Pulsed Field Gel Electrophoresis



Bulletin 6228

Pulsed Field Gel Electrophoresis Standards

Bio-Rad offers standards for all pulsed field gel electrophoresis (PFGE) applications, from field inversion gel electrophoresis (FIGE) separation of cosmid inserts to the largest chromosomal separations. Higher molecular weight standards are prepared in low-melt agarose blocks that can be cut to fit any well dimensions.

No more than four samples should be placed between lanes with standards. Placement of standards may be adjusted when fewer than 7 (for 10-well gels) or 11 (for 15-well gels) samples are loaded on a gel.

For 10-well gels, load standards in lanes 1, 5, and 10

For 15-well gels, load standards in lanes 1, 5, 10, and 15



Standard and markers. A, 5 kb ladder standard; B, 8.3–48.5 kb standard; C, lambda ladder standard; D, S. cerevisiae marker; E, H. wingei marker; F, S. pombe marker.

PulseNet Global Reference Standard: Salmonella enterica, serotype Braenderup H9812

Information in this section is intended for PulseNet members.

H9812 is used as the global reference standard by PulseNet member laboratories. It was selected because when digested with Xbal, it has an optimal number of bands (18) distributed across a wide range of sizes (20.5–1,135 kb).

- H9812 is used to normalize migration variation occurring across the gel and to accurately determine sample band sizes
- H9812 is always digested with Xbal, regardless of which enzyme is used to digest the actual samples
- No more than 4 samples should be placed between H9812 lanes. Placement of standards may be adjusted when fewer than 7 (for 10-well gels) or 11 (for 15-well gels) samples are loaded on a gel:
 - For 10-well gels, load standards in lanes 1, 5, and 10
 - For 15-well gels, load standards in lanes 1, 5, 10, and 15



Lanes 1, 5, and 10 include the H9812 standard.



Recommendations

- Prior to using a new lot of H9812 plugs, compare it with a previously used lot to confirm that the new lot produces an acceptable PFGE pattern, that is no pattern changes, no changes in band intensity, and no DNA degradation
- Pretested H9812 standard plugs serve as a positive control for Xbal digests and can be useful in troubleshooting efforts:
 - If a problem is observed in sample lanes but not the standard lanes, it was caused by something that affected only the sample (for example, sample plug preparation, Blnl digest)
 - If a problem is observed in both sample and standard lanes, it is caused by a step involving both sample and standard plugs (for example, Xbal digest, electrophoresis)

Tip: Make an H9812 plug when preparing sample plugs to control for parameters and reagents used to make plugs on that day. If H9812 and sample plugs have deteriorated but pretested H9812 standards appear appropriately, the problem occurred during plug preparation.

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S. enterica, serotype Braenderup H9812 standard.



Bio-Rad Laboratories, Inc.

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