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## **Introduction**

### **General Requirements for Human Genomic DNA**

- 1** DNA must be double-stranded (not single-stranded). This requirement relates to the restriction enzyme digestion step in the protocol.
- 2** DNA must be free of PCR inhibitors. Examples of inhibitors include high concentrations of heme (from blood) and high concentrations of chelating agents (i.e., EDTA). The genomic DNA extraction/purification method should render DNA that is generally salt-free because high concentrations of certain salts can also inhibit PCR and other enzyme reactions.
- 3** DNA must not be contaminated with other human genomic DNA sources, or with genomic DNA from other organisms. PCR amplification of the ligated genomic DNA is not human specific, so sufficient quantities of non-human DNA may also be amplified and could potentially result in compromised genotype calls. Contaminated or mixed DNA may manifest as high detection rates and low call rates.
- 4** DNA must not be highly degraded. For any particular SNP, the genomic DNA fragment containing the SNP must have Nsp I (or Sty I) restriction sites intact so that ligation can occur on both end of the fragment and PCR can be successful. The approximate average size of genomic DNA may be assessed on a 1% or 2% agarose gel using an appropriate size standard control. High quality genomic DNA will run as a major band at approximately 10-20 kb on the gel; assay performance may vary with

DNA that is substantially more degraded.

**5** Pre-amplified genomic DNA has been tested and found to give results comparable to the standard DNA preparation methods. The Repli-G® Kit (whole genome amplification kit; QIAGEN) was used to amplify 10 ng genomic DNA

### **Protocol for DNA extraction from Frozen Samples**

Affymetrix'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  $\mu$ L 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  $\mu$ L 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  $\mu$ L 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  $\mu$ L 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  $\mu$ L.

**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 Affymetrix 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  $\mu\text{L}$  of nuclease free water to blank the instrument.
- 3** Use 1.5  $\mu\text{L}$  of each gDNA sample to measure DNA concentration. Record the gDNA concentration ( $\text{ng}/\mu\text{L}$ ) for each sample. Calculate the yield ( $\mu\text{g}$ ) by multiplying DNA concentration ( $\text{ng}/\mu\text{L}$ ) by the sample volume (that is, 100  $\mu\text{L}$ ) 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  $\mu$ L 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  $\mu$ L nuclease free water in one of the wells of the E-Gel.
- 7** Mix 5  $\mu$ L TrackIt 1 Kb DNA Ladder with 95  $\mu$ L deionized water and load 10  $\mu$ L 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  $\mu$ L 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.

### **Step 3. gDNA Cleanup (optional)**

If a genomic DNA preparation is suspected to contain inhibitors, the following cleanup procedure can be used:

- 1** Add 0.5 volumes of 7.5 M NH<sub>4</sub>OAc, 2.5 volumes of absolute ethanol (stored at –20°C), and 0.5 µL of glycogen (5 mg/mL) to 250 ng genomic DNA.
- 2** Vortex and incubate at –20°C for 1 hour.
- 3** Centrifuge at 12,000 x g in a microcentrifuge at room temperature for 20 minutes.
- 4** Remove supernatant and wash pellet with 0.5 mL of 80% ethanol.
- 5** Centrifuge at 12,000 x g at room temperature for 5 minutes.
- 6** Remove the 80% ethanol and repeat the 80% ethanol wash one more time.
- 7** Re-suspend the pellet in reduced EDTA TE buffer (10 mM Tris, pH 8.0, 0.1 mM EDTA, pH 8.0).

*For further information or a quote, please contact [m.herman@dnavision.be](mailto:m.herman@dnavision.be)*