COOKE AND DUNCAN (1997) PROTOCOL

For Genomic DNA isolation of *phytophthora*

- Four days old young mycelium grown in Reibeiro's liquid medium was filtered through sterile filter paper.
- Dried and weighed
- 0.1g of mycelium was taken in an eppendorf tube with 50mg of sterile sand or glass powder and 10mg PVPP
- 750µl extraction buffer was added to the eppendorf tube
- The mixture was ground
- Centrifuged at 13,000 rpm for 5 minutes.
- After centrifugation the supernatant was taken in a sterile eppendorf
- 500µl Tris phenol: chloroform: isoamyl alcohol (25:24:1) was added to it and inverted gently for 2 minutes.
- Centrifuged at 13,000 rpm for 5 minutes.
- The aqueous layer was transferred to sterile eppendorf tube
- Fill the tube with isopropanol and gently inverted.
- Centrifuged at 13,000rpm for 10 minutes.
- Discarded the supernatant and washed the pellet in 70% ethanol and inverted gently
- Centrifuged at 13,000 rpm for 2 minutes.
- Air dried the pellet
- Resuspended I 100µl sterile distilled water.
- 3µl RNase (5mg/ml) added and incubated for 30 minutes at 37°C.
- Stored at -20°C
- Agarose Gel electrophoresis carried out with 0.8% agarose gel, added 8µl ethidium bromide (10µg/ml) into 200ml 1X TAE buffer.
- DNA quantified by using eppendorf biophotometer.

Stock Chemicals for DNA Isolation

1. 1 M Tris HCl
   - Tris base : 12.1g
   - Distilled water : 100 ml
   - pH : 7.5

2. 5M NaCl
   - NaCl : 29.22g
   - Distilled water : 100 ml

3. 0.5M EDTA
   - EDTA : 18.61g
   - Distilled water : 100 ml
   - pH : 8.0

4. 10% SDS
   - SDS :10 g
   - Distilled water : 100 ml
   - pH : 7.2

5. STE Extraction Buffer (50ml)
   - 1 M Tris HCl : 10 ml
   - 5M NaCl : 2.5 ml
   - 0.5M EDTA : 2.5 ml
   - 10% SDS : 2.5 ml
   - Distilled water : 32.5 ml