

Materials List for:

Extraction of High Molecular Weight Genomic DNA from Soils and Sediments

Sangwon Lee¹, Steven J. Hallam¹

¹Department of Microbiology and Immunology, University of British Columbia - UBC

Correspondence to: Sangwon Lee at sangwon.ubc@gmail.com

URL: https://www.jove.com/video/1569

DOI: doi:10.3791/1569

Materials

Name	Company	Catalog Number	Comments
*Denaturing solution: 10 ml in total			
Guanidine isothiocyanate (MW 118.16), 4.73 g			
1M Tris-HCl (pH 7.0), 100 μl			
0.5M EDTA, 20 μl			
Add water to 9.95 ml in total.			
Autoclave.			
Add 50 μl 2-mercapt–thanol just before use. (5 μl of 2-mercapt–thanol per 1 ml Denaturing Solution)			
Note: Keep the Denaturing solution at 4 °C. Do not use buffer older than one week. If possible, make fresh buffer to use.			
**Extraction Buffer			
1M Sodium phosphate buffer [pH 7.0]*, 100 ml			
1M Tris-HCl [pH 7.0], 100 ml			
0.5M EDTA [pH 8.0], 200 ml			
5 M NaCl, 300 ml			

Autoclave and keep it at room temperature.

Add 200ml, 5% Hexadecyltrimethylammonium bromide (CTAB, autoclaved) and 100 ml, 20% SDS (autoclaved) just before use. If CTAB was crystallized, melt it at 60 °C.

* 1M Sodium phosphate buffer: 57.7 ml, 1M Sodium phosphate monobasic (NaH_2PO_4) and 42.3 ml, 1M Sodium phosphate dibasic (NaH_2PO_4). Adjust pH to 7.0

Note: Do not use 20 % SDS if it has precipitation. It is normal to see milky suspension when you add SDS to the solution. Once you add SDS, place the extraction buffer at 60 °C to ensure SDS is well suspended.