Kit Components (100T)

component	Amount
Proteinase K	1 ml
EL Buffer	10ml
RS Buffer	10 ml
GA Buffer	20 ml
BA Buffer	20ml (add 21ml ethanol before use)
G Binding Buffer	50ml
Wash Buffer	42ml (add 63ml ethanol before use)
Elution Buffer	20 ml
Spin column	100
Handbook	1 copy

Storage

- The Proteinase K is to be stored at 2-8℃,others at 15-25℃.
- All reagents, when stored properly, are stable for 18 months.

Introduction

The Kit provides a very simple, fast and economic way for the isolating of pure high–molecular-weight genomic DNA from all kinds of bacterial, including Gram-NegativeBacterial and Gram-Positive Bacterial, adopting the Genomic DNA Buffer Set. The simplepurification procedure, based on the remarkable selectivity of Biospin membrane, allows isolation of high yields of pure genomic DNA in less than 1 hour. It requires no expensive equipment, involves only few steps, and completely avoids the use of toxic and hazardous reagents such as phenol and chloroform. In general, the Kit can get at most 30ug genomic DNA from 5×10^9 Bacterial cells.

The pure DNA can be applied extensively in PCR /Real time PCR, sequencing, Southern blot, mutant analysis, SNP and the others.

Principle

The structure of bacterial cells is destroyed completely by EL Buffer, DNA in the sample is liberated under the cooperation of RS Buffer, GA Buffer and PK solution. After lysis,. Simple processing completely removes contaminants and enzyme inhibitors such as proteins and divalent cationsgt Released DNA is bound exclusively andspecifically to the Biospin membrane inpresence of a Binding Buffer under appropriate salt iron and pH conditions. Denatured protein and other contaminants are removed by several washing procedures. The DNA is then eluted from the membrane with the Elution Buffer.

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Apparatus and Materials to Be Supplied by the User

* sterile 1.5ml micro centrifuge tubes
* 10μl/100μl/1000μl tips
* microcentrifuge capable of 14,000 × g
* Absolute ethanol
* Vortex mixer

Important notes

1. Gram negative bacteria(optional step):Please add 315mg Lysozyme to EL Buffer,mix thoroughly for 30seconds or so until the solution is clear,stored at 2-8℃.
   Gram positive bacteria:Please add 315mg Lysozyme to EL Buffer,mix thoroughly for 30seconds or so until the solution is clear,stored at 2-8℃.
2. Please add 21ml absolute ethanol to BA Buffer and mix thoroughly before use.
3. Please add 63ml absolute ethanol to Wash Buffer and mix thoroughly before use.
4. In order to optimize the effective result, the appropriate number of bacterial cells is at most 5×10^9, which OD600 is between 1.0~2.0.
5. RS Buffer may form precipitates upon storage. If a precipitate has formed, incubate the buffer at 56°C until the precipitate has fully dissolved.

Protocol

1. Harvest 0. 5～4. 0ml cells (maximum 2 x 10^9 cells) in a micro centrifuge tube by centrifuging for 1 min at maxmum speed. Discard supernatant as far as possible.
2. Resuspend pellet in 100 μl EL Buffer. Mix the mixture thoroughly with tip.
3. Incubate at 37℃ for10-60 minutes.
   For gram negative bacteria, Incubate at 37℃ for 10 15minutes.For gram positive bacteria,Incubate at 37℃ for 30-60mins or more times.
4. Add 100μl RS Buffer, ,10μl PK Solution, respectively, mix thoroughly.
   Optional: add 2μl RNase A(20mg/ml) and mix thoroughly.
5. Incubate at 56℃ for 15 minutes And remove the tube from 56℃.If the sample is difficult to be lysised,please extend the incubation time
6. Add 200μl GA Buffer and mix thoroughly.
7. Centrifuge at 13,000 x g for 1 minutes.
8. Transfer the supernatant to a new 1.5ml tube. If some mucous material exist in the supernatant,please transfer them to the new 1.5ml tube.
9. Add 400μl BA Buffer. Mix thoroughly.
10. Transfer the mixture to the spin column. Centrifuge at 13,000 x g for 1 minute. Discard flow-through.
11. Add 500μl of the G Binding Buffer into the spin column. Centrifuge at 10,000 x g for 1

minute. Discard flow-through.

12. Add 500µl Washing Buffer to the spin column. Centrifuge at 10,000 x *g* for 1 minute. Discard flow-through.
13. Repeat step 12th.
14. Centrifuge for an additional 1 minute at 10,000 x *g* and transfer the spin column to a sterile 1.5ml micro centrifuge tube.
15. Add 100µl Elution Buffer, incubate at room temperature for 1 minute.
16. Centrifuge at 10,000 x *g* for 1 minute. Remove Spin column, the buffer in the micro centrifuge tube contains the DNA.
17. The purified DNA can be used directly for kinds of downstream molecular biological experiments. Store at -20℃ if not used immediately.

FAQ

1. Which kind of bacteria we can extract genomic DNA from?

A We can extract genomic DNA from a majority of bacteria, including Gram Negative Bacterial and Gram Positive. We can break up the construct of bacteria's cell.

2. What can we do if RS buffer solution turn to muddiness ?

A Please shake up thoroughly.

3. Is RNase A necessary for the test?

A Determined by test aim. It's not necessary, if the extracted genomic DNA is used to backward test of molecular biology such as PCR, enzyme digestion and so on; It's necessary for RNA digesting adequately, if we want to get high purity genomic DNA.

4. Is there organic solution in the extraction process?

A No.

5. How long about the extracted bacteria genomic DNA fragments?

A Among 20 150KB.

6. What's the matter about the small quantity of the extracted genomic DNA?

A Pay attention to the following items

a) Please check that the bacteria is in the position of logarithmic phase when the bacteria reproduce fast. For research use only

b) Check to add lysozyme into EL buffer solution and shake up sufficient.

c) After add EL buffer solution, break up the bacteria mass at the bottom of tube and provide enough incubate time at 37℃.

d) Ensure that other extraction processes follow with the specification strictly.

7. **What's the quantity range of the bacteria for extraction?**

A Usually, the bacteria's quantity is among 10^7~10^8/ml, which was incubated on liquidculture medium and was in the position of logarithmic phase, and the max. quantity is 5×10^9 Please take count of cells if the bacteria was incubated on solid culture medium. Pay attention to enrichment when the bacteria's quantity is small.

Analysis DNA

⊕**Absorbance anlysis**

Get some DNA, diluted in a advisable factor with elution buffer. Survey the OD $_{260}$, OD $_{280}$ and OD $_{320}$.

expressions: concentration (μg/ml) $=50\times OD_{260}\times$dilution fact

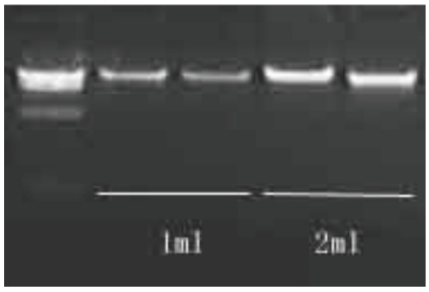
target: $2.0\geq OD_{260-320}/\ OD_{280-320}\geq 1.7$

Notice: $1.0\geq OD_{260}\geq 0.1$, the result of ratio is much reliable.

⊕**Agarose Gel Analysis**

0.8 ~ 1% Agarose gel

Example 1: different amount



Example 2: G+: gram-negative, G-: gram-positive