Agilent

SureSelect Enzymatic Fragmentation Kit

Protocol

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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 compatible SureSelect Reagent Kits.

What's New in Version B1

- New *Troubleshooting* entry for fragmentation of low concentration DNA samples (see **page 18**); also see **step 2** on **page 14**
- DNA sample dilution/concentration instructions for both intact DNA and FFPE samples relocated to **step 2** on **page 14**
- Updates to mixing method options in **step 4** and **step 6** on **page 15**
- Updates to thermal cycler and plasticware recommendations and usage instructions (see **Table 3** on page 10 and **step 3** on **page 14**)
- Removal of references to retired kit name
- Updates to Technical Support contact information (see **page 2**)

What's New in Version B0

- Support for kits relabeled as *SureSelect Enzymatic Fragmentation Kit* (see **Table 2** on page 10 and references to product name throughout document)
- New section **"SureSelect Platform Compatibility"** on page 8
- Support for enzymatic fragmentation of DNA for library construction using Agilent's SureSelect XT HS2 DNA system (see **Table 1** on page 8)
- Support for 2x150 read length NGS (see **page 14**)
- Update to *Note* on fragment analysis on **page 15**
- Updates to Technical Support contact information (see **page 2**)

Content

1
Before You Begin

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

Overview

The overall workflow for SureSelect NGS library prep and target enrichment, starting with DNA fragmentation by the SureSelect Enzymatic Fragmentation Kit, is summarized in Figure 1. The workflow segment that is detailed in this protocol publication is indicated by the shaded rectangle. For information on SureSelect platform compatibility, and for links to user manuals detailing the rest of the workflow, see "SureSelect Platform Compatibility" on page 8.

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

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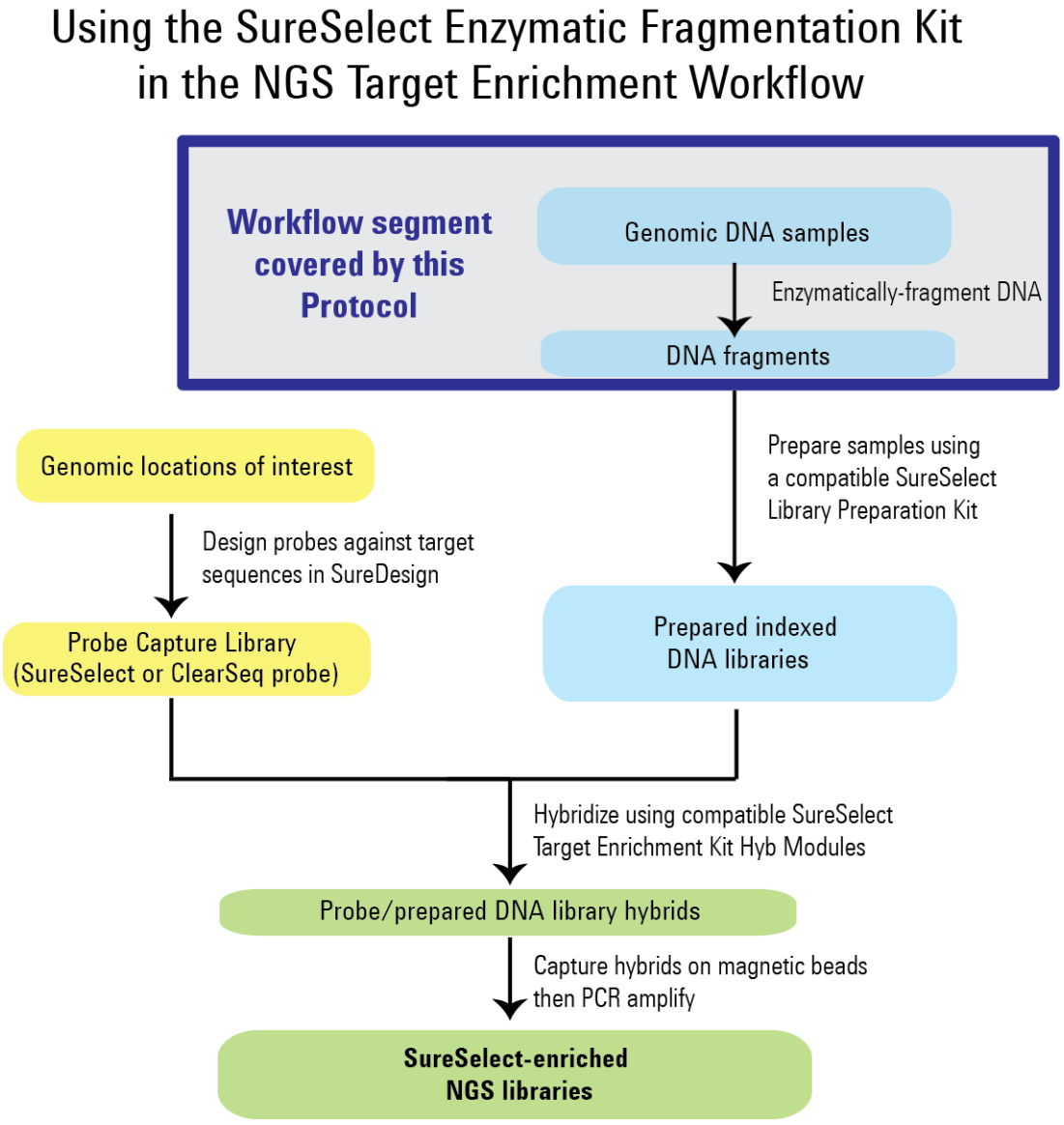

Figure 1 Overall target-enriched sequencing sample preparation workflow.

SureSelect Platform Compatibility

The SureSelect Enzymatic Fragmentation Kit is compatible with the specific SureSelect platforms and their supporting protocols listed in Table 1 below.

NOTE The SureSelect Enzymatic Fragmentation Kit has **not** been optimized for use with the SureSelect^{XT}, SureSelect^{QXT} or SureSelect^{XT2} systems.

See the appropriate SureSelect user manual listed in Table 1 for details of workflow steps downstream of enzymatic DNA fragmentation.

Table 1 Compatible SureSelect Platforms

SureSelect platform	Protocol document number (link)
SureSelect XT HS2 DNA	G9983-90000
SureSelect XT HS	G9702-90000
SureSelect XT Low Input	G9703-90000 (single-indexed libraries) G9703-90050 (dual-indexed libraries)

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 2 Required Reagents

Description	Vendor and part number
SureSelect Enzymatic Fragmentation Kit 16 Reactions 96 Reactions	Agilent p/n 5191-4079 p/n 5191-4080
Nuclease-free Water (not DEPC-treated)	Thermo Fisher Scientific p/n AM9930

Required Equipment

Table 3 Required Equipment

Description	Vendor and part number
Thermal Cycler with 96-well, 0.2 ml block	Various suppliers
Plasticware compatible with the selected thermal cycler: 96-well plates or 8-well strip tubes Tube cap strips	Consult the thermal cycler manufacturer's recommendations
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 a compatible Agilent SureSelect Reagent Kit. Enzymatic fragmentation of DNA is intended to replace the mechanical shearing of gDNA samples in certain SureSelect library preparation protocols. For an overview of the NGS library preparation workflow, see Figure 1 on page 7, and for a list of compatible SureSelect platforms, see Table 1 on page 8.

Fragmentation conditions are optimized for NGS workflows using read lengths of 2x100 or 2x150 reads.

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.

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 14.

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 4.

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 4 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 an Agilent TapeStation instrument, 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 5 to determine the recommended amount of input DNA for the sample.

Table 5 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.

Proceed to "Step 2. Enzymatically fragment the DNA" on page 14.

Step 2. Enzymatically fragment the DNA

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

2 In wells of a thermal cycler-compatible strip tube or PCR plate, dilute 10 ng to 200 ng of each gDNA sample with nuclease-free water to a final volume of 7 µl. Keep the samples on ice.

NOTE See Table 4 on page 13 or Table 5 on page 13 for FFPE DNA input guidelines based on the measured DNA quality in each sample.

If the DNA concentration is too low to supply the 10–200 ng input amount required for your workflow in 7 µl, sample volume may be reduced using a suitable concentration method. Alternatively, see *Troubleshooting* on page 18 for protocol modifications for dilute samples.

3 Preprogram a thermal cycler (with the heated lid ON) using the program in Table 6 with the optimal fragmentation duration for your sample type and NGS read length, specified in Table 7.

Immediately pause the program, and keep paused until samples are loaded in step 7.

Table 6 Thermal cycler program for enzymatic fragmentation*

Step	Temperature	Time
Step 1	37°C	Varies--see Table 7
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.

Table 7 Fragmentation duration based on sample type and NGS read length

NGS read length requirement	Target fragment size	Duration of 37°C incubation step	
		High-quality DNA samples	FFPE DNA samples
2 ×100 reads	150 to 200 bp	15 minutes	15 minutes
2 ×150 reads	180 to 250 bp	10 minutes	15 minutes

4 Prepare the appropriate volume of Fragmentation master mix by combining the reagents in Table 8.

   Mix well by pipetting up and down 20 times or seal the tube and vortex at high speed for 5–10 seconds. Spin briefly to remove any bubbles and keep on ice.

Table 8 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**

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

6 Mix well by pipetting up and down 20 times or cap the wells and vortex at high speed for 5–10 seconds. Spin the samples briefly.

7 Immediately place the plate or strip tube in the thermal cycler and resume the program in Table 6.

8 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 to the appropriate SureSelect system user manual listed in Table 1 on page 8.

NOTE

This is not a stopping point in the workflow, and analysis of the enzymatically-fragmented samples is not required prior to library preparation. Moreover, electrophoretic analysis of the fragmented samples may produce misleading results due to the presence of agents that affect DNA fragment migration.

Proceed directly to end-repair and dA-tailing using the appropriate user manual link found in Table 1 on page 8.

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 9** below.

Table 9 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 concentration of DNA samples is too low for enzymatic fragmentation

- ✔ The standard enzymatic fragmentation protocol requires 10–200 ng input DNA in a volume of 7 µl, and uses a final fragmentation reaction volume of 10 µl. For dilute samples, enzymatic fragmentation may be performed using the modified protocol below:
  - Bring samples containing 10–200 ng DNA to 17 µl final volume with 1X Low TE Buffer.
  - Prepare the Fragmentation master mix as directed in **Table 8** on page 15.
  - Add 3 µl of the master mix to each 17-µl DNA sample. Mix and spin as directed on **page 15**.
  - Run the thermal cycling program in **Table 6** on page 14 using the 37°C fragmentation duration shown in the table below.

NGS read length	High-quality DNA samples	FFPE DNA samples
2 ×100 reads	25 minutes	25 minutes
2 ×150 reads	15 minutes	25 minutes

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 library preparation and target enrichment protocols (see the protocol for your SureSelect platform in **Table 1** on page 8).
- ✔ 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 compatible SureSelect Reagent Kits.

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