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Advanced Applications of Next Generation Sequencing in Food Safety

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UCD Centre for Food Safety

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DAY 1

Monday 22nd January 2024

09:00 Stellenbosch welcome UCD to laboratory facilities

12:30 Lunch

SESSION 1: INTRODUCTION TO NEXT GENERATION SEQUENCING (NGS)
technologies, applications, and an introduction to bioinformatics

13:30 Professor Pieter A. Gouws, Stellenbosch University, South Africa

- Welcome, opening remarks and introduction to the program

14:30 Dr Guerrino Macori, University College Dublin, Ireland

- Overview of Next Generation Sequencing Technologies -what do we need to know

15:00 Professor Séamus Fanning, University College Dublin, Ireland

- Next Generation Sequencing Technologies in the context of risk assessment & food safety|

15:30 Dr Guerrino Macori, University College Dublin, Ireland

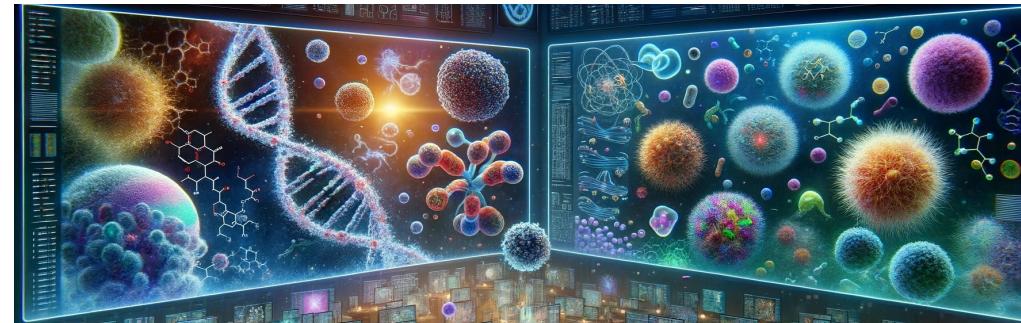
- Introduction to Bioinformatics and its applications

16:30 The industry perspective –

Karin Carstensen (Woolworths)

Dr Tynan Marais (WhiteSci)

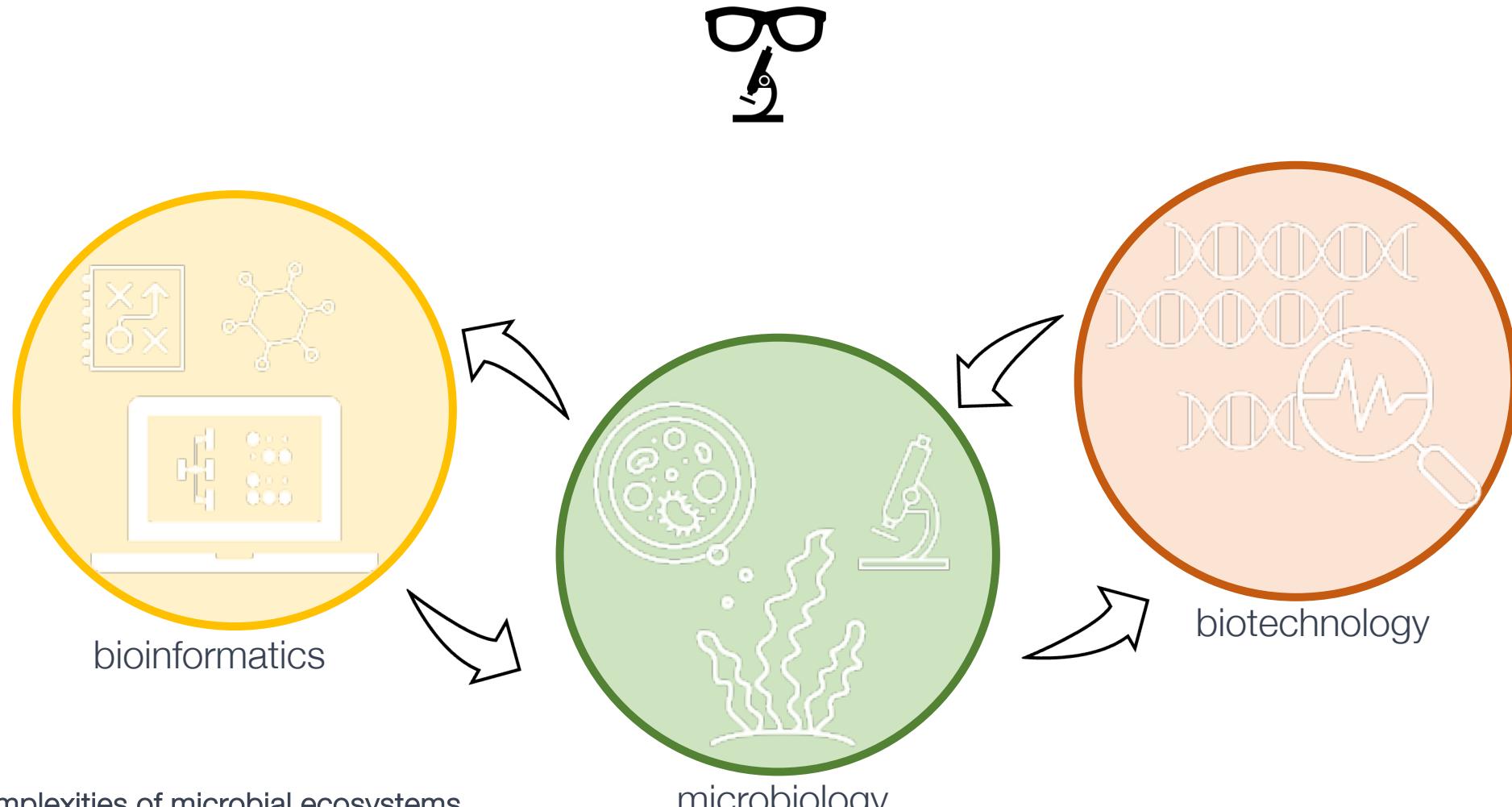




Overview of NGS Technologies - what do we need to know



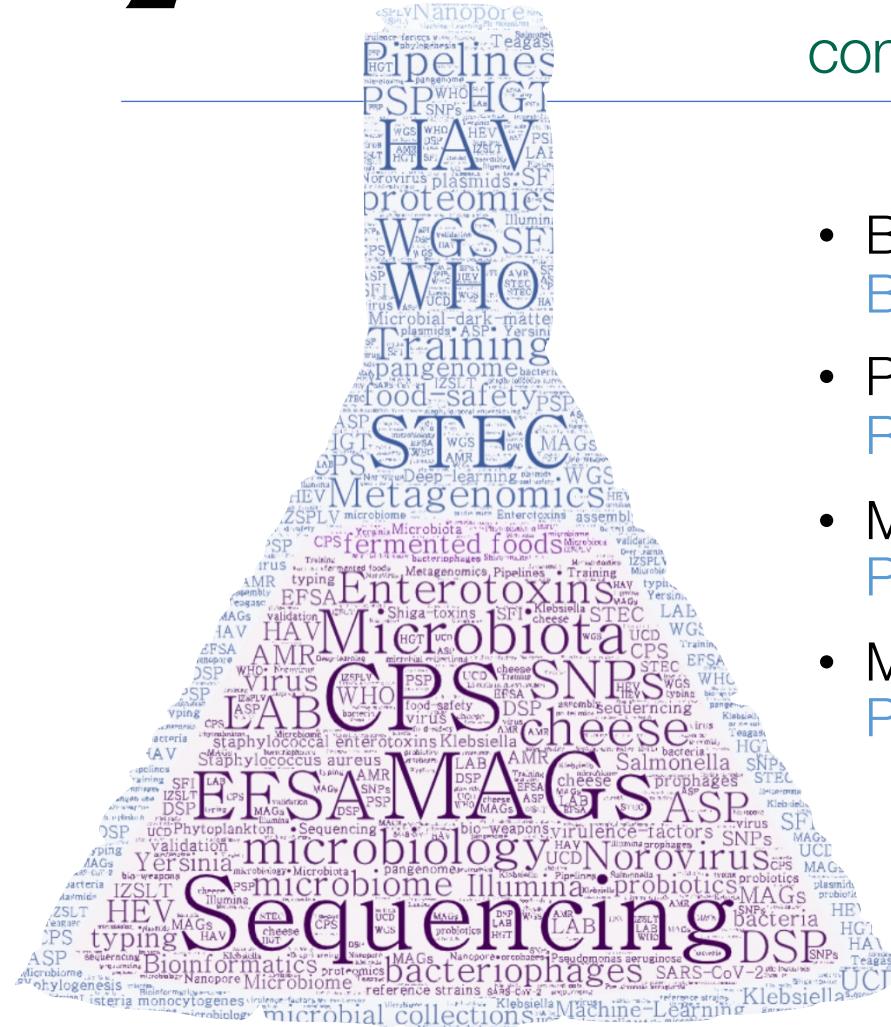
Background & History
Next Generation Sequencing
Overview of NGS Technologies
Data Generation and Applications



unravelling the complexities of microbial ecosystems
to pioneering the development of novel diagnostic
tools and **cutting-edge bioinformatics pipelines**



...introducing myself



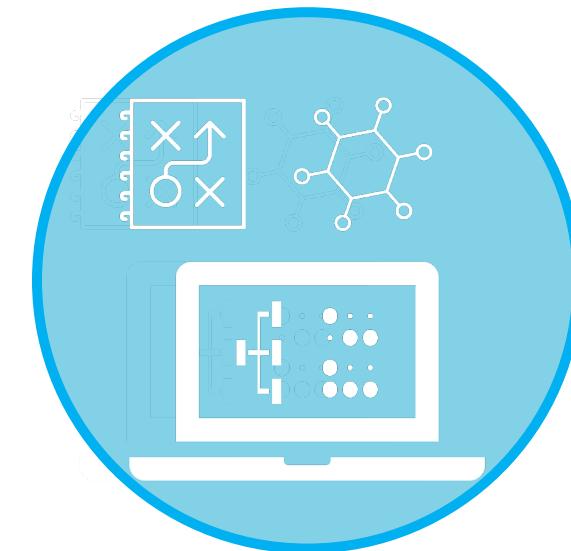
cutting-edge sequencing technologies to decrypt the complexities of microbial communities, and infectious diseases.

- Bioinformatics, food safety and genomics studies
Bioinformatician UCD-CFS
 - Population genomics, genomic surveillance
Research Scientist UCD-SPHPSS
 - Microbiome, models, harnessing novel strains, nutrition
PostDoctoral researcher APC Microbiome Ireland
 - Molecular biology and biotechnology
PhD, MSc Genomic Biotechnology, BSc Biotechnology

next wave of innovations in biological science interpretation of omics data that can be effectively harnessed to drive scientific advancements and uncover new frontiers.



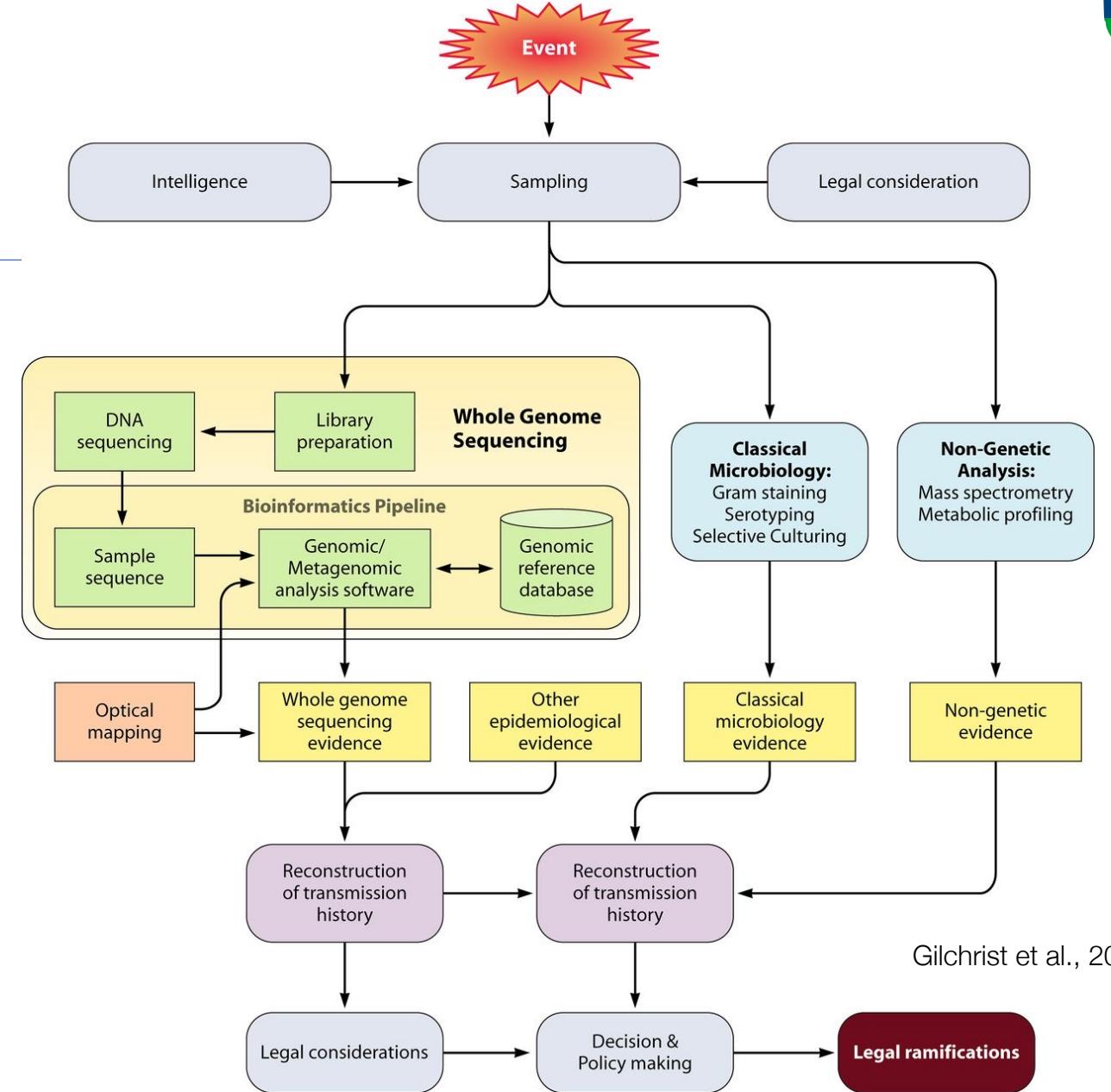
...introducing myself



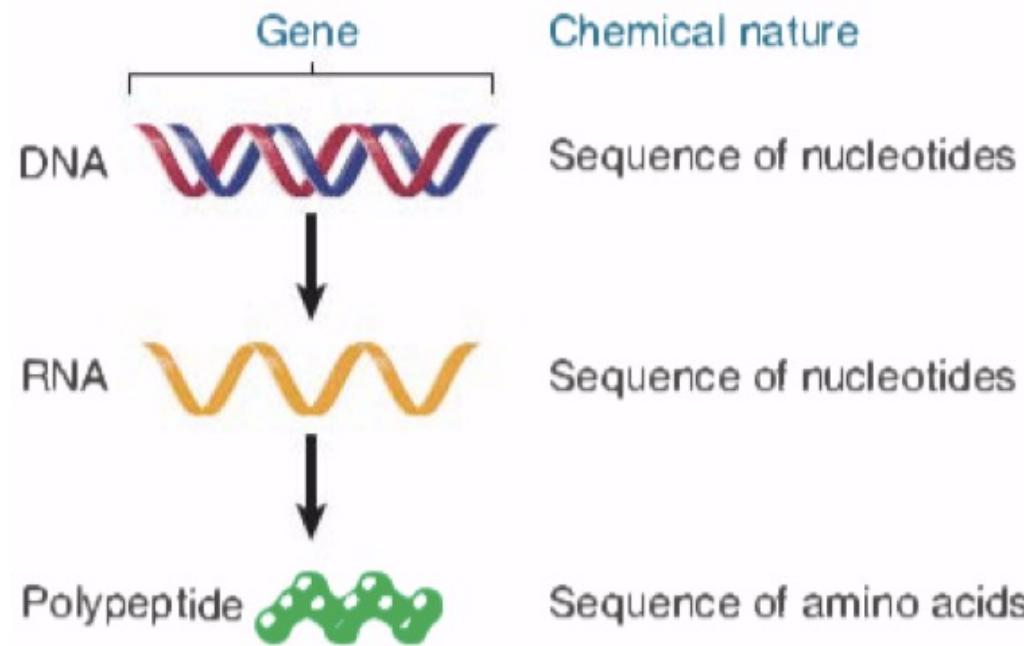
Real-world Applications and Hands-on Experience
[pathogen genomic analysis](#), [gene-disease association studies](#), or [modelling protein-protein interactions](#)

Overview

- Sanger sequencing
- Next Generation Sequencing
- Applications

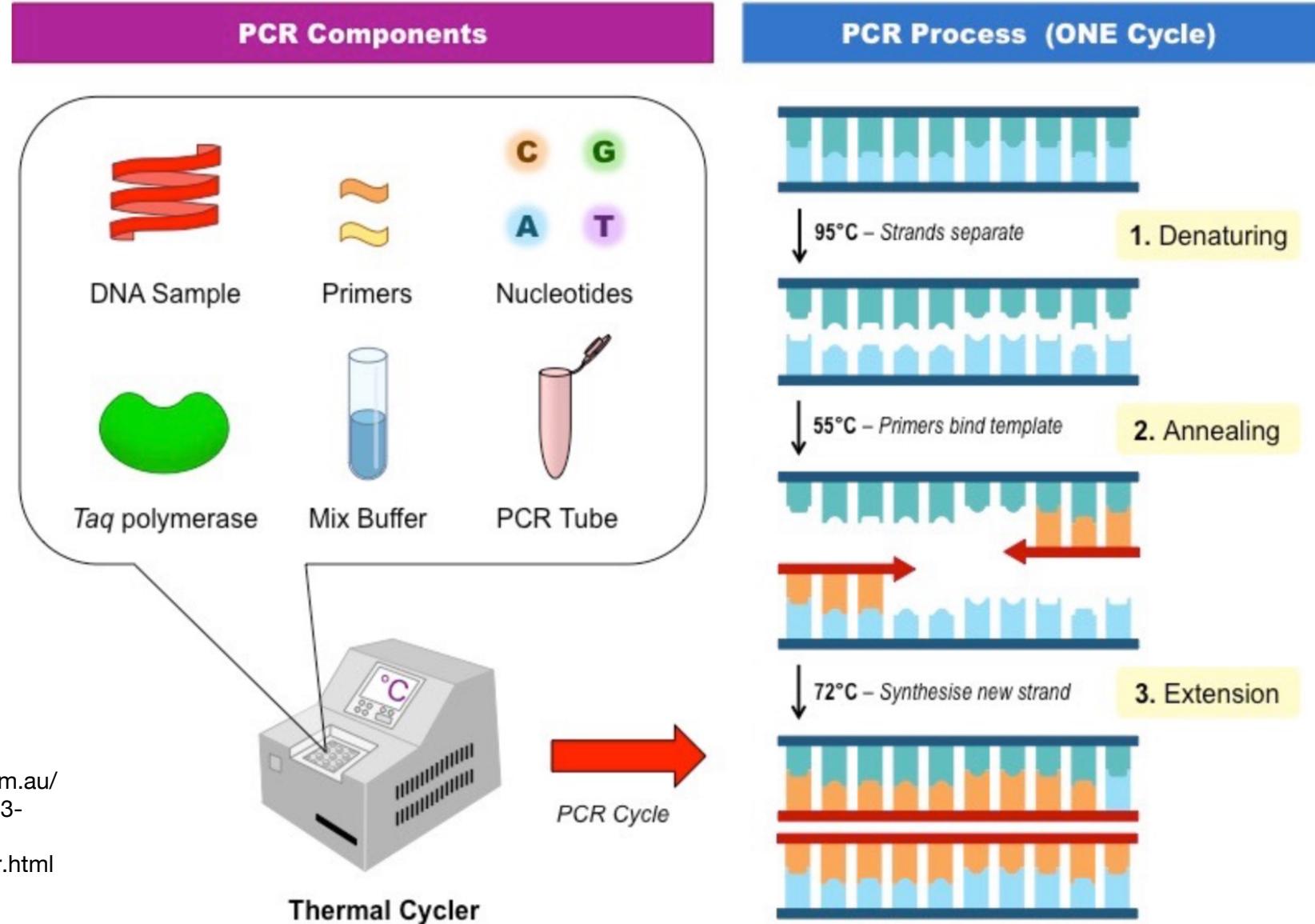


Background



AATTAAAGACAAACGGTTAGATGTGTTGGAATACCCTATTTTATA
CATGTTAGTACCTAAGAACATGAATCAAGAGAAGAATTATTTTGATG
GAGTTGCAATTAAAGACAAACGGTTAGATGTGTTGGAATACCCT
ATTTTATACATGTTAGTACCTAAGAACATGAATCAAGAGAAGAATTAT
TTTGATG

Background



<https://ib.bioninja.com.au/standard-level/topic-3-genetics/35-genetic-modification-and/pcr.html>

Sequencing

Proc. Natl. Acad. Sci. USA
Vol. 74, No. 12, pp. 5463–5467, December 1977
Biochemistry

DNA sequencing with chain-terminating inhibitors

(DNA polymerase/nucleotide sequences/bacteriophage ϕ X174)

F. SANGER, S. NICKLEN, AND A. R. COULSON

Medical Research Council Laboratory of Molecular Biology, Cambridge CB2 2QH, England

Contributed by F. Sanger, October 3, 1977

ABSTRACT A new method for determining nucleotide sequences in DNA is described. It is similar to the "plus and minus" method [Sanger, F., & Coulson, A. R. (1975) *J. Mol. Biol.* 94, 441–448] but makes use of the 2',3'-dideoxy and arabinonucleoside analogues of the normal deoxynucleoside triphosphates, which act as specific chain-terminating inhibitors of DNA polymerase. The technique has been applied to the DNA of bacteriophage ϕ X174 and is more rapid and more accurate than either the plus or the minus method.

The "plus and minus" method (1) is a relatively rapid and simple technique that has made possible the determination of the sequence of the genome of bacteriophage ϕ X174 (2). It depends on the use of DNA polymerase to transcribe specific regions of the DNA under controlled conditions. Although the method is considerably more rapid and simple than other available techniques, neither the "plus" nor the "minus" method is completely accurate, and in order to establish a sequence both must be used together, and sometimes confirmatory data are necessary. W. M. Barnes (*J. Mol. Biol.*, in press) has recently developed a third method, involving ribo-substitution, which has certain advantages over the plus and minus method, but this has not yet been extensively exploited.

Another rapid and simple method that depends on specific chemical degradation of the DNA has recently been described by Maxam and Gilbert (3), and this has also been used extensively for DNA sequencing. It has the advantage over the plus and minus method that it can be applied to double-stranded DNA, but it requires a strand separation or equivalent fractionation of each restriction enzyme fragment studied, which makes it somewhat more laborious.

a stereoisomer of ribose in which the 3'-hydroxyl group is oriented in *trans* position with respect to the 2'-hydroxyl group. The arabinosyl (ara) nucleotides act as chain terminating inhibitors of *Escherichia coli* DNA polymerase I in a manner comparable to ddT (4), although synthesized chains ending in 3' araC can be further extended by some mammalian DNA polymerases (5). In order to obtain a suitable pattern of bands from which an extensive sequence can be read it is necessary to have a ratio of terminating triphosphate to normal triphosphate such that only partial incorporation of the terminator occurs. For the dideoxy derivatives this ratio is about 100, and for the arabinosyl derivatives about 5000.

METHODS

Preparation of the Triphosphate Analogues. The preparation of ddTTP has been described (6, 7), and the material is now commercially available. ddA has been prepared by McCarthy *et al.* (8). We essentially followed their procedure and used the methods of Tener (9) and of Hoard and Ott (10) to convert it to the triphosphate, which was then purified on DEAE-Sephadex, using a 0.1–1.0 M gradient of triethylamine carbonate at pH 8.4. The preparation of ddGTP and ddCTP has not been described previously; however we applied the same method as that used for ddATP and obtained solutions having the requisite terminating activities. The yields were very low and this can hardly be regarded as adequate chemical characterization. However, there can be little doubt that the activity was due to the dideoxy derivatives.

The starting material for the ddGTP was *N*-isobutyryl-5'-

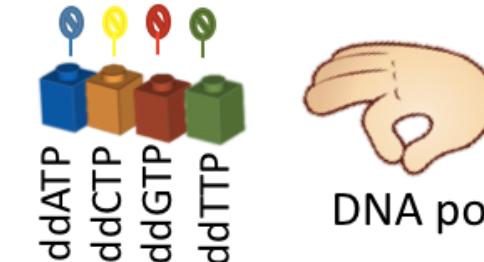
Fred Sanger
“Chain termination
sequencing”

Sequencing

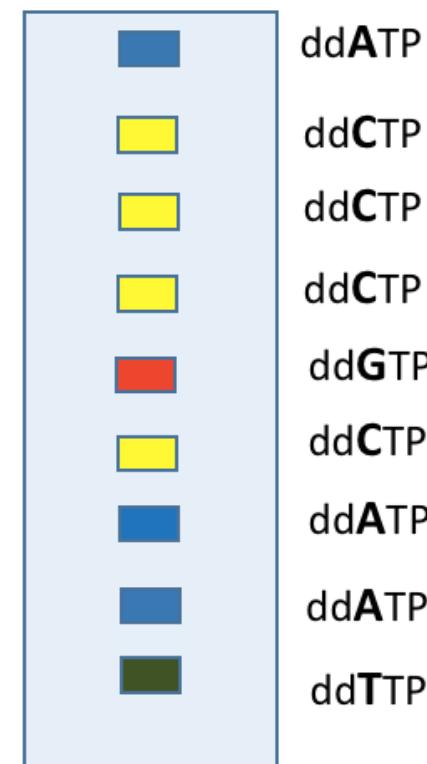
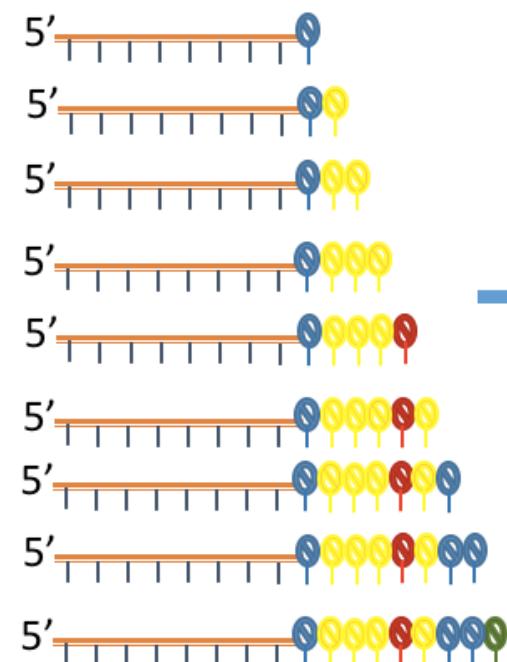
DNA template



- Dideoxynucleotides (chain-elongating inhibitors of DNA polymerase)
- DNA polymerase



gel electrophoresis



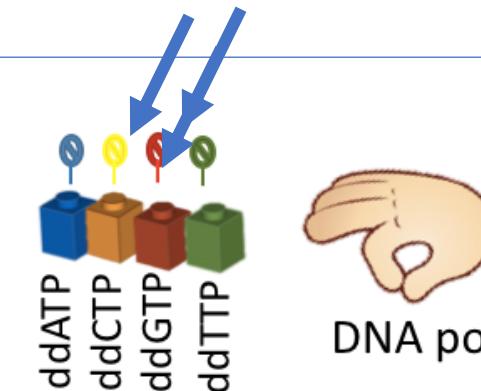
ACCCGCAAT

Sequencing

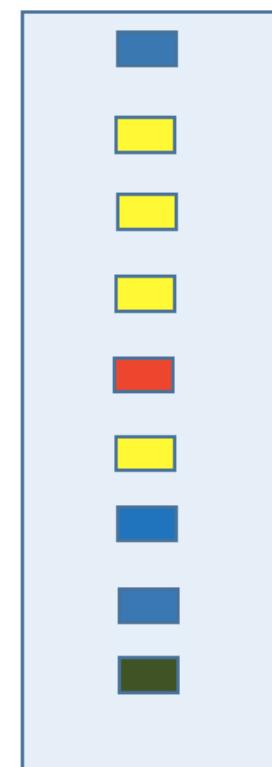
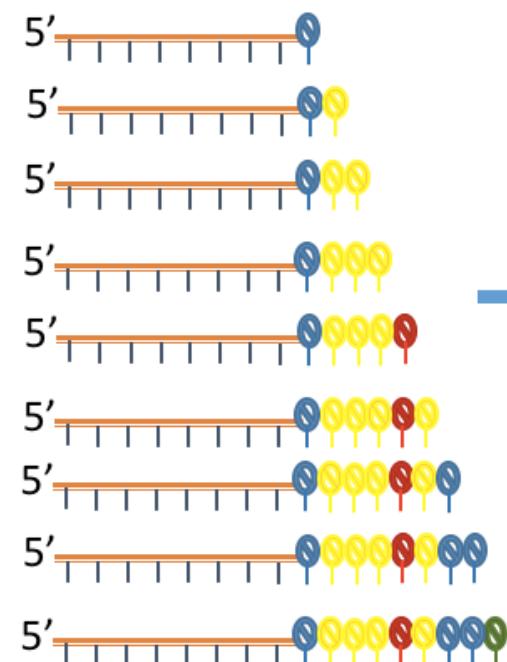
DNA template



- Dideoxynucleotides (chain-elongating inhibitors of DNA polymerase)
- DNA polymerase



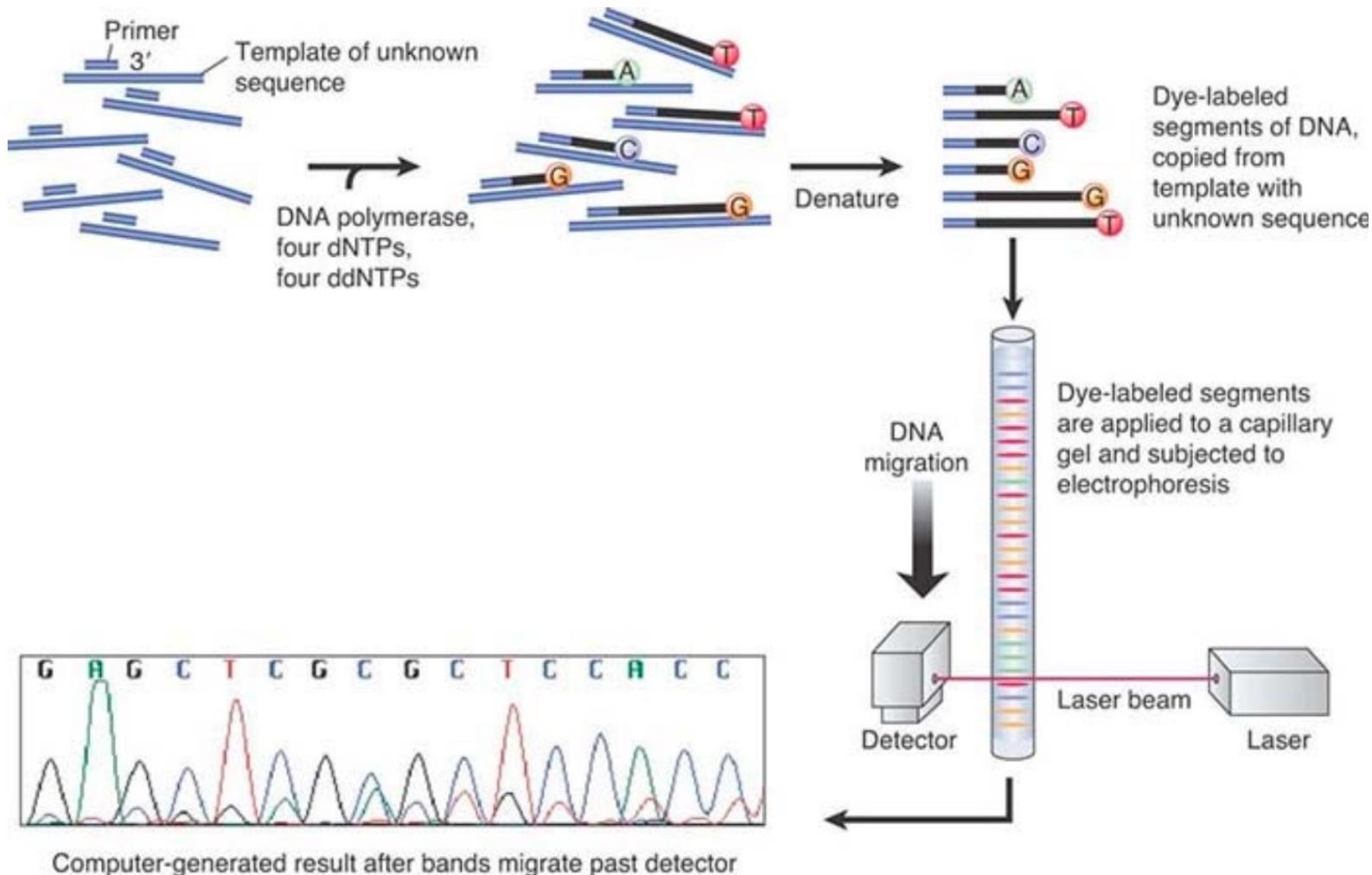
gel electrophoresis



ddATP
ddCTP
ddCTP
ddCTP
ddGTP
ddCTP
ddATP
ddATP
ddTTP

ACCCGGCAAT

Sequencing



Lewin's Genes XII, Lewin, Krebs, Goldstein, Kilpatrick. Jones & Bartlett Learning, 2018

Sequencing

AATTAAAGACAAACGG

TACCCCTATTTTATAACATGTTAG

ACAAGAGAAGAATTATTTTGTGGAGTTGC

ACGGTTAGATGTGTTGGAATA

GTTAGATGTGTTGGAAT

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TTTAGTACCTAAGAACATCAAGAGAAGAATTATTTTG

TGGAATACCTATTTTATA

GAATGAATCAAGAGAAGAATTATTTTGA

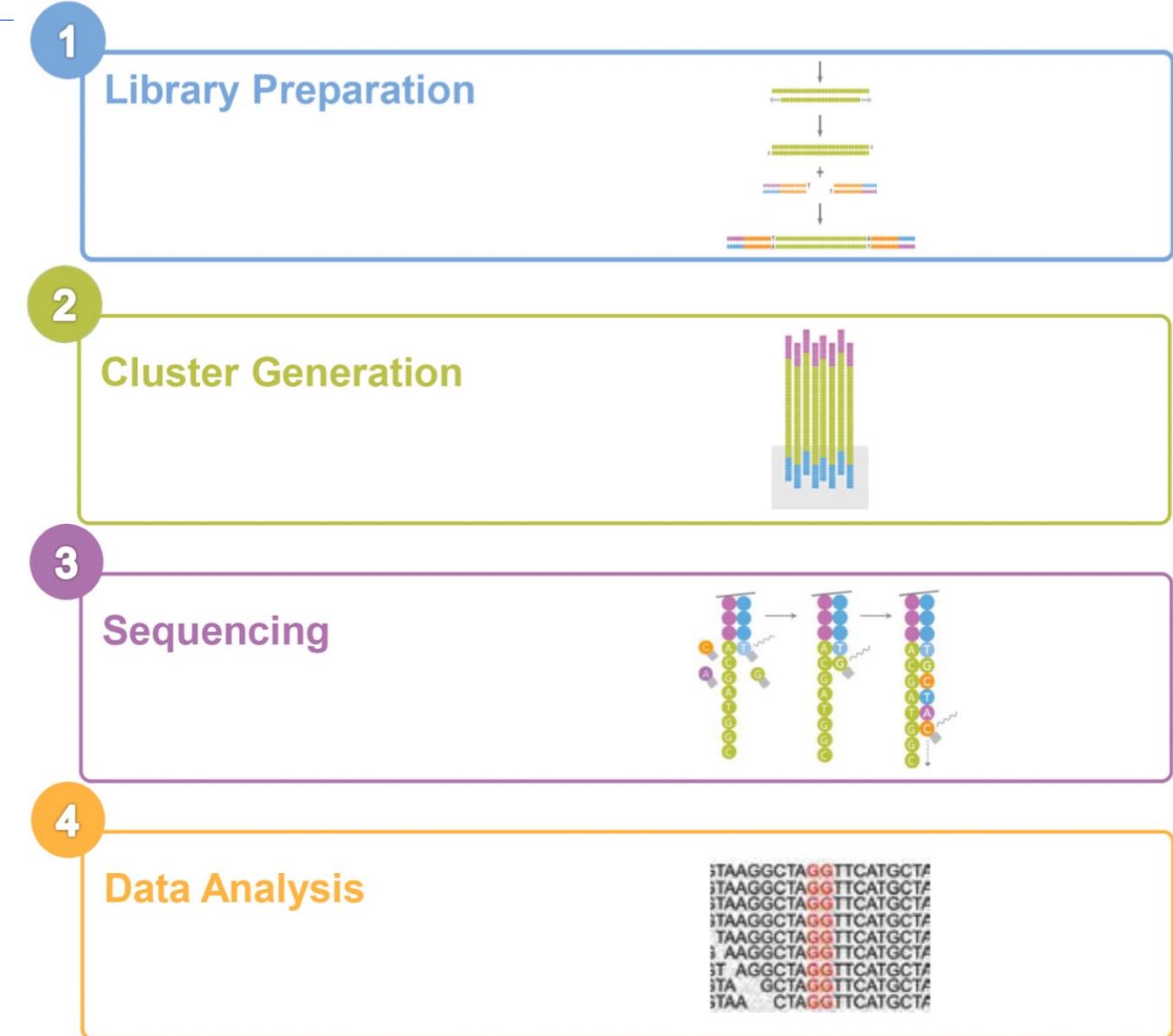
Next Generation Sequencing

- Illumina, Personal Genome Machines, Oxford Nanopore Technologies, ThermoFisher (SOLiD - 180 Gb per run, 2 x 60 bp reads)
- Single-molecule real-time (SMRT) Pacific Bioscience



Next Generation Sequencing

Illumina sequencing technology workflow



Next Generation Sequencing

Illumina sequencing technology workflow

Illumina sequencing technology workflow

Sample 1 purified
Genomic DNA

AATTAAAGACAAACGGTTAGATGTGT
TTGGAATAACCCTATTTTATACATGTTA
GTACCTAAGAACATGAATCAAGAGAAGAA
TTTATTTTGATGGAGTTGCAATTTAA
AGACAAACGGTTAGATGTGTTGGAAT
ACCCTATTTTATACATGTTAGTACCT
AAGAACATGAATCAAGAGAAGAATTATTT
TTGATGG

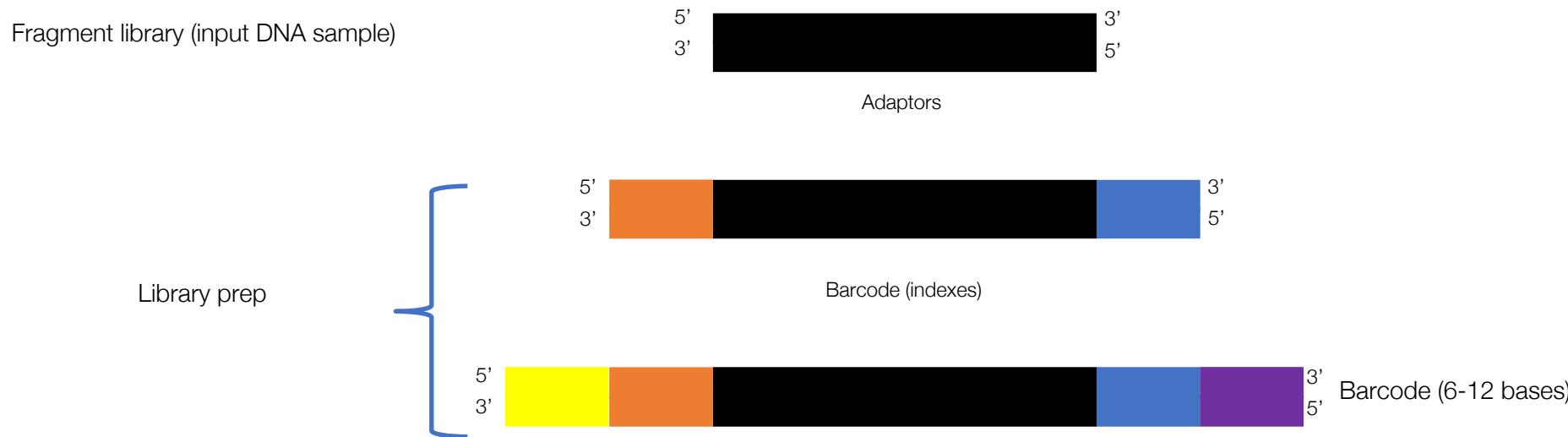


Fragment
Genomic DNA

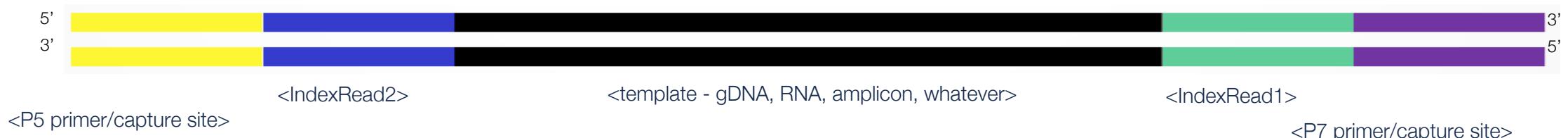
AATTAA
AAGACA
AACGGT
TAGATG
TGTTTG
GAATAC

Next Generation Sequencing

Illumina sequencing technology workflow



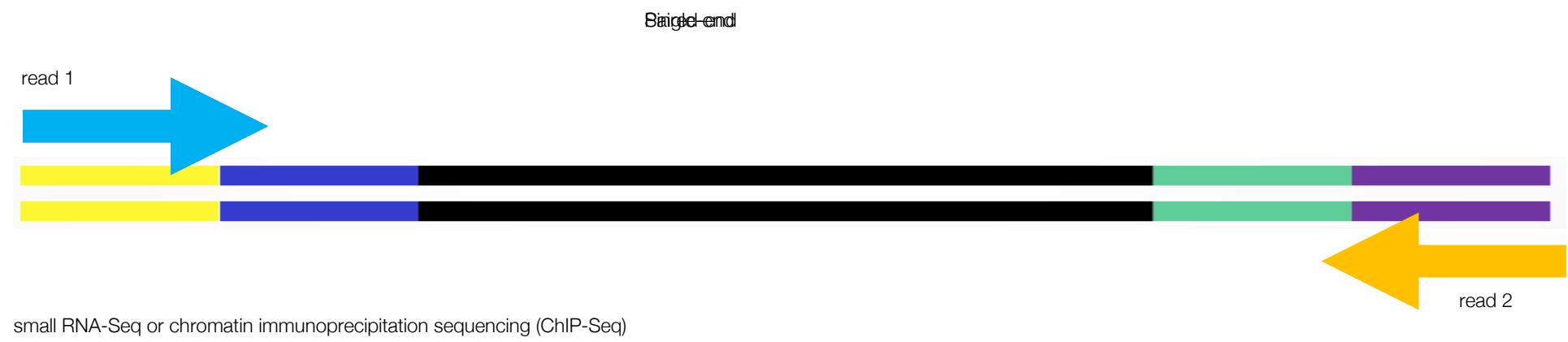
Canonical ILLUMINA library design as of January 2024 (all 5'-3')



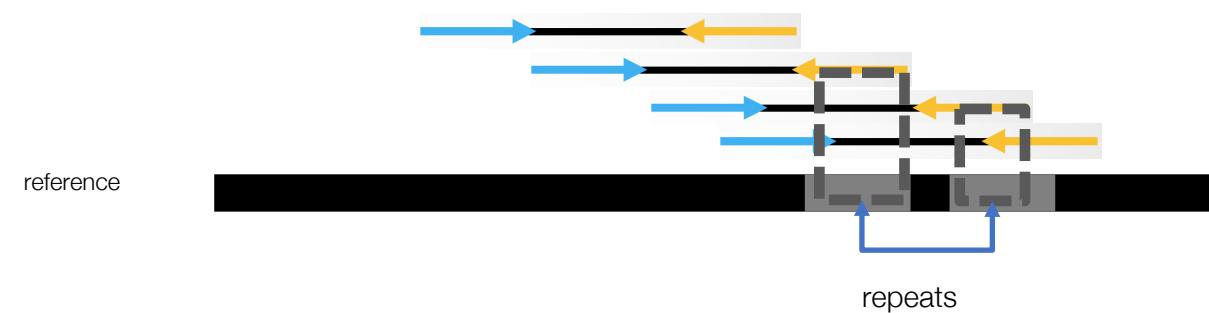
Next Generation Sequencing

Illumina sequencing technology workflow

1. Library preparation



Alignment to the Reference Sequence



Next Generation Sequencing

Illumina sequencing technology workflow

Sample 1 purified Genomic DNA

AATTAAAGACAAACGGTTAGATGTGT
TTGGAATACCCCTATTTTATACATGTTA
GTACCTAAGAACATGAATCAAGAGAAAGAA
TTTATTTTGATGGAGTTGCAATTAA
AGACAAACGGTTAGATGTGTTGGAAT
ACCCTATTTTATACATGTTAGTACCT
AAGAATGAATCAAGAGAAAGAATTATT
TTGATGG

Fragment Genomic DNA

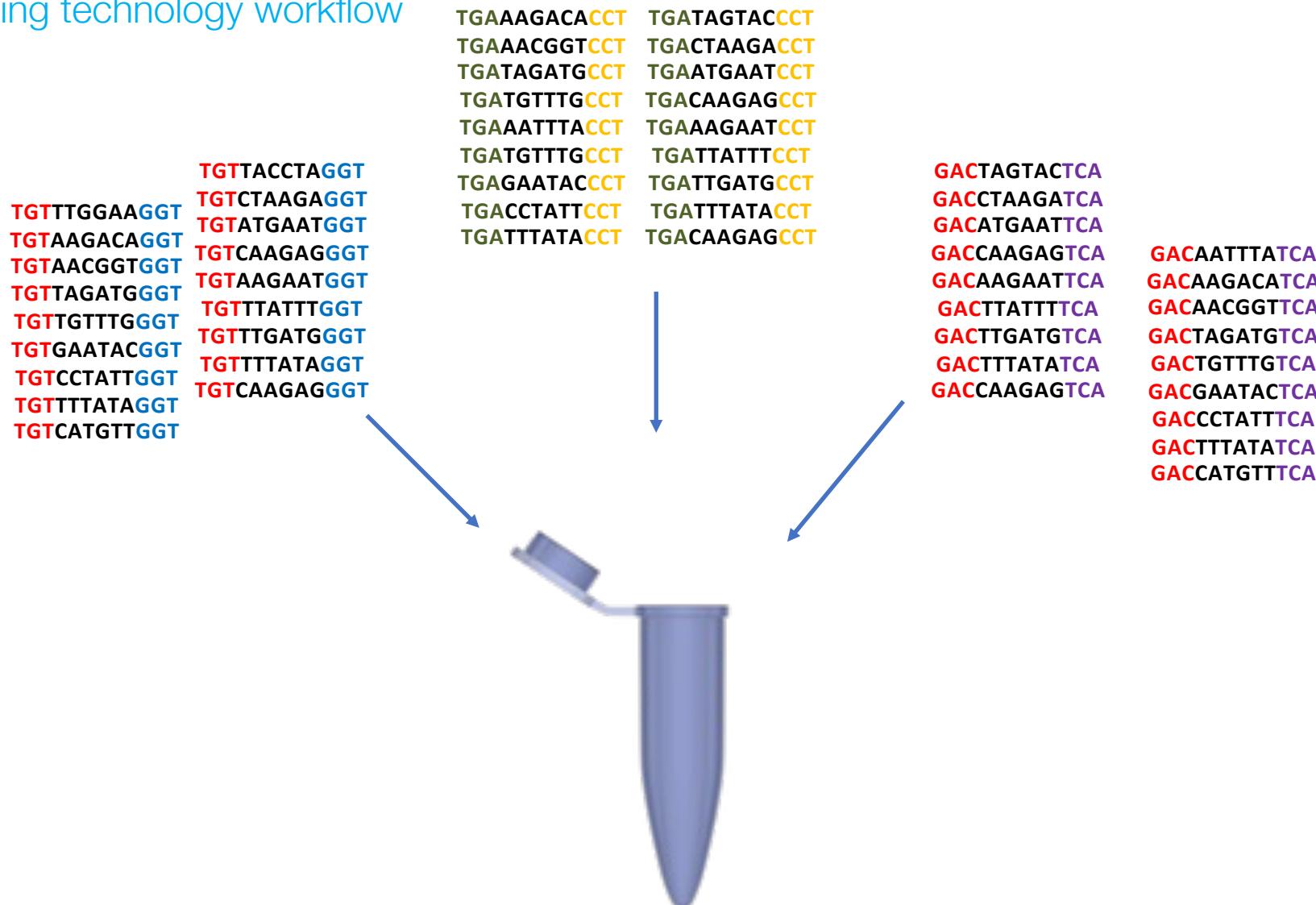
AATTAA
AAGACA
AACGGT
TAGATG
TGTTTG
GAATAC CAAGAG
AAGAAT
TTATT
TTGATG
TTTATA
CAAGAG

Ligate indexed Adapters

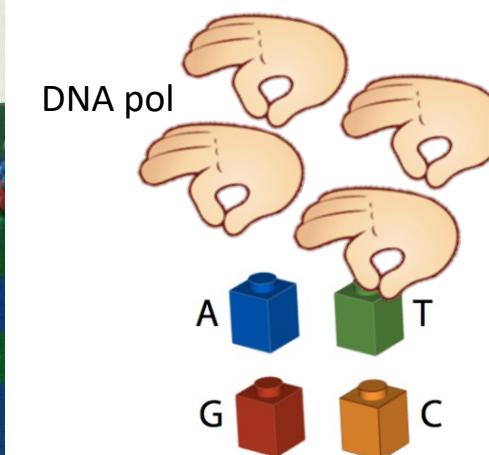
GACAATTAA**TCA**
GACAAGAC**A**TCA
GACAACGG**T**CA
GACTAGATG**T**CA
GACTGTTG**T**CA
GACGAATA**C**TA
GACCAAGAG**T**CA
GACAAGAAT**T**CA
GACTTATT**T**CA
GACTTGATG**T**CA
GACTTTATA**T**CA
GACCAAGAG**T**CA

Next Generation Sequencing

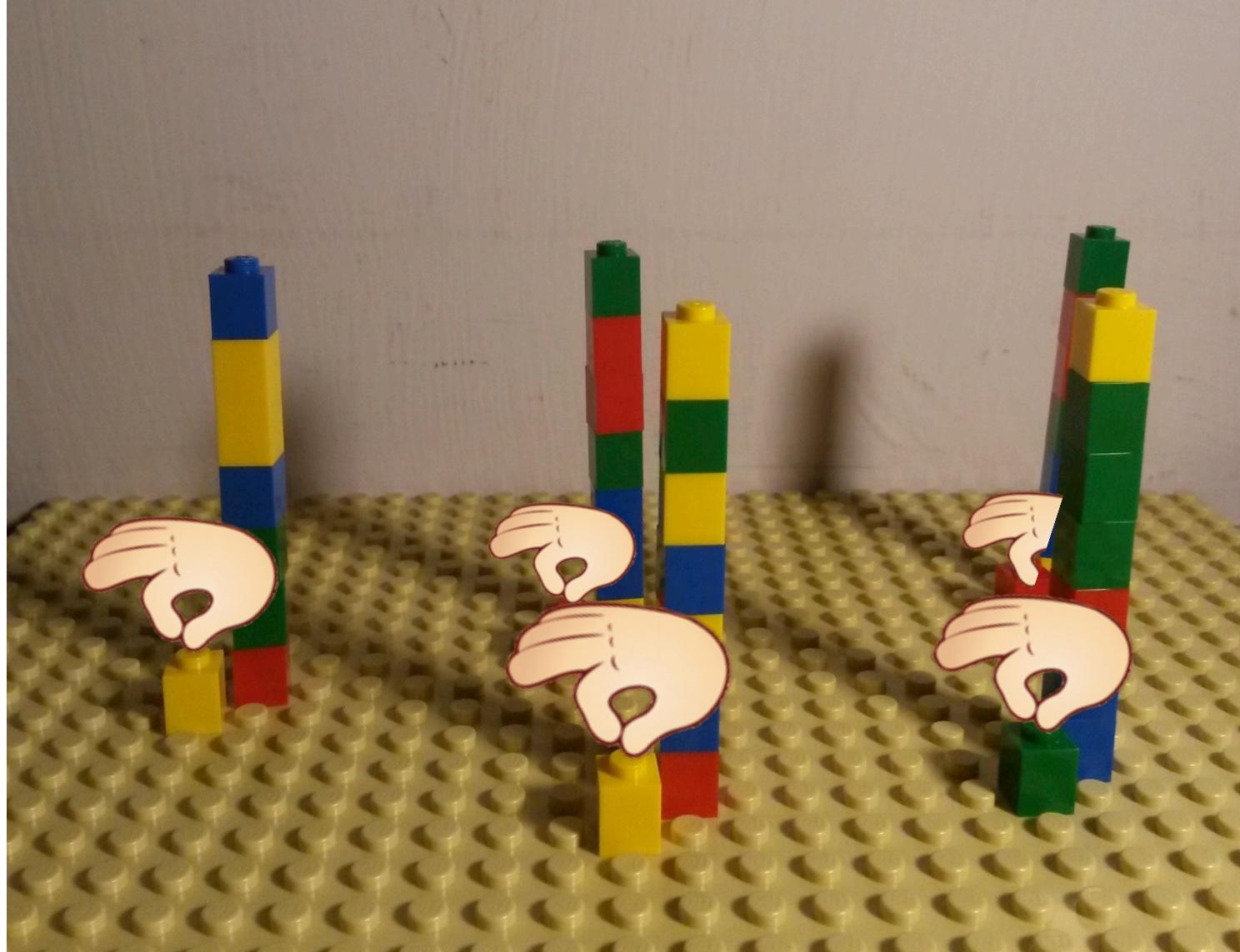
Illumina sequencing technology workflow



Next Generation Sequencing

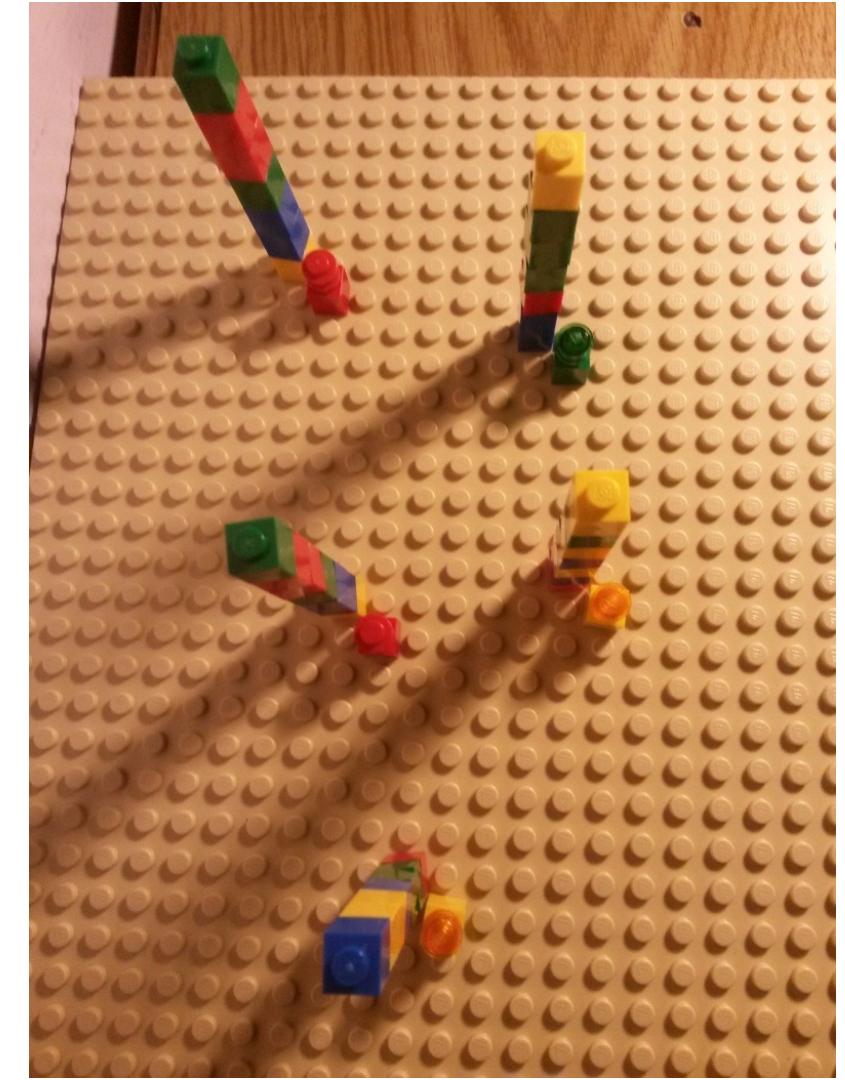
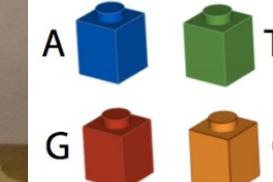
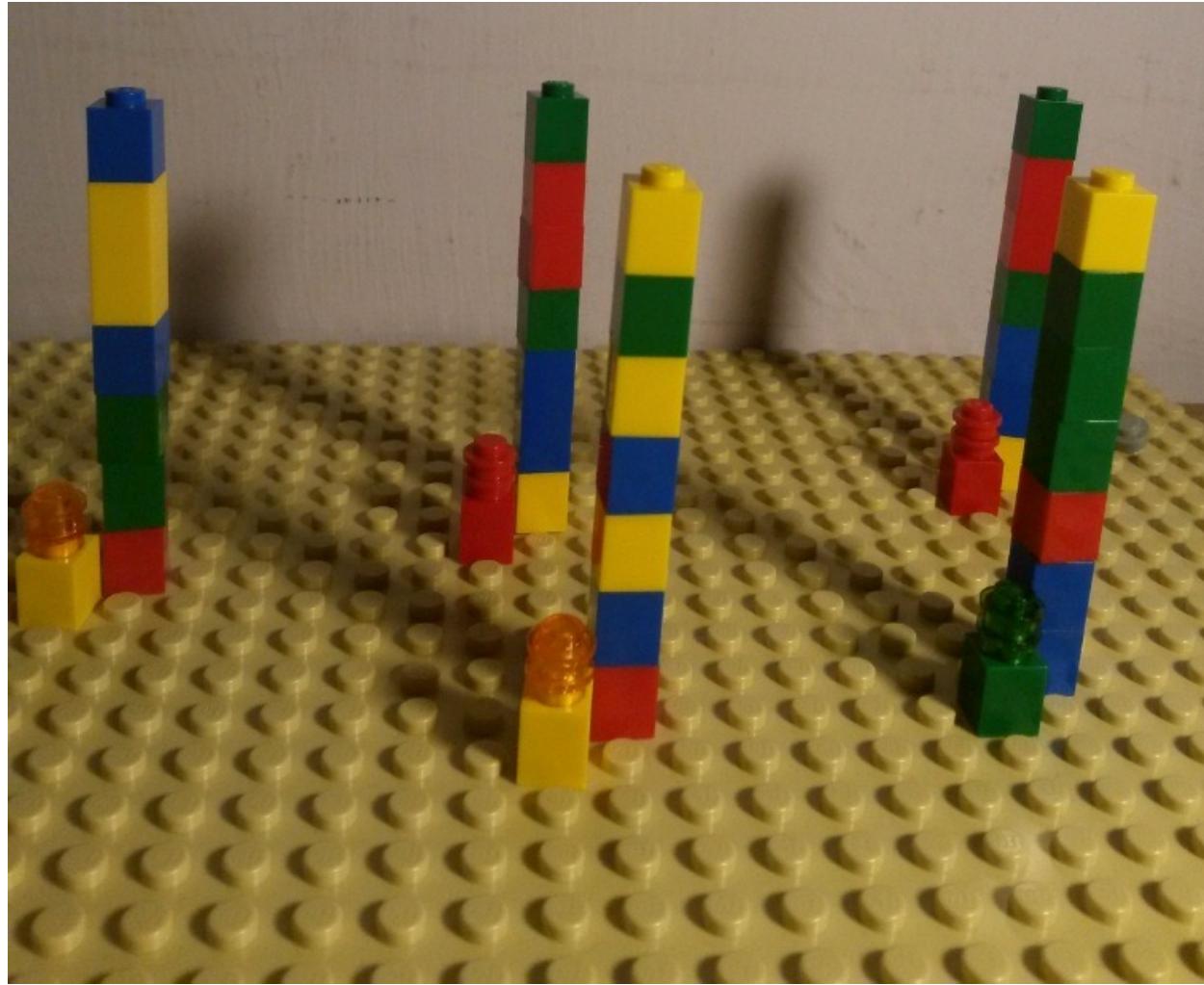


Next Generation Sequencing



Next Generation Sequencing

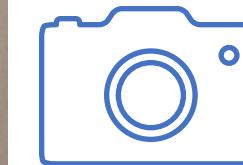
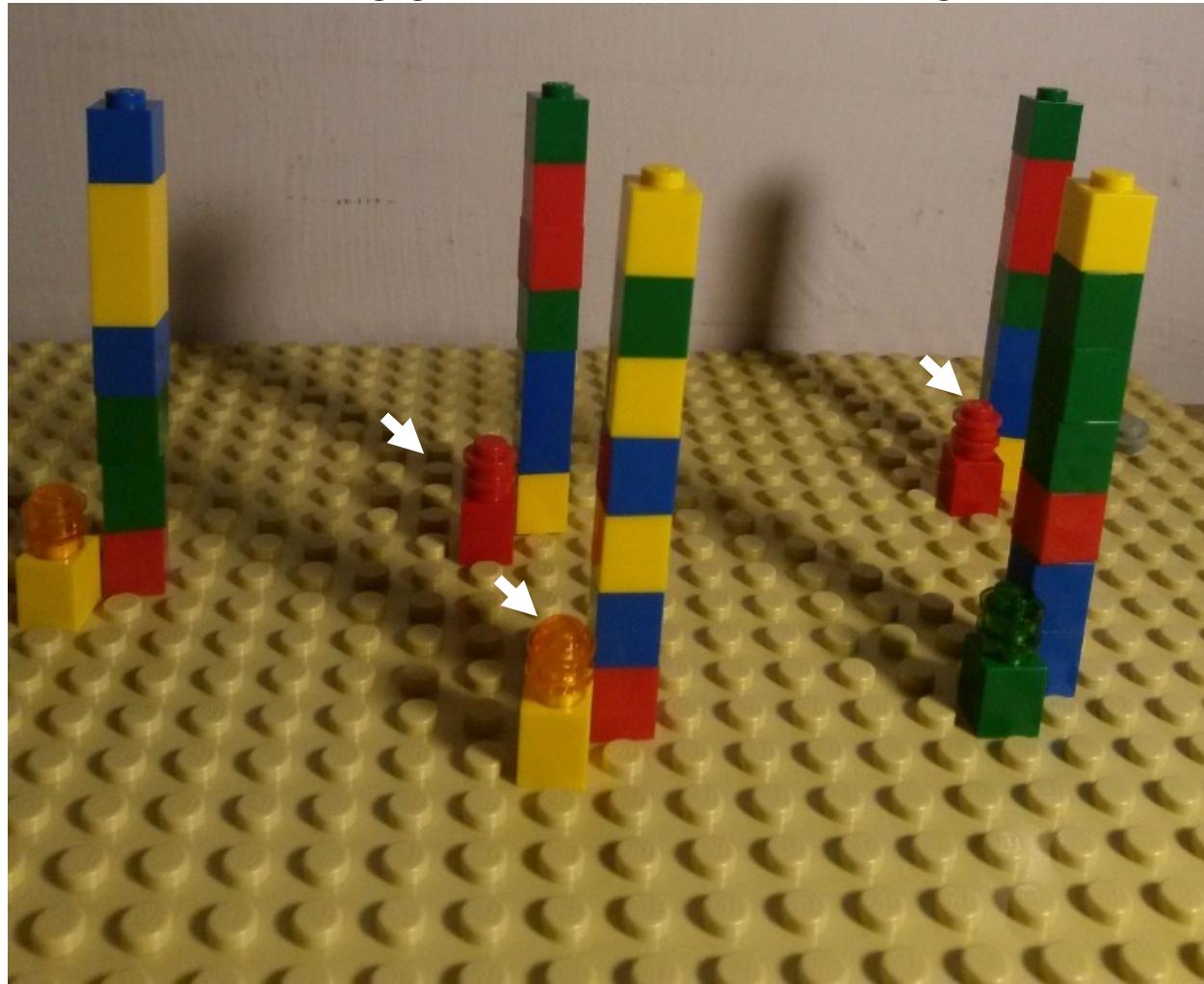
cleavable blocking groups added - sequencing-by-synthesis



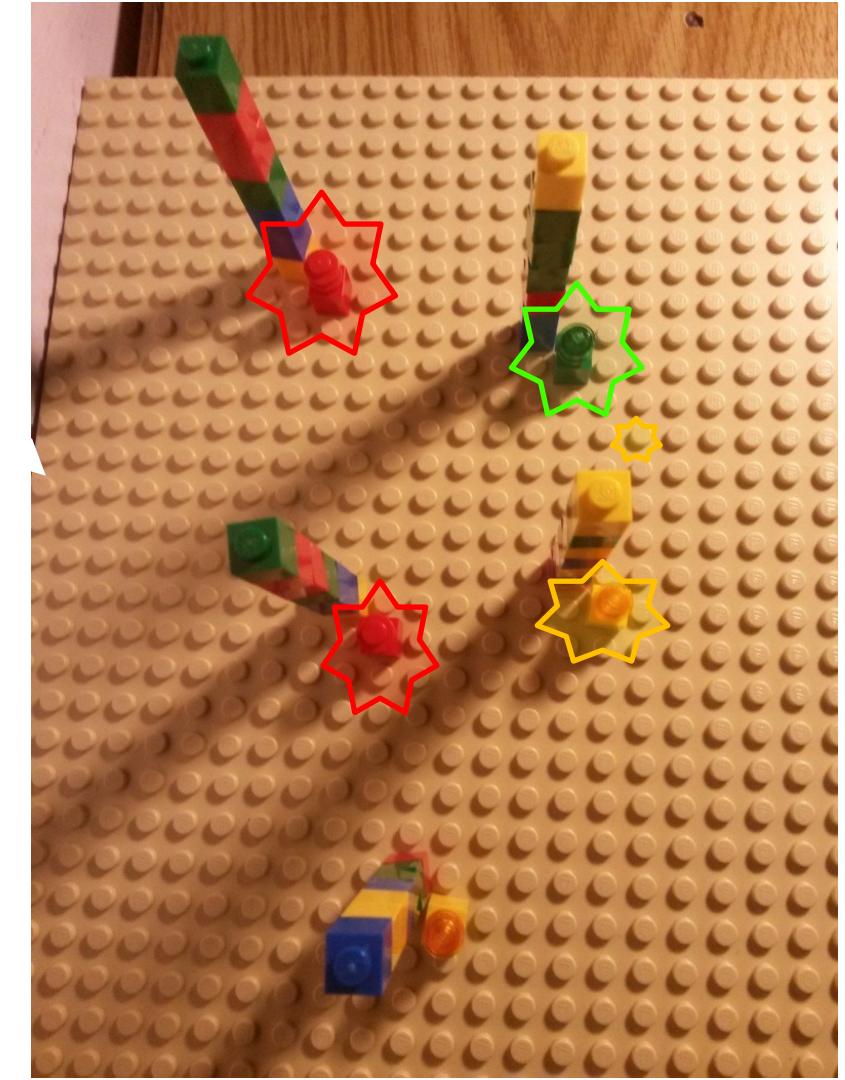
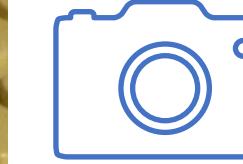
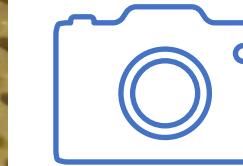
Illumina key innovation is cleavable blocking groups and fluorophores on the nucleotides and subsequent acquisition of the image instead a gel of the Sanger

Next Generation Sequencing

cleavable blocking groups added - sequencing-by-synthesis



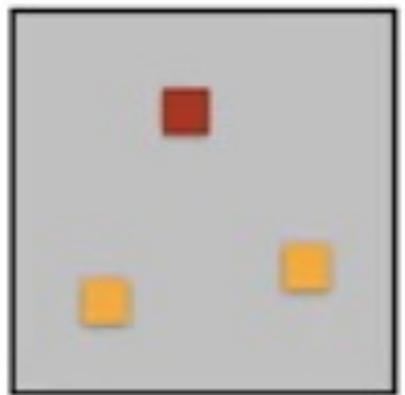
A  T
G  C



Illumina key innovation is cleavable blocking groups and fluorophores on the nucleotides and subsequent acquisition of the image instead a gel of the Sanger

Next Generation Sequencing

Following the addition of the four dNTPs to the templates, the images are recorded and the terminators are removed



Cycle 1

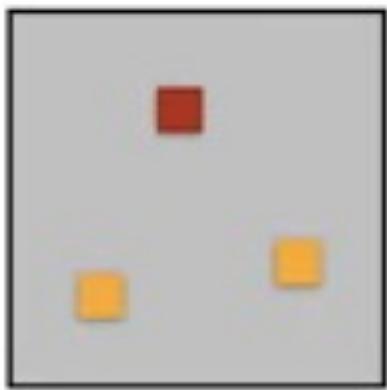
c

t

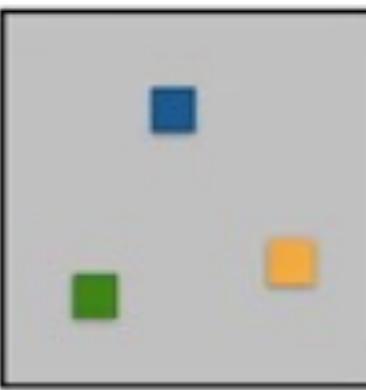
t

Next Generation Sequencing

Following the addition of the four dNTPs to the templates, the images are recorded and the terminators are removed



Cycle 1



Cycle 2

C

CA

T

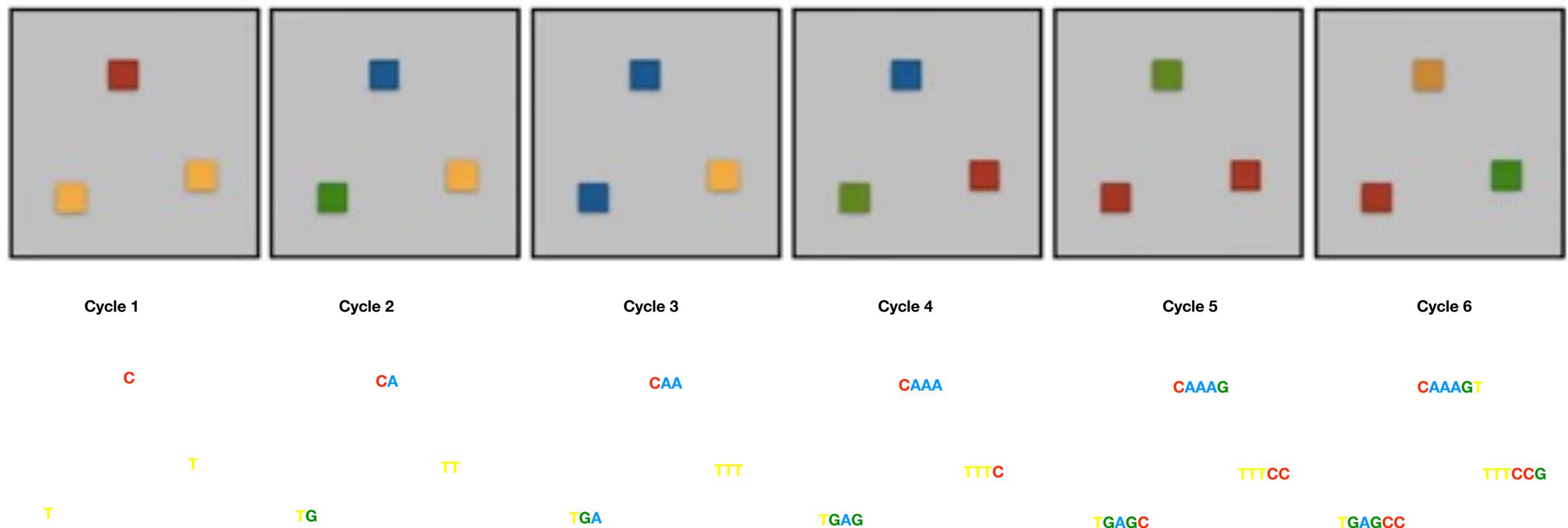
TT

T

TG

Next Generation Sequencing

Following the addition of the four dNTPs to the templates, the images are recorded and the terminators are removed



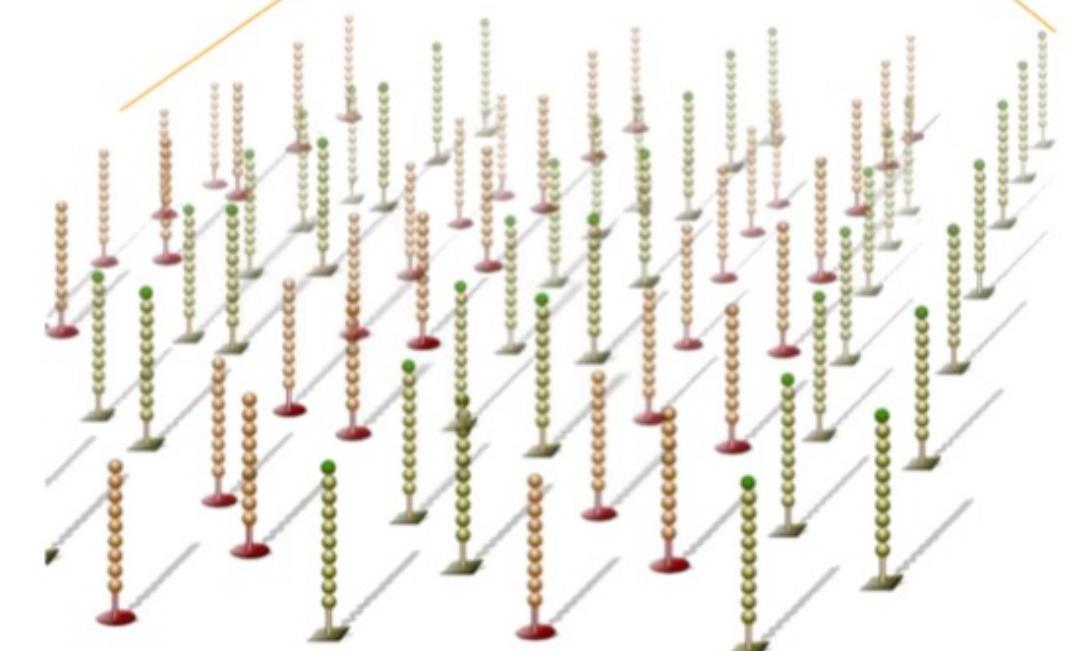
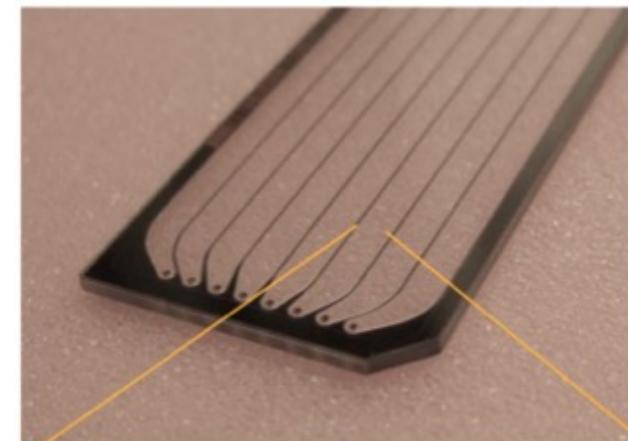
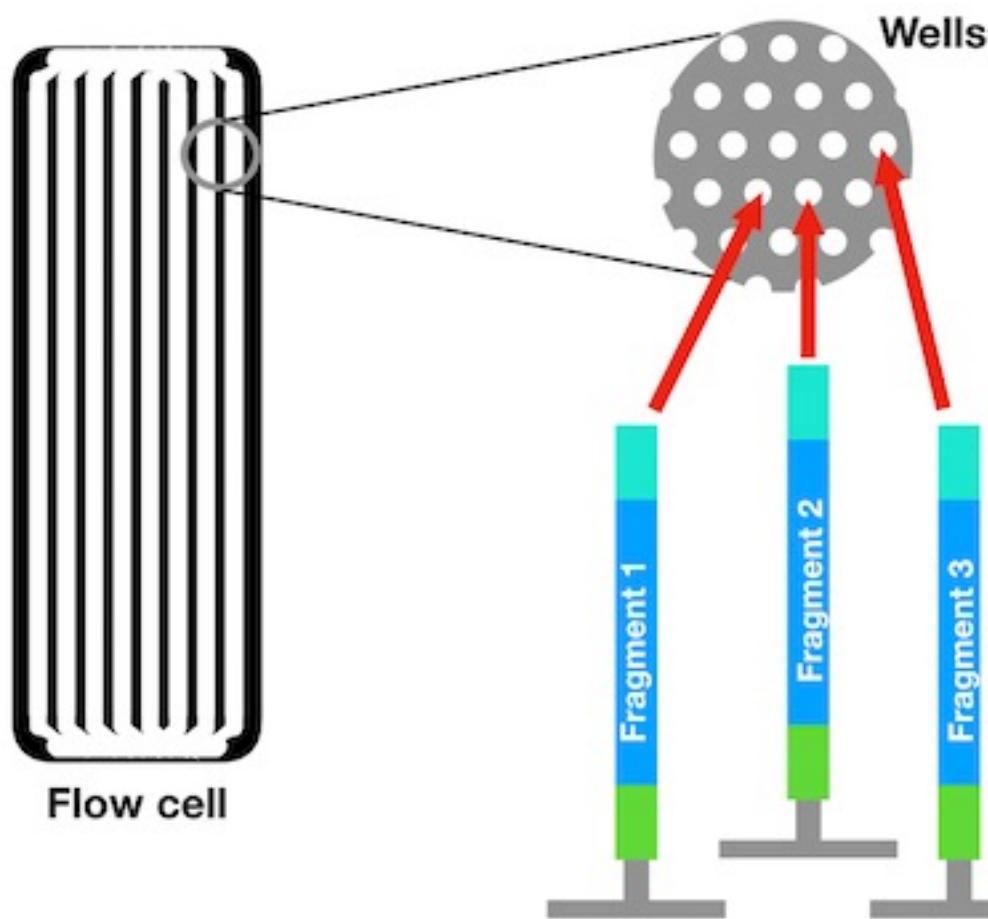
Next Generation Sequencing

Illumina sequencing technology workflow

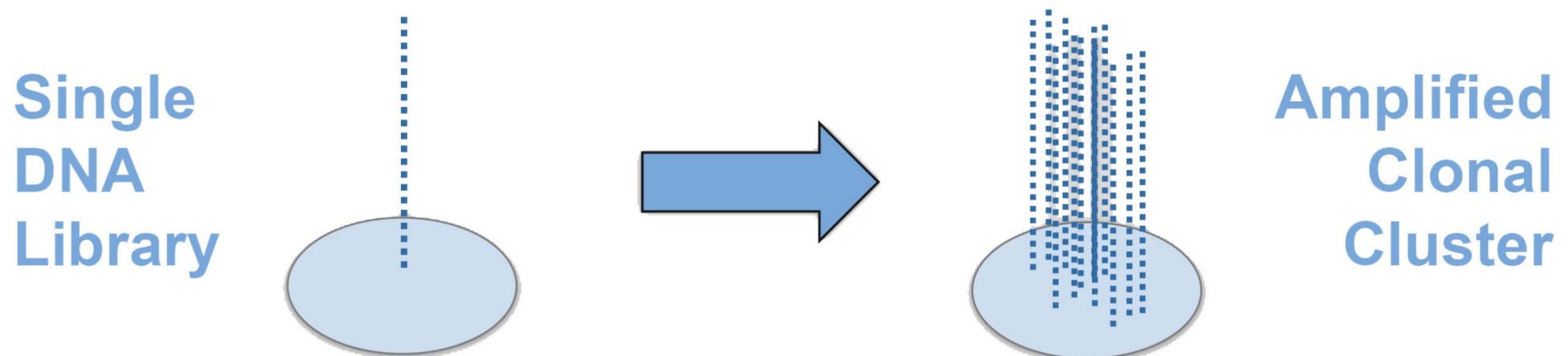
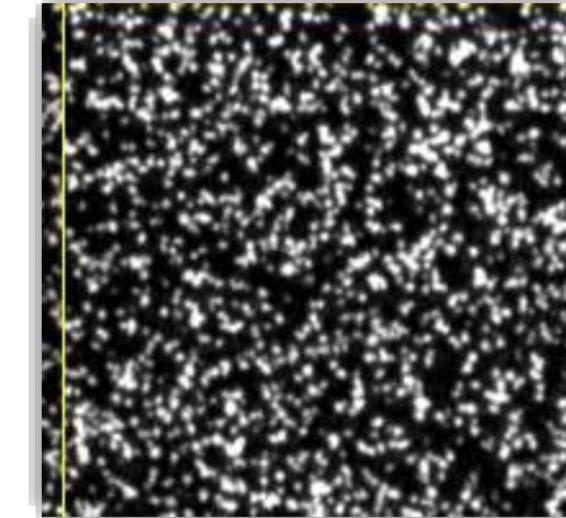
2. Cluster generation



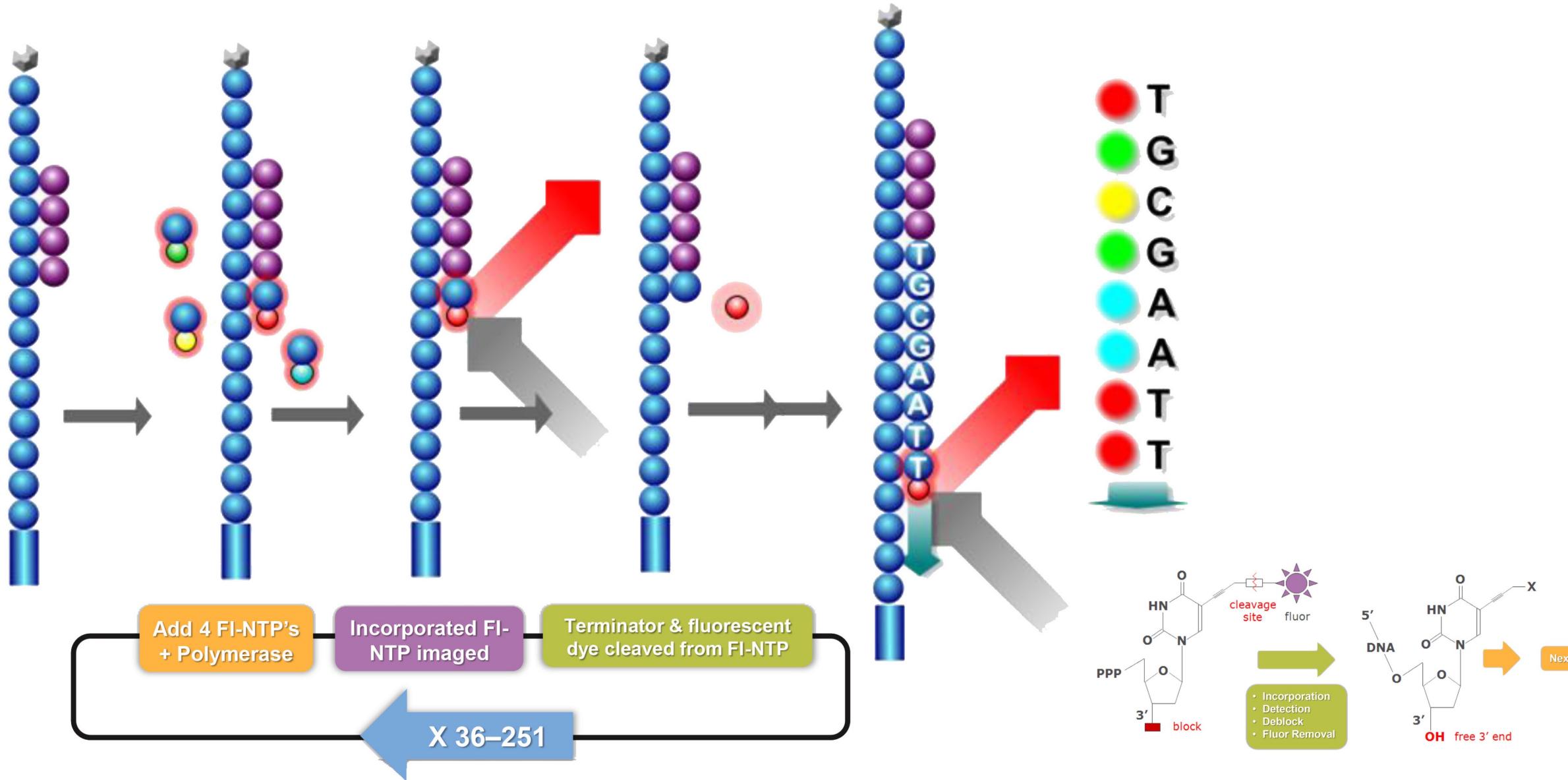
2. Cluster generation



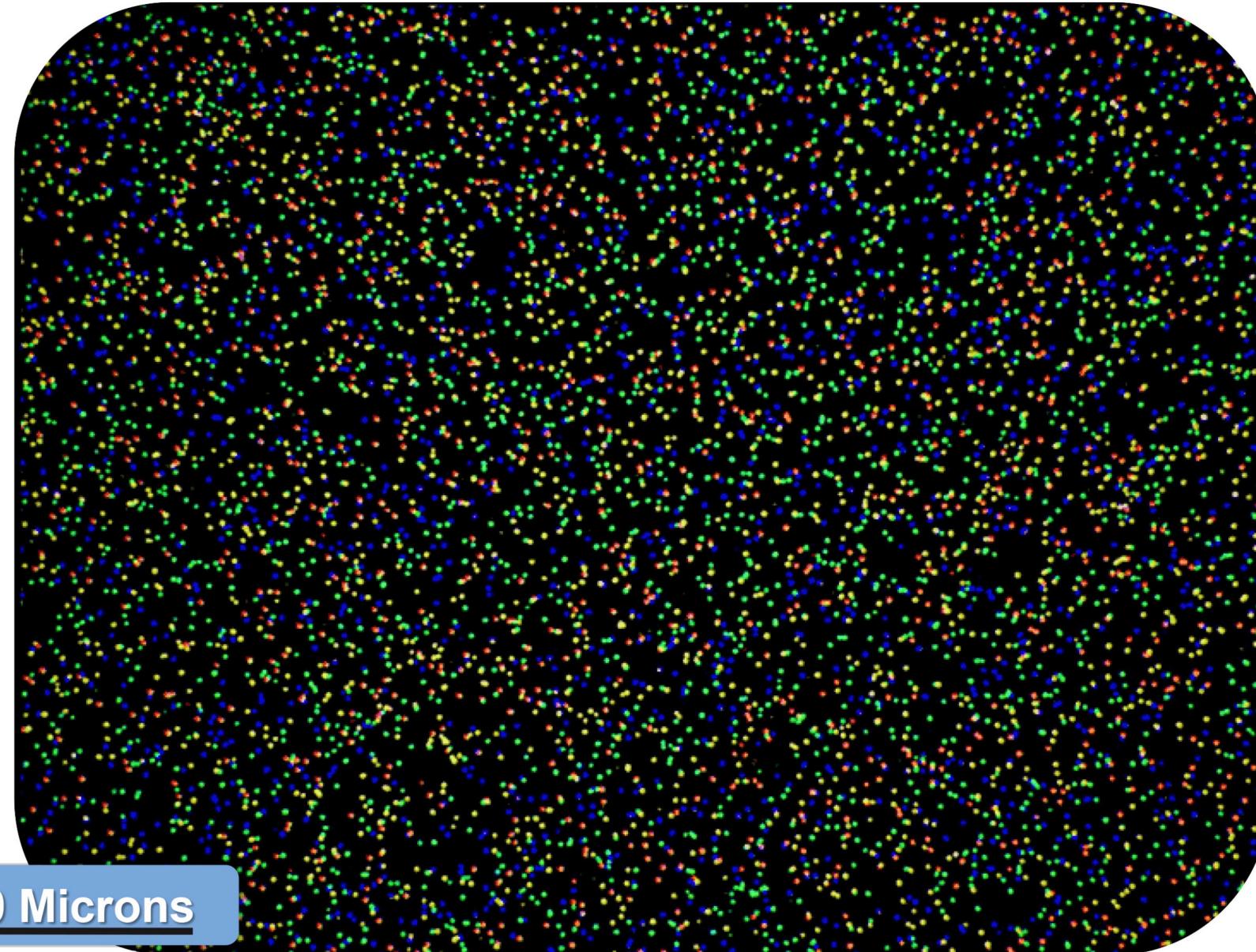
2. Cluster generation



Next Generation Sequencing



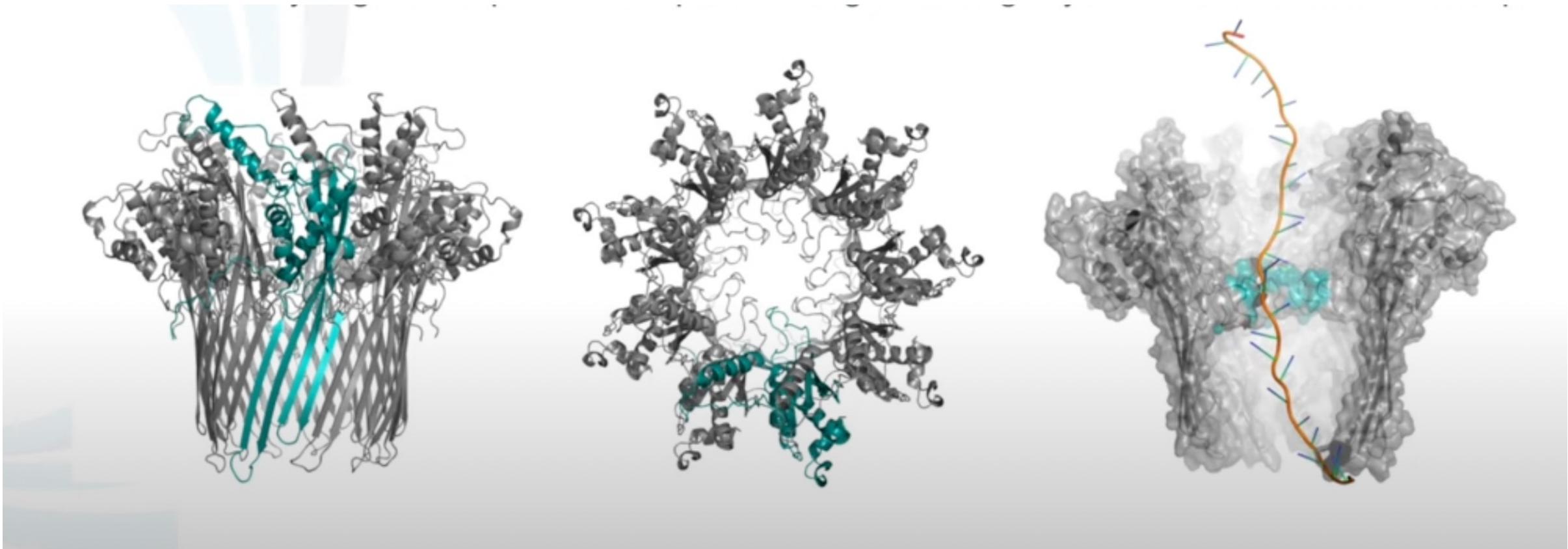
Next Generation Sequencing



Next Generation Sequencing



Next Generation Sequencing



Next Generation Sequencing



2.8 Gb

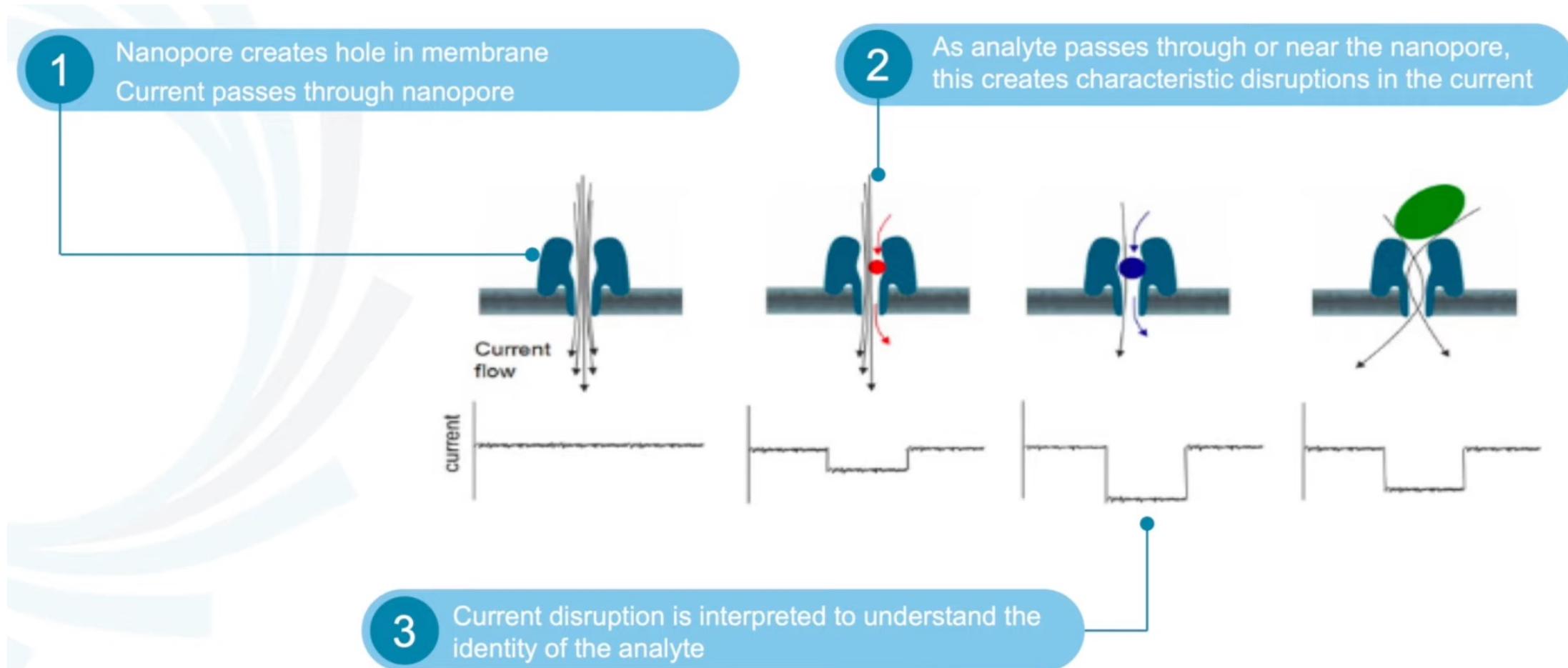


50 Gb

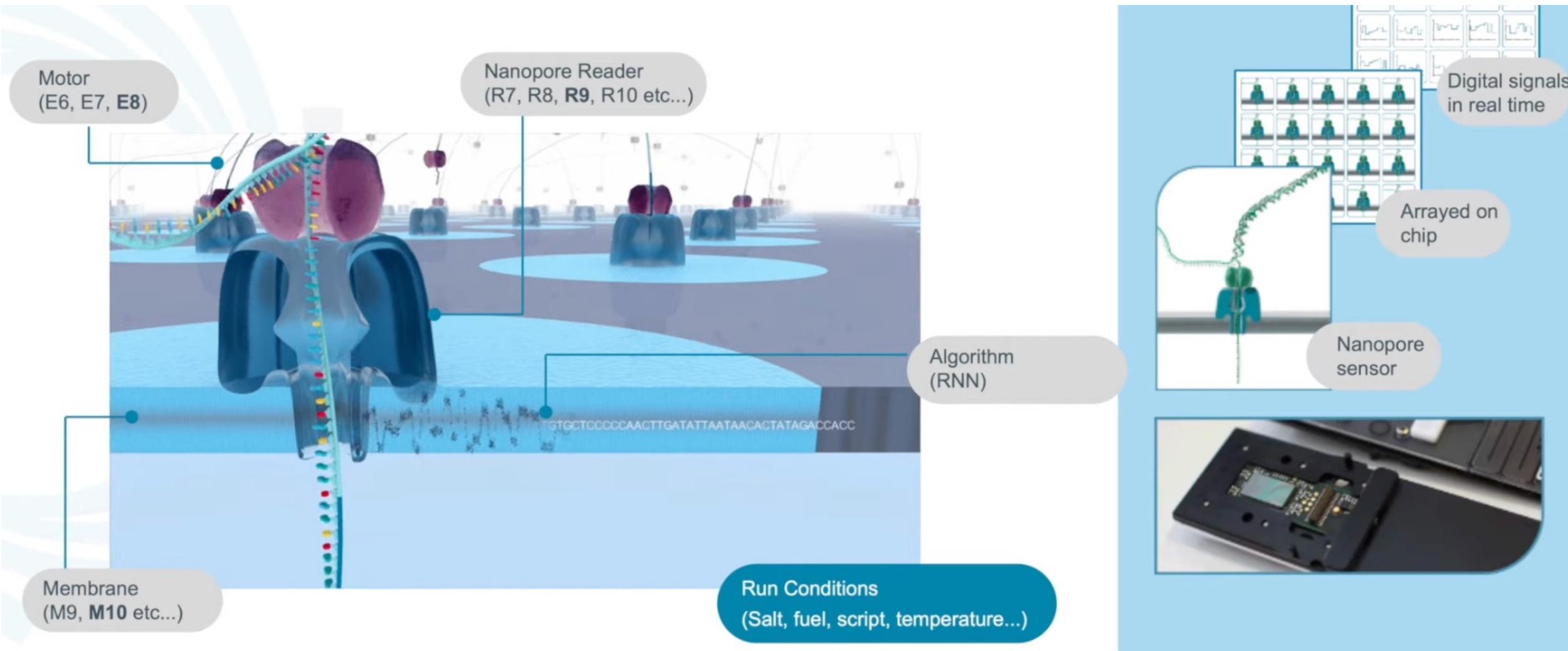


290 Gb

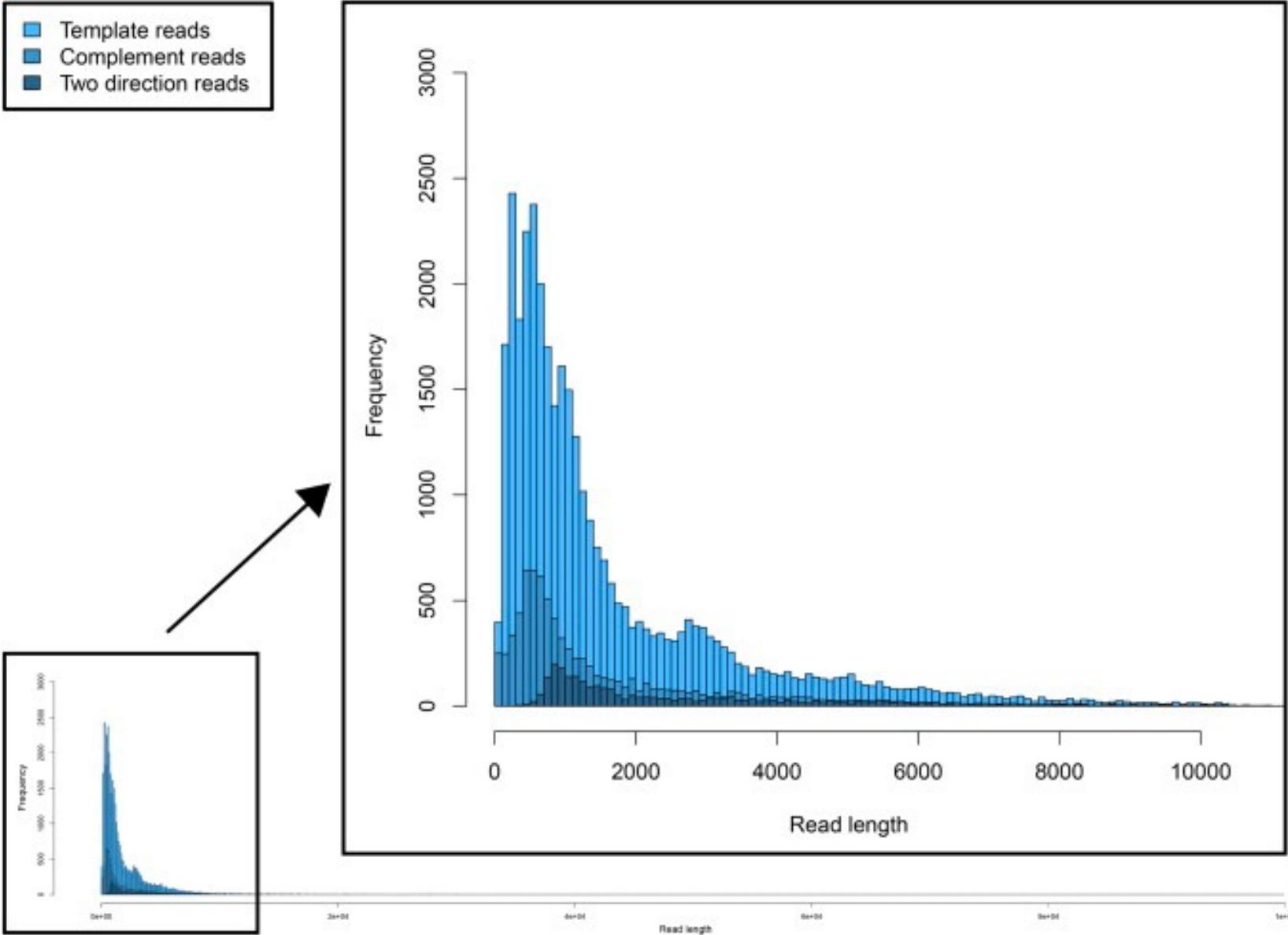
Next Generation Sequencing



Next Generation Sequencing



Next Generation Sequencing



Congratulations!

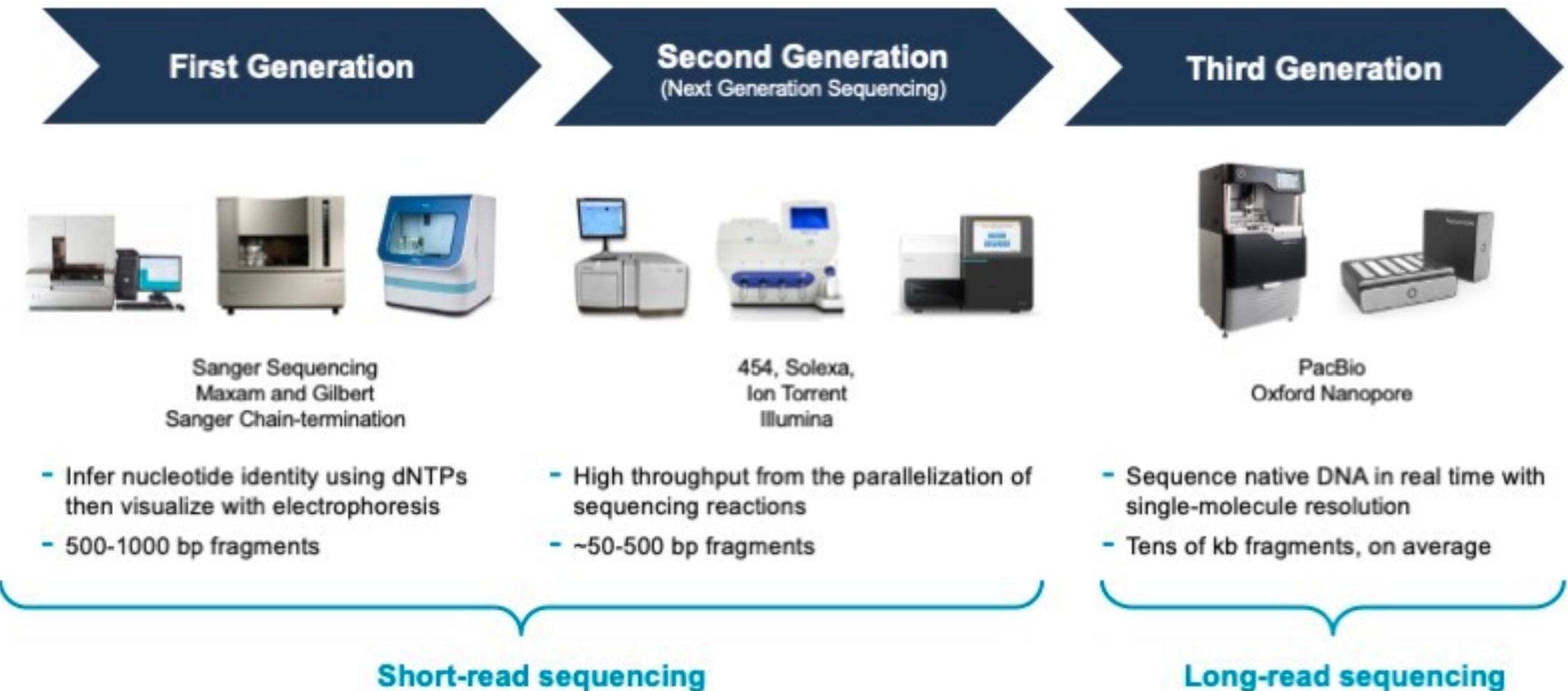
The first >2 Mb DNA read, achieved with nanopore sequencing

Matt Loose, Alex Payne, Nadine Holmes, Vardhman Rakyan & team, University of Nottingham, UK

May 2018

Why long reads, and why ultra-long reads?

Next Generation Sequencing



Applications

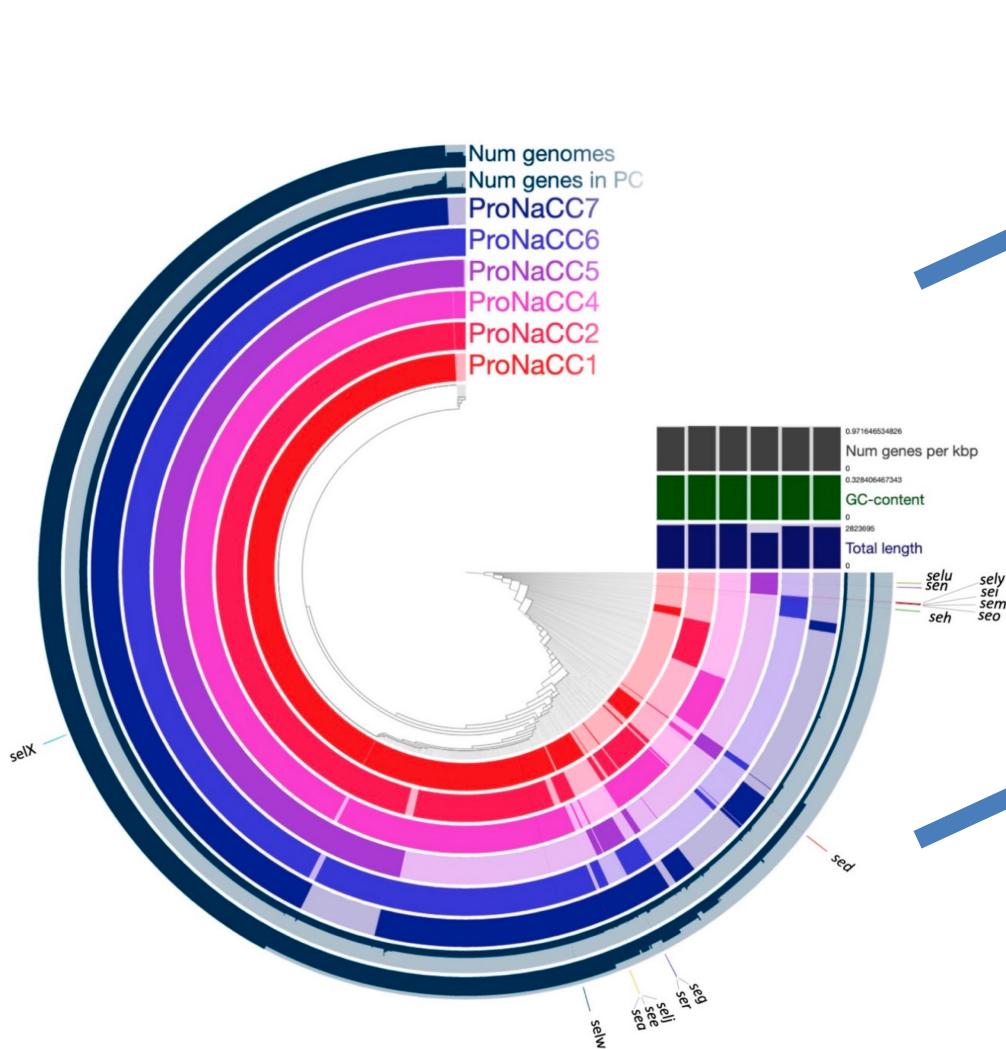
Microbiology approaches



Culture-dependent methods

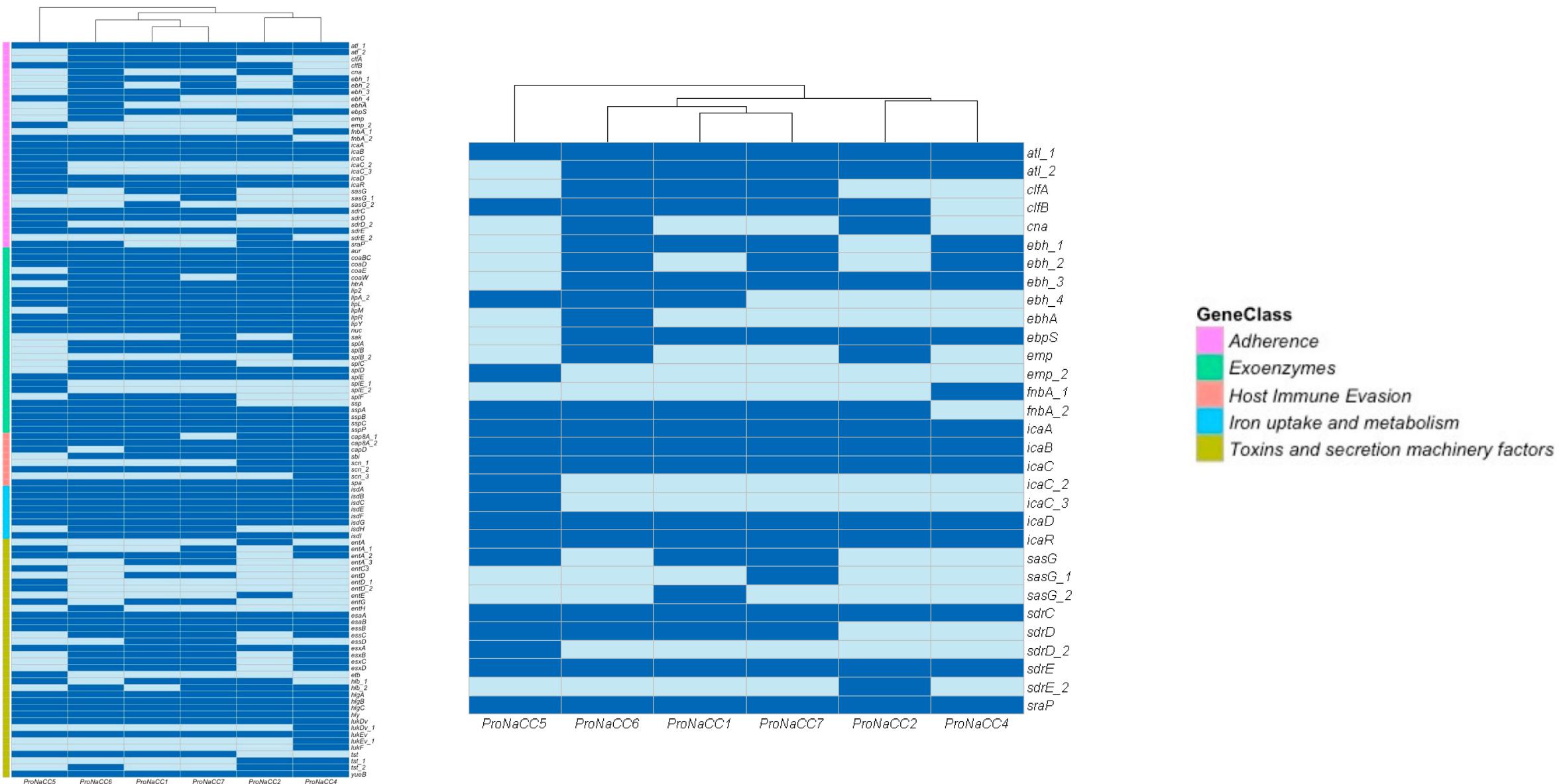
Early Microbiology and the Microscope





GGTCGTAATATCGCACGTCCATATGTTGGTGAACCAAGGAAACTTTAC
ACGTACATCTAATCGACATGACTATCGTTAAAACCTTTGGTAAAAA
CTGTCTTAGATCATTGAAAGACGGTGGTTATGATGTTATTGCCATC
GGTAAAATTAAATGACATTTATGATGGTGAAGGTGTAACAGAACGGT
TCGTACGAGAGTAACATGGACGGTATGGATCAATTGATGAAAATTG
TTAAGAAAAGATTCACAGGTATTAGCTTCTAAACTTAGTAGACTTT
GATGCATTATACGGTCATCGTCGTGATAAACCAGGTTATGCACAAGC
AATTAAAGATTCGATGATCGCTGCCAGAACTGTTAGCAACTTAA
AAGAAGACGATTTAGTAATTATTACAGCAGACCATGGTAATGACCCG
ACAGCGCCAGGTACGGACCATACGAGAGAATATATCCCAGTAATTAT
GTACAGTCCGAAATTAAAGGTGGTCATGCACTAGAAAGTGATACTA
CATTCAGTTCTATCGGTGCAACTATAGCAGATAATTCAACGTAACA
TTACCCAGAGTTCGGTAAAAGTTATTAAAGGAATTGAAATAGAATAA
ATTTAGATATTATAAAAAACAGCAGTGAAGTTAACTATAACAATAGTT
TTCTCACTGCTTTTATTATAATAGAGAAACGTAAGACCG

GGTCGTAATCGCACGTCCATATGTTGGTGAACCAAGGAAACTTTAC
ACGTACATCTAATCGACATGACTATGCGTTAAAACCTTTGGTAAAAA
CTGTCTTAGATCATTGAAAGACGGTGGTTATGATGTTATTGCCATC
GGTAAAATTAAATGACATTTATGATGGTGAAGGTGTAACAGAACGGT
TCGTACGAGAGTAACATGGACGGTATGGATCAATTGATGAAAATTG
TTAAGAAAAGATTTCACAGGTATTAGCTTCTAAACTTAGTAGACTTT
GATGCATTATACGGTCATCGTCGTGATAAACCAGGTTATGCACAAGC
AATTAAAGATTTCGATGATCCCTGCCAGAACTGTTAGCAACTTAA
AAGAGACGATTTAGTAATTATTACACCGACCATGTAATGACCCCCG
ACAGGCCAGGTACGGACCATACGAGAGAATATATCCCAGTAATTAT
GTACAGTCCGAAATTAAAGGTGGTCATGCACTAGAAAAGTGATACTA
CATTCACTTCTATCGGTGCAACTATAGCAGATAATTCAACGTAACAA
TTACCAAGAGTTCCGTTAAAGTTATTAAAGGAATTGAAATAGAATAA
ATTTAGATATTATAAAAAACAGCAGTGAAGTTAACTATAACAATAGTT
TTCTCACTGCTGTTTATTATAATAGAGAAACGTAAGACCG



Enterotoxin	Contig	Sequence Start ¹	Sequence End ²	% Coverage ³	% Identity ⁴	Reference ⁵
SED	14	12,761	13,533	99.87	84.73	UniProtKB—R9SA89
SEIJ	14	14,428	15,212	97.64	83.95	UniProtKB—O85217
SER	14	15,329	16,103	99.74	84.9	UniProtKB—Q76LS8
SEIX	3	249,399	250,007	100	84.24	UniProtKB—G0Z026
SEIW	5	93,584	94,369	100	100	GB—KX655710.1
SEE	1	409,445	410,214	99.87	83.9	GB—WP_044122767
SEIX	24	4093	4701	100	84.24	UniProtKB—G0Z026
SEIW	6	46,777	47,558	99.36	96.55	GB—KX655711.1
SEA	1	983	1744	98.57	81.15	UniProtKB—P0A0L2
SEIW	3	200,805	201,585	99.36	96.16	GB—KX655711.1
SEIX	7	89,367	89,975	100	84.4	UniProtKB—G0Z026
SEIY	2	58,778	59,440	100	84.77	UniProtKB—A0A0K2S2V0
SEIX	2	634,655	635,263	100	84.56	UniProtKB—G0Z025
SEG	9	13,778	14,550	99.87	86.16	UniProtKB—P0A0L8
SEN	9	14,836	15,609	100	85.92	UniProtKB—A0A0H3JS72
SEIU	9	15,630	16,397	98.08	84.42	UniProtKB—Q6XXM3
SEI	9	16,560	17,279	99.17	87.5	UniProtKB—O85383
SEM	9	17,320	18,033	99.58	85.01	UniProtKB—A0A0H3K005
SEO	9	18,317	19,096	100	87.82	UniProtKB—A0A0H3JS76
SEIW	1	1,068,149	1,068,933	99.87	97.84	GB—KX655711.1
SEIX	2	96,391	96,999	100	82.92	UniProtKB—G0Z026
SEH	5	98,979	99,700	99.86	86.29	UniProtKB—P0A0M0
SED	11	6058	6830	99.87	84.73	UniProtKB—R9SA89
SEIJ	11	7725	8509	97.64	83.95	UniProtKB—O85217
SER	11	8626	9400	99.74	-84.9	UniProtKB—Q76LS8
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SEIX	7	241,512	242,120	100	84.24	UniProtKB—G0Z026

databases >

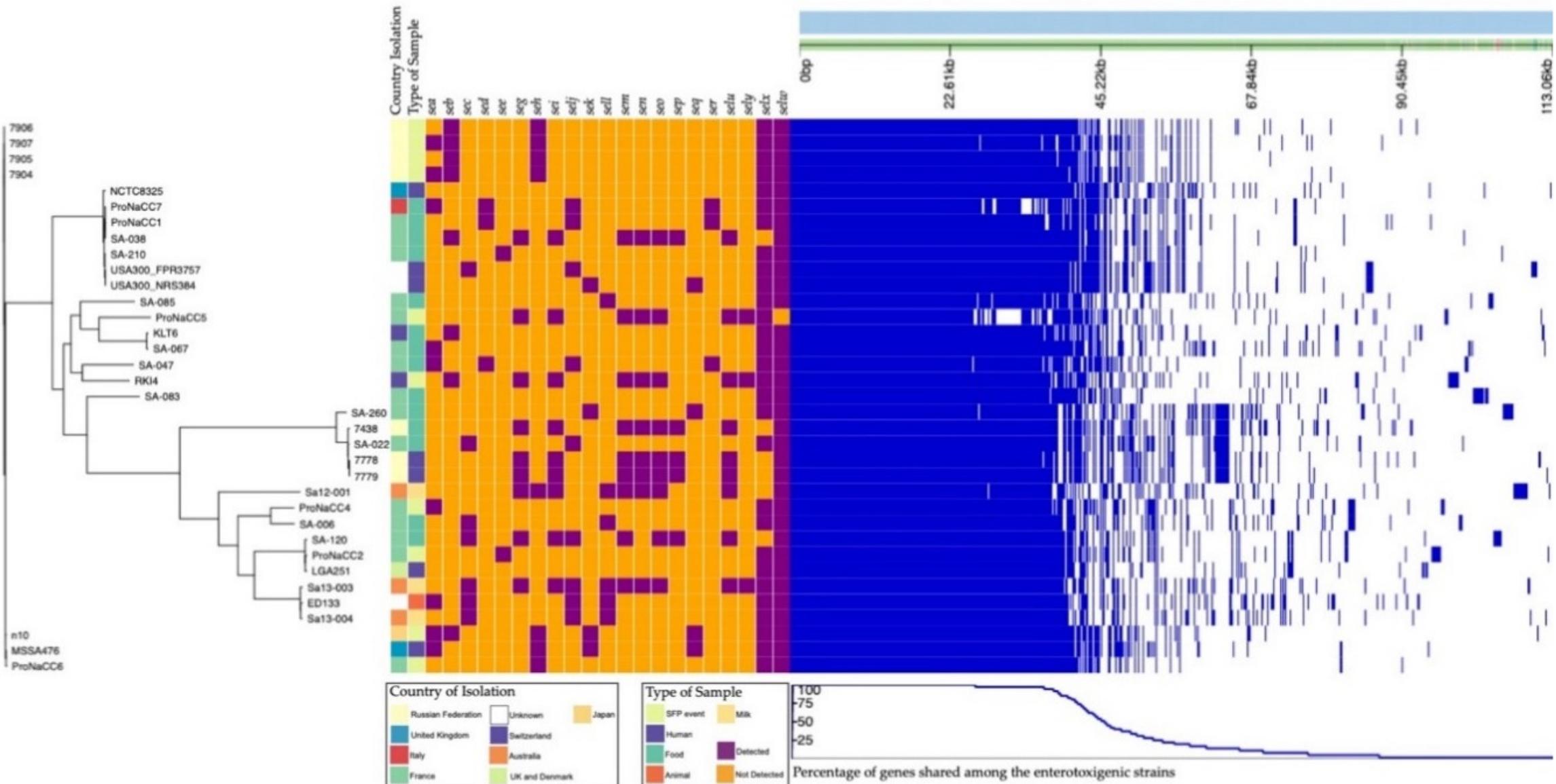
-  Database S1
-  Database S3
-  Database S5
-  Database S7

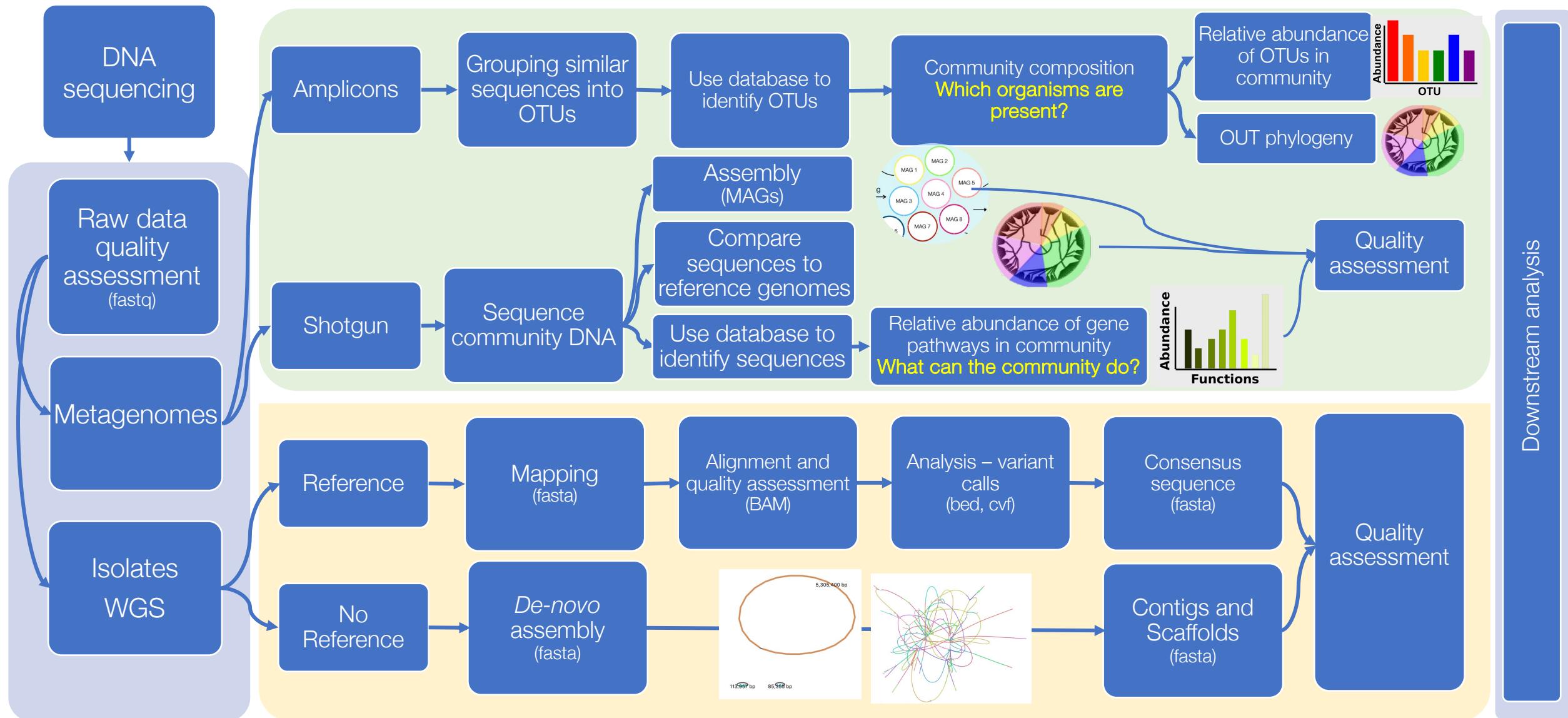
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aureus OX=1280 GN=entA PE=1
SV=1
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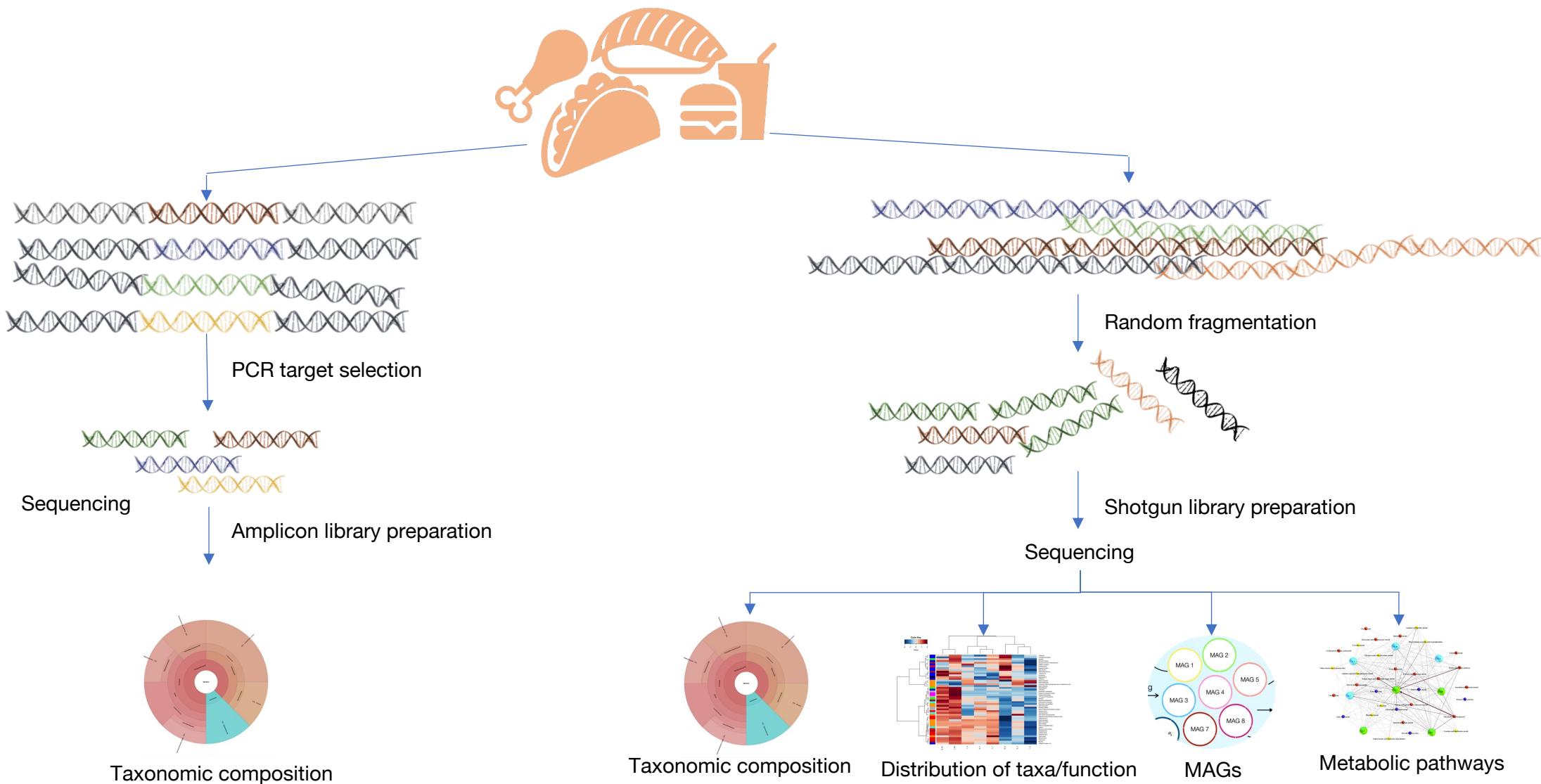
● ● ●

Database S7

>NC_012547.1 Staphylococcus aureus plasmid pG01, complete sequence
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Protected designation of origin (PDO) product: Lardo - *Valle d'Aosta Lard d'Arnad*
is a type of salumi made by curing strips of fatback with rosemary and other herbs and spices



OTU	0 days		7 days		15 days		30 days		60 days		90 days	
	Abundance (%)	SD	Abundance (%)	SD	Abundance (%)	SD	Abundance (%)	SD	Abundance (%)	SD	Abundance (%)	SD
Plant A												
<i>Acinetobacter</i>	4.12	5.94	5.67	9.44	2.49	3.99	0.04	0.04	1.07	1.85	2.40	3.73
<i>Acinetobacter guillouiae</i>	0.10	0.13	0.25	0.43	0.02	0.04	0.00	0.00	0.00	0.00	0.00	0.00
<i>Acinetobacter johnsonii</i>	35.02	0.67	23.40	37.64	31.85	42.40	3.01	2.80	0.61	0.89	8.01	12.15
<i>Acinetobacter iwoffii</i>	1.53	1.95	2.70	4.54	0.00	2.21	0.18	0.00	0.00	0.00	0.36	0.56
<i>Arenimonadaceae</i>	0.77	0.09	0.12	0.04	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
<i>Bacillus</i>	0.15	0.25	0.04	0.07	0.02	0.04	0.00	0.00	0.19	0.33	0.04	0.04
<i>Brochothrix</i>	3.21	0.36	0.38	0.65	0.27	0.47	0.00	0.00	0.31	0.54	2.70	4.62
<i>Caulobacteraeae</i>	1.84	3.19	0.06	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.31	0.54
<i>Chromohalobacter</i>	0.00	0.00	0.10	0.13	0.08	0.14	1.75	0.92	0.38	0.39	17.29	16.46
<i>Dectridium</i>	0.07	1.85	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.07
<i>Holomonsellaceae</i>	0.00	0.00	0.00	0.00	0.15	0.13	0.44	0.33	0.17	0.18	0.40	0.35
<i>Holomonsis</i>	0.00	0.00	27.00	45.89	15.43	7.63	21.53	12.68	9.77	5.27	10.35	6.57
<i>Kocuria</i>	0.13	0.22	0.00	0.00	0.04	0.07	0.04	0.00	0.00	0.00	0.06	0.11
<i>Lactobacillus</i>	0.29	0.51	0.00	0.00	0.00	0.00	0.00	0.00	0.04	0.04	0.17	0.13
<i>Listeriacae</i>	0.86	0.44	0.17	0.19	0.06	0.11	0.10	0.04	0.06	0.06	3.24	3.95
<i>Methylophilus caseolyticus</i>	0.01	0.00	0.00	0.21	0.19	0.00	0.00	0.00	0.00	0.00	0.44	0.76
<i>Methylstearina mobilis</i>	0.36	2.39	0.02	0.04	0.00	0.00	0.00	0.00	0.00	0.00	0.49	0.83
<i>Micrococcus</i>	0.19	0.33	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.08	0.04
<i>Parococcus</i>	0.63	1.09	0.04	0.00	0.00	0.00	0.03	0.00	0.02	0.04	0.44	0.60
<i>Propionibacterium acnes</i>	0.29	0.51	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
<i>Pseudomonas</i>	2.43	3.17	1.69	1.76	0.23	0.24	0.05	0.04	3.01	5.22	0.13	0.06
<i>Pseudomonas fragi</i>	0.71	3.14	0.33	38.64	0.26	20.45	0.05	0.05	1.3	10.21	21.35	
<i>Pycnochromobacter</i>	0.73	0.59	1.80	1.00	1.74	2.90	0.18	0.11	2.66	4.49	9.60	11.10
<i>Salinosporea</i>	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.36	2.35
<i>Sphingomonas</i>	0.29	0.51	0.02	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.13	0.22
<i>Staphylococcus</i>	0.04	0.07	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.40	1.96
<i>Staphylococcus equorum</i>	0.02	0.04	3.76	3.45	3.55	1.66	1.78	1.97	0.77	0.63	12.17	7.21
<i>Staphylococcus sciuri</i>	0.22	0.22	0.01	1.80	1.21	1.00	0.70	0.02	0.00	0.00	5.00	5.06
<i>Staphylococcus succinus</i>	0.00	0.00	0.17	0.24	0.08	0.10	0.08	0.07	0.02	0.04	0.52	0.44
<i>Vibrio</i>	0.06	0.11	3.60	6.23	22.92	39.59	64.50	6.16	65.73	16.10	3.79	6.34

Material and Methods:

Three plants:

1. low maturation temperature (plant A [10% NaCl, 2°C]) x3
2. using a low NaCl concentration (plant B [2.5% NaCl, 4°C]) x3
3. artisanal process (plant C [30% NaCl, 8°C]) x3

Experimental design:

Lard samples were obtained at time 0 and after 7, 15, 30, 60, and 90 days of maturation.
16s rRNA gene sequencing (V3-V4 regions) and microbiology parameters

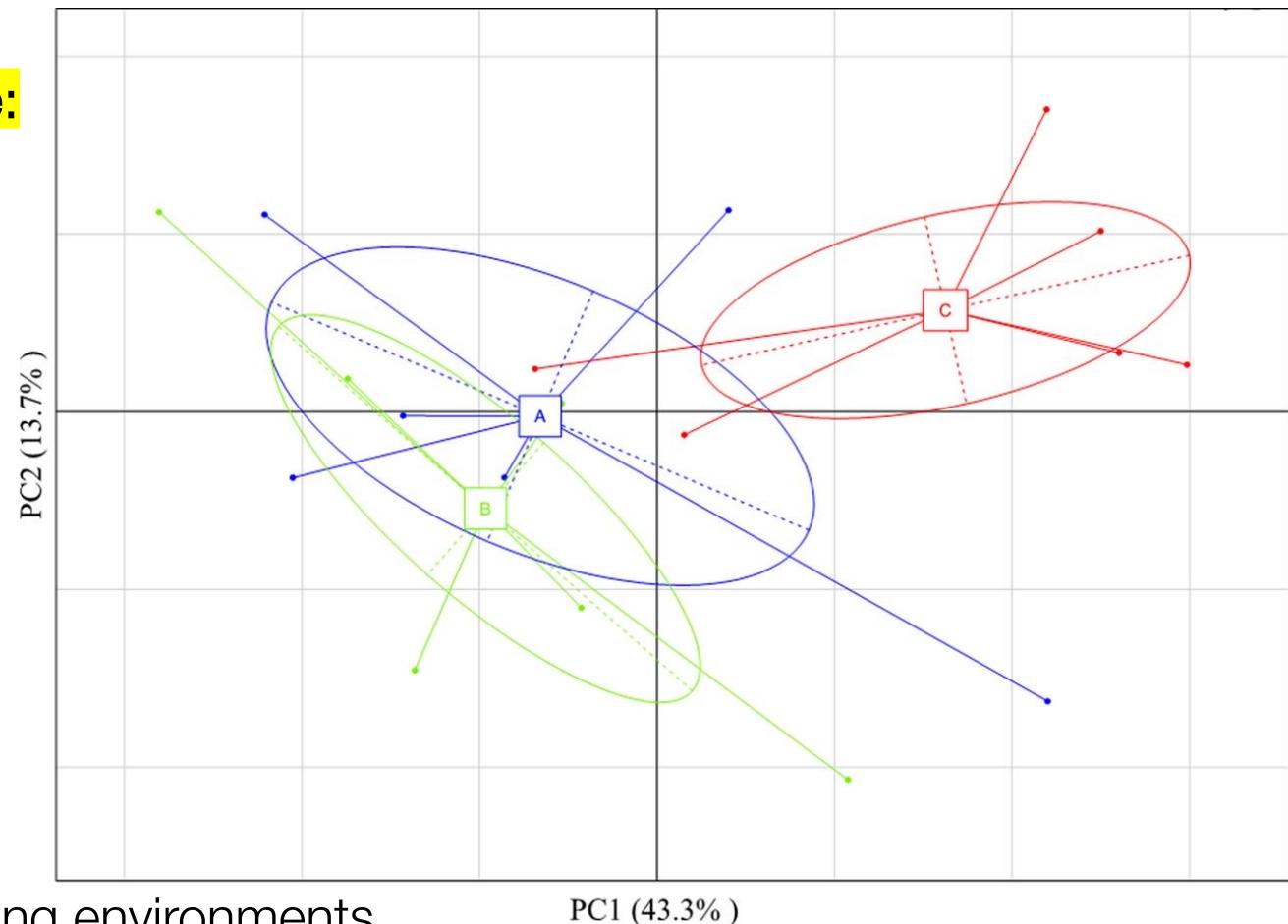
Ferrucino et al., 2017 doi:10.1128/AEM.00983-17



An example: a protected designation of origin (PDO) product

Main taxa identified by sequencing were:

Acinetobacter johnsonii,
Psychrobacter,
Staphylococcus equorum,
Staphylococcus sciuri,
Pseudomonas fragi,
Brochothrix,
Halomonas,
Vibrio



Relative abundances from the plants

A-B . Undesired bacteria in food-processing environments

Ferrocino et al., 2017 doi:10.1128/AEM.00983-17



An example: a protected designation of origin (PDO) product

Main taxa identified by sequencing were:

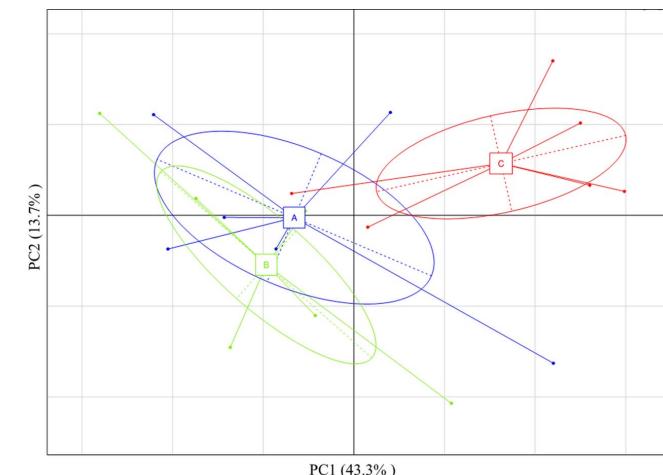
- ● *Acinetobacter johnsonii*
- *Psychrobacter,*
Staphylococcus equorum,
- *Staphylococcus sciuri,*
- ● *Pseudomonas fragi*
- ● *Brochothrix,*
- ● *Halomonas*
- *Vibrio*

-> spoilage agent: lipolytic activity

-> spoilage agent: lipolytic activity

-> spoilage agent: lipolytic potential (salt-resistant)

Acinetobacter johnsonii was also found in C samples but was not detected at the end of the ripening [concentrations of sodium chloride]



16S rRNA gene sequencing



An example: a protected designation of origin (PDO) product

The use of 16S rRNA gene sequencing for technological improvement and provide products with reduced content of salt

- Changes in the food production process can drastically affect the microbial community structure
- Impact on the final characteristics of the products

Importance:

- Reduction in the salt concentration in the brines to address a consumer demand for less salty products can negatively impact the quality of the final product due to the higher abundance of spoilage bacteria.
- Importance of the use of traditional process to produce PDO from a spoilage perspective.



Thank you for the attention!



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