RingCap®

Thyroid Cancer Gene Mutation Detection Kit

High Throughput Sequencing

Instruction for Use

Product Name

Thyroid Cancer Gene Mutation Detection Kit (High-Throughput Sequencing)

Packing Specification

16 Tests/kit, 32 Tests/kit

Intended Use

This kit uses to take the nucleic acid extracted from thyroid fine needle puncture sample or tissue sample as the test sample, including 16 genes on the DNA level and 87 genes (209 fusion forms) on the RNA lever. See attached table 1 for the specific gene list. It covers the key pathogenic genes of thyroid cancer, including single nucleotide variation, small fragment insertion / deletion, and gene fusion mutation types. The test results evaluate the molecular characteristics of patients with thyroid cancer, so as to provide clinical reference, improve the accuracy of benign and malignant judgment of thyroid cancer, optimize treatment plan, accurately guide medication and assist genetic screening.

Technological Principle

High throughput sequencing, also known as next generation sequencing (NGS), can be divided into semiconductor sequencing and DNA nanosphere sequencing according to different sequencing principles. High throughput sequencing can sequence hundreds of thousands or even millions of target nucleic acid molecules in parallel at one time. It has the characteristics of high output and high resolution. It not only provides rich sequence variation information, but also greatly reduces the sequencing cost and time-consuming. It plays a significant role in cancer multi-channel and multi-target research.

This kit is based on ordinary PCR platform, combines specific modified primers and RingCap® Loop mediated ligation amplification technique was used to detect mutant genes in nucleic acid samples. Specific modified primers were used to amplify the target sequence by precise PCR. At the same time, RingCap® Loop mediated link amplification technology was used to modify the end of the amplified product and connects the specific sequence end. Combined with the use of special PCR reaction program, ligase and high specific RingCap Taq enzyme, the target sequence in the sample nucleic acid can be constructed on the ordinary PCR platform for high-throughput sequencing, so as to realize the accurate and rapid detection of multi gene and multi-target mutations.

Kit Contents

Table1 Kit Contents

Number	Content Name	Strip Color	16 Tests/Kit			32 Tests/Kit			Note
			Volume	Tube Number	8-Tube Strip	Volume	Tube Number	8-Tube Strip	
1	**TC-DNA-1 PCR Strip**	Blue	20 μL	16 tubes	2 strips	20 μL	32 tubes	4 strips	Each tube contains same reagent.
2	**TC-DNA-2 PCR Strip**	Yellow	20 μL	16 tubes	2 strips	20 μL	32 tubes	4 strips	Each tube contains same reagent.
2	**TC-RNA PCR Strip**	Pink	20 μL	16 tubes	2 strips	20 μL	32 tubes	4 strips	Each tube contains same reagent.
3	**UDI 1-32 Reaction Strip**	White	20 μL	32 tubes	4 strips	20 μL	32 tubes	4 strips	Each tube represents an UDI.
4	**UDI 33-64 Reaction Strip**	White	——	——	——	20 μL	32 tubes	4 strips	Each tube represents an UDI.
5	**RingCap-Taq (1#)**	——	25 μL	1 tube	——	25 μL	2 tubes	——	——
6	**TC Negative Control**	——	250 μL	1 tube	——	250 μL	1 tube	——	——
7	**TC-DNA Positive Control**	——	50 μL	1 tube	——	50 μL	1 tube	——	BRAF, TERT
8	**TC-RNA Positive Control**	——	20 μL	1 tube	——	20 μL	1 tube	——	CCDC6/RET

Note 1: In UDI reaction strips, different UDI numbers respectively contain 64 different UDI recognition sequences (see Appendix table 3).

Note 2: The contents of different batches cannot be mixed.

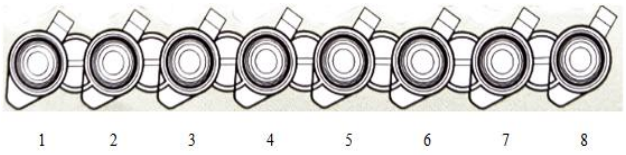

Figure 1. UDI numbers of 8-tube strips

Additional required Equipment and Materials

1. DNA/RNA Isolation Kit: For fine needle puncture (FNA) samples, AllPrep DNA/RNA Mini Kit (QIAGEN, Cat. No.80204); for FFPE or fresh tissue samples, commercial DNA/RNA extraction kits are recommended
2. RNA reverse transcription Kit: SuperScript™ VILO™ cDNA Synthesis Kit (Thermo Fisher, Cat. No. 11754-050)
3. Quantification kit of nucleic acids: QuantiFluor®dsDNA System (Promega, Cat. No. E2670) or Qubit® dsDNA HS Assay Kit (Thermo Fisher Scientific,Cat. No. Q32851/Q32854)
4. Fluorometer: Qubit™ 4 Fluorometer (Thermo Fisher Scientific, Cat. No:Q33238) or Quantus™ Fluorometer (Promega, Cat. No E6150)
5. Magnetic beads: Magnetic beads Kit (Beckman Coulter, Cat. No. A63880/A63881/A63882); SG Pure Bead (Xiamen Spacegen Co., Ltd, Cat. No. SPG-PB001/002)
6. Nuclease-Free Water
7. Magnetic rack
8. Absolute ethanol (Analytical Grade)
9. Sequencing Reagents: Selecting the corresponding sequencing reagent according to the gene sequencer
10. Illumina PhiX Control V3 (Illumina, Cat. No. FC-110-3002)
11. Nuclease-free pipette tips with filter
12. TE Buffer (pH 8.0)

Storage and Stability

1. Storage Condition. Store the kit away from light at -15℃ to -25℃, valid for 12 months. Once opened, reagents can be stored in their original packaging at -15℃ to -25℃ until the stated expiration date shown on the packaging. Repeated thawing and freezing should beavoided. Do not exceed a maximum of 5 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℃.
3. Check labels for the production date and expiration date of the kit.

Applicable Instruments

1. PCR system/ thermal cycler: ABI9700, ABI 2720, ABI Veriti, ABI Mini Amp.
2. Sequencing instruments: Illumina sequencing instruments (Miseq, NextSeq 500/550, Miniseq,etc).

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: fine needle puncture sample or thyroid tissue.
2. Fine needle puncture (FNA)samples: Ensure that at least 3 times of puncture in the thyroid nodule with strict compliance to FNA procedures specification, and was quickly placed in centrifuge tubes containing 100 μL RNA Later and transported in a foam box with ice packs at low temperature. Importantly. Quantify sample DNA with a Fluorometer, the concentration should be ≥ 1 ng/μL, the ratio of OD_{260}/OD_{280} should be within the range of 1.7-2.2, the total amount of DNA should be ≥ 10 ng; Assess the quality of sample RNA with an ultraviolet spectrophotometer, the concentration should be ≥ 2 ng/μL, the ratio of OD_{260}/OD_{280} should be within the range of 1.8-2.3, the total amount of RNA should be ≥ 20 ng.
3. Fresh tissue and FFPE samples: Fresh tissue size should be less than a green bean and the length ≥1 cm, was quickly placed in centrifuge tubes containing 100 μL RNA Later and transported in a foam box with ice packs at low temperature; it is recommended to choose FFPE samples that have not been stored for more than 2 years and at least 20% of the collected pathological tissue were tumor lesions, and use no less than 8 pieces of 5 μm section or 5 pieces of 10 μm section for nucleic acid extraction; quantify sample DNA with a Fluorometer, the concentration should be ≥ 5 ng/μL, the ratio of OD_{260}/OD_{280} should be within the range of 1.7-2.2, the total amount of DNA should

be ≥ 50 ng; Assess the quality of sample RNA with an ultraviolet spectrophotometer, the concentration should be ≥ 5 ng/μL, the ratio of OD_{260}/OD_{280} should be within the range of 1.8-2.3, the total amount of RNA should be ≥ 50 ng.

4. Once the DNA quantity or quality did not conform to the above requirements, re-extract DNA with resampling or a larger sample. Reverse transcript sample RNA to cDNA immediately after RNA is extracted. Proceed to library construction or store the DNA/cDNA at -15℃ to -25℃ for no more than 12 months.

Experimental Procedure

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

I. Library Enrichment

1. Reagent preparation: Unfreeze the **TC-DNA-1 PCR Strip** (Blue), **TC-DNA-2 PCR Strip** (Yellow), **TC-RNA PCR Strip** (Pink) at room temperature, briefly centrifuge the tubes before use; Place the **RingCap-Taq (1#)** on ice after centrifugation.
2. Tested sample preparation:
   Tissue sample: With a Fluorometer, dilute sample DNA to 5 ng/μL with TE Buffer (pH 8.0), and prepare ≥ 10 μL of the diluted sample.
   FNA: If DNA concentration based on Fluorometer > 5 ng/μL diluting sample DNA to 5 ng/μL with TE Buffer (pH 8.0), prepare ≥ 10 μL of the diluted sample; else recommending choice of original nucleic acid to the tested sample.
   cDNA sample: cDNA sample after reverse transcription.
3. Enriching reaction for TC-DNA
   1) Add 0.5 μL of **RingCap-Taq (1#)** to 10 μL of the DNA Sample, TC-DNA positive control and TC negative control, vortex slightly followed by brief centrifugation.
   2) Gently remove the cap of the TC-DNA-1 and TC-DNA-2 PCR strip, sequentially add 5 μL of the template prepared above 1) into the respective tube, and replace the cap carefully.
   3) Centrifuge the tubes slightly to dislodge bubbles.
4. Enriching reaction for TC-RNA
   1) Add 0.25 μL of **RingCap-Taq (1#)** to 5 μL of the cDNA sample, TC-RNA positive control and TC negative control, vortex slightly followed by brief centrifugation.
   2) Gently remove the cap of enriching PCR tubes/ strips, sequentially add 5 μL of the template prepared above into the respective tube, and replace the cap carefully.
   3) Centrifuge the tubes 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℃	2 minutes	1
Denaturation	98℃	15 seconds	15
Annealing	65℃	4 minutes	
Storage	4℃	∞	1

Note: Proceed to "Purification of Enriching Products" or store the products at 2-8℃ within 4 hours or at -15℃ to -25℃ within 24 hours. Storing for more than 24 hours is not suggested.

II. Purification of Enriching Products

Note: Bring magnetic bead to room temperature and vortex thoroughly to disperse magnetic bead before use; prepare fresh 70% ethanol with Nuclease-Free Water.

1. Transfer all PCR product of **TC-DNA-1 PCR Strip** and **TC-DNA-2 PCR Strip** to a new 1.5 mL centrifuge tube, following thoroughly blow and mix , add 50 μL magnetic beads to tube, pipet up and down 5 times to mix magnetic beads suspension thoroughly with the product.
2. Transfer 25 μL of the PCR enrichment product of **TC-RNA PCR Strip** to a new 1.5 mL centrifuge tube, add 25 μL magnetic beads to each tube, pipet up and down 5 times to mix magnetic beads suspension thoroughly with the product.

3. Incubate the mixture for 5 minutes at room temperature.
4. Place the tube on a magnetic rack, then incubate for 2 minutes, carefully remove and discard the supernatant without disturbing magnetic beads.
5. Add 150 µL of freshly prepared 70% ethanol into 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.
6. Repeat step 5 for a second wash.
7. Remove all the ethanol from the tube, and keep the tube on the magnetic rack for 5 minutes to air-dry magnetic beads (avoid over-dry).
8. Remove the tube from the magnetic rack, add 35 µL of TE Buffer (pH 8.0) to each tube, replace the cap, and 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. Incubate the mixture for 5 minutes at room temperature.
9. Place the tube on the magnetic rack for 2 minutes until the solution is clear, carefully remove and store the supernatant (i.e. **purified product**), store at -15℃ to -25℃ or proceed to the next reaction immediately.

III. Library Construction

Note: Use different UDI for different samples (DNA mutation or RNA fusion mutation).

1. Reagent preparation: unfreeze the **UDI Reaction Strip** based on DNA and RNA amount at room temperature**,** briefly centrifuge the tubes before use; place the **RingCap-Taq (1#)** on ice after centrifugation.
2. Construction reaction for TC-DNA
   1) Add 0.25 µL of **RingCap-Taq (1#)** to 5 µL of the purified products of DNA Sample, TC-DNA positive control and TC negative control, vortex slightly followed by brief centrifugation.
   2) Gently remove the cap of the **UDI Reaction Strip**, sequentially add 5 µL of the template prepared above 1) into the respective tube, and replace the cap carefully.
   3) Centrifuge the tubes/ strips slightly to dislodge bubbles.
3. Construction reaction for TC-RNA
   1) Add 0.25 µL of **RingCap-Taq (1#)** to 5 µL of the purified products of cDNA sample, TC-RNA positive control and TC negative control, vortex slightly followed by brief centrifugation.
   2) Gently remove the cap of the **UDI Reaction Strip**, sequentially add 5 µL of the template prepared above 1) into the respective tube, and replace the cap carefully.
   3) Centrifuge the tubes slightly to dislodge bubbles.
4. Load the **UDI Reaction Strip** tubes 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℃	2 minutes	1
Denaturation	98℃	15 seconds	25
Annealing	65℃	4 minutes	
Storage	4℃	∞	1

Note: Proceed to “Library Purification”, or store the products at 2 - 8℃ within 4 hours or at -15℃ to -25℃ within 24 hours. Storing for more than 24 hours is not suggested.

IV. Library Purification

Note: Bring magnetic bead to room temperature and vortex thoroughly to disperse magnetic bead before use; prepare fresh 70% ethanol with Nuclease-Free Water.

1. Transfer 25 µL of the PCR product to a new 1.5 mL centrifuge tube, add 25 µL of magnetic beads to each tube, pipet up and down 5 times 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 magnetic rack, then incubate for 2 minutes, carefully remove and discard the supernatant without disturbing magnetic beads.
4. Add 150 μL of freshly prepared 70% ethanol into 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 4 for a second wash.
6. Remove all the ethanol from the tube, and keep the tube on the magnetic rack for 5 minutes to air-dry 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, replace the cap, and 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; Incubate the mixture for 5 minutes at room temperature.
8. Place the tube on the magnetic rack for 2 minutes until the solution is clear, carefully remove and store the supernatant (i.e. **library**) store at -15℃ to -25℃ 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; TC negative control should not have the fragment in more than 250 bp. For TC DNA positive and all sample DNA libraries, the target fragments should be in 250-350 bp. For TC RNA positive and all sample cDNA libraries, the target fragments should be in 250-300 bp. Fuorometer quantification kit is recommended to measure the concentration of sample library and should be more than 1 ng/μL.
2. According to the library concentration measured by the fluorometer, use the following formula to convert the molar concentration of the library, where the DNA length is calculated as 300 bp, and the RNA length is calculated as 280 bp.

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

3. Per the concentration measured, dilute the sample library to 4 nM with Nuclease-Free Water.
4. The proportion of DNA and cDNA is 4:1 (Mix 20 μL of each DNA sample library with 5 μL of each cDNA sample library). 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. Sample dilution and denaturation according to the matching Illumina sequencing kit (refer to the operation manual of each equipment).
6. Store undiluted sample 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.

VI. Bioinformatics Analysis

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

Data Analysis

1. Result of TC-DNA
   1) Standard of quality: For all sample DNA libraries, the target fragments should be in 250-350 bp as well as On target and Uniformity >80%, moreover, Mean Depth >5000×.
   2) Mutated positive judging criteria: In the result of somatic variation analysis, if effective depth ≥300 and mutation frequency ≥1%, this mutation site is judged as positive mutation. Otherwise, it is judged as negative or below the detection limit.
2. Result of TC-RNA
   1) Judging criteria: For all sample cDNA libraries, the target fragments should be in 250-300 bp, as well as HMBS and TBP genes named housekeeping gene should be all detected with average end-to-end reads ≥1000; thyroid-specific genes named TG, TTF1, TPO and TSHR should be detected as least two genes with average end to end reads ≥200.
   2) Under 1) premise, if the forward and reverse of target regions are all read, and account for the total reads of HMBS and TBP genes ≥ 1%, the gene fusion is judged as positive mutation. Otherwise, it is judged as negative or below the detection limit.

Interpretation of Results

1. TC negative libraries may be ≥0.5 ng/μL, but it should be not any fragment above 250 bp. Otherwise, this test is invalidated.
2. For the DNA positive control library, the target fragment should be in 250-350 bp, as well as On target and Uniformity >80%, moreover,

Mean Depth >5000×. For the RNA positive control library, the target fragments should be in 250-300 bp as well as HMBS and TBP genes named housekeeping gene should be all detected with average end to end reads >1000. Importantly, the gene fusion result of positive control is consistent with information of appendix table 2.

3. 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 and the "Standards and Guidelines for the Interpretation of Next Generation Sequencing Clinical Reports" formulated by the Expert Group for Interpretation of Next-Generation Sequencing Clinical Reports in 2022 could divide into 4 types:
   1) Clear clinical significance(I): Diagnostic\prognostic marker of specific tumor or drugs recommended\approved in the professional guidelines.
   2) Potential clinical significance(II): Diagnostic\prognostic marker of specific tumor or drugs that have level A evidence of other tumors in the multiple small research.
   3) Unknown clinical significance(III): 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(IV): It is found higher rates of variants in the general population and not have published evidence.

Limitation of the Kit

For mutation sites that were not included in the kit or DNA/RNA extracted from samples were a small number or stored in a false situation, the results shall not be interpreted by the instruction.

Performance characteristics

1. The kit should be of neat appearance, have clear labels, and no leakage.
2. When unfrozen, the reagents shall be clear, without sediments.
3. Wide range of test samples, including FNA, fresh tissue, and FFPE.
4. The kit allows the detection of 1% of specific gene mutations in 5 ng DNA of FNA samples or 25 ng DNA of tissue samples.
5. The kit allows the detection of 200 copies/μL of fusion mutations in RNA of FNA or 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. 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 (-15℃ to -25℃) 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.

Symbols

Symbol	Symbol definition
	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 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. Thyroid Professional Committee of Guangdong Medical Education Association, Cytopathology and Molecular Diagnosis Professional Committee of Guangdong Primary Medical Society. Guangdong expert consensus on thyroid cancer gene detection and clinical application.2020.
2. Thyroid Surgeon Committee of the Surgeon Branch of the Chinese Medical Doctor Association. Consensus of Chinese experts on the diagnosis and treatment of medullary thyroid cancer[J]. 2020..
3. 2021 American Thyroid Association Guidelinesfor Management of Patients with Anaplastic Thyroid Cancer.2021
4. Cabanillas ME, Mabel R, Camilo J . Targeted Therapy for Advanced Thyroid Cancer: Kinase Inhibitors and Beyond[J]. Endocrine Reviews, 2019(6):6.
5. Jorge Alsina, Raul Alsina, Seza Gulec, MD, FACS. A Concise Atlas of Thyroid Cancer Next-Generation Sequencing Panel ThyroSeq v.2

[J]. European Thyroid Journal, 2017.

6. NCCN Clinical Practice Guidelines in Oncology:Thyroid Carcinoma, Version 2.2022.
7. Xing M . Genetic-guided Risk Assessment and Management of Thyroid Cancer[J]. Endocrinology & Metabolism Clinics of North America, 2019, 48(1):109-124.
8. 8. Yip L , Sosa JA .Molecµlar-Directed Treatment of Differentiated Thyroid Cancer: Advances in Diagnosis and Treatment[J]. Jama Surg, 2016.
9. National Health Commission of the People's Republic of China. Guidelines for the diagnosis and treatment of thyroid cancer.2022.

EC REP

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

Appendix table 1:

Gene Information Included in the Kit

DNA Gene							
KRAS	NRAS	HRAS	TP53	BRAF	TERT	CTNNB1	SPOP
PIK3CA	RET	EIF1AX	AKT1	TSHR	GNAS	ZNF148	PTEN
RNA Gene Fusion							
AFAP1	AGK	AGTRAP	AKAP13	AKAP9	ALK	BCR	TRIM33
BRAF	C2orf44	CCDC6	CD74	CDC27	CEL	CLCN6	VCL
CLIP4	CLTC	CUX1	EML4	ERC1	ESRP1	ETV6	ZKSCAN5
FAM131B	FCHSD1	FKBP15	FN1	GNAI1	GOLGA5	HIP1	TRIM27
HOOK3	IGF2BP3	KCTD7	KIAA1468	KIAA1549	KIF5B	KLC1	TRIM24
KTN1	MACF1	MAD1L1	MAP4K3	MET	MIR548F1	MKRN1	TPR
MPRIP	MSN	MYH9	NCOA1	NCOA4	NFASC	NPM1	TPM4
NTRK1	NTRK2	NTRK3	NUDCD3	PPARG	PAPSS1	PAX8	TPM3
PCM1	PLIN3	POR	PPARΓ	PPFIBP1	PRKAR1A	RAF1	TPM1
RET	RNF130	RNF213	SLC45A3	SND1	SOX6	SQSTM1	THADA
SRGAP3	SSBP2	STRN	STRN3	TAX1BP1	TBL1XR1	TFG	

Appendix table 2:

Information of Positive Control Mutations

Positive Control	Gene	Base Mutation	Amino Acid Mutation	Cosmic ID	Mutation Type
TC DNA-positive control	BRAF	c.1799T>A	p.V600E	476	SNV
	TERT	c.1-124C>T	—	—	SNV
TC RNA-positive control	RET	CCDC6/RET	—	1271	Gene fusion

Appendix table 3:

Information of 64 UDI Recognition Sequences based on Illumina Tech

Number	i7 Sequence	i5 Sequence for NovaSeq, MiSeq	i5 Sequence for iSeq, MiniSeq, NextSeq
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
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
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
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
UDI-33	GCAGTGCC	CGACGAAC	GTTCGTCG
UDI-34	TTAACGAC	CGTCTCAA	TTGAGACG
UDI-35	CATTACTT	AACGCTGT	ACAGCGTT
UDI-36	AGCACTGG	GCAAGTCG	CGACTTGC
UDI-37	TTGCGATA	TAGTAGTT	AACTACTA
UDI-38	AACTGCGT	ATCGTCGG	CCGACGAT
UDI-39	CCGGATCA	GTGACACA	TGTGTCAC
UDI-40	GGTCTAAG	TCTTAGTC	GACTAAGA
UDI-41	CAATCTGT	GACTAAGG	CCTTAGTC
UDI-42	GGCCAATT	CGTGATCG	CGATCACG
UDI-43	TTGATGCG	ACACTGAT	ATCAGTGT

UDI-44	ACTGTCAC	TAGAGCTA	TAGCTCTA
UDI-45	TCAAGTTA	ATCACTTC	GAAGTGAT
UDI-46	CGGTCGCG	CGATCACA	TGTGATCG
UDI-47	GATGACAC	GTGCGCGT	ACGCGCAC
UDI-48	ATCCGAGA	TCTGTGAC	GTCACAGA
UDI-49	GCACTTAC	GATTAACC	GGTTAATC
UDI-50	CGCAGTAT	ACAATGGC	GCCATTGT
UDI-51	AAGGACTG	GGTCGTCT	AGACGACC
UDI-52	CTTACGGA	CTCGGCAA	TTGCCGAG
UDI-53	TAATGACT	TAGCCTTG	CAAGGCTA
UDI-54	ATCCAACG	ACGAACGT	ACGTTCGT
UDI-55	GCGTCCGA	CTCGTATG	CATACGAG
UDI-56	TGTGTGTC	TGATCGAA	TTCGATCA
UDI-57	CACAGAGC	AGTACAGC	GCTGTACT
UDI-58	TAACGTCA	GATGTGTA	TACACATC
UDI-59	ACTGCGTT	CCATGTAT	ATACATGG
UDI-60	CGGTTCAG	TTCCAGCG	CGCTGGAA
UDI-61	GTTCAGCT	ACGCGCTG	CAGCGCGT
UDI-62	GTATATAG	CGAACTCA	TGAGTTCG
UDI-63	ACGACCGA	GTCGTAGT	ACTACGAC
UDI-64	TGCGTATC	TAGTACAC	GTGTACTA