

***S. cerevisiae* Transformation ~Electroporation Method~**

2006-0104

Day 1:

- 1) Culture cells that you want to transform
 - Inoculate cells by using sterilized toothpick into 10 ml YPDA/sample* in a sterilized flask at 30°C incubator-shaker for O/N.

*If you have 2 different DNA samples for one kind of yeast strain, you need to culture in 20 ml YPDA.

Day 2:

Prepare: Sterilized water and 1M Sorbitol on ice

- 1) Measure O.D.

Cells O.D. (density) should be between 0.5 ~ 1.0 O.D. If it's more than 1.0 O.D., dilute it to 0.3 ~ 0.4 O.D. with YPDA. Culture it again for 2 ~ 3 hours until 0.5 ~ 1.0 O.D. After dilution, you want cells to divide at least once or twice. WT cell's doubling time (one generation time) is about 2 hours.

- 2) Transfer cell culture into 50 ml blue cap tube (sterilized). On ice for 5 min.

<<<<<< From here cells should be always on ice >>>>>>

- 3) Collect cells by centrifugation (2400 rpm, 4°C, 3 min). Decant supernatant.
- 4) Wash cells (means: re-suspend cells) with 10 ml of sterilized H₂O (ice-cold). Vortex well. Centrifuge (2400 rpm, 4°C, 3 min) and decant supernatant.
- 5) Wash cells with 1 ml of sterilized H₂O (ice-cold), vortex and transfer to 1.5 ml tube by pipetman. Centrifuge (8000 rpm, 4°C, 10 sec) and aspirate supernatant completely.
- 6) Wash cells with 1 ml of sterilized ice-cold 1M Sorbitol. Vortex well. Centrifuge (8000 rpm, 4°C, 10 sec) and aspirate supernatant.
- 7) Re-suspend cells by pipet up and down in 200 µl/sample of sterilized 1M Sorbitol. (If you have 2 DNA samples for one strain, suspend in 400 µl)
- 8) Take 200 µl of suspended cells into a new 1.5 ml tube on ice.
- 9) Add 10 µl of purified fragment DNA (or **2 µl plasmid DNA**). (Keep the rest of DNA at -20°C)
- 10) Mix with 1000 µl pipetman and transfer all to sterilized cuvette (green cap). Keep the original empty tube on ice. You use it later.

- 11) Set the cuvette in the holder of Micro Pulser Electroporator.
 - Chose “fungi”, and find “Sc4” program using arrow.
 - Chose “Time / ms” in measurements.
- 12) Push the pulse button. Hear “buzz” sound. Read “Time / ms”, if it is between 4.0 ~ 6.0, this process is succeed.
- 13) Add 800 μ l of cold 1M Sorbitol, immediately after the pulse. Mix well by pipetting up and down. (After this, it is ok to be at RT.) Transfer all to original tube ASAP.
- 14) Plating Cells. Take 100 μ l out of 1000 μ l pulsed cells. Spread cells on a selection plate (marked “less”) evenly with 6~8 beads.
- 15) Centrifuge rest of cells (8000 rpm, 10 sec). Decant supernatant, but leave about 100 μ l of supernatant. Suspend cells with pipetman, and spread cells on another selection plate (marked “more”) with 6~8 beads. Place both plates at 30°C air incubator.

Day 3~5:

1) Grow Selected Colonies

When you get colonies on the selection plates, pick 4 (sometime more) colonies on a new same selection plate with toothpicks and re-streak each colony like below. Incubate it at 30°C for one or two days.

