

Sample preparation for Oxford Nanopore sequencing

AfricaBP
4th December 2023

Introduction



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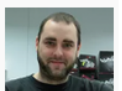
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Overview



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- Intro nanopore sequencing
- Sample preparation: a technical overview
 - gDNA
 - amplicon
 - multiplexing
- Sequencing results and applications
 - Nanopore VS Sanger
 - Metabarcoding

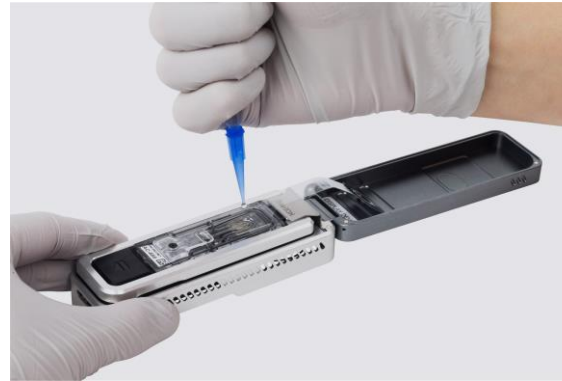
How does it work?

Nanopore Sequencing



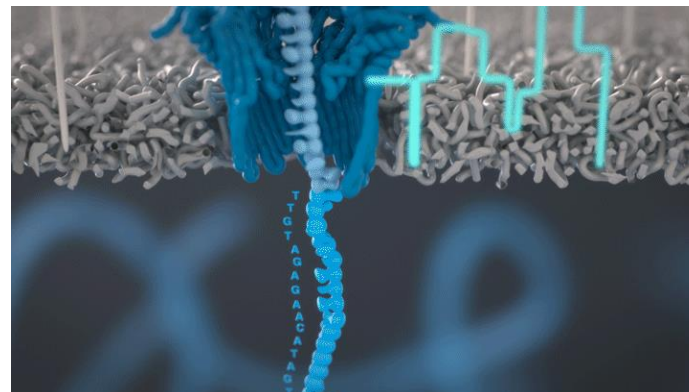
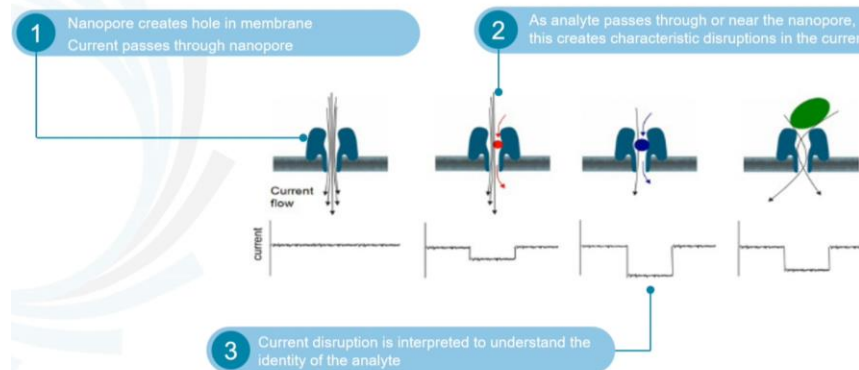
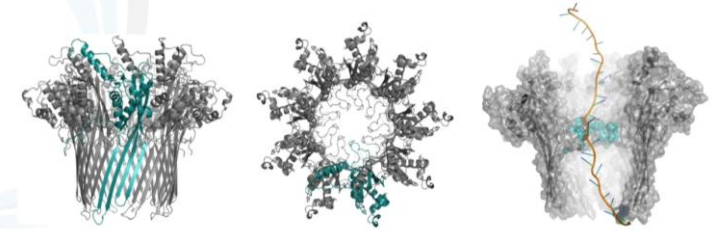
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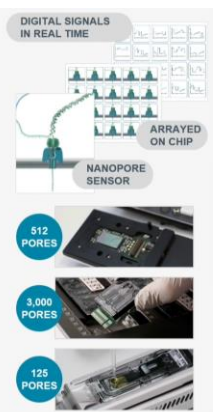
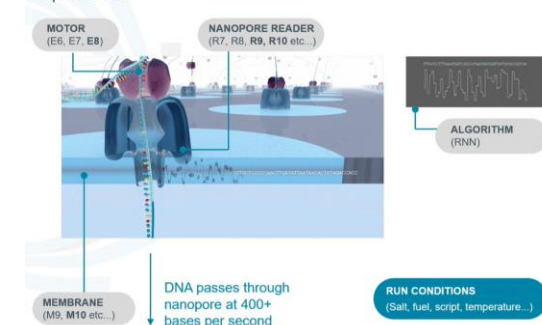
The heart of our technology

- In nature, protein nanopores function as gateways between two systems.
- We have carefully engineered protein nanopores through mutating key residues in the barrel of the pore.



Nanopore DNA/RNA sequencing: a novel, scalable method

DNA/RNA strand is passed through nanopore → signal interpreted into sequence data



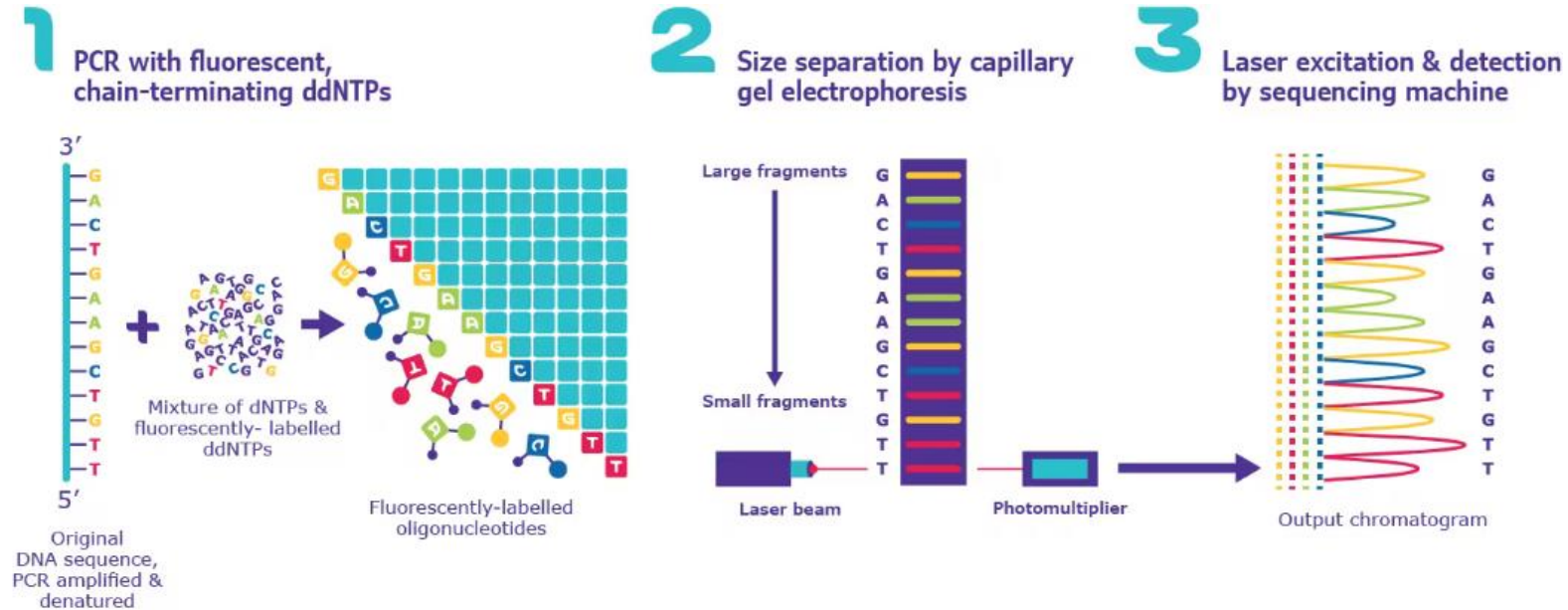
How does it
work?

Nanopore Sequencing VS Sanger

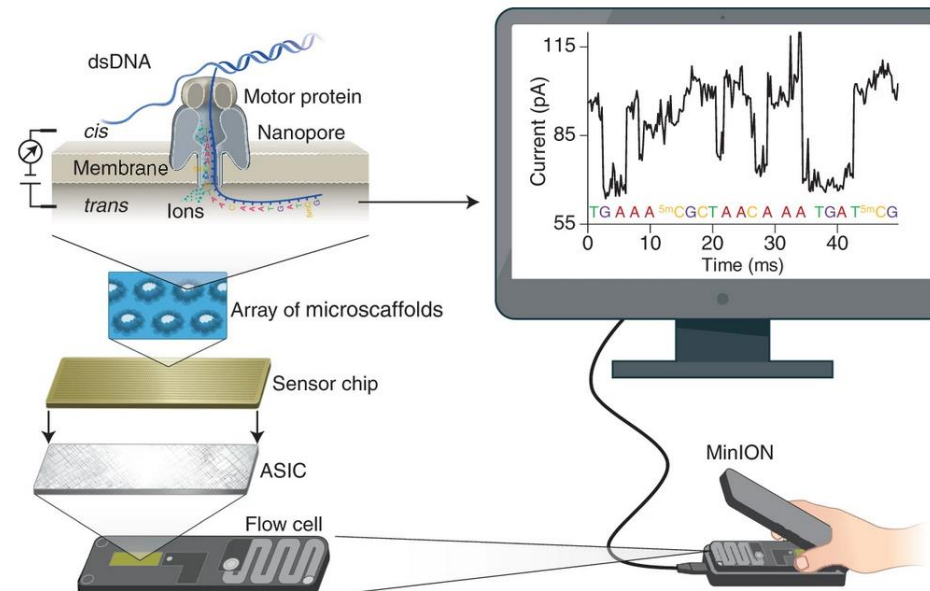


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From: [Nanopore sequencing technology, bioinformatics and applications](#)



How does it
work?






Nanopore Sequencing



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Highly scalable

			
MinION Mk1C	MinION Mk1B	GridION X5	PromethION P24 P48
Available to pre order	Commercially available	Commercially available	Commercially available
Complete sequencing, analysis and viewing device	Portable, USB powered biological analysis	Five flow cells and integrated computing	High-throughput, versatile benchtop system (P24 or P48)
512* channels For up to 30Gb/flow cell	512* channels For up to 30 Gb/flow cell	5 x 512=2,560* channels for up to 150Gb/device	P24: 24 x 3,000* channels for >3.5Tb
Flongle Adapter for MinION/GridION supports smaller single-use flow cells 128 channels, up to 1.8Gb now, towards 3Gb			

*up to this number of nanopore channels may be available for sequencing at any time

Commercial model: Place instruments (no CapEx requirement) → Sell consumables, services (Flow cells, kits)

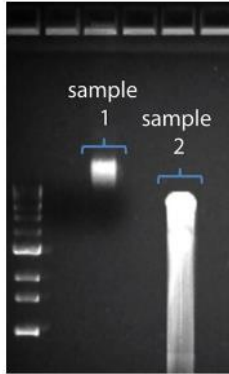
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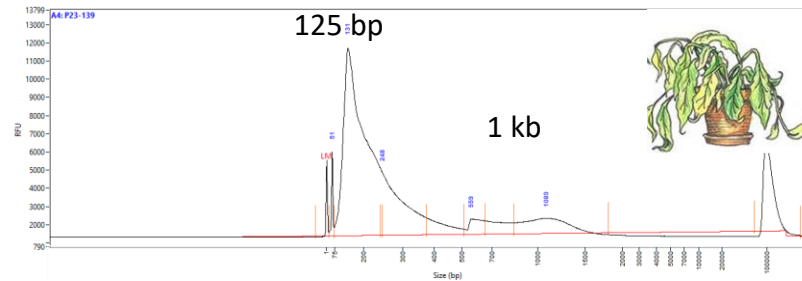


Sample preparation - DNA QC

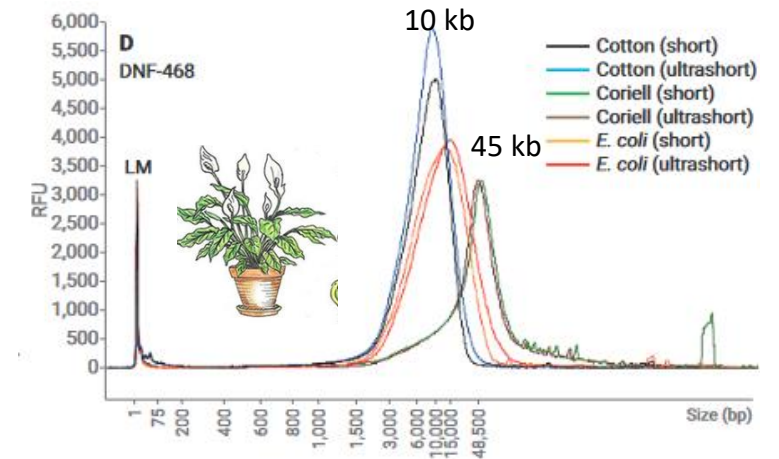


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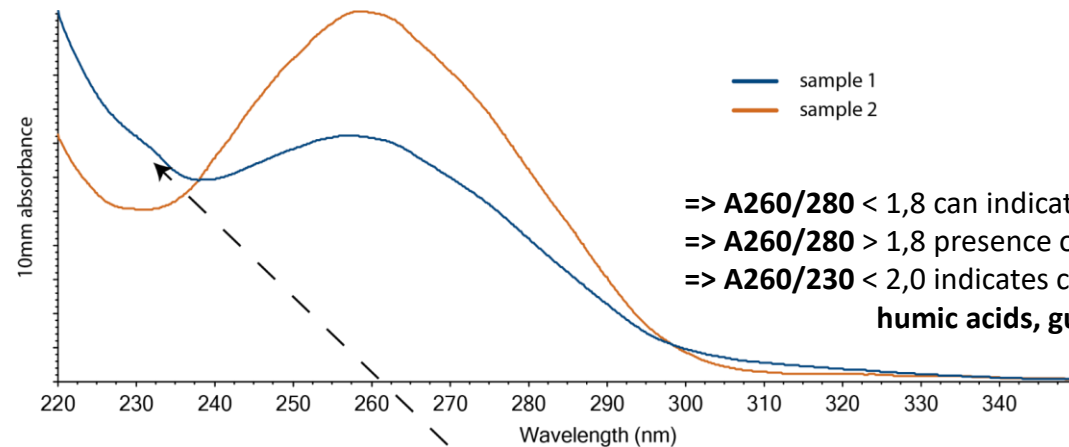
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Size and Concentration - Fluorescence & Electrophoresis



Contaminants - Nanodrop



=> $A_{260}/A_{280} < 1,8$ can indicate protein or phenol
=> $A_{260}/A_{280} > 1,8$ presence of RNA
=> $A_{260}/A_{230} < 2,0$ indicates contamination with salt, humic acids, guanidine, peptides or other chemicals.

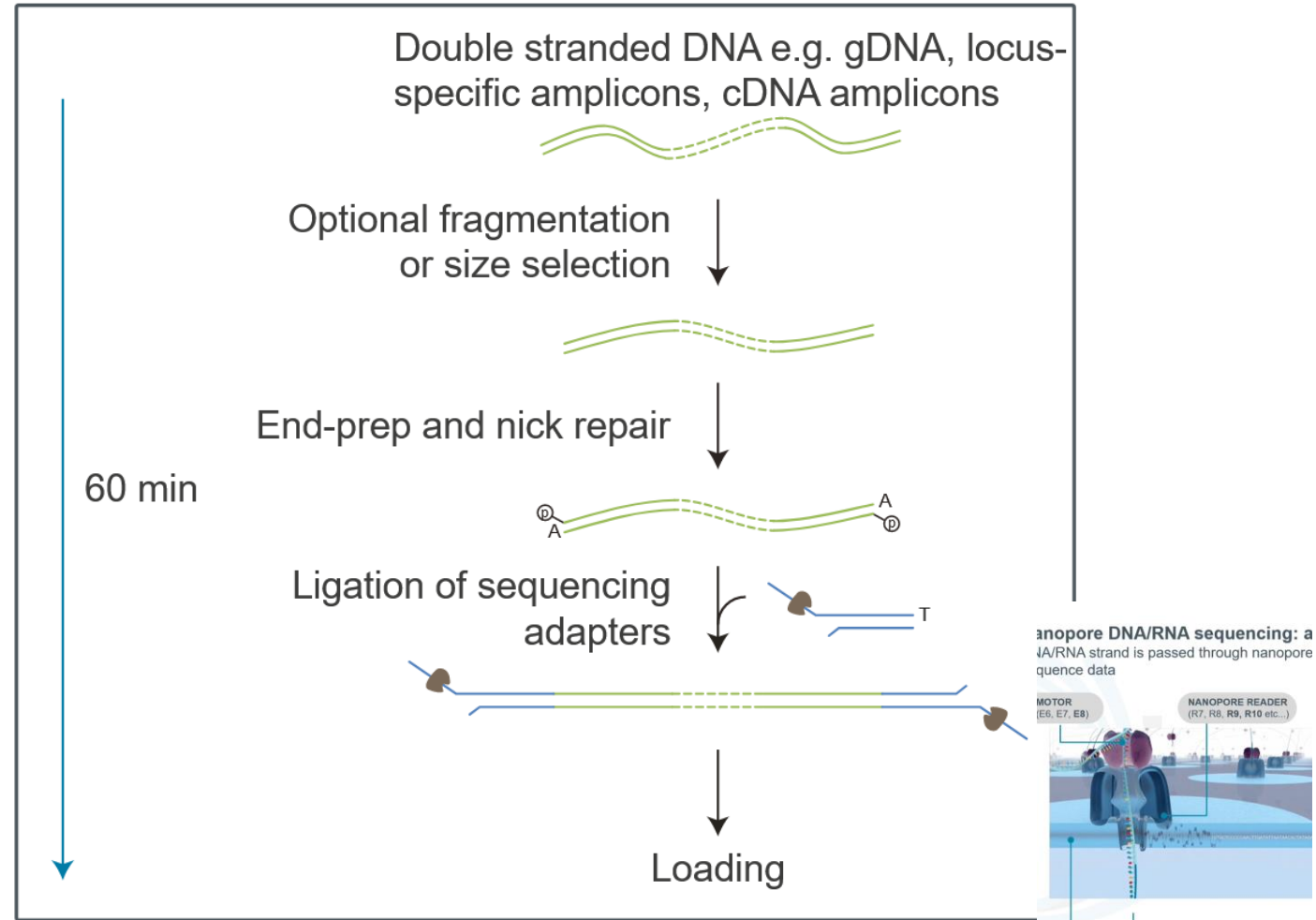
Sample ID	Nucleic Acid Conc.	Unit	A260	A280	260/280	260/230	Sample Type	Factor
sample 1	207.5	ng/μl	4.150	2.410	1.72	0.99	DNA	50.00
sample 2	1080.1	ng/μl	21.601	12.369	1.75	2.17	DNA	50.00

Sample preparation – Nanopore libraryprep



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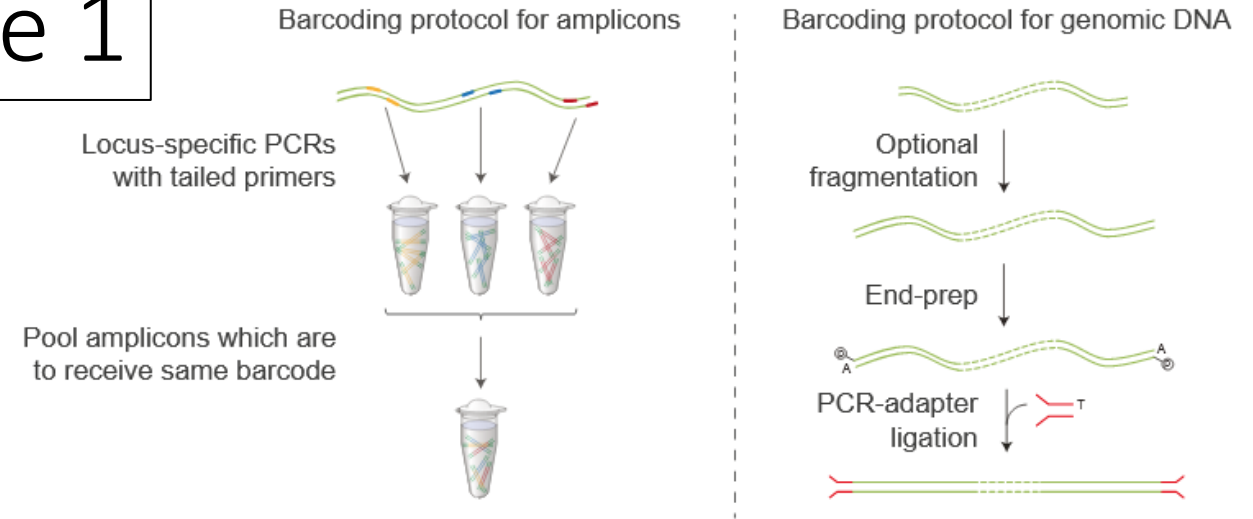
Sample preparation – Multiplexing



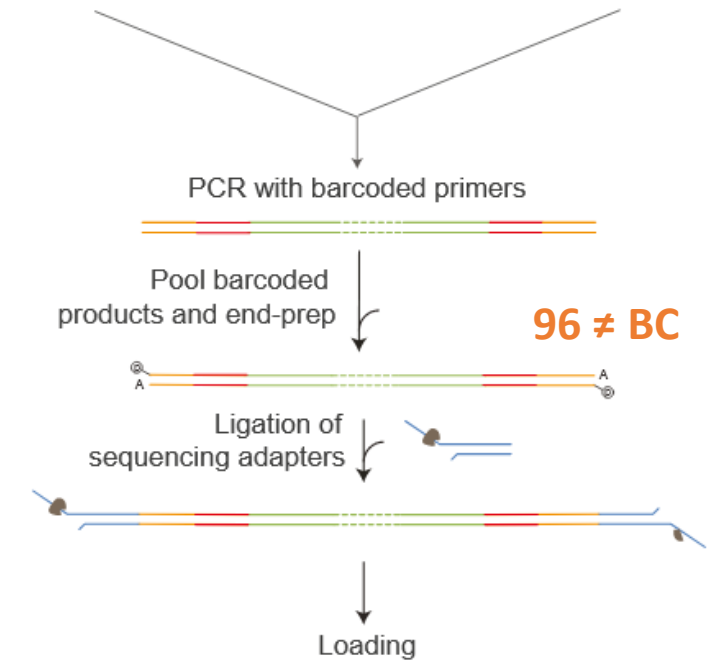
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Stage 1



Stage 2



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Applications and results – Nanopore VS SANGER



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ITP 2023-2026 - MYCOBLITZ IN BENIN: A LAB IN A BACKPACK
Capacity building VLIR project

Applications and results

-

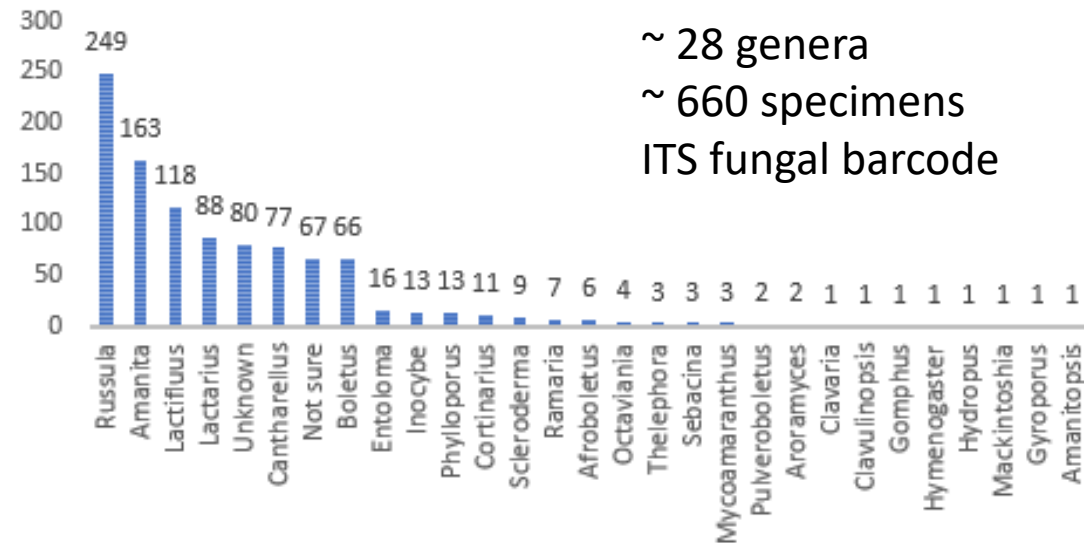
Nanopore VS SANGER



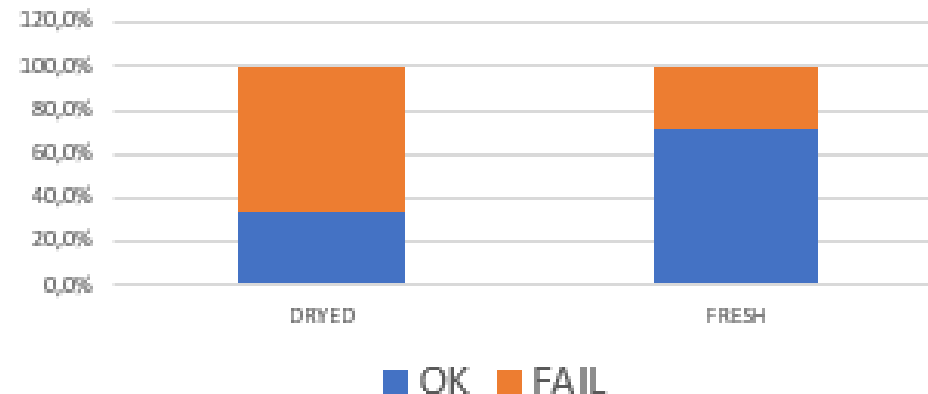
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#SPECIES VS GENERA



Amplification success rate (ITS1-4:650bp)



Applications and results

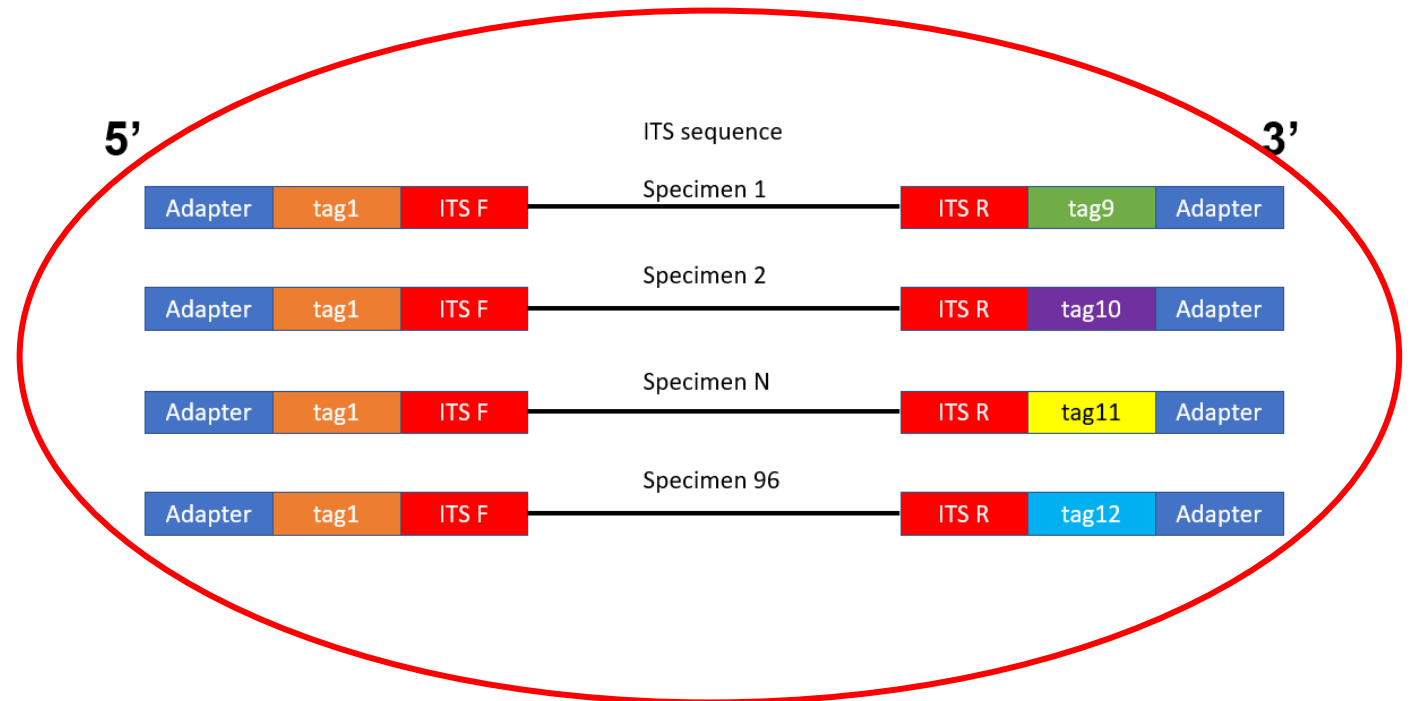
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Nanopore VS SANGER



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Applications and results

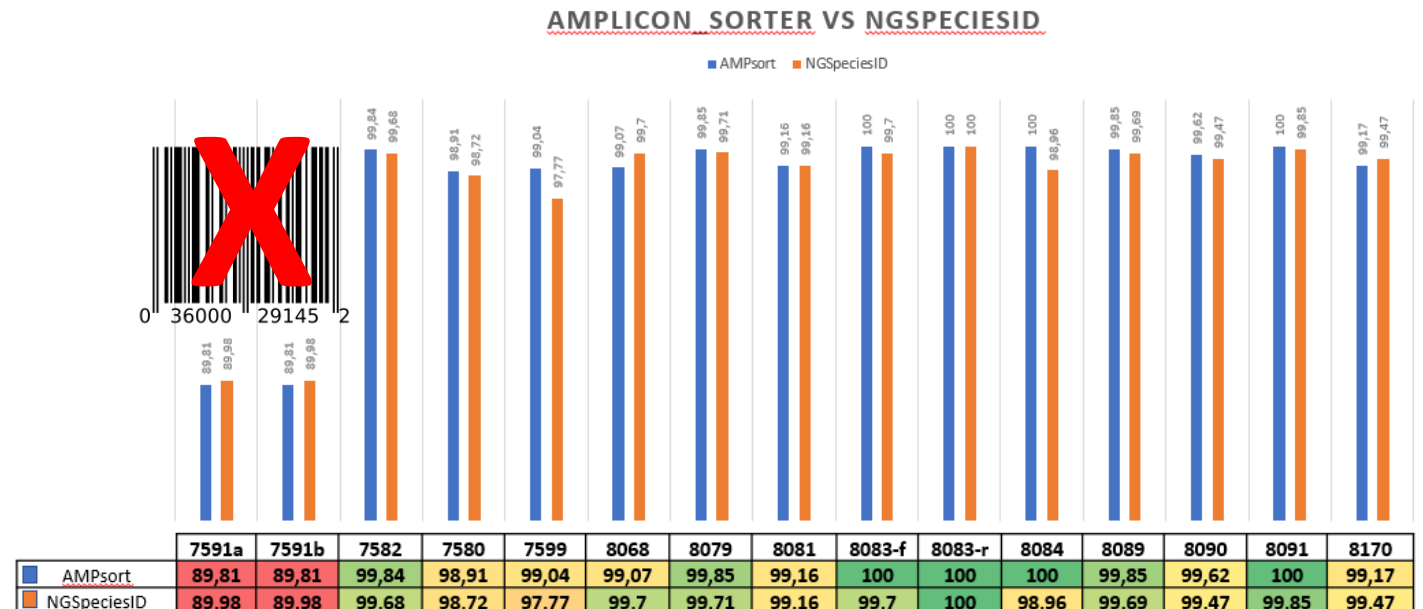
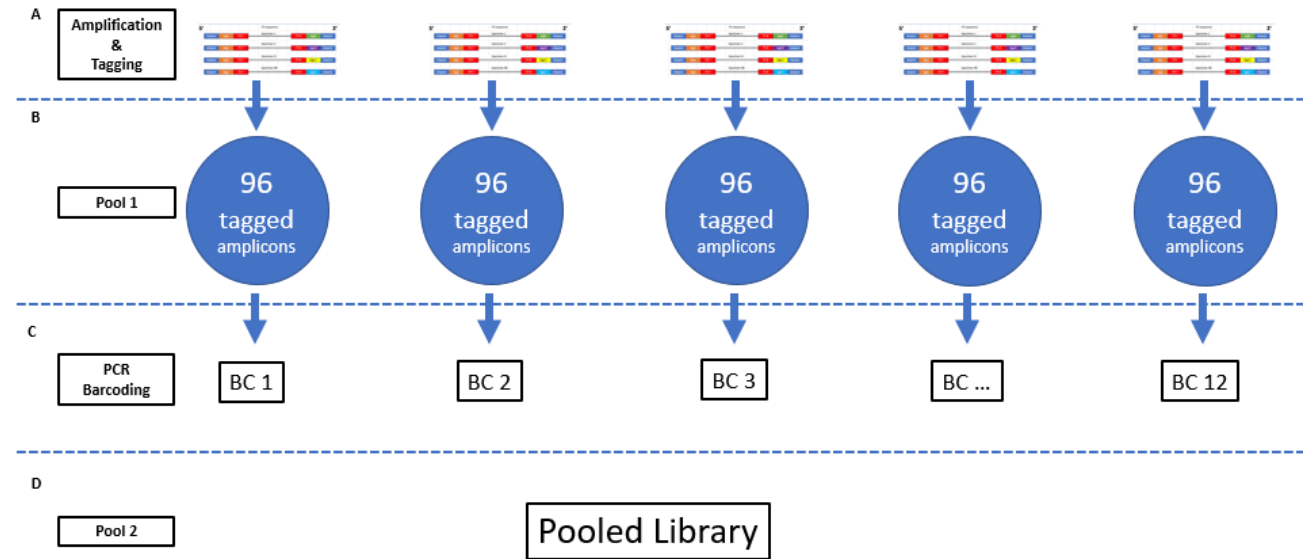
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Nanopore VS SANGER



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Applications and results – Fungal metabarcoding

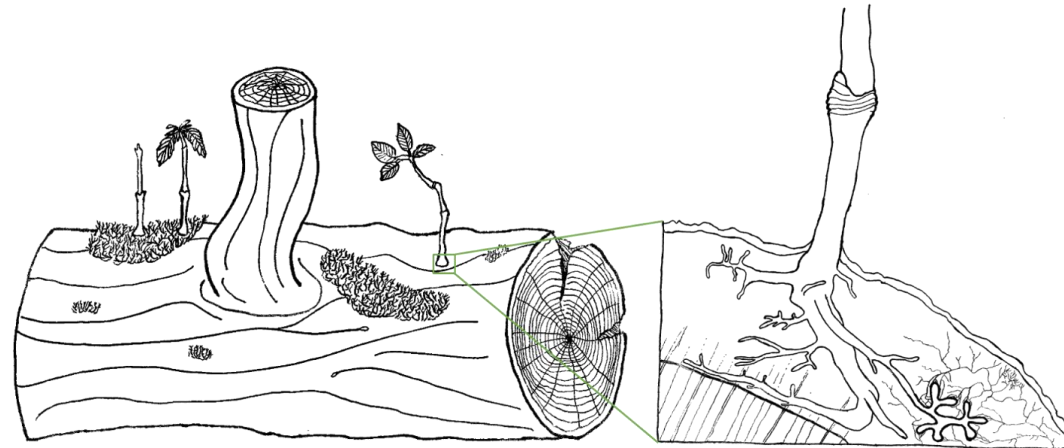


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Fagaceae
fagus sylvatica



Fungal metabarcoding using Nanopore sequencing: illuminating ectomycorrhizal diversity in *Fagus sylvatica* nurse logs (Glen Dierickx et.al., unpublished results)

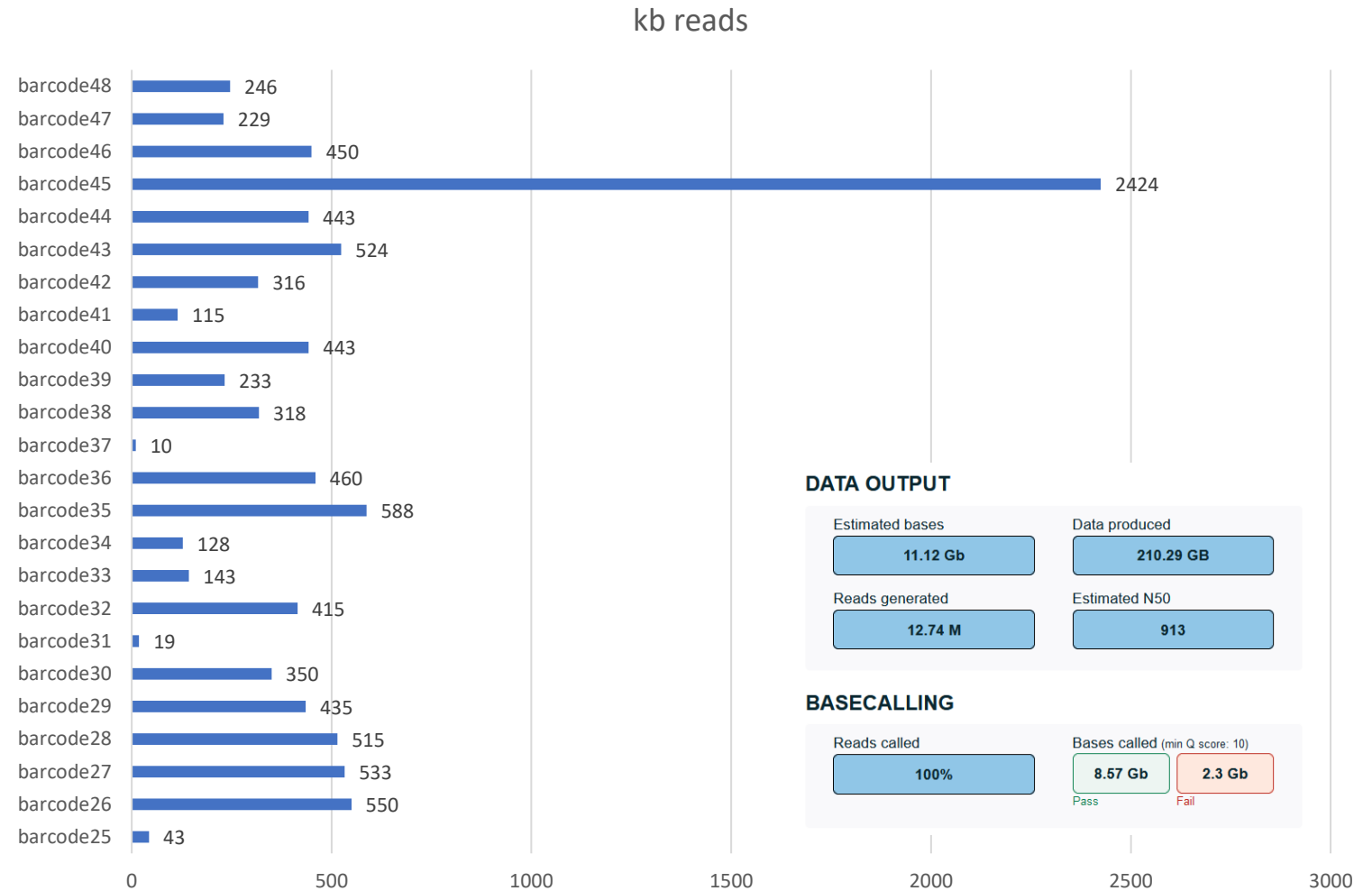
Applications and results

Fungal metabarcoding



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Applications and results - Fungal metabarcoding



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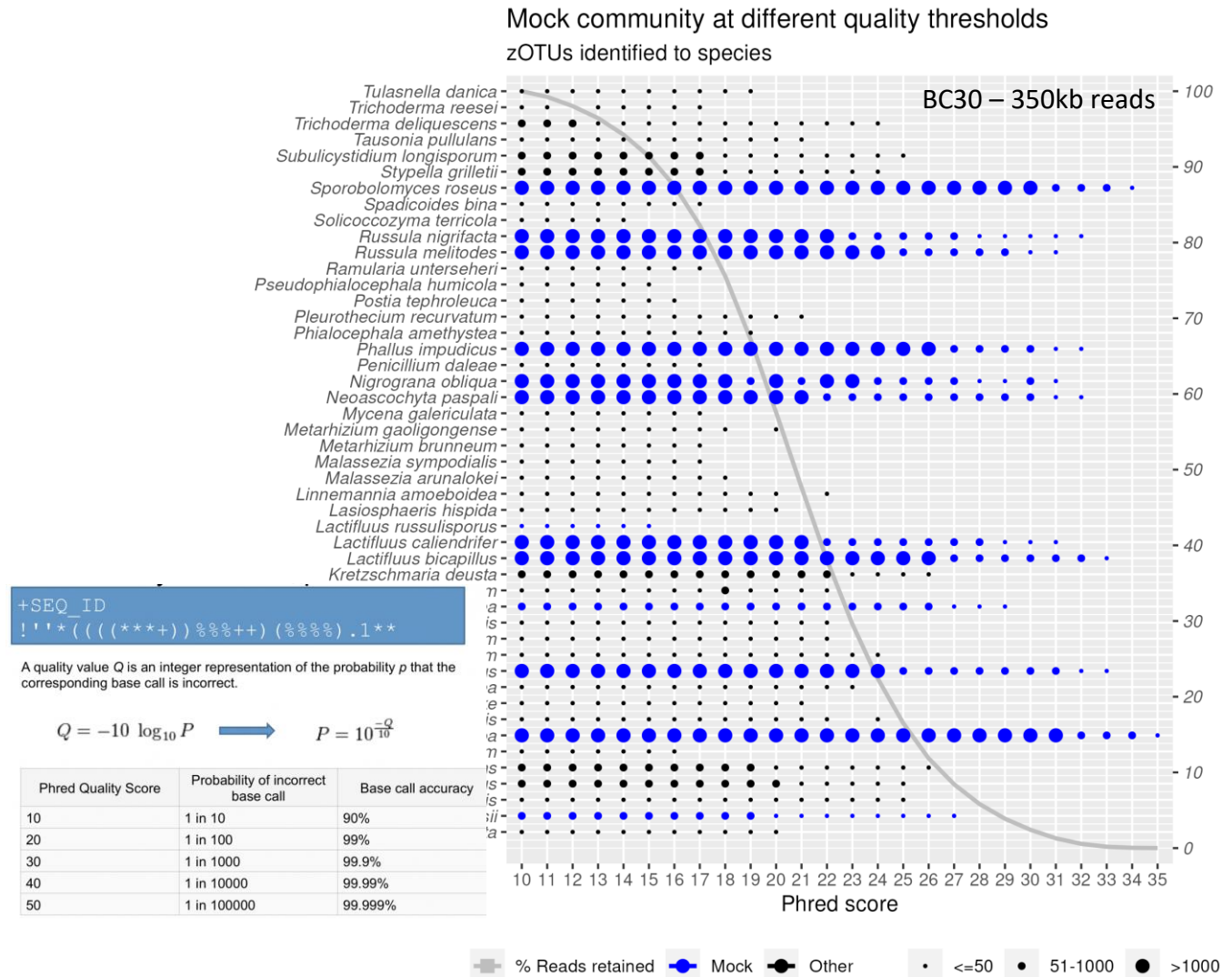


Fig 1: BC30 – Mock community -350kb reads
(Glen Dierickx et.al unpublished)

Thank you for
your attention !



<http://www.larschatrou.wixsite.com/mysite>



<http://www.mycology.be>



<https://github.com/orgs/MycoMatics/>



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