USER GUIDE

16, 32, 48 AND 96 SAMPLES

SOPHiA Custom Bundle Solution

Using the SOPHiA DNA Library Preparation Kit I

SOPHiA GENETICS™

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SUMMARY INFORMATION

Product Name	SOPHiA Custom Bundle Solution	
Product Type	Custom Bundle Solution	
Product Family	Molecular kit + analytics	
Algorithm ID	ILL1XG1G4_CNV_5	ILL1XG1G4_CNV_NextSeq_5
Sequencer	Illumina - MiSeq	Illumina - NextSeq
Gene Panel ID	HPD_v4	
Product Version	1.0	
Sample Type	Germline DNA isolated from peripheral blood	
Document ID	ID-60101-57	
Document Version	1.1	
Revision Date	24.Aug.21	

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REF

CS2302ILLRGLL01-32;
CS2302ILLRGLL01-048; CS2302ILLRGLL01-96

RUO

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REVISION HISTORY

DOCUMENT ID/ VERSION	DATE	DESCRIPTION OF CHANGE
ID-60101-57 - 1.1	24.Aug.21	• Page 46: Recommended reads per sample corrected. • Page 10, 38: Minor modifications.

ID-60101-57 - 1.0	03.Aug.21	• 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.2.1.2.16_r3en
		  • PM_T1_5.1.19_r2en
		  • PM_T1_5.1.18_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. INITIAL CONSIDERATIONS

1.1 Kit Content (16, 32, 48 or 96 samples)

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

Depending on the kit format, following components are provided:

COMPONENT	NUMBER OF ITEMS DEPENDING ON KIT FORMAT			
	16 samples kit	32 samples kit	48 samples kit	96 samples kit
BOX 1	1	1	1	2 (48 samples each)
Illumina®-compatible Adapters with Dual Index (in a 96-well plate format included in Box 1)	16	32	48	96 (Plate contained in one of the two Box 1s)
BOX 2	1	1	1	2 (48 samples each)
SOPHiA DNA Library Preparation Kit I	1	2 (16 samples each)	1	2 (48 samples each)

BOX 1 (STORE AT -25°C TO -15°C)

- IDT Blocking Oligo xGen® Universal Blockers - TS Mix (12 µl)
- Human Cot DNA (25 µl)
- Custom Bundle Solution xGen® Lockdown® 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 Adapters with Dual Index in a 96-well plate format (7 µl each):
  see Appendix 1 for adapters display and sequences.

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 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 is provided (see the table on the previous page).

SOPHiA DNA LIBRARY PREPARATION KIT I*

(STORE AT -25°C TO -15°C)

- For 32 samples, two 16 sample kits are provided.
- For 96 samples, two 48 sample kits are provided.

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

* SOPHiA GENETICS is the exclusive distributor of this library preparation kit.

IMPORTANT

xGen Hybridization Buffer Enhancer, used in the Kit Box 1, is a product suspected of causing cancer. May damage fertility or unborn child. May cause damage to organs (blood) through prolonged or repeated exposure if swallowed. Do not handle until all safety precautions have been read and understood. Do not breathe dust/fumes/gas/mist/vapors/spray. Use personal protective equipment as required. If exposed or concerned: Get medical advice/attention. Store locked up. Dispose off contents/container to an approved water disposal plant.

xGen 10X Wash Buffer 1, in the Kit Box 1, may be harmful if swallowed. May be harmful if inhaled. Causes mild skin irritation. Causes serious eye damage. Call a Poison Center or doctor/physician if you feel unwell. If skin irritation occurs: Get medical advice/attention. If in eyes: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. Immediately call a Poison Center or doctor/physician.

1.2 Material Required (not provided)

USER-SUPPLIED MATERIALS (TO BE PURCHASED SEPARATELY)

- KAPA™ Library Amplification kit KK2620 (Roche Cat. No: 07958978001)
- RNase/DNase-free 0.2 ml 8-tube strips
- DNA low binding 1.5 ml tubes
- 1.5 ml tubes
- 50 ml conical tubes
- Filter tips
- Ethanol (molecular biology grade)
- Illumina sequencing reagents

LABORATORY EQUIPMENT

To avoid sample contamination:

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

- Post-PCR zone
  - Capillary electrophoresis system
  - DNA vacuum concentrator
  - Fluorometric quantitation equipment and reagents
  - Magnetic separation rack (1.5 ml tube compatible)
  - Magnetic separation rack (96-well type)
  - Multichannel pipettes (P10 or P20; P100; P200)
  - Table top microcentrifuge (8-tube strips compatible)
  - Thermal cycler (programmable heated lid)
  - Thermoblock or water bath (1.5 ml tube compatible)
  - Vortex mixer

2. LIBRARY PREPARATION

2.1 Genomic DNA Preparation

MATERIALS

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

IMPORTANT

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 260/280 ratio, and within 1.6-2.4 for 260/230. We recommend confirmation of the sample integrity by capillary electrophoresis or an equivalent technique. In order to avoid mistakes with DNA input, an initial dilution to obtain a concentration in the 50 to 100 ng/µl range is recommended. The DNA concentration should be confirmed by a fluorometric quantitation (e.g. Qubit®, Thermo Fisher) and the obtained value used to calculate the final dilution.

PREPARATION

Remove the FX Enhancer from -20°C storage and thaw out 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 has to be taken into consideration before starting.

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

* For 96 samples two 48 sample kits are provided, which includes 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 high quality genomic DNA (gDNA) sample into the appropriate number of PCR strips, in the following manner:

gDNA DILUTION	
gDNA	200 ng
IDTE	Complete to 30 µl

- 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.

- Depending on the number of samples, proceed as follows:
  - If processing **4 samples**, add 5 µl FX enhancer to each tube of the 4-tube strip containing 30 µl gDNA 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 (in µl)	11.5	17.5	11.5	17.5	24	36

    b. Using a multichannel pipette, add 5 µl of the FX Enhancer from the above tubes to the 30 µl of gDNA 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.

3. Keep on ice until enzymatic fragmentation reaction setup.

2.2 Pre-mixes and Reagents Preparation

COMPONENTS AND REAGENTS

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

PREPARATION

- Remove the SOPHiA DNA Library Prep Kit I components from -20°C storage and thaw on ice.
- Remove the Dual Index Adapters Plate from -20°C storage and put it into 4°C refrigerator for later use.
- Remove the AMPure XP beads from 2-8°C storage and let them equilibrate at room temperature for at least 30 minutes.
- Prepare fresh 80% Ethanol (volume according to the following scheme based on number of reactions) :

80% ETHANOL							
Number of Reactions	**4**	**8**	**12**	**16**	**24**	**32**	**48**
80% Ethanol (in ml)	10	20	30	30	40	50	70

- Once the SOPHiA DNA Library Prep Kit I components are thawed, mix the reagents by inverting the tube 5-10 times and briefly spin in a microcentrifuge.

PRE-MIXES

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

FX REACTION PRE-MIX							
Number of Reactions	**4**	**8**	**12**	**16**	**24**	**32**	**48**
FX Buffer 10x (in µl)	23.6	47.1	75	95	150	190	300
FX Enzyme Mix (in 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 Ligation Buffer 5x (in µl)	95	190	300	380	600	760	1200
DNA Ligase (in µl)	47.5	95	150	190	300	380	600
Nuclease-free water (in 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 Ligation Buffer 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 (in µl)	115	230	345	460	690	920	1380
Primer Mix Illumina Library Amp (in µl)	6.9	13.8	20.7	27.6	41.4	55.2	82.8
Nuclease-free water (in 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.

2.3 Enzymatic Fragmentation, End Repair and A-Tailing

MATERIALS

- Diluted and conditioned double stranded gDNA in 35 µl
- FX reaction pre-mix
- RNase/DNase-free 0.2 ml 8-tube strips

PREPARATION

- Program the thermal cycler for FXFrag with the following settings:

	TEMPERATURE (°C)	TIME (MINUTES)
Lid	70	
Step 1	4	1
Step 2	32	5
Step 3	65	30
Hold	4	∞

- Start the FXFrag 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 Reaction 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 Reaction pre-mix (in 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 reaction pre-mix to each of the 35 µl of gDNA samples (total of 50 µl in 4 or 8-tube strips).
   - Using a pipette set to 40 µl (multichannel if processing 8 or more samples), mix thoroughly by pipetting up and down 5 times and briefly spin in a microcentrifuge.
3. Place in the thermal cycler and continue the FXFrag program.

Proceed immediately to Ligation.

2.4 Ligation

MATERIALS

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

PREPARATION

- Remove the Dual Index Adapters 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 1 for the respective plate format.
- During the FX fragmentation, prepare new PCR strips with 5 µl of different Dual Index Adapters per tube as per your indexing strategy, according to the following scheme:

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

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

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 Ligation pre-mix in a new set of PCR 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 (in µl)	100	160	100	160	200	320

2. Using a multichannel pipette, transfer the 50 µl of each FX 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.
4. Using a multichannel pipette if processing 8 or more samples, add 45 µl of Ligation pre-mix to each FX 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.**

2.5 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 3 minutes or until the liquid becomes clear.
4. Carefully discard 170 µl of 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. 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 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. Incubate at room temperature for 5 minutes and spin briefly if required.
11. Place the 4 or 8-tube strips on a 96-well plate format magnetic rack for 3 minutes or until liquid becomes clear.
12. Using a multichannel pipette, carefully transfer 100 µl of the supernatant to new, labeled 4 or 8-tube strips.

    Proceed to Dual Size Selection.

2.6 Dual Size Selection

MATERIALS

- Ligated 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 ligated 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 strips on a 96-well plate format magnetic rack for 3 minutes or until liquid becomes clear.
4. Using a multichannel pipette carefully transfer 140 µl of the supernatant to new, labeled 4 or 8-tube strips 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.
6. Place the 4 or 8-tube strips on a 96-well plate format magnetic rack for 3 minutes or until liquid becomes clear.
7. Carefully discard 150 µl of the 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.

   Let it/them stand 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.

2.7 Library Amplification

MATERIALS

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

PREPARATION

- Program the thermal cycler for LibAmp 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	
Step 4: Extension	72	30	
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**, proceed as follows:
     a. 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 (in µl)	65	100	65	100	130	200

2. Assemble the reaction as follows:
   - Using a multichannel pipette if processing 8 or more samples, add 30 µl of PCR pre-mix to the dual size selected ligation products and beads (total volume 50 µl = 30 µl + 20 µl).
   - Mix thoroughly by pipetting up and down 10 times and spin briefly.

3. Place the tubes in the thermal cycler and run the LibAmp program.

Safe stopping point overnight at 4°C.

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 3 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 it/them 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 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 -20°C for longer storage.

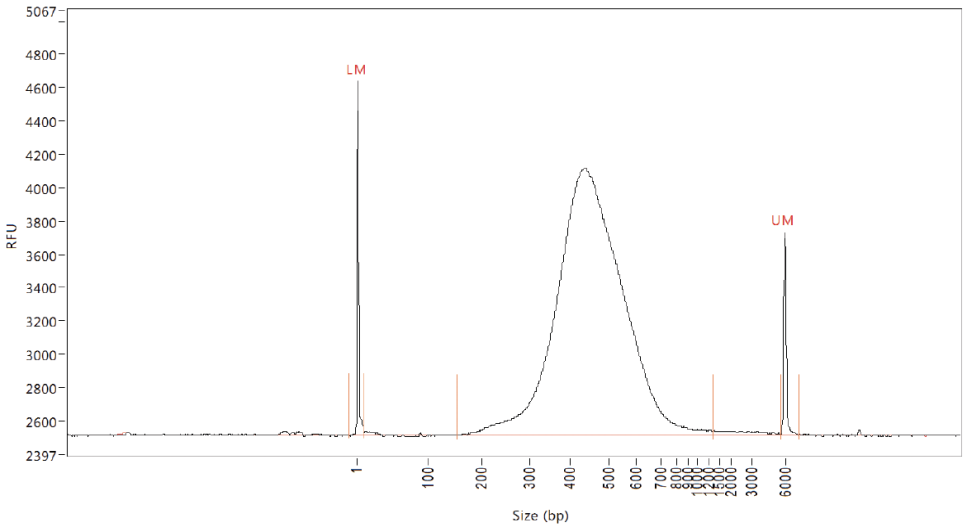
2.9 Individual Library Quantification and Quality Control

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 mentioned above).
3. Quality control the libraries by analyzing their profile via capillary electrophoresis. Library DNA fragments should have a size distribution between 300bp and 700bp.



Example of a DNA library distribution obtained with the Agilent Fragment Analyzer capillary electrophoresis system.

3. CAPTURE

3.1 Library Pooling

MATERIALS

- Individual libraries
- Human Cot DNA
- Blocking oligos xGen Universal Blockers - TS Mix
- DNA low-binding 1.5 ml tubes

PROCEDURE

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

NUMBER OF CAPTURES (Refer to the table in point 3)	1	2	3	4
Human Cot DNA (in µl)	5	11	16.5	22
Blocking oligos xGen Universal Blockers - TS Mix (in µl)	2	4.4	6.6	8.8

2. If performing two or more captures, pipette 7 µl of the above pre-mix into individual DNA low-binding tubes.
3. To the individual tubes containing the above 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

4. Mix thoroughly by pipetting up and down 10 times and spin briefly.
5. 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.

3.2 Hybridization

MATERIALS

- Lyophilized libraries
- 2x Hybridization Buffer
- Hybridization Buffer Enhancer
- Custom Bundle Solution xGen® Lockdown 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:

NUMBER OF CAPTURES	1	2	3	4
2x Hybridization Buffer (in µl)	8.5	18.7	28.05	37.4
Hybridization Buffer Enhancer (in µl)	3.4	7.48	11.22	14.96
Nuclease-free Water (in 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 3 to 5 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 pages 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**
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.

3.3 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 4-hour 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.

3.4 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 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 directly to Wash Streptavidin Beads to Remove Unbound DNA.

3.5 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 Bufer I (1/3 at 65°C and 2/3 at room temperature
- 1x Wash Buffer II
- 1x Wash Buffer III
- 1x Stringent Wash Buffer (at 65°C)
- Nuclease-free water
- IDTE

PROCEDURE

Work to 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:

- **When placing the first tube in thermoblock at 65°C for the 1st incubation of 5 min (point 5), start a timer.**
- **Begin processing the second tube.**
- **When placing the second tube at 65°C, notice the time separating the tubes and ensure to respect this time gap along steps 2 to 8 in order 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 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.
   Note: 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.

3.6 Post-capture Amplification

MATERIALS

- Streptavidin beads/nuclease-free water suspension (20 µl)
- 2x KAPA HiFi HotStart ReadyMix
- 10x Library Amplification Primer Mix
- Nuclease-free water

PREPARATION

- Program the thermal cycler for PostCapAmp using the following settings:

	TEMPERATURE (°C)	TIME (SECONDS)	
Lid	99		
Step 1: Initial Denaturation	98	45	
Step 2: Denaturation	98	15	X cycles (please see Appendix 4 for details)
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**
2x KAPA HiFi HotStart ReadyMix (in µl)	25	55	82.5	110
10x Library Amplification Primer Mix (in µl)	2.5	5.5	8.25	11
Nuclease-free water (in 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 PostCapAmp program.

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

3.7 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.
3. Place tube(s) on a magnetic rack for 3 minutes or until the liquid becomes clear.
4. Carefully discard 90 µl supernatant using a multichannel pipette.
   Keep 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 it/them 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 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.
11. Place tube(s) on a 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 -20°C for longer storage.

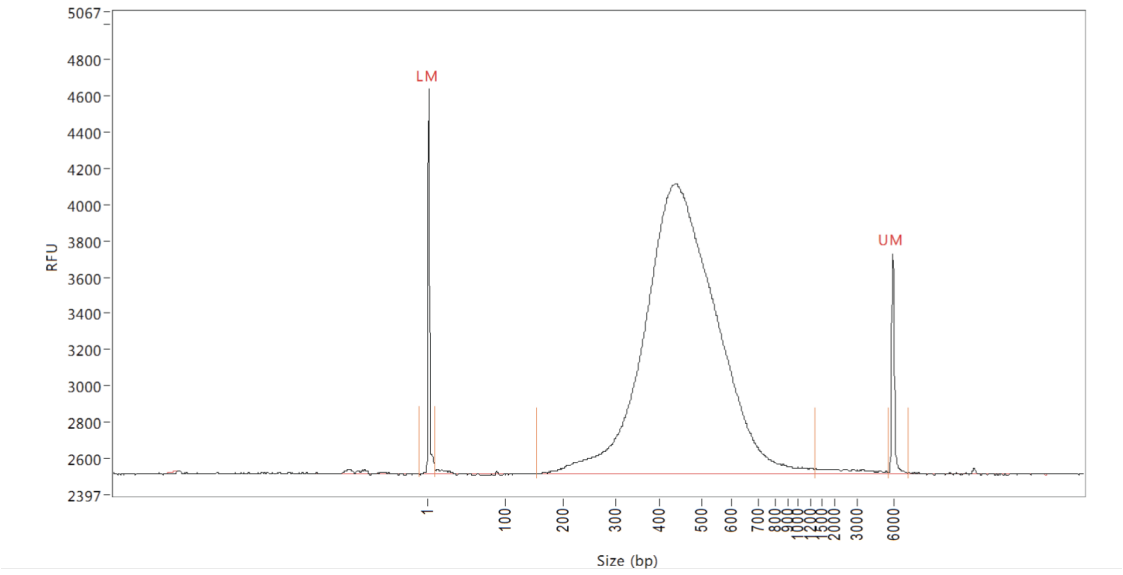
3.8 Final Library Quantification and Quality Control

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 pool of libraries by analyzing their profile via capillary electrophoresis. Library DNA fragments should have a size distribution between 300bp and 700bp.



Example of captured library pool size distribution obtained with the Agilent Fragment Analyzer capillary electrophoresis system.

4. SEQUENCING

4.1 Library Preparation for Sequencing

MATERIALS

- Illumina Sequencing Reagents and Flow Cell (please see Appendix 4 for details)
- Final captured libraries
- EBT Buffer or similar

PROCEDURE

1. Determine the molarity of each pool with average size of the library (peak size in base pairs) and concentration (ng/µl) obtained during step 3.8 as follows:

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

2. Dilute each pool to 4 nM and mix each dilution in equal amount (e.g. 5 µl of each). Mix it well and use this dilution according to Illumina standard denaturation recommendation.
3. Load an x pM dilution* of the denatured libraries**.

* For dilution details, please see Appendix 4.

** Loading concentration and library dilution depend on the sequencing instrument used (please see Appendix 4 for details).

Appendix 1 Dual Index Adapter Plates

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									

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					

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			

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

i5	i5 sequences for sample sheet NovaSeq, MiSeq, HiSeq 2000/2500	i5 sequences for sample sheet MiniSeq, NextSeq, HiSeq 3000/4000
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 sequences for sample sheet
D701	ATTACTCG
D702	TCCGGAGA
D703	CGCTCATT
D704	GAGATTCC
D705	ATTCAGAA
D706	GAATTCGT
D707	CTGAAGCT
D708	TAATGCGC
D709	CGGCTATG
D710	TCCGCGAA
D711	TCTCGCGC
D712	AGCGATAG

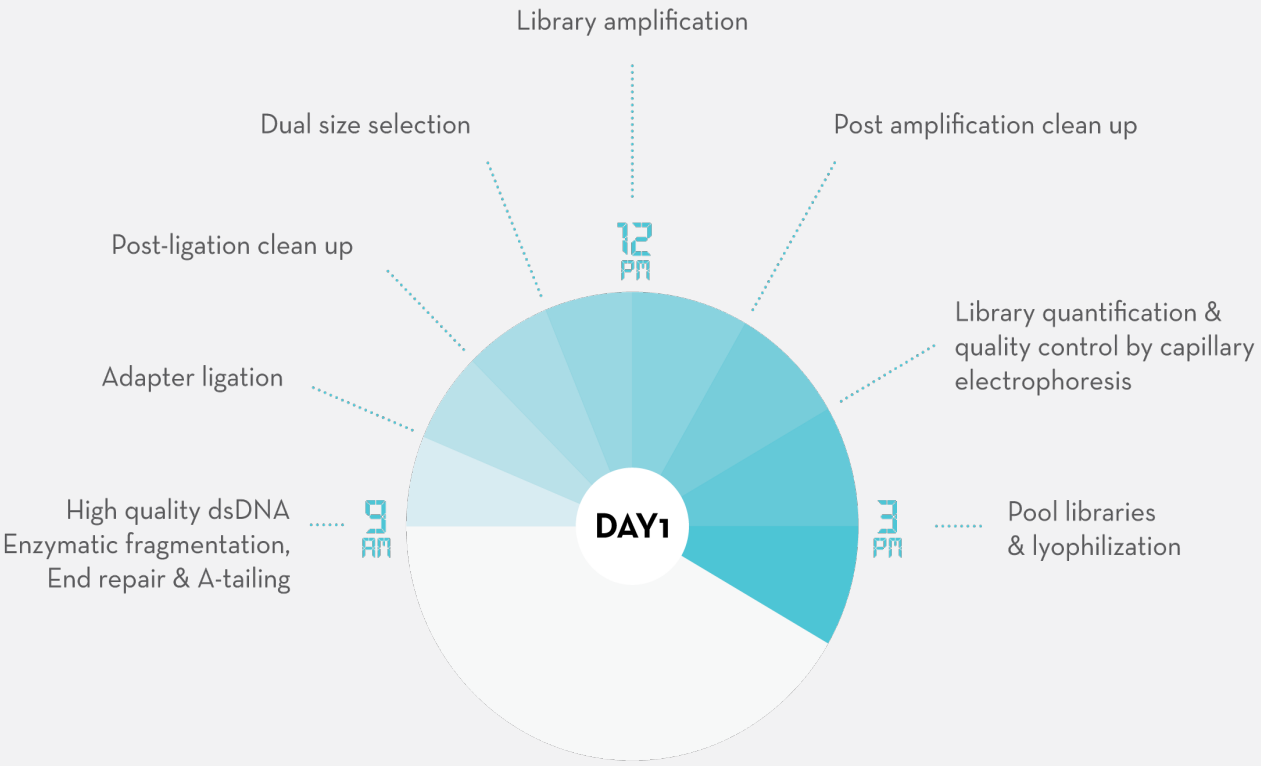
Appendix 2 Laboratory Equipment used in SOPHiA GENETICS Laboratory

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

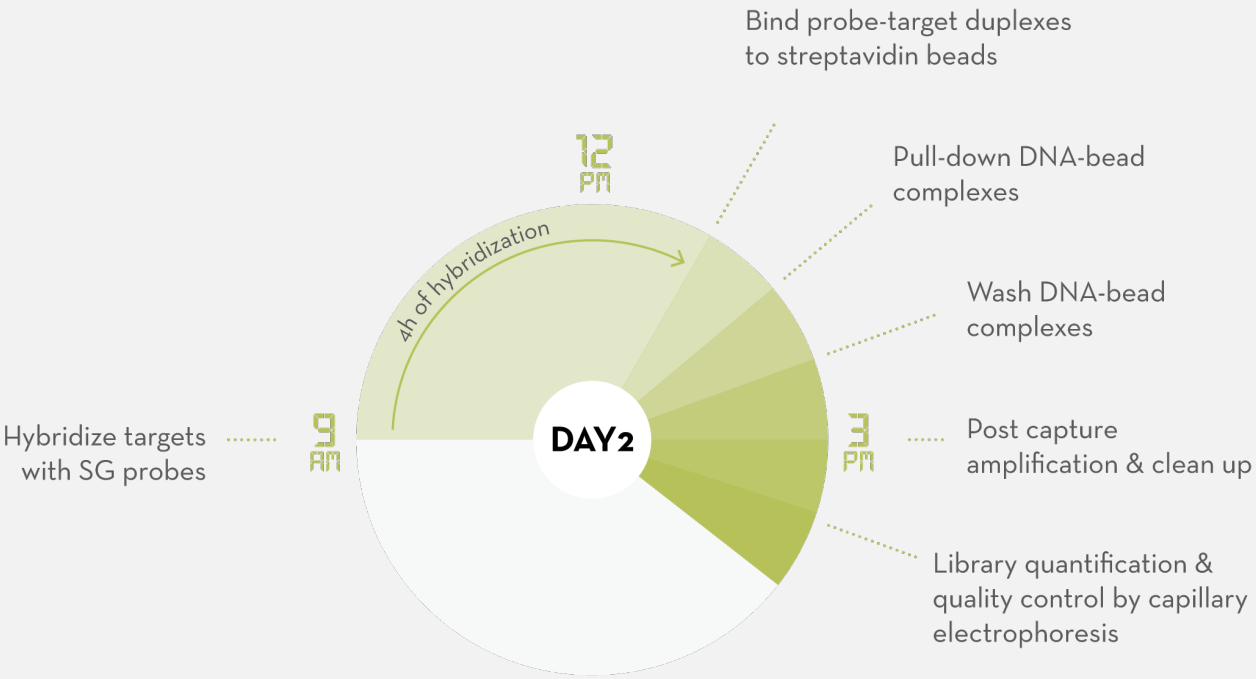
PRE-PCR ZONE	SUPPLIER	PRODUCT NAME	CATALOG N°
Vortex mixer	Scientific Industries	Vortex Genie 2	SI-0236
Table top microcentrifuge (8-tube strips compatible)	Starlab	Mini Centrifuge	N2631-0007
Magnetic separation rack 96-well type	Alpaqua	96S Super Magnet Plate	A001322
Magnetic separation rack 96-well 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 pro-grammable heated lid	Biometra	TAdvanced 96	
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

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

Appendix 3 General Workflow - SOPHiA Capture Solutions



LIBRARY PREPARATION
WITH SOPHiA DNA LIBRARY PREP KIT I



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

Appendix 4 Custom Solution Specifications

Gene Panel	**Histrionic Personality Disorder (HPD_v4)**
Algorithm ID	**MiSeq: ILL1XG1G4_CNV_5** **NextSeq: ILL1XG1G4_CNV_NextSeq_5**

Gene list/Target regions: The panel includes:

All protein-coding exons for all transcripts of a given gene for the following genes with +-25bp flanking into the intron and including 5' UTR exons that contain coding sequence:
APC, ATM, ATP7B, AXIN2, BAP1, BARD1, BMPR1A, BRCA1, BRCA2, BRIP1, CDH1, CDK4, CDKN2A, CFTR, CHEK2, COL1A1, COL1A2, COL4A3, COL4A4, COL4A5, CTNNA1, CYP21A2, DMD, EPCAM, FAM175A, FBN1, GALNT12, GREM1, HOXB13, MLH1, MRE11, MSH2, MSH3, MSH6, MUTYH, NBN, NF1, NTHL1, PALB2, PIK3CA, PKD1, PKD2, PKHD1, PMS2, POLD1, POLE, PTEN, RAD50, RAD51C, RAD51D, RNF43, RPS20, SCG5, SMAD4, SMARCA4, SPRED1, STK11, TP53, TSC1, TSC2, XRCC2.

POST CAPTURE AMPLIFICATION PCR CYCLES	13

READ LENGTH (IN BP)	RECOMMENDED READS PER SAMPLE
150	3.9 Million
≥ 200	2.9 Million

Available kit formats and sequencing recommendations*:

TOTAL REACTIONS IN THE KIT	KIT FORMAT	INSTRUMENT	READ LENGTH (IN BP)	SAMPLES PER RUN ACCORDING TO THE KIT FORMAT	TOTAL POSSIBLE SAMPLES PER RUN	LIBRARY DILUTION	LOADING DILUTION
16	4 x 4	MiSeq v3	300	16	16	4 nM	10 pM
		NextSeq 550 Mid-Output	150	16	67		1.3 pM
		NextSeq 550 High-Output	150	16	207		1.3 pM

TOTAL REACTIONS IN THE KIT	KIT FORMAT	INSTRUMENT	READ LENGTH (IN BP)	SAMPLES PER RUN ACCORDING TO THE KIT FORMAT	TOTAL POSSIBLE SAMPLES PER RUN	LIBRARY DILUTION	LOADING DILUTION
32	4 x 8	MiSeq v3	300	16	16	4 nM	10 pM
		NextSeq 550 Mid-Output	150	32	67		1.3 pM
		NextSeq 550 High-Output	150	32	207		1.3 pM
48	4 x 12	MiSeq v3	300	12	16	4 nM	10 pM
		NextSeq 550 Mid-Output	150	48	67		1.3 pM
		NextSeq 550 High-Output	150	48	207		1.3 pM
96	(4 x 12) x 2	MiSeq v3	300	12	16	4 nM	10 pM
		NextSeq 550 Mid-Output	150	60	67		1.3 pM
		NextSeq 550 High-Output	150	96	207		1.3 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 10 pM dilution.

For MiniSeq, load a 1.8 pM dilution.

6. Symbols

	User Manual, Operating Instructions
	Research Use Only
	Reference Number
	Manufacturer

7. Support

In case of difficulty using SOPHiA DDM™ please consult the troubleshooting section of the "General information SOPHiA DDM™ usage" document, or contact our support line and e-mail mentioned on the "Summary Information" page of this user guide.