

Competent Yeast Cells

1. Inoculate ~10ml YEPD Media with cells from a single yeast colony. Incubate at 30oC for 2-3 days while shaking.
2. Inoculate 10ml of fresh YEPD with 500ul of the saturated yeast culture. Incubate at 30oC overnight while shaking. OD600 will be ~1-2.
3. The next morning, inoculate 250ml YEPD with 2.5ml of the overnight culture (OD600 0.1-0.2). Incubate culture at 30oC while shaking until OD600 is 0.7 ± 0.1 (about 5 hours).
4. Transfer yeast culture to centrifuge tubes and pellet cells with a 2000 RPM spin for 5 minutes.
5. Discard supernatant. Place cells on ice. Wash cells with 50ml sterile ddH₂O. Pellet cells with a 2000 RPM spin for 5 minutes.
6. Discard supernatant. Place cells on ice. Resuspend cells in 25ml LiSORB. Incubate cells at 30oC for 15 minutes while shaking slowly.
7. Pellet cells with a 2000 RPM spin for 5 minutes. Discard supernatant. Place cells on ice. Resuspend cells in 2.5ml LiTE. Measure the volume of the resuspended cells. Add 10% vol/vol of DMSO to resuspended yeast cells. Aliquot cells into 200ul volumes and store in screw cap tubes at -80oC.