Cell Lysis: Pipetting scheme

Assume that RNases are everywhere. Steps at room temperature and without denaturating agents in solution should be performed fast. Ideally, use a Rnase-free benchtop with its own pipettes. Use RNase inhibitor if needed.

As this should be used in our device, we did explicitly not use temperatures below 23 ° C as a lysis step. Always measure your cell density before the experiment and dilute it to a common standard. In our experience, cell densities above 10^9 cells/ml are hardly usable for good, i.e. pure, results. Densities below 10^8 cells/ml do not result in enough DNA/RNA to be measured.

Aim of the experiment

This experiment is used to compare different ways of cell lysis. Heat-only lysis (too simple) and GuanidiniumSCN-lysis (common proprietary protocols) are not included.

Materials

Heat + SDS lysis:

adapted from http://ecoliwiki.net/colipedia/index.php/Methods:Genomic_DNA_prep

TES-Buffer (10mM Tris-HCl, 10mM EDTA, pH 8.0, 2% SDS)

Alkaline lysis :
adapted from
https://www.chemie.uni-kl.de/fileadmin/agpierik/Methoden/Miniprep_Birnboim_and_Doly.pdf

Solution 1
25 mM Tris-HCl, pH 8.0
10 mM Na_2EDTA
Store at 4 °C
Solution 2
0.2 M NaOH
1% SDS (mass-%)
Prepare freshly every month
Solution 3
3 M NaOAc (sodium acetate), pH 4.8 (24.6 g anhydr. NaOAc/100 ml, adjust pH with glacial acetic acid

Pipetting scheme

Method	SDS+Heat	Alkaline lysis
Prepare buffers...		
Amount:	1x	4x
Spin suspension at 300x G, 5 min (not lysis, just to remove medium)	100 mL bact-Suspension	100 mL bact-susp
Resuspend in ...	300 µl TES (0.45 ml in source)	66 µl Solution1
	Heat 15 min@75 °C lysis, vortex	Add 132 µl Solution 2, INVERT 4x
	(Addition of inhibitor if you have to wait before proceding)	Wait 0/1/3/10 minutes (no waiting / 0 min produced best/longest RNA)
		add 99 µl Solution 3, INVERT 4x
		Total volume: 297 µl (0.45 ml)
		(Addition of inhibitor if you have to wait before proceding)
Now do Phenol-chlorophorm-extraction or any other purification method.		