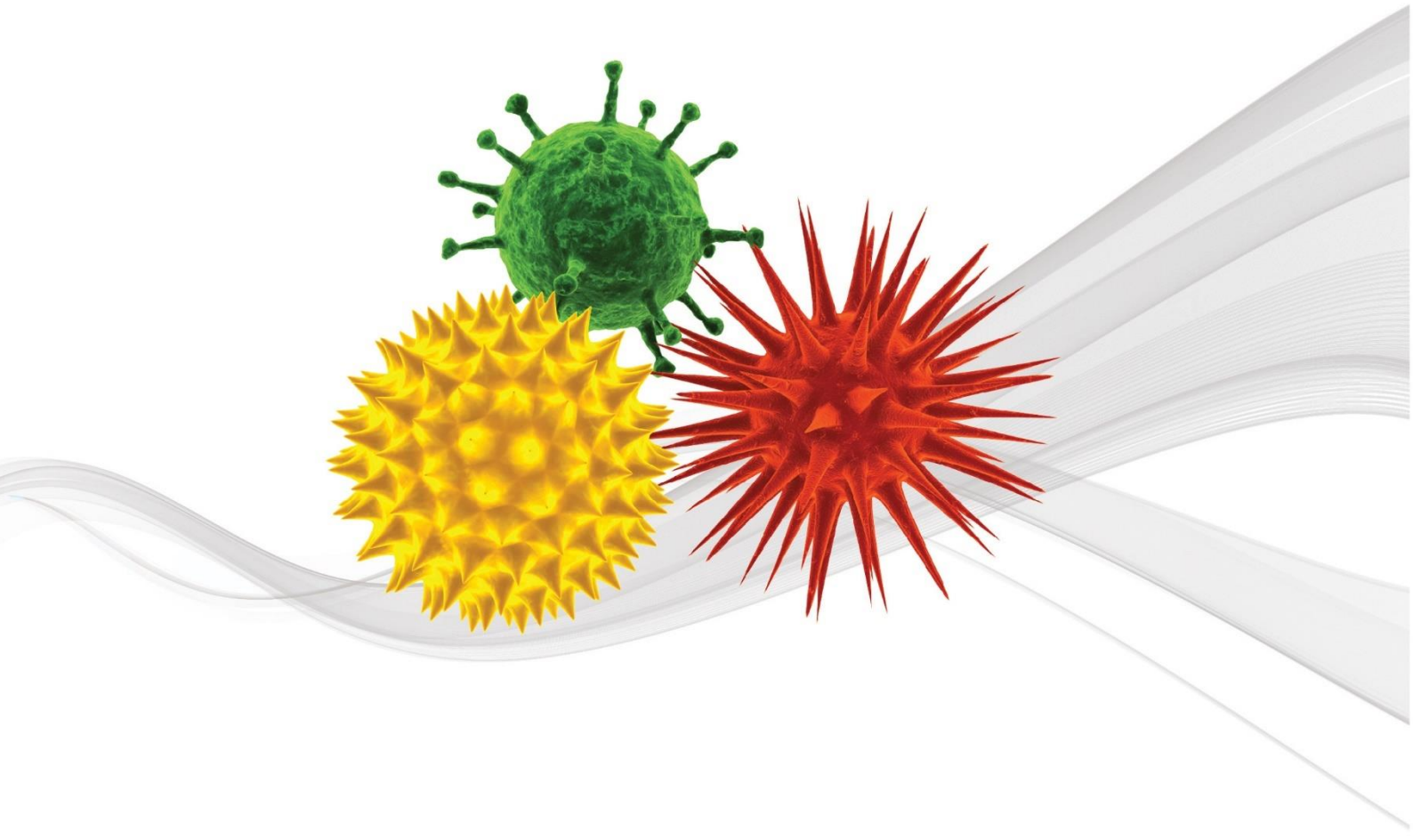
NGeneBio

SOLIDaccuTest™

User Manual for DNA Panel



IVD

Version: NGB-MG-131V(3)-01E

SOLIDaccuTest™ DNA

For clinical use in diagnostic procedures.
Please read the instructions carefully before use.

NGeneBio Co., Ltd.

Daerung Post-tower 1 Bldg, 288, Digital-ro, Guro-gu, Seoul, 08390, Republic of Korea
Tel: +82 2 867 9798 **Fax:** +82 2 866 9784 **Homepage:** www.ngenebio.com

Customer Service

For inquiries about the product and technical support requests, please contact us by telephone (+82 2 867 9798) or e-mail (support.technology@ngenebio.com).

SOLIDaccuTest™ DNA

(Cat. No. NGB131V-096, NGB131V-048)

Warranty and Liability

All products of NGeneBio Co., Ltd. are manufactured under a strict quality control process. NGeneBio Co., Ltd. warrants the quality of the product during the warranty period (marked on the product).

NGeneBio Co., Ltd. shall not be liable for any problem or issue arising from the improper use of the products other than those described in this product manual or under the methods which differ those described herein. In the event of a problem with the product, the customer should report their problems in detail within 30 days to the customer service center of NGeneBio Co., Ltd. or to supplier.

(Tel: +82 2 867 9798, Fax: +82 2 866 9784, e-mail: support.technology@ngenebio.com)

ORDER INFORMATION

Cat. No.	Kit Composition	Reactions	Storage Condition
NGB131V-096	DNA Library Prep Kit	96	-20℃
	Target Enrichment Kit	8	
NGB131V-048	DNA Library Prep Kit	48	
	Target Enrichment Kit	4	

SAFETY AND PRODUCT USE LIMITATIONS

- This product is a research use reagent and is also in use for purposes of diagnostic tests.
- This product is only for an experienced user who is qualified in the use of clinical specimens.
- The product components are not to be mixed with the components of the other lot number products.
- This product guarantees its stability within the expiry date stated on the product.
- If you need information on the material safety data sheet (MSDS) of this product, please contact the NGeneBio Co., Ltd. customer service center.
- Before using this product, be sure to read the product manual, check the components included in the product, and then perform the experiment.
- Dangerous materials (tips in contact with specimen and tubes, etc.) should be treated and disposed of in accordance with the specified legal method.
- All test procedures are carried out in accordance with the safety rules of each laboratory and regulation.

WARNING AND PRECAUTIONS

1) Before using this product, read carefully the following warnings and precautions.
2) *In vitro* diagnostic reagent SOLIDaccuTest™ DNA should be compatible with a gene-sequencing analyzer such as MiSeqDx.
3) Since the kit components and manuals are optimized and revised periodically, it is important to follow the user manual which is provided with the purchased kit.
4) NGeneBio Co., Ltd. has a limitation to provide support for any issues or problems resulting from non-adherence to this instruction for the user manual.
5) Before implementing this kit in your laboratory, highly recommend to performing a validation using the known reference materials (such as NA12878, HD701, etc.).
6) Do not use an expired kit.
7) Since the library preparation process is very sensitive to pipetting errors, highly recommended to use pipettes calibrated within 6 months.
8) Use calibrated thermocycler within 1 year.
9) As genomic DNA samples may vary in qualities, use high-quality of DNA. Severely damaged DNA might cause a failure of the library preparation process.
10) The value of purity of the gDNA used in the library production can be between 1.8 and 2.0 based on the absorbance ratio 260/280 highly recommended. Concentrations should be measured by a fluorescent dye-based quantitative method, such as Qubit® Fluorometer (Thermo Fisher Scientific).
11) Since contamination by DNA or RNA other than the specimen may affect the quantification of gDNA extracted from the specimen, keep clean the experimental tools and environments to prevent cross-contamination.
12) For gDNA fragmentation, a mechanical method using an ultra-sonicator such as Covaris and an enzymatic method can be used. Either method is not included in the kit and highly recommended for an evaluation and validation before use.
13) Contamination of reagents and equipment, and inaccurate reaction temperatures or storage conditions may affect test results.
14) Do not reuse the kit components.
15) If you have any inquiries, please contact NGeneBio Co., Ltd.

CONTENTS

1. PRODUCT INFORMATION

INTENDED USE

SOLIDaccuTest™ DNA is an excellent tool to explorer genetic variants associated with solid tumors using a comprehensive method of next-generation sequencing (NGS). It reflects the latest research trends and is optimized for the medical purpose by selecting essential genes of solid tumors including lung, colon, breast, skin, brain, gastric, ovarian cancers etc. Therefore, the ultimate purpose of SOLIDaccuTest™ DNA is to detect genetic mutations that have clinical utility in prognosis or diagnosis or therapeutic implications in various solid tumors.

PRINCIPLES AND PROCEDURE OVERVIEW

Solid tumors are an abnormal mass of tissue that typically does not contain cysts or liquid areas (such as leukemia, a type of blood cancers) [1]. Solid tumors may be benign (non-cancerous) or malignant (cancerous) and can be differentiated by the type of cells where they form. For example, lung cancer, colorectal cancer, brain tumor, breast cancer etc. One of the methods to treat solid tumor is a procedure in which a surgeon removes cancer from your body. This surgical method produces sometimes problems such as pain and infections. In addition, even if the cancer can be removed through surgery, the cancer cells may remain in an invisible state and it is a great deal to remove when metastasis to other organs occurs. In order to remove these residual cancers, chemotherapy/radiation therapy is performed. However, these therapies cause serious side effects such as affecting nearby healthy cells and/or fatigue.

One of the most important challenges in diagnosing solid tumors is the high degree of tumor heterogeneity. Genetic variations that have critical clinical implications could be present in very low levels, detecting these mutations difficult. For that reason, detection of such variations is a critical deal for accurate diagnosis and prognosis. Thus, NGS is extensively helpful and useful for resolving these challenges by providing a comprehensive genetic profile of solid tumors, which also can provide a more effective treatment strategy for each patient's cancer/tumor [2].

SOLIDaccuTest™ DNA is capable of running up to 12 samples simultaneously per run, when MiSeq Reagent Kit v3 (600 cycles) is employed for NGS run.

The multi-genes genetic testing procedure using SOLIDaccuTest™ DNA with NGS system consists of five steps: sample preparation, library preparation, hybridization & capture, NGS data generation, and variants analysis. SOLIDaccuTest™ DNA is used for preparation of libraries and hybridization & capture of target genes at STEP 2 and 3.

STEP 1. Sample preparation

- Extraction of gDNA from tumor tissues or frozen tissues (FFPE)
- Quantification and quality assessment of extracted gDNA
- Shearing gDNA and quality check

▼

STEP 2. Library preparation using SOLIDaccuTest™ DNA

- Repair end & adapter ligation
- PCR enrichment and indexing

▼

STEP 3. Hybridization and capture of target genes using SOLIDaccuTest™ DNA

- Prepare hybridization and hybridize probes
- Capture the target DNA library
- Final PCR for Illumina platform

▼

STEP 4. NGS data generation

- Quantification and quality assessment of library
- Performing sequencing using target libraries with MiSeqDx

▼

STEP 5. Multi-genes variants analysis

- Variants detection through NGS data analysis using NGeneAnalySys™ Software

REAGENTS AND STORAGE REQUIRMENT

SOLIDaccuTest™ DNA can produce up to 96 (NGB131V-096) or up to 48 (NGB131V-048) libraries and 8 or 4 NGS runs, respectively. All reagents are valid for 12 months under appropriate storage conditions. All reagents should be stored at -25 to -15℃.

Kit	No.	Product Composition	Number of tubes				Cap Color	Storage Condition
			96 rxns		48 rxns			
			No.	µL	No.	µL		
DNA Library Prep Kit	1	ER Enzyme Mix	1	288	1	144	Green	-20℃
	2	ER Buffer	1	672	1	336	Green	
	3	AL Enzyme Mix	3	960	2	720	Red	
	4	AL Enhancer	1	96	1	48	Red	
	5	Adapter	1	960	1	480	Red	
	6	UDG Enzyme	1	288	1	144	Red	
	7	Index PCR Master Mix	2	1200	1	1200	Blue	
	8	Universal Primer	1	480	1	240	Blue	
	9	Index Primer A01	1	40	1	20	Blue	
	10	Index Primer A02	1	40	1	20	Blue	
	11	Index Primer A03	1	40	1	20	Blue	
	12	Index Primer A04	1	40	1	20	Blue	
	13	Index Primer A05	1	40	1	20	Blue	
	14	Index Primer A06	1	40	1	20	Blue	
	15	Index Primer A07	1	40	1	20	Blue	
	16	Index Primer A08	1	40	1	20	Blue	
	17	Index Primer A09	1	40	1	20	Blue	
	18	Index Primer A10	1	40	1	20	Blue	
	19	Index Primer A11	1	40	1	20	Blue	
	20	Index Primer A12	1	40	1	20	Blue	
Target Enrichment Kit	1	Target Probes	1	32	1	16	Purple	-20℃
	2	Blockers Mix	1	16	1	8	Orange	
	3	Blocker DNA	1	40	1	20	Red	
	4	2X Hyb Buffer	1	68	1	34	White	
	5	Hyb Buffer Enhancer	1	22	1	11	Brown	
	6	2X Bead Wash Buffer	2	1000	1	1000	Gray	
	7	10X Wash Buffer I	1	240	1	120	Clear	
	8	10X Wash Buffer II	1	160	1	80	Clear	
	9	10X Wash Buffer III	1	160	1	80	Clear	
	10	10X Stringent Wash Buffer	1	320	1	160	Clear	
	11	2X Final PCR Mix	1	200	1	100	Green	
	12	P5 primer	1	20	1	10	Yellow	
	13	P7 primer	1	20	1	10	Yellow	

MATERIALS REQUIRED BUT NOT PROVIDED

The following equipment and reagents are recommended for using this product

1) Laboratory Equipment

	Equipment	Supplier / Catalog No.
1	MiSeq or MiSeqDx	Illumina
2	DNA shearing system	
	Focused-ultrasonicators and consumables	Covaris
3	96-well thermal cyclers	General laboratory supplier
4	Digital electrophoresis system	
	2100 Electrophoresis Bioanalyzer	Agilent Technologies, Cat # G2939AA
	- High Sensitivity DNA Kit	Agilent Technologies, Cat # 5067-4626
	2200 (or 4200) TapeStation	Agilent Technologies, Cat # G2965AA
	- High Sensitivity D1000 ScreenTape & Reagent	Agilent Technologies, Cat # 5067-5584/5067-5585
	- D1000 ScreenTape & Reagent	Agilent Technologies, Cat # 5067-5582/5067-5583
	- Genomic DNA ScreenTape & Reagent	Agilent Technologies, Cat # 5067-5365/5067-5366
5	Magnetic separation rack	
	16-tube DynaMag™-2 Magnet (for 1.5 mL tube)	Life Technologies, Cat # 12321D
	Magnet stand-96 (DynaMag-96 side) (for 0.2 mL tube)	Life Technologies, Cat # 12331D
	12x 1.5-mL Magnetic Separation Rack	PERMAGEN LABWARE, Cat # MSR12
	0.2-mL PCR Strip Magnetic Separation Rack	PERMAGEN LABWARE, Cat # MSR812
6	Microcentrifuge for PCR tubes and 1.5 mL tubes	General laboratory supplier
7	Fluorometer system	
	Qubit® Fluorometer	Life Technologies, various
	- Qubit® Assay Tubes	Life Technologies, Cat # Q32856
	- Qubit® dsDNA HS Assay Kit	Life Technologies, Cat # Q32851
	- Qubit® dsDNA BR Assay Kit	Life Technologies, Cat # Q32850
	Quantus Fluorometer	Promega, Cat # E6150
	- Thin-walled PCR tubes	Promega, Cat # E4942
	- ONE dsDNA dye kit	Promega, Cat # E4871
8	Vacuum concentrator	General laboratory supplier
9	Vortex mixer	General laboratory supplier
10	Water bath or heating block	General laboratory supplier
11	Pipettes (single or multi-channel) (2, 10, 100, 200, or 1000 µL)	General laboratory supplier

2) Reagents and Consumables

	Other Materials	Supplier / Cat. No.
1	Agencourt AMPure XP – PCR Purification beads	Beckman-Coulter, Cat # A63880
2	Dynabeads® M-270 Streptavidin	Life Technologies, Cat # 65305
3	>80% Ethanol	General laboratory supplier
4	1.5 mL microtubes	General laboratory supplier
5	0.2 mL PCR tubes and caps / or 8-strip tubes	General laboratory supplier
6	DNA LoBind® Tubes, 1.5 mL	Eppendorf, Cat # 0030108051
7	Low TE (10 mM Tris-HCl, pH 8.0, 0.1 mM EDTA)	Life Technologies, Cat # 12090015 (or general laboratory supplier)
8	Nuclease-free water	General laboratory supplier
9	Aerosol barrier pipette tips	General laboratory supplier
10	10 N NaOH	General laboratory supplier
11	MiSeq Reagent Kit, v3 (600 cycles) – up to 12 samples recommended	Illumina, Cat # MS-102-3003
12	PhiX Control Kit v3	Illumina, Cat # FC-110-3001
13	NEBNext® Ultra II FS DNA Module (Fragmentase)	NEB, Cat # E7810

STARTING MATERIALS

1) FFPE Tissue

Under the conditions in the table below, DNA samples are extracted from the FFPE tissue section using QIAGEN's FFPE DNA extraction kit (GeneRead™ DNA FFPE Kit) or the similar performance kit. Be advised to treat RNase when extracting DNA.

Condition / Specimen	Surface Area	Tumor Percentage
FFPE Tissue	≥25 mm^2	≥30%

Extracted gDNA from tumor FFPE tissue is recommended to have a DIN value of at least 3 that is measured by TapeStation (Agilent Technologies) or Bioanalyzer. The concentration of DNA is measured using a fluorescent dye method such as Qubit® Fluorometer (Thermo Fisher Scientific).

2) Whole Blood

Under the conditions in the table below, DNA samples are extracted from the whole blood stored in the EDTA tube using QIAGEN's gDNA extraction kit (QIAamp® DNA Blood Mini Kit) or the similar performance kit. Be advised to treat RNase when extracting DNA.

Condition / Specimen	Storage Temperature	Storage Period
EDTA whole blood	4℃	Up to 7 days
	18 to 25℃	Up to 48 hours

Extracted gDNA from whole blood is recommended to have a DNA Integrity Number (DIN) value of at least 7 that is measured by TapeStation (Agilent Technologies) or Bioanalyzer. The concentration of DNA is measured using a fluorescent dye method such as Qubit® Fluorometer (Thermo Fisher Scientific).

High-quality gDNA must be used, and the storage temperature should be -25 ~ -15℃. Thaw all reagents at room temperature and store on ice before use.

Sample Requirements	
A260/280	1.8-2.0
DIN* for gDNA from FFPE	≥3
DIN* for gDNA from fresh tissues or blood	≥7
Input DNA amount	FFPE DNA ≥400 ng / 53 µL
(Quantitative values based on Qubit® Fluorometer)	≥200 ng / 53 µL

* DNA Integrity Number (DIN)

2. SOLIDaccuTest™ DNA PROCEDURE

REAGENT PREPARATION

1) Each reagent should be thawed completely and be placed on ice before use.
2) Each component should be vortexed and centrifuged briefly to ensure that no residue is left on the tube's cap or wall.
3) The Agencourt AMPure XP beads should be kept at room temperature before use so that the temperature of the beads is equal to room temperature and vortexed to ensure uniform mixing.

A. SAMPLE PREPARATION

STEP A-1. gDNA FRAGMENTATION (Covaris system - Recommended)

User preparing materials (in case of Covaris system)
Covaris E-series or S-series
Covaris microTUBE
Low TE (10 mM Tris-HCl, pH 8.0, 0.1 mM EDTA)
≥400 ng of gDNA per reaction (volume up to 53 µL)

1) Make sure that gDNA samples are high quality within the range from 1.8 to 2.0 on OD 260/280 ratio.
2) Use a fluorometer such as Qubit® dsDNA Assay to measure the concentration of the gDNA samples.
3) Adjust a total volume of the gDNA (400 ng or more) samples up to 53 µL with Low TE.
4) Transfer the gDNA samples to a Covaris microTUBE for fragmentation.
5) Set up the parameters of Covaris system for that fragmented DNA size to be ~200 bp.
6) Transfer each sheared DNA sample to a strip tube.
7) Go to **STEP B. QUALITY CONTROL.**

★ Caution: As it might vary in DNA loss depending on equipment used, check the concentration of remaining DNA after shearing.

STEP A-2. gDNA FRAGMENTATION (an enzymatic method)

User preparing materials (in case of fragmentase)

NEBNext® Ultra II FS DNA Module (NEB)

Low TE (10 mM Tris-HCl, pH 8.0, 0.1 mM EDTA)

PCR tubes

A thermocycler (general laboratory equipment) or a heating block

≥400 ng of FFPE DNA per reaction (volume up to 26 µL)

1) Make sure that gDNA samples are high quality within the range from 1.8 to 2.0 on OD 260/280 ratio.
2) Use a fluorometer such as Qubit® dsDNA Assay to measure the concentration of the gDNA samples.
3) Adjust a total volume of the gDNA (400 ng or more) samples up to 26 µL with Low TE.
4) Prepare the gDNA samples in a PCR tube for fragmentation.
5) Ensure that the Ultra II FS Reaction Buffer is completely thawed. If a precipitation occurs in the buffer, dissolve completely by pipetting up and down several times and briefly vortex to mix well.
6) Vortex the Ultra II FS Enzyme Mix up to 8 seconds before use. This step is critical for optimal performance of the enzyme.
7) Perform the enzymatic reaction according to the manufacturer's instructions. The optimization for incubation time is required prior to use.
8) After the reaction completed, adjust the volume **up to 60 µL** with Low TE for the next step.
9) Proceed immediately the next step (Go to **STEP 2. ADAPTOR LIGATION**).

★ **Caution:** NEBNext® Ultra II FS DNA Module contains the enzymes and buffers converting intact genomic DNA into fragmented DNA with 5′ phosphorylated 3′ dA-tailed ends, which means the end repair is performed during the fragmentation. Depending on the quality of input DNA, the DNA loss can occur at different levels.

STEP B. QUALITY CONTROL (QC)

Before beginning

Let the TapeStation Reagent come to room temperature for at least 30 minutes

---

User preparing materials

2200 or 4200 TapeStation (Agilent Technologies)

TapeStation ScreenTape and Reagents

TapeStation 96 well plate or 8-strip tube

TapeStation Adhesive Plate Seal

Qubit® Fluorometer (Life Technologies)

Qubit® Assay Tubes

Qubit® dsDNA BR Assay Kit

53 µL of fragmented DNA (~400 ng) from STEP A-1

---

1) Quality check can be done with the 2200 or 4200 TapeStation system.
2) For analysis of sheared 400 ng DNA samples, use a TapeStation ScreenTape and reagents kit.
3) Check that a peak of fragmented DNA size is shown between 150 to 250 bp on electropherogram of TapeStation system.
4) Measure the concentration of fragmented DNA using a Qubit® Fluorometer or similar equipment. If the sheared DNA has a concentration of approximately **4 ng/µL (at least 2 ng/µL)**, proceed the next step. (**STEP 1. END REPAIR**).

B. LIBRARY PREPARATION

STEP 1. END REPAIR

SOLIDaccuTest™ DNA included materials

ER Enzyme Mix – Green Cap Tube

ER Buffer – Green Cap Tube

User preparing materials

Thermal Cycler (PCR machine)

★ NOTE : Ramp rate setting

Thermo Fisher Scientific instrument including Veriti® : 100%

Other manufacturer thermal cycler : 4℃/sec

PCR tubes

50 µL of fragmented DNA (STEP A-1)

1) Add the following components to each PCR tubes containing the fragmented DNA 50 µL. Mix well by vortexing or pipetting multiple times and spin-down briefly.

(To process multiple samples, prepare master mixes without the DNA sample and use it.)

Component	Volume (µL)
Fragmented DNA (~400 ng/50 µL)	50
ER Enzyme Mix	3
ER Buffer	7
Total volume	**60**

2) PCR tubes are placed in a thermal cycler with heating lid (Lid set to ≥75℃), and execute the following program.

Temperature (℃)	Time (min)
20	30
65	30
4	Hold

STOPPING POINT: If necessary, samples can be stored at -20°C; however, the yield may be decreased slightly (about 20%). We highly recommend to continuing the adapter ligation step.

STEP 2. ADAPTER LIGATION

SOLIDaccuTest™ DNA included materials

AL Enzyme Mix – Red Cap Tube

AL Enhancer – Red Cap Tube

Adapter – Red Cap Tube

UDG Enzyme – Red Cap Tube

User preparing materials

Thermal Cycler (PCR Machine)

Low TE (10 mM Tris-HCl, pH 8.0, 0.1 mM EDTA)

PCR tubes

60 µL of End prep reaction mixture from STEP 1 (or STEP A-2)

1) Depending on input DNA quantity, dilute the adapters with Low TE. Refer to the condition shown in the table below.

Input DNA quantity	Dilution ratio (adaptor volume : total volume)	Working concentration of adapter (µM)
101 – 1000 ng	No dilution	15.0
5 – 100 ng	10-Fold (1:10)	1.5
Less than 5 ng	25-Fold (1:25)	0.6

2) Add the following components to 60 µL of ER Mixture directly. Mix well by vortexing or pipetting multiple times and spin-down briefly.
(To process multiple samples, prepare master mixes without the mixture from STEP 1 and use it.)

Component	Volume (µL)
ER Mixture (from STEP 1 or STEP A-2)	60.0
AL Enzyme Mix	30.0
AL Enhancer	1.0
Adapter	2.5
Total	**93.5**

3) Incubate the 93.5 µL of ligation mixture in a thermal cycler at **20℃ for 15 minutes. (lid set to off)**

4) **Add 3 µL** of **UDG Enzyme** to the ligation mixture and mix well by vortexing or pipetting multiple times and spin-down briefly.

5) Incubate the ligation reactant in thermal cycler at **37℃ for 15 minutes. (lid set to ≥ 47℃)**

STOPPING POINT: The ligated DNA samples can be stored overnight at -20℃.

STEP 3. SIZE SELECTION

Before beginning

Caution

: AMPure XP Beads should be stored at 4℃ when not in use, and kept at room temperature at least 30 minutes before use so that the beads temperature should be equal to room temperature. Shake the reagent well before use. It should appear homogenous and consistent in color.

User preparing materials

Agencourt AMPure XP beads (room temperature)
Magnetic stand
PCR tubes
Low TE (10 mM Tris-HCl, pH 8.0, 0.1 mM EDTA)
80% Ethanol, freshly prepared (room temperature)

96.5 µL of adapter ligated DNA from STEP 2

---

1) **Add 45 µL (~0.5X) of Agencourt AMPure XP beads** to each ligation reaction (96.5 µL). Mix well by pipetting multiple times and spin-down briefly.
2) **Incubate** samples for **5 minutes** at room temperature.
3) Place the tubes on a magnetic stand to separate the beads from the supernatant. Wait until the solution becomes clear. (approximately 5 minutes)
4) After about 5 minutes (or when the solution becomes clear), carefully **transfer the supernatant** containing your DNA to a new tube. (★**Caution: do not discard the supernatant**) Discard the beads that contain the unwanted large fragmented DNA.
5) **Add 55 µL (~0.6X) of Agencourt AMPure XP beads** to the supernatant and mix well by pipetting multiple times and spin-down briefly.
6) **Incubate** samples for **5 minutes** at room temperature.
7) Place the tube on a magnetic stand to separate the beads from the supernatant. Wait until the solution becomes clear. (approximately 5 minutes)
8) After about 5 minutes (or when the solution becomes clear), carefully **remove the supernatant** that contains unwanted DNA. Be careful not to disturb the beads that contain the desired DNA.
9) **Add 200 µL of 80% freshly prepared ethanol** to the tubes with beads placed in the magnetic stand. Wait for 1 minute at room temperature for beads washing, and then carefully remove and discard the supernatant. Be careful not to disturb the beads that contain the desired DNA.

10) **Repeat Step 9) once**. Be sure to remove all residual ethanol after the second wash.
11) Place tubes (with caps opened) on the magnetic stand and **dry the beads at room temperature** for 5 minutes. (★**Caution: Do not over dry the beads.** The over-dried beads may result in lower recovered DNA. Elute the sample when all the liquid is evaporated and the beads are brown-colored and matte. If the beads turn into light-brown color and crack is seen, the beads are over-dried, therefore, carry out the elution step immediately)
12) Place the tubes off from the magnetic stand. **Add 17 µL of Low TE** to each sample well.
13) Resuspend by tapping or vortexing without the beads attached to the tube wall. **Incubate** it at room temperature for **3 minutes**.
14) Place the tubes on the magnetic stand. After about 5 minutes (or when the solution becomes clear), **transfer 15 µL of clear supernatant** that contains the desired DNA to a new PCR tube for the next step.

STOPPING POINT: If required, the samples can be stored overnight at -20℃.

STEP 4. PCR ENRICHMENT AND INDEXING

SOLIDaccuTest™ DNA included materials

Index PCR Master Mix – Blue Cap Tube

Index Primer (A01-A12) – Blue Cap Tube

Universal Primer – Blue Cap Tube

User preparing materials

Thermal Cycler (PCR machine)

★ NOTE : Ramp rate setting

Thermo Fisher Scientific instrument including Veriti® : 100%

Other manufacturer thermal cycler : 4℃/sec

PCR tube

15 µL of purified adapter ligated DNA from STEP 3

1) Add the following components to a PCR tube.
(To process multiple samples, prepare master mixes without the mixture from STEP 3 and use it.)

Component	Volume (µL)
Adapter-ligated, **purified DNA (from STEP** 3)	15
Index PCR Master Mix	25
Index Primer (A01-A12)	5
Universal Primer	5
Total volume	**50**

2) Mix well by vortexing or pipetting multiple times and spin-down the tubes. Place the tubes on a thermal cycler and run the following PCR program.

Cycle Step	Temperature (℃)	Time	Cycles
Initial Denaturation	98	30 sec	1
Denaturation	98	10 sec	5-9★
Annealing/Extension	65	75 sec	
Final Extension	65	5 min	1
Hold	4	Hold	

★Caution: The number of PCR cycles should be chosen referred Table 4.1 because PCR efficiency and yield are different depends on DNA quality and quantity.

Table 4.1 Expected library yield

Template condition		PCR cycles and yield	
DNA quality	Input DNA (ng)	PCR cycles	Expected library yield (ng)
FFPE (DIN ≥3)	100	8	250-1000
	200	7	250-1000
	400-500	5-6	200-1000
DIN ≥7	100	6	250-300
	200	5	250-300

STEP 5. CLEAN UP

Before beginning

Caution

: AMPure XP Beads should be stored at 4℃ when not in use, and kept at room temperature at least 30 minutes before using so that the beads temperature should be equal to room temperature. Shake the reagent well before use. It should appear homogenous and consistent in color.

User preparing materials

Agencourt AMPure XP beads (room temperature)
Magnetic stand
PCR tubes
Low TE (10 mM Tris-HCl, pH 8.0, 0.1 mM EDTA)
80% Ethanol, freshly prepared (room temperature)

50 µL of amplified DNA library from STEP 4

1) **Add 45 µL (0.9X) of Agencourt AMPure XP beads** to the PCR reaction and mix well by pipetting multiple times and spin-down briefly.
2) **Incubate** samples for **5 minutes** at room temperature.
3) Place the tube on a magnetic stand to separate the beads from the supernatant. Wait until the solution becomes clear. (approximately 5 minutes)
4) After about 5 minutes (or when the solution becomes clear), carefully **remove the supernatant** that contains unwanted DNA. Be careful not to disturb the beads that contain the desired DNA.
5) **Add 200 µL of 80% freshly prepared ethanol** to the tubes with beads placed in the magnetic stand. Wait at room temperature for 1 minute for beads washing, and then carefully remove and discard the supernatant. Be careful not to disturb the beads that contain the desired DNA.
6) **Repeat Step 5) once**. Be sure to remove all residual ethanol after the second wash.
7) Place tubes (with caps open) on the magnetic stand and **dry the beads at room temperature** for 5 minutes. (★**Caution: Do not over dry the beads.** The over-dried beads may result lower recovered DNA. Elute the sample when all the liquid is evaporated and the beads are brown-colored and matte. If the beads turn into light-brown color and crack is seen, the beads are over-dried, therefore, carry out the elution step immediately.)
8) Place the tubes off from the magnetic stand. **Add 33 µL of Low TE** to each sample well for eluting the DNA target from the beads.

9) Resuspend by tapping or vortexing without the beads attached to the tube wall. **Incubate** for at least **3 minutes** at room temperature.
10) Place the tube on the magnetic stand. After about 5 minutes (or when the solution becomes clear), **transfer 30 µL of clear supernatant** that contains the desired DNA to a new PCR tube.
11) Measure the concentration of the purified library using Qubit® Fluorometer and Qubit® dsDNA BR assay Kit or similar system.
12) Measure the average length of the purified library on TapeStation using TapeStation ScreenTape and reagents or similar system.
13) Mix and pooling as equal concentration of each libraries. The total mass of pool should be between 500 to 1,000 ng.

STOPPING POINT: If required, samples can be stored at -20℃.

C. HYBRIDIZATION AND CAPTURE

STEP 6. PREPARE HYBRIDIZATION

SOLIDaccuTest™ DNA included materials

Blockers Mix – Orange Cap Tube

Blocker DNA – Red Cap Tube

User preparation materials

Vacuum concentrator

DNA LoBind® Tubes, 1.5 mL

Pooled library (500~1000 ng) from STEP 5

1) Add the following component to a DNA LoBind® tube and mix by tapping and spin down.

Component	Per 1 Capture probe
Pooled library	500-1000 ng
Blockers Mix	2 µL
Blocker DNA	5 µL

2) Dehydrate the mixture using a vacuum concentrator. The chamber temperature set to at 70℃ or lower.

STOPPING POINT: After drying, tubes can be stored overnight at room temperature (15-25℃).

STEP 7. HYBRIDIZE PROBES WITH THE LIBRARY

SOLIDaccuTest™ DNA included materials

2X Hyb Buffer - White Cap Tube

Hyb Buffer Enhancer – Brown Cap Tube

Target Probes – Purple Cap Tube

User preparation materials

Thermal Cycler (PCR machine)

Water bath or heating block

PCR tube

Nuclease-free Water

Dried DNA library from STEP 6

1) Thaw all reagents and buffers at room temperature.

   ※ Check crystallization of salts is appeared in the tube of 2X Hyb Buffer. If crystals are present, heat the tube at 65℃ and shaking intermittently until the buffer is completely solubilized.

2) Add the following components to the tube from STEP 6, and incubate it at room temperature for 10 minutes.

Components	Volume (µL)
2X Hyb Buffer	8.5
Hyb Buffer Enhancer	2.7
Nuclease-free Water	1.8
Total volume	**13.0**

3) Mix by pipetting up and down, and transfer all to a new PCR tube.

4) Incubate the PCR tube in a thermal cycler at **95℃ (lid set at 105°C) for 10 minutes**.

5) Place the samples off from thermal cycler and immediately **add 4 µL of the target probes.** Final volume will be 17 µL.

6) Vortex and briefly spin down. Then incubate the samples in thermal cycler at **65℃ (lid set at 75℃) for 4-16 hours**.

   ※ The hybridization temperature is 65℃ that improve the efficiency of on-target capture.

STEP 8. PREPARE WASH BUFFERS

SOLIDaccuTest™ DNA included materials

2X Bead Wash Buffer – Gray Cap Tube
10X Wash Buffer I – Clear Cap Tube
10X Wash Buffer II - Clear Cap Tube
10X Wash Buffer III - Clear Cap Tube
10X Stringent Wash Buffer - Clear Cap Tube

User preparing materials

Water bath or heating block
Nuclease-free water

1) For a single capture reaction, dilute the following the Wash buffers to prepare 1X working solution as follow:

※ The 1X working solutions can be stored at room temperature (15-25℃) for up to 4 weeks.

Component	Concentrated buffer (µL)	Nuclease-free water(µL)
2X Bead Wash Buffer	250	250
10X Wash Buffer I ★	30	270
10X Wash Buffer II	20	180
10X Wash Buffer III	20	180
10X Stringent Wash Buffer	40	360

★ If necessary, preheat 10X Wash Buffer I in a 65℃-water bath or heating block to remove crystallized salts.

2) Prepare tubes of aliquoted Wash Buffer I and Stringent Wash Buffer from STEP 8 – 1), and store the buffers at the temperature specified in the following table:

Component	Volumes of 1X working solutions for each capture (µL)	Temperature for 1X working solution (℃)
Wash Buffer I	100	65★
	200	Room temperature (15-25)
Stringent Wash Buffer	400	65★

★ Preheat buffers in a 65℃-water bath or heating block for at least 2 hours before use in STEP 11.

3) Keep the remaining 1X buffers at room temperature for **STEP 11**.

STEP 9. PREPARE THE BEADS FOR CAPTURING

User preparing materials

Dynabeads® M-270 Streptavidin beads (for capture)
Magnetic Stand
DNA LoBind® 1.5 mL Tube
PCR tube

1X working solutions in STEP 8

1) Take aliquot **100 µL** of **Dynabeads® M-270 Streptavidin beads** per capture reaction into a 1.5 mL LoBind® tube.

   (For 2 captures, prepare 200 µL of beads. For more than 6 captures, you will prepare more than one tube.)

2) Place the tube in a magnetic stand, allowing beads to fully separate from the supernatant.
3) Remove the clear supernatant with a pipette, ensuring that the beads remain in the tube.
4) Perform the following washing process:

   a. Add **200 µL of 1X Bead Wash Buffer** per capture, and vortex for 10 seconds.

   b. Place the tube in the magnetic stand, allowing beads to fully separate from the supernatant.

   c. Carefully remove and discard the clear supernatant with a pipette.

5) Perform a second washing by repeating step 5).
6) **Add 100 µL of 1X Bead Wash Buffer** per capture and vortex.
7) Transfer **100 µL** of the resuspended beads into a new PCR tube for each capture reaction.
8) Place the tube in a magnetic stand, allowing beads to fully separate from the supernatant.
9) Remove and discard the clear supernatant
10) Proceed immediately to the **STEP 10** for binding hybridized target to the streptavidin beads.

STEP 10. CAPTURE THE TARGET DNA LIBRARIES

User preparing materials

Thermal Cycler (PCR machine)

17 µL of hybridization sample from STEP 7

Streptavidin beads from STEP 9

1) Transfer the hybridization samples (from STEP 7) to the tube containing capture beads (from STEP 9).
2) Mix thoroughly by pipetting up and down multiple times.
3) Incubate the PCR tube **at 65℃ for 45 minutes** in to a thermal cycler to bind the target DNA libraries to the capture beads (lid set to 75℃). Every 12 minutes, vortex the tube for 3 seconds to keep homogeneous beads reaction during the incubation.

STEP 11. BEADS WASHING

User preparing materials

Water bath or heating block

DNA LoBind® Tubes, 1.5 mL

PCR tube

Magnetic Stand

Nuclease-free water

1X working solutions from STEP 8

17 µL of the beads with bound DNA libraries from STEP 10

1. Perform 65℃ washes

1) **Add 100 µL of preheated 1X Wash Buffer I** to the tube from STEP 10.

2) Vortex briefly and spin down to collect contents at the bottom of the tube.

3) Transfer the beads mixture to a new LoBind® 1.5 mL tube.

4) Place the tube in a magnetic stand, allowing beads to fully separate from the supernatant.

5) Remove and discard the supernatant, which contains unbound DNA.

6) Perform the following washing process:

a. **Add 200 µL of preheated 1X Stringent Wash Buffer**, and gently pipetting up and down multiple times. (★Do not create bubbles while pipetting.)

b. Incubate the tube at 65℃ for 5 minutes with a water bath or a heating block.

c. Place the tube in a magnetic stand, allowing beads to fully separate from the supernatant.

d. Remove and discard the supernatant, which contains unbound DNA.

7) Repeat 6) once.

2. Perform room temperature washes

1) **Add 200 µL of room temperature 1X Wash Buffer I** and vortex for 2 minutes.

2) Place the tube in a magnetic stand, allowing beads to fully separate from the supernatant.

3) Remove and discard the supernatant.

4) **Add 200 µL of room temperature 1X Wash Buffer II** and vortex for 1 minutes.

5) Place the tube in a magnetic stand, allowing beads to fully separate from the supernatant.

6) Remove and discard the supernatant.

7) **Add 200 µL of room temperature 1X Wash Buffer III** and vortex for 30 seconds.

8) Place the tube in a magnetic stand, allowing beads to fully separate from the supernatant.

9) Remove and discard the supernatant.

3. Resuspend Beads

1) Place the tube containing the beads with captured DNA libraries off from the magnetic stand.

2) Resuspend the beads with **20 µL of Nuclease-free water**.

3) Mix well by vortexing or pipetting multiple times and ensure no bead stuck to the wall of the tube.

STEP 12. PERFORM FINAL PCR ENRICHMENT

SOLIDaccuTest™ DNA included materials

P5 Primer – Yellow Cap Tube

P7 Primer – Yellow Cap Tube

2X Final PCR Mix – Green Cap Tube

User preparing materials

Thermal Cycler (PCR machine)

★ NOTE : Ramp rate setting

Thermo Fisher Scientific instrument including Veriti® : 100%

Other manufacturer thermal cycler : 4℃/sec

PCR tubes

20 µL of beads with captured DNA libraries from STEP 11

1) Prepare the PCR mixture in PCR tube as follows:

Component	Volume (µL)
Beads with captured DNA in STEP 11	20.0
2X Final PCR Mix	25.0
P5 Primer	2.5
P7 Primer	2.5
Total volume	**50.0**

2) Briefly vortex and spin down to keep the homogeneous PCR mixture of the beads in solution.

3) Place the PCR tube in a thermal cycler and run the following program (Lid set to 105℃).

Cycle Step	Temperature (℃)	Time	Number of Cycles
Polymerase activation	98	45 sec	1
Amplification			
Denaturation	98	15 sec	12
Annealing	60	30 sec	
Extension	72	30 sec	
Final extension	72	1 min	1
Hold	4	Hold	1

STOPPING POINT: PCR products can be stored at 4℃ overnight.

STEP 13. PURIFY FINAL PCR PRODUCTS

Before beginning

Caution

: AMPure XP Beads should be stored at 4℃ when not in use, and kept at room temperature at least 30 minutes before using so that the beads temperature should be equal to room temperature. Shake the reagent well before use. It should appear homogenous and consistent in color.

User preparing materials

Agencourt AMPure XP beads (room temperature)
Magnetic stand
PCR tube
Low TE (10 mM Tris-HCl, pH 8.0, 0.1 mM EDTA)
80% Ethanol, freshly prepared (room temperature)

50 µL of PCR products from STEP 12

---

1) **Add 75 µL (1.5X) of Agencourt AMPure XP beads** to the PCR reaction, mix well by pipetting several times and spin-down briefly.
2) **Incubate** samples for **5 minutes** at room temperature.
3) Place the PCR tube on a magnetic stand to separate the beads from the supernatant. Wait until the solution becomes clear. (approximately 5 minutes)
4) After about 5 minutes (or when the solution becomes clear), carefully **remove the supernatant** that contains unwanted DNA. Be careful not to disturb the beads that contain the desired DNA.
5) **Add 200 µL of 80% freshly prepared ethanol** to the tubes with beads placed in the magnetic stand. Wait for 1 minute at room temperature for beads washing, and then carefully remove and discard the supernatant. Be careful not to disturb the beads that contain the desired DNA.
6) **Repeat Step 5) once**. Be sure to remove all residual ethanol after the second wash.
7) Place tubes (with caps opened) on the magnetic stand and **dry the beads at room temperature** for 5 minutes. (★**Caution: Do not over dry the beads.** The over-dried beads may result lower recovered DNA. Elute the sample when all the liquid is evaporated and the beads are brown-colored and matte. If the beads turn into light-brown color and crack is seen, the beads are over-dry, therefore, carry out the elution step immediately.)
8) Place the tubes off from the magnetic stand. **Add 22 µL of Low TE** to elute the target

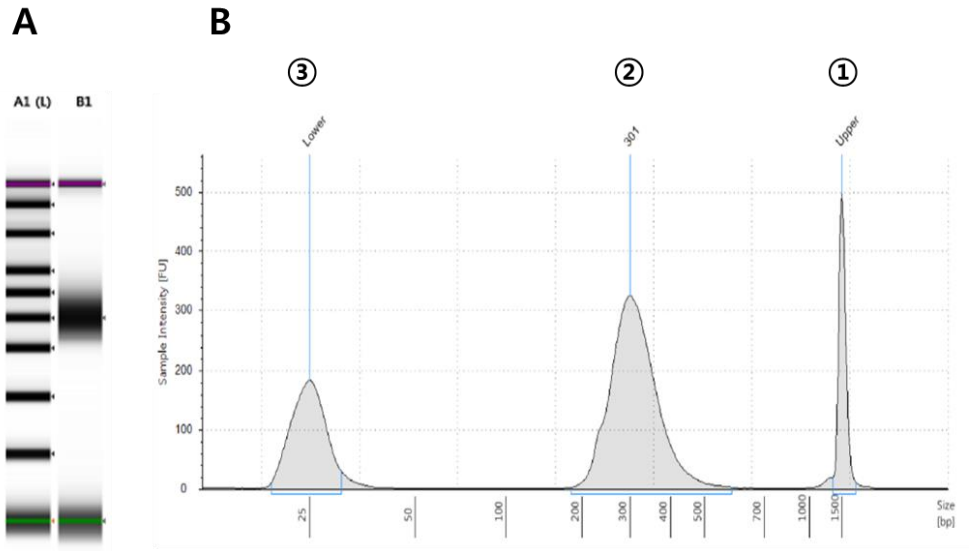
DNA from the beads.

9) Resuspend by tapping or vortexing without the beads attached to the tube wall. **Incubate** for at least **3 minutes** at room temperature.
10) Place the tube on the magnetic stand. After about 5 minutes (or when the solution becomes clear), **transfer 20 µL of the clear supernatant** that contains the desired DNA to a new PCR tube.
11) Measure the concentration of the purified library using Qubit® Fluorometer and Qubit® dsDNA BR assay Kit or similar system.
12) Measure the average length of the purified library on TapeStation using TapeStation ScreenTape and reagents or similar system.

    Convert library concentration to molality referred to the concentration from Qubit fluorometer results and the size from TapeStation results. Then dilute the library to 4 nM. Contact NGeneBio for the automatic calculation file.

STOPPING POINT: If required, samples can be stored at -20℃.

3. LIBRARY VALIDATION



[Figure] Final quality check of libraries
using Agilent 4200 TapeStation HS D1000 ScreenTape Assay

A) Gel image of libraries from 4200 TapeStation results

The size of the prepared DNA library is checked by size marker on the left side. If it is between 230 to 370 bp, the experiment was performed well.

B) Electropherogram of libraries from 4200 TapeStation results

① Upper Marker

② Libraries (230-370 bp): In case of successfully prepared libraries, the main peak appears between 230 to 370 bp.

③ Lower Marker

4. RESULT ANALYSIS

Next-generation sequencing data generated with SOLIDaccuTest™ DNA performs variants analysis using a dedicated analysis software NGeneAnalySys™ provided by NGeneBio Co., Ltd.

Caution

: After using SOLIDaccuTest™ DNA, the manufacturer is not responsible for the results analyzed other than the analysis software provided by NGeneBio Co., Ltd.

5. PERFORMANCE

ANALYTICAL PERFORMANCE TEST

The analytical performance of SOLIDaccuTest™ DNA was evaluated and the results were briefly described here. The samples for this test included a single nucleotide variant (SNV), an insertion (INS), a deletion (DEL), or/and copy number variation (CNV) which can be verified via the bioinformatics pipeline.

For the performance evaluation, genomic DNAs were extracted from reference samples and clinical specimens of solid cancer patients carrying significant mutations, and were used to determine the sensitivity (Limit Of Detection, LOD), specificity, precision (repeatability and reproducibility), and accuracy (method comparison).

1) Sensitivity & specificity

- ❒ Sensitivity: the DNA samples with specific mutations were used to determine the mutation detection rate using NGS of which libraries were generated by SOLIDaccuTest™ DNA.
- ❒ LOD: The minimum mutation allele fraction of the reference samples in which specific genetic variations (SNV or/and INDEL) can be detected using NGS with SOLIDaccuTest™ DNA-generated libraries.
  - Genomic DNA of the reference samples and clinical samples with the well-described mutation detection rates was serially diluted at four or five different levels (undiluted, 1/2, 1/4, or 1/8).
    - ❖ The result demonstrated that LOD for SNV was up to 3% and for INDEL was up to 5% when the average coverage was greater than 900X.
- ❒ Specificity: the DNA samples without specific mutations were used to determine the mutation detection rate using NGS.
  - Comparison of the mutation detection rate of the reference sample NA12878 which carries known SNVs reported in GIAB (http://jimb.stanford.edu/giab/). Also comparing the mutation detection rate of the clinical samples carrying certain mutations previously analysed.
    - ❖ Specificity = TN/(TN+FP) = 100% in average of three repeats (100, 100, 100%)

2) Accuracy

Accuracy: the analytic performance, accuracy, of SOLIDaccuTest™ DNA was determined as a positive detection rate in the samples of which specific genetic variants were previously identified as positive

and negative using NGS.

- Positive Percent Agreement (PPA): consistency between the positively detected mutations and the existing analysed result = TP/(TP+FN)
- Positive Predictive Value (PPV): the proportion of positive results in this test that are true positive results = TP/(TP+FP)
  - ❖ The result of accuracy analysis in general showed 100% of PPA and PPV.
  - ❖ Analysis results depending on mutation types (SNV or INDEL) also displayed 100% PPA and PPV.

3) Precision

Repeatability: consistency (equivalent or more than 90% identical) among the results of the test repeated under the same conditions.

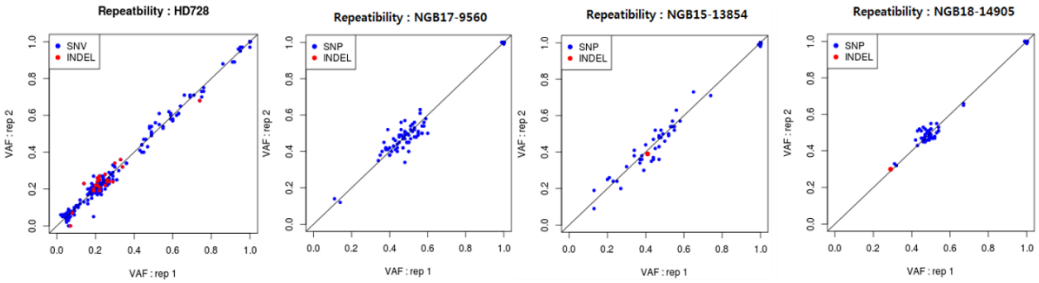
- Experiments were repeatedly performed with the same samples under the same conditions. The results revealed that all reported mutations were detected and the repeat detection rates of mutations in the test samples were confirmed [Figure 1].
- In these results, two different samples were used to confirm that all of the specific genetic mutations were precisely detected without interfering between each sample.
- Detection of all mutations in repeated experiments performed with the identical samples and verification of mutation detection without inter-sample interference
  - ❖ The precision-repeatability of SOLIDaccuTest™ DNA was evaluated as that the mutation detection using the identical sample was run under a single equipment, reagent, and researcher.
  - ❖ The result showed that the mutation detection was corresponding to each expected mutation rate (allele frequency) for positive mutation of HD728, NGB17-9560, NGB15-13854, and NGB18-14905.
  - ❖ All positive mutations were detected without interferences between samples.

Reproducibility: more than 90% consistency for variants of VAF in the results of the identical sample between runs by different researchers and different date under the identical experiment conditions [Figure 2].

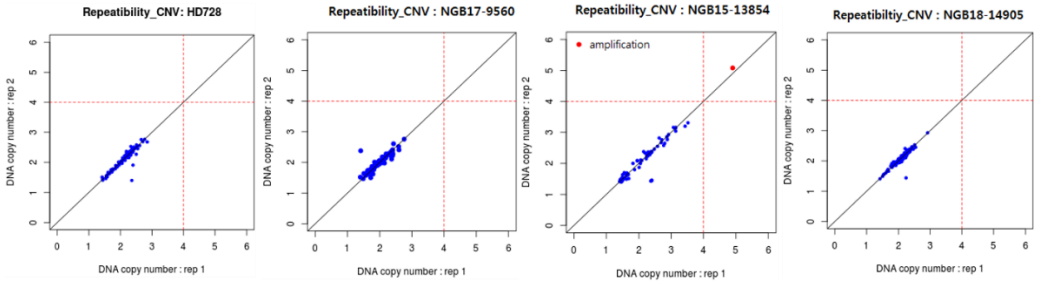
- The precision-reproducibility of SOLIDaccuTest DNA was evaluated as the concordance rate between runs using the identical sample run under the same experimental conditions by different researchers and on different date.
- The results showed the concordance rate was more than 92% for HD728, 100% for NGB17-

9560, 100% for NGB15-13854, and more than 99% for NGB18-14905.

[A] SNV & INDEL

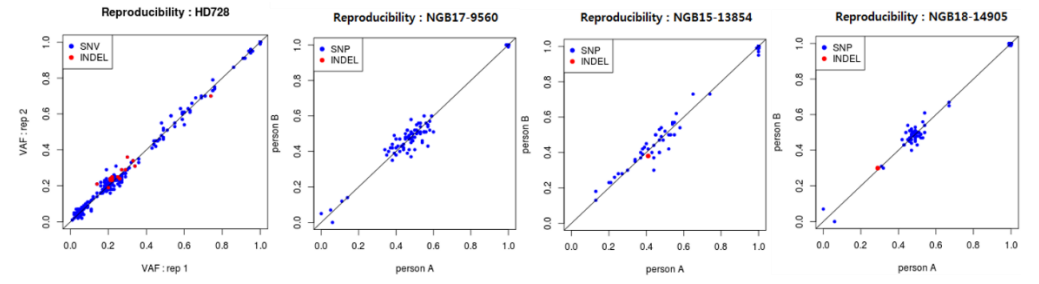


[B] CNV

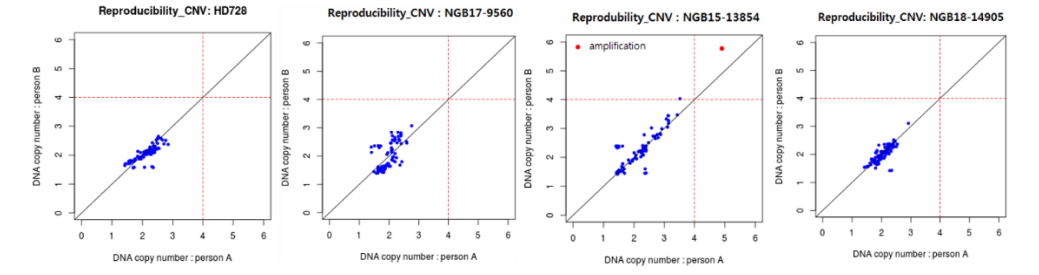


[Figure1] Precision-repeatability

[A] SNV & INDEL



[B] CNV



[Figure 2] Precision-reproducibility

6. APPENDIX

Information of Index Primer (A01~A12)

Index primer	Sequence
Index A01	ATCACG
Index A02	CGATGT
Index A03	TTAGGC
Index A04	TGACCA
Index A05	ACAGTG
Index A06	GCCAAT
Index A07	CAGATC
Index A08	ACTTGA
Index A09	GATCAG
Index A10	TAGCTT
Index A11	GGCTAC
Index A12	CTTGTA

※ Recommended index sets depending on number of samples

- 2 samples: A06, A12
- 3 samples: A04, A06, A12
- 6 samples: A02, A04, A05, A06, A07, A12
- over 6 samples: whichever indexes

Recommendation: Prepare MiSeq/MiSeqDx Running

Sample Sheet

Please contact NGeneBio support team by E-mail (support.technology@ngenebio.com) for requesting the sample sheet file.
Or prepare it using Illumina Experiment Manager software in accordance with Illumina Experiment Manager software guide (Illumina part # 15031335).

Library Denaturation, dilution, and MiSeq Reagent cartridge loading

A library of 10-12 pM loading is recommended in case of using MiSeq Reagent Kit, v3 (600 cycles).

The library prepared using SOLIDaccuTest™ DNA is diluted to a final concentration of 4 nM. Before loading into the MiSeq Reagent cartridge, the library is denatured with freshly prepared 0.2 N NaOH.

■ Library denaturation and dilution (using pre-chilled HT1 buffer on ice)

<table>
<tr><th></th><th colspan="4">Sample library (target genes)</th><th>PhiX Control</th></tr>
<tr><td rowspan="2">Denaturation</td><td colspan="4">5 µL of 4 nM library pool + 5 µL of 0.2 N NaOH</td><td>5 µL of 2 nM PhiX + 5 µL of 0.2 N NaOH</td></tr>
<tr><td colspan="5">Incubate for 5 minutes at room temperature</td></tr>
<tr><td>Dilution 1</td><td colspan="4">Add 990 µL of pre-dissolved HT1
→ 20 pM library</td><td>Add 490 µL of pre-dissolved HT1
→ 20 pM PhiX</td></tr>
<tr><td rowspan="3">Dilution 2</td><td>Final Conc.</td><td>10 pM</td><td>11 pM</td><td>12 pM</td><td rowspan="3">375 µL of 20 pM PhiX + 225 µL of HT1
→ 12.5 pM PhiX</td></tr>
<tr><td>20 pM library</td><td>350 µL</td><td>385 µL</td><td>420 µL</td></tr>
<tr><td>HT1</td><td>350 µL</td><td>315 µL</td><td>280 µL</td></tr>
<tr><td>Mixture</td><td colspan="5">665 µL of library diluted to 10 - 12 pM + 35 µL of 12.5 pM PhiX (5%)</td></tr>
<tr><td>Loading</td><td colspan="5">Load 600 µL of library mixture into the MiSeq Reagent cartridge</td></tr>
</table>

※ The library denaturation and dilution procedures are in accordance with Illumina's MiSeq System, Denature and Dilute Libraries Guide (Illumina, part # 15039740). Loading and sequencing of the library reagent cartridges is done in the MiSeq System User Guide (Illumina, part # 15027617).

Troubleshooting

Problem	Suspected cause	Solution
The final concentration of library was lower than expected.	A sample of inappropriate quality or quantity may have been used, or the quantitation or cleanup method may not be accurate.	200 - 400 ng of DNA sample is measured with the Qubit® Fluorometer and used. Refer to the recommended cleanup method to clean.
After ethanol washing, the bead does not completely dissolve in the Resuspension Buffer.	In this case, the beads have been over-dried.	Continue pipetting until the bead completely resuspended.
The cluster density was lower than expected.	There may have been a problem with the quantification of the library.	Quantitate the library with the Qubit® Fluorometer and dilute it. Then, load it on the cartridge.
A peak smaller than the expected library size in the TapeStation is observed.	The proper amount of Cleanup Beads may not have been entered, or the ethanol washing was not properly performed.	Select the size with the exact amount of Cleanup Beads and be careful not to transfer the beads with the supernatant. Remove the ethanol completely when performing ethanol washing.
The duplicate rate was higher than expected.	If a small quantity or low DIN value is used, the duplicate rate will increase as the PCR cycle number increases.	Reduce the PCR cycles using the recommended DNA quantity and quality.

7. REFERENCE

1) National Cancer Institute: https://www.cancer.gov/

2) Takeuchi & Okuda (2018) Knowledge base toward understanding actionable alterations and realizing precision oncology. *International Journal of Clinical Oncology*. DOI:10.1007/s10147-018-1378-0.

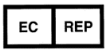
3) Yuan Lu, Yingjia Shen, Wesley Warren and Ronald Walter (2016) Next Generation Sequencing in Aquatic Models, Next Generation Sequencing - Advances, Applications and Challenges, Jerzy K Kulski, *Intech Open*, DOI: 10.5772/61657.

4) Cancer. World Health Organization. Available online: http://www.who.int/mediacentre/factsheets/fs297/en/

5) Susswein LR, Marshall ML, Nusbaum R, et al. (2016) Pathogenic and likely pathogenic variant prevalence among the first 10,000 patients referred for next-generation cancer panel testing. *Genet Med.* **18**:823-832.

6) Van Allen EM, Wagle N, Stojanov P, et al. (2014) Whole-exome sequencing and clinical interpretation of FFPE tumor samples to guide precision cancer medicine. *Nat Med.* **20**:682-688.

8. KEY TO SYMBOLS

Symbol	Description
	CE marking of Conformity
	*In vitro* diagnostic medical device
	Batch code
	Catalogue number
	Use-by date
	Temperature limit
	Caution
	Manufacturer
	Date of manufacture
	Consult instructions for use
	Authorized representative in the European community
	Do not re-use
	Contains sufficient for <n> tests

NGeneBio Co., Ltd.

Manufacturer :

Name: NGeneBio Co., Ltd.
Address: Daerung Post-tower 1 Bldg, 288, Digital-ro, Guro-gu, Seoul, 08390
Republic of Korea

SOLIDaccuTest™ DNA

EC REP

CMC MEDICAL DEVICES & DRUGS, S.L.
C/ Horacio Lengo No 18,
CP 29006, Malaga-Spain

CE
IVD

Registration Number
RPS/134/2020

NGeneBio