

Revision : ----	Document Code: WI- ML-6473	Document Title: Standard Operating Procedure (SOP) for Pulsed-Field Gel Electrophoresis (PFGE)	 وادیہ علم و تحقیق وادیہ تعلیم و تحقیق وادیہ تعلیم و تحقیق
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1. Purpose

To describe the standard operating procedure for **Pulsed-Field Gel Electrophoresis (PFGE)** and the associated processes.

2. Scope of Operation

PFGE is a specialized laboratory technique used for the separation of large DNA molecules (up to 10 Mb). This method operates by periodically changing the direction of the electric field, which allows large DNA molecules to reorient and migrate through the agarose matrix. It is particularly essential for **microbial typing** (identifying different bacterial strains) and analyzing the genetic structure of microorganisms.

3. Responsibility

The implementation of this procedure is the responsibility of the relevant laboratory experts.

4. Operating Procedure

A. Sample Preparation

- First, DNA is extracted from the target bacteria or microorganism.
- To prevent physical damage to the high-molecular-weight DNA, it is typically embedded in an **agarose plug** before processing.

B. Restriction Enzyme Digestion

- The extracted DNA is digested using specific restriction enzymes to produce manageable, albeit large, DNA fragments.

C. Loading Samples into the Gel

- An electric field with alternating, pulsed directions is applied to the agarose gel. This pulsed field is necessary to allow large DNA molecules to navigate through the pores of the gel matrix, which would otherwise be impossible with standard constant-field electrophoresis.

D. Electrophoresis Settings

- The operator must precisely configure parameters such as **voltage, pulse time (switch time), and temperature** according to the specific protocol.

E. Gel Staining and Imaging

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- Separated DNA molecules are visualized using specialized fluorescent dyes (e.g., Ethidium Bromide or safer alternatives) under appropriate lighting (UV or Blue light).

F. Data Analysis

- The resulting DNA banding patterns are compared to identify microbial strains or to analyze the genetic architecture of the microorganisms.

Advantages of PFGE:

- High-Resolution Separation:** Optimized for separating large DNA molecules that are difficult to resolve using conventional methods.
- High Reproducibility:** PFGE results are highly reproducible under standardized laboratory conditions.
- High Sensitivity:** Capable of detecting subtle genetic variations between different microbial strains.

5. Safety Instructions

A. Chemical Safety (Hazardous Substances)

- When handling chemicals such as **Acrylamide, Bis-acrylamide, Ammonium Persulfate (APS), and TEMED** (N,N,N',N',N,N,N',N'-Tetramethylethylenediamine), you **must** wear appropriate Personal Protective Equipment (PPE), including **gloves, safety goggles, and a laboratory coat**.
- Warning:** These substances are toxic and potentially mutagenic; they must be handled with extreme caution.

B. Electrical Safety

- Electrophoresis involves the use of high voltage. Avoid direct contact with electrodes and electrophoresis buffers.
- Ensure the power supply is completely disconnected before touching any part of the electrophoresis unit.

C. Fume Hood Usage

- Working with volatile or toxic substances, such as **TEMED**, must be performed inside a **chemical fume hood** to prevent the inhalation of toxic vapors.

6. Maintenance

A. Gel Care

- Polyacrylamide/Agarose gels must be stored in a refrigerator under dark conditions.
- Avoid exposing gels to high temperatures or direct light.
- Ensure all air bubbles are removed from the gel prior to use.

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- Do not use expired gels.

B. Buffer Management

- Electrophoresis buffers must be fresh and of high quality.
- Buffers should be stored at the appropriate temperature.
- Do not use contaminated or expired buffers.

C. Equipment Maintenance

- After each use, thoroughly wash and dry all components of the electrophoresis unit with **distilled water**.
- Prevent any chemical contact with the electrical components of the device.
- Perform regular inspections of the equipment; repair or replace any damaged parts immediately.

D. DNA Storage

- Extracted DNA from the gel must be stored at -20°C or lower.
- Avoid repeated freeze-thaw cycles to maintain DNA integrity.

⚠ IMPORTANT:

- In the event of any technical malfunction or error, consult the relevant laboratory expert immediately.

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