

Sterilize all material before use.  
Only open your flasks or tubes in a flow bench.

1.	Prepare an overnight 5 ml starter culture of the cells in the prescribed medium <sup>1</sup> and at the required cultivation temperature (16h±1h in shaker).
2.	Dilute the saturated culture 1/100 in 20 ml of the prescribed medium <sup>1</sup> .
3.	Incubate the cells at the prescribed cultivation temperature until the OD <sub>600</sub> reaches 0,5.
4.	Keep the culture on ice for at least 10 minutes.  It is also very important to keep the cells at 4°C or on ice for the remainder of the procedure. Any bottles, tubes or solutions used after this point, must be pre-chilled to 4°C.
5.	Transfer the culture into cooled Falcon-tubes and centrifuge them for 5 minutes at 4 °C and 5000 rpm.
6.	Remove the supernatant and wash the cells with 20 ml of ice cold sterile distilled water by pipetting up and down.
7.	Centrifuge for 5 minutes at 4 °C and 5000 rpm.
8.	Remove the supernatant and wash the cells with 10 ml of ice cold sterile distilled water by pipetting up and down.
9.	Centrifuge for 5 minutes at 4 °C and 5000 rpm.
10.	Remove the supernatant and suspend the cells in 2 ml of ice cold sterile distilled water by pipetting up and down.
11.	Per Falcon-tube with 2 ml ice cold sterile distilled water & cells, 0.3 ml ice cold glycerol needs to be added.  Calculate the total amount of glycerol needed, based on the number of tubes. Heat the glycerol stock to easily transfer the calculated amount to an empty sterile tube. Put this tube on ice until sufficiently cooled. Pool the content of all Falcon-tubes, add it to the chilled glycerol and mix by pipetting up and down. Aliquot the cell mixture in sterile pre-chilled tubes (100 µl per tube) and freeze via snapfreezing by putting the tubes in liquid nitrogen.
12.	Store the tubes at -80°C.

<sup>1</sup> Using SOB (Super Optimal Broth) or SOC (SOB + glucose) medium can increase the transformation efficiency later on.

SOB broth:	Tryptone	20 g
	Yeast extract	5 g
	NaCl	0.5 g
	Purified water	950 ml
	KCl 250 mM	10 ml

Set to pH 7.0 and add purified water to a total volume of 1 liter

Sterilize

Add 5 ml of a sterile 2 M MgCl<sub>2</sub> solution

SOC broth:

Prepare 1 liter SOB broth (including MgCl<sub>2</sub> solution)

Add 20 ml of a sterile 1 M glucose solution