User Guide

SPRIselect User Guide

SPRI Based Size Selection

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Safety Notice

Do not attempt to perform any procedure before carefully reading all instructions. Always follow product labeling and manufacturer's recommendations. If in doubt as to how to proceed in any situation, contact your Beckman Coulter Representative.

Alerts for Warning, Caution, Important, and Note

WARNING

The signal word WARNING is displayed in an orange signal panel and the associated text (in this example the definition of WARNING) is in bold-face.

⚠ WARNING

WARNING indicates a potentially hazardous situation which, if not avoided, could result in death or serious injury.

In this document the signal word WARNING is only used to indicate the possibility of personal injury. It is not used to indicate the possibility of erroneous data.

CAUTION

The signal word CAUTION is displayed in a yellow signal panel and the associated text (in this example the definition of CAUTION is in bold-face as shown below.

⚠ CAUTION

CAUTION indicates a potentially hazardous situation, which, if not avoided, may result in minor or moderate injury. It may also be used to alert against unsafe practices.

In this document the signal word CAUTION is used to indicate the possibility of damage to the instrument.

IMPORTANT

The signal word IMPORTANT is in bold-face and the associated text (in this example the definItion of IMPORTANT) is indented if it wraps.

IMPORTANT IMPORTANT is used for comments that add value to the step or procedure being performed. Following the advice in the Important adds benefit to the performance of a piece of equipment or to a process.

The signal word IMPORTANT is used to draw attention to information that is critical for the successful completion of a procedure and/or operation of the instrument.

NOTE

The signal word NOTE is in bold-face and associated text (in this example the definition of NOTE) is indented if it wraps.

NOTE NOTE is used to call attention to notable information that should be followed during installation, use, or servicing of this equipment.

Contents

Introduction to the SPRIselect User Guide

About This Manual

The information in this manual is organized as follows:

SPRI Based Size Selection
Provides an overview of SPRIselect, its uses and its key features.

Glossary
Provides definitions for terms used throughout this manual.

Intended Use

SPRIselect is intended for molecular biology research applications. It is not intended or validated for use in the diagnosis of disease or other conditions.

Warranty Disclaimer

Beckman Coulter makes no warranties of any kind whatsoever express or implied, with respect to the method, including but not limited to warranties of fitness for a particular purpose or merchantability or that the method is non-infringing. All other warranties are expressly disclaimed. Your use of the method is solely at your own risk, without recourse to Beckman Coulter.

Conventions Used

This manual uses the following conventions:

- The names of instrument manuals referred to in the text are in *italic.*
- Messages that appear on the instrument screen are in *italic*.
- Buttons that appear on the instrument screen are in **bold face.**
- Selections that appear on the instrument screen are in **bold face**.
- The software path to a specific function or screen appears with the greater than (**>**) symbol between succeeding screen options, like this: **Options > Log Configuration**.

SPRI Based Size Selection

Introduction

SPRIselect is a SPRI-based chemistry that speeds and simplifies nucleic acid size selection for fragment library preparation for Next Generation sequencing. In this process, size selection is required to produce a uniform distribution of fragments around an average size. Using SPRIselect, the size distribution can be adjusted to suit the application and platform used. The process can be scaled for low to high throughput workflows.

The guidance provided below can be used to optimize the desired size selection range. Used manually or automated on a liquid handling system such as the Biomek Laboratory Automation Workstation, SPRIselect will provide rapid and consistent size selection suitable for most applications.

SPRIselect is covered by US Patents 5705628, 6534262, and 5898071.

Materials

- 85% Ethanol, non-denatured (ethanol is hygroscopic, prepare fresh for optimal results)
- Water (molecular biology grade) or a standard buffer solution such as Tris (10 mM, pH 8) or TE (10 mM Tris, pH 8, 1 mM EDTA) for DNA elution

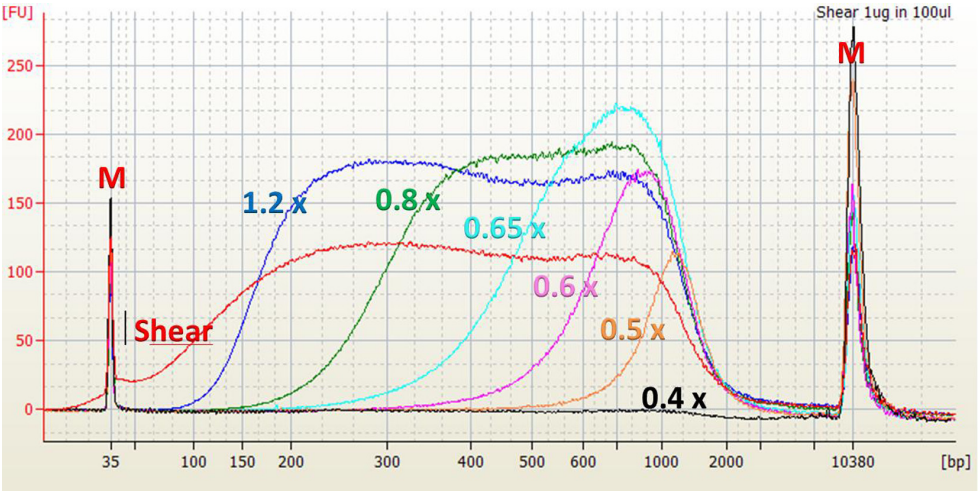
Sample Prerequisites

- Samples should be fragmented double-stranded DNA.
- Samples should be dissolved in molecular biology grade water or standard buffer solution such as Tris or TE. See *Effects of Common Laboratory Reagents on Size Selection* at end of user guide for more information.
- Sample volume should be ≥ 50 µL. A lower volume will decrease pipetting accuracy of **SPRIselect**, therefore increasing selection point variability.
- DNA fragments may be size selected in a range no smaller than 150 bp and no larger than 800 bp.
- To maximize recovery for a Left Side Size Selection, the majority of the sample's size distribution should be larger than the selection point.
- To maximize recovery for a Right Side Size Selection, the majority of the sample's size distribution should be smaller than the selection point.
- To maximize recovery for a Double Size Selection, the size distribution should be centered between the selection points.

Left Side Size Selection

As a general rule, increasing the ratio of **SPRIselect** volume to sample volume will increase the efficiency of binding smaller fragments. Figure 1 illustrates this relationship.

Figure 1 Agilent High Sensitivity DNA chip Electropherogram.



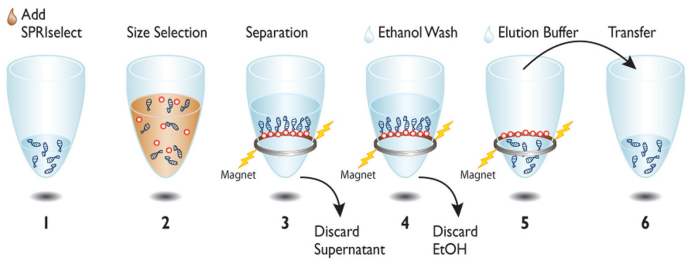
M = upper and lower markers for High Sensitivity DNA chip

Shear = 1 µL of 20 ng/µL input control sample in water

1.2x to 0.4x = 1 µL of shear, size selected with given ratio of **SPRIselect** volume to sample volume.

Figure 2

Left Side Size Selection Process Overview



1. Thoroughly shake the SPRIselect bottle to resuspend the SPRI beads. Following the trend depicted in Figure 1, add the required volume of **SPRIselect** for the desired ratio to the sample.

   TIP Volume of sample * ratio = volume of **SPRIselect**.
   Example: 50 µL sample * 0.8x ratio = 40 µL of **SPRIselect**

2. Mix the total reaction volume by pipetting 10 times and incubate at RT for 1 minute
   OR

vortex for 1 minute at an appropriate speed until homogenous (depending on labware and total volume).

NOTE Insufficient mixing of sample and SPRIselect will lead to inconsistent size selection results. Make sure to mix well.

3. Place the reaction vessel on an appropriate magnetic stand or plate and allow the SPRI beads to settle to the magnet. Settle times will vary; a higher initial sample volume, higher **SPRIselect** ratio or weaker magnets will require a longer settle time. Remove and discard the clear supernatant.

   NOTE Care should be taken not to aspirate more than a trace amount of beads during this step, as the desired library is associated with the beads. Significant bead loss will result in reduced yield.

4. With the reaction vessel still on the magnet, add 180 µL of 85% ethanol (non-denatured) and incubate at RT for 30 seconds. Remove and discard the ethanol supernatant.

   NOTE Care should be taken not to aspirate more than a trace amount of beads during this step, as the desired library is associated with the beads. Significant bead loss will result in reduced yield.

5. To elute the sample:

   a. Remove the reaction vessel from the magnet and add ≥ 20 µL of molecular biology grade water or standard buffer solution such as Tris or TE.

      NOTE Elution volume should be large enough so that the liquid level is high enough for the beads to settle to the magnet.

   b. Mix the total elution volume by pipetting 10 times to resuspend the beads and incubate at RT for 1 minute
      OR
      vortex for 1 minute at an appropriate speed until homogenous (depending on labware and total volume).

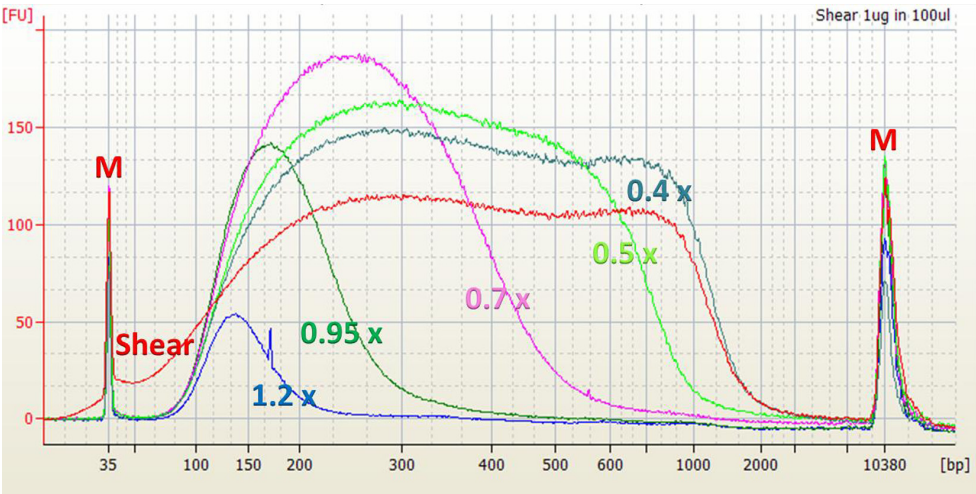
   c. Place the reaction vessel on an appropriate magnetic stand or plate and allow the SPRI beads to settle to the magnet. Settle times will vary; a higher elution volume or weaker magnets will require a longer settle time.

6. Transfer the eluate (size selected sample) to an appropriate storage vessel.

Right Side Size Selection

As a general rule, increasing the ratio of SPRIselect volume to sample volume will decrease the efficiency of binding larger fragments. Figure 3 illustrates this relationship.

Figure 3 Agilent High Sensitivity DNA chip Electropherogram.

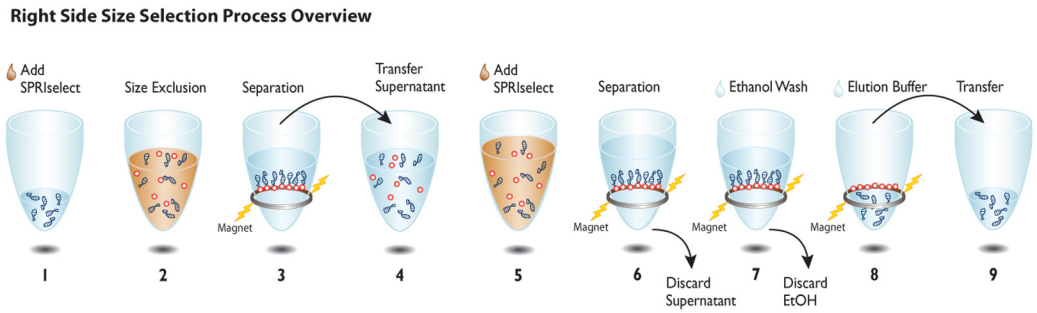


M = upper and lower markers for High Sensitivity DNA chip.

Shear = 1 µL of 20 ng/µL input control sample in water.

1.2x to 0.4x = 1 µL of shear, size selected with given ratio of **SPRIselect** volume to sample volume.

Figure 4



1. Thoroughly shake the SPRIselect bottle to resuspend the SPRI beads. Following the trend depicted in Figure 3, add the required volume of **SPRIselect** for the desired ratio to the sample.

   TIP Volume of sample * ratio = volume of **SPRIselect**.
   Example: 50 µL * 0.7x ratio = 35 µL of **SPRIselect**

2. Mix the total reaction volume by pipetting 10 times and incubate at RT for 1 minute
   OR

Vortex for 1 minute at an appropriate speed until homogenous (depending on labware and total volume).

NOTE Insufficient mixing of sample and SPRIselect will lead to inconsistent size selection results. Make sure to mix well.

3. Place the reaction vessel on an appropriate magnetic stand or plate and allow the SPRI beads to settle to the magnet. Settle times will vary; a higher initial sample volume, higher **SPRIselect** ratio or weaker magnets will require a longer settle time.
4. Transfer the clear supernatant, which contains the Right Side Size Selected sample, to a new reaction vessel. The reaction vessel with the remaining beads can be discarded.

   NOTE Care should be taken not to aspirate more than a trace amount of beads during this step as the undesired larger fragment sizes are associated with the beads. Significant bead transfer will cause tailing into the larger size range.

5. Add the required volume of **SPRIselect**, using the calculation below, to the supernatant from Step 4 above. This will bind the fragments in the supernatant to the new SPRI beads.

   TIP Sample Volume µL * (1.8x – the initial ratio) = volume of SPRIselect
   Example: 50 µL * (1.8 – 0.7) = 55 µL of **SPRIselect**

6. Perform the following:
   a. Mix the total reaction volume by pipetting 10 times and incubate at RT for 1 minute
   OR
   vortex for 1 minute at an appropriate speed until homogenous (depending on labware and total volume).

      NOTE Insufficient mixing of sample and SPRIselect will lead to inconsistent size selection results.

   b. Place the reaction vessel on an appropriate magnetic stand or plate and allow the SPRI beads to settle to the magnet. Settle times will vary; a higher initial sample volume, higher **SPRIselect** ratio or weaker magnets will require a longer settle time.
   c. Remove and discard the clear supernatant.

   NOTE Care should be taken not to aspirate more than a trace amount of beads during this step, as the desired library is associated with the beads. Significant bead loss will result in reduced yield.

7. With the reaction vessel still on the magnet, add 180 µL of 85% ethanol (non-denatured) and incubate at RT for 30 seconds. Remove and discard the ethanol supernatant.

   NOTE Care should be taken not to aspirate more than a trace amount of beads during this step, as the desired library is associated with the beads. Significant bead loss will result in reduced yield.

8. To elute the sample:
   a. Remove the reaction vessel from the magnet and add ≥ 20 µL of molecular biology grade water or standard buffer solution such as Tris or TE.

      NOTE Elution volume should be large enough so that the liquid level is high enough for the beads to settle to the magnet.

b. Mix the total elution volume by pipetting 10 times to resuspend the beads and incubate at RT for 1 minute
OR
vortex for 1 minute at an appropriate speed until homogenous (depending on labware and total volume).

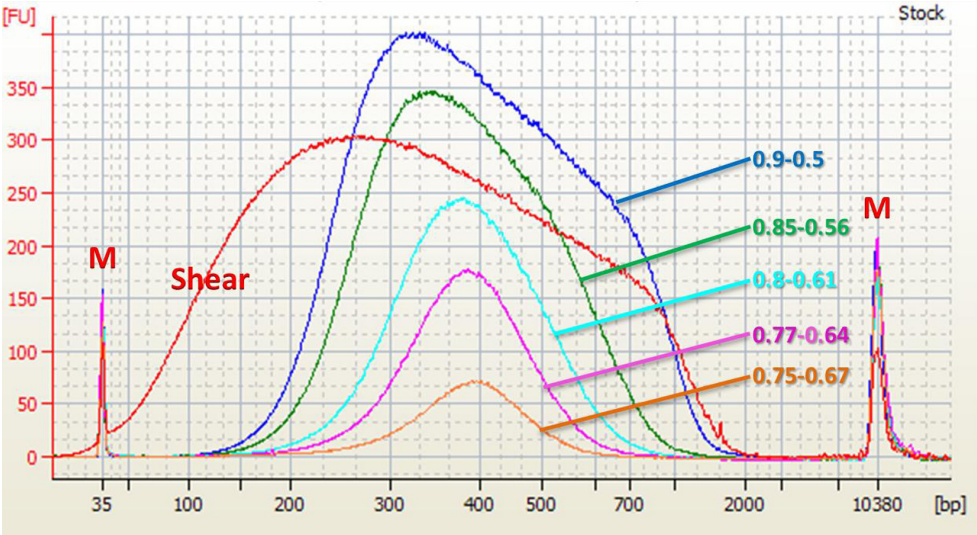
c. Place the reaction vessel on an appropriate magnetic stand or plate and allow the SPRI beads to settle to the magnet. Settle times will vary; a higher elution volume or weaker magnets will require a longer settle time.

9. Transfer the eluate (size selected sample) to an appropriate storage vessel.

Double Size Selection Considerations

As a general rule, the Left Side Size Selection ratio always needs to be greater than the Right Side Size Selection ratio. Figure 5 illustrates this relationship.

Figure 5 Agilent High Sensitivity DNA chip Electropherogram.



M = upper and lower markers for High Sensitivity DNA chip

Shear = 1 µL of 20 ng/µL input control sample in water

0.XX to 0.XX = 1 µL of shear, size selected with given Left side ratio and Right side ratio of **SPRIselect** volume to sample volume

Table 1 Typical Recovery (determined by experiment)

Ratios (Left-Right)	bp Region	Selection Delta (bp)	bp Region's % of Shear	Recovered % of bp Region	Recovered Region's % of Shear
Shear	40-3000	2960	100.0%	100.0%	100.0%
0.9-0.5	175-1300	1125	72.7%	60.4%	43.9%
0.85-0.56	200-700	500	61.8%	49.6%	30.6%
0.8-0.61	230-660	430	52.1%	33.4%	17.4%
0.77-0.64	260-575	315	40.8%	21.1%	8.6%
0.75-0.67	280-540	260	33.7%	10.1%	3.4%

Percentages calculated from Agilent 2100 Expert Smear Region analysis Conc. [pg/µL]

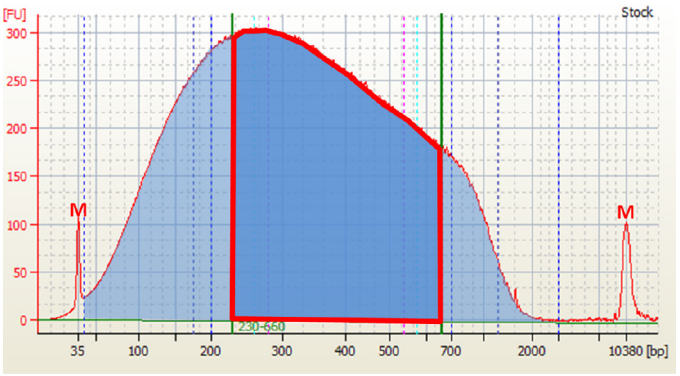
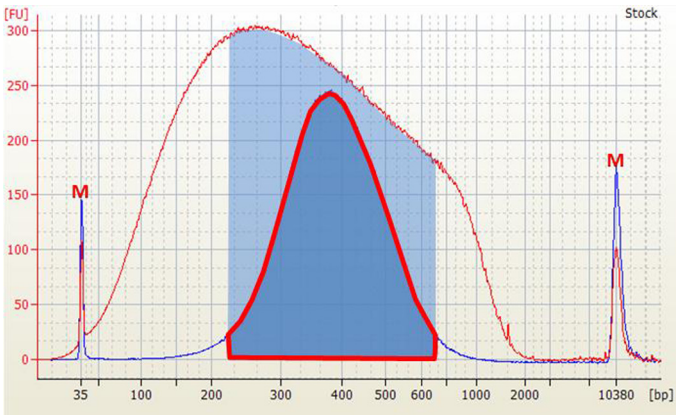
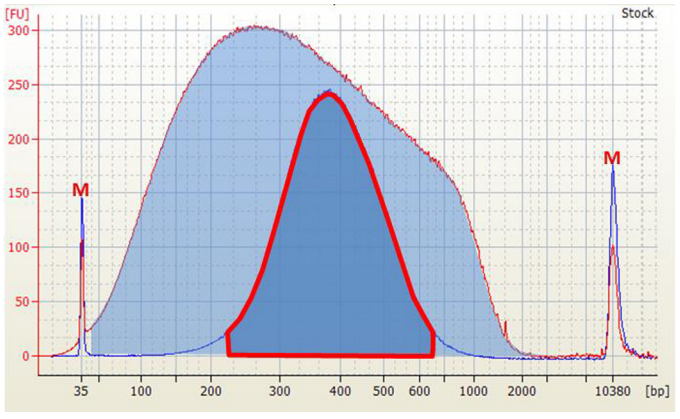
Table 1 Definitions:

Ratios (Left-Right): Left Side Size Selection ratio - Right Side Size Selection ratio

bp Region: Agilent 2100 Expert Smear Region analyzed for the given **Ratios (Left-Right)**

Selection Delta (bp): The difference between the Left and Right selection points of the **bp Region**
Example: 660 Right bp – 230 Left bp = 430 bp Delta

Table 2 Recovery of Shear- *Note: the 0.8-0.61 ratio sample seen in Table 1 will be used for all definition examples below*

	Definition	Diagram
bp Region's % of Shear	The maximum potential recovery of the Stock sample for the targeted size selection region.	
Recovered % of bp Region	Percent recovery of the targeted region	
Recovered % of Shear	Percent recovery of the Shear sample.	

Effects of Common Laboratory Reagents on Size Selection

The common laboratory reagents described in Table 3 were added to the reaction (as an additive) to determine the effect on Size Selection.

- Each Left Side Size Selection below was 1 µg sheared in 50 µL + 50 µL additive + 65 µL **SPRIselect** (0.65x ratio). Final elution in 100 µL of water. 1 µL of eluent was run on an Agilent High Sensitivity DNA chip.

  TIP If a possible reagent additive not listed below is of concern, use this dilution scheme to test if there is any effect on size selection.

- The "0.65x Control" (red trace) is the expected selection result for a 0.65x Left Side Size Selection.
- The concentration of the additive can be found in the Electropherogram table.
- The "M" is the Agilent High Sensitivity DNA assay's upper and lower markers.
- Keep in mind that additive effects are cumulative. If there are multiple additives that all move the selection the same direction, the result will be larger than any individual component. If some component(s) move the selection Left and some Right, it's possible to have little to no effect.

Table 3 Effects of Common Laboratory Reagents on Size Selection. Blue background represents wider selection. Red background represents narrower selection.

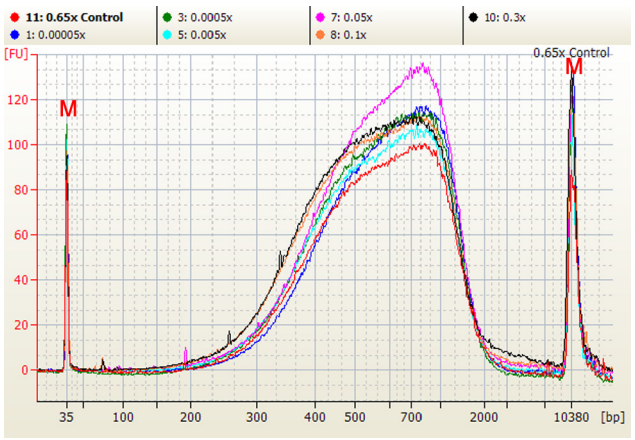
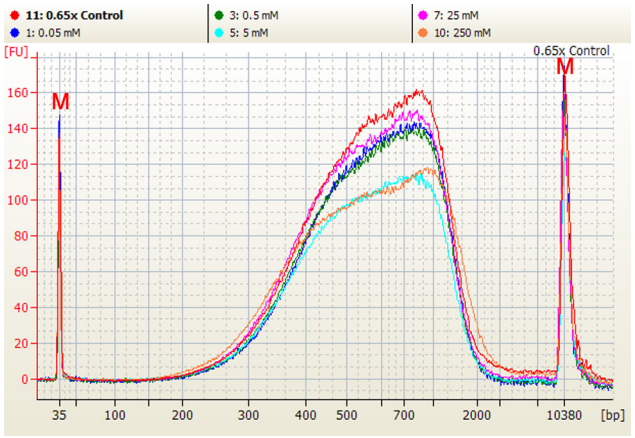
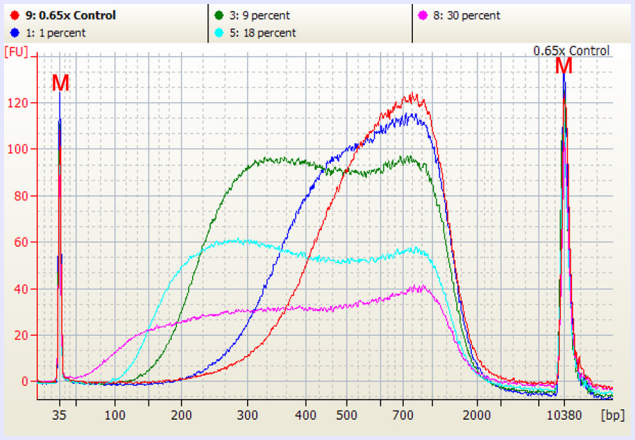
Additive	Effect	Agilent High Sensitivity Chip Trace
DMSO (Dimethyl sulfoxide)	None	
DTT (Dithiothreitol)	None	
85% Ethanol	Wider Size Selection	

Table 3 Effects of Common Laboratory Reagents on Size Selection. Blue background represents wider selection. Red background represents narrower selection.

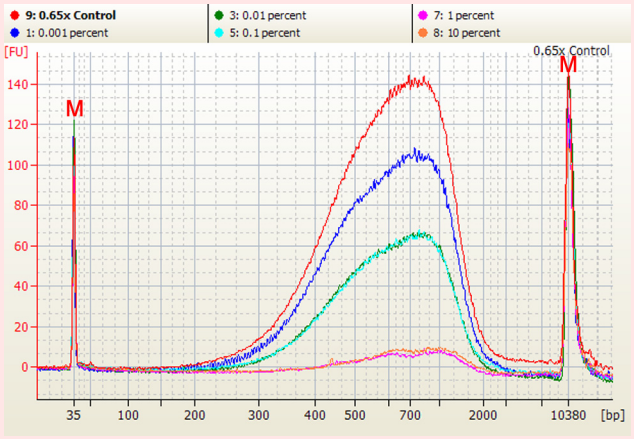
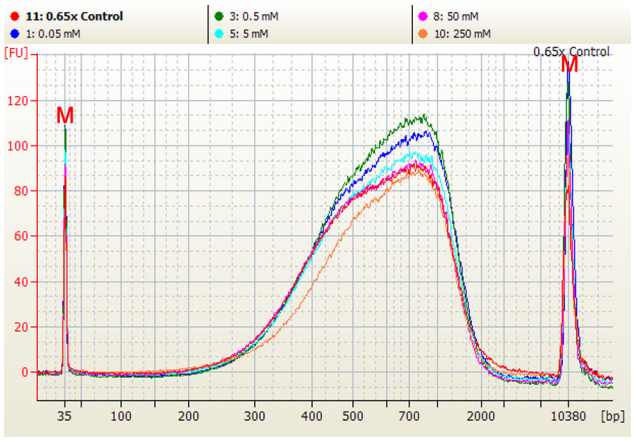
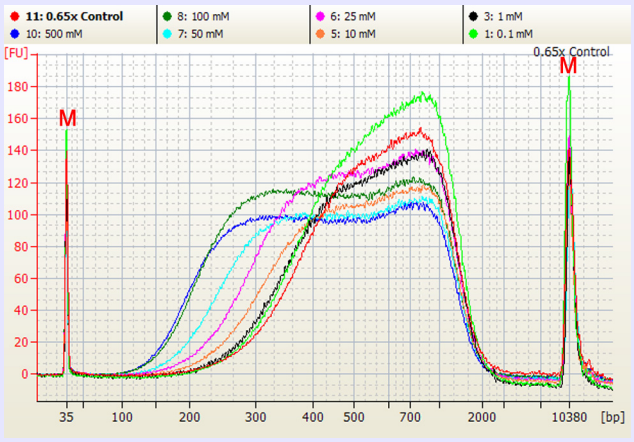
Additive	Effect	Agilent High Sensitivity Chip Trace
Glycerol	Narrower Size Selection	
KCl (Potassium chloride)	None	
$MgCl_2$ (Magnesium chloride)	Wider Size Selection	

Table 3 Effects of Common Laboratory Reagents on Size Selection. Blue background represents wider selection. Red background represents narrower selection.

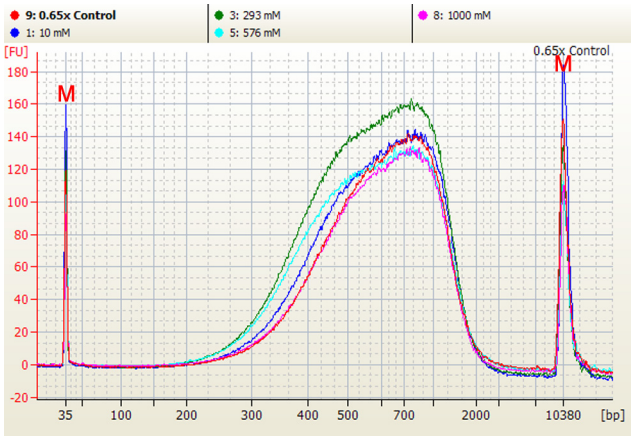
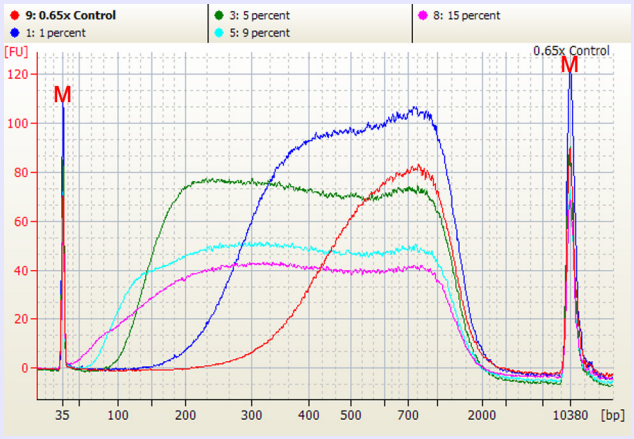
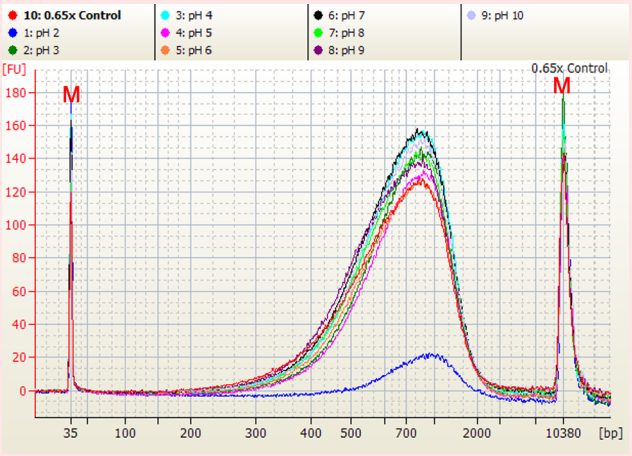
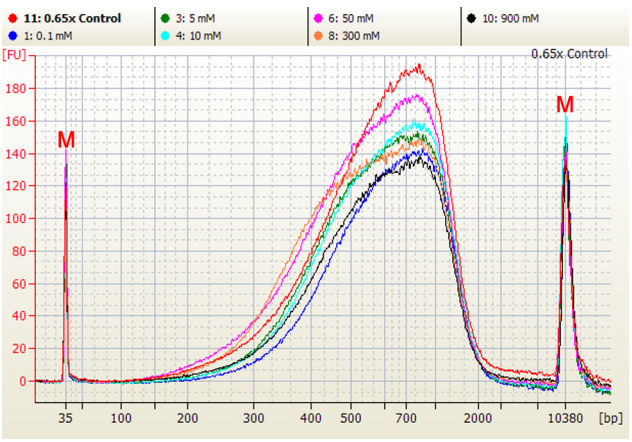
Additive	Effect	Agilent High Sensitivity Chip Trace
NaCl (Sodium chloride)	None	
PEG (Polyethylene glycol)	Wider Size Selection	

Table 3 Effects of Common Laboratory Reagents on Size Selection. Blue background represents wider selection. Red background represents narrower selection.

Additive	Effect	Agilent High Sensitivity Chip Trace
pH	Narrower Size Selection for pH < 3. No effect for pH ≥ 3.	
Sodium Acetate	None	

Glossary

bp	base pair
Double Size Selection	a Left and Right Side Size Selection performed on the same sample
Left Side Size Selection	defines the new start point of the sample's size distribution
µg	microgram
µL	microliter
ng/µL	nanograms per microliter
Right Side Size Selection	defines the new end point of the sample's size distribution
RT	Room Temperature (15-30°C)
Tris	tris(hydroxymethyl)aminomethane (10 mM, pH 8)
TE	Tris with Ethylenediaminetetraacetic acid (10 mM Tris, pH 8,1 mM EDTA)

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