



# Agilent CRISPR Discovery Gel Kit

## Kit Guide

**For Research Use Only.**

**Not for use in diagnostic procedures.**

# Notices

## Manual Part Number

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# Contents

<b>1</b>	<b>Agilent CRISPR Discovery Gel Kit</b>	<b>5</b>
<b>2</b>	<b>Additional Material and Equipment Required</b>	<b>8</b>
	Material and Equipment Required for Analysis with the Fragment Analyzer System	8
	Additional Equipment/Reagents Required (Not Supplied)	9
<b>3</b>	<b>Agilent CRISPR Discovery Gel Kit Protocol</b>	<b>10</b>
	Gel Preparation	10
	Inlet Buffer Preparation	13
	Capillary Conditioning Solution Preparation	13
	Instrument Preparation	14
	Marker/Ladder/Sample Preparation	15
	General Information	15
	1 bp/6,000 bp Marker Preparation	15
	Mutation Detection Kit DNA Ladder Preparation	16
	Sample Preparation	17
	Performing Experiments	19
	Running an Experiment	19
	Viewing and Editing Experimental Methods	22
	Processing Experimental Data	25
	Fragment Analyzer Shut Down/Storage	26
	Instrument Shut Down/Storage	26
<b>4</b>	<b>Checking Your Separation Results</b>	<b>27</b>
	Mutation Detection Kit DNA Ladder	27
	CRISPR Sample	28

## Contents

<b>5</b>	<b>Troubleshooting</b>	<b>30</b>
	<b>Appendix A: CRISPR Main Plugin for the ProSize Data Analysis Software</b>	<b>31</b>
	Plugin Setup	31
	Using the CRISPR Main Plugin	32
	<b>Appendix B: CRISPR Single Cell Line Plugin for the ProSize Data Analysis Software</b>	<b>36</b>
	Plugin Setup	36
	Using the CRISPR SCL Plugin	37
	<b>Appendix C: CRISPR Pooled Cell Line Plugin for the ProSize Data Analysis Software</b>	<b>41</b>
	Plugin Setup	41
	Using the CRISPR SCL Plugin	42

# 1

## Agilent CRISPR Discovery Gel Kit

The CRISPR Discovery Gel kit from Agilent (1,000 Samples) (Part # DNF-910-K1000CP) is for determining the presence of CRISPR mutations after heteroduplexing and T7 Endonuclease I cleavage. Sizing and relative quantification between fragments can be obtained using this kit.

**Table 1 Physical Specifications**

Type	Specifications
Sample Volume Required	2 $\mu$ L
Number of Samples per Run	12-Capillary: 11 (+ 1 well DNA Ladder) or 12 (Imported DNA Ladder) <sup>2</sup> 48-Capillary: 47 (+ 1 well DNA Ladder) or 48 (Imported DNA Ladder) <sup>2</sup> 96-Capillary: 95 (+ 1 well DNA Ladder) or 96 (Imported DNA Ladder) <sup>2</sup>
Total Electrophoresis Run Time	35 min (22-47 Array) <sup>3</sup> ; 50 min (33-55 Array)

**Table 2 Analytical Specifications**

Type	Specifications
DNA Sizing Range	100 bp – 6,000 bp
Sizing Precision <sup>1</sup>	2% CV
Sizing Accuracy <sup>1</sup>	$\pm$ 5% or better
DNA Fragment Concentration Range	0.005 ng/ $\mu$ L – 2 ng/ $\mu$ L input DNA (adjustable by dilution of sample)
Separation Resolution	10 bp @ 500 bp (22-47 Array) <sup>3</sup> 8 bp @ 500 bp (33-55 Array)

<sup>1</sup> Results using DNA Ladder or DNA Fragment standards initially prepared in 0.1x TE Buffer.

<sup>2</sup> Refer to Marker/Ladder/Sample Preparation section for information on using an imported DNA Ladder.

<sup>3</sup> The 22 cm effective, 47 cm total length capillary is only available for 12-capillary Fragment Analyzer instruments.

## Agilent CRISPR Discovery Gel Kit

**Table 3 Storage Conditions**

Store at -20°C:	Store at 2-8°C (DO NOT FREEZE):	Store at Room Temperature (DO NOT FREEZE):
Intercalating Dye	dsDNA 810 Gel	5x Capillary Conditioning Solution
Markers, 1 bp & 6000 bp, 10% Formamide	5x 930 dsDNA Inlet Buffer	Mineral Oil Dropper Bottle
Mutation Detection Kit DNA Ladder	Dilution Buffer 0.1x TE	

Ensure all reagents are completely warmed to room temperature prior to use.

**Table 4 Kit Components**

Part Number	Name	Amount
DNF-810-0500	dsDNA 810 Gel	500 mL
DNF-600-U030	Intercalating Dye	30 µL x 2
DNF-355-0300	5x 930 dsDNA Inlet Buffer	300 mL, (dilute with sub-micron filtered water prior to use)
DNF-475-0100	5x Capillary Conditioning Solution	100 mL, (dilute with sub-micron filtered water prior to use)
FA-MRK915F-0003	Markers, 1 bp & 6000 bp, 10% Formamide* <ul style="list-style-type: none"> <li>Lower Marker (Set to 1 bp) and 6,000 bp Upper Marker in 1x TE buffer with 10% Formamide</li> </ul>	3.2 mL
FS-SLR905-0001	35-400 bp DNA Ladder <ul style="list-style-type: none"> <li>100 bp – 3,000 bp; 0.1 ng/µL total concentration in 0.1x TE buffer</li> </ul>	1.2 mL x 2
FS-SM015	Mineral Oil Dropper Bottle	15 mL
DNF-494-0060	Dilution Buffer 0.1x TE	60 mL

\*These components contain 10% Formamide. Refer to the product material safety datasheets for safety and handling information

**WARNING**

**Working with Formamide**

The following kit component contains 10% Formamide and handling of this component might hold health risks:

1 bp and 6,000 bp Markers (Part # FA-MRK915F-0003)

- Refer to product material safety datasheets for further chemical and biological safety information.
- Follow the appropriate safety procedures such as wearing goggles, safety gloves and protective clothing.

---

**WARNING**

**Working with Chemicals the handling of reagents and chemicals might hold health risks.**

- Refer to product material safety datasheets for further chemical and biological safety information.
  - Follow the appropriate safety procedures such as wearing goggles, safety gloves and protective clothing.
-

## 2 Additional Material and Equipment Required

### Material and Equipment Required for Analysis with the Fragment Analyzer System

#### Hardware:

- Fragment Analyzer with LED fluorescence detection:
  - 5200 Fragment Analyzer (Part # M5310AA)
    - FA 12-Capillary Array Ultrashort, 22cm (Part # A2300-1250-2247), OR
    - FA 12-Capillary Array Short, 33cm (Part # A2300-1250-3355), OR
    - FA 12-Capillary Array Long, 55cm (Part # A2300-1250-5580)
  - 5300 Fragment Analyzer (Part # M5311AA)
    - FA 48-Capillary Array Short, 33cm (Part # A2300-4850-3355) OR
    - FA/ZAG 96-Capillary Array Short, 33cm (Part # A2300-9650-3355) OR
    - FA/ZAG 96-Capillary Array Long, 55cm (Part # A2300-9650-5580)
  - 5400 Fragment Analyzer (Part # M5312AA)
    - FA/ZAG 96-Capillary Array Short, 33cm (Part # A2300-9650-3355) OR
    - FA/ZAG 96-Capillary Array Long, 55cm (Part # A2300-9650-5580)

#### Software:

- Fragment Analyzer instrument control software (version 1.1.0.11 or higher)
- ProSize data analysis software (version 2.0.0.61 or higher)

#### Reagents:

- Capillary Storage Solution, 100 mL (Part #GP-440-0100)



### Additional Equipment/Reagents Required (Not Supplied)

- 96-well PCR sample plates. Please refer to Appendix E – Fragment Analyzer Compatible Plates and Tubes in the Fragment Analyzer User Manual for a complete approved sample plate list.
- Multichannel pipettor(s) and/or liquid handling device capable of dispensing 1 – 100  $\mu$ L volumes (sample plates) and 1,000  $\mu$ L volumes (inlet buffer plate)
- Pipette tips
- 96-well plate centrifuge (for spinning down bubbles from sample plates)
- Sub-micron filtered DI water system (for diluting the 5x 930 dsDNA Inlet Buffer and 5x Capillary Conditioning Solutions)
- Fisherbrand 96 DeepWell 1 mL Plate, Natural Polypropylene, Fisher Scientific # 12-566-120 (Inlet Buffer and waste plate)
- Reagent Reservoir, 50 mL (VWR 89094-680 or similar) (for use in pipetting Inlet Buffer plates/sample trays)
- Conical centrifuge tubes for prepared gel and/or 1x Conditioning Solution
  - 50 mL (for smaller volumes): BD Falcon #352070, available from Fisher Scientific #14-432-22 or VWR #21008-940
  - 250 mL (for larger volumes): Corning #430776, available from Fisher #05-538-53 or VWR #21008-771

## 3 Agilent CRISPR Discovery Gel Kit Protocol

### Gel Preparation

- 1 Store the dsDNA 810 Gel at 2-8°C upon arrival.
- 2 Store the dsDNA 810 Gel at 2-8°C upon arrival.
- 3 The Intercalating Dye should be stored at -20°C.
- 4 Bring the dsDNA 810 Separation Gel and Intercalating Dye to room temperature prior to mixing.
- 5 Mix appropriate volumes of Intercalating Dye and dsDNA Separation Gel necessary for less than two weeks of operation. Use a 50 mL conical centrifuge tube to allow a small minimum working volume. For larger volumes, use a 250 mL conical centrifuge tube and remove the collar of the tube holder in the instrument reagent compartment.

#### NOTE

Some loss of detection sensitivity will be observed over a two-week period after the gel/dye mixture has been prepared. For maximum detection sensitivity applications, it is recommended to prepare gel/dye mixture daily. It is not recommended to use gel/dye mixture that is more than two weeks old.

- 6 The volume of separation gel required per run varies between 12-capillary, 48-capillary and 96-capillary Fragment Analyzer systems. The volumes required are summarized below.

## Agilent CRISPR Discovery Gel Kit Protocol

**Table 5 Volume Specifications for 12-Capillary Fragment Analyzer Systems**

# of Samples to be Analyzed <sup>1</sup>	Volume of Intercalating Dye	Volume of Separation Gel	Volume of 1x Conditioning Solution
12	1.0 µL	10 mL	10 mL
24	1.5 µL	15 mL	15 mL
36	2.0 µL	20 mL	20 mL
48	2.5 µL	25 mL	25 mL
96	4.5 µL	45 mL	45 mL

<sup>1</sup> A 5 mL minimum volume should be initially added to the tube. One sample well per separation is dedicated to the ladder.

**Table 6 Volume Specifications for 48-Capillary Fragment Analyzer Systems**

# of Samples to be Analyzed <sup>1</sup>	Volume of Intercalating Dye	Volume of Separation Gel	Volume of 1x Conditioning Solution
48	2.5 µL	25 mL	25 mL
96	4.0 µL	40 mL	40 mL
144	5.5 µL	55 mL	55 mL
192	7.0 µL	70 mL	70 mL
240	8.5 µL	85 mL	85 mL
288	10.0 µL	100 mL	100 mL

<sup>1</sup> One sample well per separation is dedicated to the ladder.

**Table 7 Volume Specifications for 96-Capillary Fragment Analyzer Systems**

# of Samples to be Analyzed <sup>1</sup>	Volume of Intercalating Dye	Volume of Separation Gel	Volume of 1x Conditioning Solution
96	4.0 µL	40 mL	40 mL
192	8.0 µL	80 mL	80 mL
288	12.0 µL	120 mL	120 mL
384	16.0 µL	160 mL	160 mL
480	20.0 µL	200 mL	200 mL

<sup>1</sup> One sample well per separation is dedicated to the ladder.

## Agilent CRISPR Discovery Gel Kit Protocol

- Place the prepared separation gel/intercalating dye mixture onto the instrument and insert the desired fluid line (Gel 1 or Gel 2 pump position). Ensure the fluid line is positioned at the bottom of the conical tube to avoid introducing air bubbles, which can cause pressurization errors.
- When adding separation gel to the instrument, update the solution levels in the Fragment Analyzer control software. From the main screen, select **Utilities > Solution Levels**. A menu will be displayed to enter in the updated fluid levels (Figure 1).

Check the fluid volumes before proceeding. Ensure that the waste is empty and that the gel and conditioning solutions are full.

Record the solution volumes here:

	Volume (mL)	Solutions
Gel 1	35.3	810
Gel 2	37.5	0.5N NaOH
Conditioning Solution	34.5	
Waste	156.0	

OK Cancel

Figure 1 Solution Levels menu

- When switching applications (e.g., between kits), prime the appropriate gel fluid line after loading fresh Separation Gel. From the main screen of the Fragment Analyzer control software, select **Utilities > Prime**. Select the desired fluid line(s) (Conditioning, Gel 1, or Gel 2) and press **OK** to purge the fluid line with fresh Separation Gel (Figure 2).

Prime...

Fluid Selected

- Conditioning
- Gel 1
- Gel 2

Cycles: 1

Fill Rate: 300  $\mu\text{L/s}$

Empty Rate: 300  $\mu\text{L/s}$

OK Cancel

Figure 2 Prime menu

## Inlet Buffer Preparation

- 1 Store the 5x 930 dsDNA Inlet Buffer at 2-8°C upon arrival. Do not freeze.
- 2 Bring the 5x 930 dsDNA Inlet Buffer to room temperature prior to mixing and use.
- 3 In a clean container, add 10 mL of the 5x 930 dsDNA Inlet Buffer per 40 mL of deionized sub-micron filtered water. Agitate to mix. The entire bottle can be mixed to 1x concentration and stored at 2-8°C if desired.

## Capillary Conditioning Solution Preparation

- 1 Store the 5x Capillary Conditioning Solution at room temperature upon arrival. Do not freeze.
- 2 In a clean container (e.g. 50 mL or 250 mL conical centrifuge tube), add 10 mL of the 5x Capillary Conditioning Solution per 40 mL of deionized sub-micron filtered water. Agitate to mix. The entire bottle can be mixed to 1x concentration and stored at room temperature if desired.
- 3 Once mixed, place the 1x Capillary Conditioning Solution onto the instrument and insert the conditioning fluid line (conditioning solution pump position). Ensure the fluid line is positioned at the bottom of the conical tube to avoid introducing air bubbles, which can cause pressurization errors.
- 4 The 1x Capillary Conditioning Solution should be added to the system as use demands. Tables 6-8 illustrate the volume specifications for the conditioning solution.
- 5 When adding fresh 1x Capillary Conditioning Solution to the instrument, update the solution levels in the Fragment Analyzer instrument control software. From the main screen, select **Utilities > Solution Levels**. A menu will be displayed to enter in the updated fluid levels (Figure 1).

## Instrument Preparation

- 1 Check the fluid level of the waste bottle and waste tray daily and empty as needed.
- 2 Prepare a fresh 96 DeepWell 1mL Plate filled with 1.0 mL/well of 1x 930 dsDNA Inlet buffer daily. (12-Capillary System: Row A only; 48-Capillary System: Row A to Row D; 96-Capillary System: All Rows) Do not overfill the wells of the inlet buffer plate.

### 12-Capillary Systems:

- a In Row H of the same prepared buffer plate, place **1.0 mL/well of Capillary Storage Solution** (Part# GP-440-0100).  
Row H of the buffer plate is used for the **Store** location, and the array moves to this position at the end of the experimental sequence.

### 48-Capillary System:

- a In the Sample 3 drawer, place a sample plate filled with **100 µL/well of Capillary Storage Solution** (Part# GP-440-0100) in Row A to Row D.  
Row A to Row D of the Sample 3 is used for the **Store** location, and the array moves to this position at the end of the experimental sequence.

### 96-Capillary Systems:

- a In the Sample 3 drawer, place a sample plate filled with **100 µL/well of Capillary Storage Solution** (Part# GP-440-0100).  
Sample 3 is used for the **Store** location, and the array moves to this position at the end of the experimental sequence.

#### NOTE

Ensure Row H of the buffer tray (12-capillary systems) or the Sample 3 drawer (48-Capillary and 96-capillary systems) is always filled with Capillary Storage Solution, and the capillary array is placed against Storage Solution when not in use, to prevent the capillary tips from drying out and potentially plugging.

The Capillary Storage Solution should be replaced every 2-4 weeks, as the solution will gradually thicken following exposure to the open air via evaporation. More frequent replacement may be required in low humidity or warm lab environments.

- 3 Place the prepared inlet buffer plate into Drawer "B" (top drawer) of the Fragment Analyzer. Ensure that the plate is loaded with well A1 toward the back left on the tray.
- 4 Place an empty 96 DeepWell 1mL Plate into Drawer "W" (second from top) of the Fragment Analyzer. This plate serves as the capillary waste tray and should be emptied daily. Alternatively, the supplied open reservoir waste plate may be used.

## Marker/Ladder/Sample Preparation

### General Information

The recommended 96-well sample plate for use with the Fragment Analyzer system is a semi-skirted PCR plate from Eppendorf (#951020303). Please refer to Appendix C – Fragment Analyzer Compatible Plates and Tubes in the Fragment Analyzer User Manual for a complete approved sample plate list. The system has been designed to operate using these dimensions/styles of PCR plates.

#### NOTE

The use of PCR plates with different dimensions to the above recommended plate could lead to decreased injection quality and consistency. Damage to the capillary array cartridge tips is also possible.

### 1 bp/6,000 bp Marker Preparation

- 1 Store the 1 bp and 6,000 bp Marker solution at -20°C upon arrival.
- 2 Bring the 1 bp and 6,000 bp Marker solution to room temperature prior to use; agitate solution to ensure it is properly mixed and centrifuge vial prior to dispensing.
- 3 The Marker solution is supplied as a **ready-to-use** solution, containing a fast running lower marker (set to 1 bp), and a 6,000 bp upper marker at 0.5 ng/μL in 1x TE buffer solution. It is intended for use as an external standard marker plate.
- 4 Prepare the Marker solution plate by dispensing 30 μL/well into Row A only (12-Capillary), Rows A-D (48-Capillary) or every well (96-Capillary) of a separate sample plate. Cover the wells with 20 μL/well of the supplied mineral oil to allow reuse for at least 30+ injections.
- 5 The prepared Marker solution plate should be placed into Drawer “M” (third from top) of the Fragment Analyzer. Ensure the plate is loaded with well A1 toward the back left.

### Mutation Detection Kit DNA Ladder Preparation

- 1 Store the Mutation Detection Kit DNA Ladder solution at -20°C upon arrival.
- 2 Bring the Mutation Detection Kit DNA Ladder solution to room temperature prior to use; agitate solution to ensure it is properly mixed and centrifuge vial prior to dispensing.
- 3 The Mutation Detection Kit DNA Ladder solution is supplied as a ready-to-use solution in a 0.1x TE buffer. It is used for calibrating the size of analyzed DNA fragments and is typically added to a well of the sample plate and run in parallel with the samples. Pipette 24 µL of Mutation Detection Kit DNA Ladder solution into the respective well of the sample plate:

#### 12-Capillary System:

- a Well 12 of each row to be analyzed

#### 48-Capillary System:

- a Well D12 if samples are in Row A to Row D, or
- b Well H12 if samples are in Row E to Row H

#### 96-Capillary System

- a Well H12

- 4 Alternatively, once the Mutation Detection Kit DNA Ladder has been run under the experimental method and additional samples are to be run under the same experimental conditions, the ladder can be imported in the ProSize software, enabling use of all 12, 48 or 96 wells of the sample plate. Refer to the ProSize software User Manual for information on exporting and importing calibration ladders.



### Sample Preparation

Proper sample preparation will ensure detection of CRISPR mutations. The protocol below provides some general guidelines regarding sample preparation.

### PCR Reaction

- 1 After extracting genomic DNA, perform the PCR reaction, amplifying the region of interest from both CRISPR mutated and wild type samples. It is important to optimize PCR conditions, so that only one PCR product is detected. This kit can be used to detect PCR products before enzymatic cleavage if desired.

### Heteroduplex Formation

- 1 Quantitate the amount of PCR product using fluorometry (e.g., Qubit or similar device).
- 2 Mix CRISPR mutated DNA with wild type DNA in a 1:1 ratio (ng/ng) to improve the heteroduplexing efficiency.
- 3 Using a thermocycler, heteroduplex CRISPR mutated DNA and wild type DNA:
  - a Heat denature amplicon for 10 min at 95°C
  - b 95-85°C at -2.0°C/sec
  - c 85°C for 20 sec
  - d 85-25°C at -0.1°C /sec
  - e 25°C for 20 sec
  - f 4°C Hold

### Heteroduplex Cleavage Reaction

- 1 Agilent recommends use of the AccuCleave T7 Kit for performing the enzymatic heteroduplex cleavage reaction (Part # DNF-440-1000CP).
- 2 Follow the AccuCleave T7 Kit protocol for performing the heteroduplex digestion and stop reaction.

### Sample Plate Preparation

- 1 A minimum volume of 20 µL/well is required for proper sample injection. If the AccuCleave T7 kit was used for heteroduplex cleavage, the final 200 µL reaction mixture can be used without further dilution. If a different heteroduplex cleavage protocol is used, it is recommended to use 2 µL sample and 22 µL DNF-494 Dilution Buffer 0.1x TE per well.

### NOTE

#### Important Sample Mixing Information

When mixing sample with Dilution Buffer 0.1x TE solution, it is important to mix the contents of the well thoroughly to achieve the most accurate quantification. It is highly suggested to perform one of the following methods to ensure complete mixing:

- When adding 2  $\mu$ L of sample to the 22  $\mu$ L of diluent, **swirl the pipette tip** while pipetting up/down to further mix. OR
- After adding 2  $\mu$ L of sample to the 22  $\mu$ L of diluent, place a plate seal on the sample plate and **vortex the sample plate** at 3000 rpm for 2 min. Any suitable benchtop plate vortexer can be used. Ensure that there is no well-to-well transfer of samples when vortexing. The plate should be spun via a centrifuge after vortexing to ensure there are no trapped air bubbles in the wells. OR
- After adding 2  $\mu$ L of sample to the 22  $\mu$ L of diluent, **use a separate pipette tip set** to a larger 20  $\mu$ L volume, and pipette each well up/down to further mix. OR
- Use an **electronic pipettor capable of mixing a 10  $\mu$ L volume** in the tip after dispensing the 2  $\mu$ L sample volume. Some models enable using the pipette tip for both adding and mixing.

- 2 Samples should be loaded into every well of the Sample Plate. If less than the number of capillaries (i.e. 12, 48, or 96 capillary instruments) are to be analyzed, the user should place 24  $\mu$ L of Dilution Buffer 0.1x TE into the empty wells of the Sample Plate prior to performing the experiment.
- 3 After mixing the samples with Dilution Buffer 0.1x TE in each well, check the wells of the sample plate to ensure there are no air bubbles trapped in the bottom of the wells. If necessary, centrifuge the plate to remove any air bubbles. The presence of trapped air bubbles can lead to injection failures.
- 4 For best results, run the sample plate as soon as possible. If the sample plate will not be used immediately, cover the sample plate with a cover film, store at 2-8°C, and use as soon as possible. Alternatively, to prevent evaporation, place a mineral oil overlay on each sample (20  $\mu$ L/well).
- 5 To run the samples, place the plate in one of the available sample plate drawers (Drawers 4-6 from the top) of the Fragment Analyzer system.

### NOTE

The CCD detection system of the Fragment Analyzer system provides a high dynamic range for detection. An ideal injection range would yield peak heights from 100 – 20,000 RFUs. Overloading of sample can decrease separation resolution and saturate the detector, leading to mismatched lower/upper marker peak heights and poor results. It is important to optimize sample dilution and concentration and use experimental parameters to work within the specified RFU range.

## Performing Experiments

### Running an Experiment

- 1 To set up an experiment, from the main screen of the Fragment Analyzer control software, select the **Operation** tab (Figure 3). Select the sample tray location to be analyzed (1, 2, or 3) by left clicking the **Sample Tray #** dropdown or by clicking the appropriate sample plate tab (alternate plate view) and choosing the appropriate location.

**NOTE**

For 48-Capillary or 96-Capillary Systems: Sample 3 is typically assigned to the Capillary Storage Solution.

- 2 Left click a well of the desired sample plate row with the mouse. The selected row will be highlighted in the plate map (e.g., Row A in Figure 3). Enter the sample name if desired into the respective **Sample ID** cell by left clicking the cell and typing in the name. Alternatively, sample information can be imported from a .txt or .csv file by selecting the **Load from File...** option.

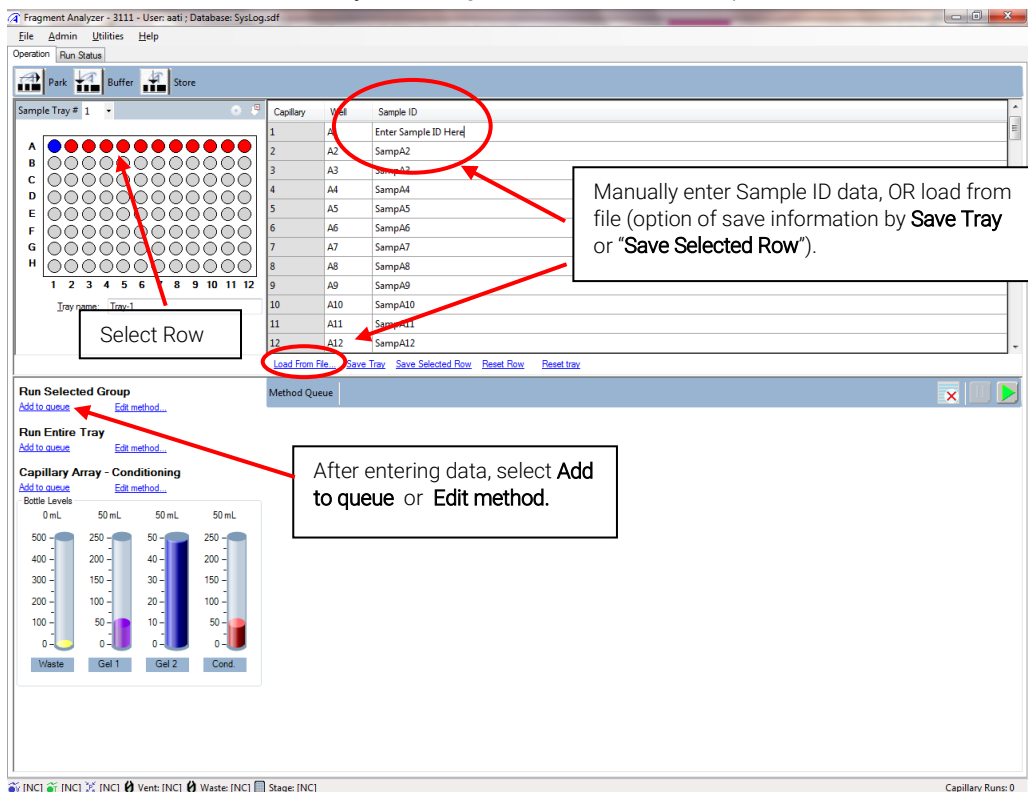
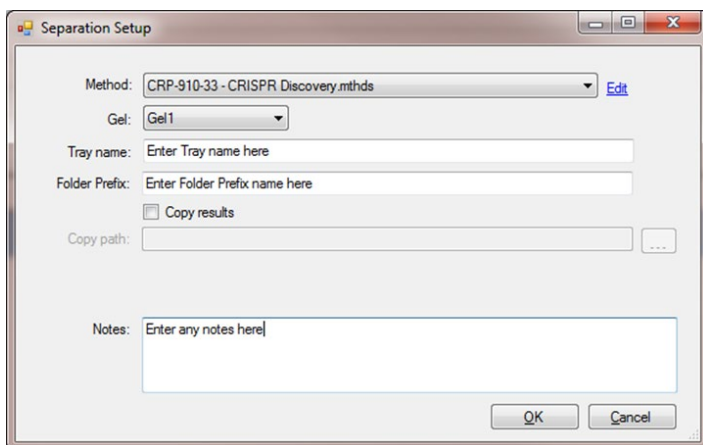


Figure 3. Main Screen showing selection of sample row and entering sample information

## Agilent CRISPR Discovery Gel Kit Protocol




- After sample information for the row or plate has been entered, under the **Run Selected Group** field press **Add to queue**. The **Separation Setup** form will be displayed enabling the user to select the experimental method and enter additional information (Figure 4).



**Figure 4.** Separation Setup form to select experimental Method and enter additional information

- In the **Separation Setup** pop-up form, left click the dropdown and select the appropriate preloaded experimental **Method** file. The available methods are sorted by kit number and are linked to the directory containing methods for the currently installed capillary array length (e.g., 22cm or 33cm). Select the following method:
  - Select **CRP-910-22 – CRISPR Discovery.mthds** for the 22 cm effective length, 47 cm total length “ultra-short” capillary array.
  - Select **CRP-910-33 – CRISPR Discovery.mthds** for the 33 cm effective length, 55 cm total length “short” capillary array.
- Select the appropriate **Gel** line being used for the experiment (Gel 1 or Gel 2) using the dropdown.
- The **Tray Name** can be entered to identify the sample plate. The **Folder Prefix** if entered will amend the folder name (normally a time stamp of HH-MM-SS from the start of the CE run).
- To copy the experimental results to another directory location in addition to the default save directory, check the **Copy results** box and select the desired **Copy path**: directory by clicking the ... button and navigating the desired save directory.
- Any **Notes** can be entered regarding the experiment; they will be saved and displayed in the final PDF report generated by the ProSize software.

## Agilent CRISPR Discovery Gel Kit Protocol

- Once all information has been entered, press **OK** to add the method to the instrument queue (press **Cancel** to abort adding the method).
- Repeat Steps 1-9 for any remaining sample rows to be analyzed.
- On 96-capillary systems, or in 12-capillary or 48-capillary systems if the entire 96-well sample tray is to be run using the same experimental method, under the **Run Entire Tray** field press **Add to queue**. A form similar to Figure 4 will be displayed for entering information and adding the run to the instrument queue for the entire 96-well sample tray.
- After a row or tray has been added to the queue, the method(s) will be listed on the main screen under the **Method Queue** field (Figure 5).
- Prior to starting the experiment, verify all trays (buffer/storage, waste, marker, sample, etc.) have been loaded into their respective drawer locations.
- Press the Play icon () to start the sequence loaded into the queue. To Pause the queue after the currently running experiment is completed, press the  button. To Clear the run queue of all loaded runs press the  button.

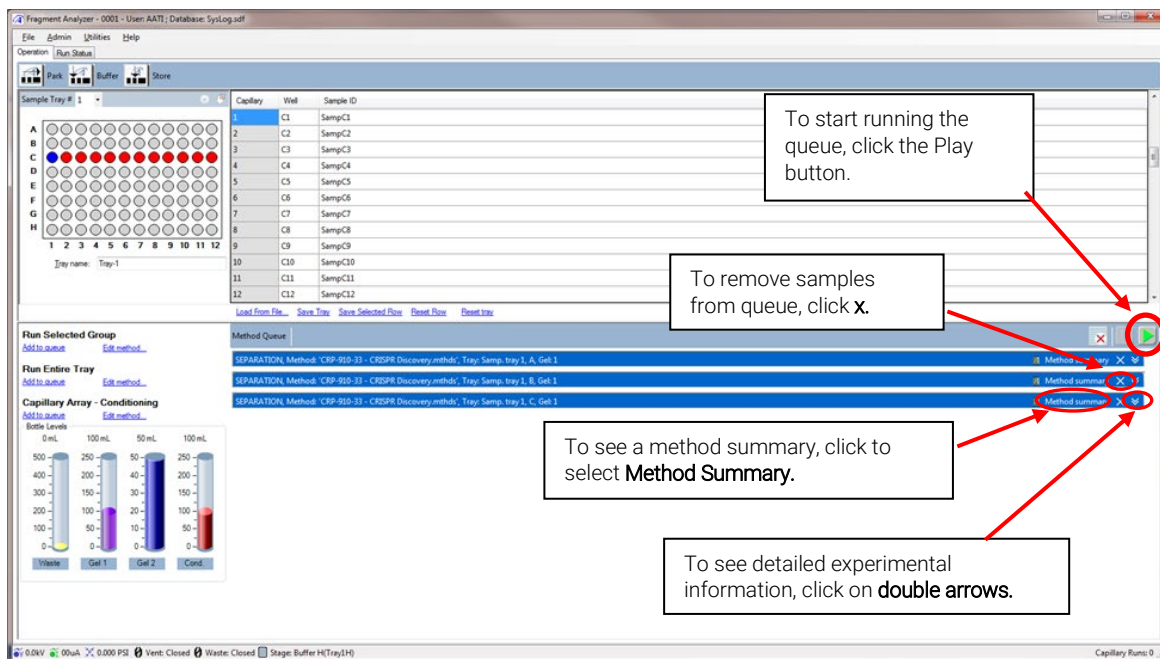


Figure 5. Main screen after selection of samples to the run queue

- Once an experiment has been loaded onto the queue, the user can view or edit the method (Administrator level only can edit a method) by pressing the **Method Summary** field. To remove the method from the queue, press the **x**

button; to view the stepwise details of the method press the double down arrow icon.

- 16 The user may add a pause or prime step into the queue by right clicking the mouse while over the queue and selecting **Insert Pause** or **Insert Prime**.
- 17 The order of the experimental queue can be rearranged by dragging down individual entries. Further information regarding the Method Queue operation is provided in the Fragment Analyzer User Manual.
- 18 Once started, the instrument performs all programmed experiments in the **Method Queue** uninterrupted, unless a Pause step is present. Note that additional experiments can be programmed and added to the **Method Queue** at any time while the instrument is running if desired. After completion of the last queued experiment, the stage will automatically move to the **Store** location (12-Capillary Systems: Row H of the inlet buffer tray containing the Capillary Storage Solution; 48-Capillary and 96-Capillary Systems: Sample 3 location).

### Viewing and Editing Experimental Methods

- 1 A user level operator can **View** the steps of the experimental method by pressing the **View** link on the **Separation Setup** screen, or by pressing the **Method Summary** option once a method has been loaded onto the experimental queue. User level operators cannot edit any steps of a queued separation method.
- 2 Administrator level operators can **Edit** certain steps of the experimental method. To open the method editor screen, press the **Edit** link from the **Separation Setup** screen (Figure 4). The method editor screen is displayed, showing the steps of the method (Figure 6).
- 3 The preloaded, optimized steps for the **CRP-910-22** (Figure 6) and **CRP-910-33** (Figure 7) methods are shown below. The **CRP-910-22** method steps are:
  - a **Full Conditioning** flushing method (Automatically enabled).  
Default Gel Selection: Gel 1.
  - b Perform Prerun (enabled) (5.5 kV, 30 sec)
  - c Rinse (disabled)
  - d Marker Injection (enabled) Voltage Injection (4 kV, 5 sec). This step injects the 1 bp/6,000 bp marker plate.
  - e Rinse (disabled)
  - f Sample Injection (enabled) Voltage Injection (5 kV, 45 sec). This step injects the prepared sample plate
  - g Separation (enabled) Voltage (5.5 kV, 35 min). This step performs the CE Separation.

## Agilent CRISPR Discovery Gel Kit Protocol

Separation Method:

Full Conditioning     Gel prime to buffer    Gel selection: **Gel 1** ▼

Gel Prime

Perform Pre-run    Voltage: **5.5** kV    Time: **30** Sec.

Rinse    Tray: **Buffer** ▼    Row: **A** ▼    # Dips: **1** ▼

Marker Injection    Row: **A** ▼

Voltage Injection    Voltage: **4.00** kV    Time: **5** Sec.

Vacuum Injection    Pressure: **-2.0** PSI

Rinse    Tray: **Marker** ▼    Row: **A** ▼    # Dips: **1** ▼

Sample Injection

Voltage Injection    Voltage: **5.00** kV    Time: **45** Sec.

Vacuum Injection    Pressure: **-2.0** PSI

Separation    Voltage: **5.5** kV    Time: **35.00** Min.

Figure 6. CRP-910-22 CRISPR Discovery method method

## Agilent CRISPR Discovery Gel Kit Protocol

- Figure 7 shows the preloaded method for the 33 cm effective length, 55 cm total length “short” array. The Prerun and Separation voltage is set to 6 kV, the Marker Injection voltage to 5 kV for 5 sec, the Sample Injection voltage to 5 kV for 45 sec, and the Separation time to 50 min.

Separation Method:

Full Conditioning     Gel prime to buffer    Gel selection: Gel 1

Gel Prime

Perform Prerun    Voltage: 6.0 kV    Time: 30 Sec.

Rinse    Tray: Buffer    Row: A    # Dips: 1

Marker Injection    Row: A

Voltage Injection    Voltage: 5.00 kV    Time: 5 Sec.

Vacuum Injection    Pressure: -2.0 PSI

Rinse    Tray: Buffer    Row: A    # Dips: 1

Sample Injection

Voltage Injection    Voltage: 5.00 kV    Time: 45 Sec.

Vacuum Injection    Pressure: -2.0 PSI

Separation    Voltage: 6.0 kV    Time: 50.00 Min.

OK    Cancel

Figure 7. CRP-910-33 CRISPR Discovery method

- An Administrator level user has the option to adjust the **Gel Selection**; **Prerun** settings; **Rinse** settings including **Tray**, **Row** and **# Dips**; **Marker Injection** settings including **Row**; **Sample Injection** settings; and the **Separation** settings. For example, if the marker solution is loaded into a row other than Row A on a 12-capillary instrument, this can be adjusted prior to or while the method is loaded on the experimental queue.
- To apply any adjustments to the method being placed on the experimental queue, press the **OK** button. To exit the editor screen without applying any changes press the **Cancel** button.

### NOTE

Any edits made to the experimental method from the **Separation Setup** or **Method Summary** screen will only apply to the currently loaded experiment in the queue. No changes are made to the original separation method file.



### Processing Experimental Data

- 1 When processing data, the ProSize software will automatically recognize the separation method performed and apply the appropriate matching configuration file from the C:\PROSize 3.0\Configurations directory:
  - The **CRP-910-22 – CRISPR Discovery** separation method will be processed using the **CRP-910-22 – CRISPR Discovery** configuration file.
  - The **CRP-910-33 – CRISPR Discovery** separation method will be processed using the **CRP-910-33 – CRISPR Discovery** configuration file.

#### NOTE

If the preloaded ProSize configuration files **CRP-910-22 – CRISPR Discovery** or **CRP-910-33 – CRISPR Discovery** are not located in the C:\PROSize 3.0\Configurations directory, contact Agilent Technical Support to obtain these files.

- 2 The data is normalized to the lower marker (set to 1 bp) and upper marker (set to 6,000 bp) and calibrated to the Mutation Detection Kit DNA Ladder run in parallel to the samples. Figure 8 shows an example of the 1 bp and 6,000 bp markers injected with the Mutation Detection Kit DNA Ladder. A total of 16 peaks should be observed.
- 3 When processing data, ProSize is set to the **DNA** mode in the **Advanced Settings**. The Quantification settings are set to **Use Ladder** for quantification with a **Final Conc. (ng/  $\mu$ L)** of 0.0083 and a **Dilution Factor** of 12 (2  $\mu$ L sample + 22  $\mu$ L Diluent Marker). Note that if a pre-dilution was performed prior to the experiment, the **Dilution Factor** setting should be changed to reflect the estimated final sample concentration.
- 4 For full information on processing data, refer to the ProSize Data Analysis Software User Manual.
- 5 In addition, several ProSize plugin programs are available for performing additional CRISPR related calculations and reporting. See **Appendices A-C** for further information.

#### NOTE

The use of ProSize plugin programs for CRISPR analysis require ProSize Version 2.0.0.61 or higher. Please contact Agilent Technical Support for information on software upgrades.

## Fragment Analyzer Shut Down/Storage

### Instrument Shut Down/Storage

After each experiment, the instrument automatically places the capillary array in the **Store** position against Capillary Storage Solution:

- 12-Capillary Systems: Row H of the buffer tray
- 48-Capillary Systems: Rows A-D of the buffer tray
- 96-Capillary Systems: Sample 3

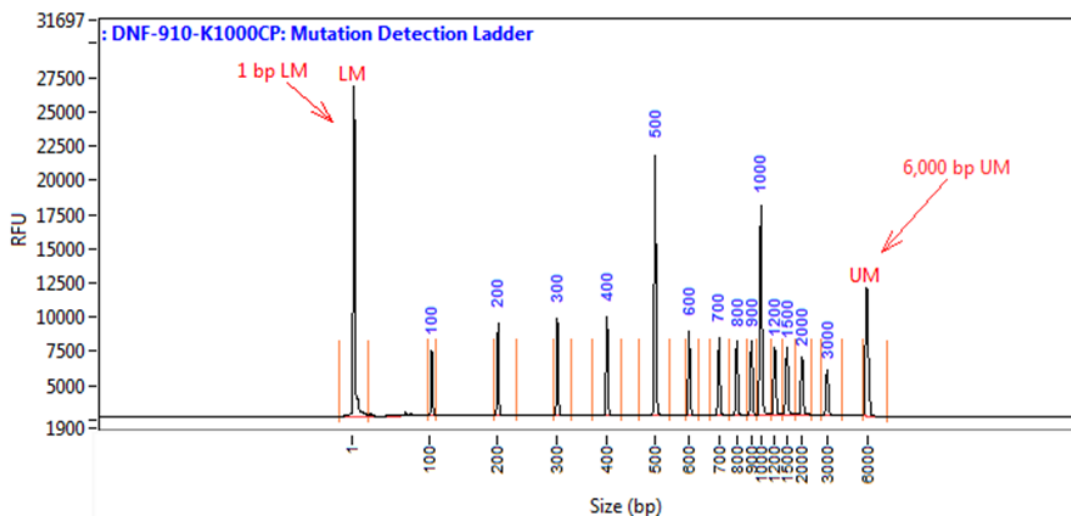
No further action is required.

If the instrument is to be idle for more than one day, it is recommended to turn off the power to the system.

## 4 Checking Your Separation Results

### Mutation Detection Kit DNA Ladder

Figure 8 shows the typical expected results for the Mutation Detection Kit DNA Ladder, co-injected with the 1 bp lower marker and 6,000 bp upper marker, and run in the 33 cm effective length, 55 cm total length “short” capillary array. A total of 16 peaks should be observed, with the sizes annotated as in Figure 8. All fragments in the ladder should be resolved.

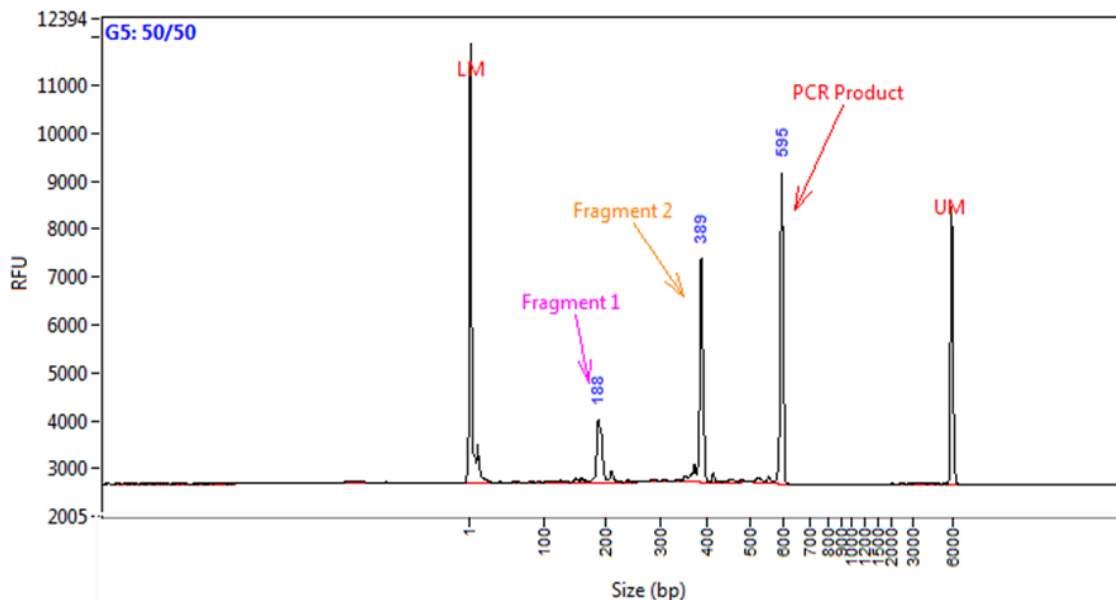


**Figure 8.** Representative Mutation Detection Kit DNA Ladder result injected with 1 bp lower marker and 6,000 bp upper marker, using the Fragment Analyzer system with the DNF-910-K1000CP reagent kit. Method: **CRP-910-33**. Peaks annotated by size (bp).

## CRISPR Sample

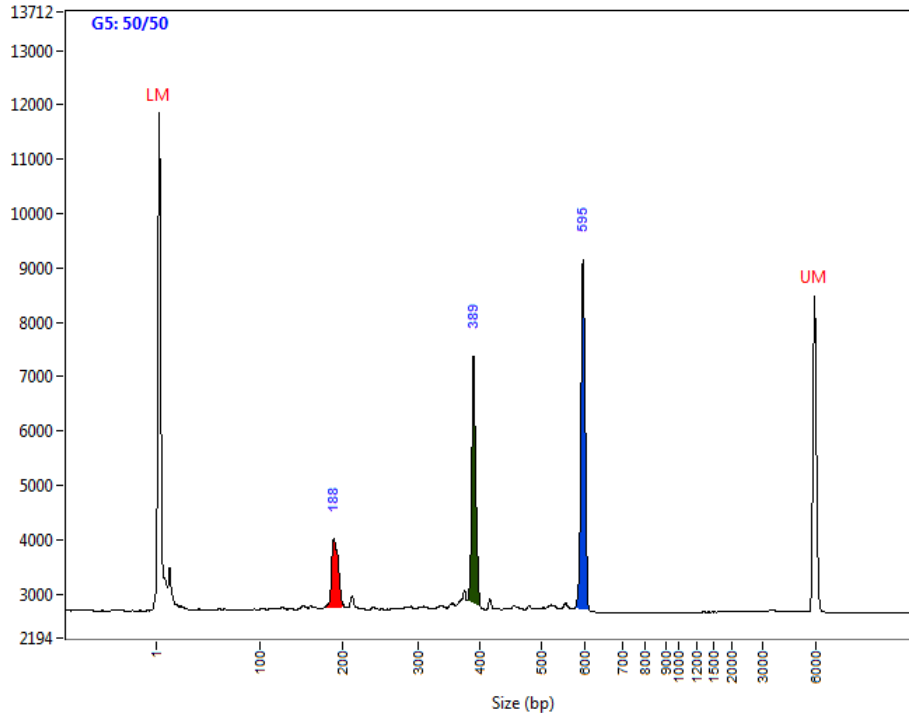
A positive CRISPR assay result is indicated by the presence of cut DNA fragments whose size sum to the intact, original DNA fragment within a reasonable sizing **% Error**. An example positive result is shown in Figures 9 and 10.

Figure 9 shows the result as viewed within the ProSize software main program; Figure 10 shows the same result viewed using the **CRISPR Main** plugin program (see **Appendixes A-C** for further information).



**Figure 9.** Example of a positive identification of a CRISPR mutation, using the DNF-910-K1000CP CRISPR Discovery kit on the Fragment Analyzer system. Data shown was generated using a synthetic gene system to model CRISPR mutations.

## Checking Your Separation Results



**Figure 10.** Sample from Figure 9 of a positive identification of a CRISPR mutation, viewed using the CRISPR Main ProSize plugin program. Cut fragments whose size sums to that of the original DNA fragment are automatically highlighted and data can be exported. Data shown was generated using a synthetic gene system to model CRISPR mutations. See **Appendixes A-C** for further information.

## 5 Troubleshooting

The following table lists several potential assay specific issues which may be encountered when using the DNF-910-K1000CP CRISPR Discovery Kit and suggested remedies. Contact Agilent technical support if you have any additional troubleshooting or maintenance questions.

**Table 8 Troubleshooting actions for assay specific issues**

Issue	Cause	Corrective Action
The peak signal is >> 20,000 RFU; upper marker peak is low or not detected relative to lower marker.	1 Input DNA sample concentration is too high.	1 Further dilute input DNA sample concentration with Dilution Buffer 0.1x TE and repeat experiment. 2 Reduce injection time and/or injection voltage and repeat experiment.
No peak observed for DNA sample when expected. Lower/Upper Marker peaks observed.	1 Sample concentration too low and out of range. 2 Sample was not added to Dilution Buffer 0.1x TE or not mixed well. 3 Wrong sample row was injected.	1 Prepare more concentrated sample and repeat experiment (e.g. 4 µL sample + 20 µL Dilution Buffer 0.1x TE); or repeat experiment using increased injection time and/or injection voltage for Sample Plate. 2 Verify sample was correctly added and mixed to sample well. 3 Verify the correct sample row was selected for the analysis.
Sample peak(s) migrate before or co-migrate with 1 bp Lower Marker.	1 Excess primer-dimer species in sample.	1 Further dilute input DNA sample concentration with Dilution Buffer 0.1x TE to minimize primer-dimer interference and repeat experiment.
Sample peak(s) migrate after or co-migrate with 6,000 bp Upper Marker.	1 DNA sample size out of range of assay.	1 Analyze samples with dsDNA 930 Reagent Kit, 75 bp – 20,000 bp (DNF-930).
No sample peak or Lower Marker peak observed for individual sample.	1 Air trapped at the bottom of sample plate well, or bubbles present in sample well. 2 Insufficient sample volume. A minimum of 20 µL is required. 3 Capillary is plugged.	1 Check sample plate wells for trapped air bubbles. Centrifuge plate. 2 Verify proper volume of solution was added to sample well. 3 Check waste plate for liquid in the capillary well. If no liquid is observed, follow the steps outlined in Appendix G—Capillary Array Cleaning of the Fragment Analyzer User Manual for unclogging a capillary array.



## Appendix A: CRISPR Main Plugin for the ProSize Data Analysis Software

The **CRISPR Main** plugin program for the ProSize data analysis software enables a user to enter expected PCR fragment size as well as the expected cleavage fragment sizes, and automatically score and highlight detected cut fragments. A PDF report or .csv file export can then be generated for the results. This plugin is designed to be used in conjunction with the Agilent AccuCleave T7 Kit (Part # DNF-440-1000CP).

### NOTE

The use of ProSize plugin programs, including the **CRISPR Main** plugin, require ProSize Version 2.0.0.61 or higher. Please contact Agilent Technical Support for information on software upgrades.

## Plugin Setup

- 1 Obtain the plugin library file **CRISPR\_2\_0.Ilb** by contacting Agilent Technical Support.
- 2 In the directory C:\PROSize 2.0 create a folder named **Plugins** if one is not already present.
- 3 Paste the file **CRISPR\_2\_0.Ilb** into the C:\PROSize 2.0\Plugins folder and restart the ProSize program.

## Using the CRISPR Main Plugin

- 1 In ProSize open the data file you wish to analyze.
- 2 From the ProSize main screen, select the dropdown **Tools** > **CRISPR Main** to open the CRISPR Main plugin (Figure A1).

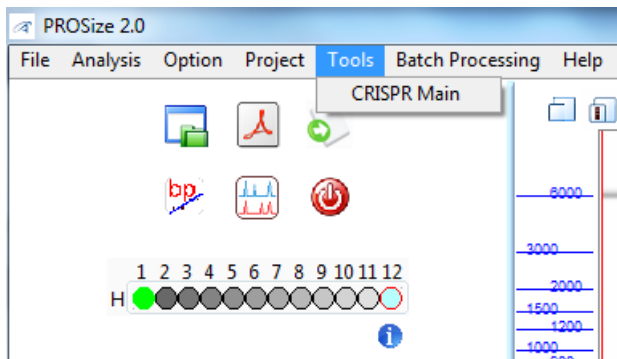


Figure A1. Launching the ProSize CRISPR Main plugin.

- 3 To configure fragment sizes for the plugin, or load previously configured and saved values, select **Configure** in the upper left hand corner of the display (Figure A2).

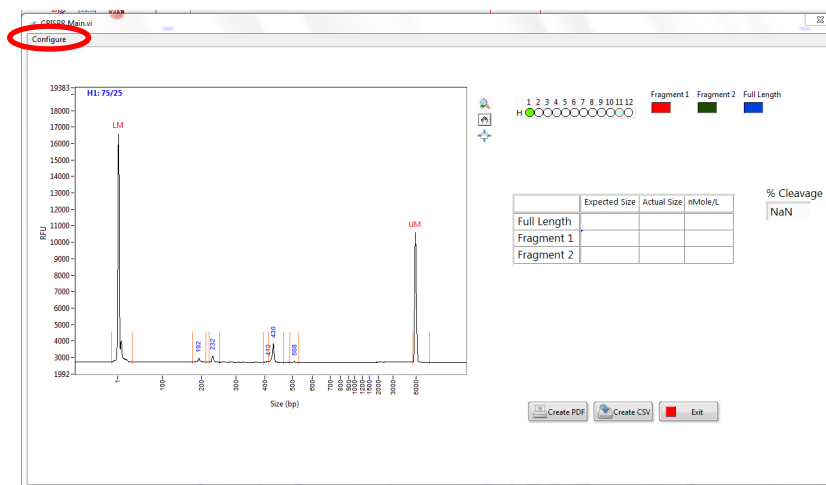


Figure A2. Select **Configure** to enter expected PCR fragment and cleaved DNA fragment sizes.



## CRISPR Main Plugin for the ProSize Data Analysis Software

- 4 In the **Configure** screen enter the expected PCR fragment size (**Full Length**) and the expected cleavage fragment sizes (**Fragment 1** and **Fragment 2**; enter in order of increasing size) for each sample well (Figure A3). Right-click over a cell and select **Apply to Column** to copy the entered value in that cell to the entire column. To apply an entire row of entered values to all rows, right click over the row with the desired values entered and select **Apply to All**. To apply to a selected set of wells, right click over the row with the desired values entered and select **Apply to Selected**, and mark the respective wells to copy the values to in the pop up dialog box (Figure A4), and click **Done**. To clear all entered values, right click and select **Clear All**.

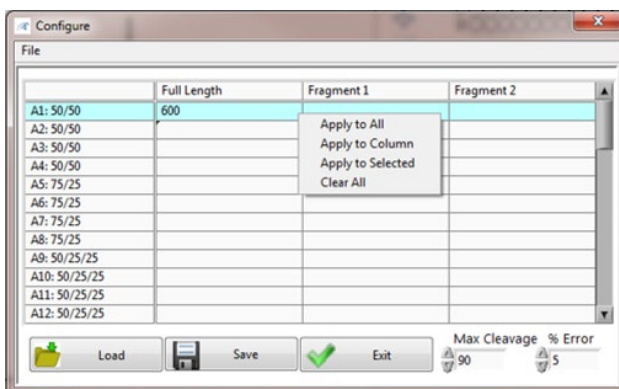


Figure A3. Setting the expected **Full Length** PCR and cleavage fragment sizes (**Fragment 1** and **Fragment 2**; in order of increasing size) in the **Configure** screen.

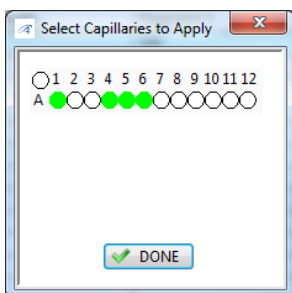


Figure A4. **Apply to Selected** pop up dialog, to select additional wells to apply size information from a specific well.

In the **Configure** screen, the **% Error** field determines what +/- percentage sizing error is allowed between the expected and measured size to be flagged by the program; this setting can be adjusted by the user as desired from the default value of 5%.

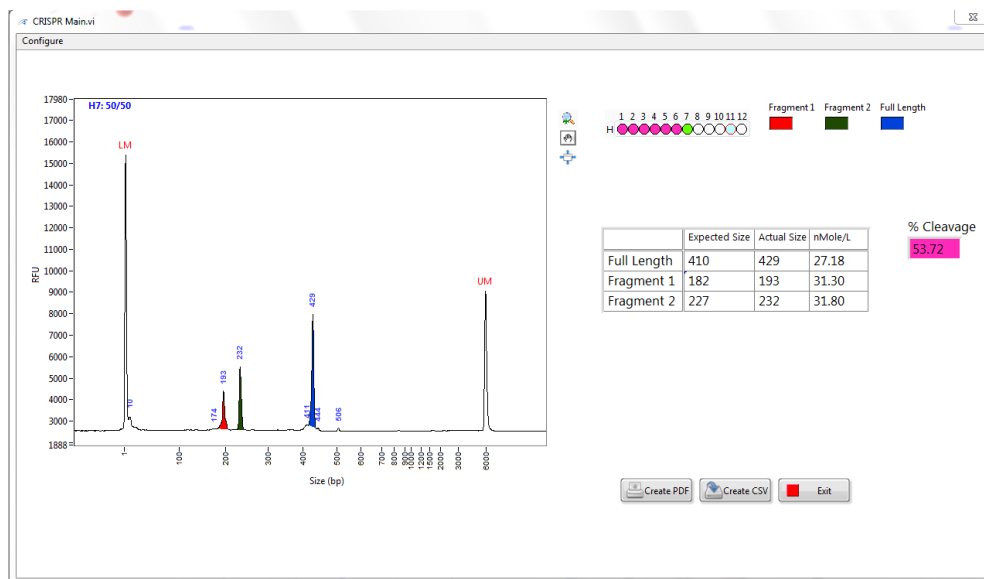
## CRISPR Main Plugin for the ProSize Data Analysis Software

The **Max Cleavage** setting determines the threshold above which the **% Cleavage** value is no longer highlighted in the **CRISPR Main** plugin screen, to avoid false positives of 100%. A recommended value for this setting is 90%.

Fragment configurations can be saved and loaded from the **Configure** screen by selecting the **Load** or **Save** buttons, or by selecting **File-Load** or **File-Save** from the dropdown, respectively.

To exit the Configure screen and apply the currently entered settings to the data, press **Exit** or select **File-Exit** from the dropdown.

- Once the configuration is set, exit back to the **CRISPR Main** plugin screen. The plugin will automatically color code the peaks corresponding to the input sizes if they fall within the **% Error** range, for quick identification as shown in Figure A5. The peak color coding can be toggled between red, green and blue by left clicking on the fragment colors in the upper right hand corner. The plugin will automatically calculate the molar concentration (in nmole/L) of each peak, as well as the **% Cleavage** value.



**Figure A5.** A positive CRISPR result as displayed in the **CRISPR Main** plugin, showing the calculated **% Cleavage**. The Full Length PCR and enzyme cleavage fragments each have a unique color code (red, green or blue). Data shown was generated using a synthetic gene system to model CRISPR mutations.

## CRISPR Main Plugin for the ProSize Data Analysis Software

- Results calculated by the **CRISPR Main** plugin can be output as a PDF report by selecting **Create PDF**. Options are provided to generate a PDF **Configuration Table**, **Capillary Table**, and/or **Capillary Graph** for all or for selected samples in the report (Figure A6). When selecting the **PDF** button to print a report, a capillary selection pop up dialog will appear, similar to Figure A4, to select the respective sample wells to include in the PDF report.

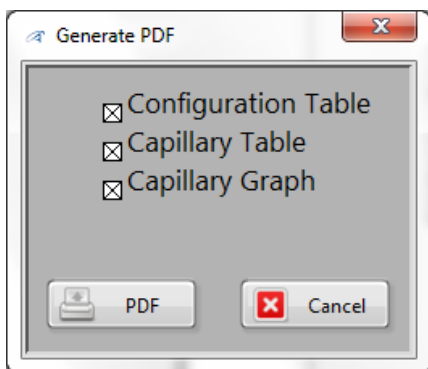


Figure A6. PDF report options for the **CRISPR Main** plugin.

Results calculated by the **CRISPR Main** plugin can be exported to a .csv file by selecting the **Create CSV** option. A standard file save dialog is displayed to save the file with the desired file name and location. All sample wells are automatically output in the csv file.



## Appendix B: CRISPR Single Cell Line Plugin for the ProSize Data Analysis Software

The CRISPR Single Cell Line (**CRISPR SCL**) plugin program for the ProSize data analysis software enables a user to enter expected PCR fragment size as well as the expected cleavage fragment sizes, and automatically score, predict zygosity, and highlight detected cut fragments. A PDF report or .csv file export can then be generated for the results. This plugin is designed to be used in conjunction with the Agilent AccuCleave T7 Kit (Part # DNF-440-1000CP).

### NOTE

The use of ProSize plugin programs, including the **CRISPR SCL** plugin, require ProSize Version 2.0.0.61 or higher. Please contact Agilent Technical Support for information on software upgrades.

## Plugin Setup

- 1 Obtain the plugin library file **CRISPR Single Cell Line 2\_0.llb** by contacting Agilent Technical Support.
- 2 In the directory *C:\PROSize 2.0* create a folder named **Plugins** if one is not already present.
- 3 Paste the file **CRISPR Single Cell Line 2\_0.llb** into the *C:\PROSize 2.0\Plugins* folder and restart the ProSize program.

## Using the CRISPR SCL Plugin

- 1 In ProSize open the data file you wish to analyze.
- 2 From the ProSize main screen, select the dropdown **Tools** > **CRISPR SCL** to open the CRISPR SCL plugin (Figure B1).

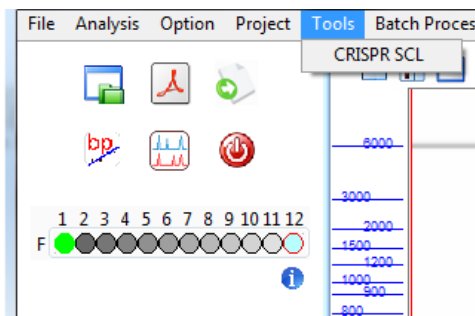


Figure B1. Launching the ProSize CRISPR SCL (Single Cell Line) plugin.

- 3 To configure fragment sizes for the plugin, or load previously configured and saved values, select **Configure** in the upper left hand corner of the display (Figure B2).

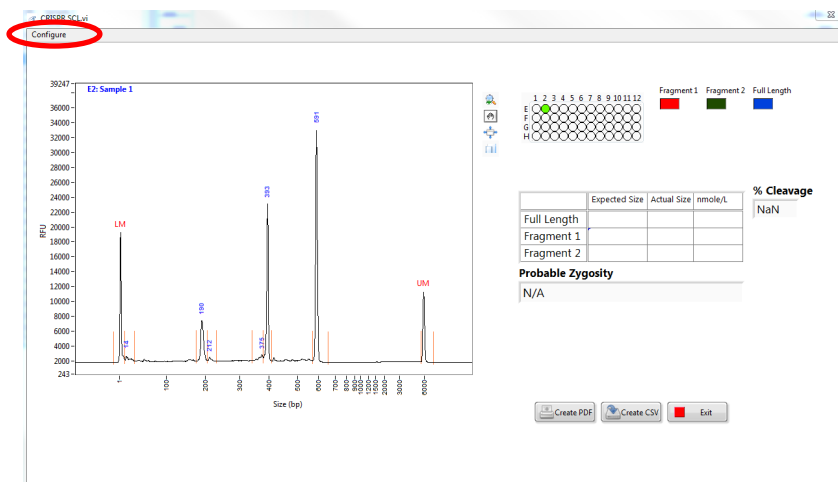


Figure B2. Select **Configure** to enter expected PCR fragment and cleaved DNA fragment sizes.

## CRISPR Single Cell Line Plugin for the ProSize Data Analysis Software

- 4 In the **Configure** screen enter the expected PCR fragment size (**Full Length**) and the expected cleavage fragment sizes (**Fragment 1** and **Fragment 2**; enter in order of increasing size) for each sample well (Figure B3). Right-click over a cell and select **Apply to Column** to copy the entered value in that cell to the entire column. To apply an entire row of entered values to all rows, right click over the row with the desired values entered and select **Apply to All**. To apply to a selected set of wells, right click over the row with the desired values entered and select **Apply to Selected**, mark the respective wells to copy the values to in the pop up dialog box (Figure B4), and click **Done**. To clear all entered values, right click and select **Clear All**.

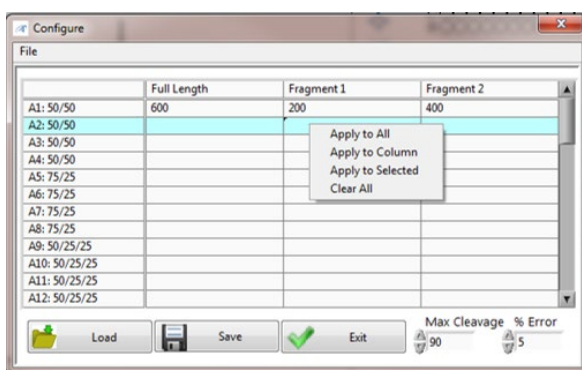


Figure B3. Setting the expected **Full Length** PCR and cleavage fragment sizes (**Fragment 1** and **Fragment 2**; in order of increasing size) in the **Configure** screen.

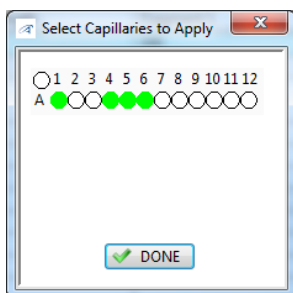


Figure B4. Apply to Selected pop up dialog, to select additional wells to apply size information from a specific well.

In the **Configure** screen, the **% Error** field determines what +/- percentage sizing error is allowed between the expected and measured size to be flagged by the program; this setting can be adjusted by the user as desired from the default value of 5%.

## CRISPR Single Cell Line Plugin for the ProSize Data Analysis Software

The **Max Cleavage** setting determines the threshold above which the **% Cleavage** value is no longer highlighted in the **CRISPR SCL** plugin screen, to avoid false positives of 100%. A recommended value for this setting is 90%.

Fragment configurations can be saved and loaded from the **Configure** screen by selecting the **Load** or **Save** buttons, or by selecting **File-Load** or **File-Save** from the dropdown, respectively.

To exit the **Configure** screen and apply the currently entered settings to the data, press **Exit** or select **File-Exit** from the dropdown

- 5 Once the configuration is set, exit back to the **CRISPR SCL** plugin screen. The plugin will automatically color code the peaks corresponding to the input sizes if they fall within the % Error range, for quick identification as shown in Figure B5. The peak color coding can be toggled between red, green and blue by left clicking on the fragment colors in the upper right hand corner. The plugin will automatically calculate the molar concentration (in nmole/L) of each peak, the **% Cleavage** value, and the **Probable Zygosity** (Monoallelic, Diallelic Homozygous, or Diallelic Heterozygous).

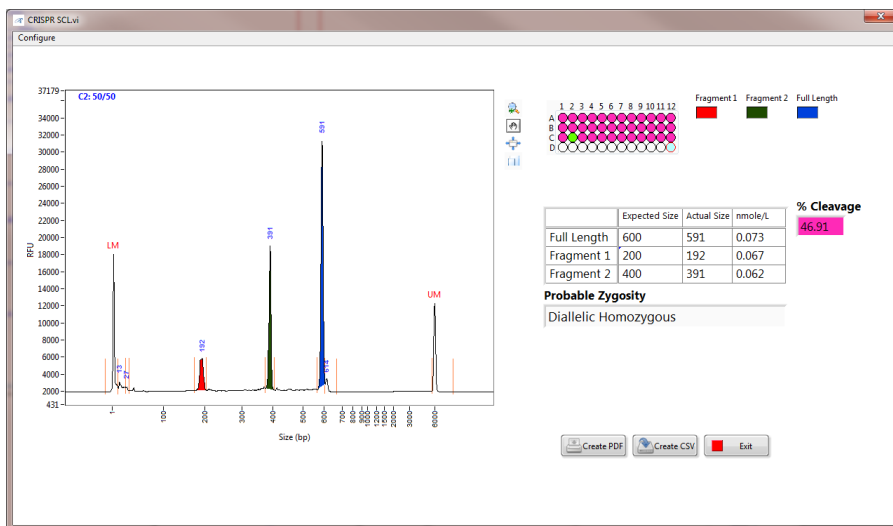
### NOTE

The **Probable Zygosity** is determined based upon the theoretical **% Cleavage** values found in the AccuCleave T7 Manual as follows:

- $\leq 44.4$  % Monoallelic Homozygous
- 44.5 % - 55.4 % Diallelic Homozygous
- $\geq 55.5$  % Diallelic Heterozygous

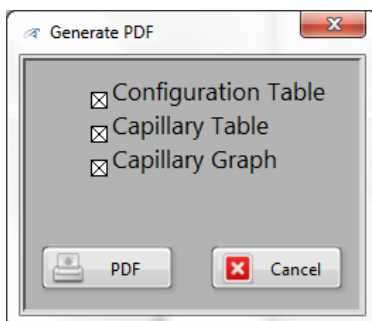
The returned zygosity should be used for screening purposes only, and is not intended for replacing sequencing.

## CRISPR Single Cell Line Plugin for the ProSize Data Analysis Software



**Figure B5.** A positive CRISPR result as displayed in the CRISPR SCL plugin, showing the % Cleavage and Probable Zygosity calculated. The Full Length PCR and enzyme cleavage fragments each have a unique color code (red, green or blue). Data shown was generated using a synthetic gene system to model CRISPR mutations.

- Results calculated by the CRISPR SCL plugin can be output as a PDF report by selecting **Create PDF**. Options are provided to generate a PDF **Configuration Table**, **Capillary Table**, and/or **Capillary Graph** for all or for selected samples in the report (Figure B6). When selecting the **PDF** button to print a report, a capillary selection pop up dialog will appear, similar to Figure B4, to select the respective sample wells to include in the PDF report.



**Figure B6.** PDF report options for the CRISPR SCL plugin.

Results calculated by the CRISPR SCL plugin can be exported to a .csv file by selecting the **Create CSV** option. A standard file save dialog is displayed to save the file with the desired file name and location. All sample wells are automatically output in the .csv file.



## Appendix C: CRISPR Pooled Cell Line Plugin for the ProSize Data Analysis Software

The CRISPR Pooled Cell Line plugin program (**CRISPR PS**, where PS stands for “Pooled Sample”), for the ProSize data analysis software, enables a user to enter expected PCR fragment size as well as the expected cleavage fragment sizes. The plugin will then automatically score, highlight detected cut fragments, predict zygosity, and calculate % Mutated of the sample. A PDF report or .csv file export can then be generated for the results. This plugin is designed to be used in conjunction with the Agilent AccuCleave T7 Kit (Part # DNF-440-1000CP).

### NOTE

The use of ProSize plugin programs, including the **CRISPR PS** plugin, require ProSize Version 2.0.0.61 or higher. Please contact Agilent Technical Support for information on software upgrades.

## Plugin Setup

- 1 Obtain the plugin library file **CRISPR Pool Sample 2\_0.llb** by contacting Agilent Technical Support.
- 2 In the directory *C:\PROSize 2.0* create a folder named **Plugins** if one is not already present.
- 3 Paste the file **CRISPR Pool Sample 2\_0.llb** into the *C:\PROSize 2.0\Plugins* folder and restart the ProSize program.

## Using the CRISPR SCL Plugin

- 1 In ProSize open the data file you wish to analyze.
- 2 From the ProSize main screen, select the dropdown **Tools** > **CRISPR PS** to open the CRISPR PS plugin (Figure B1).

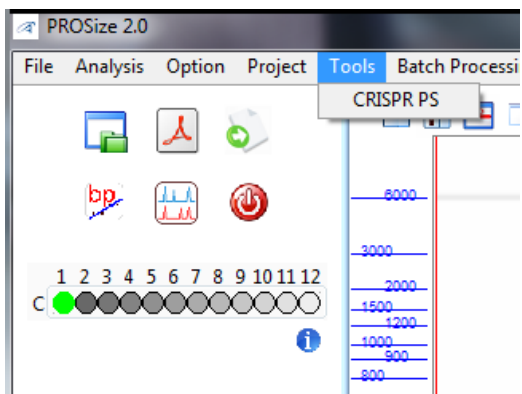


Figure C1. Launching the ProSize **CRISPR PS** (Pooled Sample) plugin.

- 3 To configure fragment sizes for the plugin, or load previously configured and saved values, select **Configure** in the upper left hand corner of the display (Figure C2).

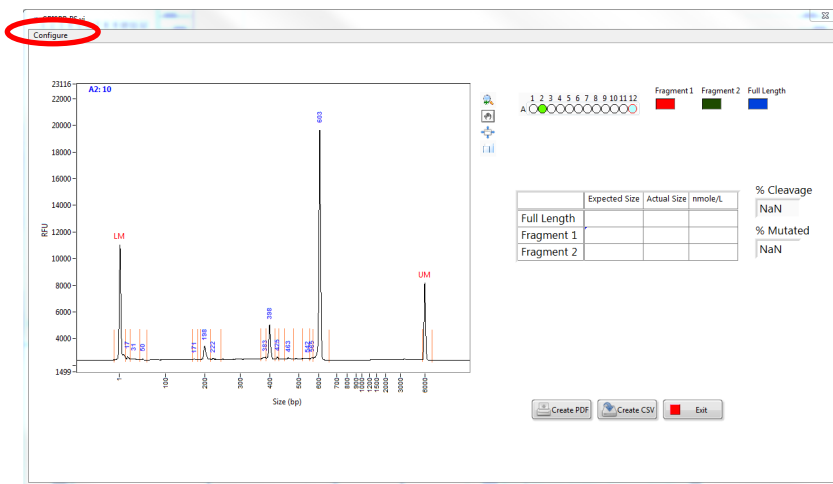


Figure C2. Select **Configure** to enter expected PCR fragment and cleaved DNA fragment sizes.

## CRISPR Pooled Cell Line Plugin for the ProSize Data Analysis Software

- 4 In the **Configure** screen enter the expected PCR fragment size (**Full Length**) and the expected cleavage fragment sizes (**Fragment 1** and **Fragment 2**; enter in order of increasing size) for each sample well (Figure C3). Right-click over a cell and select **Apply to Column** to copy the entered value in that cell to the entire column. To apply an entire row of entered values to all rows, right click over the row with the desired values entered and select **Apply to All**. To apply to a selected set of wells, right click over the row with the desired values entered and select **Apply to Selected**, mark the respective wells to copy the values to in the pop up dialog box (Figure C4), and click **Done**. To clear all entered values, right click and select **Clear All**.

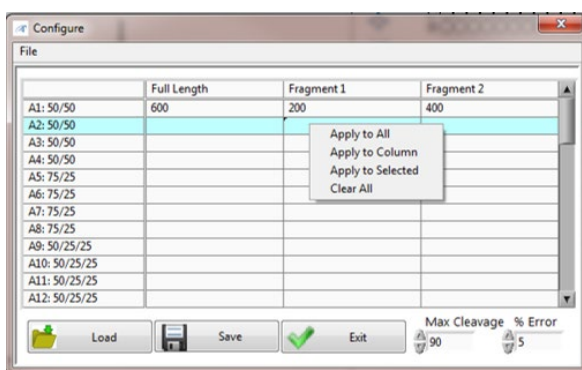


Figure C3. Setting the expected **Full Length** PCR and cleavage fragment sizes (**Fragment 1** and **Fragment 2**; in order of increasing size) in the **Configure** screen.

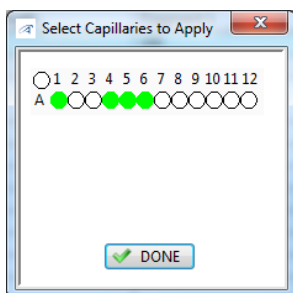


Figure C4. Apply to Selected pop up dialog, to select additional wells to apply size information from a specific well.

In the **Configure** screen, the **% Error** field determines what +/- percentage sizing error is allowed between the expected and measured size to be flagged by the program; this setting can be adjusted by the user as desired from the default value of 5%.

## CRISPR Pooled Cell Line Plugin for the ProSize Data Analysis Software

The **Max Cleavage** setting determines the threshold above which the **% Cleavage** value is no longer highlighted in the **CRISPR PS** plugin screen, to avoid false positives of 100%. A recommended value for this setting is 90%.

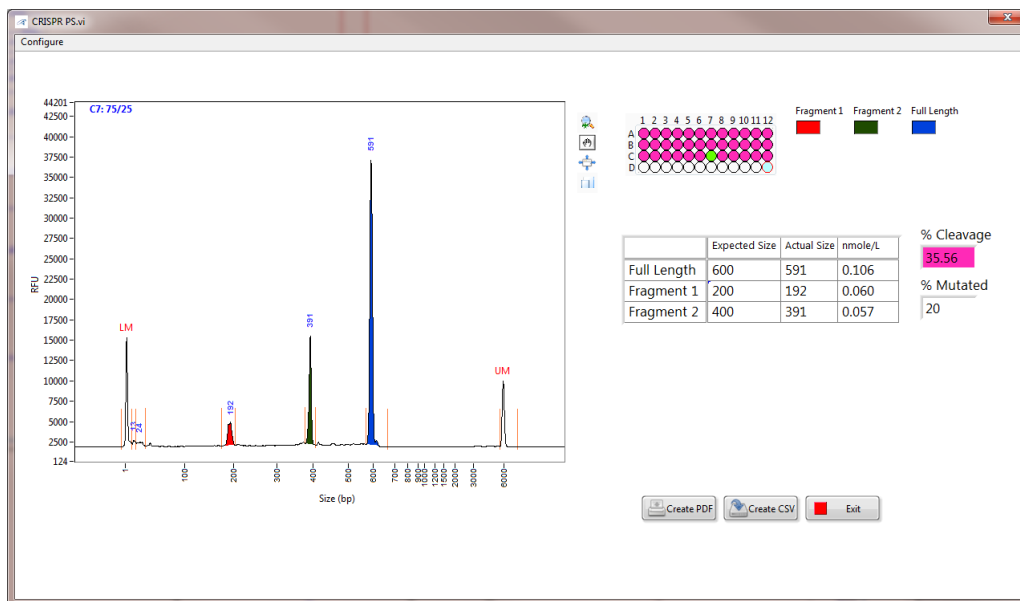
Fragment configurations can be saved and loaded from the **Configure** screen by selecting the **Load** or **Save** buttons, or by selecting **File-Load** or **File-Save** from the dropdown, respectively.

To exit the **Configure** screen and apply the currently entered settings to the data, press **Exit** or select **File-Exit** from the dropdown

- Once the configuration is set, exit back to the **CRISPR PS** plugin screen. The plugin will automatically color code the peaks corresponding to the input sizes if they fall within the **% Error** range, for quick identification as shown in Figure C5. The peak color coding can be toggled between red, green and blue by left clicking on the fragment colors in the upper right hand corner. The plugin will automatically calculate the molar concentration (in nmole/L) of each peak, the **% Cleavage** value, and the **% Mutated** value.

### NOTE

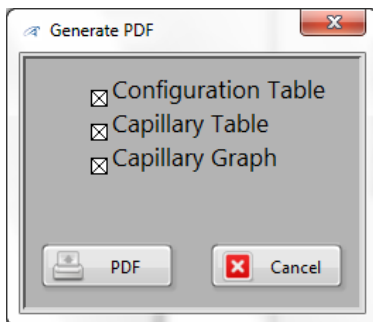
The **% Mutated** value is determined as based upon the theoretical equation found in the AccuCleave T7 Manual (see **Overview** Section).



**Figure C5.** A positive CRISPR result as displayed in the **CRISPR SCL** plugin, showing the **% Cleavage** and **% Mutated** calculated. The Full Length PCR and enzyme cleavage fragments each have a unique color code (red, green or blue). Data shown was generated using a synthetic gene system to model CRISPR mutations.

## CRISPR Pooled Cell Line Plugin for the ProSize Data Analysis Software

- Results calculated by the **CRISPR PS** plugin can be output as a PDF report by selecting **Create PDF**. Options are provided to generate a PDF **Configuration Table**, **Capillary Table**, and/or **Capillary Graph** for all or for selected samples in the report (Figure C6). When selecting the **PDF** button to print a report, a capillary selection pop up dialog will appear, similar to Figure C4, to select the respective sample wells to include in the PDF report.



**Figure C6.** PDF report options for the **CRISPR PS** plugin.

- Results calculated by the **CRISPR PS** plugin can be exported to a .csv file by selecting the **Create CSV** option. A standard file save dialog is displayed to save the file with the desired file name and location. All sample wells are automatically output in the .csv file.

# In This Book

This Kit Guide describes the following:

- Agilent CRISPR Discovery Gel Kit
- Additional Material and Equipment Required
- Agilent CRISPR Discovery Gel Kit Protocol
- Checking Your Separation Results
- Troubleshooting

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