CE

RingCap®

Oncology Multi-Gene Mutations Detection Kit

High Throughput Sequencing

Instruction for Use (Illumina)

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) in 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 to guide 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 somatic mutations of 5 genes (See Appendix Table 1), including single base mutations, insertions, and deletions [1-8]. The correlations between gene mutations and specific target drugs were mainly from literatures and were generally recognized by clinical practice [5-9].

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 one time, 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

Table 1. Kit contents

No.	Content Name	Main Content	Strip Color	16 Tests/Kit			32 Tests/Kit			Note
				Volume	Quantity	8-Tube Strip	Volume	Quantity	8-Tube Strip	
1	**Onco-DNA UDI 1 -8 PCR Strip**	Primer, UDI Primer	Purple	10 µL	8 tubes	1 strip	10 µL	8 tubes	1 strip	Each tube contains different UDI primer.
2	**Onco-DNA UDI 9 -16 PCR Strip**	Primer, UDI Primer	Green	10 µL	8 tubes	1 strip	10 µL	8 tubes	1 strip	Each tube contains different UDI primer.
3	**Onco-DNA UDI 17-24 PCR Strip**	Primer, UDI Primer	White	——	——	——	10 µL	8 tubes	1 strip	Each tube contains different UDI primer.
4	**Onco-DNA UDI 25-32 PCR Strip**	Primer, UDI Primer	Yellow	——	——	——	10 µL	8 tubes	1 strip	Each tube contains different UDI primer.
5	**Master Mix 2×**	Taq enzyme, dNTPs, Mg^{2+}, Buffer	——	170 µL	1 tube	——	170 µL	2 tubes	——	——
6	**Onco-DNA Negative Control**	Wild type DNA	——	20 µL	1 tube	——	20 µL	1 tube	——	——

7	**Onco-DNA Positive Control**	Mutation type DNA	——	20 μL	1 tube	——	20 μL	1 tube	——	——

Note 1: Containing UDI Primer reaction strips, different UDI numbers respectively contain different UDI recognition sequences (see Appendix Table 2,3).

Note 2: The reaction solutions have been pre-loaded into 8-Tube strips, as shown in Figure 1.

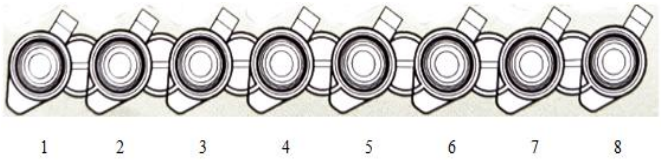


Figure 1. UDI numbers of 8-Tube Strips

Note 3: The contents of different batches of reagents cannot be mixed.

Additional required Equipment and Materials

1. Nucleic acids extraction kit: Nucleic Acid Extraction Kit (FFPE DNA) (Xiamen Spacegen Co., Ltd, Cat. No. SPG-HSD001R
2. Quantification kit of nucleic acids: Quanti Fluor® dsDNA System (Promega, Cat. No. E2670) or Qubit® dsDNA HS Assay Kit (Thermo Fisher Scientific, Cat. No. Q32851/Q32854), Qubit® ssDNA Assay Kit (Alternatively) (Thermo Fisher Scientific, Cat. No. Q10212)
3. Fluorometer: Quantus™ Fluorometer (Promega, Cat. No. E6150) or Qubit™4.0 Fluorometer (Thermo Fisher Scientific, Cat. No. Q33238)
4. Magnetic beads: SG Pure Beads (Xiamen Spacegen Co., Ltd, Cat. No. SPG-PB001R/002R) or HighPrep™ PCR (MagBio, Cat. No. AC-60005/ AC-60050/ AC-60250/ AC-60500)
5. Sequencing reagents and corollary reagents to be purchased separately: Selecting the corresponding sequencing reagent according to the gene sequencer
   (1) Illumina corollary reagents: PhiX Control V3 (Illumina, Cat. No. FC-110-3001)
   (2) MGI corollary reagents: MGIEasy universal library conversion kit (APP-A) (MGI, Cat. No. 1000004155), High throughput sequencing primer kit (App-C) (Alternatively) (MGI, Cat. No. 1000027472)
6. Magnetic rack
7. Microvolume UV-visible spectrophotometer
8. Ethanol absolute (Analytical Grade)
9. TE Buffer (pH 8.0)
10. Nuclease-Free Water
11. Nuclease-Free pipette tips with filter

Transportation, Stability and Storage

1. This product is shipped on frozen ice packs. The contents of the shipment should be stored immediately upon receipt at -15℃ to -25℃ in a constant-temperature freezer and protected from light. Repeated thawing and freezing should be avoided. Do not exceed a maximum of 5 freeze-thaw cycles. The shelf-life of the kits is 12 months.
2. Check labels for the production date and expiration date of the kit.

Applicable Instruments

1. Library preparation PCR apparatus: ABI 9700, ABI 2720, ABI Veriti, ABI Mini Amp, etc.
2. Sequencing instruments:
   (1) Illumina sequencing instruments (Miseq, NextSeq 500/550, Miniseq, etc.)
   (2) MGI sequencing instruments (MGISEQ-2000, DNBSEQ-G99RS, etc.)

Specimen Material

The quality of the nucleic acids to be detected is critical. Please collect samples according to the following recommended sample types:

1. Recommended sample types: FFPE.
2. FFPE samples: It is recommended to choose FFPE samples that have not been stored for more than 2 years and at least 30% of the collected pathological tissue is tumor lesions, and use no less than 8 pieces of 5 μm section or 5 pieces of 10 μm section for nucleic acids extraction.

Experimental Procedure

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

I. Library Preparation

1. Reagent preparation: Thaw the Onco-DNA UDI PCR Strip as needed at room temperature until no ice is present in the tubes, up and down to mix, briefly centrifuge the strip before use. Place the **Master Mix 2×** on ice after centrifugation.
2. Sample preparation:
   (1) Nucleic acid extraction and quality control: Commercial nucleic acids extraction kit is recommended to extract genomic DNA from the samples. Assess the quality of sample DNA with a Microvolume UV-visible spectrophotometer, the ratio of OD_{260}/OD_{280} should be within the range of 1.8-2.2, quantify sample DNA with a Fluorometer, the concentration should be ≥5 ng/μL, the total amount of DNA should be ≥25 ng. Once the DNA quality or quantity is not conformed with the above requirements, re-extract DNA with new and/or larger input. DNA is recommended to library construction immediately or store at -15℃ to -25℃ for no more than 12 months.
   (2) DNA Sample: Dilute DNA sample to 5 ng/μL with TE Buffer (pH 8.0) based on the effective DNA concentration measured by the Fluorometer, and the volume ≥5 μL.

Note: Use PCR Strip contains different UDI for DNA samples.

3. Enriching reaction for DNA
   (1) Prepare a volume of at least 5 μL of the DNA Sample, Onco-DNA PC and Onco-DNA NTC.
   (2) Gently remove the cap of the Onco-DNA UDI PCR Strip, add 10μL Master Mix 2× to each reaction tubes, and sequentially add 5 μL of the template prepared above (1) into the respective tube, cap the tubes carefully.
   (3) Centrifuge the tubes slightly and avoid creating air bubbles.
4. Load the PCR centrifuge tubes above into the thermal cycler, then set up and run the program according to Table 2.

Table 2. PCR Amplification Procedure

Step	Temperature	Time	Cycle Number
Pre-denaturation	98℃	2 minutes	1
Denaturation	98℃	15 seconds	15
Annealing	60℃	4 minutes	
Denaturation	98℃	15 seconds	20
Annealing	65℃	2 minutes	
Hold	4℃	∞	1

Note: Proceed to "Purification of **Library**", or store the products at 2℃ to 8℃ within 8 hours or at -15℃ to -25℃ within 24 hours. Storing for more than 24 hours is not suggested.

II. Purification of Library

Note: Transfer the magnetic beads to room temperature and vortex thoroughly to disperse magnetic beads before use. Prepare fresh 70% ethanol with Nuclease-Free Water.

1. Transfer 25 μL of PCR enrichment product of Onco-DNA UDI PCR Strip each to a new 1.5 mL centrifuge tube, add 25 μL (1×sample volume) of magnetic beads to each tube, pipet up and down 5 times to mix magnetic beads suspension thoroughly with the product.
2. Incubate the mixture for 5 minutes at room temperature.
3. Place the tubes on a magnetic rack for 2 minutes until the solution becomes clear, carefully remove and discard the supernatant without disturbing magnetic beads.

Note: The magnetic beads contain amplified library and should not be discarded.

4. Add 150 μL of freshly prepared 70% ethanol to each tube, rotate the tubes clockwise or counterclockwise five times. Place the tubes on the magnetic rack for 2 minutes until the solution becomes clear, carefully remove and discard the supernatant without disturbing magnetic beads.
5. Repeat step 5 one more time for a second wash.
6. Remove all the ethanol from the tubes, and keep the tubes on the magnetic rack for 5 minutes at room temperature to air-dry the magnetic beads (avoid over-dry).

7. Remove the tube from the magnetic rack, add 35 μL of TE Buffer (pH 8.0) to each tube to fully infiltrate the magnetic beads, and vortex thoroughly (or mix by pipetting at least half the total volume up and down at least 5 times), briefly centrifuge to collect the droplets.
8. Incubate the mixture for 5 minutes at room temperature.
9. Place the tubes on the magnetic rack for 2 minutes until the solution becomes clear, carefully transfer and store the supernatant (i.e. **library**), proceed to next step or store at -15℃ to -25℃.

III. Library Quantification

Bioanalyzer is recommended for the quality control of library fragments. For DNA NTC libraries and DNA PC library and all sample DNA libraries, the target fragments should be in 200-300 bp. Fluorometer quantification kit is recommended to measure the concentration of sample library and should be more than 0.5 ng/μL.

IV. Library dilution, mixing and sequencing

1. Illumina sequencing platform
   (1) According to the library concentration measured by the Fluorometer, use the following formula to convert the molar concentration of the library.

$$\text{Library concentration (nM)} = \frac{\text{Library concentration (ng/μL)} \times 10^6}{\text{Library length (bp)} \times 650}$$

   (2) Per the concentration measured, dilute the sample library to 4 nM with Nuclease-Free Water.
   (3) Proceed sample dilution and denaturation according to the matching Illumina sequencing kit (refer to the operation manual of each equipment).
   (4) The concentration of Phix Control V3 is more than 5% (for example: If the loading volume is 600 μL, the volume occupied by Phix Control V3 should be more than 30 μL).
   (5) Library on-machine sequencing (according to the specification of instrument and matching reagent).
2. MGI sequencing platform
   (1) The recommended amount of cyclic library input is 0.5 pmol, and the required proportion of each library in the total library of 0.5 pmol is calculated, and then the required amount of each library is calculated according to the following formula:

$$\text{Library input (ng)} = \frac{\text{Library length (bp)} \times 650 \times \text{proportion\%}}{1000}$$

   (2) According to the measured library concentration, the required input volume is calculated, and then mixed to obtain 0.5 pmol total library, the total volume is not more than 48 μL.
   (3) Denaturation and cyclization of libraries according to the MGIEasy Universal Library Conversion Kit (App-A) (no need for split-conversion PCR, see the accompanying reagent manual for instructions).
   (4) Proceed sample dilution and denaturation according to the matching sequencing kit (refer to the operation manual of each equipment).
   (5) Library DNB preparation and on-machine sequencing (operation instructions refer to the accompanying reagent manual and instrument manual).

Note: Store undiluted libraries at -15℃ to -25℃ for up to 7 days, the mixture of diluted libraries is suggested to be used right after it is ready.

V. Bioinformatics Analysis

Transfer the Fastq files obtained by sequencing to the analysis server, perform data quality control, sequence alignment and mutation annotation analysis-based on the Clinical NGS Data Analysis System of Xiamen Spacegen Co., Ltd.

Data Analysis

1. Standard of quality: For all sample DNA libraries, the target fragment should be in 200-300 bp, On Target should be ≥80%, Uniformity should be ≥75% and mean depth should be ≥5000×.
2. Mutated positive judgement criterion: In the results of variation analysis, if effective depth is ≥500×. And mutation frequency is ≥1%, this mutation site is judged as positive mutation. Otherwise, it is judged as negative or below the detection limit.

Interpretation of Results

1. For the DNA negative control library, the target fragment should be in 200-300 bp, as well as On Target should be ≥80%, Uniformity should be ≥75%, moreover, mean depth ≥5000×. Otherwise, this test is invalidated.

2. For the DNA positive control library, the target fragment should be in 200-300 bp, as well as On Target should be ≥80%, Uniformity should be ≥75%, moreover, mean depth ≥5000×. Otherwise, this test is invalidated.
3. For the DNA sample libraries, the target fragment should be in 200-300 bp, each amplicon should have coverage, as well as On Target should be ≥80%, Uniformity should be ≥75%, moreover, mean depth ≥5000×. Otherwise, this mutation detection results are invalidated.
4. The grade of somatic variation based on the "Standards and Guidelines for the Interpretation and Reporting of Sequence Variants in Cancer: A Joint Consensus Recommendation of the Association for Molecular Pathology, American Society of Clinical Oncology, and College of American Pathologists" jointly formulated by AMP/ASCO/CAP in 2017 could divide into 4 types:
   (1) Clear clinical significance: Diagnostic\prognostic marker of specific tumor or drugs recommended\approved in the professional guidelines.
   (2) Potential clinical significance: Diagnostic\prognostic marker of specific tumor or drugs that has level A evidence of another tumor in the multiple small research.
   (3) Unknown clinical significance: It is not found higher rates of variants in the general population and tumor databases, moreover, not has clear published evidence.
   (4) Harmless or may be harmless clinical significance: It is found higher rates of variants in the general population and not published evidence.

Limitation of the Kit

1. The detection results are for research use only. For mutation sites that are not included in the kit, or the nucleic acids extracted from samples are stored longer than required, the results shall not be interpreted by the instruction.
2. The negative results cannot exclude the mutations. For few tumor cells, excessive degradation, or the nucleic acids concentration is below the detection limit can also cause a negative result.
3. Unreasonable sample collection, transportation, processing, improper operation and the experimental environment may lead to false negative or positive results.
4. Tumor tissue (cells) may have large heterogeneity, different test results may be obtained by sampling different parts.

Performance characteristics

1. The kit should be neat in appearance, clearly labels, and no leakage.
2. When unfrozen, the reagents shall be clear, without sediments.
3. Negative reference conformity rate should be 100%.
4. Positive reference conformity rate should be 100%.
5. The kit allows the detection of 1% of specific gene mutations in 25 ng DNA tissue samples.

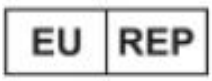
Warnings and Precautions

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. Avoid repetitively freezing and thawing the reagents in the kit. Do not exceed a maximum of 5 freeze-thaw cycles.
4. The results of this kit will be affected by sample source, collection process, quality, transportation conditions, pre-treatment, etc., as well as the quality of the extracted nucleic, instrument types, operating environment, and the limitation of current molecular biotechnology. The factors and variables mentioned above would lead to false positive or false negative results. Users must be aware of the potential errors, accuracy and limitations that may exist during the process of detection.
5. The quality of nucleic acids is crucial, and the quality control of DNA should be performed after extraction, proceed to further steps immediately or store properly at -15℃ to -25℃.
6. Do not substitute any original reagents contained in the kit. Do not mix reagents with different Lots.
7. Pay special attention to the use of positive control and the use of filter pipette tips is highly recommended to avoid false-positive results caused by contamination of reagents.
8. Be cautious of contamination from external nucleic. Ensure to add the nucleic template before operating the positive control. Segregate areas for reagent preparation and sample processing. Use dedicated pipettes and pipette tips for reagent preparation and template

addition, respectively.

9. Clean experiment areas before experiment with 10% hypochlorous acid followed by twice water rinsing. Sterilize the environment and pipettes with 10% hypochlorous acid, 75% ethanol, or UV radiation after experiment.
10. All reagents in use have potential hazard. It is recommended wearing proper protective suit and gloves. For first-use of this kit, you may receive training by our technical supports.
11. All samples including positive control in the kit should be considered as potential infectious substances which should be handled carefully.
12. Avoid using peripheral wells of PCR instrument; vacate holes or columns between samples to avoid cross-contamination.

Symbols

Symbol	Symbol definition
	Consult instructions for use.
	In vitro diagnostic medical device.
	Date of manufacture.
	Lot number.
	Temperature limitation.
	Use by.
	This way up upright position of the packages for transport or storage.
	Keep dry.
	Keep away from sunlight.
	Manufacturer.
	European Authorised Representative.
	CE mark for European conformity.

References

1. Mork TS, Wu YL, Thongprasert S, et al. Gefitinib or carboplatin-paclitaxel in pulmonary adenocarcinoma. N Engl J Med. 2009, 361 (10): 947-57.
2. Gazdar AF. Personalized medicine and inhibition of EGFR signaling 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 MM, el al. Global cancer statistics. CA Cancer J Clin. 2011, 61 (2): 69-90.
5. Soulières D, Greer W, Magliocco AM, et al. KRAS mutation testing in the treatment of metastatic colorectal cancer with anti-EGFR

therapies. Curr Oncol. 2010, 17 Suppl 1: S31-40.

6. Chapman PB, Hauschild A, Robert C, et al. Improved survival with vemurafenibin melanoma with BRAFV600E mutation. N Engl J Med. 2011, 364 (26): 2507-16.
7. De Roock W, Claes B, Bernasconi D, et al. Effects of KRAS, BRAF, NRAS, and PIK3CA mutations on the efficacy of cetuximab plus chemotherapy in chemotherapy-refractory metastatic colorectal cancer: a retrospective consortium analysis. Lancet Oncol. 2010, 11 (8): 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. Turke AB, Zejnullahu K, Wu YL, et al. Preexistence and clonal selection of MET amplification in EGFR mutant NSCLC. Cancer Cell. 2010, 17 (1): 77-88.

EU REP

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Appendix Table 1. Information of designated gene detected by the kit

Name	Covered Exons	Covered region / COSF
BRAF	15	chr7: 140453075-140453155
EGFR	18	chr7: 55241643-55241730
	19	chr7: 55242419-55242510
	20	chr7: 55248995-55249097
	21	chr7: 55259484-55259578
HER2	19	chr17: 37880183-37880280
	20	chr17: 37880965-37881063
	21	chr17: 37881291-37881385
KRAS	2	chr12: 25398233- 25398308
	3	chr12: 25380263-25380351
	4	chr12: 25378526-25378610
NRAS	2	chr1: 115258705-115258791
	3	chr1: 115256465-115256550
	4	chr1:115252179-115252259

Appendix Table 2. Information of UDI Recognition Sequences based on Illumina Tech

Strip Color	Number	i7 Sequence	i5 Sequence（NovaSeq, MiSeq）	i5 Sequence（iSeq, MiniSeq, NextSeq）
Purple	UDI-1	TGCATAGC	TAGGATTC	GAATCCTA
	UDI-2	TCTATGCA	GTCGTTGC	GCAACGAC
	UDI-3	GTACGCAT	CCTCGCAT	ATGCGAGG
	UDI-4	AGGTCCTG	AGAAGGCG	CGCCTTCT
	UDI-5	CATGAGCT	ACGTCAGA	TCTGACGT
	UDI-6	AACTCTAG	CATCTGAT	ATCAGATG
	UDI-7	CCGGATGC	GTATCACG	CGTGATAC
	UDI-8	GTACGATA	TGCAACTA	TAGTTGCA
Green	UDI-9	ATTCGATA	ATGGATCG	CGATCCAT
	UDI-10	CGTAGTAC	GCTGAATG	CATTCAGC
	UDI-11	GAGTACGT	CAACTGGC	GCCAGTTG
	UDI-12	TCAGTGCG	TGCAGCAT	ATGCTGCA
	UDI-13	CACACAGT	ACGACCAA	TTGGTCGT
	UDI-14	GTGCATCG	CATTCGGC	GCCGAATG
	UDI-15	TGCGTCAC	GTATGATT	AATCATAC
	UDI-16	ACATCGTA	TGCCTTCA	TGAAGGCA
White	UDI-17	CGGAACGA	GCTGGCTT	AAGCCAGC
	UDI-18	CCTGGCAC	ATAGAGAC	GTCTCTAT
	UDI-19	ATATCGCT	CACATTGA	TCAATGTG
	UDI-20	GACAGTTG	TGGTCACG	CGTGACCA
	UDI-21	TGCCTATG	ACCTTCGG	CCGAAGGT
	UDI-22	GTACCAGT	CGACCATC	GATGGTCG
	UDI-23	AATGTGCA	TAGCATCA	TGATGCTA
	UDI-24	TCGTATAC	GTTAGGAT	ATCCTAAC
Yellow	UDI-25	CTGTGTGT	CGTCGTCT	AGACGACG
	UDI-26	ACAGCACT	ATCCTAGC	GCTAGGAT
	UDI-27	TATCAGTG	GAAGCCTG	CAGGCTTC
	UDI-28	CGGTGTTA	TCGAAGTA	TACTTCGA
	UDI-29	GTCATCAC	ACCGGTAC	GTACCGGT
	UDI-30	GATGTCAG	CATTCAAT	ATTGAATG
	UDI-31	TCACAGCA	TGGTAGCA	TGCTACCA
	UDI-32	AGCACAGC	GTAATCGG	CCGATTAC

Appendix Table 3. Information of UDI Recognition Sequences based on MGI Tech

Strip Color	Number	Sequence
Purple	UDI-1	TAGGATTCTGCATAGC
	UDI-2	GTCGTTGCTCTATGCA

Strip Color	Number	Sequence
White	UDI-17	GCTGGCTTCGGAACGA
	UDI-18	ATAGAGACCCTGGCAC

	UDI-3	CCTCGCATGTACGCAT		UDI-19	CACATTGAATATCGCT
	UDI-4	AGAAGGCGAGGTCCTG		UDI-20	TGGTCACGGACAGTTG
	UDI-5	ACGTCAGACATGAGCT		UDI-21	ACCTTCGGTGCCTATG
	UDI-6	CATCTGATAACTCTAG		UDI-22	CGACCATCGTACCAGT
	UDI-7	GTATCACGCCGGATGC		UDI-23	TAGCATCAAATGTGCA
	UDI-8	TGCAACTAGTACGATA		UDI-24	GTTAGGATTCGTATAC
Green	UDI-9	ATGGATCGATTCGATA	Yellow	UDI-25	CGTCGTCTCTGTGTGT
	UDI-10	GCTGAATGCGTAGTAC		UDI-26	ATCCTAGCACAGCACT
	UDI-11	CAACTGGCGAGTACGT		UDI-27	GAAGCCTGTATCAGTG
	UDI-12	TGCAGCATTCAGTGCG		UDI-28	TCGAAGTACGGTGTTA
	UDI-13	ACGACCAACACACAGT		UDI-29	ACCGGTACGTCATCAC
	UDI-14	CATTCGGCGTGCATCG		UDI-30	CATTCAATGATGTCAG
	UDI-15	GTATGATTTGCGTCAC		UDI-31	TGGTAGCATCACAGCA
	UDI-16	TGCCTTCAACATCGTA		UDI-32	GTAATCGGAGCACAGC

Appendix Table 4. Positive control information of this kit

Positive Control	Gene Name	Base Mutation	Amino Acid Mutation	Cosmic ID	Mutation Type
DNA Positive Control	EGFR	c.2235_2249del	p.E746_A750del	COSMIC6223	Deletion Mutation
	KRAS	c.35G>A	p.G12D	COSMIC521	Point Mutation
	BRAF	c.1799T>A	p.V600E	COSMIC476	Point Mutation
	HER2	c.2313_2324dup	p.Y772_A775dup	COSMIC20959	Duplication Mutation