DNA Isolation from Paraffin Slices

Developed by the laboratories of Randy Johnson and David Looney, 2005

Procedure

Perform this protocol in the fume hood.

1. Add 1 mL of xylene to paraffin tissue section in an Eppendorf tube. Vortex well.
2. Spin at 10,000 *g* for *5 min*, and then aspirate supernatant.
3. Wash ***2 times*** with 1 mL of EtOH, centrifuging at 10,000 *g* for *5 min*, and aspirating supernatant.
4. Centrifuge again briefly and then aspirate remaining EtOH.
5. Add 180 µL of Qiagen buffer ATL and 20 µL of 18 mg/mL Proteinase K (Sigma).
6. Incubate *overnight* at 55°C, vortexing occasionally over the course of several hours.
7. Add 20 µL of 20 mg/mL RNAseA and let the tube sit for *1 min* at room temperature.
8. Add 200 µL of Qiagen buffer AL.
9. Vortex well and incubate for *10 min* in a heating block at 70°C.
10. Add 200 µL of 100% EtOH and vortex well.
11. Load onto Qiamp columns in collection tubes.
12. Centrifuge at 10,000 *g* for *1 min*, and then discard flowthrough volume.
13. Place columns in new collection tubes.
14. Wash with 500 µL of buffer AW1.
15. Briefly centrifuge at 10,000 *g* for *1 min*, and then aspirate flowthrough volume.
16. Add 500 µL of buffer AW2.
17. Centrifuge at 10,000 *g* for *3 min*, and then discard the wash.
18. Place columns in new collection tubes.
19. Add 200 µL of **preheated** buffer AE (70°C).
20. Incubate at room temperature for *1 min*.
21. Centrifuge at 10,000 *g* for *1 min*.
22. Transfer eluant to Eppendorf tubes.
23. Add 1 µL of 10 mg/mL glycogen at 1/10 volume in 3 M NaOAc (pH 5.2) and 2.5 volumes of EtoH. Incubate *overnight* in -20°C freezer.
24. Centrifuge in microfuge at maximum speed for *10 min*, and then remove EtOH.
25. Wash with 500 µL of 70% EtOH.
26. Centrifuge for *5 min*, and then remove EtOH.
27. Centrifuge briefly, and then remove remaining EtOH.
28. Dissolve in H_2O. Measure OD_{260} of 3.5 µL in 66.5 µL H_2O.