AmoyDx® Pan Lung Cancer Fusion PCR Panel

Instructions for Use

For Research Use Only. Not for use in diagnostic procedures.

REF	8.01.0333	24 tests/kit	For QuantStudio5, LightCycler480 II, cobas z 480, Bio-Rad CFX96, SLAN-96S

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Background

Lung cancer is one of the most common malignant tumor, and 80-85% of lung cancers are non-small cell lung cancer (NSCLC). There are many driver mutations in NSCLC. The frequency of mutations in NSCLC for *EGFR*, *HER2, KRAS* and *BRAF* genes are respectively 10-50% [1], 1-4% [2-3], 5-25% [4-6] and 1-2% [7-8]. About 3-7% [9-12], 1% [13-14], 1% [13, 15-17], 0.12% [18], 0.02% [18], 0.08% [18] of NSCLC patients have gene fusions in *ALK*, *ROS1*, *RET*, *NTRK1*, *NTRK2* and *NTRK3* genes, and approximately 1% of lung adenocarcinoma patients harbor *MET* exon 14 skipping mutations [19]. Targeted therapies have been developed and approved for use in patients whose tumors have some of the genomic alterations seen in NSCLC. For instance, there are approved *EGFR* inhibitors [20-21], *ALK* inhibitors [22-23], *ROS1* inhibitors [24-25], *NTRK* inhibitors [25-27] and *BRAF* inhibitors [7, 28] for patients with specific genomic alterations in these genes. Testing for genomic alterations is a requirement in order to identify patients that may benefit from these targeted therapies and testing of multiple genomic alterations is recommended by the NCCN guidelines [29]. Furthermore, there are many drugs in late stage development for other alterations (*RET* [30], *MET* [31], *HER2* [32], and *KRAS* [33]).

Intended Use

The **AmoyDx® Pan Lung Cancer Fusion PCR Panel** is a real-time PCR assay for qualitative detection of **82** hotspot alterations in *ALK (21)*, *ROS1(13)*, *RET (16)*, *MET exon14 skipping (1)*, *NTRK1 (18)*, *NTRK2 (5)* and *NTRK3 (8)* genes. The kit is intended to be used to aid clinicians to identify multi-gene status for NSCLC patients.

This kit is intended for research use only and must be operated by trained professionals in a controlled laboratory environment. The kit is compatible with QuantStudio5, LightCycler480 II, cobas z 480, Bio-Rad CFX96 and SLAN-96S platforms for testing and data collection. Results can be analyzed by ARAS, a specialized tool designed to assist in the interpretation of the AmoyDx® Pan Lung Cancer Fusion PCR Panel results.

Principles of the Procedure

This kit contains RNA fusion detection system in LEG Reaction Mix A1-A8. The RNA fusion detection includes two processes:

1) Reverse Transcription: extracted RNA from FFPE or fresh tumor tissue is employed in this step, reverse transcription of target RNA enables complementary DNA (cDNA) synthesis with the action of reverse transcriptase and specific primers.

2) PCR Amplification: the specific primers are designed for amplification of cDNA, and *ALK*, *ROS1*, *RET*, *MET*, *NTRK1*, *NTRK2* and *NTRK3* variant amplicon are detected by fluorescent probes.

The kit contains LEG Reaction Mix A1-A8, LEG RT Reaction Mix, sufficient positive control and enzyme.

1) **LEG Reaction Mix A1-A8** are designed for RNA fusion detection and internal control detection. The LEG Reaction Mixes A1-A8 include primers and FAM-labeled probes specific for detection of *ALK*/*NTRK1*/*NTRK2*/*NTRK3*/*ROS1*/*RET* gene fusions and *MET* exon14 skipping mutation, and the LEG Reaction Mixes A4/A8 also contain primers and VIC-labeled probe for detection of housekeeping gene *HPRT1* as reference gene to assess the RNA quality.
2) The **LEG RT Reaction Mix I** contain primers specific for reverse transcription of mRNA of *ALK*, *NTRK1*, *NTRK2*, *NTRK3* gene and reference gene into cDNA.

3) The **LEG RT Reaction Mix II** contain primers specific for reverse transcription of mRNA of *ROS1*, *RET*, *MET* gene and reference gene into cDNA.

4) The **LEG Reverse Transcriptase** is for reverse transcription of mRNA of target genes and reference gene into cDNA.

5) The **LEG Enzyme Mix A** contains the Taq DNA polymerase for PCR amplification and uracil-N-glycosylase which works at room temperature to prevent PCR amplicon carryover contamination.

6) The **LEG Positive Control** contains recombinant genes with *ALK*, *ROS1*, *RET*, *MET*, *NTRK1*, *NTRK2* and *NTRK3* alternations.

Kit Contents

This kit contains the following materials:

Table 1 Kit Contents

Tube No.	Reagent	Target to detect	Quantity	Florescence Signal
①	LEG Reaction Mix A1	ALK Fusions	1100 µL	FAM
②	LEG Reaction Mix A2	NTRK1 Fusions	1100 µL	FAM
③	LEG Reaction Mix A3	NTRK2 Fusions	1100 µL	FAM
④	LEG Reaction Mix A4	NTRK3 Fusions & HPRT1	1100 µL	FAM, VIC
⑤	LEG Reaction Mix A5	ROS1 Fusions	1100 µL	FAM
⑥	LEG Reaction Mix A6	ROS1 Fusions	1100 µL	FAM
⑦	LEG Reaction Mix A7	MET exon 14 skipping mutation	1100 µL	FAM
⑧	LEG Reaction Mix A8	RET Fusions & HPRT1	1100 µL	FAM, VIC
/	LEG RT Reaction Mix I	Primers, Mg^{2+}, dNTPs	580 µL/tube ×1	/
/	LEG RT Reaction Mix II	Primers, Mg^{2+}, dNTPs	580 µL/tube ×1	/
/	LEG Reverse Transcriptase	Reverse Transcriptase	45 µL/tube ×1	/
/	LEG Enzyme Mix A	Taq DNA Polymerase, Uracil-N-Glycosylase	90 µL/tube ×1	/
/	LEG Positive Control	Plasmid DNA	500 µL/tube ×1	/

Storage and Stability

The kit requires shipment on frozen ice packs below 25℃ for no more than one week. All components of the kit should be stored immediately upon receipt at -20±5℃ and protected from light. The shelf-life of the kit is twelve months. Tube opening doesn't affect expiration of the kit. The recommended maximum freeze-thaw cycle is five cycles.

Additional Reagents and Equipment Required but Not Supplied

1) Compatible PCR instrument: QuantStudio5, LightCycler480 II, cobas z 480, Bio-Rad CFX96 and SLAN-96S.

2) RNA extraction kit: we recommend use of AmoyDx® FFPE RNA Kit for FFPE tumor tissue or AmoyDx® Tissue RNA Kit for fresh tumor tissue).

3) Spectrophotometer for measuring RNA concentration.

4) 8-tube PCR strips and caps

- For QuantStudio5: Recommended 8-tube PCR strips: BIOplastics B69909; 8-tube PCR caps: B57801.

- For LightCycler480 II and cobas z 480: Recommended 8-tube PCR strips: BIOplastics B59909; 8-tube PCR caps: B57801.
- For SLAN-96S: Recommended 8-tube PCR strips: Axygen PCR-0208-C-O; 8-tube PCR caps: PCR-2CP-RT-C.

5) Mini centrifuge with rotor for centrifuge tubes.

6) Mini centrifuge with rotor for PCR tubes.

7) Vortexer.

8) Nuclease-free PCR tubes and caps.

9) Nuclease-free centrifuge tubes.

10) Adjustable pipettors and filtered pipette tips for handling RNA.

11) Tube racks.

12) Disposable powder-free gloves.

13) Sterile, nuclease-free water.

Precautions and Handling Requirements

Precautions

- Please read the instruction carefully and become familiar with all components of the kit prior to use, and strictly follow the instruction during operation.
- Please check the compatibility of the real-time PCR instruments prior to use.
- DO NOT use the kit or any kit component after their expiry date.
- DO NOT use any other reagents from different lots in the tests.
- DO NOT use any other reagent in the other test kits.

Safety Information

- Handle all specimens and components of the kit as potentially infectious material using safe laboratory procedures.
- As all the chemicals have potential hazard, only trained professionals can use this kit. Please wear suitable lab coat and disposable gloves while handling the reagents.
- Avoid skin, eyes and mucous membranes contact with the chemicals. In case of contact, flush with water immediately.
- DO NOT pipet by mouth.

Decontamination and Disposal

- The kit contains positive control; strictly distinguish the positive control from other reagents to avoid contamination which may cause false positive.
- PCR amplification is extremely sensitive to cross-contamination. The flow of tubes, racks, pipets and other materials used should be from pre-amplification to post-amplification, and never backwards.
- Gloves should be worn and changed frequently when handling samples and reagents to prevent contamination.
- Using separate, dedicated pipettes and filtered pipette tips when handling samples and reagents to prevent exogenous RNA contamination

to the reagents.

- Please pack the post-amplification tubes with two disposable gloves and discard properly. DO NOT open the post- amplification PCR tubes.
- All disposable materials are for one time use. DO NOT reuse.
- The unused reagents, used kit, and waste must be disposed of properly.

Cleaning

- After the operation, wipe down the work area, spray down the pipettes and equipment with 75% ethanol or 10% hypochlorous acid solution.

Instrument Setup

- Setup the reaction volume as 40 μL.
- For QuantStudio5 instrument, please set up as follows: Reporter Dye: FAM, VIC; Quencher Dye: None; Passive Reference: None
- For SLAN-96S instrument, please set up as follows: Fluorophores/Dyes: FAM, HEX. During the result interpretation, select "Selected Wells" for "Y-Axis Scaling Auto-adjust By" and "Absolute fluorescence Method" for "Normalization algorithm".
- Refer to the real-time PCR instrument operator's manual for detailed instructions.
- We recommend that all PCR instruments in use should be conducted fluorescence calibration once a year.

Assay Procedure

1. RNA Extraction

The specimen material must be human total RNA extracted from tumor tissue samples. It's better to use tumor tissue samples with more than 20% tumor content. The $OD_{260/280}$ value of extracted RNA should be between 1.7-2.1. The total RNA concentration for gene fusion detection is shown in Table 2.

Table 2 Recommended RNA concentration

Sample type	Storage time	RNA concentration	Remark
FFPE tissue	≤ 2 years	10-100 ng/μL	• If RNA is between 10-100 ng/μL, use the original RNA without dilution. • If RNA is more than 100 ng/μL, dilute the RNA to 100 ng/μL.
Fresh tissue	/	2-30 ng/μL	• If RNA is between 2-30 ng/μL, use the original RNA without dilution. • If RNA is more than 30 ng/μL, dilute the RNA to 30 ng/μL.

Note:

- *The FFPE tissue should be handled and stored properly, and the storage time should preferably be less than 2 years.*
- *The extracted RNA should be used immediately, if not, it should be stored appropriately, usually at -20±5℃ for no more than 3 months.*
- *The extracted RNA shall be measured by the spectrophotometer, the NanoDrop 1000 /2000 spectrophotometer is recommended.*
- *Before detection, dilute the extracted RNA with nuclease-free water to designated concentration.*

2. RNA Reverse Transcription

Step 1: Thaw and Mix Reagents

1) Thaw **LEG RT Reaction Mix I** and **II** at room temperature until fully liquid.

2) Vortex each thawed mix thoroughly (5–10 seconds), then centrifuge briefly (5–10 seconds) to collect liquid at the tube bottom.

3) Centrifuge **LEG Reverse Transcriptase** briefly (5–10 seconds) to collect contents.

Step 2: Prepare Reverse Transcription Reactions

4) For each RNA sample, set up two parallel reactions in separate labeled 0.2 mL PCR tubes (one for Mix I, one for Mix II). Add reagents in the order shown in Table 3 (below) to minimize foaming. Gently mix each tube by vortexing (5–10 seconds), then centrifuge briefly (5–10 seconds).

Labeling: For sample i (where i = 1 to n): Label tubes as "Si-cDNA 1" (Mix I) and "Si-cDNA 2" (Mix II).

Tip: Prepare a master mix of LEG RT Reaction Mix + LEG Reverse Transcriptase (e.g., for n samples: (n+1) × 18.5 µL Mix + (n+1) × 0.5 µL Transcriptase) if pipetting many reactions, to account for pipetting losses; then add RNA last to avoid degradation.

Table 3 RNA Reverse Transcription Solutions

Reagent	Volume per test
LEG RT Reaction Mix	18.5 µL
LEG Reverse Transcriptase	0.5 µL
Sample RNA	6 µL
Total	**25 µL**

Step 3: Thermal Cycling

5) Incubate all tubes at 42°C for 60 minutes.

6) Inactivate by heating at 95°C for 5 minutes, then immediately transfer tubes to ice for 2–5 minutes to cool.

Step 4: Storage and Use

7) The resulting S*i*-cDNA solutions are ready for immediate downstream PCR.

Note: *sample cDNA should be used immediately, if not, it should be stored at -20±5 ℃ for no more than 3 days after reverse transcription.*

3. RNA Fusion Detection

Step 1: Thaw and Mix Reagents

1) Thaw **LEG Reaction Mix A1-A8** and **LEG Positive Control (PC)** at room temperature.

2) Vortex each tube thoroughly (5-10 seconds), then centrifuge briefly (5-10 seconds).

Step 2: Prepare PCR Reaction Strips

3) Prepare (S+2) × PCR strips (one per sample + NTC + PC).

4) Dispense 35 µL of **LEG Reaction Mix A1-A8** into corresponding tubes across all 8-tube PCR strips:

- Add A1 to tube ① of every 8-tube PCR strip.

- Add A2 o tube ② of every 8-tube PCR strip.
- …
- Add A8 o tube ⑧ of every 8-tube PCR strip.

Tip: Use a multichannel pipette for efficiency when dispensing across multiple strips.

Step 3: Prepare Template-Enzyme Mixes

5) Prepare the following mixes in labeled tubes:

- Si -Mix A1: For each sample, add 1.3 µL **LEG Enzyme Mix A** to 25 µL S*i* cDNA 1 (total: 26.3 µL per sample).
- Si -Mix A2: For each sample, add 1.3 µL **LEG Enzyme Mix A** to 25 µL S*i* cDNA 2 (total: 26.3 µL per sample).
- N-Mix A: For each sample, add 2.34 µL **LEG Enzyme Mix A** to 45 µL nuclease-free water (total: 47.34 µL per sample).
- P-Mix A: For each sample, add 2.34 µL **LEG Enzyme Mix A** to 45 µL **LEG Positive Control** (total: 47.34 µL per sample).

Note:

- *Due to the viscosity of the enzyme mix, pipet slowly to ensure all mix is completely dispensed from the tip.*
- *Pipet enzyme mix by placing the pipet tip just under the liquid surface to avoid the tip being coated in excess enzyme.*
- *The prepared mixtures should be used immediately, avoid prolonged storage.*

Step 4: Assemble Reactions

6) For each prepared 8-tube PCR strip, add 5 µL of the appropriate template-enzyme mix to tubes ① – ⑧ (final reaction volume: 40 µL per well). Cap the tubes after additions:

- NTC Strip: Add 5 µL N-Mix A to all tubes (① – ⑧).
- Sample Strips: Add 5 µL of the corresponding Si -Mix A1 to tubes ① – ④ and 5 µL of Si -Mix A2 to tubes ⑤ – ⑧.
- PC Strip: Add 5 µL P-Mix A to all tubes (① – ⑧).

7) Briefly centrifuge all strips (5–10 seconds) to collect liquids at the tube bottoms.

Step 5: Run PCR

8) Load strips into the real-time PCR instrument according to the recommended plate layout (see Table 4).

Note: *Each PCR run must contain one Positive Control (PC) and one No Template Control (NTC)*

Table 4 Suggested PCR Plate Layout

RNA Detection												
Well	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
A	Sample1	Sample2	Sample3	Sample4	Sample5	Sample6	Sample7	Sample8	Sample9	Sample10	NTC	PC
B	Sample1	Sample2	Sample3	Sample4	Sample5	Sample6	Sample7	Sample8	Sample9	Sample10	NTC	PC
C	Sample1	Sample2	Sample3	Sample4	Sample5	Sample6	Sample7	Sample8	Sample9	Sample10	NTC	PC
D	Sample1	Sample2	Sample3	Sample4	Sample5	Sample6	Sample7	Sample8	Sample9	Sample10	NTC	PC
E	Sample1	Sample2	Sample3	Sample4	Sample5	Sample6	Sample7	Sample8	Sample9	Sample10	NTC	PC

F	Sample1	Sample2	Sample3	Sample4	Sample5	Sample6	Sample7	Sample8	Sample9	Sample10	NTC	PC
G	Sample1	Sample2	Sample3	Sample4	Sample5	Sample6	Sample7	Sample8	Sample9	Sample10	NTC	PC
H	Sample1	Sample2	Sample3	Sample4	Sample5	Sample6	Sample7	Sample8	Sample9	Sample10	NTC	PC

1) Setup the PCR run using the cycling parameters in Table 5/6.

Table 5 Real-Time PCR Parameters (QuantStudio5, SLAN-96S)

Stage	Cycles	Temperature	Time	Acquisitions
1	1	42℃	5 min	/
		95℃	5 min	/
2	10	95℃	25 s	/
		64℃	20 s	/
		72℃	20 s	/
3	36	93℃	25 s	/
		60℃	35 s	FAM, VIC/HEX
		72℃	20 s	/
4	1	40℃	30 s	/

Table 6 Real-Time PCR Parameters (LightCycler480 II, cobas z 480, Bio-Rad CFX96)

Stage	Cycles	Temperature	Time	Acquisitions
1	1	42℃	5 min	/
		94℃	5 min	/
2	10	94℃	25 s	/
		63℃	20 s	/
		71℃	20 s	/
3	36	92℃	25 s	/
		59℃	35 s	FAM, VIC
		71℃	20 s	/
4	1	40℃	30 s	/

2) Start the PCR run immediately.

3) When the PCR run is finished, analyze the data according to the “Results Interpretation” procedures.

4. Result Interpretation

➢ **Manual Analysis:**

Threshold Setting: For analysis, select a single reaction tube and corresponding fluorescence signal sequentially by tube number (Tubes 1-8), along with respective No Template Control (NTC) and the Positive Control (PC) and tubes. Adjust the threshold to 5% (QuantStudio5, Bio-Rad CFX96 and SLAN-96S) or 6% (LightCycler480 II and cobas z 480) of the PC’s peak fluorescence at the 36th cycle to determine the Ct values for each reaction tube.

1) **NTC (No Template Control):** The FAM and VIC signals in tubes 1-8, normally should exhibit no amplification curves.

a) If any FAM signals in tubes 1-8 show amplification, the experiment is deemed invalid and should be repeated, as this suggests potential contamination.

b) If the VIC signals in tubes 4 or 8 show occasional amplification while the FAM signals in tubes 1-8 do not, this does not impact the validity of the fusion detection results, and analysis may proceed as planned.

2) **PC (Positive Control):** The FAM signals in tubes 1-8, VIC signals in tubes 4 & 8 of the positive control should all demonstrate clear amplification. The Ct values for these signals should be < 25. If not, the data is *INVALID*. The sample should be retested.

3) **Internal Control (IC):** Check the RNA Internal control VIC signals in tube 4 & 8 for each sample.

a) If both VIC Ct values of tube 4 & 8 are < 33 and either one is < 27, continue with the analysis.

b) If both VIC Ct values of tube 4 & 8 are ≥ 27 or either one is ≥ 33, which indicates the partial fragmentation or degradation of RNA, or the presence of PCR inhibitors. The sample should be retested with increased or re-extracted RNA.

4) **RNA Fusion Analysis:** Set the threshold based on the fluorescence value of the positive control (PC) at the final cycle to accurately determine the Ct values for each fusion detection tube. Check the FAM signal amplifications in tubes 1-8. Classify the sample results as negative or positive in accordance with the detailed criteria specified in Table 7.

Table 7 RNA Fusion Result Determination

Tube	1	2	3	4	5	6	7	8	Results
Detected Target	ALK	NTRK1	NTRK2	NTRK3	ROS1	ROS1	MET	RET	
Positive Ct range	Ct<28	Ct<28	Ct<28	Ct<28	Ct<28	Ct<28	Ct<28	Ct<28	Positive
Negative Ct range	Ct≥28	Ct≥28	Ct≥28	Ct≥28	Ct≥28	Ct≥28	Ct≥28	Ct≥28	Negative or under the LOD*

* LOD: limit of detection

a) If any FAM Ct values of LEG Reaction Mix A1-A8 are in Positive Ct range, the sample is determined as corresponding fusion positive.

b) If all the FAM Ct values of LEG Reaction Mix A1-A8 are in Negative Ct range, the sample is determined as negative (No fusion detected) or under the LOD of the kit.

➢ **Automated Analysis:**

ARAS is a proprietary software developed by AmoyDx intended to be used as a tool to aid in the interpretation of AmoyDx® Pan Lung Cancer Fusion PCR Panel. Upon the PCR run completion, import the PCR data into the ARAS, to determine sample's fusion status based on the Ct value.

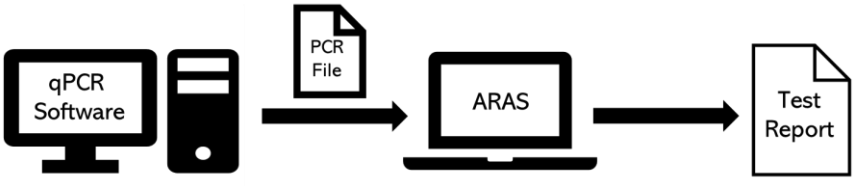

Figure 2 PCR Data to ARAS Workflow Overview

1) Enter ARAS's IP address in the Chrome browser and provide their account credentials to access ARAS.

2) Click the “Create New Analysis” button and a pop-up window will appear, select the product and instrument you wish to analyze.

3) Click the upload button to select the PCR file to be analyzed and then click “Confirm” to initiate the analysis.

   a) PCR file from QuantStudio5 should be in eds format.

   b) PCR file from LightCycler480 II, cobas z 480 or Bio-Rad CFX96 should be in zip format.

   c) PCR file from SLAN-96S should be in csv format.

4) Assign the sample layout based on the experiment, and then click the “Analysis” button to generate testing results.

5) On the result page, users can verify the accuracy of the test results by reviewing the result list, 96-well plate diagram and the amplified fluorescence curves.

6) On the report page, click the “Generate report” button to generate and download report files for the tested samples.

Note:

- *ARAS is For Research Use Only. Not for use in diagnostic procedures.*
- *PCR file generated from different instruments may necessitate distinct preparation before ARAS analysis. Please adhere the ARAS protocol to ensure that the PCR file is adequately interpretable by ARAS.*
- *For other functions provided by ARAS, please refer to the instructions of ARAS.*

Performance Characteristics

1) Sensitivity: the kit allows limit of detection (LoD) of 25 copies/μL gene variant RNA.

2) Specificity: established by testing internal negative references, the concordance rates are 100%.

3) Accuracy: established by testing internal positive references and negative references, the detection rates are 100%.

4) Precision: established by performing precision reference for 10 repeats, all results were positive, coefficient of variation for Ct values (CV, %) was less than 10%.

Limitations

1) The kit is to be used only by personnel specially trained in the techniques of PCR and the use of real-time PCR instruments.

2) The kit has been validated for use with tumor tissue samples.

3) Reliable results are dependent on proper sample processing, transport, and storage.

4) The sample containing degraded RNA may affect the ability of the test to detect the intended fusions.

5) Samples with negative results (no fusions detected) may harbor fusions not detected by this assay.

References

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Symbols

 Manufacturer

 Batch Code

 Contains Sufficient for <n> Tests

 Consult Instructions For Use

 This Way Up

 Keep Away from Sunlight

 Catalogue Number

 Use By

 Temperature Limitation

 Keep Dry

 Fragile, Handle With Care

Appendix 1

Gene Fusions Detected with LEG Reaction Mix A

Tube / Signal	Target to detect	Fusion Type	Name
① FAM	*ALK*	*EML4* exon13;*ALK* exon20	EML4-ALK-1
		EML4 exon6 ins33;*ALK* exon20	EML4-ALK-2
		EML4 exon20;*ALK* exon20	EML4-ALK-3
		EML4 exon18;*ALK* exon20	EML4-ALK-6
		EML4 exon2;*ALK* exon20	EML4-ALK-7
		EML4 exon17;ins68 *ALK* exon20	EML4-ALK-8
		EML4 exon2;ins117 *ALK* exon20	EML4-ALK-9
		EML4 exon13;ins69 *ALK* exon20	EML4-ALK-10
		EML4 exon6;*ALK* exon20	EML4-ALK-11
		EML4 exon6;*ALK* exon19	EML4-ALK-12
		EML4 exon6;ins18 *ALK* exon20	EML4-ALK-13
		EML4 exon20;ins18 *ALK* exon20	EML4-ALK-14
		EML4 exon17del58;ins39 *ALK* exon20	EML4-ALK-17
		EML4 exon17 ins65;*ALK* exon20	EML4-ALK-18
		EML4 exon17;ins30 *ALK* exon20	EML4-ALK-19
		EML4 exon17 ins61;ins34 *ALK* exon20	EML4-ALK-20
		EML4 exon3;ins53 *ALK* exon20	EML4-ALK-21
		KIF5B exon24;*ALK* exon20	KIF5B-ALK-1
		KIF5B exon17;*ALK* exon20	KIF5B-ALK-2
		KLC1 exon9;*ALK* exon20	KLC1-ALK
		TFG exon4;*ALK* exon20	TFG-ALK
② FAM	*NTRK1*	*TFG* exon5;*NTRK1* exon9	NTRK1-E9-M1
		TPM3 exon8;*NTRK1* exon10	NTRK1-E10-M1
		SQSTM1 exon5;*NTRK1* exon10	NTRK1-E10-M3
		TPR exon16 del54;*NTRK1* ins13 exon10	NTRK1-E10-M5
		TPR exon21;*NTRK1* exon10	NTRK1-E10-M6
		CD74 exon8;*NTRK1* exon10	NTRK1-E10-M7
		IRF2BP2 exon1;*NTRK1* exon10	NTRK1-E10-M8
		IRF2BP2 exon1 del48;*NTRK1* exon10	NTRK1-E10-M9
		TFG exon5;*NTRK1* exon10	NTRK1-E10-M12
		GRIPAP1 exon22;*NTRK1* exon10	NTRK1-E10-M14
		F11R exon4;*NTRK1* exon10	NTRK1-E10-M15
		SQSTM1 exon6;*NTRK1* exon10	NTRK1-E10-M17
		TPM3 exon8;*NTRK1* exon12	NTRK1-E12-M1
		MPRIP exon21;*NTRK1* exon12	NTRK1-E12-M3
		SSBP2 exon12;*NTRK1* exon12	NTRK1-E12-M4
		MPRIP exon14;*NTRK1* exon12	NTRK1-E12-M11
		MPRIP exon18;*NTRK1* exon12	NTRK1-E12-M12
		GRIPAP1 exon22;*NTRK1* exon12	NTRK1-E12-M14
③ FAM	*NTRK2*	*TRIM24* exon12;*NTRK2* exon15	NTRK2-E15-M1
		T*RIM24* exon12;*NTRK2* exon16	NTRK2-E16-M1
		SQSTM1 exon5;*NTRK2* exon16	NTRK2-E16-M3
		STRN exon3;*NTRK2* exon16	NTRK2-E16-M7
		SQSTM1 exon5;*NTRK2* exon17	NTRK2-E17-M2
④ FAM	*NTRK3*	*ETV6* exon4;*NTRK3* exon14	NTRK3-EX14-M1
		ETV6 exon5;*NTRK3* exon14	NTRK3-EX14-M2
		EML4 exon2;*NTRK3* exon14	NTRK3-EX14-M3
		SQSTM1 exon5;*NTRK3* exon14	NTRK3-EX14-M4
		RBPMS exon5;*NTRK3* exon14	NTRK3-EX14-M7
		ETV6 exon5;*NTRK3* exon15	NTRK3-EX15-M1
		ETV6 exon4;*NTRK3* exon15	NTRK3-EX15-M2
		SQSTM1 exon6;*NTRK3* exon15	NTRK3-EX15-M3
⑤ FAM	*ROS1*	*SLC34A2* exon4;*ROS1* exon32	ROS1-M1
		SLC34A2 exon13 del2046;*ROS1* exon32	ROS1-M2
		CD74 exon6;*ROS1* exon32	ROS1-M3

		SDC4 exon2;*ROS1* exon32	ROS1-M4
		SDC4 exon4;*ROS1* exon32	ROS1-M5
		SLC34A2 exon4;*ROS1* exon34	ROS1-M6
		SLC34A2 exon13 del2046;*ROS1* exon34	ROS1-M7
		CD74 exon6;*ROS1* exon34	ROS1-M8
		SDC4 exon4;*ROS1* exon34	ROS1-M9
		EZR exon10;*ROS1* exon34	ROS1-M10
⑥ FAM	*ROS1*	*TPM3* exon8;*ROS1* exon35	ROS1-M11
		LRIG3 exon16;*ROS1* exon35	ROS1-M12
		GOPC exon8;*ROS1* exon35	ROS1-M13
⑦ FAM	*MET*	*MET* Exon 14 skipping mutation	MET-M2
⑧ FAM	*RET*	*CCDC6* exon1;*RET* exon12	RET–M2
		NCOA4 exon6;*RET* exon12	RET–M5
		KIF5B exon15;*RET* exon12	RET-M15
		KIF5B exon16;*RET* exon12	RET–M16
		KIF5B exon23;*RET* exon12	RET–M17
		KIF5B exon22;*RET* exon12	RET–M19
		TRIM33 exon14;*RET* exon12	LRET-M22
		CUX1 exon10;*RET* exon12	LRET-M32
		KIAA1468 exon10;*RET* exon12	LRET-M40
		KIF13A exon18;*RET* exon12	LRET-M41
		MPRIP exon19;*RET* exon12	LRET-M42
		MYO5C exon25;*RET* exon12	LRET-M44
		PICALM exon19;*RET* exon12	LRET-M45
		RUFY2 exon9;*RET* exon12	LRET-M49
		TNIP2 exon5;*RET* exon12	LRET-M55
		WAC exon3;*RET* exon12	LRET-M57