	FOOD SAFETY LAB / MILK QUALITY IMPROVEMENT PROGRAM *Standard Operating Procedure*	MQIP Milk Quality Improvement Program

Title: Gel Electrophoresis of PCR Product			
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Gel Electrophoresis of PCR Product

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SECTION 1 INTRODUCTION

1.1 Purpose

A standard method confirming the amplification of PCR products via gel electrophoresis and imaging.

This procedure is based partially on a prior protocol *High Throughput PCR and Sequencing Reactions* by Rachel Miller and Kanika Chauhan, which can be found on the Food Safety Lab wiki.

1.2 Scope

This SOP applies to the Food Safety Lab, including the Laboratory for Molecular Typing.

1.3 Definitions

1.4 Safety

Appropriate protective measures need to be taken when working with *Listeria* spp., *Salmonella* spp., or other BSL-2 agents.
All waste from these experiments needs to be treated as BSL-2 waste.

Microwaved agar is very hot so take necessary precautions to avoid burns.

Ethidium bromide is a carcinogen. Use appropriate PPE including eye protection and nitrile gloves whenever handling anything that the ethidium bromide has contacted. Latex gloves are not to be used when handling ethidium bromide.

SECTION 2 MATERIALS

- Sterile water
- Sterile 0.2 mL tubes
- Micropipette and sterile filter tips
- Crushed Ice
- pGEM DNA ladder
- PCR product

SECTION 3 PROCEDURES

3.1 Gel Preparation

*The following produces a single gel at 1.5% agarose concentration. Adjust components as necessary if other gel densities are needed

3.1.1 Measure 1.5 grams of agarose powder into a 300ml flask.

3.1.2 Add 100ml of 0.5x TBE (located in a large container at the gel bench) to the flask, and gently swirl to mix.

3.1.3 Push a few paper towels partially into the opening of the flask as a stopper to reduce evaporation when microwaving.

3.1.4 Microwave the flask for 1 minute on full power.

3.1.5 Using a hot pad, gently swirl the flask to help dissolve the agarose.

3.1.6 Microwave for 30 more seconds, swirl again, then microwave again for 30 seconds.

3.1.7 Remove the flask from the microwave with a hot pad and set it aside for 10 minutes to cool

3.1.8 While it is cooling, prepare the gel mold, selecting the correct number of combs for your needs.

**If you have many samples, the 30-well combs allow gels to be loaded with the 10ul multi-channel pipettor. The tip spacing on that pipettor allow for filling every other well as the tips are spaced twice as far apart as the wells.*

3.1.9 When the mold is ready, slowly pour the gel into the mold from one corner of the mold, allowing the gel to spread itself across the mold. Try to maintain a constant pour and do not let the last bits of gel drip into the mold, as this could cause gel imperfections.

3.1.10 Rinse the flask with hot water as soon as possible to prevent the agar from solidifying

in the flask.

3.1.11 As soon as pouring is complete, look for any bubbles in the gel, especially in between the combs, and try to pop them with the end of a 10ul pipette tip.Allow the gel to solidify for one hour.

3.1.12 When the gel has solidified, carefully remove the combs by lifting them straight upwards from the mold

3.1.13 Remove the gel from the mold and store submerged in 0.5% TBE for up to 3 months.

3.2 **Gel Electrophoresis of PCR Products**

3.2.1 Keep the PCR products and pGEM DNA ladder on ice while loading the gel.

3.2.2 Prepare a gel by cutting it to the appropriate size for your samples. Ensure you leave enough wells for at least one pGEM DNA ladder as a well your positive and negative control PCR product.

3.2.3 Place the gel onto a chamber tray on the bench, or the gel can be placed directly into the chamber for loading

3.2.4 If you did not use a PCR buffer with loading dye in it (ie. your PCR product is colorless), add loading dye to your PCR products.

3.2.5 Load 3µL of the pGEM DNA ladder in to the first well of each row on the gel.

3.2.6 Load 3µL of PCR product per lane.

Note: this can be done using a multi-channel pipette, but every other well will be filled (as a result of the distance between each pipette tip in the multi-channel pipette).

3.2.7 Place the gel tray into the chamber oriented such that the samples will move from the negative electrode end of the chamber to the positive electrode. This is typically a left to right movement.

3.2.8 Add additional 0.5% TBE to the chamber if the gel is not completely submerged.

3.2.9 Turn on the power supply and set the intended voltage (80-120v).

3.2.10 Connect the chamber electrodes to the power supply and close the lid on the chamber. The current is running if the electrodes cause bubbling at the edges of the chamber.

3.2.11 Electrophorese PCR products at desired voltage for desired time (~20-40 minutes), or until the loading dye reaches the edge of the gel. A lower voltage will take longer to electrophorese, but will produce more clear separation between bands of different molecular weights.

3.2.12 When the gel is finished electrophoresing, turn off the power supply to stop the current, then open the lid of the chamber (Make sure no one else is using the power supply for a second chamber before turning it off. Opening the lid will stop the current if the power supply is still on)

3.2.13 Unplug the electrodes from the power supply.

3.2.14 Carefully remove your gel from the chamber and proceed to the gel imaging room

3.3 Gel imaging

3.3.1 Wearing nitrile gloves, move your gel from the electrophoresis chamber into the ethidium bromide (EtBr) stain in the gel imaging room.
3.3.2 Allow the gel to stain for 1 minute (more if the EtBr solution was prepared >2 weeks ago)
3.3.3 Remove the gel from the EtBr staining solution with the provided spatula and place in a container of fresh tap water to de-stain for 1 hour.
3.3.4 After 1 hour of de-staining, turn on the Gel Doc imager and start the ImageLab program.
3.3.5 Select the imaging protocol appropriate for your gel (see a senior lab member for assistance with this)
3.3.6 Place the gel on the imaging tray and wipe away excess liquid before closing the tray.
3.3.7 Image your gel and save the results.
3.3.8 Remove the gel from the Gel Doc tray and leave to dry in the provided space.
3.3.9 Wipe down the Gel Doc tray gently with kimwipes and turn off the device.
3.3.10 The de-stain water can be poured down the drain, while paper trash and gloves must be disposed of in the labeled bins in this room. Do not leave this room with anything that has contacted the EtBr directly or the gel itself, including gels, kimwipes, gloves, gels.

SECTION 4 TROUBLESHOOTING

4.1 No PCR product, or streaky bands present on gel

When preparing reactions using 96 well plates, a sterile basin is used to both prepare the master mix, and to aliquot the master mix into tubes using a multi-channel pipette. If multiple plates are being prepared simultaneously (i.e. >96 reactions), it is important to mix the contents of the sterile basin throughout aliquoting (this can be done by simply rocking the sterile basin back and forth a few times). Failure to ensure that the master mix remains thoroughly mixed while aliquoting may result in amplicons of multiple lengths, or may result in failed reactions.

4.2 Failed PCR due to high template concentration

Addition of too much template will inhibit the reaction by exhausting the dNTPs and primers added, before full length products can be generated. If the lysate appears very turbid, dilute the template 1:10 or 1:100 and then use 1 µL of the diluted template for the PCR.

4.3 Primer dimers present in gel electrophoresed PCR product

If primer dimers (strong bands of approximately 20-30 bp) appear, use the Qiagen PCR Product Purification kit to purify the PCR product before submitting the product for sequencing.

4.4 Unspecific products

If the gel electrophoresis yields multiple PCR products, try running the PCR using a single annealing temperature (i.e. do not run the PCR as a touchdown reaction). Alternatively, the reaction can be run performed using a hot-start enzyme. If the primers are not specific, try re-designing the primers to include fewer ambiguous bases and increase their length.

SECTION 5 REFERENCES

"High Throughput PCR and Sequencing Reactions Protocol 10 Sep 15" Cornell Food Safety Lab Wiki

SECTION 6 METHOD VERSION & CHANGES

VERSION	DATE	EDITOR	COMMENTS
Version 1	10/11/2019		Original SOP
Version 2	04/10/2020	Ser15	Added Section 6, Version Control