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Natural competence of *Bacillus subtilis* transformation

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Protocol status: Working

We use this protocol and it's working

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Keywords: bacillus subtilis transformation, natural competence of bacillus subtilis transformation, plasmid dna, ng plasmid dna, ml pellet cell, 4h incubation on incubator roller

Abstract

	final conc entr ation		Stoc k	for 50 ml		for 20 ml	
	100 mM Kaliu mph osph atpu ffer pH 7 :						
	60 mM K ₂ H PO ₄		1 M (3,4 8 g in 20 ml for Stoc klös ung)	3 ml	○	1,2 ml	○
	40 mM KH ₂ PO ₄		0,5 M (1,36 g in 20 ml for Stoc klös ung)	4 ml	○	1,6 ml	○
	3 mM Trin atriu mcit rat		0,5 M (0,7 35 g in 5 ml)	300 µl	○	120 µl	○
	20 mM Kaliu m-L- Glut amat		1 M (4,0 6 g in 20 ml)	1 ml	○	400 µl	○
	21 mM MgS O ₄		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 % Casein hydrolysate (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

