

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)

10 ml	20ml	MNGE-Medium:
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 ₄ (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)