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Transformation and Preparation of Chemically Competent Bacillus subtilis cells

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We use this protocol and it's working

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Troubleshooting

Preparation of Chemically Competent Bacillus subtilis cells

1

For 20 ml or 50 ml PARIS-Medium mix:

	final concentration	Stock	for 50 ml	for 20 ml
	100 mM potassium phosphate buffer pH 7.0			
	60 mM K2HPO4	1 M (3.48 g in 20 ml for stock)	3 ml	1.2 ml
	40 mM KH2PO4	0.5 M (1.36 g in 20 ml for stock)	4 ml	1.6 ml
	3 mM trisodium citrate	0.5 M (0.735 g in 5 ml)	300 µl	120 µl
	20 mM Potassium-L-glutamate	1 M (4.06 g in 20 ml)	1 ml	400 µl
	21 mM MgSO4	1 M (1.23 g in 5 ml)	1050 µl (7x)	420 µl (7x)
	1 % Glucose	50 % (10g in 20 ml)	1 ml	400 µl

	20 mg/ml L-Tryptophan	5 mg/ml (25 mg in 5 ml)	200 µl	80
	0.1 % Caseinhydrolysat (DIFCO!)	10 % (1 g in 10 ml)	500 µl	200 µl

2 Inoculation of precultures: Spread *B. subtilis* from Glycerol stock on LB plate and incubate over night for 37°C or over the weekend at 30°C.

3 Resuspend single clones in 5 ml Paris Medium (test tube) on incubator roller or plate washer with 1 ml Paris Medium (saves one day work)

4 Inoculation of 2.5 ml Paris Medium (test tube) from pre-culture (wash or liquid-overnight culture) to OD_{580}=0.2

5 4h incubation on incubator roller at 37°C

6 Centrifuge 1 ml pellet cells in reaction tubes for 1 min at maximum speed. Remove the supernatant.

7 Resuspend the pellet in 1 ml Paris medium with 10 % (v/v) glycerol.

8 Store 100 µl aliquots at -80 °C

Transformation of Bacillus subtilis

9 Thaw aliquots at 37 °C, add 900 µl Paris medium and 500-1000 ng plasmid DNA (test tube)

10 Incubate 6 h in the incubator roller at 37°C

11 Pellet cells, remove 800 µl, resuspend and plate the rest (for normal transformation on LB+antibiotic)