Quick Start Protocol

SureSelect XT HS2 DNA with Pre-capture Pooling

Agilent
Trusted Answers

G9985-90500 Rev A0
**For Research Use Only. Not for use in diagnostic procedures.

DNA Fragmentation and Library Preparation Workflow — 3–4 Hours

Fragment DNA: Enzymatic or Covaris

20 min
(Enzymatic fragmentation)

End repair and dA-tail fragments

40 min

Ligate MBC-tagged adaptors

40 min
Stopping point

Clean-up ligation

30 min

Amplify and dual-index

35 min

Clean-up library

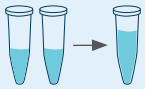
30 min

Quality control

Varies
Stopping point

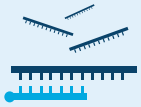
Hybridization/Capture Workflow — 4–5 Hours

Pool indexed libraries for hybridization



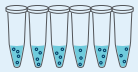
30 min

Hybridize libraries to probe



1.5–2.5 hrs
Optional overnight hold

Prepare streptavidin beads



30 min
(Concurrent)

Capture hybridized libraries

70 min

Amplify enriched libraries

30 min

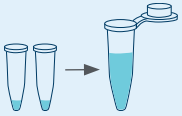
Clean-up amplified library pools

30 min

Quality control

Varies
Stopping point

Combine captured pools for multiplex NGS



Optional

Duration estimates are provided as guidelines for 16 reaction runs using 200 ng high-quality input DNA and probe design >5 Mb. Your results may vary.

This Quick Start Protocol provides key protocol details for experienced users. Visit SureSelect XT HS2 DNA System Protocol for the full User Guide with further information including:

- Safety information
- Notices to purchaser
- List of required materials
- FFPE sample qualification guidelines
- QC output examples
- Critical factors for system performance including component handling, mixing, and spinning specifications; pay special attention to the key critical factors such as mixing steps highlighted in this Quick Start Protocol
- Full protocol details including Notes and Caution statements
- Index sequences and other NGS support

DNA Fragmentation

Note: Reagent vial cap colors are indicated by colored circles adjacent to reagent use instructions.

Option 1: Enzymatic fragmentation

20 min

- ☐ Prepare DNA samples in wells: 7 µl each containing 10–200 ng DNA. Keep on ice.
- ☐ Set up thermal cycler program (Table 1). Start and pause program.

Table 1: Thermal cycler program for enzymatic fragmentation (vol 10 µl)

Step	Temperature	Time
Step 1	37 °C	See Table 2
Step 2	65 °C	5 min
Step 3	4 °C	Hold

Table 2: Duration of fragmentation at 37 °C (Step 1 in Table 1)

NGS Read Length	Duration of 37 °C Incubation Step	
	High-quality DNA	FFPE DNA
2 X 100	☐ 15 min	☐ 15 min
2 X 150	☐ 10 min	☐ 15 min

- ☐ Prepare Fragmentation master mix (Frag MM, see Table 3). Mix **>** spin **>** keep on ice.

Table 3: Frag MM reagent volumes (including excess for supported run sizes)

Reagent		16 Rxn Kits		96 Rxn Kits	
	Per Rxn	8 Rxn	16 Rxn	24 Rxn	96 Rxn
5X SureSelect Fragmentation Buffer	2 µl	18 µl	36 µl	52 µl	200 µl
SureSelect Fragmentation Enzyme	1 µl	9 µl	18 µl	26 µl	100 µl
Total	3 µl	27 µl	54 µl	78 µl	300 µl

- ☐ Add 3 µl Frag MM to DNA samples. Mix **>** brief spin.
- ☐ Place in thermal cycler and press play/continue.
- ☐ At 4 °C Hold step, remove from thermal cycler **>** add 40 µl nuclease-free water **>** keep on ice.

Proceed directly to Library Preparation.

Option 2: Covaris shearing

5 min (per sample)

Prep Ahead: Set up the Covaris E220 instrument. Refer to the instrument user guide

- ☐ Prepare DNA samples: 50 µl each containing 10–200 ng DNA in 1X Low TE. Keep on ice.
- ☐ Transfer DNA sample to the Covaris microTUBE **>** spin **>** verify all bubbles released.
- ☐ Shear DNA using following settings:

Duty factor = 10%
Peak incident power = 175
Cycles per burst = 200
Bath temp = 2–8 °C

Use shearing duration based on NGS read length and sample type (Table 4).

Table 4: Shearing duration

NGS Read Length	Shearing Duration	
	High-quality DNA	FFPE DNA
2 X 100	☐ 2 X 120 sec	☐ 240 sec
2 X 150	☐ 2 X 60 sec	☐ 240 sec

- ☐ Transfer 50 µl sheared DNA sample to PCR plate or strip well **>** keep on ice.
- ☐ Spin Covaris microTUBE **>** transfer residual liquid to same well.

Proceed directly to Library Preparation.

Library Preparation

Note: Reagent vial cap colors are indicated by colored circles adjacent to reagent use instructions. White cap is indicated by "w".

Step 1: Prepare ligation master mix

5 min

- [ ] Vortex thawed Ligation Buffer at high speed for 15 sec.
  Reagent is viscous and must be vortexed.
- [ ] Prepare Ligation master mix (Lig MM, see Table 5). Mix **>** spin **> keep at RT** during end repair/A-tailing steps.

Table 5: Lig MM reagent volumes (including excess for supported run sizes)

Reagent		16 Rxn Kits		96 Rxn Kits	
	Per Rxn	8 Rxn	16 Rxn	24 Rxn	96 Rxn
Ligation Buffer	23 µl	207 µl	414 µl	598 µl	2300 µl
T4 DNA Ligase	2 µl	18 µl	36 µl	52 µl	200 µl
Total	25 µl	225 µl	450 µl	650 µl	2500 µl

Step 2: End repair and dA-tail fragments

40 min

- [ ] Set up thermal cycler program (Table 6). Start and pause program.

Table 6: Thermal cycler program for End repair/A-tailing (vol 70 µl)

Step	Temperature	Time
Step 1	20 °C	15 min
Step 2	72 °C	15 min
Step 3	4 °C	Hold

- [ ] Vortex thawed End Repair-A Tailing Buffer at high speed for 15 sec.
  Reagent is viscous and must be vortexed.
- [ ] Prepare End repair/A-tailing master mix (ER-AT MM, see Table 7). Mix **>** spin **>** keep on ice.

Table 7: ER-AT MM reagent volumes (including excess for supported run sizes)

Reagent		16 Rxn Kits		96 Rxn Kits	
	Per Rxn	8 Rxn	16 Rxn	24 Rxn	96 Rxn
End Repair-A Tailing Buffer	16 µl	144 µl	288 µl	416 µl	1600 µl
End Repair-A Tailing Enzyme Mix	4 µl	36 µl	72 µl	104 µl	400 µl
Total	20 µl	180 µl	360 µl	520 µl	2000 µl

- [ ] Get 50 µl fragmented DNA samples from ice. Add 20 µl ER-AT MM. Mix **>** spin.
- [ ] Place in thermal cycler and press play/continue.
- [ ] At 4 °C Hold step, remove from cycler **>** keep on ice.

Step 3: Ligate MBC-tagged adaptors

40 min

- [ ] Set up thermal cycler program (Table 8). Start and pause program.

Table 8: Thermal cycler program for Ligation (vol 100 µl)

Step	Temperature	Time
Step 1	20 °C	30 min
Step 2	4 °C	Hold

- [ ] Get 70 µl DNA samples from ice. Add 25 µl Lig MM from Step 1. Mix **>** spin.
- [ ] Ⓦ Add 5 µl SureSelect XT HS2 Adaptor Oligo Mix. Mix **>** spin.
- [ ] Place in thermal cycler and press play/continue.
- [ ] At 4 °C Hold step, remove from cycler **>** keep on ice.

Stopping point

Prep Ahead: Put AMPure XP beads at RT for use in next step (30 min equilibration)

Step 4: Clean-up ligation

30 min

- [ ] Get AMPure XP beads held at RT, vortex until homogeneous.
- [ ] Add 80 µl of AMPure XP beads to each DNA sample well. Mix **>** incubate at RT 5 min.
- [ ] Collect beads using magnet (5–10 min) **>** remove and discard supernatant.
- [ ] Wash beads 2X with 200 µl freshly-prepared 70% ethanol per wash.
  Washes completed: ☐ 1 ☐ 2
- [ ] After second wash solution removed, spin briefly **>** remove residual ethanol.
- [ ] Dry samples unsealed on the thermal cycler at 37 °C (1–2 min).
- [ ] Elute by adding 35 µl nuclease-free water to each sample well. Seal wells **>** vortex **>** brief spin. Incubate at RT 2 min.
- [ ] Collect beads using magnet (~5 min).
- [ ] Transfer 34 µl cleared supernatant to fresh well **>** keep on ice.

Note: Reagent vial cap colors are indicated by colored circles adjacent to reagent use instructions. Clear cap is indicated by "c".

Step 5: Amplify and dual index (Pre-capture PCR)

35 min

- [ ] Set up thermal cycler program (Table 9). Start and pause program.

Table 9: Thermal cycler program for pre-capture PCR (vol 50 µl)

Step	Number of Cycles	Temperature	Time
Step 1	1	98 °C	2 min
Step 2	☐ 8 for 100–200 ng high-quality input library	98 °C	30 sec
	☐ 9 for 50 ng high-quality input library	60 °C	30 sec
	☐ 11 for 10 ng high-quality input library ☐ 11 for 100–200 ng FFPE input library ☐ 12 for 50 ng FFPE input library ☐ 14 for 10 ng FFPE input library	72 °C	1 min
Step 3	1	72 °C	5 min
Step 4	1	4 °C	Hold

- [ ] Prepare Pre-capture PCR master mix (Pre-PCR MM; Table 10). Mix **>** spin **>** keep on ice.

Table 10: Pre-PCR MM reagent volumes (including excess for supported run sizes)

Reagent		16 Rxn Kits		96 Rxn Kits	
	Per Rxn	8 Rxn	16 Rxn	24 Rxn	96 Rxn
ⓒ 5X Herculase II Buffer with dNTPs	10 µl	90 µl	180 µl	260 µl	1000 µl
● Herculase II Fusion DNA Polymerase	1 µl	9 µl	18 µl	26 µl	100 µl
Total	11 µl	99 µl	198 µl	286 µl	1100 µl

- [ ] Get 34 µl purified library samples from ice. Add 11 µl Pre-PCR MM.
- [ ] Add 5 µl of the appropriate SureSelect XT HS2 Index Primer Pair to each sample well. Vortex **>** spin.
- [ ] Resume thermal cycler program without adding samples. Once cycler reaches 98 °C, add sample plate/strip **>** close lid.

Prep Ahead: Put AMPure XP beads at RT for use in next step (30 min equilibration)

Step 6: Clean-up library

30 min

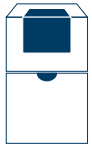
- [ ] Get AMPure XP beads held at RT, vortex until homogeneous.
- [ ] Remove samples from cycler **>** add 50 µl of AMPure XP beads to each amplified DNA well. Mix **>** incubate at RT 5 min.
- [ ] Collect beads using magnet (5 min) **>** remove and discard supernatant.
- [ ] Wash beads 2X with 200 µl freshly-prepared 70% ethanol per wash.
  Washes completed: ☐ 1 ☐ 2
- [ ] After second wash solution removed, spin briefly **>** remove residual ethanol.
- [ ] Dry samples unsealed on the thermal cycler at 37 °C (1–2 min).
- [ ] Elute by adding 15 µl nuclease-free water to each sample well. Seal wells **>** vortex **>** brief spin. Incubate at RT 2 min.
- [ ] Collect beads using magnet (2–3 min).
- [ ] Transfer cleared supernatant to fresh well **>** keep on ice.

Step 7: Quality control

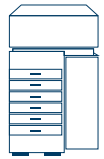
Varies

Assess quality and quantity using one of these platforms:

Agilent 2100 Bioanalyzer System with DNA 1000 Assay



Agilent 4200 or 4150 TapeStation System with D1000 Assay



Agilent 5200, 5300 or 5400 Fragment Analyzer System with NGS Fragment Kit (1–6000 bp)

Stopping point

Hybridization/Capture

Note: Reagent vial cap colors are indicated by colored circles adjacent to reagent use instructions.

Step 1: Pool indexed libraries

30 min

- ☐ Pool prepared DNA libraries in wells of a PCR plate/strip according to Table 11 (1 pool per well).

Table 11: Pre-capture pool composition

Probe	Amount of each library in pool	Libraries per pool	Total DNA per Hyb
☐ XT HS Precap Human All Exon V8/V8+UTR/V8+NCV	375 ng	8	3 µg
☐ Other All-Exon or Exome Probes and ClearSeq Inherited Disease	187.5 ng	8	1.5 µg
☐ Custom Probes and ClearSeq Comprehensive Cancer	93.75 ng	16	1.5 µg

- ☐ Reduce pool volumes in wells to <12 µl using vacuum concentrator at ≤ 45 °C. Do not overdry.
- ☐ Bring volume of each well to 12 µl with nuclease-free water.
- ☐ Seal wells **>** vortex vigorously 30 sec **>** spin briefly **>** keep on ice.

Step 2: Hybridize library pools to probe

1.5 – 2.5 hrs/overnight

- ☐ Set up thermal cycler program (Table 12). Start and pause program.

Table 12: Thermal cycler program for hybridization (vol 30 µl; heated lid ON)

Step	Probe	Number of Cycles	Temperature	Time
Step 1	All probes	1	95 °C	5 min
Step 2	All probes	1	65 °C	10 min
Step 3	All probes	1	65 °C	1 min *PAUSE HERE* *Resume after adding probe*
Step 4	All probes	60	65 °C[1]	1 min
			37 °C	3 sec
Step 5	☐ XT HS Human All Exon V8 or ☐ All other probes	1	65 °C	60 min
			65 °C	Hold briefly[2]
		1	65 °C[1]	Hold briefly[2]

Hybridization program variation footnotes:

1. Hybridization at 65 °C (steps 4 and 5) is optimal for XT HS designs. Optimal temperature may be lower for some legacy XT probe designs.
2. **For overnight workflow**, replace the final brief hold at 65 °C with hold at 21 °C for up to 16 hours.

Step 2 Continued

- ● Add 5 µl SureSelect XT HS2 Blocker Mix to sample wells. Seal wells **>** vortex 5 sec **>** brief spin **>** verify absence of bubbles in wells.
- ☐ Place in thermal cycler and press play/continue. **Run through Step 2, pausing cycler at Step 3.** During cycler Steps 1–2, do reagent prep tasks below.
- ☐ Prepare 25% RNase Block solution (see Table 13). Mix **>** keep on ice.

Table 13: 25% RNase Block prep volumes (including excess for supported run sizes)

Reagent	Per Hyb	6 Hyb	12 Hyb
● SureSelect RNase Block	0.5 µl	3.5 µl	6.5 µl
Nuclease-free water	1.5 µl	10.5 µl	19.5 µl
Total	2 µl	14 µl	26 µl

- ☐ Prepare the Probe Hyb Mix needed for your probe design. Use Table 14 for designs ≥3 Mb or use Table 15 for designs <3 Mb. Vortex **>** spin **>** use immediately (keep briefly at RT while adding to hyb).

Table 14: Probe Hyb Mix for probes ≥3 Mb (including excess for supported run sizes)

Reagent	Per Hyb	6 Hyb	12 Hyb
25% RNase Block (table 13)	2 µl	14 µl	26 µl
Probe **(≥3 Mb design)**	5 µl	35 µl	65 µl
SureSelect Fast Hybridization Buffer	6 µl	42 µl	78 µl
Total	13 µl	91 µl	169 µl

Table 15: Probe Hyb Mix for probes <3 Mb (including excess for supported run sizes)

Reagent	Per Hyb	6 Hyb	12 Hyb
25% RNase Block (table 13)	2 µl	14 µl	26 µl
Probe **(<3 Mb design)**	2 µl	14 µl	26 µl
SureSelect Fast Hybridization Buffer	6 µl	42 µl	78 µl
Nuclease-free water	3 µl	21 µl	39 µl
Total	13 µl	91 µl	169 µl

- ☐ With thermal cycler paused at Step 3, add 13 µl Probe Hyb Mix to each sample well. Pipette 8–10X to mix **>** cap wells with domed caps.

Wells must be fully sealed to prevent evaporation.

- ☐ Brief vortex **>** brief spin **>** verify absence of bubbles in wells. Immediately return samples to cycler **>** press play/continue to run Hybridization.

Note: Reagent vial cap colors are indicated by colored circles adjacent to reagent use instructions. Clear cap is indicated by "c".

Step 3: Prepare streptavidin beads

30 min

For same-day hyb and capture, begin steps below ~30 min prior to completion of hybridization program in Table 12. For overnight workflow option, begin at start of Day 2.

- ☐ Get SureSelect Streptavidin Beads or Dynabeads MyOne Streptavidin T1 Beads from storage at 4 °C, vortex until homogeneous.
- ☐ Place 50 µl of streptavidin beads in each sample well of fresh plate/strip.
- ☐ Wash beads 3X with SureSelect Binding Buffer at RT. For each wash:
  - ☐ Add 200 µl Binding Buffer per well of beads **>** mix well **>** spin briefly.
  - ☐ Collect beads using magnet (5 min) **>** remove and discard supernatant.

  Washes completed: ☐ 1 ☐ 2 ☐ 3
- ☐ Resuspend washed beads in 200 µl SureSelect Binding Buffer **>** keep at RT.

Step 4: Capture hybridized libraries

70 min

- ☐ Once streptavidin beads prepared and hybridization program reaches final hold, transfer samples briefly to RT.
- ☐ Immediately transfer hyb reactions (~30 µl) to wells containing 200 µl washed beads. Pipette 5–8X to mix **>** seal wells with fresh domed caps.
- ☐ Incubate on a 96-well plate mixer at 1400–1900 rpm for 30 min at RT.
- ☐ During 30 min hyb capture, prep pre-warmed SureSelect Wash Buffer **2** (6 aliquots per library) for post-capture washes:
  - ☐ Place 200 µl Wash Buffer **2** in fresh plate/strip wells.
  - ☐ Seal wells **>** place in thermal cycler held at 70 °C.
- ☐ After 30 min hyb capture, spin samples briefly **>** collect beads using magnet (until solution clear) **>** remove and discard supernatant.
- ☐ Resuspend capture beads at RT in 200 µl SureSelect Wash Buffer **1** by pipetting up and down 15–20X or until beads fully resuspended.
- ☐ Collect capture beads using magnet (~1 min) **>** remove and discard supernatant.
- ☐ Remove from magnet **>** keep at RT. Wash capture beads 6X using the pre-warmed SureSelect Wash Buffer **2** aliquots held at 70 °C. For each wash:
  - ☐ Add 200 µl Wash Buffer **2** at 70 °C to beads in wells.
  - ☐ Pipette up and down 15–20X.
  - ☐ Seal wells **>** vortex 8 sec **>** spin briefly (do not pellet).
  - ☐ Incubate 5 min at 70 °C in thermal cycler.
  - ☐ Collect beads using magnet (1 min) at RT **>** remove and discard supernatant.

  Washes completed: ☐ 1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 ☐ 6

Step 4 Continued

- ☐ After all wash buffer removed from final wash, add 25 µl nuclease-free water per well **>** pipette up and down 8X **>** keep on ice.

Captured DNA remains on the streptavidin beads for post-capture amplification.

Step 5: Amplify enriched libraries (Post-capture PCR)

30 min

- ☐ Set up thermal cycler program (Table 16). Start and pause program.

Table 16: Thermal cycler program for post-capture PCR (vol 50 µl)

Step	Number of Cycles	Temperature	Time
Step 1	1	98 °C	2 min
Step 2	☐ 10–11 for probe designs >5 Mb	98 °C	30 sec
	☐ 11–12 for probe designs 3–5 Mb ☐ 12–16 for probe designs 0.2–3 Mb	60 °C	30 sec
	☐ 16 for probe designs <0.2 Mb	72 °C	1 min
Step 3	1	72 °C	5 min
Step 4	1	4 °C	Hold

- ☐ Prepare Post-capture PCR master mix (Post-PCR MM; Table 17). Mix **>** spin **>** keep on ice.

Table 17: Post-PCR MM reagent volumes (including excess for supported run sizes)

Reagent	Per Rxn	6 Rxn	12 Rxn
Nuclease-free water	13 µl	91 µl	182 µl
ⓒ 5X Herculase II Buffer with dNTPs	10 µl	70 µl	140 µl
● Herculase II Fusion DNA Polymerase	1 µl	7 µl	14 µl
ⓒ SureSelect Post-Capture Primer Mix	1 µl	7 µl	14 µl
Total	25 µl	175 µl	350 µl

- ☐ Get 25 µl captured DNA bead suspensions from ice. Add 25 µl Post-PCR MM.
- ☐ Mix by pipetting until beads are in homogeneous suspension (Do **not** spin).
- ☐ Place samples in cycler **>** close lid **>** press play/continue.
- ☐ At 4 °C Hold step, remove from cycler **>** brief spin.
- ☐ Collect streptavidin beads using magnet (2 min).
- ☐ **Transfer supernatant (~50 µl) to well of fresh plate or strip >** keep on ice.

Prep Ahead: Put AMPure XP beads at RT for use in next step (30 min equilibration)

Step 6: Clean-up amplified library pools

30 min

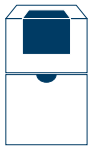
- [ ] Get AMPure XP beads held at RT, vortex until homogeneous.
- [ ] Add 50 µl of AMPure XP beads to each amplified DNA well **>** mix until beads in homogeneous suspension. Incubate at RT 5 min.
- [ ] Collect beads using magnet (3–5 min) **>** remove and discard supernatant.
- [ ] Wash beads 2X with 200 µl freshly-prepared 70% ethanol per wash.
  Washes completed: ☐ 1 ☐ 2
- [ ] After second wash solution removed, spin briefly **>** remove residual ethanol.
- [ ] Dry samples unsealed on the thermal cycler at 37 °C (1–2 min).
- [ ] Elute by adding 25 µl 1X Low TE to each sample well.
  Seal wells **>** vortex **>** brief spin. Incubate at RT 2 min.
- [ ] Collect beads using magnet (~2 min).
- [ ] Transfer cleared supernatant to fresh well **>** keep on ice.

Step 7: Quality control

Varies

Assess quality and quantity using one of these platforms:

Agilent 2100 Bioanalyzer System with High Sensitivity DNA Assay



Agilent 4200 or 4150 TapeStation System with High Sensitivity D1000 Assay

Agilent 5200, 5300 or 5400 Fragment Analyzer System with HS NGS Fragment Kit (1–6000 bp)

Stopping point

Step 8: Combine captured pools for multiplex NGS (Optional)

- [ ] Calculate the number of indexes that can be combined per lane, according to sequencer capacity and amount of sequencing data required per sample.
- [ ] Combine the captured library pools such that each index-tagged sample is present in equimolar amounts in the final pool using one of the following methods:
  - ☐ Dilute each captured pool to the concentration of the most dilute pool using Low TE, then combine equal volumes of all to make the final pool.
  - ☐ Combine the appropriate volume of each captured pool solution to achieve equimolar concentration in the final pool, then adjust mixture to the desired final volume using Low TE. See the **assay user guide** for more information on this method.

Run notes

Run Date	
Operator	
Probe Name or Design ID	
Index Pairs	
Reagent Lot Information	– Library Prep Kit: – Target Enrichment Kit: – Probe: – Enzymatic Frag Kit: – Other Materials:
Sample Information	
Library Pooling Information	
Comments	

G9985-90500 Rev A0, July 2022

Agilent
Trusted Answers