S.N.A.P.™ UV-Free Gel Purification Kit

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Version B

082701

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Kit Contents and Storage

Shipping/Storage

The S.N.A.P.™ UV-Free Gel Purification Kit is shipped and stored at room temperature.

Kit Contents

The components of the S.N.A.P.™ UV-Free Gel Purification Kit are described in the table below. Sufficient reagents are provided to pour 25 crystal violet mini gels (50 ml) and for 25 column purifications.

Component	Composition	Amount
S.N.A.P.™ Columns	--	25
S.N.A.P.™ Collection Vials	--	25
Sodium Iodide Solution (NaI Solution)	6.6 M Sodium Iodide 16 mM Sodium Sulfite	11 ml
Binding Buffer	7 M Guanidine HCl	15 ml
4X Final Wash	400 mM NaCl	6 ml
Crystal Violet	2 mg/ml	2 x 0.5 ml
6X Crystal Violet Loading Dye	30% Glycerol 20 mM EDTA 100 µg/ml Crystal Violet	2 x 0.25 ml
TE Buffer, pH 8.0	10 mM Tris-HCl 1 mM EDTA, pH 8.0	2 ml
Sterile Water	Deionized	2 x 1 ml

Methods

Gel-Purifying DNA

Introduction

It is preferential to gel-purify large DNA fragments or long PCR products to increase the cloning efficiency of these products. However in traditional ethidium bromide agarose gel electrophoresis, exposure of DNA to UV light can damage your DNA and significantly decrease cloning efficiency. To avoid damage to large DNA fragments or long PCR products by UV light, we recommend visualizing and purifying these products by agarose gel electrophoresis using crystal violet (Rand, 1996). Crystal violet is non-mutagenic and easy to use. In addition, DNA can be visualized under normal light as a thin violet band while the gel is running and excised as soon as bands are sufficiently resolved.

The procedure below is appropriate for isolating a DNA fragment or a PCR product in a volume of approximately 100 µl. We've purified a DNA fragment or a PCR product as small as 100 bp and as large as 7 kb.

Note

Crystal violet is not as sensitive as ethidium bromide. Two hundred nanograms of DNA is just visible on an agarose gel containing crystal violet. For isolating DNA fragments or long PCR products, we recommend that you load greater than 200 ng per lane.

Important

DO NOT USE loading buffers containing bromophenol blue or xylene cyanol because the dyes will react with crystal violet and the DNA bands may become distorted. Use the 6X Crystal Violet loading dye supplied in the kit.

Molecular Weight Markers

You may use 4 µg of a lambda *Hind* III digest mixed with 6X Crystal Violet loading dye as molecular weight markers for sizing of your DNA. The visible bands are 4.4 kb, 6.5 kb, 9.4 kb, and 23 kb.

Crystal violet and sodium iodide will stain skin and fabric. Be sure to wear gloves and a laboratory coat.

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Gel-Purifying DNA, Continued

Materials Supplied by the User

You will need the following reagents and equipment for gel purification:

- Apparatus for agarose mini-gel electrophoresis with 8-lane or 12-lane comb
- General purpose agarose
- 1X TAE buffer (50 mM Tris-acetate, pH 8, 1 mM EDTA) **Note: Do not use TBE gels. Borate interferes with sodium iodide**
- Clean glass flask
- Sterile 25-30 ml bottle to prepare 1X Final Wash (see below)
- 100% ethanol
- New razor blade
- Autoclaved water or TE buffer for rinsing gel apparatus
- 42°C to 50°C water bath
- Microcentrifuge
- Sterile microcentrifuge tubes
- Fluorescent light box to visualize the DNA band (optional)

Before Starting

To prepare 1X Final Wash, transfer all of the 4X Final Wash solution (6 ml) to a sterile 25-30 ml bottle. Add 18 ml of 100% **ethanol** to the 4X Final Wash solution to prepare the 1X Final Wash solution (24 ml). Store at room temperature.

Nuclease Control

It is very important to minimize the presence of nucleases to ensure purification of high quality DNA. Please follow the guidelines listed below. While some guidelines may not appear as rigorous as others they are sufficient for purifying DNA.

- Wear gloves at all times
- Use sterile plasticware and glassware
- Autoclave TAE to use as the running buffer
- Rinse agarose gel apparatus and comb with autoclaved water or TE buffer
- Use a new razor to excise gel slice*
- Use new plastic wrap (i.e. Saran® Wrap) if needed

*The same razor may be used to excise different bands in the same gel if you are careful not to bring over pieces from an earlier excision.

Gel-Purifying DNA, Continued

Preparing the Gel

Follow the instructions below to prepare a 0.8% agarose gel. The recipe will make one agarose gel with a volume of 50 ml.

1. Mix 0.4 g general purpose agarose and 50 ml 1X TAE buffer in a clean glass flask.
2. Place flask in the microwave and heat until just boiling. Swirl gently to mix and dissolve the agarose and continue to heat in this fashion for 3 minutes to destroy nucleases.

   Caution: Vigorous swirling can cause the superheated agarose to boil out of the flask.
3. Remove from the microwave and cool for 3 minutes.
4. Add 40 µl of the 2 mg/ml Crystal Violet solution to the agarose and swirl to mix. The agarose should be light to medium lavender in color.
5. Rinse the gel box and comb with autoclaved water or TE buffer. **Note**: Use a comb that will hold 1-5 µg DNA in one well. Wells should be as small as possible to minimize the volume of the gel slice and improve visibility of smaller amounts of DNA.
6. Pour the gel and set the comb in the gel.
7. When the gel has solidified, cover the gel with 1X TAE buffer and gently remove the comb. There's no need to add crystal violet to the running buffer. Proceed to the next section to prepare your sample for loading.

Loading and Running the Gel

1. Add 8 µl of 6X Crystal Violet loading dye to 40 µl of the DNA sample and load onto the gel (The amounts are for 8 mm size well.).
2. Run the gel until the crystal violet in the gel has migrated to about a quarter of the way UP the gel (crystal violet appears to migrate towards the negative pole). You should also see the thin violet band move down into the gel. If no band is visible, insufficient DNA was loaded (< 200 ng).
3. If your DNA is sufficiently resolved so that you can easily excise the fragment, turn off the power supply. Proceed directly to **Excision of DNA,** see below.

Excising DNA

1. Pour off the buffer (or transfer the gel to new Saran® Wrap). **Note**: Placing the gel on a fluorescent light box may help to visualize the fragments.
2. Using a new razor blade, carefully excise the DNA from the gel. **Note**: Razor blade may be rinsed with autoclaved water or TE prior to cutting the next band.
3. Transfer the excised plug of agarose to a sterile 1.5 ml microcentrifuge tube.

   Note: You may cut the agarose plug into small pieces to reduce the melting time and temperature.
4. Estimate the volume of the agarose (generally this is around 100 µl). Alternatively, you can weigh the gel slice and assume that 1 mg ~ 1µl.
5. Add 2.5 times its volume of Sodium Iodide solution (i.e. 250 µl) and mix by shaking vigorously by hand or vortexing.
6. Incubate at 42 to 50°C until the agarose is **completely** melted (~5 minutes). Mix the solution periodically by vortexing.
7. Place the tube at room temperature and add 1.5 volumes of Binding Buffer (i.e., 525 µl) and mix well. Proceed directly to **Purification of DNA,** next page.

Continued on next page

S.N.A.P.™ Column Purification of DNA

Introduction

After the DNA is separated and excised from the gel, it is now ready to be purified on a S.N.A.P.™ purification column. This step further purifies the DNA away from the gel components and buffers, and produces high quality DNA.

Purifying DNA

1. Assemble a S.N.A.P.™ purification column (A) and collection vial (B) and load all of the mixture from Step 7, previous page, onto the column (875 µl).
2. Centrifuge at 2,000 to 3,000 x g in a microcentrifuge for 30 seconds at room temperature.
3. Pour the liquid in the collection vial **back onto the column** and repeat Step 2.
4. Repeat Step 3 one more time to bind all the DNA to the column (i.e. load solution onto the column for a total of 3 times).
5. After the last centrifugation, discard the liquid in the collection tube.
6. Add 400 µl of 1X Final Wash to the S.N.A.P.™ column and centrifuge as in Step 2.
7. Repeat Step 6 and discard the liquid in the collection tube after the second centrifugation (800 µl).
8. Centrifuge the column again **at maximum speed (>10,000 x g) for at least 1 minute** to dry the column resin. Discard the collection vial.
9. Transfer the column to a new, sterile 1.5 ml microcentrifuge tube.
10. Add 40 µl of sterile water or TE buffer (depending on your downstream application) directly to the column material and incubate for 1 minute at room temperature to let the water or TE buffer absorb into the column.
11. Centrifuge the column at maximum speed (>10,000 x g) for 1 minute to elute the DNA into the microcentrifuge tube.
12. Place the tube on ice and discard the column.
13. Assay 10 µl by ethidium bromide agarose gel electrophoresis to estimate the DNA concentration. Concentration should be between 2 and 40 ng/µl. In most cases, there is no need to concentrate the DNA further.

Store the DNA at -20°C or proceed directly to the next application.

Appendix

Troubleshooting

Factors Affecting Purification

Use the table below to troubleshoot gel purification of your DNA.

Problem	Solution
DNA band not visible on crystal violet gel	Load more DNA. You may have to load > 200 ng of DNA to visualize using crystal violet gel. Use a smaller well to create a dense band if you have low amounts of DNA.
	Sensitivity of the crystal violet stain may be increased by destaining the crystal violet stained gel in water until DNA bands are visible or by further staining the gel in 1-10 µg/ml crystal violet in 0.1X TAE.
	If the amount of DNA is too low to be visualized by crystal violet staining and you need to recover it, then you can restain the gel with ethidium bromide after crystal violet staining. **Note**: Exposure of DNA to UV light can damage the DNA and result in reduced cloning efficiency.
Low yield of DNA from gel purification	Be sure to melt gel slices thoroughly to ensure release of DNA.
	Be sure to use TAE to prepare the crystal violet agarose gel. TBE will interfere with sodium iodide during the isolation of DNA fragments.
	Fragment may fail to elute from the S.N.A.P.™ column. Warm TE buffer or sterile water to 37°C and try eluting again.

References

Rand, N. K. (1996). Crystal Violet Can Be Used to Visualize DNA Bands During Gel Electrophoresis and to Improve Cloning Efficiency. Elsevier Trends Journals Technical Tips Online, T40022.

Technical Service

World Wide Web

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- View and download vector maps and sequences
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- Request catalog and product literature

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http://www.invitrogen.com

...and the program will connect directly. Click on underlined text or outlined graphics to explore. Don't forget to put a bookmark at our site for easy reference!

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1. On the home page, go to the left-hand column under 'Technical Resources' and select 'MSDS Requests'.
2. Follow instructions on the page and fill out all the required fields.
3. To request additional MSDSs, click the 'Add Another' button.
4. All requests will be faxed unless another method is selected.
5. When you are finished entering information, click the 'Submit' button. Your MSDS will be sent within 24 hours.

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Technical Service, Continued

Emergency Information

In the event of an emergency, customers of Invitrogen can call the 3E Company, 24 hours a day, 7 days a week for disposal or spill information. The 3E Company can also connect the customer with poison control or with the University of California at San Diego Medical Center doctors.

3E Company
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Product Qualification

S.N.A.P.™ Columns

Each lot of S.N.A.P.™ columns is qualified as follows:

Ten columns from each lot are randomly selected and tested as described below.

Binding Capacity: 20 µg of pre-purified control plasmid is bound to the column and eluted. Yield of eluted DNA is determined by the OD_{260} and should be > 10 µg.

Visual Inspection: Five samples of plasmid DNA isolated from a bacterial culture using the S.N.A.P.™ protocol were visualized on a 0.8% agarose gel. Only supercoiled plasmid should be present with no contaminating RNA.

Restriction Digest: Five 500 ng plasmid DNA samples are each digested with 4 units of *Apa* I for 90 minutes. When analyzed by agarose gel electrophoresis, the digest should be 99% complete.

Endonuclease Activity: Five 500 ng of purified plasmid DNA samples are incubated with 10 mM Mg^{2+} for 4 hours and analyzed on a 0.8% agarose gel. No degradation should be observed.

Crystal Violet Solution and 6X Loading Dye

The Crystal Violet solution and the 6X Crystal Violet loading dye must allow visualization of at least 200 ng of linear DNA fragment on a 0.8% agarose gel when used according to the standard protocol (see page 3).

S.N.A.P.™ UV-Free Gel Purification Kit

To functionally test the S.N.A.P.™ UV-Free Gel Purification Kit, a PCR product (we have tested PCR products in the range of 0.5-7 kb) was purified from a 0.8% agarose gel using the protocol on page 3. The purified PCR product was analyzed on a second 0.8% agarose gel to confirm recovery. No degradation should be observed.