Molecular Subtyping of *Listeria monocytogenes* and *Listeria species* by Pulsed-field Gel Electrophoresis (PFGE)

FILE NAME: 21MAY13_LM_PFGE_SOP

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03JUN13

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Dr. Martin Wiedmann (date)

AUTHORED BY:

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(Name) (date)

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SECTION 1 INTRODUCTION

1.1 Purpose: This protocol describes the standardized method of molecular typing by PFGE of *Listeria monocytogenes* and *Listeria* species as to be used in the FSL laboratory. This SOP is a recreation of the standardized CDC Pulsenet protocol (original PulseNet protocol can be found under the reference section of this SOP).

1.2 Scope: This procedure is used for molecular typing by PFGE of *Listeria monocytogenes* and *Listeria* species under the supervision of Martin Wiedmann within the department of Food Science at Cornell University.

1.3 Definitions/Acronyms

PFGE: Pulsed Field Gel Electrophoresis
BHI: Brain Heat Infusion Agar
CSB: Cell Suspension Buffer
CLB: Cell Lysis Buffer
SKG: SeaKem Gold agarose
PK: Proteinase K
EDTA: Ethylenediaminetetraacetic Acid
TBE: Tris Base EDTA
TE: Tris-HCl EDTA
RPM: Revolutions Per Minute
ddH_2O: double distilled/deionized H_2O
RCT: Red-capped tubes
GCT: Green-capped tubes
EtBr: Ethidium Bromide
EtOH: Ethanol

SECTION 2 MATERIALS

Reagents
1M Tris HCl, pH 8.0
0.5M EDTA, pH 8.0
TE Buffer, pH 8.0
Cell Suspension Buffer (CSB)
Cell Lysis Buffer (CLB)
10X TBE, pH 8.0
0.5X TBE
dd H_2O (autoclaved)
d H_2O
Lyophilized Proteinase K (Roche Applied Science)
Lyophilized Lysozyme (Sigma-Aldrich BioUltra)
Restriction Buffer A (Roche Applied Sciences) – buffer for *Apa*I

Restriction Enzyme *Apa*I (40U/µL, Roche Applied Sciences)
Restriction Buffer NEB4 (New England Biolabs) – buffer for *Asc*I
Restriction Enzyme *Asc*I (10U/µL, New England Biolabs)
100X Bovine Serum Albumin (BSA) (New England Biolabs)
Restriction Buffer H (Roche Applied Sciences) – buffer for *Xba*I
Restriction Enzyme *Xba*I (10U/µL, Roche Applied Sciences) **(For *Salmonella* H9812 digestion)**
SKG Agarose (LONZA)
Ethidium Bromide (1mg/mL)

Materials
BHI agar plates
Loops
Sterile wooden sticks
Red-capped tubes (RCT), Borosilicate Tubes 13x100 mm
Green-capped tubes (GCT), Borosilicate Tubes 16x125 mm
Sterile cotton swabs
Micropipettes and tips. Volumes: 20 µL, 200 µL, and 1000 µL
Weighing dishes
Razor
Spatula
Sterile 125 mL bottles
Sterile 1.5 mL Eppendorf tubes
Sterile 15 mL conical tube
Sterile 50 mL conical tube
Plug Mold (BioRad)
Screened caps (BioRad)
Microscope slides
1000 mL calibrated cylinder
100 mL calibrated cylinder
2000 mL Erlenmeyer flask
Gel mold, frame, and comb (BioRad)
Level tool
70% Ethanol

Equipment
Spectrophotometer
Analytical balance
Water baths (37 °C, 55–60 °C, and 25 °C)
Incubators (37 °C and 54 °C)
Pulsed-field electrophoresis chamber
Cooling module
Control module
Drive module
GelDoc Imaging System, BioRAD

SECTION 3 PROCEDURE

Overview of Typical Work Flow:

Day 1: Streak the isolates on BHI agar and incubate 16–18 h at 37 °C.
Day 2: Plug Preparation & Washing
Day 3: Restriction Digestion (Day 3 and 4 procedures can be finished in 1day)
Day 4: Gel Run
Day 5: Gel Staining & Photographing Gel Image

3.1. Streak isolates for overnight growth:

***Note:** Before working with BSL-2 organisms please review proper handling techniques.

1. Using a sterile wooden stick, streak out isolate(s) from glycerol frozen stock onto a BHI plate. Streak bacterial isolates in a zigzag horizontal pattern until 1/3 of the plate is covered (lawn).
   a. Rotate the plate about 90 degrees, and using a sterile loop start spreading the bacteria, at the end of the lawn, in a zigzag horizontal pattern. Repeat this step one more time. This procedure will result in isolated colonies.
2. Allow bacteria to grow in 37 °C incubator overnight (16–18 hours).
   *Note: Always use freshly streaked plates for preparation of cell suspensions as cells are easier to lyse. Overnight growth should be no longer than 18 hours.
3. Streak *S.* Braenderup (FSL W3-083) to make reference plugs. Reference plugs are made in the same manner all other *Salmonella* plugs are made; refer to “Salm_PFGE_SOP”.

3.2. Plug Preparation:

1. Turn both water baths on, the 37 °C and 55–60 °C.
2. Turn on the 54 °C incubator shaker.
3. Follow the spectrophotometer instructions on Appendix 1.
4. Briefly, turn on spectrophotometer, set wavelength to 610 nm, and allow to warm up for ~15 minutes.
5. For each isolate: label a RCT (these will be used to prepare cell suspensions), a 1.5 mL Eppendorf tube, and a 50 mL conical tube.
6. Fill RCTs with 3.5 mL TE. Fill twice as many GCTs with 2.0 mL TE (these will be used for measuring ODs and altering concentration of the cell suspensions)
7. Blank spectrophotometer with 3 mL of TE in a GCT.
8. Using a sterile cotton swab that has been pre-moistened with TE, remove

cells from the lawn on the BHI plate and suspend the cells in the TE within the RCT by gently swirling sterile swab.

9. Gently, pipette up and down the cell suspension to achieve a homogeneous suspension of cells.
10. Transfer 1.0 mL of this suspension into a GCT containing 2.0 mL TE, and pipette up and down again.
11. Measure the OD_{610} of the GCT cell suspension. Target OD_{610} range is 0.340–0.430.
12. After achieving an OD_{610} within the target range, place the RCT cell suspensions on ice. Repeat steps 8–12 for all isolates.
    *Note: If the OD_{610} is not within the target range, adjust it adding more bacterial colonies or diluting it with TE.
13. Distribute 400 µL of adjusted cell suspensions into labeled 1.5 mL Eppendorf tubes and incubate in 37 °C water bath for 10 minutes.
14. Prepare 20 mg/mL lysozyme solution:

Lysozyme solution	Amount of plugs
0.010 g lysozyme + 500 µL TE	~24 plugs
0.013 g lysozyme + 650 µL TE	~31 plugs

15. Remove cell suspensions from 37 °C water bath and add 20 µL of the Lysozyme solution to each tube and incubate tubes in 55–60 °C water bath for 20 minutes.
16. Prepare 20 mg/mL Proteinase K solution (to be used in plug prep and in lysing solution):

Proteinase K solution	Amount of plugs
0.013 g PK + 650 µL dd H_2O	~12–14 plugs
0.020 g PK + 1000 µL dd H_2O	~20–21 plugs

17. Prepare 1.0% SKG agarose in sterile bottle or flask:
    0.25 g SKG + 23.5 mL TE Buffer → Microwave until completely dissolved.
    *Note: Agarose solution has to be microwaved until all the agarose is dissolved (about 1–2 minutes). Failure to do so will result in plugs that could easily break.
18. Place the agarose solution in the 55–60 °C water bath.
19. Remove cell suspensions from 55–60 °C water bath and add 20 µL of the PK solution to each tube.
20. Mix 400 µL of the SKG agarose solution with the 400 µL cell suspension/PK solution and mix by gently pipetting up and down (no more than 3–4 times to avoid agarose solidification) and immediately cast into cleaned plug mold.
    *Note: Try to avoid bubble formation.
21. Allow the plugs to solidify for 15 minutes.
22. Prepare lysing solution master mix in sterile bottle:
    n= # of isolates

5mL CLB x (n+1) =	
25µL PK x (n+1) =	

23. Distribute 5 mL of lysing solution master mix into each 50 mL conical tube.
24. Open the plug molds carefully, and using a cleaned spatula transfer solidified plugs into corresponding conical tube.
    *Spatula can be wiped with 70 % EtOH in between plugs.
25. Lyse the bacterial cells in the plugs, within conical tubes, in shaker incubator at 54 °C/ 170 RPM for 3.5 hours.

3.3. Plug Washing:

1. Before the washes, pre-warm the ddH_2O and the TE buffer at 50 °C in the water bath.
2. After cells have been lysed, remove conical lid and replace with green screen caps.
3. Drain lysing solution from plugs and replace with 10 mL autoclaved ddH_2O. Return plugs to shaker incubator for 15 minutes at 50 °C/ 70 RPM.
4. Drain and repeat one time (Total of 2 washes with ddH_2O)
5. Drain the ddH_2O and add 10 mL TE buffer. Return to shaker incubator for 15 minutes at 50 °C/ 70 RPM.
6. Drain and repeat three more times (Total of 4 washes with TE buffer).
7. Drain the remaining buffer, remove the screen caps, add 5 mL TE, and put lid on.
8. Store the tubes at 4 °C until proceeding to next step of protocol.
   *Note: Plugs stored properly can be digested and run after up to two years.

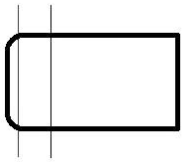
3.4. Restriction Digest of Agarose Slices (*Asc*I & *Apa*I) and the *Salmonella* Reference Agarose Slices (*Xba*I):

1. Label two 1.5 mL Eppendorf tubes for each isolate (one for *Asc*I and one for *Apa*I digestion).
2. Label two 15 mL conical tube for **A buffer solution** and *Apa*I **enzyme solution**, and other two for **NEB4 buffer solution** and *Asc*I **enzyme solution**.
3. Prepare buffer solutions:

A buffer solution (*Apa*I)	Amount/ plug slice	x (n+1)	Amount in Master mix:
Autoclaved dd H_2O	135 µL		
Restriction Buffer A	15 µL		

NEB4 buffer solution (*Asc*I)	Amount/ plug slice	x (n+1)	Amount in Master mix:
Autoclaved dd H_2O	180 µL		
Restriction Buffer NEB4	20 µL		

4. Distribute 150 µL of A buffer solution into each Eppendorf tube.
5. Distribute 200 µL of NEB4 buffer solution into each Eppendorf tube.
6. Using cleaned razor and spatula, cut rounded bottom edge of plug off and discard. Cut two ~3 mm slices from the plug (as depicted below) and place one in A buffer solution and the other in NEB4 buffer solution. Do not cut slices smaller than 2mm.



*Note: The shape of your plug slice will determine the shape of your resulting pattern, so it is important to cut a clean sharp rectangle. Spatula can be cleaned with 70% EtOH in between plugs.

7. Incubate Buffer A slices in 25 °C water bath for 10–15 minutes.
8. Incubate Buffer NEB4 slices in 37 °C water bath for 10–15 minutes.
9. Prepare restriction enzyme solutions:

***Apa*I enzyme solution**	Amount/ plug slice	x (n+1)	Amount in Master mix:
Autoclaved dd H_2O	131.75 µL		
Restriction Buffer A	15 µL		
Restriction Enzyme *Apa*I (40U/µL)	1.25 µL		
100X BSA	2 µL		

***Asc*I enzyme solution**	Amount/ plug slice	x (n+1)	Amount in Master mix:
Autoclaved dd H_2O	175.5 µL		
Restriction Buffer NEB4	20 µL		
Restriction Enzyme *Asc*I (10U/µL)	2.5 µL		
100X BSA	2 µL		

10. Take out the tubes from the water baths, and dry their external surface. Aspirate off buffer solution from plug slices and replace with 150 µL *Apa*I enzyme solution 200 µL *Asc*I enzyme solution.

11. Return tubes to respective water baths, tubes for *Apa*I at 25 °C and *Asc*I at 37 °C, for 3–7 hours.
12. After digestion, aspirate off enzyme solution in the Eppendorf tubes, replace it with 200 µL TE Buffer and store in 4 °C until next step of the protocol.
    *Note: A digested plug could be store in at 4 °C for up to two weeks.

Restriction Digest of *Salmonella* Reference Agarose Slices (*Xba*I)

1. Label one 1.5 mL Eppendorf tubes per isolate.
2. Label two 15 mL conical tube for **H buffer** solution and ***Xba*I enzyme** solution.
3. Prepare **H buffer** solution:

H buffer solution	Amount/ plug slice	x (n+1)	Amount in Master mix:
Autoclaved dd H_2O	180 µL		
Restriction Buffer H	20 µL		

4. Distribute 200 µL of restriction buffer solution into each Eppendorf tube.
5. Cut ~3 mm plugs as described in step 6 of Section 3.4.
6. Incubate Eppendorf tubes (with H buffer solution+slices) in 37 °C water bath for 10 minutes.
7. Prepare restriction enzyme solution:

***Xba*I solution**	Amount/ plug slice	x (n+1)	Amount in Master mix:
Autoclaved dd H_2O	173 µL		
Restriction Buffer H	20 µL		
Restriction Enzyme *Xba*I (10U/µL)	5 µL		
BSA	2 µL		

8. Take out the tubes from the water bath, and dry their external surface. With pipettor tip, aspirate off buffer solution from plug slices and replace with 200 µL *Xba*I enzyme solution.
9. Return slices to the 37 °C water bath, and incubate for 5.5 hours.
10. After digestion, aspirate off enzyme solution in the Eppendorf tubes, replace it with 200 µL TE Buffer and store in 4 °C until next step of the protocol.
    *Note: You can digest your reference plugs ahead of time. A digested plug could be store in at 4 °C for up to two weeks.

3.5. Gel preparation and gel run:

***Note: Please refer to Appendix 2 for screen shots of the Equipment and front panels.**

1. Turn on 55–60 °C water bath.
2. Before the gel is cast, the pulsed-field electrophoresis chamber needs to be

leveled.

a. Place the level tool in the center of the chamber. If it is not leveled, adjust the corner feet of the chamber accordingly.

3. Prepare the running buffer and pour it into the pulsed-field electrophoresis chamber.

Reagents	Volumes
dH_2O	2090 mL
10X TBE	110 mL

*Note: After diluting the 10X TBE its concentration changes to 0.5X

4. Turn on the system (CHEF-Mapper and CHEF-DRII) in the following order and make sure that the pump is set up at 70 RPM (Fig. 1):

Steps	CHEF-Mapper (Location of button)	CHEF-DRII (Location of button)
1	Control Module (front)	Drive Module (back)
2	Pump (front of Control Module)	Control Module (right side)
3	Cooling Module (front)	Pump (front of Drive Module)
4	-	Cooling Module (front)

5. The Cooling module temperature should be set up at 14 °C, and the "Actual Temperature" button (on the front of the Cooling module) should be pushed (Fig. 2).

6. Prepare 1% SKG gel in sterile flask:

Reagents	10-well gel (small gel)	15-well gel (large gel)
SKG agarose	1.0 g	1.5 g
10X TBE	5.0 mL	7.5 mL
d H_2O	95.0 mL	142.5 mL

7. To avoid additional water loss in the microwave process, weigh gel flask (containing agarose, and 10X TBE diluted in d H_2O) and zero scale. Add 8 mL autoclaved d H_2O and microwave. Microwave gel flask until these additional 8.0 mL of water has evaporated and scale returns to zero.

8. Put the flask in the 55–60 °C water bath for 10 minutes.
*Optional: Take the flask out of the water bath and let it stand on the bench for 5 minutes.

9. Assemble and clean the gel mold with 70% EtOH, and make sure it is level, using the level tool. If it is not level, move the mold over a bench where it is level. Cover the gel mold until the gel is poured.

10. Pour gel into cleaned gel mold. Save last 1.5–2.0 mL of gel for sealing the wells in an Eppendorf tube. Keep extra gel (in the tube) from solidifying by placing in 55–60 °C water bath.
*Note: Do not let gel stand for a long time before pouring as this will result in a "swirly" effect in the solidified gel.

11. Cover gel and gel mold with tupperware container to avoid dust falling into the mold.
12. Allow gel to solidify for 20–25 minutes.
13. Remove digested plug slices from 4 °C and aspirate off TE Buffer. Replace with 200 µL of 0.5X TBE.
14. Using a cleaned spatula, remove slices from Eppendorf tubes and blot slice gently with Kimwipe to remove excess of 0.5X TBE Buffer and load into well. *Note: When loading slices it is important to not damage/ crush slices, load them unevenly within the well, or create air bubbles in wells. All these will disrupt the resulting patterns.
15. Push the plugs carefully against the well's wall towards you, this will help to avoid bubbles.
16. If using 10-well gel, lanes 1 and 10 should be used for reference slices. If using 15-well gel, lanes 1, 8, and 15 should be used for reference slices.
17. When all slices are loaded, seal slices into wells by slowly pipetting saved agarose from step #10 onto slices.
18. Put the agarose gel into the frame within the electrophoresis chamber.
19. Set up the parameters on the Control module.

Parameters for PFGE (Fig. 3 & 4):

CHEF-DR II

Steps	Instructions	Numerical input
1	Push the "block" button and make sure that set up to **b-1**.	b-1
2	Push the "Volts/cm" button and set up the time.	V = 6
3	Simultaneously, push the "Block" button and the "Volts/cm" buttons to set up the Initial switch.	Initial Switch = 4.0 s
4	Simultaneously, push the "Volts/cm" button and the "Run Time" buttons to set up the Final switch.	Final Switch = 40.0 s
5	Push the "Run Time" button and set up the time.	Small 10-well gel = 17.5 hr Large 15-well gel = 18.5 hr

CHEF-Mapper

Steps	Instructions	Numerical input
1	Push the "Auto Algorithm" button.	-
2	"Low [ ]" and "High [ ]" will show on the screen of the Control module. You have to type the values as well as the units For Low: -Type 49, and then push the "Kb" button.	Low = 49 Kb

	-Push “enter” button. For High: -Type 450, and then push the “Kb” button. -Push “enter” button.	High = 450 Kb
3	Percent (%) of agarose should be set up already at 1% -Push “enter” button.	1 % Agarose
4	Gradient should be already set up at 6.0 V/cm. -Push “enter” button.	6.0 V/cm
5	“Run Time [ ]” will show on the screen. You have to type the values as well as the units. -Type 18.5, and then push the “hours” button. -Push “enter” button.	Small 10-well gel = 18.5–19 hr Large 15-well gel = 19.5–20 hr
6	“Included Angle” should be set up already at 120 ° -Push “enter” button.	120 °
7	“Initial Switch Time [ ]” and “Final Switch Time [ ]” will show on the screen. You have to type the values as well as the units For Initial Switch Time: -Type 4.0, and then push the “seconds” button. -Push “enter” button. For Final Switch Time: -Type 40.0, and then push the “seconds” button. -Push “enter” button.	 Initial Switch = 4.0 s Final Switch = 40.0 s
8	Push “Start Run”	

20. After setting up the machine, write down the Current (mA)
*mA at t=0 should be ~150

*Run times must be empirically determined, make sure every three months that the running times are still appropriate. Replace electrodes when necessary.

3.6. Staining and Photographing Gel:

1. Go to the staining room and dispose properly the Ethidium Bromide in the flask with the destaining tea bags please see **Appendix 3**.
2. Prepare the staining solution in a large container. *Note: Make sure you prepare it fresh EVERY time:

700 µL EtBr (1mg/mL)	700 mL d H_2O

3. Fill the destaining container with 2–3 L of water (not with tap water).
4. Turn off the Cooling Module, the pump, and Control Module (Appx. 2 Fig. 1)
5. Detach the hoses from the pulsed-field electrophoresis chamber, and drain it.
6. Open the chamber, remove the gel and take it to the staining container.
7. Stain for 40 minutes and destain it for ~1 hour. While staining you MUST clean the pulsed-field electrophoresis chamber as described at the end of this Section, entitled: "Cleaning the PFGE Equipment".

***Note: Please refer to Appendix 4 for screen shots of GelDoc imaging system and QuantityOne software.**

8. Take the gel to the GelDoc imaging system, turn it on (switch on the back of the device). (Fig. 1)
9. Open the drawer and place the gel on the top of it. Close it.
10. Click on the QuantityOne software icon on the computer screen. (Fig. 2)
11. Click on "Free Trial" on the screen (Fig. 3)
12. Open Live image by selecting File > GelDoc XR.
13. Push the "Epi white" button on the GelDoc system panel (Fig. 1).
14. Open the door of the GelDoc system and center gel while looking at the grid on the computer screen (Figure 4). If you do not see the grid click on "Live/Focus". It is important to center gel as this will be the final image uploaded into BioNumerics for analysis.
15. Push the "Trans UV" button on the GelDoc system panel (Fig. 1).
16. Click on "Manual Acquire" on the computer screen (Fig. 4)
17. A good starting exposure time should be ~0.500. If necessary adjust exposure and focus on the computer screen (Fig. 4).
18. Click on "Freeze" on the screen, and immediately push the "Trans UV" button on the GelDoc system panel. It is important to turn off the UV light after taking the picture to avoid overexposure.
19. Double click on "Save" and choose the folder you want to store your picture. If your isolates are from a client, save the picture within the PFGE folder in the server, >Listeria folder >Client's folder. Save it as Net ID+Date+time of the gel, for example " mw16 05-07-2013 12:36pm"
20. Double click on "Print" and paste the copy for your lab notebook (Fig. 4).
21. Once the gel picture has been captured, the file must be exported into a TIFF image file that BioNumerics recognizes.
22. To export into TIFF image follow these steps, click on: File > Export to TIFF image
    a. Under Publishing section click on "Export view excluding overlays"

b. Under Resolution section click on “Same as Scan”
c. Then click on “Export”

23. For information regarding processing gel fingerprint and labeling in BioNumerics, refer to SOP entitled “CU Naming SOP”.
24. After the image is captured and exported, return the gel into the destaining container and clean the PFGE equipment, as described below.

3.7. Cleaning the PFGE equipment

1. Make sure that the pulsed-field electrophoresis chamber is completely drained. Tilt the chamber to the right and drain the rest of the liquid through the hose in the front of the chamber.
2. Reattach the hoses to the appropriate ports and add ˜2000 mL of d H_2O (not autoclaved) into the chamber. Turn on the Control Module and Pump but not the Cooling module, and let the water recirculate into the system for 30 minutes.
3. After the chamber is rinsed, drain it. Remove all the water from the chamber as well as the hoses.

SECTION 4 REPORTING and LABELING

All data about isolates, PFGE protocol & steps, amount of reagents used, and gel results (including a picture of the gel) should be kept in your official lab notebook.

SECTION 5 TROUBLESHOOTING

Problems previously encountered with PFGE include:

(1) Smeary patterns usually results from exonuclease contamination in the plug itself or in the digestion reaction. Be careful as to not touch the plug to anything not sterilized or that has not been cleaned with ethanol (i.e. Razor blades, tubes, spatula, etc.)
(2) Poor pattern quality; incomplete digest/ partials: Use BSA in all digestion reactions; Incubate for longer periods of time (4–6 hrs); not optimal digestion reaction: check digestion calculations and reagents.

Rationales for modifications to the CDC PulseNet protocol:
(1) Increased OD yields brighter/ more intense bands even at low MW.
(2) Increased plug lysis time yields better lysis of plugs, therefore resulting in better quality patterns.
(3) Changes to digestion reaction mixtures were based on old CDC PulseNet PFGE protocols and we have found original recipes produce better patterns.
(4) In some instances, enzymes are ordered in high concentration as they are more cost effective. However, number of units per digestion rxn/ slice remains constant.
(5) Digest durations have been extended to decrease likelihood of partial/ incomplete digests.

ADDITIONAL HELP

Below is a link for additional trouble-shooting help made available by PulseNet International:

http://www.pulsenetinternational.org/SiteCollectionDocuments/pfge/PFGE_troubleshooting.pdf

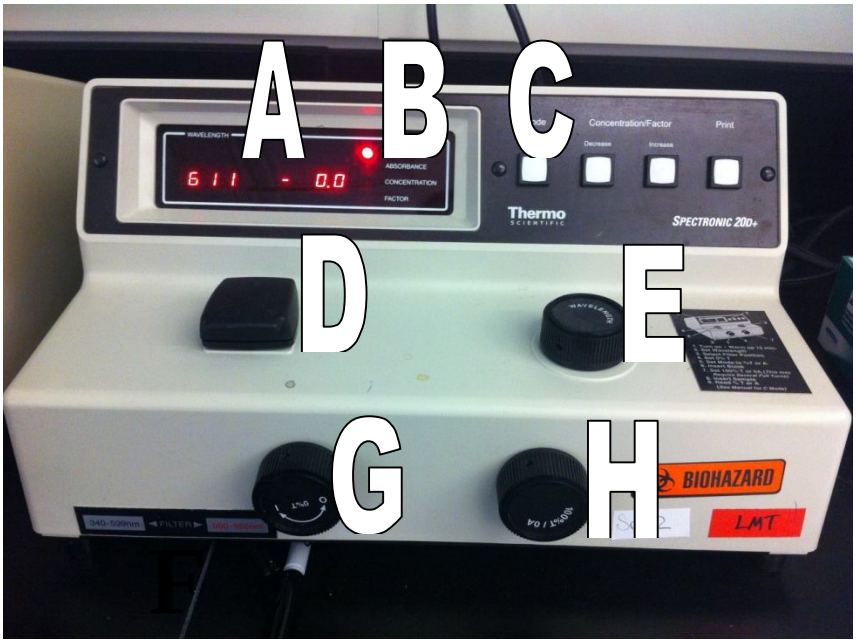
SECTION 6 REFERENCES

1. http://pulsenetinternational.org/downloads/pfge/5.3_2009_PNetStandProtLMonocytogenes.pdf
2. **Graves, L.M., B. Swaminathan.** 2001. PulseNet standardization protocol for subtyping *Listeria monocytogenes* by macrorestriction and pulsed-field gel electrophoresis. Int J Food Micro. 65: 55–62.

SECTION 7 APPENDICES

Appendix 1. To measure OD of tubes:

1. Turn on the machine on the left knob (G), let it warm up ~15 minutes.
2. It will start automatically in Transmittance mode (B), the red light is next to the Transmittance option. *Note: It is important to use the appropriate tube holder size in slot D.
3. Change the lever (F) to select the appropriate filter for the wavelength used in your experiment.
4. Use knob (E) to adjust the wavelength on the digital readout (A).
5. With no tube in use (slot D is empty), turn the left knob (G) to set the display (A) to 0.0 (0% Transmittance)
6. Put in your broth/buffer blank tube in slot D and use the right knob (H) to set the Transmittance to 100. (100% Transmittance = 0.000 Absorbance)
7. Push the "Mode" button (C) to change the status from Transmittance to Absorbance. You will see that the "100" on the screen (A) will change to 0.000.
8. Put the sample tube in slot D, remove the tube cap and shut the top of the compartment (D).
9. Record the Absorbance displayed on the screen.



Legend:

A. Digital readout
B. Status indicators
C. Mode
D. Sample compartment (slot)
E. Wavelength control
F. Wavelength filter (lever)
G. Power Switch/Zero control
H. Transmittance/Absorbance control

Appendix 2. PFGE equipment and front panels

Figure 1. PFGE Equipment

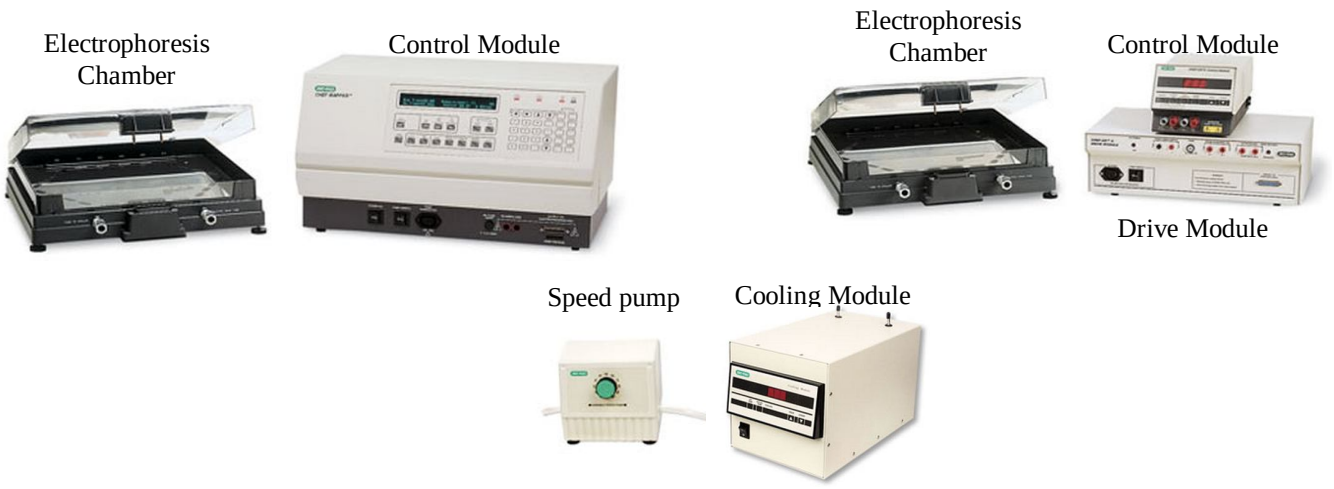


Figure 2. Front panel of Cooling Module

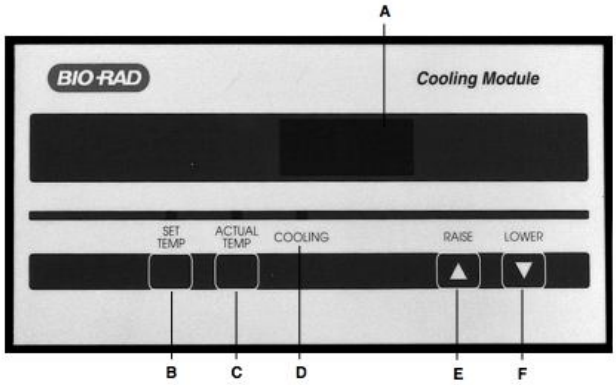
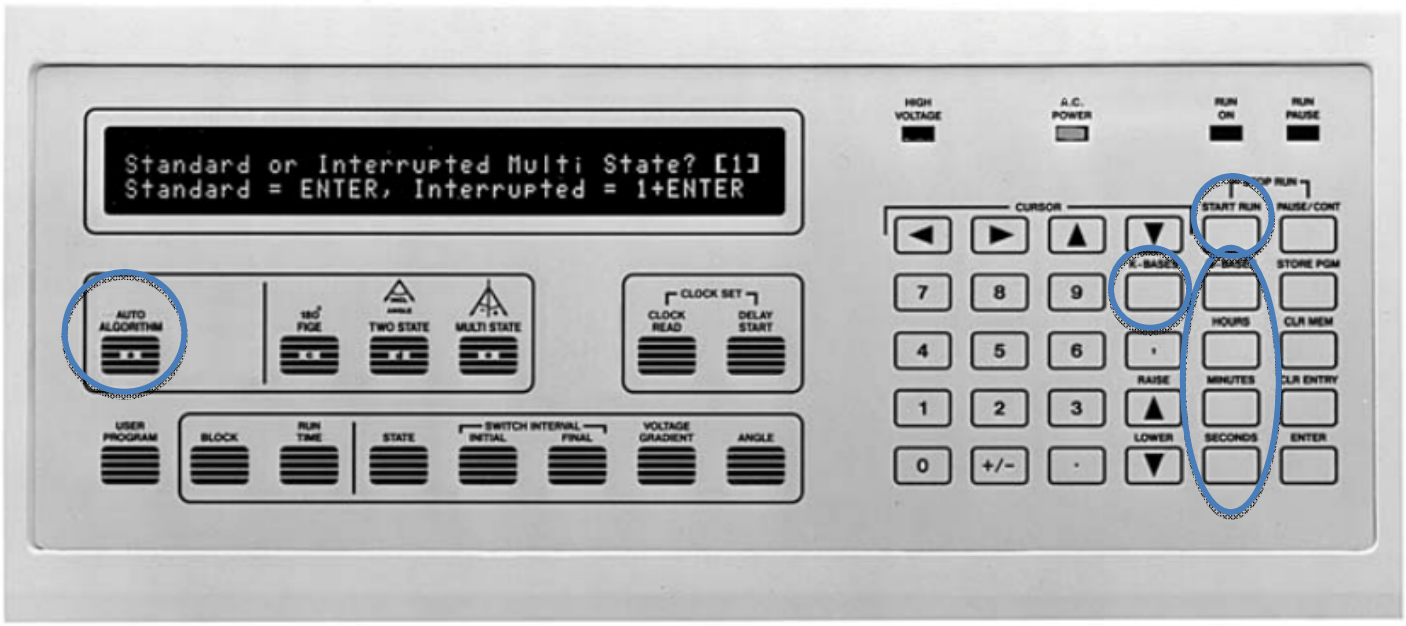


Figure 3. Front panel of the CHEF-DR II

Figure 4. Front panel of the CHEF-Mapper



Appendix 3. Proper disposal of Ethidium Bromide

1. Check the container covered with Aluminum foil to see if it has EtBr solution in it.
2. If it has then pour the content of the container into one of the two 1000 mL Erlenmeyer flasks with the destaining tea bags inside.
3. After you poured the EtBr into the container, DO NOT forget to write it in the log sheet pasted on the wall.
4. Write your initials and the date you did it.
5. Wait at least 24 hours after dumping the liquid from the flask through the drain.
6. After 7th occasion you poured the EtBr into the flask, take out the tea bag and place it in the container where the rest of used tea bags are.
7. Put a new tea bag inside the flask, and write the date and initials.

Appendix 4. Gel imaging system and software.

Figure 1. GelDoc imaging system from BioRad

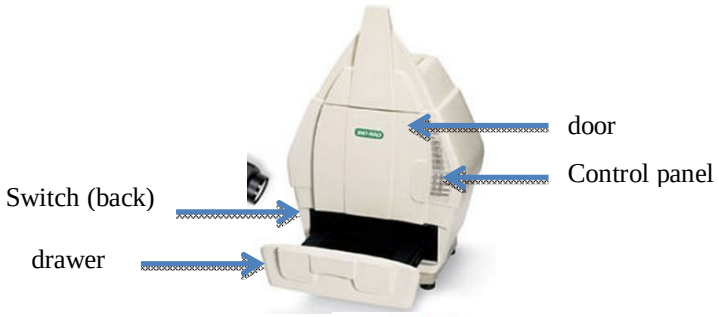


Figure 2. Quantity One software icon

Figure 3. First screen within Quantity One software

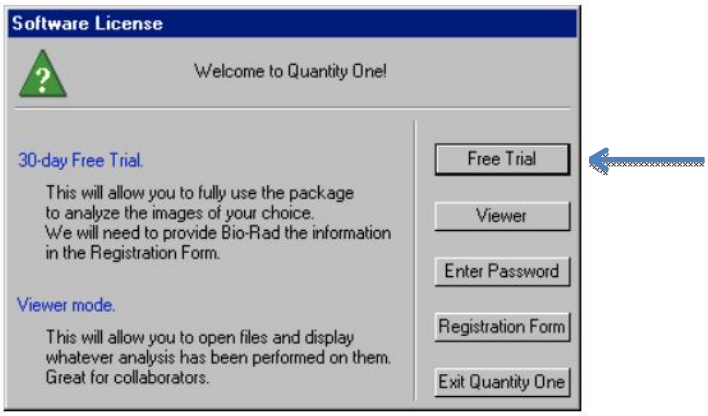


Figure 4. Main Screen within Quantity One software