Chromosomal DNA Extraction from Gram-positive Bacteria

This procedure was originally developed for *Listeria monocytogenes* but has worked w
bacteria we've tried.

1. Pellet cells from 10 ml overnight cultures in BHI or LB and wash in 5 ml of 0.1
2. Resuspend in 1 ml 10 mM Tris-HCl (pH 8.0) containing 20 % sucrose (v/v), adc
   mg/ml, and incubate at 37 C for 45 min.
3. Add 9 ml lysis buffer (10 mM Tris-HCl [pH 8.0], 1 mM EDTA, 500 mg pronas
   incubate additional 30 min at 37 C.
4. Phenol and chloroform extract lysed cells, and ethanol precipitate the DNA with
   acetate, pH 4.8 and 2 vol. 95% ethanol.
5. Spool out DNA with a glass rod, wash once with 80% ethanol before drying.

Some bacterial species may require a longer incubation in lysozyme. For *Renibacteriu*
salmon pathogen we work with), lysozyme incubations overnight at 37 C worked very
of DNA

ref: Flamm, R. K., Hinrichs, D. J., and Thomashow, M. F. 1984. Introduction of pAM|
monocytogenes by conjugation and homology between native *L. monocytogenes* plasm
44:157-161. [Abstract]

ell with other Gram+

X SSC.
l lysozyme to 2.5

e B, 1 % SDS), and

0.1 vol. 3 M sodium

n salmoninarum (a G+
well with high yields

β1 into *Listeria*
nids. Infect. Immun.