METHODS BETA

Electrophoresis

The separation of nucleic acids based upon their size is required for many common laboratory practices (e.g., subcloning, genotype diagnostics, RT-PCR). Separation of nucleic acids by agarose gel electrophoresis works by harnessing the negative charge of the phosphate backbone of nucleic acids. DNA and RNA molecules have a net negative charge spread evenly over their entire length so they will move through an agarose matrix in an electric field toward the positive pole. Shorter nucleic acids will be able to migrate through the matrix faster than larger ones during a given period of time. Depending upon the percentage of agarose used to make the gel, the range of linear separation will vary.

From: Methods in Enzymology, 2013

Related techniques:

Plasmid, Nested Gene, Immunofluorescence, DNA Template

Protocols

From: Chapter Four - Agarose Gel Electrophoresis

Laura Koontz

Methods in Enzymology Volume 529 • 2013 • Pages 35-45

4.1. Preparation

Prepare the running buffer you wish to use.

4.2. Duration

Preparation: About 2 h.

Protocol: About 1 h.

4.3. Caution

Ethidium bromide is a known mutagen and carcinogen. It, and all other nucleic acid intercalators, should be handled with care. Wear gloves. Do not microwave any buffer containing ethidium bromide or any other dye. The dyes can vaporize and be inhaled. Instead, wait until the buffer has cooled slightly and then add the dye. Wear gloves when handling the gel and solutions containing ethidium bromide. Dispose of gel and buffer in accordance with local regulations.

See Fig. 4.1 for the flowchart of the complete protocol.

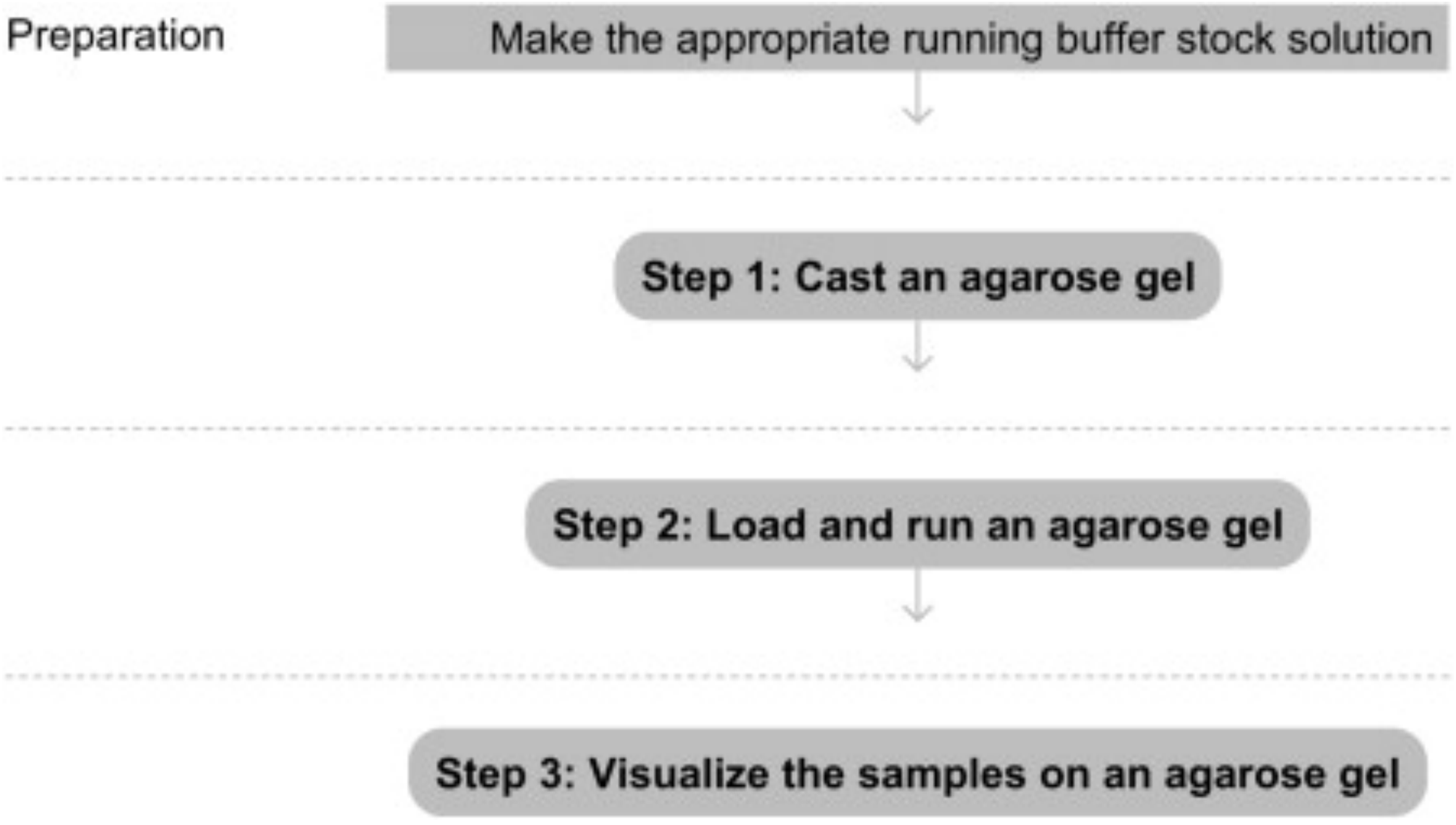

Figure 4.1. Flowchart of the complete protocol, including preparation.

5. Step 1 Casting an Agarose Gel

5.1. Overview

Cast an agarose gel for ~ 30 min before you are ready to run your samples.

5.2. Duration

30 min

1.1 First, refer to the table in (Materials section) and determine what percentage gel you need to resolve all of your samples. For example, if you are digesting DNA from plasmid minipreps to check for the insertion of a 1.5 kb fragment in a vector of 7 kb, you will need a 1% gel in order to linearly resolve all bands.
Once you have determined what percentage gel you need, add the appropriate amount of agarose to a 250 or 500 ml flask. Add 100 ml of 1× buffer.

1.2 Microwave on high for about 2 min, or until all agarose is dissolved.

1.3 Remove from microwave and allow the agarose to cool on the benchtop for a few minutes.

1.4 Once the agarose solution has cooled slightly, add your DNA dye of choice. Mix and pour into your assembled casting tray. Take care to pop any bubbles with a pipette tip. Insert the desired type of comb.

1.5 Allow gel to cool and solidify. This will take ~ 30 min.

1.6 Remove comb (and tape, if used) and place gel in electrophoresis tank filled with 1× running buffer.

5.3. Tip

Do not make your gel with H2O! Water does not have the *ionic strength* to conduct electricity through the gel and your samples will not migrate correctly.

5.4. Tip

You must run your gel in the same buffer that you use to make the gel. Otherwise it will not properly conduct electricity and your gel will not run correctly.

5.5. Tip

Assemble the gel-casting tray while microwaving or cooling your agarose solution. Take care not to move the gaskets or else your gel will leak. If you do not have a setup with a mold, secure lab tape over the ends of the gel-casting tray.

5.6. Tip

Wide combs are typically used for *purification*, while thin combs are used for diagnostics *(Fig. 4.2(a) and 4.2(b))*

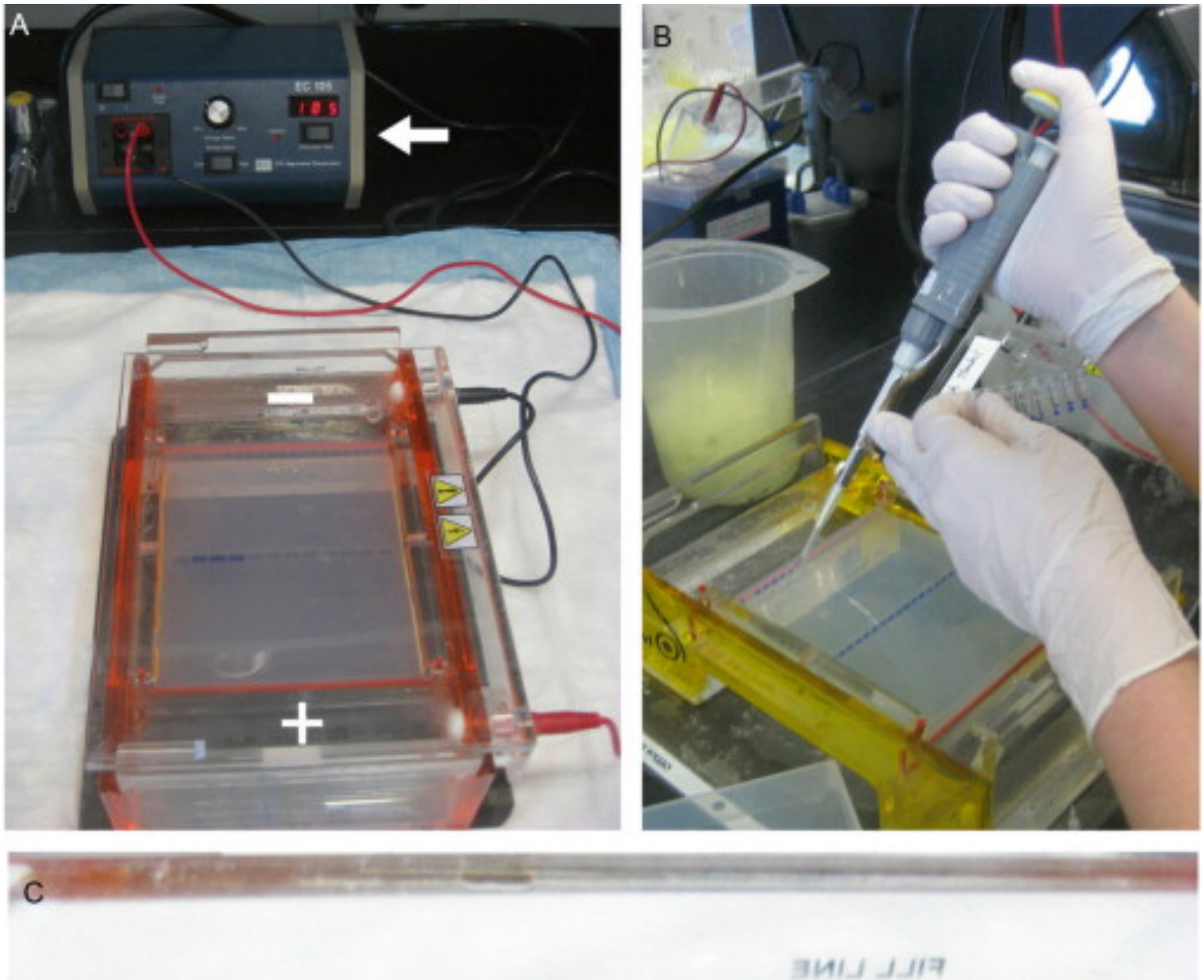

Figure 4.2. Loading and running a gel. (a) A typical gel electrophoresis apparatus consists of a gel box full of running buffer connected to a power source (arrow). Here the positive end is at the bottom of the box. Note the wide wells that the DNA is loaded in – these wells are ideal for purification because they can hold a large volume. (b) In contrast, thin wells are ideal for running a large number of diagnostic samples, such as in the case of genotyping experimental animals. (c) In order to ensure that the power source is connected correctly, look for the presence of bubbles emanating from the wire at the negative side of the gel box.

5.7. Tip

If you are in a hurry, you can cast your gel at 4 °C and it will solidify faster.

See Fig. 4.3 for the flowchart of Step 1.

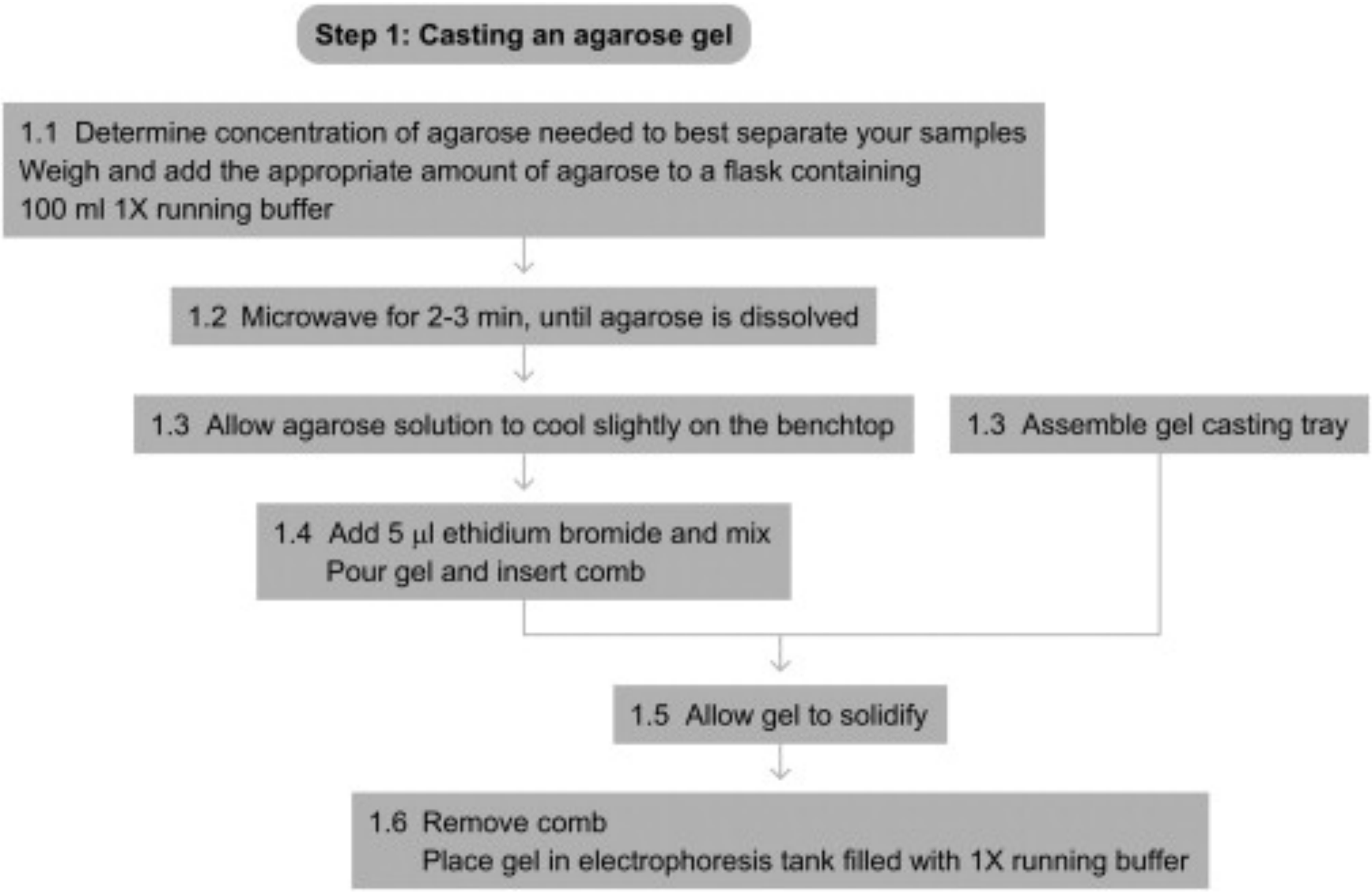

Figure 4.3. Flowchart of Step 1.

6. Step 2 Loading and Running an Agarose Gel

6.1. Overview

Here you will load your samples on the gel and separate them in an electric current.

6.2. Duration

About 1h

2.1 Determine the amount of sample you would like to run on the gel and add the appropriate amount of 6× DNA loading buffer. For example, if you are running a genotyping PCR, add 2 µl 6× DNA loading buffer to 10 µl of sample. If you are purifying a fragment for subcloning, you will want to load more.

2.2 Load an appropriate DNA ladder in one well, either 100 bp or 1 kb. Carefully pipette your samples into the wells. Be careful not to damage the wells or accidentally get your sample in adjacent wells.

2.3 Connect your gel tank to the power source. Make sure that you have connected the positive wire from your tank to the positive electrode on the power source, and likewise connected the negative to the negative. Turn your power source on (Fig. 4.2(a)).

2.4 When you are ready to visualize your gel, turn off the power supply and disconnect it from the gel tank. Carefully remove your gel. Do not put your hands in the tank while the gel is still running.

6.3. Tip

If you need to load a larger volume of sample than your well can hold, you can centrifuge it in a Speed-vac to concentrate it. Additionally, using the long thin gel loading pipet tips will allow you to load more sample than regular tips.

6.4. Tip

You can run your gel at a variety of voltages depending upon the buffer that you choose to use. Keep track of how far your sample has migrated by looking at the dye front on the gel.

6.5. Tip

To ensure that everything is connected correctly and an electric current is being supplied to the tank, check for the presence of bubbles at the positive pole of the tank (Fig. 4.2(c)).

See Fig. 4.4 for the flowchart of Step 2.

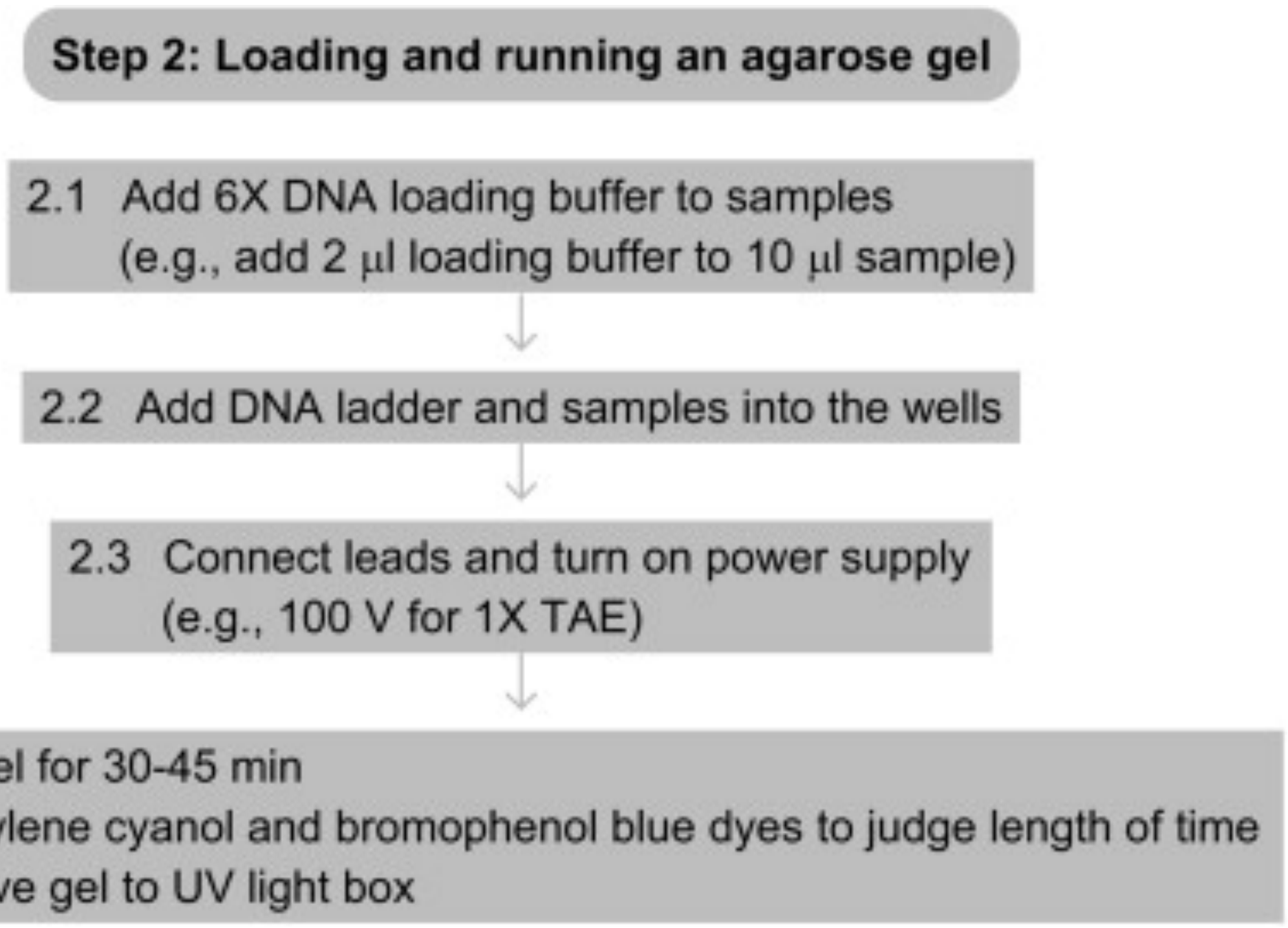

Figure 4.4. Flowchart of Step 2.

7. Step 3 Visualization of Samples on an Agarose Gel

7.1. Overview

Since your sample is stained with a fluorescent dye, you will be able to visualize it using a UV light source.

7.2. Duration

5 min

3.1 Carefully place your gel on the glass top of a UV light box. Turn the light on. You should now be able to see your DNA light up (Fig. 4.5(a) and 4.5(b).

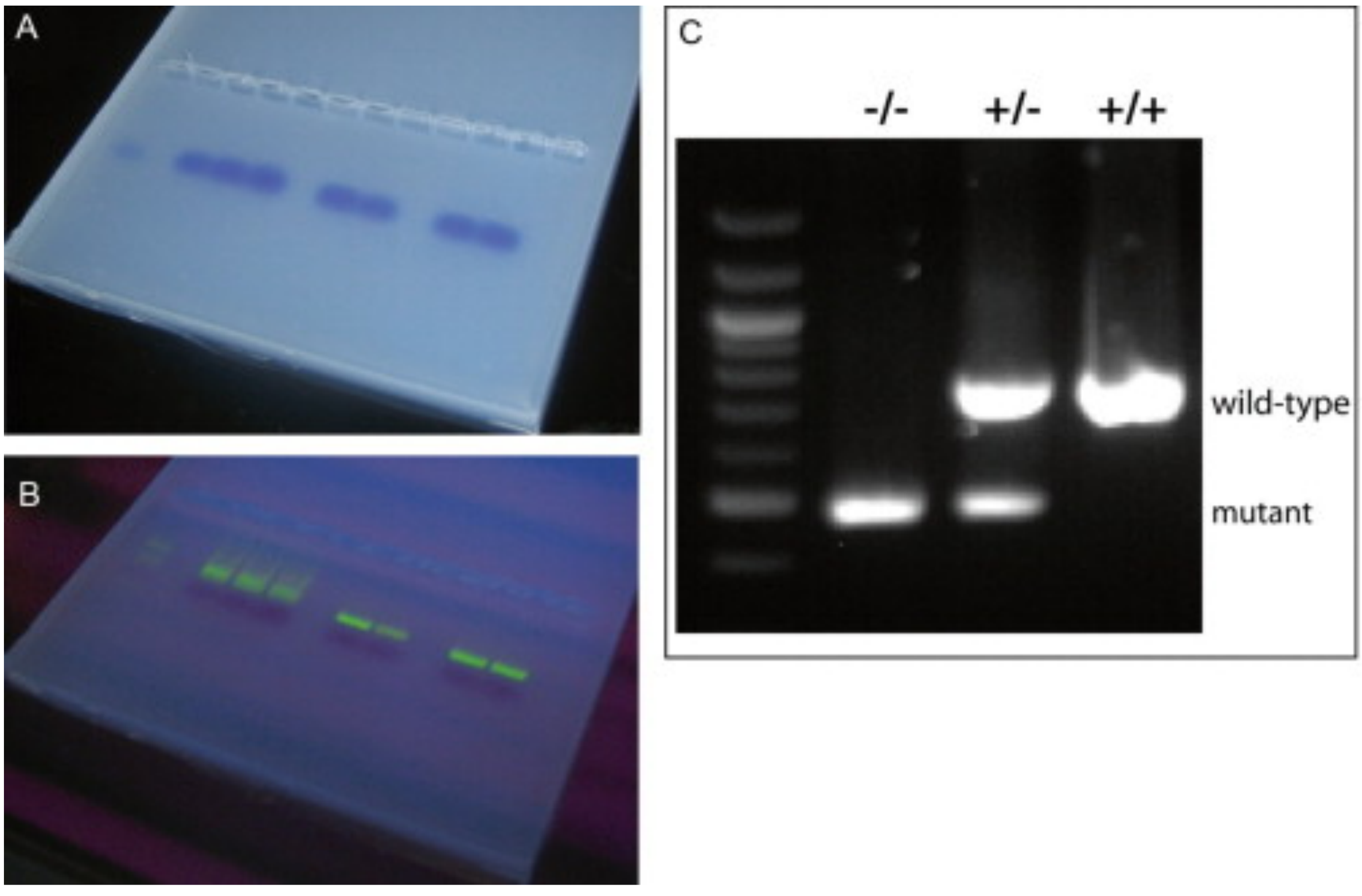

Figure 4.5. Visualization of DNA. (a) Image of an agarose gel illuminated with white light. Note the dye front. (b) Image of the same agarose gel illuminated with UV light. DNA is in green (stained with SYBR-Safe). (c) Gel electrophoresis can be used to genotype experimental animals or diseases. Here wild-type and mutant alleles can be distinguished based on a size difference.

3.2 Take a picture of your gel with a camera mounted to your UV box (Fig. 4.5(c)). If necessary, excise your DNA bands with a razor blade and transfer them to 1.5-ml microcentrifuge tubes for later gel purification and cloning.

7.3. Tip

Do not look directly at UV light. Always wear UV goggles when using UV light. Take care not to expose your skin to UV light too much as well.

7.4. Tip

Note that different DNA stains illuminate different colors – *ethidium bromide* glows orange and SYBR-Safe glows green, for example.

7.5. Tip

Minimize the amount of time that your sample is exposed to UV light. UV light induces crosslinks in DNA by creating pyrimidine dimers between adjacent cytosine and thymine residues. This is particularly crucial and therefore should be kept in mind if DNA is to be cut from your gel for purification and used for downstream sub-cloning.

Key researchers

Jerry A. Katzmann

Mayo Clinic

Rochester, United States

60 h-index | 12,209 Citations | 231 Documents

Philip Serwer

University of Texas at San Antonio

San Antonio, United States

31 h-index | 2,937 Citations | 153 Documents

Relevant methods

Staphylococcus haemolyticus prophage ΦSH2 endolysin relies on cysteine, histidine-dependent amidohydrolases/peptidases activity for lysis 'from without

Mathias Schmelcher, Olga Korobova, Nina Schischkova, Natalia Kiseleva, Paul Kopylov, Sergey Pryamchuk, David M. Donovan, Igor Abaev

Journal of Biotechnology • Volume 162, Issues 2–3 • 31 December 2012 • Pages 289-298

> #### Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and zymogram
>
> All purified proteins were analyzed with 15% SDS-PAGE, using a Kaleidoscope Precision Plus Protein Standard (BioRad, Hercules, CA). For zymograms, a 300mL culture volume equivalent of live mid-log phase (OD600nm=0.4–0.6) cells of S. aureus strain Newman was embedded in the gel during polymerization. The protein samples were boiled in Laemmli sample buffer (BioRad, Hercules, CA) with β-mercaptoethanol, and electrophoresed with identical buffers and voltage for one hour in both the SDS-PAGE and the zymogram using a Mini-Protean TETRA gel system (BioRad). SDS gels were Coomassie stained and zymograms were washed in excess water and further incubated in deionized water for 60min, followed by incubation in 10mM Tris, pH 8.0 with 150mM or 300mM NaCl for 80min. Areas of clearing in the turbid zymogram gel indicate a lytic protein in the gel.

Major allergen Phl p Va (timothy grass) bears at least two different IgE-reactive epitopes

Bufe MD, Becker PhD, Schramm, Petersen PhD, Mamat PhD, Schlaak MD

Journal of Allergy and Clinical Immunology • Volume 94, Issue 2, Part 1 • August 1994 • Pages 173-181

SDS-PAGE, Western blot, and immunologic detection

SDS-PAGE was done according to the method of Laemmli,16generally followed by electroblotting onto nitrocellulose membrane17 on a Fastblot (Biometra, Göttingen, Germany). For protein staining and immunologic detection, we used India ink and antibodies in a procedure as previously described.11For identification and estimation of the degree of IgE immunoreactivities in Western blots, intensity of allergen pattern was classified from 0 to 4.14

Proteasome components with reciprocal expression to that of the MHC-encoded LMP proteins

Mônica P. Belich, Richard J. Glynne, Gabriele Senger, Denise Sheer, John Trowsdale

Current Biology • Volume 4, Issue 9 • September 1994 • Pages 769-776

MB1 analysis by two-dimensional polyacrylamide gel electrophoresis

Proteasomes were purified from human placenta by affinity chromatography on immobilized antibody MCP21 [36] The two-dimensional gel results from non-equilibrium pH-gradient electrophoresis (NEPHGE) followed by SDS-polyacrylamide gel electrophoresis (SDS-PAGE). Proteins were transferred to membranes and stained with Ponceau-S before incubation with anti-MB1 antiserum. The secondary antibody used was peroxidase-conjugated swine anti-rabbit Ig (Dako, cat. P217). Detection was performed with tetramethyl-benzidine/hydrogen peroxide. For the peptide used to produce anti-MB1 antiserum, see below.

Related content

Chapter Four: Agarose Gel Electrophoresis

Laura Koontz

Methods in Enzymology • Volume 529 • 2013 • Pages 35-45

Chapter 3: Agarose Gel Electrophoresis

John T. Corthell

Basic Molecular Protocols in Neuroscience: Tips, Tricks, and Pitfalls • Volume • 2014 • Pages 21-25

Analysis of Amyloid Aggregates Using Agarose Gel Electrophoresis

Sviatoslav N. Bagriantsev, Vitaly V. Kushnirov, Susan W. Liebman

Methods in Enzymology • Volume 412 • 2006 • Pages 33-48

Methods for recovering nucleic acid fragments from agarose gels

Giovanni Duro, Vincenzo Izzo, Rainer Barbieri

Journal of Chromatography B: Biomedical Sciences and Applications • Volume 618, Issues 1–2 5 • August 1993 • Pages 95-104

7: Gel Electrophoresis

A. Drabik, A. Bodzoń-Kułakowska, J. Silberring

Proteomic Profiling and Analytical Chemistry (Second Edition) • 2016 • Pages 115-143