



AmoyDx[®] *NTRK* Gene Fusions Detection Kit

Instructions for Use

REF 8.0126001X024E 24 tests/kit For Stratagene Mx3000P™, ABI7500, QuantStudio 5, LightCycler480 II, SLAN-96S



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Background

Neurotrophic tropomyosin-related kinase (NTRK) is a group of high affinity nerve growth factor (NGF) receptors, including tropomyosin receptor kinases (Trk) A, B, and C which are encoded by *NTRK1*, *NTRK2*, and *NTRK3* genes, respectively. NTRK proteins, upon NGF ligand binding, function in the phosphorylation of members of the RAS/MAPK, PI3K/AKT, PLC- γ pathways that play a key role in cell proliferation, differentiation, and the development of proprioceptive neurons [1-2].

Gene translocations that involve each *NTRK1/2/3* genes lead to the constitutive tyrosine kinase activation and are shown to drive tumor growth. *NTRK* fusions have been described in a wide variety of adult and pediatric solid tumors, such as breast cancer, colorectal cancer, non-small cell lung cancer and glioblastoma [1-2]. *NTRK* fusions are found at a low frequency in common cancer types, and at high frequencies in rare cancer types, such as secretory breast carcinoma and infantile fibrosarcoma [3]. The treatment of patients with *NTRK* fusion-positive cancers with TRK tyrosine kinase inhibitors is associated with high response rates, regardless of tumor histology [4-5].

This kit has not been combined with drugs for clinical trials. It is only used for detection of *NTRK* fusions in cancer patients. The test results are for clinical reference only. The clinician should judge the test results based on the patient's condition, drug indications, treatment response and other laboratory test indicators comprehensively.

Intended Use

The AmoyDx[®] *NTRK* Gene Fusions Detection Kit is a real-time PCR assay for qualitative detection of 109 fusions of *NTRK1/2/3* genes in human total RNA extracted from solid tumor formalin-fixed paraffin-embedded (FFPE) tissue samples.

The kit is for *in vitro* diagnostic use, and intended to be used by trained professionals in a laboratory environment.

Principles of the Procedure

The kit is based on two major processes: 1) **Reverse Transcription**: extracted RNA from FFPE 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 *NTRK* variant cDNA, and mutant amplicon is detected by fluorescent probes labeled with FAM, while reference gene amplicon is detected by fluorescent probe labeled with VIC.

The kit is composed of NTRK RT Mix, NTRK PCR Mix, NTRK Positive Control, NTRK Reverse Transcriptase and NTRK Enzyme Mix .

- 1) The **NTRK RT Mix** contains primers specific for reverse transcription of both *NTRK1/2/3* gene RNA and reference gene RNA into complementary DNA (cDNA).
- 2) The **NTRK PCR Mix** ①~⑧ are consist of fusion detection and internal control systems. The fusion detection system contains primers and FAM-labeled probes specific for *NTRK1/2/3* gene fusions. The internal control system contains primers and VIC-labeled probe for detection of reference gene to reveal the RNA quality and presence of PCR inhibitors that may lead to false negative results.
- 3) The **NTRK Positive Control** contains recombinant gene with *NTRK1/2/3* gene fusions.
- 4) The **NTRK Reverse Transcriptase** is for reverse transcription of target RNA and reference gene RNA into cDNA.
- 5) The **NTRK Enzyme Mix** contains the Taq DNA polymerase for PCR amplification and uracil-N-glycosylase which works at room temperature to prevent PCR amplicon carryover contamination.

Kit Contents

This kit contains the following materials:

Table 1 Kit Contents

Tube No.	Content	Main Ingredients	Quantity	Fluorescent signal
①	NTRK PCR Mix ①	Primers, Probes, Mg ²⁺ , dNTPs	1100 μ L/tube \times 1	FAM, HEX/VIC
②	NTRK PCR Mix ②	Primers, Probes, Mg ²⁺ , dNTPs	1100 μ L/tube \times 1	FAM, HEX/VIC
③	NTRK PCR Mix ③	Primers, Probes, Mg ²⁺ , dNTPs	1100 μ L/tube \times 1	FAM, HEX/VIC
④	NTRK PCR Mix ④	Primers, Probes, Mg ²⁺ , dNTPs	1100 μ L/tube \times 1	FAM, HEX/VIC
⑤	NTRK PCR Mix ⑤	Primers, Probes, Mg ²⁺ , dNTPs	1100 μ L/tube \times 1	FAM, HEX/VIC
⑥	NTRK PCR Mix ⑥	Primers, Probes, Mg ²⁺ , dNTPs	1100 μ L/tube \times 1	FAM, HEX/VIC
⑦	NTRK PCR Mix ⑦	Primers, Probes, Mg ²⁺ , dNTPs	1100 μ L/tube \times 1	FAM, HEX/VIC

⑧	NTRK PCR Mix ⑧	Primers, Probes, Mg ²⁺ , dNTPs	1100 μL/tube ×1	FAM, HEX/VIC
/	NTRK RT Mix	Primers, Mg ²⁺ , dNTPs	1150 μL/tube ×1	
/	NTRK Reverse Transcriptase	Reverse Transcriptase	30 μL/tube ×1	/
/	NTRK Enzyme Mix	Taq DNA Polymerase, Uracil-N-Glycosylase	85 μL/tube ×1	/
/	NTRK Positive Control	Plasmid DNA	500 μL/tube ×1	/

Storage and Stability

The kit requires shipment on frozen ice packs. All contents of the kit should be stored immediately upon receipt at -20±5°C and protected from light.

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

Additional Reagents and Equipment Required but Not Supplied

- Compatible PCR instruments:
Stratagene Mx3000P™, ABI7500, QuantStudio 5, LightCycler480 II, or SLAN-96S.
- RNA extraction kit: we recommend use of AmoyDx RNA extraction kit (AmoyDx® FFPE RNA Kit for paraffin embedded specimens).
- Spectrophotometer for measuring RNA concentration.
- Mini centrifuge with rotor for centrifuge tubes.
- Mini centrifuge with rotor for PCR tubes.
- Vortexer.
- Nuclease-free centrifuge tubes.
- Nuclease-free PCR tubes and caps.
- Adjustable pipettors and filtered pipette tips for handling RNA.
- Tube racks.
- Disposable powder-free gloves.
- Sterile, nuclease-free water.

Precautions and Handling Requirements

For *in vitro* diagnostic use.

Precautions

- Please read the instruction carefully and become familiar with all components of the kit prior to use. Strictly follow the instruction during operation.
- Please check the compatible 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 contact of the skin, eyes and mucous membranes 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 results.
- 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.
- Use separate, dedicated pipettes and filtered pipette tips when handling samples and reagents to prevent exogenous DNA 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 experiment, 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 μ L.
- For Stratagene Mx3000P™, if there’s low net fluorescence signal (dR) but high background signals (R), please reduce the signal gain setting of instrument properly.
- For ABI7500 and QuantStudio 5, please set up as follows: Reporter Dye: FAM, VIC; Quencher Dye: TAMRA; Passive Reference: NONE.
- For LightCycler480 II, if there is fluorescence crossover on instrument, fluorescence calibration is also required. To run the assays on a LightCycler machine, please use the Roche 480 adaptor, available from BIOplastics, Cat No. B79480.
- For SLAN-96S, please set up as follows: Probe mode: FAM, VIC. During the result analysis, open the “Preference” window, in “Chart Options” section; select “Selected Wells” for “Y-Axis Scaling Auto-adjust By” and “Absolute Fluorescence Value Normalization” for “Amplification Curve”.
- Refer to the operation manual of the real-time PCR instrument for detailed instructions.
- We recommend that for all PCR instruments in use a fluorescence calibration should be conducted once a year.

Assay Procedure

1. RNA Extraction

The specimen material must be extracted RNA from solid tumor FFPE tissue samples. RNA extraction kit is not included in the kit. Before RNA extraction, it’s essential to use standard pathology methodology to ensure tumor sample quality. It’s better to use tumor tissue samples with more than 30% tumor cells. Carry out the RNA extraction according to the instructions of RNA extraction kit.

The OD value of extracted RNA should be measured using the spectrophotometer after extraction. The OD_{260}/OD_{280} value should be between 1.8~2.1. The total RNA concentration for NTRK fusion detection is shown in Table 2.

Table 2 Recommended RNA concentration

Sample type	RNA concentration	Remark
FFPE tissue	10~200 ng/ μ L	<ul style="list-style-type: none"> • If RNA is between 10~200 ng/μL, use the original RNA without dilution; • If RNA is more than 200 ng/μL, dilute the RNA to 200 ng/μL with nuclease-free water.

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 at -20 ± 5 °C for no more than one week.

2. Reverse Transcription

- 1) Take **NTRK RT Mix** and **NTRK Reverse Transcriptase** as need out of the kit from the freezer, and other reagents remained in freezer at -20 ± 5 °C.
- 2) Thaw **NTRK RT Mix** at room temperature. When the reagent is completely thawed, mix the reagent thoroughly by vortexing and

centrifuge for 5~10 seconds to collect all liquid at the bottom of the tube.

- 3) Centrifuge **NTRK Reverse Transcriptase** for 5~10 seconds prior to use.
- 4) Prepare a sterile nuclease-free PCR tube for each sample: pipet 43 μL **NTRK RT Mix**, 1 μL **NTRK Reverse Transcriptase** and 6 μL sample RNA to the tube. Thoroughly mix the reagents by gently pipetting up and down more than 10 times, and then centrifuge briefly.
- 5) Incubate the tubes at 42 $^{\circ}\text{C}$ for 5 minutes, 95 $^{\circ}\text{C}$ for 5 minutes, then transfer them to ice. The resulting sample cDNA are used for subsequent PCR amplification.

Note: sample cDNA should be used immediately, if not, it should be stored at -20 ± 5 $^{\circ}\text{C}$ for no more than one week after reverse transcription.

3. PCR amplification

- 1) Take **NTRK PCR Mix ①~⑧**, **NTRK Positive Control** and **NTRK Enzyme Mix** out of the kit from the freezer.
- 2) Thaw **NTRK PCR Mix ①~⑧** and **NTRK Positive Control** at room temperature. When the reagents are completely thawed, mix each reagent thoroughly by vortexing and centrifuge for 5~10 seconds to collect all liquid at the bottom of the tube.
- 3) Centrifuge **NTRK Enzyme Mix** for 5~10 seconds prior to use.
- 4) Prepare sufficient NTRK Master Mix ①~⑧ containing NTRK Enzyme Mix and each NTRK PCR Mix (NTRK PCR Mix ①~⑧, respectively) in separate sterile centrifuge tube according to the ratio in Table 3. Thoroughly mix each NTRK Master Mix by vortexing and centrifuge for 5~10 seconds.

Table 3 NTRK Master Mix

Content	Volume per test
NTRK Enzyme Mix	0.3 μL
Each NTRK PCR Mix	34.7 μL
Total	35 μL

Note:

- Every PCR run must contain one PC (Positive control) and one NTC (No template control).
 - 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.
- 5) Take out the sample cDNA and nuclease-free water for NTC (No template control).
 - 6) Prepare 8 PCR tubes for NTC: Dispense 35 μL of NTRK Master Mix ①~⑧ to each PCR tube respectively. Then add 5 μL of nuclease-free water to each PCR tube, and cap the PCR tubes.
 - 7) Prepare 8 PCR tubes for each sample: Dispense 35 μL of NTRK Master Mix ①~⑧ to each PCR tube respectively. Then add 5 μL of sample cDNA to each PCR tube, and cap the PCR tubes.
 - 8) Prepare 8 PCR tubes for PC: Dispense 35 μL of NTRK Master Mix ①~⑧ to each PCR tube respectively. Then add 5 μL of NTRK Positive Control to each PCR tube, and cap the PCR tubes.
 - 9) Briefly centrifuge the PCR tubes to collect all liquid at the bottom of each PCR tube.
 - 10) Place the PCR tubes into the appropriate positions of the real-time PCR instrument. A recommended plate layout is shown in Table 4.

Table 4 Recommended Plate Layout

Tube No.	1	2	3	...	9	10	11	12
①	Sample 1	Sample 2	Sample 3	...	Sample 9	Sample 10	PC	NTC
②	Sample 1	Sample 2	Sample 3	...	Sample 9	Sample 10	PC	NTC
③	Sample 1	Sample 2	Sample 3	...	Sample 9	Sample 10	PC	NTC
④	Sample 1	Sample 2	Sample 3	...	Sample 9	Sample 10	PC	NTC
⑤	Sample 1	Sample 2	Sample 3	...	Sample 9	Sample 10	PC	NTC
⑥	Sample 1	Sample 2	Sample 3	...	Sample 9	Sample 10	PC	NTC
⑦	Sample 1	Sample 2	Sample 3	...	Sample 9	Sample 10	PC	NTC
⑧	Sample 1	Sample 2	Sample 3	...	Sample 9	Sample 10	PC	NTC

11) Setup the PCR protocol using the cycling parameters in Table 5.

Table 5 Cycling Parameters

Stage	Cycles	Temperature	Time	Data collection
1	1	95°C	5 min	/
		95°C	25 s	/
2	15	64°C	20 s	/
		72°C	20 s	/
		93°C	25 s	/
3	31	60°C	35 s	FAM and HEX/VIC
		72°C	20 s	/

12) Start the PCR run immediately.

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

4. Result Interpretation

Before the data analysis, the following items should be checked:

- 1) For NTC: The FAM Ct values of NTRK PCR Mix ①~⑧ should be no amplification and Ct values should be > 31. If not, the data is *INVALID*. The sample should be retested.
- 2) For Positive Control: The FAM and HEX/VIC Ct values of NTRK PCR Mix ①~⑧ should be ≤ 20. If not, the data is *INVALID*. The sample should be retested.
- 3) For the reference gene assay (HEX/VIC signal) in NTRK PCR Mix ①~⑧ for each sample:
 - a) If any HEX/VIC Ct value of NTRK PCR Mix ①~⑧ is ≤ 20, continue with the analysis.
 - i. If all the HEX/VIC Ct values of NTRK PCR Mix ①~⑧ are ≤ 31, continue with the analysis.
 - ii. If any HEX/VIC Ct value of NTRK PCR Mix ①~⑧ is > 31, the data is *INVALID*. The sample should be retested.
 - b) If all the HEX/VIC Ct values of NTRK PCR Mix ①~⑧ are >20, this indicates RNA degradation or presence of PCR inhibitors. The sample should be retested with increased or re-extracted RNA, as there may be false negative results.

Analyze the fusion assay for each sample:

- 4) Record the FAM Ct value of NTRK PCR Mix ①~⑧ for each sample:
 - a) If all the FAM Ct values of NTRK PCR Mix ①~⑧ are > 25, the sample is determined as Negative (No *NTRK* fusion detected) or under the LOD (limit of Detection) of the kit.
 - b) If any FAM Ct value of NTRK PCR Mix ①~④ is ≤ 25, the sample is determined as Positive (*NTRK1* fusion detected).
 - c) If any FAM Ct value of NTRK PCR Mix ⑤~⑥ is ≤ 25, the sample is determined as Positive (*NTRK2* fusion detected).
 - d) If any FAM Ct value of NTRK PCR Mix ⑦~⑧ is ≤ 25, the sample is determined as Positive (*NTRK3* fusion detected).
- 5) The sample may contain two or more fusion patterns simultaneously.
- 6) Some cross- reactivity may occur between *NTRK1* fusions in NTRK PCR Mix ①~④, and also between *NTRK3* fusion in NTRK PCR Mix ⑦~⑧.

Performance Characteristics

The performance characteristics of this kit were validated on Stratagene Mx3000P™, ABI7500, QuantStudio 5, LightCycler480 II and SLAN-96S.

1) Limit of detection:

The limit of detection was established by testing plasmid DNA with *NTRK1/2/3* fusions. The results show the limit of detection for each *NTRK1/2/3* fusion is 125~250 copies/reaction.

2) Specificity:

The kits have been validated of non-specificity amplification at a background of 1200 ng wild-type RNA.

3) Accuracy:

Accuracy of the kit was established by testing *NTRK1/2/3* fusion-positive reference controls and 10 negative reference controls, the detection concordance rate are 100%.

4) Precision:

Precision of the kit was established by testing of the precision reference control for 10 repeats; the test gave positive results and Ct's CV (%) ≤ 10%.














Limitations

- 1) The kit is to be used only by personnel specially trained with PCR techniques.
- 2) The results can be used to assist clinical diagnosis, combining with other clinical and laboratory findings.
- 3) The kit has been validated for use with extracted RNA from cancer FFPE tissue samples.
- 4) The kit can only detect 109 *NTRK1/2/3* gene fusions listed in the appendix.
- 5) Reliable results are dependent on proper sample processing, transport, and storage.
- 6) The sample containing degraded RNA may affect the ability of the test to detect *NTRK* fusions.
- 7) Samples with negative result (No *NTRK1/2/3* Fusion Detected) may harbor *NTRK1/2/3* fusions not detected by this assay.

References

- 1) Farago, A. F., L. P. Le, *et al.* (2015). *J Thorac Oncol* 10(12): 1670-1674.
- 2) Khotskaya, Y. B., V. R. Holla, *et al.* (2017). *Pharmacol Ther* 173: 58-66.
- 3) Kheder, E. S., D. S. Hong (2018). *Clin Cancer Res* 24(23): 5807-5814.
- 4) U.N. Lassen, C.M. Albert, S. Kummar, *at,al.* (2018). ESMO.
- 5) G.D. Demetri, L. Paz-Ares, A.F. Farago, *et al.* (2018). ESMO.

Symbols

	Authorized Representative in the European Community		In Vitro Diagnostic Medical Device
	Manufacturer		Catalogue Number
	Batch Code		Use By
	Contains Sufficient for <n> Tests		Temperature Limitation
	Consult Instructions For Use		Keep Dry
	This Way Up		Fragile, Handle With Care
	Keep Away from Sunlight		

Appendix

NTRK1/2/3 Gene Fusions Detected by the Kit

Tube	Detected Target	Fusion Type	Tube	Detected Target	Fusion Type		
①	NTRK1 Fusion	TP53 exon8;ins6 NTRK1 exon8	③	NTRK1 Fusion	TPR exon6;NTRK1 exon12		
		TP53 exon9;ins6 NTRK1 exon8			GRIPAP1 exon22;NTRK1 exon12		
		TP53 exon10;ins6 NTRK1 exon8			SCYL3 exon11;NTRK1 exon12		
		TP53 exon11;ins6 NTRK1 exon8			MEF2D exon9;NTRK1 exon12		
		CTRC exon2;NTRK1 exon8			AMOTL2 exon6;NTRK1 exon12		
		IRF2BP2 exon1;NTRK1 exon8			PRDX1 exon5;NTRK1 exon12		
		④	NTRK1 Fusion	LRRC71 exon1;NTRK1 exon8	⑤	NTRK2 Fusion	MPRIP exon21;NTRK1 exon14
				LMNA exon2;NTRK1 exon11			LMNA exon2;NTRK1 exon16
				LMNA exon3;NTRK1 exon11			VCL exon16;NTRK2 exon12
				LMNA exon5;NTRK1 exon11			AFAP1 exon13;NTRK2 exon12
				LMNA exon10;NTRK1 exon11			VCAN exon6; NTRK2 exon12
				LMNA exon11 del150;NTRK1 exon11			NACC2 exon5;NTRK2 exon13
		②	NTRK1 Fusion	PPL exon21;NTRK1 exon11	⑥	NTRK2 Fusion	NOS1AP exon9; NTRK2 exon13
				GRIPAP1 exon22;NTRK1 exon11			TBC1D2 exon6; NTRK2 exon14
				BCAN exon13;NTRK1 exon11			TRIM24 exon12;NTRK2 exon15
				TFG exon5;NTRK1 exon9			TRAF2 exon9;NTRK2 exon15
TPR exon21;NTRK1 exon9	SQSTM1 exon4;NTRK2 exon15						
TFG exon4;NTRK1 exon9	ETV6 exon5;NTRK2 exon15						
TPM3 exon10;NTRK1 exon9	TLE4 exon7;NTRK2 exon15						
AFAP1 exon4;NTRK1 exon9	TRIM24 exon12;NTRK2 exon16						
TRIM63 exon8;NTRK1 exon9	AGBL4 exon6;NTRK2 exon16						
TPM3 exon8;NTRK1 exon10	SQSTM1 exon5;NTRK2 exon16						
SQSTM1 exon2;NTRK1 exon10	STRN3 exon7;NTRK2 exon16						
SQSTM1 exon5;NTRK1 exon10	WNK2 exon24;NTRK2 exon16						
TPR exon10;NTRK1 exon10	QKI exon6;NTRK2 exon16						
TPR exon16 del54;NTRK1 ins13 exon10	STRN exon3;NTRK2 exon16						
TPR exon21;NTRK1 exon10	GKAP1 exon9; NTRK2 exon16						
CD74 exon8;NTRK1 exon10	KCTD8 exon1;NTRK2 exon16						
IRF2BP2 exon1;NTRK1 exon10	PRKAR2A exon2;NTRK2 exon16						
IRF2BP2 exon1 del48;NTRK1 exon10	PAN3 exon1;NTRK2 exon17						
PPL exon21;NTRK1 exon10	SQSTM1 exon5;NTRK2 exon17						
PEAR1 exon15;NTRK1 exon10	BCR exon1;NTRK2 exon17						
TFG exon5;NTRK1 exon10	ETV6 exon4;NTRK3 exon14						
GRIPAP1 exon22;NTRK1 exon10	ETV6 exon5;NTRK3 exon14						
TFG exon6;NTRK1 exon10	EML4 exon2;NTRK3 exon14						
F11R exon4;NTRK1 exon10	SQSTM1 exon5;NTRK3 exon14						
F11 exon4;NTRK1 exon10	TFG exon6;NTRK3 exon14						
SQSTM1 exon6;NTRK1 exon10	MYH9 exon31;NTRK3 exon14						
ARHGEF2 exon21;NTRK1 exon10	RBPMS exon5;NTRK3 exon14						
CHTOP exon5;NTRK1 exon10	BTBD1 exon4; NTRK3 exon14						
NFASC exon21;NTRK1 exon10	SPECC1L exon5;NTRK3 exon14						
TPM3 exon7 del39;NTRK1 exon10	VIM exon8;NTRK3 exon14						
BCAN exon12;NTRK1 exon10	STRN exon3;NTRK3 exon14						
PPL exon11;NTRK1 exon13	STRN3 exon3;NTRK3 exon14						
③	NTRK1 Fusion	TPM3 exon8;NTRK1 exon12	⑦	NTRK3 Fusion	HNRNPA2B1 exon7;NTRK3 exon14		
		LMNA exon6 del172;NTRK1 exon12			AKAP13 exon3;NTRK3 exon14		
		MPRIP exon21;NTRK1 exon12			ETV6 exon5;NTRK3 exon15		
		SSBP2 exon12;NTRK1 exon12			ETV6 exon4;NTRK3 exon15		
		LMNA exon2;NTRK1 exon12			SQSTM1 exon6;NTRK3 exon15		
		LMNA exon4;NTRK1 exon12			ETV6 exon6;NTRK3 exon15		
		LMNA exon8;NTRK1 exon12			ETV6 exon4;NTRK3 exon12		
		LMNA exon10;NTRK1 exon12			ETV6 exon5;NTRK3 exon13		
		LMNA exon12;NTRK1 exon12			ETV6 exon4;NTRK3 exon13		
		MPRIP exon14;NTRK1 exon12			ETV6 exon5;NTRK3 exon16		
		MPRIP exon18;NTRK1 exon12					
						⑧	NTRK3 Fusion