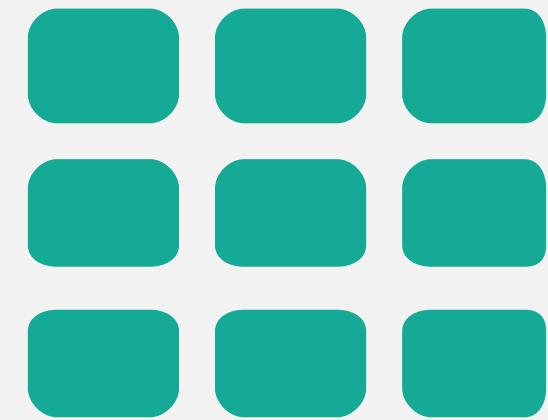
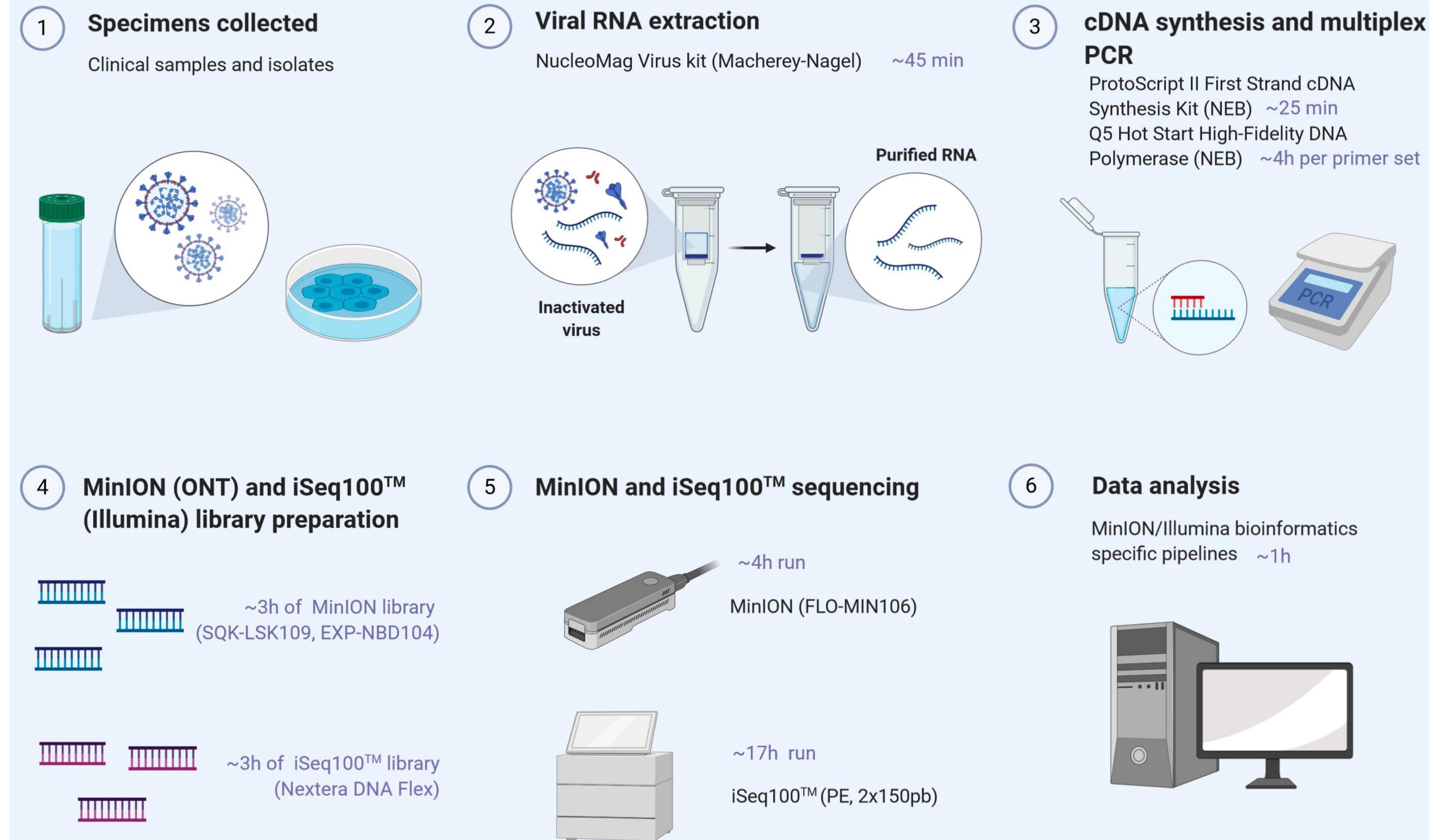
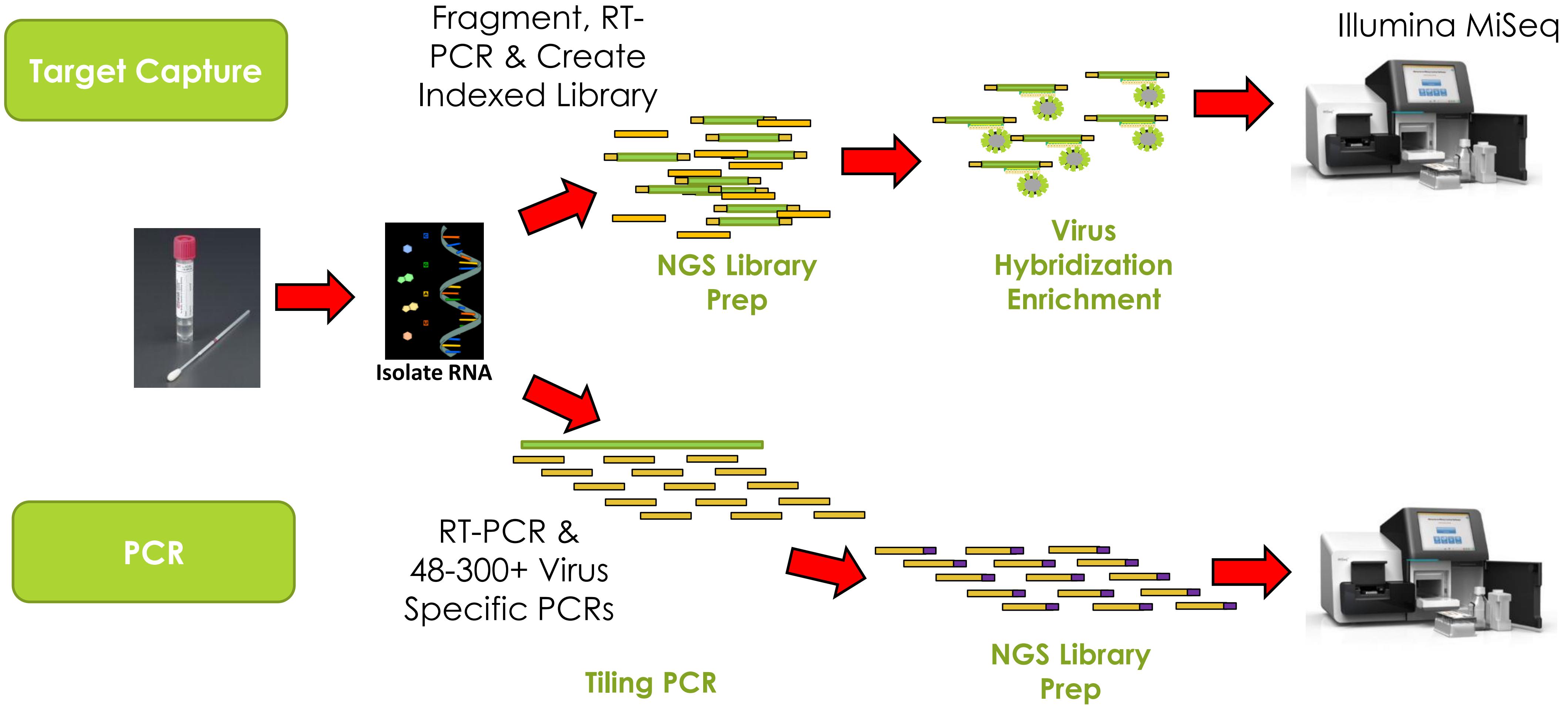


SARS-COV-2 ILLUMINA DATA

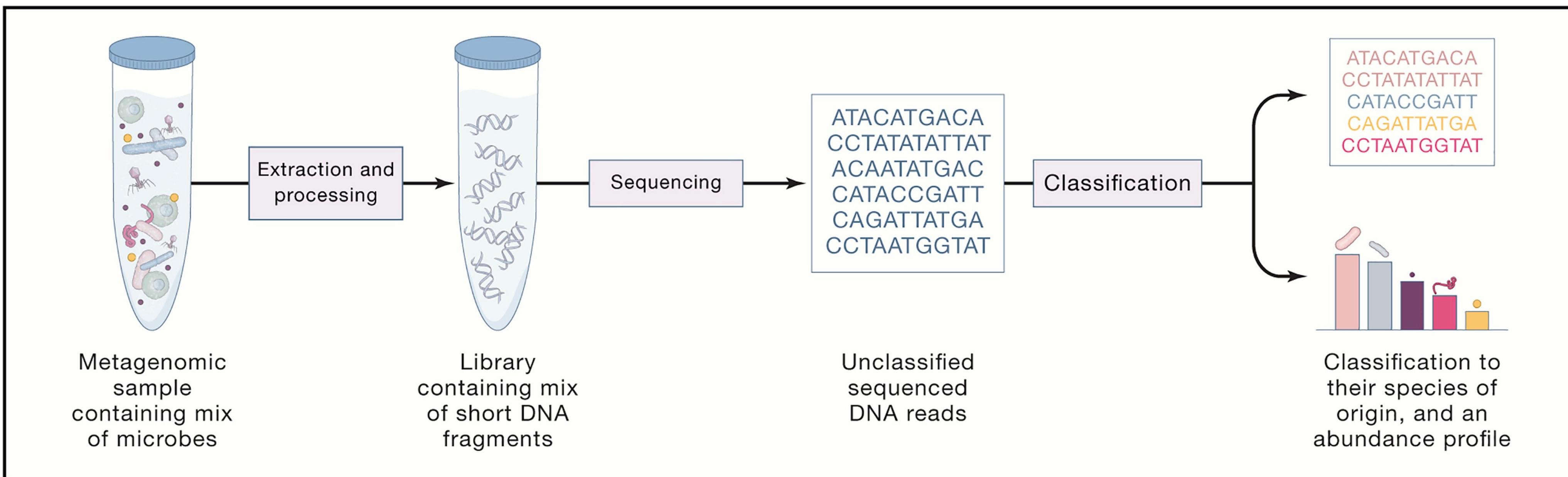


SARS-CoV-2 sequencing workflow

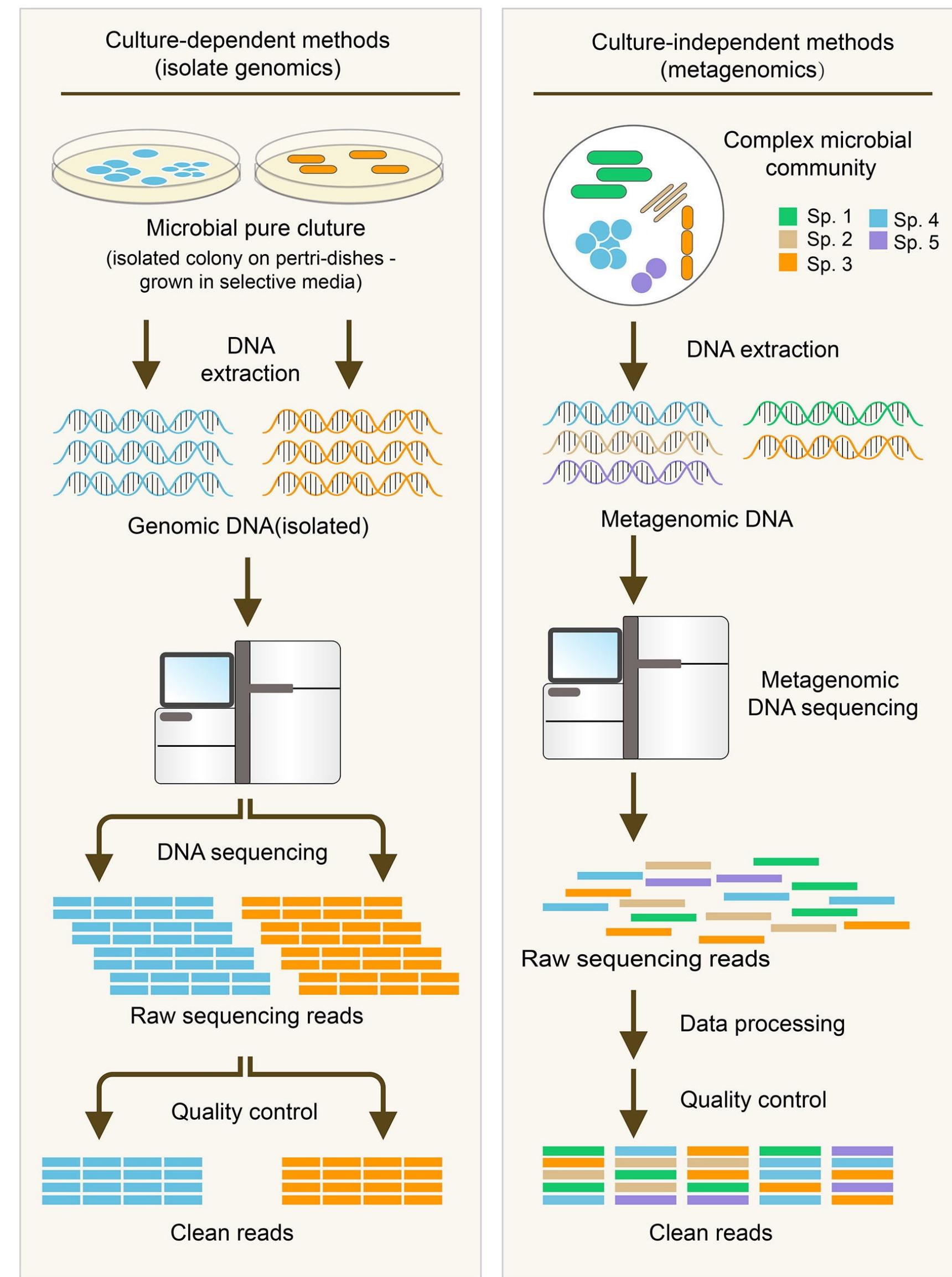


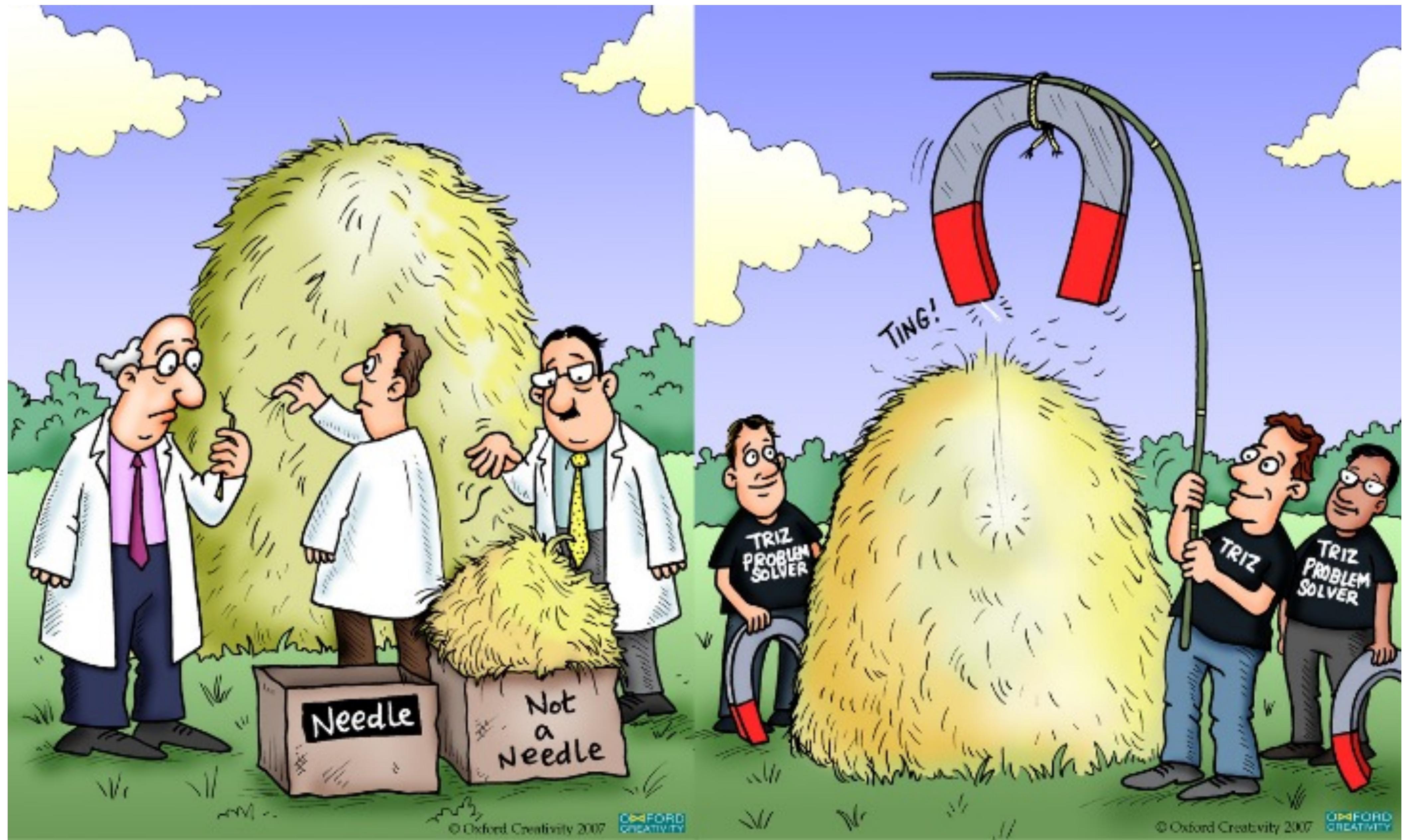


Complex samples (metagenomics)

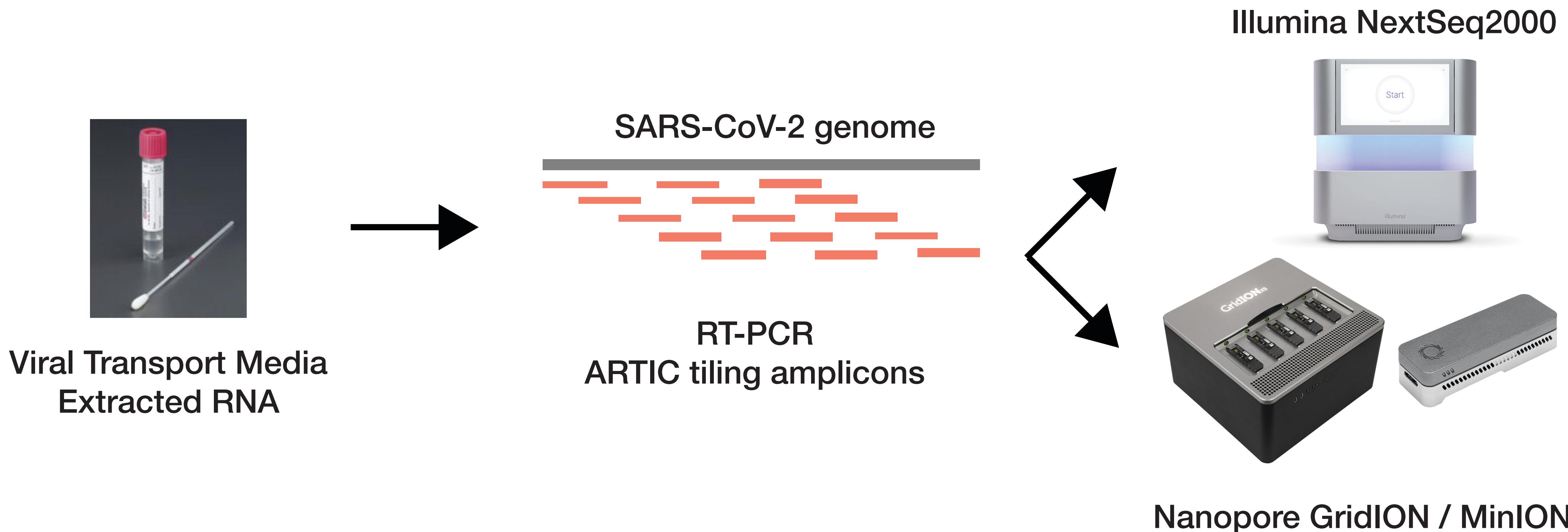


Sequencing Isolates vs Communities



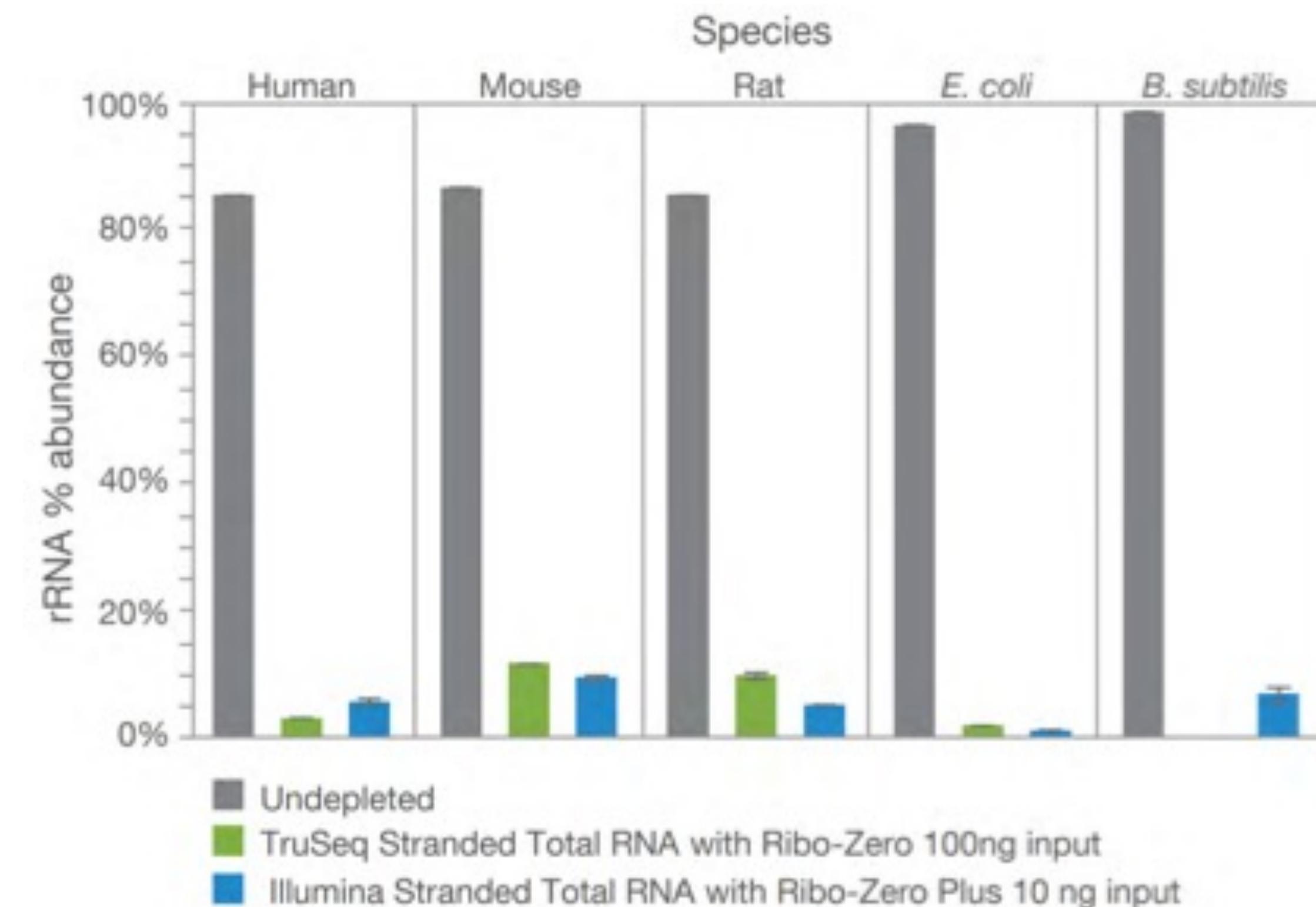
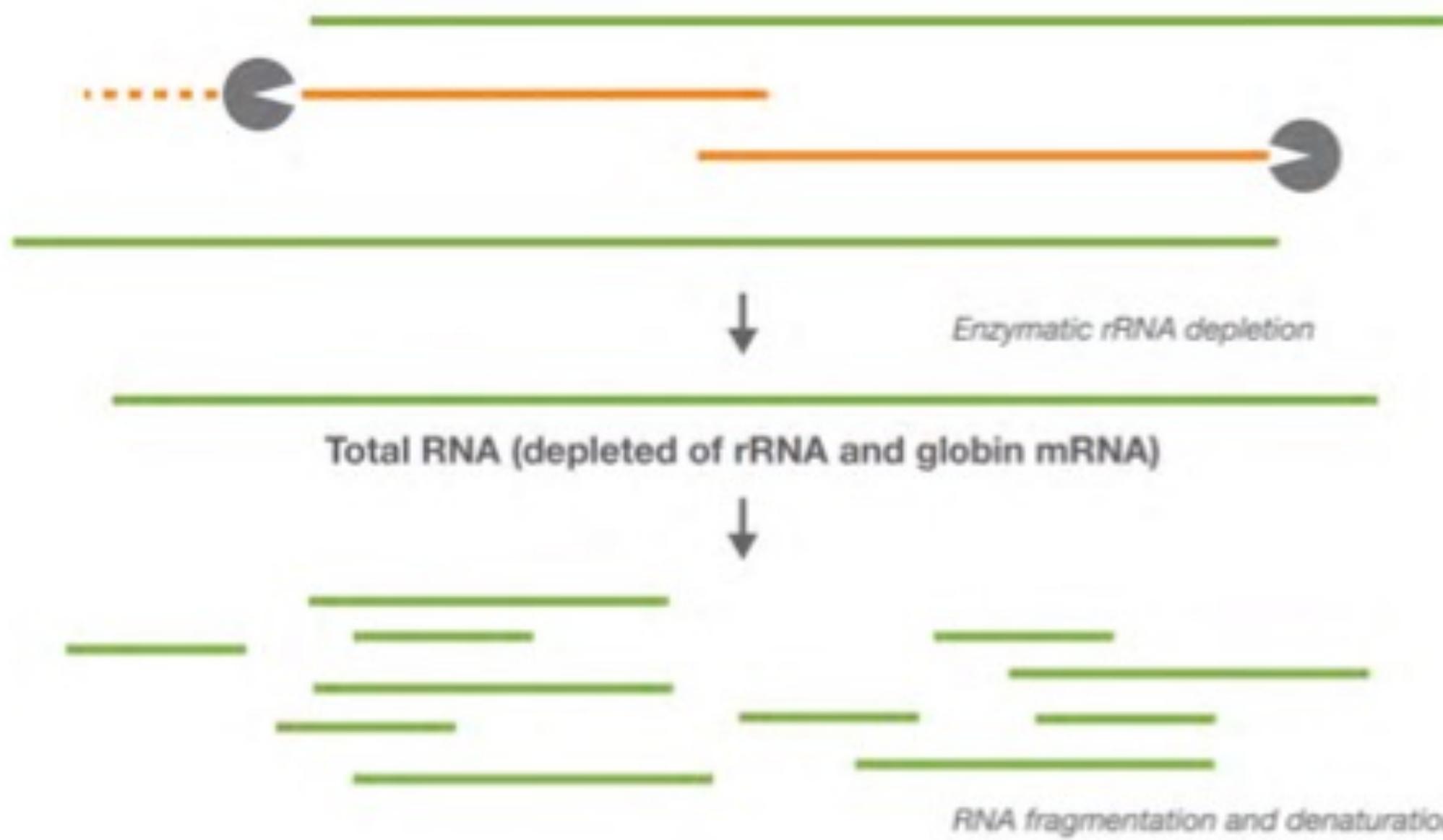


Enrich what you DO want

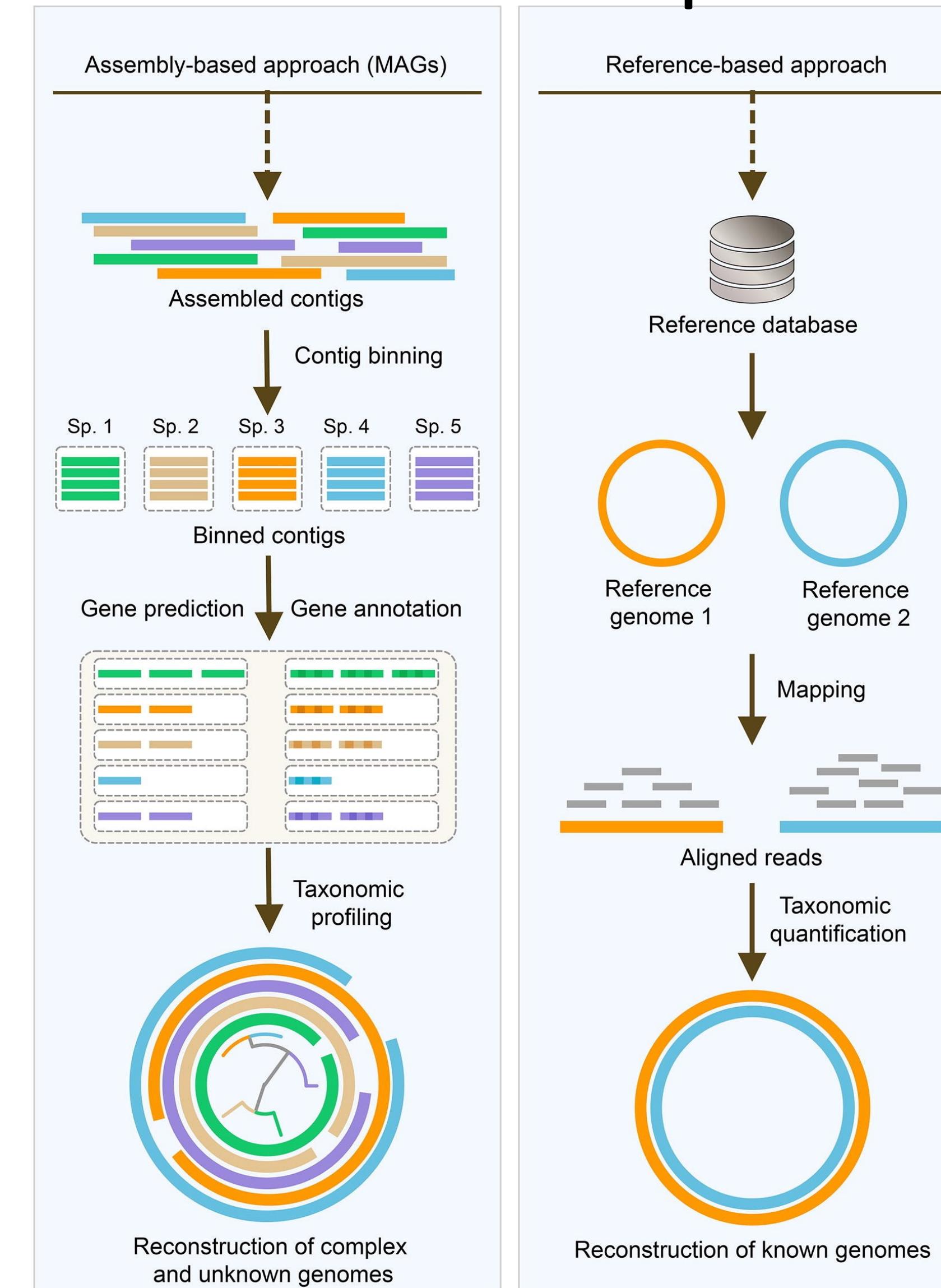


Deplete what you DON'T want

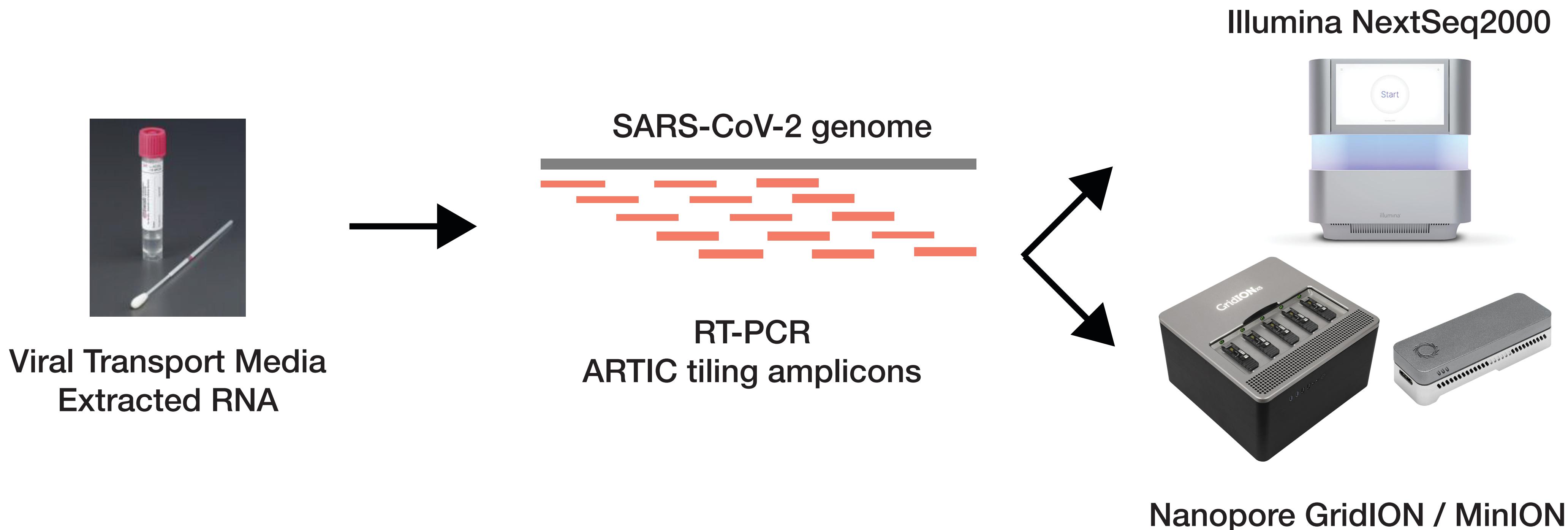
rRNA depletion



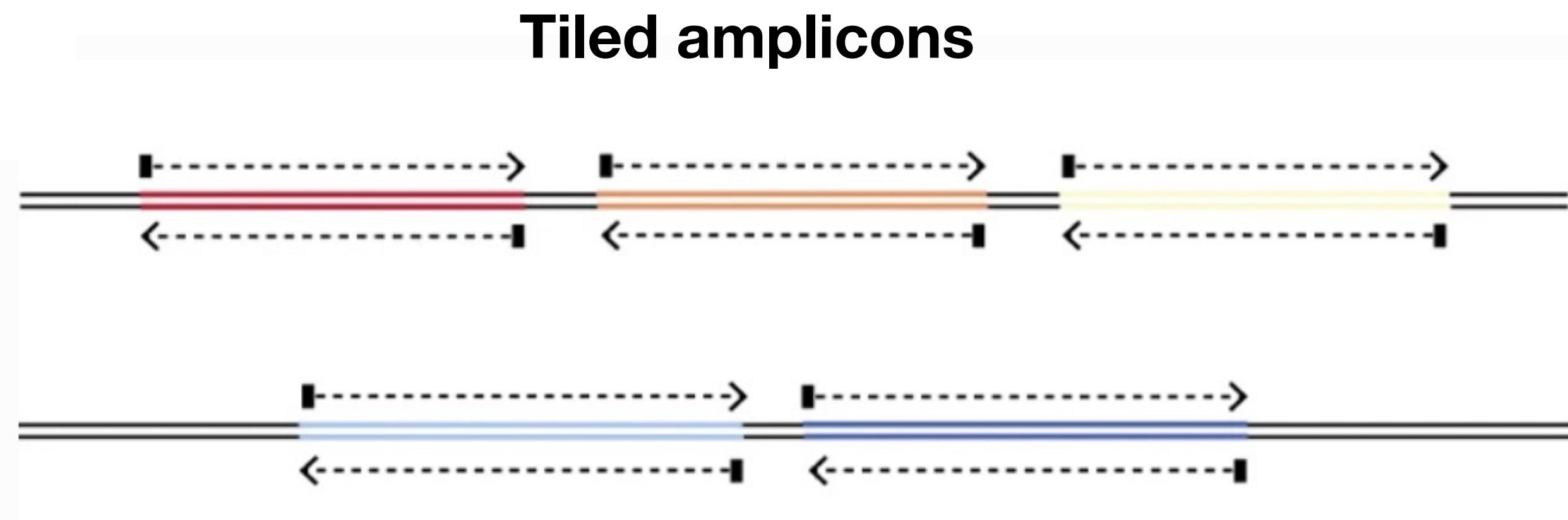
Bioinformatic approaches to extracting genomes from complex samples



Enrich what you DO want

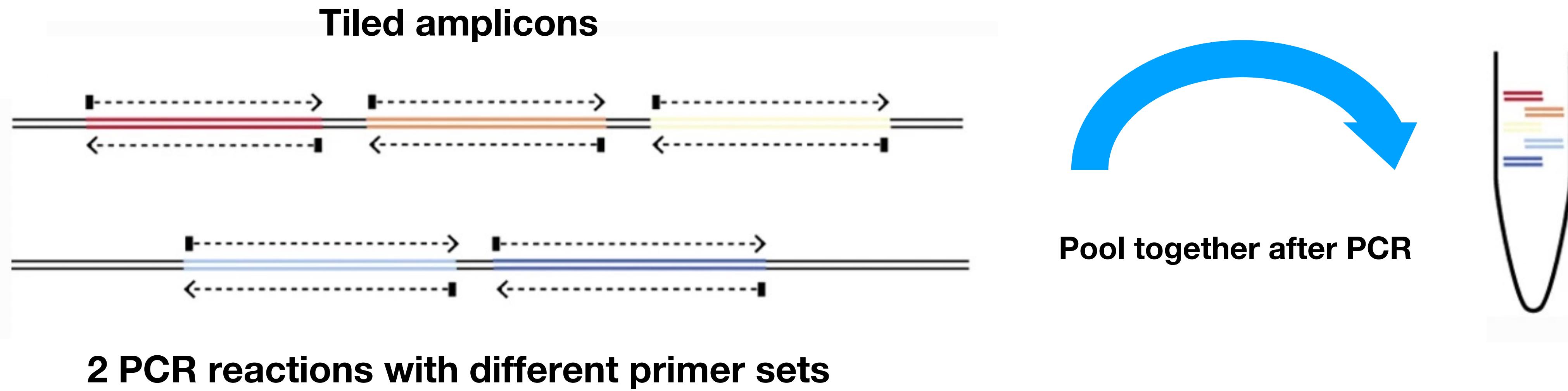


Viral amplicon sequencing

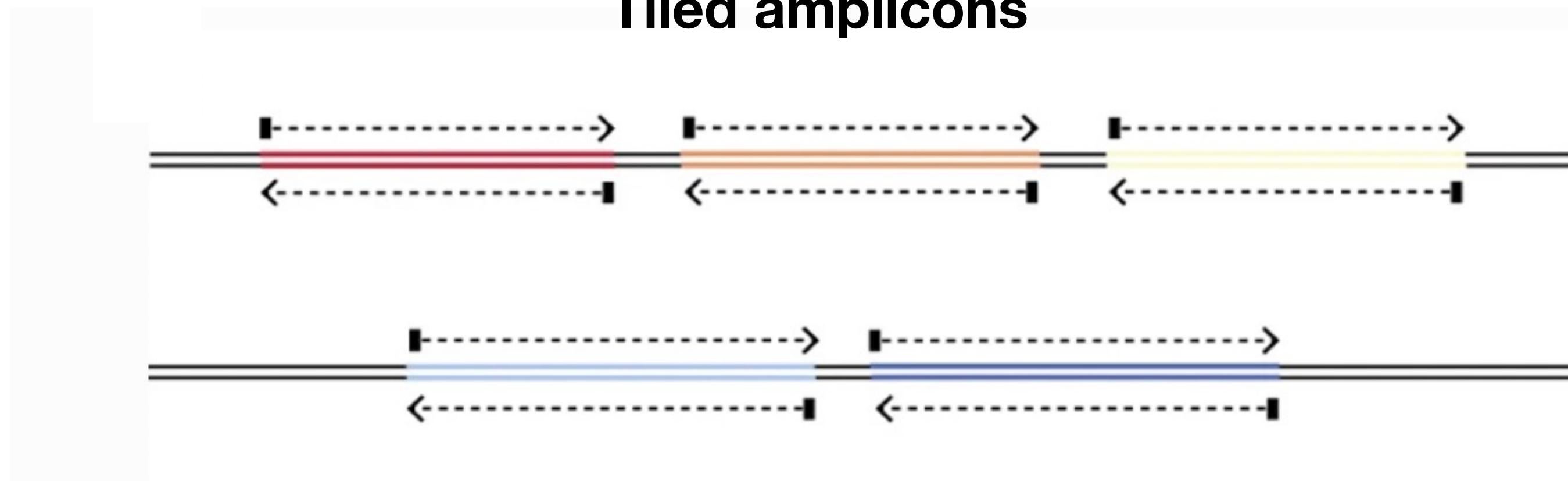


2 PCR reactions with different primer sets

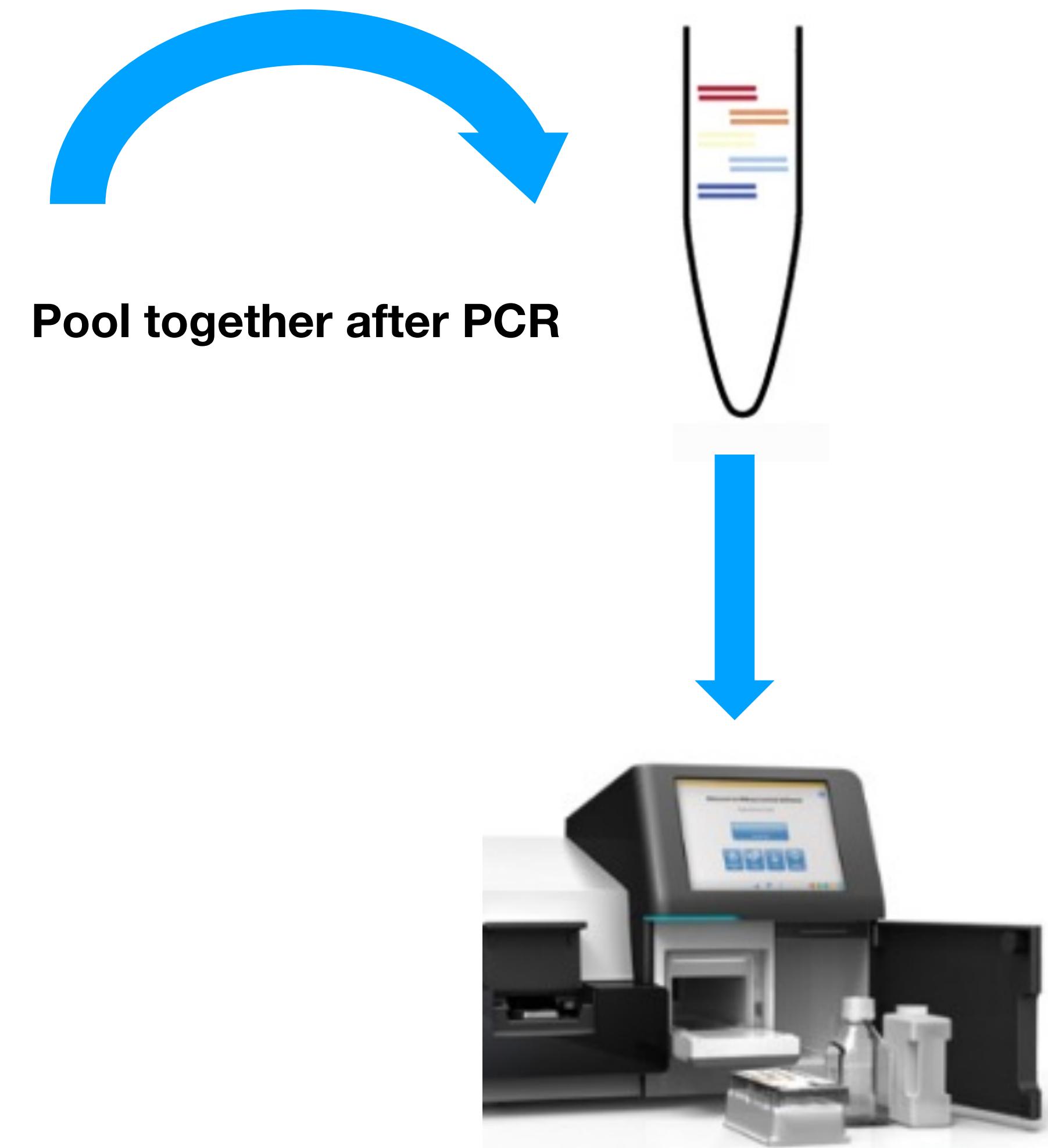
Viral amplicon sequencing



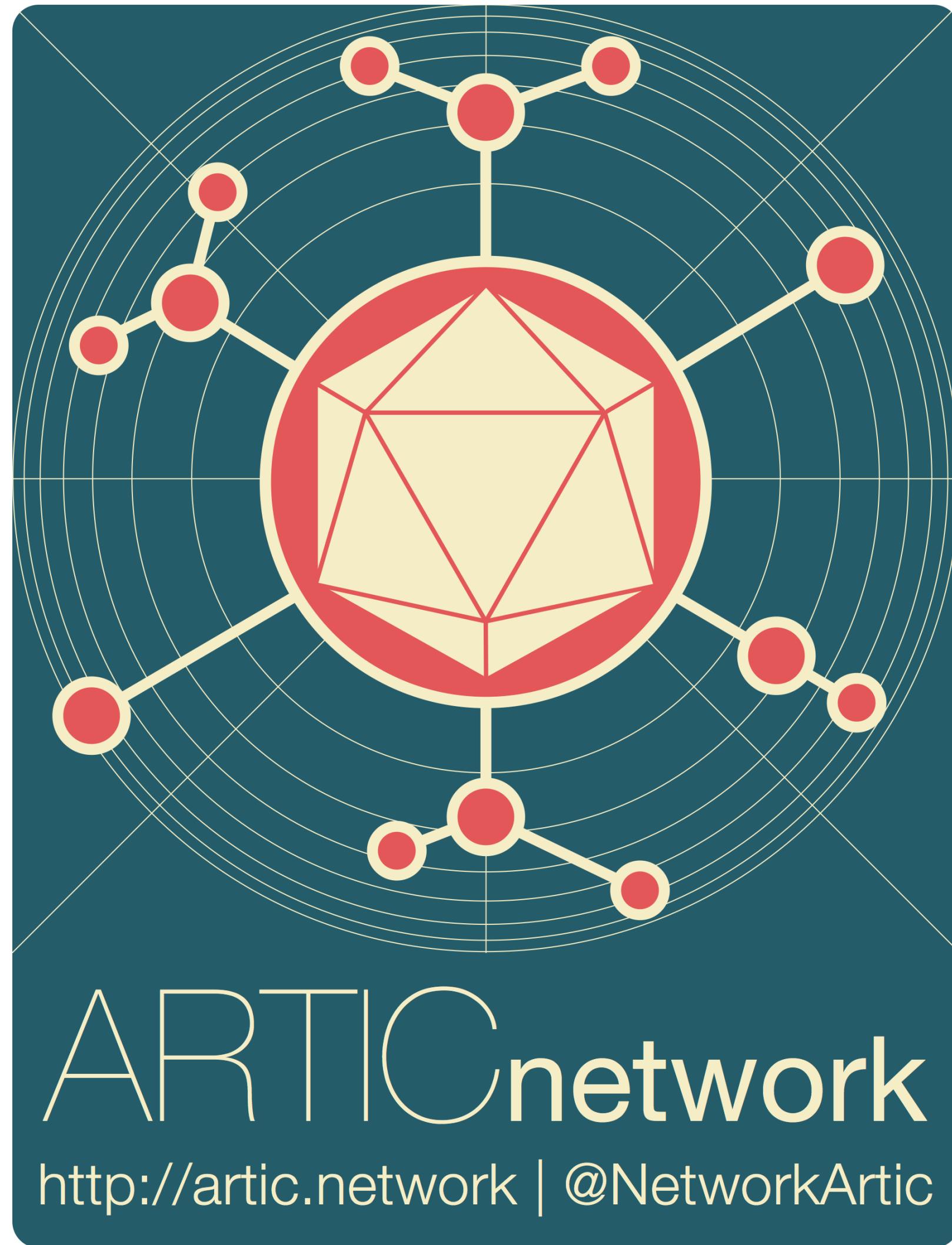
Viral amplicon sequencing



2 PCR reactions with different primer sets



Viral amplicon sequencing



- Currently have protocols for many viruses:
- SARS-CoV-2
 - Zika
 - Ebola
 - Nipah
 - Chikungunya

Design new primer schemes

← → C primalscheme.com D :

Report an issue Protocol GitHub

primalscheme

primer panels for multiplex PCR

Design a new scheme

FASTA file... Select a file...

Options

High-GC mode Pinned

Amplicon size

400

Min/max will be set at 5% either side of target.

Scheme name

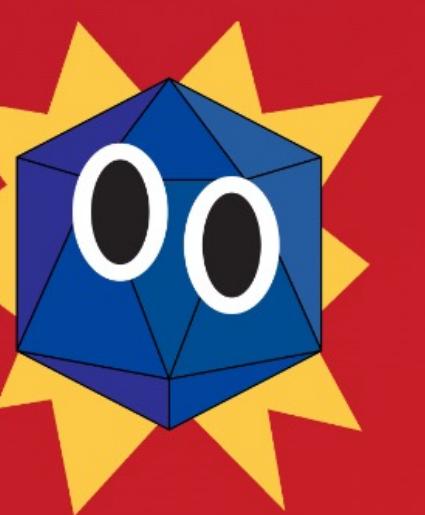
e.g., nCov-400

A short name/prefix for your scheme, no spaces.

Use the [standard protocol](#) for these settings.

Design scheme

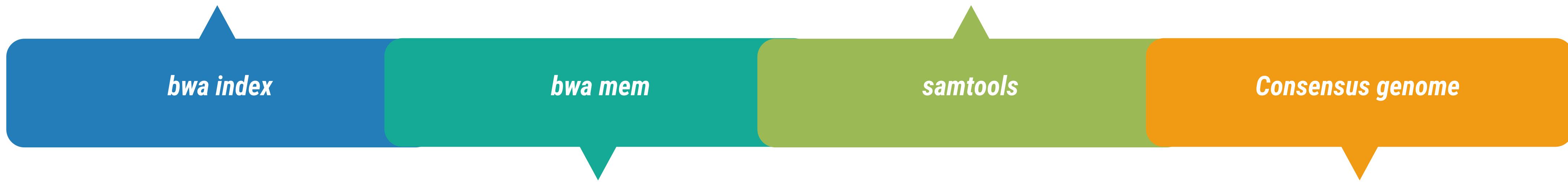
[View demo inputs](#)



Mapping to reference genome

Create index of ref genome:
`bwa index ref.fasta`

Convert sam to bam and sort the bam:
`samtools convert sam -> bam`
`samtools sort`



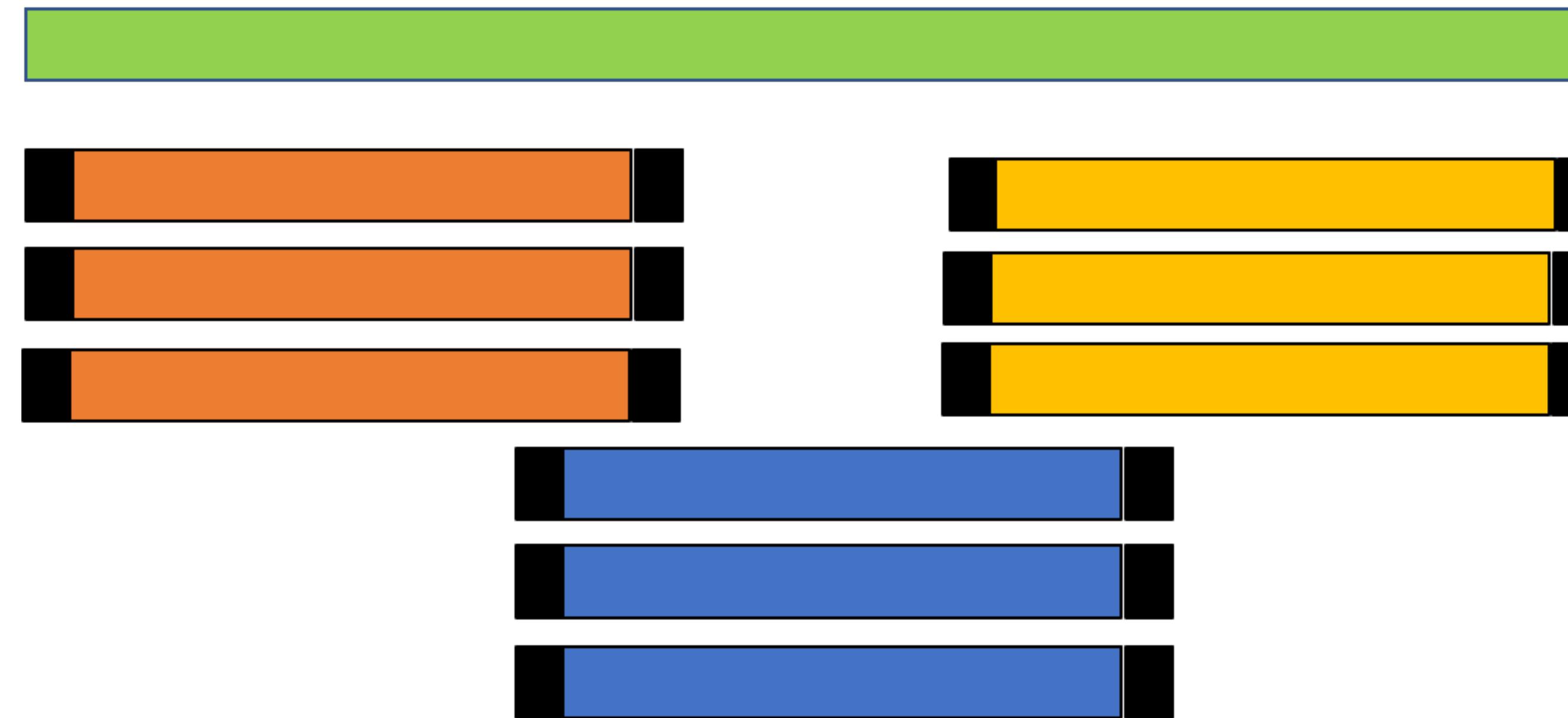
Map reads to reference:
`bwa mem ref.fa R1.fq.gz R2.fq.gz`

Call variants against ref:
`samtools mpileup`
`bcftools consensus`

You must think carefully about what reference genome you want to use!

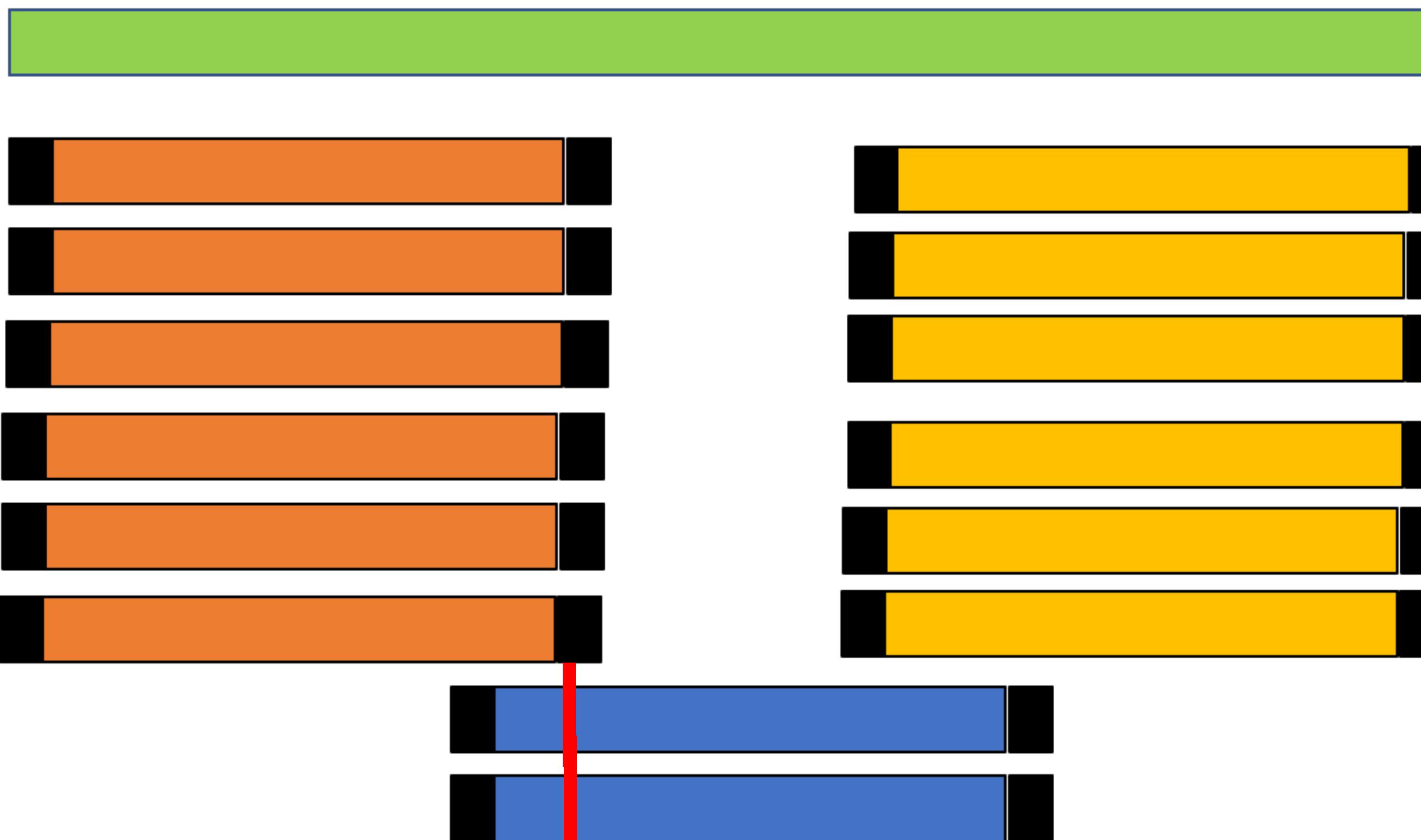
You won't find things in your sample that are not in the reference!

Primer trimming is important in this type of amplicon based approach



We will trim these primers out using *ivar*

What happens in this case?



Questions?