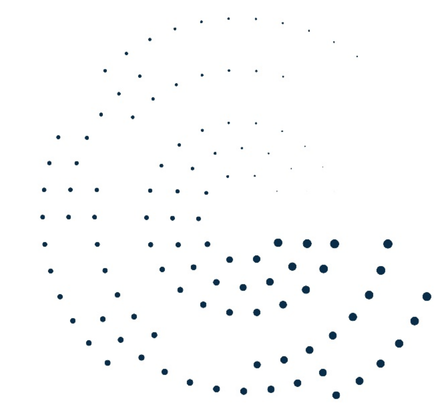
INSTRUCTIONS FOR USE

16, 32, 48 and 96 Samples

SOPHiA DDM™

Myeloid Plus Solution



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

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SUMMARY INFORMATION

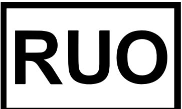
Product Name	SOPHiA DDM™ Myeloid Plus Solution
Product Type	Bundle Solution
Product Family	Molecular kit + analytics
Algorithm ID	ILL1XG1S9_CNV_2
Gene Panel ID	MYS_plus_v1
Product Version	v1.0
Release Version	Not Applicable
Sample Type	Somatic DNA / RNA isolated from peripheral blood and bone marrow
Sequencer	Illumina® MiSeq®
Document ID	SG-00244
Document Version	v3.0
Revision Date	Jan 2024

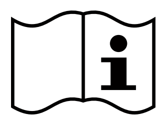
This Instructions For Use is applicable to all SOPHiA DDM™ versions.

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

SOPHiA GENETICS SA
ZA La Pièce 12
1180 Rolle
Switzerland

BS0112ILLRSMY01-016;
BS0112ILLRSMY01-032;
BS0112ILLRSMY01-048





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PRODUCT CODES

	FULL PRODUCT CODE	BOX 1	BOX 2	BOX 3	LIBRARY PREPARATION KIT
REF	BS0112ILLRSMY01-016	B1.E1.0012.R-16	B2.0012.R-16	B3.0012.R-16	700232
	BS0112ILLRSMY01-032	B1.E1.0012.R-32	B2.0012.R-32	B3.0012.R-32	700232
	BS0112ILLRSMY01-048	B1.E1.0012.R-48	B2.0012.R-48	B3.0012.R-48	700234

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REVISION HISTORY

DOCUMENT ID / VERSION	DATE	DESCRIPTION OF CHANGE
SG-00244 – v3.0	Jan 2024	• Added new sections *Product Codes* and *Product Introduction* • 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*. • Update of trademark symbols and product names • Cosmetic text and formatting changes and minor document restructuring • 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. • Added missing symbols to *Symbols* section
SG-00244 – v2.1	Apr 2023	• Incorporation of appropriate trademarks throughout.
SG-00244 – v2.0	Jun 2022	• Product Name Updated • Pipeline ID Updated • Document ID Updated

ID-60101-61 – 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.2.1.2.140_r2en
		  ◦ PM_RUO_B.2.1.2.141_r2en
		  ◦ PM_RUO_B.2.1.2.142_r2en
		• Minor changes for clarity in the following sections:
		  ◦ Section 3.1 Library Pooling
		  ◦ Section 3.5 Wash Streptavidin Beads to Remove Unbound DNA

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1 PRODUCT INTRODUCTION

The SOPHiA DDM™ Myeloid Plus Solution is a bundle solution including library preparation and capture kit, as well as a fully integrated FASTQ-to-report bioinformatic workflow for the analysis, interpretation, and reporting of sequencing data obtained on Illumina® MiSeq® instrument(s). The workflow has been optimized for the analysis and interpretation of sequencing data for the detection of somatic variants in genes known to be associated with myelodysplastic syndromes, myeloproliferative neoplasms, and leukemia.

The workflow consists of a secondary analysis pipeline and tertiary analytical tools and resources. The secondary analysis pipeline detects Single Nucleotide Variants (SNVs), Insertions/Deletions (INDELs), and Copy Number Variants (CNVs), as well as FLT3 internal tandem duplications and leukemia-associated gene fusions (via the RNA module).

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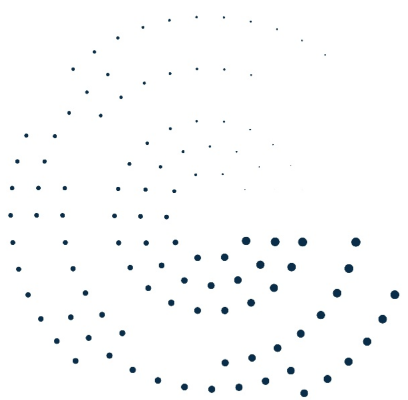
DNA PART



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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 Dual Index Adapters (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 I	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)
- Myeloid Plus Solution Probes by SOPHiA GENETICS (20 µl)
- 2x Hybridization Buffer (50 µl)

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- 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 Dual Index Adapters in 96-well plate format (7 µl adapter per well). See Appendix I for adapter display and sequences.
- Post-Capture Illumina® Primers Mix (20 µl)
- Post-Capture PCR Enhancer (20 µl)
- Post-Capture PCR Master Mix (122 µl)

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 I (Store At -25°C To -15°C)*

REAGENT	KIT FORMAT	
	16 SAMPLES	48 SAMPLES
HiFi PCR Master Mix 2x (µl)	500	1560
Primer Mix Illumina® Library Amp (µl)	30	95
FX Enzyme Mix (µl)	200	625
FX Buffer 10x (µl)	100	315
FX Enhancer (µl)	100	315
DNA Ligase (µl)	200	625
DNA Ligase Buffer 5x (µl)	400	1250

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

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* 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.

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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

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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

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PRODUCT NAME	GHS PICTOGRAM	H&P STATEMENTS	SIGNAL WORD	HAZARDOUS COMPONENT
		center/ doctor. • P501 Dispose of contents/ container in accordance with national regulations.		

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)

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- 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

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2.2 Library Preparation

2.2.1 Input Material Preparation

Materials

- Double-stranded high quality genomic DNA (gDNA)
- FX Enhancer
- IDTE
- RNase/DNase-free 0.2 ml 8-tube strips

Input Recommendations

DNA integrity, concentration, and purity are critical during this step. The purity of the DNA can be assessed using a UV spectrophotometer. Recommended absorbance ratios are between 1.8–2.0 for the 260/280 nm ratio and within 1.6–2.4 for 260/230 nm.

We recommend confirmation of the sample integrity by capillary electrophoresis or an equivalent technique.

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

Remove the FX Enhancer from -20°C storage and thaw at room temperature. After thawing, mix the reagent by gently inverting the tube 5 times and briefly spin in a microcentrifuge.

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.

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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 extracted DNA sample into the appropriate number of PCR strips, in the following manner:

DNA DILUTION	
DNA	200 ng
IDTE	Complete to 30 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.

4. Depending on the number of samples, proceed as follows:
   - If processing **4 samples**, add 5 µl of FX Enhancer to each tube of the 4-tube strip containing 30 µl of DNA samples (total of 35 µl in each tube of the 4-tube strip).
   - If processing **8 or more samples**, proceed as follows:
     a. To facilitate pipetting, create a reservoir of FX Enhancer 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**
FX Enhancer (µl)	11.5	17.5	11.5	17.5	24	36

     b. Using a multichannel pipette, add 5 µl of FX Enhancer from the above tubes to 30 µl of DNA samples (total of 35 µl in each tube of the 4 or 8-tube strips).
   - Using a multichannel pipette set to 20 µl, mix gently by pipetting up and down 5 times and briefly spin in a microcentrifuge.

5. Keep on ice until Enzymatic Fragmentation reaction set-up.

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2.2.2 Pre-Mixes and Reagents Preparation

Components And Reagents

- FX Enzyme Mix
- FX Buffer 10x
- DNA Ligase
- DNA Ligase Buffer 5x
- HiFi PCR Master Mix 2x
- Primer Mix Illumina® Library Amp
- Nuclease-free water
- AMPure® XP beads
- Ethanol

Preparation

1. Remove the SOPHiA GENETICS™ DNA Library Prep Kit I components from -20 °C storage and thaw on ice.
2. Remove the Dual Index Adapters Plate from -20 °C storage and put it into 4 °C refrigerator for later use.
3. 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.
4. 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	20	30	30	40	50	70

5. Once the SOPHiA GENETICS™ DNA Library Prep Kit I components are thawed, mix the reagents by inverting the tube 5–10 times to ensure that there is no remaining precipitate and briefly spin in a microcentrifuge. Place on ice.

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Pre-Mixes

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

FX PRE-MIX							
Number of Reactions	**4**	**8**	**12**	**16**	**24**	**32**	**48**
FX Buffer 10x (µl)	23.6	47.1	75	95	150	190	300
FX Enzyme Mix (µl)	47.1	94.2	150	190	300	380	600

- 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**
DNA Ligase Buffer 5x (µl)	95	190	300	380	600	760	1200
DNA Ligase (µl)	47.5	95	150	190	300	380	600
Nuclease-free water (µl)	71.3	142.5	225	285	450	570	900

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

The DNA Ligase Buffer 5x is highly viscous, pipette gently and make sure to obtain a homogeneous Ligation pre-mix.

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

PCR PRE-MIX							
Number of Reactions	**4**	**8**	**12**	**16**	**24**	**32**	**48**
HiFi PCR Master Mix 2x (µl)	115	230	345	460	690	920	1380
Primer Mix Illumina® Library Amp (µl)	6.9	13.8	20.7	27.6	41.4	55.2	82.8
Nuclease-free water (µl)	16.1	32.2	48.3	64.4	96.6	128.2	193.2

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

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2.2.3 Enzymatic Fragmentation, End Repair and A-Tailing

Materials

- Diluted and conditioned double stranded gDNA in 35 µl
- FX 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	70	-
Step 1	4	1
Step 2	32	5
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 FX 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**
FX pre-mix (µl)	33	52.5	33	52.5	66	105

2. Assemble the reaction as follows:
   - Using a multichannel pipette, if processing 8 or more samples, add 15 µl of FX pre-mix to each of the 35 µl of DNA samples (total of 50 µl in 4 or 8-tube strips).
   - Using a multichannel pipette set to 40 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.

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Proceed immediately to Ligation.

2.2.4 Ligation

Materials

- Fragmentation reaction products in 50 µl each
- Ligation pre-mix
- Dual Index Adapters
- RNase/DNase-free 0.2 ml 8-tube strips

Preparation

1. Remove the Dual Index Adapter plate from 4 °C (transferred from -20 °C to 4 °C earlier) and briefly spin the plate to collect all the liquid. Refer to *Appendix I* for the respective plate format.
2. During the Fragmentation, prepare new PCR strips with 5 µl of different Dual Index Adapters 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

3. 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)	100	160	100	160	200	320

2. Using a multichannel pipette, transfer the 50 µl of each Fragmentation reaction product to the 4 or 8-tube strips containing 5 µl of Dual Index Adapters.
3. Mix thoroughly by pipetting up and down 10 times and spin briefly.

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4. Using a multichannel pipette, add 45 µl Ligation pre-mix to each Fragmentation reaction product (55 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 100 µl each
- AMPure® XP beads equilibrated at room temperature
- Freshly prepared ethanol 80%
- Nuclease-free water
- RNase/DNase-free 0.2 ml 8-tube strips

Procedure

1. 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.
2. Incubate at room temperature for 5 minutes and spin briefly if required.
3. Place the 4 or 8-tube strip on a 96-well plate format magnetic rack for 5 minutes or until the liquid becomes clear.
4. Carefully discard 170 µl of 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. Incubate 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 105 µl of nuclease-free water to the beads and wait for a few seconds. Mix thoroughly by pipetting up and down 10 times.
11. Incubate at room temperature for 5 minutes and spin briefly if required.

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12. Place the 4 or 8-tube strip on a 96-well plate format magnetic rack for 5 minutes or until the liquid becomes clear.
13. Using a multichannel pipette, carefully transfer 100 µl of the supernatant to new, labelled 4 or 8-tube strip.

Proceed to Dual Size Selection.

2.2.6 Dual Size Selection

Materials

- Ligation reaction products in 100 µl each
- AMPure® XP beads equilibrated at room temperature
- Freshly prepared ethanol 80%
- IDTE
- RNase/DNase-free 0.2 ml 8-tube strips

Procedure

1. Using a multichannel pipette, add 60 µl of AMPure® XP beads to each of the 100 µl ligation 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 collect all liquid.
3. Place the 4 or 8-tube strip on a 96-well plate format magnetic rack for 5 minutes or until the liquid becomes clear.
4. Using a multichannel pipette, carefully transfer 140 µl of the supernatant to a new, labelled 4 or 8-tube strip containing 20 µl of AMPure® XP beads. Mix thoroughly by pipetting up and down 10 times.
5. Incubate at room temperature for 5 minutes and spin briefly if required to collect all liquid.
6. Place the 4 or 8-tube strip on a 96-well plate format magnetic rack for 5 minutes or until the liquid becomes clear.
7. Carefully discard 150 µl of supernatant using a multichannel pipette.

   Keep the tubes on the magnetic rack for the following steps.

8. Using a multichannel pipette, add 170 µl of 80% ethanol to the beads. Incubate for 30 seconds to 1 minute.
9. Carefully discard the ethanol using a multichannel pipette.
10. Repeat steps 8 and 9 once.
11. Remove the residual ethanol using a P10 or P20 multichannel pipette.
12. Air-dry the beads at room temperature for 4 minutes. Do not over-dry the beads because this could decrease the amount of recovered DNA.

    Remove the tubes from the magnetic rack.

13. 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.

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2.2.7 Library Amplification

Materials

- Dual size selected Ligation reaction products and beads resuspended in 20 µl IDTE each
- PCR pre-mix

Preparation

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

	TEMPERATURE (°C)	TIME (SECONDS)	
Lid	99	-	
Step 1: Initial Denaturation	98	120	
Step 2: Denaturation	98	20	8 cycles
Step 3: Annealing	60	30	8 cycles
Step 4: Extension	72	30	8 cycles
Step 5: Final Extension	72	60	
Hold	10	∞	

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 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**
PCR pre-mix (µl)	65	100	65	100	130	200

2. Assemble the reaction as follows:
    - Using a multichannel pipette, add 30 µl of PCR pre-mix to the dual size selected 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.

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2.2.8 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.

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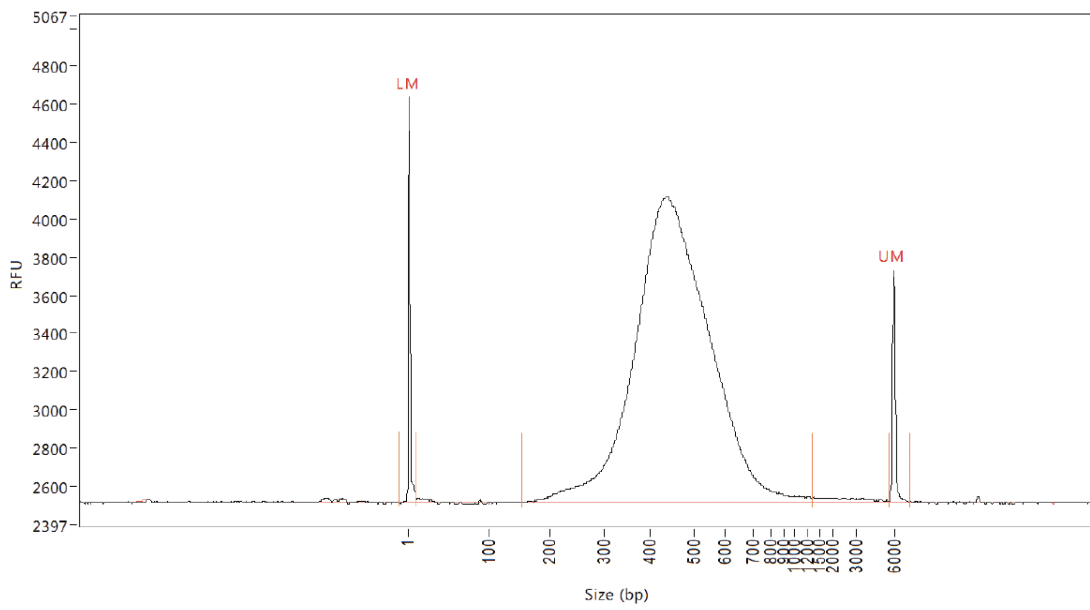
2.2.9 Individual Library Quantification and Quality Control

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 300 bp and 700 bp.



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

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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.

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2.4 Capture

2.4.1 Hybridization

Materials

- Lyophilized libraries
- 2x Hybridization Buffer
- Hybridization Buffer Enhancer
- Myeloid 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.

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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 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**

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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

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- 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.

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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.

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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

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- 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	-	
Step 1: Initial Denaturation	98	120	
Step 2: Denaturation	98	20	15 cycles
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

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- 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

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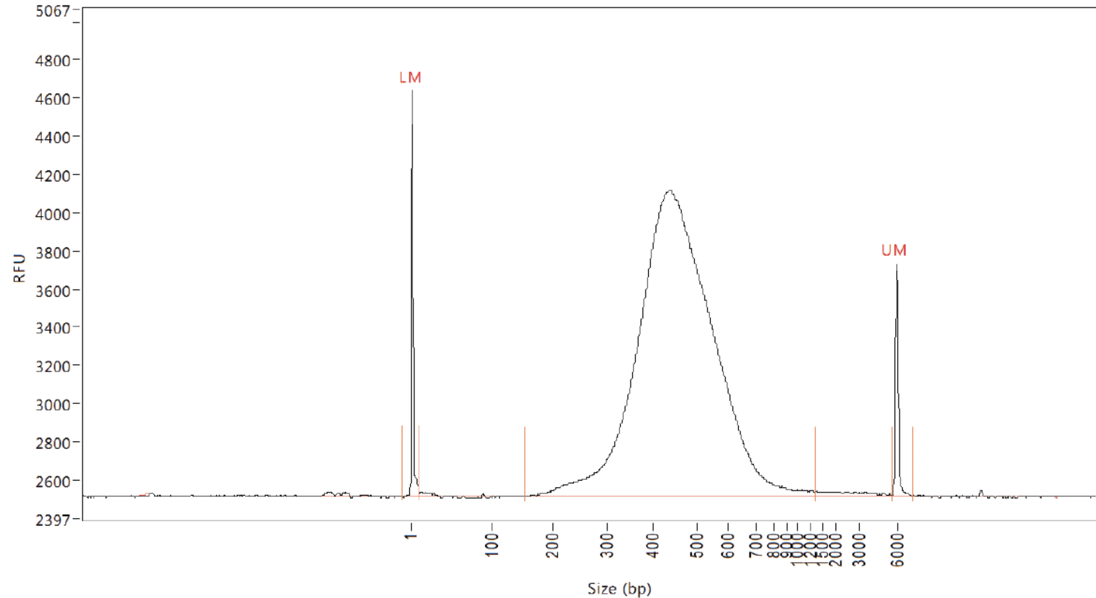
Procedure

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 300 bp and 700 bp.



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

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2.5 Sequencing

2.5.1 Sequencing Preparations

Materials

- Illumina® MiSeq® 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
MiSeq®	10 pM

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 HiSeq®, load a 10 pM dilution.
- For NextSeq® 500/550, load 1.3 pM [Adjust the dilution (1.1 pM to 1.5 pM range) according to the number of clusters obtained in the first run].
- 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	2.7 million
≥ 200	2 million

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3 APPENDICES

3.1 Appendix I: Dual Index Adapter Plates

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

	1	2	3	4	5	6	7	...	12
A	701-501	701-502	701-503	701-504					
B	702-501	702-502	702-503	702-504					
C	703-501	703-502	703-503	703-504					
D	704-501	704-502	704-503	704-504					
E									
F									
G									
H									

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

	1	2	3	4	5	6	7	...	12
A	701-501	701-502	701-503	701-504					
B	702-501	702-502	702-503	702-504					
C	703-501	703-502	703-503	703-504					
D	704-501	704-502	704-503	704-504					
E	705-501	705-502	705-503	705-504					
F	706-501	706-502	706-503	706-504					
G	707-501	707-502	707-503	707-504					
H	708-501	708-502	708-503	708-504					

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

	1	2	3	4	5	6	7	...	12
A	701-501	703-502	705-503	707-501	709-502	711-503			
B	702-501	704-502	706-503	708-501	710-502	712-503			
C	703-501	705-502	701-504	709-501	711-502	707-504			
D	704-501	706-502	702-504	710-501	712-502	708-504			
E	705-501	701-503	703-504	711-501	707-503	709-504			
F	706-501	702-503	704-504	712-501	708-503	710-504			
G	701-502	703-503	705-504	707-502	709-503	711-504			
H	702-502	704-503	706-504	708-502	710-503	712-504			

3.1.4 96 Illumina®-compatible Dual Index Adapters In 96-well Plate Format (7 µl Each)

	1	2	3	4	5	6	7	8	9	10	11	12
A	701-501	702-501	703-501	704-501	705-501	706-501	707-501	708-501	709-501	710-501	711-501	712-501
B	701-502	702-502	703-502	704-502	705-502	706-502	707-502	708-502	709-502	710-502	711-502	712-502
C	701-503	702-503	703-503	704-503	705-503	706-503	707-503	708-503	709-503	710-503	711-503	712-503
D	701-504	702-504	703-504	704-504	705-504	706-504	707-504	708-504	709-504	710-504	711-504	712-504
E	701-505	702-505	703-505	704-505	705-505	706-505	707-505	708-505	709-505	710-505	711-505	712-505
F	701-506	702-506	703-506	704-506	705-506	706-506	707-506	708-506	709-506	710-506	711-506	712-506
G	701-507	702-507	703-507	704-507	705-507	706-507	707-507	708-507	709-507	710-507	711-507	712-507
H	701-508	702-508	703-508	704-508	705-508	706-508	707-508	708-508	709-508	710-508	711-508	712-508

3.1.5 Index Sequences For The Illumina®-compatible Dual Index Adapters

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:

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- 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

i5	i5 SEQUENCE (FORWARD ORIENTATION)	i5 SEQUENCE (REVERSE ORIENTATION)
D501	TATAGCCT	AGGCTATA
D502	ATAGAGGC	GCCTCTAT
D503	CCTATCCT	AGGATAGG
D504	GGCTCTGA	TCAGAGCC
D505	AGGCGAAG	CTTCGCCT
D506	TAATCTTA	TAAGATTA
D507	CAGGACGT	ACGTCCTG
D508	GTACTGAC	GTCAGTAC

i7	i7 SEQUENCE
D701	ATTACTCG
D702	TCCGGAGA
D703	CGCTCATT
D704	GAGATTCC
D705	ATTCAGAA
D706	GAATTCGT
D707	CTGAAGCT
D708	TAATGCGC
D709	CGGCTATG
D710	TCCGCGAA

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i7	i7 SEQUENCE
D711	TCTCGCGC
D712	AGCGATAG

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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

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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

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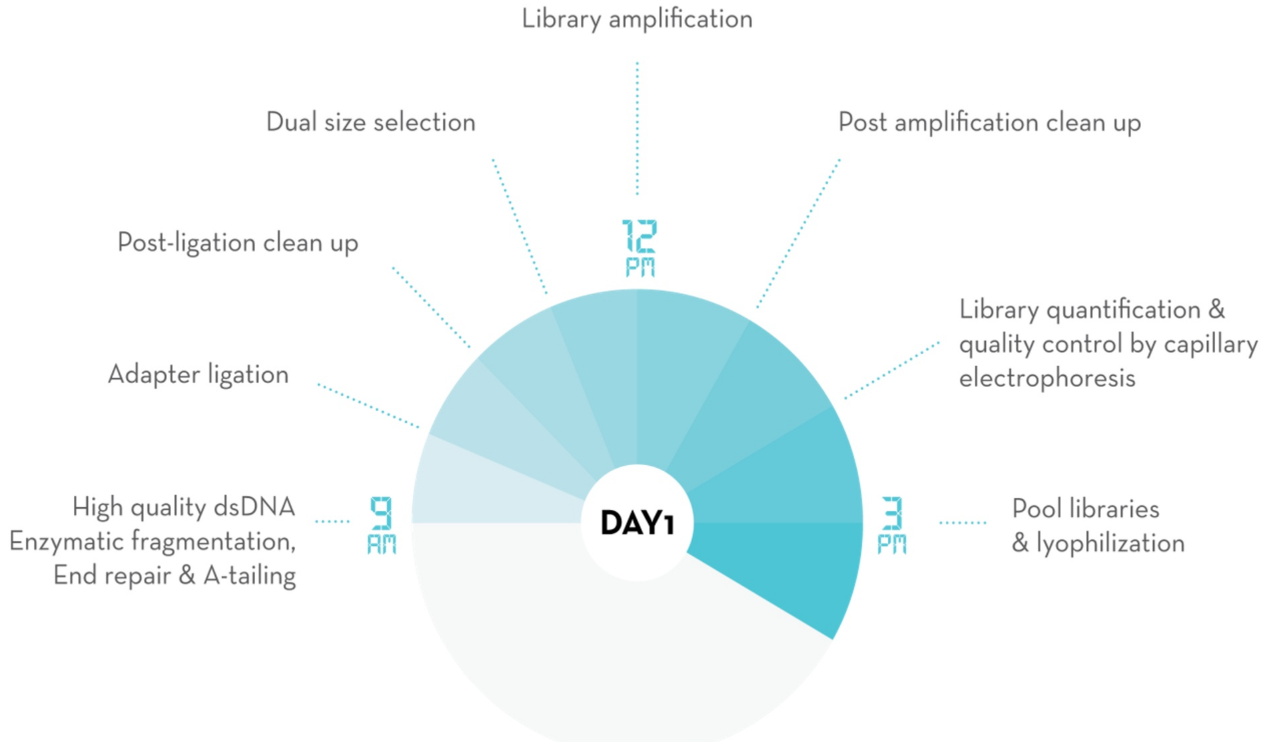
3.3 Appendix III: General Workflow SOPHiA DDM™ Capture Solution



Library Preparation with SOPHiA GENETICS™ DNA Library Prep Kit I

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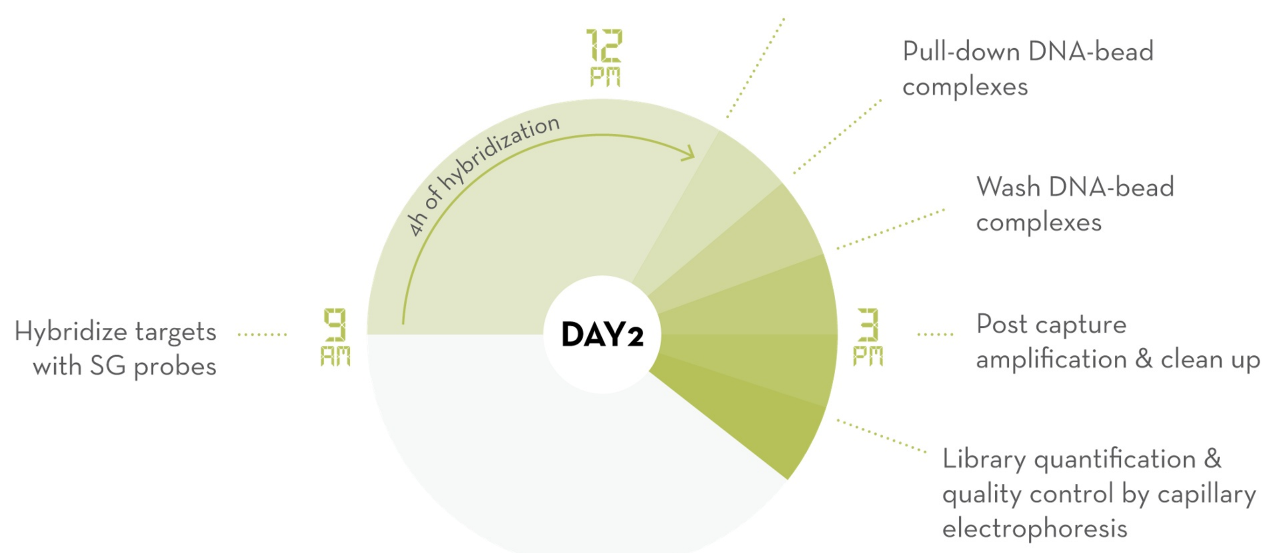

CAPTURE
EASY WORKFLOW
- ONLY 1-4 TUBES TO HANDLE (MULTIPLEX POOLED LIBRARIES)
- ONLY 3 HOURS HANDS-ON TIME

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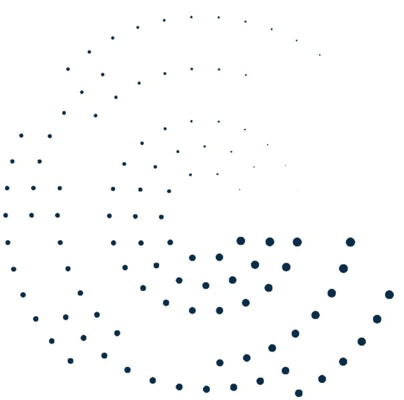
RNA PART



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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™ MYSplus 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
MYSplus 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™ MYSplus, such as Agencourt® AMPure® XP, IDTE Buffer and Nuclease-free water can be found in Box 2.

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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)

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

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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 100 to 500 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
- MYSplus Oligos
- Nuclease-free water

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

Preparation

1. Thaw the RNA samples on ice.
2. Remove the SOPHiA DDM™ MYSplus 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	500 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:

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	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
MYSplus 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.

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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:

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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.

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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:

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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.

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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	-	
Step 1: Initial Denaturation	98	180	
Step 2: Denaturation	98	10	35 cycles
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.

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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.

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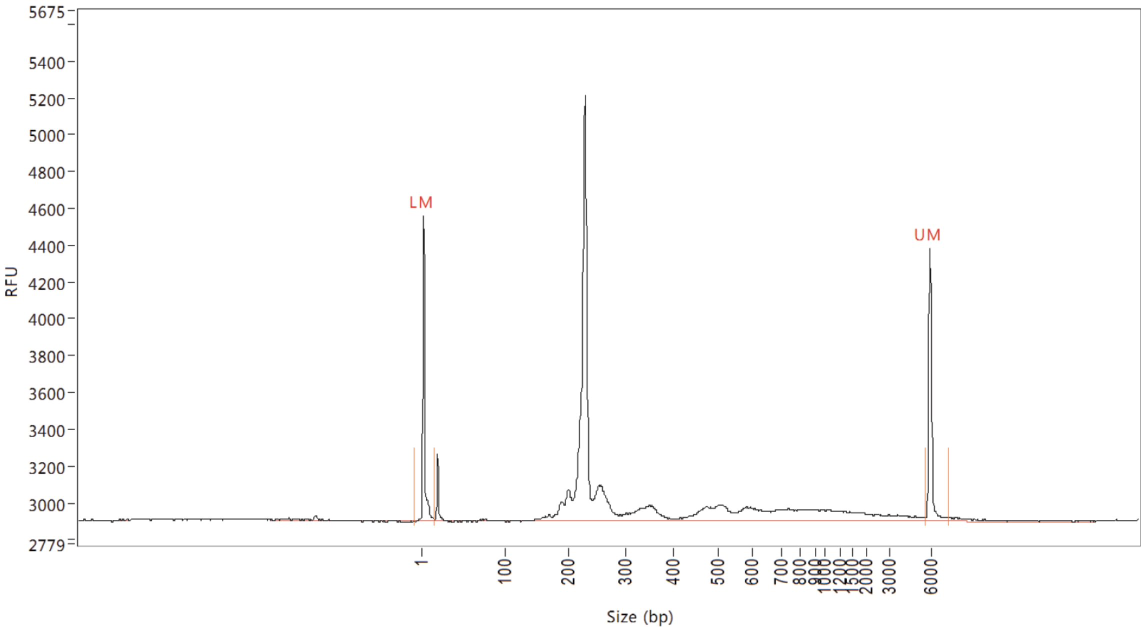
4.2.7 Individual Library Quantification And Quality Control

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 230 bp.



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

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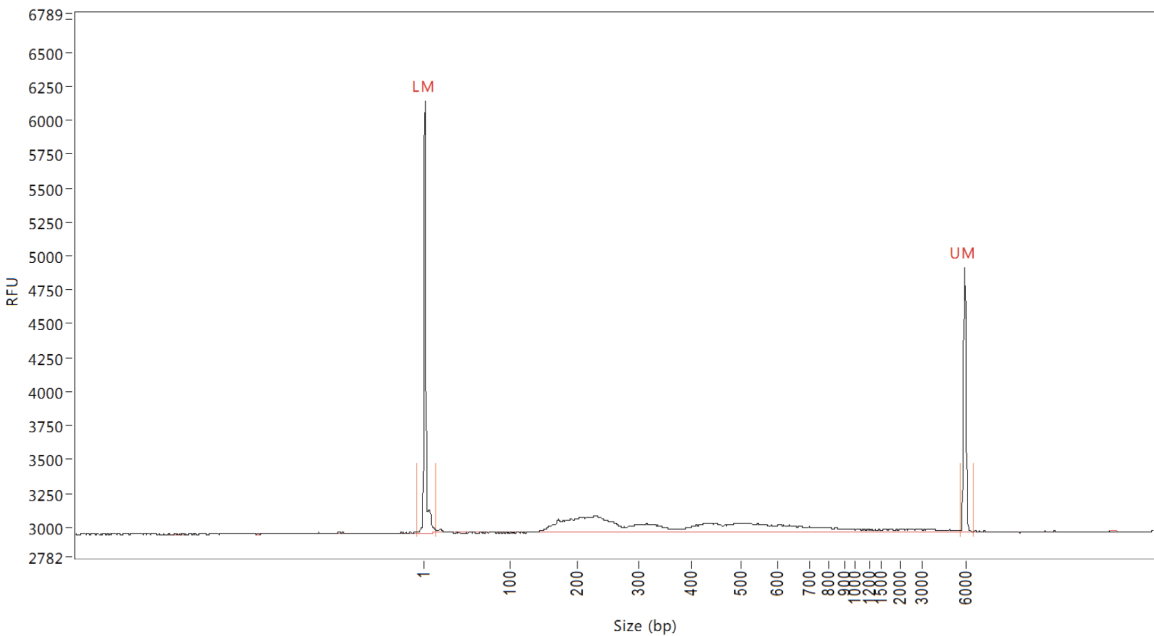

Example of capillary electrophoresis showing **fusion-negative** library obtained with the Agilent Fragment Analyzer™ capillary electrophoresis system. LM: Lower Marker, UM: Upper Marker.

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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.

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4.4 Sequencing Preparations

4.4.1 Materials

- Illumina® Sequencing Kit
- Final pooled MYSplus libraries
- Final captured MYS 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 230 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 MYSplus 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 MYSplus 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 MYSplus libraries can negatively impact the quality of data.

Below is an example for MiSeq® with 24 samples:

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

LIBRARY POOL (10 pM)	VOLUME (µl)
MYS	679
MYSplus (3%)	21

- Load 600 µl of the mix.

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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.

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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 MYSplus 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

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

	TEMPERATURE (°C)	TIME (SECONDS)	
Lid	99	-	
Step 1: Initial Denaturation	98	180	
Step 2: Denaturation	98	10	35 cycles
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.

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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.

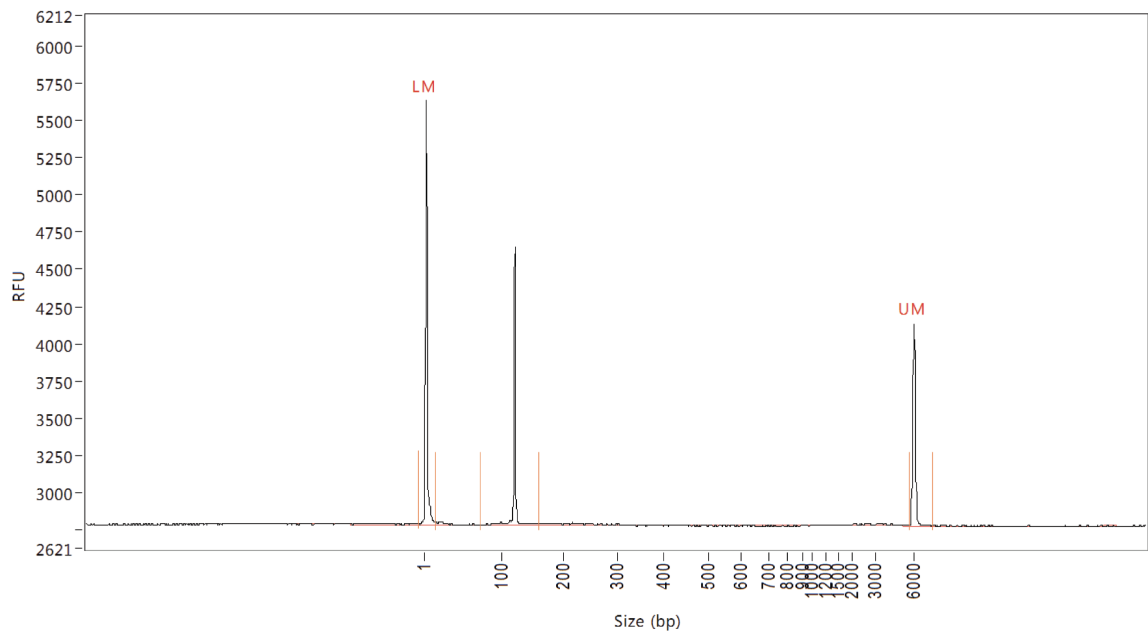
12. Carefully transfer 18 µl of the supernatant (transferring two times 9 µl is recommended at this step) to a new labeled library storage tube.

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.

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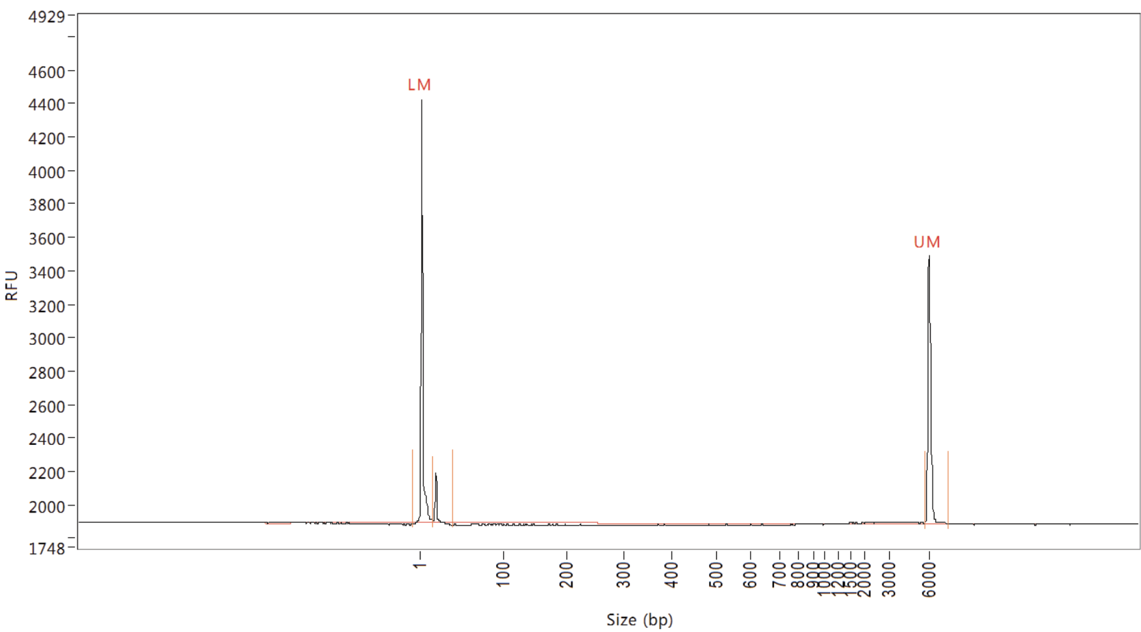

Example of capillary electrophoresis showing **negative control PCR** obtained with the Agilent Fragment Analyzer™ capillary electrophoresis system. LM: Lower Marker, UM: Upper Marker.

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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

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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

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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

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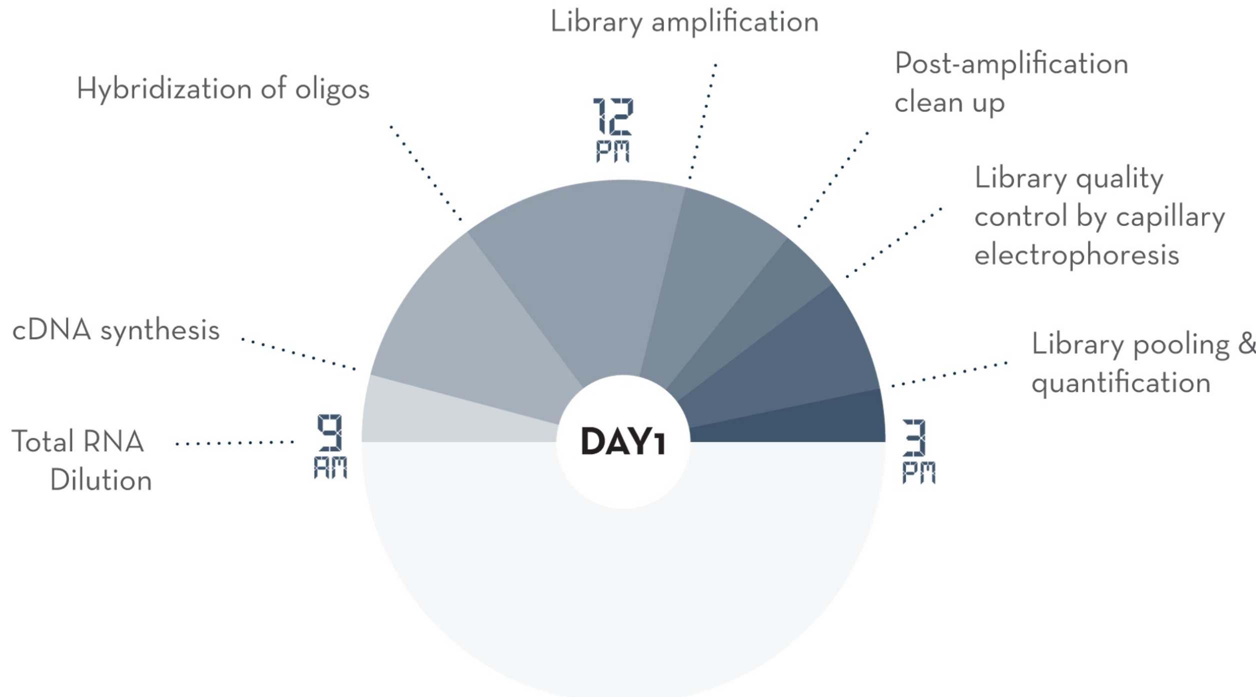
5.3 Appendix VII: General Workflow SOPHiA DDM™ Plus Solution



Library Preparation with SOPHiA GENETICS™ RNA Library Prep Kit

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6 SYMBOLS

SYMBOL	TITLE
	Consult instructions for use
REF	Catalog number
LOT	Batch code (Lot Number)
	Caution
	Manufacturer
	Date of manufacture
	Temperature Limit
	Use-by date
RUO	Research Use Only
Σ	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.

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

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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.

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Document Approvals

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Approval Verdict: Approve	Martin Fritzsche, (mfritzsche@sophiagenetics.com) Technical Approval 31-Jan-2024 17:07:21 GMT+0000

Approval Verdict: Approve	Paolo Florent, (pflorent@sophiagenetics.com) Regulatory Approval 31-Jan-2024 20:44:49 GMT+0000

QA Approval Verdict: Approve	Imane Bouras, (ibouras@sophiagenetics.com) Quality Assurance Approval 01-Feb-2024 09:50:15 GMT+0000

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