CytoSure
SureSeq

A Sysmex Group Company

OGT NGS Universal Library Preparation Handbook

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1 Introduction

The Universal NGS Complete Workflow has been developed and optimised for use with SureSeq™ and CytoSure® gene panel baits designed by Oxford Gene Technology (OGT) to deliver accurate detection of a wide range of variants.

The OGT Next Generation Sequencing (NGS) range is compatible with Illumina HiSeq™, NovaSeq™, NextSeq™ and MiSeq™ chemistries.

There are sufficient reagents contained within the 24 reaction kit to perform 3 pools of 8 samples. For the 96 reaction kit, there are sufficient reagents to perform 4 pools of 24 samples.

1.1 Pack Contents

Component	Contents	Catalogue number (24 reactions)	Catalogue number (96 reactions)
Box 1 (shipped at –20°C, store at –20°C)	Universal Library Preparation Kit	770100-24	770100-96
Box 2 (shipped at –20°C, store at –20°C)	Universal Index Adapters	770200-24	770200-96
Box 3 (shipped at –20°C, store at –20°C)	Universal Hybridisation & Wash Kit	770400-24	770400-96
Box 4 (shipped at 4°C, store at 4°C)	Universal Bead Kit	770300-24	770300-96

1.2 Storage

The kit should be used before the expiry date indicated on the kit label. The Universal Library Preparation Kit, Universal Index Adapters, SureSeq and CytoSure panel baits and Universal Hybridisation & Wash Kit should be stored at –20°C.The Universal bead kit should be stored at 4°C

1.3 Safety

Handling of the SureSeq and CytoSure panel baits should be carried out by trained laboratory staff in accordance with good laboratory practice, using the correct protective equipment such as laboratory coats, safety glasses and gloves.
The OGT Universal Hybridisation & Wash Kit does contain chemicals which are potentially hazardous when mishandled, particular care should be given to both Formamide and the Hybridisation Buffer.

Ensure that all Operators have familiarised themselves with the SDS and relevant Risk Assessments before proceeding with the Protocol.

1.4 Intended Use

These products are for Research Use Only assays. This kit is designed to be used by suitably trained personnel using DNA extracted from a variety of tissues including blood and bone marrow.

2 Equipment and reagents required

2.1 Equipment required (not supplied)

- Agilent® 4200 TapeStation (cat. no. G2991BA, or equivalent) and relevant reagents
- 2 x Thermal cyclers (e.g. BioRad MJ Research DNA Engine PTC-200) or equivalent
- Laboratory vortex mixer + adapter for a standard microwell plate (e.g. IKA™ MS 3 Digital Vortex Mixer or BioShake iQ Thermomixer)
- Microfuge for standard 1.5 ml tubes and 8-Strip PCR tubes
- Appropriate magnetic rack for 96–well microwell plates and 1.5 ml tubes
- Qubit® fluorometer (Life Technologies cat. no. Q32857)
- Thermo Scientific NanoDrop™
- 20-200 µl and 1-10 µl 8-Channel pipette
- Illumina® MiSeq, NextSeq, NovaSeq or HiSeq
- *Optional:* Hot block for 1.5 ml tubes

2.2 Reagents and consumables required (not supplied)

- Molecular Biology Grade 100% Ethanol (Sigma Aldrich, cat. no. E7023 or equivalent)
- Molecular Biology Grade water (Sigma Aldrich, cat. no. W4502-1L or equivalent)
- Quant-iT™ dsDNA HS Assay Kit and Quant-iT dsDNA BR Assay Kit (Life Technologies, cat. no. Q32850, Q32854)
- Qubit Assay Tubes (Life Technologies, cat. no. Q32856)
- Agilent D1000 and High Sensitivity D1000 Reagents and ScreenTapes (cat. no. 5067-5582, 5067-5583, 5067-5584, 5067-5585)
- DNA 1.5ml LoBind Tubes (Eppendorf, cat. no. 022431021 or equivalent)
- 96-well microwell plates (Starlab, cat. no. E1403-0100) and 8-Strip PCR tubes (Starlab cat. no. A1402-3700) or equivalent
- Sequencing reagents required for the MiSeq / HiSeq / NextSeq (e.g. Illumina, cat. no. MS–102-2002, MS-102-2022, GD-401-3001, FC-401-3001)
- 5.0 M Sodium hydroxide solution, molecular biology-grade (e.g. Sigma-Aldrich, cat. no. S8263)
- 15 ml or 50 ml Falcon Tubes or similar
- *Optional:* Gel-loading tips (1-200 µl)

2.3 Interpret Software

Raw data files generated from the Illumina sequencers can be analysed using the Interpret Software and turned into interactive reports. The Interpret Software is OGT's powerful, standalone data analysis package that is provided with the kit.

3 Workflow overview

For ordering information about OGT products visit www.ogt.com.

The following section contains instructions for sample library production specific to the Illumina sequencing platform. For each sample, an individual library preparation is performed. Each sample is tagged with an index (barcode) sequence, as well as a unique molecular identifier (UMI) for error correction and increased accuracy during sequencing. Samples are amplified and then pooled into sets of eight. Each pool is hybridized, captured, and then amplified ready for sequencing.

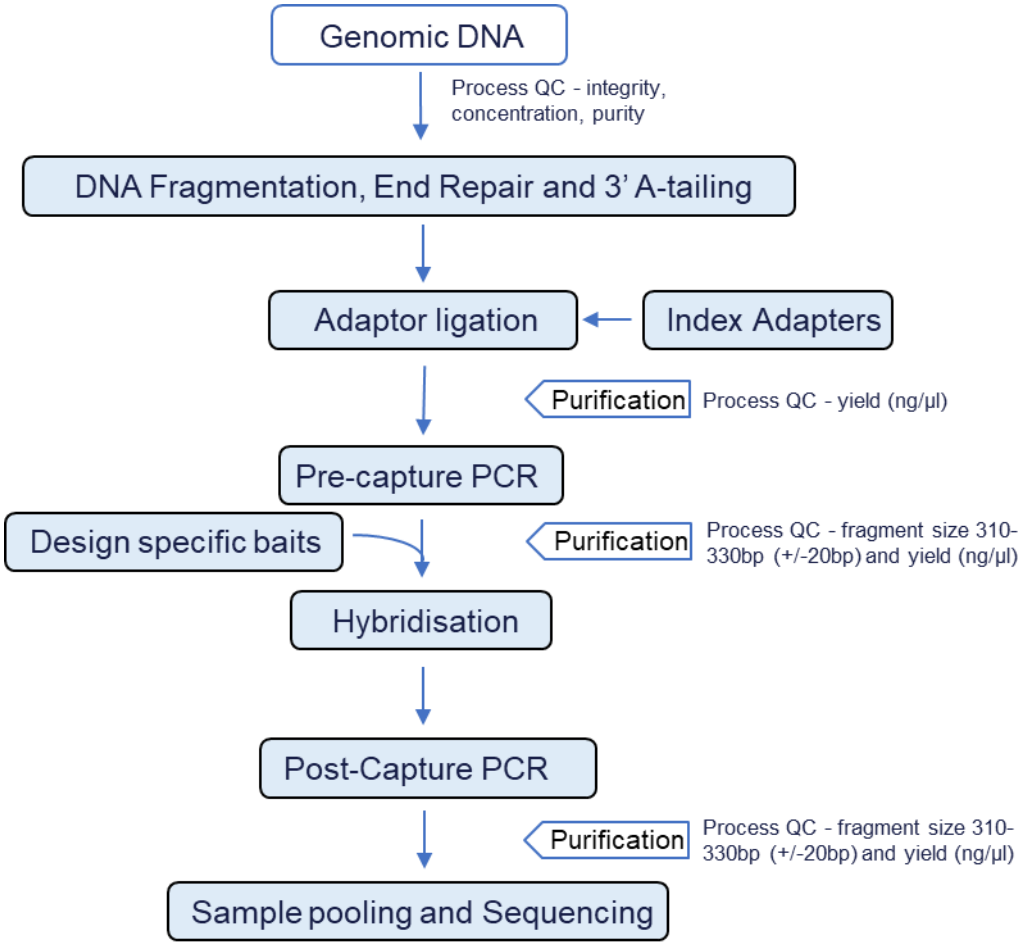


Figure 1:Workflow of sample library preparation indicating the expected DNA fragment size at each step of the procedure.

4 Sample requirements

This protocol has been optimised for DNA inputs of 500ng. However, it can be used for DNA inputs ranging from 200 ng up to 500 ng.

4.1 Sample throughput

- Low-throughput (LT) protocol: all incubations are performed in 0.2 ml tubes. Post incubation, each reaction volume is transferred to a fresh 1.5 ml tube and sample clean-up performed using the volumes highlighted in blue (marked with ♦). All wash steps are performed using a magnetic rack capable of holding 1.5 ml tubes.
- High-throughput (HT) protocol: all incubations are performed in 0.2 ml tubes or a non-skirted PCR plate. Post incubation, the sample purification is performed in the same 0.2 ml tube or non-skirted PCR plates using volumes highlighted in red (marked with •). All wash steps are performed using a magnetic rack, capable of holding 0.2 ml tubes or a 96-well non-skirted PCR plate (0.2 ml volume).

5 Sample Quality Control

Assessing sample integrity, concentration, and purity:

- Concentration — Use Life Technology Qubit (or similar)
- DNA integrity — Use Agilent 4200 TapeStation (or similar)
- Purity — Use Thermo Scientific NanoDrop™ (or similar)

Determination of the concentration of gDNA sample is mandatory for all samples prior to starting the Universal Library Preparation protocol. In addition, DNA integrity and purity assessment (steps 5.2 – 5.3) are optional for DNA derived from whole blood samples.

5.1 DNA concentration - Qubit dsDNA BR Assay Kit

Refer to Manufacturers user guide for the Life Technology Qubit. Key steps are described below:

1. Prepare the Qubit working solution by diluting the Qubit reagent 1:200 in Qubit buffer.
2. Aliquot 190 µl of Qubit working solution into the two tubes used for standards and 199 µl of Qubit working solution into each of the tubes used for samples.
3. Add 10 µl of each Qubit standard and 1 µl of sample to the appropriate tubes.
4. Mix by vortexing for 2–3 sec being careful not to generate bubbles.
5. Incubate the tubes at room temperature for 2 min.
6. Measure DNA concentrations following the onscreen prompts.
7. **Note:** Samples with an initial DNA concentration > 100 ng/µl should be prediluted to 40 – 100 ng/µl with TE Buffer provided in the kit. Confirm the DNA concentration using the Qubit dsDNA BR assay. The precise quantification of DNA input is essential for reproducible fragmentation results.

5.2 DNA integrity — Genomic DNA ScreenTape

Optional: This step is important if high quality DNA is not used. Refer to Manufacturers user guide for the Agilent Tapestation. Key steps are described below:

1. Add 1 µl of Genomic DNA Ladder into the first tube/well of the strip tube or plate.
2. For each sample under assessment, add 1 µl of DNA sample to 10 µl of Genomic DNA Sample Buffer. Seal all the tubes/wells.
3. Vortex the tubes or plate for 5 sec.
4. Briefly spin down to collect the sample at the bottom of the tubes/wells.
5. Load strip of tubes or plate into the Agilent 2200 TapeStation.
6. Highlight the required samples on the controller software and fill in the sample names in the sample sheet.
7. Select “Start” and provide a filename to save your results.

8. Check that the electropherogram shows that the integrity of the gDNA is intact with an even distribution and maximum peak size >5000 bp.

5.3 Purity — NanoDrop

Optional: This step is important if high quality DNA is not used.

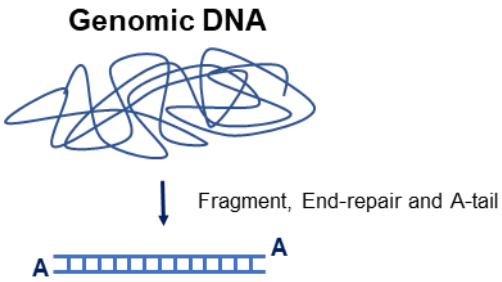
Refer to Manufacturers user guide for the NanoDrop. Key steps are described below:

1. Use the ‘Nucleic Acid’ and ‘DNA-50’ setting
2. Load 1 µl of each sample onto the pedestal.
3. Click “Measure”.
4. Record the readings for 260/230, 260/280 and the concentration (ng/µl).

An OD 260/280 ratio of 1.8 to 2.0 and OD 260/230 ratio of 2.0 to 2.2 is recommended. Use of DNA samples with lower ratios may result in poor performance.

Contact your FAS if you require any advice as to the quality of your samples.

6 DNA Fragmentation, End Repair, and 3’ End A-tailing



Genomic DNA is enzymatically fragmented. The fragmented dsDNA is repaired with enzymes in the Fragmentation and ER mix to create blunt ends. At the same time, a 3’ adenine overhang is created in preparation for Adapter ligation.

Estimated time: 60 min for 8 - 24 samples. Hands-on time: 15 min.

6.1 Preparation

- Remove TE Buffer (blue lid) from storage (–15°C to –25°C). Allow to thaw to room temperature.

  Note: Use only the TE Buffer (blue lid) provided in the kit for preparation of DNA samples (10mM Tris, 1mM EDTA). Use of other TE formulations (e.g., 0.1x TE) or water may affect fragmentation results

- Remove Step 1: Frag + ER Buffer (orange lid) from storage (–15°C to –25°C). Allow to thaw to room temperature and place on ice. Ensure that all components in the Buffer are well dissolved. If necessary, mix by pipetting or vortex until dissolved.
- Remove Step 1 Frag + ER Enzyme (orange lid) from storage (–15°C to –25°C) and place on ice.
- Prepare the thermal cycler using the settings as shown in Table 2. **Preheat** the thermal cycler to 37°C. Where possible, set the heated lid to 75°C, alternatively, have the pre-set heat lid activated.

6.2 DNA Fragmentation, End Repair and 3’ End A-Tail

1. Prepare required amount of DNA with the TE Buffer (blue lid) provided in 0.2 ml tubes/wells to a total volume of 26.5 µl. Keep the DNA samples on ice.

   Note: Samples with an initial DNA concentration > 100 ng/µl should be prediluted to 40 – 100 ng/µl with TE Buffer provided in the kit. Confirm the DNA concentration using the Qubit dsDNA BR assay. The precise quantification of DNA input is essential for reproducible fragmentation results.

2. Briefly vortex the tubes, spin down, and place on ice.
3. Label a new set of PCR tubes for each sample and place on ice.

4. Prepare the Fragmentation and ER Master Mix as shown in Table 1: Fragmentation and ER Master MixTable 1. Mix all reagents on the vortex mixer for 3 sec and spin down briefly. Keep all reagents on ice. Mix the Fragmentation and ER Master Mix on the vortex mixer and spin down.

Reagent	**1x library (µl)**	**___ x library (µl)**	**16x library (µl)** (includes excess)	**24x library (µl)** (includes excess)
DNA sample	26.5	-	-	-
Step 1: Frag + ER Buffer (orange lid)	7		119	182
Step 1: Frag + ER Enzyme (orange lid)	1.5		25.5	39
TOTAL	**35**		**144.5**	**221**

Table 1: Fragmentation and ER Master Mix

5. Pipette 8.5 µl Fragmentation and ER Master Mix into each well of the prepared empty tubes from step 3. Keep on ice.
6. Using a multi-channel pipette add 26.5 µl of DNA to each well containing the Fragmentation and ER Master Mix.

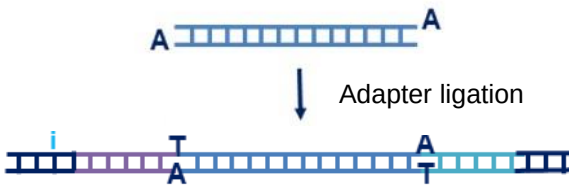
   IMPORTANT: Samples should be kept on ice until transferred to the thermal cycler
7. Mix on a vortex mixer for 3 seconds.
8. Spin the tubes briefly and immediately transfer to the **preheated** thermal cycler. Start the programme.
   IMPORTANT: Ensure thermal cycler is preheated and ready to begin before placing samples inside. See notes in 6.1 Preparation

Step	**Temperature (°C)**	**Time**
1	37	20 min
2	65	30 min
3	4	Hold

Table 2: Fragmentation and ER Incubation profile

IMPORTANT: Keep samples at 4°C or on ice until you are ready to proceed with Adapter ligation. If necessary, samples can be stored at –20°C; however, a loss in yield (~20%) may be observed. We recommend continuing with Adapter ligation immediately.

7 Adapter Ligation



Illumina compatible Adapter sequences are ligated onto the repaired dsDNA fragments with DNA ligase, using the 3' overhang created during end repair/A tailing. Adapters contain UMI (unique molecular identifier) sequences and unique sample indexes.

Estimated time: 35 min for 8 - 24 samples. Hands-on time: 15 min.

7.1 Preparation

- Take the Mag-Bind® TotalPure NGS beads out of the fridge at least 30 min before use to allow them to warm to room temperature.
- Make up fresh solution of 80% ethanol using molecular biology grade ethanol and molecular biology grade water.
- Remove the Step 2: Ligation reagents (yellow lids) from storage (–15°C to –25°C). Place the Step 2: Ligase on ice and allow the Step 2: Ligase Buffer to thaw to room temperature. Ensure that all components in the Ligase Buffer are well dissolved. If necessary, incubate at 37°C until dissolved.
- Remove the Universal Index Adapter plate from storage (–15°C to –25°C) and allow to thaw on ice just before use. Briefly spin the Adapter plate in a centrifuge to collect the liquid. Keep the plate on ice at all times. Do not heat above room temperature.
- Index Adapters are for single use only. If only using a part plate, recap the used Adapter wells to avoid spillage of excess Index Adapter.
- Assign a different Index Adapter to each sample. See Table 3 and Table 4 for the location of the Index Adapters on the plate.
- Ensure that the samples you are planning to pool in the hybridisation have indexes which are balanced. Ideally for a pool of eight samples, each pool will contain a complete column from the index plate.

	1	2	3	4	5	6	7	8	9	10	11	12
A	Index Adapter 1	Index Adapter 9	Index Adapter 17	X	X	X	X	X	X	X	X	X
B	Index Adapter 2	Index Adapter 10	Index Adapter 18	X	X	X	X	X	X	X	X	X
C	Index Adapter 3	Index Adapter 11	Index Adapter 19	X	X	X	X	X	X	X	X	X
D	Index Adapter 4	Index Adapter 12	Index Adapter 20	X	X	X	X	X	X	X	X	X
E	Index Adapter 5	Index Adapter 13	Index Adapter 21	X	X	X	X	X	X	X	X	X
F	Index Adapter 6	Index Adapter 14	Index Adapter 22	X	X	X	X	X	X	X	X	X
G	Index Adapter 7	Index Adapter 15	Index Adapter 23	X	X	X	X	X	X	X	X	X
H	Index Adapter 8	Index Adapter 16	Index Adapter 24	X	X	X	X	X	X	X	X	X

Table 3: Layout of Adapter Index Plate (1-24) (where X denotes an Empty well)

	1	2	3	4	5	6	7	8	9	10	11	12
A	Index Adapter 1	Index Adapter 9	Index Adapter 17	Index Adapter 25	Index Adapter 33	Index Adapter 41	Index Adapter 49	Index Adapter 57	Index Adapter 65	Index Adapter 73	Index Adapter 81	Index Adapter 89
B	Index Adapter 2	Index Adapter 10	Index Adapter 18	Index Adapter 26	Index Adapter 34	Index Adapter 42	Index Adapter 50	Index Adapter 58	Index Adapter 66	Index Adapter 74	Index Adapter 82	Index Adapter 90
C	Index Adapter 3	Index Adapter 11	Index Adapter 19	Index Adapter 27	Index Adapter 35	Index Adapter 43	Index Adapter 51	Index Adapter 59	Index Adapter 67	Index Adapter 75	Index Adapter 83	Index Adapter 91
D	Index Adapter 4	Index Adapter 12	Index Adapter 20	Index Adapter 28	Index Adapter 36	Index Adapter 44	Index Adapter 52	Index Adapter 60	Index Adapter 68	Index Adapter 76	Index Adapter 84	Index Adapter 92
E	Index Adapter 5	Index Adapter 13	Index Adapter 21	Index Adapter 29	Index Adapter 37	Index Adapter 45	Index Adapter 53	Index Adapter 61	Index Adapter 69	Index Adapter 77	Index Adapter 85	Index Adapter 93
F	Index Adapter 6	Index Adapter 14	Index Adapter 22	Index Adapter 30	Index Adapter 38	Index Adapter 46	Index Adapter 54	Index Adapter 62	Index Adapter 70	Index Adapter 78	Index Adapter 86	Index Adapter 94
G	Index Adapter 7	Index Adapter 15	Index Adapter 23	Index Adapter 31	Index Adapter 39	Index Adapter 47	Index Adapter 55	Index Adapter 63	Index Adapter 71	Index Adapter 79	Index Adapter 87	Index Adapter 95
H	Index Adapter 8	Index Adapter 16	Index Adapter 24	Index Adapter 32	Index Adapter 40	Index Adapter 48	Index Adapter 56	Index Adapter 64	Index Adapter 72	Index Adapter 80	Index Adapter 88	Index Adapter 96

Table 4: Layout of Adapter Index Plate (1-96)

7.2 Prepare Ligation Master Mix

1. Prepare the Ligation Master Mix as shown in Table 5 depending on your DNA input. Keep all reagents on ice. Mix well on a vortex mixer and spin down briefly.

Reagents	1x library (µl)	__ x library (µl)	16x library (µl) (includes excess)	24x library (µl) (includes excess)
DNA sample	35	-	-	-
Index Adapter	2.5	-	-	-
Step 2: Ligase Buffer (yellow lid)	9		153	234
Step 2: Ligase (yellow lid)	2		34	52
TOTAL	**48.5**		**187**	**286**

Table 5: Ligation reaction mix

2. Add 11 µl of the Ligation Master Mix to each DNA sample from previous reaction on ice.
3. Add 2.5 µl Index Adapter to each DNA sample, while ensuring to use a different Index Adapter for each sample to be sequenced in the same sequencing lane.
4. Mix by pipetting 10 times or vortex briefly.
5. Spin the tubes and immediately transfer to the thermal cycler.
6. Incubate in a thermal cycler for 20 min at 20°C. **Do not use a heated lid.**

IMPORTANT: Proceed immediately to ligated library purification.

7.3 Ligated library purification

Estimated time: 40 min for 8 - 24 samples.

1. Use only room temperature Mag-Bind® TotalPure NGS beads.
2. Vortex mix beads for at least 1 min or until the beads solution appears homogeneous and consistent in colour.
3. Add 11 µl of homogenous Mag-Bind® TotalPure NGS beads to each DNA sample. Mix well using a vortex mixer or mix by pipetting 10 times. Briefly spin down the tubes.
4. Incubate at room temperature for 5 min.

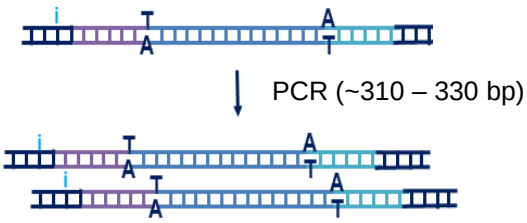
   Note: Briefly mix beads every 4 samples or every 4 rows when working with a multichannel pipette to ensure the beads are fully resuspended and ensure the correct volume of beads has been used.
5. Place the tube in the magnetic stand and wait for the solution to clear (approximately 3–5 min).
6. Keep the tube in the magnetic stand. Carefully **transfer the cleared solution containing the DNA sample** to a new tube, avoid touching the beads. Beads can be discarded.

7. Add 29 µl of homogenous Mag-Bind® TotalPure NGS beads to each sample. Mix well using a vortex mixer or mix by pipetting 10 times. Briefly spin down the tubes. Incubate at room temperature for 5 min.
8. Place the tube in the magnetic stand and wait for the solution to clear (approximately 3–5 min).
9. Keep the tube in the magnetic stand. Carefully remove the cleared solution, avoid touching the beads. Discard the solution and **keep the beads containing the DNA sample**.
10. Continue to keep the tube in the magnetic stand/rack whilst adding ♦ 500 µl / • 200 µl of 80% ethanol to each tube.
11. Incubate for 30sec and then remove the ethanol.
12. Repeat wash (step 10 and step 11) for a total of two washes.
13. Seal the tubes and briefly spin to collect the residual ethanol. Return the tubes to the magnetic stand for 30 seconds. Remove the residual ethanol with a P20 pipette.
14. Dry the samples at room temperature for 1-2mins

    IMPORTANT: Do not over-dry as this will decrease yield.
15. Add 34 µl of nuclease-free water directly to the bead pellet, mix well on a vortex mixer, spin briefly, and incubate for 5 min at room temperature.
16. Place the tube on the magnetic stand and leave for 2–3 min until the solution is clear.
17. Transfer 32 µl of the supernatant to a fresh 0.2 ml tube or 96-well plate. Tubes containing beads can be discarded at this time.
18. Assess the yield using the Qubit dsDNA High Sensitivity Kit as per manufacturer's instructions. The expected DNA concentrations after Adapter ligation are > 2 ng/µl.

OPTIONAL STOPPING POINT: If the samples are not to be used immediately, store at 4°C. If continuing proceed to Pre-capture PCR.

8 Pre-capture PCR



High fidelity PCR is used to amplify the DNA library prior to hybridisation and target capture. The number of PCR cycles is to be kept to a minimum to reduce number of duplicate reads (PCR copies of the same original DNA fragment) in the sequence data.

Estimated time: 45 - 60 mins for 8 - 24 samples. Hands-on time: 15 min.

8.1 Preparation

- Remove the Step 3: Primer Mix (red lid) and Step 3: PCR Buffer (red lid) from storage (–15°C to –25°C) and allow to thaw at room temperature.
- Ensure that all components in the PCR Buffer are well dissolved. If necessary, incubate at 37°C until dissolved.
- Remove the Step 3: PCR Polymerase (red lid) from storage (–15°C to –25°C) and place on ice.
- Set up PCR profile using the settings as shown in Table 7.

8.2 Prepare PCR Master Mix

1. Prepare the PCR Master Mix as shown in Table 6. Keep all reagents on ice. Mix well on a vortex mixer and spin down briefly.

Reagent	1x library (µl)	__ x library (µl)	16 x library (µl) (includes excess)	24 x library (µl) (includes excess)
Adapter-ligated DNA sample	31	-	-	-
Step 3: PCR buffer (red lid)	5		85	130
Step 3: Primer mix (red lid)	2.5		42.5	65
Step 3: PCR polymerase (red lid)	2		34	52
Nuclease-free water (clear lid)	9.5		161.5	247
TOTAL	**50**		**323**	**494**

Table 6: Components for PCR reaction mix

2. Add 19 µl of the PCR master mix to each Adapter-ligated DNA sample on ice. Mix by pipetting 10 times or vortex briefly and spin down the tubes.
3. Place the tubes in a thermal cycler and run the PCR programme. Set the heated lid to 105°C

Step	Temperature (°C)	Time
1	98	3 min
2	98	30 sec
3	65	30 sec
4	72	1 min
5	Repeat Step 2 to Step 4 for according to number of cycles indicated in Table 8	
6	72	10 min
7	4	Hold

Table 7: PCR Profile

DNA input	Total Number of PCR cycles
200-300 ng	6
301-500 ng	5

Table 8: Total Number of PCR cycles for recommended range of DNA inputs

8.3 PCR purification

Estimated time: 40 min for 8 - 24 samples.

1. Use only room temperature Mag-Bind® TotalPure NGS beads.
2. Mix beads for at least 1 min or until the reagent appears homogeneous and consistent in colour.
3. Add 45 µl of homogenous Mag-Bind® TotalPure NGS beads to each DNA sample (in either ♦1.5 ml LoBind tubes or • 0.2 ml tubes/96-well plate). Mix well using a vortex mixer or mix by pipetting 10 times. Briefly spin down the tubes.

   Note: Mix the beads every 4 samples or every 4 rows when working with a multichannel pipette to ensure the beads are fully resuspended and the correct volume of beads has been used.
4. Incubate at room temperature for 5 min.
5. Place the tube in the magnetic stand and wait for the solution to clear (approximately 3–5 min).
6. Keep the tube in the magnetic stand. Carefully remove the cleared solution from the tubes, avoid touching the beads.
7. Continue to keep the tube in the magnetic stand/rack whilst adding ♦ 500 µl / • 200 µl of 80% ethanol to each tube.
8. Incubate for 30 sec and then remove the ethanol.
9. Repeat wash (step 7 and step 8) for a total of 2 washes.
10. Seal the tubes and briefly spin to collect the residual ethanol. Return the tubes to the magnetic stand for 30 seconds. Remove the residual ethanol with a P20 pipette.
11. Dry the samples at room temperature for 1-2 mins.

    IMPORTANT: Do not over-dry as this will decrease yield.
12. Add 25 µl of nuclease-free water directly to the bead pellet, mix well on a vortex mixer, spin briefly, and incubate for 5 min at room temperature.
13. Place the tube on the magnetic stand and leave for 2–3 min until the solution is clear.
14. Remove 24 µl of the supernatant to a fresh 0.2 ml tube or 96-well plate. Tubes containing beads can be discarded at this time.
15. Assess the quality of the DNA with an Agilent DNA1000 ScreenTape and check that the electropherogram shows a fragment size distribution with a maximum peak size of 310 - 330 bp (+/– 20 bp) (Figure 2). Set up the instrument and prepare the tape, samples and ladder following manufacturer's instructions.

    Note: Fragment sizes outside of this range may reduce sequence data quality – contact your FAS if you require further advice
16. Assess yield using 1 µl the Qubit dsDNA HS Kit as per manufacturer's instructions. The expected yield is >18 ng/µl.
17. Calculate the volumes of samples required for 250 - 500 ng as input for the hybridisation. OGT has supplied a Pooling sheet to help with the calculations.

    Note: The hybridisation is best performed with 500 ng per sample. For lower library yields adjust the input of all samples down to the lowest yield in the sample preparation. Do not reduce inputs further than 250 ng per sample.

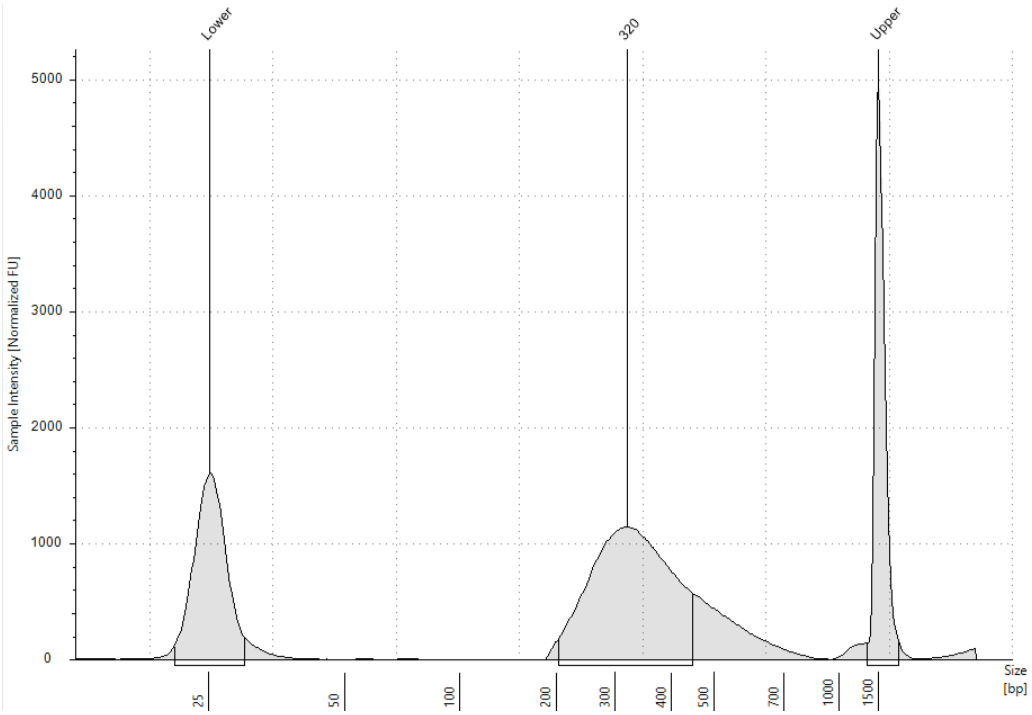
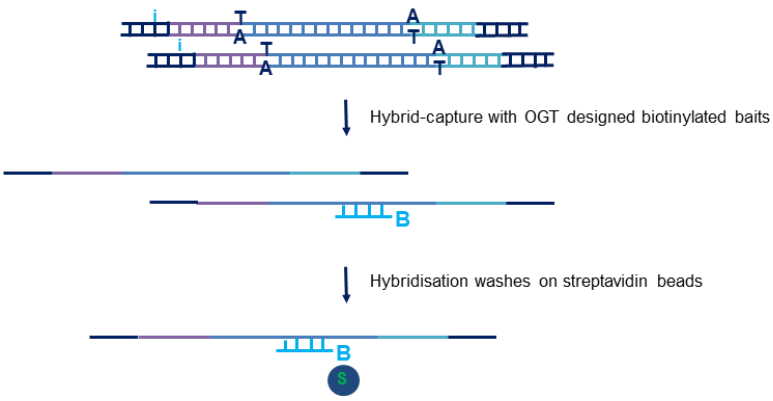


Figure 2: Analysis of amplified prepared library DNA using an Agilent D1000 ScreenTape assay. The electropherogram shows a maximum peak in the size range of 310 - 330 bp (+/– 20 bp).

OPTIONAL STOPPING POINT: If the samples are not to be used immediately, store at 4°C overnight or at –20°C for long-term storage. If continuing proceed to Hybridisation.

9 Hybridisation



The amplified library is denatured and captured by SureSeq or CytoSure (biotinylated) baits. The hybridised targets are then bound to streptavidin beads and washed to remove any off-target DNA.

Estimated time: 30 mins for 8 - 24 samples.

9.1 Preparation

- It is highly recommended to test the hybridisation conditions (thermal cycler and plasticware) to ensure minimal evaporation occurs during the overnight incubation.
- To test, add 15 µl of Nuclease free water (without DNA) in each well that you might use and use the thermocycler settings in Table 10. Check after overnight incubation that the evaporation does not exceed 1–2 µl per well.
- Take the Mag-Bind® TotalPure NGS beads out of the fridge at least 30 min before use to allow them to warm to room temperature.
- Make up fresh solution of 80% ethanol using molecular biology grade ethanol and molecular biology grade water.
- Remove the Hybridisation Buffer (red lid), Formamide (yellow lid), Cot Human DNA (green lid), Index Blockers (blue lid), and Nuclease-free Water (clear lid) from storage (–15°C to –25°C) and allow to thaw to room temperature.
- Remove the appropriate SureSeq or CytoSure baits from storage (–15°C to –25°C) and allow to thaw to room temperature.
- Ensure that all components in the Hybridisation Buffer are well dissolved. If necessary, incubate at 37°C until dissolved.
- Combine eight libraries to form a pool. For each pool, carry out one hybridisation capture. The hybridisation reaction requires equal DNA inputs from each sample to be combined in an 8-sample pool.

9.2 Sample preparation

1. OGT has supplied a Pooling sheet to help with the below calculations.
2. Aliquot 500ng of each of the eight libraries in a pool into a 1.5ml LoBind tube.

NOTE: If 500ng is not possible, a pool can be prepared with lower amounts down to 250 ng but ensure that all libraries are in equal amounts. Contact OGT Support for further help.

3. To each pool prepared add 10 µl Cot Human DNA (green lid).
4. Mix well using a vortex mixer and briefly spin the tubes.
5. Add 2X volume Mag-Bind® TotalPure NGS beads to each pool.

   Example: To a 60 µl pool (+ 10 µl Cot Human DNA) add 140 µl beads.
6. Mix well using a vortex mixer or mix by pipetting 10 times. Incubate at room temperature for 5 min.
7. Place the tube in the magnetic stand and wait for the solution to clear (approximately 3–5 min).
8. Keep the tube in the magnetic stand. Carefully remove the cleared solution from the tubes, avoid touching the beads.
9. Continue to keep the tube in the magnetic stand/rack whilst adding 500 µl of 80% ethanol to each tube.
10. Wait for 30 sec to allow any disturbed beads to settle and then remove the ethanol.
11. Dry the bead for approximately 5 min or until the residual ethanol completely evaporates.

    IMPORTANT: Do not over-dry as this will decrease yield.

    Note: The bead pellet is dry when the appearance of the surface changes from shiny to matte. Over-drying results in cracks in the bead pellet.
12. While the beads are drying, proceed with preparation of Hybridisation Master Mix (9.2.3)

9.3 Preparation of Hybridisation Master Mix

1. Prepare the Hybridisation Master Mix at room temperature as shown in Table 9. Mix well on a vortex mixer and spin down briefly.

Reagent	1x Pool (µl)	___ x Pool (µl)
Hybridisation Buffer (red lid)	7.5	
Formamide (yellow lid)	3.5	
Nuclease-free Water (clear lid)	2.5	
Total	**13.5**	

Table 9: Components of the Hybridisation Master Mix

1. Add 13.5 µl of the Hybridisation Master Mix directly to the bead pellet. Mix well on a vortex mixer, spin briefly, and incubate for 5 min at room temperature.

   Note: Make sure beads are resuspended well. Larger volumes of beads might have to be vortexed longer to ensure complete resuspension. Do not mix by pipetting.

2. Place the tube on the magnetic stand and leave for 3–5 min until the solution is clear.
3. Transfer 13.0 µl of supernatant into a fresh 0.2ml tube.
4. Add 2 µl Index Blockers (blue lid) to the hybridization reaction.
5. Add 2 µl of SureSeq or CytoSure panel-specific baits.
6. Seal the tubes, mix well on a vortex mixer and spin down briefly. The final volume should be 17 µl.
7. Place the tubes into the thermal cycler and run the following programme, with a heated lid temperature set to 105°C.

Step	Temperature (°C)	Time
1	95	5 min
2	65	Hold

Table 10: Hybridisation Profile

8. Make sure all caps are on tightly and all wells are sealed.
9. Incubate the hybridisation mixture overnight (16-20hours) at 65°C.
10. Continue to Hybridisation wash.

10 Hybridisation Wash with Universal Hyb & Wash Kit

Estimated time: 1.25 hr for 8–24 samples. Hands-on time: 45 min.

10.1 Preparation

- Pre-warm a thermal cycler to 65°C for at least 30 min before use.
- Pre-warm a thermal cycler to 35°C for at least 30 min before use.

  Note: It is important to maintain the correct temperature; it is recommended that you verify the temperature by using a calibrated thermometer.
- Allow the M270 Streptavidin magnetic beads to equilibrate to room temperature at least 30 min before use.
- Remove the Hyb Wash Buffer (blue dot) and Bead Priming Buffer (orange lid/dot) from storage (–15°C to –25°C) and allow to thaw to room temperature. These can be left on the bench overnight for defrosting.

10.2 Prepare Hyb Wash Buffer for use with CytoSure NGS Constitutional Panel

- The CytoSure NGS Constitutional Panel requires the addition of Component A (brown lid) to the Hyb Wash Buffer bottles.
- Remove Component A (brown lid) from storage (–15°C to –25°C) and allow to thaw to room temperature.
- Follow Table 11 for the volume required for the kit size.

Kit Size	Volume of Component A to add to Hyb Wash Buffer bottle (µl)
24 Reaction	100
96 Reaction	180 (per Hyb Wash Buffer bottle)

Table 11: Volume of Component A to add to the Hyb Wash Buffer bottle

- Label the bottle to indicate that Component A has been added.
- Invert the bottle 10 times after the Component A has been added.

Optional: If mixing by vortexing, pipette slowly when using to avoid introducing bubbles.

10.3 Prepare sequence capture and bead wash buffers

1. Ensure the Bead Priming Buffer and Hyb Wash Buffer are fully thawed.
   Note: Incubate at 37°C for 5-10 min to resuspend any precipitates. It is possible to defrost these buffers at room temperature during your overnight incubation.
2. Aliquot 6 x 200 µl of Wash Buffer per hybridisation pool into 0.2 ml strip tubes as shown in Figure 3 for one hybridisation pool.

3. Pre-warm the aliquots to the following temperatures in a heat block for a minimum of 30 min before use:

- 3 x 200 µl at 65°C / pool
- 3 x 200 µl at 35°C / pool

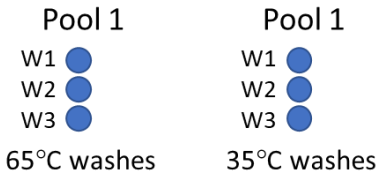


Figure 3: Set-up of Wash Buffer aliquots for one hybridisation pool.

10.4 Prepare magnetic beads

1. Vigorously resuspend the room temperature Dynabeads M270 Streptavidin magnetic beads on a vortex mixer.

   Note: It maybe necessary to vortex the tube on its side (ensure cap securely closed) or pipette mix to ensure that the beads are fully resuspended.

2. Add 100 µl M270 magnetic beads to a tube (in either ♦1.5 ml LoBind tubes or • 0.2 ml tubes/96-well plate) for each hybridisation pool (up to 400 µl of beads can be washed in a single 1.5 ml LoBind tube).

   a. Place on a magnetic rack, allow the beads to separate from the supernatant then carefully remove and discard the supernatant.

   b. Add 200 µl of 1x Bead Priming Buffer per 100 µl beads. Mix briefly on a vortex mixer and place back on the magnetic rack.

   c. Allow the beads to separate (about 10 seconds) from the supernatant then carefully remove and discard the supernatant.

   d. Repeat steps b-c twice for a total of two washes.

   e. Resuspend the beads in 1x the original volume of Bead Priming Buffer (i.e., for 100 µl of beads add 100 µl Bead Priming buffer) and mix on a vortex mixer.

   f. Transfer the beads into a new 0.2 ml tube for each capture reaction (100 µl/ pool).

   g. Place on a magnetic rack, allow the beads to separate from the supernatant then carefully remove and discard the supernatant.

Note: Proceed immediately to the next step. Small amounts of residual Bead Priming Buffer will not interfere with the downstream binding of the DNA to Dynabeads. Do not allow the beads to dry out.

10.5 Hybrid capture

1. After the overnight incubation transfer all of the hybridised sample (~17 µl volume) to the prepared streptavidin beads.
2. Mix thoroughly using a vortex mixer and ensure that all the beads are resuspended. Pulse spin to collect the contents.
3. Return the tubes to the thermal cycler set to 65°C for 45 min. Ensure that the heated lid is on (set at 105°C).
4. Every 15 minutes, mix the tubes well on a vortex mixer for 3 sec followed by a brief pulse spin to collect the contents. Place the tube back in a thermal cycler set to 65°C. This ensures the beads remain in suspension.
5. After the 45 minutes incubation, briefly spin the tubes in a centrifuge and proceed immediately to the next step.

10.6 Wash Streptavidin beads to remove unbound DNA

Note: Work quickly to ensure the temperature does not drop much below 65°C. To achieve this, we recommend performing all washes in 0.2 ml capped strip-tubes and using a multichannel pipette.

Note: After the addition of fresh buffer ensure the pellet has been fully resuspended by a brief mix on a vortex mixer followed by visual inspection. DO NOT use pipette for mixing.

1. Place the tubes on a magnetic rack, allow the beads to separate from the supernatant.
2. Carefully remove and discard the supernatant containing unbound DNA.
   Caution: The hybridisation mix contains Formamide.
3. Remove the tubes from the magnetic rack.
4. Add 200 µl of pre-warmed (to 65°C) Hyb Wash Buffer to the capture beads plus bound DNA.
5. Mix briefly on a vortex mixer to disrupt the bead pellet. Check to ensure the bead pellet has been fully resuspended.
6. Place the tubes in a plate vortex mixer set at approximately 2000 RPM and mix for 1 min at room temperature. Briefly spin in a centrifuge to collect the contents.
7. Return the samples to the thermal cycler set to 65°C for 5 min (ensure that the lid is heated above 65°C). Briefly spin in a centrifuge to collect the contents.
8. Place on a magnetic rack, allow the beads to separate from the supernatant then carefully remove and discard the supernatant.

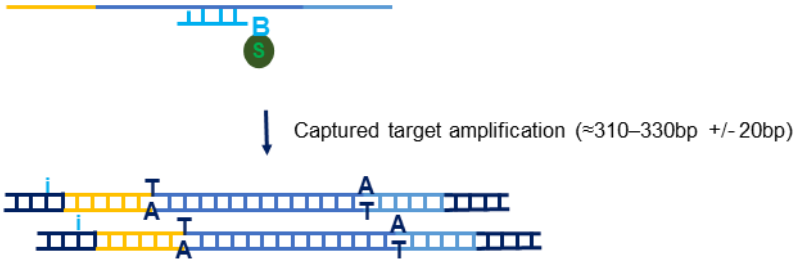
   Caution: The supernatant may contain trace amounts of Formamide.
9. Remove the tubes from the magnetic rack.
10. Repeat steps 4-9 twice, for a total of three 65°C washes.
11. Add 200 µl of pre-warmed (to 35°C) Hyb Wash Buffer and mix briefly on a vortex mixer to disrupt the bead pellet. Ensure the mixture is homogenous.
12. Place the tubes in a plate vortex mixer and mix vigorously on a plate vortex mixer set at approximately 2000 RPM for 2 min.

13. Briefly spin in a centrifuge to collect the liquid.
14. Place on a magnetic rack, allow the beads to separate from the supernatant then carefully remove and discard the supernatant.
15. Add 200 µl of pre-warmed (to 35°C) Hyb Wash Buffer and mix briefly on a vortex mixer to disrupt the bead pellet. Ensure the mixture is homogenous.
16. Place the tubes in a plate vortex mixer and mix vigorously on a plate vortex mixer set at approximately 2000 RPM for 1 min.
17. Briefly spin in a centrifuge to collect the liquid.
18. Place on a magnetic rack, allow the beads to separate from the supernatant then carefully remove and discard the supernatant.
19. Add 200 µl of pre-warmed (to 35°C) Hyb Wash Buffer and mix briefly on a vortex mixer to disrupt the bead pellet. Ensure the mixture is homogenous.
20. Place the tubes in a plate vortex mixer and mix vigorously on a plate vortex mixer set at approximately 2000 RPM for 30 sec.

    Note: Do not mix for more than 30 sec.
21. Briefly spin in a centrifuge to collect the liquid.
22. Place on a magnetic rack, allow the beads to separate from the supernatant then carefully remove and discard the supernatant.
23. Remove from the magnetic rack and resuspend the beads in 30 µl of nuclease-free water, mix thoroughly on a plate vortex mixer to form bead slurry.

OPTIONAL STOPPING POINT: If the samples are not to be used immediately, store the bead slurry at 4°C. Do not freeze the bead slurry. If continuing proceed to Post-capture PCR.

11 Post-capture PCR



After capture of target sequences, primers are added to amplify the NGS libraries for compatibility with Illumina platforms.

Estimated time: 1.75 hr for 8 – 24 samples. Hands-on time: 15 min.

11.1 Preparation

- Take the Mag-Bind® TotalPure NGS out of the fridge at least 30 mins before use to allow them to warm to room temperature.
- Make up fresh solution of 80% ethanol using molecular biology grade ethanol and molecular biology grade water.
- Remove the Step 4: Primer Mix (purple lid) and Step 4: PCR Buffer (purple lid) from storage (–15°C to –25°C) and allow to thaw at room temperature. Ensure that all components in the PCR Buffer are well dissolved. If necessary, incubate at 37°C until dissolved.
- Remove the Step 4: PCR polymerase (purple lid) from storage (–15°C to –25°C) and place on ice.
- Set up PCR profile using the settings as shown in Table 12, set the heated lid temperature to 105 °C.

11.2 Prepare PCR Master Mix

1. For multiple pools, prepare the reaction mix as shown in Table 12, on ice. Mix well on a vortex mixer.

Reagent	1x library (µl)	___x library (µl)	2x library (µl) (includes excess)	3x library (µl) (includes excess)
Captured DNA and bead slurry	14	-	-	-
Nuclease-free water	26.5		66.25	92.75
Step 4: PCR Buffer (purple lid)	5		12.5	17.5
Step 4: Primer mix (purple lid)	2.5		6.25	8.75
Step 4: PCR Polymerase (purple lid)	2		5	7
TOTAL	**50**		**90**	**126**

Table 12: Components for Post-capture PCR

2. For each pool, aliquot 36 µl of the PCR reaction mix into a fresh 0.2 ml reaction tube.
3. Pipette mix the bead slurry to ensure the beads are homogeneous. Add 14 µl of captured DNA and beads slurry to each tube.
4. Mix by pipetting 10 times or vortex briefly and pulse spin tubes.
5. Place the tubes in a thermal cycler and run the PCR programme in Table 13.

Step	Temperature (°C)	Time
1	98	3 min
2	98	30 sec
3	65	30 sec
4	72	1 min
5	Repeat Step 2 to Step 4 for a total number of cycles as specified in Table 14	
6	72	10 min
7	4	Hold

Table 13: PCR profile

Total Number of PCR cycles	Panel
18	770001 - Core MPN
16	770002 - Myeloid plus 770004 - CLL+CNV 770005 - Germline Breast Cancer + CNV
15	770003 - Pan-Myeloid
10	502003 - CytoSure Constitutional

Table 14: Post-capture PCR total cycle numbers

11.3 Post-capture PCR purification

Estimated time: 40 min for 8 - 24 samples.

1. Use only room temperature Mag-Bind® TotalPure NGS beads.
2. Vortex beads for 1 min or until the reagent appears homogeneous and consistent in colour.
3. Add 45 µl of homogenous Mag-Bind® TotalPure NGS beads to each bead slurry sample pool (in either ♦1.5 ml LoBind tubes or • 0.2 ml tubes/96-well plate). Mix well using a vortex mixer or mix by pipetting 10 times. Briefly spin down the tubes.

   Note: Mix the beads every 4 samples or every 4 rows when working with a multichannel pipette to ensure the beads are fully resuspended and the correct volume of beads has been used.
4. Incubate at room temperature for 5 min.
5. Place the tube in the magnetic stand and wait for the solution to clear (approximately 3–5 min).
6. Keep the tube in the magnetic stand. Carefully remove the cleared solution from the tubes, avoid touching the beads.
7. Continue to keep the tube in the magnetic stand/rack whilst adding ♦ 500 µl / • 200 µl of 80% ethanol to each tube.
8. Incubate for 30 sec and then remove the ethanol.
9. Repeat wash (step 7 and step 8) for a total of two washes.
10. Seal the tubes and briefly spin to collect the residual ethanol. Return the tubes to the magnetic stand for 30 seconds. Remove the residual ethanol with a P20 pipette.
11. Dry the bead pellets at room temperature for 1-2mins

    IMPORTANT: Do not over-dry as this will decrease yield.
12. Add 32 µl of nuclease-free water directly to the bead pellet, mix well on a vortex mixer, spin briefly, and incubate for 5 min at room temperature.
13. Place the tube on the magnetic stand and leave for 2–3 min until the solution is clear.
14. Transfer 30 µl of the supernatant to a fresh 0.2 ml tube or 96-well plate. The tubes containing beads can be discarded at this time.

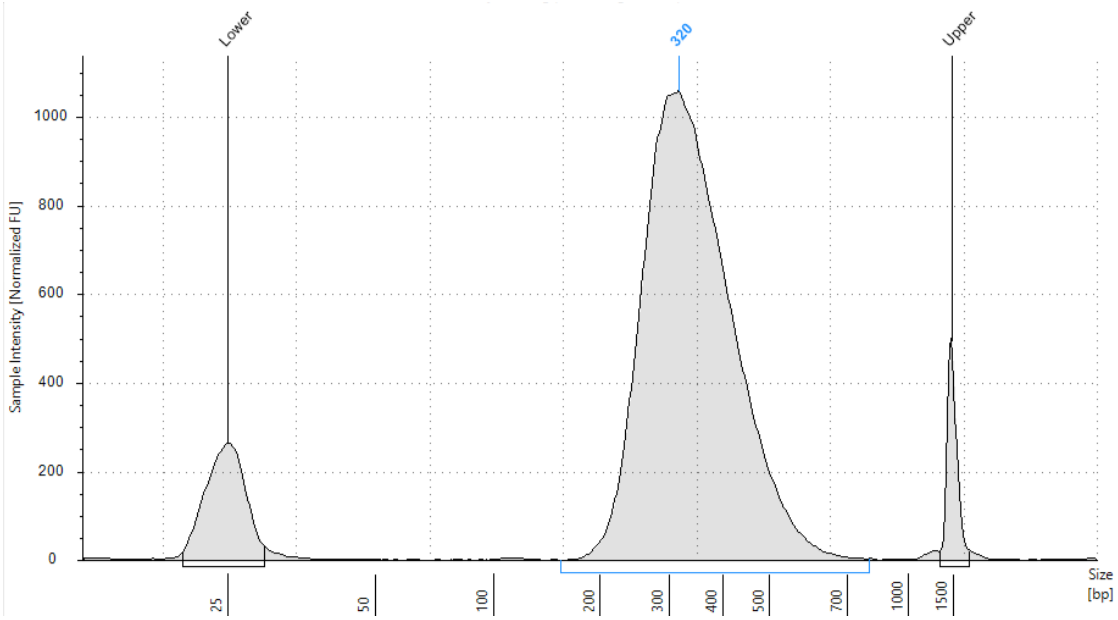
15. Assess the size of the amplified product using the Agilent High Sensitivity D1000 ScreenTape or similar. The electropherogram should show a maximum peak size of 310 - 330 bp (+/– 20 bp) (Figure 4). Set up the instrument and prepare the chip, samples and ladder following manufacturer's instructions.
16. Assess the PCR yield using High Sensitivity dsDNA Qubit assay. The expected yield is panel dependent and between 1 – 35 ng/µl - contact your FAS for further details.
17.
18. 

Figure 4: Analysis of amplified capture DNA pool using an Agilent High Sensitivity D1000 ScreenTape assay. The electropherogram shows a maximum peak in the size range of approximately 310 – 330 bp (+/– 20 bp).

OPTIONAL STOPPING POINT: If the samples are not to be used immediately, store them at 4°C overnight or at –20°C for long-term storage.

12 MiSeq Sequencing

The DNA pools prepared in the previous section (Post-capture PCR) need to be combined such that each unique index-tagged sample is present in equimolar amounts in the final sequencing pool. This requires both accurate determination of peak size (bp), provided by Agilent TapeStation (High-Sensitivity Kit), and accurate determination of sample concentration (ng/µl), provided by Life Technologies Qubit (High-Sensitivity) assay.

12.1 Preparing the sequencing pool

1. To determine the volume (µl) of each indexed DNA pool required to generate the 4 nM Sequencing Pool, use the pooling template file provided by OGT (or the formulae below).
2. Complete the “Pool Parameter” and “Samples” tables in the “Pool” tab of the pooling template. Cells in green should be manually modified as required; parameters marked with * must be provided.
   Note: This protocol has been validated with 150-base paired-end reads using the MiSeq Reagent Kit v2 300 cycle (cat. no. MS-102-2002).
3. Each indexed sample should be entered separately into the pooling template file.
4. Add the appropriate volume of each indexed Pool to a fresh 1.5 ml LoBind tube labelled “4 nM Sequencing Pool”; the volumes can be found in column C of “Volumes to pipette” tab.
5. Adjust the final volume of the Sequencing Pool with nuclease free water to the desired final concentration (4 nM). This can be found in column C of “Volumes to pipette” tab next to “Nuclease-free water”.
6. Validation of Sequencing Pool concentration — Analyse the Sequencing Pool using an Agilent TapeStation (High Sensitivity Kit) to determine peak size distribution and determine the concentration (ng/µl) yield using High Sensitivity Qubit assay. Complete the “Pool validation and dilution” tab to determine the molar concentration of the Sequencing Pool.
7. The Sequencing Pool can now be prepared for loading on to the MiSeq. Refer to the appropriate Illumina protocol.

As cluster density can vary between machines, we recommend loading a final concentration of 8-12 pM if using a v2 300 cycle kit.

12.1.1 Formula 1 – nM of each sample

$$nM = \frac{[Sample\ concentration\ (ng/\mu l)]\ \times 10\text{^}6}{([Sample\ size\ in\ bp] \times 660) + 157.9}$$

12.1.2 Formula 2 — Volume of each Indexed DNA Sample

$$Volume\ of\ indexed\ sample = \frac{Sequencing\ Pool\ (\mu l)\ \times\ Pool\ concentration\ (4nM)}{Number\ of\ samples\ in\ Pool\ \times\ nM\ concentration\ of\ the\ sample}$$

12.2 Preparing the MiSeq Sample Sheet

The MiSeq Sample sheet can be created in Excel using the MiSeq pooling template provided by OGT.

1. Open the completed MiSeq pooling sheet (from “Preparing the sequencing pool”) and click on the “Sample sheet” tab.
2. Highlight all cells with text as shown in 6. Adjust the number of rows highlighted as appropriate.

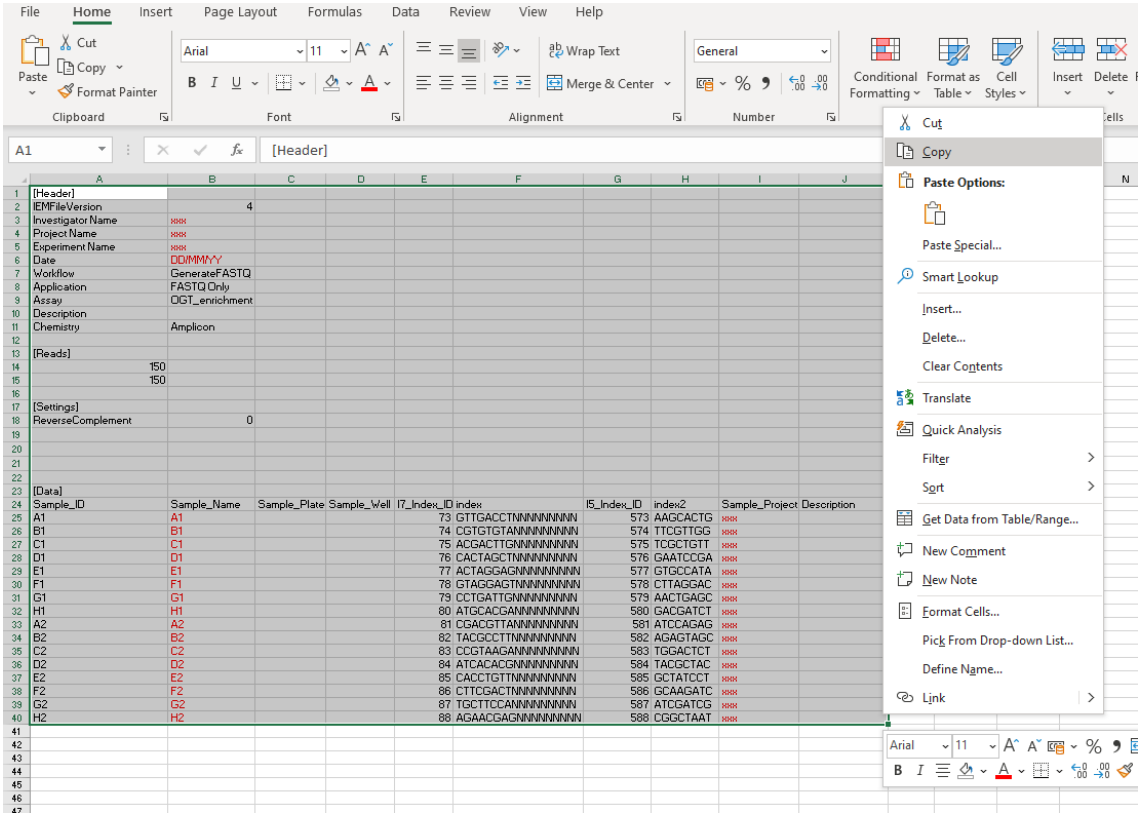


Figure 5: Example SampleSheet on the MiSeq pooling template file.

3. Copy highlighted cells and paste into a new Excel file.
   Note: All text in red is for user and sample specific information. All text in black is required to ensure that the MiSeq will recognise the file.
4. Save the new sheet as a CSV (comma delimited) file.
5. The MiSeq sample sheet can now be uploaded to the MiSeq.

13 NextSeq Sequencing

The DNA pools prepared in the previous section (Post-capture PCR) need to be combined such that each index-tagged sample is present in equimolar amounts in the final sequencing pool. This requires both accurate determination of peak size (bp), provided by Agilent TapeStation (High-Sensitivity Kit), and accurate determination of sample concentration (ng/µl), provided by Life Technologies Qubit (High-Sensitivity) assay.

13.1 Preparing the sequencing pool

To determine the volume (µl) of each indexed DNA sample required to generate the 4 nM Sequencing Pool, use the pooling template file provided by OGT (or the formulae below).

1. Complete the “Pool Parameter” and “Samples” tables in the “Pool” tab of the pooling template. Cells in green should be modified as required; parameters marked with * must be completed.

Note: This protocol has been validated with 150-base paired-end reads using the NextSeq 550 High-Output Kit 2x150bp (Illumina cat. no. 20024908).

2. Each indexed sample should be entered in separately into the pooling template file.

3. In the “Volumes to pipette” tab, column C contains the values of each 8-plex pool that should be pipetted. Pipette this volume of each hybridisation pool into a fresh 1.5 ml LoBind tube labelled “4 nM Sequencing Pool”; remembering to change tips to avoid cross-contamination.

4. Adjust the final volume of the sequencing pool with nuclease free water to the desired final concentration (4 nM). This can also be found in column C of “Volumes to pipette” tab next to “Nuclease-free water”.

Optional: If you store the library before sequencing, add Tween 20 at 0.1% v/v and store at -20°C.

5. Validation of Sequencing Pool concentration — Analyse the Sequencing Pool using an Agilent TapeStation (High Sensitivity Kit) to determine maximum peak size and determine the concentration (ng/µl) yield using High Sensitivity Qubit assay. Complete the “Pool validation and dilution” tab to determine the molar concentration of the Sequencing Pool.

6. The Sequencing Pool can now be prepared for loading on to the NextSeq. Refer to the appropriate Illumina protocol for your NextSeq kit.

NOTE: As cluster density can vary between machines, please use the recommended final loading concentration for your machine. We have used a final loading concentration of 1.4pM when using a 2x150bp NextSeq 500/550 High Output kit.

13.2 Formula 1 – Concentration (nM) of each sample

$$nM = \frac{[Sample\ concentration\ (ng/\mu l)] \times 10\text{^}6}{([Sample\ size\ in\ bp] \times 660) + 157.9}$$

13.3 Formula 2 — Volume of each Indexed DNA Sample

$$Volume\ of\ indexed\ sample = \frac{Sequencing\ Pool\ (\mu l) \times Pool\ concentration\ (4nM)}{Number\ of\ samples\ in\ Pool \times nM\ concentration\ of\ the\ sample}$$

13.4 Preparing the NextSeq

1. The NextSeq should be prepared according to manufacturer's protocols.

2. The sequencing pool should be made to a recommended final concentration of 1.4 pM with the HT1 Buffer.

3. The sequencing pool should be combined with 5% denatured PhiX before running.

4. When using BaseSpace, select + Custom Library Prep Kit in the Library Prep Kit dropdown menu.

5. Use the information in Table 15 and Table 16 to create the Universal Library prep kit.

6. The Universal NGS Library prep kit will now be available to use for the sequencing run.

14 Appendix

14.1 Adapter Sequences

Adapter	Sequence
1	AGATCGGAAGAGCACACGTCTGAACTCCAGTCA
2	AGATCGGAAGAGCGTCGTGTAGGGAAAGAGTGT

Table 15: Adapter Sequences

14.2 Index Sequences

I7_Index_ID	Index1 sequence	I5_Index_ID	Index2 sequence
1	CTGATCGTNNNNNNNNN	501	ATATGCGC
2	ACTCTCGANNNNNNNNN	502	TGGTACAG
3	TGAGCTAGNNNNNNNNN	503	AACCGTTC
4	GAGACGATNNNNNNNNN	504	TAACCGGT
5	CTTGTCGANNNNNNNNN	505	GAACATCG
6	TTCCAAGGNNNNNNNNN	506	CCTTGTAG
7	CGCATGATNNNNNNNNN	507	TCAGGCTT
8	ACGGAACANNNNNNNNN	508	GTTCTCGT
9	CGGCTAATNNNNNNNNN	509	AGAACGAG
10	ATCGATCGNNNNNNNNN	510	TGCTTCCA
11	GCAAGATCNNNNNNNNN	511	CTTCGACT
12	GCTATCCTNNNNNNNNN	512	CACCTGTT
13	TACGCTACNNNNNNNNN	513	ATCACACG
14	TGGACTCTNNNNNNNNN	514	CCGTAAGA
15	AGAGTAGCNNNNNNNNN	515	TACGCCTT
16	ATCCAGAGNNNNNNNNN	516	CGACGTTA
17	GACGATCTNNNNNNNNN	517	ATGCACGA
18	AACTGAGCNNNNNNNNN	518	CCTGATTG
19	CTTAGGACNNNNNNNNN	519	GTAGGAGT
20	GTGCCATANNNNNNNNN	520	ACTAGGAG
21	GAATCCGANNNNNNNNN	521	CACTAGCT
22	TCGCTGTTNNNNNNNNN	522	ACGACTTG
23	TTCGTTGGNNNNNNNNN	523	CGTGTGTA
24	AAGCACTGNNNNNNNNN	524	GTTGACCT
25	CCTTGATCNNNNNNNNN	525	ACTCCATC
26	GTCGAAGANNNNNNNNN	526	CAATGTGG
27	ACCACGATNNNNNNNNN	527	TTGCAGAC
28	GATTACCGNNNNNNNNN	528	CAGTCCAA
29	GCACAACTNNNNNNNNN	529	ACGTTCAG

30	GCGTCATTNNNNNNNNN	530	AACGTCTG
31	ATCCGGTANNNNNNNNN	531	TATCGGTC
32	CGTTGCAANNNNNNNNN	532	CGCTCTAT
33	GTGAAGTGNNNNNNNNN	533	GATTGCTC
34	CATGGCTANNNNNNNNN	534	GATGTGTG
35	ATGCCTGTNNNNNNNNN	535	CGCAATCT
36	CAACACCTNNNNNNNNN	536	TGGTAGCT
37	TGTGACTGNNNNNNNNN	537	GATAGGCT
38	GTCATCGANNNNNNNNN	538	AGTGGATC
39	AGCACTTCNNNNNNNNN	539	TTGGACGT
40	GAAGGAAGNNNNNNNNN	540	ATGACGTC
41	GTTGTTCGNNNNNNNNN	541	GAAGTTGG
42	CGGTTGTTNNNNNNNNN	542	CATACCAC
43	ACTGAGGTNNNNNNNNN	543	CTGTTGAC
44	TGAAGACGNNNNNNNNN	544	TGGCATGT
45	GTTACGCANNNNNNNNN	545	ATCGCCAT
46	AGCGTGTTNNNNNNNNN	546	TTGCGAAG
47	GATCGAGTNNNNNNNNN	547	AGTTCGTC
48	ACAGCTCANNNNNNNNN	548	GAGCAGTA
49	GAGCAGTANNNNNNNNN	549	ACAGCTCA
50	AGTTCGTCNNNNNNNNN	550	GATCGAGT
51	TTGCGAAGNNNNNNNNN	551	AGCGTGTT
52	ATCGCCATNNNNNNNNN	552	GTTACGCA
53	TGGCATGTNNNNNNNNN	553	TGAAGACG
54	CTGTTGACNNNNNNNNN	554	ACTGAGGT
55	CATACCACNNNNNNNNN	555	CGGTTGTT
56	GAAGTTGGNNNNNNNNN	556	GTTGTTCG
57	ATGACGTCNNNNNNNNN	557	GAAGGAAG
58	TTGGACGTNNNNNNNNN	558	AGCACTTC
59	AGTGGATCNNNNNNNNN	559	GTCATCGA
60	GATAGGCTNNNNNNNNN	560	TGTGACTG
61	TGGTAGCTNNNNNNNNN	561	CAACACCT
62	CGCAATCTNNNNNNNNN	562	ATGCCTGT
63	GATGTGTGNNNNNNNNN	563	CATGGCTA
64	GATTGCTCNNNNNNNNN	564	GTGAAGTG
65	CGCTCTATNNNNNNNNN	565	CGTTGCAA
66	TATCGGTCNNNNNNNNN	566	ATCCGGTA
67	AACGTCTGNNNNNNNNN	567	GCGTCATT
68	ACGTTCAGNNNNNNNNN	568	GCACAACT
69	CAGTCCAANNNNNNNNN	569	GATTACCG
70	TTGCAGACNNNNNNNNN	570	ACCACGAT
71	CAATGTGGNNNNNNNNN	571	GTCGAAGA
72	ACTCCATCNNNNNNNNN	572	CCTTGATC
73	GTTGACCTNNNNNNNNN	573	AAGCACTG
74	CGTGTGTANNNNNNNNN	574	TTCGTTGG

75	ACGACTTGNNNNNNNNN	575	TCGCTGTT
76	CACTAGCTNNNNNNNNN	576	GAATCCGA
77	ACTAGGAGNNNNNNNNN	577	GTGCCATA
78	GTAGGAGTNNNNNNNNN	578	CTTAGGAC
79	CCTGATTGNNNNNNNNN	579	AACTGAGC
80	ATGCACGANNNNNNNNN	580	GACGATCT
81	CGACGTTANNNNNNNNN	581	ATCCAGAG
82	TACGCCTTNNNNNNNNN	582	AGAGTAGC
83	CCGTAAGANNNNNNNNN	583	TGGACTCT
84	ATCACACGNNNNNNNNN	584	TACGCTAC
85	CACCTGTTNNNNNNNNN	585	GCTATCCT
86	CTTCGACTNNNNNNNNN	586	GCAAGATC
87	TGCTTCCANNNNNNNNN	587	ATCGATCG
88	AGAACGAGNNNNNNNNN	588	CGGCTAAT
89	GTTCTCGTNNNNNNNNN	589	ACGGAACA
90	TCAGGCTTNNNNNNNNN	590	CGCATGAT
91	CCTTGTAGNNNNNNNNN	591	TTCCAAGG
92	GAACATCGNNNNNNNNN	592	CTTGTCGA
93	TAACCGGTNNNNNNNNN	593	GAGACGAT
94	AACCGTTCNNNNNNNNN	594	TGAGCTAG
95	TGGTACAGNNNNNNNNN	595	ACTCTCGA
96	ATATGCGCNNNNNNNNN	596	CTGATCGT

Table 16: Index sequences

14.3 Recommended Sequencing Guidelines

Panel	Recommended sequencing platform	Number of samples per sequencing run	Pool size proposal	Recommended kit size purchase	Number of runs based on kit size purchase
770001 - CoreMPN	MiSeq 300V2	48	6 x 8plex	2 x 24 reactions	1 run
				1 x 96 reactions	2 runs
770002 – Myeloid Plus	MiSeq 300V2	16	2 x 8plex	2 x 24 reactions	3 runs
				1 x 96 reactions	6 runs
770004 – CLL+CNV	MiSeq 300V2	16	2 x 8plex	2 x 24 reactions	3 runs
				1 x 96 reactions	6 runs
770003 - Pan Myeloid	MiSeq 300V2	8	1 x 8plex	1 x 24 reactions	3 runs
	MiSeq 600V3	16	2 x 8plex	2 x 24 reactions	3 runs
				1 x 96 reactions	6 runs
	NextSeq Mid-Output	48	6 x 8plex	2 x 24 reactions	1 run
				1 x 96 reactions	2 runs
502003 - CytoSure Constitutional	NextSeq High-Output	24	3 x 8plex	1 x 24 reactions	1 run

Table 17: Recommended Sequencing Guidelines

Illumina Catalogue numbers are as follows:

MiSeq 300V2: MS-102-2002, MiSeq 600V3: MS-102-3003

NextSeq Mid-Output: 20024905, NextSeq High-Output: 20024908

14.4 Reagent Tube Locations

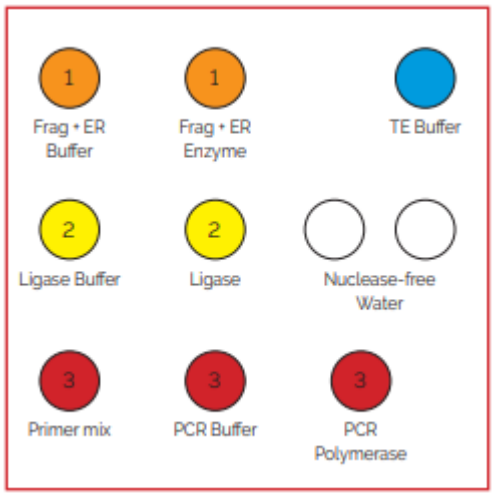


Figure 6: Location of tubes in 24 reaction Library preparation kit (770100-24)

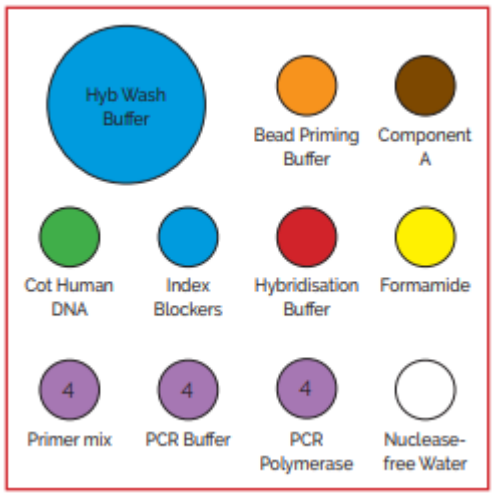


Figure 7: Location of tubes in 24 reaction Hybridisation & Wash kit (770400-24)

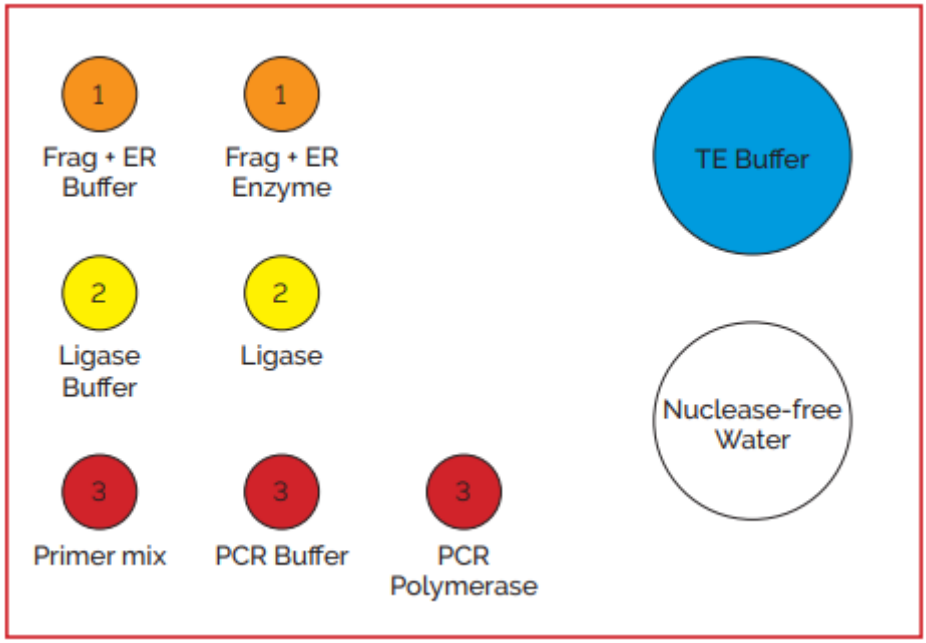


Figure 8: Location of tubes in 96 reaction Library preparation kit (770100-96)

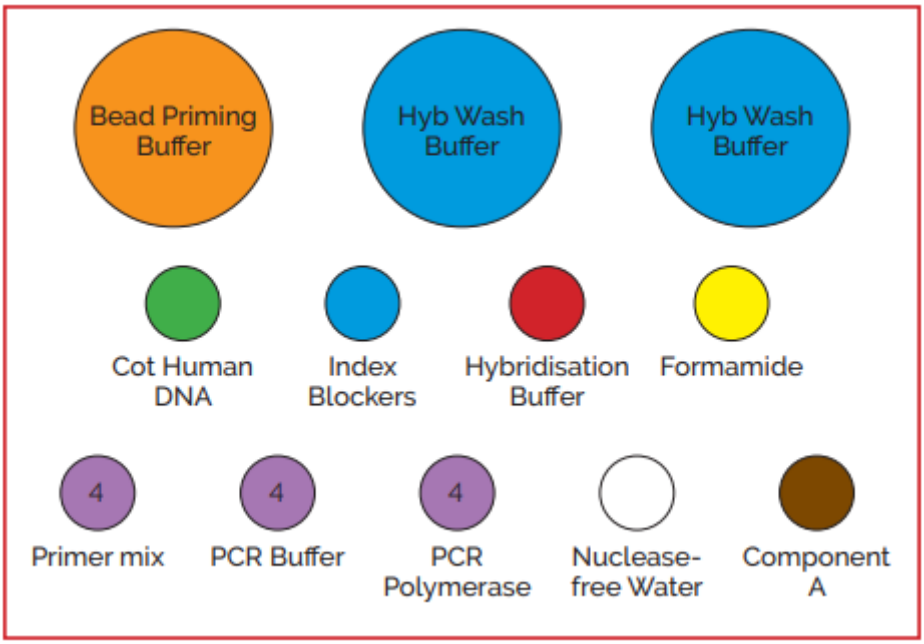


Figure 9: Location of tubes in 96 reaction Hybridisation & Wash kit (770400-96)

15 Ordering information

Table 18: Ordering information

Product Name	Product Description	Cat. No.
Universal Library Preparation Complete Solution	Bundle of 1x Universal Library Preparation Kit, 1 x Universal Index Adapters, 1x Universal Hybridisation & Wash Kit, 1x Universal Bead Kit	770500-24 770500-96

For an up-to-date product list and the latest product information, visit ogt.com

16 Legal information

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17 Contact information

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