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Supplementary Material

SUPPLEMENTARY MATERIAL TO

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Table 1. Natural transformation medium for *Bacillus subtilis*

10 X MN-Medium:	
136 g	K_2HPO_4 (× 3 H_2O)
60 g	KH_2PO_4
10 g	Na-citrat (× 2 H_2O)
MNGE-Medium:	
9,2 ml	1 x MN-Medium (920 µl 10x MN + 8,28 ml steril H_2O)
1 ml	Glucose (20%)
50 µl	K-Glutamat (40%)
50 µl	Fe[III]- ammonium-citrate (2,2 mg/ml)
100 µl	Tryptophan (5 mg/ml)
30 µl	$MgSO_4$ (1M)
Expression Mix:	
500 µl	yeast extract (5%)
250 µl	casamino-acids (CAA) (10%)
250 µl	H_2O
50 µl	Tryptophan (5 mg/ml)

Table 2. Plasmids (DNA sources) used in the study.

Name	Plasmid Content	Source
pBS1C	Empty vector; integrating into amyE gene region at *B. subtilis* genome; cm^r	(Crisp *et al.* 2015)
pBS2E	Empty vector; integrating into lacA gene region at *B. subtilis* genome; mls^r	(Crisp *et al.* 2015)

* cm^r, chloramphenicol resistant gene; mls^r, erythromycin resistant gene

Table 3. Transformation protocol for *B. subtilis* (Simple version)

- Inoculate 0.1 OD_{600} of *B. subtilis* culture into 10 ml of MNGE
- Grow the culture overnight at 37°C, 200 rpm until OD reach to 1.1-1.3
- Take 400 µl of the well-mixed culture into a test tube for transformation.
- Add the DNA. (1-2 µg linear plasmid)
- Culture it for 1 hour (37°C, 200 rpm)
- Add 100 µl Expression Mix
- Culture it for 1 hour (37°C, 200 rpm)
- Take 20-50 µl of culture to spread on selective agar plates.

References

Crisp, A., Boschetti, C., Perry, M., Tunnacliffe, A. & Micklem, G. 2015. Expression of multiple horizontally acquired genes is a hallmark of both vertebrate and invertebrate genomes. *Genome Biology*, 16(1): 1-13.