PROT_2v1

Protocol: Preparation of chromatin-enriched fraction

(Sadaie et al., MCB 2008)

performed as described previously (34) with some modifications.

- harvest cells (2.5×10^8 ~ 10 OD), wash once with **stop buffer** and place on ice for 5 min.
- resuspend cells in **PEMS buffer**
- incubate at 37°C for 20 min.
- spin at 400 x g at 4°C for 5 min,
- wash cell pellet twice with **1.2 M sorbitol**
- lyse cells with **HBS buffer** (25 mM MOPS, 60 mM -glycerolphosphate, 15 mM MgCl2, 15 mM EGTA, 15 mM p-nitrophe- nylphosphate, 0.1 mM sodium vanadate, pH 7.2) containing 1 mM PMSF, 1 Complete (Roche), and 1.5% Triton X-100.
- spin at 22,000 x g at 4°C for 15 min to obtain supernatant and pellet fractions

buffers and reagents:

- **stop buffer:** 150 mM NaCl, 50 mM NaF, 10 mM EDTA, 1 mM NaN3
- **PEMS buffer:** 100 mM PIPES [piperazine-N,N-bis(2-ethanesulfonic acid)], 10 mM EGTA, 10 mM MgSO4, 1.2 M sorbitol containing 1 mg/ml Novozyme and 1 mg/ml Zymolyase 100T
- **1.2 M sorbitol**
- **HBS buffer:** 25 mM MOPS, 60 mM glycerolphosphate, 15 mM MgCl2, 15 mM EGTA, 15 mM p-nitrophenylphosphate, 0.1 mM sodium vanadate, pH 7.2 containing 1 mM PMSF, 1 Complete (Roche), and 1.5% Triton X-100.

(34) Yuya Ogawa,[1,†] Tatsuro Takahashi,[1] and Hisao Masukata[1,2,*] (MCB 1999): Association of Fission Yeast Orp1 and Mcm6 Proteins with Chromosomal Replication Origins

For separation of chromatin-enriched insoluble fractions from soluble proteins, the method for budding yeast (18) was used with some modifications. Fission yeast cells (5×10^8 cells) placed in ice-cold STOP buffer (150 mM NaCl, 50 mM NaF, 10 mM EDTA, 1 mM NaN_3) for 5 min were incubated at 37°C for 20 min in PEMS (100 mM PIPES [pH 6.9], 1 mM EGTA, 1 mM $MgSO_4$, 1 M sorbitol) containing 1.2 mg of Lysing enzymes (Sigma) per ml and 0.4 mg of Zymolyase 20T (Seikagaku Corporation) per ml and washed with ice-cold 1 M sorbitol in 25 mM morpholinepropanesulfonic acid (MOPS; pH 7.2). Cells resuspended in HBS buffer (HB buffer supplemented with 0.4 M sorbitol) at a concentration of 10^9 cells/ml were lysed by addition of Triton X-100 at a final concentration of 0.5% for 5 min on ice, and the insoluble fraction was recovered by centrifugation for 15 min at $20,000 \times g$.