プロトプラスト融合（Protoplast fusion）法による二倍体細胞の取得

1 harvest $5x10^7$ -10^8 cells

2 wash with 1ml SP1

3 incubate in SP1 for 5 min at room temperature.

4 spin down, and suspend with 500 μl SP2 (containing 10 mg/ml Novozyme (Sigma L1412-5G))

5 incubate for 30 min at 37℃

*Avoid too long incubation in this step not to lyse the cells.

*After the treatment of Novozyme here, cells should be pelleted by 3000 rpm for 3 min, or by チビタン for several seconds.

6 mix 2 μl cell suspension with equal volume of water on a slide glass and check protoplasts under a microscope (protoplast will burst by adding water).

*If protoplasts are not observed, add newly prepared SP2 containing Novozyme , and incubate for 30 min.

7 pellet and wash cells with 1ml SP3 for 2 times

*Suspend cells moderately by pipeting.

*Although lysed cells are sometimes difficult to be pelleted, replacing SP2 with SP3 make it easy to pellet the lysed cells.

8 suspend cells in SP4, adjust cell concentration to $5x10^7$/50 μl

9 mix 50 μl of each strains into one tube, suspend moderately.

10 add 1ml SP5, incubate for more than 30 min at 26-30℃

11 pellet and suspend cells in 100-200 μl SP4.

12 spread cells on selective medium containing 1.2 M sorbitol.

Solutions

SP1 (SP2+EDTA)

1.2M D-sorbitol

50mM Citrate-phosphate (pH 5.6)

40mM EDTA

SP2

1.2M D-sorbitol

50mM Citrate-phosphate (pH 5.6)

SP3

1.2M D-sorbitol

10mM Tris-HCl (pH 7.6)

SP4 (SP3+CaCl2)

1.2M D-sorbitol

10mM Tris-HCl (pH 7.6)

10mM $CaCl_2$

SP5

20% PEG4000

10mM Tris-HCl (pH 7.6)

10mM $CaCl_2$