Purification of DNA by phenol extraction and ethanol precipitation

- Add 2 ml of **lysis buffer** and then add 5 µl of **proteinase K** and vortex gently.
- Incubate in the water bath at 50 °C for *8 h* or *overnight*.
- Add an equal volume of **phenol** to the DNA containing reaction mixture and vortex gently.
- Centrifugation at 1,000 x g for *15 min*.
- Remove the aqueous phase carefully into a fresh microfuge tube and add equal amount of 1:1 (v/v) **phenol / CIAA.**
- Centrifuge at 1,000 x g for *15 min.*
- Remove the supernatant and add equal amount of **CIAA.**
- Centrifuge at 1,000 g for *15 min.*
- Remove 500 µl in a fresh microcentrifuge tube and add 0.1 volume 3M **sodium acetate** and 2 volumes **100% Ethanol.**
- Mix vigorously by hand (do not vortex) until the precipitation of a sediment.
- Incubate at –20 °C overnight or for shorter periods at –70 °C (e.g. *60 min*).
- Recover the precipitated DNA by centrifugation in the microcentrifuge at 18,000 x g for *30 min* at 4 °C.
- Carefully pour out / aspirate supernatant (do not lose DNA-pellet).
- Carefully add 1 mL cold **70% Ethanol** (do not vortex).
- Spin at 18,000 x g for *20 min* 4°C.
- Carefully pour out / aspirate supernatant (do not lose DNA-pellet).
- Air dry *10 min* at room temperature (do not overdry, because DNA becomes hard to dissolve).
- Dissolve in 100µl ddH_2O because unbuffered DNA undergoes degradation.

Notes :

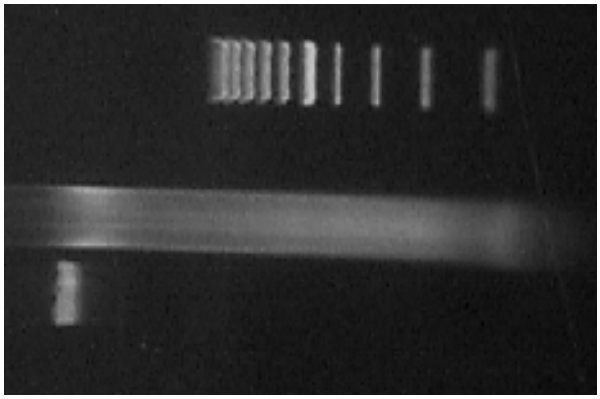
1. **CIAA** → chloroform-isoamyl alcohol (24:1)
2. **Lysis buffer** :
   100mM NaCl 5M
   10mM Tris pH 7,4 1M
   25mM EDTA 0.5M
   0.5% SDS 10%
   H_2O
   store at room temperature.
3. **Proteinase K**: commonly used in molecular biology to digest protein and remove contamination from preparations of nucleic acid. Addition of

Proteinase K to nucleic acid preparations rapidly inactivates nucleases that might otherwise degrade the DNA or RNA during purification. The enzyme is active in the presence of chemicals contained in lysis buffer.
Stored as 20 mg/ml aliquots at -20 C; Can be refrozen a few times.

4. **Phenol** → removes protein.
5. **Chloroform** → removes phenol.
6. **100% Ethanol** → precipitates DNA.
7. **70% Ethanol** → washes out salt.

Purification of DNA in Tuberculosis

Follow the above protocol. Additionally, introduce one extra step between steps 1 and 2 by adding 10 µl of lysozyme at room temperature for 2 hours.



→ DNA ladder 100 bp 5 µl

→ DNA paraffin sample

→ DNA sample

Notes:

Lysozyme disrupts the cell walls of gram positive bacteria. This type of bacteria contains a peptidoglycan covering that functions as a protective barrier. Lysozyme hydrolyzes 1,4-beta-linkages between N-acetylmuramic acid and N-acetyl-D-glucosamine residues in peptidoglycans. When these cross linkages are disrupted, the cell can no longer protect itself from the outside environment.