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Department of Genomic Medicine

Cancer Immune Monitoring and Analysis Center

Division of Cancer Medicine

The University of Texas MD Anderson Cancer Center

Director:

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SOP Title: *Manual DNA Isolation from Frozen Tissue using 50mL Tubes and the QIAamp DNA Mini Kit*

I. Purpose

The purpose of this procedure is to extract DNA from frozen tissue using the QIAamp DNA Mini Kit.

II. Materials

1. Qiagen QIAamp DNA Mini Kit (catalog # 51304)
2. 70% Ethanol
3. Dry ice
4. KimWipes
5. Spatula
6. Centrifuge
7. RNase A (Qiagen Catalog #: 949014)
8. Ice buckets
9. Homogenizer

III. Notes

Before performing protocols, set two water baths, one to 56°C and the other to 70°C. QIAamp Mini spin columns and buffers can be stored dry at room temperature (15–25°C) for up to 1 year without showing any reduction in performance.

Lyophilized QIAGEN Protease can be stored at room temperature (15–25°C) for up to 12 months without any decrease in performance. For storage longer than 12 months or if ambient temperatures constantly exceed 25°C, QIAGEN Protease should be stored dry at 2–8°C.

IV. Preparation

1. Collect enough dry ice to fill the ice bucket ¾ full.
2. Clean tweezers and spatulas thoroughly with 70% ethanol and allow them to dry.
3. Label the required number of weigh boats and place in container filled with dry ice.
4. Once the weight boat is frozen, place it on a precision balance and zero out the scale.
5. Place the frozen tissue in the frozen weigh boat and weigh again, determining the amount of tissue.
6. If there is more than 25 mg of tissue, cut the tissue and use a smaller portion. Tissue larger than 25mg will hinder the extraction.

V. Homogenization and extraction

1. Add 1080µl Buffer ATL to each 50-mL tube.
2. Add each piece of tissue to the 50-mL tube and homogenize until the tissue is completely ground.
3. Add 120µl proteinase K, mix by flicking the tube and incubate at 56°C until the tissue is completely lysed. The lysis time varies depending on the type of tissue processed but is usually complete in 1-3hrs. To ensure efficient lysis, flick the tubes 2-3 times per hour during incubation.
4. After lysis is complete, add 24µl RNase A (100mg/ml), mix by pulse-vortexing for 15 seconds and incubate for 2 mins at room temperature. Briefly centrifuge the 50-mL tube to remove drops from inside the lid before adding 1200µl Buffer AL to the sample. Mix again by pulse-vortexing for 15 seconds and incubate at 70°C for 10 minutes. Briefly centrifuge again to remove drops from inside the lid.
5. Add 1200µl ethanol (96-100%) to the sample and mix by pulse-vortexing for 15 seconds. After mixing, briefly centrifuge the 50-mL tube to remove drops from the lid.
6. Carefully apply 680µl of the mixture from step 6 (including the precipitate) to the QIAamp Mini spin column (in a 2 ml collection tube) without wetting the rim. Close the cap, and centrifuge at 6000 x g (8000 rpm) for 1 min. Place the QIAamp Mini spin column in a clean 2 ml collection tube (provided), and discard the tube containing the filtrate.
7. Continue to apply the sample mixture to the column until all is depleted – this should take 5-6 rounds of centrifugation. It is essential to apply all of the precipitate to the QIAamp Mini spin column.
8. Carefully open the QIAamp Mini spin column and add 500µl Buffer AW1 without wetting the rim. Close the cap and centrifuge at 8000 rpm for 1 minute. Place the QIAamp Mini spin column in a clean 2 ml collection tube and discard the collection tube containing the filtrate.

9. Carefully open the QIAamp Mini spin column and add 500µl Buffer AW2 without wetting the rim. Close the cap and centrifuge at full speed for 3 minutes.
10. Place the QIAamp Mini spin column in a new 2ml collection tube and discard the old collection tube with the filtrate. Centrifuge at full speed for 1 minute.
11. Place the QIAamp Mini spin column in a clean 1.5 ml microcentrifuge tube and discard the collection tube containing the filtrate. Carefully open the QIAamp Mini spin column and add 35µl Buffer AE and incubate at room temperature for 10 minutes.
12. Centrifuge at 12,000 rpm for 1 minute.
13. Add 25ul Buffer AE to the spin column and incubate at room temperature for 10 minutes.
14. Centrifuge at 12,000 rpm for 1 minute. Briefly vortex the sample and store on ice.

VI. Related Documents

Qiagen Supplementary Protocol: QIAamp DNA Mini and Blood Mini Handbook 11/2007

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