RingCap®

Human Pan-Cancer Drive Gene Mutations Detection Kit

High Throughput Sequencing

Instruction for Use

Product Name

Human Pan-Cancer Drive 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) in peripheral blood or FFPE pathological tissue collected from patients with pan-cancer drive gene mutations.

The gene mutations detected by this kit cover 56 gene hotspot somatic mutations, including single-base mutations, insertions, deletions and gene fusion mutations and other types of mutations [1-8]. These mutations are related to targeted drug use. Sexuality mainly comes from domestic and foreign literature reports, and has been generally recognized by clinical treatment. [5-10]

Technological Principles

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	Strip Color	16 Tests/Kit			32 Tests/Kit			Note
			Volume	Quantity	8-Tube Strip	Volume	Quantity	8-Tube Strip	
1	**Onco56-DNA Enriching PCR Strip**	Blue	20 μL	16 Tubes	2 Strips	20 μL	32 Tubes	4 Strips	Each Tube Contains Same Reagent.
2	**Onco56-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	——	20 μL	2 Tubes	——	——
8	**Onco56 Negative Control**	——	1.0 mL	1 Tube	——	1.0 mL	1 Tube	——	——
9	**Onco56 DNA Positive Control**	——	20 μL	1 Tube	——	20 μL	1 Tube	——	——

10	**Onco56 RNA Positive Control**	——	20 μL	1 Tube	——	20 μL	1 Tube	——	——

Note: In the Barcode ligation reaction strip, different barcode numbers respectively contain 32 different IonDx recognition sequences (see Appendix Table 3); the reaction solution has been pre-loaded in the eight 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).

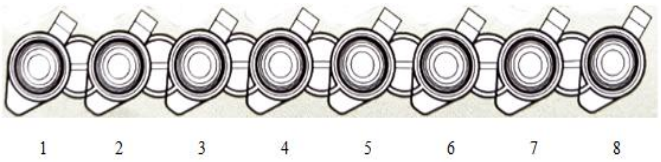

Figure 1 Barcode numbers of 8-tube strips

Note: The contents of different batches cannot be mixed.

Equipment and Reagents Required

1. Nucleic acid extraction Kit: It is recommended to use a commercial nucleic acid extraction kit;
2. RNA Reverse Transcription Kit: Use Thermofisher SuperScript® VILO™ cDNA Synthesis Kit, Cat. No. 11754-050;
3. Nucleic acid quantification Kit: It is recommended to use Promega's QuantiFluor® dsDNA System, Cat. No. E2670; Thermo Fisher Scientific's Qubit® dsDNA HS Assay Kit, Cat. No. Q32851/Q32854;
4. Fluorometer: It is recommended to use the Qubit™ 4 Fluorometer from Thermo Fisher Scientific, Cat. No.Q33238 and Quantus™ Fluorometer from Promega, Cat. No E6150;
5. Magnetic beads: use Agencourt AMPure XP Kit from Beckman Coulter, product number A63880/A63881/A63882; Xiamen Spacegen Co., Ltd SGpure beads, Cat. No. SPG-PB001;
6. Purified water without DNase and RNase;
7. Anhydrous ethanol (analytical grade);
8. Sequencing reagents: select the corresponding sequencing reagents according to the gene sequencer used;
9. DNase and RNase-free pipette tips with filter cartridges;
10. TE (pH 8.0) buffer;

Transportation, Stability, and Storage

1. Storage Condition: Store the kit away from light at - 20±5°C, valid for 12 months, and is not influenced by bottle openings, moreover, do not use the reagents after 5 repeated freeze-thaw cycles;
2. Transportation Condition.:The kit should be transported in foam cases with ice bags, with transporting time of less than one week and transporting temperature lower than 25°C;
3. Check labels for the production date and expiration date of the Kit.

Applicable Apparatus

1. Suitable PCR instruments for library preparation: ABI9700, ABI 2720, ABI Veriti, ABI MiniAmp;
2. Applicable instruments for sequencing: Ion Torrent Sequencer.

Specimen Material

The quality of the DNA/RNA to be detected is critical. In clinical operation, please collect samples according to the following recommended sample types, and then perform DNA/RNA extraction:

1. Recommended sample types: Paraffin-embedded pathological tissue or peripheral blood plasma;
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 that have not been stored for more than 3 years; extract DNA or RNA with at least 8 slices of 5 μm section or at least 5 slices of 10 μm section;
3. Peripheral blood: use a non-heparin anticoagulant blood collection tube, and collect 10 mL;
4. Commercial Kits are highly recommended to extract genomic DNA from the samples. Assess the quality of sample DNA with an ultraviolet-visible spectrophotometer (UV–vis), the ratio of OD_{260}/OD_{280} should be within the range of 1.8-2.2; quantify sample DNA with a fluorescence meter, and the concentration should be ≥ 2 ng/μL, the total amount of DNA should be ≥ 20 ng. Once the DNA

quantity or quality did not conform with the requirements above, re-extract DNA with resampling or a larger amount of sample. Proceed to library enrichment immediately or store sample DNA at -20±5 °C for up to 12 months.

Experimental Procedure

Note: Parallel library construction of Onco56-DNA Positive Control (PC), Onco56-RNA Positive Control (PC)and Onco56 Negative Control (NTC) with the tested sample is suggested.

1. Library Enrichment

a) Reagent preparation: unfreeze the **Onco56-DNA Enriching PCR strip (blue)** and **Onco56-RNA Enriching PCR strip (pink)** at room temperature, briefly centrifuge the tubes before use; place the **RingCap-Taq (1#)** on ice after centrifugation;

b) Sample preparation

i According to the effective DNA concentration measured by the fluorometer, dilute the sample DNA to 2 ng/μL, and the volume is ≥ 5 μL, which is the sample DNA to be tested; it is recommended to directly use the original solution as the template for peripheral blood plasma DNA.

ii RNA sample: cDNA sample after reverse transcription;

c) Enriching reaction for Onco56-DNA:

i. Add 0.25 μL of RingCap-Taq(1#) to 5 μL of the "DNA Sample", Onco56-DNA PC, and Onco56-NTC, vortex slightly followed by a brief centrifugation;

ii. Gently remove the cap of enriching PCR tubes/ strips, sequentially add 5 μL of the template prepared above into the respective tube, replace the cap carefully;

iii. Centrifuge the tube strips slightly to dislodge bubbles.

d) Enriching reaction for Onco56-RNA:

i. Add 0.25 μL of RingCap-Taq(1#) to 5 μL of the "RNA Sample", Onco56-RNA PC, and Onco56-NTC, vortex slightly followed by a brief centrifugation;

ii. Gently remove the cap of enriching PCR strips, sequentially add 5 μL of the template prepared above into respective tube, replace the cap carefully;

iii. Centrifuge the strips slightly to dislodge bubbles.

e) Load the PCR reaction strips into the thermal cycle; remove the reaction sub-panel of the instrument, then run the following program;

f) Open the PCR instrument setting interface, set the amplification program according to Table 2, and run PCR amplification.

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	4 °C	∞	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.

2. Purification of Enriching Products

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

a) Transfer all 25 μL PCR reaction products from the DNA and RNA enrichment PCR reaction tube to a new 1.5 ml centrifuge tube, add 25 μL (1 × sample volume) of magnetic beads, pipette up and down 5 times to mix thoroughly suspended DNA with magnetic beads;

b) Incubate the above two liquids (containing magnetic beads) at room temperature for 5 minutes;

c) Place the tube on a magnet rack, then incubate for 2 minutes, carefully remove and discard the supernatant without disturbing magnetic beads. Note: magnetic beads contain an amplified library, please do not discard them.

d) Add 150 µL of freshly prepared 70% ethanol solution (the ethanol solution should not cover the magnetic bead sample), place it on a magnet rack, turn the centrifuge tube clockwise/counterclockwise 5 times, and place it on the magnet rack to incubate for 2 minutes. Discard the supernatant until the solution is clear;

e) Repeat step d for a second wash;

f) Remove all the ethanol from the tube, and keep the tube on the magnet rack for 5 minutes to air-dry magnetic beads (avoid over-dry);

g) Remove the tube from the magnet rack, add 35 µL of TE (pH 8.0) buffer to each tube, cover the cap, and vortex thoroughly (alternatively, mix by pipetting at least half the total volume up and down at least 5 times before covering the cap), briefly centrifuge to collect the droplets; Incubate the mixture for 5 minutes at room temperature;

h) Place the centrifuge tube on the magnet rack for 2 minutes until the solution is clear, and aspirate the supernatant, which is then amplified and purified product. Store it properly or proceed to the next reaction immediately.

3. Library Construction

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

a) Reagent preparation: unfreeze the **Barcode ligation reaction strip** at room temperature**,** briefly centrifuge the tubes before use; place the "**RingCap-Taq (1#)**" on ice after centrifugal;

b) Construction reaction for Onco56-DNA:

i. Add 0.25 µL of **Ringcap-Taq (1#)** to 5 µL of the purified products of "DNA Sample", Onco56-DNA PC, and Onco56-NTC, vortex slightly followed by a brief centrifugation;

ii. Gently remove the cap of Barcode ligation reaction strip , sequentially add 5 µL of the template prepared above into the respective tube, and replace the cap carefully;

iii. Centrifuge the Barcode ligation reaction strip slightly to dislodge bubbles;

c) Construction reaction for Onco56-RNA:

i. Add 0.25 µL of **Ringcap-Taq (1#)** to 5 µL of the purified products of "RNA Sample", Onco56-RNA PC, and Onco56-NTC, vortex slightly followed by a brief centrifugation;

ii. Gently remove the cap of Barcode ligation reaction strip , sequentially add 5 µL of the template prepared above into the respective tube, and replace the cap carefully;

iii. Centrifuge the Barcode ligation reaction strip slightly to dislodge bubbles;

d) Load the PCR reaction tubes/ strips into the thermal cycle;

e) Open the setup interface of the PCR instrument, set the amplification program according to Table 3, and run PCR amplification.

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	4 °C	∞	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.

4. Library Purification

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

a) Transfer 25 µL of the PCR reaction product to a new 1.5 ml centrifuge tube, add 25 µL (1 × sample volume) of magnetic beads reagent, pipette the liquid up and down 5 times to completely re-suspend the DNA product and magnetic beads;

b) Incubate the mixture for 5 minutes at room temperature;

c) Place the tube on a magnet rack, then incubate for 2 minutes, carefully remove and discard the supernatant without disturbing magnetic beads;

d) Add 150 μL of freshly prepared 70% ethanol (The ethanol solution pass the magnetic bead sample) into each tube, rotate the tube side-to-side in the two positions of the magnet rack for 5 times to wash magnetic beads, place the tube on the magnet rack for 2 minutes, carefully remove and discard the supernatant without disturbing magnetic beads;

e) Repeat step d for a second wash;

f) Remove all the ethanol from the tube, keep the tube on the magnet rack for 5 minutes to air-dry magnetic beads (avoid over-dry);

g) Remove the tube from the magnet rack, add 35 μL of TE (pH 8.0) buffer to each tube, cover the cap, and vortex thoroughly (alternatively, mix by pipetting at least half the total volume up and down at least 5 times before covering the cap), briefly centrifuge to collect the droplets; Incubate the mixture for 5 minutes at room temperature;

h) Incubate the mixture for 5 minutes at room temperature;

i) Place the tube on the magnet rack for 2 minutes until the solution is clear, carefully remove and store the supernatant which is the library, and store it properly or proceed to "Library Quantification and Dilution".

5. Library Quantification and Dilution

a Quality control (QC) of sample library: A bioanalyzer is recommended for the quality control of library fragments; For Onco56 -NTC library, no fragments shall be detected at 250 bp or above; For Onco56-PC library and all sample libraries, the main fragments should be around 250-350 bp; for library concentration lowers than 0.5 ng/μL (measured by fluorometer), either of which is decided unqualified ;

b Per the concentration measured, dilute sample library to 34 ng/mL with Nuclease-free water;

c Mix equal amount of DNA and RNA library with 20:1, then centrifuge slightly for further use;

d Take the above-mixed library for library enrichment, sequencing (see instrument and reagent procedure).

6. Library enrichment, sequencing

a) Perform a water-in-oil PCR reaction to the above mixed library on the Ion One Touch 2 instrument, following the matching sequencing reaction universal kit; the entire program takes about 5 hours or can be run overnight, the product can be placed at room temperature for 8 hours after the operation;

b) 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;

c) Complete the cleaning and initialization of the gene sequencer according to the operation instructions of the gene sequencer;

d) 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.

e) 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";

f) 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;

g) 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 e and start sequencing;

h) After sequencing, perform a water wash and turn off the instrument.

7. Bioinformatics Analysis

Transfer the Excel files obtained by sequencing to the analysis server, followed perform data quality control, sequence alignment, and mutation annotation analysis-based on the clinical high-throughput sequencing data analysis system (abbreviated as analysis system below) of Xiamen Spacegen Co., Ltd.

Positive Judgement Value

1. Result of Onco56-DNA

a) Standard of quality: For all sample DNA libraries, On target Ratio and Uniformity should be more than75%, moreover, Mean Depth

more than1000×;

b) Mutated positive judging criteria: In the result of somatic variation analysis, if effective depth > 500× and mutation frequency > 1%, this mutation site is judged as positive mutation; Otherwise, it is judged as negative or below the detection limit.

2. Results of Onco56-RNA

a) Judging criteria: At least two of the five internal control genes must have Total Reads greater than 200 each;

b) Under a) premise, if the forward and reverse of target regions are all read and Total Reads account for the Average Total Reads of five internal control genes should be ≥2%, the gene fusion is judged as positive mutation; Otherwise, it is judged as negative or below the detection limit.

Interpretation of Results

1. The negative control library concentration may be greater than ≥ 0.5 ng/μL and should be no fragments detected at 250 bp or above on a bioanalyzer; otherwise, the current experiment result is invalid;
2. Target fragments of PC libraries should be 250 ~ 350 bp, otherwise, the current experiment result is invalid; furthermore, for PC, the homogeneity of the positive control should be ≥ 75%, and the sequencing depth should be ≥ 1000 ×, moreover, the test results of the positive control were consistent with the information in the Appendix Table 2;
3. The tested DNA library of the sample should have a strong band at 250 ~ 350 bp after the capillary electrophoresis test. Through high-throughput sequencing, each amplicon should be covered, and the homogeneity should be ≥ 75%, the sequencing depth should be ≥ 1000 ×, otherwise, the DNA test result of the sample to be tested is invalid;
4. In the " RNA quality control results " result of RNA fusion gene mutation analysis of the tested sample, if the Total Reads of 5 internal control genes have less than 2 genes is ≥ 200, the RNA fusion gene detection result is invalid.

Limitation of the Kit

The detection ability of DNA/RNA extracted from paraffin tissue samples beyond the detection site range of this kit and the storage period beyond the time limit cannot be carried out according to this manual.

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 positive reference samples are 100%.
3. The consistency rates of negative reference samples are 100%.
4. It can detect gene mutations as low as 1% in 10 ng DNA samples and fusion mutations in RNA samples as low as 100 copies.
5. Repeat the test 10 times on the same repeatable reference material, all of which should be positive for the corresponding mutation type.

Precautions and Warning

1. Please read the instruction carefully in prior to experiments.
2. Conduct experiments abide by laboratory regulations to reduce cross-contaminates 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 after the experiment.
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 the DNA/RNA used in the test is very important. After DNA extraction, it is recommended to perform magnetic bead purification to ensure the purity of DNA. At the same time, quality control should be carried out to determine the extraction quality. After the extraction is completed, the next step should be performed as soon as possible or store at the recommended temperature, RNA samples are recommended to be reverse transcribed into cDNA and stored.
8. Do not substitute any original reagents contained in the kit. Do not mix reagents of different lots.

9. It is recommended to use pipette tips with filter cartridges to prevent reagent contamination and cause false positive results.
10. Be cautious of contamination from external DNA; use specific pipettes and tips for reagents preparation and template addition. The place where the reagents are prepared should be separated from the place where the template is added.
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. The used kits are clinical waste and should be disposed properly.

Notes

Symbol	Legend
	Indicates the need for the user to consult the instructions for use.
	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.

References

1. MorkTs, Wu YL, Thongprasert S, et al. Gefitinib or carboplatin-paclitaxel in pµlmonaryademocarcinoma. N Engl J Med.2009,361 (10):947-57.
2. Gazdar AF. Personalized medicine and inhibition of EGFR singnaling in lung cancer. N Engl J Med. 2009, 361 (10): 1018-20.
3. Dancey JE. Epidermal growth factor receptor inhibitors in non-small cell lung cancer. Drugs. 2007, 67 (8): 1125-38.
4. Jemal A, Bray F, Center VI M, el al. Global cancer statistics.CA Cancer J Clin. 2011. 61 (2): 69-90.
5. Souliares D,Greer W,Maglioeco AM,et al. KRAS mutationtesting in the treatment of metastatic colorectal cancer with anti-EGFR

therapies. CurrOncol.2010,17 Suppl 1:S31-S40.

6. Paul B.Chapman,M.D.,et al. Improved Survival with Vemurafenibin Melanoma with BRAF Mutation. N Engl J Med. 2011:364,2507-16.
7. Wendy De Roock, Bart Claes,et al.Effects of KRAS,BRAF,NRAS, and PIK3CA mutationson the efficacy of cetuximab plus chemotherapy inchemotherapy-refractory metastatic colorectalcancer: a retrospective consortium analysis.Lancet Oncol.2010,11:753–62.
8. Soda M,Choi YL, Enomoto M,et al.Identification of the transforming EML4-ALK fusion gene in non-small-cell lung cancer. Nature.2007, 448 (7153): 561-6.
9. Sai-Hong Ignatius Ou, Jackie Tan, et al. ROS1 as a 'druggable' receptor tyrosine kinase: lessons learned from inhibiting the ALK pathway. Expert Rev. Anticancer Ther.2012,12 (4):447-56.

   Alexa B.Turke,KreshnikZejnullahu,et al.Preexistence and Clonal Selectionof MET Amplification in EGFR Mutant NSCLC.Cancer Cell. 2010, 17: 77-88.

EC REP

Manufacturer:Lotus NL B.V.
Address:Koningin Julianaplein 10, 1e Verd, 2595AA, The Hague, Netherlands.
E-mail:peter@lotusnl.com

Manufacturer: XIAMEN SPACEGEN CO., LTD.
Address: 4th floor, No.2041 Xizhou Road, Xike Town, Tong'an District,
Xiamen 361100, P. R. China
Tel: +86 592 7578317 Fax: +86 592 7578319
E-mail: spacegen@ispacegen.com
Website: http://www.sspacegen.com

Attached table 1

Gene Information Covered by the Kit

DNA							
MPL	PDGFRA	BRAF	FGFR2	CDH1	PTEN	IDH2	SMARCB1
NRAS	KIT	EZH2	HRAS	TP53	RET	AKT1	GNAS
ALK	KDR	FGFR1	ATM	ERBB2	NOTCH1	RB1	SRC
IDH1	FBXW7	JAK2	KRAS	SMAD4	MLH1	EGFR	PIK3CA
ERBB4	APC	CDKN2A	PTPN11	STK11	MET	FGFR3	SMO
VHL	CSF1R	GNAQ	HNF1A	GNA11	ABL1	FLT3	JAK3
NPM1	CTNNB1						
RNA(Fusion)							
ALK	ROS1	RET	NTRK1	NTRK3	MET exon14- skipping		
CNV							
HER2	MET						

Attached table 2

Positive Control Mutation Information

Positive Control	Gene	Base Mutation	Amino Acid Mutation	Cosmic ID	Mutation Type
Onco56 DNA Positive Control	EGFR	c.2235_2249del15	p.E746_A750delELREA	6223	Deletion mutation
	KRAS	c.35G > A	p.G12D	521	Point mutation
	BRAF	c.1799T > A	p.V600E	476	Point mutation
	HER2	c.2324_2325ins12	p.A775_G776insYVMA	20959	Insertion mutation
Onco56 RNA Positive Control	ALK	EML4-ALK(E13-A20)	——	463	Fusion mutation
	ROS1	SLC34A2-ROS1(S4-R32)	——	1197	Fusion mutation

Attached table 3

Information of IonDx Recognition Sequences Based on Ion Torrent Tech

Strip Color	Barcode Number	Sequence
Purple	Barcode_001	CTAAGGTAAC
	Barcode_002	TTACAACCTC
	Barcode_003	CCTGCCATTCGC
	Barcode_004	TGGAGGACGGAC
	Barcode_005	TGAGCGGAAC
	Barcode_006	CCTTAGAGTTC
	Barcode_007	TCCTCGAATC
	Barcode_008	AACCTCATTC
Green	Barcode_009	CGGACAATGGC
	Barcode_010	TCCTGAATCTC
	Barcode_011	TAAGCCATTGTC
	Barcode_012	CTGAGTTCCGAC
	Barcode_013	CGGAAGAACCTC
	Barcode_014	TCTTACACAC
	Barcode_015	AAGGAATCGTC
	Barcode_016	TAGGTGGTTC

Strip Color	Barcode Number	Sequence
White	Barcode_017	TAAGGAGAAC
	Barcode_018	AAGAGGATTC
	Barcode_019	TACCAAGATC
	Barcode_020	CAGAAGGAAC
	Barcode_021	CTGCAAGTTC
	Barcode_022	TTCGTGATTC
	Barcode_023	TTCCGATAAC
	Barcode_024	CTGACCGAAC
Yellow	Barcode_025	TCTAACGGAC
	Barcode_026	TTGGAGTGTC
	Barcode_027	TCTAGAGGTC
	Barcode_028	TCTGGATGAC
	Barcode_029	TCTATTCGTC
	Barcode_030	AGGCAATTGC
	Barcode_031	TTAGTCGGAC
	Barcode_032	CAGATCCATC