

Genomic DNA extraction

Tumor tissue and matched normal genomic DNA samples were extracted from whole blood and osteosarcoma tissue biopsy specimens, respectively, using the salting-out method (Miller et al. 1988). To extract genomic DNA from whole blood, 500 μ l of Reagent A (10 mM Tris-HCl pH 8.0, 320 mM Sucrose, 5 mM MgCl₂, and 1% Triton X-100) was added to the buffy coat from whole blood and centrifuged at 13,000 rpm for 3 minutes. The supernatant was then discarded. This step was repeated two more times with 1,000 μ l of Reagent A. Next, 340 μ l of Reagent B (400 mM Tris-HCl pH 8.0, 60 mM EDTA, 150 mM NaCl, and 1% SDS) and 100 μ l of 5 M NaClO₄ were added. The reaction was incubated at 65°C for 20 minutes and allowed to cool down. Then, 580 μ l of cold chloroform-IAA was added. The sample was inverted for 20 minutes and centrifuged at 13,000 rpm at 4°C for 3 minutes. The resultant clear lysate was transferred to a new tube, and 880 μ l of chilled ethanol was added. The lysate was then incubated at -80°C for 1 hour. Genomic DNA was pelleted out by centrifugation at 13,000 rpm, 4°C, for 10 min. The supernatant was discarded, and the DNA pellet was washed twice with 75% ethanol. The genomic DNA pellet was air-dried at room temperature for 30 minutes and then dissolved in an appropriate volume of 1X TE buffer.

To extract genomic DNA from an osteosarcoma tissue biopsy specimen (tumor tissue), approximately 30 mg of the tissue was washed twice with PBS. Then, 500 μ l of Reagent B was added and the tissue was homogenized. Next, 13 μ l of 20 mg/ml Proteinase K was added to the lysate, mixed by vortexing for 10 seconds, and incubated at 50°C overnight. Subsequently, 20 μ l of 5 mg/ml RNase was added to the reaction, followed by incubation at 37°C for 30 minutes. At the end of the incubation, 147 μ l of 5 M

NaClO₄ and 600 µl of cold phenol:chloroform:IAA (25:24:1) were added to the lysate. The mixture was then inverted for 20 minutes and centrifuged at 13,000 rpm at 4°C for 5 minutes. The clear lysate was transferred to a new microcentrifuge tube. To pellet out the genomic DNA of the tumor, 880 µl of chilled ethanol was added, followed by the steps mentioned above.

Reference:

Miller SA, Dykes DD, Polesky HF. A simple salting out procedure for extracting DNA from human nucleated cells. *Nucleic Acids Res.* 1988;16(3):1215. doi:10.1093/nar/16.3.1215