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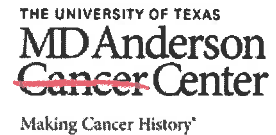

Department of Genomic Medicine

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Division of Cancer Medicine

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Director:

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SOP Title: *Extraction of DNA from FFPE Tissue*

I. Purpose

The purpose of this document is to provide instructions for the extraction of gDNA from FFPE tissue sections using QIAamp DNA FFPE Tissue Kit.

II. Materials

1. Xylene.
2. Ethanol (96%-100%).
3. Water bath @ 56˚C & heating block @ 90˚C.
4. QIAamp DNA FFPE Kit: Proteinase K, Buffer AL (Lysis), Buffer AW1 (Reconstituted), Buffer AW2 (Reconstituted), Buffer ATE (Elution).
5. RNase A (100ng/mL).
6. QIAamp MinElute columns and 2mL collection tubes.

III. Notes

1. The following procedure is employed to extract gDNA from FFPE tissue sections using QIAamp DNA FFPE Tissue Kit.
2. RNA may be co-purified with the DNA, which may inhibit downstream enzymatic reactions, although it does not affect PCR. For this reason, RNase A (100mg/mL) stock solution is applied.
3. Chemical waste should never be poured down the sink.

IV. Procedure

1. Scrape the FFPE tissue from the slides using a clean razor and pair of tweezers. Move the tissue into a labeled, 1.5-mL microcentrifuge tube.
2. In the fume hood, add 1mL xylene. Invert the tubes to mix – do NOT vortex. Incubate for 30 minutes at room temperature.
3. Centrifuge at full speed (15,000 rpm) for 3 minutes at room temperature.

4. Remove supernatant, NOT PELLET.
5. Repeat step 1-3 until the FFPE sample loses its structural integrity, which typically takes about 1-2 intervals.
6. Add 1mL ethanol and Incubate for 30 minutes at room temperature.
7. Centrifuge at full speed for 3 minutes at room temperature.
8. Remove supernatant i.e., carefully remove residual ethanol using a fine pipet tip.
9. Open the tube and incubate sample at room temperature for 10 minutes or until all residual ethanol has evaporated.
10. Re-suspend the pellet in 180µL Buffer ATL. Add 20µL proteinase K and mix by gentle vortexing.
    a. If the pellet is large and is not sufficiently covered by Buffer ATL, add an additional 180µl Buffer ATL to those tubes.
11. Incubate at 56˚C for approximately 15 hours or until the sample has been completely lysed.

    a. If the sample isn't completely lysed after overnight incubation, add 20µL proteinase K (those with 180µL Buffer ATL) or 80µL proteinase K (those with 360µL Buffer ATL). Incubate 1-2 hours or until samples is lysed completely.
12. Incubate at 90˚C for 1 hour to allow Buffer ATL to partially reverse formaldehyde modification of nucleic acid.
13. Centrifuge briefly to remove drops from inside of the lid.
14. Allow the sample to cool to room temperature. Add 4µL RNase A (100 mg/mL) and incubate for 2-5 minutes at room temperature.
15. Add 200µL Buffer AL to the sample and immediately mix thoroughly by vortexing. Then add 200µL ethanol and immediately mix again thoroughly by vortexing.
16. Centrifuge briefly to remove drops from inside the lid.
17. Transfer the entire lysate to the MinElute column, close the lid, and centrifuge at 10,000 rpm for 1 minute. Place the column in a clean 2mL collection tube, and discard the collection tube containing the flow-through.
18. Add 500µL Buffer AW1, incubate for 5 minutes at room temperature and centrifuge at 10,000 rpm for 1 minute. Place the column in a clean collection tube, and discard the collection tube containing the flow-through.
19. Add 500µL Buffer AW2, incubate for 5 minutes at room temperature and centrifuge at 10,000 rpm for 1 minute. Place the column in a clean collection tube, and discard the collection tube containing the flow-through.
20. Centrifuge at 14,000 rpm for 3 minutes to dry the membrane completely.
21. Place the column in a clean 1.5mL microcentrifuge tube and discard the collection tube containing the flow-through. Apply 12.5µL Buffer ATE to the membrane and incubate for 10 minutes at room temperature. Centrifuge at 12,500 rpm for 1 minute.

22. Apply 7.5µl Buffer ATE to the membrane and incubate for 10 minutes at room temperature. Centrifuge at 12,500 rpm for 1 minute.
23. Immediately place the samples on ice. Store DNA at -20°C.

V. Definitions

N/A

VI. Related Documents

Genomics Laboratory

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