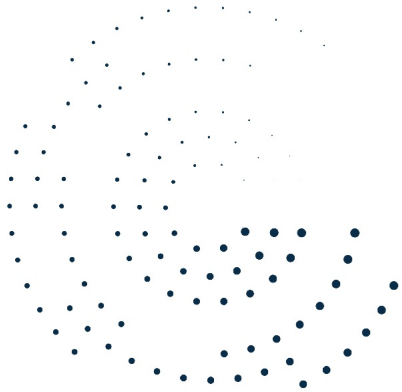
INSTRUCTIONS FOR USE

16, 32, 48 and 96 Samples

SOPHiA DDM™

Solid Tumor Plus Solution



Using the SOPHiA GENETICS™ DNA Library Prep Kit II and the SOPHiA GENETICS™ RNA Library Prep Kit

SOPHiA GENETICS™

SUMMARY INFORMATION

Product Name	SOPHiA DDM™ Solid Tumor Plus Solution
Product Type	Bundle Solution
Product Family	Molecular kit + analytics
Algorithm ID	ILL1XG1S5_FFPE_CNV_NextSeq; ILL1XG1S5_FFPE_CNV_NextSeq_1
Gene Panel ID	STS_plus_v1
Product Version	v1.0
Release Version	Not Applicable
Sample Type	Somatic DNA/RNA isolated from formalin-fixed paraffin-embedded (FFPE) tumor tissue specimens
Sequencer	Illumina® NextSeq® 500/550
Document ID	SG-00262
Document Version	v2.1
Revision Date	Feb 2024

This Instructions For Use is applicable to all SOPHiA DDM™ versions.

Please read the Instructions For Use thoroughly before using this product.

SOPHiA GENETICS SA
ZA La Pièce 12
1180 Rolle
Switzerland

REF

BS0115ILLRSMY05-16;
BS0115ILLRSMY05-32;
BS0115ILLRSMY05-48;
BS0115ILLRSMY05-96

RUO

PRODUCT CODES

	FULL PRODUCT CODE	BOX 1	BOX 2	BOX 3	LIBRARY PREPARATION KIT
REF	BS0115ILLRSMY05-16	B1.H1.0015.R-16	B2.0015.R-16	B3.0015.R-16	900232
	BS0115ILLRSMY05-32	B1.H1.0015.R-32	B2.0015.R-32	B3.0015.R-32	900232
	BS0115ILLRSMY05-48	B1.H1.0015.R-48	B2.0015.R-32	B3.0015.R-48	900234
	BS0115ILLRSMY05-96	B1.H1.0015.R-96	B2.0015.R-32	B3.0015.R-48	900234

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REVISION HISTORY

DOCUMENT ID / VERSION	DATE	DESCRIPTION OF CHANGE
SG-00262 – v2.1	Feb 2024	• Section *2.1.2 Kit Content*: Added missing information regarding Stubby Universal (SU) adapter quantities within the contents of Box 1 • Sections *3.1.5 and 5.2.2 - Index Sequences For The Illumina®-compatible Unique Dual Index Primers*: Added clarifications regarding the orientation of the i5 index sequence depending on the used sequencer to section • Section *2.2.8 Individual Library Quantification and Quality Control*: Removed misleading example of DNA library size distribution • Added missing symbols to *Symbols* section
SG-00262 – v2.0	Sep 2023	• Cosmetic text and formatting changes and minor document restructuring • Added new sections *Product Codes* and *Product Introduction* • Changes of name and incorporation of trademark symbol throughout for product and library preparation kit. • Removed mention of third-party provider's intellectual property from sections *2.2.1 Kit Content*, *2.3.1 Library Pooling for Hybridization and Capture*, and *2.4.1 Hybridization*. • Specified "NextSeq®" sequencer as "NextSeq® 500/550" • Increased incubation time on the magnetic rack during Post-Ligation Clean Up (step 11), Post-Amplification Clean Up (step 11), and Post-Capture Amplification Clean Up (step 11) from 3 min to 5 min. • Updated company address.
ID-60101-53 – v1.1	Mar 2023	• Incorporation of SOPHiA GENETICS™ and SOPHiA DDM™ throughout.

ID-60101-53 – v1.0	Aug 2021	• Title page, Company logo, Header, Footer, Last page.
		• Combined four kit size "User guides" together to include different sample numbers. Included tables and made appropriate changes as and when necessary for this purpose.
		• Following Kit "User guide" documents were combined:
		  • PM_RUO_B.2.1.2.110_r3en
		  • PM_RUO_B.2.1.2.107_r2en
		  • PM_RUO_B.2.1.2.35_r6en
		• Minor changes for clarity in the following sections:
		  • Section 3.1 Library Pooling
		  • Section 3.5 Wash Streptavidin Beads to Remove Unbound DNA
		• New Plate layout and index sequences included

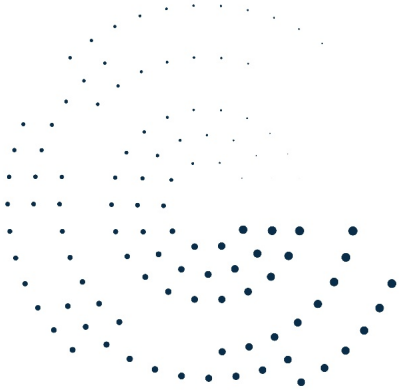
TABLE OF CONTENTS

1 PRODUCT INTRODUCTION

The SOPHiA DDM™ Solid Tumor Plus Solution is a genomic application that characterizes the complex mutational landscape of the major solid tumors by combining DNA target capture and RNA target amplicon with the analytical capabilities and advanced features of the SOPHiA DDM™ Platform.

SOPHiA DDM™ Solid Tumor Plus Solution targets DNA variants and RNA transcripts of fusion genes associated with solid tumors, including lung, colorectal, skin, and brain cancers. The DNA panel covers 42 genes including 6 unique loci to detect MSI status associated with colorectal cancer, and the RNA panel targets 137 clinically relevant gene fusions. Probe design is optimized to guarantee high on-target rate and coverage uniformity throughout the entire target regions.

DNA PART



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2 PROTOCOL

2.1 Materials

2.1.1 Initial Considerations

Upon receipt, please ensure that all tubes are physically intact and stored at recommended temperatures for optimum kit performance. Inappropriate handling and storage of the kit components under other conditions may adversely affect the kit's performance.

2.1.2 Kit Content (16, 32, 48 And 96 Samples)

Always briefly spin the tubes before use to collect all liquid.

Depending on the kit format, the following components are provided:

COMPONENT	NUMBER OF ITEMS DEPENDING ON KIT FORMAT			
	16 SAMPLE KIT	32 SAMPLE KIT	48 SAMPLE KIT	96 SAMPLE KIT
Box 1	1	1	1	2 (48 samples each)
Illumina®-compatible Unique Dual Index Primers (in 96-well plate format included in Box 1)	16	32	48	96 (Plate contained in one of the two Box 1)
Box 2	1	1	1	2 (48 samples each)
SOPHiA GENETICS™ DNA Library Prep Kit II	1	2 (16 samples each)	1	2 (48 samples each)

Box 1 (Store At -25 °C To -15 °C)

- Universal Blockers - TS Mix (12 µl)
- Human Cot DNA (25 µl)
- Solid Tumor Solution Plus Probes by SOPHiA GENETICS (20 µl)
- 2x Hybridization Buffer (50 µl)

- Hybridization Buffer Enhancer (20 µl)
- 2x Bead Wash Buffer (1250 µl)
- 10x Stringent Wash Buffer (200 µl)
- 10x Wash Buffer I (160 µl)
- 10x Wash Buffer II (110 µl)
- 10x Wash Buffer III (110 µl)
- Depending on the kit format: 16, 32, 48 or 96 Illumina®-compatible Unique Dual Index Primers V2 in a 96-well plate format (7 µl of primer per well). Please refer to Appendix I for primers display and sequences.
- Post-Capture Illumina® Primers Mix (20 µl)
- Post-Capture PCR Enhancer (20 µl)
- Post-Capture PCR Master Mix (122 µl)
- SU (Stubby Universal) Adapter (110 µl for 16 samples, 220 µl for 32 samples, 320 µl for 48 samples and 650 µl for 96 samples)

Box 2 (Store At +2 °C To +8 °C)

- Dynabeads® M-270 Streptavidin (440 µl)
- Agencourt® AMPure® XP (3 x 1.5 ml for 16 samples, 8.7 ml for 32 samples and 11.6 ml for 48 samples, see Note below for 96 samples)
- IDTE Low TE Buffer (10 ml)
- Nuclease-free water (20 ml)

Note: For 96 samples, two times Box 2 of 48 samples are provided (see table on previous page).

2.1.3 SOPHiA GENETICS™ DNA Library Prep Kit II (store At -25 °C To -15 °C)*

REAGENT	KIT FORMAT	
	16 SAMPLES	48 SAMPLES
PCR Master Mix 2x (µl)	520	2 x 750
Fragmentation Buffer (µl)	148	2 x 222
Fragmentation Enzyme Mix (µl)	43	127
Ligation Mix (µl)	634	1853
Ligation Enhancer (µl)	22	64

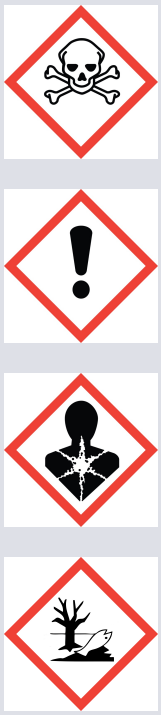
* SOPHiA GENETICS is the exclusive distributor of this Library Prep kit.

* For 32 samples, two 16-sample kits are provided.

* For 96 samples, two 48-sample kits are provided.

Refer to Warnings and Precautions below for additional details.

2.1.4 Warnings and Precautions

PRODUCT NAME	GHS PICTOGRAM	H&P STATEMENTS	SIGNAL WORD	HAZARDOUS COMPONENT
2x Hybridization Buffer		• H300 Fatal if swallowed. • H311 Toxic in contact with skin. • H315 Causes skin irritation. • H370 Causes damage to organs. • H370 Causes damage to organs (Central nervous system). • H411 Toxic to aquatic life with long-lasting effects. • P260 Do not breathe vapor/ spray. • P264 Wash contaminated skin thoroughly after handling. • P270 Do not eat, drink or smoke when using this product. • P273 Avoid release to the environment. • P280 Wear protective gloves/ protective clothing/ eye protection/face protection. • P301+P310 If swallowed: Immediately call a poison center/doctor. • P302+P352 If on skin: Wash with plenty of water. • P308+P311 If exposed or concerned: Call a poison center or doctor. • P321 Specific treatment (see medical advice on this label). • P330 Rinse mouth. • P332+P313 If skin irritation occurs: Get medical advice/ attention. • P362+P364 Take off contaminated clothing and wash it before reuse. • P391 Collect spillage. • P405 Store locked up. • P501 Dispose of contents/ container in accordance with national regulations.	Danger	Tetramethyl- ammonium chloride
Hybridization Buffer Enhancer		• H351 Suspected of causing cancer. • H360 May damage fertility or the unborn child. • H373 May cause damage to	Danger	Formamide

PRODUCT NAME	GHS PICTOGRAM	H&P STATEMENTS	SIGNAL WORD	HAZARDOUS COMPONENT
		organs through prolonged or repeated exposure. • P201 Obtain special instructions before use. • P202 Do not handle until all safety precautions have been read and understood. • P260 Do not breathe vapour/ spray. • P280 Wear protective gloves/ protective clothing/ eye protection/ face protection. • P308+P313 IF exposed or concerned: Get medical advice/ attention. • P314 Get medical advice/ attention if you feel unwell. • P405 Store locked up. • P501 Dispose of contents/ container in accordance with national regulations.		
10x Stringent Wash Buffer		• H302 Harmful if swallowed. • H315 Causes skin irritation. • H319 Causes serious eye irritation	Danger	Ethylenediaminetetraacetic acid disodium salt
10x Wash Buffer I		• H228 Flammable solid. • H302 Harmful if swallowed. • H315 Causes skin irritation. • H318 Causes serious eye damage. • H332 Harmful if inhaled. • H401 Toxic to aquatic life. • H402 Harmful to aquatic life. • H412 Harmful to aquatic life with long lasting effects. • P273 Avoid release to the environment. • P280 Wear protective gloves/ protective clothing/ eye protection/ face protection. • P305+P351+P338 If in eyes: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. • P310 Immediately call a poison	Danger	Sodium dodecyl sulfate

PRODUCT NAME	GHS PICTOGRAM	H&P STATEMENTS	SIGNAL WORD	HAZARDOUS COMPONENT
		center/ doctor. • P501 Dispose of contents/ container in accordance with national regulations.		
Fragmentation Buffer		• H302 Harmful if swallowed. • H370 Causes damage to organs. • H412 Harmful to aquatic life with long lasting effects. • P273 Avoid release to the environment. • P260 Do not breathe vapor. • P270 Do not eat, drink or smoke when using this product. • P264 Wash thoroughly after handling. • P308 + P311 IF exposed: Call a POISON CENTER or doctor. • P301 + P312, P330 IF SWALLOWED: Call a POISON CENTER or doctor if you feel unwell. Rinse mouth. • P405 Store locked up. • P501 Dispose of contents and container in accordance with all local, regional, national and international regulations.	Danger	Tetramethyl-ammonium chloride

Please use  and  as personal protective equipment.

2.1.5 Materials Required (Not Provided)

User-Supplied Materials (To Be Purchased Separately)

Lab-related plasticware

- DNA low binding 1.5 ml tubes
- 50 ml conical tubes
- Filter tips
- 1.5 ml tubes
- RNase/DNase-free 0.2 ml 8-tube strips

Reagents

- Ethanol (molecular biology grade)

Other

- Ice to ensure reagents and pre-mixes are kept at 4 °C
- Illumina® sequencing reagents

Laboratory Equipment

To avoid sample contamination, separate the workspace into:

- *Pre-PCR zone*
    - Fluorometric quantitation equipment and reagents (e.g., Qubit® or similar)
    - Magnetic separation rack (96-well type)
    - Multichannel pipettes (P10 or P20; P100; P200) with appropriate filter tips
    - Tabletop microcentrifuge (8-tube strips compatible)
    - Thermal cycler (programmable heated lid; 0.2 ml PCR tubes compatible)
    - Vortex mixer
- *Post-PCR zone*
    - Capillary electrophoresis system
    - DNA vacuum concentrator (e.g., SpeedVac™ or similar)
    - Fluorometric quantitation equipment and reagents (e.g., Qubit® or similar)
    - Magnetic separation rack (1.5 ml tube compatible)
    - Magnetic separation rack (96-well type)
    - Multichannel pipettes (P10 or P20; P100; P200) with appropriate filter tips
    - Tabletop microcentrifuge (8-tube strips compatible)
    - Thermal cycler (programmable heated lid; 0.2 ml PCR tubes compatible)
    - Thermoblock or water bath (1.5 ml tube compatible)
    - Vortex mixer

2.2 Library Preparation

2.2.1 Input Material Preparation

Materials

- DNA extracted from formalin-fixed paraffin-embedded tissue (FFPE DNA)
- IDTE
- Nuclease-free water
- RNase/DNase-free 0.2 ml 8-tube strips

Input Recommendations

The quality of FFPE-extracted DNA is variable and might impact sequencing data. Exposure to formalin damages the integrity of DNA molecules and can lead to DNA fragmentation. It also induces sequencing artifacts due to deamination events. Insufficient DNA quality can thereby confound the data analysis and cause false-positive, false-negative, and uninterpretable results.

We highly recommend to use the maximum amount of DNA stated in the protocol to generate high-quality sequencing data.

Depending on the amount of DNA used, adjust the number of PCR cycles according to the following table:

REQUIRED PCR CYCLES GIVEN DIFFERENT DNA INPUT AMOUNTS			
Amount of FFPE DNA (ng)	10 ≤ n < 50	50 ≤ n < 100	100 ≤ n < 200
PCR cycles	12	10	8

To avoid mistakes with DNA input, an initial dilution to obtain a concentration in the 50–100 ng/µl range is recommended. The DNA concentration should be confirmed by a fluorometric quantification method (e.g., Qubit®, Thermo Fisher), and the obtained value should be used to calculate the final dilution.

Preparation

Depending on the kit format, the number of DNA samples to be pooled per capture reaction will vary according to the following table. This must be taken into consideration before starting.

KIT FORMAT	16 SAMPLE KIT	32 SAMPLE KIT	48 SAMPLE KIT	96 SAMPLE KIT*
Number of individual libraries per capture	4	8	12	12

*For 96 samples, two 48 sample kits are provided which include 8 capture reactions.

Procedure

1. Prepare the following PCR strips according to the number of reactions:

NUMBER OF REACTIONS	4	8	12	16	24	32	48
PCR strip	**4-tube**	**4-tube**	**4-tube**	**8-tube**	**8-tube**	**8-tube**	**8-tube**
Number of strips	1	2	3	2	3	4	6

2. Prepare a dilution for each FFPE extracted DNA (FFPE DNA) sample into the appropriate number of PCR strips, in the following manner:

DNA DILUTION	
FFPE DNA	Amount of FFPE DNA (see table of DNA input recommendations above)
IDTE	Complete to 26 µl

3. Mix briefly by gently pipetting up and down 5 times followed by a brief spin in a microcentrifuge to collect all liquid.

Safe stopping point overnight at 4 °C.

2.2.2 Pre-Mixes and Reagents Preparation

Components And Reagents

- Fragmentation Enzyme Mix
- Fragmentation Buffer
- Ligation Enhancer
- Ligation Mix
- PCR Master Mix 2x
- Nuclease-free water
- AMPure® XP beads
- Ethanol

Preparation

1. Remove the SOPHiA GENETICS™ DNA Library Prep Kit II components from -20 °C storage and thaw on ice.
2. Remove the AMPure® XP beads from 2–8 °C storage and let them equilibrate at room temperature for at least 30 minutes. Vortex the AMPure® XP beads thoroughly, to ensure proper resuspension of the beads, prior to use in all subsequent steps.
3. Prepare fresh 80% Ethanol (volume according to the following scheme based on number of reactions):

80% ETHANOL VOLUMES							
Number of Reactions	**4**	**8**	**12**	**16**	**24**	**32**	**48**
80% Ethanol (ml)	10	10	20	20	30	40	50

4. Ensure that the Fragmentation Buffer in the SOPHiA GENETICS™ DNA Library Prep Kit II is completely thawed. If a precipitate is seen in the buffer, pipette up and down several time to break it and vortex to mix until full resuspension.
5. Mix well and spin all reagents prior to use and place on ice.

Pre-Mixes

Vortex the Fragmentation Enzyme Mix prior to use and place on ice.

Note: It is important to vortex the Fragmentation Enzyme Mix prior to use for optimal performance.

1. Prepare the **Fragmentation pre-mix** as follows:

FRAGMENTATION PRE-MIX							
Number of Reactions	**4**	**8**	**12**	**16**	**24**	**32**	**48**
Fragmentation Buffer (µl)	35	70	105	140	210	280	420
Fragmentation Enzyme (µl)	10	20	30	40	60	80	120

   - Mix thoroughly by pipetting up and down 10 times and spin briefly.
   - Keep on ice.

2. Prepare the **Ligation pre-mix** as follows:

LIGATION PRE-MIX							
Number of Reactions	**4**	**8**	**12**	**16**	**24**	**32**	**48**
Ligation Mix (µl)	145	290	435	580	870	1160	1740
Ligation Enhancer (µl)	4.83	9.66	14.5	19.33	29	38.66	58

   - Mix thoroughly by pipetting up and down 10 times and spin briefly.
   - Keep on ice.

The Ligation Mix is highly viscous, pipette gently and make sure to obtain a homogeneous Ligation pre-mix.

2.2.3 Enzymatic Fragmentation, End Repair and A-Tailing

Materials

- Diluted double stranded FFPE DNA in 26 µl
- Fragmentation pre-mix
- RNase/DNase-free 0.2 ml 8-tube strips

Preparation

1. Program the thermal cycler for Fragmentation with the following settings:

	TEMPERATURE (°C)	TIME (MINUTES)
Lid	75	-
Step 1	4	1
Step 2	37	20
Step 3	65	30
Hold	4	∞

2. Start the Fragmentation program. When the block reaches Step 1 (4 °C), pause the program.

Procedure

Always keep the samples and pre-mix on ice before and after the incubation to block the enzymatic reaction.

1. Depending on the number of samples, proceed as follows:
    - If processing **4 samples**, proceed to step 2.
    - If processing **8 or more samples**, to facilitate pipetting, create a reservoir of Fragmentation pre-mix by adding the following volumes to a new set of 4 or 8-tube strips according to the following scheme:

NUMBER OF REACTIONS	8	12	16	24	32	48
PCR strip (1 strip)	**4-tube**	**4-tube**	**8-tube**	**8-tube**	**8-tube**	**8-tube**
Fragmentation pre-mix (µl)	20.5	31	20.5	31	41	62

2. Assemble the reaction as follows:

- Using a multichannel pipette, if processing 8 or more samples, add 9 µl of Fragmentation pre-mix to each of the 26 µl of DNA samples (total of 35 µl in 4 or 8-tube strips).
- Using a multichannel pipette set to 20 µl, mix thoroughly by pipetting up and down 5 times and briefly spin in a microcentrifuge.

3. Place in the thermal cycler and continue the Fragmentation program.

Proceed immediately to Ligation.

2.2.4 Ligation

Materials

- Fragmentation reaction products in 35 µl each
- Ligation pre-mix
- SU Adapters
- RNase/DNase-free 0.2 ml 8-tube strips

Preparation

1. During the Fragmentation, prepare new PCR strips with 5 µl of SU Adapter per tube according to the following scheme:

NUMBER OF REACTIONS	4	8	12	16	24	32	48
PCR strip (1 strip)	**4-tube**	**4-tube**	**4-tube**	**8-tube**	**8-tube**	**8-tube**	**8-tube**
Number of strips	1	2	3	2	3	4	6

2. Set up the thermal cycler at 20 °C (open lid).

Procedure

Always keep the samples and pre-mix on ice before and after the incubation to block the enzymatic reaction.

1. Depending on the number of samples, proceed as follows:
   - If processing **4 samples**, proceed to step 2.
   - If processing **8 or more samples**, to facilitate pipetting, create a reservoir of Ligation pre-mix by adding the following volumes to a new set of 4 or 8-tube strips according to the following scheme:

NUMBER OF REACTIONS	8	12	16	24	32	48
PCR strip (1 strip)	**4-tube**	**4-tube**	**8-tube**	**8-tube**	**8-tube**	**8-tube**
Ligation pre-mix (µl)	70	105	70	105	140	210

2. Using a multichannel pipette, transfer the 35 µl of each Fragmentation reaction product to the 4 or 8-tube strips containing 5 µl of SU Adapters.
3. Mix thoroughly by pipetting up and down 10 times and spin briefly.
4. Using a multichannel pipette, add 31 µl Ligation pre-mix to each Fragmentation reaction product (40 µl in each tube of the 4 or 8-tube strip).
5. Mix thoroughly by pipetting up and down 10 times and spin briefly.
6. Incubate in the thermal cycler at 20 °C for 15 minutes (open lid).

Proceed to Post-Ligation Clean Up.

Do not place the strip(s) on ice at the end of the ligation as it might decrease the binding of the DNA to the beads.

2.2.5 Post-Ligation Clean Up

Materials

- Ligation reaction products in 71 µl each
- AMPure® XP beads equilibrated at room temperature
- Freshly prepared ethanol 80%
- Nuclease-free water
- IDTE
- RNase/DNase-free 0.2 ml 8-tube strips

Procedure

1. Using a multichannel pipette, add 29 µl of nuclease-free water to each of the 71 µl ligation reaction products. Mix thoroughly by pipetting up and down 10 times.
2. Using a multichannel pipette, add 80 µl of AMPure® XP beads to each of the 100 µl ligation reaction products. Mix thoroughly by pipetting up and down 10 times.
3. Incubate at room temperature for 5 minutes and spin briefly if required.
4. Place the 4 or 8-tube strip on a 96-well plate format magnetic rack for 5 minutes or until the liquid becomes clear.
5. Carefully discard 170 µl of supernatant using a multichannel pipette.

   Keep the tubes on the magnetic rack for the following steps.
6. Using a multichannel pipette, add 170 µl of 80% ethanol to the beads. Incubate for 30 seconds to 1 minute.
7. Carefully discard the ethanol using a multichannel pipette.
8. Repeat steps 6 and 7 once.
9. Remove the residual ethanol using a P10 or P20 multichannel pipette.

10. Air-dry the beads at room temperature for 5 minutes. Do not over-dry the beads because this could decrease the amount of recovered DNA.

    Remove the tubes from the magnetic rack.

11. Using a multichannel pipette, add 20 µl of IDTE to the beads. Mix thoroughly by pipetting up and down 10 times and spin briefly.

Proceed to Library Amplification.

2.2.6 Library Amplification

Materials

- Ligation reaction products and beads resuspended in 20 µl IDTE each
- PCR Master Mix 2x
- Depending on the kit format: 16, 32, 48 or 96 Unique Dual Index Primer Plate for Illumina®

Preparation

1. Program the thermal cycler for Library Amplification with the following settings:

	TEMPERATURE (°C)	TIME (SECONDS)	
Lid	99	-	n* cycles
Step 1: Initial Denaturation	98	120	
Step 2: Denaturation	98	20	
Step 3: Annealing	60	30	
Step 4: Extension	72	30	
Step 5: Final Extension	72	60	
Hold	10	∞	

* Follow the table in section *2.2.1 Input Material Preparation – Input Recommendations* to determine the number of PCR cycles based on the amount of starting material.

Procedure

1. Depending on the number of samples, proceed as follows:
    - If processing **4 samples**, proceed to step 2.
    - If processing **8 or more samples**, to facilitate pipetting, create a reservoir of PCR Master Mix 2x by adding the following volumes to a new set of 4 or 8-tube strips according to the following scheme:

NUMBER OF REACTIONS	8	12	16	24	32	48
PCR strip (1 strip)	**4-tube**	**4-tube**	**8-tube**	**8-tube**	**8-tube**	**8-tube**
PCR Master Mix 2x (µl)	60	85	60	85	120	170

2. Assemble the reaction as follows:
    - Using a multichannel pipette, add 5 µl of different Unique Dual Index Primer per tube to the ligation products and beads, according to your indexing strategy.
    - Mix thoroughly by pipetting up and down 10 times and spin briefly.
    - Using a multichannel pipette, add 25 µl of PCR Master Mix 2x to the ligation products and beads (50 µl in 4 or 8-tube strips).
    - Mix thoroughly by pipetting up and down 10 times and spin briefly.
3. Place the tubes in the thermal cycler and run the Library Amplification program.

Safe stopping point overnight at 4 °C.

2.2.7 Post-Amplification Clean Up

Materials

- PCR reaction products in 50 µl each
- AMPure® XP beads equilibrated at room temperature
- Freshly prepared ethanol 80%
- Nuclease-free water
- DNA low-binding tubes for the storage of libraries

Procedure

1. Using a multichannel pipette, add 50 µl of AMPure® XP beads to each 50 µl of the PCR product. Mix thoroughly by pipetting up and down 10 times.
2. Incubate at room temperature for 5 minutes and spin briefly if required.
3. Place the 4 or 8-tube strips on a 96-well plate format magnetic rack for 5 minutes or until the liquid becomes clear.
4. Carefully discard 90 µl supernatant using a multichannel pipette.

    Keep the tubes on the magnetic rack for the following steps.
5. Using a multichannel pipette, add 170 µl of 80% ethanol to the beads. Let the tubes stand for 30 seconds to 1 minute.
6. Carefully discard the ethanol using a multichannel pipette.
7. Repeat steps 5 and 6 once.
8. Remove the residual ethanol using a P10 or P20 multichannel pipette.
9. Air-dry the beads at room temperature for 5 minutes. Do not over-dry the beads because this could decrease the amount of recovered DNA.

    Remove the tubes from the magnetic rack.

10. Using a multichannel pipette, add 20 µl of nuclease-free water to the beads. Mix thoroughly by pipetting up and down 10 times. Incubate at room temperature for 5 minutes and spin briefly if required.
11. Place the 4 or 8-tube strip on a 96-well plate format magnetic rack for 5 minutes or until liquid becomes clear.
12. Carefully transfer 18 µl (transferring two times 9 µl is recommended at this step) of the supernatant to a new and labeled library storage tube.

Safe stopping point overnight at 4 °C or -20 °C for longer storage.

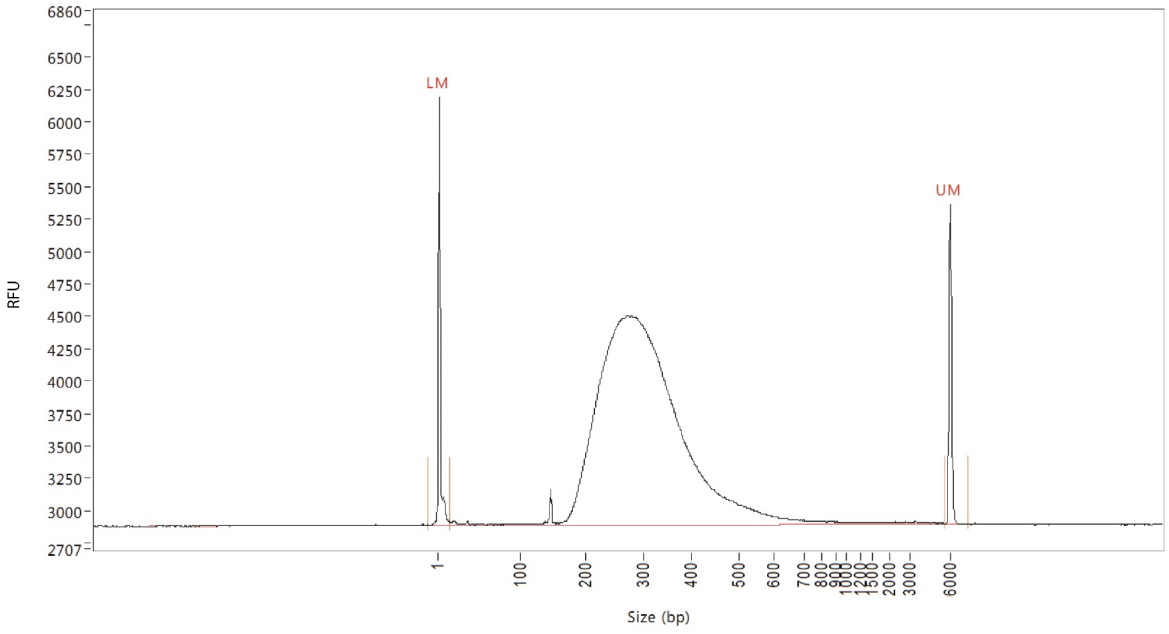
2.2.8 Individual Library Quantification and Quality Control

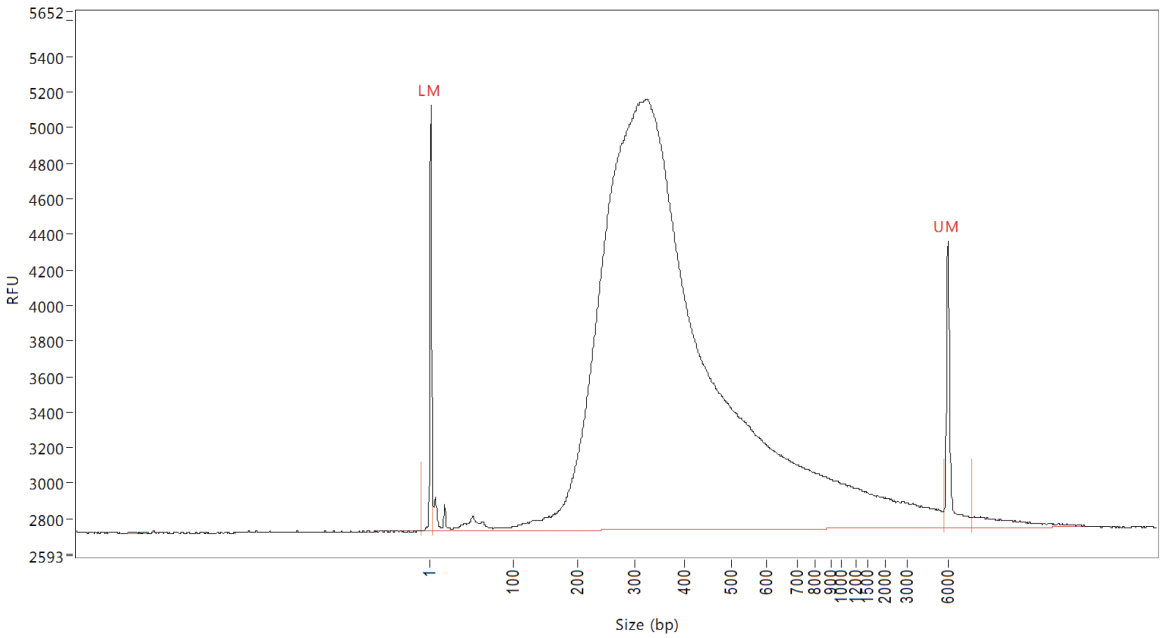
Materials

- Fluorometric quantitation equipment and reagents
- Capillary electrophoresis system
- Nuclease-free water
- RNase/DNase-free 0.2 ml 8-tube strips

Procedure

1. Prepare a 4-time dilution of each library with nuclease-free water (e.g., 2 µl of library in 6 µl nuclease-free water).
2. Quantify the libraries with a fluorometric method (e.g., Qubit HS quantification using 2 µl of the 4x library dilution prepared previously).
3. Quality control the libraries by analyzing their profile via capillary electrophoresis. Library DNA fragments should have a size distribution between 200 bp and 800 bp.





Example of a DNA library distribution obtained with the Agilent Fragment Analyzer™ capillary electrophoresis system. LM: Lower Marker, UM: Upper Marker.

2.3 Library Pooling

2.3.1 Library Pooling for Hybridization and Capture

Materials

- Individual sequencing libraries
- Human Cot DNA
- Universal Blockers – TS Mix
- DNA low-binding 1.5 ml tubes

Procedure

1. Prepare a library Capture pre-mix of the following in a DNA low-binding tube:

CAPTURE PRE-MIX				
Number of captures	**1**	**2**	**3**	**4**
Human Cot DNA (µl)	5	11	16.5	22
Universal Blockers – TS Mix (µl)	2	4.4	6.6	8.8

2. Prepare one DNA low-binding 1.5 ml tube per capture.
3. Pipette 7 µl of the above Capture pre-mix into individual DNA low-binding tubes for each capture.
4. To the individual tubes containing the above Capture pre-mix, add a pool of individual libraries according to the kit format:

KIT FORMAT	16 SAMPLES KIT	32 SAMPLES KIT	48 SAMPLES KIT	96 SAMPLES KIT
Number of individual libraries per capture	4	8	12	12
Amount of each library per capture	300 ng	200 ng	150 ng	150 ng
Total amount of libraries per capture	1200 ng	1600 ng	1800 ng	1800 ng

5. Mix thoroughly by pipetting up and down 10 times and spin briefly.
6. Dry each mix using a vacuum DNA concentrator until mix is completely lyophilized. Use mild heating (45–50 °C) to speed up the lyophilization.

Safe stopping point overnight at -20 °C.

Proceed to Hybridization.

2.4 Capture

2.4.1 Hybridization

Materials

- Lyophilized libraries
- 2x Hybridization Buffer
- Hybridization Buffer Enhancer
- Solid Tumor Plus Solution Probes
- Nuclease-free water
- RNase/DNase-free 0.2 ml 8-tube strips
- 1.5 ml Tubes
- 10x Wash Buffer I
- 10x Wash Buffer II
- 10x Wash Buffer III
- 10x Stringent Wash Buffer
- 2x Beads Wash Buffer

Preparation

1. Pre-warm the thermal cycler to 95 °C (set lid to 99 °C).
2. After the 10-minute denaturation, switch directly to 65 °C (set lid to 75 °C).

We recommend the use of different thermal cyclers for 95 °C and 65 °C incubations, if available.

Procedure

1. Prepare a Hybridization pre-mix according to the number of capture reactions:

HYBRIDIZATION PRE-MIX				
Number of captures	**1**	**2**	**3**	**4**
2x Hybridization Buffer (µl)	8.5	18.7	28.05	37.4
Hybridization Buffer Enhancer (µl)	3.4	7.48	11.22	14.96
Nuclease-free Water (µl)	1.1	2.42	3.63	4.84

2. Resuspend the lyophilized pellet in 13 µl of the Hybridization pre-mix.
3. Transfer the resuspended pellet to a PCR tube (one tube per capture reaction).
4. Incubate in the thermal cycler at 95 °C for 10 minutes.

Do not let the tube temperature drop below 65 °C from step 4 to 6 as this can lead to incorrect probe annealing.

5. Move the PCR tube from the 95 °C to 65 °C thermal cycler, then add 4 µl of probes to the mix. Using a pipette set to 13 µl, mix thoroughly by pipetting up and down 5 times.
6. Incubate in the thermal cycler at 65 °C for 4 to 16 hours.
7. Prepare the 1x working solutions of different wash buffers in advance as described in the following sections to allow them to reach equilibrium during the hybridization reaction.

Wash Buffer Preparation For 1 Reaction

BUFFER	STOCK BUFFER (µl)	WATER (µl)	FINAL VOLUME 1X (µl)
10x Wash Buffer I	33	297	**330**
10x Wash Buffer II	22	198	**220**
10x Wash Buffer III	22	198	**220**
10x Stringent Wash Buffer	44	396	**440**
2x Bead Wash Buffer	275	275	**550**

Pre-warm 1x Stringent Buffer and an aliquot of 110 µl of 1x Wash Buffer I at 65 °C in a thermoblock or water bath for at least 2 hours. Keep the remaining Wash Buffer I at room temperature.

Wash Buffer Preparation For 2 Reactions

BUFFER	STOCK BUFFER (µl)	WATER (µl)	FINAL VOLUME 1X (µl)
10x Wash Buffer I	66	594	**660**
10x Wash Buffer II	44	396	**440**

BUFFER	STOCK BUFFER (µl)	WATER (µl)	FINAL VOLUME 1X (µl)
10x Wash Buffer III	44	396	**440**
10x Stringent Wash Buffer	88	792	**880**
2x Bead Wash Buffer	550	550	**1100**

Pre-warm 1x Stringent Buffer and an aliquot of 220 µl of 1x Wash Buffer I at 65 °C in a thermoblock or water bath for at least 2 hours. Keep the remaining Wash Buffer I at room temperature.

Wash Buffer Preparation For 3 Reactions

BUFFER	STOCK BUFFER (µl)	WATER (µl)	FINAL VOLUME 1X (µl)
10x Wash Buffer I	99	891	**990**
10x Wash Buffer II	66	594	**660**
10x Wash Buffer III	66	594	**660**
10x Stringent Wash Buffer	132	1188	**1320**
2x Bead Wash Buffer	825	825	**1650**

Pre-warm 1x Stringent Buffer and an aliquot of 330 µl of 1x Wash Buffer I at 65 °C in a thermoblock or water bath for at least 2 hours. Keep the remaining Wash Buffer I at room temperature.

Wash Buffer Preparation For 4 Reactions

BUFFER	STOCK BUFFER (µl)	WATER (µl)	FINAL VOLUME 1X (µl)
10x Wash Buffer I	132	1188	**1320**
10x Wash Buffer II	88	792	**880**
10x Wash Buffer III	88	792	**880**
10x Stringent Wash Buffer	176	1584	**1760**
2x Bead Wash Buffer	1100	1100	**2200**

Pre-warm 1x Stringent Buffer and an aliquot of 440 µl of 1x Wash Buffer I at 65 °C in a thermoblock or water bath for at least 2 hours. Keep the remaining Wash Buffer I at room temperature.

2.4.2 Streptavidin Beads Preparation

Materials

- Streptavidin beads equilibrated at room temperature
- 1x Bead Wash Buffer

- 1.5 ml tubes
- RNase/DNase-free 0.2 ml 8-tube strips

Procedure

Perform these steps just before the end of the hybridization incubation.

1. Mix the beads by vortexing them for 15 seconds.
2. Transfer 100 µl of beads per capture (200 µl for 2 reactions, 300 µl for 3 reactions, 400 µl for 4 reactions) to a single 1.5 ml tube.
3. Place the tube on a magnetic rack and let it stand until the solution becomes clear. Carefully remove and discard the supernatant.
4. Add 200 µl of 1x Bead Wash Buffer per capture (400 µl for 2 reactions, 600 µl for 3 reactions, 800 µl for 4 reactions) to the tube. Vortex for 10 seconds.
5. Place the tube on a magnetic rack and let it stand until the solution becomes clear. Carefully remove and discard the supernatant.
6. Repeat steps 4 and 5 once.
7. Add 100 µl of 1x Bead Wash Buffer per capture (200 µl for 2 reactions, 300 µl for 3 reactions, 400 µl for 4 reactions) to the tube. Vortex for 10 seconds.
8. Transfer 100 µl of cleaned beads to a new PCR tube (one tube per capture reaction).
9. Place tube(s) on a 96-well plate format magnetic rack and let it/them stand until the solution becomes clear. Carefully remove and discard the supernatant.

Do not allow the beads to dry.

Proceed immediately to Binding of Hybridized Targets to the Beads.

2.4.3 Binding of Hybridized Targets to the Beads

Materials

- Cleaned Streptavidin beads in PCR tube(s)
- Hybridization reaction(s)

Procedure

Work quickly to ensure that the temperature of the sample(s) remains close to 65 °C.

1. Remove the hybridization reaction(s) from the thermal cycler and briefly spin down the tube(s) and place them back on the thermocycler.

2. Place the washed Streptavidin bead tubes in the thermocycler (no more than two tubes at a time to avoid drying of beads).
3. For each hybridization reaction, transfer 17 µl of the hybridization reaction solution to one PCR tube containing cleaned beads. Resuspend the beads by pipetting up and down until the solution is homogeneous.
4. Bind the DNA to the beads by placing the tube(s) into a thermal cycler set at 65 °C (lid at 75 °C). Incubate for 45 minutes.
5. During the incubation, gently pipette up and down the tube(s) every 15 minutes to ensure that the beads remain in suspension.

Proceed immediately to Wash Streptavidin Beads to Remove Unbound DNA.

2.4.4 Wash Streptavidin Beads to Remove Unbound DNA

Materials

- Hybridized targets on beads
- RNase/DNase-free 0.2 ml 8-tube strips
- DNA low-binding 1.5 ml tubes
- 1x Wash Buffer I (⅓ at 65 °C and ⅔ at room temperature)
- 1x Wash Buffer II
- 1x Wash Buffer III
- 1x Stringent Wash Buffer (at 65 °C)
- Nuclease-free water
- IDTE

Procedure

Ensure that the temperature remains close to 65 °C for steps 1 to 7.

Note: If working with 2 or more capture tubes, work in a staggered manner from steps 2 to step 8, including the following:

1. When placing the first tube in thermoblock at 65 °C for the first incubation of 5 min (step 5), start a timer.
2. Begin processing the second tube.
3. When placing the second tube at 65 °C, note the time separating the tubes and ensure to respect this time gap along step 2 to step 8 to ensure each tube incubates exactly 5 min at 65 °C with the stringent wash.

1. Add 100 µl of 1x Wash Buffer I (at 65 °C) to each of the hybridized target/streptavidin beads tubes.
2. Working with one tube at a time, resuspend and transfer the mix one by one to a new DNA low-binding 1.5 ml tube. If working with two or more capture tubes, work in a staggered manner as indicated above.
3. Place tube on a magnetic rack and let it stand until the solution becomes clear. Carefully remove and discard the supernatant.
4. Add 200 µl of 1x Stringent Wash Buffer (at 65 °C) to the tube. Gently resuspend the beads by pipetting up and down.

Strong mixing of beads with the stringent wash buffer could decrease the quality of the capture.

5. Incubate at 65 °C for 5 minutes.
6. Place the tube on a magnetic rack and let it stand until the solution becomes clear. Carefully remove and discard the supernatant.
7. Repeat steps 4 to 6 once.

   Work at room temperature.

8. Add 200 µl of 1x Wash Buffer I (at room temperature) to your tube. Gently resuspend the beads by pipetting up and down.

   If working with 2 or more capture tubes; from this step on, process all the tubes at the same time.

9. Vortex for 2 minutes.
10. Place tube(s) on a magnetic rack and let it/them stand until the solution becomes clear. Carefully remove and discard the supernatant.
11. Add 200 µl of 1x Wash Buffer II to each tube(s). Vortex for 1 minute.
12. Place tube(s) on a magnetic rack and let it/them stand until the solution becomes clear. Carefully remove and discard the supernatant.
13. Add 200 µl of 1x Wash Buffer III to each tube(s). Vortex for 30 seconds. Spin briefly to collect all the liquid.
14. Place tube(s) on a magnetic rack and let them stand until the solution becomes clear. Carefully remove and discard the supernatant.
15. Add 200 µl of 1x IDTE to each tube(s). Resuspend the beads. Spin briefly to collect all the liquid.
16. Place tube(s) on a magnetic rack and let it/them stand until the solution becomes clear. Carefully remove and discard the supernatant.
17. Remove all the remaining liquid by using a P10 or P20 pipette.
18. Add 20 µl of nuclease-free water to each tube(s), resuspend and transfer the beads/water mix to a new PCR tube.

2.4.5 Post-Capture Amplification

Materials

- Streptavidin beads/nuclease-free water suspension (20 µl)
- Post-Capture PCR Master Mix 2x

- Post-Capture Illumina® Primers Mix
- Post-Capture PCR Enhancer
- Nuclease-free water

Preparation

1. Program the thermal cycler for Post-Capture Amplification using the following settings:

	TEMPERATURE (°C)	TIME (SECONDS)	
Lid	99	-	15 cycles
Step 1: Initial Denaturation	98	120	
Step 2: Denaturation	98	20	
Step 3: Annealing	60	30	
Step 4: Extension	72	30	
Step 5: Final Extension	72	60	
Hold	10	∞	

Procedure

1. Prepare the PCR pre-mix as follows:

PCR PRE-MIX				
Number of Reaction(s)	**1**	**2**	**3**	**4**
Post-Capture PCR Master Mix 2x (µl)	25	55	82.5	110
Post-Capture Illumina® Primers Mix (µl)	2.5	5.5	8.25	11
Post-Capture PCR Enhancer (µl)	2.5	5.5	8.25	11

2. Add 30 µl of PCR pre-mix to each bead suspension. Mix thoroughly by pipetting up and down 10 times and spin briefly.
3. Place the tube(s) in the thermal cycler and run the Post-Capture Amplification program.

Safe stopping point overnight at 4 °C or -20 °C for longer storage.

2.4.6 Post-Capture Amplification Clean Up

Materials

- PCR reaction products in 50 µl each
- AMPure® XP beads equilibrated at room temperature

- Freshly prepared ethanol 80%
- IDTE
- DNA low-binding tubes for library storage

Procedure

1. Add 50 µl of AMPure® XP beads to each of the 50 µl PCR reaction products. Mix thoroughly by pipetting up and down 10 times.
2. Incubate at room temperature for 5 minutes and spin briefly if required to remove the liquid from the tube walls.
3. Place tube(s) on a magnetic rack for 5 minutes or until the liquid becomes clear.
4. Carefully discard 90 µl supernatant using a multichannel pipette.

   Keep the tube(s) on the magnetic rack for the following steps.
5. Using a multichannel pipette, add 170 µl of 80% ethanol to the beads. Let the tube(s) stand for 30 seconds to 1 minute.
6. Carefully discard the ethanol.
7. Repeat steps 5 and 6 once.
8. Remove the residual ethanol using a P10 or P20 pipette.
9. Air-dry the beads at room temperature for 5 minutes. Do not over-dry the beads because this could decrease the amount of recovered DNA.

   Remove the tube(s) from the magnetic rack.
10. Add 20 µl of IDTE to the beads. Mix thoroughly by pipetting up and down 10 times. Incubate at room temperature for 5 minutes and spin briefly if required to remove the liquid from the tube walls.
11. Place tube(s) on a magnetic rack for 5 minutes or until liquid becomes clear.
12. Carefully transfer 18 µl of the supernatant (transferring two times 9 µl is recommended at this step) to a new, labeled library storage tube.

Safe stopping point overnight at 4 °C or -20 °C for longer storage.

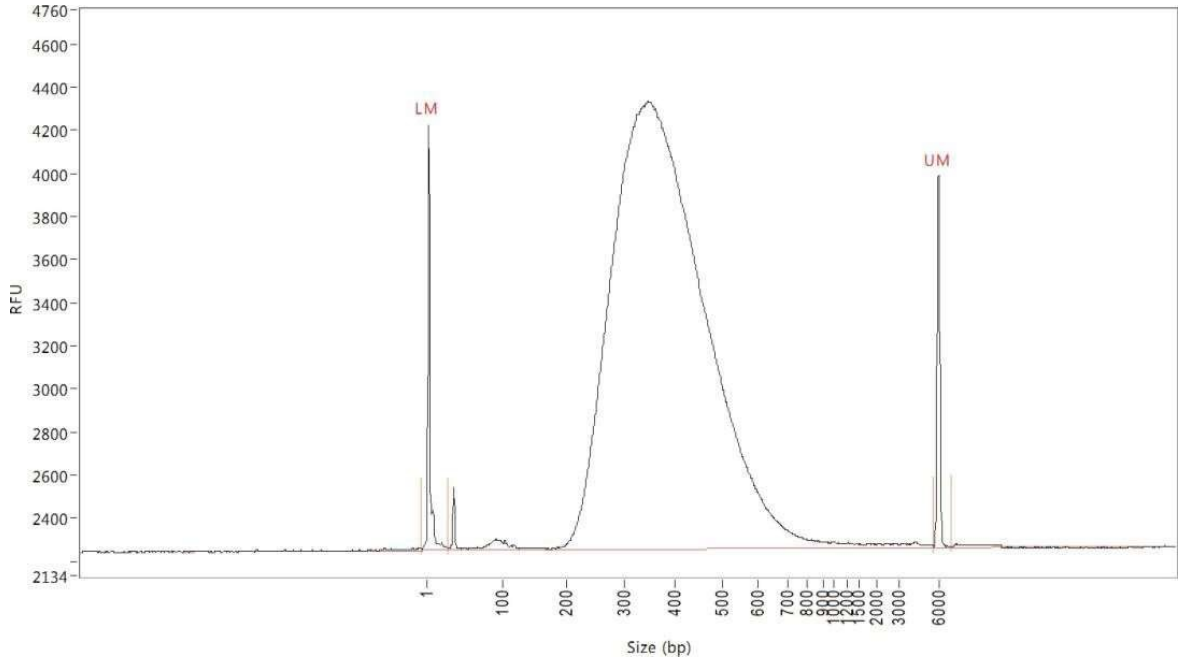
2.4.7 Final Library Quantification and Quality Control

Materials

- Fluorometric quantitation equipment and reagents
- Capillary electrophoresis system

Procedure

1. Quantify each captured library pool with a fluorometric method (e.g., Qubit® HS quantification using 2 µl of the library).
2. Control the quality of the captured pools of libraries by analyzing their profile via capillary electrophoresis. Library DNA fragments should have a size distribution between 200 bp and 800 bp.



Example of post-capture library pool size distribution obtained with the Agilent Fragment Analyzer™ capillary electrophoresis system. LM: Lower Marker, UM: Upper Marker.

2.5 Sequencing

2.5.1 Sequencing Preparations

Materials

- Illumina® NextSeq® 500/550 Sequencing Kit
- Final captured libraries
- EBT Buffer or similar

Procedure

1. Determine the molarity of each captured pool with the average size of the library (peak size in base pairs) and concentration (ng/µl) obtained during step *2.4.7 Final Library Quantification and Quality Control* as follows:

$$\text{Library molarity (nM)} = \frac{\text{Library concentration (ng/µl)}}{\text{Average size in base pairs} \times 649.5} \times 10^6$$

2. Dilute each captured pool to 4 nM.
3. If processing multiple sequencing pools, mix them in equal amounts (e.g., 5 µl of each) and use this dilution according to Illumina® standard denaturation recommendations.
4. For loading dilution, see the table below:

SEQUENCING INSTRUMENT	LOADING DILUTION
NextSeq® 500/550 (Mid-Output)	1.3 pM
NextSeq® 500/550 (High-Output)	1.4 pM [Adjust the dilution (1.1 pM to 1.5 pM range) according to the number of clusters obtained in the first run]

The loading dilutions below are the guidelines recommended by the manufacturers. Adjust them according to the number of clusters you observe for your individual machine. Sample multiplexing must be adjusted depending on the instrument used. Please note that the following sequencers are not covered by this particular pipeline and would require testing via an additional setup program:

- For MiSeq® and HiSeq®, load a 10 pM dilution.
- For MiniSeq™, load a 1.8 pM dilution.

5. For recommended reads per sample, see the table below:

READ LENGTH* (BP)	RECOMMENDED TOTAL READS PER SAMPLE
150	2.1 million

* For FFPE samples, sequencing reads longer than 150 bp are not recommended due to DNA degradation in these samples.

3 APPENDICES

3.1 Appendix I: Unique Dual Index Primer Plates

3.1.1 16 Illumina®-compatible Unique Dual Index Primers In 96-well Plate Format (7 µl Each)

	1	2	3	4	5	6	7	...	12
A	sgUDI-385	sgUDI-393							
B	sgUDI-386	sgUDI-394							
C	sgUDI-387	sgUDI-395							
D	sgUDI-388	sgUDI-396							
E	sgUDI-389	sgUDI-397							
F	sgUDI-390	sgUDI-398							
G	sgUDI-391	sgUDI-399							
H	sgUDI-392	sgUDI-400							

3.1.2 32 Illumina®-compatible Unique Dual Index Primers In 96-well Plate Format (7 µl Each)

	1	2	3	4	5	6	7	...	12
A	sgUDI-49	sgUDI-57	sgUDI-65	sgUDI-73					
B	sgUDI-50	sgUDI-58	sgUDI-66	sgUDI-74					
C	sgUDI-51	sgUDI-59	sgUDI-67	sgUDI-75					
D	sgUDI-52	sgUDI-60	sgUDI-68	sgUDI-76					
E	sgUDI-53	sgUDI-61	sgUDI-69	sgUDI-77					
F	sgUDI-54	sgUDI-62	sgUDI-70	sgUDI-78					
G	sgUDI-55	sgUDI-63	sgUDI-71	sgUDI-79					
H	sgUDI-56	sgUDI-64	sgUDI-72	sgUDI-80					

3.1.3 48 Illumina®-compatible Unique Dual Index Primers In 96-well Plate Format (7 µl Each)

	1	2	3	4	5	6	7	...	12
A	sgUDI-1	sgUDI-9	sgUDI-17	sgUDI-25	sgUDI-33	sgUDI-41			
B	sgUDI-2	sgUDI-10	sgUDI-18	sgUDI-26	sgUDI-34	sgUDI-42			
C	sgUDI-3	sgUDI-11	sgUDI-19	sgUDI-27	sgUDI-35	sgUDI-43			
D	sgUDI-4	sgUDI-12	sgUDI-20	sgUDI-28	sgUDI-36	sgUDI-44			
E	sgUDI-5	sgUDI-13	sgUDI-21	sgUDI-29	sgUDI-37	sgUDI-45			
F	sgUDI-6	sgUDI-14	sgUDI-22	sgUDI-30	sgUDI-38	sgUDI-46			
G	sgUDI-7	sgUDI-15	sgUDI-23	sgUDI-31	sgUDI-39	sgUDI-47			
H	sgUDI-8	sgUDI-16	sgUDI-24	sgUDI-32	sgUDI-40	sgUDI-48			

3.1.4 96 Illumina®-compatible Unique Dual Index Primers In 96-well Plate Format (7 µl Each) - Plate B (IPdRUdIpB96)

	1	2	3	4	5	6	7	8	9	10	11	12
A	sgUDI-97	sgUDI-105	sgUDI-113	sgUDI-121	sgUDI-129	sgUDI-137	sgUDI-145	sgUDI-153	sgUDI-161	sgUDI-169	sgUDI-177	sgUDI-185
B	sgUDI-98	sgUDI-106	sgUDI-114	sgUDI-122	sgUDI-130	sgUDI-138	sgUDI-146	sgUDI-154	sgUDI-162	sgUDI-170	sgUDI-178	sgUDI-186
C	sgUDI-99	sgUDI-107	sgUDI-115	sgUDI-123	sgUDI-131	sgUDI-139	sgUDI-147	sgUDI-155	sgUDI-163	sgUDI-171	sgUDI-179	sgUDI-187
D	sgUDI-100	sgUDI-108	sgUDI-116	sgUDI-124	sgUDI-132	sgUDI-140	sgUDI-148	sgUDI-156	sgUDI-164	sgUDI-172	sgUDI-180	sgUDI-188
E	sgUDI-101	sgUDI-109	sgUDI-117	sgUDI-125	sgUDI-133	sgUDI-141	sgUDI-149	sgUDI-157	sgUDI-165	sgUDI-173	sgUDI-181	sgUDI-189
F	sgUDI-102	sgUDI-110	sgUDI-118	sgUDI-126	sgUDI-134	sgUDI-142	sgUDI-150	sgUDI-158	sgUDI-166	sgUDI-174	sgUDI-182	sgUDI-190
G	sgUDI-103	sgUDI-111	sgUDI-119	sgUDI-127	sgUDI-135	sgUDI-143	sgUDI-151	sgUDI-159	sgUDI-167	sgUDI-175	sgUDI-183	sgUDI-191
H	sgUDI-104	sgUDI-112	sgUDI-120	sgUDI-128	sgUDI-136	sgUDI-144	sgUDI-152	sgUDI-160	sgUDI-168	sgUDI-176	sgUDI-184	sgUDI-192

3.1.5 Index Sequences For The Illumina®-compatible Unique Dual Index Primers

The table below lists the index sequences. Please note that when using manual Sample Sheets, the following Illumina® instruments require the **reverse orientation** of the i5 sequence:

- NextSeq® 500/550
- NextSeq® 1000/2000 in Standalone mode (Sample Sheet v1)

- HiSeq® 3000/4000/X
- NovaSeq™ 6000 with v1.5 reagent kits and NovaSeq™ X/X Plus
- MiniSeq™ (except when used with Rapid Reagent Kits)
- iSeq™ 100

All other Illumina® instruments (e.g., MiSeq®) and instrument configurations require the **forward** i5 orientation.

This information is subject to change. Please always refer to the Illumina® support pages for the latest information on which sequencing systems require the i5 index sequence in forward or reverse (complement) orientation:

https://support-docs.illumina.com/SHARE/AdapterSequences/Content/SHARE/AdapterSeq/Overview.htm

INDEX	i5 SEQUENCE (FORWARD ORIENTATION)	i5 SEQUENCE (REVERSE ORIENTATION)	i7 SEQUENCE
sgUDI-1	CGGTTCAA	TTGAACCG	GACTTCCT
sgUDI-2	TTCGTGAA	TTCACGAA	AGTCGATG
sgUDI-3	ATACCTGC	GCAGGTAT	CTGTCCAC
sgUDI-4	TGTAACCG	CGGTTACA	GCGAATTG
sgUDI-5	GCTTCGCT	AGCGAAGC	GGTGCTTA
sgUDI-6	GAACGATC	GATCGTTC	TCATAGGT
sgUDI-7	TGGCACCT	AGGTGCCA	ATGTCGCA
sgUDI-8	GACTGGCT	AGCCAGTC	CTCAAGTG
sgUDI-9	TCGGTTAT	ATAACCGA	TGAATCGT
sgUDI-10	ATGATCAC	GTGATCAT	TCGCCTTA
sgUDI-11	CATGACGA	TCGTCATG	GATGGAAC
sgUDI-12	CCAGCATG	CATGCTGG	ACGGTGGA
sgUDI-13	CGAGCATA	TATGCTCG	TCCTTGCG
sgUDI-14	CACTCGCA	TGCGAGTG	CTGCATGT
sgUDI-15	TCTAGTAC	GTACTAGA	AGCGGCAA
sgUDI-16	GTGCACGT	ACGTGCAC	CAAGTCGA
sgUDI-17	GGCATAGA	TCTATGCC	GCTACATC
sgUDI-18	AATCTTGG	CCAAGATT	TGAAGCAA
sgUDI-19	GATGCAAG	CTTGCATC	GGTGGATA
sgUDI-20	GACTACTG	CAGTAGTC	TTGATGGC
sgUDI-21	CACGTGGA	TCCACGTG	GTGCATCT
sgUDI-22	CCGATGAG	CTCATCGG	CACTCAGC

INDEX	i5 SEQUENCE (FORWARD ORIENTATION)	i5 SEQUENCE (REVERSE ORIENTATION)	i7 SEQUENCE
sgUDI-23	ATGCGTCT	AGACGCAT	AGATCTGG
sgUDI-24	TGATGCTC	GAGCATCA	TCTTGCAA
sgUDI-25	GTTAACGG	CCGTTAAC	CTCATTCA
sgUDI-26	ATACGGTA	TACCGTAT	TTCGAGCT
sgUDI-27	TTGCCATC	GATGGCAA	GCTCATAG
sgUDI-28	AGCCGTAC	GTACGGCT	AGAACCTC
sgUDI-29	CCGGTCTT	AAGACCGG	TTGACCGA
sgUDI-30	GAGTAACA	TGTTACTC	GACTGACA
sgUDI-31	CCAGGTTA	TAACCTGG	CACGTATC
sgUDI-32	CGTTAATG	CATTAACG	TCGAGCGA
sgUDI-33	GTTGGAAG	CTTCCAAC	TGCCACTC
sgUDI-34	AGGCTCGT	ACGAGCCT	AGTGCTGG
sgUDI-35	TACTCGAA	TTCGAGTA	GCATAGCT
sgUDI-36	TTCAGACC	GGTCTGAA	CTAGCGAG
sgUDI-37	TGAGTTCT	AGAACTCA	TACCGACC
sgUDI-38	TCATGATG	CATCATGA	ATGCTTGG
sgUDI-39	GAGGTCGA	TCGACCTC	GATCGGTT
sgUDI-40	CTGCATCG	CGATGCAG	CCGACTCA
sgUDI-41	AGTACAAG	CTTGTACT	GGATACAT
sgUDI-42	TACTAGTC	GACTAGTA	ATGGTAGG
sgUDI-43	GTCGTATG	CATACGAC	ACCATAGC
sgUDI-44	CAAGGTAT	ATACCTTG	TGCGTCCA
sgUDI-45	ACGCAGGA	TCCTGCGT	AGACCTAT
sgUDI-46	TCGTGGCT	AGCCACGA	TCTTGCTG
sgUDI-47	ATTATCGC	GCGATAAT	CATCACTC
sgUDI-48	CGTACCAG	CTGGTACG	GTGAAGTA
sgUDI-49	GCTCACTG	CAGTGAGC	ACCAAGGA
sgUDI-50	CAGGATTG	CAATCCTG	CAGACCTG
sgUDI-51	GTCTAGTT	AACTAGAC	CGAGCAAC
sgUDI-52	TGAATGGC	GCCATTCA	TCTTGACT
sgUDI-53	ACGACAAT	ATTGTCGT	GACAATGG

INDEX	i5 SEQUENCE (FORWARD ORIENTATION)	i5 SEQUENCE (REVERSE ORIENTATION)	i7 SEQUENCE
sgUDI-54	GAACGCCA	TGGCGTTC	GTTCTACG
sgUDI-55	CTGTCCTG	CAGGACAG	AACGCTGC
sgUDI-56	ATAAGGAC	GTCCTTAT	GGACATCA
sgUDI-57	TACATTCC	GGAATGTA	TTGAGCTC
sgUDI-58	GGTTAGCT	AGCTAACC	ACGTTGAG
sgUDI-59	CCTGCTGA	TCAGCAGG	CTTCAGGA
sgUDI-60	CCTCAATC	GATTGAGG	TGCCAACT
sgUDI-61	CGAAGAAT	ATTCTTCG	AGGTCATG
sgUDI-62	TAGTCGAG	CTCGACTA	TACTAGCA
sgUDI-63	ATCCTCAC	GTGAGGAT	GTAACTGT
sgUDI-64	GCTGCAGT	ACTGCAGC	TGAGTTGA
sgUDI-65	GGCAATCG	CGATTGCC	CCTTAGAC
sgUDI-66	GCATCTTA	TAAGATGC	TATCGCCA
sgUDI-67	GTGCTGAA	TTCAGCAC	GCAGAACA
sgUDI-68	ATTGACGC	GCGTCAAT	AGGAATGC
sgUDI-69	GCCGCATT	AATGCGGC	CGTGAGGT
sgUDI-70	TGTAGTCA	TGACTACA	CAATTCAG
sgUDI-71	TCCTCCGA	TCGGAGGA	GGTCCTTC
sgUDI-72	CAATCGAG	CTCGATTG	TTCCGGCA
sgUDI-73	TAAGTCCG	CGGACTTA	ATGCCTGA
sgUDI-74	GGACAGTT	AACTGTCC	TCCAGGAC
sgUDI-75	TTGAGTGA	TCACTCAA	GCTGTCAC
sgUDI-76	CGTAACAT	ATGTTACG	CGACGATT
sgUDI-77	TACGCAGT	ACTGCGTA	TCGCAACG
sgUDI-78	ACCTGACC	GGTCAGGT	GAGTTGTA
sgUDI-79	CCACCTGA	TCAGGTGG	CGCTAAGG
sgUDI-80	TCACCGTG	CACGGTGA	TTGCGTGC
sgUDI-97	GCCAGTAT	ATACTGGC	CCTATGTT
sgUDI-98	ATGATGGA	TCCATCAT	ACCGGTTA
sgUDI-99	CAATCGGC	GCCGATTG	GTAGAATG
sgUDI-100	CGACGAAG	CTTCGTCG	TCAAGCCT

INDEX	i5 SEQUENCE (FORWARD ORIENTATION)	i5 SEQUENCE (REVERSE ORIENTATION)	i7 SEQUENCE
sgUDI-101	TCTGCTTA	TAAGCAGA	GCGTATGT
sgUDI-102	ACTCCATT	AATGGAGT	TGACGTAC
sgUDI-103	GCCATGAT	ATCATGGC	TAGCTGAT
sgUDI-104	TGTCTAGG	CCTAGACA	GGTACATG
sgUDI-105	GTATGCAG	CTGCATAC	ACCGAATT
sgUDI-106	CGTTATCC	GGATAACG	CGGATGGT
sgUDI-107	ACATGCTT	AAGCATGT	CTTGCGAT
sgUDI-108	AGTACGTA	TACGTACT	TGCTTAGA
sgUDI-109	TTGCAGTT	AACTGCAA	AGGTCAGT
sgUDI-110	CCAGACAT	ATGTCTGG	AACCAATG
sgUDI-111	GTGATTAC	GTAATCAC	GACTGCTT
sgUDI-112	TCAAGAAC	GTTCTTGA	TAGTTCGG
sgUDI-113	TCATGAGC	GCTCATGA	TGATTGTC
sgUDI-114	CCGTGGTA	TACCACGG	CTCAAGGC
sgUDI-115	AGTAACGG	CCGTTACT	GTCGTACA
sgUDI-116	TCACCACG	CGTGGTGA	ACTATGCT
sgUDI-117	AACGTCAG	CTGACGTT	CCATGGAG
sgUDI-118	AGATGACG	CGTCATCT	GTATCCAT
sgUDI-119	TACTTACC	GGTAAGTA	CTCACCTG
sgUDI-120	TCTGCAAT	ATTGCAGA	TTAAGACG
sgUDI-121	GTGGAACT	AGTTCCAC	GACATGGC
sgUDI-122	TGGATCAC	GTGATCCA	GCTAGAAG
sgUDI-123	TTGACGAC	GTCGTCAA	CACTCACT
sgUDI-124	CAGTCATA	TATGACTG	CGGATGTA
sgUDI-125	ATCGCATT	AATGCGAT	TGGAACGG
sgUDI-126	AATGCCGT	ACGGCATT	TAGCATTC
sgUDI-127	ATCGTGCC	GGCACGAT	CACTGCAC
sgUDI-128	AGATCCAG	CTGGATCT	GACCTCAG
sgUDI-129	CACTTGTA	TACAAGTG	CCTACGCT
sgUDI-130	GGTTACTG	CAGTAACC	TGACAGAA
sgUDI-131	GTTCAACT	AGTTGAAC	TATCTGAC

INDEX	i5 SEQUENCE (FORWARD ORIENTATION)	i5 SEQUENCE (REVERSE ORIENTATION)	i7 SEQUENCE
sgUDI-132	ATACGGAG	CTCCGTAT	CTGGCAGA
sgUDI-133	CAATGGCG	CGCCATTG	AACCTAGC
sgUDI-134	CCACTGAG	CTCAGTGG	CGTCAAGG
sgUDI-135	TATCGATG	CATCGATA	ATGAACTC
sgUDI-136	ATAGCTGG	CCAGCTAT	AAGATTGC
sgUDI-137	AAGGTTAC	GTAACCTT	CTAGCACC
sgUDI-138	AGCAAGGT	ACCTTGCT	TCTTCGAA
sgUDI-139	GACCAAGC	GCTTGGTC	GTAGTTCA
sgUDI-140	GGCGACTA	TAGTCGCC	GTGCTCAC
sgUDI-141	CCTGGTCA	TGACCAGG	GTCCAGGT
sgUDI-142	CACGTACA	TGTACGTG	GGTCGCAT
sgUDI-143	ACCATTAC	GTAATGGT	CTGTCAGG
sgUDI-144	TAGGTCCT	AGGACCTA	GCGTATCG
sgUDI-145	ACAGTGCT	AGCACTGT	GACAGCCT
sgUDI-146	ATGTCCGC	GCGGACAT	AATACGAG
sgUDI-147	CTGTACAG	CTGTACAG	CAAGACGG
sgUDI-148	TAGCCTCA	TGAGGCTA	CCTGGCTT
sgUDI-149	CACTGCTG	CAGCAGTG	CGGATTGC
sgUDI-150	CTAGGATC	GATCCTAG	TAAGCTTG
sgUDI-151	CAGTATGG	CCATACTG	CGAACGAC
sgUDI-152	CAGGCTGA	TCAGCCTG	CTCGGCTT
sgUDI-153	TCTGAAGT	ACTTCAGA	CACGATAA
sgUDI-154	AGTCTGCA	TGCAGACT	GGATGACT
sgUDI-155	TCACGACT	AGTCGTGA	GAAGACCA
sgUDI-156	AGCAATGA	TCATTGCT	GCAGTTAA
sgUDI-157	CTTAGTTG	CAACTAAG	AGATGAGC
sgUDI-158	AACAGCCG	CGGCTGTT	TCGCCAGT
sgUDI-159	GAGTATGA	TCATACTC	TCTGGTAA
sgUDI-160	ATCAACCG	CGGTTGAT	GTATTGCA
sgUDI-161	CTTGATAC	GTATCAAG	ACTATTCG
sgUDI-162	GTAACGCC	GGCGTTAC	CCTAATTG

INDEX	i5 SEQUENCE (FORWARD ORIENTATION)	i5 SEQUENCE (REVERSE ORIENTATION)	i7 SEQUENCE
sgUDI-163	TGAAGGCT	AGCCTTCA	AGGCGATT
sgUDI-164	TGACCGCA	TGCGGTCA	TGGTATGC
sgUDI-165	GTCTGATC	GATCAGAC	TAACGACA
sgUDI-166	AACGCCGA	TCGGCGTT	GACTTGCG
sgUDI-167	CCAATAGC	GCTATTGG	GTCATCTG
sgUDI-168	ACTGTCAA	TTGACAGT	CGCACTTG
sgUDI-169	TCGCTTGC	GCAAGCGA	GCATACGG
sgUDI-170	ACTCATCA	TGATGAGT	GTCGCCAT
sgUDI-171	CGCTTGCA	TGCAAGCG	CTCGTGAC
sgUDI-172	ATCCTATG	CATAGGAT	CAGGAATA
sgUDI-173	ACGGCTCA	TGAGCCGT	CAGACTGG
sgUDI-174	TCACTGTC	GACAGTGA	GCCGATCA
sgUDI-175	TCAACGGT	ACCGTTGA	TCACCTAC
sgUDI-176	TTGCACTA	TAGTGCAA	ACCACGTT
sgUDI-177	ACAAGGAT	ATCCTTGT	CCAAGTTG
sgUDI-178	CATGGACA	TGTCCATG	CTGCTAAC
sgUDI-179	GAGGATGC	GCATCCTC	GTTCCGGT
sgUDI-180	GTGGATTA	TAATCCAC	TCATGGAT
sgUDI-181	TTGCCGAA	TTCGGCAA	ATGCAGTA
sgUDI-182	GTGAGTCC	GGACTCAC	TTCAGTTG
sgUDI-183	GGACTAAG	CTTAGTCC	AGTGCACC
sgUDI-184	GATACCGG	CCGGTATC	GCTGGCTA
sgUDI-185	CCGTACAA	TTGTACGG	TCTAAGAC
sgUDI-186	AGATTCGG	CCGAATCT	TTGGAGCG
sgUDI-187	GGAAGTAT	ATACTTCC	TGGTTGAA
sgUDI-188	TACGCTGG	CCAGCGTA	TGGCAATG
sgUDI-189	CGTAGTAT	ATACTACG	GGTAATAG
sgUDI-190	TATCCAGG	CCTGGATA	ACTTGATC
sgUDI-191	CGAATCGA	TCGATTCG	CAGGTTGC
sgUDI-192	ATAGCCTT	AAGGCTAT	TTCGGTTA
sgUDI-385	GGCGAGTT	AACTCGCC	AGGCAGCA

INDEX	i5 SEQUENCE (FORWARD ORIENTATION)	i5 SEQUENCE (REVERSE ORIENTATION)	i7 SEQUENCE
sgUDI-386	AATCGTCA	TGACGATT	GACTTCGG
sgUDI-387	TCGTTCAG	CTGAACGA	TCAGCTAT
sgUDI-388	CTAACAGC	GCTGTTAG	CTTAGATC
sgUDI-389	GTCGGCTT	AAGCCGAC	CCATTAAC
sgUDI-390	TAATCAGC	GCTGATTA	TGGACTGG
sgUDI-391	CGGCATAA	TTATGCCG	GTTGGCTA
sgUDI-392	ACTATGCG	CGCATAGT	AACCAGCT
sgUDI-393	TCGAGCTA	TAGCTCGA	GTGACGAG
sgUDI-394	CGCTATGT	ACATAGCG	TACTGTTC
sgUDI-395	ATACTGAC	GTCAGTAT	ACAGACGA
sgUDI-396	GATGCACG	CGTGCATC	CGTCTACT
sgUDI-397	AGCCGCAT	ATGCGGCT	TTCCTAGA
sgUDI-398	TAGGATCG	CGATCCTA	CGGAATCT
sgUDI-399	CTATTGTC	GACAATAG	ACATGGTC
sgUDI-400	GCTACAGA	TCTGTAGC	GATGCCAG

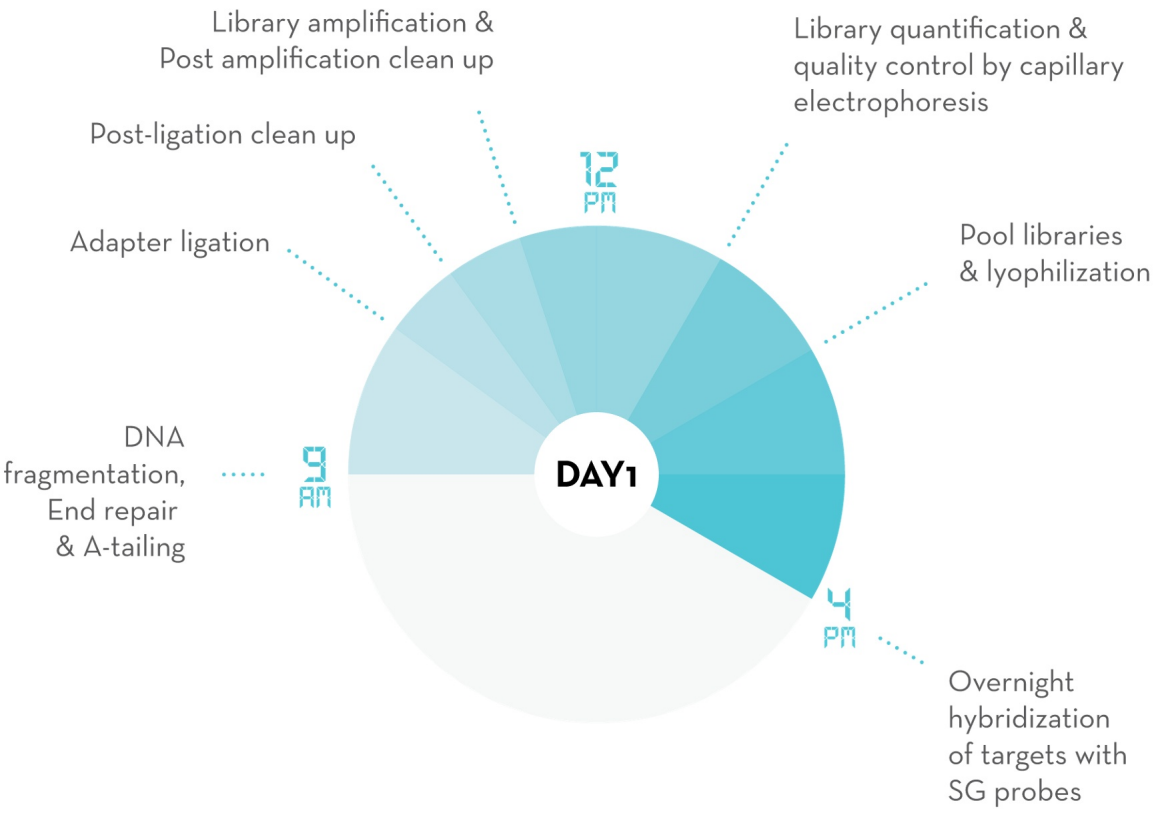
3.2 Appendix II: Laboratory Equipment Used in SOPHiA GENETICS Laboratory

USER-SUPPLIED MATERIALS	SUPPLIER	PRODUCT NAME	CATALOG N°
RNase/DNase-free 8-tube strips (0.2 ml)	Thermo Fisher Scientific	EasyStrip Snap Tubes	AB-2000
DNA low binding tubes (1.5 ml)	Axygen	MaxyClear Microcentrifuges Tubes	MCT-175-C
Tubes (1.5 ml)	Eppendorf	Eppendorf Tubes	3810X
Conical tubes (15 ml and 50 ml)	Falcon	15 ml & 50 ml Conical Centrifuge Tubes	352096 & 352070
Filter tips	Starlab	TipOne RPT	S1180-3710, S1183-1740, S1180-8710, S1180-9710, S1182-1730
Ethanol (molecular biology grade)	Merck	Ethanol Absolute	1.00983.1000
(For RNA part) Nuclease cleaning product	Invitrogen	RNaseZap™	AM9780

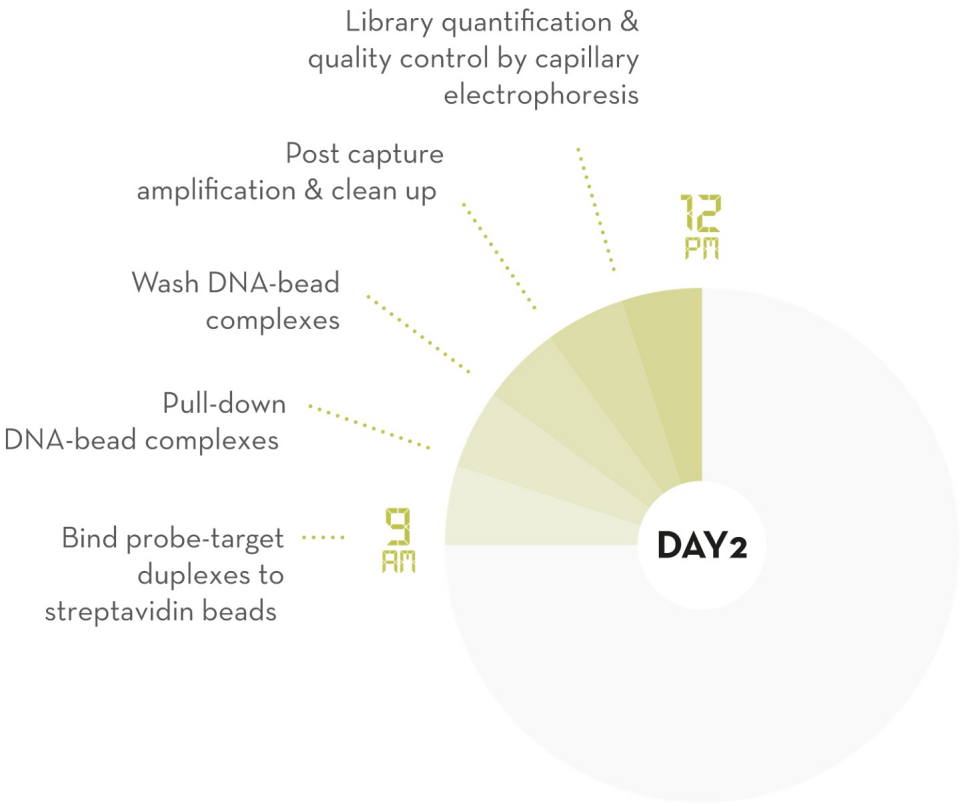
PRE-PCR ZONE	SUPPLIER	PRODUCT NAME	CATALOG N°
Vortex mixer	Scientific Industries	Vortex Genie 2	SI-0236
Table top microcentrifuge (8-tube strips compatible)	Starlab	Mini Centrifuge	N2631-0007
Magnetic separation rack 96-well type	Alpaqua	96S Super Magnet Plate	A001322
Magnetic separation rack 96-well type	Thermo Fisher Scientific	DynaMag-96 Side Magnet	12331D
Multichannel pipettes (P10; P100; P300)	StarLab	ErgoOne	S7108-0510, S7108-1100, S7108-3300
Thermal cycler with programmable heated lid	Biometra	TAdvanced 96	
Fluorometric quantitation equipment and reagents	Thermo Fisher Scientific	Qubit 3.0 Fluorometer & Qubit dsDNA HS Assay kit	Q33216 & Q32854
Single channel pipettes (P10; P100; P200; P1000)	StarLab	ErgoOne	S7100-0510, S7100-1100, S7100-2200, S7100-1000

POST-PCR ZONE	SUPPLIER	PRODUCT NAME	CATALOG N°
Thermal cycler with programmable heated lid	Biometra	TAdvanced 96	
Capillary electrophoresis system	Advanced Analytical	Agilent Fragment Analyzer	
Vacuum concentrator (SpeedVacTM or similar)	Thermo Fisher Scientific	Savant DNA120-230	
Dry block heater or water bath(1.5 ml tube compatible)	Techne	Dri-Block DB-1	
Magnetic separation rack (1.5 ml tube compatible)	Thermo Fisher Scientific	MagJET Separation Rack, 12 x 1.5 mL tube	MR02
Magnetic separation rack (96-well type)	Alpaqua	96S Super Magnet Plate	A001322
Magnetic separation rack 96-well type	Thermo Fisher Scientific	DynaMag-96 Side Magnet	12331D
Vortex mixer	Grant instrument	Multi-tube Vortex Mixer, V32	
Vortex mixer	Scientific Industries	Vortex Genie 2	SI-0236
Table top microcentrifuge (8- tube strips compatible)	StarLab	Mini Centrifuge	N2631-0007
Multichannel pipettes (P10; P100; P300)	StarLab	ErgoOne	S7108-0510, S7108- 1100, S7108-3300
Fluorometric quantitation equipment and reagent	Thermo Fisher Scientific	Qubit 3.0 Fluorometer & Qubit dsDNA HS Assay kit	Q33216 & Q32854
Single channel pipettes (P10; P100; P200; P1000)	StarLab	ErgoOne	S7100-0510, S7100- 1100, S7100-2200, S7100-1000

3.3 Appendix III: General Workflow SOPHiA DDM™ Capture Solution

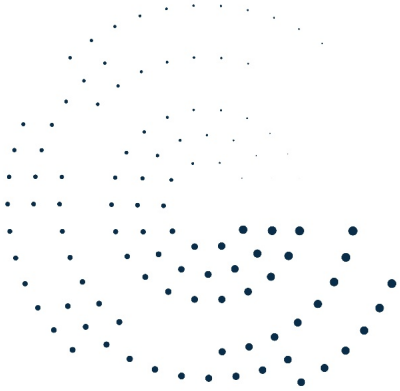


Library Preparation with SOPHiA GENETICS™ DNA Library Prep Kit II



CAPTURE
EASY WORKFLOW
- ONLY 1-2 TUBES TO HANDLE (MULTIPLEX POOLED LIBRARIES)
- ONLY 3 HOURS HANDS-ON TIME

RNA PART



SOPHiA GENETICS™

4 PROTOCOL

4.1 Materials

4.1.1 Initial Considerations

Upon receipt, please ensure that all tubes are physically intact and stored at recommended temperatures for optimum kit performance. Inappropriate handling and storage of the kit components under other conditions may adversely affect the kit's performance.

4.1.2 Kit Content (16, 32 And 48 Samples)

Always briefly spin the tubes before use to collect all liquid.

The SOPHiA DDM™ STSplus kit exists in three formats: 16, 32 and 48 reactions. The Dual Index Primer plates for RNA libraries always comes as one plate with 48 unique dual indices and it is the same for all formats.

Box 3 (Store At -25 °C To -15 °C)

COMPONENT	NUMBER OF ITEMS DEPENDING ON KIT FORMAT	
	16 SAMPLE KIT	48 SAMPLE KIT
First Strand Synthesis Reaction Buffer (µl)	77	231
Random Primers (µl)	20	60
Reverse Transcriptase (µl)	20	60
RNase Inhibitor (µl)	10	30
DNA Ligase (µl)	16	48
DNA Ligation Buffer (µl)	77	231
Polymerase Master Mix 2x (µl)	780	2340
Control Primers (µl)	120	360
Hybridization Buffer (µl)	30	90
SOPHiA DDM™ STSplus Oligos (µl)	30	90
Illumina®-compatible Dual Index Primers (in 96-well plate format, 12 µl each). See *Appendix VI* for primer display and sequences	48	48

Note: For the 32 reaction kits, two 'Box 3' of 16 reactions are provided.

Some reagents required for SOPHiA DDM™ STSplus, such as Agencourt® AMPure® XP, IDTE Buffer and Nuclease-free water can be found in Box 2.

4.1.3 Materials Required (Not Provided)

User-Supplied Materials (To Be Purchased Separately)

Lab-related plasticware

- 50 ml conical tubes
- Filter tips
- 1.5 ml tubes
- RNase/DNase-free 0.2 ml 8-tube strips

Reagents

- Ethanol (molecular biology grade)
- Nuclease-free water

Other

- Ice to ensure reagents and pre-mixes are kept at 4 °C
- Illumina® sequencing reagents

Laboratory Equipment

To avoid sample contamination, separate the workspace into:

- *Pre-PCR zone*
    - Fluorometric quantitation equipment and reagents
    - Magnetic separation rack (96-well type)
    - Multichannel pipettes (P10 or P20; P100; P200)
    - Tabletop microcentrifuge (8-tube strips compatible)
    - Thermal cycler (programmable heated lid)
    - Vortex mixer
- *Post-PCR zone*
    - Capillary electrophoresis system
    - Fluorometric quantitation equipment and reagents
    - Magnetic separation rack (96-well type)
    - Multichannel pipettes (P10 or P20; P100; P200)

- Tabletop microcentrifuge (8-tube strips compatible)
- Vortex mixer

4.2 Library Preparation

4.2.1 Input Material Preparation

Input Recommendations

RNA Handling

Ribonucleases (RNases) are highly prevalent and stable enzymes that can rapidly degrade RNA molecules. Hence, when working with RNA, certain precautions must be taken to avoid RNase contamination:

- Decontaminate your workspace and pipettes using nuclease cleaning products before working with RNA.
- Keep purified RNA on ice while working and store it at -20 °C or -80 °C.
- Wear a lab coat and change gloves frequently.
- Use disposable nuclease-free plasticware, aerosol-barrier pipette-tips, and nuclease-free reagents.

RNA Quantification

To accurately pipette the correct amount of RNA input, we recommend performing an initial dilution to obtain a concentration in the range of 50 to 100 ng/µl. The RNA concentration should be confirmed by fluorometric quantitation (e.g., Qubit®) and the obtained value used to calculate the final dilution.

4.2.2 Pre-Mixes and Reagents Preparation

Components And Reagents

- Total RNA
- First Strand Synthesis Reaction Buffer
- Random Primers
- Reverse Transcriptase
- RNase Inhibitor
- DNA Ligase
- DNA Ligase Buffer
- PCR Master Mix 2x
- Hybridization Buffer
- STSplus Oligos
- Nuclease-free water

- AMPure® XP beads
- RNase/DNase-free 0.2 ml 8-tube strips

Preparation

1. Thaw the RNA samples on ice.
2. Remove the STSplus kit components from -20 °C storage and thaw on ice.
3. Once thawed, mix the reagents by inverting the tube 5–10 times and spin briefly in a microcentrifuge.
4. Remove the Dual Index Primer plate from -20 °C storage and place in 4 °C refrigerator for later use.
5. Remove the AMPure® XP beads from 2–8 °C storage and let them equilibrate at room temperature for at least 30 minutes. Vortex the AMPure® XP beads thoroughly, to ensure proper resuspension of the beads, prior to use in all subsequent steps.
6. Prepare fresh 80% Ethanol (volume according to the following scheme based on number of reactions):

80% ETHANOL VOLUMES							
Number of Reactions	**4**	**8**	**12**	**16**	**24**	**32**	**48**
80% Ethanol (ml)	2.5	5	7.5	10	15	20	30

Procedure

1. Prepare PCR strips according to the number of reactions:

NUMBER OF REACTIONS	4	8	12	16	24	32	48
PCR strip (1 strip)	4-tube	8-tube	4-tube	8-tube	8-tube	8-tube	8-tube
Number of strips	1	1	3	2	3	4	6

2. Prepare the following dilution for each RNA sample into the appropriate number of PCR strips:

RNA DILUTION	
Total RNA	100 ng
RNase-free water	Complete to 13.5 µl

   - Mix thoroughly by gently pipetting up and down 5 times followed by a brief spin in a microcentrifuge to collect all liquid.
   - Keep the samples on ice until cDNA Synthesis.

3. To ensure a homogenous processing of the samples, we recommend that you use pre-mixes in reservoirs and multichannel pipettes. Depending on the kit format, we recommend a minimum number of reactions to be processed simultaneously in order to avoid lack of reagents. Please follow the recommendations below:

	KIT FORMAT		
Recommendation for minimum number of reactions	**16 samples kit**	**32 samples kit**	**48 samples kit**
	4 reactions	8 reactions	12 reactions

Pre-Mixes

1. Prepare the **RT pre-mix** as follows:

RT PRE-MIX							
Number of Reactions	**4**	**8**	**12**	**16**	**24**	**32**	**48**
RNase Inhibitor (µl)	2.4	4.8	7.2	9.6	14.4	19.2	28.8
First Strand Synthesis Reaction Buffer (µl)	19.2	38.4	57.6	76.8	115.2	153.6	230.4

   - Mix thoroughly by pipetting up and down 10 times and spin briefly.
   - Keep on ice.

2. Prepare the **Annealing pre-mix** as follows:

ANNEALING PRE-MIX							
Number of Reactions	**4**	**8**	**12**	**16**	**24**	**32**	**48**
Hybridization Buffer (µl)	7.2	14.4	21.6	28.8	43.2	57.6	86.4
STSplus Oligos (µl)	7.2	14.4	21.6	28.8	43.2	57.6	86.4

   - Mix thoroughly by pipetting up and down 10 times and spin briefly.
   - Keep on ice.

3. Prepare the **Ligation pre-mix** as follows:

LIGATION PRE-MIX							
Number of Reactions	**4**	**8**	**12**	**16**	**24**	**32**	**48**
DNA Ligase Buffer (µl)	19.2	38.4	57.6	76.8	115.2	153.6	230.4
Nuclease-free water (µl)	130.6	261.1	391.7	522.2	783.3	1044.4	1566.6
DNA Ligase (µl)	3.9	7.7	11.6	15.4	23.1	30.8	46.2

   - Mix thoroughly by pipetting up and down 10 times and spin briefly.
   - Keep on ice.

The Ligase Buffer is highly viscous, pipette gently and make sure to obtain a homogeneous Ligation pre-mix.

4. Prepare the **PCR pre-mix** as follows:

PCR PRE-MIX							
Number of Reactions	**4**	**8**	**12**	**16**	**24**	**32**	**48**
Polymerase Master Mix 2x (µl)	120	240	360	480	720	960	1440
Nuclease-free water (µl)	48	96	144	192	288	384	576

- Mix thoroughly by pipetting up and down 10 times and spin briefly.
- Keep on ice.

4.2.3 cDNA Synthesis

Materials

- Diluted RNA samples (13.5 µl)
- Random Primers
- Reverse Transcriptase
- RT pre-mix
- RNase/DNase-free 0.2ml 8-tube strips

Preparation

1. Pre-heat the thermal cycler to 65 °C (set lid to 99 °C).
2. Program the thermal cycler for cDNA program with the following settings:

	TEMPERATURE (°C)	TIME (MINUTES)
Lid	99	-
Step 1: Primer Extension	25	10
Step 2: Reverse Transcription	42	50
Step 3: Enzyme Deactivation	70	15
Step 4: Hold	4	∞

Procedure

Always keep the samples and pre-mix on ice before and after the incubation to prevent RNA degradation and to block the enzymatic reaction.

1. To facilitate pipetting, create a reservoir of **Random primers** in a new set of PCR strips according to the following scheme:

NUMBER OF REACTIONS	4	8	12	16	24	32	48
PCR strip (1 strip)	**4-tube**	**4-tube**	**4-tube**	**8-tube**	**8-tube**	**8-tube**	**8-tube**
Random Primers (µl)	1.1	2.3	3.5	2.3	3.5	4.6	7

2. To facilitate pipetting, create a reservoir of **RT pre-mix** in a new set of PCR strips according to the following scheme:

NUMBER OF REACTIONS	4	8	12	16	24	32	48
PCR strip (1 strip)	**4-tube**	**4-tube**	**4-tube**	**8-tube**	**8-tube**	**8-tube**	**8-tube**
RT pre-mix (µl)	5	10	15	10	15	20	30

3. To facilitate pipetting, create a reservoir of **Reverse Transcriptase** in a new set of PCR strips according to the following scheme:

NUMBER OF REACTIONS	4	8	12	16	24	32	48
PCR strip (1 strip)	**4-tube**	**4-tube**	**4-tube**	**8-tube**	**8-tube**	**8-tube**	**8-tube**
Reverse Transcriptase (µl)	1.1	2.3	3.5	2.3	3.5	4.6	7

4. Assemble the reaction as follows:
    - Using a multichannel pipette, add 1 µl of Random Primers pre-mix to the 13.5 µl of RNA samples.
    - Mix by pipetting up and down 5 times and briefly spin in a microcentrifuge.
5. Place the tubes in the thermal cycler preheated at 65 °C for 5 minutes and then place the tubes immediately on ice.

The tubes should be kept on ice before and after the incubation at 65 °C to avoid degradation of RNA molecules.

6. Continue to assemble the reaction on ice as follows:
    - Using a multichannel pipette, add 4.5 µl of RT pre-mix to your 14.5 µl of RNA samples and Random Primers.
    - Mix by pipetting up and down 5 times and briefly spin in a microcentrifuge.
7. Incubate in the thermal cycler at 25 °C for 2 minutes.
8. Assemble the rest of the reaction at room temperature as follows:
    - Using a multichannel pipette, add 1 µl of Reverse Transcriptase to your 19 µl of RNA samples, Random Primers and RT pre-mix.
    - Mix by pipetting up and down 5 times and briefly spin in a microcentrifuge.
9. Place the reaction in the thermal cycler and start the cDNA program.

Safe stopping point overnight at 4 °C or longer at -20 °C.

Proceed to Oligos Annealing.

4.2.4 Oligos Annealing

Materials

- cDNA Synthesis reaction product
- Annealing pre-mix
- Ligation pre-mix
- RNase/DNase-free 0.2ml 8-tube strips

Preparation

1. Program the thermal cycler for Annealing with the following settings:

	TEMPERATURE (°C)	TIME (MINUTES)
Lid	99	-
Step 1	95	2
Step 2	60	60
Step 3	56	∞
Step 4	98	5
Hold	4	∞

Note: Please note that for this step it is important to have a PCR machine that allows to put a program on hold and continue it at the next step.

Procedure

Always keep the samples and pre-mix on ice before and after the incubation to prevent RNA degradation and to block the enzymatic reaction.

1. To facilitate pipetting, create a reservoir of **Annealing pre-mix** in a new set of PCR strips according to the following scheme:

NUMBER OF REACTIONS	4	8	12	16	24	32	48
PCR strip (1 strip)	**4-tube**	**4-tube**	**4-tube**	**8-tube**	**8-tube**	**8-tube**	**8-tube**
Annealing pre-mix (µl)	3.5	7	10	7	10	14	20

2. To facilitate pipetting, create a reservoir of **Ligation pre-mix** in a new set of PCR strips according to the following scheme:

NUMBER OF REACTIONS	4	8	12	16	24	32	48
PCR strip (1 strip)	**4-tube**	**4-tube**	**4-tube**	**8-tube**	**8-tube**	**8-tube**	**8-tube**
Ligation pre-mix (µl)	35	70	105	70	105	140	210

3. Assemble the reaction as follows:
    - Using a multichannel pipette, add 3 µl of Annealing pre-mix to a new 8-tube strip.
    - Using a multichannel pipette, add 5 µl (out of the 20 µl) of synthesized cDNA to each tube containing 3 µl of Annealing pre-mix, mix thoroughly by pipetting up and down 10 times and spin briefly.
4. Place the reaction in the thermal cycler and start the Annealing program.
5. Once the thermal cycler has reached Step 3 at 56 °C, allow the samples to cool at 56 °C for 5 minutes. One minute before adding the Ligation pre-mix to the samples, place the Ligation pre-mix reservoir at 56 °C. With the tubes and the reservoir on the thermoblock and using a multichannel pipette, add 32 µl of Ligation pre-mix to each tube. Mix by pipetting up and down 10 times. Pipette gently to avoid creating bubbles.

    Note: If droplets or evaporation are observed in the tubes, a brief spin of the tubes before or after adding the Ligation pre-mix may be performed.

    Do not let the tube temperature drop below 56 °C during this step as this can lead to incorrect oligo annealing.
6. Incubate in the thermal cycler at 56 °C for 15 minutes and then continue the Annealing program (proceed to Step 4 at 98 °C for 5 minutes).

After this step, always keep the annealing reaction product on ice and work quickly. The product can then be stored at -20 °C. After storage at -20 °C, at 4 °C or above for longer periods of time, background noise will drastically increase in the samples.

Proceed immediately to Library Amplification.

4.2.5 Library Amplification

Materials

- Annealing reaction products
- Dual Index Primers
- PCR pre-mix
- RNase/DNase-free 0.2ml 8-tube strips

Preparation

1. Remove Dual Index Primer plate from 4 °C (transferred from -20 °C to 4 °C earlier) and briefly spin the plate to collect all liquid.

Note: Please note that we recommend a control PCR which can be done at the same time as the library amplification (see *Appendix V* for details).

2. Program the thermal cycler for Library Amplification with the following settings:

	TEMPERATURE (°C)	TIME (SECONDS)	
Lid	99	-	35 cycles
Step 1: Initial Denaturation	98	180	
Step 2: Denaturation	98	10	
Step 3: Annealing	68	30	
Step 4: Extension	72	30	
Step 5: Final Extension	72	120	
Hold	10	∞	

Procedure

1. To facilitate pipetting, create a reservoir of **PCR pre-mix** by adding the following volumes to a new set of 4 or 8-tube strips:

NUMBER OF REACTIONS	4	8	12	16	24	32	48
PCR strip (1 strip)	**4-tube**	**4-tube**	**4-tube**	**8-tube**	**8-tube**	**8-tube**	**8-tube**
PCR pre-mix (µl)	37	76	115	76	115	152	230

2. Assemble the reaction as follows:

   - Depending on the number of reactions, prepare the following number of PCR strips:

NUMBER OF REACTIONS	4	8	12	16	24	32	48
PCR strip (1 strip)	**4-tube**	**8-tube**	**4-tube**	**8-tube**	**8-tube**	**8-tube**	**8-tube**
Number of PCR strips	1	1	3	2	3	4	6

   - To the above tubes, add 10 µl of Dual Index Primers per tube according to your indexing strategy (see *Appendix VI*).
   - Using a multichannel pipette, add 5 µl of the annealing products to the tubes containing the primers.

     Store the remaining annealing products at -20 °C immediately.
   - Using a multichannel pipette, add 35 µl of PCR pre-mix to the mixture.
   - Mix thoroughly by pipetting up and down 10 times and spin briefly.

3. Place the tubes in the thermal cycler and run the Library Amplification program.

Safe stopping point overnight at 4 °C or -20 °C for longer storage.

Proceed to Post-Amplification Clean Up.

4.2.6 Post-Amplification Clean Up

Materials

- PCR reaction products in 50 µl each
- AMPure® XP beads equilibrated at room temperature
- Freshly prepared ethanol 80%
- IDTE
- DNA low-binding tubes for storage of libraries

Procedure

1. Using a multichannel pipette, add 40 µl of AMPure® XP beads to each 50 µl of the PCR product. Mix thoroughly by pipetting up and down 10 times.
2. Incubate at room temperature for 5 minutes and spin briefly if required.
3. Place the 4 or 8-tube strips on a 96-well plate format magnetic rack for 5 minutes or until the liquid becomes clear.
4. Carefully discard 90 µl supernatant using a multichannel pipette.

   Keep the tubes on the magnetic rack for the following steps.
5. Using a multichannel pipette, add 170 µl of 80% ethanol to the beads. Let the tubes stand for 30 seconds to 1 minute.
6. Carefully discard the ethanol using a multichannel pipette.
7. Repeat steps 5 and 6 once.
8. Remove the residual ethanol using a P10 or P20 multichannel pipette.
9. Air-dry the beads at room temperature for 5 minutes. Do not over-dry the beads because this could decrease the amount of recovered DNA.

   Remove the tubes from the magnetic rack.
10. Using a multichannel pipette, add 20 µl of IDTE to the beads. Mix thoroughly by pipetting up and down 10 times. Incubate at room temperature for 5 minutes and spin briefly if required.
11. Place the 4 or 8-tube strip on a 96-well plate format magnetic rack for 5 minutes or until liquid becomes clear.
12. Carefully transfer 18 µl (transferring two times 9 µl is recommended at this step) of the supernatant to a new and labeled library storage tube.

Safe stopping point overnight at 4 °C or -20 °C for longer storage.

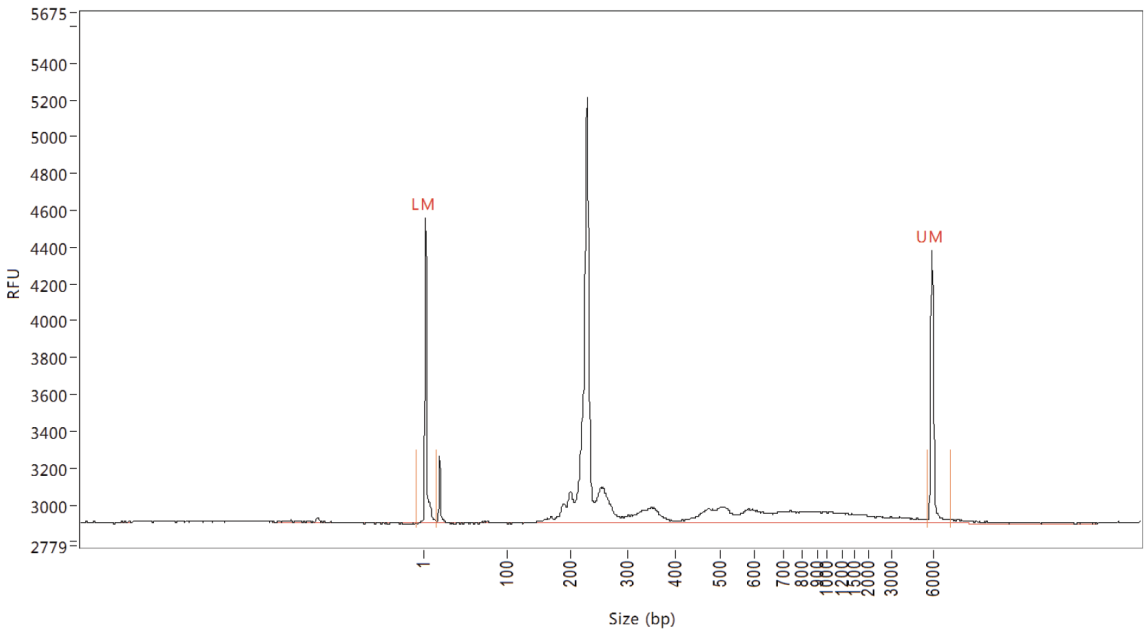
4.2.7 Individual Library Quantification And Quality Control

Materials

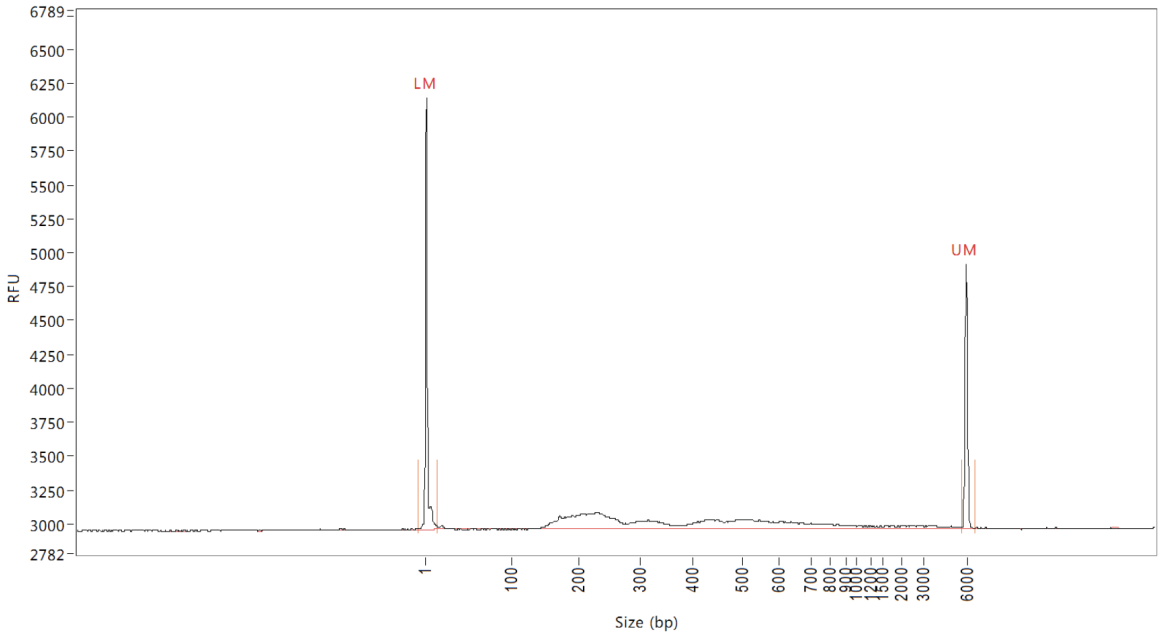
- Purified amplified libraries
- Capillary electrophoresis system

Procedure

1. Quality control the libraries by analyzing their profile via capillary electrophoresis. The expected band size showing positive fusion library is of approximately 240 bp.



Example of capillary electrophoresis showing **fusion-positive** library obtained with the Agilent Fragment Analyzer™ capillary electrophoresis system. LM: Lower Marker, UM: Upper Marker.



Example of capillary electrophoresis showing **fusion-negative** library obtained with the Agilent Fragment Analyzer™ capillary electrophoresis system. LM: Lower Marker, UM: Upper Marker.

4.3 Library Pooling And Quantification

4.3.1 Materials

- Individual sequencing libraries
- Fluorometric quantitation equipment and reagents
- DNA low-binding 1.5 ml tubes

4.3.2 Procedure

1. Pool 3 µl of each library (to be sequenced together) in a DNA low-binding tube.

   Note: If desired, each individual library can be quantified by Qubit® for reaction quality control.

2. Mix thoroughly by pipetting up and down 10 times and spin briefly.
3. Quantify the pooled libraries with a fluorometric method (e.g, Qubit® HS quantification using 2 µl of the prepared pool of libraries).

Safe stopping point overnight at -20 °C.

4.4 Sequencing Preparations

4.4.1 Materials

- Illumina® Sequencing Kit
- Final pooled STSplus libraries
- Final captured STS libraries
- Low TE Buffer or similar

4.4.2 Procedure

1. Determine the molarity of each captured pool with the average size of the library of 240 bp (corresponds to the expected fusion peak size in base pairs) and concentration (ng/µl) obtained during step *4.2.7 Individual Library Quantification and Quality Control* and *4.3 Library Pooling and Quantification* as follows:

$$\text{Library molarity (nM)} = \frac{\text{Library concentration (ng/µl)}}{\text{Average size in base pairs} \times 649.5} \times 10^6$$

2. Calculate the spike-in percentage of the STSplus libraries, depending on the number of samples and the sequencer used, according to the table below. For optimal results, it is recommended that 60,000 paired-end reads (i.e., 30,000 read pairs or "fragments") are attributed to STSplus libraries of each sample:

NUMBER OF SAMPLES	MiSeq® V3	NextSeq® 500/550 MID-OUTPUT
16	2%	0.4%
24	3%	0.6%
32	-	0.8%
48	-	1.2%

Note: It is important to follow these spike-in recommendations, as under- or over-loading STSplus libraries can negatively impact the quality of data.

Below is an example for MiSeq® with 24 samples:

- Denature STS and STSplus libraries in parallel. Post denaturation, with both pools at 10 pM, mix pools in the following manner:

LIBRARY POOL (10 pM)	VOLUME (µl)
STS	679
STSplus (3%)	21

- Load 600 µl of the mix.

Note: Please note that this example does not take into consideration the addition of PhiX. Follow Illumina® recommendations for the addition of PhiX to the final pool.

5 APPENDICES

5.1 Appendix V: Control PCR

We recommend performing an internal control. All samples can be tested with a pair of control primers to guarantee that sample loading, cDNA synthesis and Oligos Annealing were efficient. These control primers amplify control Oligos present in the STSplus Oligos mix. The control PCR can be done at the same time as the library amplification.

5.1.1 Materials

- Annealing reaction products
- Polymerase 2x Master Mix
- Control Primers
- Nuclease-free water
- Capillary electrophoresis system
- RNase/DNase-free 0.2ml 8-tube strips

Optional (depending on capillary electrophoresis system):

- AMPure® XP beads equilibrated at room temperature
- Freshly prepared 80 % ethanol
- IDTE

5.1.2 Preparation

1. Prepare the PCR mix as follows (include 1 extra reaction as a negative control).

> **Note:** Please note that the reaction volume of the control PCR is 25 µl and not 50 µl.

PCR MIX (25 µl)							
Number of reactions	**4**	**8**	**12**	**16**	**24**	**32**	**48**
Negative control	**1**	**1**	**1**	**1**	**1**	**1**	**1**
Polymerase 2x Master Mix (µl)	75	135	195	255	375	510	750
Control primers (µl)	30	54	78	102	150	204	300
Nuclease-free water (µl)	30	54	78	102	150	204	300

2. Prepare the thermal cycler for Library Amplification with the following settings:

	TEMPERATURE (°C)	TIME (SECONDS)	
Lid	99	-	35 cycles
Step 1: Initial Denaturation	98	180	
Step 2: Denaturation	98	10	
Step 3: Annealing	68	30	
Step 4: Extension	72	30	
Step 5: Final Extension	72	120	
Hold	10	∞	

5.1.3 Procedure - PCR

1. Assemble the reaction as follows:
    - Add 22.5 µl of the PCR master mix to new 8-tube strips.
    - Using a multichannel pipette, add 2.5 µl of the annealing products to the tubes containing the PCR mix.
    - In the negative control, add 2.5 µl of nuclease-free water instead of template.

      Store the remaining annealing products at -20 °C immediately.
    - Mix thoroughly by pipetting up and down 10 times and spin briefly.
2. Place the tubes in the thermal cycler and run the Library Amplification program.

Proceed to Post-Amplification Clean Up (optional).

5.1.4 Procedure - Post-Amplification Clean Up

Note: Optional – Depending on your capillary electrophoresis system, this clean up may not be necessary.

1. Using a multichannel pipette, add 20 µl of AMPure XP beads to your 25 µl PCR product. Mix thoroughly by pipetting up and down 10 times.
2. Incubate at room temperature for 5 minutes and spin briefly if required.
3. Place the 8-tube strips on a 96-well plate format magnetic rack for 3 minutes or until liquid becomes clear.
4. Carefully discard supernatant using a multichannel pipette.

   Keep tubes on the magnetic rack for the following steps.
5. Using a multichannel pipette add 170 µl of 80% ethanol to the beads. Let them stand for 30 seconds to 1 minute.
6. Carefully discard ethanol using a multichannel pipette.
7. Repeat steps 5 and 6 once.
8. Remove the residual ethanol using a P10 or P20 multichannel pipette.

9. Air-dry the beads at room temperature for 5 minutes. Do not over-dry the beads because this could decrease the amount of recovered DNA.

   Remove tubes from the magnetic rack.

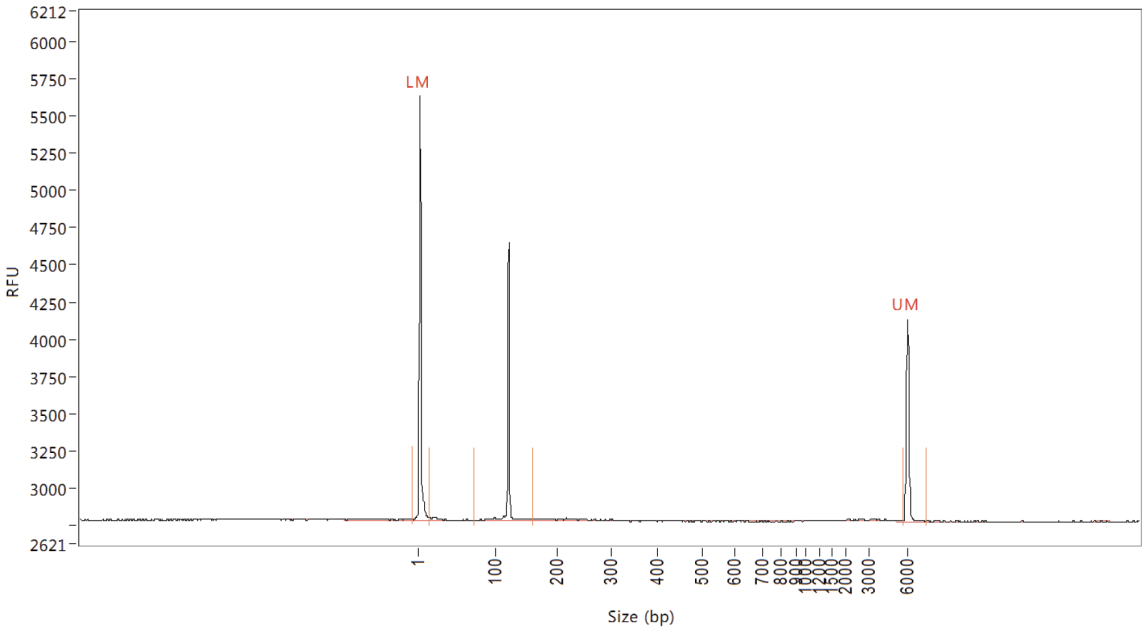
10. Using a multichannel pipette add 20 µl of IDTE to the beads. Mix thoroughly by pipetting up and down 10 times. Incubate at room temperature for 5 minutes and spin briefly if required.
11. Place the 8-tube strips on a 96-well plate format magnetic rack for 3 minutes or until liquid becomes clear.
12. Carefully transfer 18 µl of the supernatant (transferring two times 9 µl is recommended at this step) to a new labeled library storage tube.

Safe stopping point overnight at 4 °C or longer at -20 °C.

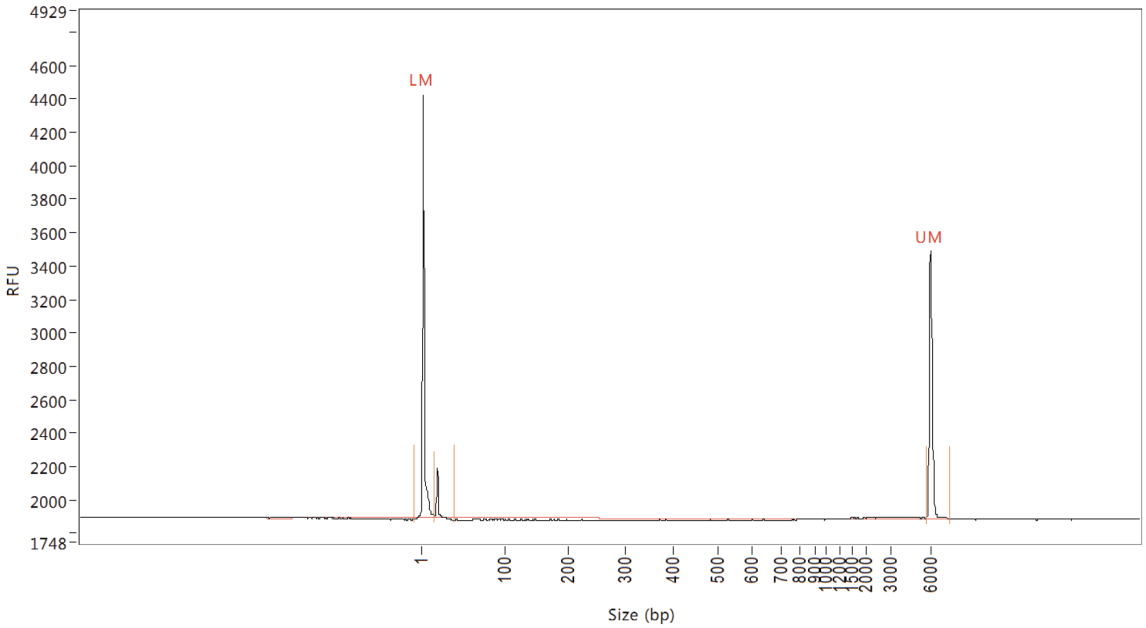
5.1.5 Procedure - Control PCR Assessment

The control PCR is not sequenced, it is only assessed using capillary electrophoresis. The presence of a band at approximately 120 bp indicates that the cDNA synthesis and the annealing steps were correctly performed.

1. Evaluate the quality of the control PCR by analyzing its profile via capillary electrophoresis. The expected band size is approximately 120 bp.



Example of capillary electrophoresis showing **positive control PCR** obtained with the Agilent Fragment Analyzer™ capillary electrophoresis system. LM: Lower Marker, UM: Upper Marker.



Example of capillary electrophoresis showing **negative control PCR** obtained with the Agilent Fragment Analyzer™ capillary electrophoresis system. LM: Lower Marker, UM: Upper Marker.

5.2 Appendix VI: Dual Index Primer Plates

5.2.1 48 Illumina®-compatible Dual Index Primers In 96-well Plate Format (7 µl Each)

	1	2	3	4	5	6	7	...	12
A	sgUN-1	sgUN-9	sgUN-17	sgUN-25	sgUN-33	sgUN-41			
B	sgUN-2	sgUN-10	sgUN-18	sgUN-26	sgUN-34	sgUN-42			
C	sgUN-3	sgUN-11	sgUN-19	sgUN-27	sgUN-35	sgUN-43			
D	sgUN-4	sgUN-12	sgUN-20	sgUN-28	sgUN-36	sgUN-44			
E	sgUN-5	sgUN-13	sgUN-21	sgUN-29	sgUN-37	sgUN-45			
F	sgUN-6	sgUN-14	sgUN-22	sgUN-30	sgUN-38	sgUN-46			
G	sgUN-7	sgUN-15	sgUN-23	sgUN-31	sgUN-39	sgUN-47			
H	sgUN-8	sgUN-16	sgUN-24	sgUN-32	sgUN-40	sgUN-48			

5.2.2 Index Sequences For The Illumina®-compatible Dual Index Primers

The table below lists the index sequences. Please note that when using manual Sample Sheets, the following Illumina® instruments require the **reverse orientation** of the i5 sequence:

- NextSeq® 500/550
- NextSeq® 1000/2000 in Standalone mode (Sample Sheet v1)
- HiSeq® 3000/4000/X
- NovaSeq™ 6000 with v1.5 reagent kits and NovaSeq™ X/X Plus
- MiniSeq™ (except when used with Rapid Reagent Kits)
- iSeq™ 100

All other Illumina® instruments (e.g., MiSeq®) and instrument configurations require the **forward** i5 orientation.

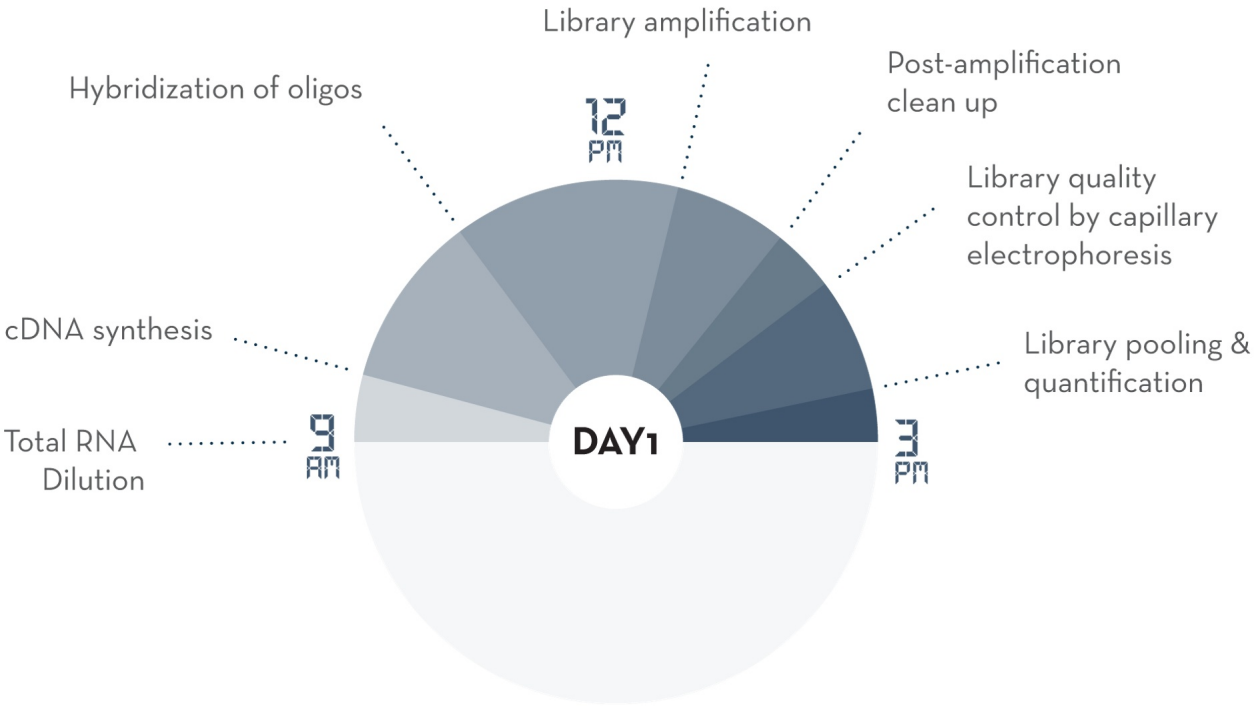
This information is subject to change. Please always refer to the Illumina® support pages for the latest information on which sequencing systems require the i5 index sequence in forward or reverse (complement) orientation:

https://support-docs.illumina.com/SHARE/AdapterSequences/Content/SHARE/AdapterSeq/Overview.htm

INDEX	I5 SEQUENCE (FORWARD ORIENTATION)	I5 SEQUENCE (REVERSE ORIENTATION)	I7 SEQUENCE
sgUN-1	AAGCGTAT	ATACGCTT	CTACCAGC
sgUN-2	GTTGGTCC	GGACCAAC	GTTATGAG
sgUN-3	CCAGGAAC	GTTCCTGG	ATAGACCT
sgUN-4	GACAGTCG	CGACTGTC	GGATTGAA
sgUN-5	TCTGACCA	TGGTCAGA	GCATCGTG
sgUN-6	ATCCTACA	TGTAGGAT	TGTCTGTA
sgUN-7	CGTAATGA	TCATTACG	CGAGTTCC
sgUN-8	TGCTACGG	CCGTAGCA	AACGCACG
sgUN-9	CTGGCTGT	ACAGCCAG	TCACCGAT
sgUN-10	TCTTAGGC	GCCTAAGA	CTGCTGGA
sgUN-11	CACGCCTA	TAGGCGTG	AGCGTGGA
sgUN-12	TACCAATC	GATTGGTA	TGTTGACG
sgUN-13	TATCTTCG	CGAAGATA	ACAAGTAG
sgUN-14	ACTGCTAC	GTAGCAGT	CGGTAAGC
sgUN-15	CACTAGCC	GGCTAGTG	TCGGCATA
sgUN-16	AGTAGCAC	GTGCTACT	AGTCCTTG
sgUN-17	CAGCAACT	AGTTGCTG	GTACAACG
sgUN-18	CTTACATG	CATGTAAG	TAGGTGCA
sgUN-19	ACGGTAGT	ACTACCGT	GTGAACGC
sgUN-20	CATCGGAG	CTCCGATG	GCCATACC
sgUN-21	TCTAGGTG	CACCTAGA	GCTGATGT
sgUN-22	TTAAGGCA	TGCCTTAA	ACGCTGAC
sgUN-23	GTAGCCAC	GTGGCTAC	GAGCTAGG
sgUN-24	CCAATGGT	ACCATTGG	GGCCAAGT
sgUN-25	GCCTATTC	GAATAGGC	AATGTCGG
sgUN-26	CTGGAGTG	CACTCCAG	ACATGACA
sgUN-27	GGCGGTAA	TTACCGCC	CAGATCAA
sgUN-28	GTCGAATA	TATTCGAC	GTCGTGCT
sgUN-29	CACTTATG	CATAAGTG	CCGAGTTA
sgUN-30	TAGTGGTC	GACCACTA	ATGTCGAG
sgUN-31	AATACGCT	AGCGTATT	CAACGTTC
sgUN-32	GCTACCAA	TTGGTAGC	TTGAGCAT

INDEX	I5 SEQUENCE (FORWARD ORIENTATION)	I5 SEQUENCE (REVERSE ORIENTATION)	I7 SEQUENCE
sgUN-33	TACATCGT	ACGATGTA	TCCTCAAC
sgUN-34	TGCCGTCT	AGACGGCA	CGCAACCT
sgUN-35	TGGAAGTT	AACTTCCA	TGAACATC
sgUN-36	GCAACTGC	GCAGTTGC	CCTTCTGG
sgUN-37	AGACCACT	AGTGGTCT	TCACGTGA
sgUN-38	CGTGACTC	GAGTCACG	CATACCGC
sgUN-39	CCTTGATT	AATCAAGG	CACGTTAT
sgUN-40	ATCACCGG	CCGGTGAT	TTGTCACT
sgUN-41	GATCAGCA	TGCTGATC	TAGGATAG
sgUN-42	TTAGGCGG	CCGCCTAA	GACTCGGT
sgUN-43	TTCCGCTA	TAGCGGAA	CTACTCAG
sgUN-44	GTGACTCA	TGAGTCAC	CGCTGGTA
sgUN-45	TGGCTACC	GGTAGCCA	ATTCATGC
sgUN-46	TAATCAGG	CCTGATTA	TGAATCCG
sgUN-47	GGATGCTT	AAGCATCC	GCACTTCT
sgUN-48	CCGTCCAT	ATGGACGG	AATCGACC

5.3 Appendix VII: General Workflow SOPHiA DDM™ Plus Solution



Library Preparation with SOPHiA GENETICS™ RNA Library Prep Kit

6 SYMBOLS

SYMBOL	TITLE
	Consult instructions for use
	Catalog number
	Batch code (Lot Number)
	Caution
	Manufacturer
	Date of manufacture
	Temperature Limit
	Use-by date
	Research Use Only
	Contains sufficient for <n> tests
	Refer to Warnings and Precautions section.
	Refer to Warnings and Precautions section.
	Refer to Warnings and Precautions section.
	Refer to Warnings and Precautions section.

SYMBOL	TITLE
	Refer to Warnings and Precautions section.
	Box 1
	Box 2

7 SUPPORT

In case of difficulty using the SOPHiA DDM™ Desktop App, please consult the troubleshooting section of the "General information about usage of SOPHiA DDM™" document or contact our support line by telephone at +41 21 694 10 60 or e-mail support@sophiagenetics.com. Please visit www.sophiagenetics.com for further details.

Any serious incident occurring in relation to the device should be promptly reported to SOPHiA GENETICS and the competent authorities of the member state where the user is established.

Do not use components that are damaged. Contact support@sophiagenetics.com if there are any concerns with the kits.

Document Approvals

Approved Date: 19 Feb 2024

Approval Verdict: Approve	Martin Fritzsche, (mfritzsche@sophiagenetics.com) Technical Approval 19-Feb-2024 12:46:47 GMT+0000

Approval Verdict: Approve	Paolo Florent, (pflorent@sophiagenetics.com) Regulatory Approval 19-Feb-2024 12:56:29 GMT+0000

QA Approval Verdict: Approve	Alexandra Bonnet, (abonnet@sophiagenetics.com) Quality Assurance Approval 19-Feb-2024 18:00:52 GMT+0000