

Best practices for success running DNA on Lonza's FlashGel® System

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The FlashGel® System offers a rapid and convenient solution for the electrophoresis of DNA and RNA. Designed for quick screening of up to 32 samples, it separates DNA in less than 5 minutes, allowing real-time observation of band migration right at the bench. Requiring 5- to 20-fold less nucleic acid load, the FlashGel® System saves time, reagents and sample volume compared to traditional agarose gel electrophoresis. The FlashGel® System supports two cassette formats: the standard FlashGel® Cassette and the FlashGel® FLX Cassette. Both systems are easy to use and deliver fast results. Deviating from the recommended protocol, however, can cause artifacts, band distortion, and difficulty

reading results. Here, we present a series of best practices for getting the most out of your FlashGel® System.



Tip 1: Flood the wells prior to sample loading

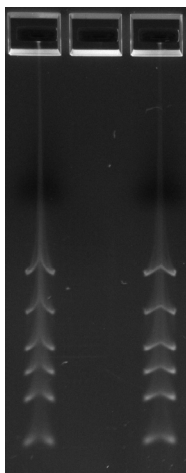


Figure 1. Samples loaded into dry wells in a FlashGel® Cassette can lead to significant distortions, feathering, smearing, or other visual artifacts that can interfere with sample interpretation.

Prior to loading samples into a FlashGel® or FlashGel® FLX Cassette, make sure to flood all sample wells with distilled or deionized water. We recommend AccuGENE® Molecular Biology Water (Lonza, Cat. No. 51200) for best results. Tilt the cassette and use a lint-free wipe or small piece of blotting paper to remove any excess. Do not blot the wells directly, as you want to make sure that the wells are filled with water for sample loading. In Figure 1 you can see that loading and running sample in a dry well can lead to significant band distortion such as “feathering”.

Tip 2: Hold pipette fully upright and carefully dispense samples into the well

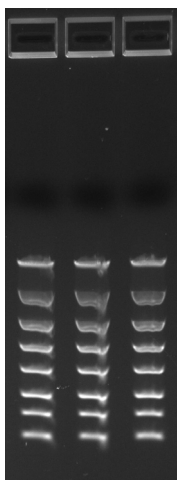


Figure 2. Touching the tip of the pipette to the gel or holding the pipette at an angle when dispensing samples into the well can lead to smearing, as seen in this image.

Tilting the pipette tip when dispensing sample and/or making contact with the gel itself when loading samples can damage the gel or cause samples to be lost or wicked between the gel and the cassette. Smearing of the sample in Figure 2 was caused by contacting the tip of the pipette with the gel when loading the sample.

Tip 3: Keep sample load volumes to 5 μ L

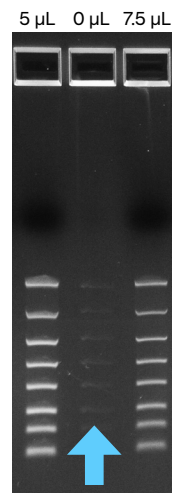


Figure 3. Do not load more than 5 μ L of sample per well. Loading 7.5 μ L, for example (rightmost lane) led to spillover that can be observed in the middle lane (blue arrow).

The FlashGel® System uses DNA stains that are 5 – 20 times more sensitive than ethidium bromide. As a result, optimal sample concentrations are about 1/5th the typical per-band concentration of an ethidium bromide gel. Because of this sensitivity, most DNA samples should be diluted (see next section) while load volume per well must not exceed 5 μ L. In Figure 3, you can see that loading 7.5 μ L of sample (rightmost lane) causes spillover into the middle lane. The left lane is properly loaded with 5 μ L of sample.

Tip 4: Diluting DNA to a final sample load level between 5 – 20 ng/band will prevent visual artifacts and distortion.

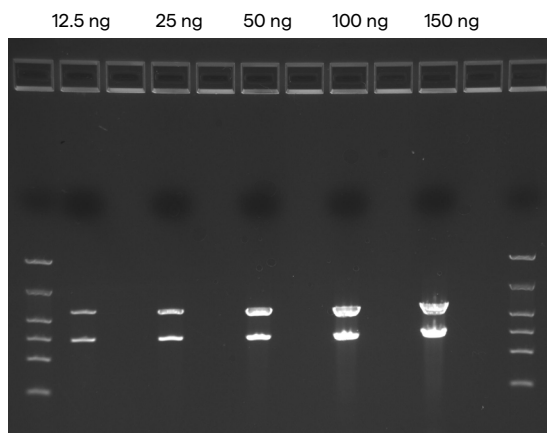


Figure 4. Effect of DNA sample load size on band quality. Load size of 12.5 ng and 25 ng per band show crisp bands with no distortion. As the load size increases, you begin to see visual distortions such as “feathering” or smearing of the band, which could complicate data interpretation.

FlashGel® Stains are much more sensitive than ethidium bromide. To obtain optimal imagery, DNA samples should be diluted with molecular biology-grade water such that each 5 µL of sample loaded into each well contains between 5 – 20 ng of DNA per band. Figure 4 shows that, above 25 ng of DNA per band, the bands begin to show artifacts such as feathering or smearing, while the bands containing 25 ng and 12.5 ng DNA are much clearer and crisper.

Tip 5: Diluting DNA samples with high salt levels, such as restriction digests, will prevent visual artifacts and distortion

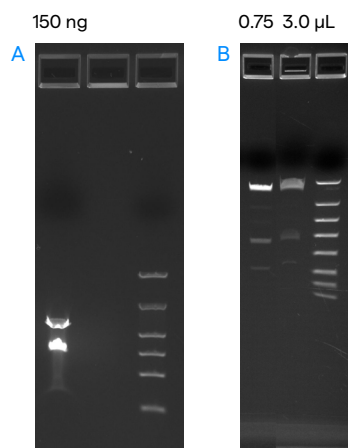


Figure 5. Examples of the impact of undiluted high-salt samples on performance of the FlashGel® System. A: a high sample load (150 ng/band) in an undiluted restriction digest causes severe distortion and smearing of bands. B: diluting the restriction digest sample to 15% (0.75 µL digest diluted to 5 µL) using molecular biology-grade water results in well-defined bands, while a more concentrated sample of 60% (3 µL digest diluted to 5 µL) causes pinching and blurring of bands, as well as distortion of the DNA ladder.

As shown in Tip 4, loading more than 20 ng/band can lead to band distortion. However, this artifact is exacerbated when there is a high salt concentration, such as when samples are derived from restriction digests (Figure 5A). Figure 5B demonstrates the difference between a restriction digest sample diluted with molecular biology-grade water (0.75 µL digest diluted to 5 µL) and a much more concentrated restriction digest sample (3 µL digest diluted to 5 µL). The higher salt concentration of the 3 µL restriction digest sample resulted in pinching and blurring of bands, as well as pulling the DNA ladder (which has low salt and therefore low conductivity) to the left.

Tip 6: Implementing a short hold time prior to initiating separation can help smooth out visual artifacts

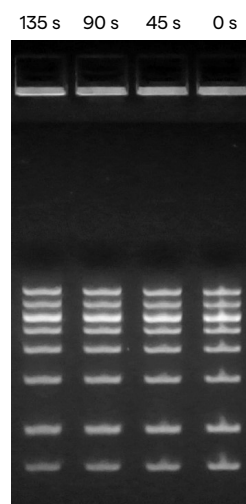


Figure 6. Band characteristics as a function of hold time. Time shown above each lane indicates the amount of time between sample loading and running the gel. For some samples, running the gel immediately after loading (0 s) can show slight distortion or “feathering” which can be easily remedied by waiting a few minutes (90 s) in between loading the gel and running the samples.

In some samples, you may observe a mild distortion or “feathering” of bands if gels are run immediately after sample loading (Figure 6, rightmost lanes). Implementing a hold time of a few minutes between loading samples and running the gel will eliminate this visual artifact (Figure 6, leftmost lanes).

Conclusion

The FlashGel® System provides a rapid and robust way to separate DNA fragments in real-time. Following the recommendations given here will help to ensure the best possible results. If you ever have questions or require troubleshooting help, you can always reach out to Lonza’s Scientific Support Team at scientific.support@lonza.com.

Ordering information

Cat. no.	Description
FlashGel® System for FlashGel® DNA Cassettes, FlashGel® RNA Cassettes and FlashGel® FLX Cassettes	
57025	FlashGel® Dock
57040	FlashGel® Camera
57068	FlashGel® Power Supply
57067	FlashGel® System, includes FlashGel® Dock, FlashGel® Camera, FlashGel® FLX Cassettes 1.2% 12+1 well single tier, FlashGel® FLX DNA Prestain Loading Buffer, and FlashGel® Marker 100 bp – 4 kb
57026	FlashGel® DNA Starter Kit, includes FlashGel® Dock, FlashGel® FLX Cassettes 1.2% 12+1 well single tier, FlashGel® FLX DNA Prestain Loading Buffer, and FlashGel® Marker 100 bp – 4 kb
FlashGel® FLX format	
317258	FlashGel® FLX Cassettes 1.2% agarose, 12+1 well single-tier format
317259	FlashGel® FLX Cassettes 1.2% agarose, 16+1 well double-tier format
317260	FlashGel® FLX Cassettes 2.2% agarose, 12+1 well single-tier format
317281	FlashGel® FLX Cassettes 2.2% agarose, 16+1 well double-tier format
316992	FlashGel® FLX DNA Prestain Loading Buffer
316993	FlashGel® FLX RNA Prestain Loading Buffer
57024	FlashGel® RNA Starter Kit includes FlashGel® FLX Cassettes 1.2% 12+1 well single tier, RNA Marker, FlashGel® FLX RNA Prestain Loading Buffer, and Molecular Biology Water
Pre-stained FlashGel® Recovery Cassette format	
57051	FlashGel® Recovery Cassettes 1.2% agarose, 8+1 double-tier format
57022	FlashGel® Recovery Cassettes 2.2% agarose, 8+1 double-tier format
57060	FlashGel® Recovery Buffer
57050	FlashGel® Recovery Kit, includes FlashGel® Recovery Cassettes 1.2% 8+1 well double tier, FlashGel® Recovery Buffer, FlashGel® Loading Dye, FlashGel® QuantLadder, and Visualization Glasses
Pre-stained FlashGel® DNA Cassette format	
57023	FlashGel® DNA Cassettes 1.2% agarose, 12+1 well single-tier format
57029	FlashGel® DNA Cassettes 1.2% agarose, 16+1 well double-tier format
57031	FlashGel® DNA Cassettes 2.2% agarose, 12+1 well single-tier format
57032	FlashGel® DNA Cassettes 2.2% agarose, 16+1 well double-tier format
50462	FlashGel® Loading Dye
50473	FlashGel® DNA Marker, 100 bp – 4 kb
57033	FlashGel® DNA Marker, 50 bp – 1.5 kb
57034	FlashGel® DNA Marker, 100 bp – 3 kb
50475	FlashGel® QuantLadder

Cat. no.	Description
Pre-stained FlashGel® RNA Cassette format	
57027	FlashGel® RNA Cassettes, 1.2% agarose, 12+1 well single-tier format
57028	FlashGel® RNA Cassettes, 1.2% agarose, 16+1 well double-tier format
50571	Formaldehyde Sample Buffer for RNA denaturation, contains bromophenol blue and xylene cyanol
50462	FlashGel® Loading Dye RNA
50577	FlashGel® RNA Marker, 0.5 bp – 9 kb
51200	AccuGENE® Molecular Biology Water (DNase/RNase free)

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