Aspergillus Transformation Protocols

Introduction

A quickoverview of the two different transformation protocols

Materials

- DNA
- Protoplasts
- Falcon tubes
- PCT
- TM molten agar
- TM plates
- ATB

Procedure

Regular protocol

1. Add at MOST 20 uL DNA (or max 25% of protoplast volume) and 100 uL of protoplasts in a 50 mL falcon tube.
2. Incubate in the falcon tube at RT for at least 30 min.
3. Add 1mL of PCT.
4. Gently mix by gently swirling the tube in a circular motion – careful they are fragile. Do not vortex or pipette mix.
5. Incubate for 5 min at RT.
6. Add 3 mL of ATB.
7. Add 12 mL of molten (40-45 °C) TM agar.
8. Immediately pour mixture directly onto TM plates, and swirl to spread mixture evenly.
9. Let the plates settle for a few minutes and incubate at 37 °C for 4 days.

Quick protocol

10. Add at MOST 25 uL (1500-5000 ng; If episomal plasmid, 10-100 ng is sufficient) DNA (or max 25% of protoplast volume) and 100 uL of protoplasts (more if difficult trans) in a 2 ml Eppendorf tube. 1 uL (100 ng) pac6 as positive control.

11. Add 150 µL PCT with large-nozzle pipette tip.
12. Gently mix by swirling – careful the protoplasts are fragile.
13. Incubate 10-30 min at room temperature.
14. Add 250 µL ATB.
15. Distribute transformation mix on osmotic-stabilized selective media and let the agar absorb the mix before incubating