EMORY UNIVERSITY

Emory Integrated Genomics Core

Emory Integrated Core Facilities

EIGC.008_General Agarose Gel Electrophoresis

Standard Operating Procedure Staff Review Page:

I have read and understand the procedure listed above.

Employee name	Date SOP review complete

Standard Operating Procedure Approval Page:

Date Implemented: 11 July 2011

	Name	Date
Updated:	Ashima Amin	18 June 2020
Supersedes:	Ashima Amin	11 April 2016

Annual Review and Approval

Michael Zwick, PhD
Laboratory Director

Changes Made:
061820: Review, Added EZ Gel info. Renumbered SOPs. Previously EIGC.010

Changes to previous procedures:

041116: Review

Test Principle: Agarose gel electrophoresis is used to size fractionate DNA fragments. An agarose gel is submerged in electrophoresis buffer in a gel box or is part of a gel cassette containing a positive and negative electrode at opposite ends. DNA (genomic or amplified PCR product) is placed in a well at the negative end of the gel. When an electrical current is applied to the gel, the negatively charged DNA migrates through the gel toward the positive electrode. The gel contains ethidium bromide, a chemical that intercalates into DNA and fluoresces under UV light. The gel is visualized and photographed on a UV light box.

Specimen Types: DNA

Required Reagents:

E-Gel Protocol

E-Gel 2% Double Comb – 16 Well (ThermoFisher, #G601802)

E-Gel PowerBase v4 (ThermoFisher, #G6200-04)

E-Gel 2% 48 Well (ThermoFisher, #G800802)

E-Gel 2% 96 Well (ThermoFisher, #G700802)

E-Gel Mother E-Base (ThermoFisher, EB-M03)

E-Gel 1 Kb Plus DNA Ladder (ThermoFisher, 10488090)

E-Gel 1 Kb Plus DNA Ladder Working Stock - 1:1 dilution of ladder stock with diH_2O

diH_2O

Manual Casting Protocol

6X DNA loading dye, Promega

DNA ladder, various (including Promega)

Agarose – Low EEO, Fisher

Agarose – Low melting, Fisher

10X TBE, Roche

1X TBE, N/A – 1:10 dilution of 10X TBE with diH_2O

1% Ethidium bromide, Fisher

diH_2O

Quality Control: A size ladder is run on each gel. DNA fragment bands can be sized by comparing the band to the ladder.

Safety: All employees are required to follow universal safety protocols when working with potentially infectious biohazardous materials. Safety procedures are outlined in the Emory University Bloodborne Pathogen Exposure Control Plan (see Employee Safety Notebook). Ethidium bromide (EtBr) is used to visualize DNA in an agarose gel and is a powerful mutagen. Gloves must be worn, and care must be exercised when handling EtBr. UV light is harmful to the skin and eyes. Protective skin and eye equipment must be worn when using a UV light box.

General Gel Guidelines:

- Keep all sample volumes uniform.
- Load deionized water into any wells left empty.
- Run the gel immediately after loading.
- Dilute samples that contain high salt concentration buffers (certain restriction enzyme and PCR buffers) 2- to 20-fold before loading.

E-Gel Protocol

E-Gel Sample Preparation:

- Use 20−100 ng DNA per band for samples containing one unique band or up to 500 ng per lane for samples containing multiple bands. In general, 10 µL of undiluted DNA will be used.

Mix the appropriate volume of deionized water and the appropriate volume of sample following the chart below. Follow the chart below for run times as well:

E-Gel Volumes and Run Times:

E-Gel	DNA Sample (µL)	diH_2O (µL)	1:1 Ladder (µL)	Run Time (minutes)
Double Comb (16)	10	10	10*	20
48	10	5	15	15
96	10	10	20	5

Note: The ladder volume for the double comb E-gel differs from the total sample volume. Run times listed above are only estimates. Gel can be imaged midrun to verify complete resolution.

Double Comb (16 Well) E-Gel Preparation:

1. **Plug the PowerBase into an electrical outlet using the adaptor plug. The base will perform a brief self-test. (With no gel in the base, no light will be illuminated on the base after the self-test).**

2. **Insert the E-Gel with the comb in place into the PowerBase right side first and then press the left side into place. There should be an audible click. A steady, red light will illuminate when the E-Gel is correctly inserted.**

3. **You must first pre-run the E-Gel for 2 minutes with the comb in place before loading your samples to ensure proper resolution of your DNA fragments.**

    a. **Press and hold either the 15- or 30-minute button until the red light turns to a flashing green light. This indicates that the 2-minute pre-run has started.**

    b. **At the end of the pre-run, current will automatically shut off. The flashing-green light will change to a flashing red light, and the PowerBase will beep rapidly.**

    c. **Press and release either button to stop the beeping (you will hear only one beep). The light will change from a flashing red light to a steady red light.**

    d. **Remove the combs by gently pushing up on the side handles of each comb and remove any fluid that is present in the wells.**

Loading and Running Double Comb (16 Well) E-Gels:

1. **Load 10 µL of 1:1 ladder working stock into each ladder well. The ladder wells are located directly in the middle of the E-gel and are designated with a letter "M".**

2. **Load appropriate volume of sample into each well for a total of 20 µL. Record the sample order for later addition to the QC report. Change the pipet tip between each sample. Note: The wells of the E-Gel are compatible for loading with a multichannel pipet.**

3. **Load 20 µL of water into any remaining empty wells.**

4. **Press and release the 30-minute button to start the 30-minute electrophoresis run. The light will change to a steady green light. Because the E-gel will only need to run for approximately 20 minutes, set a timer for 20 minutes.**

5. **Press the 30-minute button to end the run after 20 minutes. The light will turn to a steady red light. Remove the gel from the PowerBase. Note: If the bands are not fully resolved, the gel can be run for additional time but do not exceed 25 total minutes. Do not allow the top lanes to run into the lanes below.**

6. **To avoid diffusing of the bands, image the gel as soon as possible after ending the run (within 20 minutes).**

48/96 Well E-Gel Preparation:

1. **Plug the Mother E-Base into an electrical outlet.**

2. **Press and release the pwr/prg (power/program) button to select program EG. Press and hold the time button until the desired run time is displayed. (See chart above for times).**

Loading and Running 48/96 Well E-Gels:

1. **Remove the gel from the pouch. Note: Load each gel within 30 minutes of removing the gel from the pouch.**

2. **Remove the comb from the gel and remove any fluid that is present in the wells.**

3. **Slide the gel into the electrode connections on the E-Base starting with the right side of the gel. If the gel is properly inserted, a fan in the base begins to run, a red-light illuminates, and the digital display shows the run time.**

4. **Load 15 µL (48 well) or 20 µL (96 well) of 1:1 ladder working stock. Ensure that the marker salt concentration is similar to that of adjacent samples. The ladder wells are located directly before and after the sample wells and are designated with a letter "M".**

5. **Load DNA sample into each well. Load 10 µL sample and 5 µL water for the 48 well gel. Load 10 µL sample and 10 µl water for the 96 well gel. Keep all sample volumes uniform. Load samples manually or with a multichannel pipet.**

6. **Load the same volume of water to match total sample volume into any remaining empty wells.** Note: Run the gel within 15 minutes of loading.

7. **To begin electrophoresis, press and release the pwr/prg button on the E-Base. The red light will change to green.**

8. **At the end of the run (signaled by a flashing red light and rapid beeping), press and release the pwr/prg button to stop the beeping and flashing red light. Note: If the bands are not fully resolved, the gel can be run for additional time but do not exceed 25 total minutes for the 48 well or 17 total minutes for the 96 well. Do not allow the top lanes to run into the lanes below.**

9. **Remove the gel cassette from the base.**

10. **To avoid diffusing of the bands, image the gel as soon as possible after ending the run (within 20-40 minutes).**

Manual Casting Protocol

Pouring a Gel:

1. **Place the required agarose in a flask (see chart).**

2. **Measure the required volume of 1X TBE using a graduated cylinder and add to flask.**

3. **Heat the mixture in a microwave to melt the agarose. Swirl the agarose as it begins to melt or use 50% power to prevent the agarose from boiling over. Note: The agarose will be extremely hot.**

4. **When the agarose is completely melted and mixed, cool to 60ºC. This will take approximately 20 minutes. The flask will be hot but comfortable to handle.**

5. **Add EtBr solution and mix thoroughly.**

6. **Pour into gel tray with appropriate comb (see specific protocols).**

7. **Allow gel to cool and solidify completely.**

Gel Composition Guidelines:

Volume (1X TBE)	Gel %	Agarose	Low Melt Agarose	EtBr (10 mg/ml)
20 ml	1%	.2 g	0 g	0.5 µL
	2%	.4 g	0 g	0.5 µL
	3%	.3 g	.3 g	0.5 µL

40 ml	1%	.4 g	0 g	1 µL
	2%	.8 g	0 g	1 µL
	3%	.6 g	.6 g	1 µL
100 ml	1%	1 g	0 g	2 µL
	2%	2 g	0 g	2 µL
	3%	1.5 g	1.5 g	2 µL

Note: Select a gel percentage that aligns with the proper resolution for the experiment's estimated fragment lengths. 1% - 400-8000 bp, 2% - 100-2000 bp, 3% - 25-1000 bp.

Sample Preparation:

1. **Thaw the appropriate DNA ladder (1 kb DNA Ladder is stable for at least 3 months at 4°C.).**

2. **Determine the amount of 6X DNA loading dye to be used for each sample. Loading dye is used in a 1:6 dye to sample ratio. For example, to run 5 µL or sample, use 1 µL of loading dye.**

Suggested protocol for loading a sample: The following protocol is recommended for a 5 mm wide lane.

a. **Prepare loading mixture: Distilled water 4 µl 6X Blue Loading Dye 1 µl DNA Ladder 1 µl Total volume 6 µl**
b. **Mix gently**
c. **Load onto the agarose gel**

Note: The components of the mixture should be scaled up or down, depending on the width of the agarose gel.

3. **Aliquot loading dye for each sample (including the ladder) into labeled PCR strip tubes.**

4. **Add each sample to the loading dye and mix well.**

5. **Prepare the ladder by adding the ladder (catalog. number N3232S – New England Biolabs) plus enough 1X TBE to equal the volume of the samples. For example, if the protocol calls for 5 µL of sample to be loaded onto a gel, add 1 µL of ladder and 4 µL of 1X TBE to 1 µL of loading dye.**

Running the Gel:

1. **Remove the comb by pulling up vertically and slowly. Removing the comb too quickly may result in ripping the bottoms of the wells.**

2. **Submerge the gel in 1X TBE buffer in the appropriate gel box.**

3. **Pipet the samples and ladder into the wells of the gel and record the order for later addition to the QC report. Change the pipet tip between samples.**

4. **Close the gel box lid. Attach the gel box to the power supply ensuring that the DNA will migrate through the gel toward the positive electrode.**

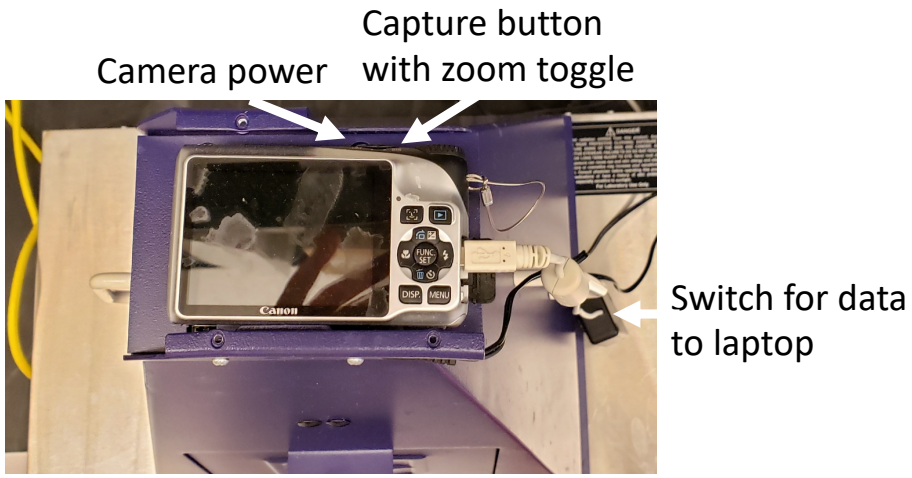
5. **Run the gel at a constant voltage for the desired time (see specific protocols). Note: For a more detailed description of electrophoresis apparatus operation, see the individual operator manuals for each gel box.**

6. **Image the gel immediately to avoid diffusing of the bands.**

Gel Imaging Protocol

1. **Use a UV light box to visualize the DNA.**

2. **Photograph the gel:**

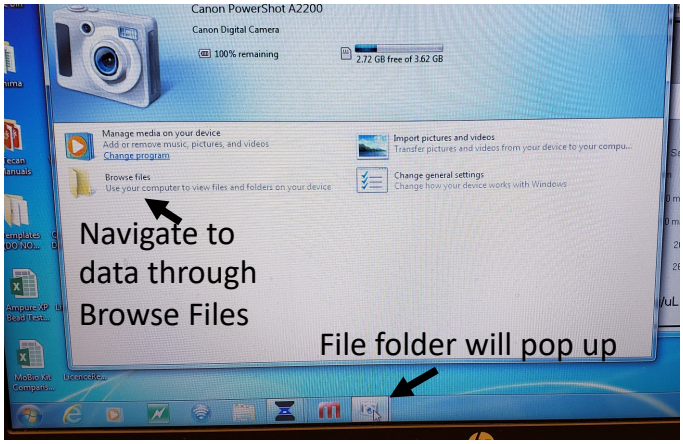


   a. **Turn on the Canon camera that is located on top of the UV box.**

   b. **Take a picture of the gel. You can zoom in using the lever that is on the capture button. You may need to orient the gel so that it fits into the camera view.**

   c. **Leaving the camera on, flip the switch to the cord that is attached to the camera. This will activate the transfer of files to the laptop.**

   d. **Open the data transfer file for the camera on the laptop.**



e. Browse to the data file and save it to the project-order file on the PPMS server.

3. Turn off the camera, switch to the laptop, and the UV box.

Interpretations: See specific protocols.

Benefits and Limitations: The benefits of agarose gel electrophoresis include being able to visualize PCR products and determine if a PCR water blank control shows evidence of contamination. The DNA fragment sizes determined by gel electrophoresis are not precise and are only an estimate.

References:

1. J. Sambrook, E.F.F., and T. Maniatis, *Molecular Cloning, A Laboratory Manual*. 2nd ed. 1989, Cold Spring Habor, NY: Cold Spring Habor Laboratory.

2. Invitorgen (2011, February 21). *E-Gel Agarose Gels.* Retrieved July 11, 2011, from http://tools.invitrogen.com/content/sfs/manuals/egel_qrc.pdf.

Troubleshooting:

E-Gel Troubleshooting

Problem	***Possible Cause***	***Possible Solution***
No current	Cassette improperly inserted or is defective.	Remove the gel cassette and re-insert the cassette correctly. Use a fresh cassette.
Poor resolution or smearing of bands	Sample overloaded.	Do not load more than 20−100 ng of DNA per band.
	High salt samples.	Dilute your samples per the above recommendations.
	Sample not loaded properly, or low sample volume loaded.	Do not introduce bubbles while loading samples. For proper resolution, keep all sample volumes uniform and load water into empty wells.
Melted gel	Increased current due to longer run times.	Do not run the gel longer than 40 minutes.
Sample leaking from wells	Wells damaged during comb removal.	Be sure to remove the comb gently without damaging the wells.
	Sample is overloaded.	Load the recommended sample volumes.

Manual Casting Troubleshooting

Problem	***Possible Cause***	***Possible Solutions***
One or more samples leaked through the bottom of the well.	The wells of the gel were broken when the comb was removed.	Repour the gel and reload the samples.
One or more samples floated out of the wells.	There is alcohol present in the sample.	Heat the genomic samples on a heat block in an open tube to allow alcohol to evaporate.
	The amount of loading dye in the sample was insufficient	Add more loading dye to the sample.
Samples and ladder did not migrate through the gel	There is no current flowing in the gel box.	Adjust the leads and check the gel box to make sure there is current flowing.
	The buffer composition is incorrect.	Remake the buffer.