

### Transformation of *Bacillus subtilis* (simple)

- inoculate 10 ml MNGE to  $OD_{600} = 0,1$  (or simply 1/100) from overnight culture
- let grow to  $OD_{600} = 1.1-1.3$  at 37°C with agitation (at least 200 rpm!)
- use 400 µl cells for transformation (in test-tube, not eppendorf!):
  - o add DNA (ca. 1-2 µg linearized plasmid or 100 µl crude-prep genomic DNA)
  - o let grow for 1 h
  - o add 100 µl Expression Mix (may need to pre-induce: Ery 0,025 µg/ml, Cm 0,125 µg/ml)
  - o let grow for 1 h
  - o plate on selective media

#### **10 X MN-Medium:**

136 g  $K_2HPO_4$  (x 3  $H_2O$ )  
60 g  $KH_2PO_4$   
10 g Na-citrat (x 2  $H_2O$ )

<b>10 ml</b>	<b>20ml</b>	<b>MNGE-Medium:</b>
920 µl	1,84 ml	10xMN-Medium
8,28 ml	16,56 ml	Sterile water
1 ml	2 ml	Glucose (20%)
50 µl	100 µl	K-Glutamat (40%)
50 µl	100 µl	Fe[III]- ammonium-citrate (2,2 mg/ml)
100 µl	200 µl	Tryptophan (5 mg/ml)
30 µl	60 µl	MgSO <sub>4</sub> (1M)
(100 µl	200 µl	threonine (5 mg/ml) for strains carrying an insertion in <i>thrC</i> )

#### **Expression Mix:**

500 µl yeast extract (5%)  
250 µl casamino-acids (CAA) (10%)  
250 µl  $H_2O$   
50 µl Tryptophan (5 mg/ml)