Natural competence of Bacillus subtilis transformation

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1 Works for me

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ABSTRACT

final concentration		Stock	for 50 ml		for 20 ml	
100 mM Kaliumphosphatpuffer pH 7 :						
60 mM K2HPO4		1 M (3,48 g in 20 ml for Stocklösung)	3 ml	□	1,2 ml	□
40 mM KH2PO4		0,5 M (1,36 g in 20 ml for Stocklösung)	4 ml	□	1,6 ml	□
3 mM Trinatriumcitrat		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 MgSO4		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 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	□

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

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