Aspergillus fumigatus Colony PCR Protocol

1. Harvest a small amount of spores in sterile water

   a. Generally, take 25 ul of sterile water in a 1.5 ml tube and scrape a small spot from a sporulating AF plate with a 1 ml pipet tip. Two scrapes seem to be plenty.
   b. Mix tip with spores in the 1.5 ml tube of water using end of pipette tip. The water should visibly have spores but NOT be a dark color or overloaded with spores, a nice light green is perfect

2. Centrifuge at max speed for 5 minutes. You should see the spores in a nice pellet the size of a pin head or two. Remove supernatant by pipetting, make sure you discard supernatant in ROCCAL.

3. Place the tube of spores (you can put the rack in also) in the microwave and heat on full power for 6 minutes (also place a 500 ml beaker of water to prevent damage to the microwave).

4. Immediately add 40 ul of 1 X TE buffer (pH 8.0) to each tube of spores and vortex vigorously. You can also use EB. Place Immediately on ICE.

5. Then centrifuge at max speed, 13,000 RPM, for 3 minutes. This is your DNA template.

6. Use 5 ul of the DNA template in the PCR reaction. You usually want to also run Beta Tubulin primers, or a primer pair with an amplicon of about the same size as your target amplicon, for each sample as a positive control to confirm that you have PCR quality DNA.

**This protocol works 90% of the time. For some reason, sometimes it does not work, but is a good quick screen for transformants. Results should always be confirmed with PCR from a good DNA extraction and Southern blot. I've found it works best with products < 2 kb.

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