Extraction of bacterial DNA and RNA

Introduction

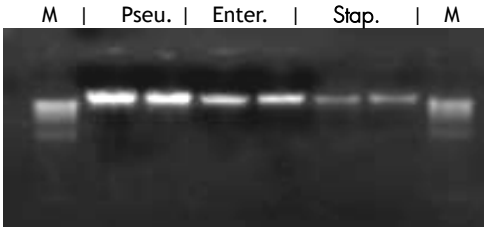
When extracting genomic DNA/RNA from bacterial samples, it is necessary to consider how to break bacterial cell wall and fully release DNA/RNA. Bacteria are divided into Gram negative and positive bacteria. Gram negative bacteria have a thin cell wall that can be fully lysed with only SDS and high temperature (65℃). Gram positive cells contain a thick cell wall, and the composition of the cell wall varies greatly, making it a difficult point for nucleic acid extraction, especially for bacteria such as Staphylococcus and Mycobacterium that are extremely difficult to lyse. Most Gram positive bacteria, such as Bacillus subtilis, can fully hydrolyze their cell walls after being treated with 1-15mg/ml lysozyme for 10-20 minutes. However, some bacteria, such as Staphylococcus aureus and Enterococcus faecalis, are not sensitive to lysozyme, and using lysozyme alone is not enough. Insufficient bacterial lysis can lead to a decrease in yield and even extraction failure. Magen's extracted HiPure Bacterial DNA Kit and HiPure Bacterial RNA Kit are designed specifically for bacterial DNA and RNA extraction using a silica gel column purification method. The kit can handle 1 x 10^9 bacteria at a time, and has successfully tested Escherichia coli, Bacillus subtilis, Pseudomonas aeruginosa, Enterococcus faecalis, Staphylococcus aureus, etc. The kit provides protease K, lysozyme and acid washed glass bead (0.1-0.2mm), which can achieve the best extraction effect through the combination of multiple wall breaking methods.

Product Name	Bacterial DNA Kit	Bacterial RNA Kit
Product	Genomic and plasmid DNA	Total RNA
Bacterial Number	<1.5 x 10^9	<1.5 x 10^9
Wall-breaking-method	Negative：SDS, 65℃ Positive: lysozyme	Negative: lysozyme Positive: lysozyme
Bacterial hard to lysis	Lysozyme + Glass Bead	Lysozyme + Glass Bead
Purification method	silica gel column	silica gel column

1. Results of bacterial DNA extraction

Extract Pseudomonas aeruginosa (negative), Enterococcus faecalis (positive, difficult to lyse) and Staphylococcus aureus (positive, extremely difficult to lyse) with HiPure Bacterial DNA Kit. After extraction, measure the OD value using Nanodrop 2000 and analyze integrity using 0.8% agarose electrophoresis. The results are as follows. According to the OD value, the purity and yield of DNA obtained by this method are ideal. From electrophoresis, it can be seen that the genomic DNA obtained by this method has good integrity and no degradation occurs.

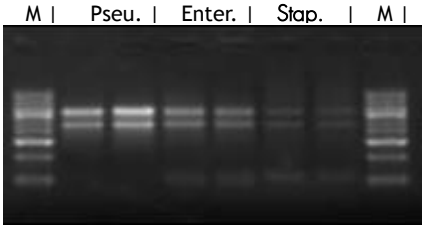
Bacterial	Cont. ng/μl	260/280	260/230	Yield μg
Pseudomonas aeruginosa	286.7	1.92	2.05	28.67
	330.4	1.92	1.95	33.04
Enterococcus faecalis	117.7	1.86	2.08	11.77
	152.7	1.85	2.11	15.27
Staphylococcus aureus	45.7	1.83	2.24	4.57
	56.3	1.82	1.71	5.63



2. Results of bacterial RNA extraction

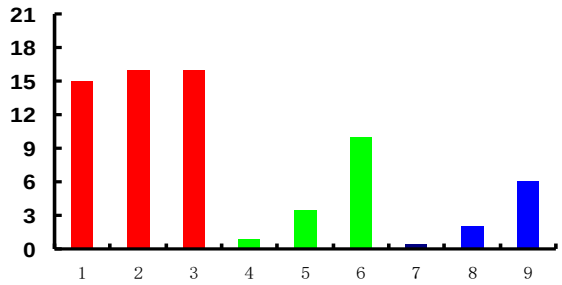
Extract Pseudomonas aeruginosa (negative), Enterococcus faecalis (positive, difficult to lyse) and Staphylococcus aureus (positive, extremely difficult to lyse) with HiPure Bacterial RNA Kit. After extraction, measure the OD value using Nanodrop 2000 and analyze integrity using 0.8% agarose electrophoresis. The results are as follows. From electrophoresis, it can be seen that the obtained RNA by this method has good integrity and no degradation phenomenon occurs. According to the OD value, the RNA yield obtained by this method is also ideal.

Bacterial	Cont. ng/μl	260/280	260/230	Yield μg
Pseudomonas aeruginosa	2.462	2.2	2.39	123.1
	1.4013	2.2	2.45	70.06
Enterococcus faecalis	0.1184	2.18	1.31	5.92
	0.0836	2.15	1.47	4.81
Staphylococcus aureus	0.0206	2	0.62	1.03
	0.022	2	0.96	1.1



3. The impact of different wall breaking methods on nucleic acid yield

Extract Pseudomonas aeruginosa (negative), Enterococcus faecalis (positive, difficult to lyse) and Staphylococcus aureus (positive, extremely difficult to lyse) with HiPure Bacterial DNA Kit. Use lysozyme, glass bead, and glass bead/lysozyme respectively to break the wall. The OD value of obtained DNA is measured using Nanodrop 2000. The results are summarized as follows.



1,4,7: Glass bead

2,5,8: Lysozyme

3,6,9: Glass bead/Lysozyme