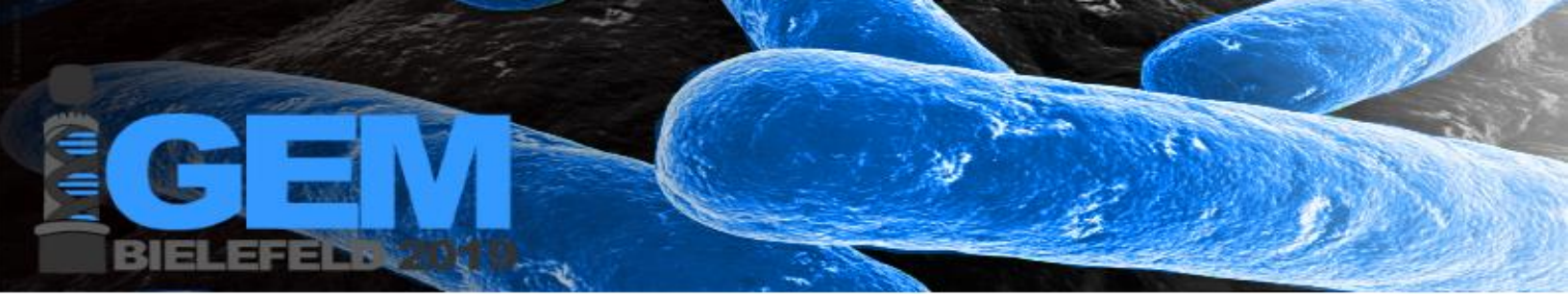

Preparation of chemically competent *Escherichia coli* cells

Materials:

- 1.5 ml microfuge tubes
- 50 ml falcon tubes

Chemicals:

- Buffer 1
- Buffer 2

Methods:

1. Inoculate 5 ml LB-Medium with the appropriate antibiotic(s) with the *E. coli* strain of interest. For DH5α strain, it is advised to use SOC-Medium instead of LB-Medium. Incubate at 37°C for minimum 6 hours.

2. Measure the OD_{600} of the incubated culture. If the OD has raised to 0.7-0.9, transfer the culture to 50 ml falcon tubes

3. Incubate for 15 minutes on ice. From this point, everything has to be done on ice.

4. Centrifuge the culture at 4°C and 4,000 x g for 10 minutes. Discard the supernatant and resuspend the cell pellet in 2 ml **Buffer 1**. Do not vortex nor use the pipette!

5. Incubate on ice for 30 minutes.

6. Centrifuge the culture at 4°C and 4,000 x g for 10 minutes. Discard the supernatant and resuspend the cell pellet in 2 ml **Buffer 2**. Do not vortex nor use the pipette!

7. Aliquot 100 µl of the cell resuspension in steril, 1.5 ml microfuge tubes. Freeze and store at -80°C.

Buffer 1: 0.1 M $CaCl_2$, 2 mM Tris-HCl → pH 7.4

Buffer 2: 0.1 M $CaCl_2$, 2 mM Tris-HCl, 10% Glycerin → pH 7.4

From: iGEM Bielefeld-CeBiTec

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