

freezeTb; detecting anti-microbial resistance in TB

WHO 2023



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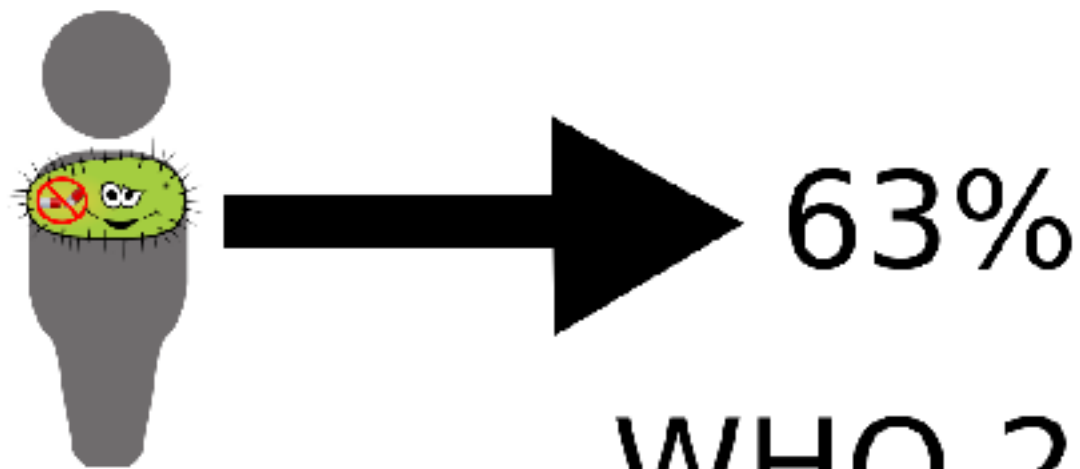
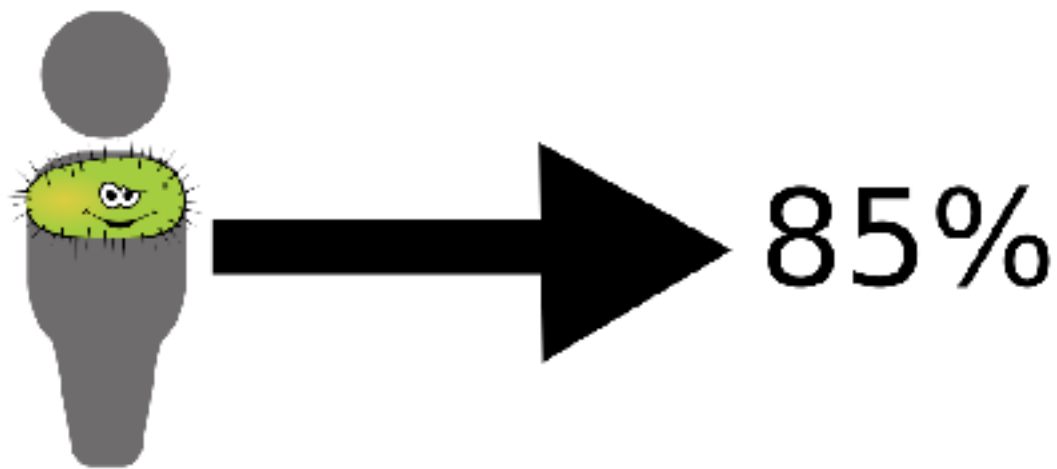
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tuberculosis

5-7 million 10 million 1 million

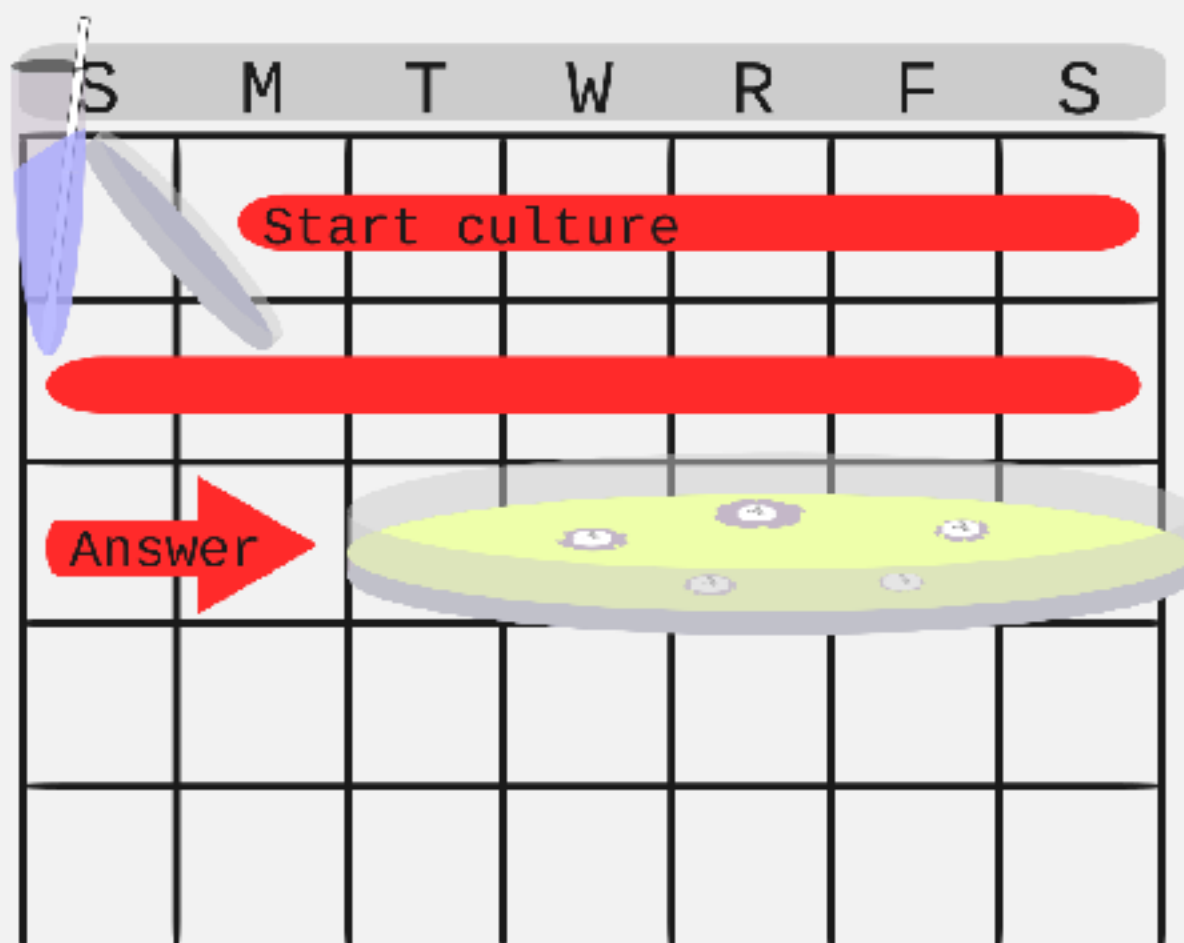
# Treatment success



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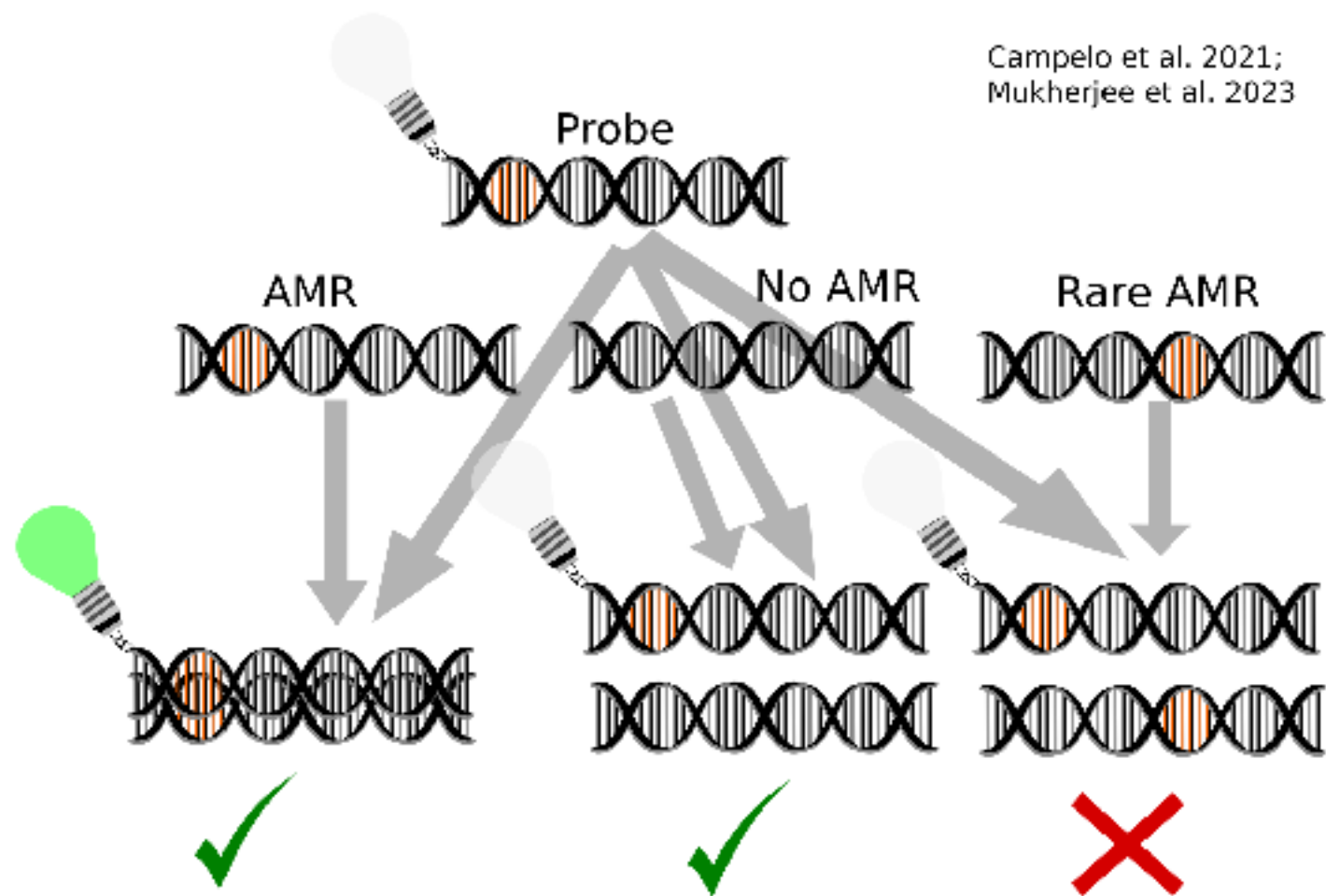
Drug resistant detection

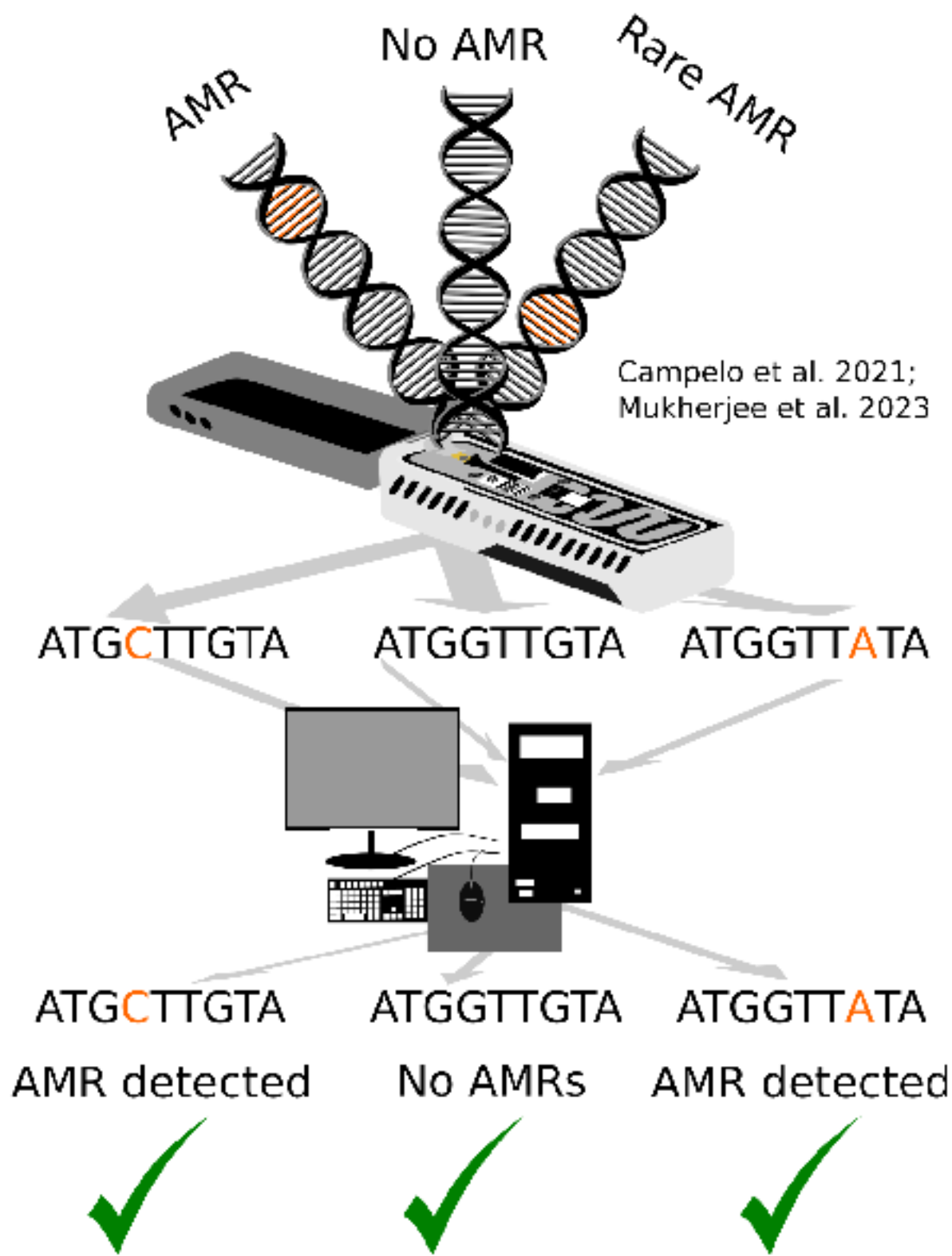
# Month

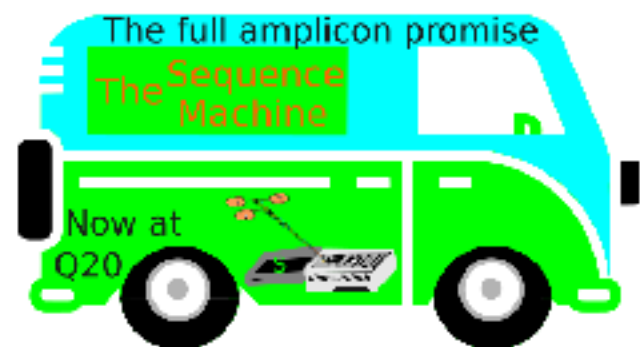
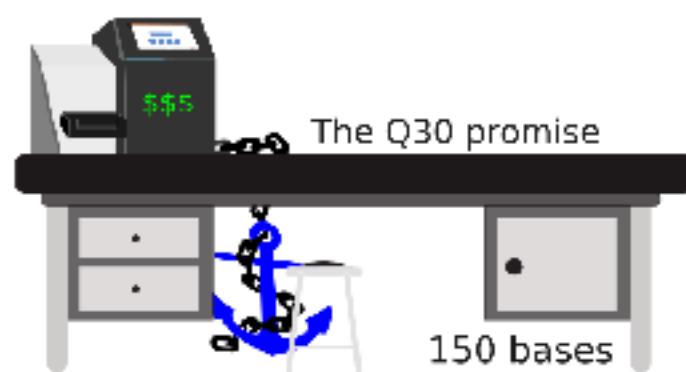


Campelo et al. 2021

Campelo et al. 2021;  
Mukherjee et al. 2023







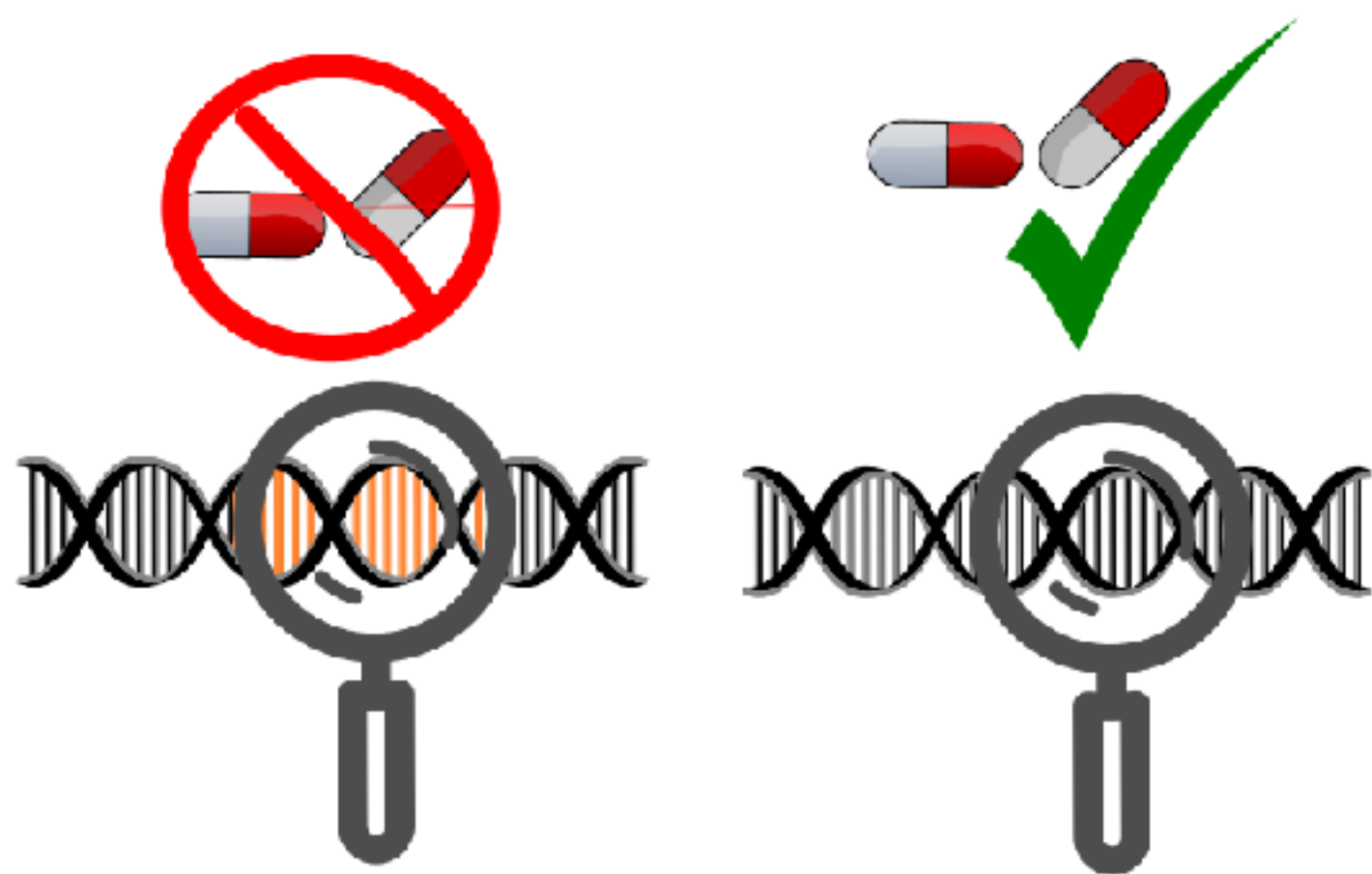
Does not include van or great danes

Xia et al. 2023

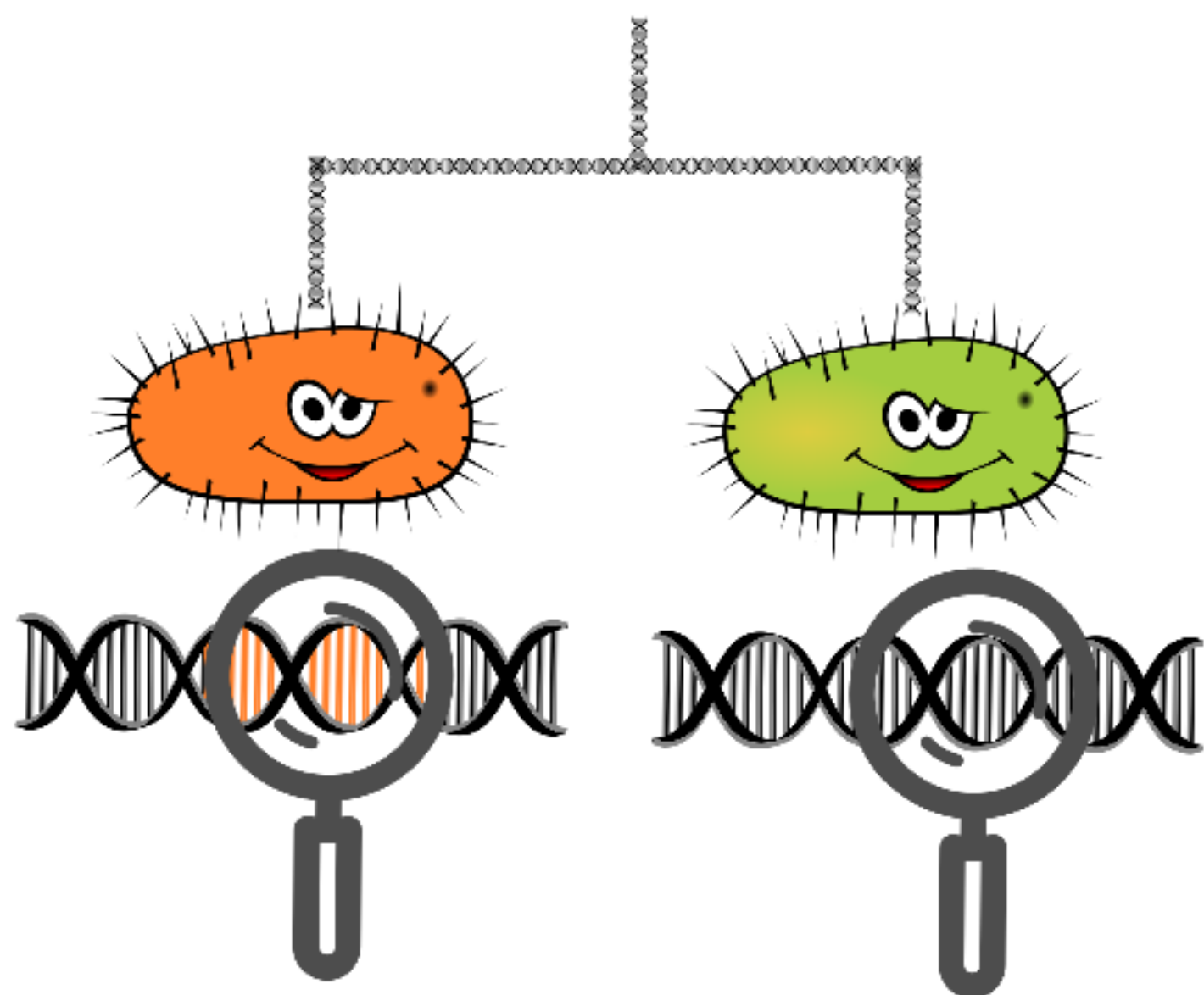


freezeTB; the software side

Expected output



Check for AMRs



Detect Lineages

## 2. a. MIRU-VNTR

- Amplifies 24 repeat regions across genome
- Lineage found by amplicon length
- Current gold standard

Direct repeat



Spacer 1

Spacer 3

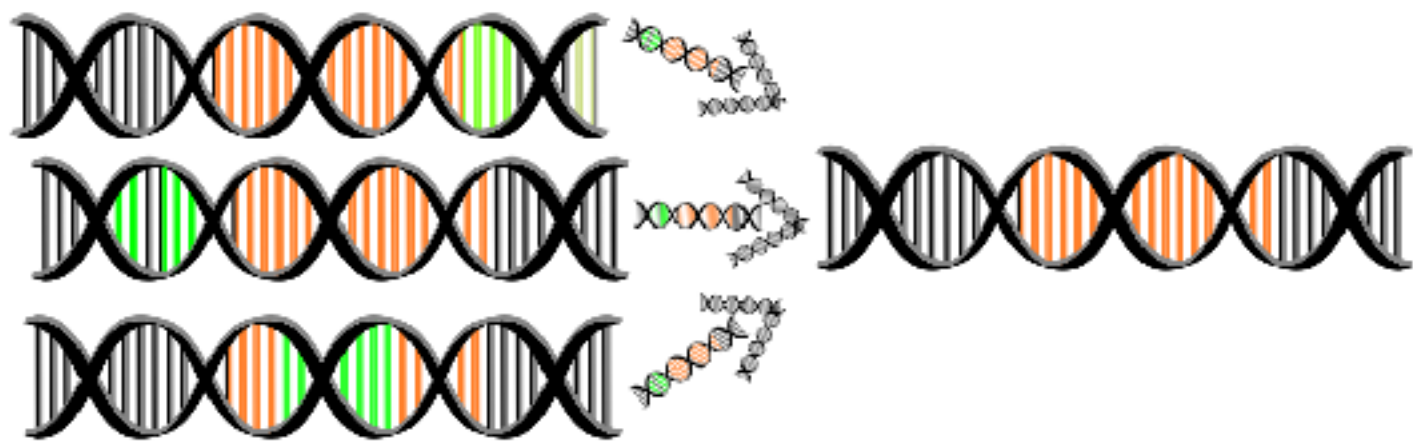


Spacer 2

101  
+



Spoligotype  
= Lineage



Build consensus

TBProfiler; the current resource



TBProfiler; pros

- AMR detection with WHO 2023 catalog
- Detects spoligotypes
- Outputs an vcf files

TBProfiler; cons

- > 5 dependencies or conda

Other solutions:

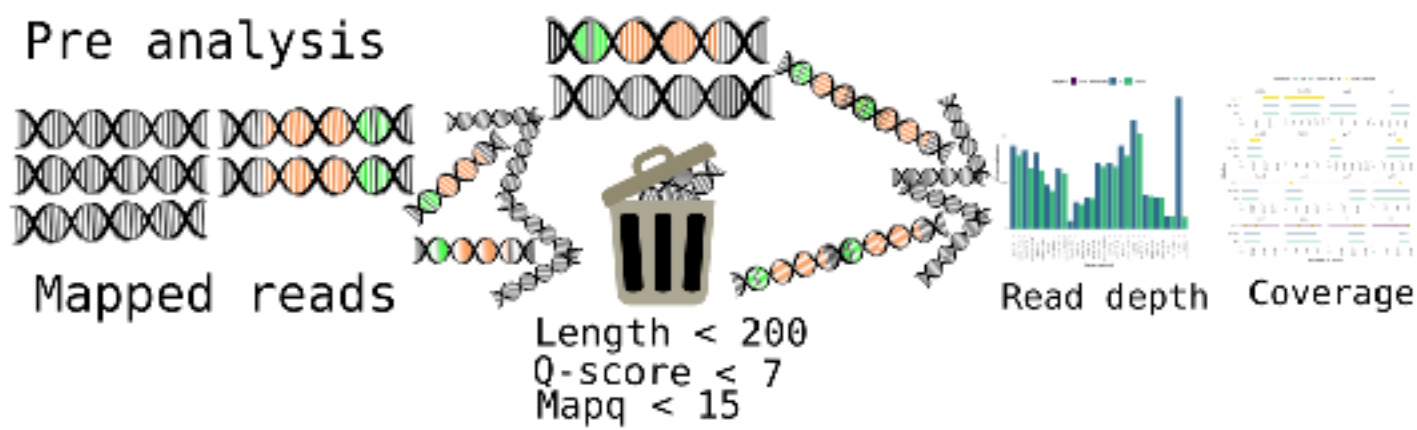
- Propitiatory (ONT)
- ??

freezeTB; the software side

- CLI: sam file
- GUI (R): fastq file + minimap2

Primer Masking [Optional]

Pre analysis



Mapped reads

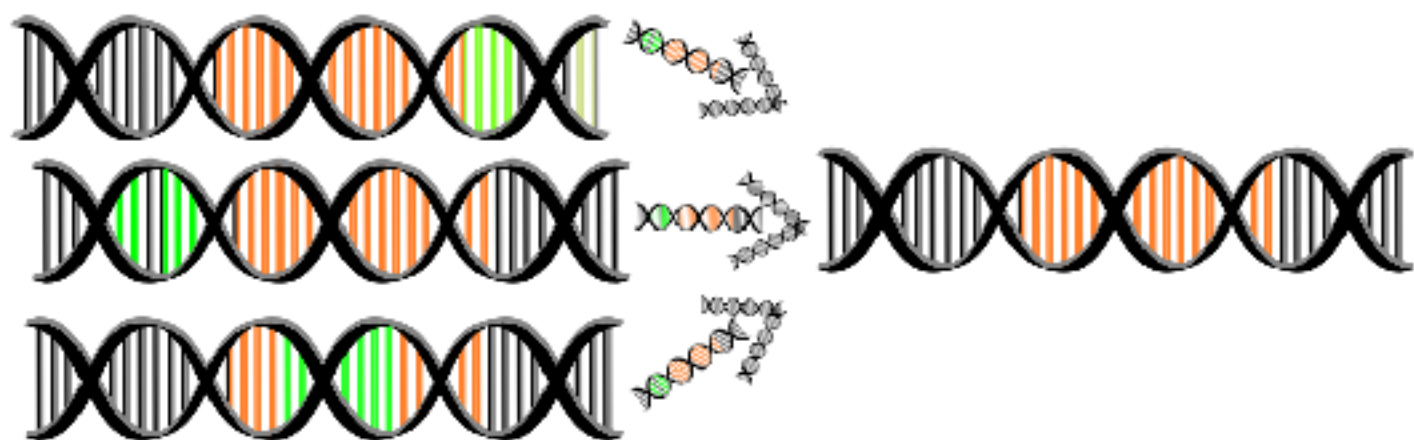


Length < 200  
Q-score < 7  
Mapq < 15

Fitler reads

Read depth

Coverage



Build consensus

Removes bases with Q-scores under 7



Break up consensus

- Fragments with read depth  $> 19x$
- Remove fragments under 200 bases

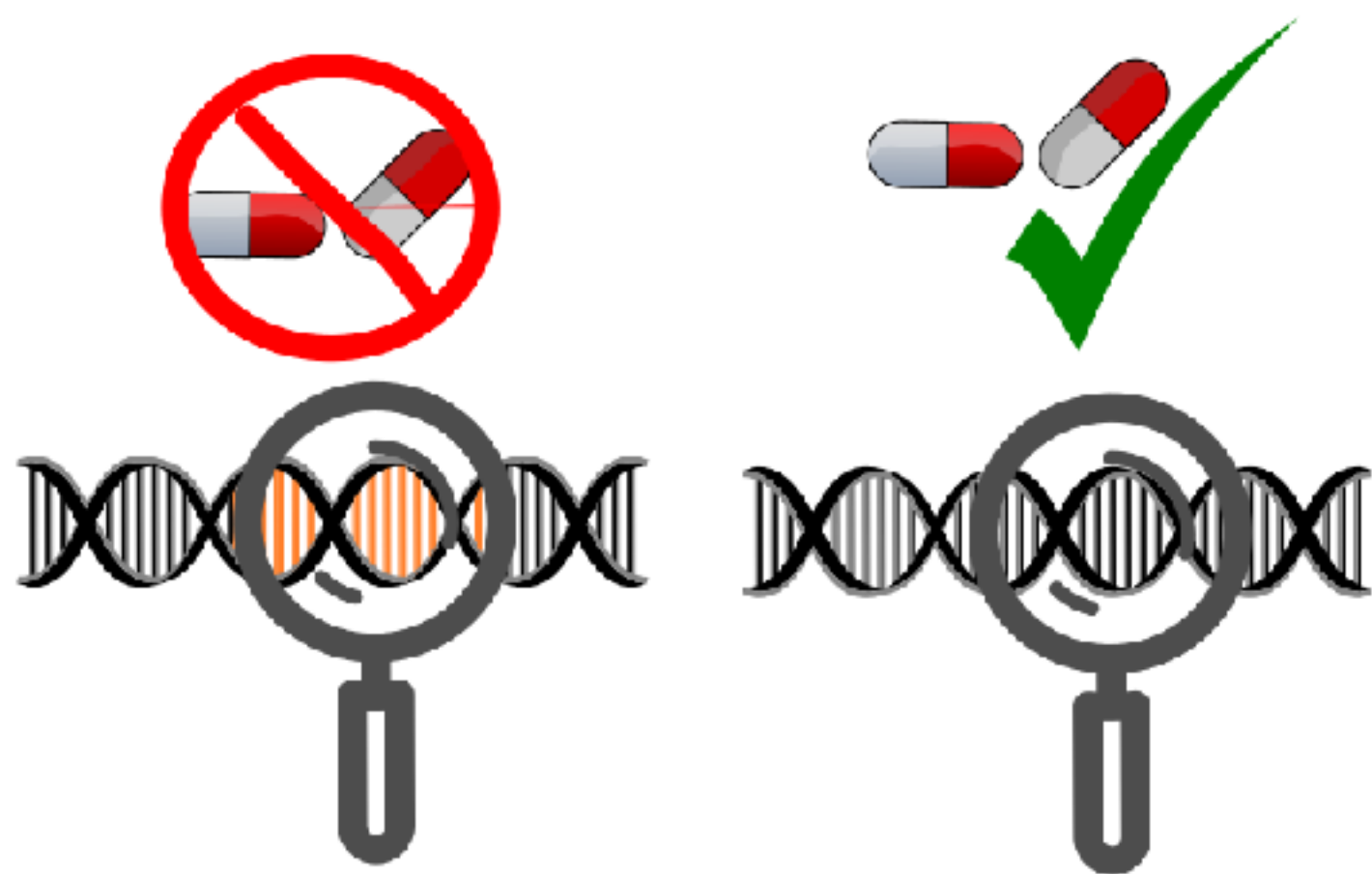
### Masking:

- Find mapped reads (A + T + G + C + dels)
- Select base with most support
- Find base support (base / mapped)
- Mask bases under  $< 50\%$  support

Indel removal:

- Find mapped reads (A + T + G + C + dels + N)
- Find indel support (indel / mapped)
- Remove indels under 70% support

Output fragments in sam file



Check for AMRs



# Tuberculosis AMR Catalog

WHO 2023

Ignore gene deletions

Frames shift and LoF:

- Only match patterns
- Less false positives from indel errors

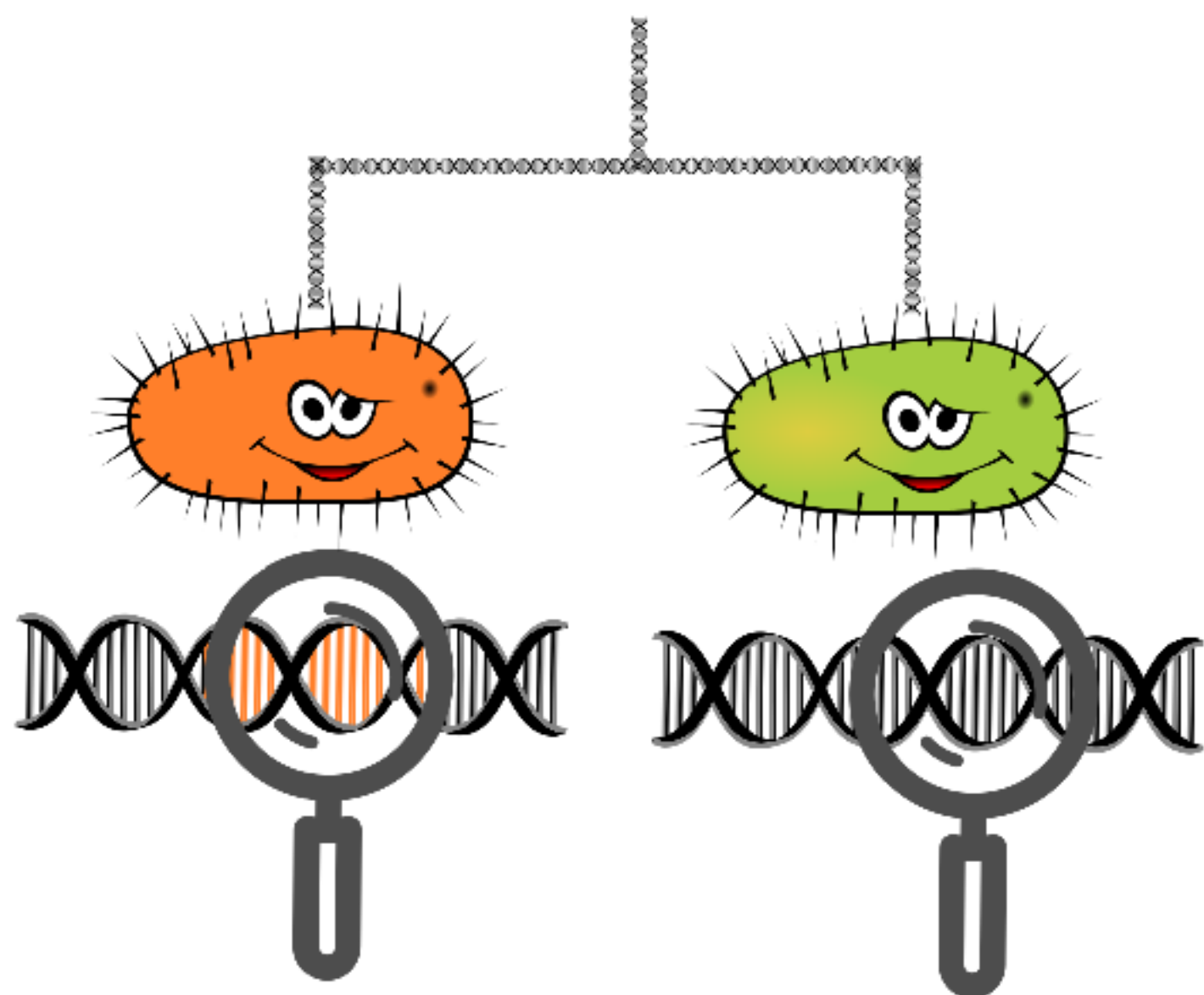


Amino acid catalog AMRs

- Translate sequence AMR region

AMR called if:

- Sequences match
- Sequence AMR region length = AMR length



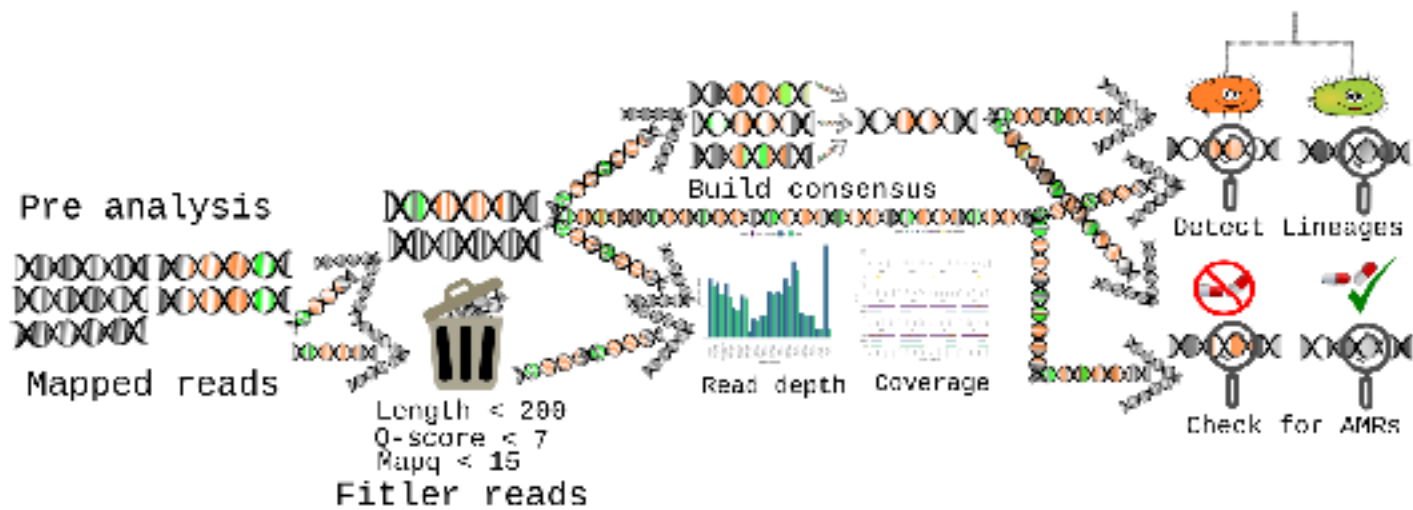
Detect Lineages

MIRU-VNTR lineages:

- See if sequence has repeat
- Check sequence repeat length
- Call lineage

## Spoligotyping:

- Get direct repeat (DR) region
- Map spacers to DR with Waterman
- Find lineage



freezeTB is modular:

- Steps are separate programs
- All programs use sam files

FreezeTB; works, but not an replacement



