UCSF Brain Tumor Center

UCSF Brain Tumor Center Biorepository and Biomarkers Laboratory

Standard Operating Procedure:

Genomic DNA Extraction from paraffin-embedded tissues*

1. Take scrolls of tissue or micordissection of slides into a 1.5 ml tube. Fill the tube with xylene, heat to 55-60 degrees for 5 min and then spin, pour off s/n.

2. Repeat 2-3 times. After washing out the xylene 2-3 times with 100% EtOH, you can get it into aqueous buffer for protease K digestion as follows:

3. Add about 600ul of extraction buffer, ExB (see below). Adjust the ExB volume based the sample amount.

4. Invert tube to ensure that sample is in suspension and not stuck to the bottom of the eppendorf tube.

5. Place tubes at 55^{o}C overnight (12-16hrs).
   Proper digestion here is critical. Inadequate digestion will lead to low yields and lower quality DNA. As a guide, the solution should look homogenous following digestion and no masses of tissue should be present. If additional digestion is required, add additional ExB (or PK) and incubate with occasional agitation until homogenous. The solution should easily pipet with a P1000.

6. Add 1ul RNase (25mg/ml) and incubate for 15min at 37^{o}C.
   This will give a final concentration of ~40ug/ml. Although some PK may still be active, this amount of RNase should be enough to get rid of all the RNA.

7. Clean DNA by phenol/chloroform extraction.
   Depending on yield, perform 2X PCI, and 2X CI. If you think yields are low, you can cut back to 1 of each.

8. Precipitate with 3M NaAcetate (pH5.2) and 2.5 volumes 100%EtOH.
   Invert tubes several times to mix. If volume is an issue, use 0.7 vol of isopropanol instead of EtOH. You may need to add 1-2ul of glycogen (20mg/ml) to increase the DNA precipitation.

9. Spin tubes at maximum speed for 30min.

10. Wash DNA pellet with 70% EtOH and re-spin 5min

11. Resuspend DNA in desired volume of TE (10mM Tris, 1mM EDTA, pH 7.6).
    20-30ul is a good rule of thumb, although this will depend on yield.

12. Quantitate DNA

Extraction Buffer (ExB).
50mM Tris (pH 8.0)
1mM EDTA (pH 8.0)
0.5% SDS
1mg/ml Proteinase K (~15mg/ml stock can be bought from Roche Cat#: 1 373 196)

*Based on the original protocols from Drs. K. Aldape, G. Hodgson, and J. Costello.