Protocol for preparation of competent cells for transformation

For two transformations

Materials:

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

Procedure:

1. Add 20 µl of the overnight bacterial 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 4 min centrifugation at 4000 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 4 min at 4000 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 4000, as a high rpm may cause the lysis of cells.

3. Competent cells prepared with this protocol are suitable for direct use only. Freezing down and storage at -70°C is not recommended.
4. The culture can be kept at 4 degree for one week and used for preparation of competent cells, but culture stored longer than 10 ten days is not suitable for competent cells.

References:

*Current protocols in molecular biology