

Cell Collection of synchronized culture for ChIP Assay

Day 1: 50ml YES culture at 25°C.

Day 2 (am): Dilute to 200ml YES. Culture at 25°C until evening.

Day 2 (pm): Dilute to 600~700ml YES. Culture at 25°C for O/N.

Prepare: 36°C water bath shaker with enough water, HU if needed, TBS (1L) at 4°C

Day 3: Adjust O.D. to 0.3/ml x 600~700ml YES. Shift temp to 36°C for 4 hours.

During the 4 hours, prepare:

- 50ml blue cap tubes (2 tubes/1 time point)
- 1.5ml screw cap tubes (1 tube/1 time point)
- 25°C water bath
- Ice water & thermometer
- Hemacytometer
- Filter funnel, Filter & forceps
- Liquid nitrogen

- person A... Collect, fix & count cells
□ person B... Quench & Wash ~ freeze cells

| Sample 0:00 | Sample 0:20 |
|---|---|
| <div>○ -0:02 Take 45ml in 50ml tube. Take 10μl to count cells. 0:00 Add 1.25ml of Formaldehyde. Count cells.</div> | <div>○ 0:18 Take 45ml in 50ml tube. Take 10μl to count cells. 0:20 Add 1.25ml of Formaldehyde. Count cells.</div> |
| <div>□ 0:20 Add 2.5ml of Glycin. 0:30 Collect cells by filter funnel. Wash cells w/ 20ml of TBS. Suspend cells in 1ml of TBS and transfer to 1.5ml screw cap tube. Centrifuge and remove liquid. Freeze by liquid nitrogen</div> | <div>□ 0:40 Add 2.5ml of Glycin. 0:50 Collect cells by filter funnel. Wash cells w/ 20ml of TBS. Suspend cells in 1ml of TBS and transfer to 1.5ml screw cap tube. Centrifuge and remove liquid. Freeze by liquid nitrogen</div> |