Real Genomics

HiYield™ Plasmid Kit Mini

Maximum Yield Mini System

Protocol Book

YPD100 // YPD300

Ver. 2017-1

Real Genomics

Precautions

I) Handling Requirements

- *Do not use a kit after its expiration date has passed.*
- *Some reagents contain the hazardous compounds guanidine thiocyanate or guanidine hydrochloride . Do not let these reagents touch your skin, eyes, or mucous membranes. If contact does occur, wash the affected area immediately with large amounts of water. If you spill the reagents, dilute the spill with water before wiping it up.*
- *Do not allow reagents containing guanidine thiocyanate to mix with sodium hypochlorite solution or strong acids. This mixture can produce a highly toxic gas.*

II) Laboratory Procedures

- *Handle all samples and the resulting waste as if potentially infectious, using safe laboratory procedures. As the sensitivity and titer of potential pathogens in the sample material varies, the operator has to optimize pathogen inactivation by the Lysis Buffer or take appropriate measures according to local safety regulations. RBC Bioscience does not warrant that samples treated with Lysis Buffer are completely inactivated and non-infectious. After sample processing is completed, remove and autoclave all disposable plastics, if you worked with potentially infectious sample material.*
- *Do not eat, drink or smoke in the laboratory work area.*
- *Wear protective disposable gloves, laboratory coats and eye protection when handling samples and kit reagents.*
- *Do not use sharp or pointed objects when working with the reagent cartridge, in order to prevent damage of the sealing foil and loss of reagent.*
- *Do not contaminate the reagents with bacteria, virus, or ribonuclease. Use disposable pipettes and RNase-free pipette tips only to remove aliquots from reagent bottles. Use the general precautions described in the literature.*
- *Wash hands thoroughly after handling samples and test reagents.*

III) Waste Handling

- *Discard unused reagents and waste in accordance with country, federal,state and local regulations.*

CONTENTS

HiYield™

Maximum Yield Mini System

Plasmid Kit Mini 1

Cat.No. YPD100//YPD300

High Copy Number Protocol 3
Low Copy Number Protocol 5
Trouble Shooting 8

Maximum Yield Mini System

HiYield™ Plasmid Kit

Cat.No. YPD100//YPD300

Kit Contents

Cat.No. YPD100
100 mini preps / kit

Component	Amount
PD1 Buffer(B008)	25ml
PD2 Buffer(B009)	25ml
PD3 Buffer(B010)	40ml
W1 Buffer	50ml
Wash Buffer (concentrated)*	25ml
Elution Buffer	10ml
RNase A (50mg/ml)(RN050)	50μl
2ml Collection Tube	100pcs
PD Cloumn	100pcs

Cat.No. YPD300
300 mini preps / kit

Component	Amount
PD1 Buffer(B046)	65ml
PD2 Buffer(B047)	75ml
PD3 Buffer(B048)	100ml
W1 Buffer	130ml
Wash Buffer (concentrated)**	40ml
Elution Buffer	30ml
RNase A (50mg/ml)(RN130)	130μl
2ml Collection Tube	300pcs
PD Cloumn	300pcs

PD1,2,3 Buffers and RNase A can be purchased separately. (Cat.No. B008~B010 / B046~B048, RN050/RN130)

Sample Source: Plasmid DNA from Bacteria
Sample Size: 1-4ml of LB broth overnight incubate bacterial cultures
Typical Plasmid Yield :
Low Copy Number : 0.5-5μg // **High Copy Number :** 10-20μg (max 30μg)

Preparation time : 20min

Add provided RNase A to PD1 Buffer and store at 4°C. If precipitates have formed in PD2 Buffer, warm the buffer at a 37°C waterbath to dissolve.
* Add 100ml ethanol (96-100%) to Wash Buffer prior to initial use.
** Add 160ml ethanol (96-100%) to Wash Buffer prior to initial use.

Description

The HiYield™ Plasmid Mini Kit is designed for rapid isolation of plamid or cosmid DNA from 1-4 ml of bacterial cultures. The modified alkaline lysis method followed by RNase treatment is utilized to obtain cleared cell lysate with minimal genomic DNA or RNA contamination. Silica spin technology coupled with chaotrophic salt provides a reliable DNA binding and elution system. Purified DNA is ready for restriction digestion, ligation, PCR and sequencing reaction.

Quality Control

The quality of HiYield™ Plasmid Mini Kit is test on a lot-to-lot basis. The kits are tested by isolation of plasmid DNA from 3ml culture of E.coli DH5 transformed with the plasmid pUC19(A_{600}>2units/ml).
More than 20μg of plasmid DNA was quantified with spectrophotometer.

Reference

Birnboim, H. C., and Doly J. (1997) Nucleic Acids Res. 7, 1513. (2) Vogelstein B., and Gillespie D. (1979) Proc. Natl Acad Sci. USA 76,615.

Note

* *For research use only. Not for use in diagnostic or therapeutic procedures.*
* *PD3 buffer contains guanidine hydrochloride which is harmful and irritant. During operation, always wear a lab coat, disposable gloves and protective goggles.*

High Copy Number Protocol

Harvesting

1. Transfer 1.5ml of bacterial culture to a microcentrifuge tube (not provided).
2. Centrifuge for 1 min at full speed (13,000 rpm) in a microcentrifuge and discard supernatant.
   (If more than1.5 ml of bacteria culture is used, repeat the Harvesting Step. For over 4ml, use multiple columns.)

Resuspension

3. Add 200μl of PD1 Buffer (RNase A added) and resuspend the cell pellet by vortexing or pipetting.

Lysis

4. Add 200μl of PD2 Buffer and mix gently by inverting the tube 10 times. **Do not vortex** , avoid shearing genomic DNA.
5. Allow mixture to stand for 2 minutes at room temperature until lysate clears.

Neutralization

6. Add 300μl of PD3 Buffer and mix immediately by inverting the tube 10 times. **Do not vortex**.
7. Centrifuge for 2 min at full speed (13,000 rpm).

DNA Binding

8. Place a PD Column in a 2ml Collection Tube.
9. Apply the clear lysate (supernatant) from Step 7 to the PD Column.
10. Centrifuge at full speed (13,000 rpm) for 30 seconds.
11. Discard the flow-through and return the PD Column back to the 2ml Collection Tube.

Wash

12.Add 400µl of W1 Buffer in the PD Column.
13.Centrifuge at full speed (13,000 rpm) for 30 seconds.
14.Discard the flow-through and return the PD Column to the 2ml Collection Tube.
15.Add 600µl of Wash Buffer (ethanol added) to PD Column.
16.Centrifuge at full speed (13,000 rpm) for 30 Seconds.
17.Discard the flow-through and return the PD Column to the 2ml Collection Tube.
18.Centrifuge again for 3 min at full speed to dry the column matrix.

DNA Elution

19.Transfer the dried PD Column to a clean 1.5ml microcentrifuge tube (not provided).
20.Add 50µl of Elution Buffer or ddH$_2$O (pH 8.0-8.5) directly onto the centre of the membrane.
Avoid residual buffer adhering to the wall of the column.
21.Allow to stand for 2 min until the liquid is absorbed.
22.Centrifuge for 2 min at full speed (13,000 rpm) to elute plasmid DNA.

Low Copy Number Protocol

Add ethanol and RNase A to buffers according to component instructions.

The typical yield is about 0.5-1.0 µg per 1ml culture when preparing low-copy-number plasmid from overnight bacterial culture in LB medium. If the plasmid is larger than 30 kb, preheat the Elution Buffer to 70℃ prior to the Elution Step.

Harvesting

1. *Harvest up to 10ml of overnight culture by centrifugation.*

Resuspension

2. *Add 400µl of PD1 Buffer (RNase A added) to the tube and resuspend the cell pellet by votexing or pipetting.*

Lysis

3. *Add 400µl of PD2 Buffer and mix gently by inverting the tube 10 times.* ***Do not vortex*** *to avoid shearing genomic DNA.*
4. *Allow mixture to stand for 2 min at room temperature until lysate clears.*

Neutralization

5. *Add 600 µl of PD3 Buffer and mix immediately by inverting the tube 10 times.* ***Do not vortex****.*
6. *Centrifuge for 3 min at full speed (13,000 rpm).*

DNA Binding

7. Place a PD Column in a 2ml Collection Tube.
8. Apply 750µl of the clear lysate (supernatant) from step 6 to the PD Column.
9. Centrifuge at 10,000 xg (13,000rpm) for 30 seconds. Discard the flow-through and return the PD Column to the 2ml Collection Tube.
10. Apply the remaining clear lysate to the same PD Column.
11. Centrifuge at 10,000 xg (13,000rpm) for 30 seconds.
12. Discard the flow-through and return the PD Column to the 2ml Collection Tube.

Wash

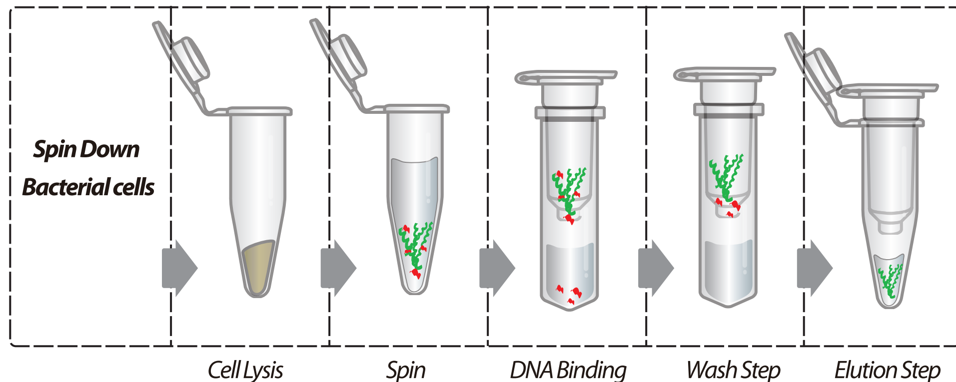
13. Add 400µl of W1 Buffer in the PD Column.
14. Centrifuge at 10,000 xg (13,000rpm) for 30 seconds.
15. Discard the flow-through and return the PD Column to the 2ml Collection Tube.
16. Add 600µl of Wash Buffer (ethanol added) in the PD Column.
17. Centrifuge at 10,000 xg (13,000rpm) for 30 seconds.
18. Discard the flow-through and return the PD Column to the 2ml Collection Tube.
19. Centrifuge again for 2 min at full speed to dry the column matrix.

DNA Elution

20. Transfer the dried PD Column onto a clean microcentrifuge tube (not provided).
21. Add 50µl of Elution Buffer to the center of the column matrix.

If plasmid DNA is larger than 10kb, use preheated Elution Buffer (70°C) during Elution Step to improve the elution eciency.

22. Allow to stand for 2 min until the liquid is absorbed by the matrix.
23. Centrifuge for 2 min at full speed to elute plasmid DNA.


Spin Down
Bacterial cells
Cell Lysis
Spin
DNA Binding
Wash Step
Elution Step

Troubleshooting

Problem	Possible Reason/Solution
Low yield	***Bacterial cells were not lysed completely*** *Too many bacterial cells were used. If using more than 10A_{600} units of bacterial culture, separate into multiple tubes. Following PD3 Buffer addition, break up the precipitate by inverting to ensure higher yield.*
	Incorrect Wash Buffer *Check to ensure Ethanol was added to Wash Buffer prior to use.*
	Incorrect DNA Elution Step *Ensure that Elution Buffer was added and absorbed to the center of PD Column matrix.*
	Incomplete DNA Elution *If plasmid DNA is larger than 10kb, use preheated Elution Buffer (70°C) during Elution Step to improve the elution efficiency.*
Eluted DNA does not perform well in downstream applications	***Residual ethanol contamination*** *After wash step, dry PD Column with additional centrifugation at top speed for 5minutes or incubation at 60°C for 5 minutes.*
	RNA Contamination *Prior to using PD1 Buffer, ensure that RNase A was added. If RNase A added PD1 Buffer is out of date, add additional RNase A. Too many bacterial cells were used, reduce sample volume.*
	Genomic DNA contamination *Do not use overgrown bacterial culture.* *During PD2 and PD3 Buffer addition, mix gently to prevent genomic DNA shearing.*
	Nuclease contamination *If host cells have high nuclease activity(e.g.,$endA^{+}$ strains), perform this Optional Wash Step to remove residual nuclease.* *After DNA Binding Step, add 200ml of PD3 Buffer to PD Column and incubate for 2 min at room temperature.* *Centrifuge at 6000 xg (8,000rpm) for 30 seconds.* *Continue from standard Wash Step.*

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