# Genomic DNA isolation from Ashbya gossypii

- Inoculate 25ml of AFM medium (eventually with G418, 50µl of a 100mg/ml solution) with 1cm2 mycelium
- Incubate at 30°C (200rpm) for 48h
- Harvest mycelium by filtration and wash once with 50ml sterile water.
- Resuspend 0.5g mycelium in 5ml SPEZ buffer.
- Incubate for 30 to 60min at 30°C
- Add 500µl 10% SDS and incubate 10min at RT.
- Add 2ml of 3M KOAc to precipitate SDS.
- Incubate for 15min on ice and spin at 10000rpm for 15 min (Sorwall, SA600 rotor).
- The clear supernatant is transferred to fresh tube. Precipitate RNA and DNA with 1 volume isopropanol.
- Incubate for 15min on ice and spin at 10000rpm for 15min.
- Resuspend the pellet in 2.5ml TE (10:10).
- Digest RNAs by adding 25µl of RnaseI (10mg/ml) and incubate 30min at 37°C.
- Add an equal volume of Phenol/Chloroform/Isoamylalcool and mix gently.
- Spin at 10000rpm for 5min. Take the aqueous phase.
- Precipitate DNA with 2.5Volume 100% ethanol (ice cold) with salt.
- Incubate on ice for 15min and spin at 10000rpm for 15min.
- Wash the DNA with 2.5Vol 70% ethanol. Spin for 5min.
- Redissolve the DNA pellet in 500µl-750µl TE (10:1).

**SPE(Z) buffer:**
1M sorbitol
10mM sodium phosphate buffer pH5.8
10mM EDTA pH=8
Zymolyase (1.7mg/ml) to add in the tube.

**SPE buffer (100ml)**
50ml 2M sorbitol
1ml 1M sodium phosphate buffer pH=5.8
1ml 1M EDTA pH=8

**Zymolyase**
Add 85µl of a 15mg/ml solution for 5ml SPE
Or add 26µl of a 50mg/ml solution for 5ml SPE