In This Section

Tips for Increasing DNA Recovery Efficiency from Agarose Gels

Introduction

This section discusses various tips which will increase the efficiency of recovery of DNA from agarose gels. These tips and recommendations can be applied to all recovery techniques.

The section is divided into the following topics:

— Choosing the appropriate agarose

— Choosing the appropriate electrophoresis buffer

— Gel casting and DNA loading tips

— Staining and recovery tips

Choosing the appropriate agarose for recovery

When recovering DNA, the choice of agarose is one of the most important factors. To avoid recovery altogether, one can choose to perform in-gel reactions.

Lonza offers Genetic Technology Grade™ (GTG®) Products that are specially prepared for demanding molecular biology applications. Lonza's GTG® Product quality tests go beyond standard nuclease assays to include enzymatic performance measurements. Our additional testing provides a more realistic index of overall product quality and reliability. You no longer need to screen agarose lots to find those which yield biologically active DNA.

Lonza agaroses and compatible recovery techniques

Cat. No.	SeaKem® GTG® Agarose	SeaPlaque® GTG® Agarose®	NuSieve® GTG® Agarose	MetaPhor® Agarose	SeaPlaque® Agarose
In-Gel		■	■		
β-Agarase		■	■		■
Phenol/ Chloroform	■	■	■	■	■
Recovery Columns	■	■	■	■	■
Electroelution	■	■	■	■	■
Freeze/ Squeeze	■	■	■	■	■

Buffer types

When recovering DNA from agarose gels, 1X Tris-acetate (TAE) Buffer is recommended for electrophoresis.

Casting and DNA loading tips

— Prepare the gel in 1X TAE Buffer

— Do not cast the gel with ethidium bromide

— Cast a gel 3 mm - 4 mm thick

— Use a combs ≤1 mm thick

— Load no more than 100 ng of DNA per band

Staining and recovery tips

When recovering DNA from agarose gels, we recommend the following:

— Stain the gel for 15 - 20 minutes

— Destain the gel in distilled water for two, 20-minute washes

— Do not expose the DNA to UV light for any longer than 1 minute; long exposure of DNA to UV light can nick the DNA

— The addition of 1 mM guanosine or cytidine to the gel and electrophoresis buffer is effective in protecting DNA against UV-induced damage

— Cut the smallest gel slice possible

It is possible to avoid staining samples which will be used for recovery by running an additional lane containing a small amount of your sample immediately next to the molecular weight marker. However, DNA is damaged by UV light in the absence of ethidium bromide so keep exposure to UV light as brief as possible. Cut the lanes containing the marker and the small amount of the sample from the rest of the gel and stain. To recover the preparative loading, line up the stained portion of the gel with the unstained portion. Check by placing on UV transilluminator and cut out the area that lines up with your sample on the unstained portion of the gel.

The FlashGel® System for Recovery, (page 20) offers a non-UV alternative for DNA recovery.

Reference

Grundemann, D. and Schomig, E., *BioTechniques* **21(5):** 898 - 903, 1996.

Introduction

ß-Agarase is an enzyme that digests the polysaccharide backbone of agarose to alcohol-soluble oligosaccharides. DNA in a low melting temperature agarose gel can be recovered after the gel is melted and digested with this enzyme. The remaining oligosaccharides will not gel or interfere with subsequent DNA manipulations such as cloning, labeling, restriction digestion or sequencing. Lonza's ß-Agarase is free of any detectable DNase, RNase and phosphatase activities.

ß-Agarase recovery is particularly useful for recovering large DNA (>10 kb) which could be sheared by other methods of recovery.

Compatible agaroses

- SeaPlaque® GTG® Agarose (certified and tested for the recovery of DNA)
- NuSieve® GTG® Agarose (certified and tested for the recovery of DNA)
- SeaPlaque® Agarose

Tips

- Transfer no more than 200 mg of the agarose gel to a microcentrifuge tube for equilibration with digestion buffer
- Completely melt the gel slice prior to the addition of enzyme
- Ethanol precipitations should be incubated at room temperature with ammonium acetate rather than sodium acetate to decrease the likelihood of co-precipitation of agarose-oligosaccharides with the DNA
- Polynucleotide kinase is inhibited by ≥7 mM ammonium ion; use sodium acetate for your precipitation if you will be phosphorylating your DNA after recovery
- The enzyme preparation retains full activity for several hours at 45°C; however, it will gradually lose activity upon longer incubations; for this reason, incubate at 40°C for overnight digestions
- For efficient recovery of small nucleic acids (<500 bp) and/or very dilute samples (<0.05 µg/ml), we recommend either carrier tRNA or nuclease-free glycogen be added; in addition, overnight precipitation at room temperature can be helpful
- The enzyme preparation has maximum activity between pH 6 - 7 and is relatively unaffected by salt concentrations between 0.1 M and 0.25 M; the equilibration of the gel slice with ß-Agarase Buffer is necessary to provide the enzyme with optimal buffer conditions; this equilibration is more important for gels prepared in TBE than in TAE Buffer due to the greater buffering capacity of TBE Buffer
- Ethanol precipitate the DNA at room temperature or on ice. The most effective DNA precipitation can be achieved at 0°C to 22°C, rather than at -20°C to -70°C. At higher temperatures, yields are more consistent and precipitation of oligosaccharides will be avoided

ß-Agarase unit definition

- One unit of Lonza ß-Agarase will completely digest 200 mg of a molten 1% SeaPlaque® GTG® Agarose gel which has been dialyzed in 1X ß-Agarase Buffer in 1 hour at 40°C; similar activities are obtained for other low melting temperature agaroses such as NuSieve® GTG® Agarose.
- Concentration: 1 unit per µl

Procedure for ß-Agarase digestion

BEFORE YOU BEGIN: Set one water bath or heating block for 70°C. Set another water bath or heating block for 45°C.

1. Electrophorese DNA in a low melting temperature agarose gel (such as SeaPlaque®, SeaPlaque® GTG®, MetaPhor® or NuSieve® GTG® Agarose) which has been prepared in TAE or TBE Buffer.
2. Briefly stain the gel with GelStar® or SYBR® Green I Nucleic Acid Stain or ethidium bromide.
3. Photograph the gel.
4. Excise a gel slice containing the DNA of interest.
5. Place the gel slice containing the DNA sample into a microcentrifuge tube.
6. Equilibrate the gel slice with 10 volumes of 1X ß-Agarase Buffer for 60 minutes at room temperature OR follow steps 6A - 6D.
   - 6a. Melt the gel slice at 70°C for 15 minutes. Make sure that the gel slice is completely melted as the enzyme preparation will not digest agarose in the gelled state.
   - 6b. Add 2 µl of the 50X ß-Agarase buffer to approximately 100 µl of melted gel solution.
   - 6c. Mix the solution.
   - 6d. Proceed to steps 9, 10 and 11.
7. Discard the buffer.
8. Melt the gel slice at 70°C (approximately 15 minutes). Make sure the gel slice is completely melted as the enzyme preparation will not digest agarose in the gelled state.
9. Cool the melted agarose solution to 45°C.
10. Add 1 unit of ß-Agarase for 200 mg (approximately 200 µl) of 1% agarose gel (add proportionally more or less of the enzyme preparation for larger or smaller gel slices, or with higher or lower agarose concentrations, respectively). **Skip to step 12.**
11. If you have followed the protocol in steps 6A - 6D, the amount of enzyme will need to be adjusted.

If the gel was prepared with . . .	Then you will need to add . . .
TAE Buffer	Twice the amount of enzyme
TBE Buffer	Seven times the amount of enzyme

12. Mix.
13. Incubate at 45°C for 60 minutes.
14. If the DNA is ≤30 kb...
    Place the solution at -20°C for 15 minutes. Centrifuge the solution for 15 minutes at 4°C.
    Continue with Step 15
    OR
    The sample can be concentrated without ethanol precipitation by centrifugation in a molecular weight-cut-off-spin column (e.g., Amicon®'s Microcon™ Micro-concentrator).
    If the DNA is >30 kb...
    The DNA/ß-Agarase solution may be used directly without precipitation for subsequent enzymatic manipulations.
    OR
    The sample can be concentrated without ethanol precipitation by centrifugation in a molecular weight-cut-off-spin column (e.g., Amicon's Microcon or Centricon™ Microconcentrators).
15. Transfer the supernatant to a new microcentrifuge tube leaving behind any undigested agarose in the pellet.
16. Add ammonium acetate to the supernatant to a final concentration of 2.5 M.
17. Add 2 to 3 volumes of 100% ethanol.
18. If the DNA is ≤0.05 µg/ml. . .
    Precipitate at room temperature for 24 hours
    OR
    Add 10 µg of RNA.
    If the DNA is >0.05 µg/ml. . .
    Precipitate at room temperature for 30 minutes.
19. Collect the precipitate by centrifugation.

Caution: Materials and methods shown here present hazards to the user and the environment. Refer to the safety information on page 210 before beginning these procedures.

ß-Agarase Recovery of DNA from Agarose Gels — continued

- Materials
  - Microcentrifuge tubes (≥1 ml)
  - Two water baths or heating blocks
  - Scalpel or razor blade
  - Microcentrifuge
- Reagents
  - SeaPlaque®, SeaPlaque® GTG®, NuSieve® GTG® or MetaPhor® Agarose
  - GelStar® or SYBR® Green I Nucleic Acid Gel Stain or ethidium bromide
  - ß-Agarase buffer
  - Supplied as a 50X concentrate; 1X concentration is: 40 mM Bis Tris/HCl 40 mM NaCl, 1 mM EDTA (pH 6.0)
  - ß-Agarase Enzyme
  - 10 M ammonium acetate
  - 100% ethanol

Caution: Materials and methods shown here present hazards to the user and the environment. Refer to the safety information on page 210 before beginning these procedures.

References

Chong, S. and Garcia, G.A., *BioTechniques* **17**: 719 - 725, 1994.
Crouse, J. and Amorese, D., *GIBCO-BRL Focus* **9**: 3 - 5, 1987.
Lamb, B.T., *et al., Nature Genetics* **5**: 22 - 29, 1993.
Morrice, L.M., *et al., Can. J. Microbiol.* **3**: 987 - 933, 1983.
Richardson, C.C., *The Enzymes,* 3rd Edition, Academic Press, 1981.
Zeugin, J.A. and Hartley, J.L., *GIBCO-BRL Focus* **7(4)**: 1 - 2, 1985.

Introduction

Electroelution is a reliable and consistent recovery method. The recovered DNA is suitable for a wide range of applications. For small fragments, a typical yield would be 50 - 85%, but as fragment length increases, yield can drop as low as 20%. The procedure below describes the electroelution of DNA into dialysis bags and has been adapted from Sambrook, *et al*. Alternatively, if you have a commercially available apparatus, follow the manufacturer's instructions.

Compatible agaroses

- SeaKem® GTG® Agarose (certified and tested for the recovery of DNA)
- SeaPlaque® GTG® Agarose (certified and tested for the recovery of DNA)
- NuSieve® GTG® Agarose (certified and tested for the recovery of DNA)
- SeaPlaque® Agarose
- NuSieve® 3:1 Agarose
- MetaPhor® Agarose

Tips

- Electrophorese DNA in 1X TAE Buffer
- Have 1 µg of DNA in your band of interest
- Minimize exposure of DNA to UV light for no more than 1 minute
- Cut the smallest gel slice possible

- Materials
  - Scalpel or razor blade
  - Spatula
  - Dialysis tubing
  - Dialysis tubing clips
  - Electrophoresis chamber
  - Hand-held, long-wavelength ultraviolet lamp
  - Disposable plastic tube
  - Pasteur pipette
- Reagents
  - 1X TAE Buffer
  - 2% w/v sodium bicarbonate, 1 mM EDTA pH 8.0, prepare in distilled water
  - 1mM EDTA, pH 8.0
  - Distilled water
  - GelStar® or SYBR® Green I Nucleic Acid Gel Stain or ethidium bromide

Caution: Materials and methods shown here present hazards to the user and the environment. Refer to the safety information on page 210 before beginning these procedures.

Procedure for preparing dialysis tubing

1. Cut the tubing into 10 cm to 20 cm long pieces.
2. Boil for 10 minutes in a large volume of 2% sodium bicarbonate/1 mM EDTA, pH 8.0.
3. Rinse the tubing with distilled water.
4. Boil for 10 minutes in 1 mM EDTA, pH 8.0.
5. Cool the tubing.
6. Store at 4°C submerged in 1 mM EDTA, pH 8.0.

Procedure for electroeluting DNA from agarose gels

1. Electrophorese DNA in an agarose gel.
2. Stain the gel with GelStar® or SYBR® Green I Nucleic Acid Stain or ethidium bromide.
3. Locate the band of interest using a UV light source. Minimize exposure of DNA to UV light to less than 1 minute.
4. Excise the band of interest using a scalpel or razor blade.
5. Wet a spatula with 1X TAE Buffer.
6. Place the agarose slice containing the DNA on a wetted spatula or a scalpel.
7. Photograph the gel for a record of which band was eluted.
8. Seal one end of treated dialysis tubing with a dialysis clip.
9. Fill the bag to the top with 1X TAE Buffer.
10. Transfer the slice of agarose into the bag with the spatula.
11. Allow the slice of agarose to sink to the bottom of the bag.
12. Remove the buffer from the dialysis bag, leaving just enough to keep the gel slice in constant contact with the buffer.
13. Clip the dialysis bag above the gel slice, avoiding air bubbles.
14. Place a shallow layer of 1X TAE Buffer in an electrophoresis chamber.
15. Immerse the bag in the electrophoresis chamber.
16. Pass electric current through the bag (4 - 5 V/cm for 2 - 3 hours). During this time, the DNA is eluted out of the gel and onto the inner wall of the dialysis tubing. This process can be monitored with a hand-held, long-wavelength UV lamp. Expose to UV light briefly.
17. Reverse the polarity of the current for 1 minute. This will remove the DNA from the wall of the bag.
18. Recover the bag from the electrophoresis chamber.
19. Gently massage the side of the bag with gloved fingers where the DNA has accumulated. This will remove the DNA from the wall of the bag. This process can be monitored with a hand-held UV lamp. Expose to UV light briefly.
20. Open the dialysis bag.
21. Transfer all of the buffer surrounding the gel slice to a plastic tube.
22. Wash out the bag with a small amount of 1X TAE Buffer.
23. Transfer the solution to the plastic tube.
24. Remove the gel slice from the bag.
25. **OPTIONAL:** Stain the slice with GelStar® or SYBR® Green I Nucleic Acid Stain or ethidium bromide to ensure all the DNA has been eluted from the slice.
26. Purify the DNA from 1X TAE Solution using phenol/chloroform extractions.*

*After electroelution, it is recommended that the DNA is further purified with a phenol/chloroform extraction followed by ethanol precipitation. Oligosaccharides and other contaminants (found in low-grade agarose) can copurify with the DNA. Phenol extractions will remove any oligosaccharides, avoiding their coprecipitation during ethanol precipitations.

References

Bostian, K.A., *et al., Anal. Biochem.* **95**: 174 - 182, 1979.
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Girvitz, S.D., *et al., Anal. Biochem.* **106**: 492 - 496, 1980.
Henrich, B., *et al., J. Biochem. Biophys. Methods* **6**: 149 - 157, 1982.
Ho, N.W.Y., *Electrophoresis* **4**: 168 - 170, 1983.
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Compatible agaroses

- SeaPlaque® GTG® Agarose (certified and tested for the recovery of DNA)
- NuSieve® GTG® Agarose (certified and tested for the recovery of DNA)
- SeaPlaque® Agarose

Tips

Recovery failures when extracting DNA from agarose using phenol/chloroform most often result from either extracting too large a piece of agarose, or precipitating agarose along with the DNA at the ethanol precipitation step. To address these difficulties, we recommend the following:

— No more than 200 mg (200 µl) of agarose should be extracted in a single tube; if your gel slice containing the DNA is larger than this, separate it into smaller pieces, then combine the extracted solutions prior to ethanol precipitation

— Ethanol precipitation of agarose can be avoided by chilling the extracted solution on ice for 15 minutes, then centrifuging the sample(s) in a cold room for 15 minutes at maximum speed in a microcentrifuge prior to adding salts and ethanol. The supernatant is then carefully decanted, and the DNA in the supernatant is precipitated following standard protocols

— Not useful for large DNA (>10kb). Vortexing will shear the DNA

Procedure

This protocol has been adapted from Sambrook, *et al.*

1. Electrophorese DNA in a low melting temperature agarose gel prepared in 1X TAE Buffer.
2. Set a water bath for 67°C.
3. Prewarm the TE at 67°C.
4. Excise the gel fragment containing the DNA.
5. Place the DNA slice in a microcentrifuge tube.
6. Estimate the volume of the slice. If the slice is significantly greater than 200 mg, break the agarose slice into smaller pieces and place each agarose piece in a separate microcentrifuge tube.
7. Melt the gel slice at 67°C for 10 minutes.
8. Add the appropriate volume of TE Buffer (prewarmed to 67°C) so that the final concentration of agarose is 0.5%.
9. Maintain the samples at 67°C until you are ready to phenol extract.
10. Add an equal volume of buffer-equilibrated phenol.
11. All subsequent steps can be done at room temperature.
12. Vortex for 15 seconds.
13. Centrifuge at maximum speed in a microcentrifuge for 3 minutes.
14. Carefully remove the top aqueous phase. The interface of white debris is the agarose, which can contain some trapped DNA. This can be back-extracted with TE to maximize yield.
15. Place aqueous phase in a clean microcentrifuge tube.
16. Repeat steps 10 - 15.
17. Add an equal volume of phenol/chloroform to the aqueous phase.
18. Vortex for 15 seconds.
19. Centrifuge at maximum speed in a microcentrifuge for 3 minutes.
20. Remove the aqueous phase and place in a clean microcentrifuge tube.
21. Repeat the extraction with an equal volume of chloroform.
22. Remove the aqueous phase and place in a clean microcentrifuge tube.
23. Chill the aqueous phase for 15 minutes on ice.
24. Centrifuge in a microcentrifuge at high speed for 15 minutes at 4°C.
25. Carefully decant the supernatant into a clean micro-centrifuge tube.
26. Ethanol precipitate the DNA in the supernatant following standard protocols.

Section VI: **Recovery of DNA from Agarose Gels**

Phenol/Chloroform Extraction of DNA from Agarose Gels — Continued

Materials

- Water bath set to 67°C
- Microcentrifuge tubes (≥1 ml)
- Ice bucket and ice
- Vortex mixer
- Microcentrifuge
- Scalpel or razor blade

Reagents

- SeaPlaque® GTG®, NuSieve® GTG® or SeaPlaque® Agarose
- TE Buffer warmed to 67°C
- Buffer-equilibrated penol
- Chloroform
- Phenol/chloroform (1:1)

Caution: Materials and methods shown here present hazards to the user and the environment. Refer to the safety information on page 210 before beginning these procedures.

References

Benson, S.A., *BioTechniques* **2**: 66 - 67, 1984.
Sambrook, J., *et al., Molecular Cloning: A Laboratory Manual,* 2nd Edition, Cold Spring Harbor Laboratory Press, 1989.

Section VI: **Recovery of DNA from Agarose Gels**

"Modified Freeze/Squeeze" Extraction of DNA from Agarose Gels

Introduction

This protocol is relatively fast and will work with low and standard melting temperature agaroses. However, like many recovery techniques, the DNA yield decreases with increasing DNA size, but should be approximately 50% for DNA 5 kb.

Compatible agaroses

- SeaKem® GTG® Agarose (certified and tested for the recovery of DNA)
- SeaPlaque® GTG® Agarose (certified and tested for the recovery of DNA)
- NuSieve® GTG® Agarose (certified and tested for the recovery of DNA)
- SeaPlaque® Agarose
- MetaPhor® Agarose
- NuSieve® 3:1 Agarose
- SeaKem® Gold Agarose

Procedure

This protocol has been adapted from Benson. For more detailed protocols on phenol chloroform extractions, refer to the previous section.

1. Electrophorese DNA in an agarose gel prepared in 1X TAE Buffer.
2. Excise gel slice containing DNA.
3. Place in a microcentrifuge tube.
4. Estimate volume based on weight.
5. Mash the gel slice with the stir rod.
6. Add an equal volume of buffer-equilibrated phenol.
7. Vortex for 10 seconds.
8. Freeze at -70°C for 5 to 15 minutes.
9. Centrifuge for 15 minutes at room temperature.
10. Remove the supernatant which contains the DNA.
11. Place the supernatant, in a clean microcentrifuge tube.
12. Phenol/chloroform extract the supernatant.
13. Follow with a chloroform extraction.
14. Ethanol precipitate following standard procedures.

Materials

- Microcentrifuge tubes (≥1 ml)
- Ice bucket and ice
- Vortex mixer
- Microcentrifuge
- Scalpel or razor blade
- Glass or plastic stir rod

Reagents

- TE Buffer warmed to 67°C
- Buffer equilibrated phenol
- Chloroform
- Phenol/chloroform (1:1)

References

Benson, S.A., *BioTechniques* **2**: 66 - 67, 1984.
Polman, J.K. and Larkin, J.M., *Biotechnology Techniques* **3**: 329 - 332, 1989.
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Ethanol Precipitation of DNA Recovered from Agarose Gels

Introduction

This method is compatible with all the recovery techniques listed in this chapter.

Tips

— Prior to adding salts and ethanol, precipitation of agarose can be avoided by chilling the supernatant on ice for 15 minutes, then centrifuging the sample(s) in a cold room for 15 minutes at maximum speed in a microcentrifuge. The supernatant is then carefully decanted, and the DNA in the supernatant is ethanol precipitated following standard protocols

— Ethanol precipitations should be incubated at room temperature with ammonium acetate rather than sodium acetate in order to decrease the likelihood of coprecipitation of agarose-oligosaccharides with the DNA or RNA

Procedure

This protocol has been adapted from Sambrook, *et al*.

1. Chill the supernatant on ice for 15 minutes.
2. Centrifuge the sample(s) in a cold room for 15 minutes at maximum speed in a microcentrifuge.
3. Carefully decant the supernatant.
4. Place in a clean microcentrifuge tube.
5. Measure the volume of the sample.
6. Add 0.2 volumes of 10 M ammonium acetate to the sample.
7. Add 2 volumes of 100% ice-cold ethanol.
8. Briefly vortex.
9. Store the mixture for 30 minutes to overnight at room temperature.
10. Centrifuge for 30 minutes at 12,000 rpm.
11. Decant the supernatant.
12. Wash the pellet three times with 70% ethanol.
13. Allow to air-dry at room temperature on the bench top.
14. Dry under vacuum for 5 - 10 minutes.
15. Dissolve the DNA in TE Buffer.

- Materials
  - Ice bucket and ice
  - Vortex mixer
  - Microcentrifuge
  - Vacuum gel dryer
  - Microcentrifuge tubes (≥1 ml)
- Reagents
  - 100% ethanol (ice cold)
  - 70% ethanol (room temperature)
  - TE Buffer, pH 8.0
  - 10 M ammonium acetate

References

Life Technologies, Inc., *GIBCO-BRL Focus* **7(4)**: 1 - 2, 1985.
Sambrook, J., *et al., Molecular Cloning: A Laboratory Manual,* 2nd Edition, Cold Spring Harbor Laboratory Press, 1989.

References for other recovery methods

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References for Recovery using glass beads
Ausubel, F.M., *et al., Current Protocols in Molecular Biology,* Red Book, Wiley & Sons, 1995.
Chen, C.W. and Thomas, C.A., *Anal. Biochem.* **101**: 339 - 341, 1980.
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