Nanopore sequencing

Workshop session 1
MB&C Course 2026

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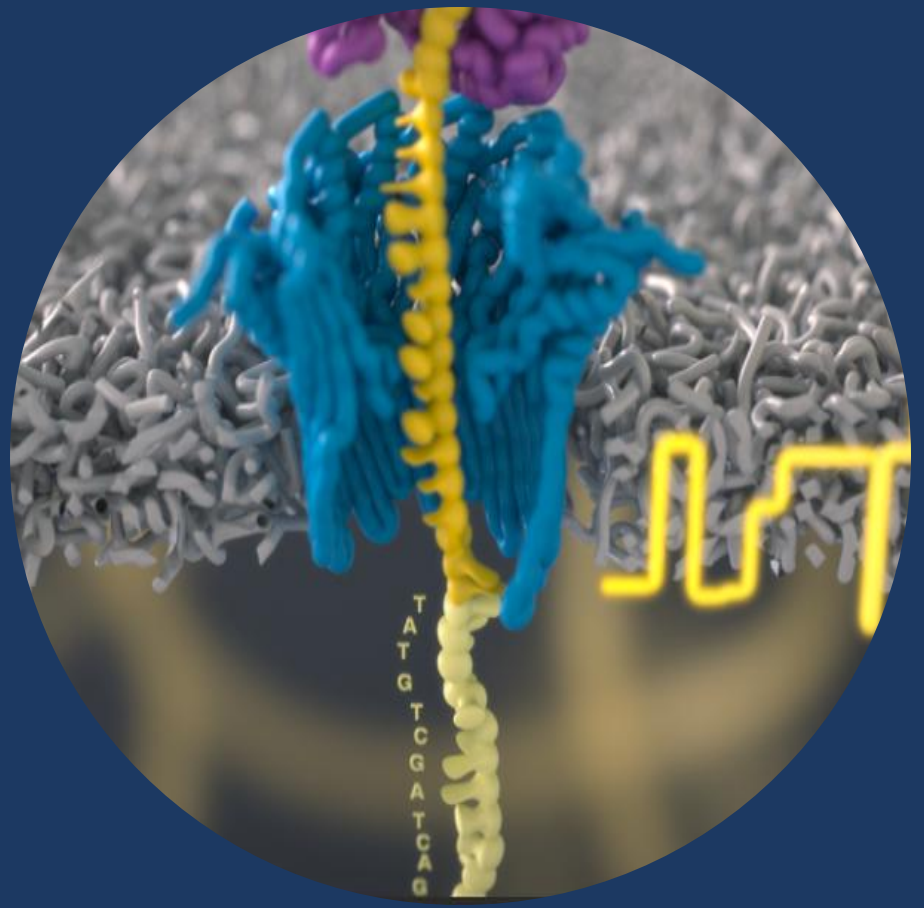
(Molecular Biologist, Jessa Hospital)



Introduction: Evolution of sequencing methods

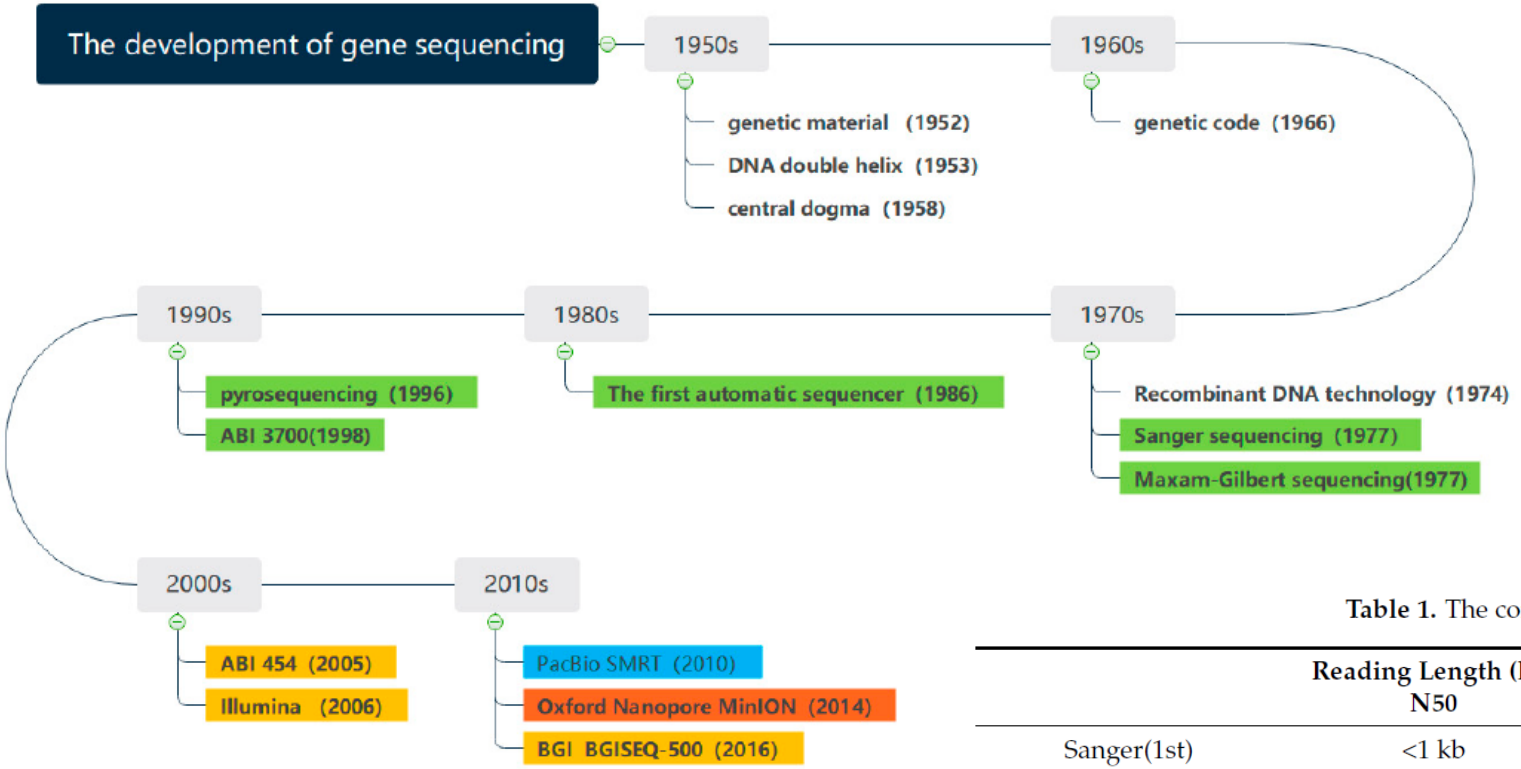
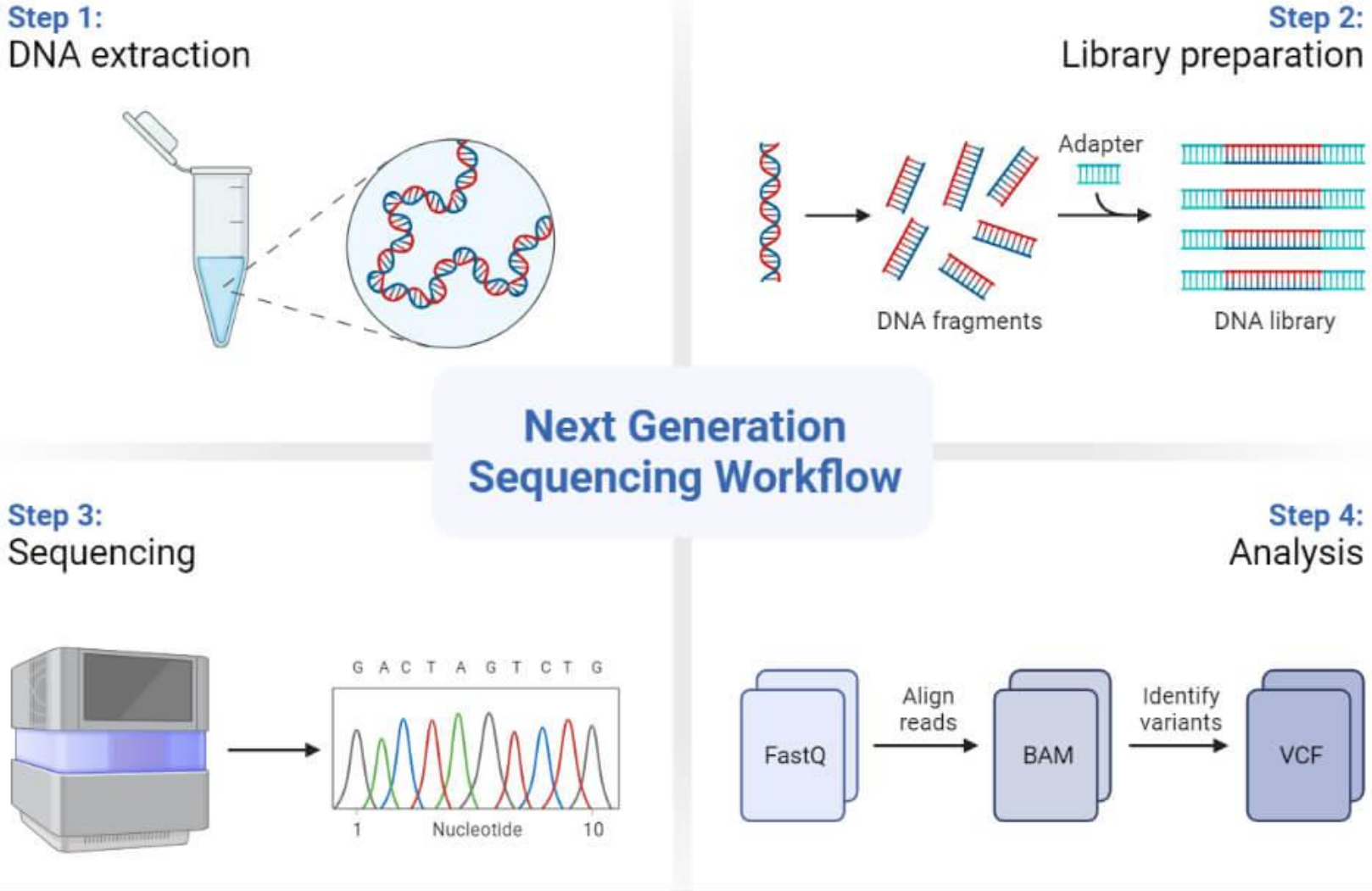


Table 1. The comparison of different generation of gene sequencing.

	Reading Length (kb) N50	Estimated Cost per Gb (US $)	Throughput per Flow Cell (Gb)	Read Accuracy (%)
Sanger(1st)	<1 kb	13,000 [d]	/	>99.9
Illumina(2nd)	0.075–0.15 [a]	50–63	16–30	>99.9
PacBio(3rd)	10–20 [b]	43–86	15–30	>99
ONT(4th)	10–60 [c]	21–42	50–100	87–98

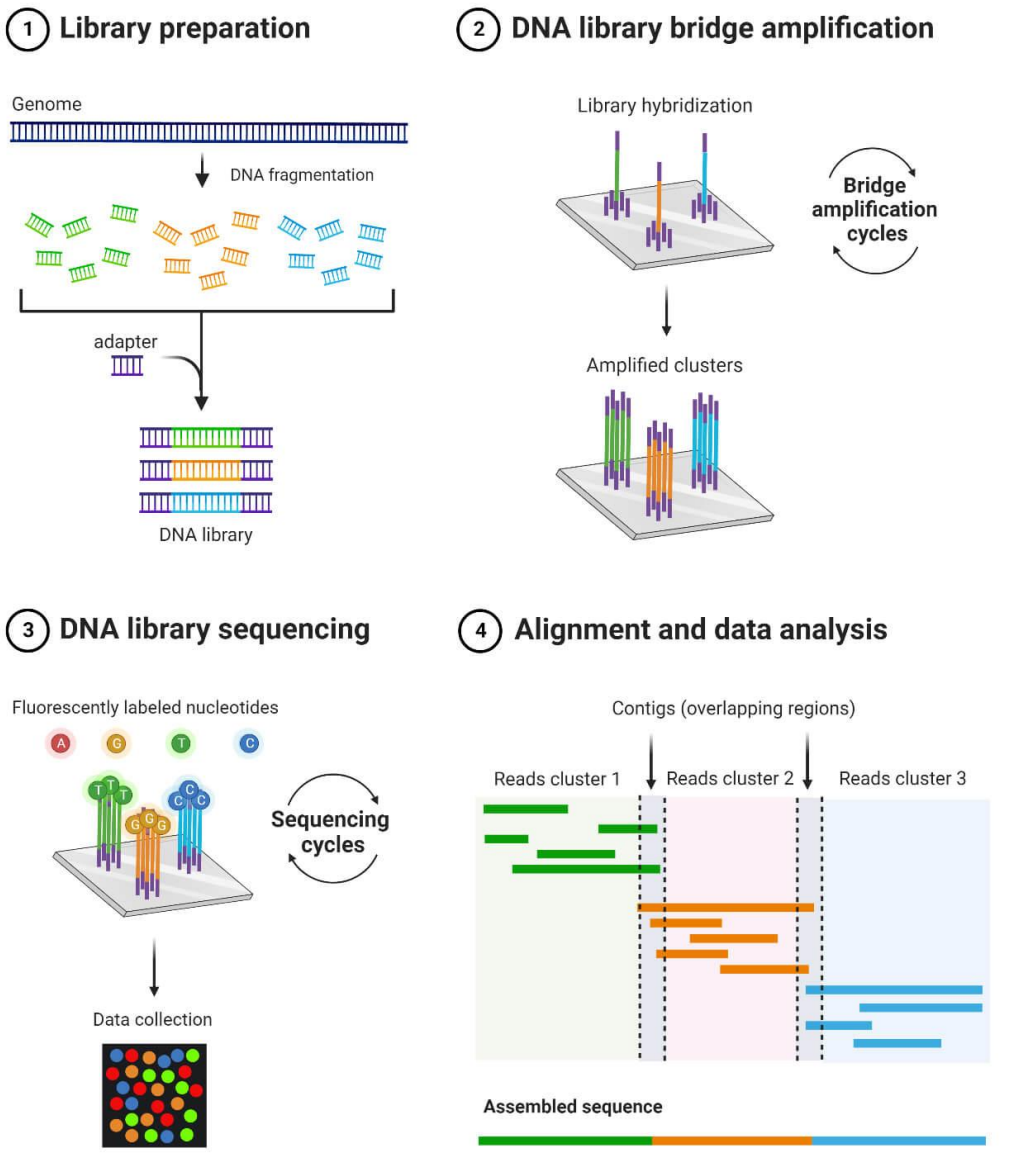
Ref: Lin, B.; Hui, J.; Mao, H. Nanopore Technology and Its Applications in Gene Sequencing. Biosensors 2021, 11, 214. https://doi.org/10.3390/bios11070214.

Introduction: Next-Generation sequencing



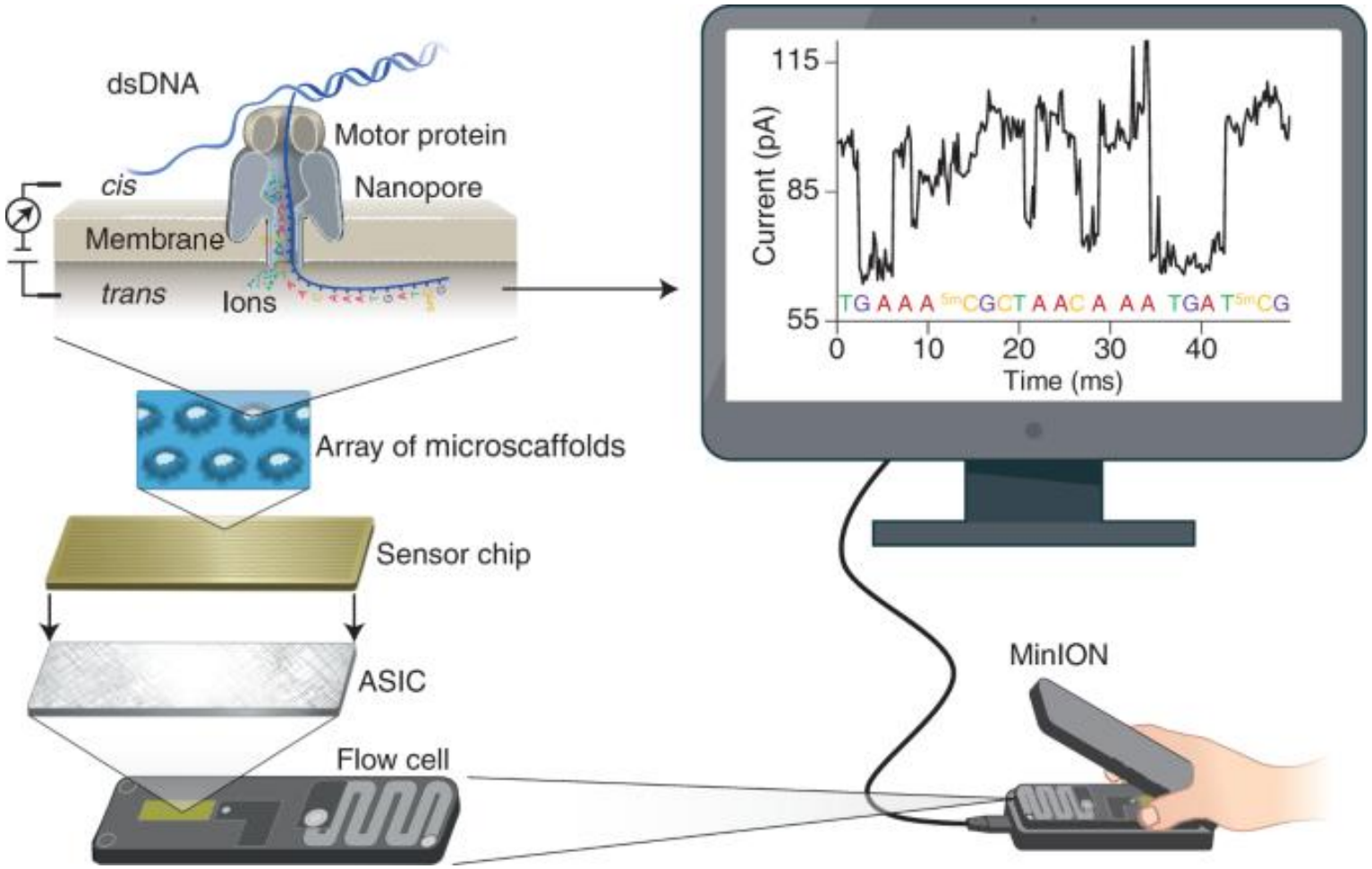
Ref: https://microbenotes.com/next-generation-sequencing-ngs/

Introduction: Illumina sequencing



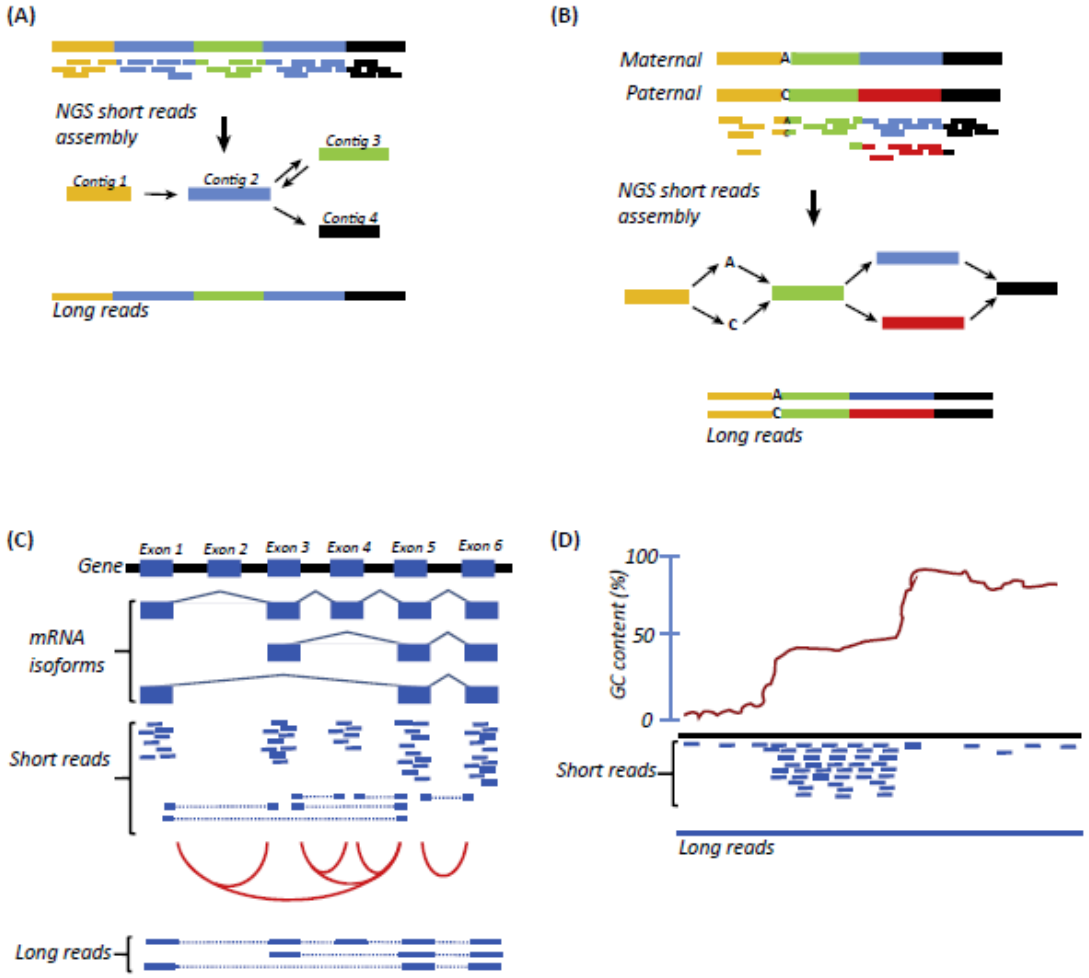
Ref: https://microbenotes.com/next-generation-sequencing-ngs/

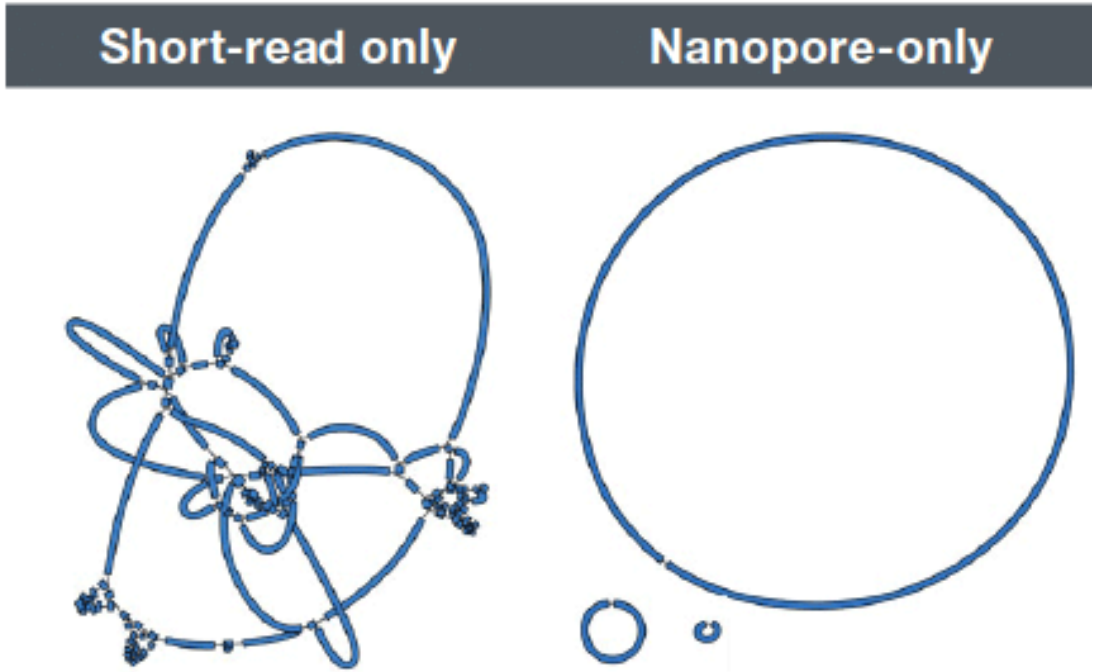
Introduction: Nanopore sequencing



Ref: Wang, Y., Zhao, Y., Bollas, A. et al. Nanopore sequencing technology, bioinformatics and applications. Nat Biotechnol 39, 1348–1365 (2021). https://doi.org/10.1038/s41587-021-01108-x

Short read vs long read sequencing





Ref: van Dijk EL, Jaszczyszyn Y, Naquin D, Thermes C. The Third Revolution in Sequencing Technology. Trends Genet. 2018 Sep;34(9):666-681. doi: 10.1016/j.tig.2018.05.008. Epub 2018 Jun 22. PMID: 29941292.

Pros and cons of Nanopore sequencing

Pros

- Long reads => complete genome coverage & assembly possible
- Fast => Data-analysis possible in real-time
- Relatively low cost
- Scalable
- No amplification or labeling

Cons

- Lower accuracy than Illumina or Sanger sequencing

Cost of Nanopore vs Illumina sequencing

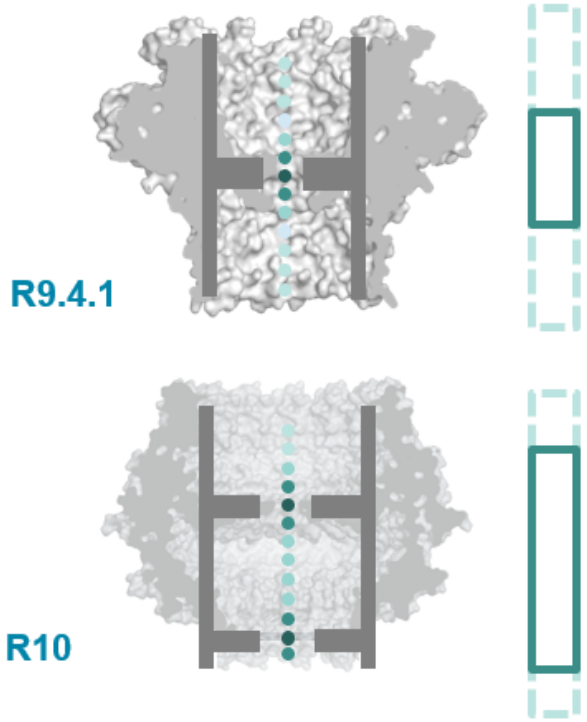
Sequencing System	iSeq™	MiniSeq™	MiSeq®	NextSeq®	HiSeq® 4000	HiSeq® X Five/Ten	NovaSeq® 6000
Output per run	1.2 Gb	7.5 Gb	15 Gb	120 Gb	1.5 Tb	1.8 Tb	1 Tb - 6 Tb[1]
Instrument price	$19.9K	$49.5K	$99K	$275K	$900K	$6M[2]/$10M[2]	$985K
Installed base[3]	NA	~600	~6,000	~2,400	~2,300[4]		~285

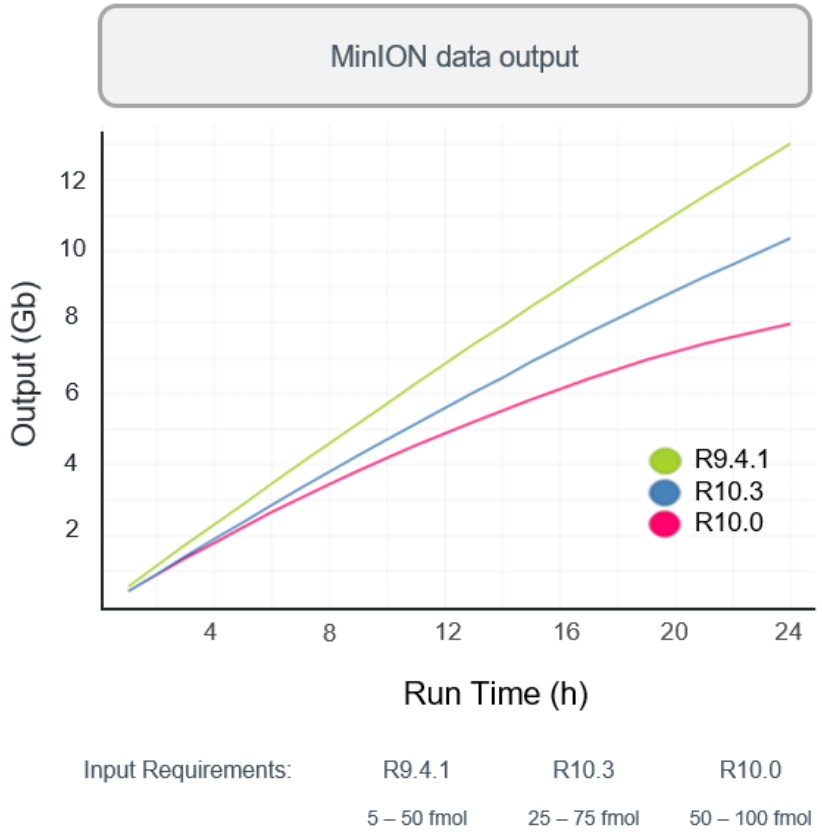
Illumina

Platform	Instrument	Average read length (kb)	Maximum read length (kb)	Instrument cost (US$)	Cost per run (US$)	Cost per Gb (US$)	Input requirement	Throughput per run	Run time	Refs
ONT	MinION	Variable[a]	Variable[a]	1000	475–900[e]	24[g]	~1 µg DNA	Up to 20 Gb	Up to 48 h	[25] https://nanoporetech.com/products#modal=comparison
	GridION	Variable[a]	Variable[a]	49 955[b] 125 000[c] Free (U$142 500 for reagents)[d]	475–900[e] per flow cell	24[g]	~1 µg DNA	Up to 100 Gb (five flow cells)	Up to 48 h	
	PromethION	Variable[a]	Variable[a]	135 000	625–2000[e] per flow cell	5[g]	~1 µg DNA	Up to 125 Gb (one flow cell) Up to 6 Tb (48 flow cells)	Up to 64 h	

Nanopore

Nanopore: tackling the accuracy problem



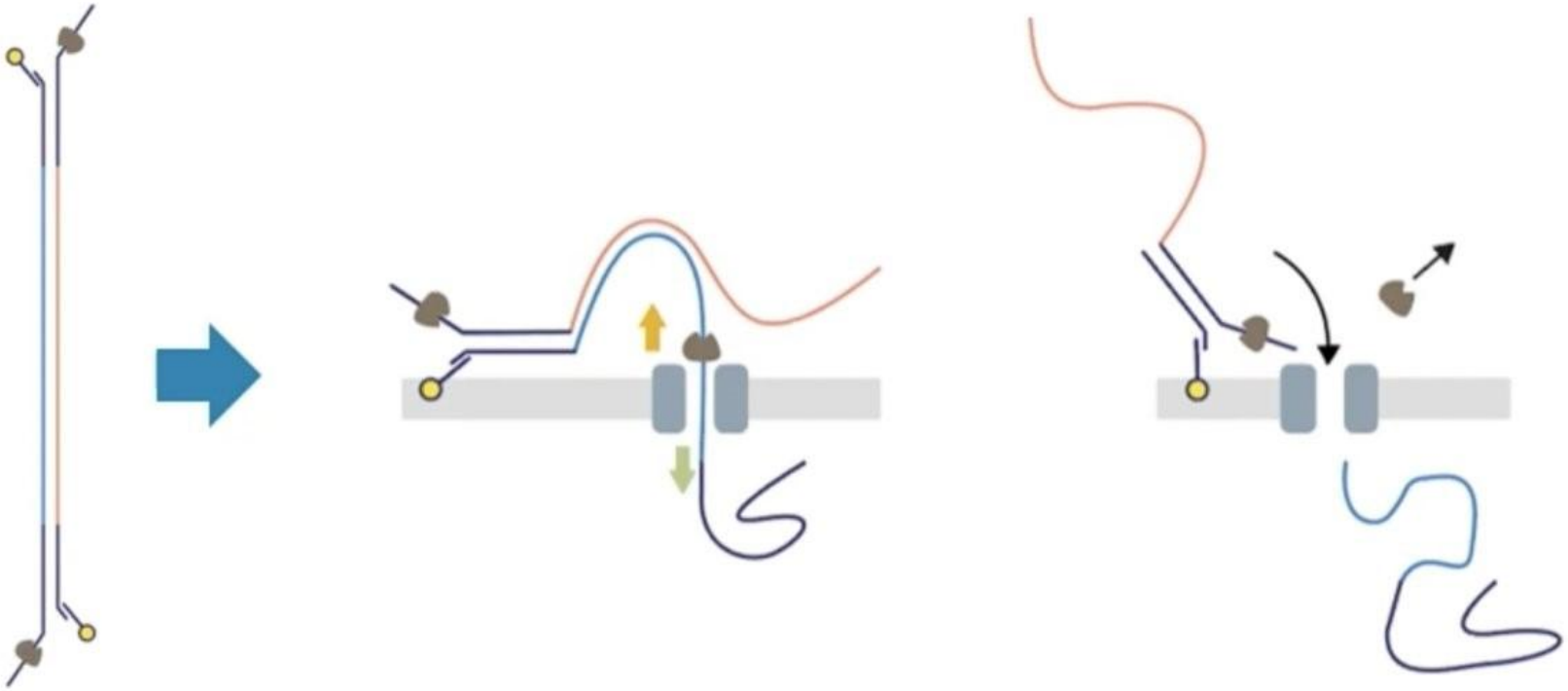


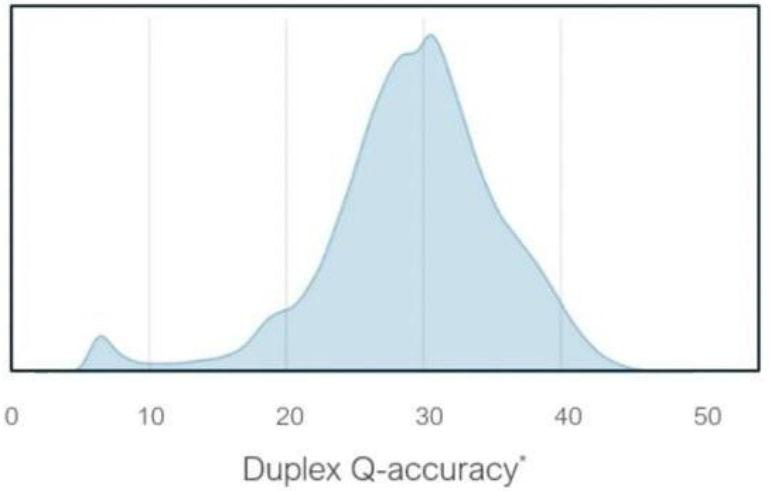
Input Requirements:	R9.4.1	R10.3	R10.0
	5 – 50 fmol	25 – 75 fmol	50 – 100 fmol

Ref: Lin, B.; Hui, J.; Mao, H. Nanopore Technology and Its Applications in Gene Sequencing. Biosensors 2021, 11, 214. https://doi.org/10.3390/bios11070214.

Nanopore: tackling the accuracy problem

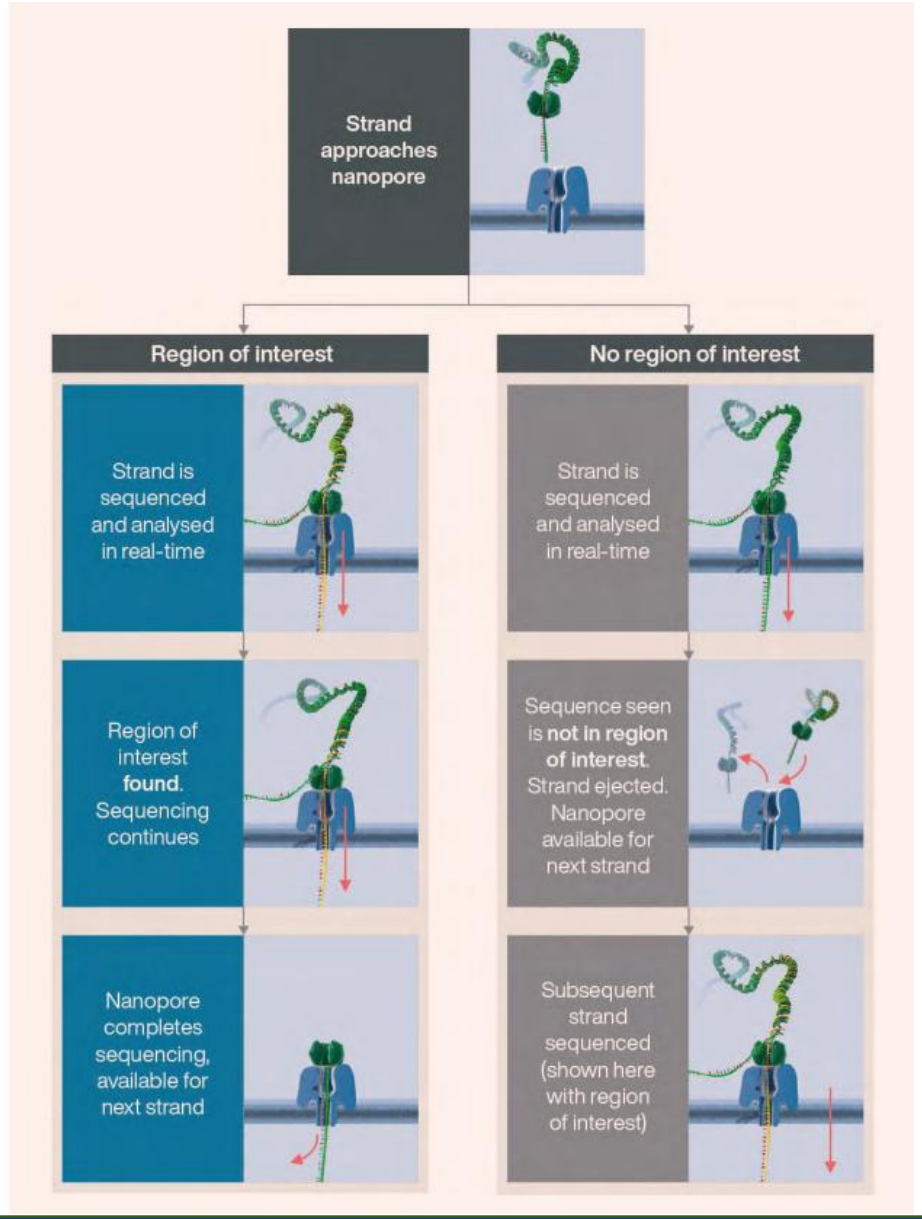
Duplex sequencing => Q30 (99,9% accuracy), but 5x slower





https://nanoporetech.com/resource-centre/clive-brown-ncm-update-2021

Adaptive sampling: finding the needle in the haystack



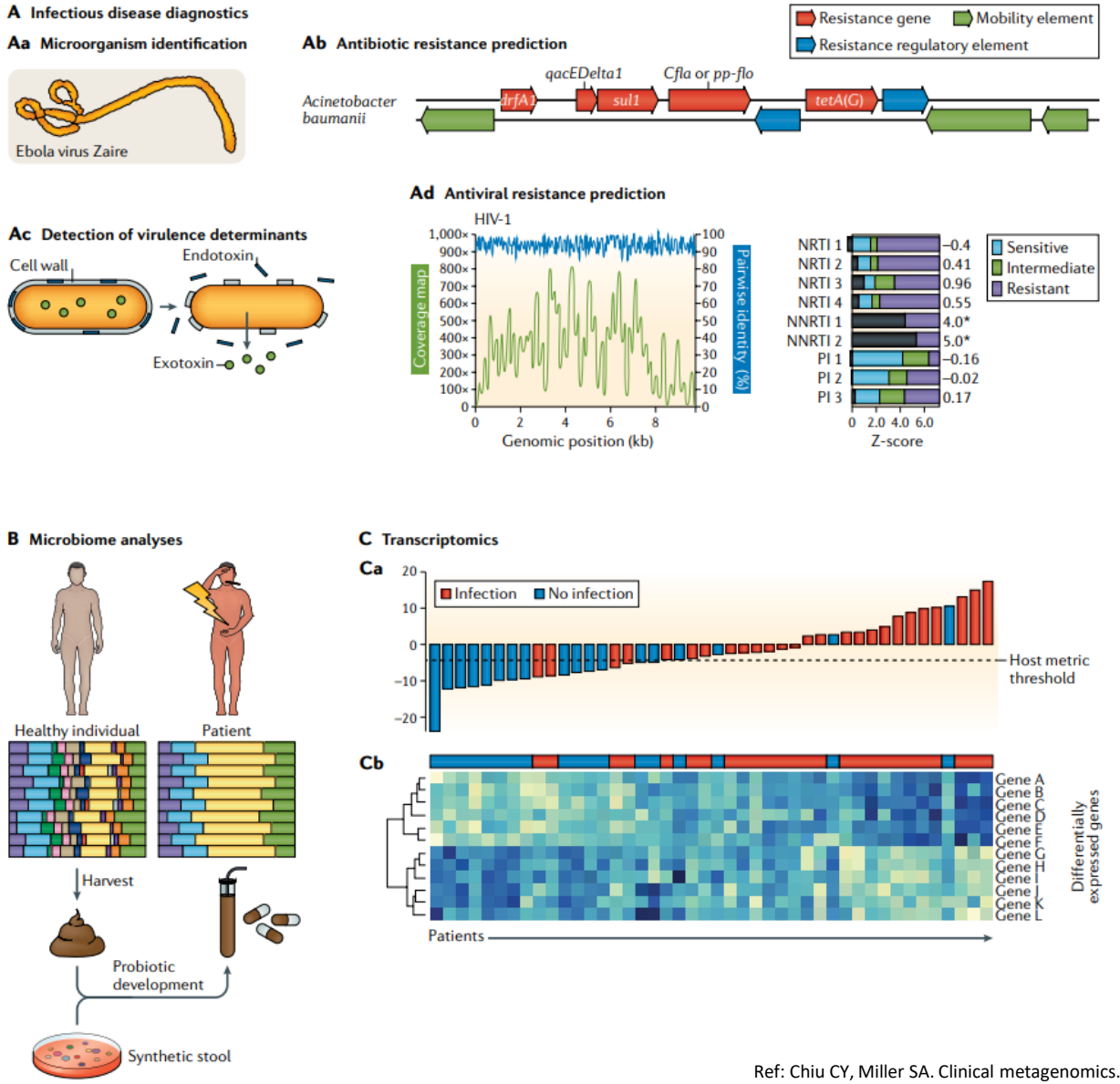
Ref: https://nanoporetech.com/sites/default/files/s3/white-papers/Cancer-research-white-paper.pdf

Overview of Nanopore devices

	Flongle	MinION & MinION Mk1C	GridION Mk1	PromethION 2/2 Solo	PromethION 24/48
Read length	Fragment length = read length. Longest read now >4 Mb[9]				
Run time	1 min – 16 hrs	1 min – 72 hrs	1 min – 72 hrs	1 min – 72 hrs	1 min – 72 hrs
Number of flow cells per device	1	1	5	2	24/48
DNA sequencing yield per flow cell*	Up to 2.8 Gb	Up to 50 Gb	Up to 50 Gb	Up to 290 Gb	Up to 290 Gb
DNA sequencing yield per device*	Up to 2.8 Gb	Up to 50 Gb	Up to 250 Gb	Up to 580 Gb	Up to 7Tb /14 Tb
Multiplexing	1 – 96 samples	1 – >2,000 samples	1 – >2,000 samples	1 – >2,000 samples	1 – >2,000 samples

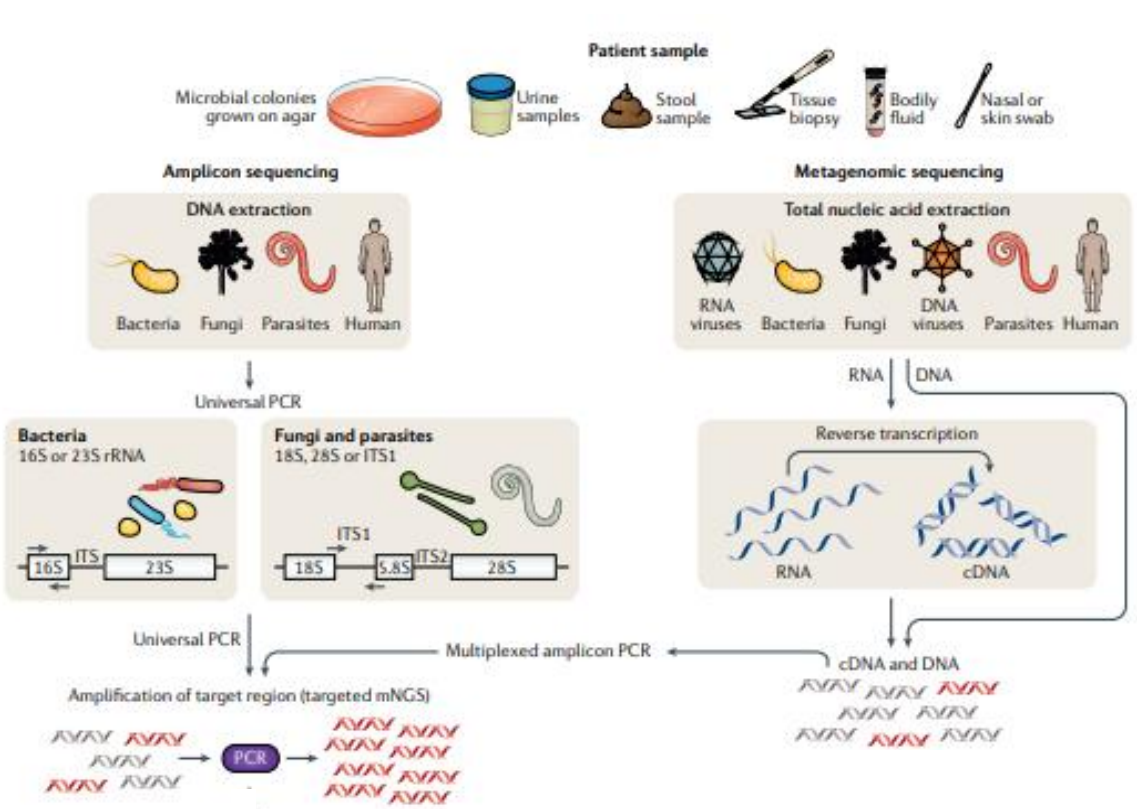
Ref: https://nanoporetech.com/sites/default/files/s3/white-papers/Cancer-research-white-paper.pdf

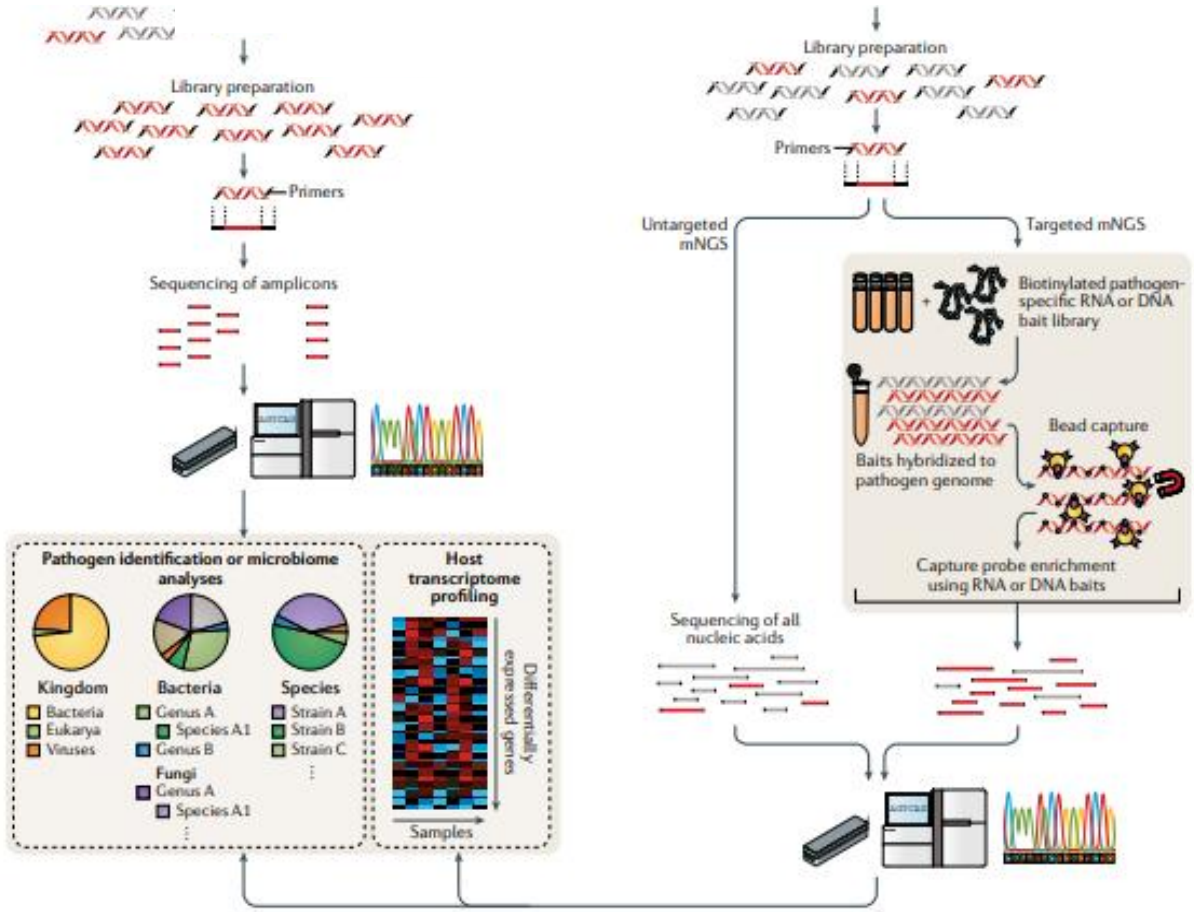
Applications of Nanopore sequencing in Microbiology



Ref: Chiu CY, Miller SA. Clinical metagenomics. Nat Rev Genet. 2019 Jun;20(6):341-355. doi: 10.1038/s41576-019-0113-7.

Applications of Nanopore sequencing in Microbiology





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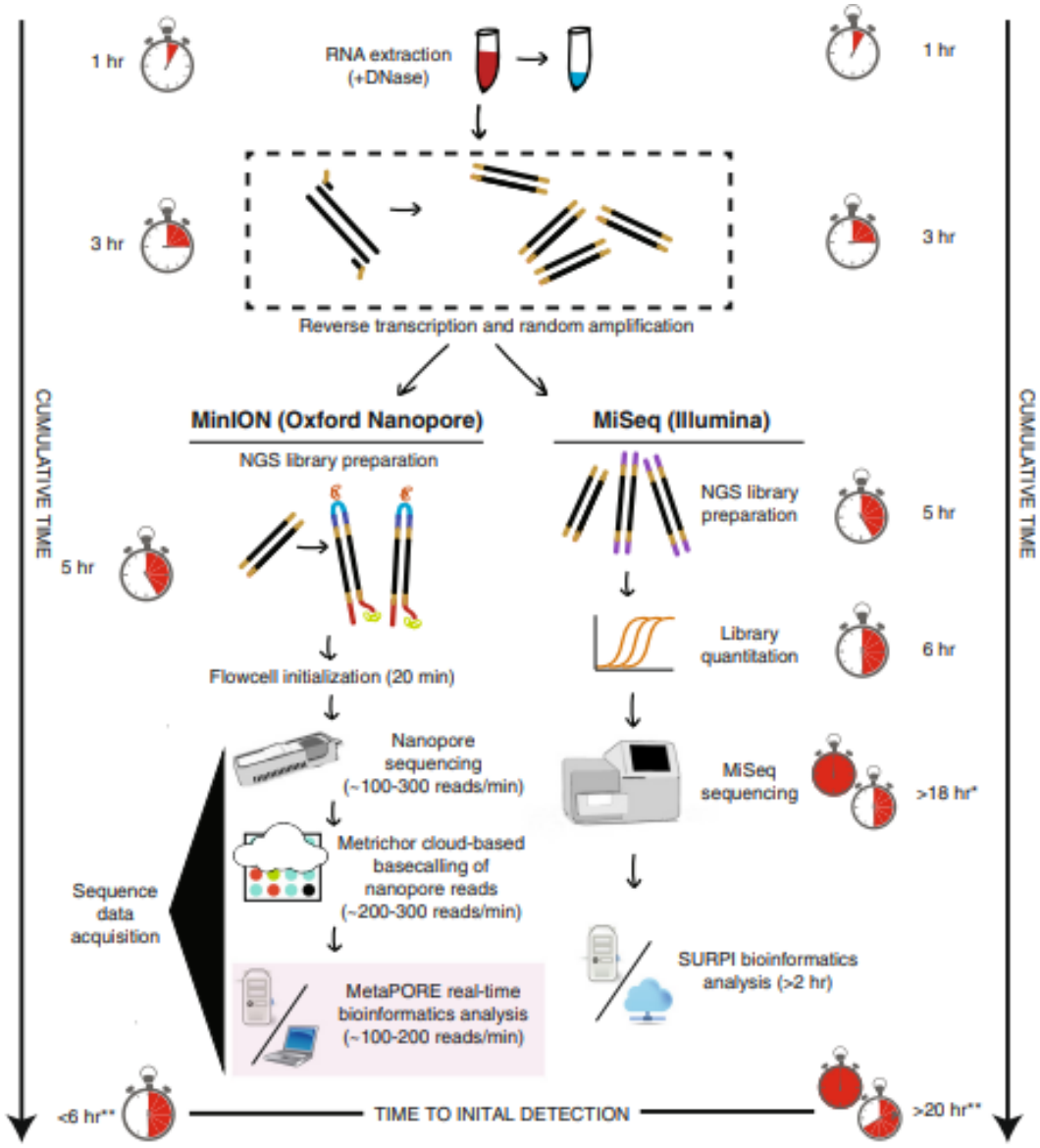


Ref: Greninger et al. Genome Medicine (2015) 7:99 DOI 10.1186/s13073-015-0220-9.

Applications of Nanopore sequencing in Oncology

Key challenges of traditional sequencing technologies

Structural variants

The short reads generated by traditional sequencing technologies cannot span large or complex structural variants (or repeat regions), requiring the use of computational analyses to infer results. As a result, many important variants may be missed or misinterpreted.

Methylation calling

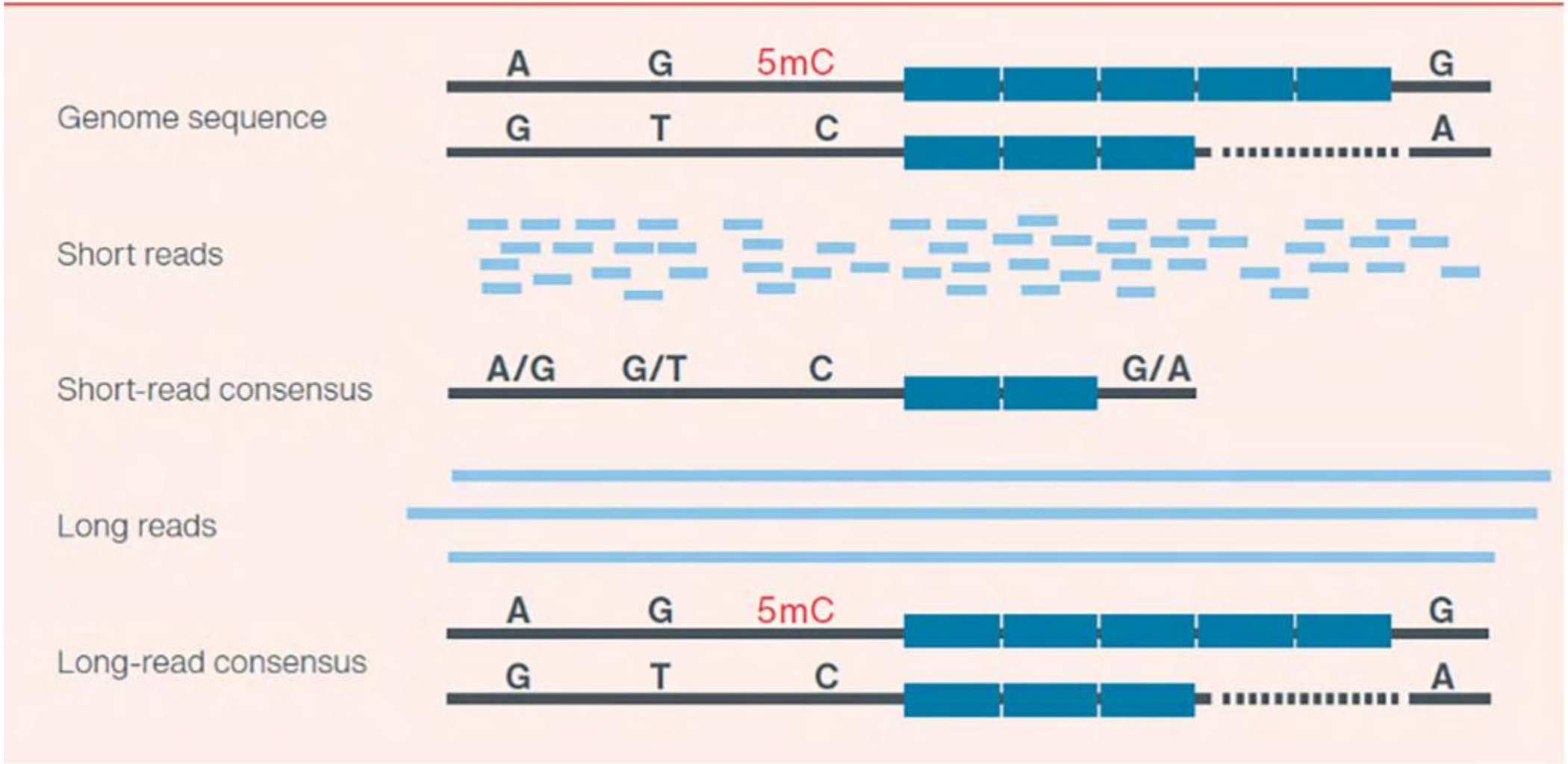
Traditional sequencing technologies require bisulphite conversion (a harsh and laborious sample prep technique) to indirectly infer methylation. Where a suitable reference sequence is not available, additional sequencing runs may also be required. Together these factors can increase experimental variability, time, and costs.

Transcript isoforms

Different transcript isoforms can be associated with different disease stages, but the short reads generated by traditional RNA-Seq techniques only partially cover a transcript's length, making it challenging to accurately assemble and quantify transcript isoforms — providing an incomplete picture of gene expression.

Ref: https://nanoporetech.com/sites/default/files/s3/white-papers/Cancer-research-white-paper.pdf

Applications of Nanopore sequencing in Oncology



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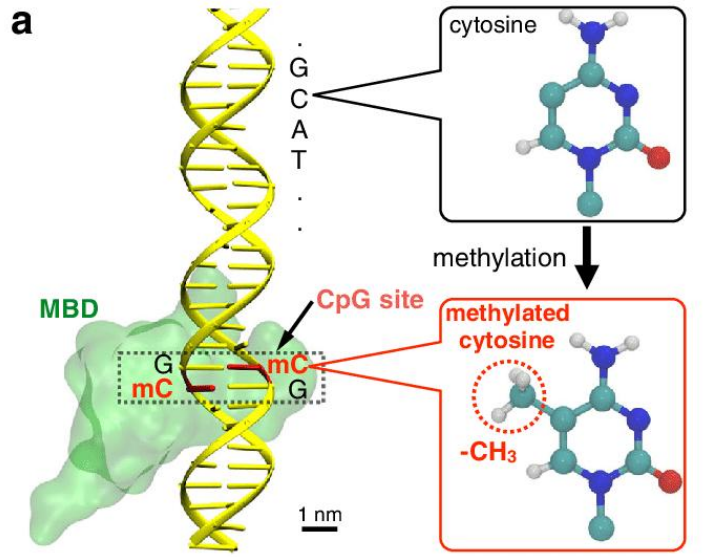
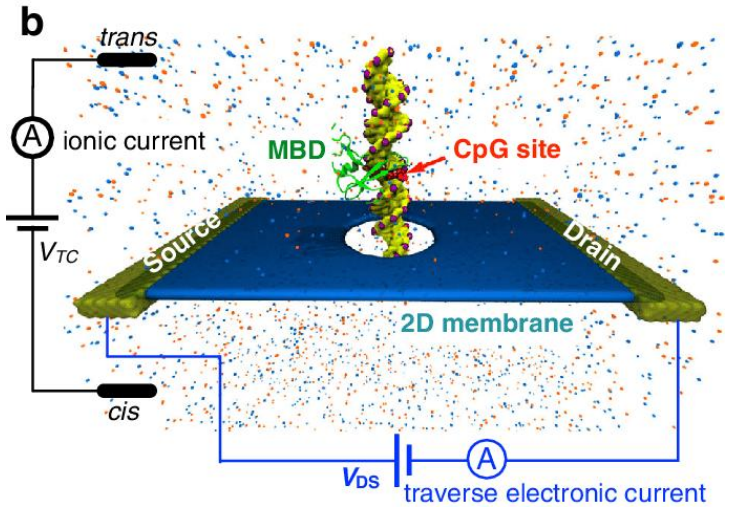
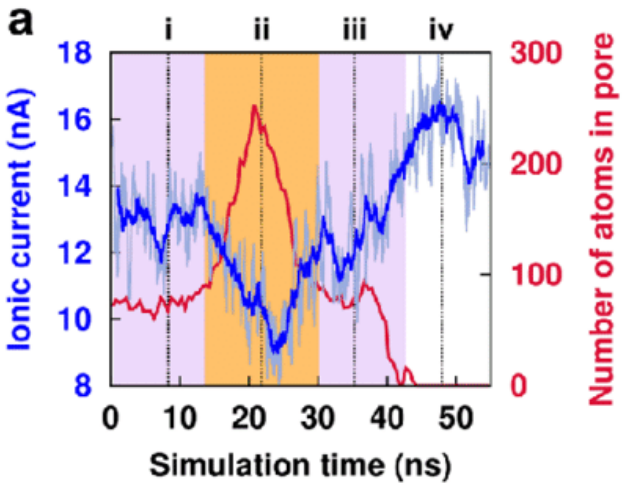
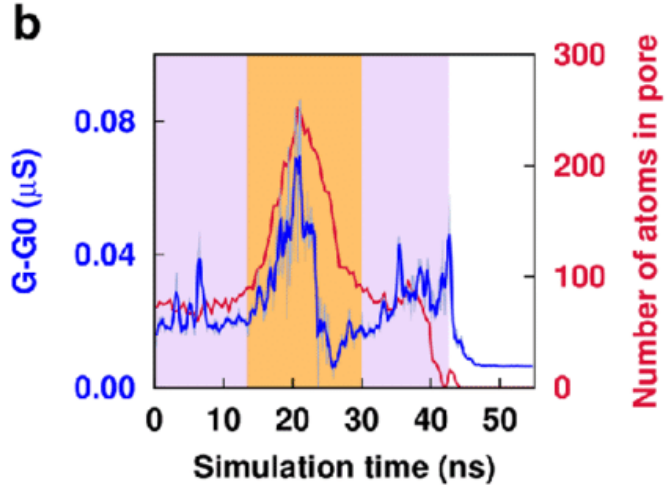
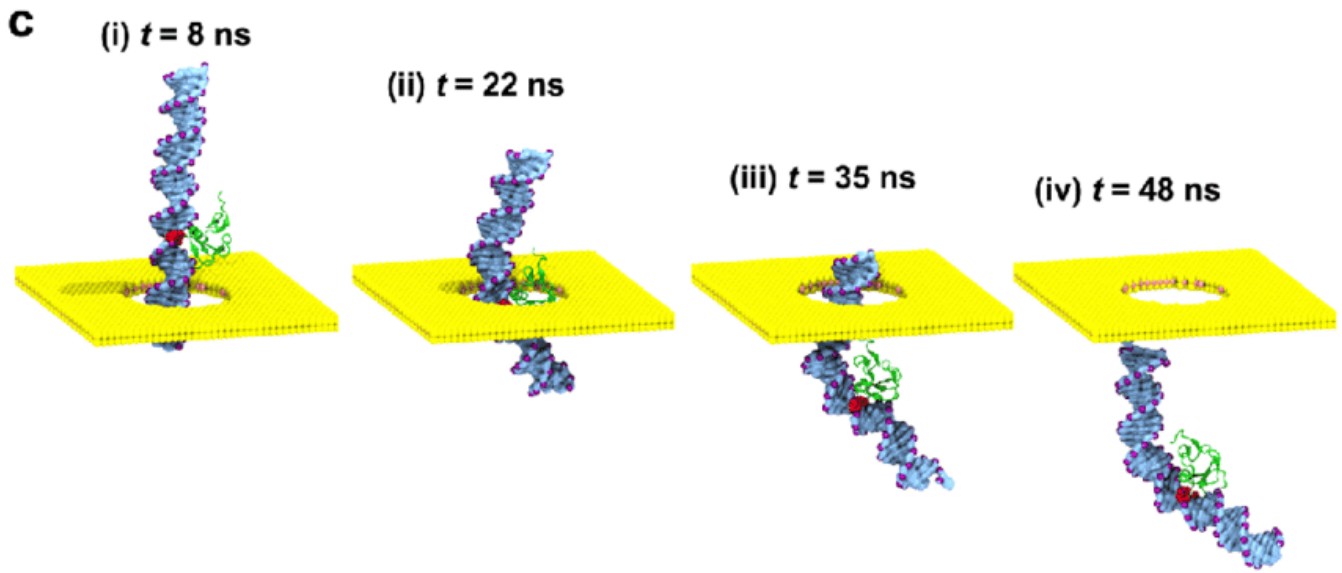
Applications of Nanopore sequencing in Oncology











Ref:Qiu, H., Sarathy, A., Schulten, K. et al. Detection and mapping of DNA methylation with 2D material nanopores. npj 2D Mater Appl 1, 3 (2017). https://doi.org/10.1038/s41699-017-0005-7.

Applications of Nanopore sequencing in Oncology

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Ultra-fast deep-learned CNS tumour classification during surgery

C. Vermeulen, M. Pagès-Gallego, L. Kester, M. E. G. Kranendonk, P. Wesseling, N. Verburg, P. de Witt Hamer, E. J. Kooi, L. Dankmeijer, J. van der Lugt, K. van Baarsen, E. W. Hoving, B. B. J. Tops ✉ & J. de Ridder ✉

Nature **622**, 842–849 (2023) | Cite this article

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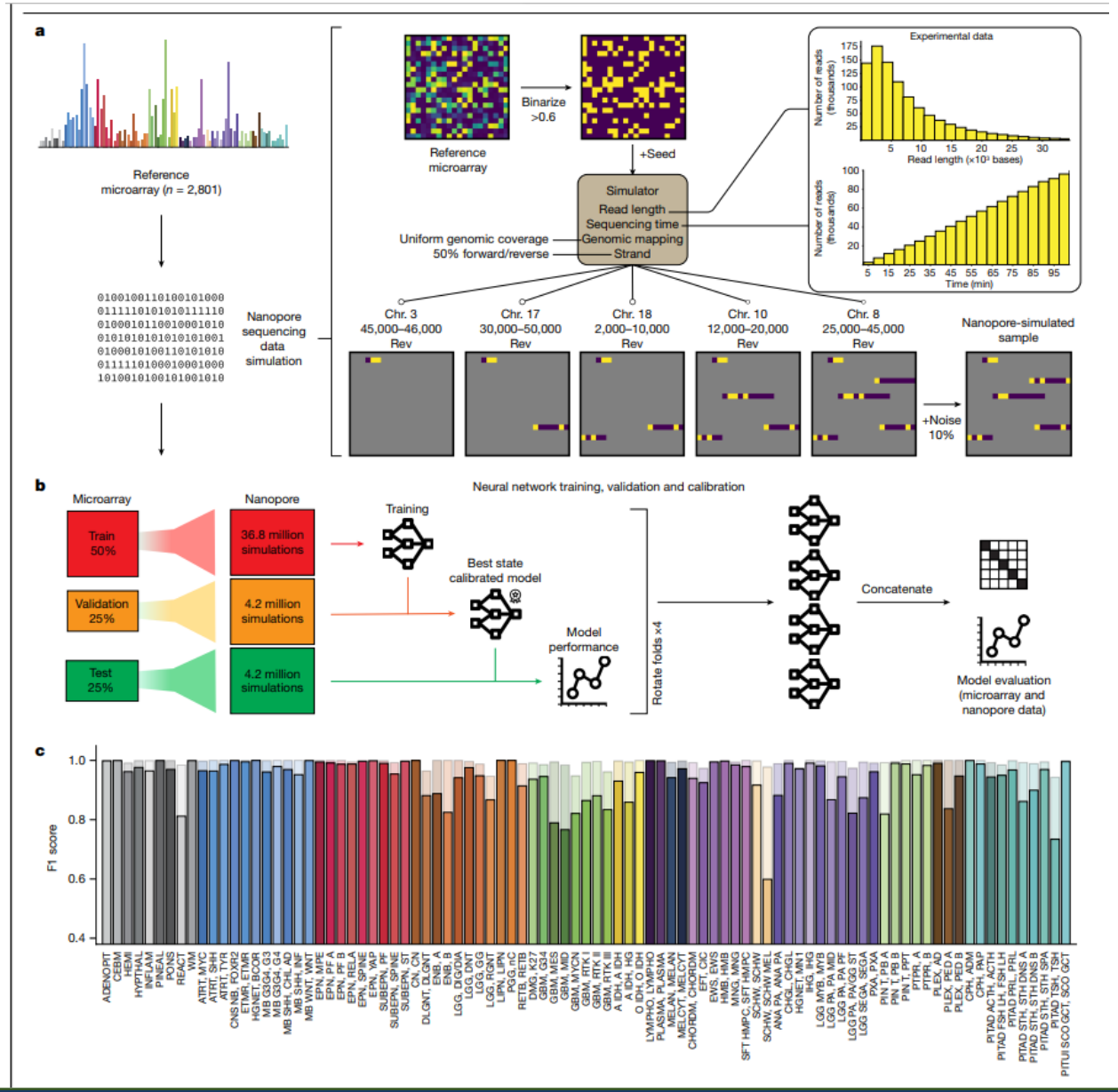
Applications of Nanopore sequencing in Oncology



Ref: Vermeulen, C., Pagès-Gallego, M., Kester, L. et al. Ultra-fast deep-learned CNS tumour classification during surgery. Nature 622, 842–849 (2023). https://doi.org/10.1038/s41586-023-06615-2

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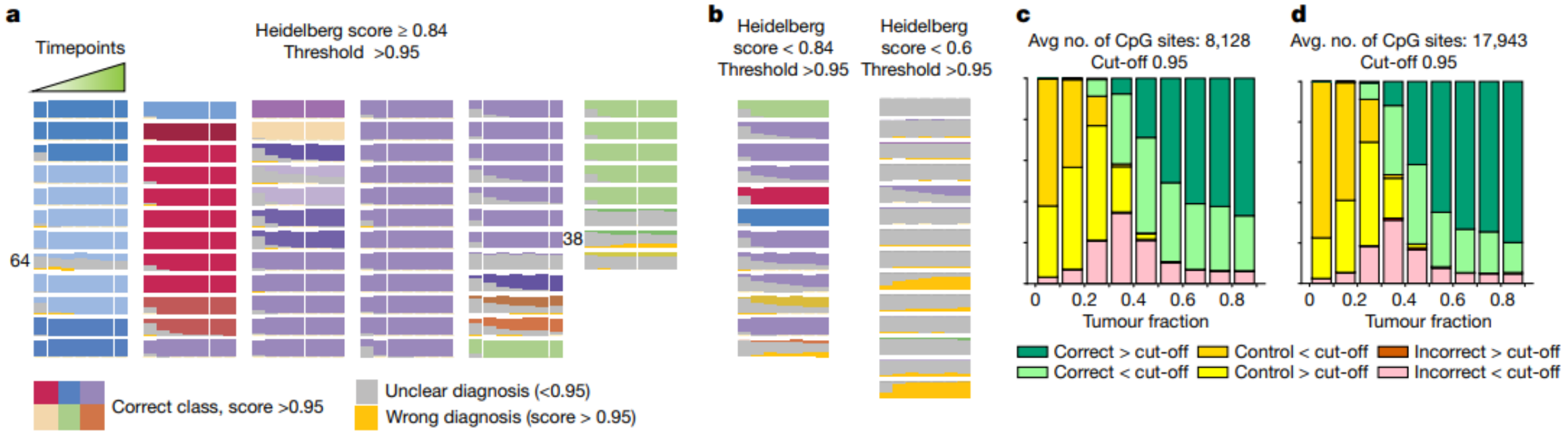

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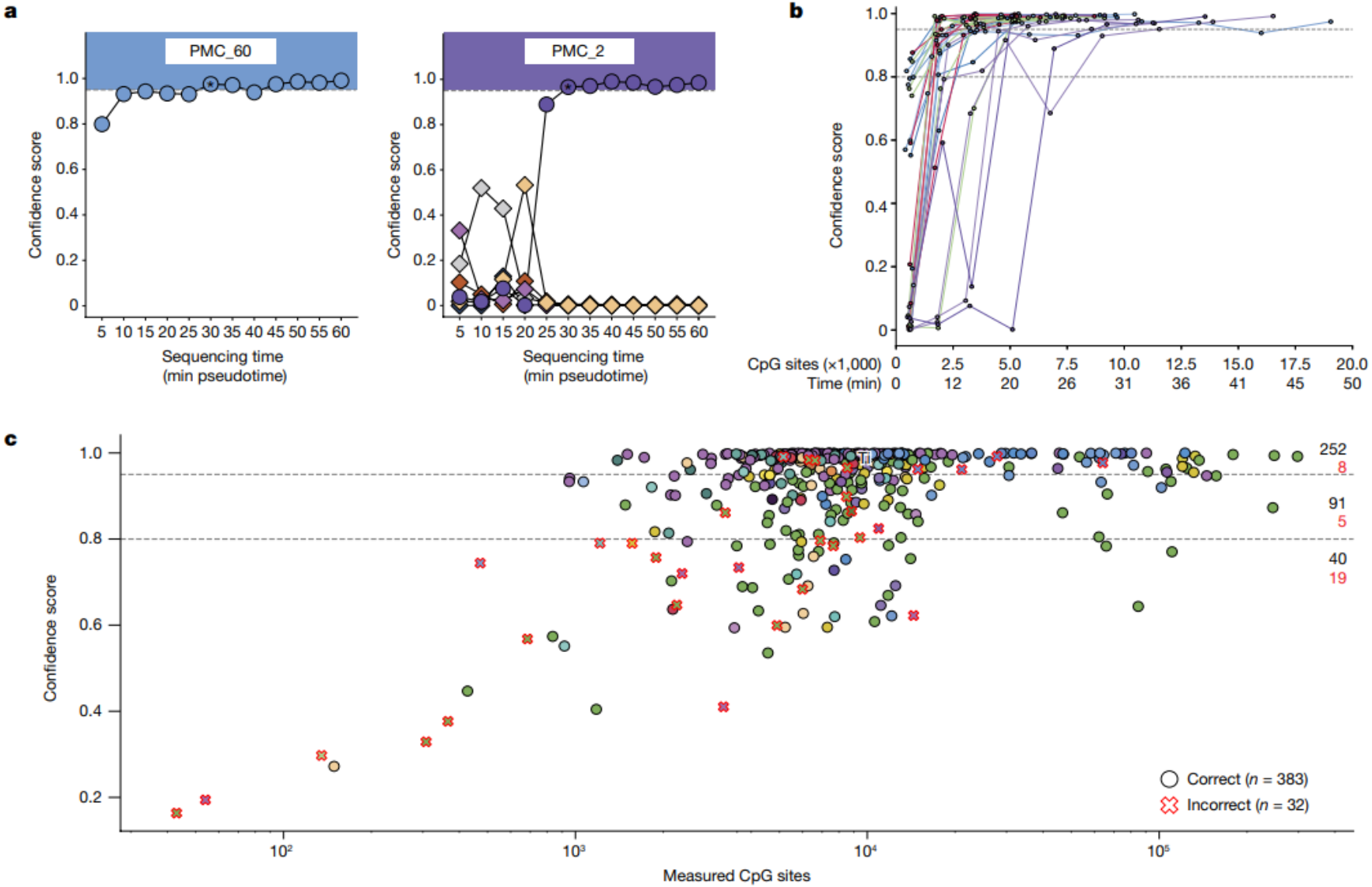

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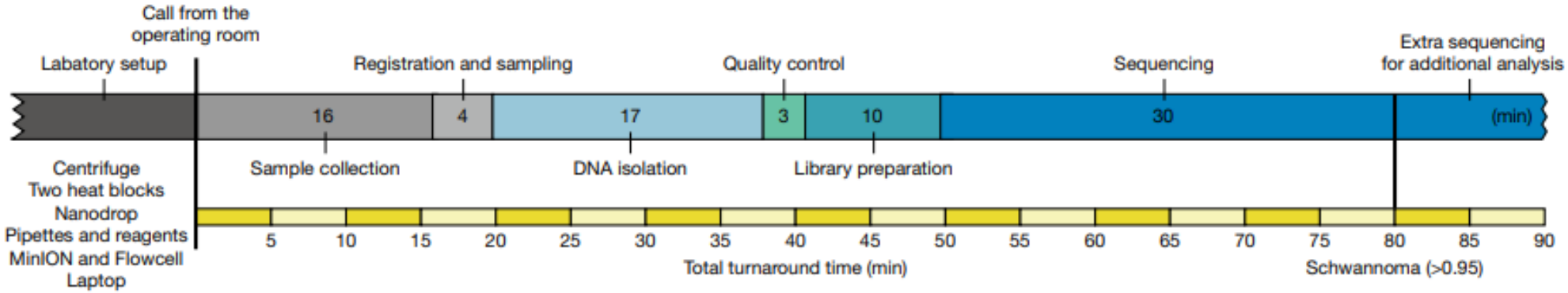

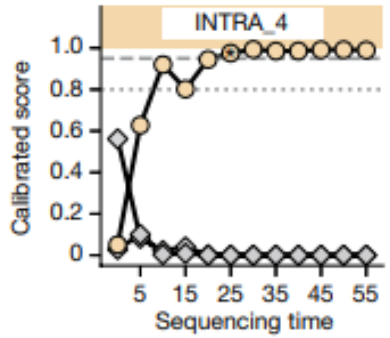

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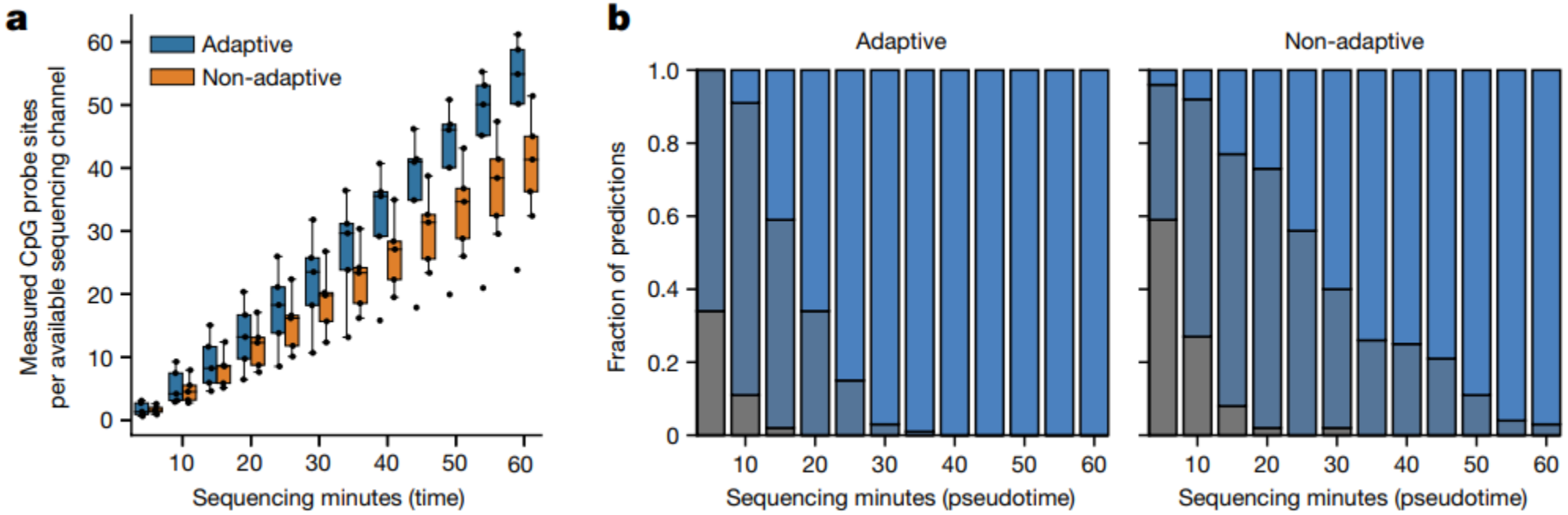

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Applications of Nanopore sequencing in Oncology

Extended Data Table 1 | Overview of the intraoperative sequencing cases

	Sturgeon	CV	ST	Heidelberg	Diagnosis	*Notes*
1.	Inconclusive	R9	-	Not performed	Adamantinomatous Craniopharyngioma	*Sample used for intraoperative sequencing not representative. Diagnosis based on histology*
2.	Medulloblastoma, non-*WNT* / non-*SHH* subtype: group 3	R9	20	Medulloblastoma, non-*WNT* / non-*SHH* subtype: group 3 (0.99)	Medulloblastoma, non-*WNT*/non-*SHH*	
3.	Inconclusive	R9	-	Pilocytic Astrocytoma, subtype: low grade glioma (0.59)	Pilocytic Astrocytoma	*Sample used for intraoperative sequencing not representative (blood clot). A later sample was used for array and integrated diagnosis*
4.	Schwannoma	R9	30	Schwannoma (0.99)	Schwannoma	*Process captured on film. (https://zenodo.org/record/8261128)*
5.	Pilocytic Astrocytoma	R10	20	Midline Pilocytic Astrocytoma (0.99)	Pilocytic Astrocytoma	*3 samples collected, two with low tumor percentage. Highest tumor percentage sample used in intraoperative sequencing*
6.	IDH-wildtype Glioblastoma RTKII	R9	35	IDH-wildtype Glioblastoma RTKII (0.87)	Glioblastoma, IDH-wildtype	*Adult Glioma case*
7.	Choroid Plexus Papiloma type A	R10	15	Plexus tumor, subtype pediatric A (0.90)	Atypical Chorioid Plexus Papiloma	
8.	Inconclusive	R10	-	Reactive tissue (0.47), Pilocytic Astrocytoma (0.43)	Pilocytic Astrocytoma	*Unusual histological features for pilocytic astrocytoma, histomolecular diagnosis partly based on KIAA1549-BRAF fusion*
9.	Pilocytic Astrocytoma	R10	15	Posterior fossa Pilocytic Astrocytoma (0.99)	Pilocytic Astrocytoma	
10.	IDH-mutant Astrocytoma	R9	10	IDH-mutant, Glioma, High- or Low-Grade Astrocytoma (0.99)	IDH-mutant, Astrocytoma, CNS WHO grade 4	*Adult Glioma case*
11.	Inconclusive	R10	-	Not performed	Germinoma	*Germinoma; tumor subtype not in reference Capper et al., 2018 datset. Diagnosis based only on intraoperative frozen section histology*
12.	Inconclusive	R9	-	White matter (0.99)	IDH-mutant, Astrocytoma, CNS WHO grade 4	*Adult Glioma case. Sample used for intraoperativesequencing was not representative, Integrated molecular diagnosis based on an other tissue sample.*
13.	Inconclusive	R10	-	Low-Grade Glioma, *MYB/MYBL1* (0.33)	Diffuse Glioma, Not Elsewhere Classified	*Case considered as 'gliomatosis cerebri' in context of complex genetic background*
14.	Inconclusive	R10	-	Not performed	Pilocytic Astrocytoma	*Biopsy sample, not tumor representative*
15.	Pilocytic Astrocytoma	R10	15	Pilocytic Astrocytoma subtype: posterior fossa	Pilocytic Astrocytoma	*Resection sample from patient 14*
16.	Adamantinomatous Craniopharyngioma	R10	10	Not performed	Adamantinomatous Craniopharyngioma	*Diagnosis based on histology*
17.	IDH-mutant Astrocytoma	R9	45	Not performed	IDH-mutant, Astrocyto - ma, CNS WHO Grade 2	*Adult Glioma case*
18.	Pilocytic Astrocytoma	R10	40	Pilocytic Astrocytoma, subtype: posterior fossa (0.95)	Pilocytic Astrocytoma	
19.	Medulloblastoma Group 4	R10	10	Medulloblastoma, Group 3 and 4, subtype: Group 4 (0.99)	Medulloblastoma, non-*WNT*/non-*SHH*	
20.	Medulloblastoma Group 3	R10	15	Medulloblastoma, non-*WNT* / non-*SHH*, subtype: Group 3 (0.99)	Medulloblastoma, non-*WNT*/non-*SHH*	
21.	Pilocytic Astrocytoma	R10	30	Pilocytic Astrocytoma (0.97)	Pilocytic Astrocytoma	
22.	Pilocytic Astrocytoma *	R10	-	Not performed	Pilocytic Astrocytoma	*Recurrent tumor, diagnosis the same as in initial tumor sample*
23.	Ependymoma, subtype *RELA* fusion	R10	10	Ependymoma, subtype *RELA* fusion (0.99)	Supratentorial Ependymoma, *ZFTA* fusion-positive **	*Classification used to support frozen section diagnosis during surgery*
24.	IDH-wildtype Glioblastoma, subtype Mesenchymal *	R9	-	IDH-wildtype Glioblastoma, subtype Mesenchymal (0.4)	Diffuse High-Grade Glioma, results further analysis pending	*Adult Glioma case; Imaging consistent with Low-Grade Diffuse Glioma*
25.	Ependymoma, posterior fossa type A	R10	25	Ependymoma, posterior fossa type A (0.99)	Ependymoma, posterior fossa type A	*Classification used to support frozen section diagnosis during surgery*

Ref: Vermeulen, C., Pagès-Gallego, M., Kester, L. et al. Ultra-fast deep-learned CNS tumour classification during surgery. Nature 622, 842–849 (2023). https://doi.org/10.1038/s41586-023-06615-2

Getting started with Nanopore sequencing

- Invest in the right type of sequencer and PC for your needs!

MinION Mk1D IT requirements

Component	Minimum	Recommended
Operating system	Windows 10/11 Ubuntu 22.04/24.04 LTS MacOS	Windows 10/11 Ubuntu 22.04/24.04 LTS
Peripheral	USB Type-C (USB 2.0 or higher)	USB Type-C (USB 2.0 or higher)
Memory	16 GB + Apple: 24 GB + Unified Memory	32 GB +
GPU	NVIDIA RTX 5060 Laptop GPU + Apple: M4 Pro +	NVIDIA RTX 5090 Laptop GPU
CPU	Intel I5 + / AMD Threadripper + / Apple M4 Pro + (6-cores +)	Intel I7 + (12-cores +)
Storage	1 TB SSD +	2 TB SSD +

Loading the Nanopore flow cell

Priming and loading the SpotON flow cell for GridION

~10 minutes

1 **Thaw the Sequencing Buffer (SQB), Loading Beads (LB), Flush Tether (FLT) and one tube of Flush Buffer (FB) at room temperature before placing the tubes on ice as soon as thawing is complete.**

2 **Mix the Sequencing Buffer (SQB), Flush Buffer (FB) and Flush Tether (FLT) tubes by vortexing, spin down and return to ice.**

3 **Slide open the GridION lid and insert the flow cell.**

Press down firmly on the flow cell to ensure correct thermal and electrical contact.

4 **Slide the priming port cover clockwise to open the priming port.**

Loading the Nanopore flow cell

Priming and loading the SpotON flow cell for GridION

~10 minutes

5 **After opening the priming port, check for a small air bubble under the cover. Draw back a small volume to remove any bubbles (a few µl):**

1. Set a P1000 pipette to 200 µl
2. Insert the tip into the priming port
3. Turn the wheel until the dial shows 220-230 µl, or until you can see a small volume of buffer entering the pipette tip

Visually check that there is continuous buffer from the priming port across the sensor array.

6 **To prepare the flow cell priming mix, add 30 µl of thawed and mixed Flush Tether (FLT) directly to the tube of thawed and mixed Flush Buffer (FB), and mix by vortexing.**

7 **Load 800 µl of the priming mix into the flow cell via the priming port, avoiding the introduction of air bubbles. Wait for 5 minutes. During this time, prepare the library for loading by following the steps below.**

8 **Thoroughly mix the contents of the Loading Beads (LB) by pipetting.**

9 **In a new tube, prepare the library for loading as follows:**

Reagent	Volume
Sequencing Buffer (SQB)	37.5 µl
Loading Beads (LB), mixed immediately before use	25.5 µl
DNA library	12 µl
Total	**75 µl**

Loading the Nanopore flow cell

Priming and loading the SpotON flow cell for GridION

~10 minutes

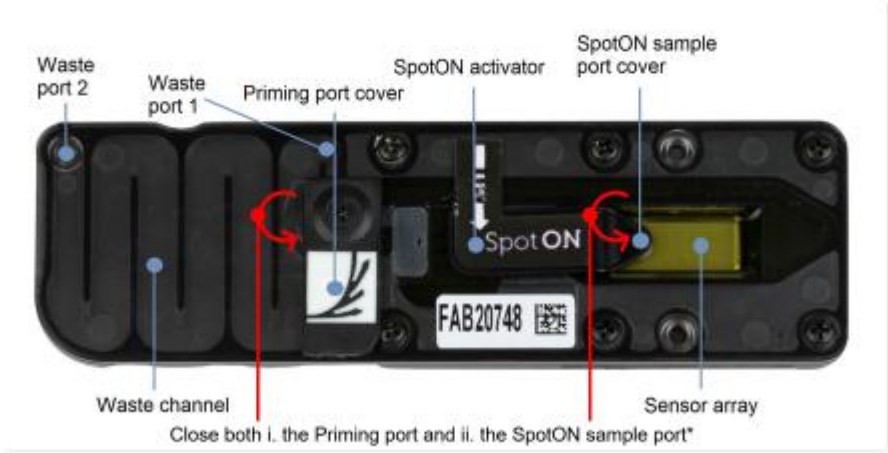
10 Complete the flow cell priming:

1. Gently lift the SpotON sample port cover to make the SpotON sample port accessible.
2. Load **200 µl** of the priming mix into the flow cell via the priming port (**not** the SpotON sample port), avoiding the introduction of air bubbles.

11 Mix the prepared library gently by pipetting up and down just prior to loading.

12 Add 75 µl of sample to the flow cell via the SpotON sample port in a dropwise fashion. Ensure each drop flows into the port before adding the next.

13 Gently replace the SpotON sample port cover, making sure the bung enters the SpotON port, close the priming port and replace the GridION lid.



*Both ports are shown in a closed position

Set-up your own experiment

- Amplicon-based? => Native barcoding kit or Ligation sequencing kit (barcoded primers)
- Untargeted approach => Rapid barcoding kit
- Ready to use (including primers, barcodes & adapters) => 16S Barcoding kit

Set-up your own experiment

Native Barcoding Kit 96 V14 SQK-NBD114.96

A versatile method of preparing barcoded sequencing libraries optimised for modal raw read accuracy of Q20+ (99%+) and long read multiplexed samples.

This uses our latest Kit 14 chemistry

- Read length: = to fragment length
- Input amount: 400 ng per sample of gDNA, 200 fmol per sample (130 ng for 1 kb amplicons)

€830.00 Buy › 3 Released

Ligation Sequencing Kit V14 SQK-LSK114

A versatile sequencing kit optimised for modal raw read accuracy of Q20+ (99%+) and long read singleplex samples.

This uses our latest Kit 14 chemistry

- Preparation time: 60 minutes
- Read length: = fragment length
- Input amount: 1000 ng dsDNA, 100+ ng DNA if performing fragmentation or PCR,100-200 fmol of amplicons or cDNA

€630.00 Buy › 3 Released

Rapid Barcoding Kit 96 V14 SQK-RBK114.96

Simple and rapid library preparation, with barcoding for up to 96 gDNA samples.

This uses our latest Kit 14 chemistry

- Preparation time: 60 minutes
- Read length: Random distribution, dependent on input fragment length
- Input amount: 200 ng dsDNA per sample

€1,030.00 Buy › 3 Released

Microbial Amplicon Barcoding Kit 24 V14 SQK-MAB114.24

Amplification and full-length sequencing of 16S rRNA for bacterial profiling and ITS for fungal profiling with barcoding for up to 24 samples.

This is an Early Access product.

This uses our Kit 14 chemistry.

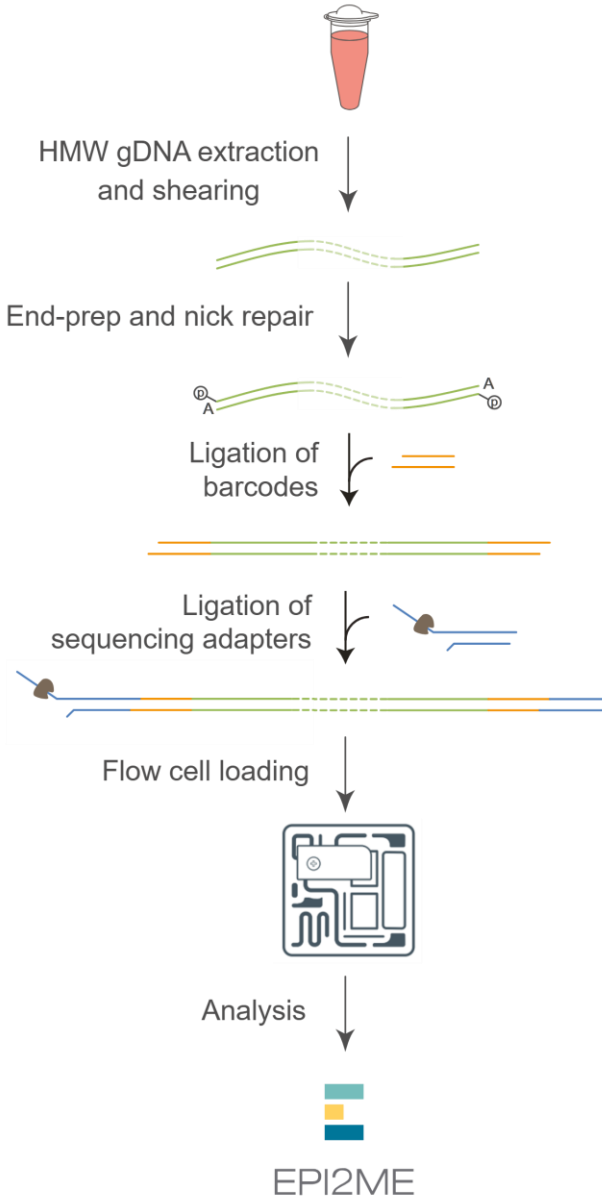
- Preparation time: 60 minutes + PCR
- Read length: Full-length 16S gene (~1.5 kb)
- Input amount: 10 ng gDNA per sample

€730.00 Buy › 2 Early Access

Hereditary cancer panel (HCP): native barcoding

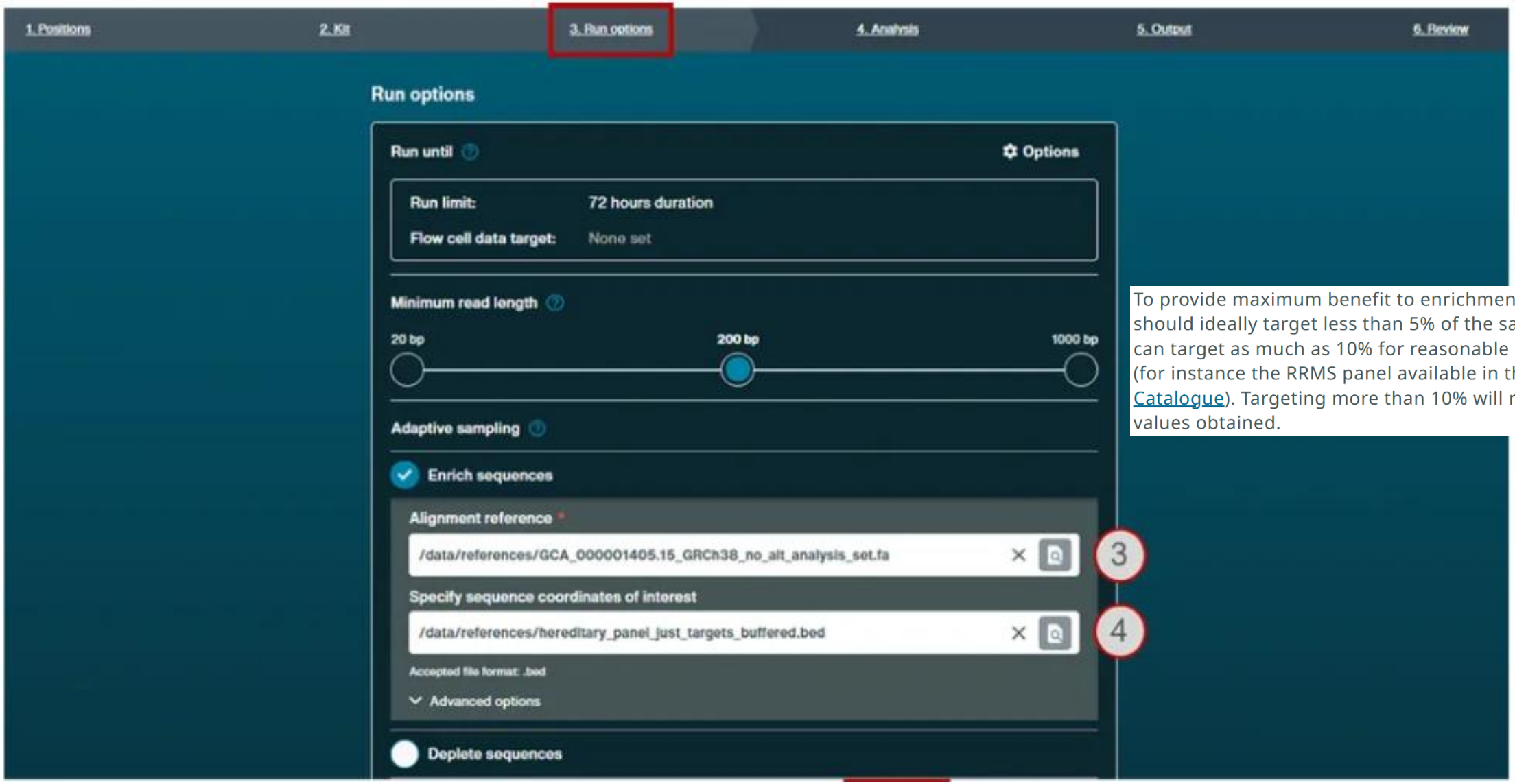
Hereditary Cancer Panel gene list														
ABRAXAS1	*ACD*	*AIP*	*AKT1*	*ALK*	*ANKRD26*	*APC*	*ARID5B*	*ATG2B*	*ATM*	*ATR*	*AXIN2*	*BAP1*	*BARD1*	*BLM*
BMPR1A	*BRAF*	*BRCA1*	*BRCA2*	*BRIP1*	*BUB1B*	*CBL*	*CDC73*	*CDH1*	*CDK12*	*CDK4*	*CDKN1B*	*CDKN1C*	*CDKN2A*	*CEBPA*
CFTR	*CHEK2*	*COL7A1*	*CSF3R*	*CTC1*	*CTNNA1*	*CTR9*	*CYLD*	*DDB2*	*DDX41*	*DICER1*	*DIS3L2*	*DKC1*	*DNAH9*	*DNAJC21*
DOCK8	*DPYD*	*EFL1*	*EGFR*	*ELANE*	*ELP1*	*EPCAM*	*ERCC1*	*ERCC2*	*ERCC3*	*ERCC4*	*ERCC5*	*ERCC6L2*	*ETV6*	*EXT1*
EXT2	*FAAP100*	*FAN1*	*FANCA*	*FANCB*	*FANCC*	*FANCD2*	*FANCE*	*FANCF*	*FANCG*	*FANCI*	*FANCL*	*FANCM*	*FAS*	*FH*
FLCN	*G6PC3*	*GALNT12*	*GATA1*	*GATA2*	*GBA1*	*GFI1*	*GPC3*	*GPR161*	*GRB10*	*GREM1*	*GSKIP*	*H19*	*HAX1*	*HLA-A*
HLA-B	*HLA-C*	*HLA-DPA1*	*HLA-DQA1*	*HLA-DQB1*	*HLA-DRB1*	*HMBS*	*HOXB13*	*HRAS*	*IGF2*	*IKZF1*	*ITK*	*KCNQ1*	*KCNQ1OT1*	*KIF1B*
KIT	*KLLN*	*KRAS*	*L3MBTL1*	*LIG4*	*LZTR1*	*MAD2L2*	*MAP2K1*	*MAP2K2*	*MAX*	*MBD4*	*MC1R*	*MECOM*	*MEN1*	*MEST*
MET	*MITF*	*MLH1*	*MLH3*	*MPL*	*MRE11*	*MSH2*	*MSH3*	*MSH6*	*MTAP*	*MUTYH*	*NAF1*	*NAPRT*	*NBN*	*NF1*
NF2	*NHP2*	*NLRP2*	*NLRP5*	*NLRP7*	*NOP10*	*NPM1*	*NRAS*	*NSD1*	*NTHL1*	*PADI6*	*PALB2*	*PALLD*	*PARN*	*PAX5*
PAX6	*PDGFRA*	*PDGFRB*	*PHOX2B*	*PIK3CA*	*PMS2*	*PMS2CL*	*POLD1*	*POLE*	*POLH*	*POT1*	*PPM1D*	*PRF1*	*PRKAR1A*	*PRSS1*
PTCH1	*PTEN*	*PTPN11*	*RAD21*	*RAD50*	*RAD51*	*RAD51B*	*RAD51C*	*RAD51D*	*RAF1*	*RB1*	*RECQL4*	*REST*	*RET*	*RFWD3*
RHBDF2	*RINT1*	*RMRP*	*RNF43*	*RPA1*	*RPL11*	*RPL15*	*RPL23*	*RPL26*	*RPL27*	*RPL31*	*RPL35A*	*RPL36*	*RPL5*	*RPS10*
RPS17	*RPS19*	*RPS20*	*RPS24*	*RPS26*	*RPS27*	*RPS27A*	*RPS28*	*RPS29*	*RPS7*	*RTEL1*	*RUNX1*	*SAMD9*	*SAMD9L*	*SBDS*
SDHA	*SDHAF2*	*SDHB*	*SDHC*	*SDHD*	*SETBP1*	*SH2B3*	*SH2D1A*	*SHOC2*	*SLX4*	*SMAD4*	*SMARCA4*	*SMARCB1*	*SMARCE1*	*SOS1*
SPINK1	*SPRED1*	*SQSTM1*	*SRP72*	*SRY*	*STAT3*	*STK11*	*STN1*	*SUFU*	*TBXT*	*TERC*	*TERF2IP*	*TERT*	*TINF2*	*TMEM127*
TP53	*TRIM28*	*TRIM37*	*TRIP13*	*TSC1*	*TSC2*	*TSR2*	*UBE2T*	*UROD*	*VHL*	*WAS*	*WRAP53*	*WRN*	*WT1*	*XPA*
XPC	*XRCC2*	*ZCCHC8*												

Hereditary cancer panel (HCP): native barcoding



Step in workflow	Process	Time	Stop option
Sample preparation	Extract gDNA from sample and quantify. Fragment the gDNA and quantify. Check the length, quantity and purity of your extracted material. **The quality checks performed during the protocol are recommended to ensure experimental success.**	4 to 6 hours	At this stage, the extracted gDNA or fragmented gDNA can be stored at –20°C for later use.
DNA repair and end-prep	Repair the gDNA, and prepare the DNA ends for adapter attachment.	60 minutes	4°C overnight
Native barcode ligation	Ligate the native barcodes to the DNA ends.	70 minutes	4°C overnight
Sequencing adapter ligation and clean-up	Ligate sequencing adapters to the DNA ends.	60 minutes	4°C for short-term storage or for repeated use, such as for reloading your flow cell. –80°C for long-term storage.
Priming and loading the flow cell	Prime the flow cell, and load your DNA library into the flow cell.	10 minutes	
Sequencing	Sequence your library using your sequencing device.	72 hours	
Data analysis	Analysis using HCP data bundle.	120 minutes	

Hereditary cancer panel (HCP): adaptive sampling



To provide maximum benefit to enrichment, the .bed files should ideally target less than 5% of the sample. However, you can target as much as 10% for reasonable returns of enrichment (for instance the RRMS panel available in the Adaptive Sampling Catalogue). Targeting more than 10% will reduce the enrichment values obtained.

Hereditary cancer panel (HCP): adaptive sampling

Hereditary cancer panel (HCP): native barcoding

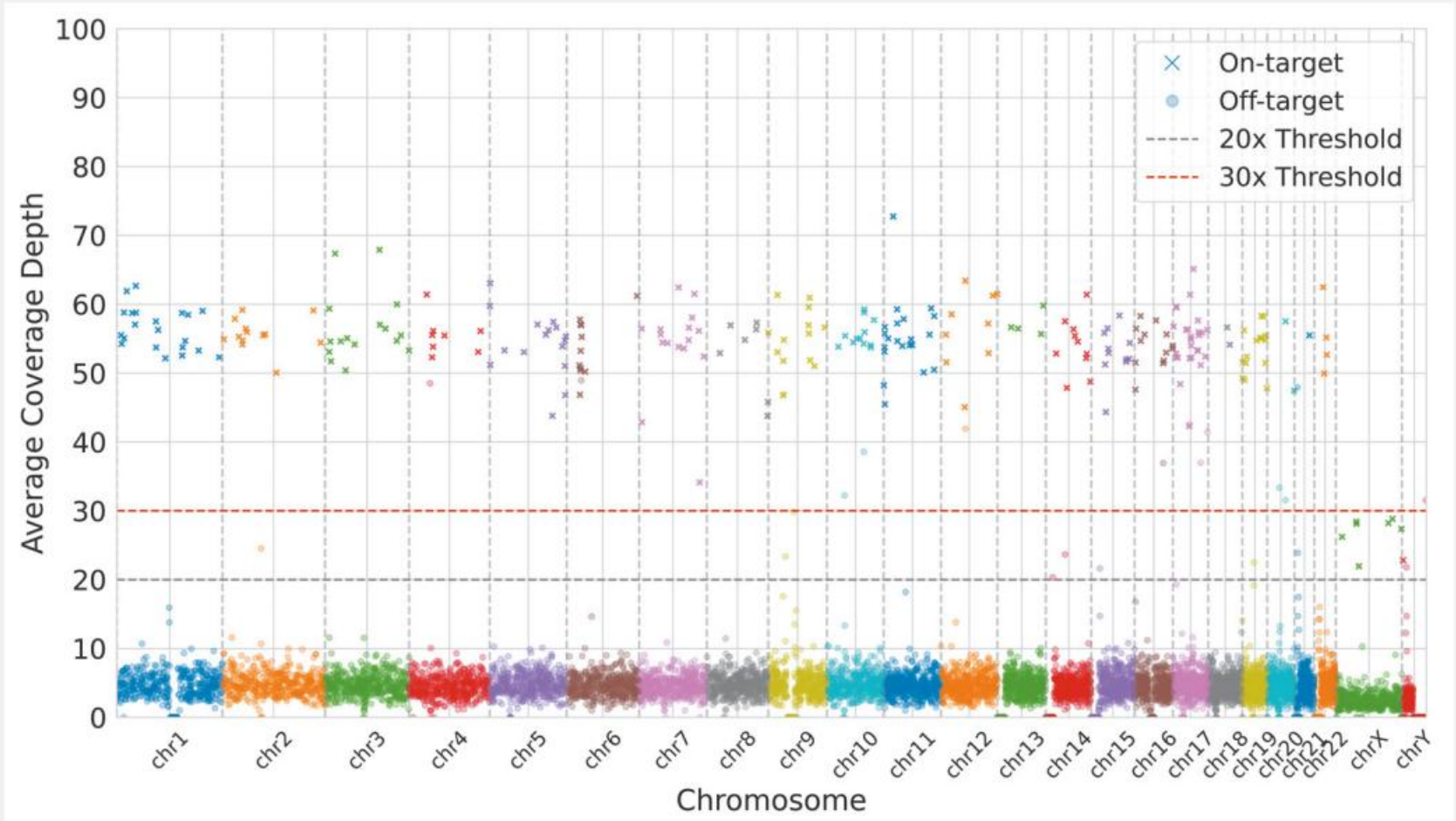
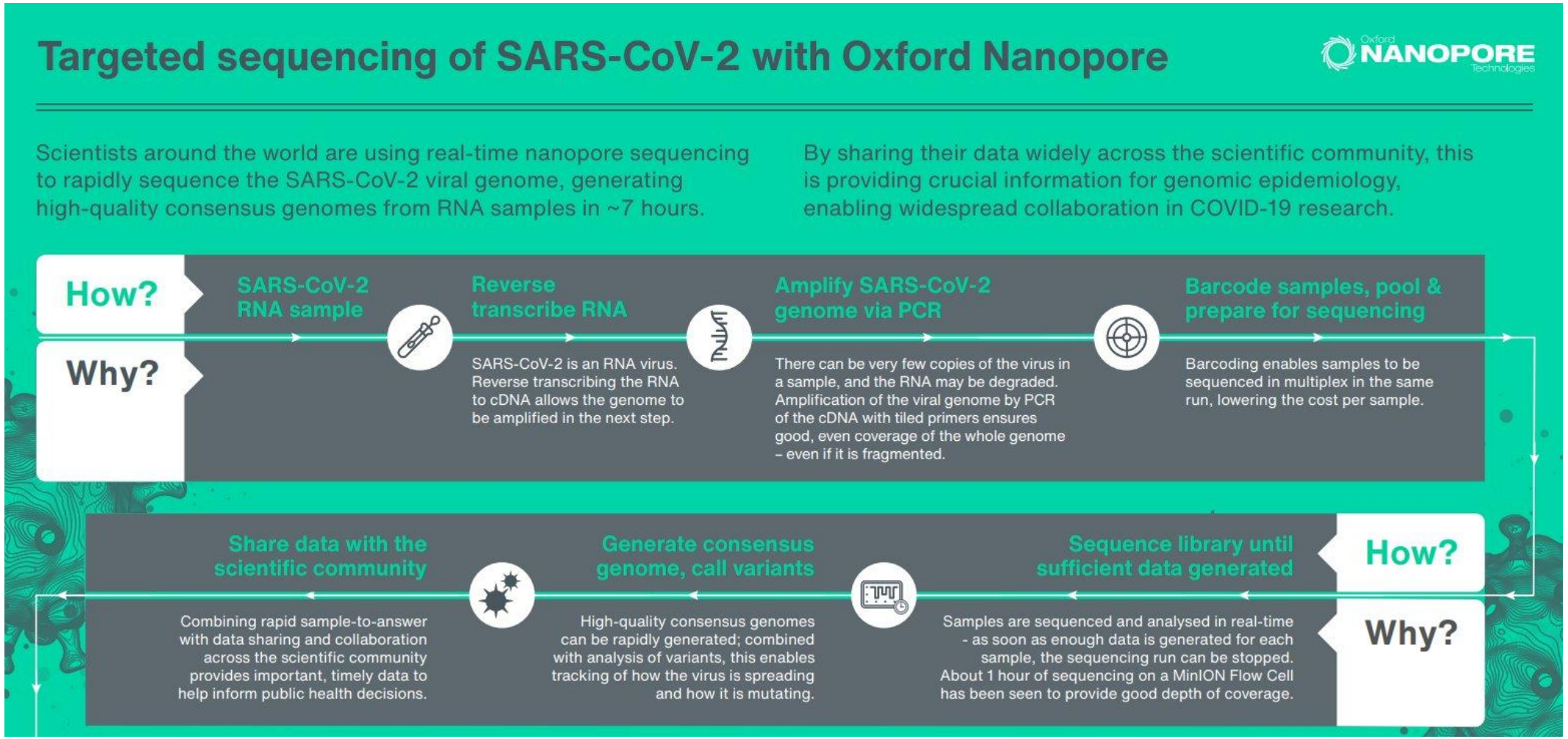


Figure 1. Sequencing coverage across the genome, depicting low-pass whole-genome coverage and >30x enrichment for target loci.

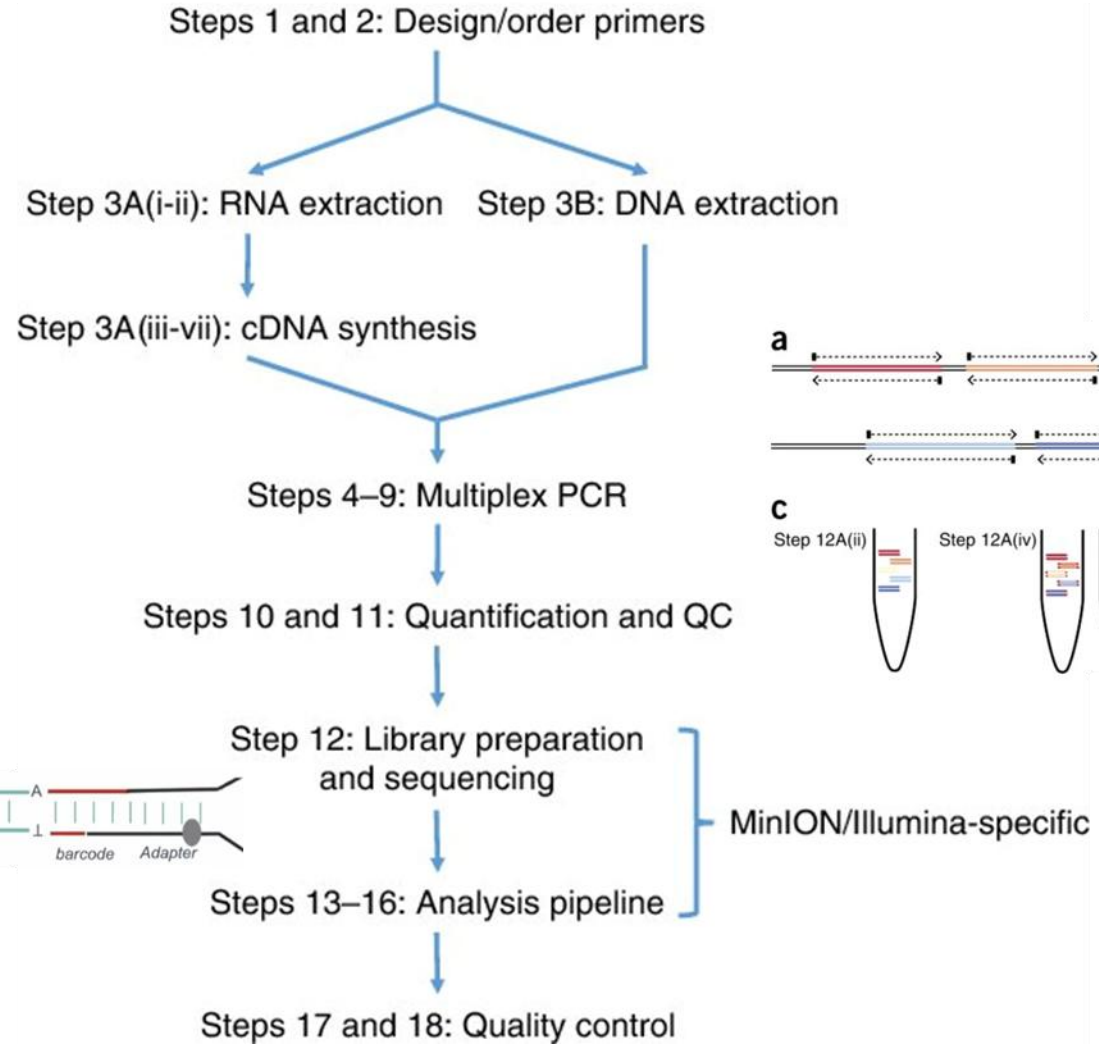
Panel specifications	
Enrichment method	Adaptive sampling
Genes	258
Reference genome	hg38
Recommended DNA input	1 µg
Sample type	Whole blood, DNA
Coverage uniformity (fold-80)	1.2
Samples per PromethION™ Flow Cell	3
Total assay time (hands-on time)	<5 days (<2 hours)

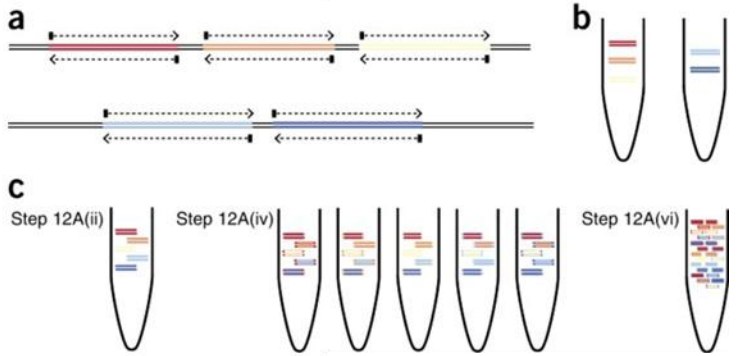
Set-up your own experiment: SARS-CoV-2 WGS



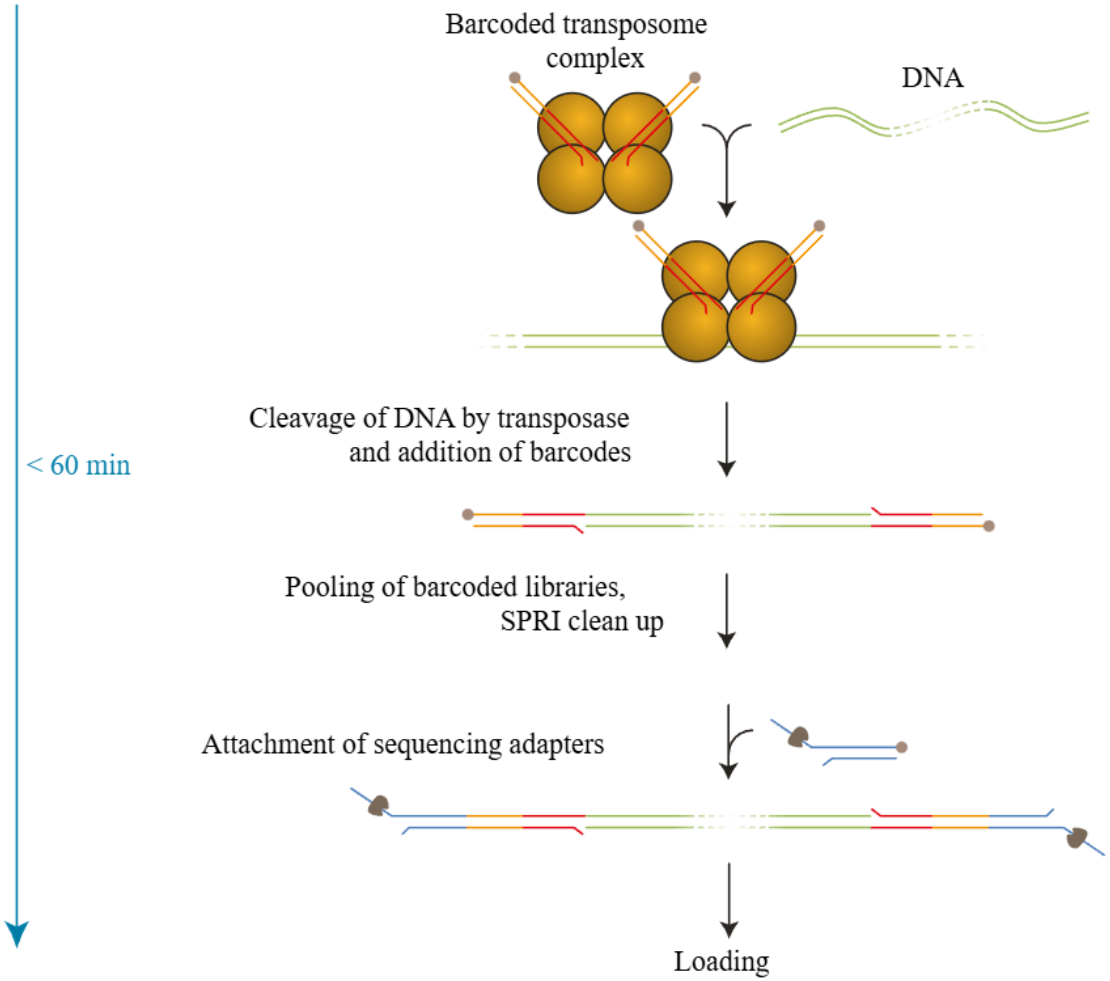
Set-up your own experiment: SARS-CoV-2 WGS

Workflow



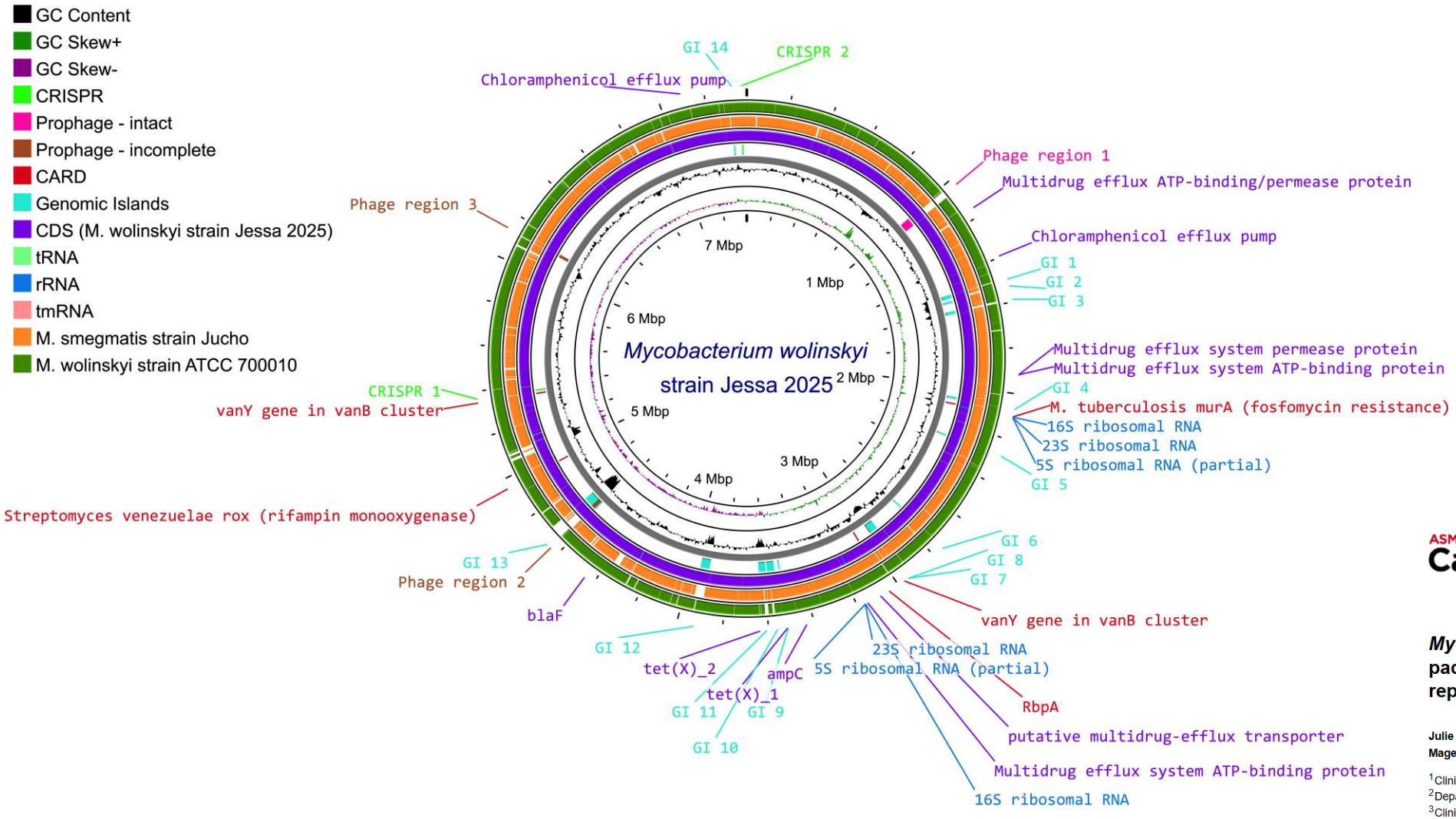


Set-up your own experiment: rapid barcoding



Library preparation step	Process	Time	Stop option
DNA barcoding	Tagmentation of the DNA using the Rapid Barcoding Kit V14.	15 minutes	4°C overnight
Sample pooling and clean-up	Pooling of barcoded libraries and AMPure XP Bead clean-up.	25 minutes	4°C overnight
Rapid adapter attachment	Attach the sequencing adapters to the DNA ends.	5 minutes	We strongly recommend sequencing your library as soon as it is adapted.
Priming and loading the flow cell	Prime the flow cell and load the prepared library for sequencing.	10 minutes	

Set-up your own experiment: rapid barcoding: bacterial WGS



- 1 contig (ca. 7.4 Mbp)
- No plasmids

ASM Case Reports
CLINICAL MICROBIOLOGY CASE REPORTS
January 2026 Volume 2 Issue 1 e00146-25
https://doi.org/10.1128/asmcr.00146-25

***Mycobacterium wolinskyi* as an emerging cause of pacemaker pocket infection and lead endocarditis: a case report and genomic characterization**

Julie Dom [1], Reinoud Cartuyvels [1], Anne Bruggemans [2], Timo Froyen [3], Petra Hilkens [1], Koen Magerman [1,4], Steven Martens [1], Britta Van Meensel [1], Jozef Dingemans [3]

[1]Clinical laboratory of Microbiology, Jessa Hospital, Hasselt, Belgium
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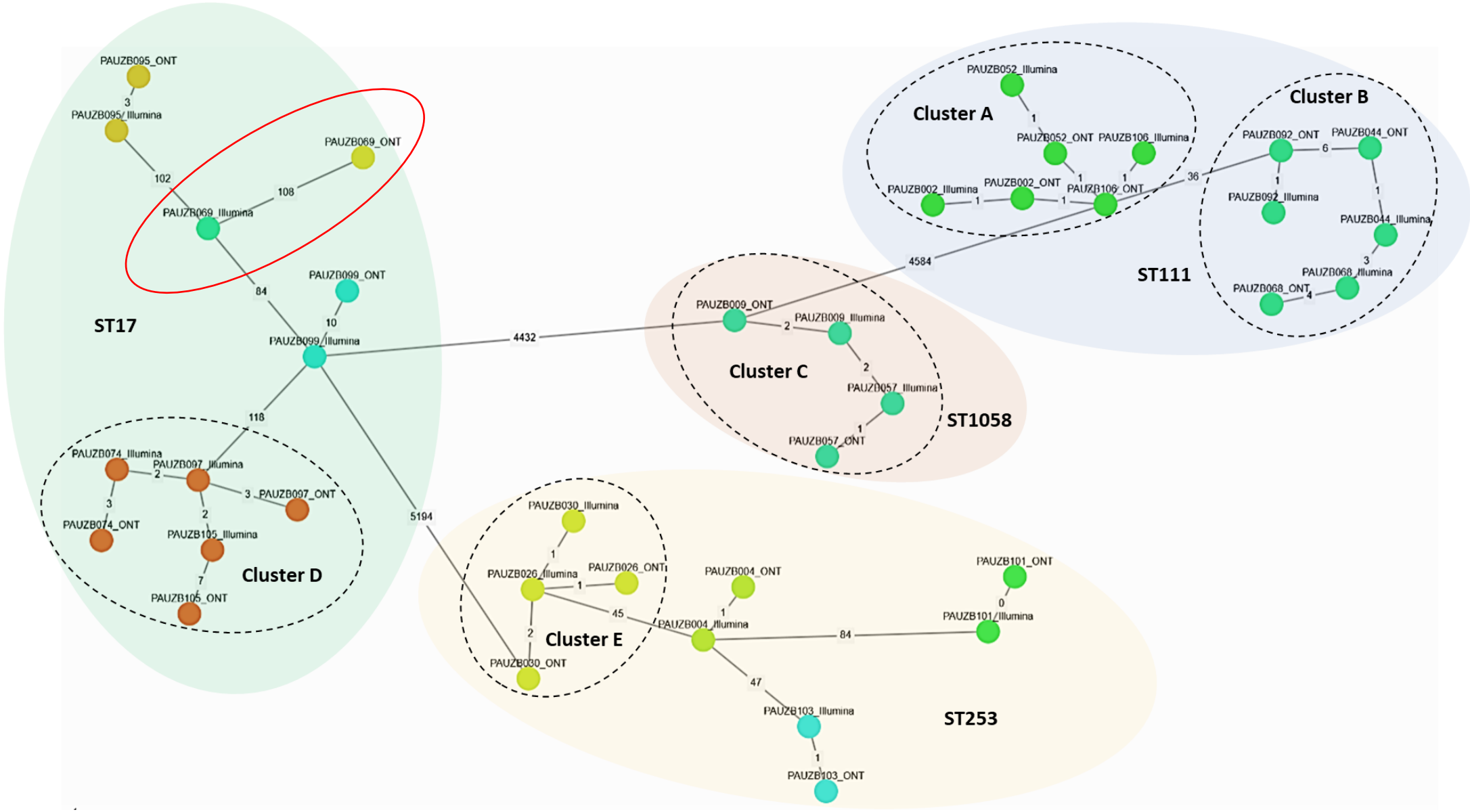
[3]Clinical laboratory of Molecular Microbiology, Jessa Hospital, Hasselt, Belgium
[4]Department of Immunology and Infection, Hasselt University, Hasselt, Belgium

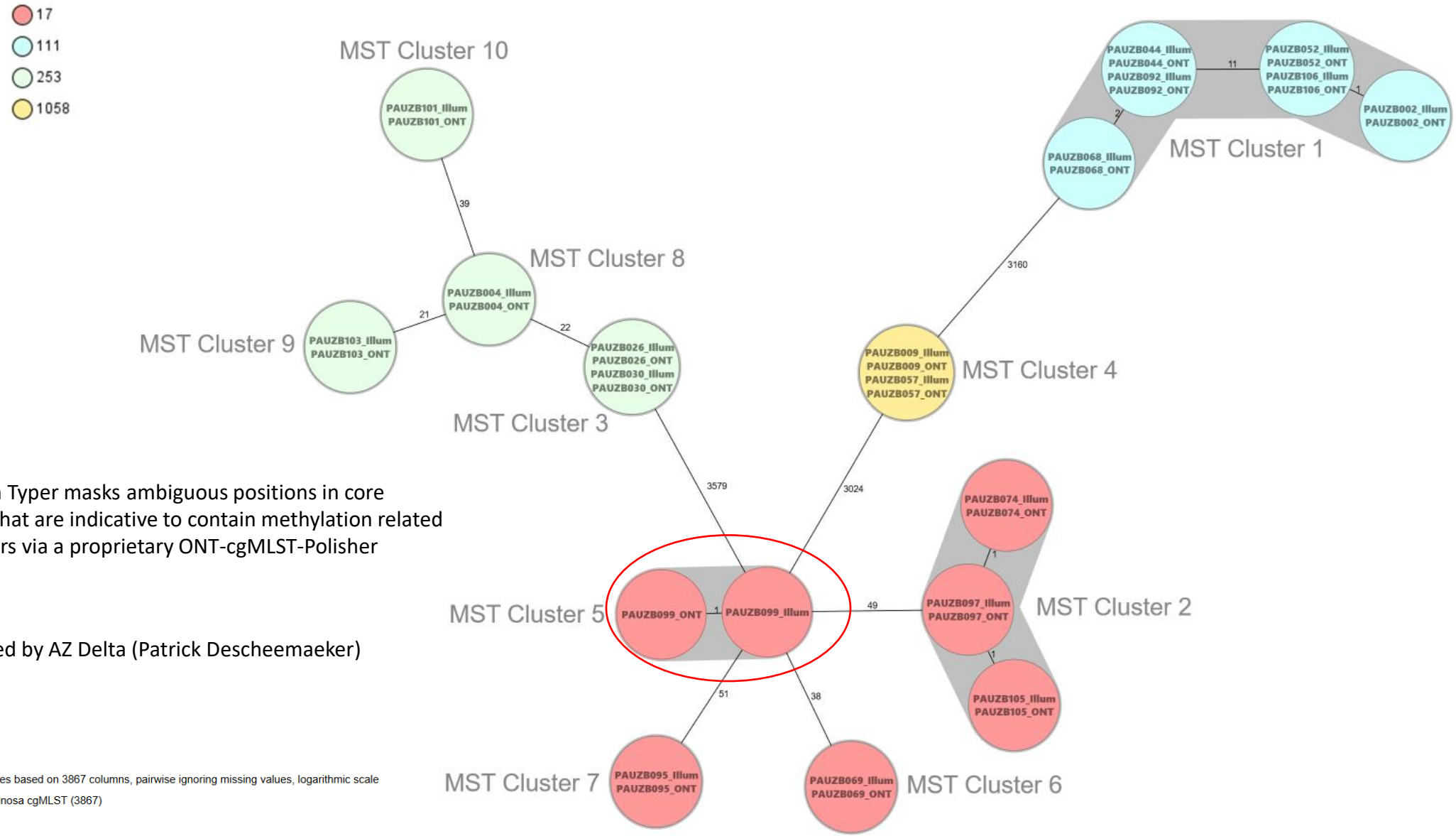
Bacterial WGS: ONT vs Illumina via BugSeq



Potential effect of methylation on ONT basecalling

Regions with heterozygous positions

Results: ONT vs Illumina via MBioSEQ Ridom Typer



MBioSEQ Ridom Typer masks ambiguous positions in core genome genes that are indicative to contain methylation related sequencing errors via a proprietary ONT-cgMLST-Polisher

Results confirmed by AZ Delta (Patrick Descheemaeker)

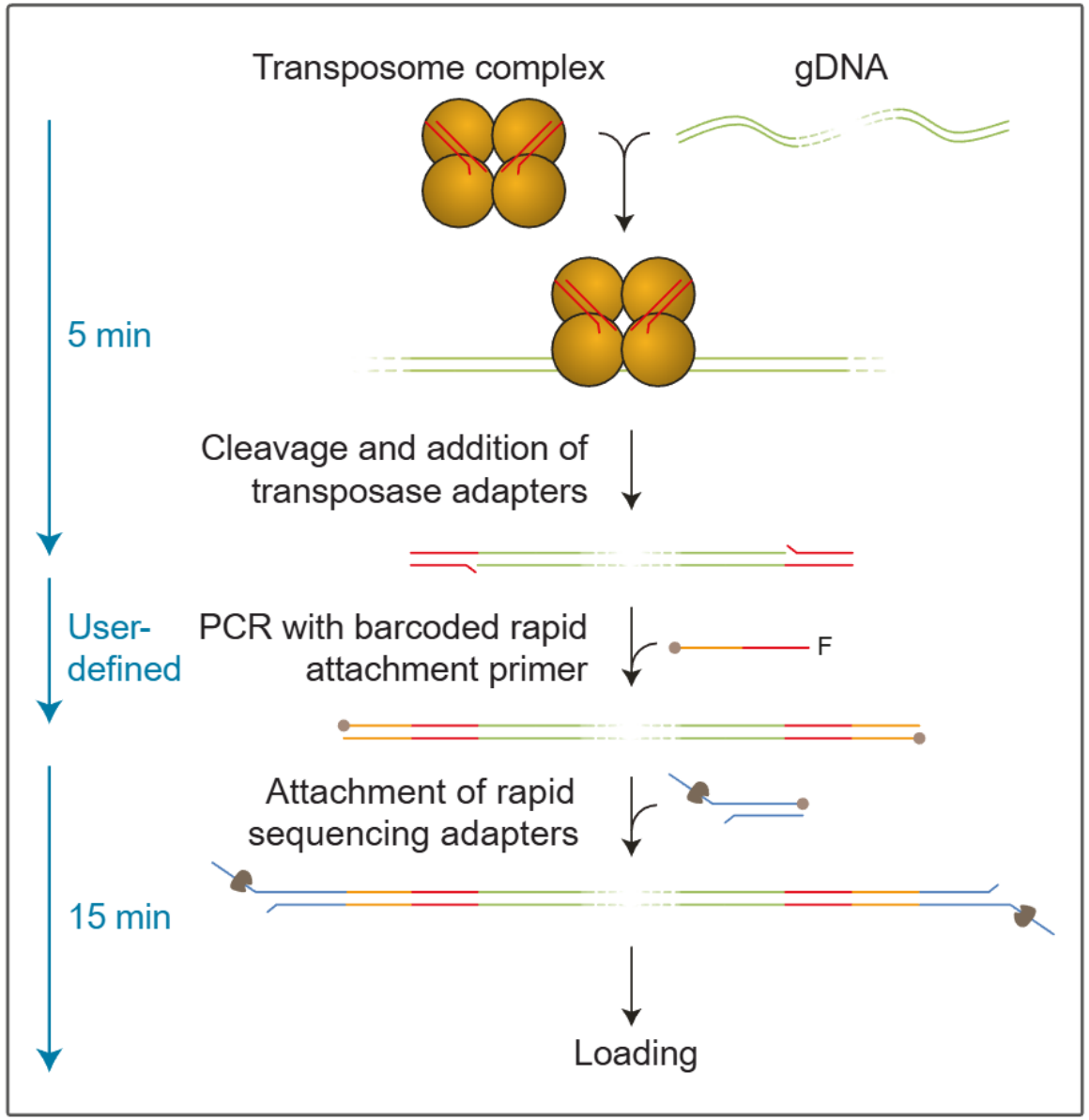
MBioSEQ Ridom Typer MST for 38 Samples based on 3867 columns, pairwise ignoring missing values, logarithmic scale

Distance based on columns from P. aeruginosa cgMLST (3867)

MST Cluster distance threshold: 12

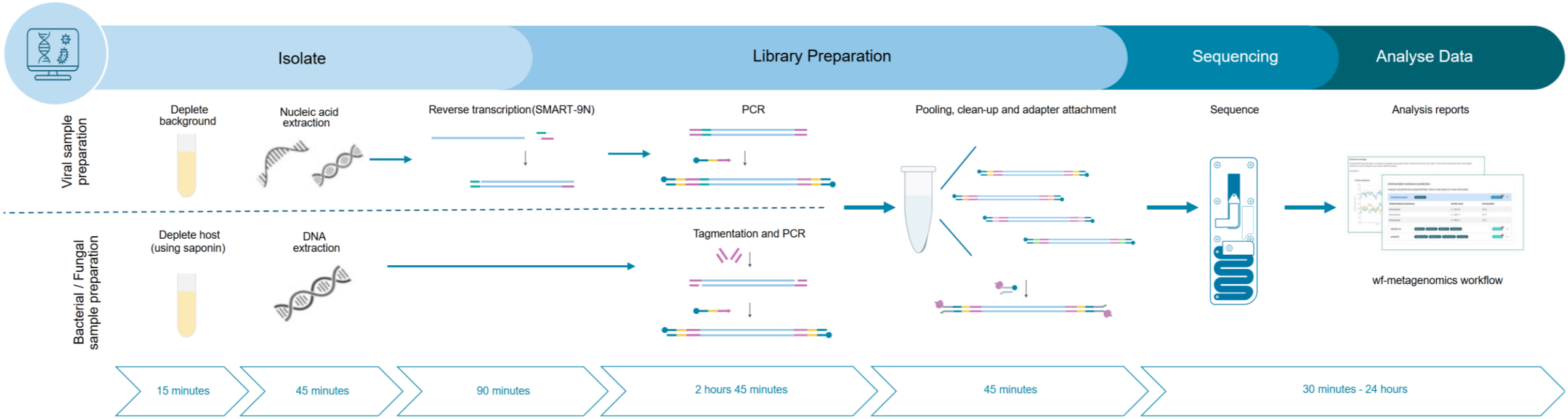
Nodes colored by column: ST

Set-up your own experiment: rapid PCR barcoding



Set-up your own experiment: rapid PCR barcoding

Shotgun metagenomics



Set-up your own experiment: rapid PCR barcoding

Shotgun metagenomics

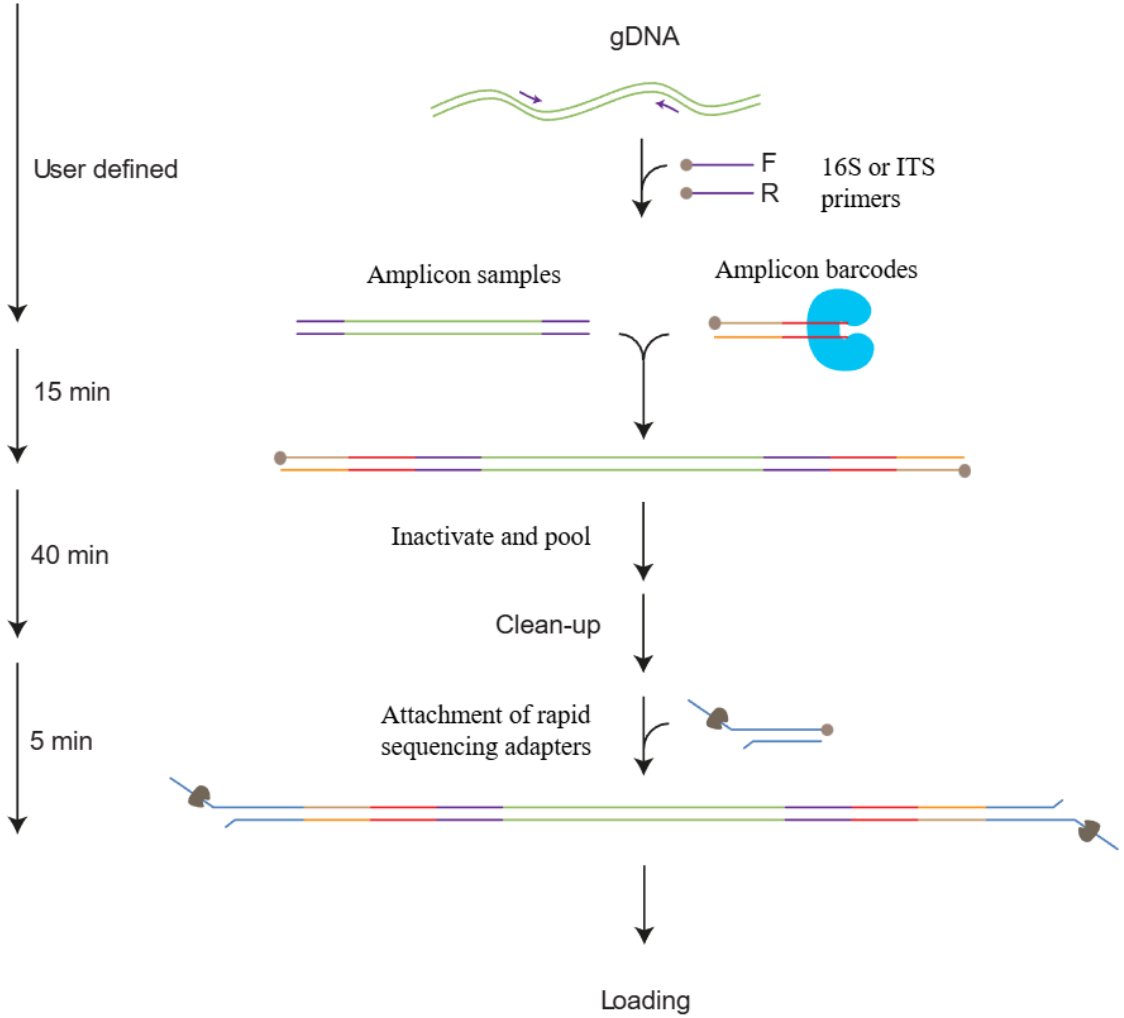
Table 1 – Impact of human DNA background on shotgun sequencing detection of *H. pylori*: comparison with 16S rRNA sequencing (EPI2ME analysis).

SampleID	11-2505-5305	11-2505-6807	11-2505-6809	11-2505-6810	11-2505-6917	11-2505-7030	11-2505-7031	11-2505-7032	11-2505-7033
16S rRNA sequencing									
H. pylori	9955	5	3	1	10051	3	0	1	4
T. radiovictrix	164	9246	1855	4702	185	6438	3184	12313	25857
I. halotolerans	47	1773	543	959	38	981	678	2947	7431
A. halotolerans	0	11	2	16	0	11	13	24	41
H. sapiens	0	0	0	0	0	0	0	0	0
Shotgun metagenomic sequencing									
H. pylori	29	0	0	0	112	0	0	0	0
T. radiovictrix	12	12	12	12	0	15	36	15	14
I. halotolerans	85	50	51	66	61	64	143	103	58
A. halotolerans	0	0	0	0	0	0	0	0	0
H. sapiens	478607	2490979	293288	254055	215115	315894	286485	221932	229219

H. pylori = *Helicobacter pylori*; *T. radiovictrix* = *Truepera radiovictrix*; *I. halotolerans* = *Imtechella halotolerans*; *A. halotolerans* = *Allobacillus halorolerans*; *H. sapiens* = *Homo sapiens*.

Set-up your own experiment: 16S/ITS sequencing

Microbial Amplicon Barcoding kit (SQK-MAB114.24) => also custom primers!



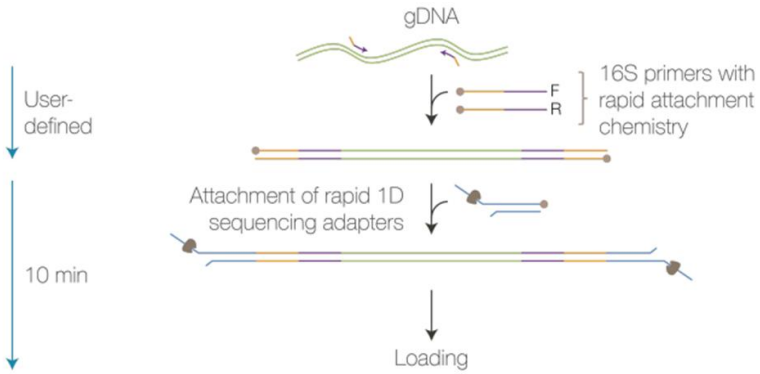
Library preparation step	Process	Time	Stop option
16S or ITS PCR amplification	Amplify the 16S or ITS gene using the primers supplied in the kit .	10 minutes + PCR	4°C overnight
Amplicon barcoding	Attach barcodes to up to 24 amplicon samples.	15 minutes	
Barcode inactivation, sample pooling and bead clean-up	Inactivate the barcoding reaction, pool your barcoded samples and perform a sample clean-up.	40 minutes	4°C short-term storage or for repeated use, such as re-loading your flow cell. -80°C for single-use long-term storage.
Rapid adapter attachment	Attach the rapid sequencing adapters to the DNA ends.	5 minutes	We strongly recommend sequencing your library as soon as it is adapted.
Priming and loading the flow cell	Prime the flow cell and load the prepared DNA library for sequencing.	10 minutes	

Set-up your own experiment: 16S sequencing

16S NGS on clinical samples

Sample spiking (ZymoBIOMICS Spike-in Control II (Low Microbial Load) & ZymoBIOMICS DNA Miniprep Kit)

16S library prep & sequencing (Oxford Nanopore)



<7 hours

Data analysis (EPI2ME 16S workflow (ONT) vs Species ID 16S tool 1928 Diagnostics)

Set-up your own experiment: 16S sequencing

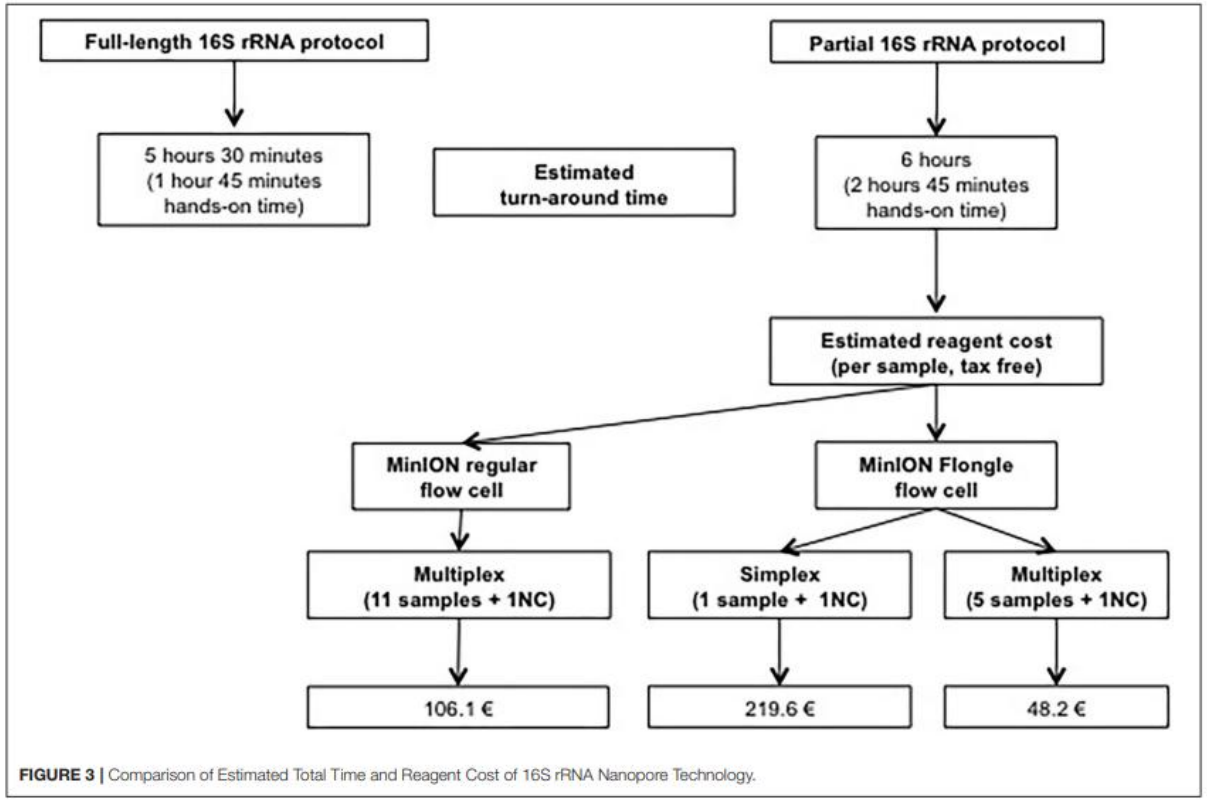


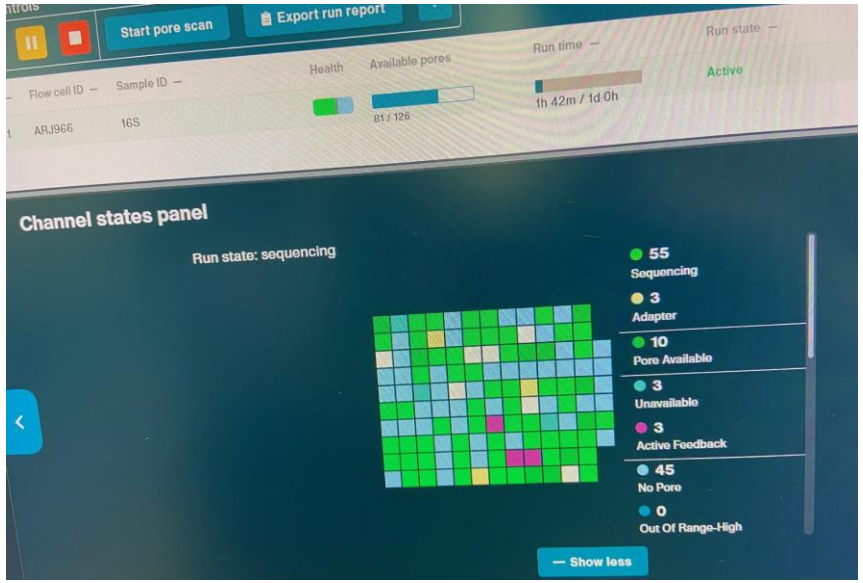
FIGURE 3 | Comparison of Estimated Total Time and Reagent Cost of 16S rRNA Nanopore Technology.

Ref: Bouchiat *et al*. Improving the Diagnosis of Bacterial Infections: Evaluation of 16S rRNA Nanopore Metagenomics in Culture-Negative Samples. Front Microbiol. 2022 Jul 14;13:943441. doi: 10.3389/fmicb.2022.943441.

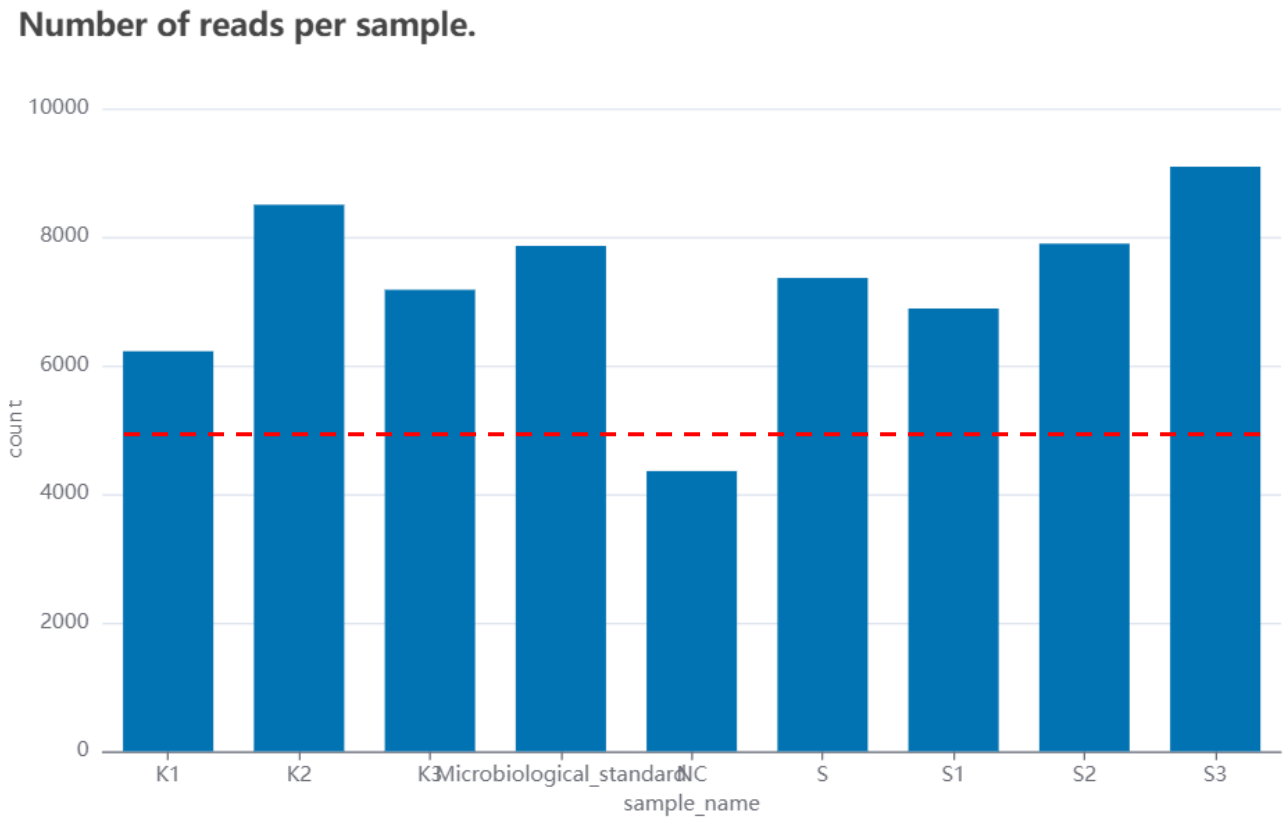
	No barcodes	12 barcodes	24 barcodes	24 barcodes (Flongle)
Flow cell price	$500	$500	$500	$90 (Flongle flow cell)
Library price	$90	$120	$150	$150
Price per sample	**$590**	**$51.67**	**$27.08**	**$10**

Set-up your own experiment: 16S sequencing

Flongle flow cell



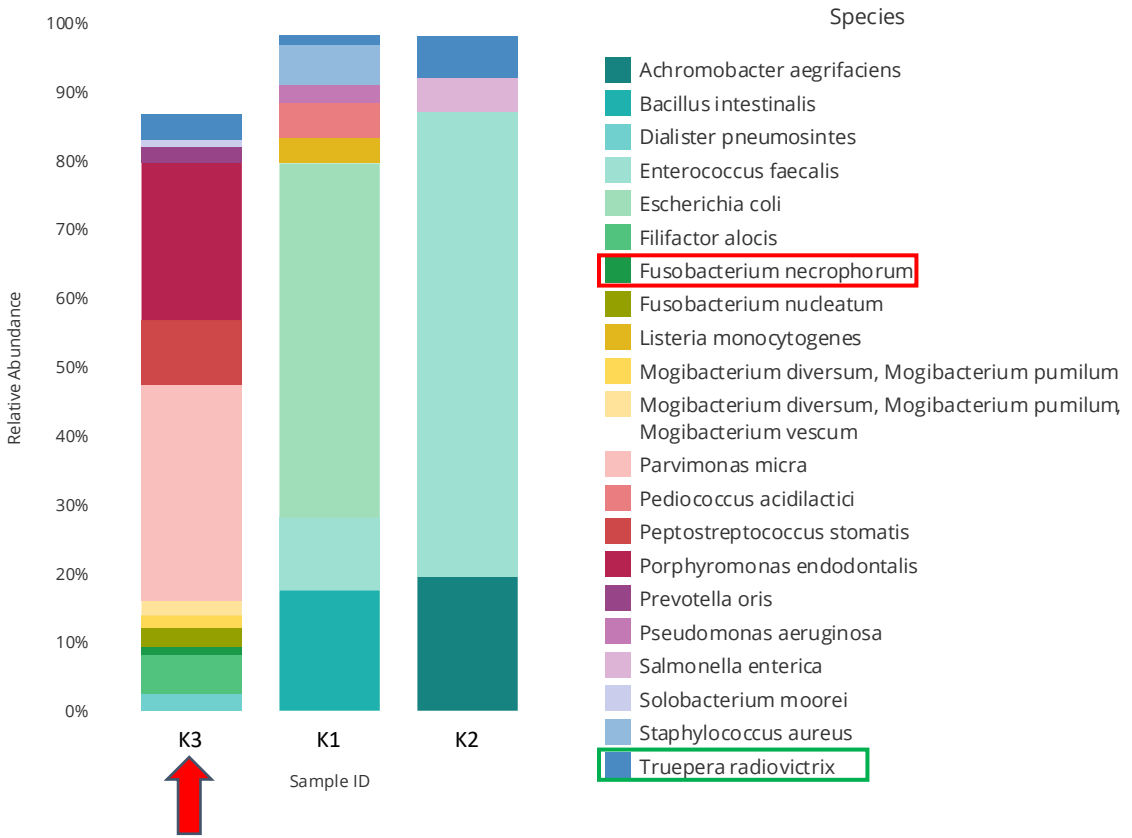
Read counts after ~4 hours of sequencing



Set-up your own experiment: 16S sequencing

16S sequencing - clinical samples			
Sample	**Sample type**	**Isolate 1**	**Isolate 2**
K1	Varia aspiraat	*Moraxella lacunata*	/
K2	Punctievocht peritoneum/ascites	*Enterococcus faecalis*	*Achromobacter insolitus*
K3	Dentaal abces	*Fusobacterium necrophorum*	*Eggerthella cathenaformis*
16S sequencing - strains			
Sample	**Sample type**	**Isolate**	**Remarks**
S1	Anaerobe hemocultuur	*Alistipes shahii*	16S Sanger failed
S2	Anaerobe hemocultuur	Anaerobe stam	/
S3	Anaerobe hemocultuur	*Parvimonas micra*	16S Sanger failed

Species	Abundance Est.	
Parvimonas micra	31.5%	
Porphyromonas endodontalis	22.9%	
Peptostreptococcus stomatis	9.4%	
Unclassified	7.7%	
Filifactor alocis	5.7%	
Truepera radiovictrix	3.8%	Spike-in control
Fusobacterium nucleatum	2.7%	
Dialister pneumosintes	2.5%	
Prevotella oris	2.3%	
Mogibacterium diversum, Mogibacterium pumilum, Mogibacterium vescum	2.1%	
Mogibacterium diversum, Mogibacterium pumilum	1.8%	
Fusobacterium necrophorum	1.2%	
Solobacterium moorei	1.1%	
Prevotella nigrescens	0.9%	
Imtechella halotolerans	0.8%	Spike-in control
Salmonella enterica	0.7%	
Slackia exigua	0.7%	
Allobacillus halotolerans	0.6%	Spike-in control
Oribacterium sinus	0.5%	
Eggerthia catenaformis	0.2%	
Dialister invisus	0.2%	



Acknowledgements

- Thank you for your attention!
- Thanks to all laboratory staff and collaborators @ Jessa!
- Thanks to the organization for the invitation!

Questions?