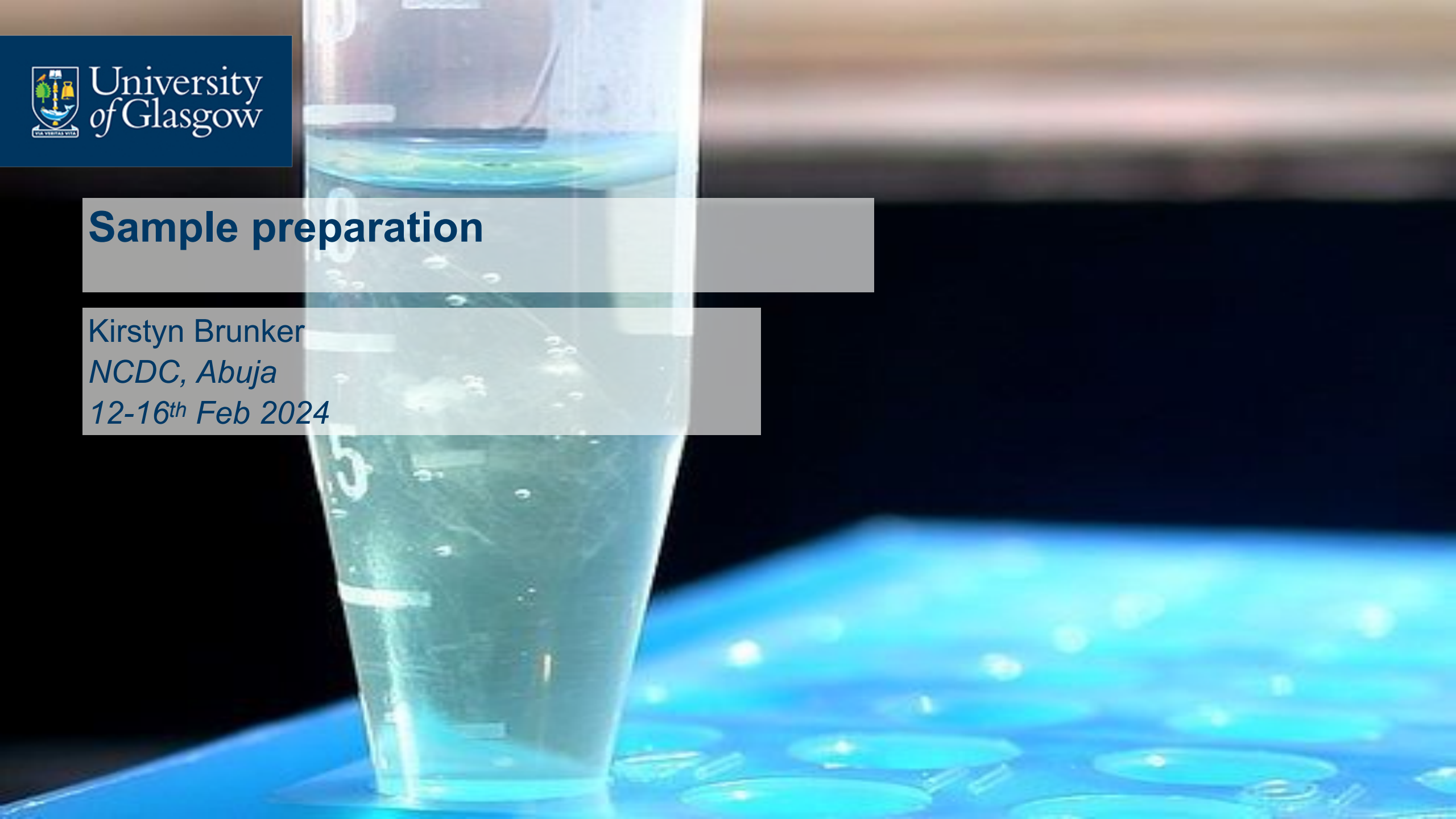


Sample preparation

Kirstyn Brunker
NCDC, Abuja
12-16th Feb 2024

University of Glasgow

Sample preparation

- Consider what you are doing at each step to optimize your desired sequence data output!

Collection and storage
- Preservation
- *Contamination*

RNA /DNA shield, RNAlater
Freeze/thaw

Make sample safe
- Biosafety
- *Contamination*

Extract nucleic acid
- Physical/chemical/ enzymatic disruption
- Quality check
- *Contamination*

Affects read length & concentration

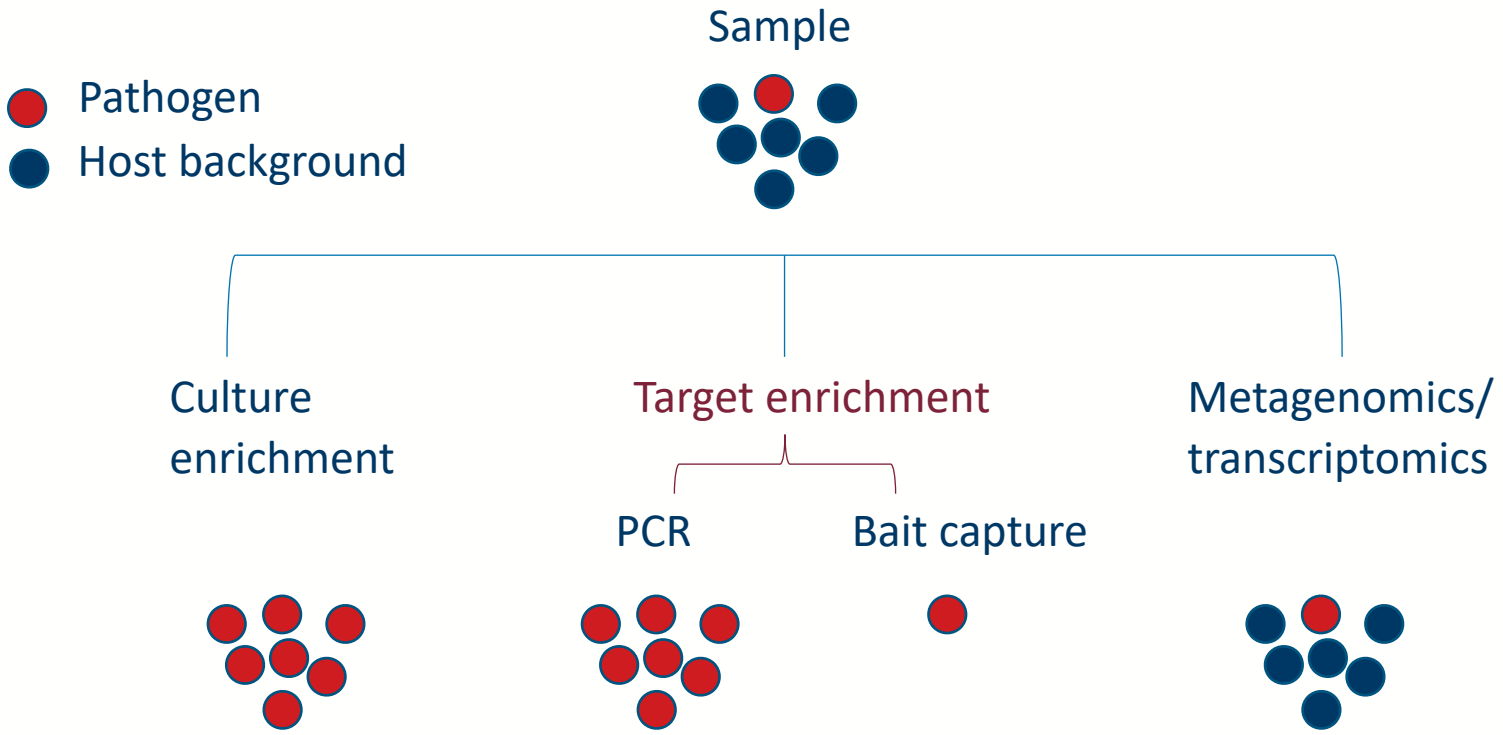
Enrich?
- Improve abundance/ sensitivity
- *Contamination*

Pros and cons of different methods

Make a sequence library
- Input material -type and amount
- *Contamination*

Determines library kit choice

Approaches



Considerations

- Time
- Cost
- Sensitivity
- Detection power
- Readiness/portability

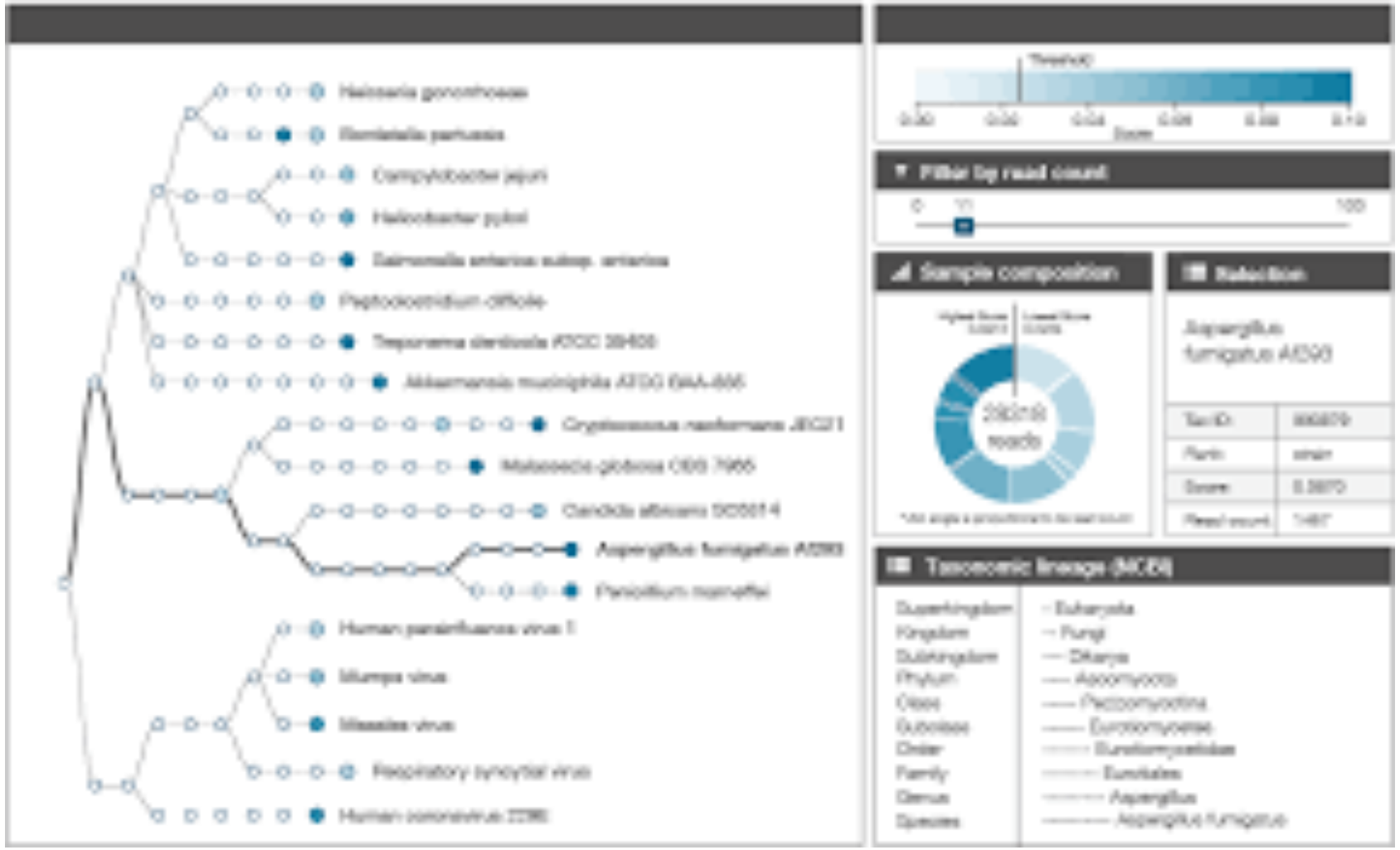
Metagenomics

Pros

- Gold standard
- Pathogen agnostic
- Unbiased

Cons

- *Lower sensitivity*
- *More expensive*
- *Complex*

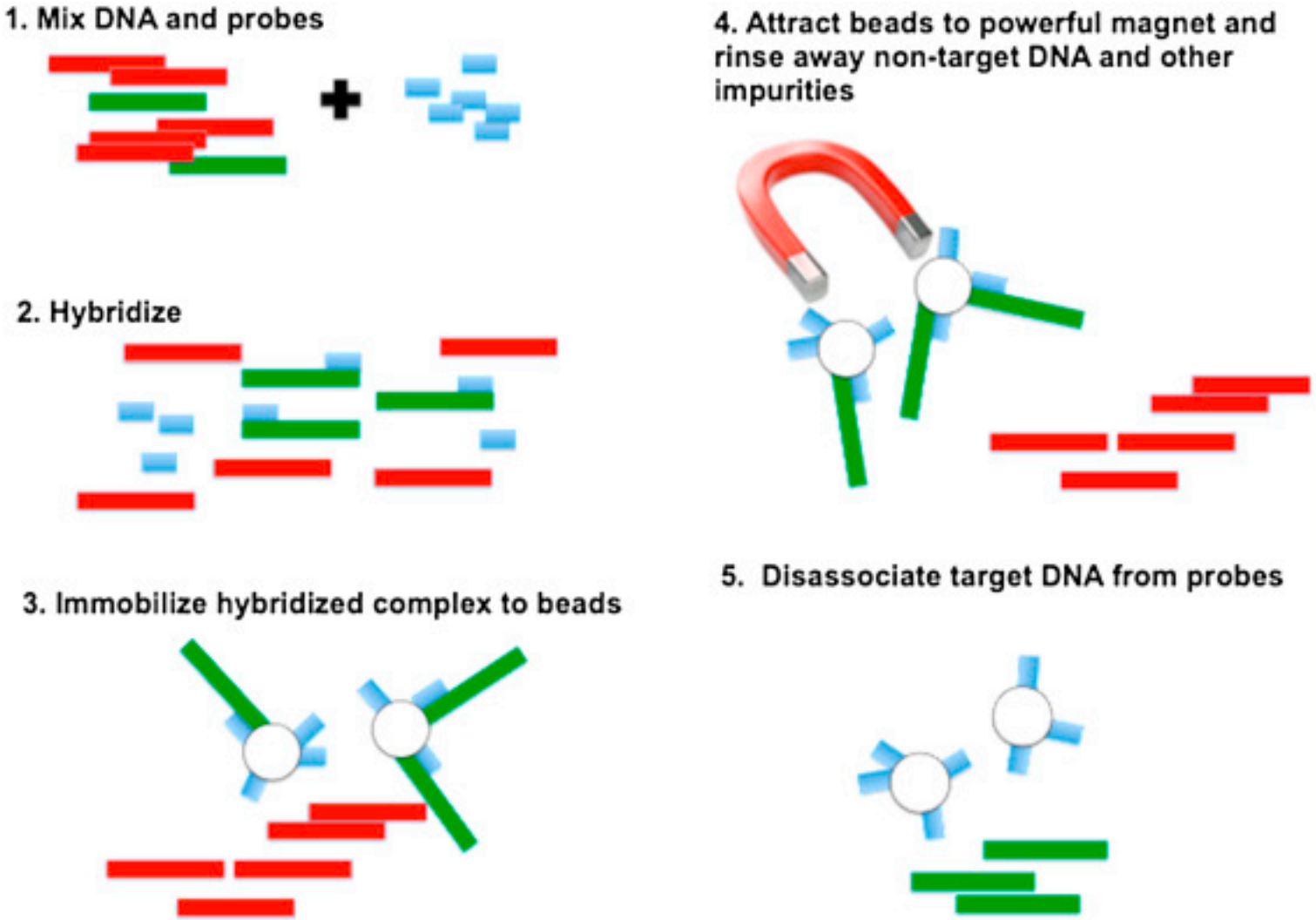


Imaeg: Oxford Nanopore

Bait capture

Pros
- Tolerant of diversity

Cons
- *A priori knowledge*
- *Expensive*
- *Slow & complex*



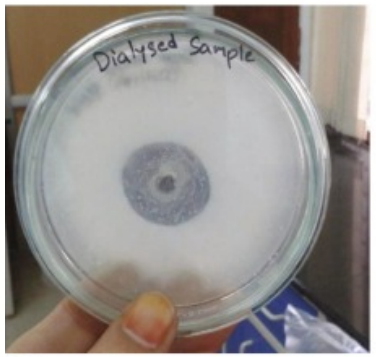
Winters et al (2017) Forensic Science International:

Culture enrichment

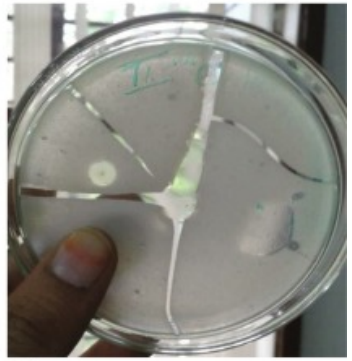
Pros
- Cheap
- Sensitive

Cons
- *A priori knowledge*
- *Slow*
- *Specific expertise*

(A)

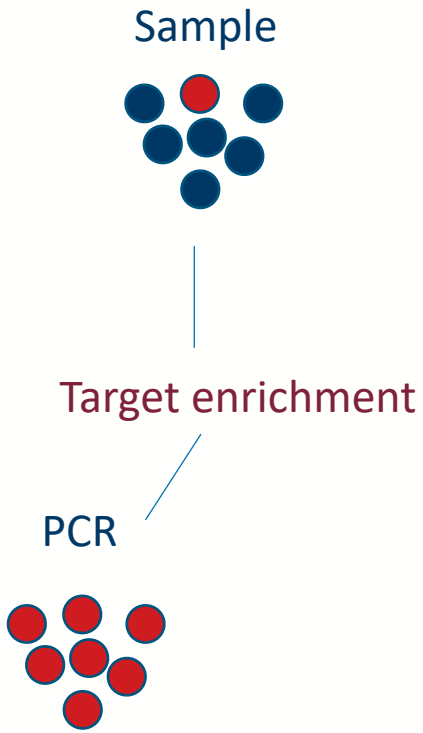


(B)


(C)

PCR enrichment

- Pathogen
- Host background



Why did we choose this?

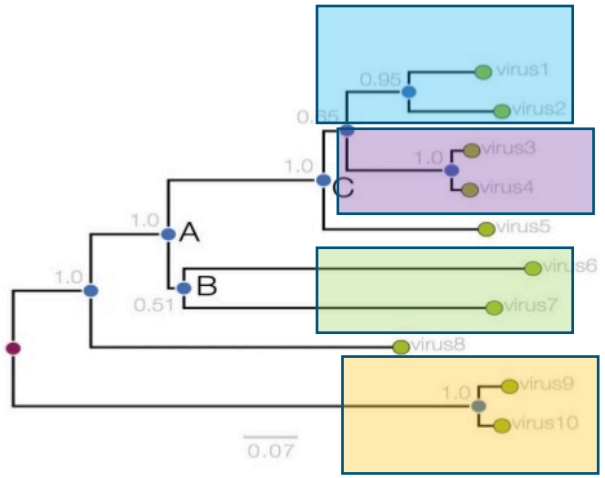
- Enriches samples with low viral material
- Helps with poor quality sampled (fragmented RNA)
- Cost-effective
- High sensitivity

Caveats:

- Requires *a priori* knowledge of pathogen
- Tolerant of limited diversity
- Potential for contamination

Designing primers

- What do I want to capture?
  - What pathogen
  - Location
  - Host variant
  - Whole or partial genome
- What *a priori* knowledge is available?



Challenges & potential solutions

- There is no existing data for study area

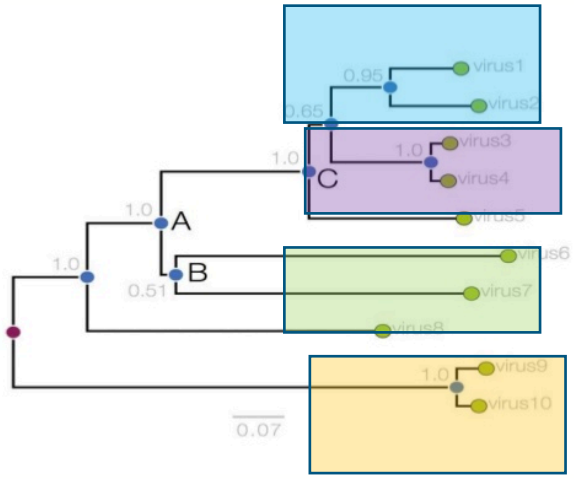
Solution: do some preliminary metagenomic sequencing to get a reference

- There are only partial genomes

Solution: use to get minor clade assignments, then use most closely related public sequences as a reference

- Diversity is too great

Solution: create multiple primer sets or try probes



Note: This is just advice not a hard set of rules!

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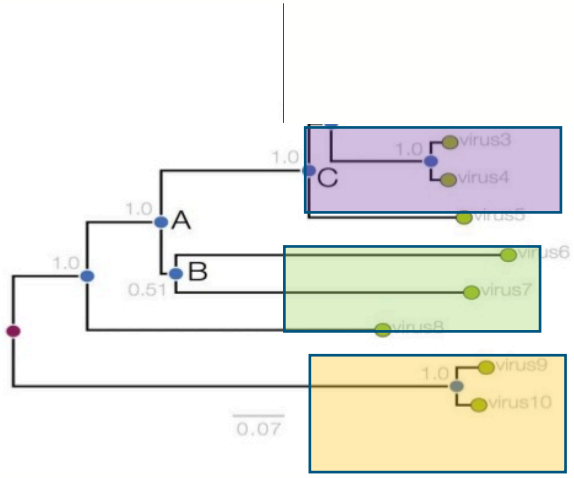

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