Imperial College London

MULTI-'OMICS ATLAS PROJECT

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DNeasy™ DNA Extraction from Post-Mortem Human Brain Tissue

Adapted from Qiagen user manual

Notes before starting

- Perform all centrifugation steps at room temperature (15-25°C).
- Redissolve any precipitates in Buffer AL and Buffer ATL.
- Add ethanol to Buffer AW1 and Buffer AW2 concentrates.
- Take previously cut tissue out of -80°C (>25mg) and equilibrate to room temperature.
- Preheat thermomixer to 56°C.
- Warm up buffer ATL to 65°C.

Protocol

1. Add 180µl Buffer ATL. Add 20µl proteinase K, mix by vortexing.
2. Let samples lyse overnight on thermomixer (56°C, 300rpm). Write down position of each sample in case labelling fades.
3. Vortex 15s directly before proceeding to the next step.
4. Add 200µl Buffer AL and mix thoroughly by vortexing.
5. Add 200µl ethanol (96-100%). Mix thoroughly by vortexing.
6. Pipette the mixture into a DNeasy Mini spin column, placed in a 2ml collection tube.
7. Centrifuge at ≥6000 x g for 1 min. Discard the flow-through and collection tube.
8. Place spin column in a new 2ml collection tube. Add 500µl Buffer AW1.
9. Centrifuge at ≥6000 x g for 1 min. Discard the flow through and collection tube. Ensure no solution is left on rim.
10. Place the spin column in a new 2ml collection tube, add 500µl Buffer AW2 and centrifuge for 3 mins at 20,000 x g.
11. Discard the flow through and collection tube. Ensure no solution is left on rim.
12. Transfer the spin column in a 1.5ml microcentrifuge tube. Add all sample info on the tube using a permanent and resistant pen.
13. Elute the DNA by adding nuclease-free H_2O to the center of the spin column membrane. Adjust volume of H_2O according to desired end volume.
14. Incubate for 10mins at room temperature (15-25°C)
15. Centrifuge for 1 min at ≥6000 x g.
16. Measure DNA concentration on nanodrop and make a note of data.
17. Measure DNA concentration on Qubit and make a note of data.
18. Store extracted DNA at -20°C.