

AmoyDx[®] Pan Lung Cancer PCR Panel

Instructions for Use

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

REF 8.01.0234 8 tests/kit For LightCycler480 II, cobas[®] z480, Bio-Rad CFX96



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> Version: P1.2 Jun 2024



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% ^[11], 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 PCR Panel is a real-time PCR assay for qualitative detection of 167 hotspot alterations in *EGFR*, *ALK*, *ROS1*, *KRAS*, *BRAF*, *HER2*, *RET*, *MET*, *NTRK1*, *NTRK2* and *NTRK3* genes. The kit is intended to be used to aid clinician to identify multigene status for NSCLC patients.

The kit is for research use only, and intended to be used by trained professionals in a laboratory environment.

Principles of the Procedure

This kit contains RNA gene fusion detection system in LEG Reaction Mix A and DNA gene mutation detection system in LEG Reaction Mix B.

The RNA gene 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 is detected by fluorescent probes.

The DNA gene mutation detection system uses ADx-ARMS technology, which comprises specific primers and fluorescent probes to detect gene mutations. During the amplification, the target mutant DNA is matched with the bases at 3' end of the primer, and amplified efficiently, then the mutant amplicon is detected by fluorescent-labeled probes. While the wild-type DNA cannot be matched with specific primers, there is no amplification occurs.

The kit contains LEG Reaction Mix A strips, LEG Reaction Mix B strips, LEG RT Reaction Mix, sufficient positive control and enzyme.

LEG Reaction Mix A strips 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) LEG Reaction Mix B strips are designed for DNA mutation detection and external control detection. The Reaction Mixes B1~B7 include primers and FAM/VIC/ROX-labeled probes specific for detection of hotspot mutations in *EGFR*, *HER2*, *KRAS* and *BRAF* genes. And the LEG Reaction Mix B8 contains DNA external control reaction mix, which is composed of primer and FAM/VIC/ROX-labeled probes for detection of a region of genomic DNA that has no known mutations or polymorphisms, to assess the DNA quality.

- 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.
- The LEG RT Reaction Mix II contain primers specific for reverse transcription of mRNA of ROS1, RET, MET gene and reference gene into cDNA.
- 5) The LEG Reverse Transcriptase is for reverse transcription of mRNA of target genes and reference gene into cDNA.
- 6) The LEG Enzyme Mix A and LEG Enzyme Mix B contains the Taq DNA polymerase for PCR amplification and uracil-Nglycosylase which works at room temperature to prevent PCR amplicon carryover contamination.
- 7) The **LEG Positive Control** contains recombinant gene with *EGFR*, *KRAS*, *BRAF*, *HER2*, *ALK*, *ROS1*, *RET*, *MET*, *NTRK1*, *NTRK2* and *NTRK3* alternations.

Kit Contents

This kit contains the following materials:

	Table 1 Kit Contents	
Content	Main Ingredients	Quantity
LEG Reaction Mix A (FU)	Primers, probe, Mg ²⁺ , dNTPs	8-tube strip* ×12
LEG Reaction Mix B (MU)	Primers, probe, Mg ²⁺ , dNTPs	8-tube strip* ×12
LEG RT Reaction Mix I	Primers, Mg ²⁺ , dNTPs	220 µL/tube ×1
LEG RT Reaction Mix II	Primers, Mg ²⁺ , dNTPs	220 μ L/tube ×1
LEG Reverse Transcriptase	Reverse Transcriptase	16 μ L/tube ×1
LEG Enzyme Mix A	Taq DNA Polymerase, Uracil-N-Glycosylase	45 μ L/tube $\times 1$
LEG Enzyme Mix B	Taq DNA Polymerase, Uracil-N-Glycosylase	45 μ L/tube $\times 1$
LEG Positive Control	Plasmid DNA	500 µL/tube ×1

Table 1 Kit Contents

*Each strip (8-tube) includes the following contents (Tables 2~3):

Table 2 Information of LEG Reaction Mix	A
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Tube No.	Reagent	Target to detect	Quantity	Florescence Signal
1)	LEG Reaction Mix A1	ALK Fusions	35 µL	FAM
2	LEG Reaction Mix A2	NTRK1 Fusions	35 µL	FAM
3	LEG Reaction Mix A3	NTRK2 Fusions	35 µL	FAM
(4)	LEG Reaction Mix A4	NTRK3 Fusions & HPRT1	35 µL	FAM, VIC
5	LEG Reaction Mix A5	ROS1 Fusions	35 µL	FAM
6	LEG Reaction Mix A6	ROS1 Fusions	35 µL	FAM
\bigcirc	LEG Reaction Mix A7	MET exon 14 skipping mutation	35 µL	FAM
8	LEG Reaction Mix A8	RET Fusions & HPRT1	35 µL	FAM, VIC



Tube No.	Reagent	Target to detect	Quantity	Florescence Signal
1	LEG Reaction Mix B1	EGFR Mutations	35 µL	FAM, VIC
2	LEG Reaction Mix B2	EGFR Mutations	35 µL	FAM, VIC
3	LEG Reaction Mix B3	EGFR Mutations	35 µL	FAM, VIC
(4)	LEG Reaction Mix B4	EGFR/HER2 Mutations	35 µL	FAM, VIC
5	LEG Reaction Mix B5	EGFR/KRAS Mutations	35 µL	FAM, VIC
6	LEG Reaction Mix B6	KRAS/HER2 Mutations	35 µL	FAM, VIC
\bigcirc	LEG Reaction Mix B7	KRAS/BRAF/EGFR Mutations	35 µL	FAM, VIC, ROX
8	LEG Reaction Mix B8	External Control	35 µL	FAM, VIC, ROX

Table 3 Information of LEG Reaction Mix B

Note: Distinguish Tube *③* from Tube *①* according to the label and hole position at the strip edge, described as follows.

For LEG Reaction Mix A:



For LEG Reaction Mix B:



Storage and Stability

The kit requires shipment on frozen ice packs. All components of the kit should be stored immediately upon receipt at $-20\pm5^{\circ}$ C and protected from light.

The shelf-life of the kit is twelve months. The maximal number of freeze-thaw cycles is five.

Additional Reagents and Equipment Required but Not Supplied

- 1) Compatible PCR instrument: LightCycler480 II, cobas[®] z480, and Bio-Rad CFX96.
- 2) DNA/RNA extraction kit: we recommend use of AmoyDx extraction kit (AmoyDx[®] FFPE DNA/RNA Kit for FFPE tumor tissue, or

AmoyDx® Tissue DNA Kit, AmoyDx® Tissue RNA Kit for fresh tumor tissue).

- 3) Spectrophotometer for measuring DNA/RNA concentration.
- 4) Mini centrifuge with rotor for centrifuge tubes.
- 5) Mini centrifuge with rotor for PCR tubes.
- 6) Vortexer.
- 7) Nuclease-free PCR tubes and caps.
- 8) Nuclease-free centrifuge tubes.
- 9) Adjustable pipettors and filtered pipette tips for handling DNA/RNA.
- 10) Tube racks.
- 11) Disposable powder-free gloves.
- 12) Sterile, nuclease-free water.
- 13) 1×TE buffer (pH 8.0).



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 DNA/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 LightCycler480 II and cobas[®] z480 instrument, it's necessary to conduct Color Compensation prior to the first use according to Color Compensation instructions. Please contact AmoyDx Technical Support or Account Manager to get the AmoyDx[®] Pan Lung Cancer Detection Reagent (V2) (for Color Compensation) and Color Compensation instructions.
- 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. DNA/RNA Extraction

The specimen material must be human genomic DNA and total RNA extracted from tumor tissue samples. DNA/RNA extraction reagents

are not included in the kit. It's better to use tumor tissue samples with more than 20% tumor content.

The OD_{260/280} value of extracted DNA and RNA should be between 1.7~2.1.

The total RNA concentration for gene fusion detection is shown in Table 4.

Table 4 Recommended RNA concentration

Sample type	Storage time	Remark		
FFPE tissue	\leq 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. 	

The amount of extracted DNA for gene mutation detection is shown in Table 5.

Table 5 Recommended DNA concentratio	Table 5	Recommended	DNA	concentratio
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Sample type	Storage time	DNA concentration	DNA amount/reaction	
	\leq 3 months	1.5 ng/µL	7.5 ng	
FFPE tissue	$>$ 3 months & \leq 1 year	2 ng/µL	10 ng	
	> 1 year & ≤ 2 years	2.5~3 ng/µL	12.5~15 ng	
Fresh tissue	/	0.5~1 ng/µL	2.5~5 ng	

Note:

- The FFPE tissue should be handled and stored properly, and the storage time should preferably be less than 2 years.
- The extracted DNA should be used immediately, if not, it should be stored appropriately, usually at -20 ± 5 °C for no more than 6 months.
- The extracted RNA should be used immediately, if not, it should be stored appropriately, usually at -20 ± 5 °C for no more than 3 months.
- The extracted DNA/RNA shall be measured by the spectrophotometer, the NanoDrop 1000 /2000 spectrophotometer is recommended.
- Before detection, dilute the extracted DNA with 1×TE buffer (pH 8.0) to designated concentration; dilute the extracted RNA with nuclease-free water to designated concentration. We recommend using at least 5 μL DNA for 10 times dilution, to ensure the validity of final concentration.

2. RNA Reverse Transcription

1) Take LEG RT Reaction Mix I, LEG RT Reaction Mix II and LEG Reverse Transcriptase out of the kit from the freezer, and



other reagents remained in freezer at -20±5°C.

- 2) Thaw the LEG RT Reaction Mix I and LEG RT Reaction Mix II at room temperature. When the reagents completely thawed, mix each reagent by vortexing and centrifuge for 5~10 seconds to collect all liquid at the bottom of the tube.
- 3) Centrifuge LEG Reverse Transcriptase for 5~10 seconds prior to use.
- 4) For each RNA sample, prepare RNA reverse transcription solutions containing LEG Reverse Transcriptase, Sample RNA, and RT Reaction Mix (LEG RT Reaction Mix I or LEG RT Reaction Mix II, respectively) in separate 0.2 mL PCR tube according to the ratio in Table 6. Thoroughly mix each reverse transcription solution by vortexing, and centrifuge for 5~10 seconds.

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

Table 6 RNA Reverse Transcription Solutions

- 5) Incubate the tubes at 42° C for one hour.
- 6) Heat the tubes at 95 ℃ for 5 minutes, then transfer the PCR tubes on the ice. The resulting Sample cDNA are ready for PCR amplification. Mark the solutions as S-cDNA 1 and S-cDNA 2, (if more samples, name as S1-cDNA 1, S2-cDNA 1, ..., Sn-cDNA 1 and S1-cDNA 2, S2-cDNA 2, ..., Sn-cDNA 2)

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

3. RNA and DNA Mutations Detection

- Note:
 - Each PCR run must contain one Positive Control (PC) and one Negative Control (NTC).
 - The prepared mixtures should be used immediately, avoid prolonged storage.
 - 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.
- Take out the LEG Positive Control (PC) and thaw the reagents at room temperature. When the reagents completely thawed, mix each reagent by vortexing and centrifuge for 5~10 seconds to collect all liquid at the bottom of the tube.
- 2) Take out the LEG Enzyme Mix A and LEG Enzyme Mix B, centrifuge for 5~10 seconds prior to use.
- 3) Take out the sample cDNA, sample DNA and nuclease-free water for NTC.
- 4) For **RNA detection**:
 - a) Prepare S-Mix A1 and S-Mix A2: Add 1.3 μL LEG Enzyme Mix A into the above 25 μL S cDNA 1 and S cDNA 2 tube respectively. Mark the solutions as S-Mix A1 and S-Mix A2. Mix each solution thoroughly by vortexing, and centrifuge for 5~10 seconds.
 - b) Prepare N-Mix A and P-Mix A: Add 2.34 μL LEG Enzyme Mix A into 45 μL nuclease-free water and 45 μL LEG Positive
 Control, respectively. Mark the solutions as N-Mix A and P-Mix A. Mix each solution thoroughly by vortexing, and centrifuge



for 5~10 seconds.

- c) Take out LEG Reaction Mix A strips (sufficient for samples, PC and NTC) and centrifuge the strips. Then gently uncover the caps prior to use.
- d) Prepare one LEG Reaction Mix A strip for NTC: Add 5 µL N-Mix A into Tube (1)~(8), cap the PCR tubes.
- e) Prepare one LEG Reaction Mix A strip for each sample: Add 5 μL S-Mix A1 into Tube ①~④, 5 μL S-Mix A2 into Tube ⑤~⑧, Cap the PCR tubes.
- f) Prepare one LEG Reaction Mix A strip for PC: Add 5 µL P-Mix A into Tube ①~⑧, cap the PCR tubes.
- 5) For **DNA detection**:
 - a) Prepare LEG Master Mix B: Add 2.7 μL LEG Enzyme Mix B into 45 μL sample DNA/45 μL nuclease-free water/45 μL LEG
 Positive Control, respectively. Mark the solutions as S-Mix B (if more samples, name as S1-Mix B, S2-Mix B, ..., Sn-Mix B),
 N-Mix B, P-Mix B. Mix each solution thoroughly by vortexing and centrifuge for 5~10 seconds.
 - b) Take out LEG Reaction Mix B strips (sufficient for samples, PC and NTC) and centrifuge the strips. Then gently uncover the caps prior to use.
 - c) Prepare one LEG Reaction Mix B strip for NTC: Add 5 μ L N-Mix B into Tube (1)~(8), and cap the PCR tubes.
 - d) Prepare one LEG Reaction Mix B strip for each sample: Add 5 μ L S-Mix B into Tube (1)~(8), and cap the PCR tubes.
 - e) Prepare one LEG Reaction Mix B strip for PC: Add 5 μL P-Mix B to Tube ①~⑧, and cap the PCR tubes.
- 6) Briefly centrifuge the PCR tubes to collect all liquid at the bottom of each PCR tube.
- Place the PCR tubes into the appropriate positions of the real-time PCR instrument. A recommended plate layout is shown in Table 7. Table 7 Suggested PCR Plate Layout

	RNA Detection							DNA Detection				
Well	1	2	3	4	5	6	7	8	9	10	11	12
Α	Sample1	Sample2	Sample3	Sample4	NTC	PC	Sample1	Sample2	Sample3	Sample4	NTC	PC
В	Sample1	Sample2	Sample3	Sample4	NTC	PC	Sample1	Sample2	Sample3	Sample4	NTC	PC
С	Sample1	Sample2	Sample3	Sample4	NTC	PC	Sample1	Sample2	Sample3	Sample4	NTC	PC
D	Sample1	Sample2	Sample3	Sample4	NTC	PC	Sample1	Sample2	Sample3	Sample4	NTC	PC
Е	Sample1	Sample2	Sample3	Sample4	NTC	PC	Sample1	Sample2	Sample3	Sample4	NTC	PC
F	Sample1	Sample2	Sample3	Sample4	NTC	PC	Sample1	Sample2	Sample3	Sample4	NTC	PC
G	Sample1	Sample2	Sample3	Sample4	NTC	PC	Sample1	Sample2	Sample3	Sample4	NTC	PC
Н	Sample1	Sample2	Sample3	Sample4	NTC	PC	Sample1	Sample2	Sample3	Sample4	NTC	PC

8) Setup the PCR Protocol using the cycling parameters in Table 8.

ratio o Cyving Falancurs									
Stage	Cycles	Temperature	Time	Data collection					
1	1	42°C	5min	/					
1	1	94°C	5min	/					
2		94℃	25s	/					
	10	63 °C	20s	/					
		71 °C	20s	/					
		92℃	25s	/					
3	36	59℃	35s	FAM, VIC and ROX					
		71 °C	20s	/					

Table 8 Cycling Parameters

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- 9) Start the PCR run immediately.
- 10) When the PCR run is finished, analyze the data according to the "Results Interpretation" procedures.

4. Result Interpretation

General recommendation for threshold setting:

It's better to adjust the threshold value manually. Adjust the threshold value by each reaction mix:

For LightCycler480 II and cobas® z480 instrument, adjust the threshold value as follows:

- a. For each reaction mix, choose Positive Control;
- b. Display the amplification curve in "Noise Band" view;
- c. Adjust the "Noise Band" value at the plateau range of the amplification curve, record the Noise Band value (Figure 1);
- d. Set the Noise Band value as 6% × Noise Band value the plateau range, click **Calculate** to obtain and read the Ct values (Figure 2);

(e.g. The Noise Band value of the Positive Control plateau range is 35.8877, the threshold value = $6\% \times 35.887 = 2.1532$.)



Figure 1



Figure 2



For Bio-Rad CFX96 instrument, adjust the threshold value as follows:

- a. For each reaction mix, choose Positive Control;
- b. Adjust the "Threshold" at the plateau range of the amplification curve, right click and select "Baseline Threshold..." to record the



"Threshold" value (Figure 3);

Figure 3

c. Set the "Threshold" value as $5\% \times$ "Threshold" value of the plateau range to obtain the Ct values (Figure 4);

(e.g. The "Threshold" value of the Positive Control plateau range is 4831.84, the threshold value = $5\% \times 4831.84 = 241.592$)





Before data analysis, the following items should be checked:

¹⁾ For the negative control (NTC): The FAM Ct values of LEG Reaction Mix A1~A8, the FAM and VIC Ct values of LEG Reaction



Mix B1~B7, and the ROX Ct value of LEG Reaction Mix B7 should be \geq 36. If not, the data is *INVALID*. The sample should be retested.

2) For Positive Control: The FAM Ct values of LEG Reaction Mix A1~A8 and VIC Ct values of LEG Reaction Mix A4/A8, the FAM and VIC Ct values of LEG Reaction Mix B1~B8, and the ROX Ct values of LEG Reaction Mix B7/B8 should be < 25. If not, the data is *INVALID*. The sample should be retested.

Note:

- Select one reaction mix and one fluorescence channel at a time for fusion / mutation analysis.
- If there is low fluorescent signal, please zoom in the amplification curve.

Analyze RNA fusion assay for each sample:

- 3) For LEG Reaction Mix A1~A8, analyze *ALK*, *NTRK1*, *NTRK2*, *NTRK3*, *ROS1*, *MET* and *RET* gene fusions status:
 - a. Check the RNA Internal control VIC signals of LEG Reaction Mix A4/A8 for each sample:
 - i. If both VIC Ct values of LEG Reaction Mix A4/A8 are < 33 and either one is < 27, continue with the analysis.
 - ii. If both VIC Ct values of LEG Reaction Mix A4/A8 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.
 - b. Check FAM signals LEG Reaction Mix A1~A8 for RNA gene variants for each sample (see Table 9):

LEG Reaction Mix A	A1	A2	A3	A4	A5	A6	A7	A8	Deculta
Detected Target	ALK	NTRKI	NTRK2	NTRK3	ROS1	ROS1	MET	RET	Kesuits
Positive Ct range	Ct<28	Positive							
Negative Ct range	Ct≥28	Negative or under the LOD*							

Table 9 Result Determination

* LOD: limit of detection

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

Analyze DNA mutation assay for each sample:

- 4) For LEG Reaction Mix B1~B8, analyze DNA gene mutations status:
 - a. Check FAM signals of LEG Reaction Mix B8 for each sample:
 - i. If FAM Ct values of LEG Reaction Mix B8 are \geq 17.5 and \leq 24, continue with the analysis.
 - If FAM Ct values of LEG Reaction Mix B8 is <17.5, it indicates the DNA is overloaded, the DNA amount should be reduced. If the mutation signals of LEG Reaction Mix B1~B7 are negative, the result is believable.
 - iii. If FAM Ct values of LEG Reaction Mix B8 >24, it indicates the partial fragmentation or degradation of DNA or the presence of PCR inhibitors. The sample should be retested with increased or re-extracted DNA.



b. Check FAM, VIC and ROX signals of LEG Reaction Mix B1~B7 for each sample (see Table 10):

LEG Reaction Mix B		B1	B2	B3	B4	B5	B6	B 7	Results
FAM	Optimal Ct range	Ct<30	Positive						
	Acceptable Ct range	30≤Ct <33	Interpret the						
	ΔCt Cut-off value	10	9	8	8	8	9	9	to the ΔCt value
	Negative Ct range	Ct≥33	Negative						
	Optimal Ct range	Ct<30	Positive						
VIC	Acceptable Ct range	30≤Ct <33	Interpret the						
	ΔCt Cut-off value	8	8	9	8	8	8	9	to the Δ Ct value
	Negative Ct range	Ct≥33	Negative						
	Optimal Ct range	/	/	/	/	/	/	Ct<30	Positive
ROX	Acceptable Ct range	/	/	/	/	/	/	30≤Ct <33	Interpret the
	ΔCt Cut-off value	/	/	/	/	/	/	9	results according to the ∆Ct value
	Negative Ct range	/	/	/	/	/	/	Ct≥33	Negative

Table 10 Result Determination

- If any FAM/VIC Ct value of LEG Reaction Mix B1~B7 or ROX Ct value of LEG Reaction Mix B7 is in Optimal Ct range, the sample is determined as corresponding mutation positive.
- ii. If any FAM/VIC Ct value of LEG Reaction Mix B1~B7 or ROX Ct value of LEG Reaction Mix B7 is in Acceptable Ct range, calculate the ΔCt value for each mutation showing positive amplification.
 - a) ΔCt = Mutant FAM (VIC/ROX) Ct value External Control FAM (VIC/ROX) Ct value. The Mutant Ct value refers to FAM/VIC/ROX Ct value of sample mutant signal, External Control Ct value refers to FAM/VIC/ROX Ct value of sample external control signal.
 - b) If the ΔCt value is less than the corresponding cut-off ΔCt value, the sample is determined as positive (Mutation detected).
 - c) If the Δ Ct value is equal or more than the corresponding cut-off Δ Ct value, the sample is determined as negative (No mutation detected) or under the LOD of the kit.
- iii. If all the FAM and VIC Ct values of LEG Reaction Mix B1~B7, ROX Ct value of LEG Reaction Mix B7 are in Negative Ct range, the sample is determined as negative (No mutation detected) or under the LOD of the kit.
- 5) Some cross-reactivity may occur between *KRAS* mutation reactions. If VIC signal in LEG Reaction Mix B5 and FAM signal in LEG Reaction Mix B6 are both positive, the reaction mix with smaller Ct value is determined as true positive, while the other reaction mix with bigger Ct value needs to be determined according to the cross-reactivity cut-off Δ Ct value criteria (see Table 11).
 - a) If the Δ Ct value is less than the cross-reactivity cut-off value, the positive curve is determined as true positive.
 - b) If the Δ Ct value is greater than or equal to the cross-reactivity cut-off value, the result is determined as negative.

Table 11 Cross-reactivity Cut-or	ff ∆Ct value
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Reaction Mix / Signal Mutation Name	B5/VIC	B6/FAM
KRAS-G12R (KRAS-M5)	5.58	
KRAS-G12C (KRAS-M6)		12.09



Note: If VIC Ct value of LEG Reaction Mix B5 is equal to FAM Ct value of LEG Reaction Mix B6, the result should be KRAS mutation positive in both LEG Reaction Mixes B5/B6 (co-occurrence).

6) The sample may contain two or more variants simultaneously.

Performance Characteristics

The performance characteristics of this kit were validated on LightCycler480 II, cobas® z480 and Bio-Rad CFX96.

- 1) Sensitivity:
 - For DNA mutation, the kit allows detection of 1~5% gene mutation in 10 ng DNA amount.
 - For RNA fusion, the kit allows detection of 25 copies/µL gene variant RNA.
- Accuracy: accuracy of the kit was established by testing internal positive references and negative references, the detection rates are 100%.
- Precision: precision of the kit was 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) The kit can only detect the 167 hotspot variants listed in the appendix.
- 4) Reliable results are dependent on proper sample processing, transport, and storage.
- 5) The sample containing degraded DNA or RNA may affect the ability of the test to detect the intended mutations or fusions.
- 6) Samples with negative result (No mutation detected) may harbor mutations or fusions not detected by this assay.

References

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Symbols



- REF Catalogue Number
 - Use By
 - Temperature Limitation
 - Keep Dry
 - Fragile, Handle With Care



Appendix 1

Tube / Signal	Target to detect	Fusion Type	Name
		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: 4LK exon20	EMI 4-ALK-11
		EML4 exon6: 4LK exon19	EML4-ALK-12
① FAM	AIK	<i>FMI 4</i> exon6:ins18 <i>41 K</i> exon20	EMI 4-AI K-13
		FMI4 exon 20 ins 18 AIK exon 20	EMI 4-AI K-14
		EML4 exon17de158 ins39 41 K exon20	EMI 4-AI K-17
		EML4 exon17 ins65: 41 K exon20	EML4-ALK-17
		EML4 exen17 ins05,4LK exen20	EML4-ALK-10
		EML4 exem 17 ins f uins 24 ALK exem 20	EMIL4-ALK-19
		EML4 exon2/ins54 ALK exon20	EMIL4-ALK-20
			ENIL4-ALK-21
		KIF5B exon24;ALK exon20	KIF5B-ALK-1
		KIF5B exon1/;ALK exon20	KIF5B-ALK-2
		KLCT exon9;ALK exon20	KLUI-ALK
		TFG exon4;ALK exon20	IFG-ALK
			NIKKI-E9-MI
			NIRKI-EI0-MI
			NTRK1-E10-M3
		TPR exon16 del54;//TRK1 ins13 exon10	NTRKT-E10-M5
		$\frac{1}{1} \frac{1}{1} \frac{1}$	NIRKI-EI0-M6
			NIRKI-EI0-M/
		IRF2BP2 exon1;NIRK1 exon10	NIRKI-E10-M8
		IRF2BP2 exon1 del48;NTRK1 exon10	NTRK1-E10-M9
2 FAM	NTRK1	TFG exon5;NTRK1 exon10	NTRK1-E10-M12
		GRIPAPI exon22;NIRKI exon10	NIRKI-E10-M14
		FIIR exon4;NIRKI exon10	NIRKI-EI0-MI5
		SQSIMI exon6;NIRKI exon10	NIRKI-EI0-MI7
		<i>IPM3</i> exon8; <i>NIRK1</i> exon12	NIRKI-EI2-MI
		MPRIP exon21;NIRK1 exon12	NTRK1-E12-M3
		SSBP2 exon12;NTRK1 exon12	NIKKI-E12-M4
		MPRIP exon14;NTRK1 exon12	NTRK1-E12-M11
		MPRIP exon18;NTRK1 exon12	NTRK1-E12-M12
		GRIPAP1 exon22;NTRK1 exon12	NTRK1-E12-M14
		TRIM24 exon12;NTRK2 exon15	NTRK2-E15-M1
		TRIM24 exon12;NTRK2 exon16	NTRK2-E16-M1
(3) FAM	NTRK2	SQSTM1 exon5;NTRK2 exon16	NTRK2-E16-M3
		STRN exon3;NTRK2 exon16	NTRK2-E16-M7
		SQSTM1 exon5;NTRK2 exon17	NTRK2-E17-M2
		ETV6 exon4;NTRK3 exon14	NTRK3-EX14-M1
		ETV6 exon5;NTRK3 exon14	NTRK3-EX14-M2
		EML4 exon2;NTRK3 exon14	NTRK3-EX14-M3
④ FAM	NTRK3	SQSIMI exon5;NTRK3 exon14	NTRK3-EX14-M4
		RBPMS exon5;NTRK3 exon14	NTRK3-EX14-M7
		EIV0 exon5;NTRK3 exon15	NIRK3-EX15-M1
		EIVO exon4;NTRK3 exon15	NTRK3-EX15-M2
		SQSIMI exon6;NTRK3 exon15	NTRK3-EX15-M3
Ann		<i>SLC34A2</i> exon4; <i>ROS1</i> exon32	ROS1-M1
5 FAM	KOSI	$SLC34A2 \exp 13 \text{ del}2046; ROS1 \exp 32$	RUSI-M2
1	1	CD/4 exono; KOS1 exon32	KUS1-M3

Gene Fusions Detected with LEG Reaction Mix A



		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
		TPM3 exon8;ROS1 exon35	ROS1-M11
6 FAM	ROSI	LRIG3 exon16;ROS1 exon35	ROS1-M12
		GOPC exon8;ROS1 exon35	ROS1-M13
⑦ FAM	MET	MET Exon 14 skipping mutation	MET-M2
		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
© FAM	DET	CUXI exon10;RET exon12	LRET-M32
8 FAM	KEI	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

Appendix 2

Gene Mutations Detected with LEG Reaction Mix B

Tube / Signal	Target to detect	Mutation	Base Change	Cosmic ID	Name	LOD
		E746_A750del (1)	2235_2249del15	6223	E-19-M1	1%
		E746_A750del (2)	2236_2250del15	6225	E-19-M2	1%
		L747_P753>S	2240_2257del18	12370	E-19-M3	1%
		E746_T751>I	2235_2252>AAT(complex)	13551	E-19-M4	1%
		E746_T751del	2236_2253del18	12728	E-19-M5	1%
		E746_T751>A	2237_2251del15	12678	E-19-M6	1%
		E746_S752>A	2237_2254del18	12367	E-19-M7	1%
		E746_S752>V	2237_2255>T(complex)	12384	E-19-M8	1%
		E746_S752>D	2238_2255del18	6220	E-19-M9	1%
		L747_A750>P	2238_2248>GC(complex)	12422	E-19-M10	1%
		L747_T751>Q	2238_2252>GCA(complex)	12419	E-19-M11	1%
		L747_E749del	2239_2247del9	6218	E-19-M12	1%
① FAM	EGFR Exon 19	L747_T751del	2239_2253del15	6254	E-19-M13	1%
		L747_S752del	2239_2256del18	6255	E-19-M14	1%
		L747_A750>P	2239_2248TTAAGAGAAG>C(complex)	12382	E-19-M15	1%
		L747_P753>Q	2239_2258>CA(complex)	12387	E-19-M16	1%
		L747_T751>S	2240_2251del12	6210	E-19-M17	1%
		L747_T751del	2240_2254del15	12369	E-19-M18	1%
		L747_T751>P	2239_2251>C(complex)	12383	E-19-M19	1%
		L747_T751del	2238_2252del15	23571	E-19-M20	1%
		L747_S752>Q	2239_2256>CAA(Complex)	12403	E-19-M21	1%
		L747_A750>P	2239_2250>CCC(Complex)	/	E-19-M24	1%
		L747_K754>QL	2239_2261>CAATT(Complex)	/	E-19-M25	1%
		E746_K754>EQHL	2238_2261>GCAACATCT(Complex)	/	E-19-M26	1%
		L747_S752>Q	2238_2256>GCAA (Complex)	26441	E-19-M27	1%
① VIC	EGFR Exon 20	S768I	2303G>T	6241	E-20-M2	1%
2 FAM	EGFR Exon 21	L858R	2573T>G	6224	E-21-M1	1%



@VR @VR @VR @VR \$COTISC21550-A0.41500.115000.21500@VR @VR @VR \$26780-210.01760.2150-710.2130.21000.2100@VR @VR \$106100.2167-100.2100.21000.21000.2100@VR \$107171710-0023100.21071.210-000.0000.000\$107171710-0023102310-0001.01201.21000.0000.000\$107171710-0023102310-0001.01201.21001.01000.0			G719A	2156G>C	6239	E-18-M1	1%
000 <th< td=""><td>2 VIC</td><td>EGFR Exon 18</td><td>G7198</td><td>2155G>A</td><td>6252</td><td>E-18-M2</td><td>2%</td></th<>	2 VIC	EGFR Exon 18	G7198	2155G>A	6252	E-18-M2	2%
③ FIGMSGP Reso 20T79M236/CT94/908-29-0072③ VICSGP Reso 21164/02357.562/16-21.4218⑤ VICSGP Reso 21154/02319.232/mcCAC12376-23.4418○ T/D ST/TimaC2311.231/mcGCACCCGT13786-23.4419○ T/D ST/TimaC2311.231/mcGCACCCGT13786-23.4419○ T/D ST/TimaC2319.232/mcCACAC13886-23.4419○ T/D ST/TimaC2319.231/mcGCACCCGT13886-23.4419○ T/D ST/TimaC2319.231/mcCACACCGTCGACA13886-23.4419○ T/D ST/TimaC2319.231/mcCACAC0635726-23.4419○ T/D ST/TimaC2319.231/mcCACAC073.056-23.4419○ T/D ST/TimaC2319.231/mcCACAC13846-23.4419○ T/D ST/TimaC2319.231/mcCACAC13846-23.4419○ T/D ST/TimaC2319.231/mcCACAC139.46-23.4419○ T/D ST/TimaC2319.231/mcCACAC139.46-23.4419○ T/D ST/TimaC2319.231/mcCACAC139.46-23.4419○ T/D ST/TimaC2319.231/mcCACAC139.46-23.4419○ T/D ST/TimaC2319.231/mcCACACCC693.0019.46-3.44○ T/D ST/TimaC2319.231/mcCACACCC693.0019.46-3.44○ T/D ST/TimaC2319.231/mcCACACCCC693.0019.419.4○ T/D ST/TimaC2319.231/mcCACACCCC693.0019.419.4			G719C	2155G>T	6253	E-18-M3	1%
(© MC) <i>LGPL</i> bas21 LB40 Signal Pip (H) T 1973 Y714ml 2319 220mc/CC 12378 F.23M4 145 (H) T 2319 2311mcGCT 12378 F.23M4 155 (H) T 2319 2311mcGCT 12378 F.23M4 155 (H) T 2319 2311mcGCT 12378 F.23M4 155 (H) T 2319 2311mcGCT 1358 F.23M4 155 (H) T 2319 2311mcGCT 1558 F.23M4 155 (H) T 771maH 2319 2310mcACCCACC 1230 E-23M10 155 (H) T 771maH 2319 230mcACCCACC 12300 E-23M10 155 (H) T 1713 771maH 2319 230mcACCCACC 693120 E-23M10 156 (H) T 1711maH 2311 2112mcACCCAC 693120 E-23M40 156 (H) T 1711maH 2311 2311mcGCC 693120 E-23M40 156 (H) T 1711maH 2310 2311mcGCCACC 693120 E-23M40 156 </td <td>③ FAM</td> <td>EGFR Exon 20</td> <td>T790M</td> <td>2369C>T</td> <td>6240</td> <td>E-20-M1</td> <td>2%</td>	③ FAM	EGFR Exon 20	T790M	2369C>T	6240	E-20-M1	2%
	③ VIC	EGFR Exon 21	L861Q	2582T>A	6213	E-21-M2	1%
ProgrammedDisplaceDisplaceDisplaceDisplaceNormalProgrammed20122012200020122		-	H773_V774insH	2319_2320insCAC	12377	E-20-M3	1%
			D770 N771insG	2310 2311insGGT	12378	E-20-M4	1%
			V769 D770insASV	2307 2308insGCCAGCGTG	12376	E-20-M5	1%
(3) FAM 23(9) 210AC-CCAGCGTGGAT 1358 4:28.040 (95) (1177) VTHamSPH 216 230imsAACCCCAC 12381 15:20.0410 (15) (1177) VTHamSPH 2312 230imsCCCAC 12381 15:20.0416 (15) (1177) VTHamSPH 2312 230imsCCCAC 12380 15:20.0416 (15) (1177) VTHamSPH 2312 230imsCCCAC 12880 15:20.0416 (15) (1177) VTHamSPH 2312 230imsCCACCTGG 18429 15:20.0416 (15) (1177) VTHimSPH 2310 231imsCGCCAC 18629 15:20.0416 (15) (1177) VTHimSP 2310 231imsCGCAC 10:20.15 15:20.0416 (15) (1177) VTHimSP 2310 231imsCGCAC 10:20.15 15:20.0416 (15) (1171) VTHIMSP 2310 231imsCGCAC 10:20.15 15:20.0416 (15) (1171) VTHIMSP 2310 231imsCGCAC 10:20.15 15:20.0416 (15) (1171) VTHIMSP 2310 231imsCGCAC 10:20.14 15:20.0416 (15) (1171) VTHIMSP 2310 231imsCGCACCC 10:20.0416 15:20.0		-	 D770 N771insSVD	 2311_2312insGCGTGGACA	13428	E-20-M8	1%
		-	V769 D770insASV	2309_2310AC>CCAGCGTGGAT	13558	E-20-M9	5%
(0) FORM 10.00 1.00.00 <td< td=""><td></td><td>-</td><td>H773 V774insNPH</td><td>2319 2320insAACCCCCAC</td><td>12381</td><td>E-20-M10</td><td>1%</td></td<>		-	H773 V774insNPH	2319 2320insAACCCCCAC	12381	E-20-M10	1%
(b) FAM 0.00.00000000000000000000000000000000		-	D770 N771insGF	2310_2311insGGGTTT	655155	E-20-M14	1%
(0) FAM (1) F13 V174min (2) F13 V174min <td></td> <td>-</td> <td>N771 D772ingH</td> <td>2211 2212/msACC</td> <td>6062572</td> <td>E 20 M16</td> <td>170</td>		-	N771 D772ingH	2211 2212/msACC	6062572	E 20 M16	170
(a) FAM 1011/2, 17/4881 1011/2, 20081/CAC (c) 1250 1250 150 (a) FAM 1173, 17/4881 1210, 22086/CCC 12850 120041 151 (b) FAM 1210, 22186/CCC 6931207 1520421 151 (b) FAM 1210, 22118/CCC 6931207 1520422 151 (c) FAM 1210, 22118/CCC 6931207 1520422 151 (c) FAT, 1771860 1210, 22118/CCC 6961207 1220421 151 (c) FAT, 1771860 1210, 22118/CCC 6961207 1220446 151 (c) FAT, 1771860 1210, 22118/CCC1 6961207 1220446 151 (c) FAT, 1771860 1210, 22118/CCC1 12304 1520446 151 (c) FAT, 1771860 1210, 22118/CCC1 12304 1520446 151 (c) FAT, 1771860 1210, 22118/CCC1 12304 150 151 (c) FAT, 1771877870 1210, 22118/CCC1 12304 150 151 (c) FAT, 1771877870 1210, 2211/22118/CCT 12304 150		-	N//1_F//2IIISH	2311_2312IIISACC	0903372	E-20-M10	170
			H//3_V//4108 Y		10200	E-20-M118	170
(*) FAM 2/18/_JAMmidA AACCITCG (*) H2/-M (*) (*) FAM EGFR Exon 20 DT/0_TTI_HIT 2311_2311msGGG (*) E20-M33 (*) (*) FAM DT/0_TTI_HIG 2310_2311msGGG (*) E20-M33 (*) (*) FAM DT/0_TTI_HIG 2310_2311msGGG (*) E20-M34 (*) (*) FT/1_TTI_HIGD 2310_2311msGGGCA 690.500 E-20-M34 (*) (*) T/0_TTI_HIGD 2310_2311msGGGGAC 8973 E-20-M34 (*) (*) T/0_TTI_HIGD 2310_2311msGGGACA 8921 E-20-M34 (*) (*) T/0_TTI_HIGD 2310_2311msGGGACA 1831 E-20-M34 (*) (*) T/0_TTI_HIGD 2310_2311msGGACAACCG1 13831 E-20-M34 (*) (*) T/1_TTI_HIGT 2311_2315mACT 643817 E-20-M44 (*) (*) T/1_TTI_HIGT 2310_2311msGGACACCC1 12389 E-20-M41 (*) (*) T/1_TTI_HIGT 2310_2311msGACACC 638147 E-20-M41 (*) (*) T/1_TTI_HIGT 2310_2311msGACACCC 6381		-	H//3_V//4insPH	2319_2320insCCCCAC	12380	E-20-M19	1%
Beta EGFR Eson 20 N771 P772miHild 2311 2311maGC 6031 C 6331 C 176 176 0 F100 N771maG 2310 2311maGGC 13004 E-20-M24 1% 0 F701 N771maG 2310 2311maGGC 69600 E-20-M24 1% 0 T700 N771maGD 2310 2311maGGGTA 4873 E-20-M36 1% 0 T700 N771maGD 2310 2311maGGGTA 4891 E-20-M36 1% 0 T710 N771maGD 2310 2311maGGGTA 4891 E-20-M36 1% N711 N771-NRGP 2311 2314A-GAGGTT 18431 E-20-M46 1% N711 N771-NRGP 2312 2315ACC-13(GGTGGAGAACCG) 1354 E-20-M46 1% N711 N771-NRT 2314 2314A-GGGTT 43814 E-20-M46 1% N711 N771-NRT 2314 2314A-GGGTT 43814 E-20-M46 1% N711 N771-NRT 2314 2314A-GGGTCCCC 13984 E-20-M46 1% N711 N771-NRT 2314 2314A-GCCCT 13984 HE2-M46 1% N711 N771-NRT 2314 2314A-GCCCCC 13984 HE2-M46<		-	V/69_D//0insGSV	2308_2309insGCAGCGTGG	18429	E-20-M21	1%
(a) FAM BG/R Eson 20 (b) 7070 X7T lineG 2310 2311 msGGG (b) E-20-M23 (b) F7070 X7T lineG 2310 2311 msGGC 13004 E-20-M26 (b) F70 X7T lineG 2310 2311 msGGC 13024 FB P772 P770 K7T lineG 2310 2311 msGGC 682859 E-20-M26 (b) FF D770 S770 K7T lineG 2310 2311 msGGGCAC 85795 E-20-M37 (b) FF D770 N7T lineGT 2310 2311 msGGGCAC 85795 E-20-M37 (b) FF D770 N7T lineGT 2310 2311 msGGCACA 123802 E-20-M37 (b) FF N7T P7TimVTN 2312 2311 msGGCACA 123802 E-20-M41 (b) FF (b) FF (b) F N7T P7TimVTN 2310 2311 msGCACACCT 12385 E-20-M41 (b) FF (b) F N7T P7TimVTN 2310 231 msGCACACCT 12385 E-20-M41 (b) FF (b) F P770 Y7T INTNN 2310 2316 msGGCTCCC 12385 HER2-M1 (b) F P770 Y7T INTSNP 2310 2340 msGGCTCCCC 12355 HER2-M1 (b) F	~		N771_P772insHH	2311_2312insACCACC	6931207	E-20-M22	1%
(a) VIC IDPO_N71imsGP 2310_2311msGCC 1904 ID-20-M24 195 (b) P172_H773imS0P 2307_2308msACAACCCC 6903050 E-20-M36 195 (c) D70_N71imsGL 2310_2311msGGGAC 87951 E-20-M36 195 (c) D70_N71imsGL 2310_2311msGGGAC 87951 E-20-M36 195 (c) N71_N71-GF 2312_312AA-GGGTT 18411 E-20-M36 195 (c) N71_N71-GF 2312_312AA-GGGTT 18431 E-20-M36 195 (c) N71_N71-R1 2312_312AA-CGGTT 13841 E-20-M41 296 (c) N71_N71-R1 2312_312AA-CGGTT 12885 E-20-M41 296 (c) N71_N71-R1 2316_CAACCCT 12384 E-20-M41 296 (c) N71_N71-R1 2316_23ACCCT 12388 E-20-M41 196 (c) N71_H77_N71-B7 2316_23ACCCT 12388 E-20-M41 196 (c) N71_H77_N71-B7 2316_2323miGT 1253 HREA-M1 196 (c) N71_H77_N71-B7 2316_232323miGT 1255 HREA-M1 196	(4) FAM	EGFR Exon 20	D770_N771insG	2310_2311insGGG	/	E-20-M23	1%
(b) YIC P772_H73mDNP 2207_2306mGACAACCCC 96900 E-20-M36 1% D770-GY 2308_2309mGTT 112427 E-20-M36 1% D770-GY 2308_2309mGTT 14247 E-20-M36 1% D770-N771mGC 2310_2311mGGGGACA 88791 E-20-M36 1% D770-N771mGC 2311_2312AA-GGGTT 48821 E-20-M36 1% N771-P72-SYDNR 2312_2314ACC1QCCTGGACAACCC0 15354 E-20-M46 1% D770-N771mGT 2310_2311mGGCACA 1238029 E-20-M46 1% N771-P72-SYDNR 2312_2313mGCT 18831 E-20-M46 1% D770-N771mGT 2313 2315-2372mGTC 12388 E-20-M56 1% P770-1771-P72-SYDNR 2316_2372mGTC 12388 E-20-M56 1% P770-1771-P72-SYDNR 2316_2372mGTC 12388 HER2-M6 1% P770-1731mGP 2316_2327mGTC 12354 HER2-M6 1% P780_Y781mGSP 2339_2406mGGCTCCCC 12554 HER2-M6 1%			D770_N771insG	2310_2311insGGC	13004	E-20-M24	1%
Price D70-CY 2308_209insGT 12427 E-20-M36 1% D700_VT1insGD 2310_2311insGGGGAC 8592 E-20-M36 1% D700_VT1insGL 2310_2311insGGGGTA 48921 E-20-M36 1% N711-CF 2311_2312AA-GGGTT 18431 E-20-M36 1% N711-CF 2312_2315ACCC>13(GGGGACAACCG) 123802 E-20-M41 1% D700_VT1insGT 2312_2315insGCACAC 123802 E-20-M36 1% D700_VT1insGT 2312_2315insGCAACCGT 12382 E-20-M31 1% P72_UT73inSP 2310_2311insGCACACCT 12388 E-20-M31 1% H773-PRPY 2317_2315insGCACC 13985 E-20-M31 1% P78_UT78insGP 2339_2340insGGCTCCC 303848 HRR2-M1 1% P78_UT78insGP 2339_2340insGGCTCCCC 12551 HR2-M1 1% G76-CV 2326_2327insTT 1252 HRR2-M1 1% G76-CVC 2326_2327insTT 156 HR2-M1 1% G776-CC 2			P772_H773insDNP	2307_2308insGACAACCCC	6962050	E-20-M26	1%
(a) VIC 1.0700 N71insGD 2310 2311msGGGGAC 88795 1.62.0.M30 19% (b) VIC 0.700 N71insGL 2310 2311msGGGTA 1481 1.52.0.M30 19% (b) N711-07CF 2311 231AA-GGGTT 1481 1.52.0.M30 19% (b) VIC (b) N711-07C N71imGT 2312 2315mACC 163840 1.52.0.M40 19% (b) VIC (b) N711-07D N71imGT 2310 2311msGGCACA 12884 1.52.0.M40 1.52.0.M40 1.55.0.M60 1.9% (c) VIC (b) N711-07D N71imGT 2310 2315mACT 1643847 1.52.0.M40 1.56.0.M60 1.9% (c) VIC (c) N711-07D N71imGT 2310 2315mACT 17388 1.52.0.M40 1.56.0.M60 1.9% (c) VIC (c) N711-07D3mVD 2310 2315mACT 1.75.6.0			D770>GY	2308_2309insGTT	12427	E-20-M34	1%
Image: Probability of the second se			.D770_N771insGD	2310_2311insGGGGAC	85795	E-20-M36	1%
NTI-OF 2311_2312ACGGGT 18431 52.0438 1% NTI_PT2-SYDNR 2312_2315ACCC-13(GGGGACACG) 10354 62.0440 1% DT70_NTIINGT 2312_2315ACCC-13(10GGACACG) 12302 52.0441 2% NTI_PT2INTON 2207_2308m5ACACACGG 6438147 62.0441 1% NTI_PT2H73mTP 2316_231m5GCACCC 12388 62.0443 1% HT73-PNPY 2317_2181m5CACCCT 1238 62.0443 1% P780_VT81m6SP 2339_2340m5GGCTCCCC 12555 HE8.244 1% P780_VT81m6SP 2339_2340m5GGCTCCCC 12555 HE8.246 1% P780_VT81m6SP 2339_2340m5GGCTCCCC 12555 HE8.246 1% G76-VC 2326_327mTT 12554 HE8.246 1% G76-VC 2326_327mTT 12554 HE8.2461 1% G76-VC 2326_327mTT 12554 HE8.2461 1% G76-VC 2326_327mTT 12554 HE8.2461 1% G766-VC 2326_327mTT 12554			D770_N771insGL	2310_2311insGGGTTA	48921	E-20-M37	1%
NTI_PT2>SVDNR 2312_2315ACCC-13(GCGTGGACAACCG) 1054 E-20-440 [1]5 DTO_NTTIINGT 2310_2311imsGGACA 123802 E-20-M10 [1]5 NTTI_PT2:sVDNR 2307_2308imsGACAACGTG 2085 E-20-M10 [1]5 PT72_HT71:mTP 2316C-AACCCT 1238 E-20-M15 [1]5 HER2-MA 715 HER2-M3 [1]5 [1]5 [1]5 GT676-VC 2335_2321miGTA [1]55 HER2-M6 [1]5 P780_YT81imGSP 2339_2340imGGGCTCCC [1]55 HER2-M6 [1]5 GT676-VC 2336_2327miGTT [1]55 HER2-M6 [1]5 GT676-VC 2336_2327miGTT [1]55 HER2-M6 [1]5 GT676-VC 2336_2327miGTT [1]55 HER2-M6 [1]55 GT676-VC 2336_2327miGTT [1]55 HER2-M10 [1]56 GT676-VC 2336_237miGTT [1]55 HER2-M10 [1]56 GT676-VC 2326_237miGTT [1]55 HER2-M10 [1]56 GT676-VC 2326_237miGT </td <td></td> <td></td> <td>N771>GF</td> <td>2311_2312AA>GGGTT</td> <td>18431</td> <td>E-20-M38</td> <td>1%</td>			N771>GF	2311_2312AA>GGGTT	18431	E-20-M38	1%
Product D770_NT7imsGT 2310_2311insGGCAA 1238029 E-20-Mil 1% N711-KL C312_231insACT 643817 E-20-Mil 2% N771_P772insVDN 2307_308insGACACGTG 2088 E-20-Mil 1% P772_H773insTP C316C-AACCCT 12388 E-20-Mil 1% H773_PNPY 2317_2318insCTAACCCT 1735761 E-20-Mil 1% P780_Y78insGSP 2339_2340 insGGCTCCCC 303948 HER2-Mil 1% P780_Y78insGSP 2339_2340 insGGCTCCCC 12556 HER2-Mil 1% P780_Y78insGSP 2340_2341insGGCTCCCC 12556 HER2-Mil 1% G776-VC 2236_2327insTT 1252 HER2-Mil 1% G776-VC 2236_2327insTCT 8995 HER2-Mil 1% G776-VC 2236_2327insTCT 8995 HER2-Mil 1% G776-VC 2236_237insTCT 8995 HER2-Mil 1% G776-VC 2236_237insTCT 8995 HER2-Mil 1% G776-LC 2326_237in			N771_P772>SVDNR	2312_2315ACCC>13(GCGTGGACAACCG)	13554	E-20-M40	1%
NTI-KL2312_2313msACT6438147E-20-M442%NTI-PTI-KL2307_2308msGACACGTG2085E-20-M521%PTZ_11773msTP2316_2AACCCCT173576E-20-M551%HT73-PNPY2317_2318msCAACCCCT173576E-20-M551%P780_Y781msGP2319_2318msCAACCCC175576E-20-M551%P780_Y781msGP2339_2340msGGGCTCCCC303948HER2-M41%P780_Y781msGP2339_2340msGGGCTCCCC12555HER2-M41%P780_Y781msGP2339_2340msGGGCTCCCC12555HER2-M11%G776-VC2336_2327msTT1/2552HER2-M11%G776-VC2336_2327msTT1/2552HER2-M11%G776-VC2336_2327msTT1/161%1%G776-VC2326_2327msTT1/161%G776-VC2326_2327msTT1/161%G776-VC2326_2327msTT1/161%G776-VC2326_2327msTT1/161%G776-VC2326_2327msTT1/161%G776-VC2326_237TAT20895HER2-M11%G776-VC2326_27TAT20895HER2-M11%G776-VC2326_27TAT19832E20-M331%G776-VC2326_27TAT19832E20-M341%MTG776-VC2326_27TAT19832E20-M331%MTG776-VC2326_27TAT12554HER2-M11%MTG776-VC2326_27TAT12554HER2-M11% <td< td=""><td></td><td></td><td>D770_N771insGT</td><td>2310_2311insGGCACA</td><td>1238029</td><td>E-20-M41</td><td>1%</td></td<>			D770_N771insGT	2310_2311insGGCACA	1238029	E-20-M41	1%
NTL PT2 PT2 PT2 PT3 			N771>KL	2312_2313insACT	6438147	E-20-M44	2%
P172_H773insTP2316C>AACCCT12388E-20-M551%H773>PSPFY2317_2318insCTAACCCT1735761E-20-M561%FRAWG776>VC2236_2327insTGT1253HER2-M31%P780_Y781insGSP2339_2340insGGCTCCCC30398HER2-M61%P780_Y781insGSP2339_2340insGGCTCCCC12556HER2-M61%G776>VC2236_2327insTTT12552HER2-M81%G776>VC2236_2327insTTT12552HER2-M101%G776>VC2236_2327insTT12552HER2-M101%G776>VC2236_2327insTT18595HER2-M101%G776>VC2236_2327insTT28895HER2-M101%G776>VC2236_2327insTT28895HER2-M101%G776>VC2236_2327insTT28895HER2-M101%G776>LC2236_237insTGT8595HER2-M101%G776>LC2236_237insTGG2895HER2-M101%G776>LC2236_237insTGG1882E-20-M211%G776>LC2236_237insTGG1882E-20-M311%WT1_G775insHV2232_232insCACGTG1882E-20-M321%WT1_G775insHV2232_232insCACGTG1842E-20-M321%WT1_G775insHV2312_232insCACGTG12382E-20-M321%WT1_G775insHV2312_232insCACGTG18432E-20-M321%WT1_G775insHV2316_2131insGTT2505E-20-M421%WT1_G775insHV2316_2131insGTT516			N771_P772insVDN	2307_2308insGACAACGTG	20885	E-20-M52	1%
HT73-PNPY2317_2318insCTAACCCT1735761E-20-M561%RegenerationG776-VC2226_2327insTCT12553HER2-M31%P780_Y781insGSP2339_2340insGGCTCCCC303948HER2-M41%P780_Y781insGSP2339_2340insGGCTCCCC12555HER2-M61%G776-VC2326_2327insTTT12552HER2-M81%G776-VC2326_2327insTTT12552HER2-M11%G776-VC2326_2327insTTT12552HER2-M11%G776-VC2326_2327insTTT1HER2-M11%G776-VC2326_2327insTTT11%1%G776-VC2326_2327insTTT11%1%G776-VC2326_2327insTTT11%1%G776-VC2326_2327insTTT12554HER2-M11%G776-VC2326_2327insTT20895HER2-M11%G776-VC2326_2377insTT12554HER2-M21%G776-VC2326_2377insTT12554HER2-M21%G776-VC2326_2377insTT19875HER2-M21%G776-VC2326_2377insTT19875HER2-M21%G776-VC2326_2377insTT19875HER2-M21%G776-VC2326_237718432E-20-M331%G776-VC2326_2377insCT18432E-20-M331%G776-VCG12C34G>C15%KRAS-M52%G12AG12A35G>C522KRAS-M32%G12AG12A35G>T <td></td> <td></td> <td>P772_H773insTP</td> <td>2316C>AACCCCT</td> <td>12388</td> <td>E-20-M55</td> <td>1%</td>			P772_H773insTP	2316C>AACCCCT	12388	E-20-M55	1%
Pressure (*) VICCOTOS>VCCOTOS>VCCOTOSHER2-M3H/HP780_V781imsGSP2339_2340 insTGGCTCCCC303948HER2-M41%P780_V781imsGSP2339_2340insGGCTCCCC12555HER2-M61%P780_V781imsGSP2340_2341insGGCTCCCC6865803HER2-M11%G776-VC2232_2327imsTT12552HER2-M11%G776-VC2326_2327imsTT12552HER2-M11%G776-VC2326_2327imsTT1/1HER2-M151%G776-VC2326_2327imsTA/HER2-M161%G776-VC2326_2327imsTA/HER2-M161%G776-VC2326_2327imsTA/HER2-M161%G776-VC2326_2327imsTA/HER2-M161%G776-VC2326_2327imsTA12554HER2-M161%G776-VC2326_2327imsTA12554HER2-M161%G776-VC2326_2327imsTA12554HER2-M161%G776-VC2326_2327imsTA12554HER2-M161%G776-VC2326_2327imsTA12554HER2-M161%G776-VC2326_2327imsTA12554HER2-M161%G776-VC2326_2327imsTA12554HER2-M161%G776-VC2326_2327imsTA12554HER2-M161%MT4_T7_G775mSVG2322_23256-CTTT12554HER2-M121%G776-VC3232_23237imsCACGTG1244F20-M321%G776-VCG12C34G>T516KRAS-M32% </td <td></td> <td></td> <td>H773>PNPY</td> <td>2317_2318insCTAACCCCT</td> <td>1735761</td> <td>E-20-M56</td> <td>1%</td>			H773>PNPY	2317_2318insCTAACCCCT	1735761	E-20-M56	1%
(a) VIC P780_Y781msGSP 2339_2340 insTGGCTCCC 303948 HER2.M4 1% (b) VIC P780_Y781msGSP 2339_2340imsGGCTCCCC 1255 HER2.M6 1% (c) VIC P780_Y781msGSP 2340_2341msGGCTCCCA 1255 HER2.M6 1% (c) VIC P780_Y781msGSP 2340_2341msGGCTCCCA 1255 HER2.M1 1% (c) VIC G776-VC 2332_2327msTTT 1252 HER2.M1 1% (c) G776-VC 2326_2327msTAT / HER2.M1 1% (c) G776-VC 2326_2327msTAT 20895 HER2.M1 1% (c) G776-VC 2326_2321msCAGGTG 20895 HER2.M1 <td></td> <td></td> <td>G776>VC</td> <td>2326_2327insTGT</td> <td>12553</td> <td>HER2-M3</td> <td>1%</td>			G776>VC	2326_2327insTGT	12553	HER2-M3	1%
Pressure (*) VICP780_Y781insGSP2339_2340insGGCTCCCC12555HER2-M61%P780_Y781insGSP2340_2341insGCTCCCCA12556HER2-M71%G776-VC2226_2327insTTT12552HER2-M81%G776-VC2326_2327insTTT1HER2-M161%G776-VC2326_2327insTTT1HER2-M161%G776-VC2326_2327insTCT4HER2-M161%G776-VC2326_2327insTCT89955HER2-M161%G776-VC2326_2327insTCT89955HER2-M161%G776-VC2326_2327insTCT89955HER2-M161%G776-VC2326_2327insTCT89955HER2-M161%G776-VC2326_2327insTCT19875HER2-M161%G776-VC2326_2327insTCT19875HER2-M211%G776-VC2326_2327insTCTG19875HER2-M211%G776-VC2326_2327insCCAGT19875HER2-M211%G776-VC2326_2327insCCAGT19875HER2-M211%MT74_C775insHV2321_232insCCAGT18432E20-M331%SVICKR45 Exon 2G12A336G-T520KRA5M61%G12A336G-T520KRA5M61%1%G12A336G-T520KRA5M62%1%G12A336G-T520KRA5M12%1%G12A336G-T520KRA5M52%1%G12A336G-T520KRA5M52%1% </td <td></td> <td></td> <td>P780_Y781insGSP</td> <td>2339_2340 insTGGCTCCCC</td> <td>303948</td> <td>HER2-M4</td> <td>1%</td>			P780_Y781insGSP	2339_2340 insTGGCTCCCC	303948	HER2-M4	1%
(a) VIC $PR8_V781insGSP$ $2340_2341insGGCTCCCA$ 12556 $HR2.M7$ 1% (b) VIC $HR2$ Exon 20 $G776-VC$ $2326_2327insTT$ 12552 $HR2.M8$ 1% $P780_V781insGSP$ $2339_2340insCGGCTCCCC$ 6865893 $HER2.M10$ 1% $G776-VC$ $2326_2327insTAT$ / $HER2.M15$ 1% $G776-VC$ $2326_2327insTCT$ 85995 $HER2.M15$ 1% $G776-VC$ 2326_3CTTAT 20895 $HER2.M16$ 1% $G776-VC$ $2326G-TTTT$ 20895 $HER2.M11$ 1% $G776-LC$ $2326G-TTTT$ 19875 $HER2.M21$ 1% $V777_0778insCG$ $2331_2332insTGTGGG$ 303939 $HER2.M21$ 1% $V774_0778insV$ $2320_2321ainsCACGT$ 18432 $E-20.M32$ 1% $V774_077 insV$ $2320_2321insCACGT$ 18432 $E-20.M33$ 1% $V774_077 insV$ $2320_2321insCACGT$ 18432 $E-20.M33$ 1% VIC $KRAS E$			P780_Y781insGSP	2339_2340insGGGCTCCCC	12555	HER2-M6	1%
(a) VIC 6776 >VC $2326_{2327insTTT}$ 1252 HER2-M8 1% (b) $7780_{1}Y81insGSP$ $2339_{2}340insCGGCTCCC$ 6865893 HER2-M10 1% (c) $G776$ >VC $2332_{2}2327insTAT$ / HER2-M15 1% (c) $G776$ >VC $2326_{2}237insTCT$ 85995 HER2-M16 1% (c) $G776$ >VC 2326_{0} -STGT 85995 HER2-M16 1% (c) $G776$ >VC 2326_{0} -STGT 20895 HER2-M16 1% (c) $G776$ -VC 2326_{0} -STGT 19875 HER2-M12 1% (c) 7774_{0} C775insHV 2321_{232} SinsCACGTG 29488 $E-20-M33$ 1% (c) KR4S Exon 2 G12			P780_Y781insGSP	2340_2341insGGCTCCCCA	12556	HER2-M7	1%
Product Product Name			G776>VC	2326 2327insTTT	12552	HER2-M8	1%
(a) VIC $HER2 Exon 20$ \Box \Box I	~		P780 Y781insGSP	2339 2340insCGGCTCCCC	6865893	HER2-M10	1%
$ \left(\begin{array}{cccccccccccccccccccccccccccccccccccc$	(4) VIC	HER2 Exon 20		2326 2327insTAT	/	HER2-M15	1%
$ \begin{array}{c c c c c c } & & & & & & & & & & & & & & & & & & &$			G776>VC		85995	HER2-M16	1%
G776-LC2226G>CTTT12554HER2-M201%G776-LC2226G>TGT19875HER2-M211%V771G776insCG2331_232insTGTGGG303939HER2-M241%% FAMY774_C775insHV2232_232insCACGTG18432E-20-M321%% FAMAT75_V774insAH2320_2321insCCACG1238028E-20-M331%% VTCKRAS Exon 2G12C34G>T516KRAS-M61%% FAMG12A35G>C522KRAS-M25%% FAMG12A34G>C518KRAS-M32%% FAMG12A34G>C518KRAS-M32%% FAMHER2 Exon 2G12R34G>C518KRAS-M32%% FAMHER2 Exon 2A775_G776insYVMA2325_2326 ins12 (TACGTGATGGCT)12558HER2-M11%% FAMHER2 Exon 3G12D35G>A521KRAS-M11%% FAMKRAS Exon 2G12D35G>A517KRAS-M15%% FAMKRAS Exon 2G12D35G>A511KRAS-M11%% FAMKRAS Exon 3G12D35G>A517KRAS-M15%% TAMKRAS Exon 15V600E1799T>A476BRAF-M11%% TAMS600E1799T>A476BRAF-M11%% TAMS600E1799T>A476BRAF-M11%% TAMS600E1799T>A4693937E-20-M61%% TAMS600E1799T>A4693937 <t< td=""><td></td><td>-</td><td>G776>LC</td><td></td><td>20895</td><td>HER2-M19</td><td>1%</td></t<>		-	G776>LC		20895	HER2-M19	1%
$ \begin{bmatrix} 1 & 1 & 1 & 1 & 1 & 1 & 1 & 1 & 1 & 1$		-	G776>LC	2326G>CTTT	12554	HER2-M20	1%
$ \begin{bmatrix} 1 & 1 & 1 & 1 & 1 & 1 & 1 & 1 & 1 & 1$			G776>LC	2326G>TTGT	19875	HER2-M21	1%
			V777 G778insCG	2331 2332jnsTGTGGG	303939	HER2-M24	1%
		+ +	V774 C775ineHV	2321_2322insCCACGT	18432	E-20-M32	1%
			V774 C775insHV	2321_2322insCACGTG	22049	F_20_M22	1 /0
$ \frac{11715_{1} \times 174113411}{1} = \frac{1230_{2}3211135CCACG}{12302} = \frac{12302}{1} = 1230$	⑤ FAM	EGFR Exon 20	н772 V/77/inc A Ц	2320_2321incCCCACC	1228020	E-20-10133	1 /0
Image: Constraint of the state of			D772 H772:	2320_2321HISCCCACG	255205	E-20-10155	170
\odot VIC KRAS EXOL 2 G12C 340 > 1 516 KRAS-M6 1% $@$ FAM $AAA3$ EXOL 2 G12A 336 > 1 522 KRAS-M2 5% $@$ G12V 35G>C 522 KRAS-M3 2% $G12V$ 35G>T 520 KRAS-M14 1% $@$ G12C G13C 37G>T 527 KRAS-M14 1% $@$ A775_G776insYVMA 2325_2326 ins12 (TACGTGATGGCT) 12558 HER2-M1 1% $@$ FAM $KRAS Exon 2$ G12D 33G>A 521 KRAS-M1 5% $@$ FAM $KRAS Exon 2$ G12S 34G>A 517 KRAS-M1 5% $@$ FAM $KRAS Exon 2$ G12S 34G>A 517 KRAS-M1 5% </td <td>© VIC</td> <td>VDAS Error 2</td> <td></td> <td>2310_231/INSO11</td> <td>233203</td> <td>E-20-IVI42</td> <td>17/0</td>	© VIC	VDAS Error 2		2310_231/INSO11	233203	E-20-IVI42	17/0
	UVIC	AAAS EXON 2	0120	34021	510	KRAS-MO	170
\mathbb{B} FAM $KRAS Exon 2$ $G12V$ $35G>1$ 520 $KRAS M3$ 2% \mathbb{B} FAM $KRAS Exon 2$ $G12R$ $35G>1$ 520 $KRAS M3$ 2% \mathbb{B} FAM \mathbb{A} Face $12V$ $G12R$ $34G>C$ 518 $KRAS M3$ 2% \mathbb{B}			GI2A	35G>C	522	KKAS-M2	5%
$ \begin{array}{c c c c c c c c c c c c c c c c c c c $	6 FAM	KRAS Exon 2	GI2V	35G>1	520	KKAS-M3	2%
$ \begin{array}{c c c c c c c c c c c c c c c c c c c $			G12R	34G>C	518	KRAS-M5	2%
$ \begin{array}{c c c c c c c c c c c c c c c c c c c $			G13C	37G>T	527	KRAS-M14	1%
$ \begin{array}{c c c c c c c c c c c c c c c c c c c $	[®] VIC	HER2 Exon 20	A775_G776insYVMA	2325_2326 ins12 (TACGTGATGGCT)	12558	HER2-M1	1%
$ \begin{array}{c c c c c c c c c c c c c c c c c c c $			A775_G776insYVMA	2324_2325 ins12 (ATACGTGATGGC)	20959	HER2-M2	1%
$ \begin{array}{ c c c c c c c c c } \hline & & & & & & & & & & & & & & & & & & $	⑦ FAM	KRAS Exon 2	G12D	35G>A	521	KRAS-M1	5%
⑦ VIC BRAF Exon 15 V600E 1799T>A 476 BRAF-M1 1% ⑦ ROX EGFR Exon 20 C797S 2389T>A 6493937 E-20-M6 1% 2390G>C 5945664 E-20-M7 1%			G12S	34G>A	517	KRAS-M4	5%
⑦ ROX EGFR Exon 20 C797S 2389T>A 6493937 E-20-M6 1% ② ROX 2390G>C 5945664 E-20-M7 1%	⑦ VIC	BRAF Exon 15	V600E	1799T>A	476	BRAF-M1	1%
2390G>C 5945664 E-20-M7 1%	(7) ROY	EGER Exon 20	C7078	2389T>A	6493937	E-20-M6	1%
	U KUA	LOFA EXOI 20	0/9/0	2390G>C	5945664	E-20-M7	1%
