GENECKER
For Genetic Diagnosis & Testing

User Manual

Instructions for Use

CRISPincette™ MultiCancer

Please read all contents in the product manual before use.

Research Use Only. Not for use in diagnostic procedures.

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1. Product Information

Important: Before using this product, read and understand the information in the "**D. Precautions for Use**" of the **Appendix** in this document.

1.1. Product Description

CRISPincette™ MultiCancer is a Next-Generation Sequencing (NGS) kit designed to detect mutations in cfDNA from the blood for cancer risk assessment such as lung, breast, colorectal, prostate, gastric, liver, ovarian, and pancreatic cancers. Utilizing GeneCker's ultra-precision CRISPR-Cas9 system, this kit effectively detects even low-frequency mutations by selectively cutting wild-type DNA and amplifying mutant DNA. This product is designed to analyze 23 hotspot mutations across 14 genes, including AKT, APC, BRAF, BRCA1, CTNNB1, EGFR, ERBB2, IDH1, KRAS, MYCN, NRAS, PDGFRA, PIK3CA, and TP53. Results from this kit are intended for clinical reference only. Clinicians should comprehensively interpret the test results in the context of the examinee's condition, drug indications, treatment plan, and other laboratory test indicators.

Major Target List		
AKT1 E17K	APC E1309Dfs*4	BRAF V600E
BRCA1 E23Vfs*17	CTNNB1 T41A	EGFR C797S
EGFR E746_A750del	EGFR L858R	EGFR T790M
ERBB2 V777L	IDH1 R132H/C	KRAS G12D
MYCN P44L	NRAS G12D	PDGFRA D842V
PIK3CA E545K	PIK3CA H1047R	TP53 R175H
TP53 M237I	TP53 R248Q	TP53 R273H
TP53 R337H	TP53 Y220C	

1.2. Product Component and Storage Condition

Important: Upon arrival, inspect all consumables and contact Warranty Service team if any of the components have been damaged during shipping, store all components under the recommended condition and in an upright position.

Kit	Type	Kit Components	Cap Color	Quantity	Volume (µL)	Storage
Box	tube	● Target primer set1	Green	1	120	-20℃
		● Target primer set2	Yellow	1	120	
		○ 2X PCR mix	White	2	2,400	
		● Remov Enzyme mix set1	Green	1	192	
		● Remov Enzyme mix set2	Yellow	1	192	
		● Remov RXN buffer	Red	1	384	
		● Stabilizer	Red	1	96	
		● 10X STOP buffer	Red	1	96	
Index primer set	Plate	Index primer set (Index 01~96)	-	1	2.5 µL/well	-20℃

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1.3. Required Equipment and Material (Not Provided)

Besides the components of the kit, the following equipment and materials are also required.

1) List of Equipment and Consumables

	Equipment	Supplier / Catalog No.
1	MiSeq or MiSeq Dx	Illumina
2	96-well Thermal cycler	General laboratory supplier
3	Digital Electrophoresis System (Can be selected from the equipment below)	
	Agilent 2100 Electrophoresis Bioanalyzer	Agilent Technologies, Cat # G2939AA
	- Agilent High Sensitivity DNA Kit	Agilent Technologies, Cat # 5067-4626
	Agilent 4150 TapeStation	Agilent Technologies, Cat # G2992AA
	(or Agilent 4200 TapeStation)	(Cat # G2991BA)
	- Agilent High Sensitivity D1000 ScreenTape & Reagent	Agilent Technologies, Cat # 5067-5584/5067-5585
	- Agilent D1000 ScreenTape & Reagent	Agilent Technologies, Cat # 5067-5582/5067-5583
	- Agilent Genomic DNA ScreenTape & Reagent	Agilent Technologies, Cat # 5067-5365/5067-5366
	- Agilent Cell-free DNA ScreenTape & Reagent	Agilent Technologies, Cat # 5067-5630/5067-5631
4	Magnetic Separation Stand (Can be selected from the equipment below)	
	- 16-tube DynaMag™-2 Magnet (for 1.5 mL tube)	Thermo Fisher Scientific, Cat # 12321D
	- 6-tube Magnetic Separation Rack (for 1.5 mL tube)	New England Biolabs, Cat # S1506S
	- DiaMag02 Magnetic Rack (for 1.5 mL tube)	Diagenode, Cat # B04000001
	- Magnet Stand-96 (DynaMag-96 side) (for 0.2 mL tube)	Thermo Fisher Scientific, Cat # 12331D
5	Fluorometer (Can be selected from the equipment below)	
	Qubit® 3.0/4.0 Fluorometer	Thermo Fisher Scientific, Cat # Q33216/Q33226
	- Qubit® Assay tubes	Thermo Fisher Scientific, Cat # Q32856
	- Qubit® dsDNA Quantitation, High Sensitivity	Thermo Fisher Scientific, Cat # Q32851
	- Qubit® 1x dsDNA HS Assay Kit	Thermo Fisher Scientific, Cat # Q33231
	Quantus Fluorometer	Promega, Cat # E6150
	- QuantiFluor® Single-tube Fluorometer Accessories	Promega, Cat # E4942
	- QuantiFluor® ONE ds DNA System	Promega, Cat # E4871
6	Micro centrifuge	General laboratory supplier
7	Vortex mixer	General laboratory supplier
8	Plate centrifuge	General laboratory supplier
9	Cooling rack	General laboratory supplier

2) List of Materials

	Other Materials	Supplier / Catalog No.
1	>99% ethanol	General laboratory supplier
2	microtubes, 1.5 mL	General laboratory supplier

3	8-strip tube	General laboratory supplier
4	DEPC-water	General laboratory supplier
5	SPRIselect, 450 mL	Beckman Coulter, Cat # B23319
6	1.0 N NaOH (molecular biology grade)	General laboratory supplier
7	MiSeq Reagent Kit	Illumina
8	50 mL conical tubes	General laboratory supplier
9	Qubit™ assay tubes	Thermo Fisher, Cat # Q32856
10	Pipette & pipette tip (10, 20, 100, 200 and 1,000 µL) * Filter tip is recommended	General laboratory supplier

2. Testing Protocol

2.1. Important Notes

1) All reagents used in this protocol should be performed briefly spin down to collect components clinging under the lid and wall of tubes.
2) For frozen reagents, they are fully thawed and mixed using a vortex mixer. Briefly spin down.
3) Keep all reagents on ice until ready for use.
4) To obtain a high concentration of cfDNA, proceed with the elution volume at the minimum volume guaranteed by the manufacturer.
5) If all reagents are repeatedly frozen and thawed, it might cause performance deterioration.
6) Ensure magnetic beads are sufficiently vortexed before use.
7) 85% ethanol for size selection is prepared and used immediately before every washing process.
8) The use of filter tips throughout the entire process is recommended.
9) Final library loading concentration of MiSeq is 6 pM, and PhiX control is not required.

2.2. Time Required for 48 Samples

Part	Step		Time (hrs)
Library Preparation	1st PCR & IVC	1st PCR	1.5
		In vitro Cleavage (IVC)	1
		Size Selection	1
	2nd PCR	2nd PCR	1.5
		Size Selection	0.5
	Library Pooling		0.5
Sequencing	MiSeq or MiSeq DX System		-

2.3. Sample Preparation

* This product uses DNA that meets the summary of recommended DNA quality and quantity standards below.

1) Recommended tool for sample quantification and qualification

- Accurate quantification of DNA is critical for producing a high-quality NGS library. We recommend using a fluorescence assay such as Qubit® Fluorometer for DNA quantification.
- To accurately determine the size distribution of DNA samples and libraries, we recommend using automated electrophoresis tools such as the TapeStation (Agilent) due to its ability to rapidly evaluate size distribution and quality.

2) Summary of recommended DNA quality and quantity

Summary of recommended DNA quality & quantity		
cfDNA	Amount of input DNA (Quantitative values based on Qubit® Fluorometer)	≥ 20 ng
	DNA quality	> 70% cfDNA

2.4. Library Preparation

Step 1. 1st PCR

Materials (Including Kit)
- ● Target primer set 1
- ● Target primer set 2
- ○ 2X PCR mix
User preparing materials
- 1.5 mL microtube
- 8-strip tube
- DEPC-water
- DNA sample
- Thermal cycler

1) Perform vortexing and spin down after thawing ● Target primer set 1, ● Target primer set 2, ○ 2X PCR mix in a cooling rack on ice.

2) Add the DNA sample and each reagent to the 8-strip tube under the conditions shown in the table below. When handling multiple samples, it is recommended to prepare and use a master mix in a 1.5 mL microtube. Prepare separate master mixes for target primer set 1 and set 2 in that case.

Reagent	Volume per sample (µL)
● Target primer set 1 or ● Target primer set 2	**2.5**
○ 2X PCR mix	**12.5**
DNA sample	**Variable**
DEPC-water	**Up to 25**
Total Volume	**25**

* The recommended amount of cfDNA for each primer set is ≥10 ng.

3) Perform vortexing and spin down after mixing each reagent.

4) Place the 8-strip tube in the thermal cycler preset under the conditions shown in the table below and run the program.

(Lid temperature: 105℃)

Step Description	Temperature (℃)	Time	Cycles
Pre-denaturation	98	1 min	1
Denaturation	98	10 sec	25
Annealing/Extension	65	40 sec	
Hold	4	∞	1

* Optional: The recommended ramp rate of thermal cycler is 0.5℃/sec.

Step 2. *In vitro* Cleavage (IVC)

Kit included materials
- 🟢 Removv Enzyme mix set 1 - 🟡 Removv Enzyme mix set 2 - 🔴 Removv RXN buffer - 🔴 Stabilizer - 🔴 10X STOP buffer
User preparing materials
- Thermal cycler - 1.5 mL microtube - 8-strip tube

* Perform the IVC product from each 1st PCR product set 1, set 2 (Step 1.). Store the part of 1st PCR product that didn't undergo the IVC procedure on ice or in a refrigerator until use for IVC control in the 2nd PCR (Step 4).

1) Perform vortexing and spin down after thawing 🔴 Removv RXN buffer, 🟢 Removv Enzyme mix set 1, 🟡 Removv Enzyme mix set 2, 🔴 Stabilizer in cooling rack on ice.

2) Add the 1st PCR product (Step 1.) and each reagent to the 8-strip tube under the conditions in the table below. When handling multiple samples, it is recommended to prepare and use a master mix in a 1.5 mL microtube. Prepare separate master mixes for Removv Enzyme mix set 1 and set 2 in that case.

	Reagent	Volume per Sample (µL)
IVC	1st PCR Product (set 1 or set 2)	1
	🔴 Removv RXN buffer	4
	🔴 Stabilizer	1
	🟢 Removv Enzyme mix set 1 or 🟡 Removv Enzyme mix set 2	4
	Total Volume	**10**

3) Perform vortexing and spin down after mixing each reagent.

4) Place the 8-strip tube in the thermal cycler preset under the conditions shown in the table below and run the program.
(Lid temperature: 60℃)

Step Description	Temperature (℃)	Time
In vitro Cleavage (IVC)	45	30 min

5) Collect 10 µL of each set 1 and set 2 products into one well of a new 8-strip Tube. At this time, be careful not to mix the IVC control product and the IVC product after the 30 minutes incubation.

6) Add 2 µL of 🔴 10X STOP buffer to IVC product 20 µL.

7) Perform vortexing and spin down.

Step 3. Size Selection

User preparing materials
- 8-strip tube - DEPC-water - >99% ethanol - magnetic stand - SPRI Beads

* This step is valid only for IVC products (Step 2.).

1) Perform vortexing and spin down sufficiently before using the SPRI beads.
2) Mix homogeneously by 10 times of pipetting after adding 40 µL of SPRI beads (Ratio: 2X) to approximately 20 µL to the IVC product (Step 2.).
3) Incubate samples for 5 minutes at room temperature.
4) Prepare fresh 85% ethanol for washing step.
5) Place the sample tube on the magnetic stand until the beads are separated in pellet form (about 2-5 minutes).
6) Remove and discard the supernatant using a pipette, without disturbing the beads pellet.
7) While keeping the 8-strip tubes on the magnetic stand, add 100 µL of 85% fresh ethanol, incubate for 1 minute at room temperature for beads washing, and then carefully remove and discard the supernatant without disturbing the beads using a pipette.
8) Remove all remaining ethanol using a pipette once more and incubate for 1 minute at room temperature for ethanol drying. Be cautious not to over dry the beads pellet.
9) Add 10 µL of DEPC-water to each well and mix homogeneously by 10 times of pipetting.
10) Place the 8-strip tubes on the magnetic stand until the supernatant is clear and the beads are separated in Pellet form (about 1-2 minutes).
11) Transfer 10 µL of the clear supernatant to new 8-strip tubes. At this point, ensure that the elution does not contain beads. If beads are present, place the tube back on the magnetic stand and transfer the elution again.
* If the used volume of DEPC-water was 10 µL, transfer 8 µL of the supernatant to the 8-strip tubes to prevent the beads from being eluted together.

Step 4. 2nd PCR

Materials (Including Kit)
- ○ 2X PCR mix - Index primer set Index 01-96 (plate)
User preparing materials
- 1.5 mL microtube - DEPC-water - Thermal cycler

* Perform each 1st PCR product set 1, set 2 (Step 1.), IVC Size Selection product (Step 3.).

1) Perform vortexing and spin down after thawing ○ 2x PCR in a cooling rack on ice.

2) Add the IVC control (Step 1.) and size selection product (Step 3.) and each reagent to the 8-strip tube under the conditions shown in the table below. When handling multiple samples, it is recommended to prepare and use a master mix in a 1.5 mL microtube.

IVC control	
Reagent	Volume per Sample (µL)
Mix of 1st PCR product set 1, set 2 (Step 1.)	**0.5 + 0.5**
○ 2X PCR mix	12.5
DEPC-water	9
Index primer set Index 01-96 (plate)	2.5
Total Volume	**25**

IVC	
Reagent	Volume per Sample (µL)
IVC size selection product (Step 3.)	**1**
○ 2X PCR mix	12.5
DEPC-water	9
Index primer set Index 01-96 (plate)	2.5
Total Volume	**25**

* Be cautious of the Index primer plate's direction.

3) Perform vortexing and spin down after mixing reagents with each 1st PCR product set 1, set 2 (Step 1.), and the IVC size selection product (Step 3.).

4) Place the 8-strip tube in the thermal cycler preset under the conditions shown in the table below and run the program.

(Lid temperature: 105℃)

Step Description	Temperature (℃)	Time	Cycles
Pre-denaturation	98	1 min	1
Denaturation	98	10 sec	25
Annealing/Extension	65	40 sec	
Hold	4	∞	1

* Optional: The recommended ramp rate of thermal cycler is 0.5℃/sec.

Step 5. Size Selection

User preparing materials
- 1.5 mL microtube - DEPC-water - >99% ethanol - Magnetic Stand - SPRI Beads

1) Perform vortexing and spin down sufficiently before using the SPRI beads.
2) Mix 2nd PCR product (Step 4.) IVC control and IVC separately.

   In this process, the IVC control product and the IVC product should be distinguished. However, size selection is performed by mixing samples of several indexes into one 1.5 mL microtube.
3) After adding SPRI beads (Ratio: 0.8X) to the 2nd PCR product (Step 4.) as mentioned in the table below, mix homogeneously by pipetting up and down 10 times.

Number of 2nd PCR Product	Volume of 2nd PCR Product (each)	Size Selection Mixture	
		Volume of 2nd PCR Product	Volume of SPRI Beads
n	10 µL	(n x 10) µL	(n x 8) µL
48 (*Maximum)	10 µL	(48 x 10) µL	(48 x 8) µL

4) Incubate samples for 5 minutes at room temperature.
5) Prepare fresh 85% ethanol for the washing step.
6) Place the sample tube on the magnetic stand until the beads are separated in pellet form (about 2-5 minutes).
7) Remove and discard the supernatant using a Pipette, without disturbing the beads pellet.
8) While keeping the 1.5 mL microtube on the magnetic stand, add a sufficient volume of 85% fresh ethanol to cover the pellets. Incubate for 1 minute at room temperature to wash the beads.
9) Carefully remove and discard the supernatant using a pipette, without disturbing the beads.
10) Remove all remaining ethanol using a pipette once more. Be cautious not to over-dry the beads pellet.
11) After adding DEPC-water to a 1.5 mL microtube as mentioned table below, mix homogeneously by pipetting up and down 10 times.

Number of 2nd PCR Product	* Elution Volume of DEPC-water
8-16	30 µL
16-24	50 µL
25-32	70 µL
33-48	100 µL

12) Incubate samples for 1 minute at room temperature.
13) Place the 1.5 mL microtubes on the magnetic stand until the supernatant is clear and the beads are separated in Pellet form (about 1-2 minutes).
14) Transfer the clear supernatant to new 1.5 mL microtubes. At this point, ensure that the elution does not contain beads. If beads are present, place the tube back on the magnetic stand and transfer the elution again.
    * If the used volume of DEPC-water used was 60 µL, transfer 58 µL of the supernatant to the 1.5mL microtubes to prevent the beads from being eluted together.

Step 6. Library Pooling

User preparing materials
- 1.5 mL microtube - DEPC-water - Fluorometer

1) Qubit® Fluorometer is recommended for the concentration of both the IVC control and IVC product from the size selection product (Step 5.). If the Qubit® Fluorometer is not available, use another type of fluorescent dye-based equipment to measure the dsDNA concentration. When using the Qubit® Fluorometer, utilize Qubit® 1x dsDNA HS Assay Kit.

   (Qubit® 1x dsDNA HS working solution : Sample = 198 µL : 2 µL)

2) Dilute each sample in a 1.5 mL microtube to a 10 ng/µL concentration using DEPC-water.

3) Mix 10 µL of the IVC control and 10 µL of the IVC product in a new 1.5 mL microtube.

Step 7. Library QC

1) Qubit® Fluorometer is recommended for the concentration of the pooled library (Step 6.). If the Qubit® Fluorometer is not available, use another type of fluorescent dye-based equipment to measure the dsDNA concentration. When using the Qubit® Fluorometer, utilize Qubit® 1x dsDNA HS Assay Kit.

   (Qubit® 1x dsDNA HS working solution : Sample = 190 µL : 10 µL)

2) Convert the measured concentration of the library to molar concentration. (Length of dsDNA: ≒ 220 bp)

   * Moles dsDNA (mol) = mass of dsDNA (ng) / ((length of dsDNA (bp) x 615.96 g/mol/bp) + 36.04 g/mol)

3) Dilute the converted molar concentration to 4 nM using DEPC-Water.

2.5. Sequencing (Refer to manufacturer's guidelines)

Step 8. Denaturation and Dilution Library

* Thaw the reagent cartridge and pre-chilled HT1 Buffer in a water bath at room temperature. Ensure not to exceed the maximum water line. Store them in the refrigerator until it's time for library denaturation.

* Dilute 1 N NaOH to 0.2 N NaOH using DEPC-treated Water. It is recommended to use the 0.2 N NaOH within 12 hours after dilution for optimal results.

1) Perform vortexing and spin down after adding 0.2 N NaOH 5 µL into 4 nM library 5 µL in a 1.5 mL microtube.

2) Incubate samples for 5 minutes at room temperature.

3) Add 990 µl prechilled HT1 to the tube containing denatured library. The result is 1 ml of a 20 pM denatured library.

4) Perform vortexing and spin down.

5) Place the library on ice until the next step.

6) Dilute to 6 pM by using the table provided below. (PhiX control is not required.)

	6 pM
20 pM library	180 µL
Prechilled HT1 Buffer	420 µL

7) Invert the library diluted to the final concentration about 5 times and spin down.

8) Place the final prepared library on Ice until it is loaded onto the MiSeq reagent cartridge.

Step 9. Sequencing

1) Load 600 µL of the final prepared library onto a MiSeq reagent cartridge.

2) Set up a MiSeq Sample Sheet using the Illumina Local Run Manager (LRM) system.
   * It is recommended to use the sample sheet (.csv) provided by GeneCker

3) Start the MiSeq run following the Illumina MiSeq control software instructions.
   * If you are requesting sequencing from another institution, we recommend that the data throughput per index does not exceed 0.2 Gb.

Warranty and Liability

GeneCker Co., Ltd. is not responsible for problems caused by using test methods other than the one suggested in this product manual. In the event of a problem with the product, the customer can report the problem within 30 days to GeneCker Warranty Service Team.

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3. Appendix

A. Index Information

Index Primer	I7 Index Sequence	I5 Index Sequence	State
Index 01	**ATTACTCG**	**TATAGCCT**	A1
Index 02	**ATTACTCG**	**ATAGAGGC**	B1
Index 03	**ATTACTCG**	**CCTATCCT**	C1
Index 04	**ATTACTCG**	**GGCTCTGA**	D1
Index 05	**ATTACTCG**	**AGGCGAAG**	E1
Index 06	**ATTACTCG**	**TAATCTTA**	F1
Index 07	**ATTACTCG**	**CAGGACGT**	G1
Index 08	**ATTACTCG**	**GTACTGAC**	H1
Index 09	**TCCGGAGA**	**TATAGCCT**	A2
Index 10	**TCCGGAGA**	**ATAGAGGC**	B2
Index 11	**TCCGGAGA**	**CCTATCCT**	C2
Index 12	**TCCGGAGA**	**GGCTCTGA**	D2
Index 13	**TCCGGAGA**	**AGGCGAAG**	E2
Index 14	**TCCGGAGA**	**TAATCTTA**	F2
Index 15	**TCCGGAGA**	**CAGGACGT**	G2
Index 16	**TCCGGAGA**	**GTACTGAC**	H2
Index 17	**CGCTCATT**	**TATAGCCT**	A3
Index 18	**CGCTCATT**	**ATAGAGGC**	B3
Index 19	**CGCTCATT**	**CCTATCCT**	C3
Index 20	**CGCTCATT**	**GGCTCTGA**	D3
Index 21	**CGCTCATT**	**AGGCGAAG**	E3
Index 22	**CGCTCATT**	**TAATCTTA**	F3
Index 23	**CGCTCATT**	**CAGGACGT**	G3
Index 24	**CGCTCATT**	**GTACTGAC**	H3
Index 25	**GAGATTCC**	**TATAGCCT**	A4
Index 26	**GAGATTCC**	**ATAGAGGC**	B4
Index 27	**GAGATTCC**	**CCTATCCT**	C4
Index 28	**GAGATTCC**	**GGCTCTGA**	D4
Index 29	**GAGATTCC**	**AGGCGAAG**	E4
Index 30	**GAGATTCC**	**TAATCTTA**	F4
Index 31	**GAGATTCC**	**CAGGACGT**	G4
Index 32	**GAGATTCC**	**GTACTGAC**	H4
Index 33	**ATTCAGAA**	**TATAGCCT**	A5
Index 34	**ATTCAGAA**	**ATAGAGGC**	B5
Index 35	**ATTCAGAA**	**CCTATCCT**	C5
Index 36	**ATTCAGAA**	**GGCTCTGA**	D5
Index 37	**ATTCAGAA**	**AGGCGAAG**	E5
Index 38	**ATTCAGAA**	**TAATCTTA**	F5
Index 39	**ATTCAGAA**	**CAGGACGT**	G5
Index 40	**ATTCAGAA**	**GTACTGAC**	H5
Index 41	**GAATTCGT**	**TATAGCCT**	A6
Index 42	**GAATTCGT**	**ATAGAGGC**	B6
Index 43	**GAATTCGT**	**CCTATCCT**	C6
Index 44	**GAATTCGT**	**GGCTCTGA**	D6

Index 45	**GAATTCGT**	**AGGCGAAG**	E6
Index 46	**GAATTCGT**	**TAATCTTA**	F6
Index 47	**GAATTCGT**	**CAGGACGT**	G6
Index 48	**GAATTCGT**	**GTACTGAC**	H6
Index 49	**CTGAAGCT**	**TATAGCCT**	A7
Index 50	**CTGAAGCT**	**ATAGAGGC**	B7
Index 51	**CTGAAGCT**	**CCTATCCT**	C7
Index 52	**CTGAAGCT**	**GGCTCTGA**	D7
Index 53	**CTGAAGCT**	**AGGCGAAG**	E7
Index 54	**CTGAAGCT**	**TAATCTTA**	F7
Index 55	**CTGAAGCT**	**CAGGACGT**	G7
Index 56	**CTGAAGCT**	**GTACTGAC**	H7
Index 57	**TAATGCGC**	**TATAGCCT**	A8
Index 58	**TAATGCGC**	**ATAGAGGC**	B8
Index 59	**TAATGCGC**	**CCTATCCT**	C8
Index 60	**TAATGCGC**	**GGCTCTGA**	D8
Index 61	**TAATGCGC**	**AGGCGAAG**	E8
Index 62	**TAATGCGC**	**TAATCTTA**	F8
Index 63	**TAATGCGC**	**CAGGACGT**	G8
Index 64	**TAATGCGC**	**GTACTGAC**	H8
Index 65	**CGGCTATG**	**TATAGCCT**	A9
Index 66	**CGGCTATG**	**ATAGAGGC**	B9
Index 67	**CGGCTATG**	**CCTATCCT**	C9
Index 68	**CGGCTATG**	**GGCTCTGA**	D9
Index 69	**CGGCTATG**	**AGGCGAAG**	E9
Index 70	**CGGCTATG**	**TAATCTTA**	F9
Index 71	**CGGCTATG**	**CAGGACGT**	G9
Index 72	**CGGCTATG**	**GTACTGAC**	H9
Index 73	**TCCGCGAA**	**TATAGCCT**	A10
Index 74	**TCCGCGAA**	**ATAGAGGC**	B10
Index 75	**TCCGCGAA**	**CCTATCCT**	C10
Index 76	**TCCGCGAA**	**GGCTCTGA**	D10
Index 77	**TCCGCGAA**	**AGGCGAAG**	E10
Index 78	**TCCGCGAA**	**TAATCTTA**	F10
Index 79	**TCCGCGAA**	**CAGGACGT**	G10
Index 80	**TCCGCGAA**	**GTACTGAC**	H10
Index 81	**TCTCGCGC**	**TATAGCCT**	A11
Index 82	**TCTCGCGC**	**ATAGAGGC**	B11
Index 83	**TCTCGCGC**	**CCTATCCT**	C11
Index 84	**TCTCGCGC**	**GGCTCTGA**	D11
Index 85	**TCTCGCGC**	**AGGCGAAG**	E11
Index 86	**TCTCGCGC**	**TAATCTTA**	F11
Index 87	**TCTCGCGC**	**CAGGACGT**	G11
Index 88	**TCTCGCGC**	**GTACTGAC**	H11
Index 89	**AGCGATAG**	**TATAGCCT**	A12
Index 90	**AGCGATAG**	**ATAGAGGC**	B12
Index 91	**AGCGATAG**	**CCTATCCT**	C12
Index 92	**AGCGATAG**	**GGCTCTGA**	D12
Index 93	**AGCGATAG**	**AGGCGAAG**	E12

Index 94	**AGCGATAG**	**TAATCTTA**	F12
Index 95	**AGCGATAG**	**CAGGACGT**	G12
Index 96	**AGCGATAG**	**GTACTGAC**	H12

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D	Index 04	Index 12	Index 20	Index 28	Index 36	Index 44	Index 52	Index 60	Index 68	Index 76	Index 84	Index 92
E	Index 05	Index 13	Index 21	Index 29	Index 37	Index 45	Index 53	Index 61	Index 69	Index 77	Index 85	Index 93
F	Index 06	Index 14	Index 22	Index 30	Index 38	Index 46	Index 54	Index 62	Index 70	Index 78	Index 86	Index 94
G	Index 07	Index 15	Index 23	Index 31	Index 39	Index 47	Index 55	Index 63	Index 71	Index 79	Index 87	Index 95
H	Index 08	Index 16	Index 24	Index 32	Index 40	Index 48	Index 56	Index 64	Index 72	Index 80	Index 88	Index 96

B. Sample Sheet

Use the sample sheet (.csv) **provided by GeneCker** or prepare a proper sample sheet for Illumina Local Run Manager (LRM) by following the Illumina Experiment Manager software guide.

* Rules for writing sample sheet: Control - Sample Name_Visit number_Control / IVC - Sample Name_Visit number_IVC

Example.

1	[Header]						
2	Experiment Name	Test1	①				
3	Date	0000-00-00	②				
4	Module	GenerateFASTQ - 2.0.0					
5	Workflow	GenerateFASTQ					
6	Library Prep Kit	Custom	③				
7	Chemistry	Amplicon					
8	[Reads]						
9	151						
10	151						
11	[Data]						
12	Sample_ID ④	Description	I7_Index_ID	index	I5_Index_ID	index2	Sample_Project
13	1		A701	ATTACTCG	A501	tatagcct	
14	2		A701	ATTACTCG	A502	atagaggc	
15	3		A701	ATTACTCG	A503	cctatcct	
16	4		A701	ATTACTCG	A504	ggctctga	
17	5		A701	ATTACTCG	A505	aggcgaag	
18	6		A701	ATTACTCG	A506	taatctta	
19	7		A701	ATTACTCG	A507	caggacgt	
20	8		A701	ATTACTCG	A508	gtactgac	

① Insert experiment title.

② Insert NGS run date.

③ Insert 'Custom'. (* The sample sheet provided by GeneCker has 'Custom' entered.)

④ Insert the Sample_ID as the example below.

* Patients' ID_Tissue or Plamsa_Visit Number_Control or IVC

** ' ' or '-' cannot be enter in sample sheet.

Ex) Patient1234_Plasma_V1_Control (If used index 4, enter 4 instead)

Patient1234_Plasma_V1_IVC (If used index 8, enter 8 instead)

☞ If necessary, the "Sample Sheet" form can be sent by e-mail (**info@genecker.com**) to request.

C. Troubleshooting Guide

Troubles	Probable Cause	Resolution
Insufficient library volume	Not following the manufacturer's recommended PCR conditions may result in improper amplification of amplicons, leading to insufficient amounts of the library being produced in subsequent steps.	Adhere to the manufacturer's recommended PCR conditions.
	Improper temperature of the PCR lid means DNA evaporation may occur, potentially resulting in insufficient production of the library.	
	If there is an issue with the temperature sensor of the PCR equipment, the proper maintenance of temperature within the PCR device may be compromised, potentially leading to a failure in the correct amplification process.	Regularly inspect the equipment to ensure its proper functioning.
In case the VAF (Variant Allele Frequency) doesn't change value is low or not after the *In vitro* Cleavage reaction	**Short Reaction Time**: If the reaction time for *in vitro* Cleavage is too short, there may be limited cleavage of wild-type DNA, resulting in difficulty obtaining enough of the desired target genes	Follow the manufacturer's recommended *in vitro* Cleavage conditions and consider re-reaction if necessary.
	Excessive Reaction Time: If the reaction time for *in vitro* Cleavage exceeds the manufacturer's recommended duration, excessive cleavage of the amplicon may occur.	
	Delayed Stop reaction: If the addition of ●10X STOP buffer is delayed after the *in vitro* Cleavage reaction, excessive cleavage of the amplicon may occur.	It is recommended to add the ●10X STOP buffer within 5 minutes after the *in vitro* Cleavage reaction.
	Using the Remov Enzyme mix with an inappropriate set configuration may lead to excessive cleavage of the amplicon.	Perform *in vitro* cleavage using a Remov Enzyme mix set with the same as the primer set.
If there is a loss of beads during the Size Selection process	Beads may be sucked into the pipette tip during the removal of supernatant.	Remove the supernatant after the magnetic beads are completely bound to a magnetic stand.
In case of inaccurate volume measurement of the beads	Inadequate vortexing of beads may lead to the capture of a non-specific size library due to inaccurate ratios during the process.	Ensure thorough vortexing of the beads and prevent any beads from remaining on the inner and outer walls of the pipette tip.
If beads do not fully dissolve in DEPC-Water after an ethanol wash	If the beads were over-dried.	Pipette until the beads are completely dissolved.
		Immediately dissolve the beads after an ethanol wash to prevent over drying.

D. Precautions for Use

1. This product may not be used for purposes other than research.
2. This product is disposable and cannot be reused.
3. It is recommended to read the following precautions before using this product.
4. This product is designed to use Next-Generation Sequencing (NGS) and requires the use of MiSeq or MiSeq Dx equipment, which are base sequencing analyzers.
5. Prepare a master mix of all reagents on ice.
6. Since the NGS library preparation step is sensitive to pipetting errors, it is recommended to use a calibrated pipette within 1 year.
7. It is recommended to use filter tips to prevent cross-contamination.
8. Since the quality of the DNA sample affects the results, it is recommended to use high-quality DNA. Severely damaged DNA can cause the failure of library construction.
9. The concentration of DNA should be measured using the fluorescent dye method.
10. Contamination by DNA and RNA other than the sample may affect the quantification of DNA extracted from the sample, so keep the surrounding environment clean to avoid cross-contamination.
11. Contamination of test reagents and equipment, reaction temperatures, or storage conditions different from those recommended may affect test results.
12. If you need help with GeneCker's product, please contact the GeneCker Co., Ltd. Support at **info@genecker.com**.

4. Documentation and Support

DISCLAIMER

TO THE EXTENT ALLOWED BY LAW, **GeneCker Co., Ltd.** WILL NOT BE LIABLE FOR SPECIAL, INCIDENTAL, INDIRECT, PUNITIVE, MULTIPLE, OR CONSEQUENTIAL DAMAGES IN CONNECTION WITH OR ARISING FROM THIS DOCUMENT, INCLUDING YOUR USE OF IT.

Symbol Descriptions

The following table describes the symbols on the shipment packaging, consumable, or consumable packaging.

Symbol	Description	Symbol	Description
	Indicates the manufacturer.		Storage temperature ranges in degrees Celsius. Store the consumables within the indicated range.
	Date of manufacture.		Caution.
	Indicates the part number so that the consumable can be identified.		The date the consumable expires. For best results, use the consumable before this date.
	Indicates the batch code to identify the manufacturing batch or a lot of the consumables.		Contains sufficient for <n> tests.
	Consult instructions for use.		Do not reuse.
	The intended use is Research Use Only (RUO).		

Revision History

Document Number	Revision	The Date of Revision	Contents of Revision
GC-IFU-N102R-EN	0	01 July 2024	Initial release

Customer and Technical support :)

For Customer and technical assistance, contact GeneCker Support.

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