Competent cells

Competent #1 - Chemo-Competent *Bacillus subtilis*

1. Inoculate a culture of *Bacillus subtilis* in 4 ml of LB-Medium
2. Let them grow overnight at 37°C, 200 rpm
3. Measure the OD_{600} of your overnight culture and dilute it in MNGE Medium to an OD_{600} of around 0.1-0.2/ml in 10 ml LB
4. Let the cells grow to an OD_{600} of 1.0-1.3/ml (37°C, 200 rpm)
5. Cells should be competent now (very motile)
6. Aliquot 400 µl of the samples in different tubes (each tube stands for one transformation)
7. You can either use the competent cells directly or add glycerol to dilute to a final concentration of 10% and freeze them in -80°C
   (Protocol from iGEM team munich 2012)

MNGE-Medium (100 ml)

9.2 ml	10x MN-Medium
82.8 ml	Sterile water
10 ml	Glucose (20% filtered)
500 µl	K-Glutamate (40%)
500 µl	Fe[III]-ammonium-citrate (2,2 mg/ml)
1 ml	Tryptophan (5 mg/ml)
300 µl	$MgSO_4$ (1 M)
(1 ml	Threonine (5 mg/ml) only for selective agar after the transformation)

Transformation of *Bacillus subtilis*

Transformation #1

Using fresh, competent *B. subtilis* cells:

1. Add 600 ng of DNA and incubate at 37°C (200 x g) for 1 h
2. Add 100 µl of expression mix to each sample
3. Incubate at 37°C for another hour
4. Plate 400 µl the samples on selective agar

Note: if you use the frozen aliquots you can also just add the DNA to the sample, don't centrifuge the competent cells!

(Protocol from iGEM team Munich 2012)

Expression mix 10,5 ml

5 ml	Yeast extract (5%)
2.5 ml	Casamino-acids (CAA)(10%)
2.5 ml	Sterile water

500 µl	Tryptophan (5 mg/ml)