Large Scale Preparation of Total DNA from Aspergillus

Solutions:
A. 10mM Tris-HCl, 100mM EDTA
B. 20% Sarkosyl
C. RNase A 10 mg/ml in 50 mM NaOAc pH 4.8 and heated in a boiling water bath for 5 minutes.
D. 20% PEG-6000, 2.5M NaCl

Grow a culture of mycelia in 250-1000 ml of YG overnight.

Harvest mycelia on Miracloth, wash with water, press dry, determine the mass and freeze either in liquid nitrogen or on dry ice.

Lyopholize the frozen mycelia overnight.

Grind the freeze dried mycelia in a mortar and pestle to a fine powder.

Add the powdered mycelia to solution A that is preheated to 65^{o}C at 1 gram wet weight to 10 ml.

Add 1 ml solution B and 0.4 ml solution C per 10 ml and place at 65^{o}C for 30 minutes after mixing.

Add proteinase K to 200 □g/ml and incubate at 37^{o}C for 3 or more hours.

Sediment the debris at 2,000 rpm for 5 minutes in IEC centrifuge.

Extract the supernatant 2X with phenol, 2X with phenol: chloroform: Isoamyl alcohol and 2X with chloroform :isoamyl alcohol.

To the final aqueous phase add an equal volume of solution D and incubate on ice for 1 hr. or overnight at 4^{o}C.

Sediment the precipitate at 9,000 rpm in a swinging bucket rotor for 15 minutes.

Dissolve the pellet in 4.5 ml TE, add 0.5 ml 3M NaOAc and 13 ml ethanol and incubate at $^{-}$80^{o}C for 15 minutes.

Sediment the DNA at 9,000 rpm for 15 minutes in a swinging bucket rotor, wash once and dry.

Dissolve the DNA in TE.

Alternatively you can band the DNA on CsCl ethidium bromide gradients after the proteinase K step.

To do this add 1 gram CsCl per ml of solution and ethidium bromide to 0.5 mg/ml of final volume.

Spin the gradients to equilibrium as for a plasmid.