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Instruction Manual

CloneMiner™ cDNA Library Construction Kit Web Appendix

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Overview

Introduction

The CloneMiner™ cDNA Library Construction Kit Web Appendix is intended for use with the CloneMiner™ cDNA Library Construction Kit. Use this kit to construct high-quality cDNA libraries without the use of traditional restriction enzyme cloning methods.

You may size fractionate your *att*B-adapted cDNA by column chromatography or gel electrophoresis. Protocols are provided in this manual to size fractionate radiolabeled and non-radiolabeled cDNA by gel electrophoresis. You should have the following steps completed prior to using the protocols provided in this Web Appendix:

- First strand synthesis
- Second strand synthesis
- *att*B1 Adapter ligation

Gel Electrophoresis

Perform the gel electrophoresis method to increase the average insert size of your cDNA or to select cDNA of a particular size. Note that other size fractionation protocols are suitable. Refer to the CloneMiner™ cDNA Library Construction Kit manual for protocols to size fractionate cDNA by column chromatography. This manual is provided with the CloneMiner™ cDNA Library Construction Kit and is also available from our Web site (www.invitrogen.com) or by contacting Technical Service (page 15).

Starting mRNA

To successfully construct a cDNA library, it is crucial to start with high-quality mRNA. For guidelines on isolating mRNA, refer to the CloneMiner™ cDNA Library Construction Kit manual. The amount of mRNA needed to prepare a library depends on the efficiency of each step. Generally, 1 to 5 µg of mRNA will be sufficient to construct a cDNA library containing 10^6 to 10^7 primary clones in *E coli*. If you will be selecting for large cDNA size inserts (>4 kb), we recommend starting with a minimum of 2 µg of mRNA.

Note

This Web Appendix only contains protocols for size fractionating your cDNA by gel electrophoresis. Refer to the CloneMiner™ cDNA Library Construction Kit manual for detailed protocols on performing the first and second strand synthesis, ligating the *att*B1 Adapter, performing the BP recombination reaction, and transforming competent cells.

Purchaser Notification

The CloneMiner™ cDNA Library Construction Kit Web Appendix is intended for use with the CloneMiner™ cDNA Library Construction Kit. This kit is covered by multiple Limited Use Label Licenses. By use of this kit, you accept the terms and conditions of the Limited Use Label Licenses outlined in the CloneMiner™ cDNA Library Construction Kit manual.

Advance Preparation

Introduction

You will need to supply many of the reagents and materials required to perform size fractionation by gel electrophoresis. Refer to the list below to help you prepare or acquire these materials in advance.

Note

Refer to the section entitled **Before Starting** at the beginning of each procedure for a complete list of required reagents.

Materials Required

You should have the following materials on hand before performing size fractionation by gel electrophoresis:

- Phenol:chloroform:isoamyl alcohol (25:24:1)
- 7.5 M NH_4OAc (ammonium acetate)
- 100% ethanol
- 70% ethanol
- TE buffer (10 mM Tris-HCl, pH 8.0; 1 mM EDTA)
- Gel electrophoresis apparatus and reagents
- Low melting agarose (Invitrogen, Catalog no. 15517-014)
- 1 Kb Plus DNA Ladder, recommended (Invitrogen, Catalog no. 12302-011). Other DNA ladders are suitable.
- Gel phenol, prepared in advance (see page 14 for a recipe)
- 5 M NaCl
- Scintillation vials (for radiolabeled cDNA only)
- Scintillation counter (for radiolabeled cDNA only)

Size Fractionating Radiolabeled cDNA by Gel Electrophoresis

Introduction

This section provides guidelines on size fractionating your *att*B-flanked cDNA by gel electrophoresis. To use this procedure, you will need to have performed the protocols outlined in the sections entitled **Synthesizing the First Strand, Synthesizing the Second Strand, and Ligating the *att*B1 Adapter** of the CloneMiner™ cDNA Library Construction Kit manual.

Use extreme caution when working with radioactive material. Follow all federal and state regulations regarding radiation safety. For general guidelines when working with radioactive material, refer to the CloneMiner™ cDNA Library Construction Kit manual.

Before Starting

You should have the following materials on hand before beginning:

Supplied with kit:

- UltraPure™ Glycogen (20 µg/µl)

Supplied by user:

- *att*B-flanked cDNA (from the *att*B1 Adapter ligation reaction)
- Thermocycler (recommended) or water bath, heated to 70°C
- Phenol:chloroform:isoamyl alcohol (25:24:1)
- 7.5 M NH_4OAc (ammonium acetate)
- 100% ethanol
- Dry ice or a -80°C freezer
- 70% ethanol
- TE buffer (10 mM Tris-HCl, pH 8.0; 1 mM EDTA)
- Scintillation vials
- Scintillation counter
- Gel electrophoresis apparatus and reagents
- Low melting agarose (Invitrogen, Catalog no. 15517-014)
- 1 Kb Plus DNA Ladder, recommended (Invitrogen, Catalog no. 12302-011). Other DNA ladders are suitable.
- Water bath, heated to 65°C
- Gel phenol, prepared in advance (see page 14 for a recipe)
- Water bath, heated to 37°C
- 5 M NaCl

Stopping the Ligation Reaction

1. Incubate the tube containing the cDNA adaptor ligation reaction (from the section entitled **Ligating the *att*B1 Adapter** of the CloneMiner™ cDNA Library Construction Kit manual) at 70°C for 10 minutes to inactivate the ligase.
2. Place the tube on ice.

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Size Fractionating Radiolabeled cDNA by Gel Electrophoresis, continued

Phenol/Chloroform Extraction

1. To the 50 µl cDNA adaptor ligation reaction, add 50 µl of phenol:chloroform:isoamyl alcohol (25:24:1) and shake by hand thoroughly for approximately 30 seconds.
2. Centrifuge at room temperature for 5 minutes at 14,000 rpm. Carefully remove the upper aqueous phase to a fresh 1.5 ml tube.
3. Proceed to **Ethanol Precipitation**, below.

Ethanol Precipitation

1. To the aqueous phase, add the reagents in the following order:

Glycogen (20 µg/µl)	1 µl
7.5 M NH_4OAc	25 µl
100% ethanol	190 µl

   Note: You may stop at this point and store the tube at -20°C overnight if necessary.
2. Place the tube in dry ice or at -80°C for 10 minutes. Centrifuge the sample at +4°C for 25 minutes at 14,000 rpm.
3. Carefully remove the supernatant while trying not to disturb the cDNA pellet. Add 150 µl of 70% ethanol.

   Note: Use a Geiger counter to monitor the supernatant for the presence of radioactivity. The majority of the radioactivity should be found in the pellet and not in the supernatant.
4. Centrifuge the sample at +4°C for 2 minutes at 14,000 rpm. Carefully remove the supernatant. Repeat the 70% ethanol wash. Remove as much of the remaining ethanol as possible.
5. Dry the cDNA pellet in a SpeedVac® for 2-3 minutes or at room temperature for 5-10 minutes.
6. Resuspend the pellet in 15 µl of TE buffer by pipetting up and down 30-40 times. Transfer the sample to a fresh tube.

   Note: Use a Geiger counter to make sure you have resuspended and transferred all of the cDNA pellet. The majority of the radioactivity should be found in the fresh tube and not in the old tube.

Preparing the cDNA for Electrophoresis

Follow the recommendations listed below to ensure proper cDNA separation:

- Use a well size of approximately 0.25 x 1.2 cm.
- Load 1 lane per µg of starting mRNA. For example, if you started with 2 µg of mRNA, divide your cDNA sample and run over two lanes. Do not load more than 300 ng of cDNA (based on the first strand cDNA yield) per lane.
- If you have more than 300 ng of cDNA (based on the first strand cDNA yield), split the sample and run in multiple wells. You may need to add additional TE buffer or loading dye to run a minimum volume of 10 µl per well.

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Size Fractionating Radiolabeled cDNA by Gel Electrophoresis, continued

Fractionating the cDNA

1. Prepare a 1% low melting agarose/1X TBE-buffered gel. We recommend using a low ethidium bromide concentration (~140 ng/ml) to preserve the integrity of the cDNA.
2. Load your cDNA sample according to the guidelines outlined on the previous page along with a 1 kb DNA standard. Electrophorese samples at 60-70 volts for 3-5 hours. If you wish to electrophorese your samples overnight, run them at 60 volts for 15-20 minutes followed by 20 volts overnight.
3. Visualize the gel under UV light. Using the DNA standard as a reference, mark the gel at the desired size. Work quickly to minimize UV exposure.
4. Using a sterile razor blade or scalpel, cut the gel from the mark all the way to the top of the well or to the desired range of insert size.
5. Cube the gel slice and place approximately 0.5 ml of gel pieces into 1.5 ml microcentrifuge tubes. Depending on the size of your gel slice, you may have up to 10-15 tubes. Centrifuge tubes at 14,000 rpm for 20 seconds. Proceed to **Gel Phenol Extraction**, below.

Gel Phenol Extraction

1. Melt gel pieces by incubating tubes at 65°C for 10 minutes. At the same time, incubate the 50 ml tube of gel phenol at 37°C for 10 minutes.
2. Transfer the tubes containing the gel pieces to 37°C and incubate for 6 minutes.
3. Working quickly, add 0.5 volume (~250 µl) of gel phenol to each tube of melted agarose. We recommend removing no more than 5 tubes from the 37°C water bath at a time to ensure that the agarose does not solidify.
4. Shake tubes vigorously by hand for 20-30 seconds. Do not vortex. Centrifuge at room temperature for 5 minutes at 14,000 rpm.
5. Remove the top aqueous phase (approximately ~0.6 ml) and transfer to a fresh tube. Be careful to avoid the flocculent interface between the two phases. It is normal for the top aqueous phase to become cloudy due to the presence of residual phenol.

   Note: You may back extract by adding 250ul of TE buffer to the remaining gel phenol. Repeat Step 4. Remove the top aqueous phase and add to the existing aqueous phase.
6. Add a 1X volume of gel phenol to the aqueous phase and repeat Step 4.
7. Remove the top aqueous phase and transfer to a fresh tube. Distribute the aqueous phase among the tubes so that each tube contains 450 µl. Proceed to **Ethanol Precipitation**, next page.

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Size Fractionating Radiolabeled cDNA by Gel Electrophoresis, continued

Ethanol Precipitation

1. Add the following reagents to each 450 µl aliquot and mix by inverting the tubes. The cloudy aqueous phase will become clear after addition of ethanol. Note that you will use 5 M NaCl in this ethanol precipitation procedure to minimize the final volume in each tube.

Glycogen (20 µg/µl)	1 µl
5 M NaCl	9 µl
100% ethanol	1.15 ml

Note: You may stop at this point and store the tubes at -20°C overnight if necessary.

1. Place the tubes in dry ice or at -80°C for 10 minutes. Centrifuge the samples at +4°C for 25 minutes at 14,000 rpm.
2. Carefully remove the supernatant while trying not to disturb the cDNA pellets. Add 150 µl of 70%.

   Note: Use a Geiger counter to monitor the supernatant for the presence of radioactivity. The majority of the radioactivity should be in the pellet and not in the supernatant.
3. Centrifuge the sample at +4°C for 2 minutes at 14,000 rpm. Carefully remove the supernatant. Repeat the 70% ethanol wash. Remove as much of the remaining ethanol as possible.
4. Dry the cDNA pellets in a SpeedVac® for 2-3 minutes or at room temperature for 5-10 minutes.

Resuspending the cDNA

1. You now have multiple cDNA pellets for one cDNA library. Resuspend one cDNA pellet in 5 µl of TE buffer by pipetting up and down 30-40 times.
2. Transfer the 5 µl suspension to the next cDNA pellet and resuspend by pipetting up and down 30-40 times. Repeat until all pellets for one library are resuspended. Transfer the 5 µl suspension to a fresh tube.
3. Using a fresh 5 µl aliquot of TE buffer, repeat the resuspension steps from above to collect any remaining cDNA from the tubes. Add this 5 µl suspension to the 5 µl suspension from Step 2. **The entire cDNA for one library should now be resuspended in a total volume of 10 µl.**

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Size Fractionating Radiolabeled cDNA by Gel Electrophoresis, continued

Calculating the Double Strand cDNA Yield

1. Place the capped tube containing the resuspended cDNA from Step 3, previous page directly into a scintillation vial. Do not add scintillation fluid. Obtain Cerenkov counts.
2. Determine the cDNA yield. Use the specific activity (SA) determined from the first strand reaction sample and the equation below to calculate the yield of double-stranded cDNA.

$$\text{Amount of ds cDNA (ng)} = \frac{(\text{Cerenkov cpm}) \times 2 \times (4\ \text{pmol dNTP/pmol dCTP}) \times (1{,}000\ \text{ng/}\mu\text{g ds cDNA})}{\text{SA (cpm/pmol dCTP)} \times (1{,}515\ \text{pmol dNTP/}\mu\text{g ds cDNA})}$$

$$= \frac{(\text{Cerenkov cpm}) \times 8}{\text{SA} \times (1.515)}$$

What You Should See

You should have a final cDNA yield of approximately 30-40 ng to perform the BP recombination reaction. Using 30-40 ng of cDNA in the BP reaction should produce a library containing 5-10 million clones.

Note: If you are selecting for large cDNA size inserts (>4 kb), we recommend using 60-80 ng of cDNA in the BP recombination.

The Next Step

Proceed to the section entitled **Performing the BP Recombination Reaction with Radiolabeled cDNA** in the CloneMiner™ cDNA Library Construction Kit manual.

Size Fractionating Non-Radiolabeled cDNA by Gel Electrophoresis

Introduction

This section provides guidelines on size fractionating your *att*B-flanked cDNA by gel electrophoresis. To use this procedure, you will need to have performed the protocols outlined in the sections entitled **Synthesizing the First Strand, Synthesizing the Second Strand, and Ligating the *att*B1 Adapter** of the CloneMiner™ cDNA Library Construction Kit manual.

Because your cDNA is not labeled with [α-^{32}P]dCTP, you will need to estimate your cDNA yields using a plate spotting assay. You will be performing this assay throughout the size fractionation procedure. We recommend that you read the section entitled **Performing the Plate Spotting Assay** in the CloneMiner™ cDNA Library Construction Kit manual before size fractionating your cDNA.

Before Starting

You should have the following materials on hand before beginning:

Supplied with kit:

- UltraPure™ Glycogen (20 µg/µl)

Supplied by user:

- *att*B-flanked cDNA (from the *att*B1 Adapter ligation reaction)
- Thermocycler (recommended) or water bath, heated to 70°C
- Phenol:chloroform:isoamyl alcohol (25:24:1)
- 7.5 M NH_4OAc (ammonium acetate)
- 100% ethanol
- Dry ice or a -80°C freezer
- 70% ethanol
- TE buffer (10 mM Tris-HCl, pH 8.0; 1 mM EDTA)
- Gel electrophoresis apparatus and reagents
- Low melting agarose (Invitrogen, Catalog no. 15517-014)
- 1 Kb Plus DNA Ladder, recommended (Invitrogen, Catalog no. 12302-011). Other DNA ladders are suitable.
- Water bath, heated to 65°C
- Gel phenol, prepared in advance (see page 14 for recipe)
- Water bath, heated to 37°C
- 5 M NaCl

Stopping the Ligation Reaction

1. Incubate the tube containing the cDNA adaptor ligation reaction (from the section entitled **Ligating the *att*B1 Adapter** of the CloneMiner™ cDNA Library Construction Kit manual) at 70°C for 10 minutes to inactivate the ligase.
2. Place the tube on ice.

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Size Fractionating Non-Radiolabeled cDNA by Gel Electrophoresis, continued

Phenol/Chloroform Extraction

1. To the 50 µl cDNA adaptor ligation reaction, add 50 µl of phenol:chloroform:isoamyl alcohol (25:24:1) and shake by hand thoroughly for approximately 30 seconds.
2. Centrifuge at room temperature for 5 minutes at 14,000 rpm. Carefully remove the upper aqueous phase to a fresh 1.5 ml tube.
3. Proceed to **Ethanol Precipitation**, below.

Ethanol Precipitation

1. To the aqueous phase, add the reagents in the following order:

Glycogen (20 µg/µl)	1 µl
7.5 M NH_4OAc	25 µl
100% ethanol	190 µl

   Note: You may stop at this point and store the tube at -20°C overnight if necessary.
2. Place the tube in dry ice or at -80°C for 10 minutes. Centrifuge the sample at +4°C for 25 minutes at 14,000 rpm.
3. Carefully remove the supernatant while trying not to disturb the cDNA pellet. Add 150 µl of 70% ethanol.
4. Centrifuge the sample at +4°C for 2 minutes at 14,000 rpm. Carefully remove the supernatant. Repeat the 70% ethanol wash. Remove as much of the remaining ethanol as possible.
5. Dry the cDNA pellet in a SpeedVac® for 2-3 minutes or at room temperature for 5-10 minutes.
6. Resuspend the pellet in 15 µl of TE buffer by pipetting up and down 30-40 times. Transfer the sample to a fresh tube.

Preparing the cDNA for Electrophoresis

Follow the recommendations listed below to ensure proper cDNA size separation:

- Use a well size of approximately 0.25 x 1.2 cm.
- Load 1 lane per µg of starting mRNA. For example, if you started with 2 µg of mRNA, divide your cDNA sample and run over two lanes.
- Add additional TE buffer and loading dye, if necessary, to run a minimum volume of 10 µl per well.

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Size Fractionating Non-Radiolabeled cDNA by Gel Electrophoresis, continued

Fractionating the cDNA

1. Prepare a 1% low melting agarose/1X TBE-buffered gel. We recommend using a low ethidium bromide concentration (~140 ng/ml) to preserve the integrity of the cDNA.
2. Load your cDNA sample according to the guidelines outlined on the previous page along with a 1 kb DNA standard. Electrophorese samples at 60-70 volts for 3-5 hours. If you wish to electrophorese your samples overnight, run them at 60 volts for 15-20 minutes followed by 20 volts overnight.
3. Visualize the gel under UV light. Using the DNA standard as a reference, mark the gel at the desired size. Work quickly to minimize UV exposure.
4. Using a sterile razor blade or scalpel, cut the gel from the mark all the way to the top of the well or to the desired range of insert size.
5. Cube the gel slice and place approximately 0.5 ml of gel pieces into 1.5 ml microcentrifuge tubes. Depending on the size of your gel slice, you may have up to 10-15 tubes. Centrifuge tubes at 14,000 rpm for 20 seconds. Proceed to **Gel Phenol Extraction**, below.

Gel Phenol Extraction

1. Melt gel pieces by incubating tubes at 65°C for 10 minutes. At the same time, incubate the 50 ml tube of gel phenol at 37°C for 10 minutes.
2. Transfer the tubes containing the gel pieces to 37°C and incubate for 6 minutes.
3. Working quickly, add 0.5 volume (~250 µl) of gel phenol to each tube of melted agarose. We recommend removing no more than 5 tubes from the 37°C water bath at a time to ensure that the agarose does not solidify.
4. Shake tubes vigorously by hand for 20-30 seconds. Do not vortex. Centrifuge at room temperature for 5 minutes at 14,000 rpm.
5. Remove the top aqueous phase (approximately ~0.6 ml) and transfer to a fresh tube. Be careful to avoid the flocculent interface between the two phases. It is normal for the top aqueous phase to become cloudy due to the presence of residual phenol.

   Note: You may back extract by adding 250ul of TE buffer to the remaining gel phenol. Repeat Step 4. Remove the top aqueous phase and add to the existing aqueous phase.
6. Add a 1X volume of gel phenol to the aqueous phase and repeat Step 4.
7. Remove the top aqueous phase and transfer to a fresh tube. Distribute the aqueous phase among the tubes so that each tube contains 450 µl. Proceed to **Ethanol Precipitation**, next page.

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Size Fractionating Non-Radiolabeled cDNA by Gel Electrophoresis, continued

Ethanol Precipitation

1. Add the following reagents to each 450 µl aliquot and mix by inverting the tubes. The cloudy aqueous phase will become clear after addition of ethanol. Note that you will use 5 M NaCl in this ethanol precipitation procedure to minimize the final volume in each tube.

Glycogen (20 µg/µl)	1 µl
5 M NaCl	9 µl
100% ethanol	1.15 ml

   Note: You may stop at this point and store the tubes at -20°C overnight if necessary.
2. Place the tubes in dry ice or at -80°C for 10 minutes. Centrifuge the samples at +4°C for 25 minutes at 14,000 rpm.
3. Carefully remove the supernatant while trying not to disturb the cDNA pellets. Add 150 µl of 70%.

   Note: Use a Geiger counter to monitor the supernatant for the presence of radioactivity. The majority of the radioactivity should be in the pellet and not in the supernatant.
4. Centrifuge the sample at +4°C for 2 minutes at 14,000 rpm. Carefully remove the supernatant. Repeat the 70% ethanol wash. Remove as much of the remaining ethanol as possible.
5. Dry the cDNA pellets in a SpeedVac® for 2-3 minutes or at room temperature for 5-10 minutes.

Resuspending the cDNA

1. You now have multiple cDNA pellets for one cDNA library. Resuspend one cDNA pellet in 5 µl of TE buffer by pipetting up and down 30-40 times.
2. Transfer the 5 µl suspension to the next cDNA pellet and resuspend by pipetting up and down 30-40 times. Repeat until all pellets for one library are resuspended. Transfer the 5 µl suspension to a fresh tube.
3. Using a fresh 5 µl aliquot of TE buffer, repeat the resuspension steps from above to collect any remaining cDNA from the tubes. Add this 5 µl suspension to the 5 µl suspension from Step 2. **The entire cDNA for one library should now be resuspended in a total volume of 10 µl.**

Preparing Aliquots for the Plate Spotting Assay

1. Remove 1 µl of your 10 µl cDNA sample and add to 9 µl of TE buffer to make a 1:10 dilution.
2. Remove 5 µl of the 1:10 dilution and add to 5 µl of TE buffer to make a 1:20 dilution.
3. Proceed **to Estimating the cDNA Yield**, next page.

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Size Fractionating Non-Radiolabeled cDNA by Gel Electrophoresis, continued

Estimating the cDNA Yield

You will be estimating the concentration and yield of your cDNA sample using the plate spotting assay. Refer to the section entitled **Performing the Plate Spotting Assay** in the CloneMiner™ cDNA Library Construction Kit manual for detailed guidelines on preparing the plates and staining the DNA.

1. Using the **DNA Spotting Assay** protocol, spot 1 µl of your 1:10 dilution and 1 µl of your 1:20 dilution onto a prewarmed plate.
2. Estimate the cDNA concentration of the diluted sample. Multiply this concentration by the dilution factor to get the cDNA concentration of your size fractionated cDNA.
3. Determine the final cDNA yield by multiplying the cDNA concentration by the total volume in the tube.
4. You may need to prepare additional dilutions of your samples for the plate spotting assay if your spots appear saturated (see Important Note in the section entitled **Performing the Plate Spotting Assay**).

What You Should See

You should have a final cDNA yield of approximately 75-100 ng to perform the BP recombination reaction. Using approximately 75-100 ng of cDNA in the BP reaction should produce a library containing 5-10 million clones.

Note: If you are selecting for large cDNA size inserts (>4 kb), we recommend using 150-200 ng of cDNA in the BP recombination.

Note

If you have previously performed the BP recombination reaction using radiolabeled cDNA, note that the amount of non-radiolabeled cDNA required for the BP recombination reaction is greater (see page 7). This larger amount is due to the difference in scale between quantifying DNA by radioactivity using a scintillation counter and quantifying DNA by the plate spotting assay using the DNA standard. Thus, 30 ng of cDNA as measured by counts is roughly equivalent to 50-100 ng of cDNA as measured by comparison to the DNA standard.

The Next Step

Proceed to the section entitled **Performing the BP Recombination Reaction with Non-Radiolabeled cDNA** in the CloneMiner™ cDNA Library Construction Kit manual.

Troubleshooting

Introduction

The following table lists some potential problems and possible solutions that may help you troubleshoot various steps during the size fractionation procedure.

Problem	Cause	Solution
Gel phenol is cloudy	Phenol is not clean	Use a different lot of phenol.
	50 ml plastic tube is not phenol-resistant	Use phenol-resistant plastic tubes or glass.
	Improper storage	Make sure to protect gel phenol from light. Store at +4ºC for up to 6 months.
Poor cDNA recovery	Incorrect agarose used	Make sure to use low melting point agarose.
	Too much gel placed in tube	Place no more than 0.5 ml of a gel piece in each tube.
	Gel re-solidified during gel phenol extraction	Work quickly during this procedure to minimize re-solidification. We recommend removing no more than 5 tubes at a time from the 37ºC water bath.

Recipes

Gel Phenol

Phenol, 500 ml (Invitrogen, Catalog no. 15509-037)
Sterile water
5 M NaCl
1 M Tris-HCl, pH 8.0

Prepare the gel phenol one day before you intend to use it. If you observe any flocculence or an interface, discard the gel phenol and prepare a new stock.

1. Loosen the cap on the phenol bottle. Place the bottle of phenol in a 65ºC water bath under a fume hood to dissolve phenol crystals. This step will take at least 1 hour.
2. Tighten the cap and mix by turning the bottle upside down to ensure all the phenol crystals have dissolved.
3. To a 50 ml tube, add the following reagents:

Sterile water	15 ml
5 M NaCl	100 µl
1 M Tris-HCl, pH 8.0	400 µl
Phenol (dissolved)	25 ml

4. Mix solution vigorously by inverting the 50 ml tube several times. Cover tube with foil and store at +4ºC for up to 6 months.

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...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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Technical Service, continued

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