Isolation of genomic DNA (for PCR…)
(by Tina Wecke, 2010)
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1. Inoculate 10 ml LB medium from a fresh overnight culture and incubate at 37°C in a shaker
2. At OD600 of 0.8-1.0 harvest cells by centrifugation (10 min, 5000 rpm, RT)
3. Resuspend cell pellet in 400 µl TEN and transfer the solution to a 2 ml eppendorf cup
4. Ad 20 µl lysozyme and incubate for 20 min at 37°C
5. Add 2 µl RNase and incubate for 3 min 65°C
6. 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
7. Add 900 µl phenol (equilibrated with TE buffer, pH 7.5-8.0) and mix well by inverting the tube
8. Centrifuge (5 min, 13000 rpm, RT) and transfer the upper phase into a new 1.5 ml eppendorf cup
9. Repeat the extraction once with phenol and twice with chloroform:isoamylalcohol (24:1)
10. Transfer the aqueous phase to 10 ml -20°C cold ethanol in a test tube
11. Coil up the precipitated DNA with the bended tip of a Pasteur pipette
12. Air dry the DNA
13. Dissolve DNA in 100 µl TEN* overnight at 4°C
14. 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)