Pulsed Field Gel Electrophoresis

Protocol

Bulletin 6224

Plug Preparation

This plug preparation protocol describes how to prepare intact, chromosome-sized DNA for pulse field gel electrophoresis (PFGE). It includes guidelines about preparing the cell suspension, adjusting the cell density, casting the plug, cell lysis, and washing the plug.

Preparing the Cell Suspension

Purpose

A cell suspension is prepared to evenly suspend the cells within a buffer solution that will maintain the cells in a viable state until the plugs are cased. The appropriate buffer may vary from organism to organism. There is no "universal buffer" for making cell suspensions and preventing cell lysis. Testing may be required to identify the most appropriate buffer for the organism, if it is not already known.

Process

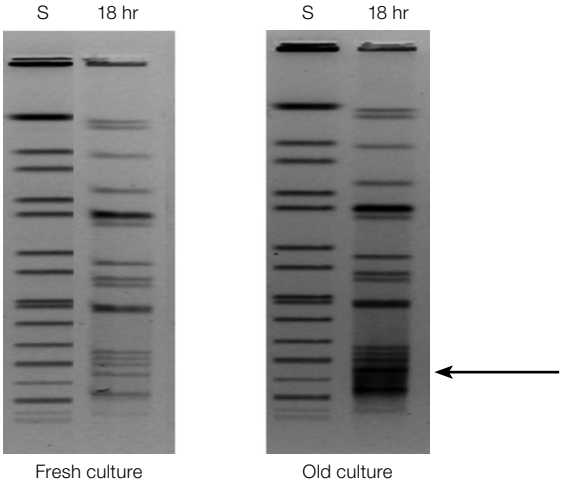
Transfer bacterial cells from the growth media to a cell suspension buffer.

Potential Problems

Undue stress to bacterial cells prior to casting the plug can cause cell lysis, leading to DNA degradation, and is characterized by "smearing" on the gel.

Recommendations

- Grow cultures on nonselective media
- Use fresh cultures grown for 14–18 hr at appropriate temperature and conditions
- Procedure
  - — Do not let cultures sit at room temperature for more than 1 hr before making cell suspensions
  - — Do not store cultures at 4°C before making cell suspensions
  - — Do not use cultures grown for more than 18 hr (contain lysed cells)
- **Do not** centrifuge or vortex cell suspensions
- **Do not** leave bacterial cell suspensions at room temperature for extended periods of time prior to casting plugs



PFGE gel from a fresh culture shows no smearing and all bands are distinct. Lysed cells are present in 48-hr old cultures (same organism), characterized by smears on the PFGE gel. S, standard.

Adjusting the Cell Density

Purpose

Cell density needs to be adjusted to ensure an optimal amount of bacterial cells is present in the agarose plug.

Process

As the cells are transferred to the cell suspension buffer, the optical density (OD) or turbidity of the solution should be measured. Make adjustments as needed by adding additional cells or cell suspension buffer until an appropriate value within the optimized range for that organism is reached.

Potential Problems

- If the cell suspension concentration is too low, not enough DNA is present and the resulting pattern will be faint and difficult to analyze
- If the cell suspension concentration is too high, increased amounts of lytic and restriction enzymes are necessary to achieve complete lysis and DNA digestion, respectively
- As the amount of DNA increases, the band thickness increases; doublets of bands are difficult to discriminate and closely migrating bands of similar sizes may appear as a single band

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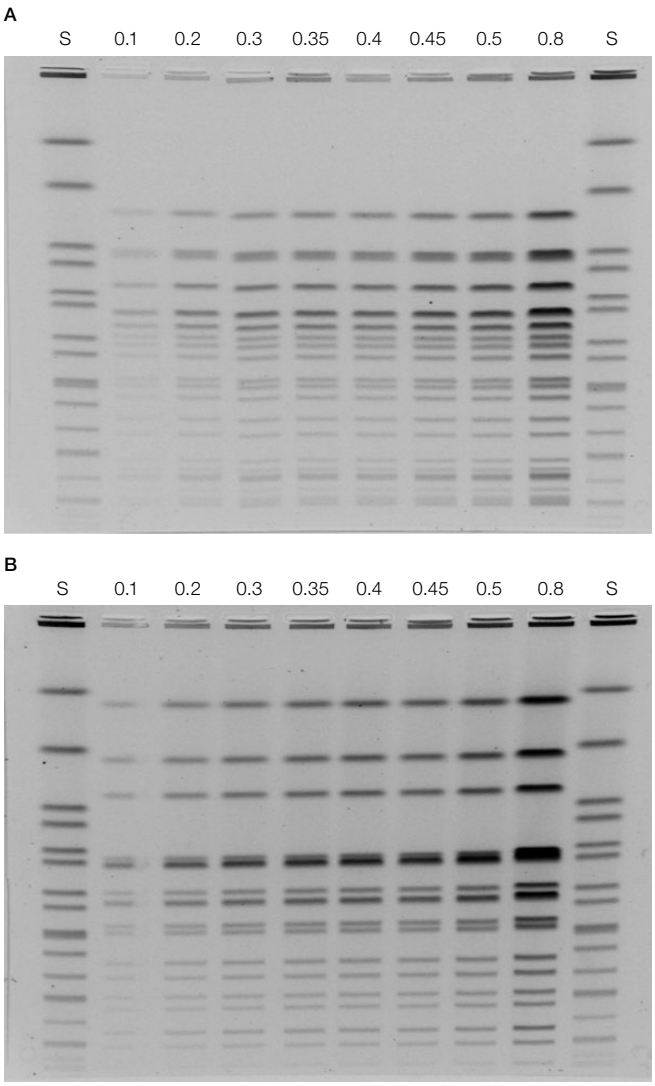
Recommendations

- Test several different cell suspension concentrations over a wide range. Even with standardized protocols, the optimal range may vary slightly across laboratories, depending on the equipment and reagents used
- Look for the range that results in consistently clean patterns with limited background and sharp, clear bands



Examples of cell suspensions with cell concentration increasing from left to right. Lanes with 0.3–0.5 OD show similar band intensities in cell suspensions of *Escherichia coli* (**A**) and *Salmonella* (**B**). S, standard.

Casting the Plug

Purpose

Encasing the cells prior to cell lysis within an agarose matrix is necessary to stabilize them and allow for isolation of intact DNA molecules.

Process

1. Transfer an aliquot (typically 400 ml) of the cell suspension to a 1.5 ml centrifuge tube.
2. Add proteinase K (20 ml of 20 mg/ml stock) to inactivate damaging nucleases.
3. Quickly add an equal volume (400 ml) of 1% PFGE-quality agarose that has been equilibrated to ~54°C.
4. Pipet gently to mix and dispense into plug mold(s).
5. Allow plugs to solidify (~10 min).

Reusable Plug Mold (catalog #170-3622)

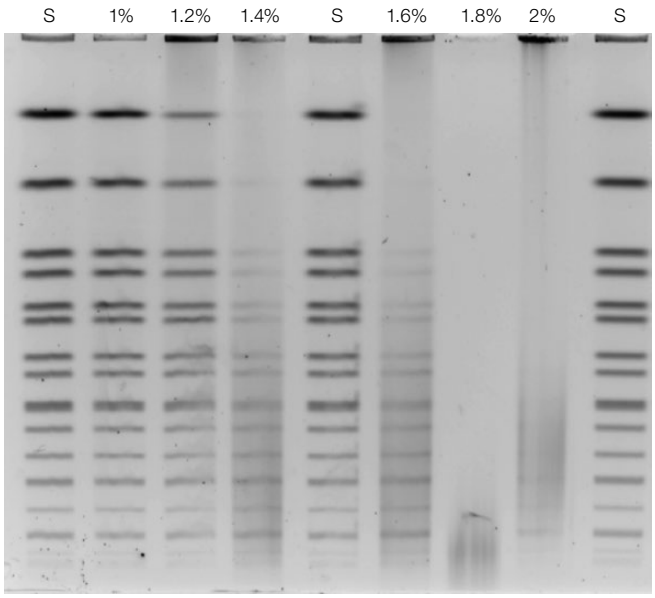
50-Well Disposable Plug Mold (catalog #170-3713)

Potential Problems

- Agarose that is too hot may damage the cells
- Agarose thickens and solidifies as it cools, making pipetting difficult and leading to an uneven distribution of cells within plugs

Recommendations

- Equilibrate melted agarose to 50–54ºC and keep it warm while casting plugs
- Limit mechanical force (pipetting) when mixing melted agarose with cell suspensions
- Do not re-use plug agarose more than 3 or 4 times; repeated heating results in loss of fluid and increases the agarose concentration



Gel showing effects of re-using plug agarose. When agarose is reheated, water evaporates and the agarose concentration increases, resulting in plugs that do not have the expected agarose percentage. Reheating the agarose six times increased the concentration from 1 to 2%. S, standard.

Cell Lysis

Cell lysis is necessary to break the bacterial cell open so DNA is accessible to enzymes during the subsequent DNA restriction step. As cells lyse, endogenous endonucleases are released that must be inactivated to prevent random DNA degradation.

Process

1. Place 1–3 plugs (same sample) in 5 ml of cell lysis buffer (50 mM Tris:50 mM EDTA, pH 8.0 + 1% sarcosyl + 100 µg/ml proteinase K).
2. Lyse plugs in a shaking (~175 rpm) water bath or incubator at 54ºC.

Note: Some organisms lyse more quickly or efficiently than others; maximal cell lysis occurs within 1–4 hr. Plugs typically clear as cells lyse.

Potential Problems

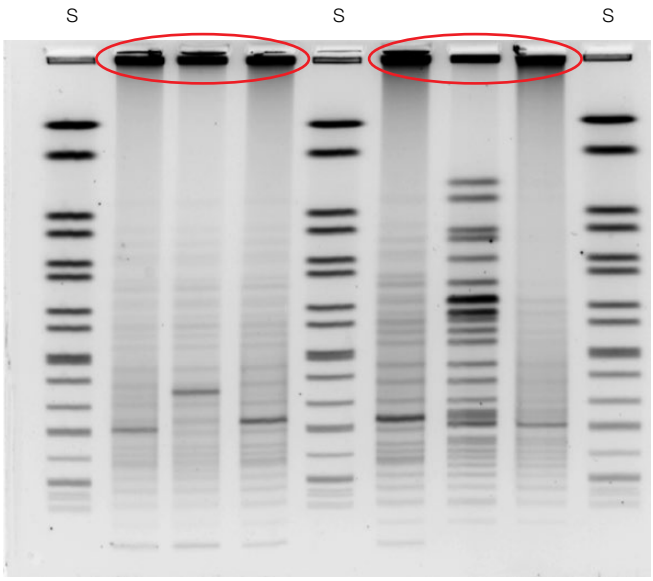
If lysis is incomplete, DNA will only be partially digested and faint bands, smearing, and significant fluorescence in the plug slice will be observed in the gel.

Note: The amount of material remaining in the wells is a good indicator of the efficiency of cell lysis.

Recommendations

- Use fresh (not expired) reagents that are kept sterile and uncontaminated
- Decrease cell density
- Follow correct temperature and shaking recommendations
- Ensure plugs are completely covered by lysis buffer

Note: When working with a strain for the first time, getting information from published protocols/articles might help avoid failed experiments. If you are a pioneer with a strain you can observe it under the microscope to determine lysis time. If the strain does not lyse, try a new enzyme or higher temperature. Optimal lysis temperature is usually similar to optimal temperature for strain growth.



Examples of incomplete cell lysis. Samples in the indicated lanes were not lysed properly. These lanes display DNA smearing, few visible bands, and cell material in the wells. S, standard.

Washing the Plug

Purpose

Washing plugs removes proteinase K, cellular debris, lytic enzymes, or proteases present during the lysis step that may interfere with subsequent DNA digestion.

Process

Wash in 10–15 ml at 50ºC for 10–15 min with constant agitation.

- 2 times with sterile clinical laboratory reagent grade water
- 4 times with TE buffer (10 mM Tris:1 mM EDTA, pH 8.0)

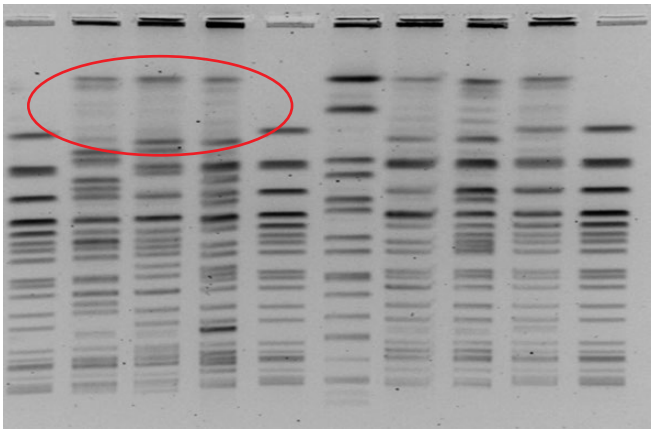
Potential Problems

Inadequate plug washing typically results in incomplete digestion or smearing.

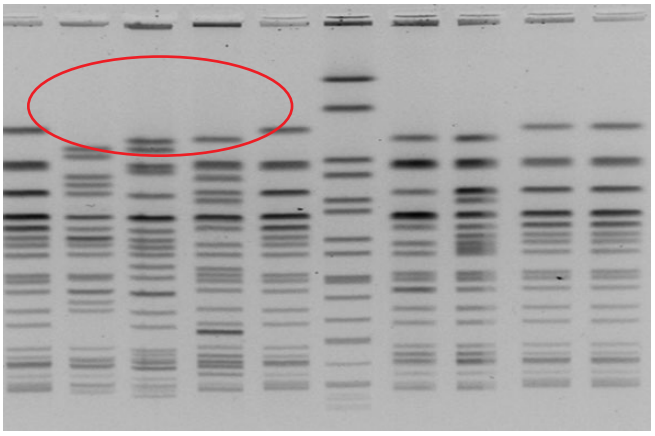
Recommendations

- Wash plugs 2 more times with TE buffer
- Digest and run another plug slice

A



B



Smearing and incomplete restriction resulting from insufficient washing. **A**, the indicated plugs were washed four times with TE buffer; **B**, the same plugs were washed six times with TE buffer.

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