Yeast Transformation by Electroporation Protocol

Preparation of solutions:

10 x TE buffer (pH 7,5): 100 mM Tris-HCl, 10 mM EDTA
10 x LiAc: 1 M LiAc, pH 7,5 (adjusted using HAc). Filter-sterilized or autoclaved for 20 min.
1 M DTT: Stored at -20˚C

Procedure:

Day 1

Pick up single colonies on plate and transfer to 5 ml YPD medium. Culture at 30˚C for 12-16 h.

Day 2

1. Inoculate the yeast into 50 ml YPD medium in flask. Culture at 30˚C for 6-9 h.

   Put sterilized water on ice

2. When OD is 0,5-1,2, transfer cell culture into a cap tube (50 ml).

   From here cells should always be kept on ice

3. Collect cells by centrifugation (1100 x g, 4˚C, 5 min). Decant supernatant.
4. Re-suspend cells with 20 ml of dH_2O (ice-cold). Mix by pipetting up and down. Centrifuge and decant supernatant.
5. Treat cells with 20 ml of 0,1 M LiAc (16 ml 1 M sorbitol + 2 ml 10 x TE buffer + 2 ml ! M LiAc) at 30˚C for 30 min. Add 0,2 ml 1 M DTT and keep cells at 30˚C for 15 min. Centrifuge and decant supernatant.

   Put 1 M sorbitol on ice

6. Wash cells twice with 20 ml of 1 M sorbitol (ice-cold). Centrifuge and decant supernatant.
7. Re-suspend cells with 100-200 µl of sterilized ice-cold 1 M sorbitol (final OD = 100-200).
8. Transfer 50 µl of suspended cells into a new 1,5 ml tube on ice.
9. Add 5 µl fragment DNA (> 200 ng/µl). Mix by pipetting up and down, and keep on ice for 15 min. Transfer all to a sterilized cuvette (green cap). Add 1 ml of cold 1 M sorbitol to new labeled tubes (used later).
10. Set the cuvette in the holder of a Micro Pulser Electroporator. Chose "manual", and set voltage at 1,5 kV. Push the pulse button. Read "Time / ms", if it is between 4,0 - 6,0 the process is successful.
11. Add 1 ml of cold 1 M sorbitol immediately after the pulse. Mix well by pipetting up and down (After this step it is OK to be at room temperature). Transfer all to the sorbitol tube as soon as possible.
12. Incubate at 30˚C for 1-3 h. Centrifuge (3000 x g, 1 min)