Contents

Important Notes

Plasmid Copy Numbers: The yield of plasmid DNA depends on the origin of the replication and the size of the plasmid. 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 3 to 5 times. Please contact our customer service for further information and refer to the table below for the commonly used plasmids.

Plasmid	Origin	Copy Numbers	Expected Yield (µg per 200 mL)
pSC101	pSC101	5	10-15
pACYC	P15A	10-12	20-25
pSuperCos	pMB1	10-20	20-40
pBR322	pMB1	15-20	30-40
pGEMR	Muted pMB1	300-400	400-500
pBluescriptR	ColE1	300-500	400-600
pUC	Muted pMB1	500-700	600-1200

Host Strains: The strains used for propagating plasmid have significant influence on yield. Host strains such as Top 10, DH5a, and C600 yield high-quality plasmid DNA. *endA*+ strains such as JM101, JM109, JM110, HB101, TG1 and their derivatives, normally have low plasmid yield due to either endogenous endonucleases or high carbohydrates released during lysis. We recommend transform plasmid to an *endA*- strain if the yield is not satisfactory. For purifying plasmid DNA from *endA*+ strains, we recommend use product number PD1711.

Culture Medium: This procedure is designed for isolating plasmid grown in standard LB medium (Luria Bertani) to density of OD_{600} 2.0 to 3.0. If rich medium such as TB or 2xYT are used, make sure the cell density doesn't exceed 3.0 (OD600). A high ratio of cell density over lysis buffers result in low DNA yield and purity. For over amount of cell numbers, either reduce the biomass or scale up the volumes of Buffer A1, B1 and N3.

Introduction

Key to the kit is our proprietary Buffer RET that selectively removes endotoxin without phase separation. The unique DNA binding system allows the high efficient binding of DNA to our ezBind™ matrix while proteins and other contaminates are removed under certain optimal conditions.

Kit Contents

Catalog#	PD1624-00	PD1624-01	PD1624-02
Preps	1	2	10
DNA Unit	1	2	10
Filter Unit	1	2	10
Replacement Cup	2	4	20
Buffer A1	110 mL	210 mL	2×510 mL
Buffer B1	110 mL	210 mL	2×510 mL
Buffer N3	55 mL	110 mL	550 mL
Buffer RET	110 mL	210 mL	2×510 mL
DNA Wash Buffer*	2×24 mL	3×24 mL	4×80 mL
RNase A（20mg/mL）	550 μL	1.1 mL	4×1.5 mL
Endofree Elution Buffer	30 mL	60 mL	270 mL
User Manual		1	1

*Buffer RET contains chaotropic salts, Buffer N3 contains acetic acid, wear gloves and protective eyewear when handling.

*Add 96 mL (PD1624-00) or 96 mL (PD1624-01) or 320 mL (PD1624-02) 96-100% ethanol to each DNA Wash Buffer bottle before use.

Before Starting

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

Important:

- RNase A: Spin down RNase A vials briefly. Add all RNase A to Buffer A1 and mix well before use.
- Buffer B1 precipitates below room temperature, it is critical to warm up the buffer at 37℃ to dissolve the precipitates before use.
- Keep the cap tightly closed for Buffer B1 after use.

Materials supplied by users:

- 100% ethanol and isopropanol.
- Vacuum system.
- 250 mL or 500 mL bottle (Corning# 430282) or 1,000 mL bottle (#430518) or equivalent.
- 50 mL conical tubes.

Storage and Stability

Buffer A1 should be stored at 4℃ once RNase A is added. All other materials can be stored at room temperature (15-25℃). The guaranteed shelf life is 12 months from the date of production.

EZgene ™ Plasmid Megaprep 10 Protocol

1. Inoculate **1000-1500 mL** LB containing appropriate antibiotic with 500 µL fresh starter culture. Grow at 37°C for 14-16 h with vigorous shaking. Harvest 500-1500 mL overnight bacterial cells by centrifugation at 5,000 x g for 10 minutes at room temperature. Decant or aspirate medium and discard.
   Note: The best way to prepare a starter culture: Inoculate a single colony from a freshly grown selective plate into 2 mL LB medium containing the appropriate antibiotic and grow at 37°C for 6-8 h with vigorous shaking (~250 rpm).

2. Resuspend the bacterial pellet in **100 mL Buffer A1** (Add RNase A into Buffer A1 before use). Pipet or vortex till the bacterial pellet dispersed thoroughly (Complete resuspension is critical for optimal yields).

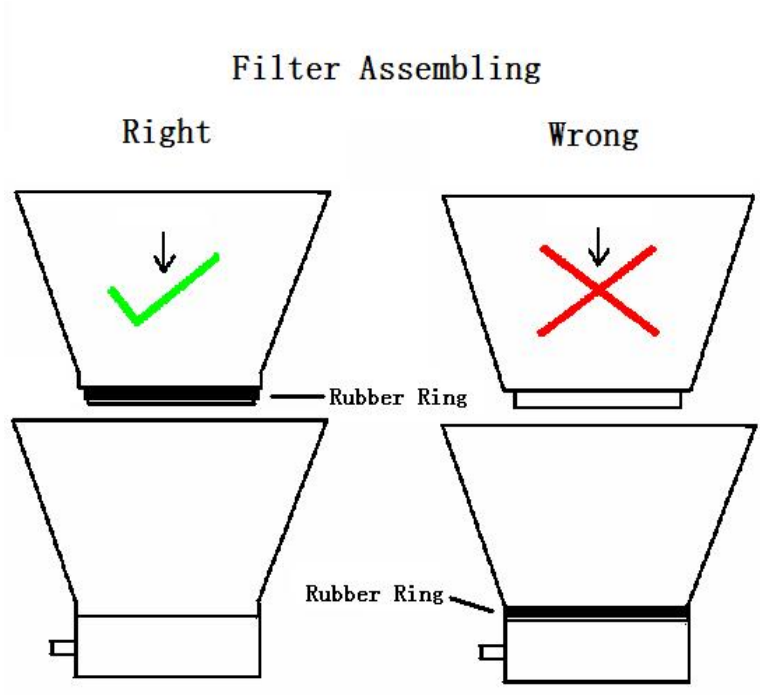
3. Add **100 mL Buffer B1**, mix thoroughly by inverting 10 times with mild shaking. Incubate for 10 min to obtain a slightly clear lysate. Complete lysis is critical for DNA yield. The mixture of completely lysed bacteria looks transparent.
   Note: Buffer B1 forms precipitation below room temperature, if solution becomes cloudy, warm up at 37°C to dissolve before use.

4. Add **50 mL Buffer N3** and mix immediately by inverting vigorously 5 -10 times till a flocculent white precipitate forms.
   Note: It is critical to mix the lysate well, if the mixture still appears conglobated, brownish or viscous, more mix is required to completely neutralize the solution.

5. Attach the 2-layer **Filter Unit** to a sterile 500 mL or 1000 mL standard bottle (Corning# 430518 or 430282 or equivalent) and screw tight. Connect the unit to a pump-driven vacuum system.

6. Transfer the relatively clear lysate from step 5 (use a 50 mL serological pipet) to the **Filter Unit**. Stand by for 2 minutes and turn on the vacuum. Load the remaining lysate till all lysate passes through the **Filter Unit**.
   Note 1: Use a 50 mL serological pipet to transfer the relatively clear to the Filter Unit. Normally around 220 mL lysate can be filtered through the Filter Unit within 10 minutes.

Note 2: If the flow through gets too slow, turn off the vacuum and wait for 1 minute. Carefully detach the upper filter cup and replace it with a Filter Unit Replacement Cup. Assemble the unit as instructed in page 6. Pour the lysate from the original cup to the Filter Unit Replacement Cup. Turn on the vacuum and filter the rest of the lysate.



8. When most of the lysate has been filtered through the unit, turn off the vacuum, wait for 1 minute, detach the unit and discard the **Filter Unit**.
Note: The DNA is in the collection bottle.

9. Connect a **DNA Unit** to a clean 500 mL bottle and screw tight. Connect the **DNA Unit** to the vacuum with the vacuum off. Add **100 mL Buffer RET** to the sample, add **90 mL 100% ethanol** to the lysate bottle. Mix well by sharp hand shaking 3-5 times and immediately. Pour lysate/ethanol mixture to the **DNA Unit** to the top level and turn on the vacuum.

10. Transfer the remaining lysate/ethanol mixture into the **DNA Unit**. When all the lysate pass through the **DNA Unit**, vacuum for another 2 minutes.

11. Add **75 mL DNA Wash Buffer** *(Add ethanol to DNA Wash Buffer before use)* evenly to the DNA membrane and allow the liquid to pass through the DNA membrane.Wash the DNA membrane with another **75 mL DNA Wash Buffer** *(Add ethanol to DNA Wash Buffer before use)* and vacuum for 1 minute at maximum force.

12. Add **80 mL 100% ethanol** evenly to the DNA membrane and allow the liquid to pass through the DNA membrane. Turn off the vacuum, wait for 1 min, and discard the flow through liquid from the collection bottle. Reconnect the **DNA Unit** to the collection bottle.

13. Turn on the vacuum for 20 minutes at maximum force (It is critical to dry the residual ethanol for optimal yield). Turn off the vacuum, incubate at 65°C for 20 min will help to remove the ethonal and increase the elution efficiency.

14. Turn off the vacuum, wait for 1 minute, and replace the 500 mL or 1,000 mL bottle with a sterile 50 mL conical tube, screw tight.

15. Add **10-15 mL Endofree Elution Buffer** evenly to the membrane and incubate at room temperature for 2 minutes. Turn on vacuum to elute DNA. Typically 5-8 mL of DNA containing solution can be collected. Turn off the vacuum. Add the eluted DNA back to the **DNA Unit** for another elution.

Note: The first elution normally yield 60-70% of the DNA while the second elution recovers another 20% of the DNA bound to the membrane.

16. The DNA is ready for downstream application such as transfection of difficult- to-transfection cells and primary cells.

Limited Use and Warranty

This product is warranted to perform as described in its labeling and in Biomiga'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 Biomiga. Biomiga's sole obligation and purchaser's exclusive remedy for breach of this warranty shall be, at the option of Biomiga, to replace the products, Biomiga 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.

For technical support or learn more product information,please contact us at 400-115-2855 or visit our website at www.biomiga.com.cn

Trouble Shooting Guide

Problems	Possible Reasons	Suggested Improvements
Low Yield	Poor cell lysis.	• Resuspend pellet thoroughly by votexing and pipeting prior adding Buffer B1. • Make fresh Buffer B1 if the cap had not been closed tightly. (Buffer B1: 0.2 M NaOH and 1% SDS).
Low Yield	Bacterial culture overgrown or not fresh.	Grow bacterial 12-16 hours. Spin down cultures and store the pellet at -20℃ if the culture is not purified the same day. Do not store culture at 4℃ overnight.
Low Yield	Low copy-number plasmid.	Increase culture volume (up to 10 mL for Minipreps, 100 mL for Midipreps, 200 mL for Maxipreps and 3 L for Megapreps). Increase the volume of Buffer A1, B1, N3 and ethanol proportionally with the ratio of 1:1:1.2:1.2.
No DNA	Plasmid lost in host *E. coli*.	Prepare fresh culture.
Genomic DNA contamination	Over-time incubation after adding Buffer B1.	Do not vortex or mix aggressively after adding Buffer B1. Do not incubate more than 5 minutes after adding Buffer B1.
RNA contamination	RNase A not added to Buffer A1.	Add RNase A to Buffer A1.
Plasmid DNA floats out of wells while running in agarose gel, DNA doesn't freeze or smell of ethanol	Ethanol traces not completely removed from column.	Make sure that no ethanol residual remaining in the silicon membrane before elute the plasmid DNA. Re-centrifuge or vacuum again if necessary.