Pulsed-Field Gel Electrophoresis (PFGE): The Molecular Epidemiologists Tool

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The study of epidemiology related to bacterial outbreaks has come a long way since Mary Mallon (Typhoid Mary) was sent to a tuberculosis sanatorium at North Brother Island (East River of New York City) because city officials did not know what to do with her. However, it was just these types of unfortunate situations which spurred epidemiologists and microbiologists to develop techniques to answer the basic question posed to a molecular epidemiologist which is whether "strain A is related to strain B." The main hypothesis of molecular epidemiology is the following: within the "epidemiologic window," the isolates being considered will have either indistinguishable characteristics (i.e. the same genetic fingerprint) and thus be considered clonally related (same strain), or the isolates will have distinguishable characteristics and be considered different strains. Currently, multiple molecular techniques have been developed to assess this question. Although it is believed that DNA sequencing of highly variable genes will become the method of choice for molecular epidemiologists in the future, currently the "gold standard" technique used in this discipline is pulsed-field gel electrophoresis (PFGE). PFGE is currently used by the Nebraska Public Health Laboratory (NPHL) for molecular epidemiology of foodborne and nosocomial pathogens.

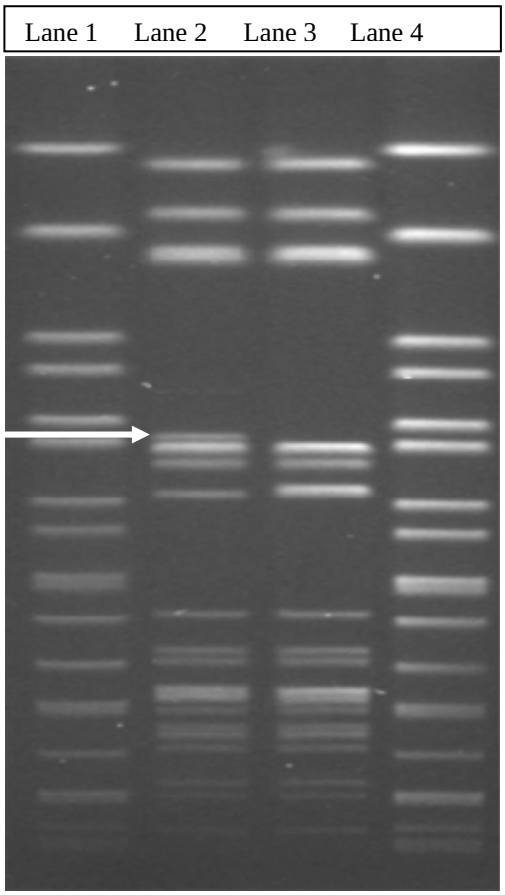
PFGE is essentially the comparison of large genomic DNA fragments after digestion with a restriction enzyme. Since the bacterial chromosome is typically a circular molecule, this digestion yields several linear molecules of DNA. The basic concept of interpretation of this experiment is the following: if one is comparing two strains that are clonal (i.e. the same strain), the sites at which the restriction enzymes act on the DNA and the length between these sites would be identical. Therefore, after digestion of the DNA and electrophoresis through an agarose gel, if the DNA banding patterns between any two isolates is identical, then these isolates are considered the same strain. Conversely, if two isolates are not the same strain, then the sites at which the restriction enzymes act on the DNA and the length between these sites would be different; thus their DNA banding patterns will be different.

The preparation of genomic DNA suitable for PFGE begins by lysing bacteria that are encased in agarose blocks. After multiple washes, the DNA within the agarose is digested with restriction enzymes and electrophoresed using PFGE. PFGE differs from conventional agarose electrophoresis in that the orientation of the electric field across the gel is periodically changed in contrast to being unidirectional and constant in standard electrophoresis. The variability in the electric field allows PFGE to resolve the very large fragments (>600 kb) associated with this analysis.

Figure 1 (on page 2) shows a PFGE gel of *Escherichia coli* O157:H7. Note that lanes 1 and 4 have indistinguishable banding patterns and are thus considered the same strain. However, lanes 2 and 3 have significantly divergent banding patterns from lanes 1 and 4 and thus are considered separate strains. In addition, note that although the DNA banding patterns in lanes 2 and 3 are very similar, there is an additional band found in lane 2 (identified by the white arrow). Therefore, one would interpret the data as stating that the isolates in lanes 1 and 4 are indistinguishable by PFGE (and divergent from the isolates in lanes 2 and 3) whereas the isolates in lanes two and three are highly related strains but distinguishable by PFGE.

One remarkable aspect of performing molecular epidemiology testing by PFGE is that all state public health laboratories as well as the Centers for Disease Control and Prevention (CDC) perform these protocols using the same methodology. Therefore, using common software and normalization protocols, the NPHL can assess whether certain foodborne pathogens (*E. coli* O157:H7, *Salmonella enterica* serotype Typhimurium, etc.) are not only indistinguishable from certain isolates within Nebraska but also from any other isolate in the United States as well as Canada. This system and database, which was developed at the CDC, is called "PulseNet." Currently, the NPHL can perform PFGE testing on a variety of foodborne (*E. coli, Salmonella, Campylobacter, Listeria,* etc.) as well as nosocomial pathogens (*Staphylococcus aureus, Enterococcus faecium, Klebsiella pneumoniae, Pseudomonas aeruginosa,* etc.).

Figure 1



Please call either Robert Wickert at 402-559-2123 or Dr. Paul D. Fey at 402-559-2122 with questions concerning PFGE testing.

Selected reviews on PFGE

Goering, R.V. Pulsed-field gel electrophoresis. In: Persing, D.H., Tenover, F.C., Versalovic, J., Tang, Y-W., Unger, E.R., Relman, D.A., and White, T.J., editors. Molecular Microbiology; Diagnostic Principles and Practice. Washington, D.C.: American Society for Microbiology; 2004: **185-196.**

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