Chapter 1

Pulsed Field Gel Electrophoresis and Genome Size Estimates

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Abstract

Pulsed field gel electrophoresis (PFGE) is a quick and reliable procedure to resolve DNA molecules larger than 30 kb by applying an electric field that periodically changes direction. This technique can be used to estimate genome size of a microorganism, to reveal if a genome is circular or linear, to indicate the presence of megaplasmids, and to show if a strain contains only one or more chromosomes.

Key words Genome size, Genome topology, Multi-replicons, Megaplasmids

1 Introduction

Pulsed field gel electrophoresis (PFGE) is an electrophoretic technique to resolve DNA fragments from 30 kb to various Mb by applying an electric field that periodically changes direction, overcoming the size limitations, due to running DNA molecules in a conventional gel electrophoresis, where a static electric field is applied. The concept that large DNA molecules could be separated by using alternating electric fields was introduced in 1982 [1]. The pulsed electrophoresis effect has been utilized by a variety of instruments (FIGE, TAFE, CHEF, OFAGE, PACE, and rotating electrode gel) to increase the size resolution of both large and small DNA molecules [2–5]. Contour-clamped homogeneous electric field (CHEF) is the most widely used apparatus that produces homogeneous electric fields so that all lanes of a gel run straight and allow separation of molecules up to 10,000 kb.

General applications of PFGE can be the separation of whole chromosomes, the resolution of megaplasmids, and the determination of genome and plasmid size and topology. Here, methods to resolve and size high-molecular-weight DNA fragments are described.
Bacteria exhibit a large variability concerning genome size; among all completely sequenced 2,805 archaeal and bacterial genomes (NCBI Complete Microbial Genomes http://www.ncbi.nlm.nih.gov/ genomes/lproks.cgi) thus far, the 139 kb of Candidatus Tremblaya princeps represents the smallest genome [6] and the 14.78 Mb of Sorangium cellulosum So0157-2 is the largest one [7], followed by 13.7 Mb of Ktedonobacter racemifer SOSP1-21 T [8], 13.03 Mb of Sorangium cellulosum So ce56 [9], and most actinomycetes that usually have a genome larger than 8 Mb, i.e., Streptomyces bingchenggensis, 11.9 Mbp [10], Catenulispora acidiphila, 10.5 Mbp [11], and Streptosporangium roseum, 10.4 Mbp [12]. Table 1 shows the limits, so far known, of genome size (0.036–14.78 Mb) and GC% content (13.5–74.9 %) of Archaea and Bacteria.

### Table 1  
Microbial genome size and GC % content

<table>
<thead>
<tr>
<th>Organism</th>
<th>Size (Mb)</th>
<th>GC %</th>
<th>Relevant feature</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nanoarchaeum equitans Kin4-M</td>
<td>0.49</td>
<td>31.6</td>
<td>Smallest genome</td>
</tr>
<tr>
<td>Methanosarcina acetivorans C2A</td>
<td>5.75</td>
<td>42.7</td>
<td>Largest genome</td>
</tr>
<tr>
<td>Methanospirillum stadtmanae DSM 3091</td>
<td>1.77</td>
<td>27.6</td>
<td>Lowest GC% content</td>
</tr>
<tr>
<td>Salinarchaeum sp. Harcht-Bsk1</td>
<td>3.26</td>
<td>66.6</td>
<td>Highest GC% content</td>
</tr>
<tr>
<td><strong>Archaea</strong></td>
<td></td>
<td></td>
<td><strong>Bacteria</strong></td>
</tr>
<tr>
<td>Candidatus Tremblaya princeps PCIT</td>
<td>0.139</td>
<td>58.8</td>
<td>Smallest genome</td>
</tr>
<tr>
<td>Sorangium cellulosum So0157-2</td>
<td>14.78</td>
<td>72.1</td>
<td>Largest genome</td>
</tr>
<tr>
<td>Candidatus Zinderia insecticola CARI</td>
<td>0.21</td>
<td>13.5</td>
<td>Lowest GC% content</td>
</tr>
<tr>
<td>Anaeromyxobacter dehalogenans 2CP-C</td>
<td>5.01</td>
<td>74.9</td>
<td>Highest GC% content</td>
</tr>
</tbody>
</table>

Data are from http://www.ncbi.nlm.nih.gov/genome/browse/. Only complete sequences were taken onto account

**a** 165 sequences  
**b** 2,640 sequences

### 1.1 Determination of Genome Size

Bacteria exhibit a large variability concerning genome size; among all completely sequenced 2,805 arcahael and bacterial genomes (NCBI Complete Microbial Genomes http://www.ncbi.nlm.nih.gov/ genomes/lproks.cgi) thus far, the 139 kb of Candidatus Tremblaya princeps represents the smallest genome [6] and the 14.78 Mb of Sorangium cellulosum So0157-2 is the largest one [7], followed by 13.7 Mb of Ktedonobacter racemifer SOSP1-21 T [8], 13.03 Mb of Sorangium cellulosum So ce56 [9], and most actinomycetes that usually have a genome larger than 8 Mb, i.e., Streptomyces bingchenggensis, 11.9 Mbp [10], Catenulispora acidiphila, 10.5 Mbp [11], and Streptosporangium roseum, 10.4 Mbp [12]. Table 1 shows the limits, so far known, of genome size (0.036–14.78 Mb) and GC% content (13.5–74.9 %) of Archaea and Bacteria.

Pulsed field gel electrophoresis (PFGE) and/or complete genome sequencing are the predominant applied methods to determine bacterial genome size. Different methods of complete genome sequencing will be presented in the following chapters.

If the electrophoretic method is used, cells are grown at the exponential phase in a liquid broth, embedded in agarose plugs, and lysed; after washing steps, genomic DNA, protected in the agarose, is digested with an appropriate restriction enzyme, and fractionated by PFGE.

The choice of the suitable restriction enzyme is a challenging issue and, mainly, depends upon the base composition (%G+C content) of the DNA of the microorganism of interest. Indeed, it
is advisable to use enzymes, which recognize relatively few sites on the genome and give a resolvable and informative number of DNA fragments on the PFGE gel. After staining of the gel, the size of the bacterial chromosome is consistently calculated from the sums of restriction fragment lengths. To get a more accurate determination of the genome size the use of different restriction enzymes is worthwhile. Figure 1 shows a schematic example, in which two enzymes A and B were used to determine the genome size of a microorganism. Enzyme A gave two bands, of 3 and 1.5 Mb, for a total of 4.5 Mb, while enzyme B three bands of 2.5, 2, and 1.5 Mb, for a total of 6 Mb. This size discrepancy is due to the fact that the enzyme A gave a 1.5 Mb band corresponding to two DNA fragments, evident by the higher intensity of this band. Thus, the genome size can be supposed to be 6 Mb.

In the case of GC-rich bacteria, like actinomycetes, enzymes that recognize specific base sequences rich in A and T nucleotides might be suitable for generating a distribution of DNA fragments that would be useful for analysis of genomic DNA, i.e., AseI (ATTAAT), DraI (TATTAA), and SspI (AATATT); on the contrary, in the case of low GC bacteria, enzymes cutting sequences rich in G and C nucleotides are preferred, like SmaI (CCCGGG) and NotI (GCGGCCGC).

Another tough issue is to get a good resolution of all fragments in one track that requires optimal adjustment of the pulse time conditions and that sometimes cannot be obtained only in a run, but different runs, changing key parameters, will be needed to run. It is convenient to perform different runs optimizing electrophoretic conditions for separation in the low-, intermediate-, and
high-molecular-weight range. On the basis of the expected size of DNA fragments, different PFGE conditions can be applied. In Table 2 examples of run parameters (pulse time, run time, set voltage, gel strength, buffer), that we used for separation of large-molecular-weight DNA, are indicated. These parameters were used with Gene Navigator® system from Amersham Biosciences.

### 1.2 Construction of a Physical Map

Besides the utility of PFGE to determine microbial genome size, PFGE and restriction endonuclease digestion were used to construct physical maps, when genetic linkage maps could not be determined. After appropriate restriction of an intact genome and PFGE discrimination of restricted DNA, it is necessary to deduce the linkages between DNA fragments and various approaches can be applied. The most commonly used method is the hybridization of complete single or double digestions with gene probes, containing the rare-cutter site used to generate the digested sample (Fig. 2). A probe containing the restriction site will hybridize with two discrete DNA bands that correspond to adjacent DNA fragments along the chromosome. The example in Fig. 2 shows the hybridization signals of a blot of digested genomic DNA with two probes (p1 and p2), revealing that DNA fragments of 3 and 2 Mb are close, since both are positive to probe p1, while DNA fragments of 2 and 1 Mb are adjacent since both gave a hybridization signal with probe p2.

### 1.3 Genome Topology

By far the majority of bacterial genomes exist as a single circular chromosome, like most studied model bacteria, like *Escherichia coli* and *Bacillus subtilis*. Relatively recently, linear and/or multiple replicons were found also in many bacteria, i.e., linear chromosomes have been found in Streptomyces, i.e., *Streptomyces coelicolor* [13], *S. lividans* [14], and *S. hygroscopicus* [15], while a mixture of linear and circular replicons were identified in *Borrelia burgdorferi* [16], *Agrobacterium tumefaciens* [17], *Rhodococcus fascians* [18], and related species.

**Table 2**

Examples of running parameters to discriminate different DNA fragments

<table>
<thead>
<tr>
<th>DNA size range (kb)</th>
<th>Pulse time</th>
<th>Run time (hours)</th>
<th>Voltage</th>
<th>Buffer</th>
<th>% Agarose</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5–200</td>
<td>20″ + 4″</td>
<td>14 + 4 = 18</td>
<td>160</td>
<td>0.5× TBE</td>
<td>1</td>
</tr>
<tr>
<td>50–1,000</td>
<td>90″</td>
<td>30</td>
<td>200</td>
<td>0.5× TBE</td>
<td>0.8</td>
</tr>
<tr>
<td>150–2,200</td>
<td>70″ + 120″</td>
<td>15 + 11 = 26</td>
<td>200</td>
<td>0.5× TBE</td>
<td>0.8</td>
</tr>
<tr>
<td>200–5,000</td>
<td>200″ + 20″</td>
<td>20 + 4 = 24</td>
<td>160</td>
<td>0.5× TBE</td>
<td>1</td>
</tr>
</tbody>
</table>
However, linear chromosomes are kept as circular ones inside the cell because of covalently bound terminal proteins. Both naturally circular and protein-covalently bound linear chromosomes remain trapped in the slot and will not enter the gel and thus nothing other than the well is stained with ethidium bromide. To discriminate between these two different topologies, a straightforward procedure including proteinase K (PK) treatment can be applied. PK treatment of a circular chromosome will not change its mobility into the gel, while, in the case of linear chromosomes kept circular by covalently bound terminal proteins, PK will cause the dissociation of the proteins, rendering the chromosome linear and able to enter the gel. To evaluate genome topology, genomic DNA, embedded in an agarose plug, is prepared using a procedure including proteinase K treatment. In parallel, two controls are usually performed: a plug is treated without PK, but with sodium dodecyl sulfate (SDS) to remove non-covalently bound proteins from DNA. Without PK treatment, the lysis might be incomplete or some binding proteins might still be present, thus retarding the mobility of the free chromosome and rendering it unable to enter the gel. The other control is performed incubating the plug of the same stock preparation with a restriction enzyme to generate several bands and to rule out the possibility of not having enough

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**Fig. 2** Southern hybridization of complete digestion with known probes can be used to link adjacent clones. Probes p1 and p2, indicated by red lines, are necessary to recognize linked DNA fragments. A indicates the sites for the rare cutting restriction enzyme A. M molecular marker (color figure online)
In the last years it was demonstrated that bacteria can contain more than one chromosome (Rhizobium, Burkholderia, Vibrio cholera, Borrelia burgdorferi) and/or megaplasmids greater than 100 kb in size (Streptomyces, Rhizobium, Agrobacterium).

Megaplasmids have been described in a variety of microorganisms and many are responsible for distinctive and significant bacterial traits, including virulence, root nodulation, nitrogen fixation, antibiotic and heavy metal resistance, conjugation, and plant tumor induction.

A challenging test to distinguish if the smaller replicon(s) is a plasmid or a chromosome may be to consider whether the bacterium can grow without the second replicon. If yes, it is a plasmid that is commonly considered as accessory genetic material, not necessary for bacterial growth. Anyway, the elimination of the second replicon can be hard to obtain. Thus, a more straightforward method is to investigate if the second replicon contains genes encoding functions essential for bacterial metabolism that is indicative of a chromosome. Probes made from both 16S rRNA PCR products or metabolic genes can be used in hybridization experiments. The presence of 16S rDNA or metabolically essential genes, particularly if in a unique copy, is a strong proof that the replicon is a chromosome.
To determine the size of a plasmid, linear forms are preferred, in that they migrate at rates that allow size determination by comparison with linear markers. For the size determination of linear plasmid, a PK treatment will eliminate terminal covalently bounded proteins. Differently, circular megaplasmids with their closed-circular supercoiled forms move very slowly in PFGE and relaxed or nicked open-circular forms remain trapped in the sample wells. In addition, their migration depends upon running conditions and their size cannot be easily calculated. For an accurate determination of their sizes, one could perform plasmid purification away from the chromosomal DNA, selection of an appropriate restriction enzyme for digestion, and summation of the sizes of the resulting fragments after gel electrophoresis, but serious technical challenges are encountered when working with very large extrachromosomal DNA molecules.

To size circular megaplasmids, S1 nuclease treatment of DNA embedded in agarose plugs to convert the plasmids into unit-length linear molecules can be carried out and PFGE of the S1-treated plug can be performed [19]; indeed S1 nuclease first nicks the supercoiled plasmid DNA, and then it cuts the intact strand opposite to one of the nick, where the DNA actually is single stranded, resulting in a molecule of linearized plasmid DNA. Usually, treatment with SDS buffer of DNA embedded in agarose plugs is performed as control, to remove non-covalently bound proteins that could interfere with DNA mobility. Expected results are shown in Fig. 4.

![Fig. 4](image-url)  
**Fig. 4** Determination of size of a circular plasmid after treatment of DNA embedded in an agarose plug with S1 nuclease (+S1). Treatment with SDS buffer of DNA (+SDS) is performed as negative control. UN undigested DNA; M molecular marker.
2 Materials

2.1 Solutions and Buffers for Plug Preparation

STE buffer: 10 mM Tris pH 8.0, 50 mM NaCl, 100 mM EDTA.
Lysis solution: 10 mM Tris pH 8.0, 50 mM NaCl, 100 mM EDTA, 0.2 % Na-deoxycholate (Sigma), 0.5 % sarkosyl (Sigma).
ESP buffer: 0.5 M EDTA pH 8.0, 1 % sarkosyl, 1 mg/ml proteinase K (added fresh).
TE buffer: 10 mM Tris pH 8.0, 100 mM EDTA.
NDS buffer: 0.5 M EDTA pH 8.0, 1 % sodium lauroyl sarcosine.
PMSF: 40 mg phenyl methyl sulfonate in 1 ml isopropanol.
SDS buffer: 2 % SDS in 0.5 M EDTA, pH 8.0.

2.2 Running Buffer and Agarose Gel

10× TBE: 108 g Tris base, 55 g boric acid, 9.3 g disodium EDTA \(2H_2O\), water to 1 l.
10× TAE: 242 g Tris base, 57.1 ml glacial acetic acid, 100 ml 0.5 M EDTA pH 8.0, water to 1 l.
Staining solution: 0.5× TBE containing 1 \(\mu\)g/ml EtBr.
Destaining solution: 0.5× TBE or distilled water.

3 Methods

To perform PFGE analysis, particular care in preparing high-molecular-weight DNA is necessary. Large-molecular-weight DNA has to be handled with extreme care, so it is normally prepared by embedding the cells in agarose prior to solubilization and enzymatic digestion of the non-DNA components. Individual cells are embedded in agarose, which protects the DNA against breakage while allowing the free flow of solutions necessary for lysis and digestion. High concentrations of EDTA are used to inhibit nuclease activity in the presence of Proteinase K that will digest cellular proteins. Material released by this digestion diffuses out of the agarose during the washes while the DNA remains trapped. DNA prepared in agarose is stable and remains available as a substrate for enzymatic restriction.

3.1 Preparation of DNA Embedded in Agarose Plugs

1. Grow cells in 10 ml rich medium to mid or late log phase (see Note 1).
2. Harvest cells by spinning at 400–4,000 \(x_g\) for 10 min at 4 °C (see Note 2).
   - Heat the pellet for 20 min at 75 °C in case of virulent strains.
3. Wash twice cell pellet by resuspending in 2–4 ml of 10 % glycerol, decant the supernatant off very carefully, and recentrifuge at 400–4,000 \(x_g\) for 10–30 min (see Note 3).
4. Resuspend the cell pellet in one-fifth the original culture volume (2 ml) of STE buffer (see Note 4).

5. Prepare molten 1.6 % low-melting-point agarose made in 1× TE, pH 8 (see Note 5) and keep it in a warm bath at 45 °C to avoid premature gelification.

6. Mix 600 μl cells with 600 μl molten low-melting-point agarose (see Note 6).

7. Pipette well to mix, and then add 100 μl of the suspension cells/agarose to disposable plug moulds. Let the agarose harden on ice for 10–20 min (see Note 7).

8. Push plugs into 10 ml of lysis solution (see Note 8).

9. Incubate at 37 °C with gentle agitation for 2–4 h.

10. Remove the lysis solution and transfer the plugs to new tubes containing 10 ml of ESP buffer.

11. Incubate for 1–2 days at 50 °C with gentle agitation (see Note 9).

12. Add 50 μl of 0.1 M PMSF, mix gently, and place the tube on ice for 1 h. PMSF destroys residual Proteinase K in the plugs (see Note 10).

13. Wash plugs three to four times in 20 ml of TE buffer at 4 °C for 30 min.

14. Store 10–12 plugs in 10 ml of NDS buffer (see Note 11).

3.2 Genomic Treatment with Enzymes

1. Wash the number of the plugs you need in TE buffer (2 ml per plug) at 4 °C for 1 h to overnight. The last wash can be done with sterile water.

2. Put one plug per a 1 ml microcentrifuge tube.

3. Digest the plugs in 1× buffer with 20–30 U of enzyme for 4 h to overnight (see Note 12). If S1-PFGE is carried out, treat total DNA embedded in agarose gel plug with 20 U of S1 nuclease and separate the DNA by pulsed field gel electrophoresis.

4. Stop the reaction by adding 1 ml of 50 mM EDTA (pH 8.0) or by directly loading the samples in PFGE apparatus.

3.3 Gel Preparation

Gels are cast and prepared using the same conditions and reagents used for conventional electrophoresis, but they are usually prepared without ethidium bromide and are stained after the run; this is due to the large volume of buffer that is used and that should be discarded later, and to the fact that intercalation of ethidium bromide slows DNA migration.

1. Add the desired amount of agarose to the correct amount of electrophoresis buffer (see Note 13). 0.5× TBE buffer (Tris–borate–EDTA) and 1× TAE buffer (Tris–acetate–EDTA) are the two buffers most frequently used for PFGE (see Note 14).

2. Heat the flask to boiling in a microwave oven. Avoid boilover (see Note 15).
3. Cool agarose to 40–50 °C before pouring (see Note 16).
4. Prepare the gel casting mould with the appropriate comb.
5. Pour delicately the agarose solution into the rubber casting frame, supplied with the apparatus (see Note 17). Leave a few ml of agarose solution for sealing the wells in the next step.
6. Remove very delicately the comb.

### 3.4 Gel Loading

Samples prepared in agarose plugs are loaded before the gel is placed in the chamber and the wells are sealed with the left agarose, prepared and used for the gel, to avoid their escape from the wells and floating in the running buffer.

1. Prepare a working area by placing some parafilm over the bench and providing a clean scalpel and a clean needle (see Note 18).
2. Decide the order of the samples. Do not forget an appropriate size marker (see Note 19).
3. Let the plug sliding from the microcentrifuge tube to some parafilm, take the plug with the scalpel, removing the excess of liquid, and let the plug sliding from the scalpel to the well; if necessary, softly push the plug into the well (see Note 20).
4. Seal the wells with the agarose left and wait till it hardens.
5. Remove the rubber casting frame (see Note 21) and transfer the gel, solidified into the plastic tray, to the gel chamber, filled with the cold running buffer (see Note 22).
6. Insert the electrode in the right position and close the lid of the electrophoretic chamber (see Note 23).
7. Connect the electrodes, balance the electrophoresis chamber, switch on the pump (see Note 24), and start the run (see Note 25).
8. After the run, take the tray containing the gel and put carefully on the bench. Push the gel to one side and let the gel slide to a glass plate bigger than the gel.
9. Put the glass plate with the gel in a staining solution (see Note 26) and incubate for 30 min to ON (see Note 27).
10. Destain for 1 h in 0.5× TBE (see Note 28).
11. Pump old buffer out from electrophoresis chamber. Rinse with ca. 2.5 l MilliQ water.

### 4 Notes

1. Standard cell OD ensures that each sample contains approximately the same amount of DNA. OD$_{600}$ of 0.6–1 gives usually good DNA quality. For Gram positive, glycine to a final...
concentration of 0.2 M is usually added to the growth medium to facilitate following cell wall degradation. Use the appropriate volume of culture on the basis of the plugs you need. Volumes from 5 to 25 ml are suggested.

2. If DNA quality is not good enough, a smearing will be visible in the absence of incubation with a restriction enzyme. Try to harvest cells earlier. Some bacteria, like actinomycetes, produce a lot of nucleases; to get good-quality DNA, it is suggested to preheat the cells at 65 °C or to reduce nucleases by using a phenol/chloroform treatment.

3. Centrifugation gravity depends upon the kind of bacterial cells.

4. It is more convenient to use plugs at three different DNA concentrations. If the samples are too concentrated, DNA will be difficult to be completely digested and analyzed. If this is the case, try to use half plug.

5. Use high-quality pulsed field gel electrophoresis (PFGE)-grade agarose. Make sure that agarose is completely melted; use a microwave and pulse it in short bursts, but do not boil it over. Discard expired agarose.

6. Cell pellets can be also kept at 45 °C. Other percentages of agarose and other ratios of agarose/cells can be used. Usually, final 0.8–1 % of agarose allows to easily handle plugs. A lower agarose concentration can cause breakage of the plug.

7. Other methods of pouring agar plugs, such as using plastic syringes as moulds, can be used. When non-disposable moulds are used, before pouring agarose suspension, close them on the bottom with paper tape. After gel solidification, remove delicately the tape. Wash the moulds in 0.2 % SDS for 1–2 h and rinse with water.

8. Use 50 ml conical screw-cap tubes and put 10–12 plugs per 10 ml of lysis solution. In case of Gram-positive bacteria, lysozyme (1 mg/ml) is added. For *Staphylococcus aureus*, the incubation with 50 µg/ml of lysostaphin is preferred. Consider that more resistant cell walls need stronger treatment, for example 1 % Triton X-100 or 1 % SDS, to render the bacteria more susceptible to lysis. RNase A (DNase free) can be added at 10 mg/ml.

9. NDS treatment for 48 h is suggested for actinomycetes.

10. PMSF treatment can be avoided, but this could inhibit downstream restriction analysis.

11. If the plugs will be used soon, let two plugs in TE buffer, so their analysis will require fewer washes before restriction.

12. A total volume of 160 µl of liquid keeps the plug submerged. Consider that the volume of the plug is 100 µl, so that the total volume is 260 µl. Pay attention if the enzyme has star effect, i.e., *DraI*, or if it works better at less than 37 °C.
13. 0.8–1 % gel is usually used. Use only glassware, combs, and gel forms that are clean.
14. 0.5× TBE is the most commonly used buffer; it does not need to be changed, even over multi-day runs; 1× TAE buffer is more useful when separating megabase-sized DNA fragments (>3 Mb). Use high-quality water to make the 0.5× TBE buffer used for the gel and running buffer. Some bacteria have a fragile DNA that undergoes DNA degradation in the presence of Tris-containing buffer. In this case, Hepes-containing buffer can be used.
15. Adjust the volume with the buffer after boiling. Make sure that the agarose is uniformly melted by swirling the flask. Pay attention: Overheated solutions can boil and over suddenly when swirled.
16. Too hot temperature can cause leaking of the agarose solution and can weaken or distort the casting mould. We pour the gel when we can keep the flask by hands.
17. Remove air bubbles, lint, dust, and visible particulates from the gel before it solidifies. Pay attention on how to prepare the gel cast. Every cast has only a way to be mounted. If you are wrong, the run will not start.
18. We use the tip of a disposable inoculating loop and needle.
19. Different markers exist in the market. It is advisable to load the border lanes with the same marker.
20. Avoid bubbles in loading plugs; a clean needle can be helpful.
21. We remove the rubber cast after loading the samples, so that the gel is more stable.
22. Cold buffer restricts premature cell lysis and subsequent DNA degradation. If running buffer is stored in cold room, allow to stand at room temperature for ca. 1 h before adding to electrophoresis chamber. 2.8 l of buffer is usually enough to cover the gel. Switch on the chiller. Temperature of 12 °C is usually used.
23. If electrodes are wet or wrongly positioned or the buffer is insufficient in the electrophoresis chamber, the run will not start. Try to disassemble and reassemble the electrodes and the lid and make sure that the buffer covers the gel. Otherwise add more buffer.
24. Ensure that the pump is working. Otherwise, your run will be unsuccessful.
25. Examples of run conditions are shown in Table 2, but many factors, such as voltage, switch interval, running time, agarose concentration of the gel, running temperature, running buffer, and angle of the alternating electric field, affect DNA migration in PFGE gels, so that different experimental attempts can be necessary.
26. The running buffer can be used for preparation of the staining solution.

27. We recycle the staining solution 3-4 times to reduce the ethidium bromide-containing waste.

28. Destaining can be done more quickly with distilled water.

References


related strain *Streptomyces coelicolor* A3(2). J Bacteriol 175:3422–3429


