Spore-PCR

PCR is made directly on conidia added to the mixed reactions. The method generally shows high specificity giving a single PCR product only. The use of high fidelity DNA polymerases such as PfuX7 or Phusion is highly recommended. The PCR is optimized on *Aspergillus nidulans* spores.

40 µL reactions have shown highest success rate - add per reaction;
1x suitable PCR buffer (8 µL of 5x)
0.2 mM dNTPs (4 µL of 2 mM solutions)
0.25 µM primers (1 µL of 10 µM solution)
~0.25-0.5 U High fidelity DNA Polymerase (PfuX7)
Ultrapure H_2O to a total of 40 µL

Spores are picked with a sterile yellow pipette tip and transferred to the PCR reaction mix, ensuring that no PCR solution remains in the tip. Tooth picks are dry and have a tendency to remove volume from the reactions.
Picking spores should be done by gently touching (the encounter should barely be visible) the conidia/colony.
Dilution series of the PCR reaction after the spores have been added can be made initially to adjust the picking routine and to ensure success. Alternatively, the pipette tip with spores is dipped sequentially (spore gradient) into, e.g. three, PCR tubes containing the reaction mix. This usually gives DNA amplification, a result, in at least one of the three reactions. **Importantly, include a control reaction in the PCR run with reference strain genomic DNA (gDNA) as template DNA and optionally also ref strain conidia, even though you would expect a negative result**.

The recommended program is as follows:

1. Initial denaturation: 98 °C for 15 min
2. 98 °C for 10 sec
3. At least follow the lowest T_m in the primer pair for 30 sec or lower the annealing
4. 72 °C for X min (X = Use 1 min pr. kb[*]) - Repeat steps 2.-4. 35 times for max amplification
5. 72 °C for 5 min
6. Cooling at 12 °C

[*] Though 10 kb products have been obtained with only 3 min extension

Load 15-20 µL of spore-PCR sample (incl. loading dye) on the agarose gel, and maximum 5 µL of the control reaction having gDNA as template.
Note: If colonies of black Aspergilli are tested, make them grow on rich medium, e.g. YPD, which will stall formation of the black pigment inhibitory to the PCR enabling a PCR run on white spores. Yellow pigment, i.e. *yA*Δ strains in *A. nidulans*, also inhibits the reaction, though it is possible with very few spores. Here the spore gradient is recommended.