DNA extraction protocol (Mag-Bind Environmental DNA 96)

Chang-Yu Chang

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Aim

Extract DNA of pellets from isolates using magnetic beads.

Materials

- 96-plate magnetic rack
- Thermomixer (eppendorf)
- Water bath set to 70C
- Deep well plate
- PCR 96 plate
- Mag-Bind Environmental DNA96 kit (Omega Bio-Tek)

Procedure

Preparation steps:

☐ Take 10ml ($100\mu l \times 100 samples$) of elution buffer into a falcon tube and warm it to 70C.

☐ Put **P2** buffer and samples on ice.

☐ Prepare 70% ethanol ($1ml/sample \approx 100ml/plate$)

☐ Make sure VHB buffer has ethanol. Otherwise add 56ml per bottle.

☐ Make mastermix of SLX-Mlus Buffer and RNase A.

- SLX-Mlus Buffer $525\mu l \times 100 = 52.5ml$
- RNase A $2\mu l \times \times 100 = 200\mu l$

Lysis

☐ Briefly centrifuge the E-Z disruptor plate (1 min at 2000 rpm) to remove ceramic beads from the walls of the wells .
☐ Re-suspend pellet samples in $527\mu l$ of mastermix.
☐ Seal the plate with caps.
☐ Shake a maximum speed in the thermomixer for 5min.
☐ Centrifuge at **500rcf** for 10 seconds. Remove and discard caps.
☐ Add $53\mu l$ of **DS Buffer.** Seal the plate with new caps.
☐ Shake a maximum speed in the thermomixer for 1min to mix.

☐ Incubate at 70C shaking very slowly (300 rpm) for 10min.
☐ For gram-positive bacteria, perform a second incubation at 95C for 2min more.
☐ Centrifuge **2500rcf** for **10 min**.
☐ Transfer 200μl suppernatant to a new set of 96-well racked tubes.
☐ Add 67μl of pre-chilled P2 buffer and 100μl of cHTR Reagent (shake before using).
☐ Seal the plate with new caps.
☐ Centrifuge at **2500rcf** for **5 min**.

DNA extraction

☐ Transfer supernatant to a 96-well plate that fits in the magnetic rack. *Our normal plates do not work. I am using the ones from the QIAGEN kit*
☐ Add 1 volume XP1 buffer and 20μl of Mag-bind particles (fridge). Mix by pippetting up and down well.
☐ Mix at room temperature at **500rpm** for **10min**.
☐ Place plate on magnetic rack for 5 minutes, until particles migrate to the bottom. *If particles do not migrate, pipette up and down a little to move them closer to the magnetic field.*
☐ Aspirate and discard cleared supernatant.
☐ Remove plate from magnetic rack and add 500μl of VHB buffer (diluted with ethanol).
☐ Mix for 1min at **2000rpm**.
☐ Place plate in magnetic rack and let sit at room temerature until particles are cleared from solution.
☐ Aspirate and discard the cleared supernatant without distrubing the magnetic beads.
☐ Remove plate from magnetic rack and add 500μl of 70% ethanol.
☐ Mix for 1min at **2000rpm**.
☐ Place plate in magnetic rack and let sit at room temerature until particles are cleared from solution.
☐ Aspirate and discard the cleared supernatant without distrubing the magnetic beads.
☐ Repeat for a second ethanol wash.
☐ Leave plate in magnetic rack for 1 min. Remove any residual liquid with a pipette.
☐ Leave the magnetic beads to dry for 10 minutes (in the magnetic rack).
☐ Add 100μl elution buffer preheated to 70C.
☐ Mix for 5min at **2000rpm**.
☐ Place the plate into magnetic rack until beads are cleared from liquid.
☐ Tranfer supernatant to a PCR skirted plate and seal with film. Store at -20C.