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Protocol for DNA extraction from Frozen Samples

Agilent's recommended procedure to isolate genomic DNA (gDNA) from frozen tissue using the Qiagen DNeasy Blood & Tissue Kit (p/n 69504).

1 Equilibrate a thermomixer to 55°C and heat block or water bath to 70°C.

2 Cut up to 25 mg frozen tissue (up to 10 mg for spleen tissue) into small pieces, place into a 1.5 mL microfuge tube, and add 180 µL Buffer ATL.

3 Add 20 µL proteinase K, mix well on a vortex mixer, and incubate in a thermomixer at 55°C shaking at 450 rpm until the tissue is completely lysed. Lysis time varies depending on the type of tissue processed. Usually lysis is complete in 1 to 3 hours. If it is more convenient, samples can be lysed overnight.

4 Let the sample cool to room temperature and spin in a microcentrifuge for 30 seconds at 6,000 x g to drive the contents off the walls and lid.

5 Add 4 µL of RNase A (100 mg/mL), mix on a vortex mixer, and incubate for 2 minutes at room temperature. Spin in a microcentrifuge for 30 seconds at 6,000 x g to drive the contents off the walls and lid.

6 Add 200 µL Buffer AL to each sample, mix thoroughly on a vortex mixer, and incubate at 70°C for 10 minutes in a heat block or water bath. Spin in a microcentrifuge for 30 seconds at 6,000 x g to drive the contents off the walls and lid.

7 Add 200 µL 100% ethanol to each sample, and mix thoroughly on a vortex mixer. Spin in a microcentrifuge for 30 seconds at 6,000 x g to drive the contents off the walls and lid.

8 Transfer the sample mixture onto a DNeasy Mini spin column placed in a 2 mL collection tube (provided). Centrifuge at 6,000 x g for 1 minute. Discard the flow-through and collection tube. Place the DNeasy Mini spin column in a new 2 mL collection tube (provided).

9 Before using for the first time, prepare Buffer AW1 by adding 100% ethanol to the Buffer AW1 bottle (supplied with Qiagen DNeasy Blood & Tissue Kit; see bottle label for volume). Mark the appropriate check box to indicate that ethanol was added to the bottle.

10 Add 500 µL Buffer AW1 onto the column, and spin in a microcentrifuge for 1 minute at 6,000 x g. Discard the flow-through and collection tube. Place the DNeasy Mini spin column in a new 2 mL collection tube (provided).

11 Prepare a fresh 80% ethanol solution by adding 40 mL 100% ethanol to 10 mL nuclease-free water.

12 Add 500 µL 80% ethanol onto the column, and centrifuge for 3 minutes at 20,000 x g to dry the DNeasy membrane. Discard the flow-through and collection tube.

13 Place the DNeasy Mini spin column in a clean 1.5 mL microcentrifuge tube, and pipet 200 µL of nuclease free water directly onto the center of the DNeasy column membrane.

14 Incubate at room temperature for 1 minute, and then spin in a microcentrifuge for 1 minute at 6,000 x g to elute the DNA.

15 Repeat elution with nuclease free water once as described in step 13 and step 14. Combine the duplicate samples in one microcentrifuge tube for a final volume of 400 µL.

16 Measure gDNA concentration and purity, and analyze on an agarose gel As described in "Step 2. gDNA Quantitation and Quality Analysis"

Step 2. gDNA Quantitation and Quality Analysis

Accurate assessment of gDNA quantity and quality are crucial to the success of an Agilent Oligo aCGH experiment. High quality gDNA should be free of contaminants such as carbohydrates, proteins, and traces of organic solvents, and should also be intact with minimal degradation. gDNA isolated from FFPE samples typically exhibits varying degrees of degradation depending on the age of the tissue and the paraffin embedding protocol used.

Use the NanoDrop ND-1000 UV-VIS Spectrophotometer (or equivalent) to assess gDNA concentration and purity. Use the agarose gel electrophoresis to assess gDNA intactness and the average molecular weight for each sample.

This information will be important for the subsequent labeling reaction.

1 Select **Nucleic Acid Measurement**, then select **Sample Type** to be **DNA-50**.

2 Use 1.5 µL of nuclease free water to blank the instrument.

3 Use 1.5 µL of each gDNA sample to measure DNA concentration. Record the gDNA concentration (ng/µL) for each sample. Calculate the yield (µg) by multiplying DNA concentration (ng/µL) by the sample volume (that is, 100 µL) and dividing by 1000.

4 Record the **A260/A280** and **A260/A230** ratios. High-quality gDNA samples should have an **A260/A280** ratio of 1.8 to 2.0, indicating the absence of contaminating proteins, and an **A260/A230** ratio of >2.0, indicating the absence of other organic compounds such as guanidinium isothiocyanate, alcohol and phenol as well as cellular contaminants such as carbohydrates.

5 Load 20 ng gDNA for each sample in a volume of 10 µL nuclease-free water in the well of a single-comb 1.2% Clear E-Gel. (No need to add loading buffer in this system).

6 As a control, load 20 ng of commercial Human Genomic DNA in a volume of 10 µL nuclease free water in one of the wells of the E-Gel.

7 Mix 5 µL TrackIt 1 Kb DNA Ladder with 95 µL deionized water and load 10 µL of the diluted ladder in one of the wells of the E-Gel.

8 Run the gel for 30 minutes as described in Invitrogen's instructions.

9 Open the gel cassette with E-Gel Opener as described in Invitrogen's instructions.

10 Stain the gel with SYBR Gold Nucleic Acid Gel Stain (diluted 1:10,000 by adding 10 µL of SYBR Gold Nucleic Acid Gel Stain to 100 mL of nuclease-free water) in a plastic tray for 15 minutes.

11 Visualize the gel on the UV-transilluminator using a SYBR Gold photographic filter.

For further information or a quote, please contact m.herman@dnavision.be