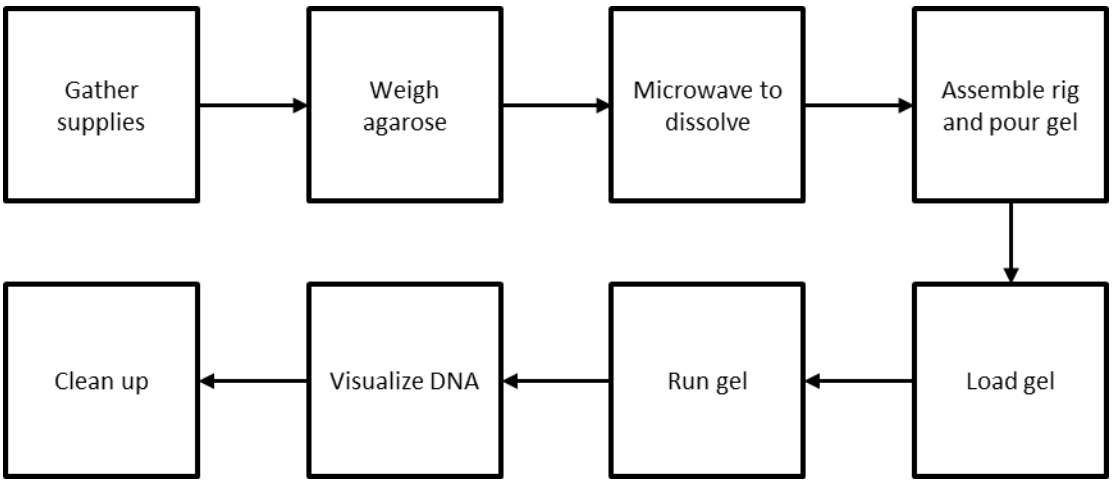
Agarose Gel for Electrophoresis

Description

This procedure describes how to make, load, and run an agarose gel for visualizing DNA fragments using the equipment available at the Las Cruces Test Site (Thermo Scientific EasyCast gel electrophoresis systems). The DNA fragments can then be visualized using the MultiDocIt Imaging system.

Flow Diagram



Procedure

Gather supplies

Supplies required: Flask (up to xxxml will fit in the microwave), 1X TAE buffer (diluted from 50X stock with RO water), agarose, balance, microwave, heat resistant gloves or suitable method for handling hot liquids in flask, gel rigs, combs and casting trays, DNA ladder diluted as per manufacturers guidelines, DNA loading dye, pipettes + tips, spatula, ethanol spray bottle, powerpac and leads for supplying power to gel rig.

The %w/v agarose to use is dependent on the size of DNA fragment that you are trying to visualize or separate. For most purposes (i.e. visualizing a PCR product) a 0.75-1.25% gel is suitable. When using the medium and large gel rigs the gels can be fragile and until you are comfortable handling the gels it is recommended to use a higher % (≥1.25%) gel to prevent breakage during handling.

Gel size/type	Typical number of samples*	Volume / gel (ml)
Small	22	~50
Medium	96	~200
Large	288	~500

*Medium and large gels have combs that have more lanes but the number shown in the table os the number that can be loaded with a 12-chanel pipette. Small gels have multiple comb types.

Weigh agarose

1. Place flask on balance and zero
2. Add agarose to amount required using a spatula

3. A 1-1.25% w/v agarose gel is suitable for most applications but other percentages may be required for certain applications (e.g. when visualizing particularly large or small DNA fragments
4. Clean balance area
5. Measure 1XTAE required in a measuring cylinder
6. Add approximately half the volume of TAE to the flask containing the agarose powder and swirl to mix

Microwave to dissolve

1. Microwave on full power until solution boils
2. Take care when removing the flask from the microwave since liquid could be super-heated and may violently boil over. Let flask sit to cool before agitating and point flask mouth away from face before agitating. (One way to get around this is to use boiling beads in the solution at this step)
3. Swirl the solution hold up to the light and observe for any undissolved agarose (agarose will appear as tiny suspended beads until fully dissolved)
4. Continue too microwave until all agarose is fully dissolved. (This may take up to 2-3 minutes for larger volumes)
5. Once fully dissolved, remove from microwave and leave to cool slightly
6. If you are not able to pour gels immediately, add remaining buffer and incubate gel at 55-60°C until ready to use

Assemble rig and pour gel

Large gels: for up to 3 96-well plates of samples

1. Inspect and clean rubber seal around end plates and in recess of mold, and teeth of combs. Remove any dried agarose from all parts.
2. Insert end plates into recess and apply firm pressure. Ensure that rubber gasket has inserted into recess and is not sticking out anywhere. This process can be aided by first spraying rubber seal with 70% ethanol spray.
3. Add remaining buffer to flask of agarose solution and allow to cool to ~50-60°C on the bench or in an incubator/water bath. If you can hold the gel in your hand comfortably then it is cool enough to pour. Pouring gel when it is too hot may warp the apparatus.
4. Add DNA stain. Currently we are using “SYBR safe DNA Gel Stain” (manufacturer recommends stock as 10,000X but we have used half this amount i.e. assume stock = 20,000X)
5. Swirl to mix
6. Pour agarose into gel casting tray with a steady motion to prevent introduction of bubbles
7. Add combs in desired spacing
8. If many bubbles are introduced during this process they can be removed by spraying 70% ethanol from a spray bottle directly over the gel. The bubble should burst once the ethanol spray lands on the gel.
9. Pour excess gel into a trash can and immediately rinse flask with 1 volume of hot water. Alternatively gel may be kept molten at 55-60 °C for later use, or allowed to cool and solidify for later re-melting and use. If it is kept for a long period of time, more DNA stain may need to be added before use.
10. Wait until gel is set. Once set the gel will become opaque.

11. If gel is to be used at a later date, wrap in cling wrap and refrigerate
12. Remove combs and place gel and tray into gel box
13. Add buffer until gel is just submerged

Medium gels: for up to 96 samples

1. Inspect and clean rubber seal around gel tray, casting tray, and teeth of combs. Remove any dried agarose from all parts.
2. Check the casting tray is level and adjust as necessary by turning adjustment knobs.
3. Insert gel tray into casting tray. Ensure that rubber gasket has formed a good seal and is not kinked or misplaced anywhere. This process can be aided by first spraying rubber seal with 70% ethanol spray.
4. Add remaining buffer to flask of agarose solution and allow to cool to ~50-60°C on the bench or in an incubator/water bath. If you can hold the gel in your hand comfortably then it is cool enough to pour. Pouring gel when it is too hot may warp the apparatus.
5. Add DNA stain. Currently we are using "SYBR safe DNA Gel Stain" (manufacturer recommends stock as 10,000X but we have used half this amount i.e. assume stock = 20,000X)
6. Swirl to mix
7. Pour agarose into gel tray with a steady motion to prevent introduction of bubbles
8. Add combs in desired spacing
9. Pour excess gel into a trash can and immediately rinse flask with 1 volume of hot water. Alternatively gel may be kept molten at 55-60 °C for later use, or allowed to cool and solidify for later re-melting and use. If it is kept for a long period of time, more DNA stain may need to be added before use.
10. If many bubbles are introduced during this process they can be removed by spraying 70% ethanol from a spray bottle directly over the gel. The bubble should burst once the ethanol spray lands on the gel.
11. Wait until gel is set. Once set the gel will become opaque.
12. If gel is to be used at a later date, wrap in cling wrap and refrigerate
13. Remove combs and place gel and tray into gel box
14. Add buffer until gel is just submerged

Small gels: for up to x samples – for this type of gel the gel rig is both the casting apparatus and the electrophoresis apparatus.

1. Inspect and clean rubber seal around gel tray and teeth of combs. Remove any dried agarose from all parts.
2. Insert tray into gel rig sideways. Ensure that rubber gasket has made a good seal and is not kinked or sticking out anywhere. This process can be aided by first spraying rubber seal with 70% ethanol spray.
3. Add remaining buffer to flask of agarose solution and allow to cool to ~50-60°C on the bench or in an incubator/water bath. If you can hold the gel in your hand comfortably then it is cool enough to pour. Pouring gel when it is too hot may warp the apparatus.
4. Add DNA stain. Currently we are using "SYBR safe DNA Gel Stain" (manufacturer recommends stock as 10,000X but we have used half this amount i.e. assume stock = 20,000X)
5. Swirl to mix

6. Pour agarose into gel casting tray with a steady motion to prevent introduction of bubbles
7. Add combs in desired spacing
8. If many bubbles are introduced during this process they can be removed by spraying 70% ethanol from a spray bottle directly over the gel. The bubble should burst once the ethanol spray lands on the gel.
9. Pour excess gel into a trash can and immediately rinse flask with 1 volume of hot water. Alternatively gel may be kept molten at 55-60 °C for later use, or allowed to cool and solidify for later re-melting and use. If it is kept for a long period of time, more DNA stain may need to be added before use.
10. Wait until gel is set. Once set the gel will become opaque.
11. If gel is to be used at a later date it can be wrapped in cling wrap and refrigerated at this point
12. Remove combs and turn gel 90 degrees in gel box
13. Add buffer until gel is just submerged

Load gel

1. Orient the gel/gel box so that the wells are positioned in the upper part of the gel (furthest away from you)
2. The red lead will be attached at the plug nearest you and the black lead at the plug furthest from you. The DNA will migrate through the gel towards the red electrode.
3. Add loading dye to sample to a final concentration of 1-2X, mix and pipette into well
4. If loading 96-well plate samples, it is customary to load rows A-D on the left hand side, ladder in the middle and rows E-H on the right hand side of the gel.
5. Load ladder. Load in middle well for large or medium gels if all wells are being used, or in wells near samples in other cases. Follow manufacturers guidelines for loading volume.
6. Attach and double check cables are plugged into correct sockets (red to red, black to black)

Run gel

1. Turn on gel powerpac. The biorad powerpac has a main power switch on the right hand side towards the back.
2. Select constant V
3. use the up arrow to raise V to desired level
4. Adjust any other levels (such as run time etc)
5. press run.
6. Bubbles should appear rising from each electrode
7. Loading dye contains dye indicators. Visualize bands after dye has migrated approximately halfway down the lane. The time that this takes will be dependent on the gel % and the voltage. The gel can always be run longer if needed.
8. Suggested voltage ranges for different gels:
    a. Small gels: 75-100V
    b. Medium gels: 100-150V
    c. Large gels: 150-250V

Visualize DNA using gel box (description elsewhere)

1. Remove leads and transfer gel to MultiDocIt Imaging system.
2. Turn on camera, and launch the DocIT-LS program.

3. Turn on the UV light and power
4. Once launched open “View” menu, select the “Plugins” option and activate the “Canon camera plug-in”. The “Canon” tab should appear in the lower right corner; click on this tab and activate the “Preview” option.
5. Adjust the zoom factor until the gel is properly centered and in focus. Click on “Capture” to take the image, which will appear in the main window.
6. To save image, right-click on the tab at the top left of the image, navigate to Unless high resolution images are required, save as jpeg

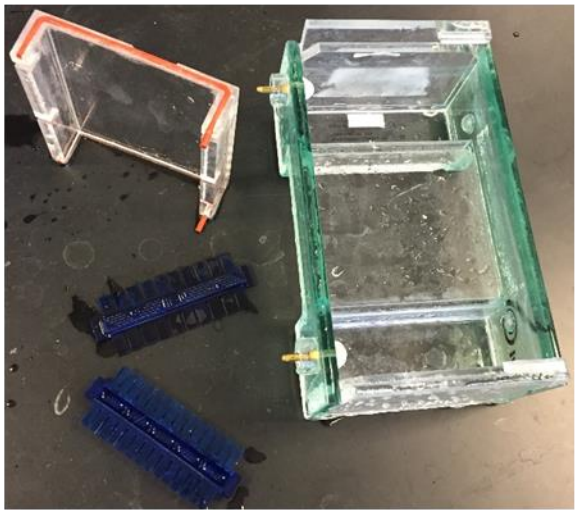
Clean up

1. Running buffer (TAE) can be reused multiple times so may be left in the gel rig for the next use. Cover the rig to prevent excessive evaporation.
2. If no gels are to be run in the near future or the buffer has been used multiple times dispose of down the sink and rinse the rig and leave to dry. Make sure a filter is in place in the sink to catch small pieces of agarose.
3. Wipe down the bench, gel rigs, casting trays, and combs
4. Used gels can be handled without gloves and disposed of in the regular trash (no ethidium bromide is in use at this time)

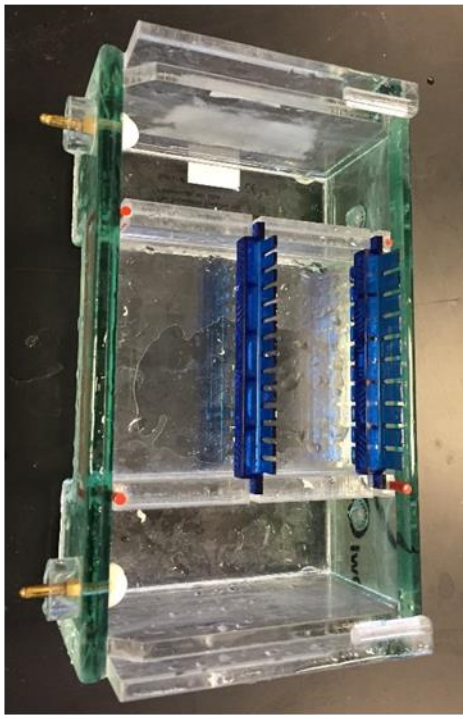
Appendix

The following picture show how the different sized gel apparatus is set up for both casting gels and for electrophoresis.

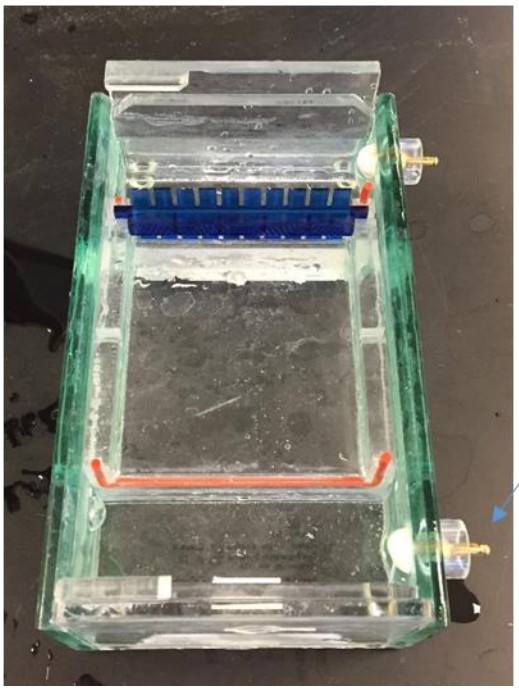
Small gel tray, combs and rig



Rig for casting



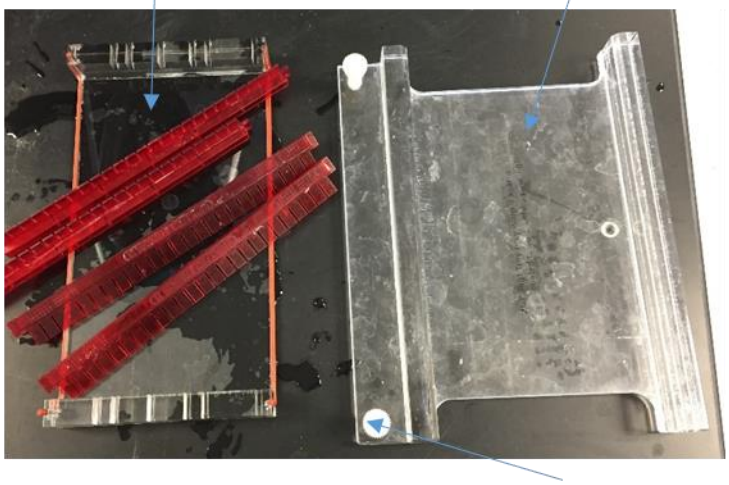
Rig for electrophoresis



Cable/lead attachments

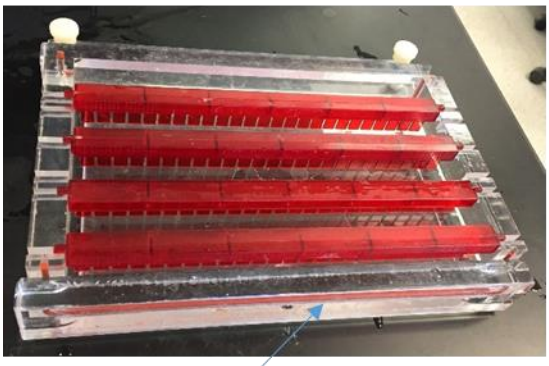
Medium gel tray and combs

Casting tray



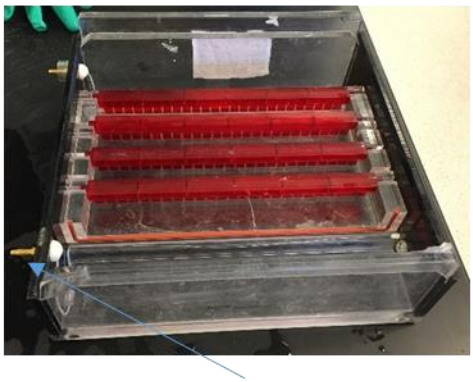
Adjustment knob

Set up for casting



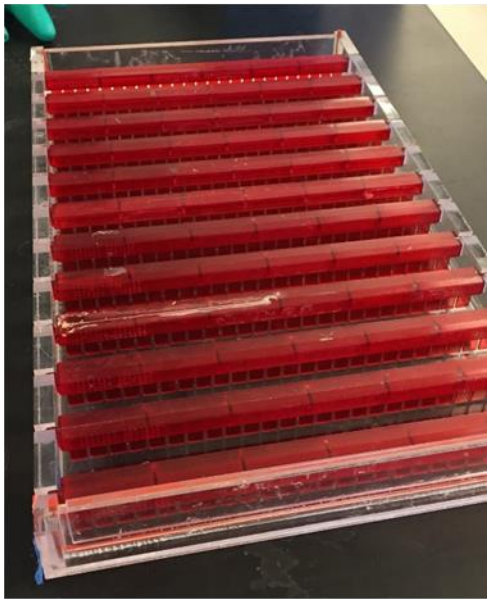
Rubber seal

Set up for electrophoresis



Cable/lead attachments.
- Combs are removed prior to loading

Large gel tray, combs and end plates



Level for levelling tray/rig

Cable/lead attachments