Lonza

The FlashGel® System

For Rapid Analysis of Fast Restriction Enzyme Digests

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FlashGel® DNA Cassettes for gel electrophoretic analysis in combination with fast restriction digests reduce the workflow from restriction digestion to gel analysis to 15 minutes.

Introduction

Since their discovery in the early 70s [1, 2] restriction enzymes have become an indispensable tool for many common applications in Molecular Biology research. As the availability of restriction enzymes has evolved, the number of enzymes has grown to well over 200 options available commercially from a variety of vendors. Additionally, manufacturers have worked to improve the ease of use of restriction enzymes by increasing the number of enzymes that work in common buffers and also developing or certifying enzymes able to be used in fast digest reactions. The FlashGel® System from Lonza includes precast gels and accessories that enable fast (2 – 7 minutes), high sensitivity separations of DNA samples. This application note shows that reaction products from fast restriction digests can be effectively analyzed on FlashGel® Cassettes leading to a significant decrease in the time needed for the entire workflow (from restriction digestion to gel analysis).

Materials and Methods

Plasmid DNA (pBR322) purchased from New England BioLabs (NEB) was used as test substrate for restriction reactions. Unless noted otherwise, restriction reactions were assembled in 50 µL reaction volumes containing 1 µg of substrate DNA that was cut with 1 µL of each restriction enzyme. Fast reactions with Thermo Scientific™ FastDigest™, Anza™ enzymes and NEB High-Fidelity (HF®) enzymes used "common" reaction buffers for each of the different enzymes. For reactions with Time-Saver™-qualified standard NEB enzymes NEB 3.1 reaction buffer was used. Standard reactions with Thermo Scientific enzymes used 2X Tango reaction buffer. Reactions were incubated at 37°C in a thermal cycler for the indicated times. Double and triple digestions were run for testing: *Pst*I/*Bam*HI double digest generated 1129 and 3232 bp fragments and *Pst*I/*Bam*HI/*Eco*RI triple digest generated 377, 752, and 3232 bp fragments. Reactions were stopped by addition of 10 µL of 6X sample buffer containing EDTA and SDS (NEB, B7021) unless indicated otherwise. Samples for separation on FlashGel® Cassettes (1.2%, Lonza, 57023) were prepared using aliquots of the reactions (1 or 1.2 µL) containing approximately 20 ng of DNA. Samples were loaded after adding the fast digest samples to 1X FlashGel® Sample Buffer (Lonza, 50462) for final 5 µL load volumes, or in some cases samples were loaded directly. FlashGel® separations were run at 275 V for 5 or 6 minutes and cassettes were imaged on a CCD imaging system using UV transillumination. Control separations were performed using a 1%, 1X TBE, Reliant® Agarose Mini-Gel (Lonza, 54280). Control reaction samples were prepared for separation by combining 5 µL of digest samples with 1X sample buffer for a final load volume of 12 µL/lane.

Results

Restriction digestion and gel analysis by standard methods can require >2 hours, which is typically split relatively evenly between restriction digestion and gel analysis steps (Figure 1). Times needed for gel analysis vary depending upon the size of the gel used and the number of samples evaluated. Results shown with one hour analysis of restriction products on an agarose mini-gel are typical.

Example of Standard Restriction Digestion and Agarose Gel Separation

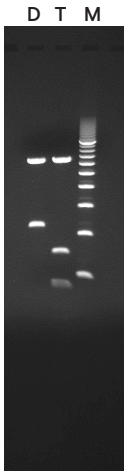

Figure 1.
Plasmid DNA was digested in *Pst*I/*Bam*HI double digest (D) or *Pst*I/*Bam*HI/*Eco*RI triple digest (T) using "standard" restriction enzymes from Thermo Scientific™. Reactions used 2X Tango reaction buffer and 1 µL of each enzyme in the reactions. Reactions were run at 37°C for 60 minutes and then stopped by adding 10 µL of 6X sample buffer (NEB) containing EDTA and SDS. Samples were prepared by combining 5 µL of the reactions with 7 µL of 1X sample loading buffer (Lonza). Marker lane (M) used a 500 bp ladder (Lonza). Restrictions and markers were loaded on a 1%, 1X TBE Reliant® Precast Mini-Gel. The gel was run at 90 V (7 V/cm) for 60 minutes and then imaged on a transilluminator.

Comparison Results for Fast Digest Reactions with 4 Enzyme Variations

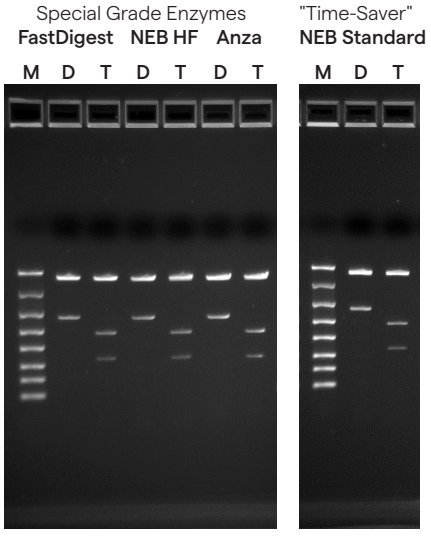

Figure 2.
Example of 5 minute double (D) and triple (T) digests using enzymes from 4 types of enzymes from 2 sources: Thermo Scientific's FastDigest and Anza enzymes and New England BioLabs' High-Fidelity (NEB HF) enzymes and standard Time-Saver Qualified enzymes (Time-Saver). Samples were prepared as described in Materials and Methods combining 1.2 µL of restriction samples and 3.8 µL 1X FlashGel® Sample Buffer. Marker lanes (M) used 2.5 µL loads of FlashGel® 100-4000 bp DNA Marker. Samples were separated on 1.2% FlashGel® Cassettes run at 275 V for 6 minutes.

Analysis of the different 5 minute fast restriction digests shows that aliquots of these reactions can be successfully resolved using 5-6 minute separations on FlashGel® Cassettes (Figure 2), irrespective of the enzyme and vendor. Additionally, optimal separations were obtained in the FlashGel® Cassettes using only 20 ng of DNA from restriction reactions. The combination of fast digest enzyme reactions and separation on FlashGel® Cassettes decreases the total time for digestion and gel analysis to <15 minutes. FlashGel® Cassettes enable the visualization of as little as 20 ng of restriction products, which is considerably less than what is typically used in standard gel electrophoresis.

FlashGel® Cassettes were applied to analyze different reaction times used for fast restriction digests (Figure 3). Using FlashGel® Cassettes it was quickly determined that 5 minute reaction times were sufficient to complete the digest.

FlashGel® Cassettes were also found to be effective for separations with direct loads of fast restriction digests using reaction buffers containing density agents in the formulation (Figure 4). This allows even faster assessment of restriction results as it simplifies sample preparation. Optimal results were seen with minimal volumes of the restriction reactions.

Summary

FlashGel® DNA Cassettes for gel electrophoretic analysis of fast restriction digests substantially reduced the time of the restriction-analysis workflow to as little as 15 minutes. FlashGel® DNA Cassettes were compatible with all the tested restriction enzymes and their sources yielding fragments within a range of 100 to 4,000 bp.

References:

1. Roberts R. (2005). How restriction enzymes became the workhorses of molecular biology. PNAS 102 (17): 5905-5908

2. Loenen WA, Dryden DT, Raleigh EA, Wilson GG, Murray NE. (2014). Highlights of the DNA cutters: a short history of the restriction enzymes. Nucleic Acids Res. 42 (1): 3-19.

Use of FlashGel® Cassette to Quickly Test Cutting Conditions

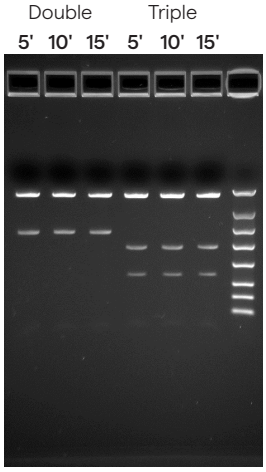

Figure 3.
Double and triple digest reactions were assembled using NEB High-Fidelity enzymes. Reactions were stopped at 5, 10, and 15 minutes by addition of 10X sample buffer and aliquots (1.2 µL) containing 20 ng of digested DNA were separated on FlashGel® Cassettes. Samples were prepared by combination of 1.2 µL of the reactions and 3.8 µL 1X FlashGel® Sample Buffer. Samples were separated on a 1.2% FlashGel® Cassette run at 275 V for 5 minutes.

Direct loads of FastDigest™ and Anza™ Restriction Reactions

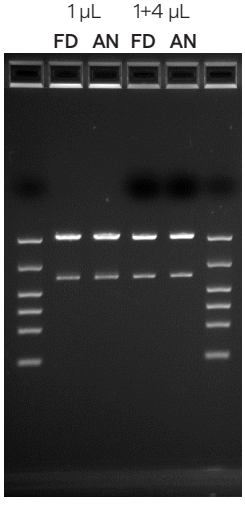

Figure 4.
Double restrictions with FastDigest™ (FD) and Anza™ (AN) enzymes were tested using Green and Red reaction buffers, respectively, containing density agents and colored tracking dyes, which enable direct gel loading and analysis after reactions. *Pst*I/*Bam*HI double digestions were run at 37°C for 10 minutes and then reaction samples were analyzed by electrophoresis on 1.2% FlashGel® Cassettes. Samples used were either direct loads (1 µL aliquots) of the restriction reactions, or samples prepared by combination of 1 µL of the restriction reactions with 4 µL (labeled as 1+4 µL in Figure) of 1X FlashGel® Sample Buffer. The gel was run at 275 V for 6 minutes and then imaged.

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MB-SP008 06/22

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