

5 PRIME

PerfectPrep™ Spin Mini Kit Manual

Purification of molecular biology grade DNA

PerfectPrep Spin Mini Kit Manual, July 2007

© 2007 5 PRIME, all rights reserved.

This document and the product it describes are subject to change without prior notice. This document does not represent a commitment on the part of 5 PRIME GmbH or its distributors.

Trademarks: PerfectPrep is a trademark of 5 PRIME GmbH; DH5 α is a trademark of Invitrogen; pBluescript is a trademark of Stratagene; pGEM is a trademark of Promega Corp.; Beckman is a trademark of Beckman Instruments, Inc; Finntip, Multistep are trademarks of Thermo Electron Oy Corporation; Impact is a trademark of Matrix Technologies Corporation, USA; Minifuge is a trademark of Heraeus Instruments GmbH.

Contents

Product specifications	4
Product description	4
Product limitations	4
Materials supplied	4
Additional materials	5
Shipping and storage conditions	5
Safety information	6
Quality assurance	6
Product warranty	6
Protocols	8
Introduction	8
Principle	8
Protocol: Plasmid DNA purification using the PerfectPrep Spin Mini Kit	11
Supporting information	13
Background Information	13
Purified sample guide	17
Special applications	17
Troubleshooting guide	19
Ordering information	23
5 PRIME distributors	23

Product specifications

Product description

The PerfectPrep Spin Mini Kit provides the components and procedures necessary for purification of molecular biology grade plasmid DNA.

Product limitations

PerfectPrep Spin Mini Kit is developed, designed, and sold for research purposes only. It is not to be used for human diagnostic or drug purposes or to be administered to humans unless expressly cleared for that purpose by the Food and Drug Administration in the USA or the appropriate regulatory authorities in the country of use. All due care and attention should be exercised in the handling of the materials described in this text.

The yield and quality of nucleic acids obtained with this product depend on culture volume, culture medium, plasmid copy number size of insert, and host strain.

As with all column-based purification methods, overloading the system with excess starting material might clog the column. Reduced yields might occur if recommendations for starting materials are not followed.

Materials supplied

Kit	Order/ref. no.	Size
PerfectPrep Spin Mini Kit (50)	2300100	50 plasmid minipreps
PerfectPrep Spin Mini Kit (200)	2300110	200 plasmid minipreps

Kit contents

PerfectPrep Spin Miniprep Kit	(50)	(200)
Order/ref. no.	2300100	2300110
RNase A Solution (10 mg/ml)	150 µl	4 x 150 µl
Buffer BL	30 ml	120 ml
Buffer PR1	15 ml	60 ml
Buffer PL2	15 ml	60 ml
Buffer PN3	20 ml	4 x 20 ml
Buffer PD	30 ml	4 x 30 ml
Buffer PW	15 ml	50 ml
Buffer PEB	15 ml	2x 15 ml
PerfectPrep Spin Mini Columns CS	50	4 x 50
PerfectPrep Spin Mini Columns CB3	50	4x 50
Collection Tubes (2 ml)	2 x 50	8 x 50

* Buffers PN3 and PD contain chaotropic salts which are irritants and not compatible with disinfecting agents containing bleach. Take appropriate laboratory safety measures and wear gloves when handling. See page 9 for further information.

Additional materials

To perform the protocols described in this manual, the following additional materials must be provided by the user:

- Ethanol (95–100%)

Shipping and storage conditions

The PerfectPrep Spin Mini Kit is shipped at ambient temperature.

The PerfectPrep Spin Mini Kit and components should be stored dry at room temperature (15–25°C) up to one year.

For long-term storage the PerfectPrep Spin Mini Columns CB3 should be stored at 2–8°C. Alternatively the complete PerfectPrep Spin Miniprep Kit can be stored at 2–8°C. When stored at this temperature, buffers should be redissolved and warmed to room temperature before use.

Component	Storage instructions
Buffer PR1	Upon receipt, store room temperature (15–25°C). For long-term storage place at 2–8°C. After addition of RNase A, store at 2–8°C
Other components	Store room at temperature (15–25°C). For long-term storage, place at 2–8°C.

Safety information

All due care and attention should be exercised in the handling of this product. We recommend all users of 5 PRIME products to adhere to the NIH guidelines that have been developed for recombinant DNA experiments, or to other applicable guidelines. Specifically, always wear a suitable lab coat, disposable gloves, and protective goggles when working with chemicals.

Additional safety information is available from www.5PRIME.com in material safety data sheets (MSDSs) for 5 PRIME products and 5 PRIME product components.

Quality assurance

5 PRIME products are manufactured using quality chemicals and materials that meet our high standards. All product components are subjected to rigorous quality assurance testing process:

- **Component testing:** each component is tested to ensure the composition and quality meet stated specifications.
- **Performance testing:** each product is tested to ensure it meets the stated performance specification.

Additional quality information is available from www.5PRIME.com including certificate of analysis sheets for 5 PRIME products and 5 PRIME product components.

Product warranty

5 PRIME is committed to providing products that improve the speed, ease-of-use and quality of enabling technologies.

5 PRIME guarantees the performance of all products in the manner described in our product literature. The purchaser must determine the suitability of the product for its particular use.

This warranty is in place of any other warranty or guarantee, expressed or implied, instituted by law or otherwise. 5 PRIME provides no other warranties of any kind, expressed or implied, including warranties of merchantability and fitness for a particular purpose. Under no circumstance shall 5 PRIME be

responsible for any direct, indirect, consequential or incidental damages or loss arising from the use, misuse, results of use or inability to use its products, even if the possibility of such loss, damage or expense was known by 5 PRIME.

Protocols

Introduction

The PerfectPrep Miniprep system provides a fast, simple, and cost-effective plasmid miniprep method using silica membrane technology. The PerfectPrep Spin Mini Kit is designed for quick and convenient processing of 1–24 samples simultaneously in less than 30 minutes.

For high throughput applications, 5 PRIME offers the PerfectPrep™ Plasmid 96 Vac Kit, which enables the purification of 96 minipreps on a vacuum manifold and is compatible with liquid handling and pipetting workstations.

High-quality plasmid DNA is eluted in a small volume of Tris buffer PEB (included in each kit) or water. As phenol extraction and ethanol precipitation are not required, plasmid DNA purified with PerfectPrep Miniprep Kits is immediately ready for use.

Applications using PerfectPrep purified DNA

Plasmid DNA prepared using the PerfectPrep system is suitable for a variety of routine applications including:

- Sequencing
- Ligation and transformation
- Restriction enzyme digestion
- Library screening
- In vitro translation
- Transfection of robust cells

Principle

The PerfectPrep Miniprep procedure consists of three basic steps:

- Preparation and clearing of a bacterial lysate
- Adsorption of DNA onto the PerfectPrep membrane
- Washing and elution of plasmid DNA

All steps are performed without the use of phenol, chloroform, CsCl, ethidium bromide, and without alcohol precipitation.

In a single step, bacteria are lysed under alkaline conditions, and the lysate is subsequently neutralized and adjusted to high-salt binding conditions. After lysate clearing, the sample is ready for purification on the PerfectPrep silica membrane.

The silica membrane provides selective adsorption of plasmid DNA in high-salt buffer and elution in low-salt buffer. The optimized buffers in the lysis

procedure, ensure that only DNA will be adsorbed, allowing RNA, cellular proteins, and metabolites to flow through.

Washing with Buffer PD removes endonucleases and is essential when working with *endA+* strains such as the JM series, HB101 and its derivatives, or any wild-type strain. The Buffer PD wash step is also necessary when large culture volumes are used for purifying low-copy plasmids.

Washing with Buffer PW removes salts.

Buffer PEB (10 mM Tris·Cl, pH 8.5) or water is used to elute the high-quality plasmid DNA from the PerfectPrep. When using water, ensure the pH is between 7.0 and 8.5, and store DNA at -20°C .

The purified DNA is ready for immediate use without precipitation, concentration, or desalting.

DNA yield

Yield depends on plasmid copy number (see page 39), insert, culture conditions (see pages 39–42), elution volume (Table 1), and elution incubation time (Table 2).

A 1.5 ml overnight culture can yield from 5 to 15 μg of plasmid DNA (see table below). Standard Luria-Bertani (LB) media should be used to obtain the optimum combination of DNA quality, yield, and concentration (for composition see page 41). Plasmid DNA should be eluted in a volume of at least 50 μl with a short incubation.

Table 1. Effect of elution volume

Elution volume, μl	Concentration, $\text{ng}/\mu\text{l}$	Recovery, %
50	149	62
100	80	88
150	69	97

10 μg pUC18 DNA was purified using the PerfectPrep Spin protocol and eluted with the indicated volumes of Buffer PEB.

Table 2. Effect of incubation time on recovery

Incubation time, min	Recovery, %	
	100 μ l	50 μ l
0	82	71
1	88	78
2	85	77
3	84	75
4	93	82
5	90	88
10	83	80
30	90	83

10 μ g pBluescript DNA was purified using the PerfectPrep Spin Miniprep protocol and eluted after the indicated incubation times with either 50 μ l or 100 μ l Buffer PEB.

Table 3. Effect of medium on DNA yield

Culture media	Yield
LB (containing 10 g/liter NaCl)	11.5 μ g
LB (containing 5 g/liter NaCl)	9.5 μ g

PerfectPrep Spin Mini Kit was used to purify DNA from 1.5 ml LB overnight cultures of XL1-Blue containing pBluescript[®]. Elution was performed according to the standard protocol (50 μ l Buffer PEB and 1 min incubation). Use of the recommended LB composition (with 10 g/liter NaCl) provides optimal plasmid yield.

Protocol: Plasmid DNA purification using the PerfectPrep Spin Mini Kit

This protocol is designed for purification of up to 20 µg of high-copy plasmid DNA from 1–5 ml overnight cultures of *E. coli* in LB (Luria-Bertani) medium.

Before starting

- ➔ For purification of low-copy plasmids and cosmids, large plasmids (>10 kb), and DNA prepared using other methods, refer to the recommendations on page 44.
- ➔ Note: All protocol steps should be carried out at room temperature.
- ➔ Add RNase A solution to Buffer PR1 to a final concentration of 100µg/µl. Add 150 µl RNase A solution(10 mg/ml) to 150 ml Buffer PR1 (order/reference number 12300100) or add 600 µl RNase A (10mg/ml) solution to 60 ml Buffer PR for order/reference number 2300110.
- ➔ Add 60 ml ethanol (96–100%) to 15 ml Buffer PW.
- ➔ Check Buffers PL2 and PN3 for salt precipitation and redissolve at 37°C if necessary.

Protocol

1. Add 500 µl Buffer BL to PerfectPrep Spin Column CB3 to equilibrate the column.
2. Centrifuge the column for 1 min at 12,000 rpm, and discard the flow-through.
3. Harvest 1–5 ml bacterial culture by centrifuging for 1 min at 12,000 rpm.
4. Discard the supernatant of the bacterial culture.
5. Resuspend pelleted bacterial cells in 250 µl Buffer PR1 (final concentration of RNase A 100 µg/ml).
6. Add 250 µl Buffer PL2, and mix thoroughly by inverting gently the tube 4–6 times.
7. Add 350 µl Buffer PN3, and mix immediately and thoroughly by inverting the tube 4–6 times.
8. Centrifuge for 10 min at 12,000 rpm in a tabletop microcentrifuge.
9. Insert a PerfectPrep Mini Column CS (filter) into a new, clean collection tube.
10. Apply the supernatant by pipetting to a Filter Column CS.
11. Centrifuge for 2 min at 12,000 rpm, and apply the clear lysate to the equilibrated PerfectPrep Spin Column CB3.
12. Centrifuge for 1 min at 12,000 rpm. Discard the flow-through.
13. Add 500 µl Buffer PD to the PerfectPrep Spin Column CB3 and centrifuge for 1 min at 12,000rpm. Discard the flow - through.

Note: The step is for further removing proteins contamination and is necessary when using *endA*⁺ strains such as the JM series, HB101 and its

derivatives, or other bacterial strains with high nuclease activity or carbohydrate content.

14. Add 700 μ l Buffer PW to the PerfectPrep Spin Column CB3 for washing to remove salts contamination and centrifuge for 1 min at 12,000 rpm.
15. Discard the flow-through and add 500 μ l Buffer PW to wash again, centrifuge for 1 min at 12,000 rpm.
16. Discard the flow-through, and centrifuge for an additional 2 min at 12,000 rpm to remove residual wash buffer.
17. Place the column with open cap in air for several minutes to dry the membrane
18. To elute DNA, place the column in a clean 1.5 ml microcentrifuge tube. Add 50–100 μ l PEB buffer (10 mM Tris·Cl, pH 8.5) or water to the center of the PerfectPrep Spin Column CB3, incubate at room temperature for 2 min, and centrifuge for 1 min at 12,000 rpm.

Note: When using sterilized water for elution, make sure that the pH value is between pH 7.0 and 8.5.

Supporting information

Background Information

Bacterial cultures

The bacterial culture influences yield and quality of plasmid DNA. The following method is recommended.

1. Pick 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 h at 37°C with vigorous shaking.

Growth for more than 16 h is not recommended since cells begin to lyse and plasmid yields may be reduced. Use a tube or flask with a volume of at least 4 times the volume of the culture.

2. Harvest the bacterial cells by centrifugation at > 8000 rpm ($6800 \times g$) in a conventional, table-top microcentrifuge for 3 min at room temperature (15–25°C).

The bacterial cells can also be harvested in 15 ml centrifuge tubes at $5400 \times g$ for 10 min at 4°C. Remove all traces of supernatant by inverting the open centrifuge tube until all medium has been drained.

Plasmid copy number

The origin of replication influences the plasmids copy number per cell. Often, very large plasmids and cosmids are maintained at very low copy numbers.

Table 4. Origins of replication and copy numbers of various plasmids

DNA construct	Origin of replication	Copy number	Classification
Plasmids			
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
Cosmids			
SuperCos	ColE1	10–20	Low copy
pWE15	ColE1	10–20	Low copy

* The pMB1 origin of replication is closely related to that of ColE1 and falls in the same incompatibility group. The high-copy-number plasmids listed here contain mutated versions of this origin.

Host strains

Host strains influence on the quantity and quality of the purified DNA:

- Host strains such as DH1, DH5a®, and C600 yield high-quality DNA with 5 PRIME protocols.
- The slower growing strain XL1-Blue also yields DNA of very high quality.
- Large amounts of carbohydrates are released during lysis of strain HB101 and its derivatives, such as TG1 and the JM100 series.
- Some strains, such as JM101, JM110, and HB101, have high levels of endonuclease activity and yield DNA of lower quality.

The methylation and growth characteristics of the strain should also be taken into account when selecting a host strain. XL1-Blue and DH5α are highly recommended for reproducible and reliable results.

Inoculation

Plates should be freshly poured and contain the appropriate selective agent so that single colonies can be isolated. It is recommended to streak from stocks, choose a colony and streak again to ensure the clone is antibiotic resistant. Note that subculturing may lead irregularities in yield. Inoculate from a single colony into 1–5 ml media containing the appropriate selective agent. Incubate for 12–16 hours with vigorous shaking. Do not use cells that have been cultivated for more than 16 hours as the cells begin to lyse and plasmid yields may be reduced.

Antibiotics

Bacteria should be kept in the presence of antibiotic selection at all stages of growth to avoid loss of plasmid. Ensure fresh stocks of antibiotics are prepared and stored correctly as many selection agents are instable.

In particular, note that ampicillin in the culture medium is continually being hydrolyzed by resistant bacterial. Thus, "satellite colonies" appear as the ampicillin is hydrolyzed in the vicinity of a growing colony on ampicillin plates. In addition, ampicillin solution should be stored frozen in single-use aliquots as it is temperature sensitive.

Recommendations for commonly used antibiotics are given in the following table.

Table 5. Preparation and use of antibiotics

Antibiotic	Stock solutions		Working concentration (dilution)
	Concentration	Storage	
Ampicillin (sodium salt)	50 mg/ml in water	-20°C	100 µg/ml (1/500)
Chloramphenicol	34 mg/ml in ethanol	-20°C	170 µg/ml (1/200)
Kanamycin	10 mg/ml in water	-20°C	50 µg/ml (1/200)
Streptomycin	10 mg/ml in water	-20°C	50 µg/ml (1/200)
Tetracycline HCl	5 mg/ml in ethanol	-20°C	50 µg/ml (1/100)

Culture media

Overloading the PerfectPrep membrane may cause contamination with bacterial genomic DNA and reduce yields. Determine the cell density of the culture and adjust the volume accordingly.

It is recommended to use standard Luria-Bertani (LB) broth culture medium with PerfectPrep Kits. The extremely high cell densities obtained with rich broths such as TB (Terrific Broth) or 2x YT can overload the purification system. If rich media are used, smaller culture volumes must be used.

Some fast growing host cells may also grow to very high cell densities. When using such hosts, the volumes of Buffers RP1, PL2, and PN3 can be doubled.

LB culture broth is prepared with recipes that vary, primarily in the amount of NaCl. For the best plasmid yields, the following formulation is recommended: 10 g Tryptone, 5 g yeast extract, 10 g NaCl.

Preparation of cell lysates

Bacteria cells are lysed in NaOH/SDS (Buffer PL2) in the presence of RNase A. Phospholipid and protein components of the cell membrane are solubilized. As cells are lysed, the cell contents are released, and chromosomal and plasmid DNA, and proteins are denatured. When the lysis time is optimized, plasmid DNA is released but chromosomal DNA is not. Long lysis times may cause the plasmid to become irreversibly denatured. The denatured form of the plasmid is resistant to restriction enzyme digestion and runs faster on agarose gels.

With the addition of Buffer PN3, the lysate is neutralized. The high-salt binding conditions created by the buffer causes denatured proteins, chromosomal DNA, cellular debris, and SDS to precipitate. Gently mixing ensures complete precipitation. Plasmid DNA renatures correctly and remains in solution.

Chromosomal DNA is coprecipitated with the cell to which it is bound. Avoid vigorous stirring and vortexing during lysis to prevent shearing and subsequent contamination with genomic DNA.

Buffers

Add the provided RNase A solution to Buffer PR1, mix, and store at 2–8°C.

Add ethanol (96–100%) to Buffer PW before use.

Check Buffers PL2 and PN3 before use for salt precipitation. Redissolve any precipitate by warming to 37°C. Do not shake Buffer PL2 vigorously.

Close the bottle containing Buffer PL2 immediately after use to avoid acidification of Buffer PL2 from CO₂ in the air.

Buffers PL2, PN3, and PD contain irritants. Wear gloves when handling these buffers.

Centrifugation

All centrifugation steps are carried out at 12,000 rpm (~13,400 x g) in a conventional, table-top microcentrifuge.

Elution

Consider the following guidelines for elution:

- The elution buffer should be dispensed directly onto the center of the PerfectPrep membrane.
- When 50 μ l is applied, the average eluate volume is 48 μ l.
- Increasing the elution volume can increase DNA yield. For increased DNA concentration, use a lower elution-buffer volume.
- Water used for elution should have a pH of 7.0–8.5 for maximum elution efficiency. Avoid pH <7.0 as this can decrease yield. Store DNA eluted with water at -20°C , as DNA may degrade in the absence of a buffering agent.
- DNA can also be eluted in TE buffer (10 mM Tris·Cl, 1 mM EDTA, pH 8.0), but the EDTA may inhibit subsequent enzymatic reactions.

Purified sample guide

Analyzing plasmid DNA on an agarose gel

The best way to check the procedure when a problem occurs is to prepare an analytical agarose gel with fractions saved from each step of the purification procedure. Precipitate the nucleic acids with 1 volume of isopropanol, rinse the pellets with 70% ethanol, drain well, and resuspend in 10 μ l TE buffer, pH 8.0. Run 2 μ l of each sample on a 1% agarose gel.

Figure 1 illustrates analysis of DNA purified with the PerfectPrep Spin method showing that the eluted pure plasmid DNA shows no contamination with other nucleic acids.

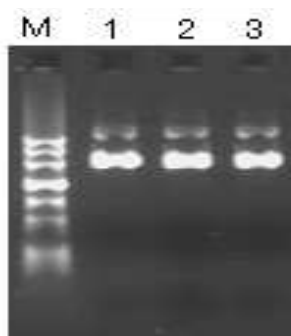


Figure 1. Agarose gel analysis of the PerfectPrep Miniprep procedure. 1-3: eluted plasmid from 3 independent experiments; M: markers.

Special applications

Purification of low-copy plasmids and cosmids

Low copy-number plasmid or cosmids can be prepared from 1–10 ml overnight *E. coli* cultures grown in LB medium. When using more than 5 ml culture volume,

double the volumes of Buffers PR1, PL2, and PN3. The Buffer PD wash is required for all strains. When preparing plasmid or cosmids that are >10 kb, pre-heat the elution buffer to 70°C prior to eluting DNA from the PerfectPrep membrane. Using these modifications, a 10 ml overnight LB culture typically yields around 5 µg DNA.

Repurifying plasmid DNA prepared by other methods

The PerfectPrep Spin Mini Kit can be used to repurify plasmid DNA isolated by other methods.

1. Add 500 µl BL buffer to a PerfectPrep Spin Column CB3 to equilibrate the column.
2. Centrifuge the column for 1 min at 12,000 rpm, and discard the flow-through.
3. Add 5 volumes of Buffer PD to 1 volume of the DNA solution, and mix.
For example, add 500 µl Buffer PD to 100 µl of DNA sample.
4. Apply the samples to PerfectPrep membrane. Draw the samples through the PerfectPrep membrane by centrifugation, and continue the protocol at the Buffer PW wash step. The optional wash step with Buffer PD is not necessary.

Troubleshooting guide

The following troubleshooting recommendations are designed to address unexpected or undesired results. To ensure optimal use, follow the guidelines and recommendations in the manual.

Observation	Low or no yield
--------------------	------------------------

Possible cause	General
-----------------------	---------

Avoiding	Low yields may be caused by a number of factors. To find the source of the problem, analyze fractions saved from each step in the procedure on an agarose gel. A small amount of the cleared lysate and the entire flow-through can be precipitated by adding 0.7 volumes isopropanol and centrifuging at maximum speed (13,000 rpm or ~17,000 x g) for 30 minutes. The entire wash flow-through can be precipitated by adding 0.1 volumes of 3 M sodium acetate, pH 5.0, and 0.7 volumes of isopropanol.
-----------------	---

Observation	No DNA in the cleared lysate before loading
--------------------	--

Possible cause	Plasmid did not propagate
-----------------------	---------------------------

Avoiding	Read the section beginning on page 15 and check that the conditions for optimal growth were met.
-----------------	--

Possible cause	Lysate prepared incorrectly
-----------------------	-----------------------------

Avoiding	Check storage conditions and age of buffers.
-----------------	--

Possible cause	Buffer PL2 or PN3 precipitated
-----------------------	--------------------------------

Avoiding	Redissolve by warming to 37°C.
-----------------	--------------------------------

Possible cause Cell resuspension incomplete

Avoiding Pelleted cells should be completely resuspended in Buffer PR1. Do not add Buffer PL2 until an even suspension is obtained.

Observation DNA is found in the flow-through of cleared lysate

Possible cause PerfectPrep membrane overloaded

Avoiding If rich culture media, such as TB or 2x YT are used, culture volumes must be reduced. It may be necessary to adjust LB culture volume if the plasmid and host strain show extremely high copy number or growth rates. See the section beginning on page 15.

Possible cause RNase A digestion omitted

Avoiding Ensure that RNase A is added to Buffer PR1 before use.

Possible cause RNase A digestion insufficient

Avoiding Reduce culture volume if necessary. If Buffer PR1 containing RNase A is older than 6 months, add additional RNase A.

Observation DNA is found in the wash flow-through

Possible cause Ethanol omitted from wash buffer

Avoiding Repeat procedure with correctly prepared wash buffer (Buffer PW).

Observation	Little or no DNA in eluate
Possible cause	Elution buffer incorrect
Avoiding	DNA is eluted only in the presence of low-salt buffer (e.g., Buffer PEB or water). Elution efficiency is dependent on pH. The maximum efficiency is achieved between pH 7.0 and 8.5. When using water for elution, make sure that the pH value is within this range.
Possible cause	Elution buffer incorrectly dispensed onto membrane
Avoiding	Add elution buffer to the center of the PerfectPrep membrane to ensure that the buffer completely covers the surface of the membrane for maximum elution efficiency.
Observation	Low DNA quality: DNA does not perform well in downstream applications
Possible cause	Eluate salt concentration too high
Avoiding	Modify the wash step by incubating the column for 2 to 5 minutes at room temperature after adding 0.7 ml of Buffer PW and then centrifuging.
Possible cause	Nuclease contamination
Avoiding	When using <i>endA+</i> host strains such as HB101 and its derivatives, the JM series, or any wild-type strain, ensure that the wash step with Buffer PD is performed.
Possible cause	Eluate contains residual ethanol
Avoiding	Ensure that air-drying step is performed correctly.

Observation	RNA in the eluate
Possible cause	RNase A digestion omitted

Avoiding Ensure that RNase A is added to Buffer PR1 before use.

Possible cause	RNase A digestion insufficient
-----------------------	--------------------------------

Avoiding Reduce culture volume if necessary. If Buffer PR1 containing RNase A is more than 6 months old, add additional RNase A.

Observation	Genomic DNA in the eluate
--------------------	----------------------------------

Possible cause	Buffer PL2 added incorrectly
-----------------------	------------------------------

Avoiding The lysate must be handled gently after addition of Buffer PL2 to prevent shearing. Reduce culture volume if lysate is too viscous for gentle mixing.

Possible cause	Buffer PN3 added incorrectly
-----------------------	------------------------------

Avoiding Upon addition of Buffer PN3 in step 3, mix immediately but gently.

Possible cause	Lysis too long
-----------------------	----------------

Avoiding Lysis in step 2 must not exceed 5 minutes.

Possible cause	Culture overgrown
-----------------------	-------------------

Avoiding Overgrown cultures contain lysed cells and degraded DNA. Do not grow cultures for longer than 12–16 hours.

Ordering information

Kit	Order/ref. no.	Size
PerfectPrep Spin Miniprep Kit (50)	2300100	50
PerfectPrep Spin Miniprep Kit (200)	2300110	200
PerfectPrep Plasmid 96 Vac Kit	2300200	2 plates
PerfectPrep Plasmid 96 Vac Kit	2300210	10 plates
PerfectPrep Plasmid 96 Vac Base Kit	2300220	50 Plt ¹
PerfectPrep BAC 96 Kit	2300300	2 plates
PerfectPrep BAC 96 Kit	2300310	10 plates
PerfectPrep BAC 96 Base Kit	2300320	50 Plt ¹
Water, Mol Bio grade	2500000	1 l
Collection Plates	2300230	50 plates
Culture Plates	2300240	50 plates
Water, Mol Bio grade	2500010	10 x 50 ml
Water, Mol Bio grade	2500020	5 l
DNA Gel Loading Buffer 10x	2500070	6 x 500 µl
TBE 5x	2500050	5 l
TAE 50x	2500060	5 l
Agarose GelExtract Mini Kit	50 preps	2300500
Agarose GelExtract Mini Kit	200 preps	2300510
PCRExtract Mini Kit	50 preps	2300600
PCRExtract Mini Kit	200 preps	2300610

¹ without plates, please order Culture Plate, Order No. 2300240, and Collection Plate, Order No. 2300230, separately

5 PRIME distributors

A complete list of 5 PRIME distributors is available from www.5PRIME.com.

