RingCap®

Oncology Multi-Gene Mutations Detection Kit

High-Throughput Sequencing

Instruction for Use

Product Name

Oncology Multi-Gene Mutations Detection Kit (High-Throughput Sequencing)

Packing Specification

16 Tests/Kit, 32 Tests/Kit

Intended Use

The kit is intended for the detection of gene somatic mutations (see Appendix Table 1, 2) in peripheral blood or FFPE pathological tissue collected from patients with non-small cell lung cancer or colorectal cancer. The results are indicated only to aid in the individualized therapy of non-small cell lung cancer or colorectal cancer patients. The results shall not be regarded as the only evidence whether a patient suits individualized therapy; determinants such as, but not limited to patients' condition, drug indications, therapeutic response and other laboratory detection indexes should also be considered before making comprehensive judgments.

The kit facilitates the detection of 482 somatic mutations of 13 genes (See Appendix Table 2), including single base mutations, insertions, deletions, and gene fusion [1-8]. The correlations between gene mutations and specific target drugs were mainly from literatures and were generally recognized by clinical practice [5-10].

Technological Principle

High-Throughput Sequencing, also known as Next Generation Sequencing (NGS), can be divided into semiconductor sequencing, DNA nanosphere sequencing and so on according to different sequencing principles. NGS enables the sequencing of up to millions of target nucleic acids at once, provides abundant variation information in short time and at relatively low cost. Highlighting the characteristics of high output and high resolution, NGS has drawn more and more attention in multiple signaling pathways and target studies of cancer. The feasibility of NGS-based multi-pathways/targets detection as an aid in the diagnosis of disease has been supported by numerous clinical trials (e. g. Lung-MAP1, CRUK, WIN Consortium, and NCI-MATCH) [1-6].

The construction of sample library relies on specific modified primers and RingCap® mediated amplification technology with the employment of PCR apparatus. Specific modified primers enable the precise PCR amplification of target sequences, RingCap® mediated amplification allows terminal modification of the products with specific sequences. With the combination of particular PCR program and Ring-Cap® polymerase, library construction of target sequences could be achieved on common PCR apparatus before they are ready for high-throughput sequencing.

Kit Contents

Table1 Kit Contents

No.	Content Name	Strip Color	16 Tests/Kit			32 Tests/Kit			Note
			Volume	Quantity	8-Tube Strip	Volume	Quantity	8-Tube Strip	
1	**Onco-DNA enriching PCR strip**	Blue	20 μL	16 tubes	2 strips	20 μL	32 tubes	4 strips	Each tube contains same reagent.
2	**Onco-RNA enriching PCR strip**	Pink	20 μL	16 tubes	2 strips	20 μL	32 tubes	4 strips	Each tube contains same reagent.
3	**Barcode 1-8 ligation reaction strip**	Purple	20 μL	8 tubes	1 strip	20 μL	16 tubes	2 strips	Each tube represents a barcode.
4	**Barcode 9-16 ligation reaction strip**	Green	20 μL	8 tubes	1 strip	20 μL	16 tubes	2 strips	Each tube represents a barcode.
5	**Barcode 17-24 ligation reaction strip**	White	20 μL	8 tubes	1 strip	20 μL	16 tubes	2 strips	Each tube represents a barcode.
6	**Barcode 25-32 ligation reaction strip**	Yellow	20 μL	8 tubes	1 strip	20 μL	16 tubes	2 strips	Each tube represents a barcode.

7	**RingCap-Taq (1#)**	——	20 μL	1 tube	——	40 μL	1 tube	——	——
8	**Onco DNA Negative Control**	——	20 μL	1 tube	——	20 μL	1 tube	——	——
9	**Onco RNA Negative Control**	——	20 μL	1 tube	——	20 μL	1 tube	——	——
10	**OncoDNA Positive Control**	——	20 μL	1 tube	——	20 μL	1 tube	——	——
11	**OncoRNA Positive Control**	——	20 μL	1 tube	——	20 μL	1 tube	——	——

Note: In barcode reaction strips, different barcode numbers respectively contain 32 different IonDx recognition sequences (see Appendix Table 3); the reagents contain various barcodes have been pre-packaged in strips; the left oblique position of the cap of the strip is oriented in the forward direction, from left to right followed by barcode 1, 2, 3, 4, 5, 6, 7, 8 (Figure 1).

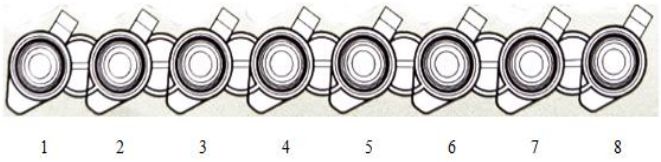

Figure 1 Barcode numbers of 8-tube strips

Note: The contents of different batches cannot be mixed.

Equipment and Reagents Required

1. Microvolume UV-Vis spectrophotometer;
2. PCR system/ thermal cycler: ABI9700, ABI 2720, ABI Veriti;
3. Fluorescence meter: Promega Quantus™ Fluorometer, Cat. No. E6150, or Thermo Fisher Scientific Qubit® 2.0 Fluorescence Meter, Cat. No. Q32866;
4. Nucleic acid extraction kit: commercial nucleic acid extraction kits are recommended;
5. RNA reverses transcription kit: Thermo Fisher Scientific, Super Script® VILO™ cDNA Synthesis Kit, Cat. No. 11754-050;
6. Quantification kit of nucleic acids: Promega, QuantiFluor® dsDNA System, Cat. No. E2670, or Thermo Fisher Scientific, Qubit® dsDNA HS Assay Kit, Cat. No. Q32851/Q32854;
7. Nucleic acids purification kit (magnetic beads method): Beckman Coulter, Agencourt AMpure XP Kit, Cat. No. A63880/ A63881/ A63882;
8. Sequencing Reagents: 1) for in use with Ion PGM: Ion PGM™ Hi-Q™ View OT2 Kit, Cat. No. A29900, Ion PGM™ Hi-Q™ View Sequencing Kit, Cat. No. A30044, or 2) for in use with Ion Proton or DA8600: Ion PI™ Hi-Q™ OT2 200 Kit, Cat. No. A26434, Ion PI™ Hi-Q™ Sequencing 200 Kit, Cat. No. A26433 or A26772;
9. TE buffer solution (pH 8.0);
10. Absolute ethanol (Analytical Grade);
11. Nuclease-free water;
12. Nuclease-free pipettes and tips.

Storage and Stability

1. Storage Condition. Store the kit away from light at -20±5 °C, valid for 9 months. Store the kit upright. Do not use the reagents after 5 freeze-thaw cycles. Once opened, the kit is stable at -20±5 °C until the stated expiration date.
2. Transportation Condition. The kit should be transported in foam cases with ice bags, with transporting time less than one week and transporting temperature lower than 25 °C.
3. Check labels for production date and expiration date of the kit.

Applicable Instruments

Sequencing instruments: Gene Sequencer Ion PGM; Ion Proton; DA8600.

Specimen Material

Collect samples according to the following recommended sample types and requirements:

1. Recommended sample types: FFPE pathological tissue or slices, peripheral blood;
2. FFPE samples: Ensure that at least 30% of the collected pathological tissue were tumor lesions; ensure that the FFPE tissue or slices contain tumor cells; choose FFPE samples which have not been stored for more than 3 years; extract DNA or RNA with at least 8 slices of 5μm section or at least 5 slices of 10 μm section;
3. Peripheral blood: with a non-heparin anti-coagulated blood collection tube, collect 10 mL of peripheral blood;
4. Commercial kits are highly recommended to extract genomic DNA/RNA from the above samples. The total volume of sample DNA extracted should be at least 10 μL. Assess the quality of sample DNA with an ultraviolet spectrophotometer, the ratio of OD_{260}/OD_{280} should be within the range of 1.8 - 2.0; quantify sample DNA with a fluorescence meter, the concentration should be >2 ng/μL. Once the DNA quantity or quality was not in conformity with the above requirements, re-extract DNA with new and/or larger amount of samples. Reverse transcript sample RNA to cDNA immediately after RNA is extracted. Proceed to library construction or store the DNA/cDNA at -20±5 °C for no more than 12 months.

Experimental Procedure

Note: Parallel library construction of Onco Positive Control (**PC**) and Onco Negative Control (**NTC**) with tested sample is suggested.

I. Library Enrichment

1. Reagent preparation: unfreeze the **Onco-DNA enriching PCR strip (blue)** and/ or **Onco-RNA enriching PCR strip (pink)** at room temperature, briefly centrifuge the tubes before use; place the **RingCap-Taq (1#)** on ice after centrifugation;
2. Sample preparation: DNA sample: dilute sample DNA to 2 ng/μL with TE buffer solution (pH 8.0), prepare 10 μL of the diluted sample; RNA sample: cDNA sample after reverse transcription (10 μL);
3. Enriching reaction for Onco-DNA
   a) Add 0.25 μL of **RingCap-Taq(1#)** to 5 μL of the "DNA Sample", Onco DNA PC and Onco DNA NTC, vortex slightly followed by a brief centrifugation;
   b) Gently remove the cap of enriching PCR tubes/ strips, sequentially add 5 μL of the template prepared above into respective tube, replace the cap carefully;
   c) Centrifuge the tubes/ strips slightly to dislodge bubbles;
4. Enriching reaction for Onco-RNA
   a) Add 0.25 μL of RingCap-Taq(1#) to 5 μL of the "RNA Sample", Onco RNA PC and Onco RNA NTC, vortex slightly followed by a brief centrifugation;
   b) Gently remove the cap of enriching PCR tubes/ strips, sequentially add 5 μL of the template prepared above into respective tube, replace the cap carefully;
   c) Centrifuge the tubes/ strips slightly to dislodge bubbles;
5. Load the PCR reaction tubes/ strips into the thermal cycler; remove the reaction subpanel of the instrument, then run the following program:

Table 2 PCR amplification procedure

Step	Temperature	Time	Cyclic Number
Pre-denaturation	98 °C	2 minutes	1
Denaturation	98 °C	15 seconds	15
Annealing	65 °C	4 minutes	
Storage	10 °C	2 minutes	1

Note: Proceed to "Purification of Enriching Products" or store the products at 2 - 8 °C within 4 hours or at -20±5 °C within 24 hours. Storing for more than 24 hours is not suggested.

II. Purification of Enriching Products

Note: Bring the beads to room temperature and vortex thoroughly to disperse the beads before use; prepare fresh 70% ethanol with nuclease-free water.

1. Transfer 25 μL of the PCR enrichment product to a new 1.5 mL Eppendorf tube, add 37.5 μL beads to each tube, pipet up and down to

mix the beads suspension thoroughly with the product;

2. Incubate the mixture for 5 minutes at room temperature;
3. Place the tube on a magnet, then incubate for 2 minutes, carefully remove and discard the supernatant without disturbing the beads;
4. Add 150 µL of freshly prepared 70% ethanol into each tube, rotate the tube side-to-side in the two position of the magnet for 5 times to wash the beads, place the tube on the magnet for 2 minutes, carefully remove and discard the supernatant without disturbing the beads;
5. Repeat step 4 for a second wash;
6. Remove all the ethanol from the tube, keep the tube on the magnet for 5 minutes to air-dry the beads (avoid over-dry);
7. Remove the tube from the magnet, add 35 µL of TE buffer solution (pH 8.0) to each tube, replace the cap, vortex thoroughly (alternatively, mix by pipetting at least half the total volume up and down at least 5 times before replacing the cap), briefly centrifuge to collect the droplets;
8. Incubate the mixture for 5 minutes at room temperature;
9. Place the tube on the magnet for 2 minutes until the solution is clear, carefully remove and store the supernatant (i.e. **purified product**) or proceed to "Library Construction".

III. Library Construction

Note: Use different barcodes for different samples/mutations (DNA mutation or RNA fusion mutation).

1. Reagent preparation: unfreeze the **Barcode ligation reaction tubes/ strips** at room temperature**,** briefly centrifuge the tubes before use; place the "**RingCap-Taq (1#)**" on ice after centrifugation;
2. Construction reaction for Onco-DNA
   a) Add 0.25 µL of **RingCap-Taq (1#)** to 5 µL of the purified products of "DNA Sample", Onco DNA PC and Onco DNA NTC, vortex slightly followed by a brief centrifugation;
   b) Gently remove the cap of barcode ligation tubes/ strips, sequentially add 5 µL of the template prepared above into respective tube, replace the cap carefully;
   c) Centrifuge the tubes/ strips slightly to dislodge bubbles;
3. Construction reaction for Onco-RNA
   a) Add 0.25 µL of **RingCap-Taq (1#)** to 5 µL of the purified products of "RNA Sample", Onco RNA PC and Onco RNA NTC, vortex slightly followed by a brief centrifugation;
   b) Gently remove the cap of barcode ligation tubes/ strips, sequentially add 5 µL of the template prepared above into respective tube, replace the cap carefully;
   c) Centrifuge the tubes/ strips slightly to dislodge bubbles;
4. Load the PCR reaction tubes/ strips into the thermal cycler; remove the reaction subpanel of the instrument, then run the following program:

Table 3 PCR amplification procedure

Step	Temperature	Time	Cyclic Number
Pre-denaturation	98 °C	2 minutes	1
Denaturation	98 °C	15 seconds	20
Annealing	65 °C	4 minutes	
Storage	10 °C	2 minutes	1

Note: Proceed to "Library Purification", or store the products at 2 - 8 °C within 4 hours or at -20±5 °C within 24 hours. Storing for more than 24 hours is not suggested.

IV. Library Purification

Note: Bring the beads to room temperature and vortex thoroughly to disperse the beads before use; prepare fresh 70% ethanol with nuclease-free water.

1. Transfer 25 µL of the PCR library product to a new 1.5 mL Eppendorf tube, add 37.5 µL of beads to each tube, pipet up and down to mix the bead suspension thoroughly with the product;
2. Incubate the mixture for 5 minutes at room temperature;

3. Place the tube on a magnet, then incubate for 2 minutes, carefully remove and discard the supernatant without disturbing the beads;
4. Add 150 μL of freshly prepared 70% ethanol into each tube, rotate the tube side-to-side in the two positions of the magnet for 5 times to wash the beads, place the tube on the magnet for 2 minutes, carefully remove and discard the supernatant without disturbing the beads;
5. Repeat step 4 for a second wash;
6. Remove all the ethanol from the tube, keep the tube on the magnet for 5 minutes to air-dry the beads (avoid over-dry);
7. Remove the tube from the magnet, add 35 μL of TE buffer solution (pH 8.0) to each tube, replace the cap, vortex thoroughly (alternatively, mix by pipetting at least half the total volume up and down at least 5 times before replacing the cap), briefly centrifuge to collect the droplets;
8. Incubate the mixture for 5 minutes at room temperature;
9. Place the tube on the magnet for 2 minutes until the solution is clear, carefully remove and store the supernatant (i.e. **library**) or proceed to “Library Quantification and Dilution”.

V. Library Quantification and Dilution

1. Quality control of sample library: bioanalyzer is recommended for the quality control of library fragments; for PC, NTC and all sample libraries, the target fragments should be 200~300 bp; fluorometer or qPCR library quantification kit is recommended to measure the concentration of sample library; for library concentration lowers than 34 ng/mL (measured by fluorometer) or lowers than 100 pM (measured by qPCR quantification kit), either of which is decided unqualified;
2. Per the concentration measured, dilute sample library to 34 ng/mL with nuclease-free water;
3. For all the DNA or RNA sample libraries which have been diluted to 34 ng/mL, mix 8 μL of each DNA sample library with 2 μL of each RNA sample library (DNA: RNA = 4: 1), thoroughly mix the libraries and centrifuge slightly for further use;
4. Draw up an appropriate amount of the mixed library for further water-in-oil PCR reaction and sequencing (refer to operation manual of each equipment).
5. Store undiluted sample libraries at -20±5 °C for up to 7 days; the mixture of diluted libraries is suggested to be used right after it is ready.

VI. Library enrichment, sequencing

1. Perform a water-in-oil PCR reaction to the above mixed library and on the Ion One Touch 2 instrument, follow the matching sequencing reaction universal kit; the entire program takes about 5 hours, the product can be placed at room temperature for 8 hours after the operation, and can be run overnight;
2. The product obtained by the water-in-oil PCR reaction can be transferred to the Ion One Touch ES instrument for purification, the operation should be performed according to the instructions of the matching sequencing reaction general kit ; the instrument automatically completes the purification operation, the running time takes about 40 minutes;
3. Complete the cleaning and initialization of the gene sequencer according to the operation instructions of the gene sequencer;
4. Log in to the gene sequencer server, click the "Plan" tab, select the "Templates" module, and find the corresponding template program (for the first use, you can create a template program and save it with the help of technical support), select "Templates" from the drop-down menu. "Plan Run" to run the experimental program;
5. Name the experiment in the "Run Plan Name" text box, enter the number of samples, information and the corresponding Barcode number, and click "Plan Run";
6. At the end of the operation of the Ion One Touch ES instrument, the purified product is automatically transferred to a 0.2 mL EP tube. Refer to the instruction manual of the gene sequencer to complete the chip calibration and loading, add sequencing polymerase, and incubate at room temperature for 5 mins;
7. Transfer the chip loaded with the library template to the chip holder and fix it, close the chip chamber, start to run the experimental program set in step 5 and start sequencing;
8. After sequencing, perform a water wash and turn off the instrument.

Data Analysis

1. After the sequencing is completed, the sequencing data is transmitted to the server, perform bases analysis, identification, sequence alignment and variant analysis by the programs "Alignment", "Variant Caller" and "Coverage Analysis" in the Torrent Suite analysis software.
2. The server wil1 firstly performs base calling analysis and converts the electrical signal files in the sequencing data into uBAM files that store unaligned sequences.
3. Align the uBAM file with the human reference genome (hg19 version), and rearrange the indel regions to obtain a rearranged-corrected

BAM file.

4. Variation identification is performed on the region within the detection range and a VCF file for storing the variant site and an EXCEL file for allelic variation are generated.
5. After the analysis, the server will generate the "Completed Runs & Reports" information of this experiment in the "Data" tab, view the overall quality of the chip and the quality control data of the sample; Aligned raw data bam, bai files, and raw results marked with mutation information such as COSMIC ID.

Interpretation of Results

1. Through the analysis software "Alignment", "Variant Caller" and "Coverage Analysis" in the Torrent Server, compare the data obtained f rom the sample sequencing ,"hg19",the DNA amplification region provided by this kit "OncoDNA_target.region_V1.0.bed", the DNA mutation informationdocument"OncoDNA_hotspot_V1.0.bed", the RNA targeting sequence document "OncoRNA_sequence_V1.0.fasta ", and the RNA alignment interval document "OncoRNA_target_V1.0.bed".
2. Judgment of results:
   a) Onco-DNA Results Judgment: Variant Caller result analysis: filter "Allele Call “for” Heterozygous" and “Allele Source” for “Hotspot "to get the COSMIC information of the mutation samples from “Allele Name”;
      i. If there is COSMIC ID, which is for the sample mutation positive COSMIC ID;
      ii. If there is not COSMIC ID, which means the detection result is negative or lower than the detection limit.
   b) Onco-RNA Results Judgment:
      i. There are 5 internal control genes in this kit. In the results of RNA fusion gene mutation analysis "Coverage Analysis", there should be at least 2 genes of the 5 internal control genes has the alignment sequence data records (Total reads) ≥ 200;
      ii. The interpretation method of fusion mutation provided by this kit is "specific site detection", and the sequence data obtained from transcription analysis is recorded as several reads per target, and unlike other analysis platforms, background signal levels should be considered when interpreting results.
         1) If the sample is not read through in the forward and reverse, record the specific fusion site as negative;
         2) If the sample has read-through in both forward and reverse, and total reads < 200, it means that the fusion close to the background signal is detected, the library load needs to be increased. After re-detection, if total reads<200, record the specific fusion site as negative or below the detection limit;
         3) If the sample has read-through in both forward and reverse, and Total reads≥200, the specific fusion site is judged as positive.

Limitation of the Kit

The results shall not be regarded as exclusive diagnostic evidence on clinic; they shall be references only when clinical practice requires. For mutation sites that were not included in the kit or DNA/RNA was extracted from FFPE samples or peripheral blood which were not collected according to designated requirements, the results shall not be interpreted by the instruction.

Performance of Products

1. The kit should be of neat appearance, clear labels, and of no leakage. When unfrozen, the reagents shall be clear, without sediments.
2. The consistency rates of both positive reference samples and negative reference samples are 100%.
3. The kit allows the detection of 5% of specific gene mutations in 10 ng DNA sample and 20 copies/μL of fusion mutations in RNA sample.
4. The repeatability is 100% by detecting designated sample for 10 repetitive times.

Precautions and Warning

1. Please read the instruction carefully in prior to experiments.
2. Conduct experiments abided by laboratory regulations to reduce cross-contaminations of products or reagents; divide experiment areas into different function zones if possible.
3. Clean experiment areas before experiment with 10% hypochlorous acid followed by water rinsing. Sterilize the environment and pipettes with 10% hypochlorous acid, 75% ethanol, or UV radiation.
4. Avoid using peripheral wells of PCR instrument; vacate holes or columns between samples to avoid cross-contamination.
5. Testing results might be influenced by sample sources, sampling process, sample quality, carriage conditions, sample handling, etc; also might it be limited by the quality of DNA, instrument types, operating environment, and the limitation of current molecular

biotechnology, all of which may lead to false positive/ negative results. The users should thoroughly be informed of potential errors as well as the limitation of accuracy.

6. Avoid unnecessary freezing-thawing the reagents, the reagents were allowed to undergo no more than 5 freeze-thaw cycles.
7. The quality of DNA/RNA matters experimental results to a great extent, hence, purification of extracted DNA with magnet beads is highly suggested. Purified DNA should be stored as required (-20±5 °C) or proceed to further steps immediately; RNA is recommended to be reverse transcript to cDNA before storage.
8. Do not substitute any original reagents contained in the kit. Do not mix reagents of different lots.
9. The use of filter tips is highly recommended to avoid false-positive results caused by contamination of reagents.
10. Be cautious of contamination from external DNA; use specific pipettes and tips for reagents preparation and template addition.
11. All reagents in use have potential hazard. Only people who have work permit for PCR laboratories are allowed to use this kit. For first-use of this kit, you may receive training by our technical supports. All used contents of the kit should be considered as clinical dessert and should be disposed properly.
12. All samples including positive control in the kit should be considered potential infectious substances. They should be handled carefully.

Notes

Symbol	Legend
	Indicates the need for the user to consult the instructions for use.
	Indicates a medical device that is intended to be used as an in vitro diagnostic medical device.
	Indicates the date when the medical device was manufactured.
	Indicates the manufacturer’s batch code so that the batch or lot can be identified.
	Indicates the temperature limits to which the medical device can be safely exposed.
	Indicates the date after which the medical device is not to be used.
	This is the correct upright position of the distribution packages for transport and / or storage.
	Indicates a medical device that needs to be protected from moisture.
	Indicates a medical device that needs protection from light sources.
	Indicates the medical device manufacturer.
	Indicates the authorized representative in the European Community/European Union.
	The product meets the basic requirements of European in vitro diagnostic medical devices directive 98/79/EC.

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EC REP

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Appendix table:

Appendix Table 1 Gene COSMIC information covered by the kit

No.	Name	Gene Type	Covered Exons	Number of Mutations
1	EGFR	DNA	18,19,20,21	102
2	KRAS		2,3,4	56
3	BRAF		15	41
4	PIK3CA		10,14,21	51
5	NRAS		2,3	32
6	HER2		19,20,21	16
7	MET		2,14,16,19,20	11
8	AKT1		2	1
9	c-KIT		9,11,13,17	100
10	PDGFRA		12,18	21
11	ALK	RNA	Fusion mutation	21
12	RET		Fusion mutation	15
13	ROS1		Fusion mutation	15

Appendix Table 2 482 kinds of mutation information covered in the kit

No.	Gene	Covered Exons	COSMIC ID
1	NRAS	2	COSM577、COSM576、COSM573、COSM574、COSM575、COSM569、COSM570、COSM571、COSM567、COSM564、COSM565、COSM566、COSM12723、COSM561、COSM562、COSM563、COSM558
		3	COSM589、COSM585、COSM586、COSM587、COSM30646、COSM33693、COSM582、COSM583、COSM584、COSM12725、COSM579、COSM580、COSM581、COSM12730、COSM28673
2	PIK3CA	10	COSM759、COSM17442、COSM760、COSM762、COSM249872、COSM125370、COSM27133、COSM763、COSM12458、COSM27155、COSM764、COSM27374、COSM765、COSM6147、COSM766、COSM12459、COSM25041、COSM767、COSM24712
		14	COSM778、COSM5030972
		21	COSM12590、COSM771、COSM36285、COSM772、COSM21451、COSM36286、COSM17445、COSM29110、COSM25085、COSM12591、COSM12463、COSM29313、COSM773、COSM94984、COSM94985、COSM27134、COSM12592、COSM25086、COSM27273、COSM27156、COSM774、COSM775、COSM776、COSM94986、COSM94987、COSM24714、COSM36289、COSM12597、COSM777、COSM27158
3	PDGFRA	12	COSM21973、COSM741、COSM739、COSM28053、COSM12417、COSM12418
		18	COSM12405、COSM12396、COSM12398、COSM12397、COSM12406、COSM12401、COSM737、COSM12411、COSM736、COSM96892、COSM12408、COSM12400、

			COSM12407、COSM12402、COSM12399
4	KIT	9	COSM1326、COSM96885
		11	COSM23418 、 COSM1204 、 COSM1205 、 COSM1210 、 COSM1330 、 COSM1211 、 COSM1213 、 COSM1221 、 COSM1216 、 COSM1219 、 COSM1220 、 COSM24748 、 COSM1217 、 COSM1218 、 COSM1223 、 COSM1332 、 COSM1227 、 COSM1226 、 COSM1229 、 COSM21978 、 COSM1233 、 COSM1232 、 COSM30551 、 COSM28637 、 COSM1235 、 COSM1234 、 COSM1239 、 COSM1238 、 COSM29015 、 COSM1241 、 COSM18896 、 COSM21976 、 COSM1243 、 COSM1245 、 COSM27069 、 COSM1251 、 COSM1247 、 COSM1248 、 COSM1250 、 COSM1249 、 COSM1255 、 COSM1252 、 COSM1253 、 COSM1254 、 COSM1256 、 COSM36293 、 COSM1257 、 COSM1260 、 COSM1333 、 COSM1258 、 COSM1264 、 COSM1265 、 COSM17946 、 COSM29442 、 COSM1270 、 COSM23560 、 COSM1273 、 COSM1275 、 COSM19029 、 COSM1277 、 COSM1334 、 COSM1285 、 COSM133754 、 COSM96888 、 COSM96883 、 COSM33966 、 COSM1289 、 COSM1290 、 COSM1293 、 COSM1294 、 COSM36305 、 COSM36313 、 COSM1297、COSM1299
		13	COSM1304、COSM25064、COSM12706
		17	COSM27910 、 COSM1310 、 COSM1311 、 COSM21979 、 COSM1312 、 COSM12711 、 COSM1314 、 COSM19285 、 COSM1315 、 COSM12710 、 COSM22379 、 COSM1317 、 COSM1316 、 COSM12709 、 COSM19109 、 COSM1321 、 COSM1322 、 COSM18681 、 COSM18682、COSM19110、COSM1323
5	EGFR	18	COSM41905 、 COSM28508 、 COSM28511 、 COSM12988 、 COSM12371 、 COSM13009 、 COSM13427 、 COSM41603 、 COSM28601 、 COSM18441 、 COSM6252 、 COSM6253 、 COSM18425、COSM6239、COSM12373、COSM22992、COSM28510、COSM13979
		19	COSM13432 、 COSM53194 、 COSM13181 、 COSM13182 、 COSM27041 、 COSM17570 、 COSM26509 、 COSM26038 、 COSM28517 、 COSM6223 、 COSM13184 、 COSM6225 、 COSM12728 、 COSM133189 、 COSM12678 、 COSM12386 、 COSM12367 、 COSM12384 、 COSM23571 、 COSM12419 、 COSM6220 、 COSM24267 、 COSM6218 、 COSM12382 、 COSM12383 、 COSM6254 、 COSM6255 、 COSM133197 、 COSM12387 、 COSM26704 、 COSM6210 、 COSM12369 、 COSM12370 、 COSM13185 、 COSM133207 、 COSM96856 、 COSM13556、COSM29274、COSM6256、COSM6268、COSM85993、COSM12423
		20	COSM26445 、 COSM6241 、 COSM6242 、 COSM12376 、 COSM14068 、 COSM12427 、 COSM12378 、 COSM13428 、 COSM13005 、 COSM13433 、 COSM12377 、 COSM13006 、 COSM22954 、 COSM6226 、 COSM22940 、 COSM13007 、 COSM28513 、 COSM13189 、 COSM27110 、 COSM6240 、 COSM13190 、 COSM22951 、 COSM20891 、 COSM27568 、 COSM133565、COSM5945664
		21	COSM12366 、 COSM26129 、 COSM6224 、 COSM12429 、 COSM12675 、 COSM12374 、 COSM6213 、 COSM14070 、 COSM13197 、 COSM28607 、 COSM53292 、 COSM33725 、 COSM28605、COSM13008、COSM13199、COSM26438
6	MET	2	COSM706
		14	COSM707
		16	COSM696、COSM698、COSM703、COSM702、COSM697、COSM701
		19	COSM699、COSM700、COSM691

7	BRAF	15	COSM1138、COSM1137、COSM1136、COSM1135、COSM21542、COSM1134、COSM6267、COSM33729、COSM1132、COSM6265、COSM478、COSM1133、COSM475、COSM477、COSM476、COSM6137、COSM18443、COSM249889、COSM473、COSM474、COSM1130、COSM33808、COSM219798、COSM144982、COSM1128、COSM30730、COSM472、COSM26625、COSM21549、COSM1124、COSM471、COSM1125、COSM1126、COSM470、COSM26506、COSM469、COSM468、COSM1123、COSM467、COSM466、COSM27639
8	KRAS	2	COSM543、COSM12703、COSM20818、COSM542、COSM538、COSM12722、COSM219781、COSM535、COSM536、COSM537、COSM12721、COSM531、COSM87280、COSM532、COSM533、COSM534、COSM527、COSM528、COSM529、COSM12655、COSM523、COSM524、COSM14209、COSM515、COSM519、COSM522、COSM520、COSM521、COSM25081、COSM34144、COSM512、COSM514、COSM516、COSM517、COSM518、COSM87301、COSM511、COSM510、COSM12654、COSM507
		3	COSM554、COSM555、COSM551、COSM552、COSM553、COSM549、COSM550、COSM87298、COSM28518、COSM547、COSM546、COSM87288、COSM1667043
		4	COSM19900、COSM19404、COSM19905
9	AKT1	2	COSM33765
10	HER2	19	COSM683、COSM5029269、COSM14060、COSM51317、COSM13170
		20	COSM20959、COSM12558、COSM303938、COSM12552、COSM12553、COSM18498、COSM18609、COSM14062、COSM26681、COSM303948
		21	COSM14065
11	ALK	Fusion mutation	COSF463、COSF412、COSF734、COSF465、COSF1376、COSF1063、COSF731、COSF480、COSF1543、COSF491、COSF1366、COSF1367、COSF733、COSF1064、COSF1065、COSF1297、COSF475、COSF413、COSF1545、COSF1542、COSF1540
12	ROS1	Fusion mutation	COSF1201、COSF1295、COSF1251、COSF1203、COSF1266、COSF1279、COSF1260、COSF1197、COSF1268、COSF1270、COSF1274、COSF1198、COSF1261、COSF1672、COSF1280
13	RET	Fusion mutation	COSF1272、COSF1492、COSF1233、COSF1512、COSF1610、COSF1254、COSF1235、COSF1263、COSF1256、COSF1242、COSF1504、COSF1510、COSF1514、COSF1482、COSF1341

Appendix Table 3 Information of 32 IonDx recognition sequences based on Ion torrent tech

Strip Color	Barcode Number	Sequence
Purple	IonDx_001	CTAAGGTAAC
	IonDx_002	TTACAACCTC
	IonDx_003	CCTGCCATTCGC
	IonDx_004	TGGAGGACGGAC
	IonDx_005	TGAGCGGAAC
	IonDx_006	CCTTAGAGTTC
	IonDx_007	TCCTCGAATC
	IonDx_008	AACCTCATTC
Green	IonDx_009	CGGACAATGGC
	IonDx_010	TCCTGAATCTC
	IonDx_011	TAAGCCATTGTC
	IonDx_012	CTGAGTTCCGAC
	IonDx_013	CGGAAGAACCTC
	IonDx_014	TCTTACACAC
	IonDx_015	AAGGAATCGTC
	IonDx_016	TAGGTGGTTC

Strip Color	Barcode Number	Sequence
White	IonDx_017	TAAGGAGAAC
	IonDx_018	AAGAGGATTC
	IonDx_019	TACCAAGATC
	IonDx_020	CAGAAGGAAC
	IonDx_021	CTGCAAGTTC
	IonDx_022	TTCGTGATTC
	IonDx_023	TTCCGATAAC
	IonDx_024	CTGACCGAAC
Yellow	IonDx_025	TCTAACGGAC
	IonDx_026	TTGGAGTGTC
	IonDx_027	TCTAGAGGTC
	IonDx_028	TCTGGATGAC
	IonDx_029	TCTATTCGTC
	IonDx_030	AGGCAATTGC
	IonDx_031	TTAGTCGGAC
	IonDx_032	CAGATCCATC