Pulsed Field Gel Electrophoresis

Protocol

Bulletin 6223

General Considerations for Troubleshooting PFGE Gels

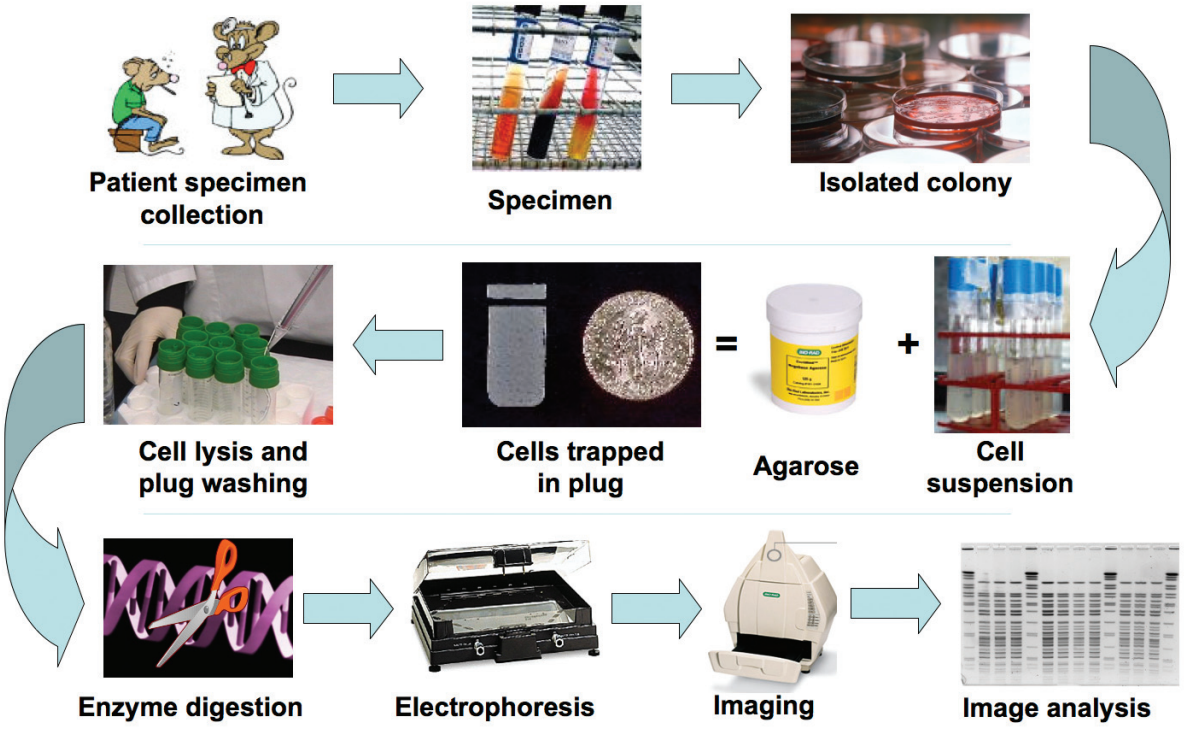
Pulsed field gel electrophoresis (PFGE) has enabled progress in cancer research, food safety, public health, quality control, and genome mapping. It is widely used in molecular epidemiology for strain typing and it has been adopted by PulseNet, a global network of health and food regulatory agency laboratories coordinated by the Centers for Disease Control and Prevention (CDC).

After obtaining a poor PFGE result, troubleshooting is necessary to resolve the problem and ultimately achieve high-quality PFGE gels again. High-quality, reproducible PFGE data allow laboratories to exchange and compare accurate information. For PulseNet member laboratories, PFGE data help prevent disease through real-time subtyping of foodborne pathogens and cluster detection followed by a swift and focused public health response.

Major procedural steps of PFGE include:

- Preparation of cell suspension
- Preparation of agarose plugs
- Lysis of cells in agarose plugs
- Washing of agarose plugs
- Restriction digestion of DNA trapped in agarose plugs
- Gel electrophoresis of digested DNA
- Documentation of PFGE gel

PulseNet Standardized Protocol for PFGE



All images are courtesy of Kara Cooper and Molly Freeman, Centers for Disease Control and Prevention, Atlanta, GA.

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Troubleshooting PFGE gels can be very complex and depends on a variety of factors:

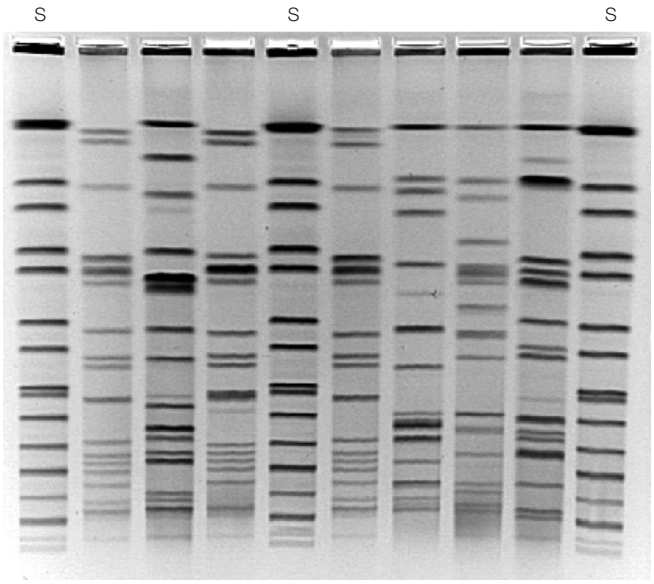
- Individual laboratory skills and technique
- Laboratory setup and equipment
- Reagents
- Water quality
- Interruptions and distractions during experiment

Many problems with poor PFGE results can be solved by correcting poor laboratory practices:

- Measure weights and volumes accurately
- Make accurate calculations
- Confirm all instruments and equipments are working properly
- Check reagents for precipitation, discoloration, cloudiness, and expiration date
- Use reagents from commercial suppliers
- Wear gloves throughout the entire procedure
- Make sure glassware is clean without any residual detergents or other debris, which can interfere with plug preparation and lysis or cause specks in the background
- **Do not** reuse plasticware unless it can be washed and rinsed properly
- **Do not** overheat agarose solutions
- Mix enzyme and buffer mixtures well
- Use appropriate reagents, enzyme, and electrophoresis conditions for each organism

Become familiar with the purpose of each step within the protocol and the potential impact of failure within each step to facilitate the review of PFGE gels. When reviewing PFGE gels:

- Determine if there have been any changes since the last good gel in:
  - Procedure
  - Equipment
  - Reagents
  - Personnel
- Review all steps of the protocol and consider if the failure could be the result of problems within one of those steps
- Examine all areas of the PFGE gels to identify the ones that are unusual because those areas may indicate the root cause of the failure



Example of a PFGE gel. Colored brackets indicate different areas of the gel that should be analyzed during troubleshooting. S, standard.

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