RingCap®

Human FGFR1/2/3 Gene Fusions Detection Kit

High-Throughput Sequencing

Instruction for Use

Product Name

Human FGFR1/2/3 Gene Fusions Detection Kit (High-Throughput Sequencing)

Packing Specification

16 Tests/Kit, 32 Tests/Kit

Intended Use

The kit is intended for the detection of FGFR1/2/3 gene fusions with FFPE pathological tissue samples from patients with cholangiocarcinoma (appendix table 1). The test results could evaluate cholangiocarcinoma patients molecular characteristics for scientific reference.

Cholangiocarcinoma (CCA) is an epithelial cell malignancy originating from biliary epithelial cells, spreading throughout the capillary and bile ducts. Multidisciplinary management currently is the main treatment including surgical resection, radiation therapy, and chemotherapy for cholangiocarcinoma patients, etc. The only eradication therapy for intrahepatic cholangiocarcinoma (ICC)continues to be surgery, in the form of liver transplantation. If the best surgical opportunity is missed, radiation, chemotherapy or targeted therapy are usually used. Cisplatin/gemcitabine combination chemotherapy has emerged as the standard treatment regimen, but the effect still needs to be improved, and are more prone to drug resistance. With the development of high-throughput techniques, more and more aberrant genes have been discovered, which shed new light on drug therapy and development.

The full names of the FGFR are Fibroblast Growth Factor Receptor that is part of the transmembrane receptor tyrosine kinases signaling pathways leading to cell proliferation, and differentiation, including FGFR1, FGFR2, FGFR3, and FGFR4. Several targeted drugs have positive developments and already entered clinical and approval stages, such as Pemigatinib approved by the FDA on April 17, 2020, which is a selective inhibitor for FGFR1/2/3 of mTOR and the response rate may be as high as 35.5%. Therefore, detecting novel gene fusions in patients with cholangiocarcinoma to guide targeted therapy could offer survival benefits.

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 the different sequencing principles. NGS enables the sequencing of up to millions of target nucleic acids at once, and provides abundant variation information in a short time and at a relatively low cost. Highlighting the characteristics of high output and high resolution, NGS has drawn more and more attention in multiple signaling pathways and targets studies of cancer.

The construction of the sample library relies on specific modified primers and RingCap® mediated amplification technology with the employment of PCR apparatus could detect FGFR gene fusions in nucleic acid. 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 a particular PCR program and Ring-Cap Taq 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	**FGFR enriching PCR strip**	Primer, dNTPs, Mg^{2+}, buffer	Pink	20 μL	16 tubes	2 strips	20 μL	32 tubes	4 strips	Each tube contains same reagent.
2	**Index 1-8 ligation reaction strip**	Barcode, dNTPs, Mg^{2+}, buffer	Purple	20 μL	8 tubes	1 strip	20 μL	8 tubes	1 strip	Each tube represents an Index.

3	**Index 9-16 ligation reaction strip**	Barcode, dNTPs, Mg^{2+}, buffer	Green	20 μL	8 tubes	1 strip	20 μL	8 tubes	1 strip	Each tube represents an Index.
4	**Index 17-24 ligation reaction strip**	Barcode, dNTPs, Mg^{2+}, buffer	White	20 μL	8 tubes	——	20 μL	8 tubes	1 strip	Each tube represents an Index.
5	**Index 25-32 ligation reaction strip**	Barcode, dNTPs, Mg^{2+}, buffer	Yellow	20 μL	8 tubes	——	20 μL	8 tubes	1 strip	Each tube represents an Index.
6	**RingCap-Taq (1#)**	Taq enzyme	——	12 μL	1 tube	——	25 μL	1 tube	——	——
7	**FGFR Negative Control**	Purified water	——	20 μL	1 tube	——	20 μL	1 tube	——	——
8	**FGFR Positive Control**	Wild-type cDNA	——	20 μL	1 tube	——	20 μL	1 tube	——	——

Note: In index reaction strips, different index numbers respectively contain 32 different IlIDx recognition sequences (see Appendix Table 2); 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 index 1, 2, 3, 4, 5, 6, 7, 8

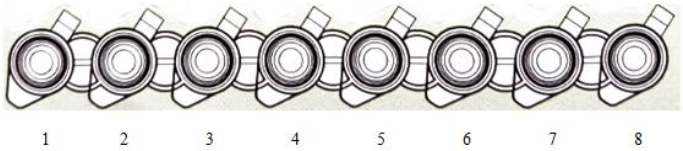

Figure 1. Index numbers of 8-tube strips

Note: The components of different batches of reagents cannot be mixed.

Equipment and Reagents Required

1. Nucleic acid extraction kit;
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/Q3285;
4. Quantification kit of nucleic acids: It is recommended to use the Qubit™ 4.0 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. Sequencing Reagents: Selecting the corresponding sequencing reagent according to the gene sequencer;
7. Nuclease-free pipettes and tips;
8. TE buffer (pH 8.0);
9. Illumina PhiX Control v3 (Illumina), Cat. No. FC-110-3002;
10. Nuclease-free water;
11. Anhydrous ethanol (Analytical Grade).

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 under 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 MiniAmp;
2. Sequencing instruments: Illumina Sequencer.

Specimen Material

1. Recommended sample types: FFPE pathological tissue;
2. FFPE samples: ensure that at least 20% of the collected pathological tissue is tumor lesions; choose FFPE samples that have not been stored for more than 2 years; extract RNA with at least 8 slices of 5 μm section or at least 5 slices of 10 μm section and assess the quality of sample RNA with an ultraviolet spectrophotometer, the ratio of OD_{260}/OD_{280} should be within the range of 1.8 - 2.2;
3. Reverse transcript sample RNA to cDNA immediately after RNA is extracted. Proceed to library construction or store the cDNA at -20±5°C for no more than 12 months.

Experimental Procedure

Note: Parallel analysis of FGFR Positive Control (PC) and FGFR Negative Control (NTC) with tested sample is suggested.

I. Library Enrichment

1. Reagent preparation: Unfreeze the **FGFR enriching PCR strip (pink)** at room temperature according to the total number of samples, briefly centrifuge the tubes before use; Place the **RingCap-Taq (1#)** on ice after centrifugation;
2. RNA sample preparation: cDNA sample after reverse transcription by RNA;
3. Enriching PCR reaction:
    a) Add 0.25 μL **RingCap-Taq (1#)** to 5 μL of the cDNA Sample, PC and NTC, vortex slightly followed by a brief centrifugation;
    b) Gently remove the cap of enriching PCR strips, sequentially add 5 μL of the template prepared above into the respective tube, and replace the cap carefully;
    c) Centrifuge the strips slightly to dislodge bubbles;
4. Load the PCR reaction strips into the PCR instrument, set up and perform the amplification procedure according to Table 2.

Table 2. PCR amplification procedure

Phase	Temperature	Time	Cyclic number
Predenaturation	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 8 hours or at - 20±5°C within 24 hours. Storing for more than 24 hours is not suggested.

II. Purification of Enriching Products

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

1. Transfer 25 μL of the PCR product to a new 1.5 mL eppendorf tube, add 25 μL of magnetic beads to each tube, and 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 (pH 8.0) to each tube, and vortex thoroughly (alternatively, mix by pipetting at least half the total volume up and down at least 5 times), briefly centrifuge to collect the droplets; Incubate the mixture for 5 minutes at room temperature.

8. Place the tube on a magnetic rack for 2 minutes until the solution is clear, carefully remove and store the supernatant (i.e. **purified product**) at - 20±5°C or proceed to "Library Construction".

III. Library Construction

Note: Using different Indexs for different samples is suggested.

1. Reagent preparation: Unfreeze the **Index ligation reaction tubes/ strips** at room temperature, briefly centrifuge the tubes before use; Place the **RingCap-Taq (1#)** on ice after centrifugation;
2. FGFR library construction:
    a) Add 0.25 μL **RingCap-Taq (1#)** to 5 μL of the purified products of cDNA samples, FGFR- PC and FGFR-NTC, vortex slightly followed by brief centrifugation;
    b) Gently remove the cap of **Index ligation reaction strips**, sequentially add 5 μL of the template prepared above into the respective tube, and replace the cap carefully;
    c) Centrifuge the strips slightly to dislodge bubbles;
3. Load the PCR reaction tubes/ strips into the PCR instrument, Set up and perform the amplification procedure according to Table 3.

Table 3. PCR amplification procedure

Phase	Temperature	Time	Cyclic number
Predenaturation	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 8 hours or at - 20±5°C within 24 hours. Storing for more than 24 hours is not suggested.

IV. Library Purification

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

1. Transfer 25 μL of the PCR product to a new 1.5 mL eppendorf tube, add 25 μL of magnetic beads to each tube, and 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 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 (pH 8.0) to each tube, and vortex thoroughly (alternatively, mix by pipetting at least half the total volume up and down at least 5 times), briefly centrifuge to collect the droplets; Incubate the mixture for 5 minutes at room temperature;
8. Place the tube on a magnetic rack for 2 minutes until the solution is clear, carefully remove and store the supernatant (i.e. library) at - 20±5°C or proceed to "Library Quantification and Dilution".

V. Library Quantification, Dilution and Storage

1. Quality control of the library: Bioanalyzer is recommended for the quality control of library fragments. For the NTC library, no fragments shall be detected. For PC and all sample libraries, the target fragments should be around 200 ~ 300 bp. The concentration of

fluorometer ≥ 0.5 ng/μL can meet the requirements;

2. Per the concentration measured, dilute the sample library to 4 nM with Nuclease-free water; the concentration ratio of PhiX Control V3 is at least 5% (e.g.the percentage of Phix Control V3 shoule be more than 30 μL in the 600 μL loading volume);
3. Dilute and denature the sample according to the matching Illumina sequencing kit (for details, refer to the operation steps of the instrument and matching reagents).
4. 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. Sequencing

Library sequencing was carried out according to the procedure of instrument and matching reagent.

VII. Bioinformatics Analysis

Transfer the Fastq 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 Judgment Value

1. Judging criteria: all 6 internal control genes(GUSB、LMNA、LRP1、MYC、PUM1and SNRPD3) forward and reverse of target regions are all read, and the average total reads ≥1000;
2. Under 1 premise, if the forward and reverse of target regions are all read which account for the total six internal control genes should be ≥0.5%, the gene fusion is judged as positive mutation; if the forward and reverse of target regions are all read, and the 0.01%≤ratio≤0.5%, the gene fusion is judged as positive mutation exclude contamination. 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 NTC should be no fragments detected above 200bp; on a bioanalyzer otherwise, the current experiment result is invalid;
2. Target fragments of PC libraries should be 200 ~ 350 bp, otherwise, the current experiment result is invalid; furthermore, for PC, the homogeneity of the positive control should be ≥ 80%, and the sequencing depth should be ≥ 2000 ×;
3. In the "Coverage Analysis" result of FGFR fusion gene mutation analysis of the tested sample, if the number of reads recorded in the alignment data of 6 internal control genes average total reads < 1000, the RNA fusion gene detection result is invalid.

Limitation of the Kit

Testing results obtained from the kit should only be taken as a scientific reference. For sites not included in the kit, or FFPE samples that were more than storage age, the results shall not be interpreted by the instruction.

Physical Performance of Products

1. The kit should be of neat appearance, clear markings, and no leakage. When unfrozen, the reagents shall be clear, without sediment.
2. The consistency rates of positive reference samples are 100%.
3. The consistency rates of negative reference samples are 100%.
4. The kit allows the detection of 200 copies/μL, of gene fusions in RNA samples.
5. The repeatability is 100% by detecting the designated sample for 10 consecutive times, and all of those tested were corresponding gene fusion-positive.

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 RNA, 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 RNA matters experimental results to a great extent, hence, purification of extracted DNA with magnetic beads is highly suggeste; 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 nucleic acid; Use specific pipettes and tips for reagents preparation and template addition.
11. All reagents in use have potential hazard. 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 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.

References

1. Abou-Alfa G K, Sahai V, Hollebecque A, et al. Pemigatinib for previously treated, locally advanced or metastatic cholangiocarcinoma: a multicentre, open-label, phase 2 study[J]. The Lancet Oncology, 2020.
2. Javle M, Lowery M, Shroff R T, et al. Phase II study of BGJ398 in patients with FGFR-altered advanced cholangiocarcinoma[J]. Journal of Clinical Oncology, 2018, 36(3): 276.
3. Shiao M S, Chiablaem K, Charoensawan V, et al. Emergence of intrahepatic cholangiocarcinoma: how high-throughput technologies expedite the solutions for a rare cancer type[J]. Frontiers in genetics, 2018, 9: 309.
4. Banales J M, Cardinale V, Carpino G, et al. Cholangiocarcinoma: current knowledge and future perspectives consensus statement from the European Network for the Study of Cholangiocarcinoma (ENS-CCA)[J]. Nature Reviews Gastroenterology & Hepatology, 2016, 13(5): 261-280.
5. Krook M A, Lenyo A, Wilberding M, et al. Efficacy of FGFR inhibitors and combination therapies for acquired resistance in FGFR2-fusion cholangiocarcinoma[J]. Molecular Cancer Therapeutics, 2020, 19(3): 847-857.

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

The information of FGFR1/2/3 Gene fusions

Gene	Gene fusion	Gene	Gene fusion
FGFR1	BAG4-FGFR1.B2F6.1	FGFR3	FGFR3-TACC3.F17intron17T4.1
	TRIM24-FGFR1.T11F10.1		ETV6-FGFR3.E5F9.1
	FN1-FGFR1.F22F4		FGFR3-TACC3.F18T9
	FN1-FGFR1.F25F4		FGFR3-TACC3.F18T11del5.1
	SQSTM1-FGFR1.S6F10.1		FGFR3-TACC3.F17T8.COSF1353
	CUX1-FGFR1.C11F10.1		FGFR3-TACC3.F17Intron17T9.1
	FN1-FGFR1.F23F4.1		FGFR3-TACC3.F18T7.NGS
	BCR-FGFR1.B4F10.1		FGFR3-TACC3.F17T10.COSF1434
	RANBP2-FGFR1.R20F10int9.1		FGFR3-TACC3.F17T11.COSF1348.1
	WHSC1L1-FGFR1.W14F5.1		FGFR3-TACC3.F18T4and5.1
	BAG4-FGFR1.B1F8.1		FGFR3-TACC3.F17ins1T10
	FN1-FGFR1.F22F3		FGFR3-TACC3.F17T9.2
	FN1-FGFR1.F23F3.1		FGFR3-TACC3.F18T1.1
	FN1-FGFR1.F27F5		FGFR3-TACC3.F17T14
	LRRFIP1-FGFR1.L8F10.1		FGFR3-TACC3.F17T11.2
	TPR-FGFR1.T22F10.1		FGFR3-TACC3.F17Int6ins36T7del4.1
	BAG4-FGFR1.B1F2		FGFR3-TACC3.F17T13.NGS
	FGFR1-PLAG1.F2P2.COSF1111		FGFR3-TACC3.F18T10.2
	FGFR1-PLAG1.F2P3.COSF1113		FGFR3-TACC3.F17T14.1
	FGFR1-ZNF703.F14Z2.COSF720		FGFR3-TACC3.F18Int136T11.1
	FGFR1-PLAG1.F1P2.COSF1108		FGFR3-TACC3.F17Int9T11.1
	FGFR1-PLAG1.F1P3.COSF1110		FGFR3-TACC3.F17T11.1
	CNTRL-FGFR1.C40F10		FGFR3-TACC3.F17T6
	FGFR1OP-FGFR1.F5F10		FGFR3-TACC3.F18T10.1
	FGFR1OP-FGFR1.F6F10		FGFR3-BAIAP2L1.F17B2.COSF1346
	FGFR1OP-FGFR1.F7F10		FGFR3-TACC3.F17T4.1
	MYO18A-FGFR1.M33F10		FGFR3-TACC3.F17T9.1
	CPSF6-FGFR1.C7F9		FGFR3-TACC3.F18T8.1
	CPSF6-FGFR1.C8F9		FGFR3-TACC3.F17T4
FGFR2	APIP-FGFR2.A6F10		FGFR3-TACC3.F18T8
	FGFR2-BICC1.F17B18.1		FGFR3-TACC3.F15T11
	COL25A1-FGFR2.C3F3		FGFR3-TACC3.F16T10.COSF1359
	SLC45A3-FGFR2.S1F2		FGFR3-TACC3.F16T11.COSF1348
	SLC45A3-FGFR2.S1F1		FGFR3-TACC3.F17T5
	FGFR2-BICC1.F17B2.1		FGFR3-TACC3.F17T9
	FGFR2-BICC1.F17B16		FGFR3-TACC3.F18T8.2
	APIP-FGFR2.A1F10		
	APIP-FGFR2.A1F6		
	FGFR2-TACC3.F17T11.1		
	FGFR2-BICC1.F17B3.2		
	FGFR2-AHCYL1.F17A5		
	FGFR2-AHCYL1.F17A2		
	FGFR2-AFF3.F17A8		
	FGFR2-CASP7.F17C2		
	FGFR2-CIT.F17C23		
	FGFR2-KIAA1967_CCAR2.F17C4		
	FGFR2-OFD1.F17O3		

Appendix table 2:

Information of 32 Index Recognition Sequences based on Illumina

Strip Color	Index Number	i7 Sequence	i5 Sequence
Purple	Index_001	TAAGGCGA	CTCTCTAT
	Index_002	CGTACTAG	TATCCTCT
	Index_003	AGGCAGAA	GTAAGGAG
	Index_004	TCCTGAGC	ACTGCATA
	Index_005	GGACTCCT	AAGGAGTA
	Index_006	TAGGCATG	CTAAGCCT
	Index_007	CTCTCTAC	CGTCTAAT
	Index_008	CGAGGCTG	TCTCTCCG
Green	Index_009	TAAGGCGA	TATCCTCT
	Index_010	CGTACTAG	GTAAGGAG
	Index_011	AGGCAGAA	ACTGCATA
	Index_012	TCCTGAGC	AAGGAGTA
	Index_013	GGACTCCT	CTAAGCCT
	Index_014	TAGGCATG	CGTCTAAT
	Index_015	CTCTCTAC	TCTCTCCG
	Index_016	CGAGGCTG	CTCTCTAT

Strip Color	Index Number	i7 Sequence	i5 Sequence
White	Index_017	TAAGGCGA	GTAAGGAG
	Index_018	CGTACTAG	ACTGCATA
	Index_019	AGGCAGAA	AAGGAGTA
	Index_020	TCCTGAGC	CTAAGCCT
	Index_021	GGACTCCT	CGTCTAAT
	Index_022	TAGGCATG	TCTCTCCG
	Index_023	CTCTCTAC	CTCTCTAT
	Index_024	CGAGGCTG	TATCCTCT
Yellow	Index_025	TAAGGCGA	ACTGCATA
	Index_026	CGTACTAG	AAGGAGTA
	Index_027	AGGCAGAA	CTAAGCCT
	Index_028	TCCTGAGC	CGTCTAAT
	Index_029	GGACTCCT	TCTCTCCG
	Index_030	TAGGCATG	CTCTCTAT
	Index_031	CTCTCTAC	TATCCTCT
	Index_032	CGAGGCTG	GTAAGGAG