

Bacillus subtilis transformation**Materials and Equipment:**

- LB plate with a desired strain
- gDNA or plasmid
- X10 MC (stored at -20°C)
- 1M MgSO₄ (solution E from resuspension salts)
- sDDW
- Sterile tube (15ml)
- Sterile wooden stick
- Selective marker plates
- Sterile beads
- Freezing tubes (nunc)
- 1ml Liquid LB
- 50% glycerol stock

Preparation of materials:

- 50% glycerol stock
Add 25ml 100% glycerol to 25ml SDDW and autoclave.
- X10MC

K ₂ HPO ₄ ·3H ₂ O	14.036gr
KH ₂ PO ₄	5.239gr
Glucose	20gr
Trisodium citrate	10ml 300mM
Ferric ammonium citrate (X1000)	1ml 22mg/ml
Casein Hydrolysate	1gr
Potassium Glutamate	2gr

* Mix with sDDW for final volume of 100ml

* Filter

* Dispense to 1ml aliquots

* Store at -20°C

Procedure:

1. Pick a single colony from an LB plate with a sterile wooden stick to a sterile tube containing the following:
 - * 900µl sDDW
 - * 100µl X10MC
 - * 10µl 1M MgSO₄
2. Mix by vortex

3. Incubate in a roller-drum shaker at 37°C for 3.5h

4. Dispense sample to sterile tubes each containing 300µl
5. Add to the tube 3µl gDNA or 5µl plasmid (1ug DNA)
- * One tube should contain no DNA for negative control
6. Incubate in a roller-drum shaker at 37°C for another 3h
7. Plate sample on two different selective marker plates with sterile beads on:
 - A. 250µl
 - B. 50µl
8. Incubate plate at 37°C over night
9. Perform differential plating from a single colony with sterile wooden stick to the same selective plate as in step 7.
10. Incubate plate at 37°C over night
11. If required perform differential plating from a single colony on another selective marker plate.
12. Incubate plate at 37°C over night
13. Pick a single colony with a wooden sterile stick into 1ml Liquid LB
14. Incubate at 23°C over night or at 37°C for 3h in a roller-drum shaker
15. Add 900µl sample to 600µl 50% glycerol in a freezing tube (nunc)
16. Vortex well
17. Freeze the strain at -80°C.
18. Add your strain's genotype and phenotype to the filemaker.

GOOD LUCK!!

(This protocol was kindly provided by the lab of Dr. Avigdor Eldar, Tel Aviv University)