Pichia Yeast Transformation protocol

Restriction Digest

1. Digest ~5－10 μg of plasmid DNA with one of the restriction enzymes Sac Ⅰ or Sal Ⅰ.
2. Check a small aliquot of your digest by agarose gel electrophoresis for complete linearization.
3. If the vector is completely linearized, heat inactivate or add EDTA to stop the reaction, phenol/chloroform extract once, and ethanol precipitate using 1/10 volume 3 M sodium acetate and 2.5 volumes of 100% ethanol.Centrifuge the solution to pellet the DNA, wash the pellet with 80% ethanol, air-dry, and resuspend in 10 μl sterile, deionized water. Use immediately or store at －20° C.

Preparing *Pichia* for Electroporation

Follow the procedure below to prepare your Pichia pastoris strain for electroporation.

1. Grow 5 ml of your Pichia pastoris strain in YPD in a 50 ml conical tube at 30° C overnight.
2. Inoculate 500 ml of fresh medium in a 2 liter flask with 0.1－0.5 ml of the overnight culture. Grow overnight again to an OD600 = 1.3－1.5.
3. Centrifuge the cells at 1500 × g for 5 minutes at 4° C. Resuspend the pellet with 500 ml of ice-cold (0° C), sterile water.
4. Centrifuge the cells as in Step 3, then resuspend the pellet with 250 ml of ice-cold (0° C), sterile water.
5. Centrifuge the cells as in Step 3, then resuspend the pellet in 20 ml of ice-cold (0° C) 1 M sorbitol.
6. Centrifuge the cells as in Step 3, then resuspend the pellet in 1 ml of ice-cold 1 M sorbitol for a final volume of approximately 1.5 ml. Keep the cells on ice and use that day. Do not store cells.

Transformation by Electroporation

1. Mix 80 μl of the cells from Step 6 (above) with 5－10 μg of linearized pPICZα DNA (in 5－10 μl sterile water) and transfer them to an ice-cold (0° C) 0.2 cm electroporation cuvette.
2. Incubate the cuvette with the cells on ice for 5 minutes.
3. Pulse the cells according to the parameters for yeast (Saccharomyces cerevisiae) as suggested by the manufacturer of the specific electroporation device being used.
4. Immediately add 1 ml of ice-cold 1 M sorbitol to the cuvette. Transfer the cuvette contents to a sterile 15 ml tube.
5. Let the tube incubate at 30° C without shaking for 1 to 2 hours.
6. Spread 50－200 μl each on separate, labeled YPDS plates containing the appropriate concentration of corresponding antibiotic.
7. Incubate plates for 2 to 3 days at 30° C until colonies form.
8. Pick 10－20 colonies and purify (streak for single colonies) on fresh YPD or YPDS plates containing the appropriate concentration of corresponding antibiotic.