

## **Isolation of chromosomal DNA from *Bacillus subtilis* for transformation**

1. Overnight culture separate into 0.8ml three parts into three 2ml tubes
2. Into each tube add 0.8ml SC buffer
3. Harvest cell by centrifugation at 13,300rpm, 1 min
4. Resuspend cell pellet in total 1ml SC buffer
5. Add 20  $\mu$ l 15mg/ml lysoenzyme 37°C 15min
6. Add 0.8 ml 5M NaCl and 0.2ml H<sub>2</sub>O
7. Filtrate DNA solution with 0.45  $\mu$ m filter
8. Use 100 $\mu$ l for *Bacillus subtilis* and 20 $\mu$ l/100 $\mu$ l cell on selection plates

SC buffer(pH 7.0) NaCl 0,15M sodium citrate 0,01M

Lysoenzyme 15mg/ml in SC buffer

NaCl 5 M