

Vlad's transformation of *B.subtillis*.

1. Grow recipient strain(s) O/N in LB medium, supplemented with antibiotics if necessary.
2. In the morning take 2 ml O/N culture and centrifuge at 5000g for 5 min.
3. Wash one time with 1 ml of M1 medium, and centrifuge at 5000g for 5 min.
4. Resuspend in 1ml of M1 medium and inoculate 0.8ml in 10 ml of M1 .Grow for 4.5h at 37°C.
5. Transfer 1.5ml of culture in 10ml of M2. Grow for 2.5h at 37°C.
6. Add to 1ml of culture approximately 1µg of DNA. And Grow 15min at 37°C.
7. Plate the cells on selective LB plates with appropriate antibiotics (plate with 100µl, 300µl, 600µl).

Medium	M1	M2
MMx10	5 ml	5 ml
H ₂ O	41.85 ml	43.125 ml
Glucose 50%	0.5 ml	0.5 ml
Mg ₂ So ₄ 1M	0.3 ml	0.3 ml
Yeast extract 5%	2 ml	1 ml
Casein hydrolysate 10 %	100µl	50µl
Tryptophan 1%	250µl	25µl

Prepare 1 L MM x 10 :

K ₂ HPO ₄	140 g
KH ₂ PO ₄ · 3H ₂ O	60 g
(NH ₄) ₂ SO ₄	20 g
C ₆ H ₅ NaO ₇ · 2H ₂ O	
(Sodium Citrate Dihydrate)	10 g

1 L