Sucrose Gradients

Buffer A : Extraction Buffer		Buffer B : Gradient Buffer	
100mM Tris pH 9.0	- 1ml 1M Tris pH 9.0	10mM HEPES pH 7.8	- 500µl 1M HEPES pH 7.8
100mM NaCl	- 1ml 1M NaCl	5mM Na_2HPO_4	- 250µl 1M Na_2HPO_4
5mM KCl	- 50µl 1M KCl	5mM KCl	- 250µl 1M KCL
0.5mM $MgCl_2$	- 5µl 1M $MgCl_2$	0.5mM $MgCl_2$	- 25µl 1M $MgCl_2$
1mM $CaCl_2$	- 10µl 1M $CaCl_2$	1mM DTT	- 500µl 100mM DTT
0.5% NP40	- 500µl 10% NP40	dd H_2O	- to 10 mls
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* Add 100µl PMSF (100mM) just before use

- Pellet cells 50-100 million cells 1500 rpms for 5 mins
- Wash cells once in 25 mls of cold PBS and centrifuge as above
- Aspirate cells to dryness keeping on ice
- Add 200µl of Buffer A and resuspend cells thoroughly. Transfer to douncer and dounce 20-30 strokes on ice.
- Transfer to 1.5ml eppendorf and allow to extract on ice for 20 minutes.
- Centrifuge at 4°c for 20 mins 45,000 xg (13,200 rpms) to remove cell debris and nuclei.
- layer supernatant gently and immediately onto precooled 5-30% sucrose gradients (~12 mls each tube)

* - Set up gradient using peristaltic pump. continuous gradient 5-30%

5% Sucrose - 5g/100mls Buffer B
30% Sucrose - 30g/100mls Buffer B

- Centrifuge in SW41 swinging bucket rotor at 4°c.

260,000 xg for 6 hours

110,000 xg for 14 hours

- Collect 14 fractions and the pellet