

DNA Preparation of test material for CGH analysis

The sample used to prepare the test DNA should have high tumor cellularity (> 50%) for the CNAs to be detectable by aCGH. Alternatively, solid tumor tissue can be enriched through amplification techniques or microdissection from frozen or paraffin sections. Material from hematologic malignancies can be enriched by flow sorting for specific markers.

The DNA preparation of cells and blood is based on Qiagen FlexiGene DNA kit protocol (Cat# 51206).

The complete protocols are included in the Qiagen FlexiGene DNA kits. For specific details on DNA preparation of cells and blood, see the sections “Protocol for Isolation of DNA from 4-14 ml Whole Blood” and “Protocol for isolation of DNA from 1-2 x 10⁶ Cultured Cells” in the handbook

The DNA preparation of tissue and tumors is based on Qiagen Dneasy Tissue kit protocol (Cat# 69504).

The complete protocol is included in the Qiagen Dneasy Tissue kits. For specific details on DNA preparation of tissue and tumors, see the section “Dneasy Protocol for Animal Tissue” in the handbook.

DNA Extraction from Formalin-Fixed Paraffin-Embedded (FFPE) Tissue is based on Puregene (Gentra D70KA) DNA purification protocol.

Paraffin removal:

- 1 Place 5-10 mg finely minced tissue (10-20µm FFPE curls) into a 1.5 ml tube. Add 300µl Xylene and incubate 5 minutes at room temperature with gentle shaking.
- 2 Centrifuge 13,000-16,000 x g for 1-3 minutes to pellet tissue. Discard xylene.
- 3 Repeat above steps twice (for a total of three washes).
- 4 Add 300µl 100% ethanol and incubate with constant mixing at room temperature.
- 5 Centrifuge at 13,000-16,000 x g for 1-3 minutes to pellet tissue. Discard ethanol.
- 6 Repeat above steps (for a total of two ethanol washes).

Cell lysis:

- 7 Add 300 µl Cell lysis solution and homogenize using 30-50 strokes with a Kontes microfuge tube pestil (Fisher# K749521-0590).

- 8 Add 1.5µl Proteinase K solution (20mg/ml) to the lysate, mix by inverting twenty-five times and incubate at 55°C for 3 hours to overnight. If tissue is not completely digested after overnight incubation, add an additional 1.5µl Proteinase K solution and continue incubation for 3 hours to overnight. If possible, invert tube periodically during incubation.

RNase treatment:

- 9 Add 1.5µl RNase (4mg/ml) to cell lysate.
- 10 Mix the sample by inverting the tube 25 times, and incubate 15-60 minutes at 37°C

Protein Precipitation:

- 11 Cool sample to room temperature
- 12 Add 100µl Protein precipitation solution to cell lysate
- 13 Vortex vigorously at high speed for 20 seconds.
- 14 Centrifuge at 13,000-16,000 x g for 3 minutes. The precipitated proteins will form a tight pellet. If the protein pellet is not tight, re vortex, cool on ice for 5 minutes and re-centrifuge as above.

DNA Precipitation:

- 15 Pour the supernatant containing the DNA (leaving behind the precipitated protein pellet) into a clean 1.5 ml microfuge tube containing 300 µl isopropanol. If the DNA yield is expected to be less than 1 µg, add a DNA carrier such as glycogen. Add 0.5µl glycogen solution (20mg/ml Gentra R-5010) per 300 µl isopropanol.
- 16 Mix by inverting 50 times.
- 17 Centrifuge 13,000-16,000 x g for 5 minutes.
- 18 Pour off supernatant and drain tube on clean absorbent paper. Add 300µl 70% ethanol and invert tube to wash pellet.
- 19 Centrifuge at 13,000-16,000 x g for 1 minute. Carefully pour off ethanol.
- 20 Invert and drain tube on clean absorbent paper and air dry 10-15 minutes.

DNA hydration and storage

- 21 Add 20µl DNA hydration solution.

- 22 Rehydrate DNA by incubating 1 hour at 65°C or overnight at room temperature. Tap tube periodically.
- 23 Following resuspension, all DNA samples are quantitated with a NanoDrop ND1000 spectrofluorimeter (NanoDrop Technologies) or equivalent method and stored at 4°C.