S. pombe Transformation ~Electroporation Method~

2005-1109

Day 1:

1) Culture cells that you want to transform
   - Inoculate cells by using sterilized toothpick into 10 ml YES/sample* in a sterilized flask at 25˚C (30˚C) water bath 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 YES.

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 YES. 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 ice-cold H_2O. Vortex well. Centrifuge (2400 rpm, 4˚C, 3 min) and decant supernatant.

5) Wash cells with 1 ml of sterilized ice-cold H_2O, 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 1.5 ml tube on ice.

9) Add 10 µl of purified fragment DNA (or plasmid DNA). (Keep the rest of DNA at -20˚C)

10) Mix with 1000 µl pipetman and transfer all to sterilized cuvett (green cap). Keep the original empty tube on ice. You use it later.

11) Set the cuvett in the holder of Micro Pulser Electroporator.
   - Chose "fungi", and find "ShS" 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 μl 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.

14) Plating Cells. Take100 μl out of 1000 μl pulsed cells. Spread cells on a YES plate (marked "less") evenly with 6~8 beads.

15) Centrifuge rest of cells (8000 rpm, 10 sec). Decant supernatant, but leave about 100 μl of supernatant. Suspend cells with pipetman, and spread cells on another YES plate (marked "more") with 6~8 beads. Place both plates at 32˚C air incubator.

Day 3:

1) Replicate to drug Plate

After 24 hours of incubation, replicate cells on to drug plates with replicator.

- Warm a drug plate to RT.
- Wipe your bench with 70% EtOH.
- Wipe the replicator and collar w/ 70% EtOH too.
- Take a piece of velvet on top of replicator and hold with collar.
- Place the YES plate w/ cells and transfer cells to velvet and remove the YES plate.
- Then place a drug plate and transfer cells to it.
- Take this drug plate out and incubate it at 32˚C for a couple of days.

Day 4~6:

1) Grow Anti-drug Colonies

When you get colonies on the drug plates, pick 4 (sometime more) colonies on a new drug plate with toothpicks and re-streak each colony like below. Incubate it at 32˚C for one or two days.