PECF EPFL

Taq polymerase isolation from *E. coli*

Solutions to prepare:

Bacterial growth media
LB
Turbo/2XY or Terrific broth
Ampicillin 1000x stock 100mg/ml
IPTG 1000x stock 500mM stock
Lysozyme

buffer A: 50mM Tris pH8, 50mM Glucose, 1mM EDTA
buffer B: 10mM Tris pH8, 50mM KCl, 1mM EDTA, 0.5%Tween 20, 0.5%Nonidet P-40 or IGEPAL
10x PCR buffer: 500mM Tris pH 9.2, 17.5mM MgCl2, 150mM (NH4)2SO4

- Culture an overnight stock with glycerol (10ml LB + ampicillin 100μg/ml)
- Innoculate 200ml with Turbo broth (or a rich medium 2XY or Terrific broth + ampicillin 100μg/ml) with the 2ml o/n culture at 37°C until the OD 600= 0.6
- Induce the Taq expression with IPTG 0.5mM and incubate 16h at 250rpm 30°C (37°C)
- Centrifuge the bacteria for 15' in 50ml tubes
- Resuspend in 11.2ml (4 tubes x2.8ml/tube) buffer A
- Transfer to 15ml Falcon tube
- Prepare 2ml of buffer A, which contains 64mg Lysozyme
- Add 0.4ml in each tube (Lysozyme 4mg/ml final)
- Mix by inversion
- Incubate 15' RT
- Add 12.8ml (3.2ml/tube) buffer B
- Mix by inversion
- Incubate 1h at 75°C in a water bath and agitate time to time
- Centrifuge at max speed for 20' 4°C
- Keep 5 min on ice
- Take supernatant, and add glycerol (50/50 with water)
- Freeze at -80°C
- Without Glycérol, stable for 1month à 4°C

- From the glycerol stock, titrate against commercially available Taq (range of 0.5 ~ 4μl per PCR reaction)