Aspergillus DNA Minipreparation

Solutions:
A. 10mM Tris-HCl, 100mM EDTA, 0.5% SDS pH8.
B. RNase A 10mg/ml in 50mM sodium acetate pH4.5 and heated in a boiling water bath for 5 minutes.

Pour YG into a petri dish, add spores and grow for 20 hours at 37^{O}C.

Harvest growth on a small square of Miracloth, rinse with water, press dry, place in a microfuge tube and freeze on dry ice.

Lyophilize for 1 day after placing microcentrifuge caps with holes punched in them on the tubes.

Transfer freeze dried mycelia to a test tube and grind with pestle, transfer (~50 µl) ground material to a microfuge tube, add 0.7 ml of solution A preheated to 65^{O}C, vortex to disperse dried mycelia and place tube at 65^{O}C for 30 minutes.

It is critical to not try and extract DNA from to much powdered mycelia. If you have 50 to 100 □l powdered mycelia this will give you a reasonable yield of DNA. Trying to extract it from more leads to DNA that will not digest and often is contaminated with nucleases.

Remove the tube from the 65^{O}C water bath and extract 1X with phenol and 1X with phenol: chloroform. Use 10 minute spins for each extraction.

Add 10µl solution B and incubate at 37^{O}C for 30 minutes, extract 1X with phenol: chloroform and then chloroform.

Add 70µl of 3M sodium acetate and fill the tube with ethanol, vortex to mix and incubate 5 minutes at room temperature.

Sediment high molecular weight DNA in microfuge for 5 mins. wash with 70% ethanol and dry.

Dissolve the final DNA pellet in 100µl TE.

Estimate the DNA concentration by running 2-5µl on a minigel so that you digest and run approximately equal amounts of DNA for the different samples.

Digest up to 20µl in a final volume of 30µl and run all of it for a genomic Southern.

from the May Laboratory