EasyPrep™ Plasmid Miniprep Manual

Catalog#: PD01-01, PD01-02

Bioland

For research use only

(April 2025)

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Introduction

The EasyPrep™ Plasmid Miniprep Kit provides a simple, fast and cost effective method to purify plasmid DNAs from bacterial culture, especially useful for low-copy-number plasmid miniprep or applications that amount of DNA needed is about 10-20 µg. The kit applies DNA-binding-specific matrix, which allows the high efficient binding of DNA, while proteins and other cellular components are easily removed during washing. Plasmid DNAs are easily eluted with Tris buffer or pure water.

The yield of the high-copy plasmid from this kit is about 5-15µg from 1.5ml culture. Plasmid DNAs purified from this kit can be used directly for downstream applications such as transfection, cloning, sequencing, as well as *in vivo* applications.

Important Notes

The yield of plasmid DNA using the Miniprep kit depends on:

1) The origin of the replication. Samples of high-copy-number and low-copy-number plasmids are shown in the table. The protocols are optimized for high-copy-number plasmid purification. For low-copy-number plasmids, both the culture volume and the buffer volume need to be scaled up 2 to 3 times (Page 11).

2) The size of the plasmids. In general, larger plasmids have higher yields than smaller plasmids if the copy-numbers are similar. For example, the yield of pcDNA3.1 (5.4kb) > pBluescript (3kb) > pUC18/19 (2.7kb).

Plasmid	Origin	High copy	Low copy
pACYC	P15A		10-12
pSC101	pSC101		5
pSuperCos	pMA2		10-20
pBR322	pMA2		15-20
pUC	Muted pMA2	500-700	
pGEM®	Muted pMA2	300-400	
pBluescript®	ColE1	300-500	

3) Culture condition. For maximal yields, the culture should be in late log or early stationary phase of bacteria growth. If the culture overgrows, you will be harvesting more dead bacteria than live cells, which leads to genomic DNA contamination. If the culture is undergrown, then of course, yields are lower than expected. In practice:

- Start culture with a fresh colony, no more than a few days old.
- Add antibiotics to the LB media just before the culture.
- Shaker speed, 250 rpm for 1 - 3.5ml LB medium in 14ml culture tubes. Use larger tubes or flasks if low-copy number is expected.
- Culture at 37°C for 12-16 hours. Growth over 16 hours is not recommended because cells begin to lyse.
- OD_{600} of the culture should be 2.0-3.5.

Before Starting

Prepare all components and get all necessary materials ready by examining this manual and become familiar with each step.

- **RNase A**: Spin down RNase A vial briefly. Add the whole tube of RNase A to Buffer A1 and mix well before use. Store Buffer A1 at 4°C after use. RNase A final concentration ≥ 100 µg/ml.
- **Buffer A2** could form precipitate below room temperature. It is critical to warm up the buffer at 37°C to dissolve the precipitates before use.
- Keep Buffer A2 bottle cap tightly closed to avoid acidification.
- **Add ethanol to DNA Washing Buffer bottle before use**:
  Cat#: PD01-01: Add 48 mL 96-100% ethanol and mix
  Cat#: PD01-02: Add 200 mL 96-100% ethanol and mix
- Carry out all centrifugations at **room temperature.**

Materials supplied by users:

- 96-100% ethanol
- 1.5 ml microcentrifuge tubes
- High speed microcentrifuge

Kit Contents

Catalog#	PD01-01	PD01-02
Miniprep DNA Columns	50	250
Buffer A1	20 ml	80 ml
Buffer A2	20 ml	80 ml
Buffer N1	25 ml	110 ml
DNA Wash Buffer (Concentrate)	12 ml	50 ml
Elution Buffer	10 ml	20 ml
Column Buffer	8 ml	30 ml
RNase A	70 µl	280 µl
2ml Collection Tubes	50	250
Manual	1	1

- *Add ethanol to DNA Washing Buffer bottle (see Page 4) before use*
- *Elution Buffer: 10mM Tris.HCl, 1mM EDTA, pH8.5*
- *Buffer KB is not included. The alternative is Qiagen's PB buffer*
- *Buffers N1 and KB contain chaotropic salts which are irritants. Take appropriate laboratory safety measures and wear gloves when handling.*

Storage and Stability

Buffer A1 should be stored at 4°C once RNase A is added. All other components can be stored at room temperature. Shelf life is 12 months from the date of purchase.

EasyPrep™ Plasmid Miniprep Protocol

(Centrifuge Method)

1. **Harvest 1-3 ml overnight bacterial culture by centrifugation at 10,000 rpm for 3 min in a tabletop centrifuge. Pour off the supernatant and blot the inverted tube on a paper towel to remove excess media.**
2. **Add 250 µl Buffer A1 and completely resuspend bacterial pellet by vortexing or pipetting.** *Ensure that RNase A is added into Buffer A1 before use. Complete resuspension is critical for optimal yields.*
3. **Add 250 µl Buffer A2, mix gently but thoroughly by inverting 5-6 times, the solution becomes slightly clear.** *Do not incubate longer than 5 min. Over-incubation causes genomic DNA contamination and plasmid damage. Buffer A2 may form precipitates below room temperature. Warm up at 37°C water-bath to dissolve the precipitates before use.*
4. **Add 350 µl Buffer N1, mix immediately and thoroughly by inverting 5-6 times. Don't vortex!** *It is critical to mix the solution well, if it appears conglobated, more mix is required.*
5. **Centrifuge at 13,000 rpm for 5 min at room temperature.** *If the lysate doesn't appear clear, reverse the tube angle, centrifuge for 5 min and then transfer the lysate to the DNA column.*
6. ***Optional:*** **Column equilibration: during centrifugation, add 100 µl Column Buffer onto the DNA column, incubate for 2 min, centrifuge at 13,000 rpm for 30-60s.** *This step ensures column having optimal binding capacity, and is needed especially when the kit is stored over 6 months.*
7. **Carefully transfer up to 800µl clear lysate into a DNA column with the collection tube** (do not bring any floating material into the tube)**. Centrifuge at 13,000 rpm for 30-60s. Discard the flow-through.**

   Note: when using end+ bacteria strains, such as JM series and HA201 derivatives, it's important removes trace nuclease. Add

500 µl KB or PB Buffer onto the DNA column, centrifuge at 11,000 rpm for 30-60 s. Discard the flow-through. End⁻ strains such as DH5 α, Top10 and XL-1 blue do not require this step.

8. **Add 750 µl DNA Wash Buffer into the DNA column, centrifuge at 13,000 rpm for 30-60s. Discard the flow-through.** *Ensure that the 96-100% ethanol has been added to DNA Wash Buffer according to instructions before use.*

9. **Centrifuge at 13,000 rpm for additional 1 min.** *It is critical to remove residual ethanol for optimal elution. Residual ethanol may interfere subsequent applications.*

10. **Place the DNA column in a clean 1.5 ml microcentrifuge tube** *(Be careful not to transfer any of the ethanol with the DNA column)*. **Add 50-100 µl Elution Buffer (TE buffer, pH8.5) to the center of the column, incubate for 1 min, centrifuge at 13,000 rpm for 1 min to elute the plasmid DNA.** *Elution buffer can be replaced with 10mM Tris.HCl, pH8.5 if EDTA interferes subsequent enzyme digestions. Proper pH is essential.*

DNA storage:

Plasmid DNA is stable in TE Buffer if stored at -20°C or below .

Yield and quality of DNA

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

DNA concentration = A_{260} x 50 x dilution factor (µg/ml)

EasyPrep™ Plasmid Miniprep Protocol

(Vacuum Method)

1. **Harvest 1-3 ml overnight bacterial culture by centrifugation at 10,000 rpm for 3 min in a tabletop centrifuge. Pour off the supernatant and blot the inverted tube on a paper towel to remove excess media.**

2. **Add 250 µl Buffer A1 and completely resuspend bacterial pellet by vortexing or pipetting.** *Ensure that RNase A is added into Buffer A1 before use. Complete resuspension is critical for optimal yields.*

3. **Add 250 µl Buffer A2, mix gently but thoroughly by inverting 5-6 times, the solution becomes slightly clear.** *Do not incubate longer than 5 min. Over-incubation causes genomic DNA contamination and plasmid damage. Buffer A2 may form precipitates below room temperature. Warm up at 37°C water-bath to dissolve the precipitates before use.*

4. **Add 350 µl Buffer N1, mix immediately and thoroughly by inverting 5-6 times. Don't vortex!** *It is critical to mix the solution well, if it appears conglobated, more mix is required.*

5. **Centrifuge at 13,000 rpm for 5 min at room temperature. During centrifugation.**

6. **Prepare the vacuum manifold as instructed by manufacturer. Insert DNA columns into the luer connector of the vacuum manifold. *Optional*: Add 100 µl Column Buffer onto the DNA column, incubate for 2 min, switch on vacuum to draw the solution through the columns.** *This step ensures column having optimal binding capacity and is needed especially when the kit is stored over 6 months.*

7. **Carefully transfer up to 800 µl clear lysates into Miniprep DNA columns** *(Do not bring any floating material into the tube)*. **Switch on vacuum to draw the solution through the columns completely, and then switch off vacuum.**

   Note: when using end+ bacteria strains, such as JM series and HA201 derivatives, it's important to remove trace nuclease. Add 400 µl KB or PB Buffer into the DNA column, Switch on vacuum to

to draw the solution through the columns. End⁻ strains such as DH5 α, Top10 and XL-1 blue do not require this step.

8. **Add 750 µl DNA Wash Buffer into the DNA columns. Switch on vacuum source to draw the solution through the columns completely, and then switch off vacuum source.** *Ensure that the 96-100% ethanol has been added to DNA Wash Buffer according to instructions before use.*

9. **Place each DNA column in a clean 1.5 ml microcentrifuge tube, centrifuge at 11,000 rpm for 1 min.** *Be careful not to transfer any of the ethanol with the DNA column. It is critical to remove residual ethanol for optimal elution. Residual ethanol may interfere subsequent applications.*

10. **Place each DNA column in a clean 1.5 ml microcentrifuge tube. Add 50-100 µl Elution Buffer (TE buffer, pH8.5) to the center of the column, incubate for 1 min, centrifuge at 11,000 rpm for 1 min to elute the plasmid DNA.** *Elution buffer can be replaced with 10mM Tris.HCl, pH8.5 if EDTA interferes subsequent enzyme digestions.*

DNA storage:

Plasmid DNA is stable in TE Buffer if stored at -20°C or below .

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Trouble Shooting Guide

Problem	Possible Reason	Suggested Improvement
Low Yield	Poor Cell lysis	Resuspend pellet thoroughly by vortexing and pipetting before adding Buffer A2. Make fresh Buffer A2 if the cap had not been closed tightly.
	Bacterial culture over-grown or not fresh	Grow bacterial 12-16 h. If the culture is not used the same da, spin down cultures and store the pellet at -20°C. Do not store culture at 4°C overnight.
	Low copy-number plasmid	Increase culture volume (up to 15 ml for Miniprep-IIs). Increase the volume of Buffer A1, A2, N1 as instructed in the protocol.
No DNA	Plasmid lost in Host *E. coli*	Prepare fresh culture from fresh colonies.
Genomic DNA contamination	Over incubation after adding Buffer A2	Do not vortex or mix aggressively after adding Buffer A2. Do not incubate more than 5 min after adding solution A2.
RNA contamination	RNase A not added to Buffer A1 or Buffer A1 not stored at 4°C after adding RNase A.	Add RNase A to Buffer A1: 100 µl RNase A (30mg/ml) for 30 ml Buffer A1.
Plasmid DNA floats out of wells while running in agarose gel, DNA doesn't freeze or smell of ethanol	Residual ethanol not completely removed from column	Centrifuge or vacuum again after washing step. Make sure that no residual ethanol remaining on the membrane before eluting the plasmid DNA.

Purification of low-copy plasmid DNA and cosmids

The Miniprep protocols can be used for preparation of low-copy-number plasmid DNA or cosmids from 5–10 ml overnight E. coli cultures grown in LB medium.

The following slight modifications to the protocols are required:

- Doubling the volumes of Buffers A1, A2 and N1.
- The wash step with Buffer KB is required for all strains.
- When plasmid DNA or cosmids are >10 kb, preheat Buffer EB (or water) to 70°C prior to eluting DNA.
- A 10 ml overnight LB culture typically yields 5–10 µg DNA. For higher yield, plasmid miniprep II kit is recommended.

Plasmid DNA Cleaning

Plasmid DNA isolated by other methods can be further purified using this kit.

1. Add 5 volumes of Buffer KB to 1 volume of the DNA solution and mix (e.g., add 500 µl Buffer KB to 100 µl of DNA sample).
2. Add the samples to the Columns, draw the samples through the membrane by centrifugation or vacuum.
3. Add 750ul DNA Wash Buffer, centrifugation or vacuum.
4. Centrifuge the columns at 13,000rpm for 1 min.
5. Elute with 50µl Buffer EB.

Limited use and warranty

This product is warranted to perform as described in its labeling and in Bioland's literature when used in accordance with instructions. No other warranties of any kind, express or implied, including, without limitation, implied warranties of merchantability or fitness for a particular purpose, are provided by Bioland. Bioland's sole obligation and purchaser's exclusive remedy for breach of this warranty shall be, at the option of Bioland, to replace the products, Bioland shall have no liability for any direct, indirect, consequential, or incidental damage arising out of the use, the results of use, or the inability to use it product.

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