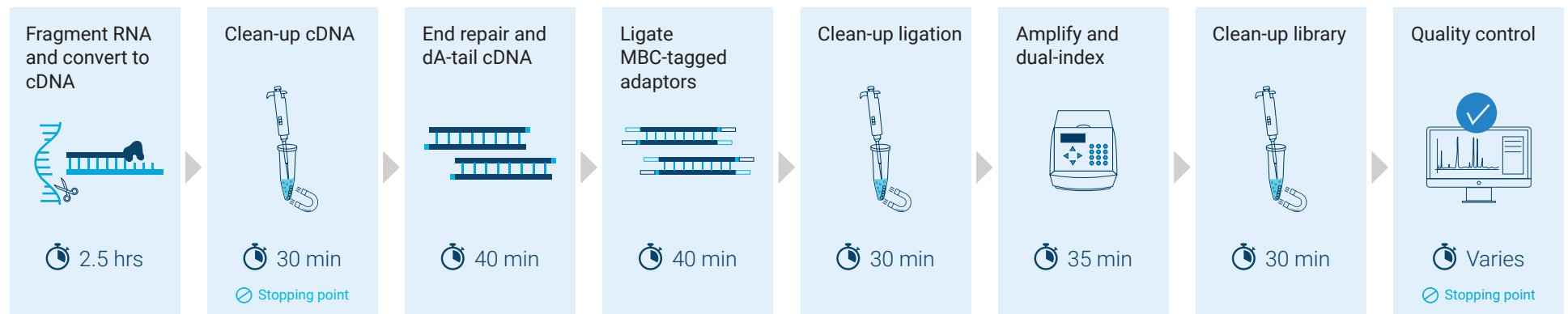


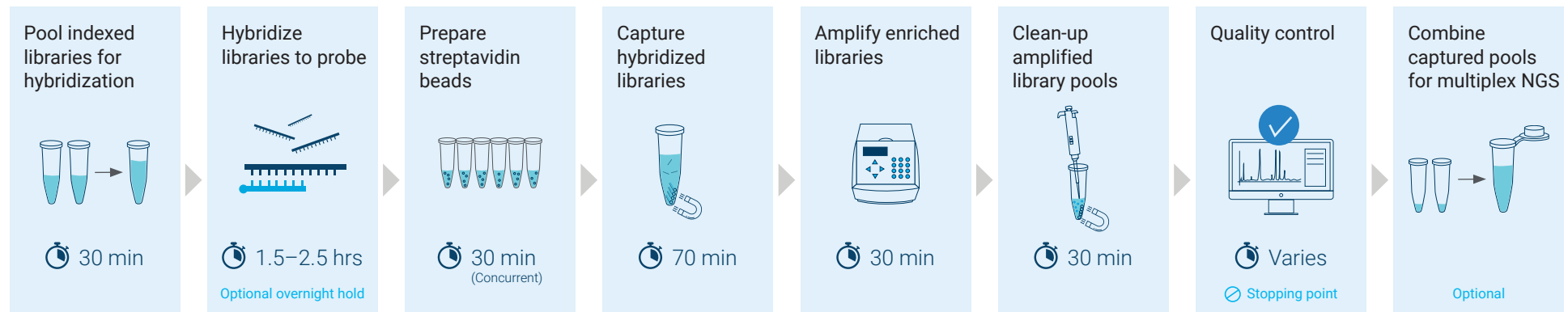
G9993-90500 Rev A0

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

## cDNA Conversion and Library Preparation Workflow 6 Hours



## Hybridization/Capture Workflow 4–5 Hours



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

This Quick Start Protocol provides key protocol details for experienced users. Visit [SureSelect XT HS2 RNA 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

## RNA Fragmentation and Conversion to cDNA

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

### Step 1: Add cDNA primers and fragment intact (non-FFPE) RNA samples 10 min

The 2X Priming Buffer includes both cDNA primers and heat-driven fragmentation agents. Intact (non-FFPE) RNA samples are fragmented by exposure to elevated temperature while FFPE RNA samples are kept on ice to prevent further fragmentation.

- If the run includes intact (non-FFPE) samples, set up thermal cycler program (Table 1). Start and pause program.

Table 1: Thermal cycler program for RNA fragmentation (vol 20  $\mu$ l)

Step	Temperature	Time
Step 1	94 °C	4 min
Step 2	4 °C	1 min
Step 3	4 °C	Hold

- Prepare RNA samples in wells: 10  $\mu$ l each containing 10–200 ng total RNA in nuclease-free water. Keep on ice.  
*Use  $\geq$  50 ng RNA for poor quality FFPE samples (DV200 of 20%–50%).*
- Add 10  $\mu$ l 2X Priming Buffer to all RNA samples. Mix  $\blacktriangleright$  brief spin.
- Place FFPE RNA samples on ice. Place intact (non-FFPE) samples in thermal cycler and press play/continue.
- At 4 °C Hold step, remove fragmented non-FFPE samples from thermal cycler  $\blacktriangleright$  keep on ice.

### Step 2: Synthesize first-strand cDNA 55 min

- Set up thermal cycler program (Table 2). Start and pause program.

Table 2: Thermal cycler program for first-strand synthesis (vol 28  $\mu$ l)

Step	Temperature	Time
Step 1	25 °C	10 min
Step 2	37 °C	40 min
Step 3	4 °C	Hold

- Vortex thawed First Strand Master Mix at high speed for 5 sec.  
*Reagent is viscous and must be vortexed.*
- Add 8.5  $\mu$ l First Strand Master Mix to RNA samples. Mix  $\blacktriangleright$  brief spin.
- Place in thermal cycler and press play/continue.
- At 4 °C Hold step, remove from thermal cycler  $\blacktriangleright$  keep on ice.

### Step 3: Synthesize second-strand cDNA 65 min

Set up thermal cycler program (Table 3). Start and pause program.

Table 3: Thermal cycler program for second-strand synthesis (vol 58  $\mu$ l)

Step	Temperature	Time
Step 1	16 °C	60 min
Step 2	4 °C	Hold

Vortex thawed Second Strand Enzyme Mix and Second Strand Oligo Mix at high speed for 5 sec.

*Reagent is viscous and must be vortexed.*

● Add 25  $\mu$ l Second Strand Enzyme Mix to sample wells > keep on ice.

● Add 5  $\mu$ l Second Strand Oligo Mix to sample wells > keep on ice.

Mix>brief spin>place in thermal cycler and press play/continue.

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

### Step 4: Clean-up cDNA 30 min

Get AMPure XP beads held at RT, vortex until homogeneous.

Add 105  $\mu$ l of AMPure XP beads to each cDNA sample well.  
Mix > incubate at RT 5 min.

Collect beads using magnet (2–5 min) > remove and discard supernatant.

Wash beads 2X with 200  $\mu$ l freshly-prepared 70% ethanol per wash.  
Washes completed:  1  2

After second wash removed, spin briefly > remove residual ethanol.

Dry samples unsealed on the thermal cycler at 37 °C (1–2 min).

Elute by adding 52  $\mu$ l nuclease-free water to each sample well.  
Seal wells > vortex > brief spin. Incubate at RT 2 min.

Collect beads using magnet (~5 min).

Transfer 50  $\mu$ l cleared supernatant to fresh well > keep on ice.

 **Stopping point**

# Library Preparation

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

## 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 4). Mix > spin > **keep at RT** during end repair/A-tailing steps.

Table 4: Lig MM reagent volumes (including excess for supported run sizes)

Reagent	Per Rxn	16 Rxn Kits		96 Rxn Kits	
		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
<b>Total</b>	<b>25 µl</b>	<b>225 µl</b>	<b>450 µl</b>	<b>650 µl</b>	<b>2500 µl</b>

## Step 3: Ligate MBC-tagged adaptors

40 min

- Set up thermal cycler program (Table 7). Start and pause program.

Table 7: 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 XT HS2 RNA Adaptor Oligo Mix. Mix > spin.
- Place in thermal cycler and press play/continue.
- At 4 °C Hold step, remove from cycler > keep on ice.

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

## Step 2: End repair and dA-tail fragments

40 min

- Set up thermal cycler program (Table 5). Start and pause program.

Table 5: 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 6). Mix > spin > keep on ice.

Table 6: ER-AT MM reagent volumes (including excess for supported run sizes)

Reagent	Per Rxn	16 Rxn Kits		96 Rxn Kits	
		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
<b>Total</b>	<b>20 µl</b>	<b>180 µl</b>	<b>360 µl</b>	<b>520 µl</b>	<b>2000 µl</b>

- Get 50 µl cDNA 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 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 cDNA 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.

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

35 min

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

Table 8: 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	<b>For high-quality RNA input libraries:</b>		
	<input type="checkbox"/> 10 for 100–200 ng RNA input	98 °C	30 sec
	<input type="checkbox"/> 11 for 50 ng RNA input	60 °C	30 sec
	<input type="checkbox"/> 12 for 10 ng RNA input		
	<b>For good-quality FFPE RNA input libraries:</b>		
	<input type="checkbox"/> 12 for 100–200 ng RNA input	72 °C	1 min
	<input type="checkbox"/> 13 for 50 ng RNA input		
	<input type="checkbox"/> 14 for 10 ng RNA input		
	<b>For poor-quality FFPE RNA input libraries:</b>		
	<input type="checkbox"/> 13 for 100-200 ng RNA input		
	<input type="checkbox"/> 14 for 50 ng RNA input		
Step 3	1	72 °C	5 min
Step 4	1	4 °C	Hold

- Prepare Pre-capture PCR master mix (Pre-PCR MM; Table 9). Mix > spin > keep on ice.

Table 9: Pre-PCR MM reagent volumes (including excess for supported run sizes)

Reagent	Per Rxn	16 Rxn Kits		96 Rxn Kits	
		8 Rxn	16 Rxn	24 Rxn	96 Rxn
<input type="checkbox"/> 5X Herculase II Buffer with dNTPs	10 µl	90 µl	180 µl	260 µl	1000 µl
<input type="checkbox"/> Herculase II Fusion DNA Polymerase	1 µl	9 µl	18 µl	26 µl	100 µl
<b>Total</b>	<b>11 µl</b>	<b>99 µl</b>	<b>198 µl</b>	<b>286 µl</b>	<b>1100 µl</b>

- 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

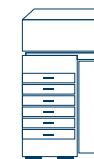
Assess quality and quantity using one of these platforms:



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 cDNA libraries



Pool prepared cDNA libraries in wells of a PCR plate/strip according to Table 10 (1 pool per well).

Table 10: Pre-capture pool composition

Probe	Amount of each library in pool	Libraries per pool	Total DNA per Hyb
<input type="checkbox"/> XT HS Precap Human All Exon V8/V8+UTR	150 ng	8	1.2 µg
<input type="checkbox"/> Other All-Exon or Exome Probes and ClearSeq Inherited Disease	75 ng	8	600 ng
<input type="checkbox"/> Custom Probes and ClearSeq Comprehensive Cancer	37.5 ng	16	600 ng

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



Set up thermal cycler program (Table 11). Start and pause program.

Table 11: 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 <i>PAUSE HERE</i> <i>Resume after adding probe</i>
Step 4	All probes	60	65 °C <sup>1</sup>	1 min
			37 °C	3 sec
Step 5	<input type="checkbox"/> XT HS Human All Exon V8 or	1	65 °C	Hold briefly <sup>2</sup>
	<input type="checkbox"/> All other probes		65 °C <sup>1</sup>	

### 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 12). Mix > keep on ice.

Table 12: 25% RNase Block prep volumes (including excess for supported run sizes)

Reagent	Per Hyb	6 Hyb	12 Hyb
<input checked="" type="checkbox"/> SureSelect RNase Block	0.5 µl	3.5 µl	6.5 µl
Nuclease-free water	1.5 µl	10.5 µl	19.5 µl
<b>Total</b>	<b>2 µl</b>	<b>14 µl</b>	<b>26 µl</b>

Prepare the Probe Hyb Mix needed for your probe design. Use Table 13 for designs ≥3 Mb or use Table 14 for designs <3 Mb. Vortex > spin > use immediately (keep briefly at RT while adding to hyb).

Table 13: Probe Hyb Mix for probes ≥3 Mb (including excess for supported run sizes)

Reagent	Per Hyb	6 Hyb	12 Hyb
25% RNase Block (table 12)	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
<b>Total</b>	<b>13 µl</b>	<b>91 µl</b>	<b>169 µl</b>

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 12)	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
<b>Total</b>	<b>13 µl</b>	<b>91 µl</b>	<b>169 µl</b>

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.

### 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 11. 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 15). Start and pause program.

Table 15: 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	<input type="checkbox"/> 12 for probe designs >5 Mb	98 °C	30 sec
	<input type="checkbox"/> 13 for probe designs 3–5 Mb	60 °C	30 sec
	<input type="checkbox"/> 14 for probe designs 0.2–3 Mb		
	<input type="checkbox"/> 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 16). Mix > spin > keep on ice.

Table 16: 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
<input checked="" type="checkbox"/> 5X Herculase II Buffer with dNTPs	10 µl	70 µl	140 µl
<input checked="" type="checkbox"/> Herculase II Fusion DNA Polymerase	1 µl	7 µl	14 µl
<input checked="" type="checkbox"/> SureSelect Post-Capture Primer Mix	1 µl	7 µl	14 µl
<b>Total</b>	<b>25 µl</b>	<b>175 µl</b>	<b>350 µl</b>

- 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



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

- cDNA Module
- Library Prep Kit:
- Target Enrichment Kit:
- Probe:
- Other Materials:

Sample Information

Library Pooling Information

Comments