HIGH OSMOLARITY ELECTROPORATION

Adapted from: **Xue, G.-P., J. S. Johnson, and B. P. Dalrymple.** 1999. High osmolarity improves the electro-transformation efficiency of the gram-positive bacteria *Bacillus subtilis* and *Bacillus licheniformis*. J. Microbiol. Meth. **34:**183–191.

COMPETENT CELL PREPARATION

DAY 1

Step	Action	Material	Parameters
1	Inoculate	a single *B. subtilis* colony into broth in test tube	2 ml LB
2	Incubate	test tube	37°C, aeration, ON

DAY 2

Step	Action	Material	Parameters
1	Dilute	overnight culture into fresh broth in a 250 ml flask	1.5 ml into 24 ml GM
2	Incubate	culture	37°C, aeration, until OD_{600}=0.85-0.95
3	Chill	culture	ice water bath, 10 min
3	Dispense	aliquot of culture into centrifuge tube	16 ml
4	Centrifuge	centrifuge tube	4°C, 5000×g, 5 min
5	Suspend	cell pellet	ice cold EM, 10 ml
6	Repeat	steps 4-5	three additional washes
7	Suspend	cell pellet	Ice cold EM, 0.4 ml
8	Store	aliquots	-80°C

(You may also proceed directly to electroporation.)

ELECTROPORATION

Step	Action	Material	Parameters
1	Mix	competent cells with plasmid DNA	60 µl cells, 1 µl plasmid DNA (50 µg/ml)
2	Chill	mixture	ice bath, 60-90 s
3	Transfer	mixture	Ice-cold cuvette (1 mm gap)
4	Pulse	cuvette with Bio-Rad Gene Pulser	25 µF, 200 Ω, and 2.1 kV; time constant 4.5-5.0 ms
5	Add	recovery medium	1 ml RM, **immediately**
6	Incubate	electroporation mix	37°C, 3 hr, aeration
7	Plate	electroporation mix onto selective media	50-200 µl, LB + antibiotics
8	Incubate	plates	37°C, overnight

RECIPES

- GM LB containing 0.5 M sorbitol
- EM 0.5 M sorbitol, 0.5 M mannitol and 10% glycerol
- RM LB containing 0.5 M sorbitol and 0.38 M mannitol