B. subtilis transformation procedure

1. Inoculate *B. subtilis* culture in 10 ml MM broth. Incubate overnight at 37°C with vigorous aeration.
2. Use 0,6 ml overnight culture to inoculate 10 ml fresh MM broth.
3. Culture for 3 hours.
4. Add 10 ml pre-warmed 'starving' medium with the culture and continue the incubation with mixing for 2 more hours (the cells should acquire competence after 2 hours. Various starvation time can be tried).
5. Mix 10 ul of DNA to 0,4 ml competent cells and shake at 37 °C for 45 min or more (depends on expected antibiotic resistance)
6. After the transformation, plate cells onto appropriate selective media.

Media:

1. SMM (Spizizen Minimal Medium) – 1 liter:

- 0,2 % (w/v) ammonium sulphate (O 9)	2 g
- 1,4 % dipotassium phosphate (O 13)	14 g
- 0,6 % monopotassium phosphate (O 7)	6 g
- 0,1 % sodium citrate dihydrate (O 3)	1 g
- 0,02% magnesium sulphate 7H2O (O 53)	0,2 g

2. MM (competency medium)

- 10 ml SMM
- 0,125 ml 40% glucose
- 0,06 ml 1M MgSO4
- 0,01 ml 20% Casamino Acids
- 0,005 ml 0,22% iron ammonium citrate

3. „Starving" medium

- 10 ml SMM
- 0,125 ml 40% glucose
- 0,06 ml 1M MgSO4