MAGBIO

"WE MAKE NGS BETTER"

GENSeq™ Hema Profiler Kit

Hybridization based target enrichment for mutation detection in blood cancer

Manual Revision v1.0
Catalog nos. GSHP-02, GSHP-16, GSHP-48, GSHP-96

PROTOCOL

Contents

For Research Use Only. Not for use in diagnostic procedures.

Information in this document is subject to change without notice.

Product Description

GENSeq™ Hema Profiler Kit enables target enrichment and sequencing of the genes involved in blood cancer. Enrichment by hybridization capture is based on GENSeq™ target enrichment panel; the GENSeq™ Hema Plus Panel. Enrichment panels are pre-designed probes for targeted next generation sequencing (NGS). GENSeq™ Hema Plus Panel spans 1.03Mb of the human genome and covers a total of **266** blood cancer related genes. The total number of probes in GENSeq™ Hema Plus Panel is 8,655.

GENSeq™ universal blockers bind to adapter sequences to prevent non-specific binding, improving the number of reads on-target. The blockers are compatible with Illumina indexed adapters.

For custom designed probes for a target of your interest, contact Support@magbiogenomics.com.

Kit Contents and Storage

GENSeq™ Consumables : Blood Cancer Profiling			
Item	**Description**	**Catalog Number**	**STORAGE**
Target Capture			
Blood Cancer Panel	GENSeq™ Hema Plus Panel	GSH-16	-20°C
		GSH-48	
		GSH-96	
Custom Panels	Custom designed to customer's needs		
Blocking oligos for TruSeq libraries	GENSeq™ Universal Blocking Oligo	GSBO-5016	-20°C
		GSBO-5048	
		GSBO-5096	
Hybridization and Wash Kit	GENSeq™ Hybridization & Wash Buffer	GSHW-16	Box 1
		GSHW-48	-20°C
		GSHW-96	
	GENSeq™ Hybridization & Wash Buffer	GSHW-16	Box 2
		GSHW-48	4°C
		GSHW-96	

Stability

• All components are stable for 12 months when stored accordingly.

Safety Information

• Wear gloves and take precautions to avoid sample contamination.
• Perform all centrifugation at room temperature (+15 to +25°C) unless indicated otherwise.
• Unless otherwise specified, all mixing steps are listed as mix thoroughly and indicate that the sample should be mixed by either vortexing for 10 seconds or pipetting up and down 10 times.
• If liquid has remained in a tube's cap after mixing, gently tap or briefly spin the sample to collect the liquid into the tube's bottom, ensuring that the mixture remains homogeneous before progressing to the next step.
• It is recommended to perform Thermal Cycler incubations using a Thermal Cycler with a heated lid set to track 10°C above the block temperature.

Important Notice

This protocol is designed for a maximum of 6 capture reactions using individual tubes. The tubes need to be sealed tightly to avoid evaporation during the 4hr incubation. Excessive evaporation during hybridization can lead to capture failure.

Equipment and Reagents to Be Supplied by User

When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, please consult the appropriate material safety data sheets (MSDSs) from each product supplier.

Item	Description
Two thermal cyclers	General laboratory supplier
Water bath or heating block	General laboratory supplier
Micro centrifuge	General laboratory supplier
Vortex mixer	General laboratory supplier
Magnet	MagBio MagStrip Magnet Stand (1.5ml x 12) (MBMS-12)
qPCR system or fluorescence-based DNA quantitation system	Thermo Fisher Qubit™ 3.0 Fluorometer (Cat # Q33216) or equivalent
Vacuum concentrator	Eppendorf Concentrator plus (Cat # 5305000193) Thermo Fisher Scientific SpeedVac system (Cat # SC210A-115) or equivalent
Digital electrophoresis system	Agilent 2100 Electrophoresis Bioanalyzer® system (Cat # G2939AA) Agilent 2200 Tapestation® system (Cat # G2965AA) or equivalent
Ethanol (Absolute)	General laboratory supplier
KAPA HiFi HotStart Ready Mix	Kapa Biosystems (Cat # KK2601)
Digital electrophoresis chips	Agilent High Sensitivity DNA Kit (Cat #5067-4626) Agilent High Sensitivity D1000 ScreenTape (Cat # 5067-5584) or equivalent
Nuclease-Free Water	General laboratory supplier
TE Solution	Integrated DNA Technologies, IDTE, pH8.0(Cat # 11-01-02-05) or equivalent (10 mM Tris, 0.1 mM EDTA)
Microtubes, 1.5 mL	Axygen MAXYMum Recovery® Microtubes, 1.7 ml (Cat # 22234-046) or equivalent
Microtubes, 0.2 mL	Axygen MAXYMum Recovery™ PCR Tubes, 0.2 ml flat cap (Cat # PCR-02-L-C) Or equivalent

Workflow

Step	Process		Time
1	Isolate genomic DNA (HighPrep™ Blood and Tissue kit)	Isolate cfDNA (cfKapture kit)	30 min
	↓	↓	
2	Shear DNA (If necessary)		30 min
	↓		
3	Prepare GENSeq™ DNA Library: Repair, add adapters, amplify		30 min
	↓		
4	Cleanup with HighPrep™ PCR		30min
	↓		
5	Combine DNA with blocking oligos		15 min
	Dry down DNA		Variable
	Perform hybridization reaction		4-16 hours
	↓		
6	Prepare buffer		15 min*
	↓		
7	Wash streptavidin beads		15 min
	↓		
8	Perform beads capture		30 min
	↓		
9	Perform washes		30 min
	↓		
10	Perform post-capture PCR		30 min
	↓		
11	Purify post-capture PCR fragments		30 min
	↓		
12	Sequence		

* Perform during hybridization reaction

Before You Start

1. Set two thermal cyclers at different incubation temperatures for target enrichment & hybridization capture protocol.

HYB program (lid set to 100°C)	
95°C	**30 sec**
65°C	**4 hr - 16 hr**
65°C	**HOLD**
WASH program (lid set at 70°C *)	
65°C	**HOLD**

*For WASH program, reduce the lid temperature to 70°C

2. Equilibrate **2X Hybridization Buffer** and **Hybridization Buffer Enhancer** to room temperature**.** Mix well and centrifuge briefly.

3. Make sure the SpeedVac system is preheated to 60°C.

1. Perform Hybridization Reaction

1.1 In a Lo-Bind 1.5 mL microtube add **libraries, Human Cot DNA** and **GENSeq™ Universal Blocking oligos** according to the table below.

Components	Volume per reaction (µL)
Libraries	Up to 6 µg (500ng/library). If multiplexing samples, use 500 ng of each library
Human Cot DNA	5
GENSeq™ Universal Blocking Oligo	2

⚠ *Make sure that >50% of libraries have been applied in the hybridization step.*
⚠ *It's recommended that total number of libraries is no more than 5 when multiplexing cfDNA and FFPE samples.*

1.2 Dry down the mixture in a SpeedVac system at 60°C. This is done to get high sample concentration.

STOP Safe to stop here.

⚠ *Make sure that the sample tube is tightly sealed to avoid losing the sample.*
⚠ *You may store the sample at room temperature overnight, but for longer storage -20°C is recommended.*

1.3 Thaw all contents of the **GENSeq™ Hybridization & Wash Buffer** to room temperature.

⚠ **2X Hybridization Buffer** *tends to crystallize. If there is crystallization then heat the tube at 65°C while shaking intermittently until the crystals are solubilized. The dissolution of the crystals may take several hours.*

1.4 Prepare Hybridization Master Mix in the tube from step 1.1 (dried hybridization tube) as shown below.

Components	Volume per reaction (µL)
GENSeq™ 2X Hybridization Buffer	8.5
GENSeq™ Hybridization Buffer Enhancer	2.7
Nuclease-free Water	1.8
GENSeq™ Plus Panel or Custom Probe	4
Total	**17 µL**

1.5 Mix gently by pipetting up and down15-20 times and incubate at 25°C for 5-10 min.

1.6 Vortex to mix well and then centrifuge briefly.

1.7 Pipette 17 µL of the capture into a 0.2 mL Lo-Bind PCR tube and then centrifuge briefly.

1.8 Place the sample tube in the thermal cycler and start the **HYB program.**

2. Buffer Preparation

⚠ Before preparing the buffers, equilibrate **Dynabeads M-270 Streptavidin beads** to room temperature. Remove the beads from 4°C storage at least 30 min before performing the washes.

2.1 Make 1X working solutions by diluting the buffers as indicated below.

Components	Nuclease-free Water (µL)	Buffer (µL)	Total (µL)
GENSeq™ 2X Bead Wash Buffer	160	160	**320**
GENSeq™ 10X Wash Buffer I	252	28	**280**
GENSeq™ 10X Wash Buffer II	144	16	**160**
GENSeq™ 10X Wash Buffer III	144	16	**160**
GENSeq™ 10X Stringent Wash Buffer	288	32	**320**

⚠ *If **Wash Buffer I** looks cloudy, resuspend by heating the bottle in a 65°C water bath.*
⚠ *The 1X working solutions are stable at room temperature (15°C-25°C) for up to 4 weeks.*

2.2 Aliquot the following solutions into separate tubes and heat to 65°C. Keep the solutions at 65°C for at least 15 min.
- one tube (110 µL aliquot) of **1X Wash Buffer I**
- two tubes (160 µL aliquot) of **1X Stringent Wash Buffer**

⚠ ***1X Wash Buffer I** (110 µL aliquot) and **1X Stringent Wash Buffer** (2 of 160 µL aliquots) will be used during the **Heated washes**, it is recommended to start this incubation at the same time as the bead capture reaction, so that the buffers will be at the required temperature when needed.*

2.3 In a Lo-Bind tube, prepare the following Bead Resuspension Mix:

Bead resuspension mix components	Volume per reaction (µL)
GENSeq™ 2X Hybridization Buffer	8.5
GENSeq™ Hybridization Buffer Enhancer	2.7
Nuclease-free Water	5.8
Total	**17**

3. Wash Streptavidin beads

⚠ **Important!** Streptavidin beads must be equilibrated to room temperature before performing bead washes.

3.1 Vortex the **streptavidin beads** thoroughly to mix well for at least 15 sec. Aliquot 50 µL of streptavidin beads into a single 1.5 mL Lo-Bind microcentrifuge tube.

3.2 Add 100 µL of **1X Beads Wash Buffer** (prepared in step 2.1) per tube containin**g streptavidin beads.** Gently pipet up and down 10 times to mix and centrifuge briefly. Place the tube on a magnetic device to allow the beads to fully separate from the supernatant. Aspirate and discard the clear supernatant, ensuring that there is no bead loss. Remove the tube from the magnetic device.

3.3 Repeat Step 3.2 twice.

3.4 Add 17 µL per capture of **Bead Resuspension Mix** (prepared in step 2) to resuspend the beads.

3.5 Pipette up and down to mix well and ensure that the beads are not left to dry in the tube. If needed, briefly centrifuge the tube at 25 x g (400 rpm).

3.6 Pipette 17 µL of resuspended beads into a new 0.2 mL Lo-Bind microtube for each capture reaction.

3.7 Incubate at 65°C for 5 min.

4. Perform bead capture

4.1 Take the sample tube out of the thermal cycler after 4 hr~16 hr incubation. Stop the **HYB program** and start the **WASH program.**

4.2 Transfer resuspended **streptavidin beads** to the 0.2 mL tube containing the sample. Vortex to ensure that the sample is fully resuspended. Briefly centrifuge the sample tube if needed (10 sec at 25 x g).

⚠ *Use Lo-Bind tips in Step 2. The operation should be quick.*

4.3 Place the sample tube in the thermal cycler under **WASH program** and set a timer for 45 min.

⚠ *It is safe to place the sample tubes in the thermal cycler before the lid temperature has fully cooled to 70°C when starting the incubation.*

4.4 Every 10-12 min remove the tube from the thermal cycler and gently vortex to ensure the sample is fully resuspended. At the end of the 45 min incubation, take the sample off the thermal cycler. Proceed immediately to heated washes.

5. Perform washes

5.1.1 Heated washes

⚠ Make sure that the buffers have reached 65°C before starting the **Heated washes.** All the operations should be quick. Avoid making bubbles during pipetting.

5.1.2 Add 100 µL of 65°C heated **Wash Buffer I** to the sample and pipet up and down 10 times to mix. Avoid making bubbles during pipetting.

5.1.3 Put the tube on the magnetic device for 1 min until supernatant is clear. Discard the supernatant.

5.1.4 Remove the tube from the magnetic device and add 150 µL of 65°C heated **Stringent Wash Buffer** to the sample. Pipet up and down 10 times to mix. Avoid introducing bubbles during pipetting.

5.1.5 Incubate the tube in the thermal cycler at 65°C for 5 min.

5.1.6 Repeat Step 5.3 and Step 5.4.

5.2.1 Room temperature washes

⚠ **Important!** Vigorously mix the samples during the room temperature washes to ensure the beads remain fully resuspended.

5.2.2 Place the tube on the magnetic device for 1 min or until the beads are completely cleared from the solution and the solution looks clear. Discard the supernatant and add 150 µL of **Wash Buffer I** equilibrated to room temperature. Resuspend the beads by vortexing thoroughly. Incubate the tube for 2 min while alternating between vortexing for 30 sec and resting for 30 sec; this ensures the mixture remains homogenous. Briefly centrifuge the tube at the end of the 2 mins incubation.

5.2.3 Place the tube on the magnetic device for 1 min until or until the beads are completely cleared from the solution and the solution looks clear. Discard the supernatant. Add 150 µL of **Wash Buffer II.** Resuspend the beads by vortexing thoroughly. Incubate the tube for 2 min while alternating between vortexing for 30 sec and resting for 30 sec; this ensures the mixture remains homogenous. Briefly centrifuge the tube at the end of the 2 mins incubation.

5.2.4 Place the tube on the magnetic device for 1 min or until the beads are completely cleared from the solution and the solution looks clear. Discard the supernatant. Add 150 µL of **Wash Buffer III.** Resuspend the beads by vortexing thoroughly. Incubate the tube for 2 min while alternating between vortexing for 30 sec and resting for 30 sec; this ensures the mixture remains homogenous. Briefly centrifuge the tube at the end of the 2 mins incubation.

5.2.4 Place the tube back on the magnetic device for 1 min or until supernatant is clear. Remove the supernatant. Use a fine pipette tip to remove any residual **Wash Buffer III** from the tube.

5.2.5 Remove the tube from the magnetic device. Add 20 µL of **Nuclease-free water** to each capture tube. Pipet up and down 10 times to mix and resuspend any beads stuck to the side of the tube. Pipette the sample into a fresh 0.2 mL Lo-Bind PCR microtube.

⚠ *Do not discard the beads. Use the entire 20 µL of resuspended beads with captured DNA in Performing post-capture PCR.*

6. Perform post-capture PCR

6.1 Thaw the **Amplfication Reaction Mix** components on ice (in the table below). Prepare the Amplification Reaction Mix as indicated below:

Amplification Reaction Mix Components	Volume per reaction (µL)
2X KAPA HiFi HotStart ReadyMix*	25
GENSeq™ Amplification Primer Mix	5
Beads with captured DNA	20
Total	**50**

** If using a PCR master mix other than Kapa HiFi, the magnesium concentration may need to be optimized for on-bead PCR.*

6.2 Put the tube in a thermal cycler, and run the following program with the heated lid set at 105°C.

Step	Temperature (°C)	Time	Number of cycles
Polymerase activation	98	45 sec	1
Denaturation	98	15 sec	6-10 cycles
Annealing	60	30 sec	
Extension	72	30 sec	
Final extension	72	1 min	1
Hold	4	Hold	1

⚠ *The number of PCR cycles should be optimized per Panel size and the number of pooled libraries per capture, to ensure there is enough yield for sequencing.*

Panel Size	1-plex	4-plex	8-plex	12-plex
10,000-100,000 probes	12 cycles	10 cycles	9 cycles	8 cycles

STOP Optional stopping point. Amplified captures may be stored at 4°C overnight.

7. Post-amplification Cleanup

⚠ **HighPrep™ PCR Beads** must be equilibrated to room temperature before use.

7.1 Prepare fresh **80% ethanol.**

7.2 Add 60 µL of **HighPrep™ PCR Beads** to each amplified capture (transfer to a larger 2 mL tube, if needed). Mix thoroughly and incubate for 5-10 min at 25 °C.

7.3 Put the sample tube on the magnetic device for 5 min or until the supernatant is clear. Take out the supernatant without disturbing the beads.

7.4 While the tube is on the magnetic device, add 150 µL of **80% ethanol** without disturbing the beads. Incubate for 1 min. Discard the ethanol.

7.5 Repeat Step 7.4

7.6 Place the sample tube back on the magnetic device and use a fine pipet tip to discard the remaining ethanol without disturbing the beads.

7.7 Allow the beads to air dry for 2-5 min. Do not over dry the beads.

7.8 Remove the sample tube from the magnet and elute in 20-22 µL low EDTA **TE buffer /TE solution.** Pipette up and down to mix. Incubate for 2-5 min at room temperature.

7.9 Place the tube on the magnetic device until the supernatant is clear. Pipette 20 µL of the clear eluate to a fresh tube avoiding bead carry-over.

Safe to stop here. Purified PCR fragments can be stored at -20°C for up to 1 week.

8. Validate and quantify library

8.1 Determine the concentration of the captured library using a fluorescence-based method for DNA quantification (such as Qubit dsDNA HS Assay kit) or qPCR.

8.2 Measure the average fragment length of the captured library on a digital electrophoresis system (e.g. the Agilent 2100 Bioanalyzer using a high sensitivity DNA chip, or the Agilent 2200 TapeStation system using a DNA tape or other similar system).

9. Perform sequencing

Perform sequencing according to the instructions for your Illumina instrument.

Ordering Information

GENSeq™ Library Prep, Hema/Onco Library Enrichment, Hybridization and Target Capture			
GENSeq™ DNA Library Prep	GENSeq™ DNA Library Prep Kit-test	GSDL-1002	2 rxn
	GENSeq™ DNA Library Prep Kit	GSDL-1024	24 rxn
	GENSeq™ DNA Library Prep Kit	GSDL-1048	48 rxn
	GENSeq™ DNA Library Prep Kit	GSDL-1096	96 rxn
GENSeq™ Tumor Profiling Kit **(for Cancer genes mutation profilng)**	GENSeq™ Onco Profiler Kit-test	GSOP-02	2 rxn
	GENSeq™ Onco Profiler Kit	GSOP-16	16 rxn
	GENSeq™ Onco Profiler Kit	GSOP-48	48 rxn
	GENSeq™ Onco Profiler Kit	GSOP-96	96 rxn
GENSeq™ Blood Cancer Profiling Kit **(for Cancer genes mutation profilng)**	GENSeq™ Hema Profiler Kit-test	GSHP-02	2 rxn
	GENSeq™ Hema Profiler Kit	GSHP-16	16 rxn
	GENSeq™ Hema Profiler Kit	GSHP-48	48 rxn
	GENSeq™ Hema Profiler Kit	GSHP-96	96 rxn
Fixed Panels	GENSeq™ Hema Plus- test	GSH-02	2 rxn
	GENSeq™ Hema Plus Panel	GSH-16	16 rxn
	GENSeq™ Hema Plus Panel	GSH-48	48 rxn
	GENSeq™ Hema Plus Panel	GSH-96	96 rxn
	GENSeq™ Onco Plus- test	GSH-02	2 rxn
	GENSeq™ Onco Plus Panel	GSH-16	16 rxn
	GENSeq™ Onco Plus Panel	GSH-48	48 rxn
	GENSeq™ Onco Plus Panel	GSH-96	96 rxn

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