iGEM LMU-Munich 2012
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Protocol for enhancement of mature spore numbers

used for **Sporo**bead production

1. Overnight culture
   - inoculate your culture in 5 ml LB-Medium
   - let them grow over night at 37 °C

2. Exponential growth
   - messure the OD_{600} of your overnight culture (if necessary delute a sample 1:100 before measurement)
   - inoculate 2 ml LB-Medium to OD_{600} = 0.1 from overnight culture

     Calculation:

     $$\frac{0{,}1\ OD_{600}}{x\ OD_{600}} \times y\ ml$$
   - let the cells grow to an OD600 = 0.8

3. Sporulation
   - centrifuge the 2 ml of cells at 13,000 rpm for 1 minute
   - wash the pellet with bi-destilled water
   - resuspend the pellet in 1 ml DS-Medium
   - let the cell grow at 37 °C for minimum 24 hours

4. Lysozym treatment
   - dilute the cells 6:1 in lysozyme solution (15 mg/µl)
   - incubate them for 1 hour at room temperature

5. Used Puffer
   - Difco Sporulation Medium (DSM):

Nutrient Broth	8 g
KCl	1 g
MgSO4 (1 M)	1 ml
MnCl2 (10 mM)	1 ml
H2O (bidest)	ad 1000 ml

Add after autoclave:

CaCl2 (1 M)	0.5 ml
FeSO4 (1mM)	1ml

- Luria-Bertani (LB) Medium:

Tryptone	10 g
Yeast extract	5 g
NaCl	10 g
H2O (bidest.)	add 1000 ml

  - for LB plates: add 15 g/l of agar
  - important: cool down the agar solution to 50°C before adding antibiotics