Rapid Plasmid DNA Extraction Mini Kit Handbook

Prepare plasmid DNA of molecular biology grade purity in 8 min.

Sample Amount: 1-5 ml of bacteria cultured

Specially added neutralizing indicator

Binding capacity: up to 50 μg

Ver：2024/09

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Composition

Rapid Plasmid DNA Extraction Mini Kit Cat. No.	5 Preps 1005005	50 Preps 1005050	250 Preps 1005250
Spin Columns	5	50	250
2 ml Collection Tubes	5	50	250
RNase A	*	28 μl	140 μl
Buffer I	1.5 ml	14 ml	70 ml
Buffer II	1.5 ml	14 ml	70 ml
Buffer N8	2 ml	20 ml	100 ml
Buffer W2 (concentrate)	2.5 ml	25 ml	65 ml×2
Buffer E	0.6 ml	6 ml	30 ml
Handbook	1	1	1

* RNase A from 5 Preps has been added to Buffer I.

Storage

1. RNase A can be transported at room temperature (0~30℃), please store RNase A at 2~8℃ after receiving the product.
2. Buffer I with RNase A added need to be stored at 2~8℃. If stored for more than 6 months, RNase A should be re-added in Buffer I to a final concentration of 100 μg/ml.
3. All the reagents and components can be stored for up to 2 years at room temperature (0~30℃). For longer storage, it is recommended to keep at 2~8℃.
4. The product stored at 2~8℃ should be restored to room temperature before use.

Equipment and Reagents to Be Supplied by User

1. Absolute ethanol.
2. Buffer W1 may needed.
3. 1.5 ml and 2 ml centrifuge tubes, pipettes, and tips.
4. Protective equipment such as disposable latex gloves and paper towels.
5. Microcentrifuge(s) (with rotor for 1.5 ml and 2 ml centrifuge tubes).
6. Vortexer.

Quality Assurance

Hangzhou Simgen Biotechnology Co., Ltd. guarantees that the products provided are qualified products that have passed the quality inspection. If user finds that the product cannot meet the experimental requirements, please stop using the product immediately and contact Simgen technical support for help. E-mail: technical@simgen.cn, Tel: 400-0099-857.

Introduction

The Rapid Plasmid DNA Extraction Mini Kit adopts the latest invention of special neutralization solution Buffer N8, which allows users to quickly extract up to 50 μg high purity plasmid DNA from 1~5 ml of bacteria cultured (LB medium) in 8 min. The plasmid DNA obtained is suitable for molecular biology experiments such as restriction enzyme digestion, sequencing, library screening, ligation and transformation, in vitro transcription and translation, and transfection of robust cells. If purified plasmid DNA is used for transfection of sensitive cells, choose the Endo-free Plasmid DNA Extraction Mini Kit (Simgen Cat. No. 1009050), Endo-free Plasmid DNA Extraction Double Mini Kit (Simgen Cat. No. 1006050) or Endo-free Plasmid DNA Extraction Midi Kit (Simgen Cat. No. 1016025）。

Safety Information

Buffer II and Buffer N8 contain irritating compounds, please wear latex gloves and protective goggles during operation to avoid staining the skin and eyes and beware of inhalation into the mouth and nose. If the skin or eyes are contaminated, rinse immediately with plenty of water or saline, and seek medical help if necessary.

Applicable Samples

Rapid Plasmid DNA Extraction Mini Kit is most suitable for use in experiments such as plasmid construction, clone identification, and sequencing, these experiments usually use high-copy plasmids, and the host bacteria are DH5α or other endA- bacterial. This product can extract plasmid DNA from 1~5 ml overnight bacteria cultured (LB medium) and the operation is very simple and fast.

Rapid Plasmid DNA Extraction Mini Kit is suitable for the extraction of plasmid DNA from Gram-negative bacteria(such as *E. coli*). If it is necessary to extract plasmid DNA from Gram-positive bacteria, please first digest the wall of the bacteria with lysozyme, refer to "Appendix 1: Extraction of Gram-positive Bacterial Plasmid DNA" on page 12 for details.

Operation Step Analysis and Explanation

1. Start sample

Pick a monoclonal colony from freshly cultured plates and inoculate into 3~5 ml of LB medium containing the corresponding antibiotic, 37℃ with vigorous shaking for 12~16 h.

Cautious:

(1) **Bacteria in glycerol stocks should not be directly cultured.** Bacteria in long-stored glycerol stocks may have undergone genotypic differentiation between individuals and may be mixed with bacteria that have lost plasmids. Glycerol stocks must be streaked on plates with antibiotics for screening, and well-grown single colonies should be selected for

inoculation and culture.

(2) **Do not pick multiple single clones into one liquid medium for culture.** There may be survival competition between different colonies due to genotypic differences, which may reduce the harvest of bacteria.

(3) **Colonies stored in a 2-8℃ refrigerator for more than two weeks have begun to die** and are no longer suitable for inoculation and culture.

(4) **Bacteria cultured time should not exceed 16 hours.** Bacteria cultured for more than 16 hours begin to dissolve, which may reduce the yield of plasmid DNA.

(5) The volume of the medium used for bacteria cultured should not exceed 1/4 of the container.

(6) By increasing the NaCl content in LB medium to 10 g/L, the recovery efficiency of plasmid DNA per milliliter of LB bacteria cultured can be increased by about 2 μg.

2. Bacteria lysis

The rapidly collected bacteria (centrifuge at 12000 rpm for 30 sec) are usually packed tightly and not easily suspended. Instead, centrifuge at 3000 rpm for 5 min can be used to collect bacteria for subsequent suspension steps.

Bacteria must be fully suspended with Buffer I, and bacteria that are not dispersed by suspension cannot be lysed by Buffer II. After bacteria lysis, both plasmid DNA and genomic DNA will release and be in a denatured state.

The solution should appear viscous and transparent after the bacteria lysis, and **if the solution still presents turbidity after adding Buffer II and does not show viscous, the possibility of fungal contamination in the culture should be considered.**

3. Precipitation of bacteria fragments and genomic DNA

Buffer N8 neutralizes the alkaline material in Buffer II. The plasmid DNA will revert to the supercoiled structure and remain in solution, and large fragments of genomic DNA will be entangled with the bacteria fragments during the renaturation process to form a precipitate. After full speed centrifugation, genomic DNA and bacteria fragments will be packed at the bottom of a 1.5 ml centrifuge tube to achieve separation from plasmid DNA.

4. Neutralizing Indicator

Due to the growth conditions of bacteria or differences between bacteria strains, it is difficult for users of plasmid DNA extraction for the first time to obtain the best neutralization results. This kit has added a neutralization indicator to Buffer I, when add Buffer N8 and invert the centrifuge tube, the blue precipitate will gradually change color until it completely transforms into a light-

yellow precipitate. At this point, it can be determined that the effect of sufficient neutralization is achieved.

5. Column purification

1) DNA binding

Pour the supernatant containing plasmid DNA into a Spin Column and centrifuge for a few secs, the DNA can be bonded to the silica membrane of the Spin Column, and residual proteins or impurities that inhibit downstream molecular biological reactions are filtered out.

2) Wash

Buffer W2 can wash away the remaining salts and pigments on the membrane, ensuring that pure plasmid DNA is bonded to the Spin Column. In the process of plasmid DNA binding and washing, there are no strict requirements on centrifugation speed and centrifugation time. The "short run" or "quick" mode in the centrifuge can be selected to save operation time.

The Spin Column may contain trace amounts of protein, which are not affect the results of typical molecular biology experiments. However, when extraction plasmid DNA from endA+ (wild type) bacteria, trace residual proteins including Non-specific endonuclease I can interfere with enzyme cutting. At this point, Buffer W1 (not provided) needs to be added for washing before Buffer W2 washing to remove trace proteins remaining on the Spin Column. The following steps need to be added between step 5 and step 6: Discard the filtrate, place the Spin Column back into the 2 ml Collection Tube, add 500 µl Buffer W1 to the Spin Column, close the lid, and centrifuge at 12000 rpm for 30 sec.

The following table shows the endA genotypes of common host bacteria.

EndA*- Strains of *E. Coli							
DH5α	DH1	DH21	JM106	JM109	SK2267	SRB	XLO
TOP10	DH10B	JM103	JM107	SK1590	MM294	Stbl2™	XL1-Blue
BJ5182	DH20	JM105	JM108	SK1592	Select96™	Stbl4™	XL10-Gold
EndA*+ Strains of *E. Coli							
C600	JM110	RR1	ABLE® C	CJ236	KW251	P2392	BL21(DE3)
HB101	TG1	TB1	ABLE® K	DH12S™	LE392	PR700	BL21(DE3) pLysS
JM101	JM83	TKB1	HMS174	ES1301	M1061	Q358	BMH 71-18
All NM strains				All Y strains			

3) High-speed spin dry

Place the washed Spin Column back into the 2 ml Collection Tube, and centrifuge at full speed (≥12,000 rpm) for 1 min. Allow Buffer W2 to be sufficiently centrifuged to remove.

During the discarding of the Buffer W2 filtrate, if the filtrate accidentally contaminates the Spin Column, it can also be removed by centrifugation again.

4) Elution of plasmid DNA

A. We recommend elute plasmid DNA using Buffer E (5 mM Tris-HCl, pH 8) provided in the kit for long-term stable storage of plasmid DNA, or elution with deionized water (pH＞7), otherwise the elution efficiency of the DNA will be affected.

B. Increasing the volume of Buffer E or deionized water used to elute plasmid DNA increases the recovery efficiency of plasmid DNA. Decreasing the elution volume increases the concentration of plasmid DNA obtained. If the volume of Buffer E or deionized water used to elute plasmid DNA is too small (e.g. 20 μl), although it is possible to increase the concentration of plasmid DNA obtained, the total amount of plasmid DNA obtained will be severely reduced because the eluate could not effectively soakage the Spin Column membrane.

C. Pre-warmed Buffer E or deionized water can improve the elution efficiency of plasmid DNA.

D. Extending the incubating time (extended to 3-5 min) after adding Buffer E or deionized water to the Spin Column can improve the elution efficiency of plasmid DNA.

E. The spin-dried Spin Column can be directly added to Buffer E to elute plasmid DNA, without volatilizing residual ethanol, and the over-drying Spin Column is not conducive to the elution of plasmid DNA.

F. For the safety of product use, if the centrifuge does not have a leak-proof lid, we recommend changing the conditions for eluting plasmid DNA to 8000 rpm centrifugation for 1 min to prevent the 1.5 ml centrifuge tube lid from falling off and damaging the centrifuge.

6. Extraction efficiency and purity of plasmid DNA

The amount of plasmid DNA obtained from bacteria depends primarily on the number of copies of plasmid DNA in the organism, and for high-copy plasmids, 5~15 μg plasmid DNA is typically obtained from 5 ml LB bacteria cultured. For low-copy plasmids or if you want to extract the maximum amount of plasmid DNA at once, we recommend using Plasmid DNA Double Mini Extraction Kit (Simgen Cat. No. 1007050) to extract Plasmid DNA from 15 ml LB bacteria cultured.

The following table shows the copy numbers of common plasmids:

plasmid	Origin of replication	Copy number	Classification
pUC vectors	pMB1	500–700	high copy
pBluescript vectors	ColE1	300–500	high copy
pGEM® vectors	pMB1	300–400	high copy
pTZ vectors	pMB1	>1000	high copy
pBR322 and derivatives	pMB1	15–20	low copy
pACYC and derivatives	p15A	10–12	low copy
pSC101 and derivatives	pSC101	~5	very low copy

When the plasmid DNA contained in the bacteria lysate is sufficient, the limiting factor of DNA extraction is determined by the Spin Column. If the plasmid DNA in the bacteria lysate is less than 30 μg, the Spin Column can approach 100% efficient adsorption plasmid DNA. If the plasmid DNA in the solution is ≥30 μg, the Spin Column can still adsorb more plasmid DNA, but some plasmid DNA will be lost in the filtrate. If plasmid DNA is continuously loaded, the saturation loading of the Spin Column can reach up to 50 μg or more (note: When the DNA adsorbed on the Spin Column reaches saturation, more than 100 μl Buffer E should be used to elute the plasmid DNA. Otherwise, a large amount of plasmid DNA will remain on the Spin Column and not be fully eluted).

The Spin Columns (Simgen Cat. No.7001050, available separately) are very stable and can maintain the original DNA adsorption capacity at room temperature for 2 years.

The quality of DNA is usually estimated by measuring the light absorption value at 260 nm, which is translated into 1 OD optical density equivalent to 50 ng/μl double-stranded DNA. The purity of DNA is usually estimated by A260/A280, and the A260/A280 ratio of pure DNA should be between 1.7 and 1.9. DNA salinity is usually estimated by A260/A230, and the A260/A230 ratio for pure DNA should be between 1.8 and 2.5.

Preparation Before Use

1. If the centrifuge has refrigeration function, please set the temperature to 25°C.
2. Add 1 ml Buffer I to the cryogenic tube containing RNase A, mix well, and then transfer the solution back into the bottle containing Buffer I, and tick the box on the label to mark "RNase A Added", and store at 2~8°C.
3. Add absolute ethanol to Buffer W2 according to the instructions on the label of the reagent bottle and tick the box on the label to mark "Ethanol Added".
4. When the room temperature is below 15°C, check if white precipitates appeared in Buffer II before use. If there is precipitate, dissolve it by incubating at 37°C.

Quick Protocol Flowchart

Scan the QR code to watch the operation video



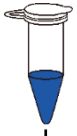
Collect 1~5 ml bacteria.

Add 250 µl Buffer I with RNase A added.



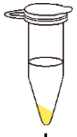
Vortex or pipet to suspend bacteria.

Add 250 µl Buffer II.



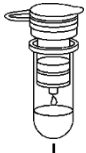
Gently invert to Mix.

Add 350 µl Buffer N8, gently invert until the blue precipitate turns completely yellow.



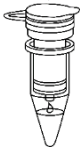
Centrifuge at full speed for 2 min.

Transfer the supernatant to a spin column.



Add 800 µl Buffer W2.

High-speed spin dry.



Elute plasmid DNA.

Protocol:

1. Collect 1~5 ml overnight cultures of bacteria cells in LB (Luria-Bertani) medium by centrifuging at 12,000 rpm for 30 sec, discard the medium. Fully suspend bacteria pellet in 250 µl Buffer I with RNase A added.

* The pellet could be suspended completely by vortexing or pipetting until no pellet left. Otherwise, it would seriously affect the final plasmid DNA yield.

2. Add 250 µl Buffer II, gently invert the tube 4~6 times to mix well.

* Before using Buffer II, make sure that no visible precipitate exists in the solution. After using Buffer II, tighten the lid to avoid prolonged contact with air.
* Do not vortex, as this will result in residue of genomic DNA.
* The solution should be viscous and slightly clear when the bacteria are lysed sufficiently, if too many bacteria were used, continue inverting the tube until the solution becomes viscous and slightly clear.
* Do not allow this step to proceed for more than 5 min.

3. Add 350 µl Buffer N8, gently invert the tube several times until the color of precipitate changes from blue to light yellow completely.

* Do not vortex, as this will result in residue of genomic DNA.
* Continue to gently invert 4~6 times after the color of precipitate changes from blue to light yellow completely, if the bacteria culture medium used was more than 3 ml.

4. Centrifuge at full speed (≥12,000 rpm) for 2 min.

* Prolong the centrifuge time to 5 min if more than 3 ml bacteria cultured was used.

5. Decanting the supernatant from step 4 into a Spin Column (the Spin Column is placed in a 2 ml Collection Tube), close the lid and centrifuge at 12,000 rpm for 30 sec.

* Decanting the supernatant into the Spin Column immediately after centrifugation, stay for a long time may cause the precipitate to float, which makes it hard to operate in following procedure.

6. Discard the filtrate, place the Spin Column back to the 2 ml Collection Tube. Add 800 µl Buffer W2 to the Spin Column. Closed the lid and centrifuge at 12,000 rpm for 30 sec.

* Repeat step 6 is recommend ensuring high quality of plasmid, if plasmid will be used for cell transfection.
* Ensure absolute ethanol has been added into Buffer W2.
* For endA+ host bacteria (e.g. wild-type E.coli, HB101, BL21), washing the Spin Column by adding Buffer W1 (Order separately, Simgen Cat. No. B025-01) is necessary to remove trace nuclease activity.

7. Discard the filtrate, place the Spin Column back to the 2 ml Collection Tube. Centrifuge at full speed (≥ 12,000 rpm) for 1 min.

* Do not omit this step, as this will cause problems in downstream applications due to the residual ethanol in the eluate.

8. Discard the 2 ml Collection Tube, place the Spin Column in a new 1.5 ml centrifuge tube, add 60~100 µl Buffer E to the center of the Spin Column, close the lid, incubate at room temperature for 1 min, and centrifuge at 12,000 rpm for 30 sec.

* The deionized water can also be used to elute the DNA, but the pH should be ensured at 7.0~8.5, otherwise it may affect the elution efficiency.

9. Discard the Spin Column. Eluted DNA can be used for various molecular biology experiments or store at -20℃ for later use.

Troubleshooting Guide

1. Plasmid DNA is low or no yield

Possible causes:

1) Absolute ethanol is not added in Buffer W2, absolute ethanol should be added according to the instructions on the label of the reagent bottle. If other reagent was added by mistake, please contact our technical support for help.
2) 70% ethanol was added in Buffer W2 by mistake. Please contact our technical support for help.
3) **Bacteria cultured are contaminated with fungi or other miscellaneous bacteria (note: antibiotics that work only against prokaryotes, not effective against fungi).** Make sure to pick monoclonal colonies from freshly prepared antibiotic-containing plates for inoculation culture.
4) The plasmid is not transformed into the host bacteria. Make sure to pick up monoclonal colonies from freshly prepared antibiotic-containing plates for inoculation and culture and make sure to add the appropriate antibiotics to the medium.
5) **Do not aspirate bacteria in glycerol stocks directly for bacteria cultured.** Please refer to the "Start Sample" on page 2 for details.
6) The purified plasmid is a low copy plasmid. Please select Plasmid DNA Double Mini Kit (Simgen Cat. No. 1007050), increase the amounts of bacteria cultured to extract plasmid DNA.
7) Buffer II has a white precipitate. If there is a precipitate, Buffer II should be incubated at 37℃ until the precipitate is dissolved before use.
8) Buffer II is not tightly closed after use, and long-term contact with air leads to a decrease in lysing efficiency. Prepare fresh Buffer II: 1% SDS, 0.2 M NaOH.
9) The bacteria are not completely suspended, and have small cell clumps remain. Do not add Buffer II until the bacteria are completely suspended.
10) Poor elution efficiency of DNA. Refer to point 4 "Elution of plasmid DNA" in "Column purification" on page 5 to optimize the elution protocol for DNA.
11) The expression products of the gene inserted on the plasmid are toxic to bacteria and affect the normal growth of bacteria.

2. Anomalies in the protocol:

1) Bacteria are deposited at the bottom of the tube, which is difficult to fully suspend with vortex shaking. If you are collecting more than 5 ml bacteria cultured and still use the rapid collection method (centrifugate at 12000 rpm for 30 sec), which may make it difficult to suspend the collected bacteria, try suspending the bacteria by blowing with a pipette tip, or centrifuge at 3000 rpm for 5 min instead.

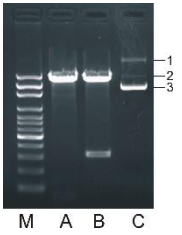
2) After adding Buffer II, the solution becomes very viscous and cannot flow. Usually the above reasons are excessive bacterial dosage, please reduce the amount of bacteria appropriately.
3) After adding Buffer II, the solution did not appear viscous and transparent, but maintained the cloudiness of bacteria in Buffer I, without stickiness. The above phenomenon is usually caused by contamination of bacteria cultured with a large number of fungi or gram-positive bacteria, which cannot be dissolved by Buffer II. Be sure to pick up monoclonal colonies from freshly prepared antibiotic-containing plates for inoculation rather than inoculation with bacteria in glycerol stocks.
4) After centrifugation, the precipitate cannot be deposited to the bottom of the tube and suspended in the liquid in the form of an expander. Usually caused by insufficient neutralization after adding Buffer N8, or because the bacterial concentration is too high, invert the tube 10 times and centrifuge again.
5) During plasmid DNA loading electrophoresis, the DNA solution added to the sample well cannot sink, but floats upwards and spreads. It is usually caused by excessive ethanol content in the eluted plasmid without operation in the full speed centrifuge step.

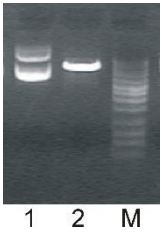
3. Restriction enzyme digestion failed

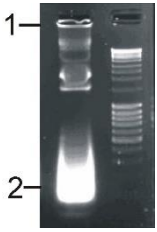
1) After restriction digestion, the electrophoresis plasmid disappears without bands, or the electrophoretic band has severe tailing.
   A. It may be due to the selection of wild-type or endA+ host bacteria, and the Spin Column was not washed with Buffer W1. Be sure to wash the column with Buffer W1 or perform further purification of plasmid DNA as described in the appendix 2.
   B. The electrophoresis buffer in the electrophoresis bath has not been changed for many times, resulting in reduced buffering capacity and easy activation of the star activity of restriction enzymes left after digestion. Change to a newly formulated electrophoresis buffer on time.
   C. The digestion product should be mix with Loading Buffer containing SDS (generally provided in restriction enzyme products), SDS can inactivate the residual restriction enzyme activity to ensure that the DNA band pattern does not change during loading and electrophoresis.
2) Digestion incompletely.
   D. The plasmid DNA concentration is too high, try to reduce the amount of plasmid DNA, or increase the amount of restriction enzyme and extend the digestion time appropriately.
   E. The eluted plasmid may contain too much ethanol due to the absence of a full speed centrifuge step, try to reduce the amount of plasmid DNA to reduce the inhibition effect of ethanol, or repurified the plasmid DNA according to the method in the appendix 2.

4. Plasmid DNA electrophoresis band type abnormalities

Figure 1 Figure 2 Figure 3







1) Normal plasmid DNA banding, as Figure 1:
   Lane A: empty vector pUC19 single enzyme cutting. Lane B: pUC19 with inserts double enzyme cutting. Lane C: empty vector pUC19. 1: dimer plasmid DNA. 2: plasmid DNA with incision. 3: supercoiled plasmid DNA.
2) Abnormal plasmid DNA banding, as Figure 2:
   The plasmid DNA dimer content obtained is high, or the purified plasmid DNA is stored at -20℃ for a period and the dimer increases (Figure 2, lane 1). The increase in plasmid dimer or polymer content is related to the structure of the plasmid DNA itself and the selected host bacteria, but the presence of plasmid dimer or polymer does not affect the electrophoretic band type of plasmid DNA digestion (Figure 2, lane 2). Elution of plasmid DNA with TE solution (10 mM Tris-HCl, 1 mM EDTA, pH 8.0) and storage at -20℃ helps reduce plasmid dimer production.

5. Plasmid DNA contains RNA contamination

Possible causes （Figure 3, lane 2 indicates location）

1) RNase A activity contained in Buffer I is reduced. If Buffer I with RNase A is stored at 2~8℃ for more than 6 months, RNase A should be supplemented to a final concentration of 100 μg/ml.
2) The amounts of bacteria are too much or the characteristics of the bacteria itself lead to excessive RNA content and cannot be completely digested by RNase A. It is possible to increase the concentration of RNase A in Buffer I.
3) The low ambient temperature in winter will lead to a decrease in RNase A activity, and the neutralization lysate can be incubated at 37℃ for 5-15 min after the neutralization step before moving to the next step to improve the digestion efficiency of RNase A.

6. Plasmid DNA containing bacterial genomic DNA

Possible causes of contamination(Figure 3, lane 1 indicates location)

1） The lysis step is too drastic: After adding Buffer II, gently invert the centrifuge tube to dissolve the bacteria.

2） Lysis step is too long: The lysis step should not exceed 5 min after add Buffer II.

3） The neutralization step is too vigorous: After adding Buffer N8, the centrifuge tube should be gently inverted to neutralize the solution.

4） The culture time of bacteria exceeds 16 hours, resulting in some bacteria showing lysis.

5） Bacteria are left in the refrigerator for too long after collection.

Appendix 1: Extraction of Gram-positive Bacterial Plasmid DNA

1. Centrifuge at 12000 rpm for 30 sec to collect 1-5 ml of bacteria cultured overnight, discard the medium. Add 50 µl sterile water and suspend the pelleted bacteria thoroughly.

* Certain divalent cations can inhibit the activity of lysozyme, so if the bacteria cultured contains divalent cations (e.g. MRS medium), an additional washing step should be performed after collecting the bacteria: add 1 ml sterile water, vortex to suspend the bacteria, then centrifuge at 12,000 rpm for 30 sec, discard the supernatant, and then add 200 µl Buffer TE and vortex to fully suspend the bacteria.

* Some specific bacteria may change the pH of the medium after cultivation (e.g. Lactobacillus), which can inhibit the activity of lysozyme, it also needs to be washed once after collecting the bacteria in the same way as above.

2. Add 50 µl lysozyme (100 mg/ml), vortex for about 15 sec to mix well, incubated at 37 °C for 30-60 min.

* Most of the bacteria have been fully wall-broken after 30 min in the water bath, but some bacteria with thicker cell walls (e.g. Staphylococcus aureus) need to be incubated for 1-2 hours to be fully wall-broken. Please adjust the water bath time appropriately according to different categories of bacteria.

3. Add 150 µl Buffer I with addition of RNase A, gently invert the tube 4-6 times. The subsequent operation can be followed by the step 2 of the protocol.

Appendix 2: Concentration and Purification of Plasmid DNA

Note: The plasmid DNA used for concentration or purification should not exceed 30 µg.

Plasmid DNA samples applicable this method:

a) Further purification of plasmid DNA harvested by conventional alkaline lysis and phenol/chloroform extraction. Residual protein (indicator 1 in Figure 3 on page 11) and degraded RNA (indicator 2 in Figure 3) can be removed.

b) Plasmid DNA (e.g., low-copy plasmid) solutions at low concentrations.

c) Plasmid DNA solution suspected of containing non-restriction enzyme.

1. **Add 5× volume of Buffer W1 to the plasmid DNA solution, do not discard the tip, mix directly with pipetting several times, and transfer the mixture to a Spin Column (Spin Column is placed in a 2 ml Collection Tube) and close the lid.**

* For example, if 100 µl plasmid DNA needs to be purified, 500 µl Buffer W1 needs to be added。

2. **Centrifuge at 12,000 rpm for 30 sec. Discard the filtrate. Place the Spin Column back to the 2 ml Collection Tube.**

* The filtrate does not need to be completely discarded, if you want to avoid the contamination of the centrifuge by the filtrate adhering to the nozzle of the Collection Tube, you can slap the 2 ml Collection Tube upside down on a paper towel once.

3. **Add 700 µl Buffer W2 to the Spin Column, close the lid, and centrifuge at 12,000 rpm for 30 sec. Discard the filtrate. Place the Spin Column back into the 2 ml Collection Tube.**

* Make sure absolute ethanol has been added into Buffer W2.

4. **Centrifuge at full speed (≧12,000 rpm) for 1 min.**

* Do not omit this step, otherwise, it may cause problems in downstream applications due to the residual ethanol in the eluate.

5. **Discard the 2 ml Collection Tube and place the Spin Column in a new 1.5 ml centrifuge tube. To elute DNA, add 50~100 µl Buffer E to the center of the column, close the lid, incubate for 1 min at room temperature, and centrifuge at 12,000 rpm for 30 s.**

* If the centrifuge does not have a leak-proof lid, change the centrifugation condition to 8000 rpm for 1 min to avoid damaging the centrifuge by falling off the lid of the 1.5 ml centrifuge tube.

* DNA can also be eluted with deionized water, but make sure that the pH of the deionized water used is 7.0-8.5, otherwise the elution efficiency of the DNA will be affected.

6. **Discard the Spin Column. Eluted DNA can be used for various molecular biology experiments or store at -20℃ for later use.**