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User's Guide

Global Genomics Partner

DNA *PrepMate* II

Cat. No.: K-3011

BIONEER
bioneer corporation

V109C3

Safety Warnings and Precautions

For research use only. Not recommended or intended for diagnosis of disease in humans or animals. Do not use internally or externally in humans or animals.

Wear gloves when you handle irritant or harmful reagents.

Warranty and Liability

All BIONEER products undergo extensive Quality Control procedures and are warranted to perform as described when used correctly. Any problems should be reported immediately. Any liability incurred by BIONEER to the customer is limited to the replacement of the products. Liability is conditional upon the customer providing details of the problem to BIONEER within 30 days and returning the product to BIONEER for full examination.

Quality Management System ISO 9001 Certified

Every aspect of our quality management system, from product development and production to quality assurance and supplier qualification, meets world-class standards.

QC Testing

Each lot of BIONEER products is tested in our quality control team as raw material prior to purchase. Acceptable lots are processed and retested as finished products.

Trademarks

PrepMate™ is a trademark of Bioneer Corporation.

Contents

DNA *PrepMate*™ II
Technical Manual

I. Description

This kit is designed for the purification of DNA fragment or plasmid DNA from low-melting, TAE and TBE agarose gel, high salt solution, and lysed bacterial cell. The size range for effective purification is 40 bp – 50 kb. Elution volume is as little as 20μl when concentrated product is needed.

The principle of this kit is based on the adsorption of DNA onto silica surfaces. Chaotropic salt enhances not only melting of agarose gel but the binding of DNA onto a silica surfaces. Adsorption of DNA is so selective that molten agarose and salts are not adsorbed. Washing eliminates salts and residual agarose gel. High-purity DNA fragments are eluted with provided E Buffer (10 mM Tris-Cl, pH 8.5) or distilled water.

Purified DNA fragment can be applied to cloning, sequencing and other molecular biological application.

This kit has several advantages over the other competitor's column-type. First, DNA samples from many different sources can be purified. Second, wide range of the DNA fragments, especially large size of fragment, is effectively recovered. Third, you can easily scale up the amount of purification if you need.

II. Kit Components

The product has been designed for 150 purifications (10 μl SM Suspension per prep), and will retain performance for at least one year.

K-3011 DNA *PrepMate*™ II

Reagents	
SM Suspension (silica-based matrix)	4 X 0.5 ml
SB Buffer Store at room temperature. Handle carefully! This buffer contains chaotropic reagent that is irritant.	2 X 100 ml
W Buffer Add 80ml of absolute ethanol to each bottle before use. Store at room temperature.	2 X 20 ml
E Buffer (10 mM Tris-Cl, pH 8.5) Store at room temperature.	15 ml

III. Protocol

A. AGAROSE GEL EXTRACTION PROTOCOL

Before you proceed, check if the following requirements are met.

i. Heating block (or water bath) at 50°C is required.

ii. 3M Sodium acetate buffer (pH 5.0) may be necessary.

iii. Did you add the specified volume of absolute ethanol to W Buffer? If not, add 80 ml before use.

The speed of all the centrifuge steps is set at 10,000 x g (13,000 rpm) in a table-top microcentrifuge.

※ For DNA fragments larger than 10 kb, mix by flicking the tube gently to avoid shearing the DNA. Do not vortex.

1. **Excise the DNA band of agarose gel as small as possible and weigh the gel slice in a clean microcentrifuge tube.**

2. **Add SB Buffer 3 volumes of the gel slice for the DNA fragment of 100 bp – 2 kb. Otherwise, refer the table below.**

DNA < 100 bp and > 2% agarose gel	6 volumes of SB Buffer
100 bp ≤ DNA < 2 kb	3 volumes of SB Buffer
2 kb ≤ DNA < 4 kb	3 volumes of SB Buffer plus 1 volumes of distilled water
4 kb ≤ DNA < 6 kb	3 volumes of SB Buffer plus 2 volumes of distilled water
DNA ≥ 6 kb	3 volumes of SB Buffer plus 3 volumes of distilled water

For example, if the weight of the gel is 150 mg that contains 1 kb fragment, add 450 μl of SB Buffer.

3. **Resuspend the SM Suspension by vortexing for 30 sec.**

4. **Add SM Suspension to the sample according to the table below and mix.**

DNA ≤ 2 μg	10 μl of SM Suspension
2 μg < DNA ≤ 10 μg	30 μl of SM Suspension
DNA > 10 μg	additional 30 μl of SM Suspension per additional 10 μg DNA

5. **Incubate at 50°C for 10 min. After incubation, ensure that there are no unwanted particles of agarose gel.**
Vortexing the tube every 2-3 min enhances the melting efficiency. If the gel is not melted, increase incubation time. Residual agarose gel may inhibit subsequent enzymatic

reaction.

6. **Check if the color of the mixture is yellow.**
   If the color of the mixture is orange or red, add 10 μl of 3M sodium acetate (pH 5.0) and mix. The color should be turned into yellow. In this case, increase the incubation time (additional more than 5 min).

7. **Centrifuge the sample for 30 sec.**

8. **Remove supernatant with a pipette carefully.**

9. **Add 500 μl SB Buffer and resuspend the pellet by vortexing※.**

10. **Centrifuge the sample for 30 sec and carefully remove supernatant with a pipette.**
    Remove all traces of supernatant with a pipette. This wash step removes residual agarose contaminants.

11. **Add 500 μl of W Buffer and resuspend the pellet by vortexing※.**

12. **Centrifuge the sample for 30 sec and carefully remove supernatant with a pipette.**
    Remove all traces of supernatant with a pipette. This wash step removes residual salt contaminants.

13. **Repeat the step 11. and 12.**

※ For DNA fragments larger than 10 kb, mix by flicking the tube gently to avoid shearing the DNA. Do not vortex.

14. **Air-dry the pellet until the pellet becomes white.**
    Do not vaccum dry. Overdrying the Silica based matrix pellet may decrease elution efficiency and make it difficult to resuspend the pellet.

15. **Add 20 μl of E Buffer and resuspend the pellet by vortexing※. Incubate the sample 5 min at least.**
    If DNA fragments are larger than 4 kb, increase incubation time to 10 min and temperature to 50°C.
    Maximum yield is achieved with the buffer at pH 7.0-8.5. In case of pure water, eluted DNA may be denatured and unstable. Provided E Buffer satisfies these requirements and does not cause any problems in ordinary enzymatic reactions such as sequencing, restriction enzyme digestion, and ligation. Common TE buffer (pH 8.0) does also give satisfactory result.
    Caution : EDTA may inhibit subsequent enzymatic reactions.

16. **Centrifuge the sample for 1 min and carefully transfer the supernatant into a clean tube.**

17. **Optional: If you want more quantity, repeat steps 15. and 16.**

※ *For DNA fragments larger than 10 kb, mix by flicking the tube gently to avoid shearing the DNA. Do not vortex.*

B. PCR PURIFICATION PROTOCOL

Before you proceed, check if you have added the specified volume of absolute ethanol (80 ml) to W Buffer.

The speed of all the centrifuge steps is set at 10,000 x g (13,000 rpm) in a table-top microcentrifuge.

1. **Remove mineral oil from the PCR reaction.**

2. **Add SB Buffer 5 volumes of the PCR reaction for DNA fragment 100 bp – 2 kb. Otherwise, refer the table below.**

100 bp ≤ DNA < 2 kb	5 volumes of SB Buffer
2 kb ≤ DNA < 6 kb	5 volumes of SB Buffer plus 3 volumes of distilled water
DNA ≥ 6 kb	5 volumes of SB Buffer plus 5 volumes of distilled water

   For example, if a PCR reaction containing 1 kb fragment of DNA is 20 μl, add 100 μl of SB Buffer.

3. **Resuspend the SM Suspension by vortexing for 30 sec.**

4. **Add SM Suspension to the sample according to the table below and mix.**

DNA ≤ 2 μg	10 μl of SM Suspension

2 μg < DNA ≤ 10 μg	30 μl of SM Suspension
DNA > 10 μg	additional 30 μl of SM Suspension per additional 10 μg DNA

5. **Incubate the sample at room temperature for 10 min. Mix by inverting the tube occasionally 2-3 times.**

6. **Centrifuge the sample for 30 sec.**

7. **Remove supernatant with a pipette carefully.**

8. **Add 500 μl of W Buffer and resuspend the pellet by vortexing.**

9. **Centrifuge the sample for 30 sec and carefully remove supernatant with a pipette.**
   Remove all traces of supernatant with a pipette. This step removes residual salt contaminants.

10. **Repeat the step 8. and 9.**

11. **Air-dry the pellet until the pellet becomes white.**
    Do not vaccum dry. Overdrying the Silica based matrix pellet may decrease elution efficiency and make it difficult to resuspend the pellet.

12. **Add 20 μl of E Buffer and resuspend the pellet by vortexing. Incubate the sample for 5 min at least.**
    If DNA fragments are larger than 4 kb, increase incubation time to 10 min and temperature to 50°C.
    Maximum yield is achieved with the buffer at pH 7.0-8.5. In case of pure water, eluted DNA may be denatured and

unstable. Provided E Buffer satisfies these requirements and does not cause any problems in ordinary enzymatic reactions such as sequencing, restriction enzyme digestion, and ligation. Common TE buffer (pH 8.0) does also give satisfactory result. Caution : EDTA may inhibit subsequent enzymatic reactions.

13. **Centrifuge the sample for 1 min and carefully transfer the supernatant into a clean tube.**

14. **Optional: If you want more quantity, repeat steps 12. and 13.**

C. PLASMID DNA PURIFICATION PROTOCOL

Before you proceed, check if the following requirements are met.

i. Ice-cold Solution I for resuspention (ref. 5.).

50 mM Glucose
25 mM Tris-Cl (pH 8.0)
10 mM EDTA (pH 8.0)

ii. Solution II for bacterial cell lysis (ref. 5.).

0.2 N NaOH
1% SDS

iii. Ice-cold Solution III for neutralization (ref. 5.).

5 M Potassium Acetate	60 ml
Glacial acetic acid	11.5 ml
Distilled water	28.5 ml

iv. Did you add the specified volume of absolute ethanol to W Buffer? If not, add 80 ml before use.

The speed of all the centrifuge steps is set at 10,000 x g (13,000 rpm) in a table-top microcentrifuge.

This protocol is optimized for 1~5 ml of bacterial culture. If the volume of the culture is larger than that, change the volume of requirements accordingly.

※ For plasmid DNA larger than 10 kb, mix by flicking the tube gently to avoid shearing the DNA. Do not vortex.

1. **Centrifuge 1~5 ml of bacterial culture at 4℃ for 1 min in a microcentrifuge tube.**

2. **Remove the culture medium as much as possible.**

3. **Add 100 μl of Solution I to the pellet and fully resuspend the pellet by vigorous vortexing or pipetting.**
   The yield of plasmid may vary according to cell amount and plasmid type. Lysis efficiency is highly dependent on complete resuspension.

4. **Add 200 μl of Solution II and gently invert several times to lysis. Incubate the mixture on ice for 5 min.**
   Do NOT vortex! Vortexing may cause shearing of genomic DNA and contaminating plasmid DNA prep. It is important to invert gently.

5. **Add 150 μl of Solution III and gently invert several times. Incubate the mixture on ice for 5 min.**
   Genomic DNA and cell debris will aggregate into a white mass. Again, be cautious not to shear genomic DNA .

6. **Centrifuge at 4℃ for 5-10 min.**

7. **Transfer the supernatant into a new clean tube.**
   Don't take any white mass.

8. **Add an equal volume of SB Buffer to the supernatant.**

9. **Resuspend the SM Suspension by vortexing for 30 sec.**

10. **Add 20 μl of SM Suspension per 1~5 ml of bacterial culture.**

11. **Incubate the sample at room temperature for 10 min and vortex※ it every 2-3 min.**
    Plasmids are bound onto the surface of the Silica based matrix.

12. **Centrifuge the sample for 30 sec.**

13. **Carefully remove supernatant with a pipette.**

14. **Add 500 μl of W Buffer and resuspend the pellet by vortexing※.**

15. **Centrifuge the sample for 30 sec and carefully remove supernatant with a pipette.**
    Remove all traces of supernatant with a pipette. This step removes residual salt contaminants and soluble impurities.

16. **Repeat the step 14. and 15.**

17. **Air-dry the pellet until the pellet becomes white.**
    Do not vaccum dry. Overdrying the Silica based matrix pellet may decrease elution efficiency and make it difficult to resuspend the pellet.

18. **Add 30 μl of E Buffer and resuspend the pellet by vortexing※. Incubate the sample for 5 min at least.**
    If DNA fragments are larger than 4 kb, increase incubation time to 10 min and temperature to 50°C.

※ For DNA fragments larger than 10 kb, mix by flicking the tube gently to avoid shearing the DNA. Do not vortex.

Maximum yield is achieved with the buffer at pH 7.0-8.5. In case of pure water, eluted DNA may be denatured and unstable. Provided E Buffer satisfies these requirements and does not cause any problems in ordinary enzymatic reactions such as sequencing, restriction enzyme digestion, and ligation. Common TE buffer (pH 8.0) does also give satisfactory result.
Caution : EDTA may inhibit subsequent enzymatic reactions.

19. **Centrifuge the sample for 1 min and carefully transfer the supernatant into a clean tube.**

20. **Optional: If you want more quantity, repeat steps 18. and 19.**

※ For DNA fragments larger than 10 kb, mix by flicking the tube gently to avoid shearing the DNA. Do not vortex.

IV. Trouble Shooting

For Agarose gel extraction and PCR purification protocol

1. Low yield

1) Incomplete melting lowers the yield. Not only inadequate concentration of chaotropic salts affect the adsorption but also DNA encased in agarose gel does not bind to the surfaces of Silica-based matirx.

2) Did you add adequate amount of ethanol to the W Buffer? Concentrated W Buffer might wash the adsorbed DNA away.

3) Incorrect elution buffer may reduce the yield. Elution buffer should not contain too much salt. The pH of the buffer should be adjusted to 7.0-8.5.

4) Incorrect binding conditions like high pH reduces the yield. SB buffer contains pH indicator which color is yellow but it turns to red or orange when the pH is out of range. In this case, several drops of sodium acetate buffer adjust the pH of the solution appropriately.

5) Too much gel slice is loaded. More than 400mg agarose gel is loaded, binding efficiency of DNA may be lowered.

2. Subsequent enzymatic reaction does not work well

1) High salt concentration of the sample prevents enzyme from working. In this case, wash with W Buffer twice.

2) Sample contains residual W buffer. Remaining ethanol

inhibit enzymatic reactions. You must always dry the pellet (SM Suspension) completely but not overdry.

3) Incomplete removal of Silica-based matrix reduce enzyme activity in subsequent reaction, because the matrix tend to bind proteins.

For Plasmid DNA purification protocol

3. Low yield of plasmid

1) Did you harvest a sufficient amount of cells? The yield is dependent on the type of the host strain. An overload of cells may decrease the yield.

2) Did you completely resuspend cells in Solution I ? Incomplete suspension decreases the efficiency of lysis.

3) Is there precipitated salt in the Solution II, III, or SB Buffer? Vortex or shake well to redissolve the precipitant. An improper concentration of the chaotrophic agent will decrease the yield. If it does not redissove easily, warm it to 50℃.

4. Contamination of chromosomal DNA (The appearance of unwanted bands at agarose gel electrophoresis).

In step 4. the sample should not be vortexed or shaken too vigorously. The time for lysis should not exceed longer than 5 min. Both can shear the chromosomal DNA and contaminate the sample.
Handle the lysate gently!

5. Sample floats upon loading in agarose gel

Sample may contain residual W Buffer. Remaining ethanol in the sample causes floating. You must always dry the pellet (SM Suspension) completely but not overdry.

6. Too many background bands appear in sequencing analysis.

Did you check the endonuclease activity of the host strain of *E. coli*? HB101, JM series and normal wild-type hosts have high endonuclease activity and interrupt the sequencing reaction by degrading the plasmid DNA. Increase the incubation time of the step 11. in this case.

7. Sample contains RNA

Too many cells were harvested. We recommend using the appropriate volume of bacterial culture.

V. Appendix

1. Quality of DNA after purification

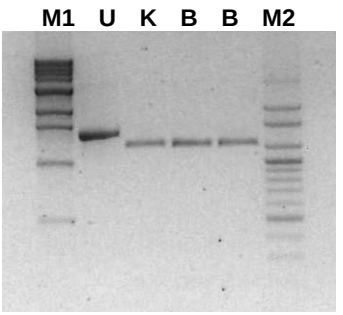

Figure 1. 1.4 kb DNA was purified from agarose gel. It was digested with *Hind* III and separated by 2% agarose gel electrophoresis. DNA was digested completely in 1.5 hrs in standard conditon.
B ; Bioneer's DNA *PrepMate*™ II, K ; competitor's kit, M1 ; 1 kb ladder, M2 ; 100 bp ladder, U ; uncut (1.4 kb).

2. Ligation test of gel-purified DNA

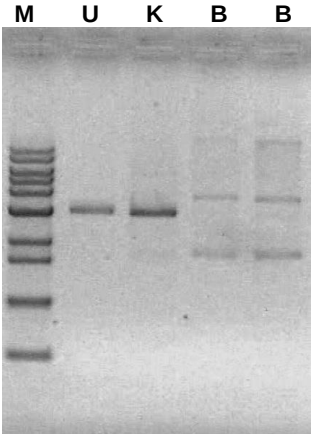

Figure 2. Re-ligation of gel-purified DNA. Gel-purified DNA was ligated by T4 DNA ligase after purification using Bioneer's DNA *PrepMate*™ II (B) and competitor's kit (K). M ; 1 kb ladder, U ; negative control (no ligation). A plasmid DNA (pBluescript II, 2.96 kb, Stratagene®) was digested with *Hind* III and gel-purified.

3. Sequencing quality of gel-purified DNA

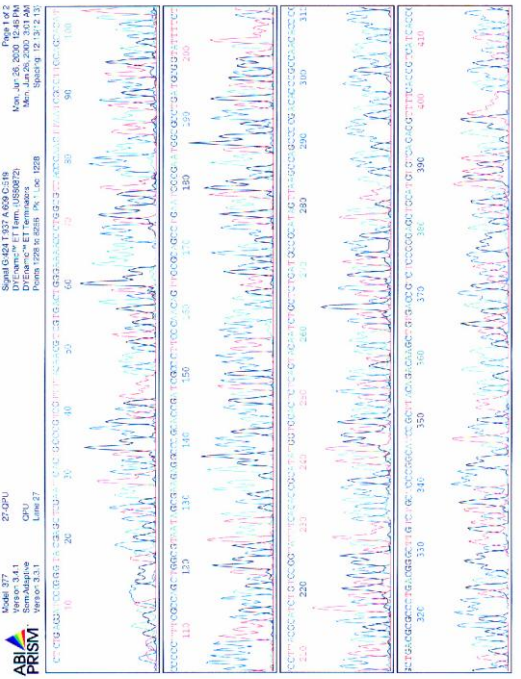

Figure 3. The sequence was analyzed by ABIPRISM™ #377 with DYEnamic™ ET Terminators (Perkin-Elmer Corp.). Up to 600 base of readable sequence is routinely obtained.

4. Preparation yield of plasmid DNA

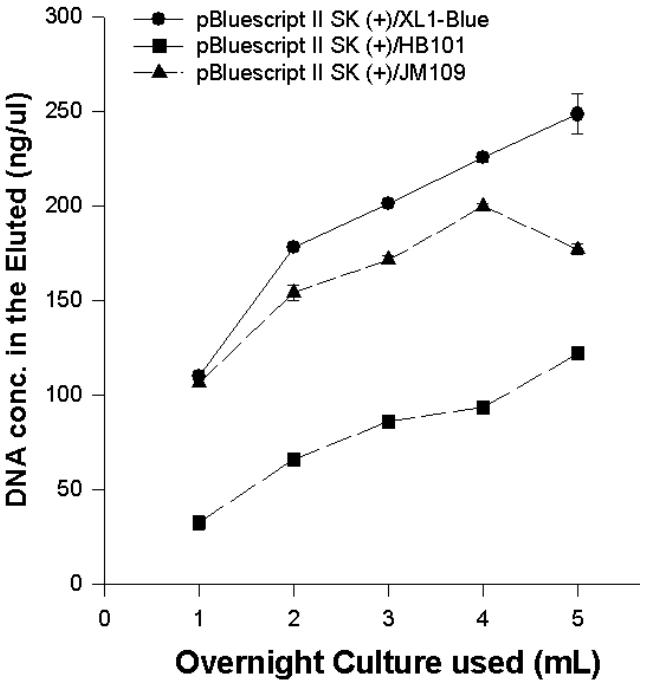

Figure 4. Preparation yield of the same plasmid with different bacterial host with different starting volume of overnight bacterial culture. It was linearly increased up to 5 ml.

5. Preparation yield of plasmid DNA

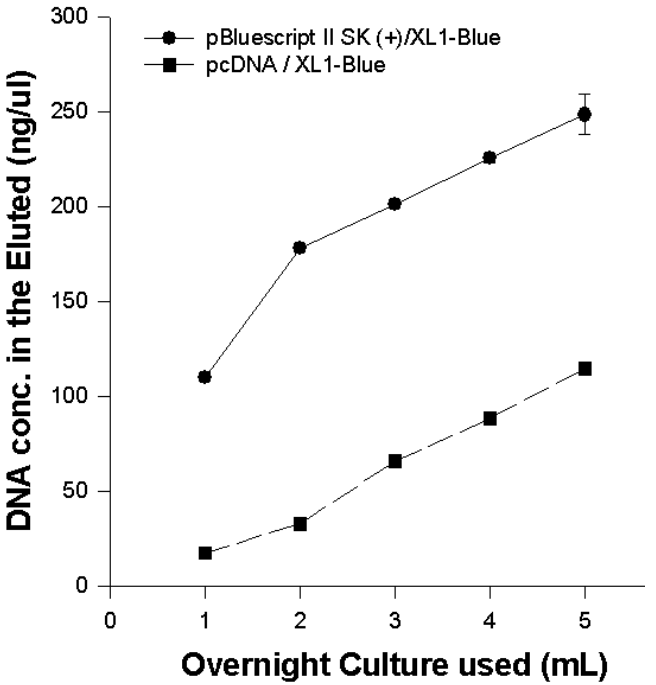

Figure 5. Preparation yield of various size of DNA with the same bacterial host. pBluescript II SK (+) (2.96 kb, Stratagene®) and pcDNA (8.6 kb, Invitrogen®) was prepared from different starting volume of overnight bacterial culture. It was linearly increased up to 5 ml.

VI. Reference

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