

Preparation of *S.cerevisiae* competent cells

Materials

YPD medium

LDST

Eppendorf centrifuge

Refrigerator(4°C and -80°C)

Protocol

1. Inoculate 100ml YPD with an aliquot of an overnight culture and grow to an O.D.600 of 1.2-1.5 (about 1×10^8 cell/ml) at 30°C with 250 rpm.
2. Harvest by centrifugation at 1500g for 5 min, at 4°C, in 50ml Eppendorf tubes.
3. Remove the supernatant and add 40ml LDST. Incubate the yeast for 30min at 30°C.
4. Harvest by centrifugation at 1500g for 5min at 4°C and remove the supernatant. Resuspend in 1ml 1M ice-cold sorbitol, then transfer the cell into 2ml Eppendorf tubes.
5. Wash in 1ml 1M ice-cold sorbitol for 3 times
6. Resuspend in 800ul 1M sorbitol. Divide it into 1.5ml Eppendorf tubes, 80ul per tube, stored at -80°C.