Spore-PCR on *Aspergillus* conidia

In 1993 Aufauvre-Brown et al performed PCR directly on *A. fumigatus* conidia, but the method has not gained much popularity. We have successfully modified and adapted the method for use with *A. nidulans* and *A. oryzae* conidia. The method allows rapid PCR screening of colonies, for example after transformation or a counter-selection event for loop-out of markers.

Spore-PCR was made directly from conidia. The method generally results in specific reactions with a single PCR product only. The Spore-PCR was mainly performed with *Taq* polymerase (Sigma), however it is also possible to use a low-cost polymerase *Paq* 5000 (Stratagene).

The *Taq* polymerase program was performed on a Perkin Elmer 2400:

1. 94 °C - 15 min
2. 94 °C - 30 sec
3. T_m-5 °C - 30 sec
4. 72 °C - x min (Use 1 min pr. Kb) - Repeat steps 2.-4. 35 times
5. 72 °C - 5 min
6. 12 °C - hold

Load at least 15-20 µL of sample on the agarose gel.

The reaction mixture for a *Taq* based reaction was as follows (25-50 µL reactions have routinely been used); 1x PCR buffer (1.5 mM $MgCl_2$), 0.2 mM dNTPs, primers at 0.2 µM, polymerase 0.03 U/µL.
Spores were picked with a sterile pipette tip and transferred to the PCR reaction mix, ensuring that no PCR solution remains in the tip. Picking spores should be done gently by barely touching the conidia at the periphery of the colonies (the spores on the tip should be almost invisible to the naked eye). The number of spores in the PCR tube should be in the vicinity of and not more than 800 spores per 50 µL. A dilution series of the PCR reaction can be made initially to adjust the picking routine of spores. Furthermore, include a reaction with purified genomic DNA as a control.

The direct transfer avoids additional dilution of conidia in water or buffer, and the long primary denaturation step (15 min.) makes pre-processing of conidia unnecessary.

The procedure described here has proven to be efficient for green *A. nidulans* conidia resulting in an average success rate of 90%, and has worked for several different reactions tested (up to 4 kb reactions has successfully been tested so far). For unknown reasons yellow conidia were not as efficient with a success rate of only 10%, however we are trying to improve this.

Reference:

Aufauvre-Brown et al, 1993. Detection of gene-disruption events in *Aspergillus* transformants by polymerase chain reaction direct from conidiospores. Current Genetics 24: 177-178.

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