

Optimizing Sample Resolution

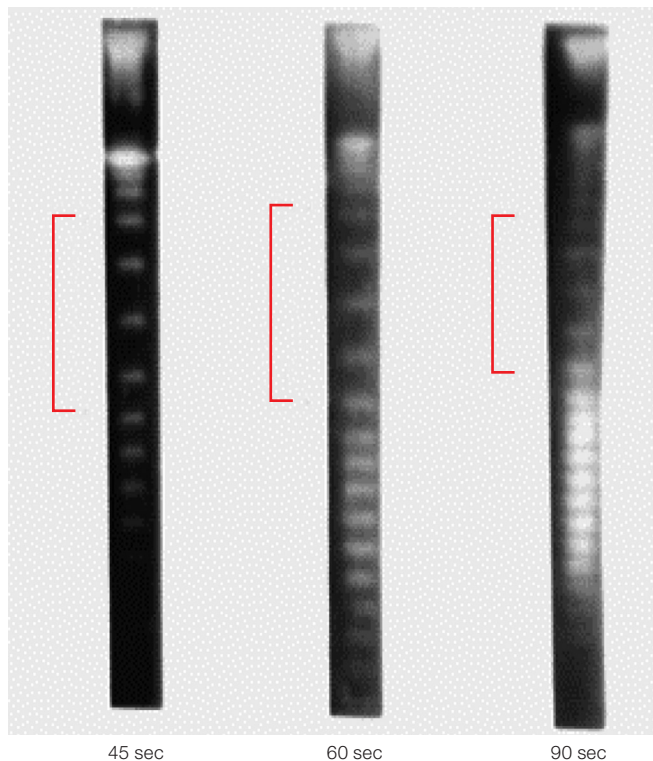
Switch Time and Switch Time Ramping

Switch time is the critical parameter in achieving the best pulsed field gel electrophoresis (PFGE) resolution. It is the length of time the electrical field is pulsed in a single direction. For example, a 60 sec switch time means that the electrical field will be pulsed in one direction for 60 sec and then switched to the other direction for 60 sec.

The switch time is generally shorter for samples with small DNA fragments and longer for samples with large DNA fragments. Samples with a wide range of DNA fragment sizes, for example the *Saccharomyces cerevisiae* chromosomes, can be resolved by changing the switch time over the course of the run. This is referred to as switch time ramping because the switch time will “ramp up” from a short switch time to a longer one.

The ramp of the switch time can be linear, that is, increasing in exact increments over the course of a run, or nonlinear, so that the user can concentrate the switch times on the largest or smallest regions as necessary to achieve better resolution. The nonlinear switch time ramping expands the linear range of fragment separation to 50–700 kb, yielding more precise fragment size measurements.

The nonlinear ramp is described by how much of the run time has elapsed by the time the user has ramped up to 50% of the final switch time. In a 21% nonlinear gradient, only 21% of the total run time would have elapsed even though the user has already achieved 50% of the final switch time. Choosing a nonlinear ramp <50%, results in greater separation of larger DNA fragments and compression of smaller DNA fragments. Choosing a nonlinear ramp >50%, results in greater separation of smaller DNA fragments and compression of larger DNA fragments.

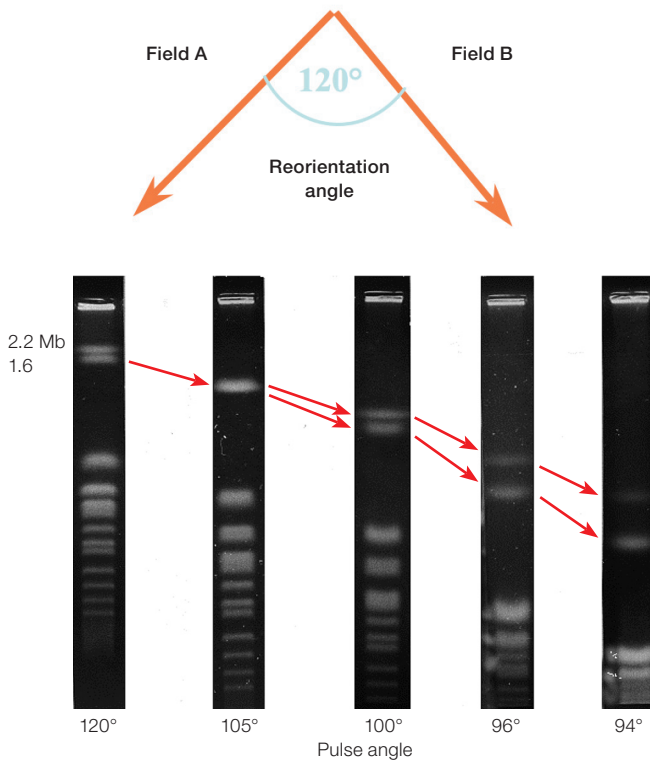


Effect of switch time on sample resolution. As the switch time increases, the larger DNA fragments move farther into the gel during the same time period. The brackets indicate the same region of the lane for each gel. The sizes of the fragments in this region become larger as the switch time increases.

Pulse/Reorientation Angle

Angle is an important factor in achieving optimal resolution, most protocols are optimized for the 120° angle.

Separations of larger sizes of DNA, such as chromosomal DNA, are greatly improved by the use of a smaller included angle (106°). Using a smaller included angle for large DNA fragments (over 1 Mb in size) results in a 50% savings in run time. As the angle is decreased, the resolution of the largest fragments generally improves and the smaller fragments become crowded.



Effects of different pulse angles on sample resolution. The space between the largest two fragments increased with the smaller angle, but the remaining 14 fragments became compressed. If the total analysis was in the range of these largest two fragments, then the user may choose a smaller pulse angle.

Voltage Gradient

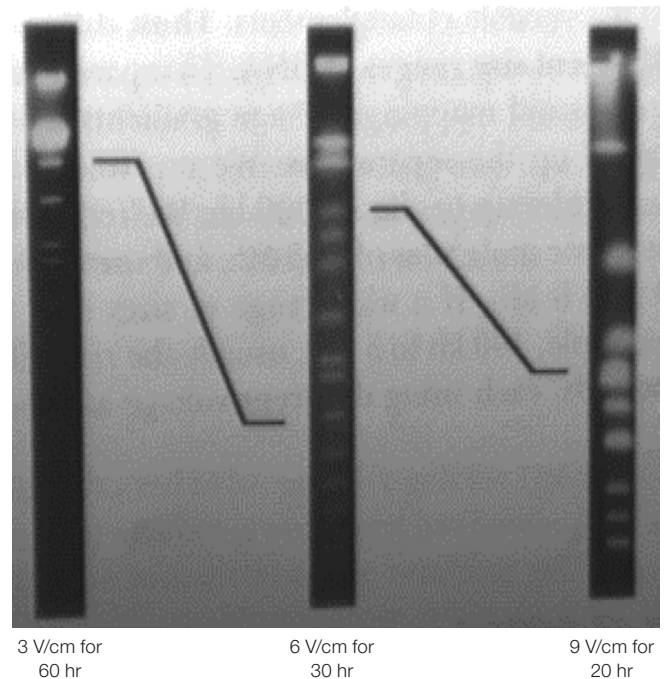
The voltage gradient describes the strength of the electrical field and is represented as V/cm, where the total voltage is divided over the distance between two electrodes.

Since that distance in a CHEF gel box is approximately 33 cm, a 200 V run is approximately 6 V/cm. Most CHEF protocols are optimized for 6 V/cm gel runs.

Typically, the best resolution of chromosomal-sized DNA fragments (>3 Mb) is achieved when using low-voltage gradients, 1.5–3 V/cm, combined with a narrow angle of electrophoresis.

High resolution of DNA fragments up to 250 kb can be achieved by using high-voltage gradients at 9 V/cm. This voltage can be combined with a narrow angle of electrophoresis to resolve samples in very short run times, 4 hr or less.

Note: Voltage alone will not improve resolution. Switch times must also be adjusted to produce the best results.



Effect of voltage gradient on sample resolution. The lines identify the largest resolved fragment in each gel and the position of that fragment in the next gel. The 3 V/cm gel separated ~400 kb fragments, the 6 V/cm gel separated up to 850 kb fragments, and the 9 V/cm gel separated up to 1.2 Mb fragments in the same V-hr. All gels used the same switch times. The resolution of the gel is diminished even though the range is greater in the 9 V/cm gel. Switch times must also be adjusted to produce better resolution.

Buffer Type, Concentration, and Temperature

Buffer Type

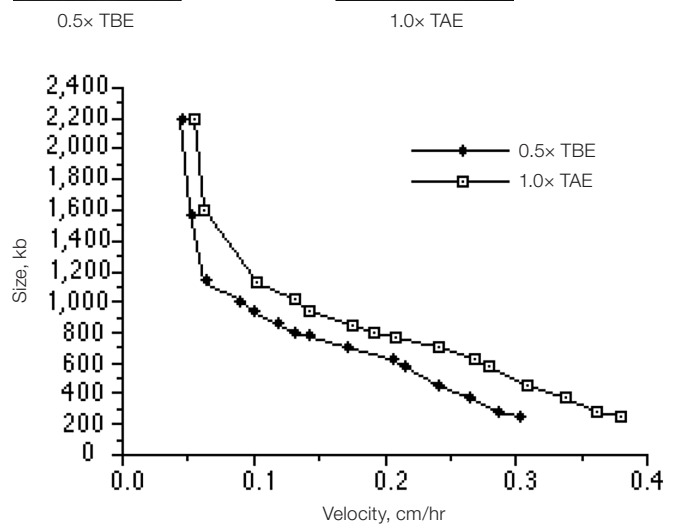
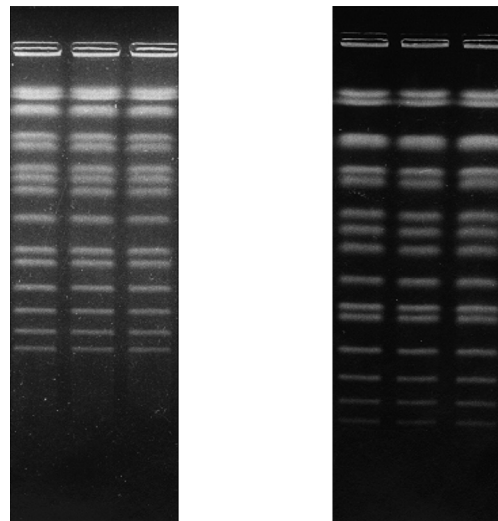
In general, the lower the ionic strength of the buffer, the faster the run. The run time advantage of low ionic strength buffer must be weighed against the limited buffering capacities of dilute buffers and the loss of resolution due to buffer breakdown.

The two buffers most frequently used for PFGE are 0.5x TBE buffer (Tris-Borate-EDTA) and 1.0x TAE buffer (Tris-Acetate-EDTA). 1x TAE buffer is very useful when separating megabase-sized DNA fragments (>3 Mb). 0.5x TBE is the most commonly used buffer. It is useful for most separations up to megabase-sized DNA and does not need to be changed, even over multi-day runs.

Buffer Temperature

Buffer recirculating during the electrophoresis run can be chilled to as low as 4°C or not chilled at all. Buffer temperature will affect run time. The warmer the buffer temperature, the faster the run. Resolution decreases as the temperature increases.

Buffer temperatures at room temperature will decrease the run time by approximately 50% when compared to chilling to 14°C, but the resolution loss will be so great it might not be worth the time savings. Buffer temperatures at 4°C will provide the sharpest resolution but at a greatly increased run time. Generally, buffer is cooled to between 12 and 15°C and this is the best compromise between run time and resolution.



Effects of buffer on sample resolution. Fragments of all sizes migrate farther, with a greater velocity, in the TAE gel than the TBE gel. The TAE buffer is lower in ionic strength (40 mM buffering agent) than the TBE buffer (90 mM buffering agent).

Agarose Type and Concentration

Agarose type affects both sample migration and ease of handling for PFGE gels. Agaroses for PFGE must be very pure, have a high tensile strength, and have a minimum of charged contaminants (usually described by the low electroendosmosis [EEO] or [Mr] agarose).

Generally, 0.8–1% agarose is ideal for PFGE separations up to 3 Mb and 0.5–0.9% is useful for the range above 3 Mb. As the agarose concentration decreases, the DNA migrates faster, a larger range of DNA can be resolved on one gel, the DNA is less well resolved, and the gel becomes more difficult to handle.

Bio-Rad agaroses are available specifically for PFGE that provide superior resolution (pulsed field Certified™ agarose) and decrease run time while maintaining good resolution (Certified megabase agarose).

Run Time

Several strategies may be employed to minimize or extend run time. In general, it is best to optimize for the shortest run time that allows clear sample resolution. This will maximize the efficiency of PFGE-related experiments.

It is possible to resume a run after staining the gel if it appears that the separation is not complete. This is not generally recommended because the ethidium bromide in the staining solution will migrate in the reverse direction of the DNA, retarding its progress.

If necessary, it is safe to leave the gel for several hours after run completion because the DNA is too large to diffuse out (>10 kb) and the presence of EDTA in the buffer and the cool buffer will inhibit nucleases.



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