

## DNA extraction – miniprep from spores for PCR

1. With a loop, pick conidia from a single colony and resuspend in 0.8ml of sterile water until suspension becomes turbid.
2. Vortex and centrifuge to sediment conidia, discard supernatant.
3. Add 0.1ml of buffer1.
4. Add 0.1ml of buffer2. Mix well and incubate for 20 mins at 65°C. Allow to cool at RT.
5. Add 0.1 ml of buffer 3 and mix. Top speed centrifuge at 6°C for 8 min. Without disturbing the pellet transfer 0.25ml of clear supernatant to a new tube and discard the rest.
6. Add 0.15 ml of distilled water. Precipitate DNA with 40ul of isopropanol. Centrifuge at top speed for 20 min at 6°C.
7. Wash with 0.3ml of 70% ethanol. Centrifuge 5min at RT, discard supernatant.
8. Allow the pellet to air dry for some minutes and resuspend pellet (invisible ) in 10 ul of distilled water.
9. Use 2ul per PCR reaction in a final volume of 50ul.

Buffer1 (resuspension)  
50mm Tris-HCl pH 8.0  
10mm EDTA  
100ug/ml Rnase A  
(store at 4°C.)

Buffer2 (lysis)  
200mm NaOH  
1%SDS  
Store RT

Buffer3 (neutralisation)  
3.0M Potassium acetate pH 5.5  
store RT

Method of Dr J A Calera-Abad, kindly supplied by Raquel Lopez:Raquel@imb.usal.es