

TOTAL DNA EXTRACTION (using freeze dried mycelium)

Material list for DNA isolation:

- Cell lysis solution (Biozym, cat.no. D-5002)
- Protein precipitation solution (Biozym, cat.no. D-5003)
- 70% Ethanol (-20°C)
- 2-propanol
- Rnase A (10 mg/ml)
- TE-buffer (pH 8.0)
- Sea sand
- Sterile potters
- Eppendorf table centrifuge
- Vortex
- Eppendorf microtubes (1.5 ml)
- Freeze dried mycelium
- Thermomixer

Material list for estimation of DNA concentration:

- Agarose
- Loadingbuffer (Bromophenolblue/xyleencyanol)(5x)
- Milli Q
- DNA marker (λ HindIII/EcoR1)
- Ethidiumbromide (0.5 ug/ml)
- 0.5 x TBE

Method

- Grind 15-20 mg freeze-dried mycelium in a 1.5 ml microtube with help of a little bit of sea sand. and 450 μ l Cell suspension solution.
- Incubate at 65 °C for 1 hour (450 rpm)
- Add 150 μ l Protein precipitation solution and vortex at high speed for 20 seconds to mix well.
- Centrifuge at 14.000 rpm for 5 minutes to pellet the proteins. The precipitated proteins will form tight white pellet.
- Transfer supernatant to a new 1.5 ml microtube.
- Centrifuge at 14.000 rpm for 5 minutes to pellet the cells. There is probably still a tight pellet formed. Discard the pellet.
- Transfer supernatant to a 1.5 ml microtube. If there was still a pellet formed, centrifuge again en transfer supernatant to a 1.5 ml microtube.

- Add 450 ml isopropanol (2-propanol) to the clear supernatant.
- Mix by inverting gently 50 times (by hand).
- Centrifuge at 14.000 rpm for 5 minutes.
- Remove supernatant. The DNA should be visible as a small white pellet.
- Wash pellet with 200 μ l 70% ethanol (-20°C)
- Remove supernatant and dry pellet in flow cabinet.
- Add 100 μ l TE buffer to pellet.
- When the pellet is dissolved, add 5 μ l 10 mg/ml Rnase and incubate 1 hour 37°C .
- Store DNA at 4°C (for short time) or -20°C (for long time)
- Put 5 μ l DNA together with 5 μ l MQ and 2 μ l loadingbuffer on a 1% agarose gel (with ethidiumbromide). Don't forget to put the DNA marker (λ -HindIII/EcoR1) on gel also.
- Estimate the DNA concentration.