# SureSelect XT HS and XT Low Input Enzymatic Fragmentation Kit

# Protocol

*For Research Use Only. Not for use in diagnostic procedures.*

Revision A0, September 2018

# Notices



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# In This Guide…

This guide provides an optimized protocol for enzymatic fragmentation of gDNA samples, replacing mechanical shearing of gDNA samples, prior to DNA library preparation for next-generation sequencing (NGS) using either SureSelect$^{XT\ HS}$ Reagent Kits or SureSelect$^{XT}$ Low Input Reagent Kits.

# Content

# 1 Before You Begin



This chapter contains information for you to read and understand before you start.

# Overview

The overall workflow for SureSelect$^{XT\ HS}$ or SureSelect$^{XT}$ Low Input target enrichment is summarized in Figure 1, with the workflow segment detailed in this protocol indicated by the shaded rectangle. For information on the rest of the workflow, refer to the SureSelect$^{XT\ HS}$ system manual or SureSelect$^{XT}$ Low Input system manual.

Enzymatic fragmentation of DNA samples, as detailed in this protocol, replaces the mechanical shearing of DNA samples, prior to preparation and enrichment of sequencing libraries.

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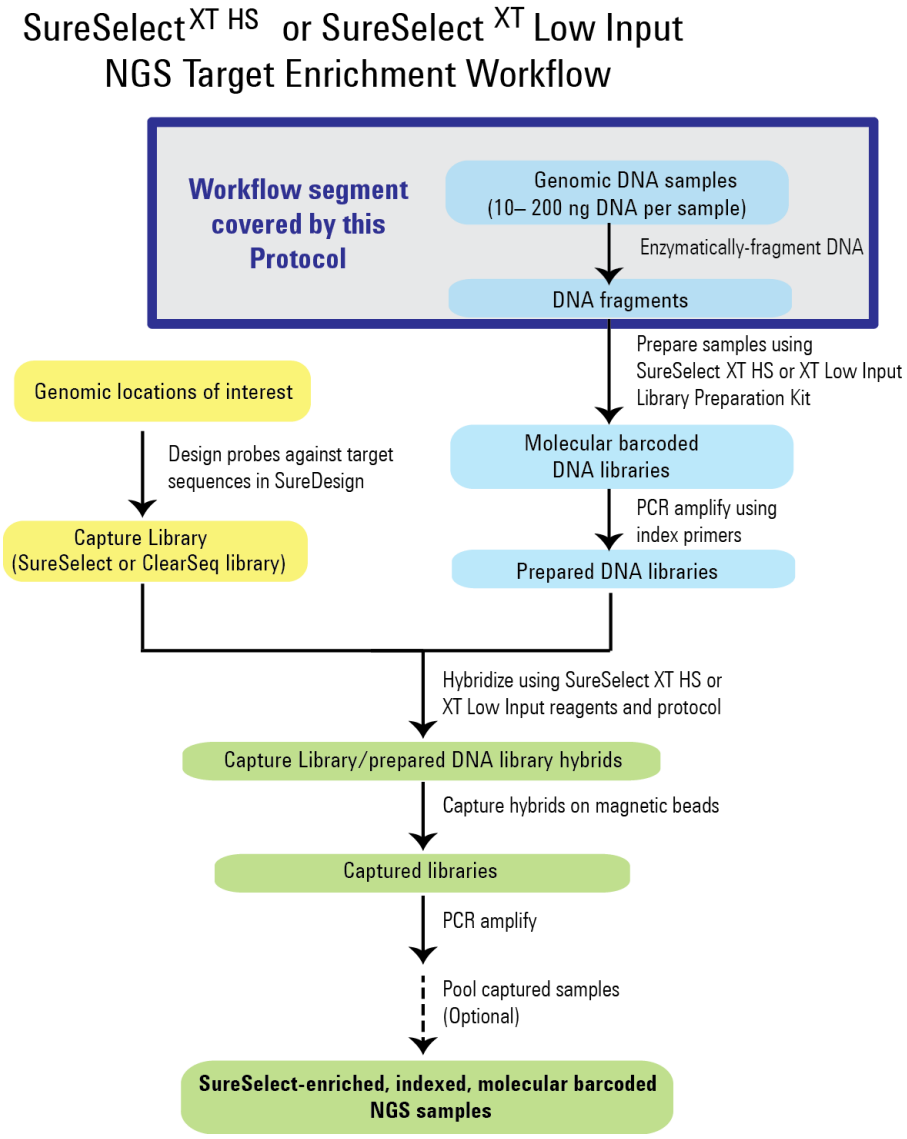


**Figure 1** Overall target-enriched sequencing sample preparation workflow.

# Procedural Notes

- To prevent contamination of reagents by nucleases, always wear powder-free laboratory gloves and use dedicated solutions and pipettors with nuclease-free aerosol-resistant tips.
- Use best-practices to prevent PCR product contamination of samples throughout the workflow:
  1. Assign separate pre-PCR and post-PCR work areas and use dedicated equipment, supplies, and reagents in each area. In particular, never use materials designated to post-PCR work areas for pre-PCR segments of the workflow.
  2. Maintain clean work areas. Clean pre-PCR surfaces that pose the highest risk of contamination daily using a 10% bleach solution, or equivalent.
  3. Always use dedicated pre-PCR pipettors with nuclease-free aerosol-resistant tips to pipette dedicated pre-PCR solutions.
  4. Wear powder-free gloves. Use good laboratory hygiene, including changing gloves after contact with any potentially-contaminated surfaces.

# Safety Notes

**CAUTION**

- Wear appropriate personal protective equipment (PPE) when working in the laboratory.

# Required Reagents

**Table 1** Required Reagents

| Description | Vendor and part number |
|---|---|
| SureSelect XT HS Enzymatic Fragmentation Kit, 16 Reactions<br>**OR**<br>SureSelect XT HS and XT Low Input Enzymatic Fragmentation Kit, 96 Reactions | Agilent p/n 5191-4079<br><br>Agilent p/n 5191-4080 |
| Nuclease-free Water (not DEPC-treated) | Thermo Fisher Scientific p/n AM9930 |

# Required Equipment

**Table 2** Required Equipment

| Description | Vendor and part number |
|---|---|
| SureCycler 8800 Thermal Cycler, or equivalent | Agilent p/n G8800A |
| 96 well plate module for SureCycler 8800 Thermal Cycler | Agilent p/n G8810A |
| SureCycler 8800-compatible plasticware:<br>96-well plates<br>OR<br>8-well strip tubes | <br>Agilent p/n 410088<br><br>Agilent p/n 410092 |
| Tube cap strips, domed | Agilent p/n 410096 |
| Plate or strip tube centrifuge | Labnet International MPS1000 Mini Plate Spinner, p/n C1000 (requires adapter, p/n C1000-ADAPT, for use with strip tubes) or equivalent |
| Pipettes (20-, and 200-µl capacity) | Rainin Pipet-Lite Pipettes or equivalent |
| Sterile, nuclease-free aerosol barrier pipette tips | general laboratory supplier |
| Vortex mixer | general laboratory supplier |
| Ice bucket | general laboratory supplier |
| Powder-free gloves | general laboratory supplier |

# 2 Enzymatic DNA Fragmentation Protocol



This protocol is used to produce enzymatically-fragmented gDNA samples prior to preparation of next-generation sequencing (NGS) libraries using Agilent's SureSelect$^{XT\ HS}$ system or SureSelect$^{XT}$ Low Input system. Enzymatic fragmentation of DNA is intended to replace the mechanical shearing of gDNA samples described in the SureSelect$^{XT\ HS}$ system manual or SureSelect$^{XT}$ Low Input system manual. For an overview of the NGS library preparation workflow, see Figure 1 on page 6.

# Step 1. Prepare and analyze quality of genomic DNA samples

The library preparation protocol requires 10 ng to 200 ng input gDNA, starting with a DNA sample volume of 7 µl for enzymatic DNA fragmentation.

DNA input amounts or quantification methods may require adjustment for some FFPE samples, as detailed below. For optimal sequencing results, use the maximum amount of input DNA available within the recommended range.

## Preparation of high-quality gDNA from fresh biological samples

1 Prepare high-quality gDNA using a suitable purification system, such as Qiagen's QIAamp DNA Mini Kit, following the manufacturer's protocol.

**NOTE** Make sure genomic DNA samples are of high quality with an OD 260/280 ratio ranging from 1.8 to 2.0.

2 Use the Qubit BR dsDNA Assay Kit to determine the concentration of each gDNA sample. Follow the manufacturer's instructions for the instrument and assay kit.

3 Place 10 ng to 200 ng of each gDNA sample in wells of a PCR plate or strip tube and dilute each sample with nuclease-free water to a final volume of 7 µl. Vortex well to mix, then spin briefly to collect the liquid. Keep the samples on ice.

Additional qualification of DNA samples is not required for DNA derived from fresh biological samples. Proceed to "Step 2. Enzymatically fragment the DNA" on page 13.

## Preparation and qualification of gDNA from FFPE samples

1 Prepare gDNA from FFPE tissue sections using Qiagen's QIAamp DNA FFPE Tissue Kit and Qiagen's Deparaffinization Solution, following the manufacturer's protocol. Elute the final gDNA samples from the MinElute column in two rounds, using 30 µl Buffer ATE in each round, for a final elution volume of approximately 60 µl.

**NOTE** If tissue lysis appears incomplete after one hour of digestion with Proteinase K, add an additional 10 µl of Proteinase K and continue incubating at 56°C, with periodic mixing, for up to three hours.

Store the gDNA samples on ice for same-day library preparation, or at –20°C for later processing.

2 Assess the quality (DNA integrity) for each FFPE DNA sample using one of the methods below.

### Option 1: Qualification using the Agilent NGS FFPE QC Kit (Recommended Method)

The Agilent NGS FFPE QC Kit provides a qPCR-based assay for DNA sample integrity determination. Results include a ΔΔCq DNA integrity score and the precise quantity of amplifiable DNA in the sample, allowing direct normalization of DNA input for each sample. DNA input recommendations based on ΔΔCq scores for individual samples are summarized in Table 3.

**a** Use the Qubit BR dsDNA Assay Kit to determine the concentration of each gDNA sample. Follow the manufacturer's instructions for the instrument and assay kit.

**b** Remove a 1 µl aliquot of the FFPE gDNA sample for analysis using the Agilent NGS FFPE QC Kit to determine the ΔΔCq DNA integrity score. See the kit user manual at www.genomics.agilent.com for more information.

**c** For all samples with ΔΔCq DNA integrity score ≤1, use the Qubit-based gDNA concentration determined in step a, above, to determine the amount of the sample needed for the protocol.

**d** For all samples with ΔΔCq DNA integrity score >1, use the qPCR-based concentration of amplifiable gDNA, reported by the Agilent NGS FFPE QC Kit results, to determine the amount of the sample needed for the protocol.

**Table 3** SureSelect XT HS DNA input modifications based on ΔΔCq DNA integrity score

| Protocol Parameter | non-FFPE Samples | FFPE Samples | |
|---|---|---|---|
| | | **ΔΔCq≤1*** | **ΔΔCq >1** |
| DNA input for Library Preparation | 10 ng to 200 ng DNA, based on Qubit Assay | 10 ng to 200 ng DNA, based on Qubit Assay | 10 ng to 200 ng of amplifiable DNA, based on qPCR quantification |

* FFPE samples with ΔΔCq scores ≤1 should be treated like non-FFPE samples for DNA input amount determinations. For samples of this type, make sure to use the DNA concentration determined by the Qubit Assay, instead of the concentration determined by qPCR, to calculate the volume required for 10–200 ng DNA.

### Option 2: Qualification using Agilent's Genomic DNA ScreenTape assay DIN score

Agilent's Genomic DNA ScreenTape assay, used in conjunction with Agilent's 4200 TapeStation, provides a quantitative electrophoretic assay for DNA sample integrity determination. This assay reports a DNA Integrity Number (DIN) score for each sample which is used to estimate the appropriate normalization of DNA input required for low-integrity DNA samples.

**a** Use the Qubit BR dsDNA Assay Kit to determine the concentration of each gDNA sample. Follow the manufacturer's instructions for the instrument and assay kit.

**b** Remove a 1 µl aliquot of the FFPE gDNA sample and analyze using the Genomic DNA ScreenTape assay. See the user manual at www.genomics.agilent.com for more information.

c Using the DIN score reported for each sample in the Genomic DNA ScreenTape assay, consult Table 4 to determine the recommended amount of input DNA for the sample.

**Table 4** SureSelect XT HS DNA input modifications based on DNA Integrity Number (DIN) score

| Protocol Parameter | non-FFPE Samples | FFPE Samples | | |
|---|---|---|---|---|
| | | DIN > 8* | DIN 3–8 | DIN<3 |
| DNA input for Library Preparation | 10 ng to 200 ng DNA, quantified by Qubit Assay | 10 ng to 200 ng DNA, quantified by Qubit Assay | Use at least 15 ng for more intact samples and at least 40 ng for less intact samples. Use the maximum amount of DNA available, up to 200 ng, for all samples. Quantify by Qubit Assay. | Use at least 50 ng for more intact samples and at least 100 ng for the least intact samples. Use the maximum amount of DNA available, up to 200 ng, for all samples. Quantify by Qubit Assay. |

* FFPE samples with DIN>8 should be treated like non-FFPE samples for DNA input amount determinations.

3 Transfer the appropriate amount of each qualified DNA sample to wells of a PCR plate or strip tube in a volume of 7 µl. Keep the samples on ice until they are used in step 4 on page 13.

NOTE

When required, the input DNA sample volume may be adjusted to 7 µl by dilution with nuclease-free water or by volume reduction using a suitable concentration method.

See Table 3 or Table 4 above for FFPE DNA input guidelines based on the measured DNA quality in each sample.

# Step 2. Enzymatically fragment the DNA

1 Before beginning the protocol, thaw the vial of 5X SureSelect Fragmentation Buffer, vortex, then place on ice.

2 Preprogram a SureCycler 8800 thermal cycler (with the heated lid ON) using the program in Table 5. Start the program, then immediately press the *Pause* button, allowing the heated lid to reach temperature while you set up the reactions.

**Table 5** Thermal cycler program for enzymatic fragmentation*

| Step | Temperature | Time |
|---|---|---|
| Step 1 | 37°C | 15 minutes |
| Step 2 | 65°C | 5 minutes |
| Step 3 | 4°C | Hold |

* When setting up the thermal cycling program, use a reaction volume setting of 10 µl.

3 Prepare the appropriate volume of Fragmentation master mix by combining the reagents in Table 6.

Mix well by pipetting up and down 20 times. Spin briefly to remove any bubbles and keep on ice.

**Table 6** Preparation of Fragmentation master mix

| Reagent | Volume for 1 reaction | Volume for 8 reactions (includes excess) |
|---|---|---|
| 5X SureSelect Fragmentation Buffer | 2 µl | 18 µl |
| SureSelect Fragmentation Enzyme | 1 µl | 9 µl |
| **Total** | **3 µl** | **27 µl** |

4 Add 3 µl of the Fragmentation master mix to each sample well containing 7 µl of input DNA.

5 Mix well by pipetting up and down 20 times. Cap the wells, then briefly spin the samples.

6 Immediately place the plate or strip tube in the SureCycler 8800 thermal cycler. Press the *Play* button to resume the thermal cycling program in Table 5.

7 When the program reaches the 4°C Hold step, remove the samples from the thermal cycler, add 40 µl of nuclease-free water to each sample, and place the samples on ice.

The 50-µl reactions are now ready for NGS sequencing library preparation, beginning with end repair/dA-tailing. Proceed directly to *Step 3. Repair and dA-Tail the DNA Ends* in the SureSelect$^{XT\ HS}$ system manual or SureSelect$^{XT}$ Low Input system manual.

**NOTE** This is not a stopping point in the workflow, and analysis of the enzymatically-fragmented samples is not required before they are used for library preparation. Proceed directly to end-repair and dA-tailing using the appropriate user manual link above.

# 3 Reference



This chapter contains kit contents and troubleshooting information.

# Kit Contents

The contents of the Enzymatic Fragmentation Kits supported by this user manual are listed in **Table 7** below.

**Table 7** Kit Content

| Component | Format |
|---|---|
| 5X SureSelect Fragmentation Buffer | Tube with blue cap |
| SureSelect Fragmentation Enzyme | Tube with green cap |

# Troubleshooting Guide

### If recovery of gDNA from samples is low

- ✔ Using excess tissue for gDNA isolation can reduce yield. Use only the amount of each specific tissue type recommended by the gDNA isolation protocol.
- ✔ Tissue sample lysis may not have been optimal during gDNA isolation. Monitor the extent of sample lysis during the Proteinase K digestion at 56°C by gently pipetting the digestion reaction every 20–30 minutes, visually inspecting the solution for the presence of tissue clumps. If clumps are still present after the 1-hour incubation at 56°C, add another 10 µl of Proteinase K and continue incubating at 56°C, with periodic mixing and visual inspections, for up to two additional hours. When the sample no longer contains clumps of tissue, move the sample to room temperature until lysis is complete for the remaining samples. Do not over-digest. Individual samples may be kept at room temperature for up to 2 hours before resuming the protocol. Do not exceed 3 hours incubation at 56°C for any sample.

### If DNA fragment size is not within the expected range after pre-capture PCR

- ✔ Verify that sample analysis was performed at the correct protocol step and not immediately after the enzymatic fragmentation steps detailed in this user manual. DNA fragment size is first assessed after pre-capture amplification using Agilent's 2100 Bioanalyzer or 4200 TapeStation, as described in the SureSelect$^{XT\ HS}$ system manual or SureSelect$^{XT}$ Low Input system manual.
- ✔ Verify that the enzymatic fragmentation reaction was performed using the following parameters:
  - Initial DNA input amount of 10–200 ng
  - Fragmentation conditions of 15 minutes at 37°C
  - Use of 1 µl of SureSelect Fragmentation Enzyme and 2 µl of 5X SureSelect Fragmentation Buffer in a final reaction volume of 10 µl.
- ✔ If DNA appears underfragmented, consider repeating the experiment using a control DNA sample to verify that the experimental DNA samples do not contain inhibitors of the fragmentation reaction.

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## In This Book

This guide provides an optimized protocol for enzymatic fragmentation of gDNA samples, replacing mechanical shearing of DNA samples, prior to DNA library preparation for next-generation sequencing (NGS) using either SureSelect$^{XT\ HS}$ Reagent Kits or SureSelect$^{XT}$ Low Input Reagent Kits.



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