A. gossypii transformation

Things to keep in mind

- Negative controls: 150 µl myc. + 50 µl H_2O
  150 µl myc. + 45 µl H_2O + 5 µl template (digested plasmid)
- Make sure 2 days before that you have enough STM
- Take enough selective plates out of the fridge before you start the transformation
- Cover the filter with an empty petri dish to prevent contamination
- keep mycelium on ice before electroporation, at 30 °C after electroporation

Electroporation Buffer (STM)

- Sucrose 46.2 g in 200 ml H_2O → autoclave
- 5 ml of 1 M Tris pH 7 – 8
- 500 µl of 1 M $MgCl_2$ (sterile filtered)
- fill up to 500 ml
- store at 4 °C

Procedure

1. Inoculate 2 x 100 ml AFM in baffled flasks with a fresh spore prep from 1 plate. Incubate for 16 hours at 30 °C and 150 rpm. Check the quality of the mycelium by microscopy (should not contain vacuoles).

2. Harvest the mycelium by vacuum filtration (paper filter) and wash with 200 ml dH_2O sterile (fill filter 4 times). Don't apply full vacuum to prevent loss of mycelium.

3. Scratch the mycelium off the filter using a sterile spatula and resuspend the mycelium in a 50 ml Falcon containing 40 ml of 50 mM potassium phosphate buffer pH 7.5 containing 25 mM DTT (37 ml H_2O + 2 ml 1M phosphate buffer + 1 ml DTT in H_2O).
   Incubate at 30 °C for 30 min with mild shaking (20 rpm or see-saw shaker).
   The DTT destabilizes the cell wall and makes the mycelia competent.

4. During incubation, prepare 1.5 ml tubes containing 50 µl of transforming DNA. Keep on ice. Put the same amount of electroporation cuvettes (Eurogentec 4 mm) on ice.

5. Harvest the mycelium by filtration. Wash with 200 ml ice-cold electroporation buffer STM (fill filter 4 times) and let it nearly dry after the last washing step.
   The sucrose protects the mycelium during electroporation.

6. Weigh an empty 15 ml Falcon tube, add the mycelium to it, weigh again and add 3.6 ml of ice-cold STM per g of mycelium. Keep the solution on ice.

7. Add 150 µl of competent mycelium to the prechilled DNA tubes using a cut blue pipette tip.

8. Take with you for electroporation:
   - ❍ Ice bucket containing DNA-mycelium mixes and cuvettes
   - ❍ 200 µl and 1 ml pipette (+ tips)
   - ❍ clean tissue to clean the cuvette
   - ❍ small bottle of room temperature AFM (or 30 °C)
   - ❍ labeled 2 ml tubes (room temperature) to put the transformed mycelium

9. Set the electroporator to 100 Ω, 1.5 kV and 25 µF (should result in a pulse length of 2 – 3 ms = time const). If you are superstitious: switch the machine three times on and off ;) Prepare everything else to be fast afterwards

10. Transfer the DNA-mycelium mix to the bottom of the cuvette using the 200 µl pipette. Avoid bubbles. If bubbles are formed, tap the cuvette on the table to get rid of them. Use the tissue to clean the metal sides of the cuvette before pressing it into the electroporation sleigh. Press both buttons until it beeps.

11. Add 1 ml of warm AFM to the cuvette as fast as possible (the faster you are, the more mycelia will survive). Mix and transfer to the labeled 2 ml tubes (room temperature). Be sure to flush the edges of the cuvette to get all of the mycelium.

12. Let the mycelium recover at 30 °C on the see-saw shaker. Open the tubes from time to time to allow gas exchange.

13. After 5 – 6 hours, distribute the content of each tube on three pre-warmed selective plates (300 µl/plate) and spread thoroughly using a sterile 5 ml glass pipette. Let the plates incubate at 30 °C.

The first heterokaryotic colonies can be seen after 24 – 36 hours of incubation.