Extraction of DNA from spores for PCR (M. Peñalva Lab, slightly modified by Oakley lab)

1. Collect the conidiospores from the surface of colonies using a toothpick (Fig. 1) and suspend them in 0.1 ml of breaking buffer (2% Triton X-100, 1% SDS, 100 mm NaCl, 1 mm EDTA, and 10 mm Tris-HCl, pH 8) in screwcap 1.5 ml microcentrifuge tubes. The amount of spores shown on the toothpick in Figure 1 is typical for a single DNA prep. Alternatively spores can be collected with a wire loop wetted with the buffer.

2. Add 150 mg of 0.45-0.5 mm glass beads and vortex for 30 sec.

3. Incubate for 30 min at 65°C (or 70ºC) vortexing 30 sec every 10 min.

4. Add 0.1 ml of phenol/chloroform/isoamyl alcohol, (25:24:1) (This mixture is known as phenol-SEVAG in some countries and Leder-phenol in others).

5. Mix by vortexing for 5 min.

6. Centrifuge at maximum speed in a microcentrifuge for 5 min. The g force is probably not critical as long as the aqueous and Leder-phenol phases are separated.

7. Collect 80 µl from the upper phase and transfer it to a new tube. Dilute an aliquot in 1:10 in TE buffer (10 mM Tris-HCl pH 7.5, 1 mM EDTA) and use 2 µl as a template for a 50 µl PCR reaction.

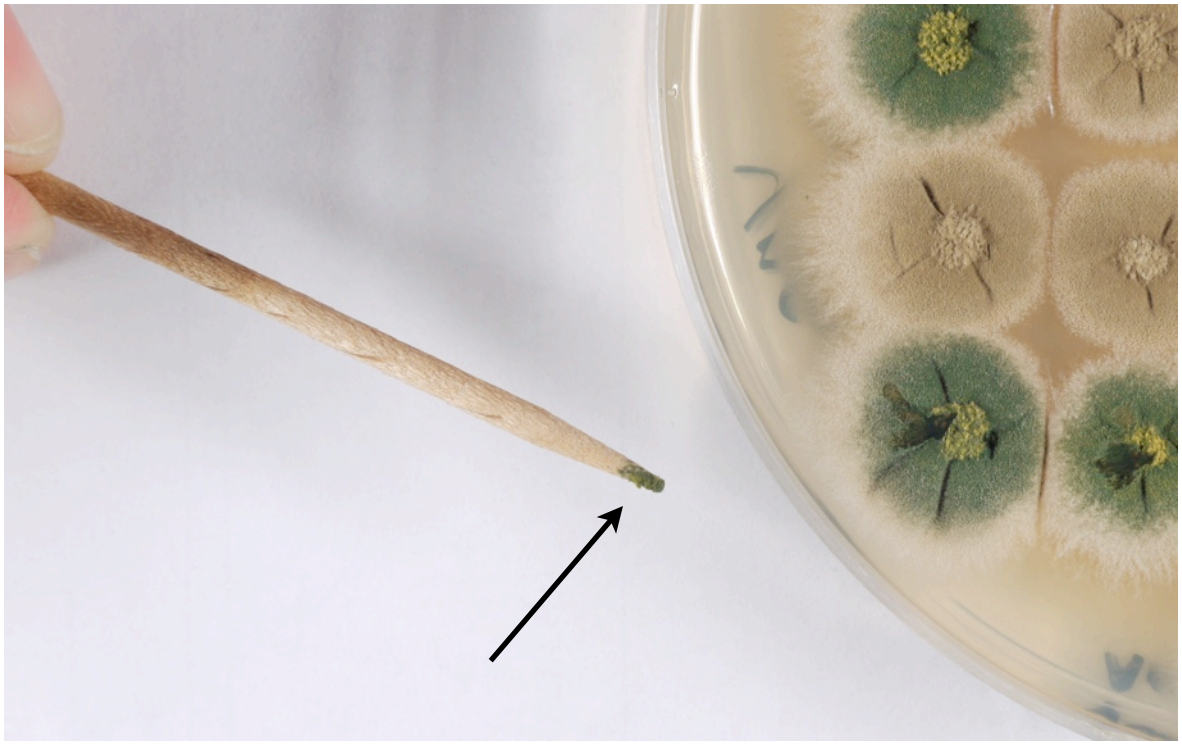
Reference:

Hervás-Aguilar A, Rodríguez JM, Tilburn J, Arst HN Jr, Peñalva MA. (2007) *Evidence for the direct involvement of the proteasome in the proteolytic processing of the Aspergillus nidulans zinc finger transcription factor PacC*. **J Biol Chem**. 282:34735-47.

Notes:

The DNA purification is reliable. The PCR amplification works in the great majority of cases (but not always). The procedure has worked reliably with Platinum taq and Accuprime taq HiFi and with non-commercially produced Taq polymerase. Other enzymes may work, but we have not tried them. The DNA is of high molecular weight (Fig. 2). There is contaminating RNA but much less than is often seen with hyphal preps. It is important to note, however, that the DNA solution does contain SDS and probably some residual phenol. This is the reason for diluting the DNA before using it for PCR. If you use very much of the undiluted DNA solution for a PCR reaction, the SDS and phenol will kill the reaction.

Figure 1. The spores are at the tip of the toothpick (arrow).



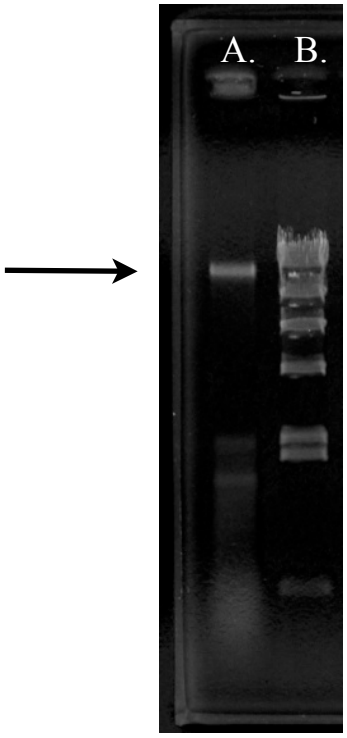


Figure 2. Lane A is 5 µl from a spore DNA extraction (from a total of 80 µl). The DNA (arrow) is of high molecular weight. The lower molecular weight material appears to be RNA with the two prominent bands being ribosomal RNAs. Lane B is bacteriophage λ digested with HinDIII.

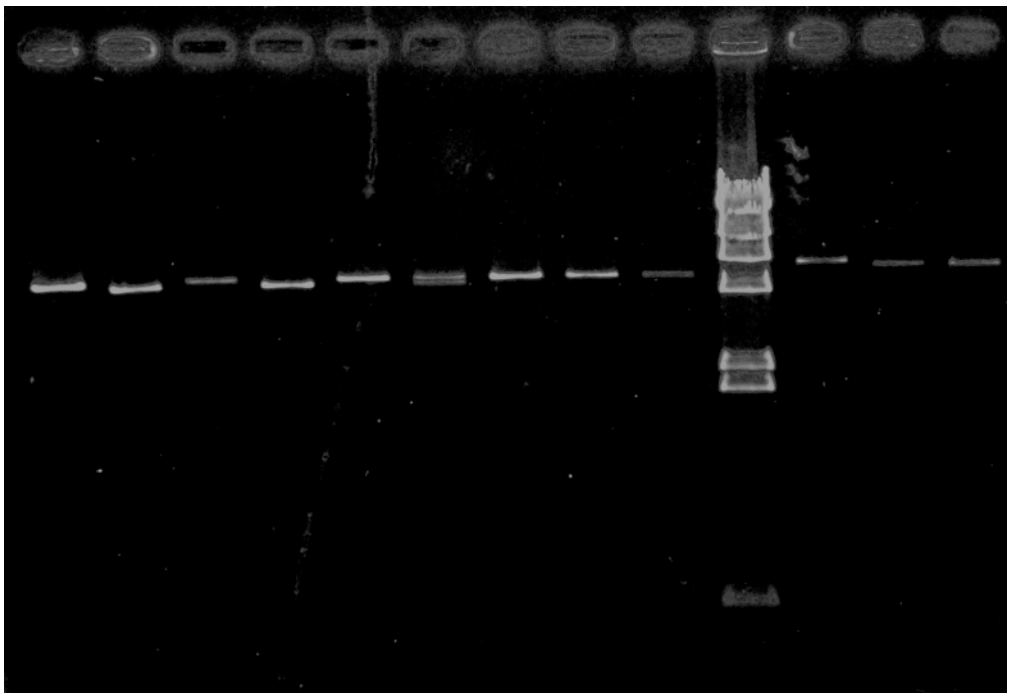


Figure 3. Representative PCR results from DNA produced from spores. The DNA was prepared from six different transformants and consecutive lanes are amplifications with different primer pairs of the same DNA sample. Lanes 1 and 2 are, thus, different amplifications from the same sample. Lane 10 is a bacteriophage λ HinDIII digest.