Application Note

WES on MGI sequencing platforms

Authors

Hui Kang
Associate Science Value Manager

Yi-Wen Yan
Senior Science Value Manager

Roche Diagnostics (Shanghai) Limited

Jacqueline Meyer
Support & Training Senior Specialist, Global Customer Support

Roche Diagnostics Cape Town, South Africa

Andrea Wijeweera
Product Manager, Sequencing Solutions

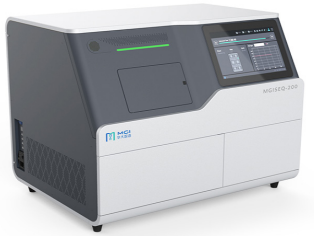
Kui Qian
Bioinformatics Consultant

Roche Diagnostics Asia Pacific, Singapore

Implementation of the KAPA HyperCap Workflow and KAPA HyperExome Probes for whole exome sequencing on MGI sequencing platforms

MGI sequencing platforms employ DNBSEQ™ technology and provide a cost-effective and flexible alternative to other short-read sequencing technologies. The KAPA HyperCap Workflow, utilizing KAPA library preparation reagents and KAPA HyperExome Probes, provide a high-performance sample preparation solution for routine human whole exome sequencing on MGI sequencing platforms.

Introduction



High-throughput whole exome sequencing (WES) is a versatile tool now widely used in basic, translational and clinical research. Exome panels may be expanded to interrogate non-coding genome content of interest, at a fraction of the cost and analysis burden of whole genome sequencing (WGS). When appropriate, WES data may be masked in different ways to focus on specific gene sets ("digital panels"), thereby eliminating the need to perform target enrichment with multiple custom panels.

Short-read sequencing remains the preferred technology for high-throughput WES, in part due to accessibility and cost-effectiveness. MGI sequencing platforms, based on DNBSEQ technology, have become a viable alternative to established short-read systems for a variety of reasons. These include accuracy, achieved through innovative single-stranded circularization (ssCir) and DNA Nanoball (DNB) template preparation strategies and combinatorial probe anchor synthesis (cPAS) sequencing chemistry.[1] In addition, the platform offers flexibility, ease-of-use, fast turnaround times, and competitive per-base sequencing cost.

The core library construction process for MGI sequencing is very similar to that used in established short-read workflows, e.g., for Illumina® sequencing. Nevertheless, very few MGI-compatible third-party library preparation solutions are currently available.

Roche is an industry leader in sample preparation, offering the KAPA portfolio of high-performance library preparation and KAPA HyperCap target enrichment reagents.[2] We have previously demonstrated the utility of the KAPA HyperPrep library preparation kit for human whole genome sequencing on MGI platforms.[3] In this Application Note, we demonstrate the KAPA HyperCap Workflow and KAPA HyperExome Probes to be suitable for the production of high-quality WES data in MGI sequencing pipelines. Results generated from a HapMap sample (100 ng input, 6-plex exome capture), as well as a step-by-step protocol, are included.

Materials and methods

Experimental design

Whole exome libraries were prepared from a characterized HapMap sample using the KAPA HyperPrep and KAPA HyperPlus kits, the KAPA HyperCap Workflow v3.1[4] and KAPA HyperExome Probes.[5] The standard KAPA HyperCap Workflow was modified to ensure MGI-compatible libraries. A workflow overview, as well as step-by-step protocol, are included in the Appendix. Single-stranded circularization (ssCir), DNA Nanoball (DNB) preparation and DNBSEQ™ using a DNBSEQ-G400 sequencer* was performed by MGI Tech Co., Ltd (Shenzhen) using standard protocols.[6,7] Data analysis was performed with open source bioinformatics tools.

Library construction and sequencing

Input DNA: High-quality human NA12891 genomic DNA (International HapMap Project, CEPH/UTAH pedigree1463)[8] was obtained from the Coriell Institute of Biomedical Research.

Mechanical shearing: Six 100 ng aliquots of NA12891 DNA were diluted to the appropriate concentration and sheared using a Covaris® M220 Focused Ultrasonicator. Of each sheared DNA sample, 50 µL were recovered for library construction with the KAPA HyperPrep Kit. The KAPA HyperPrep End Repair & A-Tailing Enzyme Mix was used in the End Repair & A-Tailing reaction.

Enzymatic DNA fragmentation: For library construction with the KAPA HyperPlus Kit, six 100 ng aliquots of NA12891 DNA were fragmented for 25 min at 37°C using KAPA Frag Enzyme and Buffer. Fragmentation products were used directly in the End Repair and A-Tailing reaction, containing KAPA HyperPlus End Repair & A-Tailing Enzyme Mix.

Adapter Ligation: To ensure MGI-compatible libraries, 5 µL of 10 µM MGIEasy DNA Adapter (MGI Tech Co., Ltd) was used (instead of KAPA Universal Adapter) in each ligation reaction. A unique adapter (barcode) was chosen for each library (according to manufacturer's instructions)[9] to support multiplexed sequencing. Ligation was performed for 30 min at 20°C.

Pre-capture PCR: Pre-capture PCR was performed with KAPA HiFi HotStart ReadyMix and 5 µL of MGI Primer Mix[10] (10 µM of each primer per reaction; provided by MGI Tech Co., Ltd). Eight cycles of pre-capture amplification were performed for KAPA HyperPrep libraries, and six cycles for KAPA HyperPlus libraries.

Pre-capture Library QC: The fragment size distribution of amplified, bead-purified Sample Libraries was determined with an Agilent® Bioanalyzer 2100 instrument and DNA 1000 Kit (Agilent Technologies). Libraries were quantified using a Qubit® Fluorometer and dsDNA HS Assay Kit (Thermo Fisher Scientific).

Target enrichment: Three Multiplex DNA Sample Library Pools were constructed (as outlined in Table 1 in the next section), by pooling 250 ng of each of six uniquely indexed KAPA HyperPrep or KAPA HyperPlus libraries, for a combined mass of 1.5 µg per 6-plex pool. Pools were hybridized to KAPA HyperExome Probes, as outlined in the KAPA HyperCap Workflow v3.1[4] for panels with a capture target size ≥40 Mb. To obtain MGI-compatible libraries, MGI Blocking Oligos (Block 3 and Block 4) were used (instead of KAPA Universal Enhancing Oligos). Preparation of the Blocking Oligos is described in detail in the Appendix. The Hybridization Master Mix was prepared according to instructions for designs with a ≥40 Mb capture target size.

Subsequent wash steps and recovery of captured Multiplex DNA Sample Pools were performed as per the standard KAPA HyperCap Workflow. Post-capture PCR was performed with KAPA HiFi HotStart ReadyMix and 5 µL of MGI Primer Mix[10] (10 µM of each primer per reaction; provided by MGI Tech Co., Ltd). Eight cycles of post-capture amplification were performed for all three pools.

Post-capture Library QC: The fragment size distribution of enriched, amplified Multiplex DNA Sample Libraries was determined with an Agilent Bioanalyzer 2100 instrument and DNA 1000 Kit (Agilent Technologies). Libraries were quantified using a Qubit Fluorometer and dsDNA HS Assay Kit (Thermo Fisher Scientific).

DNA Nanoball (DNB) Preparation: Library denaturation, ssCir, Exo digestion and cleanup, and DNB generation were performed at MGI Tech Co., Ltd (Shenzhen), using standard protocols. The recommended input amount (1 pmol per sequencing-ready library) was used for ssCir with the MGIEasy Circularization Kit (MGI Tech Co., Ltd).[6] Of each purified ssCir library, 40 fmol was converted to DNBs using the DNBSEQ-G400RS High-throughput Sequencing Set (MGI Tech Co., Ltd).[7]

Sequencing: Paired-end Sequencing (2 x150 bp) was performed on a DNBSEQ-G400 instrument (MGI Tech), using standard combinatorial probe anchored synthesis (cPAS) technology.

Alignment: Read quality was assessed using FASTQC (v0.10.1). Adapter- and quality-trimmed sequencing reads (obtained with SOAPnuke, v2.1.2) were aligned to the human reference genome (build GRCh37/hg19) using BWA-MEM (v0.7.12) with default parameters. Duplicates were marked and removed with Picard (v1.134). The output SAM file was converted to BAM format as input for GATK (v4.17.0) analysis and variant calling tools.

Quality control analysis: Quality control metrics (library insert size and alignment and genome coverage metrics) were calculated with Picard. Picard AlignmentSummaryMetrics was used to summarize the alignment metrics.

Variant calling: Germline variants (SNPs and indels) were called using GATK (HaplotypeCaller), and compared to a NA12891 truth variant list from GATK resource bundle (Broad Institute)[11] using the GATK GenotypeConcordance tool.

*In parts of Asia-Pacific, e.g., China, the DNBSEQ-G400/G400 Fast is known as MGISEQ-2000/2000 Fast and the DNBSEQ-G50 as the MGISEQ-200. All references to the DNBSEQ-G400 also apply to the MGISEQ-2000.

Results and discussion

Library construction metrics

Key library construction parameters and QC metrics are summarized in Table 1. With the KAPA HyperPrep Kit, pre-capture library yields ranged between 654 and 1,008 ng, whereas yields obtained with the KAPA HyperPlus Kit ranged between 954 and 1,236 ng. These yields were slightly lower than expected from the standard KAPA HyperCap Workflow, and were attributed to a lower than usual primer concentration in the pre-capture PCR (when using MGI-compatible reagents, as compared to using the standard KAPA HyperCap reagents for Illumina® sequencing). Nevertheless, both kits produced sufficient amplified Sample Library for pre-capture QC, target enrichment and archiving.

A smear analysis performed with the Bioanalyzer software (across the region of 150 – 1,000 bp) yielded an average fragment size in the range of 320 – 330 bp for all six pre-capture libraries prepared with the KAPA HyperPrep Kit (Figure 1A). The six libraries prepared with the KAPA HyperPlus Kit had a slightly larger average fragment size (ranging from 334 – 399 bp). Fragment length is tunable with both kits (by modifying Covaris® parameters, or adjusting fragmentation time when using the KAPA HyperPrep and KAPA HyperPlus Kits, respectively). Longer fragment lengths offer access to more intronic content, with a small impact on on-target rates. Post-capture libraries (Figure 1B) displayed the expected average fragment size (320 – 330 bp) for both the KAPA HyperPrep and KAPA HyperPlus workflows.

Sequencing metrics

Important sequencing metrics are shown in Figure 2 and Table 2 on the next page.

On-target rates: The percentage of reads on the primary target was high (>65% for KAPA HyperPlus libraries and >70% for KAPA HyperPrep libraries). The percentage bases in the padded capture target exceeded 80% for KAPA HyperPrep libraries, and was slightly lower (>78%) for KAPA HyperPlus libraries. If desired, on-target rates may be improved by tuning fragmentation parameters to yield shorter library inserts.

Duplication rates: The overall process from enzymatic fragmentation through ligation is more efficient (results in higher conversion rates) with the KAPA HyperPlus Kit, as compared to workflows employing mechanical shearing. As a result, fewer pre-capture amplification cycles are needed to achieve the amount of material needed for target enrichment. Since the same target enrichment workflow was performed for all libraries, the lower duplication rates for KAPA HyperPlus libraries (5.4% average vs. 7.8% average for KAPA HyperPrep libraries) was mainly attributed to the improved library construction efficiency of the KAPA HyperPlus Kit.

Fold-80 base penalty: This important metric of coverage uniformity is defined as the fold of additional sequencing required to ensure that the mean coverage is achieved for 80% of the target bases. Fold-80 values were low (~1.6) and consistent across both KAPA HyperPrep and KAPA HyperPlus libraries.

Table 1. Library construction parameters and QC metrics

Input	Library Preparation Kit	Fragmentation parameters	Replicate numbers	Adapter IDs	Pre-capture amplification cycles	Average pre-capture library yield	Capture pool[1]	Average post-capture library yield
Human HapMap NA12891 DNA (100 ng)	KAPA HyperPrep (Covaris shearing)	For average fragment size of 180 – 220 bp (see Section 4.1.3)	1, 2, 3	1, 2, 3	8	815 ng ±153 ng	1	488 ng ±40 ng
			4, 5, 6	15, 16, 103			2 & 3	
	KAPA HyperPlus (enzymatic fragmentation)	25 min at 37°C	1, 2, 3	4, 13, 14	6	1,083 ng ±104 ng	1	
			4, 5, 6	101, 102, 104			2 & 3	

[1]Three KAPA HyperPrep and three KAPA HyperPlus libraries (250 ng each) were pooled to create each Multiplex (6-plex) DNA Sample Library Pool. Pools 1 and 2 were constructed from different uniquely indexed KAPA HyperPrep and KAPA HyperPlus libraries, whereas Pools 2 and 3 were exact technical replicates. Eight cycles of post-capture amplification were performed for all pools.

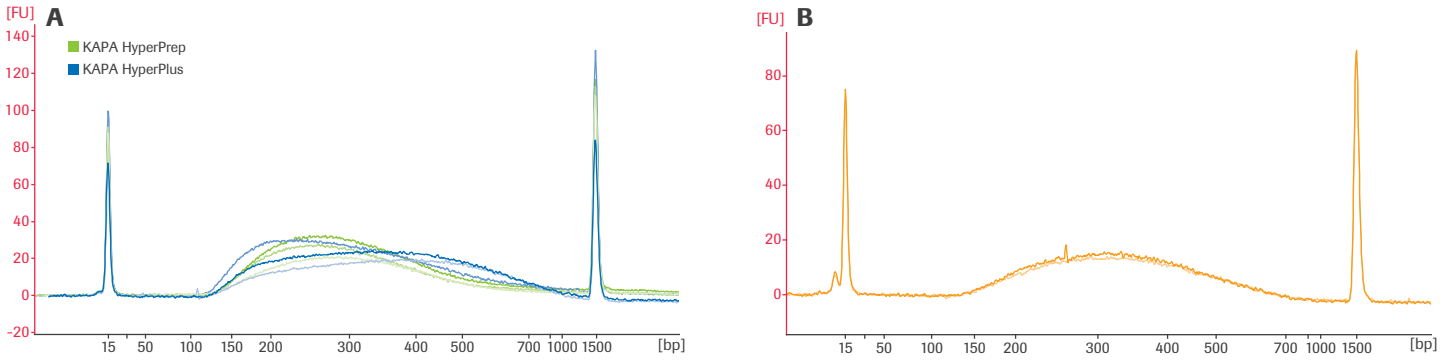


Figure 1. Fragment size distributions of selected amplified (pre-capture) Sample Libraries (A) and enriched, amplified (post-capture) Multiplex DNA Sample Library Pools (B). Libraries were prepared as described in Materials and Methods and the Appendix, and represent the variation in library fragment size distribution observed across all replicates. Library fragment size distributions were determined with an Agilent® 2100 Bioanalyzer instrument.

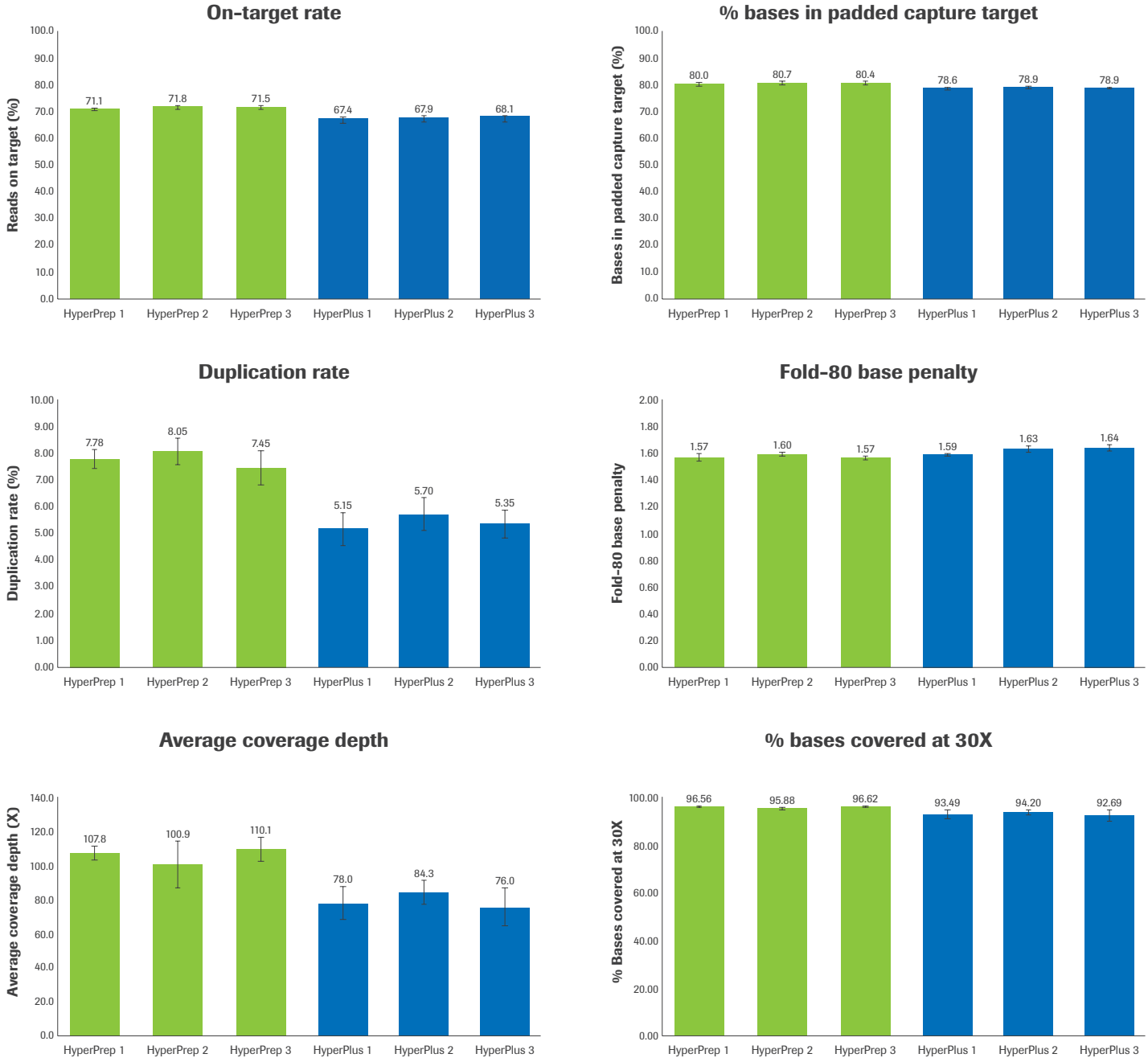


Figure 2. Key sequencing metrics for human whole exome libraries prepared with the KAPA HyperCap Workflow and sequenced on a DNBSEQ™-G400 instrument. Each bar represents a uniquely indexed KAPA HyperPrep (green) or KAPA HyperPlus (blue) library in a 6-plex Multiplex DNA Sample Library Pool, and represents the results from triplicate captures. Libraries were prepared and sequenced, and data were analyzed as described in Materials and Methods and the Appendix.

Table 2. Additional sequencing metrics[1]

Metric	HyperPrep 1	HyperPrep 2	HyperPrep 3	HyperPlus 1	HyperPlus 2	HyperPlus 3
Mean insert size (bp)	231 ±3.4	230 ±2.2	231 ±2.3	252 ±13.4	250 ±11.3	248 ±14.3
Mapping rate	99.81 ±0.03	99.80 ±0.02	99.80 ±0.03	99.78 ±0.03	99.79 ±0.02	99.79 ±0.02
Number of reads after R1/R2 trimming	47,087,890 ±1,642,109	43,970,025 ±5,521,748	47,717,259 ±3,445,385	33,790,971 ±4,481,539	36,837,378 ±3,297,830	33,111,986 ±5,298,316
Percentage high-quality reads (≥Q30)	86.06 ±1.76	83.92 ±0.50	86.15 ±1.13	86.14 ±1.95	86.75 ±2.52	85.38 ±3.56
% bases covered with at least 1 read	98.36 ±0.02	98.36 ±0.01	98.38 ±0.03	98.42 ±0.03	98.43 ±0.03	98.39 ±0.03

[1]Results for each of the three uniquely-indexed KAPA HyperPrep and KAPA HyperPlus libraries in each capture pool are the average of triplicate experiments.

Coverage depth: An average of 92.5 million high-quality reads were obtained for each KAPA HyperPrep library, yielding an average coverage depth of 106X. The average coverage depth for KAPA HyperPlus libraries was 79X (proportionately lower, in accordance with the average of 69.2 million reads available for each KAPA HyperPlus library). For both kits, the percentage of bases covered at 30X exceeded 92%, and was sufficient to support high-confidence variant calling.

Variant Calling: Variant calling results and concordance to the NA12891 truth variant set from the GATK resource bundle (Broad Institute) are given in Figure 3. SNPs were detected with a very high degree of sensitivity (>99%) and specificity (>98.7%) in all libraries. The KAPA HyperPrep and KAPA HyperPlus workflows performed comparably with respect to all variant calling metrics.

Further optimization: Data from another study suggest that significant improvements in on-target rates, coverage depth, and coverage uniformity can be achieved with modifications to the hybridization reaction setup and increasing the temperature used for hybridization, capture, and the two hot washes from 55ºC to 65ºC. Data, as well as an optimized protocol, are presented in an Application Note entitled *The KAPA HyperCap Workflow enables high-performance whole exome sequencing of FFPE DNA on MGI sequencing platforms.*[12]

Conclusions

The aim of this study was to demonstrate the utility of the KAPA HyperCap Workflow from Roche—with the KAPA HyperPrep or KAPA HyperPlus Kit and KAPA HyperExome Probes—for high-quality human whole exome sequencing on the MGI sequencing platform.

Libraries prepared from 100 ng inputs of a commercial preparation of HapMap (NA12891) genomic DNA, and enriched in a 6-plex exome capture, yielded high-quality sequencing data. Key metrics, including on-target and duplication rates, fold-80 base penalty, and various coverage metrics were comparable for libraries prepared from Covaris®-sheared or enzymatically fragmented DNA, and supported high-confidence variant calling.

Only a few minor modifications to the standard KAPA HyperCap Workflow are required to generate MGI-compatible libraries that can be used directly in standard ssCir, DNB generation and sequencing protocols. Mainly, standard barcoded adapters, library amplification primers, and blocking oligos (designed for Illumina® sequencing) have to be replaced with MGI-compatible reagents. Care must be taken to maintain the recommended working concentrations of all these reagents to ensure optimal library conversion rates, capture efficiency and library quality.

The detailed protocol included in the Appendix provides all the instructions needed to unlock the industry-leading performance of KAPA Sample Prep reagents when performing human WES on the MGI platform.

Figure 3. Variant calling results for whole exome libraries sequenced on the MGI platform. Each green bar represents a KAPA HyperPrep library and each blue bar a KAPA HyperPlus library in a 6-plex capture. Results are the average of triplicate experiments.

Sensitivity: TP/(TP+FN) for homozygous and heterozygous variants; *Specificity:* TN/(FP + TN); *Concordance:* [number of times truth and call states match exactly]/[all truth and call combinations made]. TP: true positives; TN: true negatives; FP: false positives; FN: false negatives.

Appendix

Detailed sample preparation workflow

1. Required reagents

Reagents supplied by Roche:

- KAPA HyperPrep Kit
  - Roche PN: 07962312001 (8 libraries)
  - Roche PN: 07962347001 (24 libraries)
  - Roche PN: 07962363001 (96 libraries)
- KAPA HyperPlus Kit
  - Roche PN: 07962401001 (24 libraries)
  - Roche PN: 07962428001 (96 libraries)
- KAPA HyperPure Beads
  - Roche PN: 08963835001 (5 mL)
  - Roche PN: 08963843001 (30 mL)
  - Roche PN: 08963851001 (60 mL)
  - Roche PN: 08963878001 (4 x 60 mL)
  - Roche PN: 08963860001 (450 mL)
- KAPA HyperCapture Reagent Kit
  - Roche PN: 9075810001 (24 reactions)
  - Roche PN: 9075828001 (96 reactions)
- KAPA HyperCapture Bead Kit
  - Roche PN: 9075780001 (24 reactions)
  - Roche PN: 9075798001 (96 reactions)
- KAPA HyperExome Probes
  - Roche PN: 9062548001 (12 reactions)
  - Roche PN: 9062556001 (24 reactions)
  - Roche PN: 9062564001 (48 reactions)
  - Roche PN: 9062572001 (96 reactions)
  - For larger pack sizes, please contact your Roche sales representative
- KAPA HyperChoice Probes
  - Different custom probe options are available (see https://sequencing.roche.com/en/products-solutions/products/sample-preparation/dna-reagents/target-enrichment/hybridization/custom-design.html).
  - HyperDesign Tool: free online custom design portal (www.hyperdesign.com)
- KAPA Probes Resuspension Buffer
  - Roche PN: 9075879001 (1 mL)
  - Roche PN: 9075887001 (5 mL)

The following reagents are not supplied by Roche, and must be sourced from general laboratory stocks or third-party suppliers:

- MGIEasy DNA Adapters Kit V1.0
  - MGI PN: 1000005284 (16 rxn, 16 barcodes)
  - MGI PN: 1000005282 (96 rxn, 96 barcodes)
- MGI-compatible library amplification primer mixes for pre- and post-capture PCR*
- Compatible blocking oligos for MGIEasy DNA Adapters*
- Freshly prepared 80% ethanol
- TE Buffer (10 mM Tris-HCl, pH 8.0 at 25ºC + 0.1 mM EDTA)
- Elution Buffer (10 mM Tris-HCl, pH 8.0 at 25ºC)
- Nuclease-free or PCR-grade water

The following equipment and plasticware are required for this workflow:

- Covaris® M220 instrument
- Covaris microTUBE-50 AFA Fiber Screw-Cap
- Pipettes and suitable, sterile barrier/filter tips
- PCR tubes/96-well plates (0.2 mL)
- Low DNA-binding microtubes (1.5 mL)
- Magnetic block e.g., DynaMag™-96 Side Magnet (Thermo Fisher Scientific Cat. No. 12331D)
- Themocyclers (for standard PCR)
- Agilent® Bioanalyzer 2100 or similar instrument and appropriate reagent kits
- Qubit® Fluorometer and appropriate assays (Thermo Fisher Scientific)

*Primer and blocking oligo sequences may be found on the MGI website, in the document entitled *Oligos and primers for BGISEQ&DNBSEQ NGS system*.[11] Order primers as ready-to-use solutions, or lyophilized and resuspend according to supplier's recommendations. For both pre- and post-capture amplification, prepare an amplification primer mix consisting of 10 µM of each primer in 10 mM Tris-HCl, pH 8.0 at 25ºC.

Both Block 3 and 4 are prepared with nuclease-free water to a final concentration of 300 µM. Block 4 is prepared as in the following example: resuspend each barcoded blocking oligo "Ad153_newBC_indexblock_xx" (depending on the barcoded adapters used for the libraries that are pooled in the hybridization) to a working concentration of 300 µM with nuclease-free water. Prepare a mix of the respective blocking oligos by using equal volume per oligo. This mix is the "Block 4". Use 10 µL of Block 4 per hybridization.

2. Workflow overview

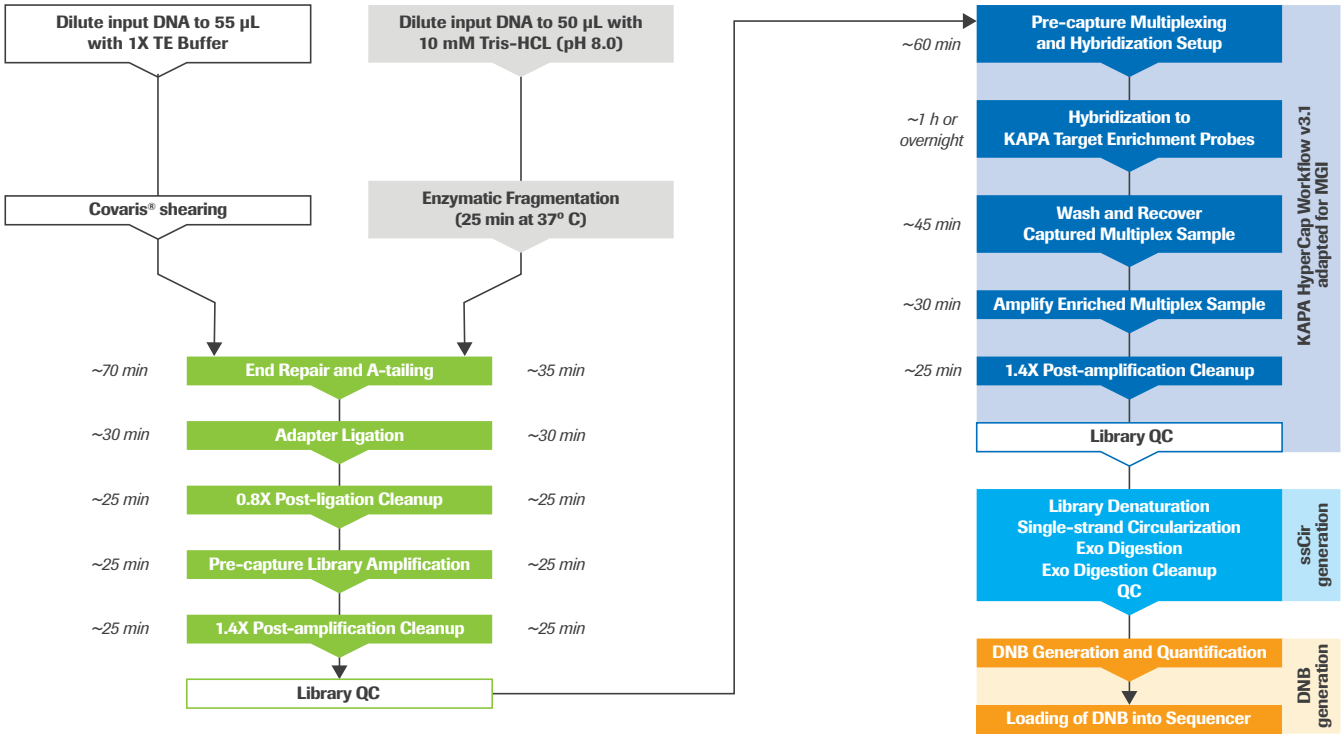


Figure A1. KAPA HyperCap Workflow for the preparation of MGI-compatible whole exome libraries from high-quality DNA. Replicate aliquots of NA12891 human genomic DNA (100 ng) were sheared to a median fragment size of 200 bp, using a Covaris® M220 instrument, or subjected to enzymatic fragmentation for 25 min at 37ºC (as described in **Sections 4.1 and 4.2**, respectively). Depending on the fragmentation method, pre-capture libraries were prepared with the KAPA HyperPrep or KAPA HyperPlus Kit as described in **Sections 4.3 – 4.7**. After library QC (**Section 4.8**), 6-plex capture pools were prepared and exome capture was performed as described in **Sections 4.9 – 4.12**. The standard KAPA HyperCap Workflow was essentially followed, exchanging Illumina®-specific adapters, library amplification primers, and blocking oligos for reagents compatible with the MGI platform. Standard protocols and MGI reagents were used for ssCir and DNB generation. The total workflow time (excluding hybridization, library QC, ssCir, and DNB generation) is 5 – 6 hours. Hybridization time may vary from 1 hour (demonstrated on the Illumina platform) to overnight (16 – 20 h).

3. Quality control of input DNA

Quality control of input DNA is strongly recommended. In this study, DNA was quantified using an Qubit® Fluorometer and Qubit dsDNA HS (High Sensitivity) Assay Kit (Thermo Fisher Scientific). These assays are designed to provide accurate quantification of double-stranded DNA molecules present in the sample.

4. Library construction protocol

This protocol describes construction of individual *Sample Libraries* using the KAPA HyperPrep or KAPA HyperPlus Kit and is based on the protocol outlined in the **KAPA HyperCap Workflow v3.1 User Guide**.[4] Optimal shearing parameters for Covaris instruments other than the M220 have to be determined empirically.

4.1 Covaris Shearing (if using the KAPA HyperPrep Kit)

4.1.1 Prepare a Covaris M220 instrument as per the manufacturer's instructions.

4.1.2 Transfer 100 ng of DNA in a total volume of 55 µL of 1X TE Buffer into a Covaris microTUBE-50 AFA Fiber Screw-Cap.

4.1.3 Shear using the following settings:

- Peak Incident Power (Watt): 50
- Duty Factor (percent): 20
- Cycles/Burst (count): 200
- Duration (seconds): 175

4.1.4 Transfer 50 µL of sheared DNA into a new plate/tube(s) and proceed with **End Repair and A-Tailing**.

4.2 Enzymatic Fragmentation (when using the KAPA HyperPlus Kit)

4.2.1 Prepare 100 ng of DNA in Elution Buffer for Enzymatic Fragmentation. Assemble each Fragmentation Reaction on ice, as outlined below:

Component	Volume
Input DNA (100 ng)	35 µL
KAPA Frag Buffer (10X)[1]	5 µL
KAPA Frag Enzyme[1]	10 µL
Total volume	**50 µL**

[1]The KAPA Frag Buffer and Enzyme may be pre-mixed and kept on ice prior to reaction setup, and dispensed as a single solution. Please note the volume of buffer is less than the volume of enzyme in this reaction.

4.2.2 Vortex gently and spin down briefly. Return the plate/tube(s) to ice. Proceed immediately to the next step.

4.2.3 Incubate the plate/tube(s) in a thermocycler, pre-cooled to 4ºC and programmed as outlined below. A heated lid is not required for this step. If used, set the temperature of the heated lid to ≤50ºC.

Step	Temp	Time
Pre-cool block	4ºC	N/A
Fragmentation	37ºC	25 min
HOLD	4ºC	∞

4.2.4 Quickly transfer the reaction to ice and proceed immediately to **End Repair and A-Tailing**.

NOTE: Both the Covaris® shearing and enzymatic fragmentation parameters are expected to yield DNA with a median fragment size of approximately 200 bp. Parameters may be tuned to achieve shorter inserts (to improve coverage metrics) or longer inserts (to obtain more information about intronic content).

4.3 End Repair and A-Tailing

4.3.1 Assemble each End Repair and A-Tailing reaction on ice in a tube or well of a PCR plate, as outlined below:

Component	Volume	
	KAPA HyperPrep	KAPA HyperPlus
Fragmented dsDNA	50 µL	50 µL
KAPA End Repair & A-Tailing Buffer[1]	7 µL	7 µL
KAPA Hyper**Prep** End Repair & A-Tailing Enzyme Mix[1,2]	3 µL	—
KAPA Hyper**Plus** End Repair & A-Tailing Enzyme Mix[1,2]	—	3 µL
Total volume	**60 µL**	**60 µL**

[1]The buffer and enzyme mix should preferably be premixed and added in a single pipetting step. Premixes are stable for <24 hr at room temperature, for ≤3 days at 4ºC, or for ≤4 weeks at -20ºC.

[2]Always use the KAPA Hyper**Prep** End Repair & A-Tailing Enzyme mix for Covaris-sheared DNA. Always use the KAPA Hyper**Plus** End Repair & A-Tailing Enzyme Mix for enzymatically fragmented DNA.

4.3.2 Vortex gently and spin down briefly. Return the plate/tube(s) to ice. Proceed immediately to the next step.

4.3.3 Incubate in a thermocycler programmed as outlined below:

KAPA HyperPrep:

Step	Temp	Time
End Repair and A-Tailing	20ºC	30 min
	65ºC[1]	30 min
HOLD	4ºC	∞

KAPA HyperPlus:

Step	Temp	Time
End Repair and A-Tailing	65ºC[1]	30 min
HOLD	4ºC	∞

[1]A heated lid is required for this incubation. If possible, set the lid temperature to 85ºC, instead of the default ~105ºC.

4.3.4 Centrifuge the plate/tubes(s) briefly and proceed immediately to **Adapter Ligation**.

4.4 Adapter Ligation

4.4.1 In the same plate/tubes(s) in which End Repair and A-Tailing was performed, assemble each Adapter Ligation reaction as follows:

Component	Volume
End Repair and A-Tailing Reaction Product	60 µL
MGIEasy DNA Adapter (10 µM)[1]	5 µL
Ligation Buffer[2]	30 µL
DNA Ligase[2]	10 µL
Nuclease-free Water[2]	5 µL
Total volume	**110 µL**

[1]Libraries that will be pooled for sequencing must each receive a unique Adapter ID or index.

[2]The water, buffer and ligase enzyme should preferably be premixed and added in a single pipetting step. Premixes are stable for ≤24 hr at room temperature, or for ≤3 days at 4ºC.

4.4.2 Mix thoroughly and centrifuge briefly.

4.4.3 Incubate in a thermocycler programmed as outlined below:

Step	Temp	Time
Set heated lid	50ºC	On
Adapter Ligation	20ºC	30 min
HOLD	4ºC	∞

4.4.4 Centrifuge plate/tube(s) briefly and proceed immediately to **Post-ligation Cleanup**.

4.5 Post-ligation Cleanup

4.5.1 Make sure that the KAPA HyperPure Beads have been equilibrated to room temperature and thoroughly resuspended to obtain a homogeneous mixture.

4.5.2 In the same plate/tube(s) from step 4.4.4, perform a 0.8X bead-based cleanup by combining the following:

Component	Volume
Adapter Ligation Reaction Product	110 µL
KAPA HyperPure Beads	88 µL
Total volume	**198 µL**

4.5.3 Mix thoroughly by vortexing and/or pipetting up and down multiple times.

4.5.4 Incubate the plate/tube(s) at room temperature for 5 min to bind DNA to the beads.

4.5.5 Place the plate/tube(s) on a magnetic stand to capture the beads. Incubate until the liquid is clear.

4.5.6 Carefully remove and discard the supernatant.

4.5.7 Keeping the plate/tube(s) on the magnetic stand, add 200 µL of freshly prepared 80% ethanol.

4.5.8 Incubate the plate/tube(s) on the magnetic stand at room temperature for ≥30 sec.

4.5.9 Carefully remove and discard the ethanol.

4.5.10 Keeping the plate/tube(s) on the magnetic stand, add 200 µL of 80% ethanol.

4.5.11 Incubate the plate/tube(s) on the magnetic stand at room temperature for ≥30 sec.

4.5.12 Carefully remove and discard the ethanol. Try to remove all residual ethanol without disturbing the beads.

4.5.13 Dry the beads at room temperature for 3 – 5 min, or until all of the ethanol has evaporated. Be careful not to over dry the beads as this may result in reduced yield.

4.5.14 Remove the plate/tube(s) from the magnetic stand.

4.5.15 Thoroughly resuspend the beads in 22 µL of Elution Buffer (10 mM Tris-HCl, pH 8.0).

4.5.16 Incubate the plate/tube(s) at room temperature for 2 min to elute DNA off the beads.

4.5.17 Place the plate/tube(s) on a magnetic stand to capture the beads. Incubate until the liquid is clear.

4.5.18 Transfer 20 µL of each eluate to a fresh well/tube and proceed with **Pre-capture library amplification**.

4.6 Pre-capture Library Amplification

4.6.1 Assemble the pre-capture library amplification reaction as follows:

Component	Volume
Purified, Adapter-ligated DNA	20 µL
KAPA HiFi HotStart ReadyMix (2X)[1]	25 µL
MGI-compatible Pre-capture Library Amplification Primer Mix[1,2]	5 µL
Total volume	**50 µL**

[1]KAPA HiFi HotStart ReadyMix and primers should be premixed and added in a single pipetting step.

[2]Refer to Section 1 (materials not supplied by Roche).

4.6.2 Mix well by pipetting up and down several times.

4.6.3 Amplify the library using the following thermocycling profile:

Step	Temp	Time	Cycles[1]
Initial Denaturation	98ºC	45 sec	1
Denaturation	98ºC	15 sec	8 for KAPA HyperPrep 6 for KAPA HyperPlus
Annealing	60ºC	30 sec	
Extension	72ºC	30 sec	
Final Extension	72ºC	1 min	1
HOLD	4ºC	∞	1

[1]The number of PCR cycles may require optimization depending on the input amount.

4.6.4 Proceed immediately to **Library amplification cleanup**.

4.7 Library Amplification Cleanup

4.7.1 In the same plate/tube(s), perform 1.4X bead-based cleanup by combining the following:

Component	Volume
Amplified Pre-capture Library DNA	50 µL
KAPA HyperPure Beads	70 µL
Total volume	**120 µL**

4.7.2 Thoroughly resuspend the beads by pipetting up and down multiple times.

4.7.3 Incubate the plate/tube(s) at room temperature for 5 min to bind DNA to the beads.

4.7.4 Place the plate/tube(s) on a magnetic stand to capture the beads. Incubate until the liquid is clear.

4.7.5 Carefully remove and discard the supernatant.

4.7.6 Keeping the plate/tube(s) on the magnetic stand, add 200 µL of 80% ethanol.

4.7.7 Incubate the plate/tube(s) on the magnetic stand at room temperature for ≥30 sec.

4.7.8 Carefully remove and discard the ethanol.

4.7.9 Keeping the plate/tube(s) on the magnetic stand, add 200 µL of 80% ethanol.

4.7.10 Incubate the plate/tube(s) on the magnetic stand at room temperature for ≥30 sec.

4.7.11 Carefully remove and discard the ethanol. Try to remove all residual ethanol without disturbing the beads.

4.7.12 Dry the beads at room temperature for 3 – 5 min, or until all of the ethanol has evaporated. Be careful not to over dry the beads as this may result in reduced yield.

4.7.13 Remove the plate/tube(s) from the magnetic stand.

4.7.14 Thoroughly resuspend the beads in 32 µL of Elution Buffer (10 mM Tris-HCl, pH 8.0) or nuclease-free water.

4.7.15 Incubate the plate/tube(s) at room temperature for 2 min to elute DNA off the beads.

4.7.16 Place the plate/tube(s) on a magnetic stand to capture the beads. Incubate until the liquid is clear.

4.7.17 Transfer 30 µL of each eluate to a new well/tube and proceed with **Pre-capture Library QC**.

NOTE: At this point, the amplified, purified pre-capture libraries may be stored at 2ºC to 8ºC for 1 – 2 weeks, or at -15ºC to -25ºC (long term storage).

4.8 Pre-capture Library QC

Evaluate the success of library construction by determining the library fragment size distribution with an electrophoretic method (e.g., with an Agilent® Bioanalyzer 2100 instrument and DNA 1000 or DNA High Sensitivity Kit). Pre-capture libraries should have a fragment size distribution of 150 – 1,500 bp. KAPA HyperPlus libraries may display a broader size distribution (as shown in Figure A2 on the next page).

A Qubit® Fluorometer and dsDNA HS Assay Kit (Thermo Fisher Scientific) are recommended for the quantification of pre-capture libraries.

If capturing a single sample, utilize 1000 ng of a uniquely indexed amplified DNA Sample Library per capture. For multiplexed capture, mix equal amounts (by mass) of the appropriate number of uniquely indexed DNA Sample Libraries to obtain a combined DNA mass of 1.5 µg. At least 250 ng of each individual library is required for 6-plex exome capture.

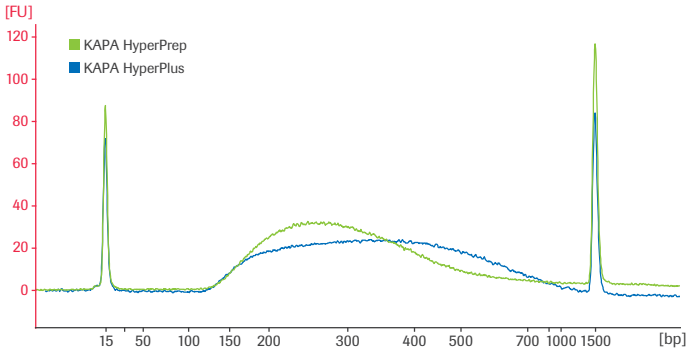


Figure A2. Expected size distribution of pre-capture libraries. Adapter-ligated libraries generated with the KAPA HyperPrep Kit (green curve) or KAPA HyperPlus Kit (blue curve) were amplified for 6 or 8 cycles using KAPA HiFi HotStart ReadyMix and MGI-compatible Library Amplification Primers. The size distribution was assessed using an Agilent® 2100 Bioanalyzer instrument and DNA 1000 Kit.

4.9 Preparing for Hybridization

4.9.1 Thaw the appropriate number of 4 µL KAPA Target Enrichment Probes aliquots (one aliquot per capture or hybridization reaction).

4.9.2 Prepare capture pools by combining equal amounts (by mass) of uniquely indexed pre-capture libraries, to obtain a combined mass of 1.5 µg. For exome capture with KAPA HyperExome Probes, 6-plex capture pools (6 x 250 ng pre-capture library) are recommended. This mixture will subsequently be referred to as the *Multiplex DNA Sample Library Pool.*

4.9.3 Add nuclease-free water to adjust the final volume of each pool to 45 µL.

NOTE: If the volume of the Multiplex DNA Sample Library Pool exceeds 45 µL, please refer to the Troubleshooting section of the KAPA HyperCap Workflow v3.1 User Guide.

4.9.4 Prepare the hybridization sample as follows:

- Add 20 µL of COT Human DNA to the Multiplex DNA Sample Library Pool, to bring the total volume up to 65 µL.

NOTE: When working with non-human DNA, the KAPA Hybrid Enhancer Reagent should be used instead of COT Human DNA. Some optimization of the protocol may be required for non-human species.

- Add 130 µL of KAPA HyperPure Beads to each well or tube containing Multiplex DNA Sample Library/ COT Human DNA mixture.
- Mix thoroughly by vortexing for 10 sec and spin down briefly.
- Incubate at room temperature for 10 min to allow the Multiplex DNA Sample Library and COT Human DNA to bind to the beads.
- Place the plate/tube(s) on the magnetic stand to capture the beads. Incubate until the liquid is clear.
- Carefully remove and discard the supernatant.
- Keeping the plate/tube(s) on the magnetic stand, add 200 µL of freshly prepared 80% ethanol.
- Incubate the plate/tube(s) on the magnetic stand at room temperature for at least 30 sec.
- Carefully remove and discard the ethanol. Try to remove any residual ethanol without disrupting the beads.
- Dry the beads at room temperature for 3 – 5 min, or until all of the ethanol has evaporated. Be careful not to over dry the beads as this may result in reduced yield.
- Prepare the MGI Blocking Oligos as follows:

Component	Volume
Block 3	1.0 µL
Block 4 (see bottom of p. 6)	10.0 µL
Nuclease-free Water	2.4 µL
Total volume	**13.4 µL**

- Remove the plate/tube(s) from the magnetic stand and add 13.4 µL of MGI Blocking Oligos to the bead-bound DNA sample in each well/tube.
- Mix thoroughly by vortexing to ensure a homogeneous mixture.

4.9.5 Prepare the Hybridization Master Mix according to the capture target size as shown in the tables below:

- *For KAPA Target Enrichment Designs >40 Mb in capture target size:*

Component	Volume
Hybridization Buffer	28 µL
Hybridization Component H	9 µL
Nuclease-free water	6 µL
Total volume	**43 µL**

- *For KAPA Target Enrichment Designs <40 Mb in capture target size:*

Component	Volume
Hybridization Buffer	28 µL
Hybridization Component H	12 µL
Nuclease-free Water	3 µL
Total volume	**43 µL**

4.9.6 Add 43 µL of the Hybridization Master Mix to the mixture of Multiplex DNA Sample Library Pool, COT DNA and Blocking Oligos (from Step 4.9.4).

4.9.7 Mix thoroughly and spin down briefly. Incubate at room temperature for 2 min.

4.9.8 Place the plate/tube(s) on the magnetic stand to capture the beads. Incubate until the liquid is clear.

4.9.9 Transfer the full volume of each eluate (56.4 µL) to a fresh well/tube containing 4 µL of KAPA Target Enrichment Probes.

4.9.10 Mix thoroughly by vortexing for 10 sec and perform a quick spin. Perform the hybridization incubation in a thermocycler using the following program (with the lid temperature set to 105ºC):

Step	Temp	Time
Denaturation	95ºC	5 min
Incubation	55ºC	HOLD

4.9.11 Incubate at 55°C for 16 – 20 hours to hybridize the KAPA Target Enrichment Probes to the Multiplex DNA Sample Library Pool before proceeding to **Wash and Recover the Captured Multiplex DNA Sample Library Pools**. The sample must be kept at 55°C until it is transferred to the Capture Beads in Step 4.10.5.

4.10 Wash and Recover the Captured Multiplex DNA Sample Library Pools

4.10.1 Before completion of the hybridization incubation, thaw the Hybridization Wash Buffers. Mix well before using the buffers.

4.10.2 Dilute the 10X Wash Buffers (I, II, III and Stringent) and the 2.5X Bead Wash Buffer from the KAPA HyperCapture Reagent Kit, to create 1X working solutions. Volumes listed in the table below are sufficient for **one capture**. These should be scaled up when performing multiple captures in parallel.

Buffer	Vol. of conc. buffer	Vol. of nuclease-free water	Total volume of 1X buffer
Stringent Wash Buffer (10X)	40 µL	360 µL	400 µL[1]
Wash Buffer I (10X)	10 µL	90 µL	100 µL[1]
	20 µL	180 µL	200 µL[2]
Wash Buffer II (10X)	20 µL	180 µL	200 µL[2]
Wash Buffer III (10X)	20 µL	180 µL	200 µL[2]
Bead Wash Buffer (2.5X)	120 µL	180 µL	300 µL[2]

[1]Place the 1X Stringent Wash Buffer and the smaller (100 µL) aliquot of 1X Wash Buffer I at 55°C for Step 4.10.8.

[2]1X working solutions of these buffers may be stored at room temperature (15°C to 25°C) for up to 2 weeks.

4.10.3 Pre-warm the 1X Stringent Wash Buffer and 100 µL aliquot of 1X Wash Buffer I at 55°C for at least 15 min.

4.10.4 Prepare the Capture Beads as follows:

- Allow the Capture Beads to equilibrate to room temperature before use.
- Vortex Capture Beads for 15 sec to ensure a homogeneous mixture before use.
- Aliquot the appropriate amount of beads per capture into a new well/tube, as outlined below:

Panel size	Volume of beads per capture
>40 Mb Capture Target Size	100 µL
<40 Mb Capture Target Size	50 µL

- Place the plate/tube(s) on the magnetic stand to capture the beads. Incubate until the liquid is clear.
- Carefully remove and discard the supernatant without disturbing the beads.
- Keeping the plate/tube(s) on the magnetic stand, add two times the initial volume of beads of 1X Bead Wash Buffer.
- Remove the plate/tube(s) from the magnetic stand and mix thoroughly by vortexing. Spin down briefly.
- Place the plate/tube(s) on the magnetic stand to capture the beads. Incubate until the liquid is clear.
- Carefully remove and discard the supernatant, without disturbing the beads.
- Keeping the plate/tube(s) on the magnetic stand, perform a second wash by adding two times the initial volume of beads of 1X Bead Wash Buffer.
- Remove the plate/tube(s) from the magnetic stand and mix thoroughly by vortexing. Spin down briefly.
- Place the plate/tube(s) on the magnetic stand to capture the beads. Incubate until the liquid is clear.
- Carefully remove and discard the supernatant, without disturbing the beads.
- Add the initial volume of beads of 1X Bead Wash Buffer.
- Remove the plate/tube(s) from the magnetic stand and mix thoroughly by vortexing. Spin down briefly.
- Aliquot the appropriate amount of resuspended beads per capture as shown in the table below in a new well/tube:

Panel size	Volume of resuspended beads per capture
>40 Mb Capture Target Size	100 µL
<40 Mb Capture Target Size	50 µL

- Place the plate/tube(s) on the magnetic stand to capture the beads. Incubate until the liquid is clear.
- Carefully remove and discard the supernatant, without disturbing the beads. The Capture Beads are now ready to bind to the hybridized DNA. Proceed immediately to the next step.

4.10.5 Transfer each hybridization sample (from step 4.9.11) into a single well/tube with the prepared Capture Beads.

4.10.6 Mix thoroughly by vortexing for 10 sec and spin down briefly.

4.10.7 Incubate the capture reaction in the thermocycler at 55°C for 15 min (set lid temperature to 105°C).

4.10.8 Add 100 µL of pre-warmed 1X Wash Buffer I to each capture reaction.

4.10.9 Mix thoroughly by vortexing for 10 sec to ensure that the mixture is homogeneous. Spin down briefly.

4.10.10 Place the plate/tube(s) on the magnetic stand to capture the beads. Incubate until the liquid is clear.

4.10.11 Carefully remove and discard the supernatant, without disturbing the beads.

4.10.12 Keeping the plate/tube(s) on the magnetic stand, add 200 µL of pre-warmed 1X Stringent Wash Buffer.

4.10.13 Remove plate/tube(s) from the magnetic stand and mix thoroughly by vortexing for 10 sec to ensure that the mixture is homogeneous. Spin down briefly.

4.10.14 Incubate the mixture in a thermocycler pre-heated to 55°C for 5 min (set lid temp to 105°C).

4.10.15 After incubation, remove the plate/tube(s) from the thermocycler and place on the magnetic stand to capture the beads. Incubate until the liquid is clear.

4.10.16 Carefully remove and discard the supernatant, without disturbing the beads.

4.10.17 Repeat steps 4.10.12 through 4.10.16 once, for a total of two washes with the pre-warmed 1X Stringent Wash Buffer.

4.10.18 To each well/tube, add 200 µL of room temp 1X Wash Buffer I.

4.10.19 Mix thoroughly by vortexing for 10 sec to ensure that the mixture is homogeneous. Spin down briefly.

4.10.20 Incubate the plate/tube(s) at room temperature for 1 min.

4.10.21 Place the plate/tube(s) on the magnetic stand to capture the beads. Incubate until the liquid is clear.

4.10.22 Carefully remove and discard the supernatant, without disturbing the beads.

4.10.23 To each well/tube, add 200 µL of room temp 1X Wash Buffer II.

4.10.24 Mix thoroughly by vortexing for 10 sec to ensure that the mixture is homogeneous. Spin down briefly and transfer the contents to a new tube.

4.10.25 Incubate the plate/tube(s) at room temperature for 1 min.

4.10.26 Place the plate/tube(s) on the magnetic stand to capture the beads. Incubate until the liquid is clear.

4.10.27 Carefully remove and discard the supernatant, without disturbing the beads.

4.10.28 To each well/tube, add 200 µL of room temp 1X Wash Buffer III.

4.10.29 Mix thoroughly by vortexing for 10 sec to ensure that the mixture is homogeneous. Spin down briefly.

4.10.30 Incubate the plate/tube(s) at room temperature for 1 min.

4.10.31 Place the plate/tube(s) on the magnetic stand to capture the beads. Incubate until the liquid is clear.

4.10.32 Carefully remove and discard the supernatant, without disturbing the beads.

4.10.33 Remove the plate/tube(s) from the magnet.

4.10.34 To each well/tube, add 20 µL of nuclease-free water and mix thoroughly. Spin down briefly.

4.10.35 Proceed to **Amplify Enriched Multiplex DNA Sample**.

4.11 Amplify Enriched Multiplex DNA Sample

4.11.1 Refer to Section 1 (reagents not supplied by Roche) for guidelines on preparing the post-capture amplification primer mix.

4.11.2 In fresh well(s)/tube(s), prepare one post-capture PCR master mix for each Enriched Multiplex DNA Sample by thoroughly mixing the components listed in the following table:

Component	Volume
KAPA HiFi HotStart ReadyMix (2X)	25 µL
MGI-compatible Post-capture Library Amplification Primer Mix	5 µL
Total volume	**30 µL**

4.11.3 Retrieve the bead-bound DNA from Section 4.10.34, and mix thoroughly by vortexing for 10 sec. Spin down briefly.

4.11.4 Transfer 20 µL of the bead-bound DNA (PCR template) to each well/tube containing 30 µL Post-Capture PCR Master Mix. Mix well.

4.11.5 Amplify the enriched Multiplex DNA Samples using the thermocycling profile given in the table below. The total number of Post-Capture PCR cycles is dependent on the Capture Target Size.

Step	Temp	Time	Cycles[1]
Initial Denaturation	98ºC	45 sec	1
Denaturation	98ºC	15 sec	Variable (see table below)[1]
Annealing	60ºC	30 sec	
Extension	72ºC	30 sec	
Final Extension	72ºC	1 min	1
HOLD	4ºC	∞	1

Capture Target Size	Number of Post-capture PCR Cycles[1]
<100 kb	18
100 kb - 2 Mb	16
>2 Mb - 40 Mb	10
>40 Mb	8

[1]Numbers of post-capture PCR cycles are recommendations and may have to be optimized depending on individual experimental needs.

4.11.6 Once post-capture amplification has been completed, proceed immediately to **Purification of Amplified, Enriched Multiplex DNA Sample**.

4.12 Purification of Amplified, Enriched Multiplex DNA Sample

4.12.1 Equilibrate the KAPA HyperPure Beads to room temperature before use. Mix well to obtain a homogeneous solution before use.

4.12.2 Place the plate/tube(s) containing the amplified, enriched Multiplex DNA Sample(s) on the magnetic stand to capture the beads. Incubate until the liquid is clear. Transfer each supernatant (50 µL) to a new well or tube.

4.12.3 Add 70 µL of KAPA HyperPure Beads to each amplified, enriched Multiplex DNA Sample.

4.12.4 Mix thoroughly by vortexing for 10 sec to ensure that the mixture is homogeneous. Spin down briefly. Incubate at room temp for 5 min to allow the Multiplex DNA Sample to bind to the beads.

4.12.5 Place the plate/tube(s) containing the bead-bound DNA on the magnetic stand to capture the beads. Incubate until the liquid is clear.

4.12.6 Carefully remove and discard the supernatant without disturbing the beads.

4.12.7 Keeping the plate/tube(s) on the magnetic stand, add 200 µL of 80% ethanol.

4.12.8 Incubate the plate/tube(s) on the magnetic stand at room temperature for ≥30 sec.

4.12.9 Carefully remove and discard the ethanol.

4.12.10 Keeping the plate/tube(s) on the magnetic stand, add 200 µL of 80% ethanol.

4.12.11 Incubate the plate/tube(s) on the magnetic stand at room temperature for ≥30 sec.

4.12.12 Carefully remove and discard the ethanol. Try to remove all residual ethanol without disturbing the beads.

4.12.13 Dry the beads at room temperature for 3 – 5 min, or until all of the ethanol has evaporated. Be careful not to over dry the beads as this may result in reduced yield.

4.12.14 Remove the plate/tube(s) from the magnetic stand.

4.12.15 Thoroughly resuspend the beads in 22 µL of Elution Buffer (10 mM Tris-HCl, pH 8.0) or nuclease-free water.

4.12.16 Vortex for at least 10 sec to ensure that all of the beads are resuspended. Briefly spin down.

4.12.17 Incubate the plate/tube(s) at room temperature for 2 min to elute the DNA off the beads.

4.12.18 Place the plate/tube(s) on a magnetic stand to capture the beads. Incubate until the liquid is clear.

4.12.19 Transfer 20 µL of each eluate to a new well/tube and proceed with **Post-capture Library QC**.

NOTE: At this point, the amplified, purified, post-capture libraries may be stored at 2ºC to 8ºC for 1 – 2 weeks, or at -15ºC to -25ºC (long term storage).

4.13 Post-capture Library QC

Evaluate post-capture libraries by determining the library fragment size distribution and post-capture library yield. For these assays, a 10-fold dilution of each final library (2 µL diluted to 20 µL in nuclease-free water) is recommended.

To determine the library fragment size distribution, analyze 1 µL of each diluted, post-capture library with an Agilent® Bioanalyzer 2100 instrument and DNA 1000 or DNA High Sensitivity Kit. Post-capture libraries are expected to have a fragment size distribution in the range of 150 – 1,000 bp, with an average fragment size of ~320 bp, as shown in Figure A3.

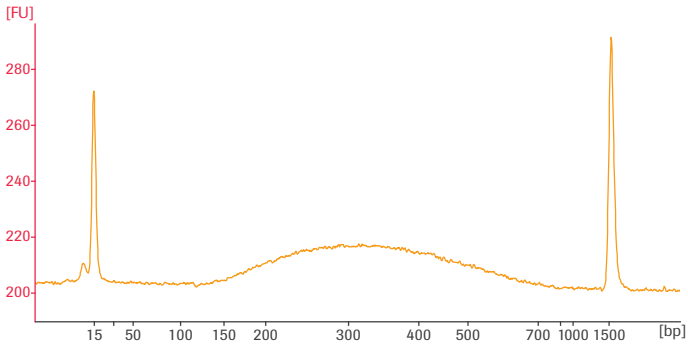


Figure A3. Expected size distribution of post-capture libraries. Sequencing-ready amplified, enriched Multiplex DNA Samples were prepared as described. The size distribution was assessed using an Agilent 2100 Bioanalyzer instrument and DNA 1000 Kit.

Use 5 µL of the 10-fold diluted library to determine the library concentration with a Qubit® Fluorometer and dsDNA HS Assay Kit (Thermo Fisher Scientific). Convert the concentration to mass. The final yield of each Multiplex DNA Sample library should be >100 ng.

DNA Sample libraries that meet all of the above criteria may be used for sequencing on MGI Sequencing Platforms. Refer to MGI protocols for ssCir and DNB generation.

References

1. Drmanac R, Sparks AB, Callow MJ, et al. Human Genome Sequencing Using Unchained Base Reads on Self-Assembling DNA Nanoarrays. Science. 2009;327(78). doi:10.1126/science.1181498.
2. https://sequencing.roche.com/. Accessed 9 September 2021.
3. Kang H, Wang, T, Yan Y-W, et al. Implementation of KAPA HyperPrep Kit for for whole genome sequencing on MGI sequencing platforms employing DNBSEQ.™ Roche Application Note, MC--05005.
4. Roche Sequencing Solutions, Inc. https://pim-eservices.roche.com/eLD/api/downloads/fd9ee7b1-46f9-eb11-0b91-005056a772fd?countryIsoCode=pi. KAPA HyperCap Workflow v3.1 User Guide. Accessed 9 September 2021.
5. Roche Sequencing Solutions, Inc. https://sequencing.roche.com/content/dam/rochesequence/worldwide/resources/KAPA-HyperExome-Probes-Sellsheet.pdf. KAPA HyperExome Probes Sell Sheet. Accessed 9 September 2021.
6. MGI Tech Co., Ltd. en.mgitech.cn/Uploads/Temp/picture/20191016/5da6dbb28f8ed.pdf. MGIEasy Circularization Kit User Manual. Accessed 9 September 2021.
7. MGI Tech Co., Ltd. en.mgitech.cn/Uploads/Temp/file/20191223/5e007f7f6da8c.pdf. DNBSEQ-G400RS High-throughput Sequencing Set User Manual. Accessed 9 September 2021.
8. Coriell Institute for Medical Research. https://catalog.coriell.org/0/Sections/Search/Sample_Detail.aspx?Ref=NA12891&Product=CC. Accessed 9 September 2021.
9. MGI Tech Co., Ltd. https://en.mgi-tech.com/Download/download_file/id/27. MGIEasy Universal DNA Library Prep Set User Manual. Accessed 9 September 2021.
10. MGI Tech Co., Ltd. en.mgitech.cn/Download/download_file/id/71. Oligos and primers for BGISEQ/DNBSEQ/MGISEQ library preparation. Accessed 9 September 2021.
11. Broad Institute. https://gatk.broadinstitute.org/hc/en-us/articles/360035890811. Truth variant dataset for NA12891: CEUTrio.HiSeq.WGS.b37.bestPractices.hg19.vcf.gz. Accessed 9 September 2021.
12. Kang H, Yan Y-W, Meyer J, et al. The KAPA HyperCap Workflow enables high-performance whole exome sequencing of FFPE DNA on MGI sequencing platforms. Roche Application Note, MC--08123.

Acknowledgments

The authors wish to thank staff from the MGI Tech Co., Ltd in Shenzhen for generously providing us with the MGIEasy DNA Adapters Kit, MGIEasy Primer Mix and sequencing services (DNBSEQ-G400) used in this project.

Published by:

Roche Sequencing Solutions, Inc.
4300 Hacienda Drive
Pleasanton, CA 94588

sequencing.roche.com

MC--07579 09/2021