Extracting TB DNA

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We will record these sessions and put them online so you can refer back to them later on

We will also put the slides up online so you can access the notes (links and image credits)

Why do we need to extract DNA?

Sequencing relies on good DNA:

- Good quality (no impurities)
- Quantity
- High molecular weight (long reads)

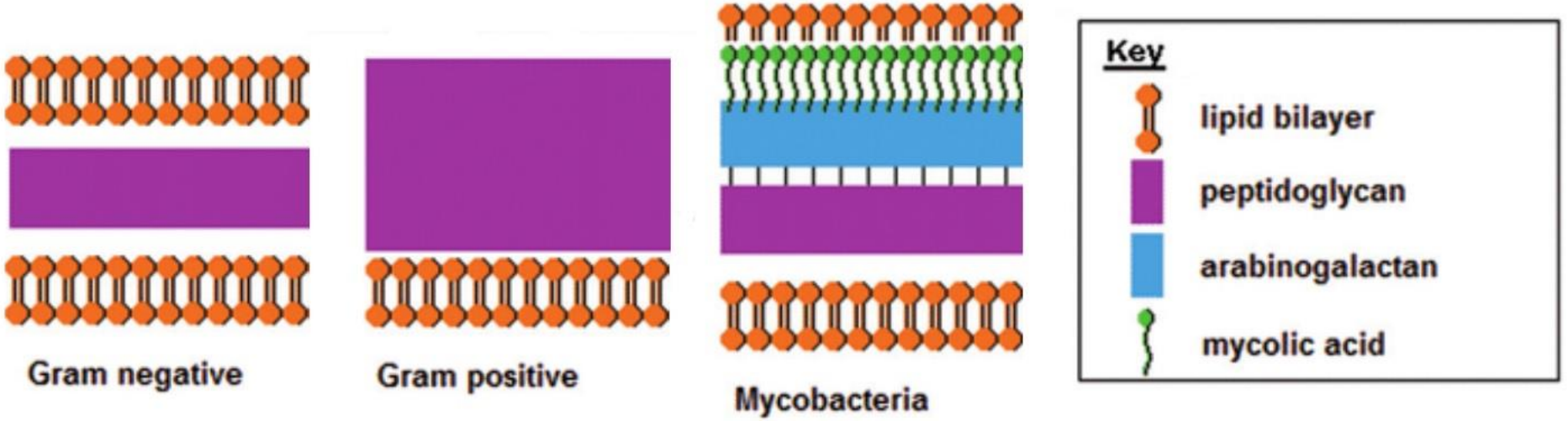
DNA extraction options

- There are a number of commercial kits available
- It can be difficult to extract (enough) good quality TB DNA…

Why is TB so difficult to extract?

- It has a thick, complicated cell wall
- To extract DNA, we have to balance between opening up enough bacterial cells to get good quality, but also being gentle enough to ensure good quality (and long strands of) DNA

Why is TB so difficult to extract?



Adapted from Naidoo et al. Biomarkers (2006)

Biological safety requirements

- *M. tuberculosis* is a hazard group 3 organism
- It should be processed only in the appropriate level of laboratory (unless validated as killed)
- Heat killing before removal (or full chemical lysis)

Basic protocol

1. Heat killing (80°C vs 95°C)
2. Sonication
3. Chemical lysis
4. DNA precipitation

1. CTAB – heat killing

- Debate as to which temperature is best (lower temperatures protect DNA better, but higher temperatures ensure killing of MTB)
- Things to consider (regarding effectiveness):
  - Type of sample you are heat killing (protective elements)
  - Concentration of your sample (how many bacteria there are)
  - Volume of your sample
  - Water bath vs heat blocks
- **Need to ensure you have validated your heat killing method**

2. CTAB – Sonication

- Sonication uses pulsed, high frequency vibrations to disrupt cell membranes and burst cells
- Can use an ultrasonic bath or probe (see notes for link to comparison)
- Baths are generally cheaper than probes

3. CTAB – chemical lysis

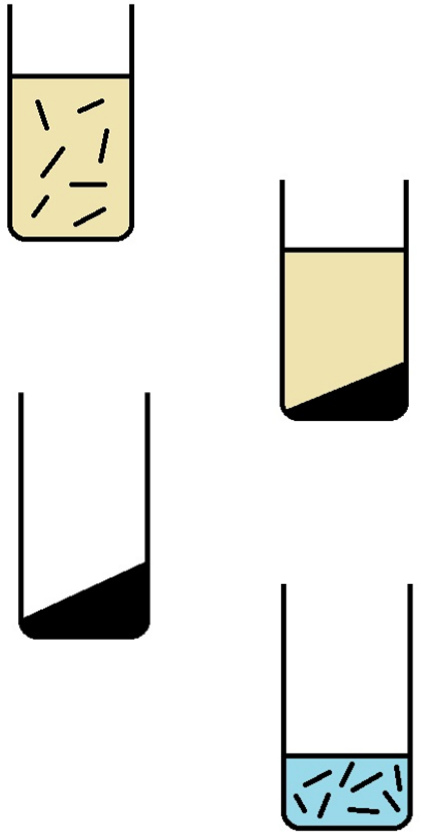
- **Lysozyme** – enzyme that breaks down cell walls
- **Proteinase K** – Breaks down proteins in cell lysates (tissue, cell culture cells) and releases nucleic acids (effectively inactivates DNases and RNases)
- **Cetyltrimethylammonium bromide (CTAB)** - detergent that denatures and precipitates the cell wall lipopolysaccharides and proteins (in presence of Na+ concentrations above 0.5 M, the DNA will remain soluble)
- **Sodium dodecyl sulphate (SDS)** – detergent that solubilizes membrane proteins and lipids

4. CTAB – DNA precipitation

- DNA is hydrophilic and dissolves in water but not in alcohol
- To separate the DNA from the cell debris, alcohols and salts are used
- Organic solvents are used to separate the DNA to an aqueous phase, leaving lipids and proteins in the organic phase. The aqueous phase is then concentrated using isopropanol, this concentrates the DNA and removes excess salt
- Isopropanol is used in preference to ethanol as a lower volume for precipitation can be used (1:1 rather than 2:1)
- Yeast tRNA is used during the alcohol precipitation step as it is an effective co-precipitant to aid in recovery of small amounts of nucleic acids and improve the DNA yield

4. CTAB – DNA precipitation

1. Add salt and alcohol to DNA
2. Centrifuge to get DNA pellet
3. Remove supernatant
4. Resuspend in water



DNA storage

- DNA can be stored at 4°C for short periods (e.g. weeks)
- If you are keeping it for longer, store at -20°C or -70°C
- Try not to freeze-thaw it too often (breaks down strands of DNA, long strands are important for ONT sequencing