

## ISOLATION OF NUCLEI/DNase I DIGESTION

1. Thaw frozen tissues\* on ice in a petri dish
2. Mince into small pieces with clean razor (not necessary for small choroid plexus papillomas)
3. Transfer tissue to Dounce homogenizer (hand-held) and homogenize in ~5 ml cold (4°) Homogenization Buffer:

10 mM Tris-HCl (pH 7.5)  
5 mM MgCl<sub>2</sub>  
0.5 M sucrose  
0.15 mM spermine  
0.5 mM spermidine

Homogenize tissue with several strokes until particulate matter is no longer visible.

4. Layer homogenate onto a sucrose cushion consisting of 0.88 M sucrose in Homogenization Buffer (10-25 ml cushion depending on size of centrifuge tube used)
5. Spin at 5000 rpm in an HB-4 rotor at 4° for 5 minutes
6. Resuspend nuclear pellet in several mls of Homogenization Buffer and layer onto another 0.88 M sucrose cushion; spin again (step 5 above)
7. Resuspend nuclei in 1-2 ml Nuclei Resuspension Buffer:

10 mM Tris-HCl (pH 7.5)  
2.5 mM MgCl<sub>2</sub>  
0.25 M sucrose

8. On ice, dispense nuclei into 50-100ul aliquots for DNase I digestion
9. Serially dilute DNase I using Nuclei Resuspension Buffer to concentrations appropriate for next step
10. Add diluted DNase I to nuclei to final concentrations of 0.1 - 50 ug/ml (don't forget a negative control with NO DNase I added)
11. Incubate digests in 25°C water bath for 5 minutes
12. Place immediately on ice and stop digestion by adding 2 volumes of Stop Buffer:

10 mM Tris-HCl (pH 8)

1% SDS  
5 mM EDTA  
50 ug/ml proteinase K

Incubate at 37°C overnight

13. To isolate the DNA, phenol-chloroform extract (2X) and chloroform extract once; EtOH precipitate

\* For tissues such as liver and spleen, a typical prep involves ~3-5 tissues (several grams of material). For choroid plexus, use as much tissue as possible (> 0.25 grams).

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