

Transformation of *Bacillus subtilis* (simple)

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

1 Liter 10 X MN-Medium:

136 g K₂HPO₄ (x 3 H₂O)

60 g KH₂PO₄

10 g Na-citrat (x 2 H₂O)

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 H₂O

50 μl Tryptophan (5 mg/ml)

Protocol generously provided by the lab
Prof. Thorsten Mascher
Großhadernerstr. 2-4
82152 Planegg-Martinsried
www.syntheticmicrobe.bio.lmu.de