

DNA Extraction Protocol

Preparation

Note: freeze-drying the samples will store them longer and better than -80°C

96-well format: (Two mL tube or other size tube protocols are the same with higher volumes of solutions, 2.0 mL tube volume shown.)

1. Place samples in -80°C for at least one hour prior to freeze drying samples.
2. Freeze dry samples in plates, 15 or 50 mL conical tubes (best), or cheesecloth for 1 to 1 ½ days.
3. Deliver small beads [Fisher #11-312B] to individual wells via cover strip (put one large bead [Fisher #11-312D] into 2.0 mL tubes, more beads for 15 or 50 mL tubes)

Grinding

1. Place a spacer between individual plates (6-96 well plates or 2 Beckman racks per run) before grinding.
2. Grind samples in paint shaker for 2 mins.
3. Centrifuge plates for 10 min @ 4000 rpm*. *Put a rubber pads to avoid plates cracking* (No need to centrifuge tubes)

Cell Lysis

1. Using an 8-channel pipette transfer 150 uL (600uL- manual pipette) of 1X Lysis buffer [5% SDS, 50mM EDTA, 50mM Tris, pH 8.0] from a solution basin into wells.
2. Cover with cover strip (make sure it is tight) and vortex for 15 to 20 sec.
3. Centrifuge for one min @ 4000 rpm*. (Not with tubes)
4. Incubate sample plates in a 65°C water bath for 1½ hour to overnight (if needed.)
5. Centrifuge samples for 2 mins @ 4000 rpm*. (Not with tubes)

Protein Precipitation

1. Add 50 uL (200 uL) of 5 M Ammonium Acetate to wells
2. Seal with cover strip and vortex for 20 sec.
3. Centrifuge for 10 min @ 4000 rpm*. (Not with tubes)

DNA Precipitation

1. Transfer supernatant to new well of another rack and add 150 uL (600uL) of 100% isopropanol. With 96 well format, add 2-5 uL of 20 mg/mL glycogen to each well. Mix gently.
2. Centrifuge for 10 min @ 4000 rpm*.
3. Pour off supernatant and carefully drain tubes of excess solution.
4. Add 150 uL (600 uL) of 70-75% ethanol, Centrifuge for 5 min @ 4000 rpm*.
5. Pour off supernatant and drain excess solution.
6. Dry pellets until dry (time varies 30 – 60 min) when using 96 well format or larger tubes. (Dry pellets in 2.0 mL tubes for **only 10 to 15 Min!**) Pellets may be clear so be careful!
7. Add 150 uL of DNASE free water (2.0 mL tubes use 10 mM Tris) to dissolve the DNA pellets. (You may incubate samples at 65°C for 5 to 60 min to help dissolving process)
8. DNA can now be stored in -20°C or -80°C (4°C is alright for short periods of time no more than 2 days)
9. RNASE samples by adding 10 units (1 uL) of RNASE ONE™ and incubating in a 37°C water bath for 1 hour.

YOU ARE DONE EXTRACTING!!

* Refers to 4 arm rotor for Eppendorff Centrifuge 5810R