A. gossypii Verification PCR

Dirty genomic DNA isolation

- Add 0.2 g of 0.5 mm glass beads into a 1.5 ml tube
- Add 200 µl DNA extraction buffer
- Add a two pinhead big piece mycelium from the border of a colony and vortex quickly
- Add 100 µl of water saturated phenol/chloroform
- Vortex for 10 min @ 4 °C
- Centrifuge @ 17000 x g (i.e. 13000 rpm in Eppendorf 5417 R) for 15 min @ RT
- Carefully take 10 µl of the upper aqueous phase and dilute with 90 µl dH_2O

DNA Extraction Buffer

- 50 mM NaCl, 1 mM EDTA pH 8, 10 mM Tris · HCl pH 8, 0.5 % Triton X-100
- 2.922 g NaCl (M_r 58.44 g/mol)
  2 ml 0.5 M EDTA pH 8
  10 ml 1 M Tris HCl pH 8
  50 ml 10 % Trition X-100
  Fill up with dH_2O to 1000 ml
- Autoclave
- Store @ RT

PCR reaction

- 1 µl primer 1 (pmol/µl)
- 1 µl primer 2 (pmol/µl)
- 5 µl "dirty" genomic DNA isolate
- 5 µl dNTPs (2mM each)
- 5 µl 10x PCR Buffer
- 3 µl $MgCl_2$ (25 mM)
- 15 µl Betaine 5 M
- 0.5 µl *Taq* DNA polymerase 5 U/µl
- 14.5 µl dH_2O

PCR Cycle

- 95 °C for 5 min
- 95 °C for 45 s
- 51 °C + 0.3 °C per cycle for 2 min
- 72 °C for 1 min
- Repeat steps 2 – 4 34x
- 4 °C unlimited time