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Miniprep DNA Purification Kit

I. Description

Takara’s Miniprep DNA Purification Kit is designed to allow simple, quick and reliable isolation of plasmid DNA, utilizing silica membrane to which DNAs are bound. This system is suitable to efficiently isolate plasmid of less than 20 kbp in size. The purified plasmid can be used directly for DNA sequencing as well as for other molecular biology techniques, such as enzyme reactions, without further manipulation. This kit can yield 1 - 20 µg of plasmid DNA from 1 - 10 ml of overnight cultures of E. coli. Also as this kit employs alkaline protease, highly purified plasmid DNA without contamination of nuclease can be prepared from endA- E. coli strain, like HB101.

II. Kit component (for 50 reactions)

1. Cell Suspension Buffer ....................................... 20 ml
2. Cell Lysis Buffer ................................................. 20 ml
3. Neutralization Solution ....................................... 30 ml
4. Wash Buffer* ...................................................... 20 ml
5. Miniprep Spin Column ....................................... 50 pcs
6. Collection Tube(2 ml) ........................................ 50 pcs
7. Alkaline Protease Solution ................................ 550 µl
8. Nuclease-free dH₂O ........................................... 13 ml

* Prior to use, prepare Wash Buffer to be 55 ml by adding 35 ml of ethanol.

III. Storage

Room temperature (Recommended at 22 - 25°C)

IV. Required Reagents & Instruments other than Kit

- Microcentrifuge (available at 14,000 x g)
- Sterile 1.5 ml microcentrifuge tubes
- Centrifuge and tubes for recovery of E. coli cells from culture broth
- Micropipette
- Ethanol
- Luria-Bertani (LB) medium including antibiotic (plate and liquid medium)

V. Protocol

A. Preparation of E. coli

Take a single colony from a fresh LB plate containing antibiotic and inoculate into LB liquid medium*1 containing same antibiotic. Incubate by shaking at 37°C overnight (12 - 16 hours).*2 When using high-copy plasmids, up to 5 ml*3 of bacterial culture can be available for this kit. When using low-copy plasmids, this kit can process up to 10 ml of bacterial culture.*4

*1: It is recommended to use LB medium. Plasmid DNA may not be efficiently prepared due to the overload of cells, if rich media for high density culture, such as Terrific Broth, are used.
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*2: After incubation, the absorbance at 600 nm would become 2 - 4.

*3: If more than 5 ml of bacterial culture is applied for a single column, the yield of plasmid DNA will be lowered since it exceeds the capacity of Miniprep Spin Column.

*4: If larger volume than 10 ml of bacterial culture is used, bacterial lysis cannot be done sufficiently. Accordingly, the purity of plasmid DNA would be lowered.

B. Preparation of a Cleared Lysate

1) Centrifuge the bacterial culture* at 10,000 x g for 5 minutes.
   * In case of high-copy plasmid, the volume should be 1 - 5 ml. In case of low-copy plasmid, the volume should be 10 ml.

2) Discard the supernatant and remove excess media by blotting the inverted tube on a paper towel.

3) Add 250 µl of Cell Resuspension Buffer and completely suspend the pellets by vortexing or pipetting. Transfer the suspension into a 1.5 ml microtube.

4) Add 250 µl of Cell Lysis Buffer and mix by inverting a tube 4 times. Do not vortex. When the cell suspension solution gets clear, proceed to the next step. If not, incubate it at room temperature for 1 - 5 minute. Do not incubate longer than 5 minutes.

5) Add 10 µl of Alkaline Protease Solution and mix by inverting the tube 4 times. Do not vortex. Incubate at room temperature for 5 minutes.*1,*2

   *1: In order to prevent the damage of plasmid DNA, do not incubate longer than 5 minutes after addition of Alkaline Protease Solution.

   *2: This step is essential for endA+ strains such as HB101. When using endA- strains such as JM109 or DH5α, highly purified plasmid DNA can be obtained even if this step is removed.

6) Add 350µl of Neutralization Solution and immediately mix by inverting a tube 4 times. Do not vortex.

7) Centrifuge at 14,000 x g for 10 minutes at room temperature.
**C. Plasmid of Plasmid DNA**

Prepare Wash Buffer (20 ml) to be 55 ml by adding 35 ml of ethanol before use.

1) Set Miniprep Spin Column in a Collection Tube.

2) Transfer approximately 850 µl of the supernatant of bacterial lysate prepared at B-7 into Miniprep Spin Column. Pay attention not to take in white precipitate together.*1

*1: When white precipitate is brought in, transfer the solution in Miniprep Spin Column into a 1.5 ml microcentrifuge tube. Centrifuge for 5-10 minute and pour the supernatant into Miniprep Spin Column again.

3) Centrifuge at 14,000 x g for 1 minute at room temperature. Take off Miniprep Spin Column from Collection Tube. Discard the filtered solution and set the Miniprep Spin Column in the Collection Tube again.

4) Add 750 µl of Wash Buffer added with Ethanol into the Miniprep Spin Column.

5) Centrifuge at 14,000 x g for 1 minute at room temperature. Take off Miniprep Spin Column from Collection Tube. Discard the filtered solution and set the Miniprep Spin Column in the Collection Tube again.

6) Add 250 µl of Wash Buffer added with Ethanol into the Miniprep Spin Column.

7) Centrifuge at 14,000 x g for 2 minutes at room temperature.

8) Transfer the Miniprep Spin Column into a new 1.5 ml microcentrifuge tube.*2

*2: Pay attention not to take Wash Buffer in. In case it is brought in, centrifuge for 1 minute and transfer the Miniprep Spin Column into a fresh microcentrifuge tube.

9) Add 100 µl of Nuclease-Free dH₂O into Miniprep Spin Column.

10) Centrifuge at 14,000 x g for 1 minute at room temperature to elute DNA.

11) Take off Miniprep Spin Column, cap the microcentrifuge tube, and store the purified plasmid DNA at -20°C.*3

*3: When storing at 4°C, add 10 µl of 10 X TE buffer. However it is not recommended to add TE buffer when applying the prepared plasmid DNA directly for sequencing. Store it at -20°C.
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VI. Yields of plasmid DNA

The yield of plasmid DNA varies depending on a number of factors; such as the volume of bacterial culture, plasmid copy number, type of culture medium, bacterial strain used. When using the plasmid of high-copy number, 3.5 - 5.0 µg of plasmid DNA can be obtained from 1.5 ml overnight culture of LB medium using DH5α as a host. When using the plasmid of low-copy number, 1.5 - 3.0 µg of plasmid DNA can be obtained from 10 ml of LB bacterial culture.

VII. Trouble shooting

<table>
<thead>
<tr>
<th>Symptoms</th>
<th>Possible Causes</th>
<th>Remedial measures</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clear cell lysis is not obtained.</td>
<td>Too many bacterial cells in culture medium was applied.</td>
<td>Reduce the applied volume of bacterial culture to be applied. Maximum volume; 10 ml for low-copy plasmid, 5 ml for high-copy plasmid. Use LB medium added with antibiotic for culture.</td>
</tr>
<tr>
<td>Insufficient suspension of bacterial cell pellet.</td>
<td></td>
<td>Suspend the cell pellets thoroughly before cell lysis. Thorough vortexing or pipetting should be done after addition of Cell Suspension Buffer. If cell clumps are visible, suspension is still insufficient.</td>
</tr>
<tr>
<td>Plasmid DNA can not be recovered.</td>
<td>Ethanol was not added to Wash Buffer.</td>
<td>Add Ethanol into Wash Buffer before starting protocol.</td>
</tr>
<tr>
<td>The yield of plasmid DNA is low.</td>
<td>Nontransformed cells without plasmid were grown.</td>
<td>Confirm that all liquid and solid medium contain antibiotic.</td>
</tr>
<tr>
<td>Bacterial culture was old.</td>
<td></td>
<td>Innoculate a fresh single colony overnight-cultured on a plate into a liquid medium added with antibiotic.</td>
</tr>
<tr>
<td>Plasmid of less copy number was used.</td>
<td></td>
<td>Check the copy number of used plasmid. It is recommended to use of higher copy number of plasmids.</td>
</tr>
<tr>
<td>Plasmid DNA is damaged.</td>
<td>Cell lysate was over incubated during alkaline lysis step.</td>
<td>As soon as the cell lysate becomes clear after added and mixed with Cell Lysis Buffer, proceed to the next step. Do not incubate longer than 5 minutes after added with Alkaline Protease Solution.</td>
</tr>
</tbody>
</table>
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<table>
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<tr>
<th>Symptoms</th>
<th>Possible Causes</th>
<th>Remedial measures</th>
</tr>
</thead>
<tbody>
<tr>
<td>DNA yields measured by electrophoresis is</td>
<td>Contaminants in the eluted DNA impeded the spectrophotometric readings.</td>
<td>Even when Wash Buffer is brought in at the step C-8, the measured value of spectrophotometric readings are influenced. Extract the recovered plasmid DNA with phenol/chloroform and recover again with ethanol precipitate. Then repeat spectrophotometric readings.</td>
</tr>
<tr>
<td>lower than spectrophotometric readings.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Contamination of genomic DNA.</td>
<td>Genomic DNAs were fragmented.</td>
<td>Do not mix by vortexing after adding Cell Lysis Buffer in order to prevent genomic DNAs from damaged.</td>
</tr>
<tr>
<td>Insufficient result of analysis by automated</td>
<td>Too little DNA was added to the sequencing reaction.</td>
<td>Inoculate a fresh single colony of <em>E. coli</em> into a fresh LB medium. As an accurate spectrophotometric readings may not be obtained, quantitate DNA also by agarose gel electrophoresis for the most accurate quantitation.</td>
</tr>
<tr>
<td>sequencing.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TE buffer was used for elution of DNA.</td>
<td></td>
<td>Re-purify plasmid DNA and elute in Nuclease-free dH₂O.</td>
</tr>
<tr>
<td>Restriction digestion is not performed.</td>
<td>Concentration of restriction enzyme and length of digestion time were not appropriate.</td>
<td>Increase the amount of restriction enzyme or extend the incubation time. Perform restriction digestion at the suitable temperature and in the optimized buffer to the enzyme used. Especially when using an enzyme which has lower optimized salt concentration, ethanol precipitation of plasmid DNA is recommended to thoroughly remove salts that may have carried over.</td>
</tr>
</tbody>
</table>
VIII. Endonuclease I

Endonuclease I is a 12 kDa periplasm protein of *E. coli* and it degrades double-stranded DNA. This protein is encoded by the gene *endA*. The *E. coli* strains with the genotype *endA*1 have a mutation in the wildtype gene *endA*, so they produce an inactive form of Endonuclease I. The strains without the genotype *endA*1 are a wildtype which produce active Endonuclease I. The strains having the mutation are referred to as *endA−*, and the ones without the mutation is referred to as *endA+*. If plasmid DNA is extracted from *endA−* strain with a simple procedure, Endonuclease I may be contaminated, which would affect the purity of plasmid. As Takara's Miniprep Kit employs Alkaline Protease Solution in the process of cell lysis, highly purified plasmid DNA can be obtained from both *endA−* and *endA+* by inactivating Endonuclease I.

Alkaline protease can help to raise the purity of plasmid DNA because Alkline Protease degrades other *E. coli* proteins as well as Endonuclease I. However, when applying the extracted plasmid directly to fluorescene automated sequencing, it is recommended to use *endA−* *E. coli* strains. The supplied Alkaline Protease was prepared from *Bacillus licheniformis*. The optimum pH of this enzyme is > pH9, so It does not show activity after added with Neutralization solution.

<table>
<thead>
<tr>
<th></th>
<th><em>endA−</em></th>
<th><em>endA+</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>DH5 α</td>
<td>CJ236</td>
<td></td>
</tr>
<tr>
<td>JM109</td>
<td>HB101</td>
<td></td>
</tr>
<tr>
<td>NovaBlue</td>
<td>MV1184</td>
<td></td>
</tr>
<tr>
<td>XL1-Blue</td>
<td>TH2</td>
<td></td>
</tr>
</tbody>
</table>

IX. Composition of Buffer and Medium

10X TE buffer

- 100 mM Tris-HCl (pH 7.5)
- 10 mM EDTA

LB medium

- 1% casein peptone
- 0.5% yeast extract
- 0.5% NaCl
- 1.5% agar (for plates only)
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X. Reference


Note: For Food and Environmental Testing use only.
Not for use in human and animal diagnostic or therapeutic.