



In This Section

Amplification of Plasmid cDNA Libraries with SeaPrep® Agarose	150
Preparing Agarose for use in Cell Culture Applications	152
References	154

Section IX: Special Applications in Agarose Gels

Amplification of Plasmid cDNA Libraries with SeaPrep® Agarose

Introduction

This section describes an amplification technique developed to address the common problem of disproportionate amplification of plasmids seen when expression cloning mRNAs of very low abundance encoding cytokines, receptors, and cell surface molecules from plasmid cDNA libraries derived from highly complex tissue sources. This amplification technique allows amplification of a plasmid cDNA library in a representative fashion, decreasing the possibility that less abundant clones would vanish during the amplification due to differential rates of replication.

The technique below describes a method where bacterial transformants are suspended, not plated, in low gelling temperature agarose. With this method, it has been found that, from generation to generation of amplification, the relative abundance of bacterial cells containing plasmids encoding selected hematopoietic factors varies less than two fold.

Preparing the bacterial growth media plus agarose

NOTE: Alternatively, prepared solutions of L Broth, LB Broth or Super Broth can also be used.

1. In a 2 liter Erlenmeyer flask, add the following reagents to 1 liter of double distilled water:
 - 1a. 20.0 g bacto-tryptone
 - 1b. 10.0 g bacto-yeast extract
 - 1c. 10.0 g NaCl
2. Measure pH of solution.
3. If necessary, adjust to pH 7.0 using 1 N NaOH or 1 N HCl.
4. Add 3.0 g of SeaPrep® Agarose to the solution.
5. Autoclave the solution on a liquid cycle for 15 - 20 minutes.
6. Cool the solution to 40°C.
7. Store in a 37°C water bath until ready-for-use.
8. Prior to use add 2 ml of 50 mg/ml ampicillin.

cdNA/vector annealing reactions

1. Prepare vector using a tailing method.
2. Prepare and purify cDNA following standard procedures.
3. Optimize the tailing of cDNA inserts following standard procedures.
4. Scale up the appropriate cDNA/vector-annealing reaction to generate up to 2.5×10^6 cfu/2000 ml.
5. Perform the appropriate number of standard bacterial transformations following standard procedures.
6. Pool all the transformations after the 37°C incubation.

NOTE: General procedures for vector and cDNA preparation, ligation and transformation can be found in Sambrook, J., *et al.* and Kriegler, M.

■ Materials

- Autoclave
- 2 L Erlenmeyer flasks
- Water bath set to 37°C
- pH meter
- Sterile 50 ml polypropylene centrifuge tubes
- Ice water bath
- Sterile 500 ml centrifuge bottle
- Centrifuge
- Sterile Nunc® Tubes
- 0.2 µm filter

■ Reagents

- SeaPrep® Agarose
- 2X L Broth (LB)
- 50 mg/ml ampicillin
- Double distilled water
- Bacto-tryptone
- Bacto-yeast extract
- NaCl
- 1 N NaOH or 1 N HCl
- pH standards
- LB-amp plates
- Filter sterilized glycerol

Section IX: Special Applications in Agarose Gels

Amplification of Plasmid cDNA Libraries with SeaPrep® Agarose — continued

Plasmid cDNA library amplification

1. Add ampicillin to a final concentration of 100 µg/ml (2.0 ml of 50 mg/ml stock solution) to the 2X LB-agarose solution.
2. Add transformants (1.25 x 10⁶ cfu) to the LB-agarose solution.
3. Gently swirl to avoid foaming.
4. Aliquot the transformation mix into 25 ml aliquots in 50 ml polypropylene centrifuge tubes.
5. Place the tubes in an ice-water bath for 20 - 60 minutes to allow the agarose gel to set.
6. Incubate overnight at 37°C undisturbed.
7. Plate 100 µl of the cell-agarose suspension directly on LB-amp plates for titer determination or prepare the suspension for library storage.

Library Storage

1. Prepare 12.5% glycerol by diluting in double distilled water.
2. Filter solution through a 0.2 µm filter into a sterile container.
3. Pour the colony-containing gel into a sterile 500 ml centrifuge bottle.
4. Pellet the cells at 8,000 rpm for 20 minutes at room temperature. **It is not necessary to melt the gel, the cells will pellet through the soft agarose.**
5. Decant the media from the cell pellets.
6. Resuspend the cell pellets in 100 ml of 12.5% glycerol in 2X L broth.
7. Aliquot into sterile Nunc® tubes.
8. Store library at -70°C.

■ Materials

- Scalpel or razor blade
- GelBond® Film
- Glass plate or press board
- Clamps or elastic bands
- A forced hot-air oven

■ Reagents

- SeaKem® LE or GTG® Agarose
- SeaKem® Gold Agarose
- NuSieve® 3:1 or NuSieve® GTG® Agarose
- MetaPhor® Agarose

References

Kriegler, M., *Gene Transfer and Expression: A Laboratory Manual*, Stockton Press, 1990.
Perez, C. and Kriegler, M., *Resolutions*, 6(4): 1 - 2, 1990.
Ausubel, F.M., et al., *Current Protocols in Molecular Biology*, Wiley & Sons, 1995.
Sambrook, J., et al., *Molecular Cloning: A Laboratory Manual*, 2nd Edition, Cold Spring Harbor

Section IX: Special Applications in Agarose Gels

Preparing Agarose for use in Cell Culture Applications

Introduction

Agarose has unique properties that make it especially useful in cell culture media applications. Agarose can form stable gels at concentrations as low as 0.3% (w/v), has very low ionic concentrations and is free of contaminating impurities that may affect the growth characteristics of cells in culture. Agarose could be used directly without supplementation for plaque assays ensuring high cell variability. The use of agarose is suggested for media where the absence of a nutrient is mandatory.

Advantages

- Highly purified, eliminating contaminants
- Excellent optical transparency which enhances colony observation
- Gelling temperatures below 28°C allowing manipulation of cells in solution
- Can be used to produce a 'defined' media system

Applications

- Cell culture media
 - Mammalian
 - Plant
 - Bacterial
 - Viral
- Overlay assays
- Plaque hybridization assays
- Soft agarose hybridoma cloning
- Attachment dependent cell culture
- Colony lift assays
- Analysis of matrix and cell matrix interactions

The concentration and type of agarose to use largely depends on your cell system, the application, and the lot specific gel strength of the agarose. There are three types of agarose which can be used for cell culture media, each having unique properties making one more suitable for a given application than another.

- **SeaKem® LE Agarose** – A standard melting and gelling temperature agarose which can be used as a solid medium to support cell growth by supplementation with growth factors and nutrients, top agar enriched with magnesium for baculovirus screening, substrate for bacterial growth and for colony lifts
- **SeaPlaque® Agarose** – A low gelling temperature agarose (28°C) that remains a liquid at 37°C allowing the manipulation of cells within the solution; SeaPlaque® Agarose can be used as a semi-solid media for anchorage independent assays, plaque assays or overlays; SeaPlaque® Agarose has also been found to be very effective as a medium for protoplast culture
- **SeaPrep® Agarose** – Unique ultra-low gelling temperature (15°C) and gel strength agarose (>75 g/cm²); SeaPrep® Agarose is ideal for hybridoma cloning; Cells can be recovered from the gel by increasing the temperature slightly, allowing transfer to a viable cell suspension for subsequent growth in liquid medium

Section IX: Special Applications in Agarose Gels

Preparing Agarose for use in Cell Culture Applications — continued

Suggested Agarose Products and Concentration Guidelines

The table below provides general guidelines on agaroses and gel concentrations for given applications. General guidelines and specific information pertaining to a given cell type and application can be obtained from the current literature and *Cell Biology: A Laboratory Manual*.

Agarose Preparation

When preparing agarose for cell culture work, it is always best to prepare the agarose in water suitable for cell culture and separate from any growth media or nutrients. Agarose solutions and media solutions should be prepared at 2X concentrations (i.e., if desired final agarose concentration is 0.6%, prepare a 1.2% agarose solution), autoclaved separately, and aliquoted into useable aliquots.

Application	SeaKem® LE Agarose	SeaPlaque® Agarose	SeaPrep® Agarose
Mammalian cell culture	0.3% - 1%	0.25% - 1.5%	
Baculovirus screening		0.3% - 0.6%	
Bacterial culture	0.7% - 1.0%	1.0%	
Colony lifts	0.7% - 1.0%	1.0%	
Anchorage independent assays	0.25% - 0.5%		
Plaque assays	0.3% - 1%	0.50% - 1.5%	
Overlays	0.3% - 0.6%	0.3% - 1%	
Protoplast culture		0.35% - 0.7%	
Hybridoma cloning			0.8% - 1.5%
Virus enumeration			1%
Cell matrix interactions		1.0% - 3.0%	
Attachment dependent cell culture		0.6%	
<i>In vitro</i> cloning – mammalian	0.5% - 2%		1% - 4%

NOTES: Higher agarose concentration gels can affect and possibly restrict cell proliferation. Optimal gel concentrations should be determined for each culture system on a case-by-case basis. In some cases, such as hybridoma cloning using SeaPrep® Agarose, it is advisable to sample different lots of agarose for the desirable gel strength qualities.

Section IX: Special Applications in Agarose Gels

Preparing the Agarose for Use in Cell Culture Applications — continued

Procedure for Autoclaving Agarose

1. Choose a flask that is 2 - 4 times the volume of the solution.
2. Add water to the flask.
3. Sprinkle in the pre-measured agarose powder at a 2X final agarose concentration.
4. Cover the flask with aluminum foil.
5. Place the flask in the autoclave.
6. Sterilize the agarose by autoclaving for 10 minutes at 15 lb/in². If using SeaPrep® Agarose, autoclave for no longer than 5 minutes.

NOTE: Agarose may lose gel strength when exposed to longer periods of time in the autoclave.

7. Once the agarose solution has cooled, aliquot into useable aliquots and store at 4°C prior to use.

■ Materials

- Flask that is 2-4 times the volume of the solution
- Autoclave
- Aluminum foil
- Sterile flasks or culture tubes

■ Reagents

- Agarose powder
- Water suitable for cell culture

General Procedure for Using Agarose in Culture Medium

1. Remelt the agarose by placing in a hot water bath or microwave.
2. Allow the agarose solution to cool to 37°C.
3. Prewarm the 2X media solution to 37°C.
4. Mix equal volumes of the sterile 2X agarose solution with sterile 2X media containing growth factors and nutrients.
5. Cast the agarose/media solution into plates or sterile culture tubes.
6. Allow the agarose solution to gel for 20 minutes if using as a feeder or overlay or maintain the solution at 37°C if using as a liquid culture.

NOTE: A solution containing 1% SeaPlaque® Agarose will stay liquid for approximately 18 hours. The amount of time an agarose solution will stay in a liquid state at 37°C, largely depends on the agarose concentration (increased agarose concentrations will decrease the time the solution stays in a liquid state), the age of the agarose and the particular lot of agarose you are using. When purchasing a new lot of agarose, we recommend you test this prior to culturing cells.

■ Materials

- Microwave or hot water bath

■ Reagents

- Gelled agarose solution
- 2X media solution

References

- Borden, E.C., et al., *Appl. Microbiol.* **20**: 289 - 291, 1970.
 Prescott, D.M., *Methods in Cell Biology*, 1st edition, Academic Press, 1975.
 Saunders, F.J.K. and Smith, J.D., *Nature* **227**: 513 - 515, 1970.
 Mack, D.O., et al., *BioTechniques*, **17**(5): 860 - 863, 1994.
 Buschmann, M.D., et al., *J. of Orthopaedic Research*, **10**: 745 - 758, 1992.
 Calba, H., et al., *Plant and Soil* **178**: 67 - 74, 1996.
 Shantze, K.A., et al., *Cancer Research* **45**: 4416 - 4421, 1985.
 Celis, J.E., *Cell Biology: A Laboratory Manual*, 2nd edition, Academic Press, 1998.
 Bagasra, O., et al., *Cancer Immunol Immunother*, **20**: 55 - 60, 1985.
 Civin, C.I., et al., *J. Immunol. Methods*, **61**: 1 - 8, 1983.
 Buschmann, M.D., et al., *J. of Cell Science*, **108**: 1497 - 1508, 1995.