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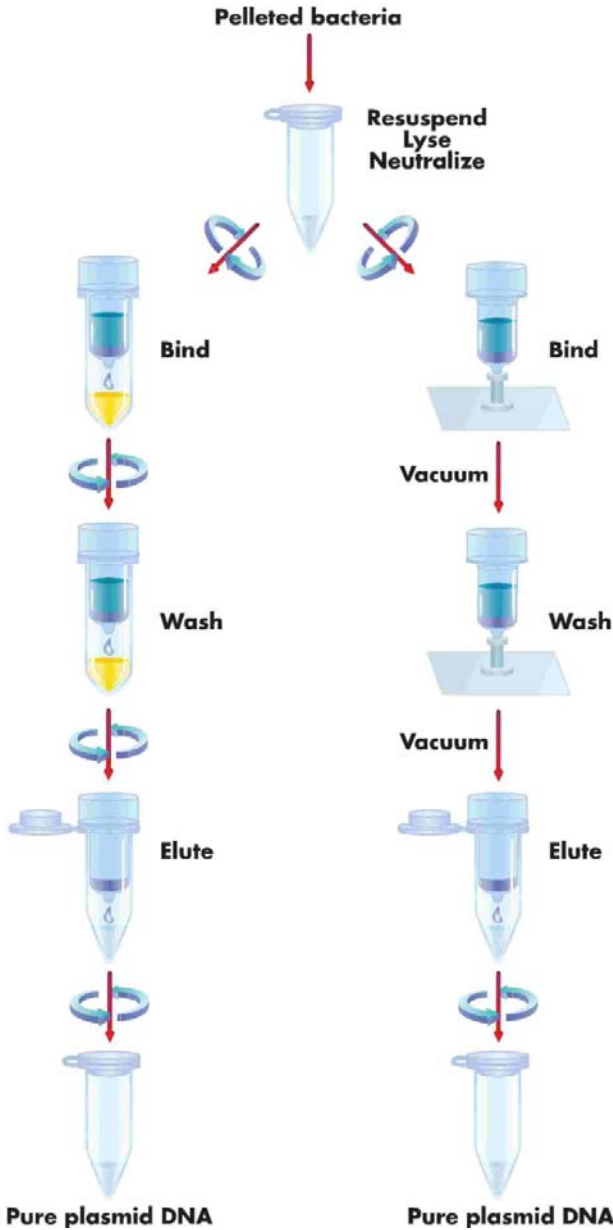
PurElute™ Miniprep Kit

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PurElute Steps

Microcentrifuge

Vacuum



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Storage and Stability Conditions

All kit components can be stored at room temperature for up to 1 year. After addition of RNase A and optional PurBlue™ Solution into EB1 Resuspension Buffer, store the mixture at 4°C. This mixture is stable at 4°C for up to six months.

Kit Components

PurElute™ Miniprep Kit	(5) 91202	(50) 91204	(250) 91206
PurElute™ Spin Columns/Collection Tubes (2 ml)	5	50	250
EB1 Resuspension Buffer	1.5 ml	20 ml	73 ml
EB2 Lysis Buffer	1.5 ml	20 ml	73 ml
EB3 Neutralization Buffer	2 ml	30 ml	140 ml
EB4 Wash Buffer I	3 ml	30 ml	150 ml
EB5 Wash Buffer II	800 µl	2 x 6 ml	55 ml
EB6 Elution Buffer	300 µl	15 ml	55 ml
RNase A Solution (10mg/ml)	15 µl	200 µl	730 µl
PurBlue™ Solution	10 µl*	20 µl	73 µl
Handbook	1	1	1

*10 µl of PurBlue™ is provided in the PurElute™ Miniprep Kit (5), transfer ONLY 2 µl of PurBlue into 1.5 ml of EB1 Resuspension Buffer.

Quality Control

DNA purified with the PurElute™ Miniprep Kit is tested for purity and quality by A_{260}/A_{280} , agarose gel electrophoresis and restriction analysis.

Introduction

The PurElute™ Miniprep Kit has been developed for rapid isolation and purification of high-copy and low-copy plasmids as well as cosmids. The kit utilizes a modified alkaline lysis protocol and can be used in a centrifuge or a vacuum manifold in less than 20 minutes processing time.

An innovative spin basket design ensures enough volume under the drip director to avoid the need for a second spin during binding.

Purified DNA can be used for most molecular biology downstream applications including PCR, cloning, transformation, DNA sequencing, and restriction analyses.

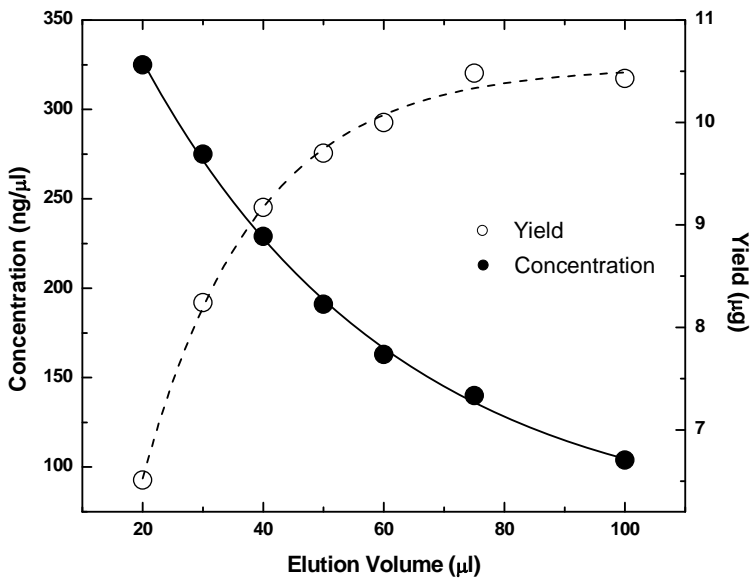
Benefits

- **High Purity DNA** - Endonuclease-Free
- **High Yield** - Up to 20 µg
- **Rapid Protocol** - Less than 20 minutes
- **Excellent Recovery** - 85-95%
- **Flexible Format** - Centrifuge or vacuum
- **User-Friendly** - Simple Spin Column Format
- **Large Plasmids** - up to 50kb
- **PurBlue™** - Visualize complete lysis for maximum DNA yield

Features of PurElute™ Miniprep Kit

Reservoir capacity	850 µl
Culture volume	1 - 10 ml
Yield (depends on plasmid size, copy number, bacterial host and growth medium)	up to 20 µg
A_{260}/A_{280}	1.81-1.99
RNA contamination (agarose gel analysis)	Not visible
Recovery Rate	85-95%

The Effect of Elution Volume on Yield and Concentration



pUC19 vector grown in EB10B *E.coli* cells was pooled and divided into 5 ml aliquots. The aliquots were purified with the PurElute™ Miniprep kit and eluted in different volumes.

Before Starting

Equipments and Materials Needed

- Microcentrifuge or Vacuum Manifold with luer adaptors (for vacuum processing)
- Microcentrifuge tubes
- Ethanol (96-100%)

Prepare EB5 Wash Buffer II

Prepare EB5 Wash Buffer II by adding appropriate volume (see bottle label) of 96-100% w/v ethanol to EB5 Wash Buffer II. Label 'ethanol added'.

Prepare RNase A/PurBlue™/EB1 Buffer Mix

- Centrifuge the RNase A and PurBlue™ vials briefly before opening.
- For the PurElute™ Miniprep Kit (5), transfer only 2 µl of PurBlue™ into the bottle of 1.5 ml of EB1 Resuspension Buffer.
- For the PurElute™ Miniprep Kit (50) and (250), transfer the entire content of PurBlue™ into the bottle of EB1 Resuspension Buffer.
- Check the "RNase A/PurBlue™ added" box on the bottle cap label. Write the date on the bottle.
- Store RNase A/PurBlue™/EB1 Buffer Mix at 4°C for up to six months.

General Information

1. If using low-copy plasmids and cosmids, culture volume can be increased to 10 ml without overloading the column. When processing 10 ml culture volume, double the volume of the EB1 Resuspension Buffer, EB2 Lysis Buffer and EB3 Neutralization Buffer.
2. If using large plasmids i.e. >10Kb, pre-heat elution buffer to 70°C for efficient elution from the spin column.

3. For troubleshooting purposes, aliquots from step 5 (cleared lysate) and 6 (aliquot from flow-through) of the centrifugation protocol can be saved for gel analysis.
4. When using the vacuum protocol, adjust flow rate to about 10-15 $\mu\text{l}/\text{second}$. A higher flow rate may lead to contamination of the eluate.

Usage Guidelines

- The PurBlue™ Solution should be added to the EB1 Resuspension Buffer at 1000x dilution i.e. 1 μl to 1 ml of EB1 Resuspension Buffer.
- PurBlue™ Solution may precipitate after addition to EB1 Resuspension Buffer. Always mix before use to re-dissolve the precipitate.
- Check EB2 Lysis and EB3 Neutralization Buffers for salt precipitation and re-dissolve by placing the bottle in a 37°C water bath for a few minutes if necessary.
- Upon addition of EB2 Lysis Buffer to the resuspended cells, the suspension mix turns blue and should be homogenous after mixing.
- After addition of EB3 Neutralization Buffer, the suspension turns colorless and neutralization is complete when the blue color completely disappears forming an evenly distributed precipitate.

Centrifugation Protocol

1. Inoculate 1 – 5 ml of LB containing the appropriate antibiotic with a single colony or 5 μ l of glycerol stock. Incubate at 37 °C while shaking at 300 rpm for approximately 16 hrs.
2. Pellet the cells of a 1 – 5 ml culture volume by centrifugation at 6,800 x g (> 8,000 rpm) in a microcentrifuge for 3 minutes at room temperature.
 - Larger culture volumes, 5 – 10 ml, can be harvested at 3,000 rpm for 10 minutes.
3. Decant supernatant and ensure that all of the liquid is removed by placing the tube face down on a paper towel.
4. Add 250 μ l of EB1 Resuspension Buffer/PurBlue™/RNase A to the cell pellet and vortex to completely resuspend the pellet leaving no clumps behind.
 - If PurBlue™ Solution is added to the resuspension buffer, always mix well before use.
 - If the vial used for harvesting the cells is larger than a microcentrifuge tube, transfer the resuspended cells into microcentrifuge tubes.
5. Set a timer for 5 minutes and add 250 μ l of EB2 Lysis Buffer to lyse the cells. Mix by turning the microcentrifuge tube upside down gently about 6 times or until the solution is clear. The solution will turn blue if PurBlue Solution is present. Do not allow the reaction to proceed for more than 5 minutes.
 - The color of the mixture will change to blue.
 - Mix gently to avoid shearing of genomic DNA.
6. Add 350 μ l of EB3 Neutralization Buffer. Mix immediately by turning the microcentrifuge tube upside down for about 6 times or up to 10 times for 5 ml or larger culture volume. The mix turns colorless if PurBlue™ is present.
 - Mix until a white precipitate without blue clumps is evenly distributed in the solution.

7. Spin the cell suspension in a microcentrifuge at 18,000 x g for 10 minutes at room temperature to pellet the precipitate.
8. Transfer the supernatant to the PurElute™ Spin Column.
9. Spin the column at 3,000 x g for 1 minute. Discard the flow-through.
10. Recommended: Add 500 µl of EB4 Wash Buffer I to the column and centrifuge at 3,000 x g for 1 minute. Discard the flow-through.
 - This wash step is necessary when using *endA*⁺ strains, low-copy plasmids and cosmids.
11. Add 750 µl of EB5 Wash Buffer II to the column and centrifuge at 3,000 x g for 1 minute. Discard the flow-through.
12. Spin the column for an additional minute at 18,000 x g to remove excess wash buffer.
13. Transfer the column into a clean microcentrifuge tube for elution. To elute DNA, add 50 µl EB6 Elution Buffer (10mM Tris-HCl, pH 8.5) or water to the center of each column, let stand for 1 minute, and centrifuge for 1 minute at 18,000 x g.
 - When using vectors that are > 10kb, pre-heat EB6 Elution Buffer or water to 70°C before eluting.

Vacuum Manifold Protocol

- 1. Follow Steps 1–7 from the Centrifugation Protocol and set up the vacuum manifold.**
- 2. Place the columns in the appropriate ports on the vacuum manifold.**
- 3. Transfer the supernatant from step 7 of the centrifugation protocol into the columns. Apply vacuum.**
 - To ensure correct adsorption and washing when using the vacuum, adjust the flow rate to 10-15 $\mu\text{l}/\text{second}$ (60-90 seconds for 850 μl).
- 4. Recommended: Add 500 μl of EB4 Wash Buffer I to the column. Apply vacuum.**
 - This wash step is necessary when using *endA*⁺ strains, low-copy plasmids and cosmids.
- 5. Add 750 μl of EB5 Wash Buffer II to the column. Apply vacuum.**
- 6. Transfer the column to a microcentrifuge and spin at 18,000 x g for 1 minute to remove excess wash buffer.**
- 7. Transfer the column into a clean microcentrifuge tube for elution. To elute DNA, add 50 μl EB6 Elution Buffer (10mM Tris-HCl, pH 8.5) or water to the center of each spin column, let stand for 1 minute, and centrifuge for 1 minute at 18,000 x g.**
 - When using vectors that are > 10kb, pre-heat EB6 Elution Buffer or water to 70°C before eluting.

Troubleshooting Guide

PROBLEM	POSSIBLE CAUSE	SUGGESTED SOLUTIONS
Poor Yield	Failure to pellet all cells	Increase centrifugation time.
	Failure to resuspend bacterial cells completely	Increase vortexing or mixing time until pellet is completely resuspended. Incomplete resuspension will result in poor cell lysis.
	Processing too many bacterial cells in culture media	Decrease culture volume if culture OD ₆₀₀ is high (OD ₆₀₀ of 3.0 is optimum, 5.0 is too high). Decrease culture volume when using richer media such as 2x YT and TB so as not to overload the column.
	Precipitates in EB2 Lysis or EB3 Neutralization buffers	Redissolve precipitates in the EB2 Lysis or EB3 Neutralization Buffers by placing the bottle in the 37°C water bath for a few minutes.
	Poor cell lysis	Make sure the EB2 Lysis buffer is fully mixed into the resuspended cells. Do not add EB3 Neutralization Buffer until the lysate is homogeneous and relatively clear. Do not exceed 5 minutes incubation time during lysis.

PROBLEM	POSSIBLE CAUSE	SUGGESTED SOLUTIONS
<p>Poor yield <i>cont...</i></p>	<p>Low-copy plasmids or cosmids</p>	<p>Use highest recommended culture volume and appropriate protocol for LB media. If using richer media such as 2x YT and TB, reduce culture volume based on culture OD₆₀₀.</p>
	<p>Antibiotics may be inactive</p>	<p>Some antibiotics are sensitive to temperature and must be stored appropriately in order to retain their activity. When inactive antibiotics are used in culture, they may lead to absence of plasmid in some daughter cells during replication. Daughter cells that do not receive plasmids will replicate faster than the ones that did and may overtake the culture.</p>
	<p>Absence of appropriate antibiotics in culture</p>	<p>In the absence of appropriate antibiotics, plasmids may not be maintained in the daughter cells during replication.</p>
	<p>No ethanol in EB5 Wash Buffer II</p>	<p>Remember to add appropriate volume of 96-100% w/v ethanol to the EB5 Wash Buffer II concentrate as stated on the bottle. Absence of ethanol in this buffer will lead to premature elution of DNA at this step.</p>

PROBLEM	POSSIBLE CAUSE	SUGGESTED SOLUTIONS
<p>Poor yield <i>cont...</i></p>	<p>Elution buffer incorrect</p>	<p>Use the EB6 Elution Buffer provided in the kit or water for elution. DNA will not be eluted from the column with a high salt buffer.</p>
	<p>No DNA in cleared lysate</p>	<p>Absence of DNA in cleared lysate could be due to plasmid not propagated in culture (absence of antibiotics in culture) or poor or no lysis of cells (check age and storage conditions of the buffers).</p> <p><i>Note:</i> DNA in cleared lysate to be analyzed should be precipitated with 0.7 volumes of isopropanol and centrifuged at about 10,000 x g for 15-30 minutes, washed with 70% ethanol and centrifuged again at the same speed for 3 minutes, Air dry the pellet and resuspend in appropriate but very small volume. Aliquot from the small volume should then be analyzed by agarose gel electrophoresis.</p>

PROBLEM	POSSIBLE CAUSE	SUGGESTED SOLUTIONS
<p style="text-align: center;">RNA contamination in eluted product</p>	<p>Addition of RNase A solution omitted</p>	<p>Check to make sure that RNase A is added to EB1 Resuspension Buffer.</p>
	<p>RNase A inactive in EB1 Resuspension Buffer</p>	<p>RNase A/PurBlue™/EB1 Resuspension Buffer mixture must be stored at 4°C for up to six months to prevent inactivation of added RNase A solution.</p>
	<p>Insufficient RNase A in resuspension buffer</p>	<p>Spin down the RNase A vial briefly to collect all the solution at the bottom of the tube before transferring tube content to the EB1 Resuspension Buffer.</p>
<p style="text-align: center;">Nicked or degraded plasmid DNA</p>	<p>Plasmid DNA nicked during handling</p>	<p>Add and mix solutions <u>gently</u> after addition of EB2 Lysis and EB3 Neutralization Buffers to prevent nicking of plasmid DNA.</p>
	<p>Lysis step was longer than 5 minutes</p>	<p>Do not allow lysis to proceed longer than 5 minutes in order to prevent irreversible denaturation of plasmid DNA.</p>

PROBLEM	POSSIBLE CAUSE	SUGGESTED SOLUTIONS
<p>Poor performance in downstream applications</p>	<p>Ethanol contamination of the eluate</p>	<p>Do not skip the extra spin step necessary for removing excess EB5 Wash Buffer II from the spin column. Remember to discard the flow-through from the previous spin step before spinning the column. This will ensure efficient excess buffer removal during spinning. Wash buffer in eluate may affect downstream applications such as sequencing reactions and restriction enzyme digestion.</p>
	<p>TE used for elution of DNA</p>	<p>EDTA may inhibit downstream applications such as sequencing reactions when present in the eluate.</p>
	<p>Use of <i>endA</i>⁺ strains</p>	<p>Perform the recommended EB4 Wash Buffer I step so as to remove residual nucleases (which may degrade plasmid over time) or carbohydrates (which may interfere with restriction enzyme digestion) that may be present in the eluate when <i>endA</i>⁺ strains are used for plasmid purification.</p>

PROBLEM	POSSIBLE CAUSE	SUGGESTED SOLUTIONS
<p>Genomic DNA in eluted product</p>	<p>Genomic DNA present in eluted product</p>	<p>Mix cell suspension <u>gently</u> after addition of EB2 Lysis and EB3 Neutralization Buffers to prevent shearing of genomic DNA which may contaminate the cell suspension. The genomic DNA may later be eluted together with the plasmid. Also do not allow lysis to proceed for more than 5 minutes.</p>
	<p>Use of overgrown culture</p>	<p>Do not grow culture for more than 12-16 hours. Overgrown culture contains lysed cell and degraded DNA which may decrease the efficiency of the purification system and may lead to contamination of the eluate with genomic DNA.</p>

Frequently Asked Questions

Growth Conditions

What is the recommended culture media?

Luria-Bertani (LB) is the recommended media. Richer growth media such as TB or 2x YT have the obvious advantage of producing more bacteria cells (up to 5 times), however this does not necessarily lead to greater yield or higher quality DNA. Excess bacteria cells can lead to inefficient lysis, inefficient neutralization and overloading of the column.

Can I use the PurElute™ kit for low-copy plasmids and cosmids?

Yes, however the yield will be lower. The culture volume may be increased to compensate for lower yield. We recommend using a 10 ml overnight culture and doubling the volumes of EB1 Resuspension buffer, EB2 Lysis buffer and EB3 Neutralization buffer used for low copy plasmids and cosmids. To maximize yield, use cells that are known to enhance plasmid and cosmid replication and use the highest recommended volume of media.

What is the maximum culture volume that can be used with the PurElute™ Miniprep kit?

The PurElute™ Miniprep kit is intended for use in the purification of DNA from 1-10 ml culture volumes – depending on the vector used.

What should the O.D. of my culture be?

The optical density of the culture - OD₆₀₀ before processing should be about 3.0, 5 is too high. If it is > 5 reduce the volume of culture processed. If using richer media such as 2x YT or TB volume of culture should also be decreased to avoid overloading the column.

What is the recipe for LB Medium?

10g Tryptone, 5g Yeast extract, 10g NaCl, diH₂O to 1 Liter.
Autoclave at 121°C for 20 minutes to sterilize.

General

How can I avoid clogging the column?

Too many cells can clog the column. Do not culture bacterial cells for more than 16 hours to prevent an overgrown culture. If the OD₆₀₀ is > 5 reduce the volume of culture processed.

How long can I store my DNA

For long-term storage, store at -20°C. Avoid repeated freezing and thawing of DNA.

What are the compositions of your buffers?

The composition of Buffer EB 1 Resuspension Buffer is 50mM Tris-HCl pH 8.0 and 10mM EDTA; EB2 Lysis Buffer is 1% SDS w/v and 200mM NaOH; EB6 is 10mM Tris-HCl pH 8.5. EB3 Neutralization Buffer is proprietary.

How long does it take to purify DNA using the PurElute™ Miniprep Kit?

The processing time depends on the number of samples. However the process is rapid, taking less than 20 minutes total time and less than 10 minutes of hands on time.

How pure is my DNA?

In general, DNA purified using PurElute™ Miniprep Kit has an A_{260}/A_{280} of >1.8. This ratio indicates that the DNA is free of proteins that could interfere with downstream applications.

What is PurBlue™ solution?

PurBlue™ solution contains a pH indicator, which allows you to visualize the alkaline lysis process. It is used as an indicator in EB 1 Buffer. After the addition of EB2 Buffer in cell resuspension, the mixture changes color to blue. After neutralization of the lysed cells with EB3 Buffer, the color of the lysate and precipitates changes to clear and white respectively.

Yield

What is the expected yield?

The yield is highly dependent on the cells, the media, the growth conditions and the plasmid used. High-copy plasmids can yield up to 20µg of DNA. Yields with low-copy plasmids are about 20-40% lower than those obtained with high-copy plasmids.

How can I improve my yield using the PurElute™ Miniprep kit?

Optimize culture conditions including media, shaker speed, and incubation time. Ensure that the cell pellet is fully resuspended before adding EB2 Lysis Buffer. Do not add EB3 Neutralization Buffer until the lysate is clear (but do not allow the lysis step to proceed for more than 5 minutes). Also avoid using excessive amounts of starting materials which can result in insufficient bacterial cell lysis and column overloading.

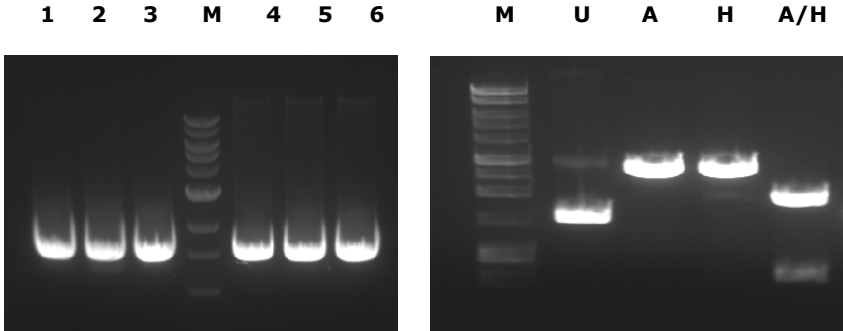
What is the total volume of the eluate?

The recommended volume for elution is 50 µl. The actual volume of the eluate obtained is about 48 µl. See chart on page 6.

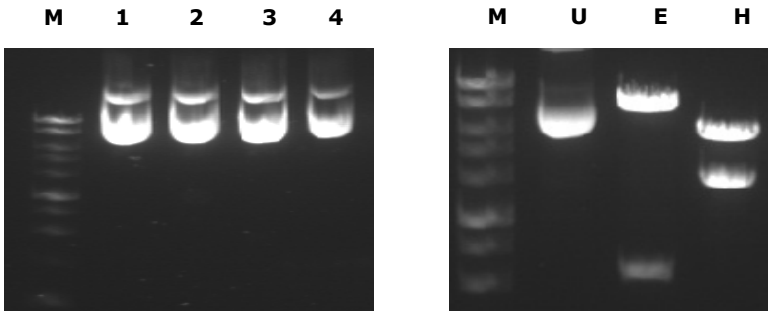
What is the recommended optimal elution volume for isolation of DNA using the PurElute™ Miniprep Kit?

An elution volume of 50 µl is recommended. Lesser volumes may result in lesser yield while larger volumes may result in a more dilute sample.

Sample Results



High-copy plasmid pUC19 (2.7Kb) purified from EB10B cells using PurElute™ Miniprep Kit (left) and Restriction Digestion with AlwNI (A) and HindIII (H). A/H is a double digestion. M is a DNA Step Ladder (Promega Corporation) and U is undigested control plasmid.



Cosmid pDO2 (9.4Kb, ATCC) purified using PurElute™ Miniprep Kit and Restriction Digestion with EcoRI (E) and HindIII (H). M is a DNA Step Ladder (Promega Corporation) and U is undigested control cosmid.

References

1. Vogelstein, B., and Gillespie, D. (1979) Preparative and analytical purification of DNA from agarose. Proc. Natl. Acad. Sci. USA **76**, 615-619.
2. Sambrook, J. et al., eds. (1989) Molecular cloning: A laboratory manual. 2nd ed., Cold Spring Harbor Laboratory Press.

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