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Protocol for DNA extraction from Formalin Fixed Tissue and Tissue Embedded in Paraffin

Methodology received from Dr. Chris Walsh and Modified by Kimberly White
Approved for sharing

(QIAamp DNA FFPE Tissue Kit)
Cat # 56404

Procedure (Paraffin Removal)

1. Trim excess paraffin of sample block
2. ***Cut 10 sections @18um thickness. Thin sections are critical for the procedure.**
    a. First few sections to should be discarded due to their exposure to air
3. Place sections into a 2ml microcentrifuge tube and add 1ml Xylene to sample. Vortex vigorously for 20 seconds.
4. Incubate in xylene with occasional vortexing for 15/20 minutes (or until there is no more paraffin pieces floating around
5. Centrifuge at full speed for 1 minute at room temperature
6. Remove supernatant and add another 1 ml of Xylene and vortex and incubate for a further 5 minutes.
7. Centrifuge at full speed for 2 minutes at room temperature
8. Remove supernatant and add 1ml of ethanol (100%) to the pellet and mix by vortexing. Leave for 1 minute at room temperature.
9. Centrifuge at full speed for 1 minute at room temperature
10. Remove supernatant by pipetting insuring that the pellet remains undisturbed.
11. Add 1ml of ethanol (70%) to the pellet and mix by vortexing. Leave for 1 minute at room temperature.
12. Centrifuge at full speed for 1 minute at room temperature
13. Remove supernatant
14. Remove excess ethanol as best as possible and then leave tube open at room temperature until dry (~15 minutes)

Formalin Fixed Tissue (Not Embedded in Paraffin)

<u>Omit Steps 1-14 for Formalin-Fixed Tissues</u>

*** (10 sections @ 18 um thick)**

Important to start with very thin slices to reverse cross-linked DNA

Wash samples 2X with PBS to remove fixative- After what step?

15. Add 390 ul of Buffer ATL (Qiagen FFPE kit) and 10ul of Proteinase K (NEB 800 units/ml-Molecular Biology grade)—Vortex to resuspend pellet
16. Place at 56^{0}C in Thermomixer R (Eppendorf) at 500rpm for 24 hours.
    (tubes in aluminum heat block on orbital shaker @ 150 rpm O/N-16 hrs)
    (check to see if lysate is clear).
17. Add another 195ul of Buffer ATL and 5ul of Proteinase K and leave on Thermomixer R at 56^{0}C and 500rpm for a further 20 hours.
    (total vol. Buffer ATL/Prot K = 600 ul) (check to see if lysate is clear).

18. Leave sample at room temperature and allow Thermomixer R to reach 90^{0}C
19. Incubate sample at 90^{0}C @ 500rpm for 1 hour (equilibrate QIAamp column to RT during incubation).
20. For RNase –free gDNA, let sample cool RT—add 2ul of RNase A (100 mg/ml) incubate for 2min at RT.
21. Add 600ul Buffer AL (Qiagen) and vortex
    (vol of both Buffer AL & ETOH as to be adjusted to total vol. Buffer ATL lysis buffer)
22. Add 600 ul 100% ethanol and vortex immediately
23. Spin down on benchtop microcentrifuge for ~20s to allow any remaining tissue to pellet at the bottom (if the intact tissue is loaded into column it may block it)
24. Transfer half of solution (excluding PELLET) to QIAamp MinElute column (in a 2ml collection tube) and centrifuge @ 10,000g for 3 min.
25. Discard flow through and add the remaining sample to column and centrifuge again at 10,000g for 3 min.
    a. If the entire sample has not passed through column then repeat spin with higher speed until the entire sample has passed through column.
26. Discard flow through and add 500ul Buffer AW1. Centrifuge @ 6000g for 1 min. Discard flow through
27. Add 500ul Buffer AW2. Centrifuge @ 6000g for 1 min. Discard flow through
28. Dry column by centrifuging at full speed for 3 minutes
29. Place column into a clean 1.5ml low-bind DNA tube (Eppendorf)
30. Add 25ul nuclease-free water directly to membrane of column and leave at room temperature for 2-5 min
31. Centrifuge at full speed for 1 min.
32. Discard column and store 1.5ml low-bind tube (now containing sample) @ -20C.

NOTES:

- QIAamp DNA FFPE Tissue Kit (Qiagen # 56404) (QIAamp columns store @ 4^0C)
- ThermoMixer R –Eppendorf (We attached a heat block to an orbital shaker @ 150 rpm)
- Proteinase K (800 U/ml) (NEB # P8107S)
- RNase A (100 mg/ml) Qiagen #19101)
- Low-bind DNA tubes (Costar #3207)
- Equilibrate QIAamp column to RT during 1hr - 90^0C incubation.
- Remember to adjust Buffer AL & ETOH to total vol of lysis buffer.

Information from Qiagen: For Formalin-Fixed Tissue, omit the first paraffin removal steps and proceed to lysis. They suggest you wash the tissue in PBS 2x to remove fixative, then proceed on with protocol.

Just to note. We tried the PBS pretreatment wash suggested in DNeasy Blood & Tissue kit to no avail. It is important to have the tissue thinly sliced with a microtome in order to reverse the fomalin-fixed crosslinking of DNA.