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TRUST THE OUTCOME

EB Pure Plasmid DNA Mini-Prep Kit

SS-EB-PMK-5	5 preps
EB-PMK-50	50 preps
EB-PMK-200	200 preps



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EB Pure Plasmid DNA Mini-Prep Kit

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Introduction

The EB Pure Plasmid DNA Mini-Prep Kit combines mini spin columns with the time-tested consistency of alkaline-SDS lysis of bacterial cells to deliver high-quality DNA in less than 30 minutes. High-Bind DNA Mini Columns facilitate the binding, washing, and elution steps thus enabling multiple samples to be processed simultaneously.

Typically, a 1.5 mL overnight culture in LB medium produces 3-12 µg plasmid DNA; although yields may vary according to plasmid copy number, *E. coli* strain, and growth conditions. The EB Pure Plasmid DNA Mini-Prep Kit is used to isolate plasmid DNA from 1-5 mL cultures. Purified plasmid DNA can be directly used for most downstream applications including automated fluorescent DNA sequencing and restriction enzyme digestion.

Protocols

The EB Pure Plasmid DNA Mini-Prep Kit is designed for fast and efficient processing. Depending on the protocol, the EB Pure Plasmid DNA Mini-Prep Kit can be used with any microcentrifuge or vacuum manifold with standard luer connectors.

Yield and Quality of DNA

Determine the absorbance of an appropriate dilution (20- to 50-fold) of the sample at 260 nm and then at 280 nm. The DNA concentration is calculated as follows:

$$\text{DNA concentration} = \text{Absorbance 260} \times 50 \times (\text{Dilution Factor}) \mu\text{g/mL}$$

A ratio greater than 1.8 indicates greater than 90% nucleic acid. Alternatively, quantity (as well as quality) sometimes can be determined best by agarose gel/ethidium bromide electrophoresis by comparison to DNA samples of known concentrations. Typically, the majority of the DNA eluted is in monomeric supercoil form, though concatemers may also be present.

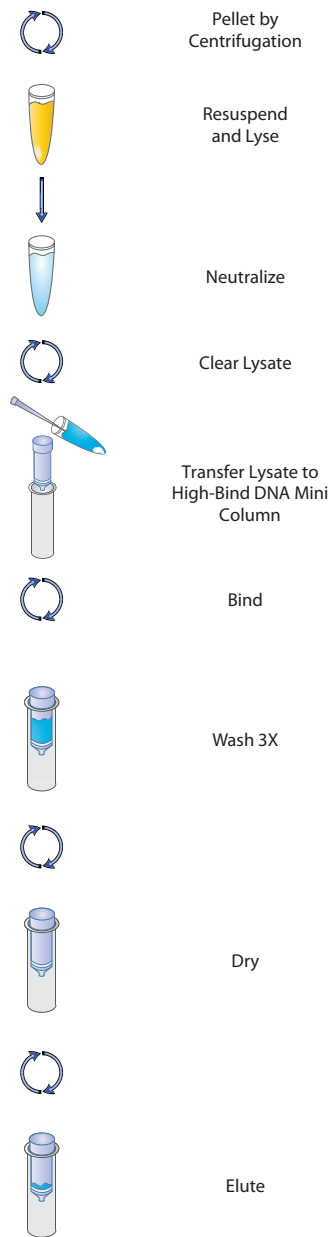
Plasmid Copy Number and Expected Yield

Yield and quality of the plasmid DNA obtained depends on a number of factors including plasmid copy number, size of insert, host strain, culture volume, culture medium, and binding capacity of the kit. Of these factors, the vector copy number, culture volume, and kit binding capacity are most important. Plasmid copy number ranges from one copy to several hundred copies per cell as dictated by their origin of replication. Very large plasmids often display a very low copy number per cell. The expected yield of 5 mL overnight cultures (LB medium) with the EB Pure Plasmid DNA Mini-Prep Kit is indicated in the following table.

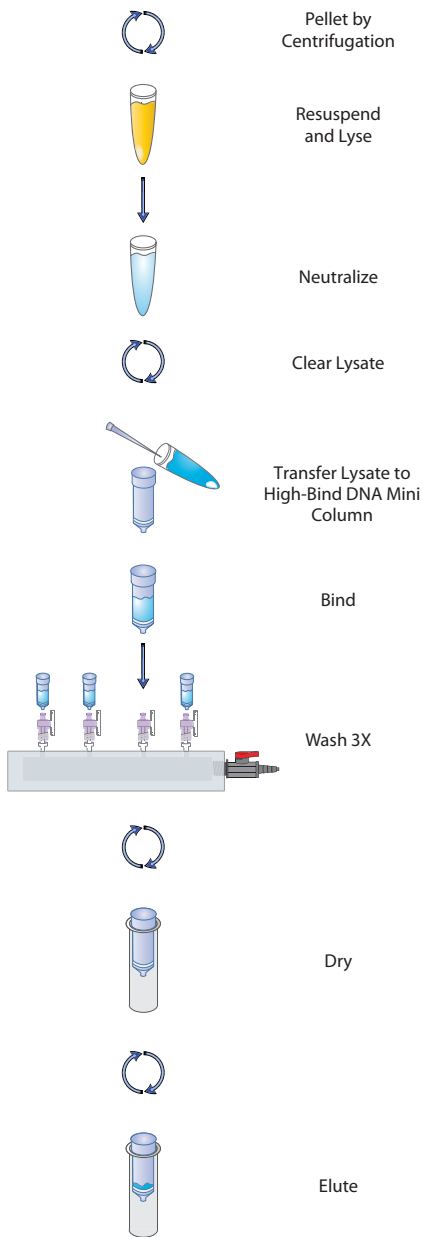
Sample yields from a 5 mL starting culture.

Plasmid	Replicon	Copy Number	Expected Yield
pUC vectors	pMB1	500-700	15-25 μg
pBluescript® vectors	ColE14	300-500	10-18 μg
pGEM® vectors	pMB1	300-400	10-20 μg
pBR322 and its derivatives	pMB1	15-20	1-2 μg
ColE14	ColE14	15-20	1-2 μg
PACYC and its derivatives	p15A	37540	0.5-1 μg
pSC101 and its derivatives	pSC101	~5	0.5 μg
pGEM	pMB1	300-700	10-20 μg

Spin Protocol



Vacuum/Spin Protocol



Kit Contents

Product	SS-EB-PMK-5	EB-PMK-50	EB-PMK-200
Purifications	5	50	200
High-Bind DNA Mini Columns	5	50	200
Collection Tubes (2 mL)	5	50	200
Solution I	3 mL	20 mL	60 mL
Solution II	3 mL	20 mL	60 mL
Solution III	3 mL	20 mL	80 mL
HBC Buffer	4 mL	25 mL	80 mL
DNA Wash Buffer	1.5 mL	15 mL	3 x 25 mL
Elution Buffer	1.5 mL	10 mL	30 mL
RNase A	Pre-Added	100 μ L	400 μ L

Preparing Reagents

1. Add the vial of RNase A to the bottle of Solution I and store at 2-8°C. (50 and 200 prep size only).
2. Dilute HBC Buffer with 100% isopropanol as follows and store at room temperature.

Kit	100% Isopropanol to be Added
SS-EB-PMK-5	1.6 mL
EB-PMK-50	10 mL
EB-PMK-200	32 mL

3. Dilute DNA Wash Buffer with 100% ethanol as follows and store at room temperature.

Kit	100% Ethanol to be Added
SS-EB-PMK-5	6 mL
EB-PMK-50	60 mL
EB-PMK-200	100 mL per bottle

4. Check Solution II and Solution III for precipitation before use. Redissolve any precipitation by warming to 37°C.

Storage and Stability

All of the EB Pure Plasmid DNA Mini-Prep Kit components are guaranteed for at least 12 months from the date of purchase when stored as follows. Solution I (once RNase A is added) should be stored at 2-8°C. All other materials should be stored at room temperature. Solution II must be tightly capped when not in use.

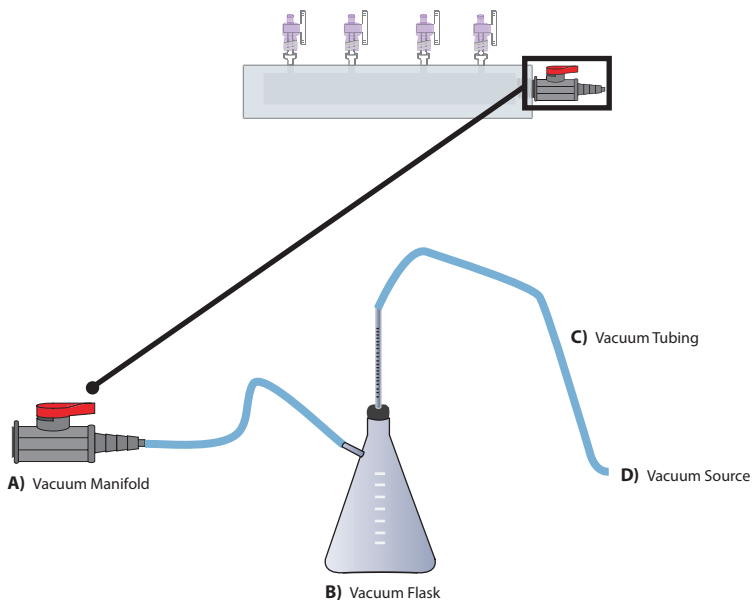
Guidelines for Vacuum Manifold

The following is required for use with the Vacuum/Spin Protocol:

- A) Compatible Vacuum Manifolds: Qiagen QIAvac24, Sigma Aldrich VM20, Promega Vacman®, or manifold with standard Luer connector
- B) Vacuum Flask
- C) Vacuum Tubing
- D) Vacuum Source (review tables below for pressure settings)

Conversion from millibars:	Multiply by:
millimeters of mercury (mmHg)	0.75
kilopascals (kPa)	0.1
inches of mercury (inHg)	0.0295
Torr (Torr)	0.75
atmospheres (atm)	0.000987
pounds per square inch (psi)	0.0145

Illustrated Vacuum Setup:



Recommended Settings

Growth and Culture of Bacteria

Bacterial Strain Selection

It is strongly recommended that an end A negative strain of *E. coli* be used for routine plasmid isolation. Examples of such strains include DH5 α [™], DH1, and C600. These host strains yield high-quality DNA with EB Pure Plasmid DNA Mini-Prep Kit protocols. XL1-Blue, although a slower growing strain is also recommended due to its yield of high-quality DNA.

Host strains derivatives from HB101 such as TG1 and the JM100 series release large amounts of carbohydrates during lysis, which may inhibit enzyme activity when not completely removed. Some strains may also lower DNA quality due to having high levels of endonuclease activity, and therefore are not recommended (i.e. JM101, JM110, HB101). One may reduce the amount of culture volume or double the volumes of Solution I, Solution II, and Solution III, if problems are encountered with strains such as TG1 and Top10F.

Inoculation

Bacterial cultures for plasmid preparations should always be grown from a single colony picked from a freshly streaked plate. Subculturing directly from glycerol stock or liquid cultures may lead to uneven yields or plasmid loss. Optimal results are obtained by using one single isolated colony from a freshly transformed or freshly streaked plate to inoculate an appropriate volume of starter culture containing the appropriate antibiotic, and then incubated for 12-16 at 37°C with vigorous shaking (~300 rpm; shaking incubator).

Note: Aeration is very important. The culture volume should not exceed 1/4 the volume of the container.

Culture Media

The EB Pure Plasmid DNA Mini-Prep Kit are specially designed for use with cultures grown in Luria Bertani (LB) medium. Richer broths such as TB(Terrific Broth) or 2xYT lead to high cell densities that can overload the purification system, and therefore are not recommended. If rich media has to be used, growth times have to be optimized, and the recommended culture volumes must be reduced to match the capacity of the High-Bind DNA Mini Column.

Note: As culture ages, DNA yield may begin to decrease due to cell death and lysis within the culture.

Recommended Settings

Culture Volume and Cell Density

Do Not Exceed Maximum Recommended Culture Volumes

For optimal plasmid yields, the starting culture volume should be based on culture cell density. A bacterial density between 2.0 and 3.0 at OD₆₀₀ is recommended. When using nutrient-rich media, care should be taken to ensure that the cell density does not exceed an OD₆₀₀ of 3.0. Using a high-density culture outside of the recommended OD range may overload the purification system.

EB Pure Plasmid DNA Mini-Prep Kit Spin Protocol

EB Pure Plasmid DNA Mini-Prep Kit - Spin Protocol

All centrifugation should be performed at room temperature unless otherwise noted. For low copy number plasmids refer to Page 17. This protocol is designed to isolate plasmid DNA from *E. coli* grown in an overnight 1-5 mL LB culture.

Materials and Equipment to be Supplied by User:

- 100% ethanol
- 100% isopropanol
- Vortexer
- Microcentrifuge capable of at least 13,000 x g
- Nuclease-free 1.5 mL or 2 mL microcentrifuge tubes
- Culture tubes
- Optional: sterile deionized water
- Optional: water bath or incubator capable of 70°C
- Optional: 3M NaOH solution

Before Starting:

- Heat Elution Buffer to 70°C if plasmid DNA is >10 kb
- Prepare Solution I, HBC Buffer, and DNA Wash Buffer according to the instructions in the Preparing Reagents section on Page 6

1. Isolate a single colony from a freshly streaked selective plate, and inoculate a culture of 1- 5 mL LB medium containing the appropriate selective antibiotic. Incubate for ~12-16 hours at 37°C with vigorous shaking (~ 300 rpm). Use a 10-20 mL culture tube or a flask with a volume of at least 4 times the volume of the culture. It is strongly recommended that an endA negative strain of *E. coli* be used for routine plasmid isolation. Examples of such strains include DH5a® and JM109®.
2. Centrifuge at 10,000 x g for 1 minute at room temperature.
3. Decant or aspirate and discard the culture media.
4. Add 250 µL Solution I/RNase A. Vortex or pipet up and down to mix thoroughly. Complete resuspension of cell pellet is vital for obtaining good yields.
Note: RNase A must be added to Solution I before use. Please see the instructions in the Preparing Reagents section on Page 6.

EB Pure Plasmid DNA Mini-Prep Kit Spin Protocol

5. Transfer suspension into a new 1.5 mL microcentrifuge tube.
6. Add 250 μ L Solution II. Invert and gently rotate the tube several times to obtain a clear lysate. A 2-3 minute incubation may be necessary.
Note: Avoid vigorous mixing as this will shear chromosomal DNA and lower plasmid purity. Do not allow the lysis reaction to proceed more than 5 minutes. Store Solution II tightly capped when not in use to avoid acidification from CO₂ in the air.
7. Add 350 μ L Solution III. Immediately invert several times until a flocculent white precipitate forms.
Note: It is vital that the solution is mixed thoroughly and immediately after the addition of Solution III to avoid localized precipitation.
8. Centrifuge at maximum speed ($\geq 13,000 \times g$) for 10 minutes. A compact white pellet will form. Promptly proceed to the next step.
9. Insert a High-Bind DNA Mini Column into a Collection Tube (2 mL).

Optional Protocol for Column Equilibration:

1. Add 100 μ L 3M NaOH to the High-Bind DNA Mini Column.
 2. Centrifuge at maximum speed for 30-60 seconds.
 3. Discard the filtrate and reuse the collection tube.
10. Transfer the cleared supernatant from Step 8 by CAREFULLY aspirating it into the High-Bind DNA Mini Column. Be careful not to disturb the pellet and that no cellular debris is transferred to the High-Bind DNA Mini Column.
 11. Centrifuge at maximum speed for 1 minute.
 12. Discard the filtrate and reuse the collection tube.
 13. Add 500 μ L HBC Buffer.
Note: HBC Buffer must be diluted with 100% isopropanol before use. Please see Page 6 for instructions.

EB Pure Plasmid DNA Mini-Prep Kit Spin Protocol

14. Centrifuge at maximum speed for 1 minute.
15. Discard the filtrate and reuse collection tube.
16. Add 700 μ L DNA Wash Buffer.
Note: DNA Wash Buffer must be diluted with 100% ethanol prior to use. Please see Page 6 for instructions.
17. Centrifuge at maximum speed for 1 minute.
18. Discard the filtrate and reuse the collection tube.

Optional: Repeat Steps 16-18 for a second DNA Wash Buffer wash step.

19. Centrifuge the empty High-Bind DNA Mini Column at maximum speed for 2 minutes to dry the column matrix.
Note: It is important to dry the High-Bind DNA Mini Column matrix before elution. Residual ethanol may interfere with downstream applications.
20. Transfer the High-Bind DNA Mini Column to a clean 1.5 mL microcentrifuge tube.
21. Add 30-100 μ L Elution Buffer or sterile deionized water directly to the center of the column membrane.
Note: The efficiency of eluting DNA from the High-Bind DNA Mini Column is dependent on pH. If using sterile deionized water, make sure that the pH is around 8.5.
22. Let sit at room temperature for 1 minute.
23. Centrifuge at maximum speed for 1 minute.
Note: This represents approximately 70% of bound DNA. An optional second elution will yield any residual DNA, though at a lower concentration.
24. Store eluted DNA at -20°C .

EB Pure Plasmid DNA Mini-Prep Kit Vacuum Protocol

EB Pure Plasmid DNA Mini-Prep Kit - Vacuum Protocol

All centrifugation should be performed at room temperature unless otherwise noted. For low copy number plasmids refer to Page 17. This protocol is designed to isolate plasmid DNA from *E. coli* grown in an overnight 1-5 mL LB culture. See Page 7 for guidelines on preparing the vacuum manifold used in this protocol.

Materials and Equipment to be Supplied by User:

- Vacuum Manifold
- 100% ethanol
- 100% isopropanol
- Vortexer
- Microcentrifuge capable of at least 13,000 x g
- Nuclease-free 1.5 mL or 2 mL microcentrifuge tubes
- Appropriate centrifuge and centrifuge tube for Step 2
- Optional: sterile deionized water
- Optional: water bath or incubator capable of 70°C
- Optional: 3M NaOH solution

Before Starting:

- Heat Elution Buffer to 70°C if plasmid DNA is >10 kb
 - Prepare Solution I, HBC Buffer, and DNA Wash Buffer according to the instructions in the Preparing Reagents section on Page 6
1. Isolate a single colony from a freshly streaked selective plate, and inoculate a culture of 1- 5 mL LB medium containing the appropriate selective antibiotic. Incubate for ~12-16 hr at 37°C with vigorous shaking (~ 300 rpm). Use a 10-20 mL culture tube or a flask with a volume of at least 4 times the volume of the culture. It is strongly recommended that an endA negative strain of *E. coli* be used for routine plasmid isolation. Examples of such strains include DH5a® and JM109®.
 2. Centrifuge at 10,000 x g for 1 minute at room temperature.
 3. Decant or aspirate and discard the culture media.

EB Pure Plasmid DNA Mini-Prep Kit Vacuum Protocol

4. Add 250 μL Solution I/RNase A. Vortex or pipet up and down to mix thoroughly. Complete resuspension of cell pellet is vital for obtaining good yields.
Note: RNase A must be added to Solution I before use. Please see the instructions in the Preparing Reagents section on Page 6.
5. Transfer suspension into a new 1.5 mL microcentrifuge tube.
6. Add 250 μL Solution II. Invert and gently rotate the tube several times to obtain a clear lysate. A 2-3 minute incubation may be necessary.
Note: Avoid vigorous mixing as this will shear chromosomal DNA and lower plasmid purity. Do not allow the lysis reaction to proceed more than 5 minutes. Store Solution II tightly capped when not in use to avoid acidification from CO_2 in the air.
7. Add 350 μL Solution III. Immediately invert several times until a flocculent white precipitate forms.
Note: It is vital that the solution is mixed thoroughly and immediately after the addition of Solution III to avoid localized precipitation.
8. Centrifuge at maximum speed ($\geq 13,000 \times g$) for 10 minutes. A compact white pellet will form. Promptly proceed to the next step.
9. Prepare the vacuum manifold according to manufacturer's instructions.
10. Connect the High-Bind DNA Mini Column to the vacuum manifold.

Optional Protocol for Column Equilibration:

1. Add 100 μL 3M NaOH to the High-Bind DNA Mini Column.
 2. Turn on the vacuum source to draw the NaOH through the column.
 3. Turn off the vacuum.
-
11. Transfer the cleared supernatant from Step 8 by CAREFULLY aspirating it into the High-Bind DNA Mini Column. Be careful not to disturb the pellet and that no cellular debris is transferred to the High-Bind DNA Mini Column.

EB Pure Plasmid DNA Mini-Prep Kit Vacuum Protocol

12. Turn on the vacuum source to draw the sample through the column.
13. Turn off the vacuum.
14. Add 500 μ L HBC Buffer.
Note: HBC Buffer must be diluted with 100% isopropanol before use. Please see Page 6 for instructions.
15. Turn on the vacuum source to draw the buffer through the column.
16. Turn off the vacuum.
17. Add 700 μ L DNA Wash Buffer.
Note: DNA Wash Buffer must be diluted with 100% ethanol prior to use. Please see Page 6 for instructions.
18. Turn on the vacuum source to draw the buffer through the column.
19. Turn off the vacuum.
20. Repeat Steps 17-19 for a second DNA Wash Buffer wash step.
21. Transfer the High-Bind DNA Mini Column to a Collection Tube (2 mL).
22. Centrifuge the empty High-Bind DNA Mini Column at maximum speed for 2 minutes to dry the column matrix.
Note: It is important to dry the High-Bind DNA Mini Column matrix before elution. Residual ethanol may interfere with downstream applications.

EB Pure Plasmid DNA Mini-Prep Kit Vacuum Protocol

23. Transfer the High-Bind DNA Mini Column to a clean 1.5 mL microcentrifuge tube.

24. Add 30-100 μ L Elution Buffer or sterile deionized water directly to the center of the column membrane.

Note: The efficiency of eluting DNA from the High-Bind DNA Mini Column is dependent on pH. If using sterile deionized water, make sure that the pH is around 8.5.

25. Let sit at room temperature for 1 minute.

26. Centrifuge at maximum speed for 1 minute.

Note: This represents approximately 70% of bound DNA. An optional second elution will yield any residual DNA, though at a lower concentration.

27. Store eluted DNA at -20°C .

Low Copy-Number Plasmid and BAC DNA Protocol

EB Pure Plasmid DNA Mini-Prep Kit Protocol - Low Copy Number Plasmid and BAC DNA Protocol

Low copy number plasmids generally give 0.1-1 µg DNA per mL overnight culture. For the isolation of plasmid DNA from low copy number plasmids (0.1-1 µg/mL culture) or low copy number plasmid (1-2 µg/mL culture) bacteria, use the following modified protocol.

Note: The EB Pure Plasmid DNA Mini-Prep Kit comes with enough Solution I, Solution II, and Solution III to perform the standard protocols. Additional Solution I, Solution II, and Solution III are needed to perform the Low Copy Number Plasmid and BAC DNA Protocol. These buffers can be purchased separately.

1. Increase the volume of starting culture from that of high copy number plasmids. Use 5-10 mL bacterial culture.
2. Pellet the bacterial cells by centrifugation.
3. Decant or aspirate and discard the culture media.
4. Perform Steps 4-8 in the standard protocols with **double the volumes of Solution I, Solution II, and Solution III.**
5. Continue with Step 9 of the standard protocols by following the wash, drying, and elution steps. **There is no need to increase the volumes of HBC Buffer, DNA Wash Buffer, or Elution Buffer.**

Troubleshooting Guide

Please use this guide to troubleshoot any problems that may arise.

Possible Problems and Suggestions

Low DNA yields	
Poor cell lysis	Only use LB or YT medium containing ampicillin. Do not use more than 5 mL (high copy number plasmids) or 10 mL (low copy number plasmids) culture with the basic protocols.
	Cells may not have been dispersed adequately prior to the addition of Solution II. Vortex to completely resuspend the cells.
	Increase Solution II incubation time to obtain a clear lysate.
	Solution II, if not tightly closed, may need to be replaced.
Culture is overgrown or not fresh	Do not incubate cultures for more than 16 hours at 37°C. Storage of cultures for extended periods prior to plasmid isolation is detrimental.
Low elution efficiency	The pH of Elution Buffer or water must be pH 8.0-9.0.
Low copy-number plasmid used	Such plasmids may yield as little as 0.1 µg DNA from a 1 mL overnight culture. Double the culture volume and follow the low copy number plasmid protocol on Page 17.
Column matrix lost binding capacity during storage	Follow the Optional Protocol for Column Equilibration prior to transferring the cleared lysate to the High-Bind DNA Mini Column. Add 100 µL 3M NaOH to the column prior to loading the sample. Centrifuge at 10,000 x g for 30 seconds. Discard the filtrate.
No DNA eluted	
DNA Wash Buffer not diluted with ethanol	Prepare DNA Wash Buffer according to instructions on Page 6.
HBC Buffer not diluted with isopropanol	Prepare HBC Buffer according to instructions on Page 6.

Troubleshooting Guide

High molecular weight DNA contamination of product	
Over mixing of cell lysate upon addition of Solution II	Do not vortex or mix aggressively after adding Solution II.
Culture overgrown	Overgrown cultures contain lysed cells and degraded DNA. Do not grow cell longer than 16 hours.
Plasmid DNA floats out of well while loading agarose gel	
Ethanol was not completely removed from column following wash steps	Centrifuge column as instructed to dry the column before elution.
Absorbance of purified DNA does not accurately reflect quantity of the plasmid (A_{260}/A_{280} ratio is high or low)	
DNA Wash Buffer is diluted with ethanol containing impurities	Check the absorbance of the ethanol between 250 nm and 300 nm. Do not use ethanol with high absorbance. Traces of impurities may remain on the binding column after washing and contribute to the absorbance in the final product.
Plasmid DNA is contaminated with RNA; RNase A treatment is insufficient	Confirm that the RNase A Solution was added to Solution I prior to first use. The RNase A solution may degrade due to high temperatures (>65 °C) or prolonged storage (> 6 months at room temperature).
Background reading is high due to silica fine particulates	Spin the DNA sample at maximum speed for 1 minute; use the supernatant to repeat the absorbance readings.
Purification is incomplete due to column overloading	Reduce the initial volume of culture.