

Chromatin binding (updated 2-9-11)

Pellet 50 mL of cells at 3000 rpm for 5'

Add 3 mL of pre-spheroplasting buffer

1 M PIPES-KOH, pH 9.4	1 mL
1 M DTT	0.1 mL
10% sodium azide	<u>0.1 mL</u>
	10 mL

Incubate at RT for 10'

Pellet cells and add 2 mL spheroplasting buffer

Zymolase 100T	10 mg
200 mM KPO ₄ , pH 7.5	1.25 mL
2 M sorbitol	1.5 mL
1 M DTT	<u>50 uL</u>
	5 mL

Incubate at 37 deg, 15'

Pellet cells and wash in 1 mL cold Wash buffer

1 M HEPES-KOH, pH 7.5	0.25 mL
1 M KCl	0.5 mL
1 M MgCl ₂	12.5 uL
2 M sorbitol	<u>1 mL</u>
	5 mL

Spin at 4000 rpm at 4 deg for 1'

Resuspend in 1 pellet volume of EB

1 M HEPES-KOH	50 uL
1 M KCl	100 uL
1 M MgCl ₂	2.5 uL
1 M DTT	1 uL
100 mM PMSF	10 uL
25x PIC	<u>40 uL</u>
	1 mL

Add 1% Triton X-100

Incubate on ice 5' with mixing

Take 25% of sample as WCE, add 2x SB

Underlay with 50% remaining volume of EBSX

Sucrose	0.3 g
1 M HEPES-KOH	50 uL
1 M KCl	100 uL
1 M MgCl ₂	2.5 uL
1 M DTT	1 uL
10% Triton X-100	25 uL
100 mM PMSF	10 uL
25x PIC	<u>40 uL</u>
	1 mL

Spin 12000 rpm at 4 deg for 10'

Take SUPE, add 2x SB

Wash pellet with 25% volume of EB+0.25% Triton

Spin 10000 rpm at 4 deg for 5'

Adjust volume with EB+0.25% Triton, add 2x SB to PELLETT, boil

Wash spheroplasts 3x with 1 M sorbitol

Resuspend in 18% ficoll in 0.02 M phosphate pH 6.8 with 0.5 mM MgCl₂

Spin 5' 4000g to pellet whole cells

Take supernatant

Spin 30,000g for 20 min to pellet nuclei

Resuspend nuclei in 3-4 volumes of cold 0.025 M NaCl with 10 mM EDTA