Lab (3): Fungal genomic DNA extraction

Introduction

The most difficult steps in the isolation of fungal DNA is to disrupt the cell wall without causing damage to genomic DNA. Most methods use mechanical or enzymatic techniques for the disruption of the cell wall, such as disruption with glass beads, grinding in liquid nitrogen, alternating freezing and thawing, ultrasound in combination with lysis buffer containing (SDS) and microwave radiation

Enzymes can also be used for digesting the cell wall. Depending on the chemical composition of the cell wall, lysozyme, cellulase, or chitinase are used.

(A):DNA extraction by the CTAB method

fungal strains (molds or yeasts) are cultivated on agar plates or broth, incubated (2-3 days at 25^0C), Once a strain had formed a colony, being ready for DNA extraction.

1-Break down cell walls of fungal mycelia by grinding with glass rods or in the presence of liquid nitrogen, **the mycelium is frozen with liquid nitrogen and ground using a mortar**

2- Add 500µl of CTAB extraction buffer and incubate the mixture at 65 ^{0}C for 1hr.

(1 M Tris, pH 8.0, 5 M NaCl, 0.5 M EDTA, CTAB (Cetyltrimethyl ammonium bromide)

3-Purify DNA by adding 500µl of phenol:chloroform:isoamyl alcohol (25 : 24 : 1) and mix well by shaking tubes.

4. Centrifuge for 5-10 minutes at maximum speed.

a. Following centrifugation, you should have three layers: top: aqueous phase, middle: debris and proteins, bottom: chloroform.

b. Go on to the next step quickly so the phases do not remix

5. Pipette off the aqueous phase taking care not to suck up any of the middle or chloroform phases. Pipetting slowly helps with this.

6. Place the aqueous phase into a new labeled eppendorf tube.

7. Estimate the volume of the aqueous phase.

8-precipitate DNA by adding equivalent volume of cold isopropanol, Mix gently.

9. Let sit in freezer for 15 min to overnight.

a. Longer times will tend to yield more DNA, but also more contaminants.

10. Centrifuge for 3 min at maximum speed.

11. Pour or pipette off the liquid, being careful not to lose the pellet with your DNA.

12. Add 700µl of cold 70% Ethanol and mix

13. Centrifuge for 1 min at maximum speed.

14. Pour or pipette off the liquid, being careful not to lose the pellet with your DNA wash with 70% ethanol

15- invert samples on a filter paper and let stand until dry.

16. Resuspend samples with 100µl of TE buffer. Allow to resuspend for 1hr at 55°C or overnight in refrigerator.

(B): DNA extraction by the thermolysis method

1-Add 5 ml of sterile distilled water into agar plate surface of cultivated fungal isolates.

2-fungal spores and hyphae are separated by using sterile loop and collected into test tube.

3-Transfere one ml of spore and mycelia suspension to microfuge tubes.

4- place the tubes in boiling water bath for 60 min.

5-centrifuge the tubes at 13000 r.p.m. and separate the supernatant that contain DNA into new eppendorf tube.