

## PREPARATION OF NUCLEAR PROTEINS FROM TISSUE

Tissues should be snap frozen in liquid nitrogen and stored at  $-80^{\circ}\text{C}$  until use

Handle tissues on dry ice or  $\text{LN}_2$  until sonication

All manipulations from this point at  $4^{\circ}\text{C}$

To 20 mg tissue, add 1 ml ice cold SHB+

Sonicate (50% duty cycle, output 4, 30 pulses, Branson Sonifier 250) on ice  
(or use glass-teflon tissue grinder)

Spin 20 min at  $1000 \times g$  (4000 rpm in microfuge) to pellet nuclei/unbroken cells

For nuclear pellet – take original pellet and resuspend in TNET+

Spin 20 min at  $1000 \times g$  (4000 rpm in microfuge) to pellet nuclei

Resuspend in RIPA+

Shear DNA by drawing through syringe  $\sim 18\text{g}$

Aliquot and freeze at  $-80$

Quantitate protein using the Pierce BCA kit (see protocol)

### SHB (sucrose homogenization buffer)

0.255 M sucrose

1 mM EDTA

20 mM Tris pH 7.4

### TNET

1% Triton X100

150 mM NaCl

50 mM Tris pH 7.4

2 mM EDTA

### RIPA

60  $\mu\text{l}$  NP40

150  $\mu\text{l}$  20% SDS

6  $\mu\text{l}$  PMSF 17 mg/ml MeOH

240  $\mu\text{l}$  25x complete

6 mls

### Notes:

SHB, TNET, and RIPA need PMSF and 1X Complete (Roche) protease inhibitors before use.

(Designated by a +)

The sonication time is variable. Just until the tissue is completely obliterated. If the tube starts to feel warm it needs to rest and sit on ice.

The resuspension is also variable for the TNET and RIPA resuspension steps depending upon the pellet size. At least 2-3 times the volume of the pellet is a good rule of thumb.