Yeast competent cells

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ABSTRACT

This is a protocol to make yeast competent cells, which can be then stored at 4 ºC for up to a week.

MATERIALS

NAME	CATALOG #	VENDOR
YPD Broth	SD7022.SIZE.250g	Bio Basic Inc.

1 Pick a yeast colony from a plate and place it inside 10 mL YPD medium.

2 Incubate the cells overnight at 30 ºC and 180 rpm.

3 The next day, measure the OD600 of the liquid culture.

4 Inoculate a flask with 50 mL YPD medium with enough volume of the overnight culture to reach an OD600 of 0.4.

5 Incubate the cells for 3-4 hours at 30 ºC and 180 rpm until an OD600 of 2.0 is reached.

6 Place the 50 mL of liquid culture inide a 50 mL tube and centrifuge the cells at 3000x g for 5 minutes and 20 ºC.

7 Discard the supernatant and resuspend the cells in 25 mL of sterile MQ water. Centrifuge cells at 3000x g for 5 minutes and at 20 ºC. Repeat this step another time.

8 Discard the supernatant and resuspend the cells in 1 mL sterile MQ water. Transfer the volume into a 1.5 mL tube.

9 Centrifuge the cells at 3000x g for 5 minutes and discard the supernatant.

10 Resupend cells in 1 mL sterile MQ water and aliquote them by 100 µL in different 1.5 mL tubes. Each tube can be used for a transformation and may be stored at 4 ºC for up to a week.