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Natural competence of Bacillus subtilis transformation

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ABSTRACT

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 We use this protocol and it's working

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final concentration		Stock	for 50 ml		for 20 ml	
100 mM Kaliumphosphat puffer pH 7 :						
60 mM K ₂ HPO ₄		1 M (3,48 g in 20 ml for Stocklösung)	3 ml	☒	1,2 ml	☒
40 mM KH ₂ PO ₄		0,5 M (1,36 g in 20 ml for Stocklösung)	4 ml	☒	1,6 ml	☒
3 mM Trisodiumcitrat		0,5 M (0,735 g in 5 ml)	300 µl	☒	120 µl	☒
20 mM Kalium-L-Glutamat		1 M (4,06 g in 20 ml)	1 ml	☒	400 µl	☒
21 mM MgSO ₄		1 M (1,23 g in 5 ml)	1050 µl (7x)	☒	420 µl (7x)	☒
1 % Glukose		50 % (10g in 20 ml)	1 ml	☒	400 µl	☒

20 mg/ml IL-Tryptophan		5 mg/ml (25 mg in 5 ml)	200 µl	☒	80	☒
0,1 % Caseinhydrolysate (DIFCO!)		10 % (1 g in 10 ml)	500 µl	☒	200 µl	☒

Inoculation VK: Spread *B. subtilis* out of cryo on LB plate and apply üN 37°C or üW 30°C

Single clone in 5 ml Paris Medium (test tube) on incubator roller or plate washer with 1 ml Paris Medium (saves one day work)

Inoculation of 2,5 ml Paris Medium (test tube) from VK (dishwasher or liquid-ÜK) to OD580=0,2

4h incubation on incubator roller at 37°C

1 ml pellet cells in Eppi (1min, max, table centrifuge)

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

Store 100 µl aliquots at -80 °C

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

Incubate 6 h in the incubator roller at 37°C

Pellet cells, remove 800 µl, resuspend and plate the rest (for normal transformer on LB+antibiotic, for upp mutants FB medium with 5-fluorouracil)

concentrations of antibiotics: Kanamycin 50 yg/ml, Neomycin 5 yg/ml, Erytromycin 1 yg/ml, Tetracycline 20 yg/ml, Chloramphenicol 5 yg/ml, Linkomycin 25 yg/ml