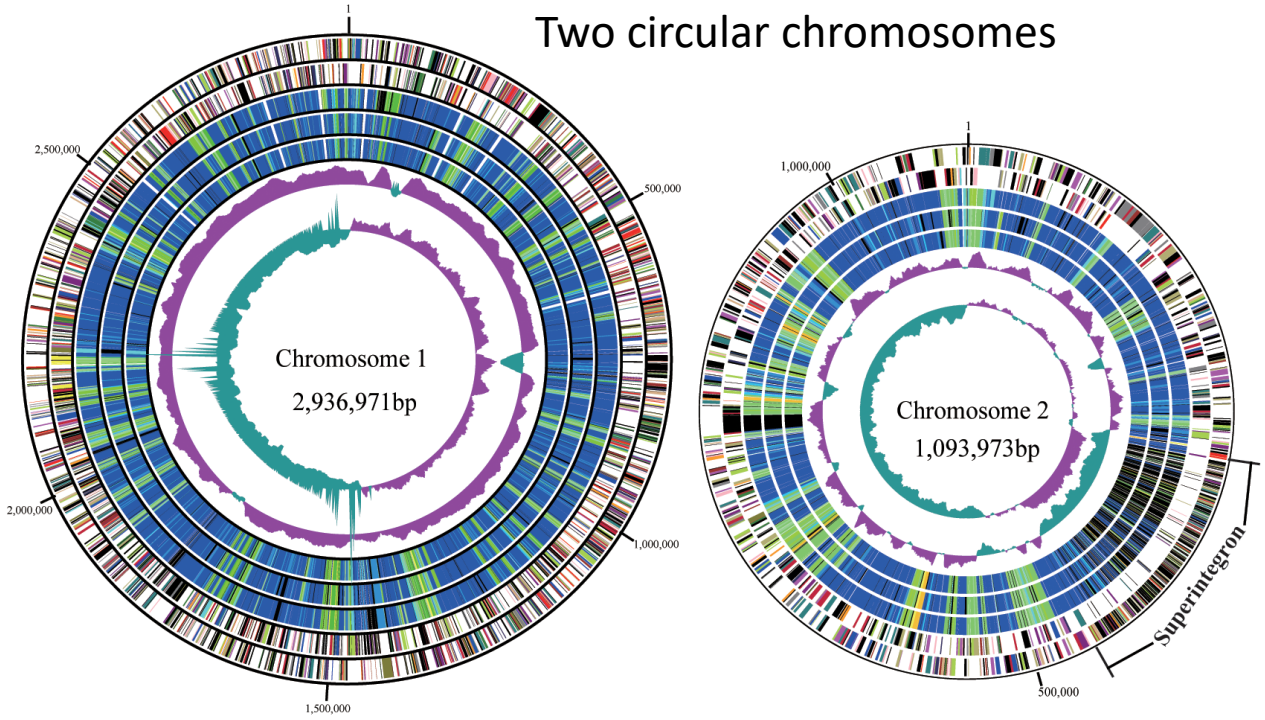
Plasmid pOSAK 3.3K

https://doi.org/10.1371/journal.pone.0202775.g003

Bacterial Genomes

Vibrio cholerae

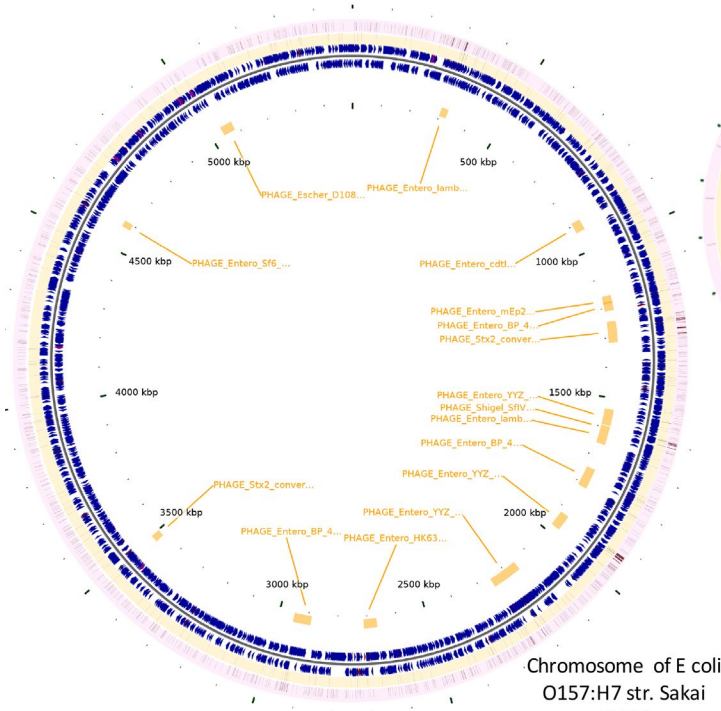
Two circular chromosomes



~4.9 Mb

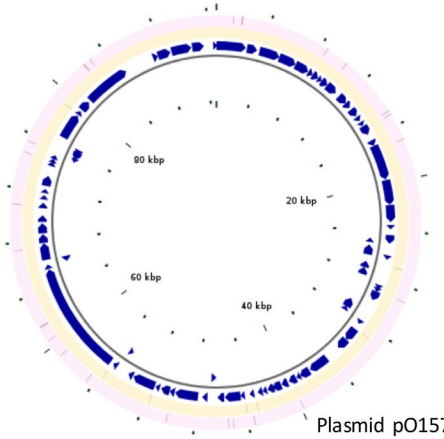
Escherichia coli

Circular chromosome

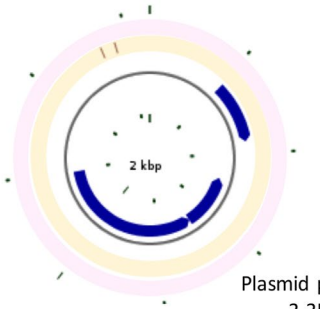


Chromosome of E coli
O157:H7 str. Sakai
5.4M

Plasmids



Plasmid pO157
92.7K
Antibiotic resistance



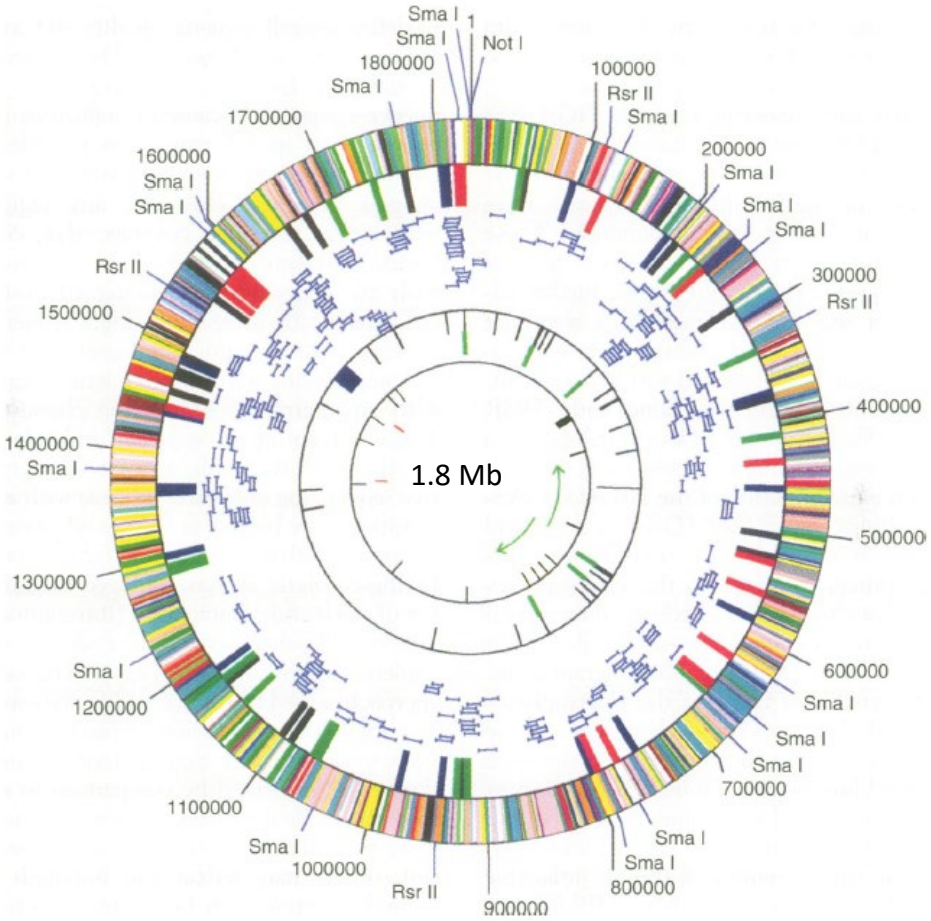
Plasmid pOSAK
3.3K
Metal resistance

~5.5 Mb

https://doi.org/10.1371/journal.pone.0098120.g001

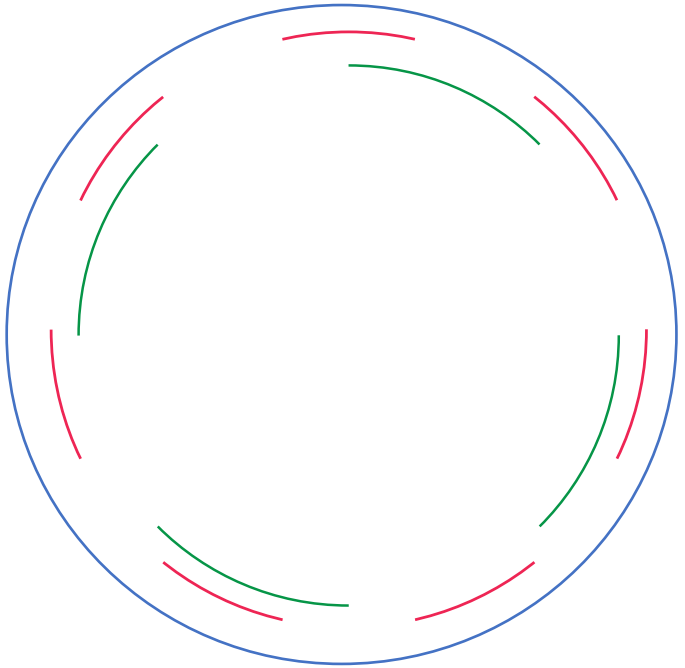
Sequencing a bacterial genome

Haemophilus influenzae RD



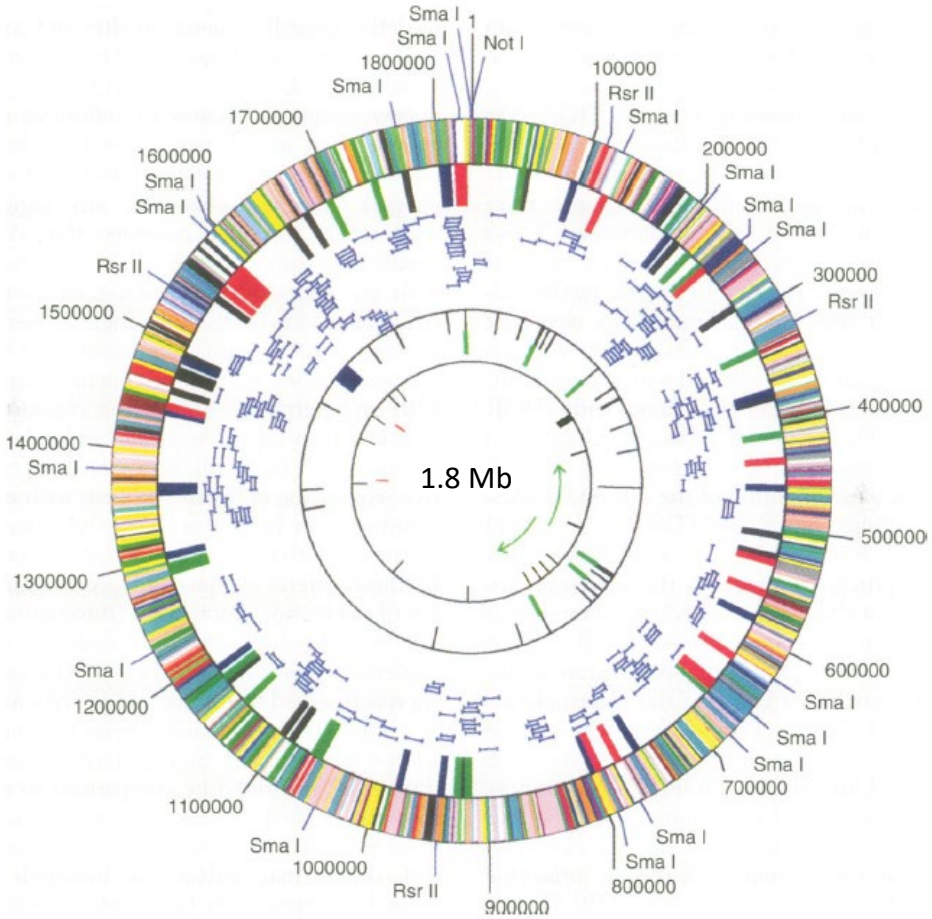
DOI: 10.1126/science.7542800

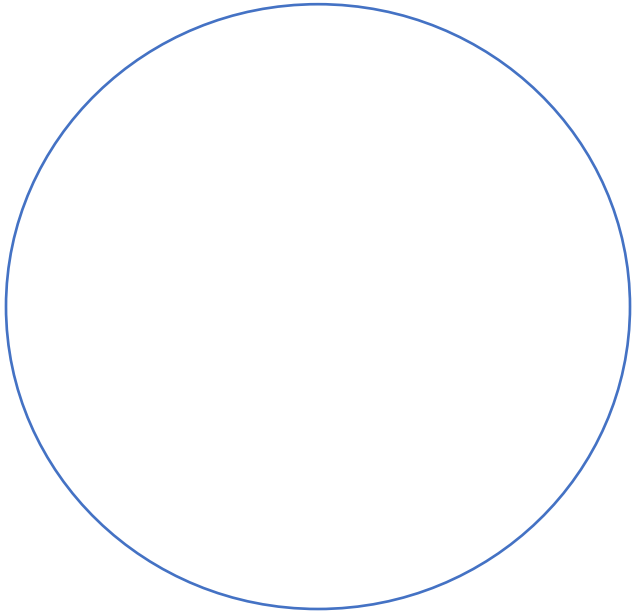
Too slow and expensive!!



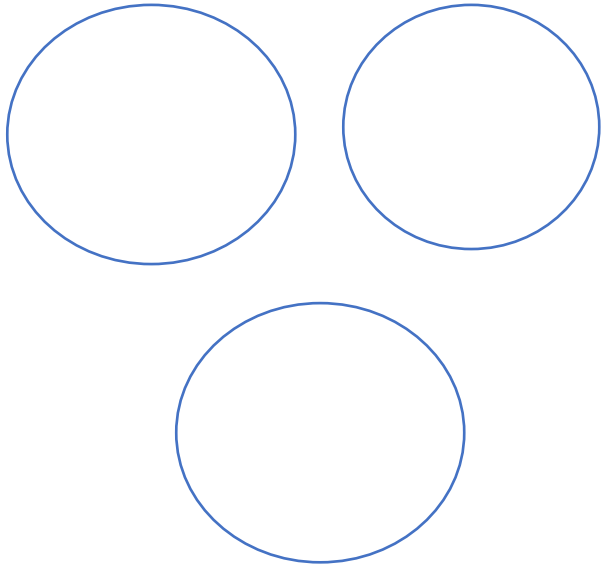
Sequencing a bacterial genome

Haemophilus influenzae



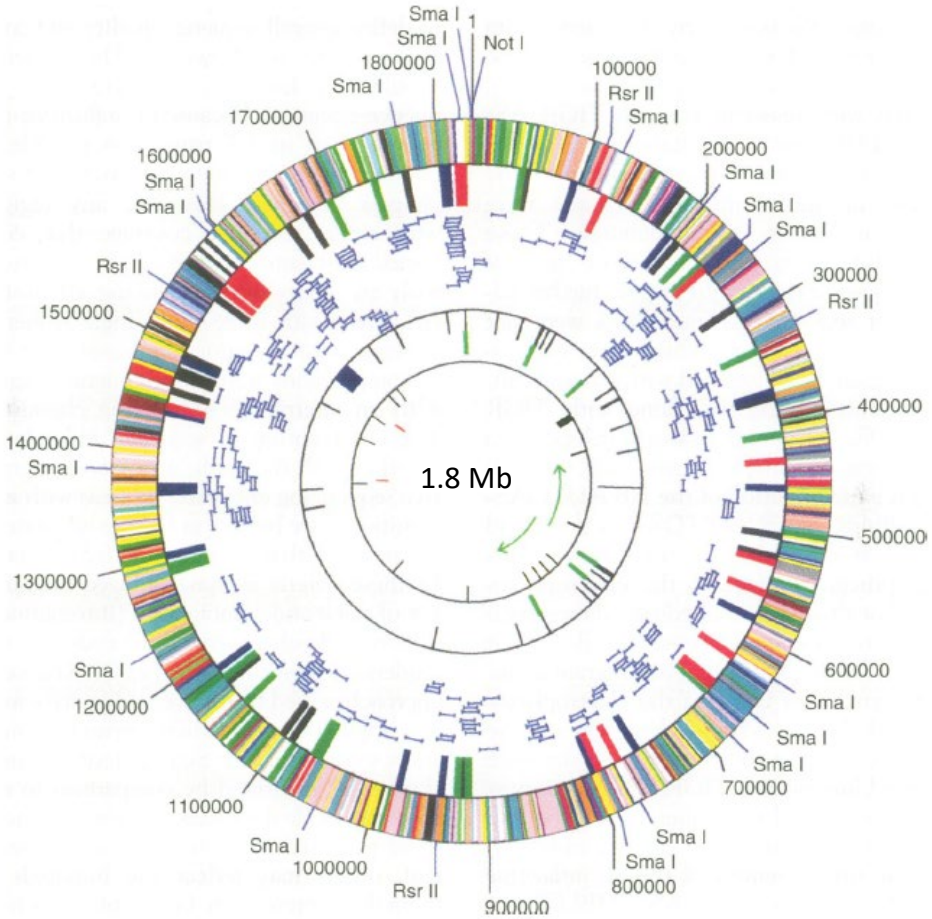


Multiple copies of the genome

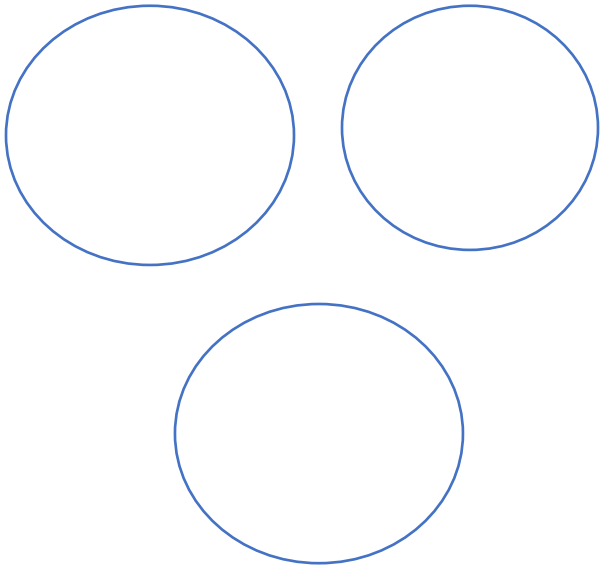


Sequencing a bacterial genome

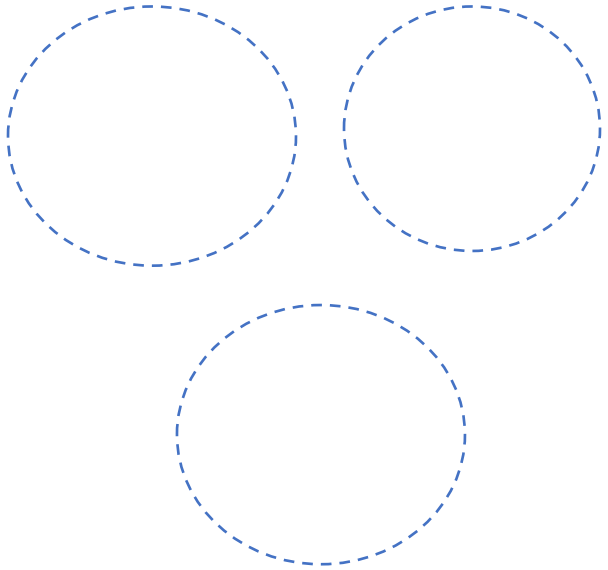
Haemophilus influenzae



Multiple copies of the genome



Fragmentation and sequence the fragments

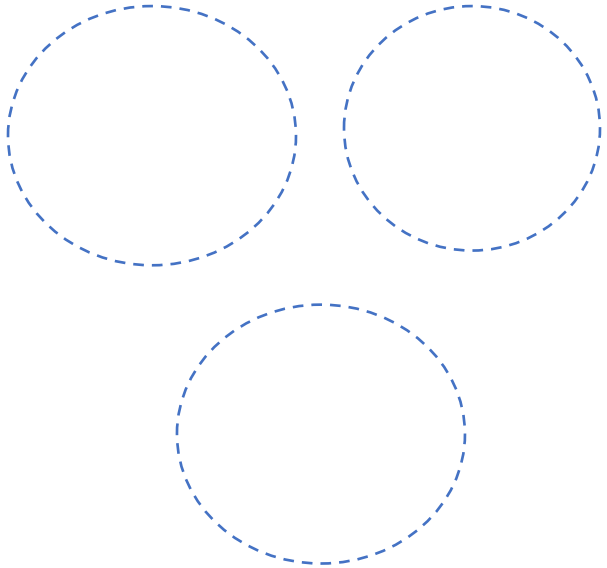


100 bp 500bp

How many fragments?

Sequencing a bacterial genome

Fragmentation and
sequence the fragments



100 bp 500 bp

How many fragments?
Or
Wat coverage do we need???

The Poisson distribution!!!!

"The probability that a base is not sequenced is $\mathbf{P_0 = e^{-m}}$*"*

m=1 equal 1 x Coverage
$P_0 = e^{-1}$=0.37 or 37 % of genome not sequenced

What does happen with 5x Coverage (~9500 clones)?

$P_0 = e^{-5}$=0.0067 or 0.67 % of genome not sequenced

And if we have 10x ???
$P_0 = e^{-10}$=4.539993e-05 or 0.000045 % of genome not sequenced
~99.995 % of the genome complete

DOI: 10.1126/science.7542800

Sequencing a bacterial genome

C=nl/L
C: Coverage
n: number of reads
l: length of the read
L: length of the genome

How many reads with Illumina (~100 bp) to obtain 10x coverage for a 1Mb genome?

$10x=n(100\ bp)/1x10^6$
$n=10(1x10^6)/100$
$n=1x10^5$ reads

Error rate ~ 1%

How many reads with MiniON (~50 kb) to obtain 10x coverage for a 1Mb genome?

$10x=n(5x10^4\ bp)/1x10^6$
$n=10(1x10^6)/\ 5x10^4$ n=200 reads

Error rate ~ 7%

Sequencing a bacterial genome

MICROBIAL GENOMICS

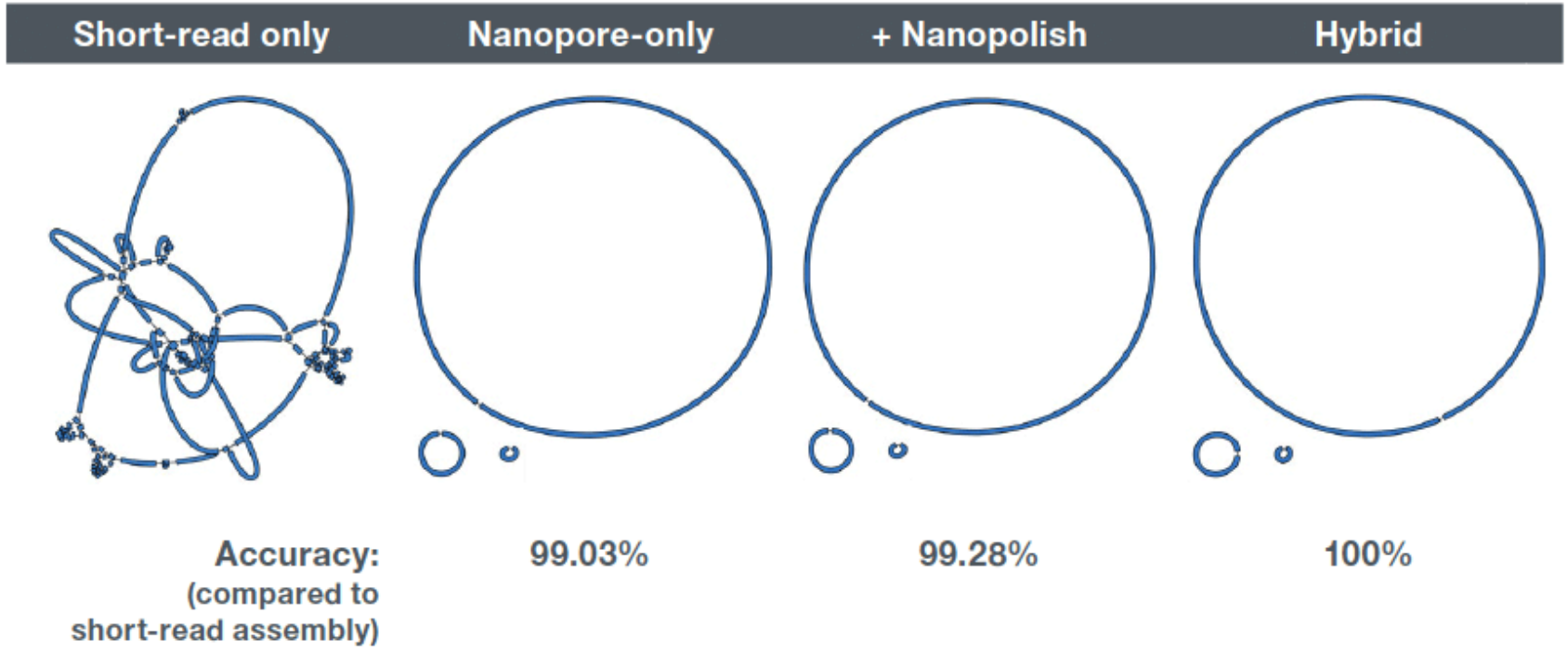
METHODS PAPER

Wick *et al.*, *Microbial Genomics* 2017;3

DOI 10.1099/mgen.0.000132

Completing bacterial genome assemblies with multiplex MinION sequencing

Ryan R. Wick,*† Louise M. Judd,† Claire L. Gorrie and Kathryn E. Holt



Sequence file formats

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

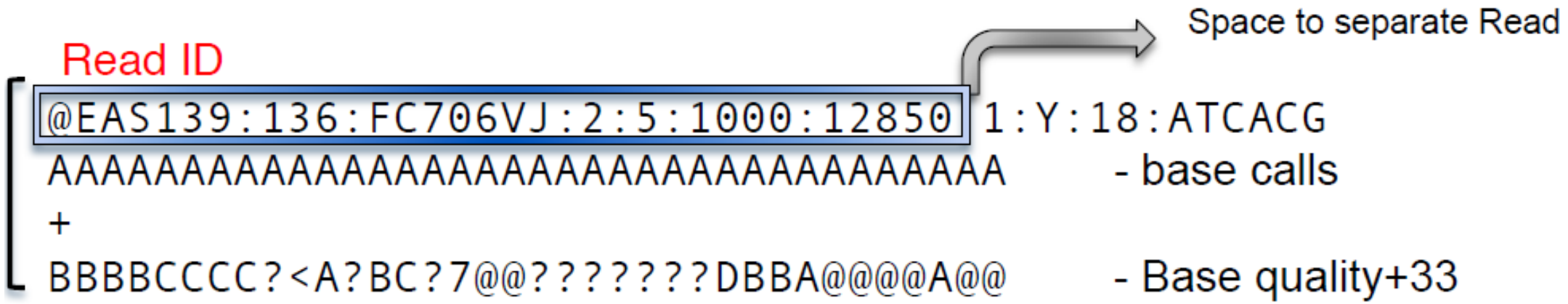
  FASTA format

  >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)



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
AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA      - 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

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

> 10^(-33/10)

[1] 0.0005011872 = 1/5000

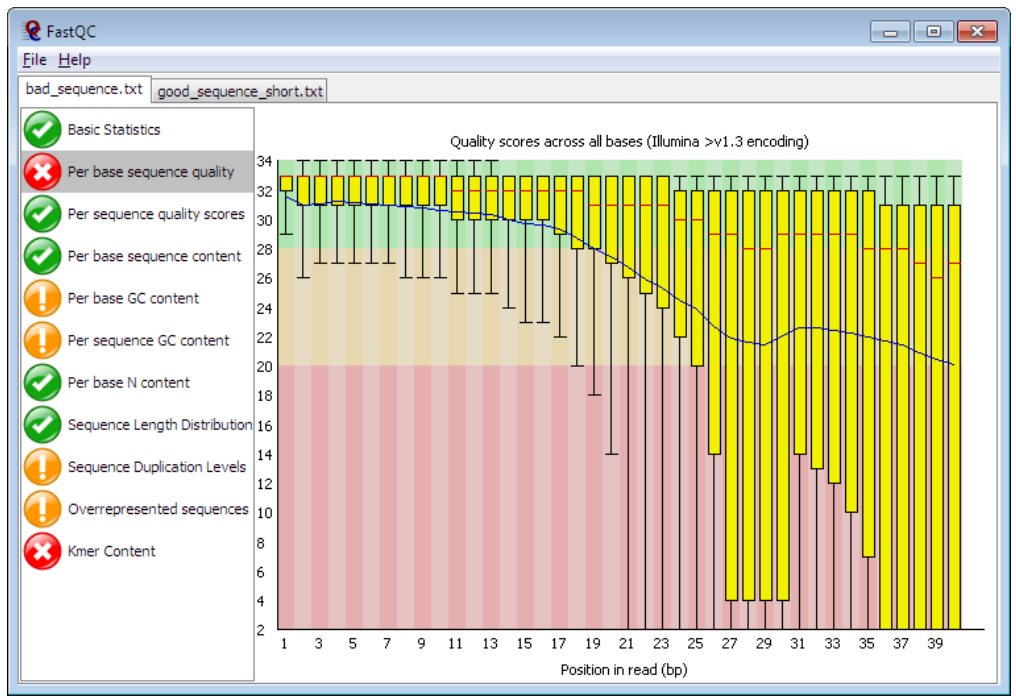
> 10^(-34/10)

[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/