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	**Index 1-32 reaction strip**	White	20 μL	32 tubes	4 strips	20 μL	32 tubes	4 strips	Each tube represents an index.
4	**Index 33-64 reaction strip**	White	——	——	——	20 μL	32 tubes	4 strips	Each tube represents an index.
5	**RingCap-Taq (1#)**	——	20 μL	1 tube	——	20 μL	2 tubes	——	——
6	**TC Negative Control**	——	500μL	1 tube	——	500μL	1 tube	——	——
7	**TC-DNA Positive Control**	——	50 μL	1 tube	——	50 μL	1 tube	——	BRAF，TERT
8	**TC-RNA Positive Control**	——	50 μL	1 tube	——	50 μL	1 tube	——	CCDC6/RET

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

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

Equipment and Reagents Required

1. DNA/RNA Isolation Kit: QIAGEN AllPrep DNA/RNA Mini Kit, Cat. No.80204;
2. RNA reverse transcription Kit: Thermo fisher Super Script® VILO™ cDNA Synthesis Kit, Cat. No. 11754-050;
3. Quantification kit of nucleic acids: Promega QuantiFluor®ds DNA System, Cat. No. E2670, 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 Magnetic beads Kit from Beckman Coulter, product number A63880/A63881/A63882; Xiamen Spacegen Co., Ltd SGpure beads, Cat. No. SPG-PB001;
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 pipettes and tips;
12. TE buffer solution (pH 8.0).

Storage and Stability

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 Instruments

1. PCR system/ thermal cycler: ABI9700, ABI 2720, ABI Veriti, ABI Mini Amp.
2. Sequencing instruments: Illumina sequencing platforms.

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 of RNA Later and transported in a foam box with ice packs at low temperature. Importantly, Nucleic acid extraction for DNA/RNA Isolation Kit is recommended in Equipment and Reagents Required. Quantify sample DNA with a Fluorometer, the concentration should be ≥ 1 ng/μL, 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 - 2.2, 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 of RNA Later and transported in a foam box with ice packs at low temperature; Recommend choice of FFPE samples which have not been stored for more than 2 years and at least 30% of the collected pathological tissue were tumor lesions; Extract DNA or RNA with at 5-10 slices of 5 - 10 μm section; Quantify sample DNA with a Fluorometer, the concentration should be ≥ 5 ng/μL, 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 - 2.2, 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 - 20±5°C for no more than 12 months.

Experimental Procedure

Note: Parallel library construction of TC Positive Control (**PC**) and TC Negative Control (**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 solution (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 solution (pH 8.0), prepare ≥ 10 μL of the diluted sample; else recommending choice of original nucleic acid to the tested sample.

   RNA sample: cDNA sample after reverse transcription;
3. Enriching reaction for TC-DNA
   a) 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;
   b) Gently remove the cap of the TC-DNA-1and TC-DNA-2 PCR strip, sequentially add 5 μL of the template prepared above a) into the respective tube, and replace the cap carefully;
   c) Centrifuge the tubes/ strips slightly to dislodge bubbles;
4. Enriching reaction for TC-RNA
   a) Add 0.25 μL of RingCap-Taq (1#) to 5 μL of the "RNA Sample", TC-RNA positive control and TC negative control, vortex slightly followed by brief centrifugation;
   b) 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;
   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	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.

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 and TC-DNA-2 PCR strip to a new 1.5 mL Eppendorf 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 Eppendorf 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 tube side-to-side in the two positions of the magnetic rack 5 times to wash magnetic beads, place the tube on the magnetic rack for 2 minutes, 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 solution (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 - 20±5°C or proceed to the next reaction immediately

III. Library Construction

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

1. Reagent preparation: unfreeze the **Index 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
   a) 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;
   b) Gently remove the cap of the Index reaction strip, sequentially add 5 μL of the template prepared above a) into the respective tube, and replace the cap carefully;
   c) Centrifuge the tubes/ strips slightly to dislodge bubbles;
3. Construction reaction for TC-RNA
   a) Add 0.25 μL of RingCap-Taq (1#) to 5 μL of the purified products of "RNA Sample", TC-RNA positive control and TC negative control, vortex slightly followed by brief centrifugation;
   b) Gently remove the cap of the Index reaction strip, sequentially add 5 μL of the template prepared above a) into the respective tube, and 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	25
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.

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 Eppendorf 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 tube side-to-side in the two positions of the magnetic rack for 5 times to wash magnetic beads, place the tube on the magnetic rack for 2 minutes, 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 solution (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 - 20±5°C 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 RNA 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 RNA is 4:1(Mix 20 μL of each DNA sample library with 5 μL of each RNA 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 - 20±5°C 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 high-throughput sequencing data analysis system (abbreviated as analysis system below) of Xiamen Spacegen Co., Ltd.

Positive Judgment Value

1. Judgment result of TC-DNA
   a) Standard of quality: For all sample DNA libraries, the target fragments should be in 250 ~ 350 bp as well as Ontarget Ratio and Uniformity should be more than 80%, moreover, Mean Depth more than 5000;
   b) 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. Judgment result of TC-RNA
   a) Judging criteria: For all sample RNA 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 of more than 1000; thyroid-specific genes named TG, TTF1, TPO and TSHR should be detected as least two genes with average end to end reads ≥ 200;
   b) Under a) premise, if the forward and reverse of target regions are all read, moreover, the total end-to-end reads of HMBS and TBP ≥ 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 in 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 Ontarget Ratio and Uniformity should be more than 80%, moreover, Mean Depth more than 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 more than 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:

a) Clear clinical significance(I): Diagnostic\prognostic marker of specific tumor or drugs recommended\approved in the professional guidelines;
b) 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;
c) 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;
d) 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 of Products

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.

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 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 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 IlldDx Recognition Sequences based on Illumina Tech

Number	I7 sequence	I5 sequence	Number	I7 sequence	I5 sequence
Index-1	TAAGGCGA	CTCTCTAT	Index-33	TAAGGCGA	AAGGAGTA
Index-2	CGTACTAG	TATCCTCT	Index-34	CGTACTAG	CTAAGCCT
Index-3	AGGCAGAA	GTAAGGAG	Index-35	AGGCAGAA	CGTCTAAT
Index-4	TCCTGAGC	ACTGCATA	Index-36	TCCTGAGC	TCTCTCCG
Index-5	GGACTCCT	AAGGAGTA	Index-37	GGACTCCT	CTCTCTAT
Index-6	TAGGCATG	CTAAGCCT	Index-38	TAGGCATG	TATCCTCT
Index-7	CTCTCTAC	CGTCTAAT	Index-39	CTCTCTAC	GTAAGGAG
Index-8	CGAGGCTG	TCTCTCCG	Index-40	CGAGGCTG	ACTGCATA
Index-9	TAAGGCGA	TATCCTCT	Index-41	TAAGGCGA	CTAAGCCT
Index-10	CGTACTAG	GTAAGGAG	Index-42	CGTACTAG	CGTCTAAT
Index-11	AGGCAGAA	ACTGCATA	Index-43	AGGCAGAA	TCTCTCCG
Index-12	TCCTGAGC	AAGGAGTA	Index-44	TCCTGAGC	CTCTCTAT
Index-13	GGACTCCT	CTAAGCCT	Index-45	GGACTCCT	TATCCTCT
Index-14	TAGGCATG	CGTCTAAT	Index-46	TAGGCATG	GTAAGGAG
Index-15	CTCTCTAC	TCTCTCCG	Index-47	CTCTCTAC	ACTGCATA
Index-16	CGAGGCTG	CTCTCTAT	Index-48	CGAGGCTG	AAGGAGTA
Index-17	TAAGGCGA	GTAAGGAG	Index-49	TAAGGCGA	CGTCTAAT
Index-18	CGTACTAG	ACTGCATA	Index-50	CGTACTAG	TCTCTCCG
Index-19	AGGCAGAA	AAGGAGTA	Index-51	AGGCAGAA	CTCTCTAT
Index-20	TCCTGAGC	CTAAGCCT	Index-52	TCCTGAGC	TATCCTCT
Index-21	GGACTCCT	CGTCTAAT	Index-53	GGACTCCT	GTAAGGAG
Index-22	TAGGCATG	TCTCTCCG	Index-54	TAGGCATG	ACTGCATA
Index-23	CTCTCTAC	CTCTCTAT	Index-55	CTCTCTAC	AAGGAGTA
Index-24	CGAGGCTG	TATCCTCT	Index-56	CGAGGCTG	CTAAGCCT
Index-25	TAAGGCGA	ACTGCATA	Index-57	TAAGGCGA	TCTCTCCG
Index-26	CGTACTAG	AAGGAGTA	Index-58	CGTACTAG	CTCTCTAT
Index-27	AGGCAGAA	CTAAGCCT	Index-59	AGGCAGAA	TATCCTCT
Index-28	TCCTGAGC	CGTCTAAT	Index-60	TCCTGAGC	GTAAGGAG
Index-29	GGACTCCT	TCTCTCCG	Index-61	GGACTCCT	ACTGCATA
Index-30	TAGGCATG	CTCTCTAT	Index-62	TAGGCATG	AAGGAGTA
Index-31	CTCTCTAC	TATCCTCT	Index-63	CTCTCTAC	CTAAGCCT
Index-32	CGAGGCTG	GTAAGGAG	Index-64	CGAGGCTG	CGTCTAAT