

Isolation of genomic DNA from *Bacillus* (for PCR...)

(by Tina, 2010)

- Inoculate 10 ml LB medium from a fresh overnight culture and incubate at 37°C in a shaker
- At OD₆₀₀ of 0.8-1.0 harvest cells by centrifugation (10 min, 5000 rpm, RT)
- Resuspend cell pellet in 400 µl TEN and transfer the solution to a 2 ml eppendorf cup
- Add 20 µl lysozyme and incubate for 20 min at 37°C
- Add 2 µl RNase and incubate for 3 min 65°C
- Add 40 µl SDS, small amount (covering the tip of a spatula) of proteinase K and 550 µl TEN*, mix and incubate for 2 hours at 60°C
- Add 900 µl phenol (equilibrated with TE buffer, pH 7.5-8.0) and mix well by inverting the tube
- Centrifuge (5 min, 13000 rpm, RT) and transfer the upper phase into a new 1.5 ml eppendorf cup
- Repeat the extraction once with phenol and twice with chloroform:isoamylalcohol (24:1)
- Transfer the aqueous phase to 10 ml -20°C cold ethanol in a test tube
- Coil up the precipitated DNA with the bended tip of a Pasteur pipette
- Air dry the DNA
- Dissolve DNA in 100 µl TEN* overnight at 4°C
- For PCR, dilute the chromosomal DNA 1:50

TEN	10 mM	Tris/HCl pH 8.0
	10 mM	EDTA
	150 mM	NaCl

TEN*	10 mM	Tris/HCl pH 8.0
	1 mM	EDTA
	50 mM	NaCl

Lysozym	20 mg/ml
RNase A	20 mg/ml
SDS	10 % (w/v)