Protocol for preparation of competent cells for transformation

For two transformations

Materials:

- 0.1 M Calcium Chloride chilled on the ice;
- Overnight bacteria l culture or bacteria l colonies;

Procedure:

1. Add 20 μl of the overnight bacteria l culture or pick a colony to 1 ml of LB antibiotic liquid medium, Incubate at 37 degree in a shaker till the OD600 value reaches 0.4—0.6.

2. Put the tubes on ice to incubate for 5 min.

3. Pellet bacterial cells by 5 min centrifugation at 5000 rpm, discard the supernatant.

4. Resuspend cells in 600 μl of ice—chilled 0.1 M Calcium Chloride solution. Incubate on ice for 30 min.

5. Centrifuge for 5 min at 5000 rpm in a microcentrifuge tube , discard the supernatant.

6. Resuspend the pelleted cells in 100 ul of ice—chilled 0.1 M Calcium Chloride solution. Incubate on ice.

7. Add 50 μl of the prepared cells to each tube containing DNA sample , mix and incubate on ice for 30 min.

8. Transform subsequently as the transformation protocol.

Note:

1. Make sure the cells are not left in the centrifuge at ambient temperature for more than 5 min as this will significantly decrease the transformation efficiency.

2. The rpm at centrifugation is not higher than 5000, as a high rpm ma y cause the lysis of cells.