CRISPR/Cas9 Gene Editing

Fragment Analyzer™ Automated CE System

Identify Single-Cell Mutations and Determine Mutation Frequency

Mutation analysis by capillary electrophoresis provides significant benefits over slab gel methods. The Fragment Analyzer detects mutations generated from Non-Homologous End Joining (NHEJ) and Homology Directed Repair (HDR) modifications to targeted gene sequences. A multi-channel capillary electrophoresis instrument that uses performance tested non-denaturing Gel Kits coupled with a sensitive intercalating dye, the Fragment Analyzer automates workflow, reduces hands-on time, and decreases time to results while providing high detection sensitivity and separation resolution. PROSize® Data Analysis Software accelerates detection of mutation events by efficiently calculating percent cleavage and mutation frequency.

For gene editing experiments, the AccuCleave™ T7CE and the associated Gel Kit will show the mutation frequency of a pooled cell population, determine the presence/absence of a mutation from a single cell compared to its wild-type complement, and under certain circumstances, assist in calling the zygosity of alleles. Additionally, researchers can determine the quality and quantity of isolated genomic DNA prior to amplification. They can measure amplicons post-PCR to assist in combining equimolar amounts of pre-heteroduplexed wild-type and experimental amplicons.

In addition to mutation detection, the Fragment Analyzer can be used to QC single guide RNA (sgRNA) and Cas9 mRNA. Furthermore, time course studies or changes to cell lines during cell selective passage can be monitored.
Fragment Analyzer Workflow

Eliminate Slab Gel Usage
With its ease of use, ability to hold multiple 96 well sample plates, fast electrophoresis times, sensitivity, and versatility, the Fragment Analyzer is the ideal instrument to analyze CRISPR/Cas9 mutated cells and cell lines.

Ease of Use
Simply “load and go”. With a long reagent shelf-life and room temperature stable reagents, prepared gel can be held worry-free on the instrument and pumped into the capillaries as needed. The long lasting capillary array does NOT require daily handling or maintenance and can be used for thousands of injections. Variable capillary array length (22 cm, 33 cm, and 55 cm) and number of capillaries (12, 48, or 96) provide the ultimate flexibility in experiment design.

Sample Loading
Holding up to three, 96-well plates, sample plates can be queued to run while new plates are loaded into the instrument as soon as samples have been injected. And with a single addition of cleavage products, sample processing requires minimal effort and can be automated if desired. Due to the minimum uptake of sample, the same sample can be injected multiple times if warranted for confirmation or reproducibility studies.

Time to Results
With an automated workflow, loaded samples will run unattended until complete. Available electrophoresis methods are designed for optimum resolution of fragments with some runs as short as 20 minutes. Electrophoresis voltage can be adjusted by the user to decrease total electrophoresis time as conditions and resolution requirements demand.

Sensitivity
Cleaved fragments can be detected down to 5 pg/μL under the right experimental conditions. High sensitivity allows for the potential to reduce the number of amplification cycles and/or reduce the amount of cleavage enzyme used.

Versatility
With many Gel Kits to choose from, users can qualify or quantify all types of DNA fragments. Specifically, Gel Kits are available to quantify and qualify genomic DNA prior to amplification. Post-amplification, amplicons can be sized and the concentration determined for correct pooling prior to heteroduplex formation and enzymatic cleavage.
Data Output

Data can be quickly exported into either PDF or CSV format. The CSV output, as shown below, provides immediate access to the pertinent information regarding the samples and can be used for further analysis when necessary. The PDF output provides a page by page report for each sample. All or some of the sample data can be exported in either report.

Data output showing the expected sizes and the observed sizes. Full length and cut fragments are color coded for easy identification. The % cleaved (highlighted in pink) is shown for each sample.

CSV output of data. Expected and measured fragment size is shown as well as the molarity of the fragments. The % cleaved is calculated using the formula shown on the previous page.
Data Analysis Software

Determining which cells have been modified by the CRISPR/Cas9 machinery is a critical first step post-mutagenesis. Slab gel-based methods, which can lack the sensitivity and resolution needed to see all mutation types as well as automation for large-scale screening and analysis, also lack an integrated software analysis package.

PROSize® Data Analysis Software effortlessly calculates fragment sizes, fragment concentration, and fragment molarity. A software plugin, designed in collaboration with Integrated DNA Technologies, Inc.®, for CRISPR/Cas9 mutation analysis shows the mutation frequency of pooled samples and zygosity of individual samples (diploid analysis).

![PROSize interface](CSV upload of expected uncut and cut fragment sizes. The % error can be adjusted as needed to aid in fragment identification.)

**Mutation Frequency Calculator**

Users can input the expected uncut amplicon size and cleaved fragment sizes either manually for each capillary or through a CSV upload. The expected fragments are automatically compared to the observed fragment sizes and color coded for easy identification. The % cleaved is calculated from the molarity of identified fragments based on the following formula:

\[
\% \text{ Cleaved} = \frac{\text{Average Molarity (Fragment #1 and Fragment #2)}}{\text{Average Molarity (Fragment #1 and Fragment #2) + Molarity Fragment Uncut}}
\]
CRISPR / Mutation Discovery Kits

The kits listed below have been optimized for detection of point mutations and other small mutations post-heteroduplexed amplicon cleavage. The Gel Kit is available in a 1,000 sample kit size.

**CRISPR Discovery Gel Kit, 1 bp – 6,000 bp, DNF-910-1000CP**
- Markers at 1 bp and 6,000 bp; the fast running lower marker eliminates problems with primer dimers.
- Input Concentration Range: 5 ng/μL – 100 ng/μL
- Gel formulation suited for a wide range of fragments

**AccuCleave Kits**

**AccuCleave T7<sup>CE</sup> Kit, DNF-440-1000CP**
- T7 Endonuclease I based assay for the detection of CRISPR gene editing events
- For use with the CRISPR Gel Discovery Kit and the Fragment Analyzer

**AccuCleave C2 Control DNA Kit, DNF-443-0002CP**
- Provides two DNA controls for use with the AccuCleave T7<sup>CE</sup> Kit

**Other Available Gel Kits**

**RNA Analysis**

**Small RNA Analysis Kit, DNF-470**
- Sizing Range: 15 nt - 200 nt
- Input Concentration Range: 50 pg/μL – 2,500 pg/μL

**Standard Sensitivity RNA Analysis Kit (15 nt), DNF-471**
- Sizing Range: 200 nt – 6,000 nt
- Input Concentration Range: 5 ng/μL – 500 ng/μL

**High Sensitivity RNA Analysis Kit (15 nt), DNF-472**
- Sizing Range: 200 nt – 6,000 nt
- Input Concentration Range: 50 pg/μL – 5,000 pg/μL

**Genomic DNA Analysis**

**Genomic DNA 50 Kb Analysis Kit, DNF-467**
- Sizing Range: 75 bp – 60,000 bp
- Input Concentration Range: 25 ng/μL – 250 ng/μL

**DNA / NGS Fragment Analysis**

**Standard Sensitivity NGS Fragment Analysis Kit (1 bp - 6,000 bp), DNF-473**
- Sizing Range: 100 bp – 6,000 bp
- Input Concentration Range: 5 ng/μL – 100 ng/μL

**High Sensitivity NGS Fragment Analysis Kit (1 bp - 6,000 bp), DNF-474**
- Sizing Range: 100 bp – 6,000 bp
- Input Concentration Range: 50 pg/μL – 5,000 pg/μL
Specifications

**Maximum Sample Throughput:** Array dependent, either 12, 48, or 96 samples

**Maximum Unattended Sample Capacity:** Up to 288 samples

**Minimum Sample Volume:** 20 μL of liquid for injection; as little as 1 μL of actual sample required

**Resolution:** Gel dependent; to as low as 3 bp

**Sizing Accuracy:** Typically 5% or better

**Detection Sensitivity:** As low as 5 pg/μL for fragments and 50 pg/μL for a smear

**Light Source:** 700 mA, 10 W LED, 470 nm excitation wavelength

**Detector:** High sensitivity CCD; 500-600 nm emission wavelength

**Software:** Fragment Analyzer instrument control, PROSize® Data Analysis Software

**Data Export Format:** CSV, PDF, flexible numerical or binary output options

**Environmental Conditions:** Indoor use, normal laboratory environment 20-23°C

**Relative Humidity Range:** < 80% (non-condensing)

**Electrical:** 100-200 VAC; 50-60 Hz; 15 A (alternate configurations available)

**Instrument Dimensions:** Fully configured 61 cm H x 38 cm W x 51 cm D (24 x 14 x 20 in)

**Instrument Weight:** 38.5 Kg (85 lbs)