High Molecular Weight DNA isolation protocol

(Judelson lab 2018)

Preparing protoplasts

- Mycelia was digested in 0.4M mannitol, 20 mM KCl, 20 mM MES pH5.7, 10 mM $CaCl_2$ containing 5 mg/ml cellulase (Sigma, from Trichoderma reesi) and 10 mg/ml β-glucanase. Gently shake at 50 rpm for 30 min at room temperature.
- Filter through Miracloth and harvested by centrifugation at 700 x*g* for 4 min.
- Wash pellet three times in 0.4M mannitol, 20 mM KCl, 20 mM MES pH5.7, 10 mM $CaCl_2$ using centrifugation at 700 x*g.*
- Wash once in MCE buffer (1M mannitol, 0.1 M NaCitrate pH 7.0 and 60 mM EDTA).
- Resuspend protoplasts in MCE buffer at 8 x 10^7 per ml.

Embedding protoplasts in agarose plugs

- Precool a plug mold at 4C (Bio-Rad, Cat # 170-3713).
- Prepare 2% low melting temperature agarose in MCE buffer and equilibrated in a 43°C water bath.
- Warm the protoplast suspension at 43°C for 30 s.
- Mix the protoplasts with the low melting agarose in varying proportions, keeping the final concentration of agarose at 0.75%. Mix using a 200 ul pipet tip with the tip cut off to make it "wide-bore", avoiding bubble formation, and transfer to the plug mold. *(try 1.6 parts protoplast to 1 part agarose; 0.8 parts protoplast, 0.8 parts MCE buffer, 1 part agarose: different ratios are tested since it is hard to identify the "ideal" ratio that will both have sufficient physical strength and a good DNA concentration).*
- Allow the plug to solidify at 4°C for at least 45 min.
- Dislodge the plugs (using a plug mold plunger from the Biorad product) and place 5 plugs into a 50 ml conical tube containing 2.5 ml of freshly made lysis buffer (200 µl Proteinase K, 0.5 M EDTA pH 9.3, 1% (w/v) sodium lauroyl sarcosine, 25 µl β-mercaptoethanol). Scale up if you have more plugs.
- Digest overnight at 50°C, and then carefully remove the liquid with a pipette.
- Add 2.7 ml of new proteinase K buffer and incubate for 2 h at 50°C.
- Equilibrate to room temperature for 5 min, and then add 50 µl of DNAase-free RNase, and incubate for 1 h at 37 °C.
- Remove the liquid, and add 10 ml of wash buffer (10 mM Tris pH 8.0, 50 mM EDTA pH 8.0). Gently shake the tube on a rocker at 180 rpm for 30 min. Repeat for a total of four washes.
- Store in the final wash.