



25, ave Georges Lemaître - B-6041 Gosselies (Belgique)  
Tel : +32 (0)71 37 85 27 - Fax : +32 (0)71 34 78 79

## **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 FFPE Samples**

### **Step 1. Paraffin Removal**

- 1** Equilibrate a heat block or water bath to 90°C and a thermomixer to 37°C.
- 2** Place up to 5 20-micron FFPE sections into a 1.5 mL nuclease-free microfuge tube.
- 3** Prepare 10% Tween 20, by adding 100 µL Tween 20 to 900 µL of nuclease-free water. Solution can be prepared in advance and stored up to 6 months at room temperature.
- 4** Add 480 µL PBS and 20 µL 10% Tween 20 to the FFPE sections in the 1.5 mL nuclease-free microfuge tube.
- 5** Transfer the sample tube to a circulating water bath or heat block at 90°C. Incubate at 90°C for 10 minutes.
- 6** Spin immediately for 15 minutes at 10,000 x g in a microcentrifuge.
- 7** Place the sample tube on ice for 2 minutes.
- 8** Remove wax disc with a pipette tip or tweezers. Remove and discard the supernatant without disturbing the pellet.
- 9** Add 1 mL of 100% ethanol to the pellet and vortex briefly.
- 10** Spin for 5 minutes at 10,000 x g in a microcentrifuge.
- 11** Remove ethanol without disturbing the pellet and let the sample tube sit at room temperature with the lid open until residual ethanol has completely evaporated.
- 12** Prepare a 1M NaSCN solution by adding 10 g of NaSCN to 123 mL of nuclease free water. Solution can be prepared in advance and stored up to 1 month at room temperature.
- 13** Add 400 µL 1M NaSCN to the dry pellet and briefly mix on a vortex

mixer.

- 14** Transfer the sample tube to a thermomixer at 37°C. Incubate overnight at 37°C shaking at 450 rpm.



## **Step 2. Proteinase K Treatment**

- 1** Equilibrate a thermomixer to 55°C.
- 2** Transfer the sample tube to a microcentrifuge. Spin for 20 minutes at 10,000 x g.
- 3** Remove and discard the supernatant without disturbing the pellet.
- 4** Add 400 µL PBS to the pellet and vortex briefly.
- 5** Spin again for 20 minutes at 10,000 x g in a microcentrifuge.
- 6** Remove and discard the supernatant without disturbing the pellet.
- 7** Add 360 µL of Qiagen buffer ATL (supplied with Qiagen DNeasy Blood & Tissue Kit).
- 8** Add 40 µL proteinase K (supplied with Qiagen DNeasy Blood & Tissue Kit), mix well on a vortex mixer, and incubate overnight in a thermomixer at 55°C shaking at 450 rpm.
- 9** Transfer the sample tube to a microcentrifuge. Spin for 30 seconds at 6,000 x g to drive the contents off the walls and lid.
- 10** Add 40 µL proteinase K, mix well on a vortex mixer, and incubate in a thermomixer for approximately 6 to 8 hours at 55°C shaking at 450 rpm.
- 11** At the end of the day, transfer the sample tube to a microcentrifuge and spin for 30 seconds at 6,000 x g to drive the contents off the walls and lid.
- 12** Add 40 µL proteinase K, mix well on a vortex mixer, and incubate Overnight in a thermomixer at 55°C shaking at 450 rpm.

### **Step 3. gDNA Extraction**

- 1** Equilibrate a heat block or water bath to 70°C.
- 2** Let samples 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.
- 3** Add 8 µL of RNase A (100 mg/mL), mix on a vortex mixer, and incubate for 2 minutes at room temperature. Transfer the sample tube to a microcentrifuge and spin for 30 seconds at 6,000 x g to drive the contents off the walls and lid.
- 4** Add 400 µL Buffer AL (supplied with Qiagen DNeasy Blood & Tissue Kit), mix thoroughly on a vortex mixer, and incubate in a circulating water bath or heat block at 70°C for 10 minutes. Transfer the sample tube to a microcentrifuge and spin for 30 seconds at 6,000 x g to drive the contents off the walls and lid.
- 5** Add 440 µL 100% ethanol, and mix thoroughly on a vortex mixer. Transfer the sample tube to a microcentrifuge and spin for 30 seconds at 6,000 x g to drive the contents off the walls and lid.
- 6** Place two DNeasy Mini spin columns in two clean 2 mL collection tubes (provided). Split the entire sample mixture onto two DNeasy Mini spin columns (i.e. 660 µL each).
- 7** Spin in a microcentrifuge for 1 minute at 6,000 x g. Discard the flow-through and collection tube. Place the DNeasy Mini spin columns in fresh 2 mL collection tubes (provided).
- 8** 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 appropriate check box to indicate that ethanol was added to the bottle.

- 9** Add 500  $\mu$ L Buffer AW1 onto each spin column, and centrifuge for 1 minute at 6,000 x g. Discard the flow-through and collection tube. Place the DNeasy Mini spin columns in fresh 2 mL collection tubes (provided). Use 2 DNeasy Mini spin columns per sample to prevent clogging.
- 10** Prepare a fresh 80% ethanol solution by adding 40 mL 100% ethanol to 10 mL nuclease-free water.
- 11** Add 500  $\mu$ L 80% ethanol onto each column, and spin in a microcentrifuge for 3 minutes at 20,000 x g to dry the column membrane. Discard the flow-through and collection tube.
- 12** Place the DNeasy Mini spin column in a clean 1.5 mL microcentrifuge tube, and add 50  $\mu$ L of nuclease free water directly to the center of each spin column.
- 13** Let stand at room temperature for 1 minute, and then spin in a microcentrifuge for 1 minute at 6,000 x g to elute the DNA.
- 14** Combine the purified DNA from the same sample in one microcentrifuge tube with a final total volume of 100  $\mu$ L.

**CAUTION**

Do *not* use Buffer AW2 supplied with the Qiagen DNeasy Blood & Tissue Kit for the subsequent step because salt from Buffer AW2 will interfere with the subsequent labeling reaction.

## **Step 4. gDNA Cleanup**

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).

## Step 4. 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  $\mu\text{L}$  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.

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