Preparation of *Taq*-Polymerase (Updated 13. April 2016 Nico Dissmeyer)

Adapted from Desai *et al.* BioTechniques 19:780-784 (1995)

Please note that the following setup is calculated for 200 mL inoculated medium that gives 50 mL of Taq solution.

Day 1

- ☐ inoculate 3 ml LB containing Carbenicillin (25 µg/mL) with glycerol stock
- ☐ grow overnight or from 8 am till 3 pm at 37°C

Day 2

- ☐ take 2 ml of the preculture, inoculate 200 ml LB/Carb in a 2 L Erlenmeyer flask or scale up appropriately
- ☐ immediately prepare a fresh glycerol stock culture from the remaining mL for further use
- ☐ let grow till culture reaches OD_{600} to 0.3 (approx. 3 hrs)
- ☐ add 0.5 mM IPTG (1 ml/200 mL culture 100 mM IPTG)
- ☐ let the culture grow o/n @ 37°C (16 h)

Day 3

- ☐ harvest the cells by centrifugation 10 min @ 4000 rpm and discard the supernatant
- ☐ store the pellet at -20°C (essential step!) , at least 1 h or overnight to break the cells

Day 4

- ☐ resuspend the cells in buffer A: 6 ml/200 mL culture
  (OVERALL VOL. is now approx. 6 mL/200 mL culture)
- ☐ sonify suspension on ice for 2 min @ cycle 5 and 20% efficiency
- ☐ add 10 mM $MgCl_2$ (60µl/200 mL culture 1 M $MgCl_2$)
- ☐ add DNase I. For 200 mL culture, we used one tenth of a complete vial RNase-Free DNase Set (Qiagen, 1500 Units, Cat. No. 79254, 65,00 €, take up in 550 µL of the provided RNase-free water) or 0.2 µl/ml DNAse (unknown provider/concentration)
- ☐ incubate 30 min at 37°C in shaking sterilized waterbath or on the roller (suspension gets yellowish)
- ☐ add buffer B: 6 ml/200 mL culture
  (OVERALL VOL. is now approx. 12 mL/200 mL culture)
- ☐ heat to 75°C for 60 min in a shaking sterilized water bath until precipitatation occurs
- ☐ centrifuge at 13.000 rpm @ 4°C for 20 min
- ☐ mix the yellowish supernatant with one volume of storage buffer 50% glycerol: approx. 12 mL/200 mL culture
  (OVERALL VOL. is now approx. 24 mL/200 mL culture)
- ☐ mix now with one volume of the cloudy storage buffer 75% glycerol approx. 24 mL/200 mL culture (2 times the initial supernatant volume)
  (OVERALL VOL. is now approx. 48 mL/200 mL culture)
- ☐ aliquot to 500 µL and store the Taq solution (final glycerol conc. is 50%) at -20°C

Day 5

- ☐ check the quality by using different concentrations of the freshly prepared Taq compared to the previous stock

Normally 0.3-0.5µl of this preparation in a 50µl PCR reaction is sufficient. High amounts of *Taq* are inhibitory

Buffer A

10 ml (needed for 200 mL culture are 6 mL)

			check here:
50 mM Tris/HCL pH 7.9	1 M Tris pH 7.9 →	500 µl	☐
50 mM Glucose (M = 180,16 g/mol)	→	90,8 mg	☐
1 mM EDTA	0,5 M EDTA →	20 µl	☐
4 mg/ml Lysozyme	→	40 mg	☐
	ad 10 ml H_20		☐

Buffer B

10 ml (needed for 200 mL culture are 6 mL)

			check here:
10 mM Tris/HCL pH 7.9	1 M Tris pH 7.9 →	100 µl	☐
50 mM KCL	2,5 M KCL →	200 µl	☐
1 mM EDTA	0,5 M EDTA →	20 µl	☐
0,5% Nonidet P-40	10 % Nonidet →	500 µl	☐
0,5% Tween 20	10 % Tween 20 →	500 µl	☐
	ad 10 ml H_20		☐

10X Storage buffer
(used to prepare the two following glycerol containing buffers)

10 ml (needed for 200 mL culture are 5 mL)

final conc. in 1X storage buffer (not in the 10X stock!):

			check here:
50 mM Tris/HCL pH 8	1 M Tris pH 8 →	2.5 ml	☐
100 mM NaCl	5 M NaCl →	1 ml	☐
1 mM EDTA	0,5 M EDTA →	100 µl	☐
0,5 mM DTT (M:154,25 g/mol)	50 mM DTT →	500 µl	☐
1% Triton X-100	100% Triton X-10 →	500 µl	☐
	ad 5 ml H_20		☐

50% Glycerol storage buffer

20 mL (needed for 200 mL culture are 12 mL)

	check here:
12,5 g Glycerol (= 10 ml; density = 1,249 g/mL)	☐
2 ml 10X storage buffer	☐
ad 20 ml H2O	☐

75% Glycerol storage buffer *(sometimes very cloudy appearance)*

30 mL (needed for 200 mL culture are 24 mL)

	check here:
28,125 g Glycerol (= 22,5 ml; density = 1,249 g/mL)	☐
3 ml storage buffer (10x)	☐
ad 30 ml H2O	☐