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Importance of pulsed-field gel electrophoresis: A short view

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Pulsed-field gel electrophoresis (PFGE) is an advanced version of the agarose gel electrophoresis, which is used to separate the large DNA fragments by applying the electricity that changes direction in the gel matrix and helps to reserve the large segment of the DNA. In PFGE, the DNA genome is cut by the restriction enzymes into the fragments to identify the specific species. In this method, a lower concentration of the agarose gel (approximately 1%) is used to make the large pores which help in trapping the larger DNA fragments. Firstly, the bacteria which are cultured in the plate are transferred into the buffer to make a suspension followed by which they are inoculated into the melted agarose to prepare the agarose plugs. These agarose plugs are treated with a lysate solution (lysozyme) to degrade the bacterial cell and extract the DNA. During this step, the agarose plugs are subjected to 37°C for one hour. Thereafter, the DNA (larger) obtained is treated with different restriction endonuclease enzymes to breakdown the DNA into fragments. These agarose plugs, which contain digested products are loaded in an agarose gel, and electricity is applied to separate the DNA fragments based on their size. Following this, the gel is stained and observed under ultraviolet radiation. This technique is widely used by scientists to subtype the bacteria based on their genetic material. The DNA restriction patterns that are generated by PFGE are stable and reproducible. However, the drawbacks related to this method are time consumption and the requirement of trained and skilled technicians. In spite of its drawbacks, it is regarded as the best practical and applicable typing tool for subtyping a broad range of bacteria. Also, this technique is being a reliable and standard method for vaccine preparation, and for controlling, preventing and monitoring diseases in different populations.

Keywords: Pulsed-field gel electrophoresis, PFGE, Agarose gel, DNA plugs, Lysozyme, Restriction endonuclease, Bacteria, Subtyping, Vaccine, Diseases, Prevention

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