Pulsed Field Gel Electrophoresis

Preparing and Running a Gel

This protocol for preparing and running a gel includes guidelines about casting an agarose gel, gel electrophoresis, recommendations for avoiding bubbles in the tubing and preventing curved or slanted lanes, electrophoresis run length, effect of voltage gradient and using incorrect electrophoresis conditions, and equipment maintenance.
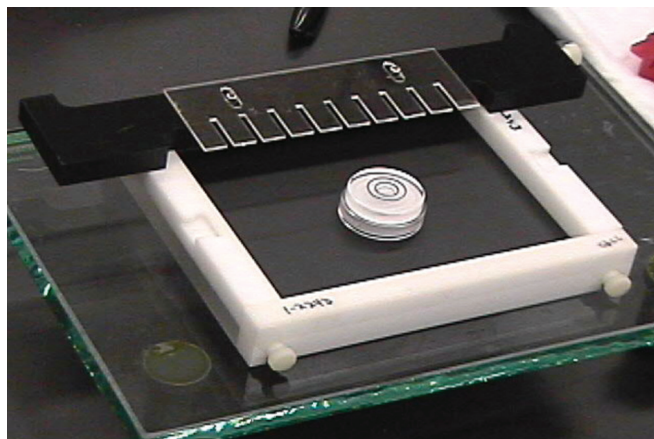
Casting an Agarose Gel

Purpose

An agarose matrix must be prepared through which the DNA molecules will be separated.

Process

- Melt a 1% agarose solution completely and allow it to equilibrate to 55–60ºC for 15 min before use

  — Use 1 g agarose in 100 ml 0.5× TBE buffer for 10-well gels

  — Use 1.5 g agarose in 150 ml 0.5× TBE buffer for 15-well gels

  — Apply short pulses to the solution in a microwave without boiling over

- Tighten the screws and level the gel form before pouring the gel

- Load the plug slices on the comb and pour the gel around (preferred) or into the wells of a solidified gel

- Allow the agarose to solidify for ~30 min, but do not leave it for more than 40 min because the gel will begin to dehydrate


A video showing how to cast an agarose gel is available at **http://biorad-ads.com/09-0398_CHEF_mapper/09-0398_pop-up.html.**

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Potential Problems

DNA fragments may not run evenly and spots or debris may appear on gel images.

Recommendations

- Use high-quality pulsed field gel electrophoresis (PFGE) grade agarose
- Make sure agarose is completely melted; use a microwave and pulse it in short bursts, but do not boil it over
- Discard expired agarose
- Keep track of lot numbers and discard deteriorated lots that can be correlated with poor results
- Use only glassware, combs, and gel forms that are clean
- Use high-quality water to make the 0.5x TBE buffer used for the gel and running buffer
- Remove air bubbles from the gel before it solidifies
- Remove lint, dust, and visible particulates from the gel before it solidifies
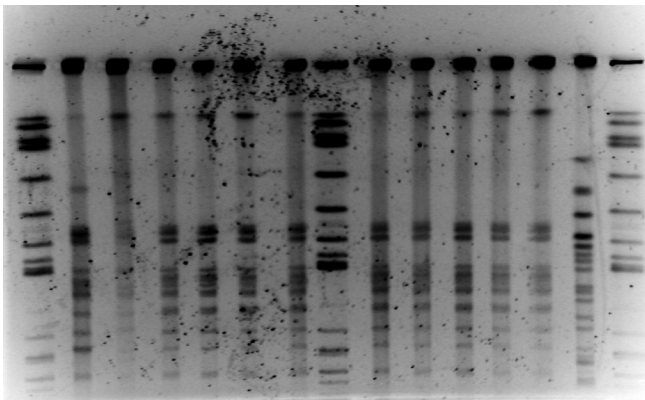
A variety of problems may lead to speckles or spots on the gel image.
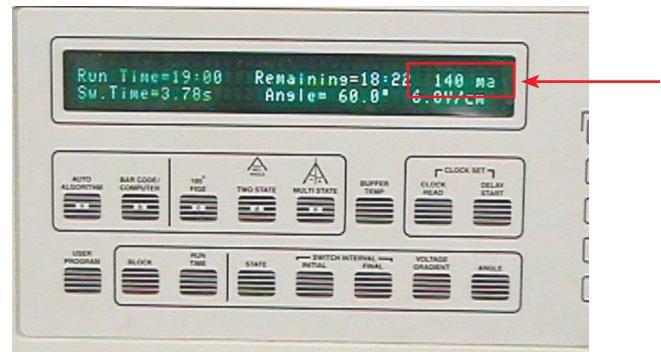
Gel Electrophoresis

Recommendations

1. Check the milliamps (mA) reading at the start of each run. A reading of 120–165 mA is normal. A high (>165 mA) or low (<100 mA) reading suggests a problem with the 0.5× TBE buffer and/or poor water quality.

- Possible reasons for a high mA value (>165):
    - Buffer dilution or formulation problems
    - Poor-quality water was used to prepare the buffer
- Possible reason for a low mA value (<100):
    - Buffer dilution or formulation problem

Note: The mA value will increase during a run (~130–165 mA)

2. Make sure high-quality water is used for the reagents.

- Change filters in the water system regularly
- Avoid prolonged storage in plastic carboys
- Use **sterile**, **ultrapure** (type I or reagent grade) water for all reagents
- Nonsterile, ultrapure water can be used for:
    - 0.5× buffer for 1% agarose gels
    - 0.5× electrophoresis running buffer
    - Destaining gels

Electrophoresis instrument screen showing a normal reading, 140 mA.

Electrophoresis instrument screen showing a low reading, 105 mA.

Avoiding Bubbles in the Tubing

Potential Problems

When Tygon tubing is too long, bubbles can collect inside the tubing. This interferes with steady buffer flow, leading to an uneven flow rate and temperature fluctuations.

Recommendations

- Cut the tubing to an appropriate length so it does not hang at an angle that allows bubbles to collect

- Check that the tubing is relatively straight and free of kinks

- Check for leaks in the tubing and keep connections tight

- Turn on the pump first before the chiller; ice in the line can also affect the circulation and cooling of the buffer

- Confirm the flow-rate is 1 L/min once a year and adjust the pump accordingly

- Confirm the temperature is 14 ± 2°C once a year and replace the probe if necessary

Cooling module and variable-speed pump for CHEF systems.

## Preventing Curved or Slanted Lanes

Factors contributing to lane curvature:

- Gel frame was not level when the agarose was poured

- Gel was not flush against the black frame

- Electrophoresis chamber was not level

- Buffer flow was uneven or interrupted

- Insufficient buffer in the electrophoresis chamber and lines
  - CHEF Mapper® system requires ~2.2 L of buffer
  - Older models require ~2.0 L of buffer

- Broken electrode(s)

Recommendations

- Balance the electrophoresis chamber before starting a run

- Make sure the frame is sealed and the screws are tightened before pouring a gel so the agarose does not leak

- Use a leveling platform to ensure the gel is level when the agarose is poured

- Ensure the pump is working and the flow rate is correct (1 L/min)

- Reverse the connections and flush out debris clogging the lines

For more information, watch the instructional video at **http://biorad-ads.com/09-0398_CHEF_mapper/09-0398_pop-up.html**.
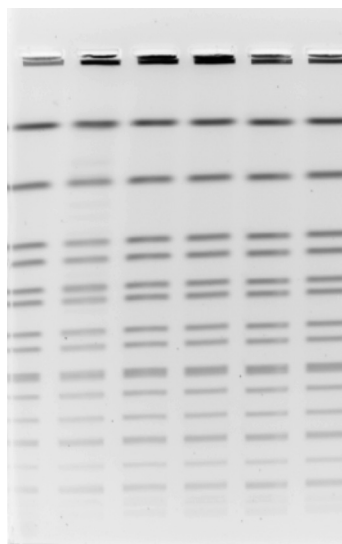
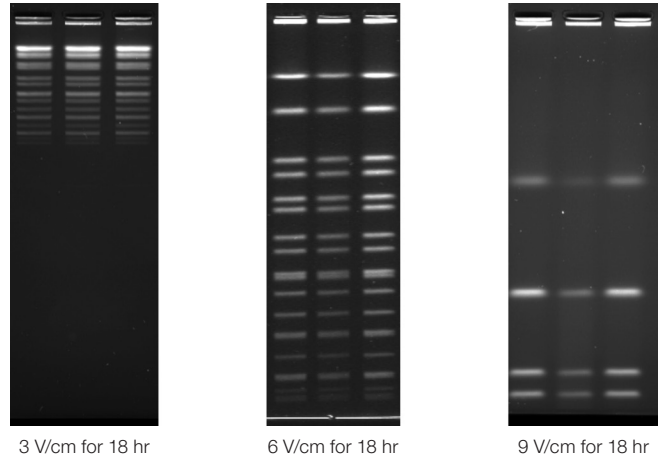Example of a gel with slanted lanes.

Electrophoresis Run Length

The bottom band of the standard (20.5 kb if using PulseNet's universal standard strain) should be 1–1.5 cm from the bottom of the gel. If the gel pattern is too short, increase the run time. This will improve band separation, but the run time should not be increased so much that the smallest band runs off the gel.
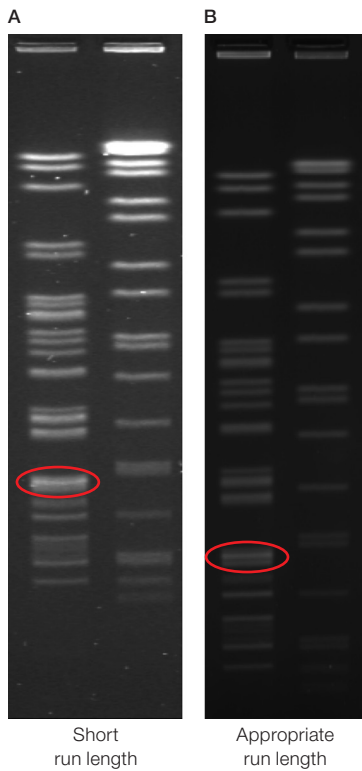
Potential Problems

1. If the run time is too short:

- Pattern is compressed

- Decreased resolution of closely migrating bands

- Normalization of the pattern may be compromised

2. If the run time is too long:

- Bottom band of the standard runs off the gel

- Unable to perform normalization

3. Critical factors that affect run times:

- Composition of 0.5× TBE buffer
  — Commercial buffer vs. in-house

- Buffer pH

- Buffer temperature
  — Ambient temperature
  — Length of tubing

- Gel concentration

A   B

Short   Appropriate
run length   run length

Examples of short and appropriate electrophoresis run lengths.
A, the run time was too short and the doublet was not resolved in the gel;
B, the run time was increased and the doublet was resolved in the gel.

Effect of Voltage Gradient

The voltage gradient also affects band resolution and band appearance in gels and must be adjusted as needed.

3 V/cm for 18 hr   6 V/cm for 18 hr   9 V/cm for 18 hr

The PulseNet universal standard strain, Salmonella enterica, serotype Braenderup H9812, run with different voltage conditions. A, bands are compressed; **B**, appropriate band patterns; **C**, bands run off the gel.
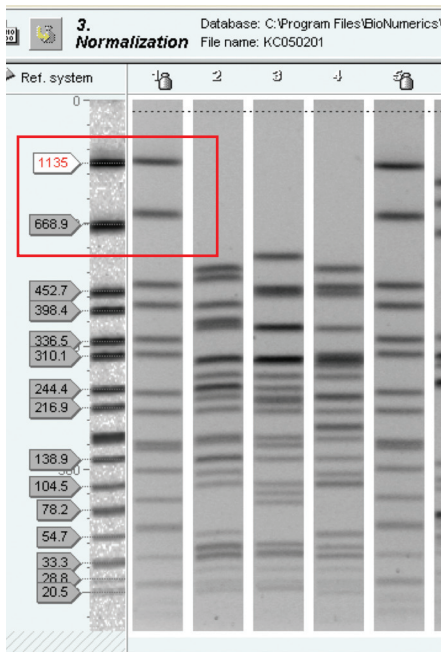
Using Incorrect Electrophoresis Conditions

An example of performing gel electrophoresis using the wrong protocol is shown below. In this case, *E. coli* isolates were run using Salmonella electrophoresis conditions (2.16–63.80 sec instead of 2.16–54.17 sec). This type of error is likely to go unnoticed until analysis. It is important to check for distortion bars (circled). Organisms run using inappropriate conditions must be re-run because it is not possible to correct for inappropriate conditions in the analysis software after a run.

E. coli **isolates run using Salmonella electrophoresis conditions.**

Electrophoresis conditions are similar, however dark bars reveal incorrect band separation and poor normalization.

Equipment Maintenance
Gel Trap Maintenance

1. Remove any small pieces of agarose from the top of the gel trap and the floor of the electrophoresis chamber after the buffer is drained.

2. Check the gel trap is flush with the bottom of the electrophoresis chamber to prevent small pieces of agarose circulating through the lines and clogging the drain port(s), which may affect buffer circulation.

3. Replace broken "feet" in the gel trap as needed.

4. Rinse the chamber and lines with 2 L distilled water to remove residual buffer after a run is complete and the buffer has been drained.

5. Spray the equipment down with 70% ethanol and wipe gently.

For more information, view the instructional video at **http://biorad-ads.com/09-0398_CHEF_mapper/09-0398_pop-up.html.**
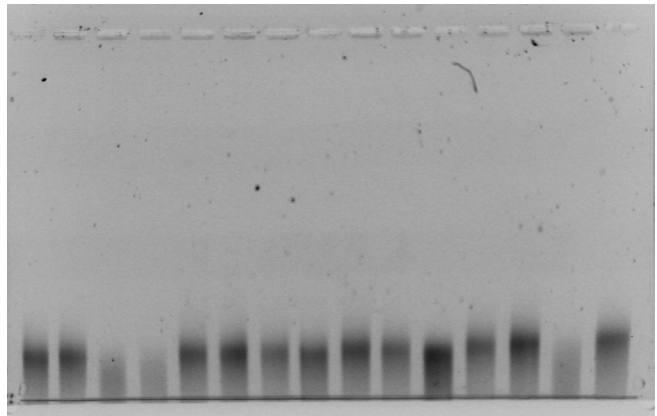
Electrophoresis Chamber

Potential

ProblemsThe chamber and/or tubing can become contaminated, affecting the entire gel. Additionally, a single tube or reagent may be contaminated, affecting only one or several lanes. In either instance, DNA will be a degraded smear on a gel.

Recommendations

Keep the chamber clean and dry between runs.
To decontaminate electrophoresis chambers:

1. Circulate 2 L of 5–10% bleach through the electrophoresis chamber and tubing for 30 min. **Leave chiller off**.

2. Drain the bleach solution from the chamber and tubing.

3. Circulate 2 L distilled water for 15–30 min through the chamberand tubing. Drain and repeat.

4. Run the gel with previously tested PFGE plugs.

5. Repeat cleaning if necessary.

A

B
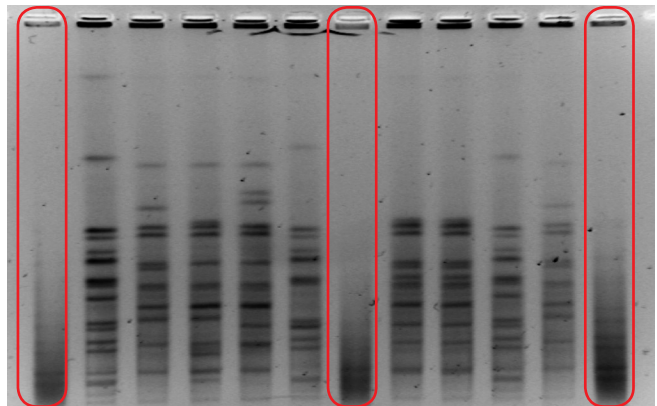
Examples showing how contamination of an electrophoresis chamber impacts gels. A, plug samples were re-run after cleaning the chamber and lines with bleach and rinsing them with water. An additional cycle of decontamination is needed and replacing the tubing may also be necessary. **B**, contamination of tubes containing plugs of Salmonella enterica, serotype Braenderup H9812 (━).

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