Plate extraction

Plates: Abgene™ 96 Well 0.8mL Polypropylene Deepwell Storage Plate8 (Thermo Fisher Scientific, Catalog number:  AB0765)

Lids: Abgene Storage Plate Caps, strips of 8 (Thermo Fisher Scientific, Catalog number:  AB0981)

1 PREPARATIONS

* Prepare the plate plan
* Leave at least 3 empty wells per plate for controls (extraction control and positive and negative and PCR control for PCR (can be at the end))
* If using several plates, try to distribute samples evenly between the plates so that they have the same weight
* Extract with the salting out method
* Plates and lids for sampling are in one of the cupboards in the corridor in front of the aphid lab, further plates for use during extraction are in the molecular lab

2 EXTRACTION

1. **Aphid sampling**
* Add 1 aphid to each well, close with lid, store at -20°C.
* Include at least one extraction control per plate (well without aphid), leave 2 additional empty wells to use as positive and negative controls for PCR
1. **Add proteinase K and TNES**
* Remove lids taking care not to contaminate or mix up lids to preserve them for future use
* 5 μL proteinase K (10mg / mL)
* Crush each aphid with a clean pipet tip
* add 300μL of TNES to each well
* return lids
* repeat for each column of the plate
* mix (vortex each corner) & spin down (centrifuge)
1. **Incubation**: overnight at 37°C.
2. **Protein Precipitation**: (weigh the plates before centrifugation, readjust with water in the empty wells if they differ by more than 1g)
- Centrifuge quickly
- Remove the lids carefully, preserve for later use
- Add 85μL of 5M NaCl to each well and reapply lids
- vortex or mix by shaking
3. **Centrifugation** : 4°C ; 4000 rpm, 1h (gel room centrifuge)
4. **Preparation of new plates:** during this time, dispense 280μL of (ice-cold) 100% ethanol into the wells of a new storage plate (800μL). Store the plates in the freezer at -20 ° C until use. Use cheap seal film.
5. **Recovery of the supernatant** (spin longer if supernatant is not clear): take 290 μl of supernatant and mix in the new plate by pipetting back and forth. Reapply lids. Leave for 1 hour at -20 ° C, or 15 min at -80 ° C. [Stopping point: leave overnight at -20°C]
6. **Centrifugation** : 4°C ; 4000 rpm, 30 min (gel room centrifuge)
7. **Removal supernatant** by gently turning the plate back over to pour the supernatant into the sink. BEFORE turning over, press some tissue against the open plate, to prevent the supernatant from flowing back into the wrong well. Then, with the tissue still on the plate turn over.Rinse the pellet with 200 μL of 70% (ice-cold) ethanol. Reapply lids.
8. **Centrifugation**: 4°C ; 4000 rpm, 30 min (gel room centrifuge)
9. **Removal of supernatant & air-drying** by gently turning the plate over (see step 9). Air dry for several hours or overnight at RT covered with a tissue to avoid contamination. Conserve lids.
10. **Resuspension** in 100 µl of H2O (adjust depending on intended use), then seal with old lids. Store at 4 ° C (if using the next day) or at -20°C.

**Plate plans**

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