Use of FlashGel™ Cassettes for separations in gene editing workflows

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Abstract

The ability to perform genome editing has expanded tremendously in recent years and has been facilitated by the development of systems based on clustered regularly interspersed short palindromic repeats (CRISPR) and CRISPR-associated protein 9 (Cas9) that allow targeted cleavage of genomic loci. We have tested the use of FlashGel™ DNA Cassettes as tools to monitor the results of several electrophoretic separation steps that maybe involved in the CRISPR/Cas9 workflow: mismatch cleavage assays, single guide RNA (sgRNA) screening, and single stranded DNA (ssDNA) production. Mismatch cleavage assays are a quick and inexpensive tool to evaluate the efficiency of editing. FlashGel™ DNA Cassettes allowed rapid (five minutes) separation of cleavage products with high sensitivity. Separation performance was confirmed using control reagents from seven commercially available mismatch cleavage kits. Testing additionally showed that FlashGel™ DNA Cassettes worked well for rapid separation of cleavage products generated using control reagents from two sgRNA screening kits. ssDNA is becoming more widely used as a donor for knock-in CRISPR/Cas9 experiments involving homology directed repair. Testing in conjunction with control reagents from a commercially available ssDNA production system showed that FlashGel™ DNA Cassettes allowed monitoring of ssDNA generation and gave the added benefit of being able to distinguish ssDNA and dsDNA based upon observation of the color of the bands. The use of FlashGel™ DNA Cassettes in gene editing workflows offers increased separation speed and detection sensitivity relative to separations using conventional agarose gel formats.

Summary of kits tested with FlashGel™ Cassettes

Vendor	Mutation detection kits	sgRNA screening kits	ssDNA production kit
A	X		
B	X		
C	X		
D	X	X	
E	X		
F	X	X	X
G	X	X	

Run condition test and optimization

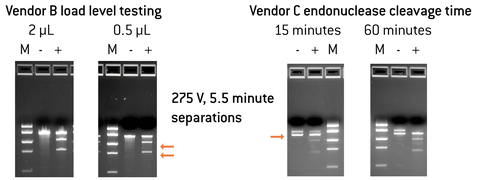

Figure 1. Examples of initial test separations of endonuclease cleavage reaction separations using controls from vendors B and C. Initial testing showed clear detection of cleavage products (in + lanes) as indicated by the presence of two smaller cleavage fragments (see arrows) running ahead of the uncleaved control fragments. The left hand images show testing of controls from vendor B using different volumes of endonuclease cleavage reactions for samples separated on FlashGel™ Cassettes. The results show improved separation with the use of smaller sample aliquots. The right hand images show comparisons of different incubation times for cleavage with reagents from vendor C. FlashGel™ Cassettes allowed a quick assessment of cleavage completion (arrow in 15' reaction indicates uncleaved heteroduplex band in sample).

Sensitivity testing with control templates

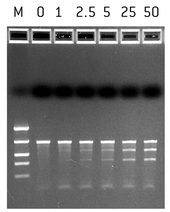

Figure 2 . Control amplicons A&B from endonuclease kit B were blended in different ratios (labels on the image indicate the percentage of amplicon B) to assess ability to detect cleavage with low levels of sequence variation in samples. Test results show the separation of 1 µL aliquots from cleavage reactions on a 1.2% FlashGel™ Cassette. This testing shows detection of heteroduplex cleavage products in samples with as little as 2.5% amplicon B in the sample.

Comparison with standard precast gels

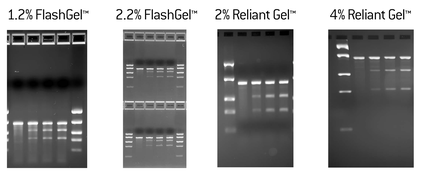

Figure 3. Comparison of endonuclease cleavage products separated on FlashGel™ Cassettes versus Reliant mini-gels. Samples containing control amplicons from endonuclease kit B (0, 5, 25, 50% amplicon B) were separated on different gel systems after the endonuclease reaction. Samples for the 1.2% FlashGel™ Cassette and the Reliant™ Gels used 2 µL of the reaction and samples for the 2-tier format FlashGel cassette used 1 µL of the reaction. Results of this testing showed that the FlashGel™ Cassettes allowed higher sensitivity detection with substantial time savings and that double tier FlashGel™ Format (allowing up to 32 samples) could also be used.

Survey of endonuclease cleavage reactions

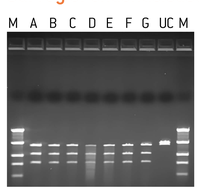

Figure 4. FlashGel™ Cassette separation of heteroduplex cleavage products using cleavage enzymes/kits from seven different vendors (designated as A-G). Control template was amplified using reagents in kit from vendor G. Aliquots of this control template were used to form heteroduplexes that could be used to test cleavage reagents from all vendors. Aliquots (1 µL) from each cleavage reaction were then used to prepare samples (1 µL cleavage product/4 µL 1X sample buffer) that were separated on a 1.2% FlashGel™ Cassette by electrophoresis at 275 V for 5 minutes. Samples also included an uncut (UC) aliquot of the heteroduplex. The results show that the FlashGel™ Cassette gave good separation of cleavage products with all kits tested.

Testing of sgRNA screening kits

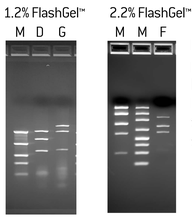

Figure 5. Separation of cleavage products from sgRNA screening kits. Control reagents (sgRNA, target amplicon, and Cas9) were tested using kits from vendors F, D, and G and 1 µL of the cleavage reaction products (and markers M) were tested by separation on 1.2% (vendor D&G samples) or 2.2% (vendor F sample) FlashGel™ Cassettes. Separation using FlashGel™ Cassettes allowed clear visualization of residual uncut target amplicons and cleavage products with apparent sizes that agreed with the values expected from the vendor protocols.

FlashGel™ Cassettes to monitor ssDNA production

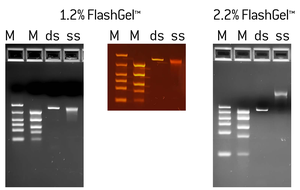

Figure 6. Testing of ssDNA generated with a ssDNA production kit from vendor F. Control reagents provided in the ssDNA production kit were used to generate a 2 kb control amplicon that was then processed using the kit reagents to convert the dsDNA amplicon to ssDNA. Aliquots (1µL) of the dsDNA template and putative ssDNA were examined by separation on 1.2 or 2.2% FlashGel™ Cassettes. The separation in the 1.2% FlashGel™ Cassette was run at 275 V for 3.5 minutes and the separation in the 2.2% cassette was run for 5 minutes. Separation on FlashGel™ Cassettes allowed rapid checks on the success of ssDNA generation; ssDNA bands showed different mobility than corresponding dsDNA and additionally, as shown in the center image, the ssDNA bands show a noticeable color difference from dsDNA. In this comparison with a 2 kb DNA template use of the 2.2% gel formulation maximized the differential migration of ss versus ds DNA molecules.

Conclusions/summary

- FlashGel™ Cassettes enabled rapid high sensitivity separation of T7 endonuclease cleavage products using reagents from all vendors tested (seven vendors)
- Cleavage products could be resolved on either single tier or double tier format FlashGel™ Cassettes enabling testing of up to 32
- samples on a single cassette
- Cleavage products from sgRNA screening tests were well resolved on FlashGel™ Cassettes
- FlashGel™ Cassettes could be used to monitor production of ssDNA, both migration differences and color differences of ssDNA bands enabled detection/differentiation of the ssDNA