AmoyDx® HANDLE OncoPro NGS Panel

Instructions for Use

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

REF 8.06.0186 24 tests/kit For Illumina NextSeq 500, NextSeq 550, NextSeq 550Dx

Amoy Diagnostics Co., Ltd.
No. 39, Dingshan Road, Haicang District,
361027 Xiamen, P. R. China
Tel: +86 592 6806835
Fax: +86 592 6806839
E-mail: sales@amoydx.com
Website: www.amoydx.com

Version: B3.0

Aug 2025

Background

Solid tumors, such as breast, lung, and colorectal cancers, are among the most common and deadly types of cancer, affecting millions of people worldwide each year.[1] Genomic alterations of tumor driver or suppressor genes, including point mutations, amplifications, deletions, and rearrangements, etc., are critical drivers of solid tumor cancer development and progression. Next-Generation Sequencing (NGS) has revolutionized cancer genetics by enabling the simultaneous sequencing of multiple genes, eliminating the need for serial testing and providing a more comprehensive tumor profiling compared to traditional single-gene or small-panel testing. [2-3]

Intended Use

The AmoyDx® HANDLE OncoPro NGS Panel is a qualitative next-generation sequencing (NGS) assay that provides comprehensive genomic profiling using both DNA and RNA isolated from formalin-fixed paraffin embedded (FFPE) tumor tissue specimens. The panel covers a total of 195 genes (see Table S1), enabling the detection of somatic single-nucleotide variants (SNV), insertions and deletions (InDel), gene fusions (on the RNA level), copy number amplifications (CNA), and homozygous deletions (HD). In addition, the panel also enables the evaluation of tumor microsatellite instability (MSI) status, homologous recombination deficiency (HRD) status, and the genetic polymorphisms of drug-metabolising enzymes in cancer chemotherapy.

The kit is intended to be used by trained professionals in a laboratory environment. The test results are for research use only, not for use in diagnostic procedures.

Principles of the Procedure

The test kit is based on Halo-shape ANnealing and Defer-Ligation Enrichment system (HANDLE system) technology to capture the target gene region (Figure 1). During the library construction process, each individual DNA molecule is tagged with a unique molecular identifier (UID) at both ends, which allows high sensitivity in variant detection by eliminating any library amplification and sequencing bias.

The test kit uses both DNA and RNA extracted from FFPE samples, and it offers a time saving protocol that can be completed within 6 hours and requires just about 1 hour of hands-on time. Firstly, the RNA is reverse transcribed into cDNA with help of the Reverse Transcriptase and the RT primers. Secondly, the cDNA product and the genomic DNA are combined in one tube for hybridization. The probe contains an extension arm and a ligation arm which are complementary to the target gene region, and the probe anneals onto the DNA or cDNA template of the target region. Thirdly, the DNA is extended from the extension arm to the ligation arm with help of the DNA polymerase, then the nicks are repaired to generate the circular products with help of the DNA ligase. Next, the remaining linear probes, single-strand and double-strand DNA are digested with help of the exonuclease, and only the target circular DNA will be kept for PCR amplification. Finally, the universal PCR amplification is performed to enrich the target libraries, and the magnetic bead-based purification is performed to obtain the final library.

After quality control (QC), the qualified libraries could be sequenced on Illumina sequencing platform. The sequencing data can be analyzed by AmoyDx NGS data analysis system (ANDAS) to detect the genomic variants in the target region.

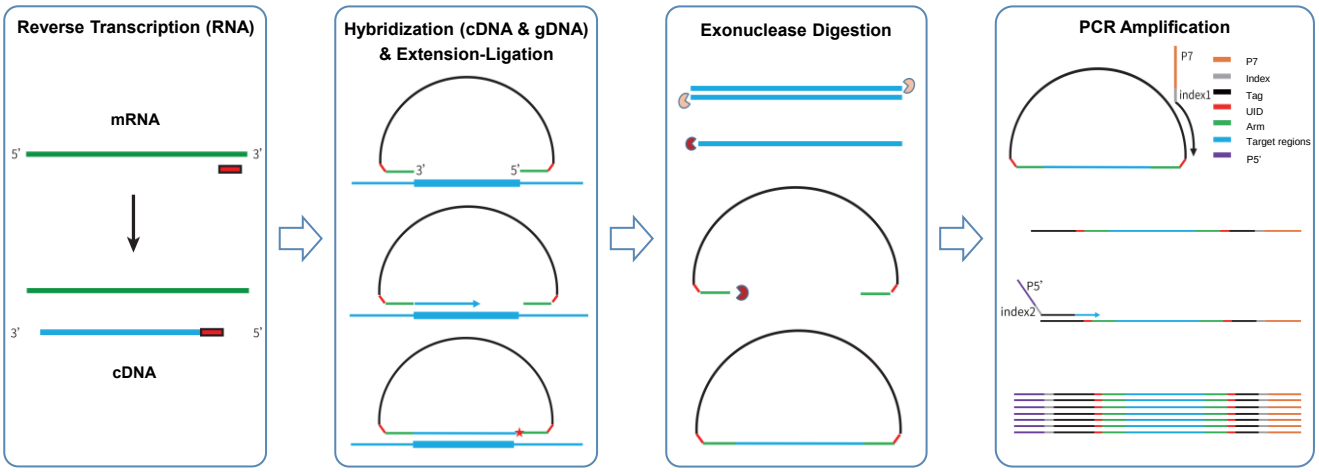

Figure 1 Principle of library construction (HANDLE system)

Kit Contents

This kit contains the following components in Table 1.

Table 1. Kit Contents

Serial No.	Components	Main Ingredient	Quantity
1-RT	HOP-RT Primers	Oligonucleotides	28 μL/tube × 1
2-RT	HOP-RT Reaction Mix	Tris-HCl, K^+, Mg^{2+}, dNTPs	35 μL/tube × 1
3-RT	HOP-Reverse Transcriptase	Reverse transcriptase	7 μL/tube × 1
4-Hyb	HOP-Probe	Oligonucleotides	56 μL/tube × 1
5-Hyb	HOP-Hybridization Buffer	Tris-HCl, Mg^{2+}	28 μL/tube × 1
6-EL	HOP-Extension Ligation Master Mix	DNA polymerase, dNTPs, DNA Ligase, Ligation buffer	40 μL/tube × 1
7-ED	HOP-Exonuclease A	DNA Exonuclease	40 μL/tube × 1
8-ED	HOP-Exonuclease B	DNA Exonuclease	28 μL/tube × 1
9-Amp	HOP-PCR Master Mix	Tris, Mg^{2+}, dNTPs, DNA polymerase	600 μL/tube × 1
502-517	HOP-S5 Primer *	Oligonucleotides	5 μL/tube × 10
707-729	HOP-N7 Primer *	Oligonucleotides	5 μL/tube × 12
PC-D	HOP-Positive Control-DNA	DNA	60 μL/tube × 1
PC-R	HOP-Positive Control-RNA	RNA	60 μL/tube × 1

* For labeling and sequence information of the indexed primers, refer to Appendix Table S3.

** The variants in the HOP-Positive Controls are listed in Appendix Table S5.

Storage and Stability

The kit requires shipment on frozen ice packs and the shipping time should be less than one week. All contents of the kit should be stored at -25℃ to -15℃ immediately upon receipt.

The shelf-life of the kit is twelve months. Repeated thawing and freezing should be avoided. Do not exceed a maximum of five freeze–thaw cycles.

Materials Required but Not Supplied

1) DNA/RNA extraction kit: The AmoyDx® Magnetic FFPE DNA Extraction kit (Amoy Diagnostics) is recommended for DNA extraction; the AmoyDx® FFPE RNA kit (Amoy Diagnostics) is recommended for RNA extraction.
2) DNA quantification kit: QuantiFluor® dsDNA System (Promega) or Qubit™ dsDNA HS Assay Kit (Thermo Fisher Scientific) is recommended.
3) RNA quantification kit: QuantiFluor® RNA System (Promega) or Qubit™ RNA HS Assay Kit (Thermo Fisher Scientific) is recommended.
4) Fluorometer: Quantus™ Fluorometer (Promega), or Qubit™ Fluorometer (Thermo Fisher Scientific) is recommended.
5) Capillary electrophoresis analyzer and related kit: Agilent 2100 Bioanalyzer system and Agilent DNA 1000 Reagents (Agilent Technologies) or Agilent High Sensitivity DNA Kit (Agilent Technologies); or Agilent 2200 TapeStation and D1000 ScreenTape/Reagents (Agilent Technologies) or High Sensitivity D1000 ScreenTape/Reagents (Agilent Technologies); or LabChip GX Touch and DNA High Sensitivity Reagent Kit (PerkinElmer); or E-Gel™ Power Snap Electrophoresis System (Thermo Fisher Scientific) and E-Gel™ EX Agarose Gels, 2% (Thermo Fisher Scientific) are recommended.
6) DNA purification kit: Agencourt AMPure XP Kit (Beckman Coulter) or equivalent is recommended.
7) PCR instrument: Applied Biosystems™ 2720 Thermal Cycler or Applied Biosystems™ MiniAmp (or equivalent).
8) Illumina PhiX Control v3, or TG PhiX Control Kit v3.
9) Sequencing reagent: Illumina 300 cycles (Paired-End Reads, 2×150 cycles) is recommended.
10) Sequencer: Illumina NextSeq 500/550, NextSeq 550Dx.
11) Magnetic stand: DynaMag™-2 Magnet is recommended, or equivalent.
12) Water bath or heating block: Bioer ThermoCell Mixing and Heating (Bioer Technology) or equivalent.
13) Mini centrifuge.
14) Vortex mixer.
15) Ice box for 0.2 mL and 1.5 mL tubes.
16) 1.5 mL nuclease-free centrifuge tubes (Axygen is recommended, or equivalent).
17) 0.2 mL nuclease-free PCR tubes, and colorless 0.5 mL PCR tubes (Axygen is recommended, or equivalent).
18) Nuclease-free filtered pipette tips.
19) Absolute ethanol (AR).
20) Nuclease-free water.
21) TE-low solution (10 mM Tris, 0.1 mM EDTA, 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 compatible PCR instruments prior to use.

- DO NOT use the kit or any kit component after their expiry date.
- DO NOT mix or combine reagents from different lots in the tests.
- DO NOT use reagents from other test kits.

Safety Information

- Handle all specimens and components of the kit as potentially infectious material using safe laboratory procedures.
- 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.

Decontamination and Disposal

- The kit includes positive control. It is essential to clearly differentiate distinguish the positive control from other reagents to avoid contamination, which may cause false positive results.
- PCR amplification is highly susceptible to cross-contamination. Therefore, all materials, including tubes, racks, and pipettes, should be handled in a strictly unidirectional flow from pre-amplification to post-amplification areas, ensuring that no reverse movement occurs.
- 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 nucleic acid contamination to the reagents.
- All consumables should be disposable. DO NOT reuse.
- Unused reagents, used kit, and waste must be disposed properly.
- Waste disposal procedures must comply with all applicable local regulations.

Cleaning

- Upon completion of the experiment, thoroughly clean the work area and disinfect pipettes and equipment using 75% ethanol or a 10% hypochlorous acid solution.

Specimen Preparation

- Sample DNA and RNA should be extracted from FFPE tumor tissue specimens. The DNA will be used for the detection of SNV, InDel, CNA, HD, MSI and HRD, and the RNA will be used for the detection of gene fusion/splicing, and MET exon14 skipping.
- The FFPE tissue sample should be fixed in 10% neutral buffered formalin for 6-24 hours. The storage time for the FFPE tissue should be no more than 5 years (recommend within 3 years).
- For FFPE surgical resection samples, it is recommended to use a total of 2-6 slices with 5-10 μm thickness. For FFPE core needle biopsy samples, it is recommended to use a total of 10-15 slices with 5 μm thickness. Users can adjust the number of FFPE slices according to the sample quality and tumor area. The freshly cut sections of FFPE tissue should be used for DNA and RNA extraction at the earliest convenience.
- Prior to testing, each FFPE tissue specimen should be subjected to independent pathology review to confirm to the presence and percentage of tumor cells. For the detection of SNV, InDel, CNA, Fusion, and MSI, the tumor cell content should be no less than 20%;

For the detection of HD and HRD, the tumor cell content should be no less than 30% (at least 30% for HD detection at gene level, at least 40% for HD detection at exon level, and at least 30% for HRD detection). For samples with a tumor cell content lower than the requirement, it is recommended to perform microdissection or macrodissection to enrich the tumor cells or re-collect samples if necessary. Microdissection/macrodissection is not applicable to core needle biopsy (CNB) samples.

- It is recommended to use a commercialized extraction kit to perform the DNA and RNA extraction and use RNase A to degrade RNA during the DNA extraction. After extraction, measure the concentration of extracted DNA and RNA using Quantus® or Qubit™. The DNA concentration should be no less than 4.3 ng/μL, and the total DNA should be no less than 30 ng. The RNA concentration should be no less than 4 ng/μL, and the total RNA should be no less than 30 ng. For unqualified samples, re-collection or re-extraction are required.
- The qualified DNA and RNA should be used for library preparation immediately. If not, the DNA should be stored at -25℃ to -15℃ and the RNA should be stored at -85℃ to -75℃ for no more than 12 months. Avoid repeated freezing and thawing.

Assay Procedure

Note:

- *It is recommended to include a HOP-Positive Control-DNA (PC-D) and a HOP-Positive Control-RNA (PC-R) in the process of library preparation, sequencing and data analysis.*
- *When using the kit for the first time, or when necessary, it is recommended to use a no template control (NTC, using Nuclease-free water instead of RNA and DNA) to verify the absence of contamination. The NTC can be used for the quality control of the library construction process and is not needed to run the sequencing or data analysis process.*
- *During the following library preparation process, please assemble each reaction mixture on ice to avoid non-specificity, and use the auxiliary plate compatible with the PCR instrument for non-strip PCR tubes to prevent the evaporation of PCR product.*
- *It is recommended to use fluorescent dye method (Quantus® or Qubit™ Fluorometer) for all the DNA/RNA concentration measurement steps.*

1. Reverse Transcription

1.1 Take out the (1-RT) **HOP-RT Primers** and thaw the reagents at room temperature. When the reagents are completely thawed, mix well on a vortex mixer for 10-15 sec and centrifuge briefly, then keep the tube on ice.

1.2 Assemble the pre-reverse transcription reaction on ice in a nuclease-free 0.2mL PCR tube by adding the following components according to Table 2.

Table 2. Pre-reverse Transcription Reaction

Reagent	Volume
Nuclease-free water	7.5-χ μL
RNA / PC RNA	χ μL
(1-RT) HOP-RT Primers	1 μL
Total	**8.5 μL**

Note:

- *"χ" represents the volume of a RNA sample for a total input amount of 30-400 ng RNA (The RNA input amount for reverse transcription depends on RNA quality and can be adjusted accordingly. For initial library construction, 200 ng is recommended.).*

- *For PC RNA, χ=7.5 μL, take 7.5 μL (PC-R) HOP-Positive Control-RNA to construct library. For NTC, χ=0 μL.*

1.3 Mix the solution thoroughly by vortexing for 10-15 sec or pipetting, and centrifuge briefly, then place the sample in a thermocycler, set the reaction volume as 9 μL and perform the following program: 65°C for 5 min, then immediately transfer the tubes to ice for at least 1 min. Then proceed directly to step 1.4.

1.4 Take out the (2-RT) **HOP-RT Reaction Mix** and thaw the reagents at room temperature. When the reagents are completely thawed, mix well on a vortex mixer for 10-15 sec and centrifuge briefly, then keep the tube on ice. Take out the (3-RT) **HOP-Reverse Transcriptase**, mix well on a vortex mixer for 10-15 sec and centrifuge briefly, then keep the tube on ice. Assemble the reverse transcription reaction on ice by adding the following components according to Table 3.

Table 3. Reverse Transcription Reaction

Reagent	Volume
(2-RT) HOP-RT Reaction Mix	1.25 μL
(3-RT) HOP-Reverse Transcriptase	0.25 μL
Pre-reverse transcription products (from step 1.3)	8.5 μL
Total	**10 μL**

Note:

*It is recommended to prepare **freshly ready-to-use premix** of HOP-RT Reaction Mix and HOP-Reverse Transcriptase for precise pipetting when processing three or more samples simultaneously.*

1.5 Mix the solution thoroughly by vortexing for 10-15 sec or pipetting, and centrifuge briefly, then place the sample in a thermocycler, set the reaction volume as 10 μL and perform the following program: 50°C for 50 min, 98°C for 10 min, 4°C hold.

Note:

The reverse transcription products should be stored at 2~8°C for no more than 20 hours if not proceed to the next step immediately.

2. Hybridization

2.1 Take out the (4-Hyb) **HOP-Probe** and (5-Hyb) **HOP-Hybridization Buffer** and thaw the reagent at room temperature. When the reagents are completely thawed, mix well on a vortex mixer for 10-15 sec and centrifuge briefly, then keep the tube on ice.

2.2 Assemble the hybridization reaction on ice by adding the following components according to Table 4.

Table 4. Hybridization Reaction (for DNA and RNA sample)

Reagent	Volume
Nuclease-free water	7-χ μL
DNA / PC DNA	χ μL
(4-Hyb) HOP-Probe	2 μL
(5-Hyb) HOP-Hybridization Buffer	1 μL
Reverse transcription product (from step 1.5)	10 μL
Total	**20 μL**

Note:

- *"χ" represents the volume of a DNA sample for a total input amount of 30-100 ng DNA (100 ng is recommended).*
- *For PC DNA, χ=7 μL, take 7 μL (PC-D) HOP-Positive Control-DNA to construct library. For NTC, χ=0 μL.*
- *It is recommended to prepare **freshly ready-to-use premix** of HOP-Probe and HOP-Hybridization Buffer for precise pipetting*

when processing three or more samples simultaneously.

2.3 Mix the solution thoroughly by vortexing for 10-15 sec or pipetting, and centrifuge briefly, then place the sample in a thermocycler. Set the reaction volume as 20 μL and perform the following program: 98℃ for 5 min, 60℃ for 2 hours, 4℃ hold.

***Note**:*

- ***Important!** The hybridization duration at 60℃ must be exactly 2 hours. Overnight hybridization is not recommended; extending or shortening the hybridization time may result in abnormal test results.*
- *Keep the tubes at low temperature (2~8℃) after hybridization is finished, as high temperature (eg. room temperature) may increase the non-specificity. It is recommended to place the ice box besides the thermocycler in advance, when hybridization step is finished, take out the reaction tube and put it in the ice box immediately.*
- *The hybridization products should be stored at 2~8℃ for no more than 20 hours if not proceed to the next step immediately.*

3. Extension-Ligation

3.1 Take out the (6-EL) **HOP-Extension Ligation Master Mix** and thaw the reagent on ice. When the reagents are completely thawed, mix well on a vortex mixer for 10-15 sec and centrifuge briefly, then keep the tube on ice.

3.2 Take out the above hybridization product from the thermocycler and keep the tube on ice. Add 1.5 μL (6-EL) **HOP-Extension Ligation Master Mix** into the PCR tubes, mix the solution thoroughly by vortexing for 10-15 sec or pipetting, and centrifuge briefly, then place the sample in a thermocycler. Set the reaction volume as 22 μL and perform the following program: 60°C for 10 min, 4°C hold. Then proceed directly to the exonuclease digestion step.

***Note**:*

- *After initiating the PCR program, it is recommended to wait until the thermocycler has heated to above 50°C before placing the reaction tube inside.*
- *Keep the tubes at low temperature after extension-ligation is finished, as high temperature like room temperature may increase the non-specificity. It is recommended to place the ice box besides the thermocycler, and when the extension-ligation program is finished, immediately transfer the tubes on ice box.*
- ***Perform the subsequent exonuclease digestion step immediately when the extension-ligation step is finished.***

4. Exonuclease Digestion

4.1 Take out the (7-ED) **HOP-Exonuclease A** and (8-ED) **HOP-Exonuclease B**, mix well on a vortex mixer for 10-15 sec and centrifuge briefly, then keep the tube on ice.

4.2 Assemble the exonuclease digestion reaction on ice by adding the following components according to Table 5.

Table 5. Exonuclease Digestion Reaction

Reagent	Volume
(7-ED) HOP-Exonuclease A	1.5 μL
(8-ED) HOP-Exonuclease B	1 μL
Extension-Ligation product (from step 3.2)	21.5 μL
Total	**24 μL**

4.3 Mix the solution thoroughly by vortexing for 10-15 sec or pipetting, and centrifuge briefly, then place the sample in a thermocycler. Set the reaction volume as 24 μL and perform the following program: 37°C for 30 min, 95°C for 10 min, 4°C hold.

Note:

- *It is recommended to prepare **freshly ready-to-use premix** of HOP-Exonuclease A and HOP-Exonuclease B for precise pipetting when processing three or more samples simultaneously.*
- *The products of exonuclease digestion should be stored at 2~8℃ for no more than 20 hours if not proceed to the next step immediately.*

5. PCR Amplification

5.1 Take out the **HOP-N7 Primer, HOP-S5 Primer**, and (9-Amp) **HOP-PCR Master Mix,** and thaw the reagents at room temperature. When the reagents are completely thawed, mix well on a vortex mixer for 10-15 sec and centrifuge briefly, then keep the tube on ice.

5.2 Assemble the PCR amplification reaction on ice by adding the following components according to Table 6.

Table 6. PCR Amplification Reaction

Reagent	Volume
(9-Amp) HOP-PCR Master Mix	25 μL
(707~729) HOP-N7 Primer	1.5 μL
(502~517) HOP-S5 Primer	1.5 μL
Exonuclease digestion product (from step 4.3)	24 μL
Total	**52 μL**

***Note**:*

- *There are 12 tubes of HOP-N7 Primer and 10 tubes of HOP-S5 Primer, and each of the HOP-N7 Primer or HOP-S5 Primer has a different index sequence, supporting a maximum of 120 dual index combinations. Use different combination of HOP-N7 Primer and HOP-S5 Primer for each sample library. Do not use the same combination of index for two or more sample libraries in one sequencing run. The detailed information for the index sequence is shown in Appendix Table S3.*
- *It is recommended to transfer the prepared tubes to the amplification room to perform PCR amplification and the following purification to avoid contamination.*

5.3 Mix the solution in each PCR tube thoroughly by vortexing for 10-15 sec or pipetting, and centrifuge briefly, then place the sample in a thermocycler. Set the reaction volume as 50 μL, and then perform the following program according to Table 7.

Table 7. PCR Program

Temperature	Time	Cycles
98°C	1 min	1
98°C	20 s	19
61°C	30 s	
72°C	20 s	
72°C	5 min	1
4°C	∞	1

Note:

The PCR products should be stored at 2~8℃ for no more than 20 hours if not proceed to the next step immediately.

6. Purification

6.1 Take out the AMPure XP beads, and equilibrate them to room temperature for 30 min, and vortex the bottle of the beads for 10-15 sec to resuspend any magnetic particles that may have settled.

6.2 Add **40 μL AMPure XP beads** and **40 μL PCR products** into a clean 1.5 mL centrifuge tube, mix thoroughly by vortexing for 10-15 sec or pipetting, then incubate the mixture at room temperature for 5 min.

6.3 Place the centrifuge tubes onto the magnetic stand for 3~5 min until the solution turns clear. Gently remove and discard the supernatant while the centrifuge tube is on the magnetic stand. Do not disturb the beads with pipette tip.

6.4 Keep the tubes on the magnetic stand, add 200 μL of freshly prepared 80% ethanol to the tube while in the magnetic rack. Incubate at room temperature for at least 30 seconds, and then carefully remove and discard the supernatant.

6.5 Repeat step 6.4 once.

6.6 Briefly spin the tube and put the tube back in the magnetic rack. Completely remove the residual ethanol, and air dry the beads for 2~3 min while the tube is on the magnetic stand with the lid open.

***Note: Do not** over-dry the beads. This may result in lower recovery of DNA target.*

6.7 Remove the tube from the magnet. Elute DNA target from the beads by adding 30 μL TE-low solution, mix thoroughly by vortexing for 10-15 sec or pipetting, and incubate at room temperature for 3 min.

6.8 Put the tube in the magnetic rack for 3~5 min until the solution turns clear. Without disturbing the bead pellet, transfer the supernatant into a clean 1.5 mL centrifuge tube to obtain the final library.

***Note:** It is recommended to store the library at -25℃ to -15℃ if not proceed directly to sequencing.*

7. Library Quality Control (QC)

1) Library concentration QC: Quantify the library concentration using Quantus™ or Qubit™ Fluorometer, sample library concentration should be no less than 5 ng/μL, and NTC library concentration should be less than 5 ng/μL.

2) Library fragment QC (optional): Assess the library fragment size using the Agilent 2100 Bioanalyzer System and the Agilent DNA 1000 Kit (or other equivalent capillary electrophoresis analyzer and relevant kit which can assess DNA fragment size), the main peak of the library fragment should be at 210-400 bp, with no significant presence of overly large or small off-target peaks, as shown in Figure 2.

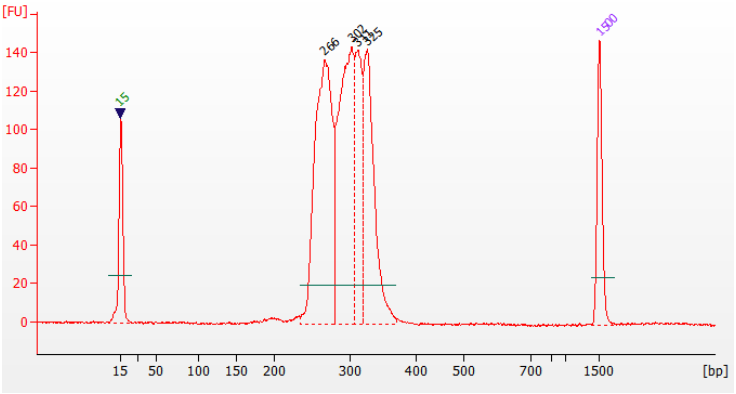

Figure 2. Example of Library Size Distribution on Agilent 2100 Bioanalyzer

Note:

- *The library distribution shown in the Figure 2 above was assessed using Agilent 2100 Bioanalyzer system and Agilent DNA 1000 Reagents. The peak at 15 bp stands for the lower marker, and the peak at 1500 bp stands for the upper marker.*
- *The no template control (NTC) library should have a concentration below 5 ng/μL, and no peak between the targeted library size (210~400 bp). If not, there may be contamination during the experiment process, the test is unqualified and the test should be repeated.*
- *If the sample library concentration is less than 5 ng/μL, it fails library QC. This could be a result of poor quality of the input DNA/RNA or inaccurate measurement of the DNA/RNA concentrations, or operational errors during the experiment, in which case library re-construction is required. If operational reasons have been ruled out, it is recommended to re-extract the nucleic acid and reconstruct the library. For samples with failed experiments and no possibility of re-sampling, it is recommended to try parallel multi-tube library construction (e.g., use 200 ng RNA and 100 ng DNA per tube and perform parallel library construction with three tubes for the same sample). When constructing parallel multi-tube libraries, use the same N7+S5 index combination for library amplification of the same sample, and combine the PCR-amplified products for purification and subsequent operations. Sequence the library according to the recommended data amount.*

8. Sequencing:

Illumina NextSeq 500/550, NextSeq 550Dx platforms and corresponding Illumina 300 cycles (Paired-End Reads, 2×150 cycles) reagent is recommended for sequencing. The recommended spike-in percentage of Illumina PhiX Control v3 is no less than 1% and no greater than 50%. The PhiX percentage must be properly adjusted to avoid its over-sequencing. The data output per sample (or PC) should be no less than 6 Gb. The suggested sample quantity per run is listed in Table 8.

Table 8. Recommended Sequencer and Sample Quantity per Run

Sequencer	Flow Cell	Read Length	Sample Quantity/Run
NextSeq 500/550, NextSeq 550Dx	Mid Output	2×150 bp	6
	High Output	2×150 bp	20

Perform the denaturation and dilution of the libraries according to the instrument's instructions. The final concentration of sequencing library is recommended in Table 9, which can be adjusted according to practical experiences.

Table 9. Recommended Final Concentration of Sequencing Library

Illumina Sequencer	Final Concentration
NextSeq 500/550, NextSeq 550Dx	0.7-1.2 pM

Note: *The concentration converting formula:*

$$Library\ \ Concentration\ [nM]\ = \frac{Library\ Concentration\ [ng/\mu L] \times 10^6}{660 \times 295}$$

9. Bioinformatics Analysis and Result Interpretation:

9.1 Check Q30 value for the sequencing data:

- If the Q30 value of the sequencing run is ≥75%, the sequencing run is qualified. If not, the sequencing run is unqualified, re-sequencing or re-construct library is required.
- If the Q30 value of each library is ≥75%, the library sequencing data is qualified. If not, the library sequencing data is unqualified, re-sequencing or re-construct the library is required.

9.2 Data Analysis:

For the sequencing data that pass the above-mentioned Q30 criteria, adopt the AmoyDx ANDAS Data Analyzer to analyze the sequencing data. Select the module ADXHS-OncoPro-HRD for bioinformatics analysis.

9.3 Data Quality Control (QC):

- Once data analysis is completed, check whether the CleanQ30 of each library is no less than 75%. If the CleanQ30 value of a library is ≥75%, the library is qualified. If not, the library is unqualified, re-sequencing or re-construct the library is required.
- For libraries that meet the CleanQ30 standard, continue to check whether each category in Table 10 meets the corresponding QC criteria. For categories that pass QC, proceed with result interpretation; for categories that do not pass QC, result interpretation cannot be performed, and it is recommended to re-construct the library or re-sequence the respective sample.

Table 10. Acceptance Criteria for Data QC

Sample Type	Category	QC Acceptance Criteria
DNA	SNV/InDel, MSI	Depth_CDS ≥ 400×
	CNA	Depth_CDS ≥ 400× and Coverage(50×)_SNP ≥ 90%
	HD	Coverage(50×)_SNP ≥ 90%
	GSS	BAFNoise ≤ 0.06 and DepthNoise ≤ 0.45
RNA	RNA samples (inner control)	RNA-Control ≥ 10 copies

Note:

- *Q30: the percentage of bases with Phred quality scores ≥30, indicating a base call accuracy of 99.9%.*
- *CleanQ30: the percentage of bases with Phred quality scores ≥30 in clean data, indicating a base call accuracy of 99.9%.*
- *Depth_CDS: The average depth of the CDS target region after UID calibration.*
- *Coverage(50×)_SNP: The proportion of the sequencing data mapped to the SNP sites with a coverage depth of no less than 50×.*
- *GSS: genomic scar score.*
- *BAFNoise: The background BAF noise of the whole genome regions.*

- *DepthNoise: The background depth noise of the whole genome regions.*
- *MSI: microsatellite instability.*
- *If the RNA level test result is negative and the inner RNA-Control is less than 10 copies, it may be mainly due to poor RNA quality or insufficient amount of RNA input, or there may be some inhibitors in the RNA sample. It is recommended to re-extract RNA and reconstruct the library.*
- *Each category in Table 10 is independent, with different quality control parameters and requirements. When interpreting the results, they can be independently interpreted according to their corresponding parameters.*

9.4 Result Interpretation:

For the variants and biomarkers that pass their corresponding QC criteria as in Table 10, the variants are detected, and the biomarkers are deemed as positive if meeting the following cut-off metrics listed in Table 11.

Table 11. Cut-Off Metrics

Sample Type	Parameter		Cut-off
DNA	**SNVs/ InDels**	HotSpot mutations in core regions (Representative mutations illustrated in **Table S4**)	Depth ≥ 60×, Freq ≥ 0.5%, AltDepth ≥ 5
		HotSpot mutations in non-core regions	Depth ≥ 60×, Freq ≥ 1%, AltDepth ≥ 10
		Non-HotSpot mutations (on non-polymer or non-STR regions)	Depth ≥ 60×, Freq ≥ 3%, AltDepth ≥ 10
		Non-HotSpot mutations (on polymer or STR regions)	Depth ≥ 60×, Freq ≥ 5%, AltDepth ≥ 10 or Depth ≥ 60×, Freq ≥ 3%, AltDepth ≥ 30
	CNA (*MET*)		CopyNumber ≥ Ploidy+2 and CopyNumber ≥ 4
	CNA (Other genes for CNA detection)		CopyNumber ≥ Ploidy+2 and CopyNumber ≥ 5
	HD		MCS > 1
	MSI		MSI_Ratio ≥ 15%
	GSS		GSS ≥ 45.0
RNA	**Fusion**		Copies ≥ 5 copies
	AR-V7, *BRAF* Splicing, EGFRvIII, *MET* Skipping		Copies ≥ 40 copies

Note:

- ***For MSI status:***

  If the detected MSI_Ratio value is between 12% and 24%, it is recommended to confirm the MSI status by PCR and capillary electrophoresis method

- ***For HRD status:***
  - A positive HRD status result is defined by either the presence of a pathogenic/likely pathogenic variant in *BRCA1* or *BRCA2* genes or a positive GSS (GSS ≥ 45.0).
  - A negative HRD status is defined by negative results in both *BRCA1/2* variants and GSS.

Table 12. HRD Status Determination

***BRCA* Status**	**GSS Status**	**Sample HRD Status**
BRCA1/2 Positive	GSS Positive	HRD Status Positive
BRCA1/2 Positive	GSS Negative	HRD Status Positive
BRCA1/2 Negative	GSS Positive	HRD Status Positive

BRCA1/2 Negative	GSS Negative	HRD Status Negative

- Variant Classification Standards for *BRCA1/2*:

  According to the classification standards of the International Agency of Research on Cancer (IARC) and the American College of Medical Genetics (ACMG), the variants of BRCA1/2 can be divided into 5 classes: pathogenic variant (5), likely pathogenic variant (4), variant of uncertain significance (VUS) (3), likely benign variant (2), benign variant (1). Only pathogenic variant (5) or likely pathogenic variant (4) is defined as BRCA1/2 Positive.

- ***For Positive Controls:***

  The positive controls should be detected as positive results for the corresponding variants as shown in Appendix Table S5. Otherwise, the testing is unqualified, and it is necessary to check if there is any operational error and repeat the test.

- ***The Following are the Descriptions for the Parameters as shown above:***
  - *Depth: The effective depth of the variant site after UID calibration.*
  - *Freq: Frequency of mutant allele.*
  - *AltDepth: Depth of mutant allele.*
  - *Polymer: The regions with 5 or more consecutive identical nucleotides.*
  - *STR (short tandem repeat): The regions with 5 or more consecutive repeat units comprising of 2-6 bases.*
  - *CopyNumber: Gene copy number in tumor cells.*
  - *Ploidy: The estimated tumor cell ploidy of the sample.*
  - *MCS (Model comparison score): A Bayes factor–based indicator used to identify homozygous deletions.*
  - *MSI_Ratio: Percentage of microsatellite instability sites.*
  - *Copies: Copies of the Fusion sequence.*

Performances

The limit of Detection (LoD) is listed in Table 13.

Table 13. The LoD of the HANDLE OncoPro Panel

Sample Type	Parameter		LoD
DNA	**SNVs/InDels**	HotSpot mutations in core regions (Representative mutations illustrated in **Table S4**)	1% VAF
		HotSpot mutations in non-core regions	2% VAF
		Non-HotSpot mutations	5% VAF
	CNA		5 copies
	HD		Gene level: 30% tumor content Exon level: 40% tumor content
	MSI		10% tumor content
	GSS		30% tumor content
RNA	**Fusion**		200 copies
	AR-V7, *BRAF* Splicing, EGFRvIII, *MET* Skipping		250 copies

Limitations

1) The kit is to be used only by personnel specially trained in the techniques of PCR and NGS.

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

3) The test results from this kit are intended for research purposes only.

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

5) Users must follow the instructions strictly, any changes in operation may affect the testing results.

6) The ANDAS database is updated on a regular basis, however, data representing the latest research, including literature evidences, related databases, and etc, could take additional time to be incorporated into the database. The ANDAS software classification results might need to be further verified by representatives from medical services, genetic counselors, or other trained professionals, to evaluate the variants' classifications referring to updated databases and latest literatures, according to ENIGMA, ACMG or applicable local guidelines. Interpretation guideline from AmoyDx can be provided as reference if requested, please send such request to sales@amoydx.com for a copy.

7) Different parts of the tumor tissue or different sampling times may cause different mutation results due to tumor heterogeneity.

8) This assay identifies germline and somatic variants in the tumor but does not distinguish between the two.

9) A negative result does not completely rule out the presence of mutations in the target genes. Insufficient tumor content in the sample, heavy degradation of the sample, or a mutation level below the detection limit can also result in a negative testing result.

10) This assay outputs MSI-H or MSS for microsatellite instability status, without providing detailed classification within the MSS group to identify the MSI-L subtype.

11) InDels larger than 26 bp has not been fully validated, the detection ability may decrease as the length of the InDels increases.

12) False negative results may occur when the deletion occurs on two adjacent probes of two consecutive amplicons.

13) When two variants occur on the same amplicon, and one of the variants is located in the probe-binding region (either extension arm or ligation arm binding region), and the other variant is located in the non-probe binding region, in this situation, it may affect the binding efficiency of the probe, resulting in insufficient depth of the variants which located in the non-probe binding region, which may cause its frequency to be lower than expected and may fail to pass the filtering threshold.

14) A small portion of regions (corresponding amplicons) are difficult to amplify due to the low amplification depth, which cannot meet the detection requirements, which may lead to false negative results. False negative results may occur in tandemly repeated regions, low-complexity regions of the human genome, or low-quality sequence.

References

[1] Bray, F., Laversanne, M., Sung, H., Ferlay, J., Siegel, R. L., Soerjomataram, I., & Jemal, A. (2024). Global cancer statistics 2022: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA: a cancer journal for clinicians, 74(3), 229–263.

[2] Chakravarty, D., & Solit, D. B. (2021). Clinical cancer genomic profiling. Nature reviews. Genetics, 22(8), 483–501.

[3] Gagan, J., & Van Allen, E. M. (2015). Next-generation sequencing to guide cancer therapy. Genome medicine, 7(1), 80.

Symbols

	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

Appendix

Table S1. Gene Lists

No.	Gene	Transcript ID	Type of Mutations	Coverage Regions	Partially Covered Exons*
01	*ABRAXAS1*	NM_139076	SNV, InDel	Exon1-9	/
02	*ABTB2*	NM_145804	SNP	rs6484711	/
03	*AKT1*	NM_001382430	SNV, InDel	Exon3-5,11-12	/
04	*AKT2*	NM_001626	SNV, InDel, CNA	Exon2-14	Exon7
05	*AKT3*	NM_005465	SNV, InDel	Exon2-3	/
06	*ALK*	NM_004304	SNV, InDel, Fusion	Exon21-25	Exon21-22,24
07	*APC*	NM_000038	SNV, InDel	Exon2-16	/
08	*AR*	NM_000044	SNV, InDel, AR-V7	Exon1-8	Exon1
09	*ARAF*	NM_001654	SNV, InDel	Exon7,11,14	/
10	*ARID1A*	NM_006015	SNV, InDel	Exon1-20	Exon1,3,18
11	*ARID1B*	NM_001374828	SNV, InDel	Exon1-20	Exon1,11
12	*ARID2*	NM_152641	SNV, InDel	Exon1-21	/
13	*ATM*	NM_000051	SNV, InDel, HD	Exon2-63	/
14	*ATR*	NM_001184	SNV, InDel	Exon1-47	/
15	*ATRX*	NM_000489	SNV, InDel	Exon1-35	Exon15
16	*AURKA*	NM_198437	SNV, InDel	Exon2-9	/
17	*AXIN1*	NM_003502	SNV, InDel	Exon2-11	/
18	*B2M*	NM_004048	SNV, InDel	Exon1-3	/
19	*BAP1*	NM_004656	SNV, InDel	Exon1-17	/
20	*BARD1*	NM_000465	SNV, InDel, HD	Exon1-11	/
21	*BMPR1A*	NM_004329	SNV, InDel	Exon3-13	/
22	*BRAF*	NM_004333	SNV, InDel, Fusion, Splicing	Exon1,3-6,11-18	/
23	*BRCA1*	NM_007294	SNV, InDel, HD	Exon2-3,5-24	/
24	*BRCA2*	NM_000059	SNV, InDel, HD	Exon2-27	/
25	*BRD3*	NM_007371	Fusion	/	/
26	*BRD4*	NM_058243	Fusion	/	/
27	*BRIP1*	NM_032043	SNV, InDel, HD	Exon2-20	/
28	*CCND1*	NM_053056	SNV, InDel	Exon1,5	/
29	*CCNE1*	NM_001238	SNV, InDel, CNA	Exon2-12	/
30	*CD274*	NM_014143	CNA	/	/
31	*CDH1*	NM_004360	SNV, InDel, CNA	Exon1-16	/
32	*CDK12*	NM_016507	SNV, InDel, HD	Exon1-14	Exon2,14
33	*CDK4*	NM_000075	SNV, InDel, CNA	Exon2	/
34	*CDK6*	NM_001145306	SNV, InDel, CNA	Exon2-8	/
35	*CDKN1B*	NM_004064	SNV, InDel	Exon1-2	/
36	*CDKN2A*	NM_000077	SNV, InDel, HD	Exon1-3	/
37	*CDKN2B*	NM_004936	SNV, InDel, HD	Exon1-2	/
38	*CHD1*	NM_001270	SNV, InDel	Exon2-36	/
39	*CHEK1*	NM_001114122	SNV, InDel, HD	Exon2-13	/
40	*CHEK2*	NM_007194	SNV, InDel, HD	Exon2-15	/
41	*CLDN18*	NM_016369	Fusion	/	/

No.	Gene	Transcript ID	Type of Mutations	Coverage Regions	Partially Covered Exons*
42	*CTNNB1*	NM_001904	SNV, InDel	Exon3,7	/
43	*CYP17A1*	NM_000102	SNV, InDel	Exon6	/
44	*CYP2D6*	NM_000106	SNP	rs1065852	/
45	*DDR2*	NM_006182	SNV, InDel	Exon5,8,13-18	/
46	*DICER1*	NM_177438	SNV, InDel	Exon2-27	/
47	*DNAJB1*	NM_006145	Fusion	/	/
48	*DNMT3A*	NM_022552	SNV, InDel	Exon2-23	/
49	*DPYD*	NM_000110	SNPs	rs3918290,rs55886062,rs67376798, rs75017182,rs1801265,rs1801159	/
50	*EGFR*	NM_005228	SNV, InDel, CNA, EGFRvIII	Exon1-28	Exon7,12,14,21
51	*EIF1AX*	NM_001412	SNV, InDel	Exon1-7	/
52	*ELK4*	NM_001973	Fusion	/	/
53	*EPCAM*	NM_002354	SNV, InDel	Exon1-9	/
54	*ERBB2*	NM_004448	SNV, InDel, CNA	Exon1-27	Exon9
55	*ERBB3*	NM_001982	SNV, InDel	Exon18-24	/
56	*ERCC1*	NM_001983	SNPs	rs11615, rs3212986	/
57	*ERCC2*	NM_000400	SNV, InDel	Exon2-23	/
58	*ERG*	NM_182918	Fusion	/	/
59	*ESR1*	NM_000125	SNV, InDel, CNA	Exon1-8	/
60	*ETV1*	NM_004956	Fusion	/	/
61	*ETV4*	NM_001079675	Fusion	/	/
62	*ETV5*	NM_004454	Fusion	/	/
63	*EZH2*	NM_004456	SNV, InDel	Exon2-20	/
64	*FANCA*	NM_000135	SNV, InDel, HD	Exon1-43	/
65	*FANCC*	NM_000136	SNV, InDel	Exon2-15	/
66	*FANCD2*	NM_001018115	SNV, InDel	Exon2-44	/
67	*FANCL*	NM_018062	SNV, InDel, HD	Exon1-14	/
68	*FAT1*	NM_005245	SNV, InDel	Exon2-27	/
69	*FBXW7*	NM_001349798	SNV, InDel	Exon4-14	/
70	*FGF19*	NM_005117	CNA	/	/
71	*FGFR1*	NM_023110	SNV, InDel, CNA,Fusion	Exon4-5,7,9-18	Exon4-5,7,9-11,13,15-18
72	*FGFR2*	NM_000141	SNV, InDel, CNA, Fusion	Exon3-9,11-16,18	Exon3-6,9,11-12,18
73	*FGFR3*	NM_000142	SNV, InDel, CNA, Fusion	Exon2-4,7-17	Exon2,9-10,13-14,16-17
74	*FGFR4*	NM_213647	SNV, InDel	Exon3,6,9-10,12-13,15-16	/
75	*FH*	NM_000143	SNV, InDel	Exon1-10	/
76	*FLT3*	NM_004119	SNV, InDel, CNA	Exon1-24	Exon1
77	*FOXA1*	NM_004496	SNV, InDel	Exon1-2	/
78	*GATA3*	NM_002051	SNV, InDel	Exon2-6	Exon3
79	*GATA6*	NM_005257	CNA	/	/
80	*GEN1*	NM_001130009	SNV, InDel	Exon2-14	/
81	*GLI1*	NM_005269	CNA	/	/
82	*GLI2*	NM_001374353	SNV, InDel, CNA	Exon2-14	Exon4,7,14
83	*GNA11*	NM_002067	SNV, InDel	Exon4-5	/
84	*GNA14*	NM_004297	SNV, InDel	Exon4-5	/

No.	Gene	Transcript ID	Type of Mutations	Coverage Regions	Partially Covered Exons*
85	*GNAQ*	NM_002072	SNV, InDel	Exon4-5	Exon5
86	*GNAS*	NM_000516	SNV, InDel	Exon8,13	/
87	*GSTP1*	NM_000852	SNPs	rs1695, rs1138272	/
88	*GTF2I*	NM_032999	SNV, InDel	Exon15	/
89	*H3-3A*	NM_002107	SNV, InDel	Exon2	/
90	*H3C2*	NM_003537	SNV, InDel	Exon1	/
91	*H3C3*	NM_003531	SNV, InDel	Exon1	/
92	*HDAC2*	NM_001527	SNV, InDel, HD	Exon1-14	/
93	*HOXB13*	NM_006361	SNV, InDel, HD	Exon1-2	/
94	*HRAS*	NM_005343	SNV, InDel	Exon2-4	Exon2
95	*HSD3B1*	NM_000862	SNV, InDel	Exon4	Exon4
96	*IDH1*	NM_005896	SNV, InDel	Exon3-10	/
97	*IDH2*	NM_002168	SNV, InDel	Exon1-11	/
98	*IFNGR1*	NM_000416	SNV, InDel	Exon1-7	/
99	*IFNGR2*	NM_005534	SNV, InDel	Exon1-7	/
100	*JAK1*	NM_002227	SNV, InDel	Exon2,12-25	/
101	*JAK2*	NM_004972	SNV, InDel	Exon12-25	Exon18,24
102	*KDM5C*	NM_004187	SNV, InDel	Exon1-26	Exon23,26
103	*KDM6A*	NM_021140	SNV, InDel	Exon1-29	Exon1
104	*KEAP1*	NM_203500	SNV, InDel	Exon2-6	/
105	*KIT*	NM_000222	SNV, InDel	Exon9,11,13-14,17-18	/
106	*KMT2B*	NM_014727	SNV, InDel	Exon1-37	Exon1,3,18,27-28
107	*KMT2C*	NM_170606	SNV, InDel	Exon1-59	Exon18
108	*KMT2D*	NM_003482	SNV, InDel	Exon2-55	Exon11-12,32,43,49
109	*KRAS*	NM_004985	SNV, InDel	Exon2-4	Exon3
110	*LTK*	NM_002344	Fusion	/	/
111	*MAP2K1*	NM_002755	SNV, InDel	Exon2-3,6	/
112	*MAP2K4*	NM_003010	SNV, InDel	Exon2-11	/
113	*MDM2*	NM_002392	SNV, InDel, CNA	Exon1-11	/
114	*MDM4*	NM_002393	CNA	/	/
115	*MET*	NM_000245	SNV, InDel, CNA, Exon14 Skipping, Fusion	Exon2-12,14-15,17-21	Exon2,12,15
116	*MLH1*	NM_000249	SNV, InDel	Exon1-19	/
117	*MLH3*	NM_001040108	SNV, InDel	Exon2-13	Exon5
118	*MMS22L*	NM_001350599	SNV, InDel, HD	Exon2-25	/
119	*MRE11*	NM_005591	SNV, InDel	Exon2-20	/
120	*MSH2*	NM_000251	SNV, InDel	Exon1-16	/
121	*MSH3*	NM_002439	SNV, InDel	Exon1-24	/
122	*MSH6*	NM_000179	SNV, InDel	Exon1-10	/
123	*MTAP*	NM_002451	SNV, InDel, HD	Exon1-8	/
124	*MTOR*	NM_004958	SNV, InDel	Exon2-58	/
125	*MUTYH*	NM_001048174	SNV, InDel	Exon2-16	/
126	*MYB*	NM_001130173	Fusion	/	/
127	*MYC*	NM_002467	CNA	/	/

No.	Gene	Transcript ID	Type of Mutations	Coverage Regions	Partially Covered Exons*
128	*MYCN*	NM_005378	SNV, InDel	Exon2-3	Exon3
129	*NBN*	NM_002485	SNV, InDel	Exon1-16	/
130	*NF1*	NM_001042492	SNV, InDel, HD	Exon1-58	/
131	*NF2*	NM_000268	SNV, InDel	Exon1-16	/
132	*NFE2L2*	NM_006164	SNV, InDel	Exon2	/
133	*NKX2-1*	NM_001079668	CNA	/	/
134	*NRAS*	NM_002524	SNV, InDel	Exon2-4	/
135	*NRG1*	NM_013964	Fusion	/	/
136	*NSD3*	NM_023034	Fusion	/	/
137	*NTRK1*	NM_002529	SNV, InDel, Fusion	Exon14-15	Exon14-15
138	*NTRK2*	NM_006180	SNV, InDel, Fusion	Exon16-19	Exon16-17
139	*NTRK3*	NM_001012338	SNV, InDel, Fusion	Exon16-17	Exon16
140	*NUTM1*	NM_001284292	Fusion	/	/
141	*PALB2*	NM_024675	SNV, InDel, HD	Exon1-13	/
142	*PAX8*	NM_003466	Fusion	/	/
143	*PBRM1*	NM_018313	SNV, InDel	Exon2-30	/
144	*PDCD1LG2*	NM_025239	CNA	/	/
145	*PDGFRA*	NM_006206	SNV, InDel	Exon12,14,18	/
146	*PIK3CA*	NM_006218	SNV, InDel	Exon2-3,5-6,8-10,14,20-21	Exon6,10,14
147	*PIK3R1*	NM_181523	SNV, InDel	Exon2-16	/
148	*PMS2*	NM_000535	SNV, InDel	Exon1-15	/
149	*POLD1*	NM_002691	SNV, InDel	Exon2-27	Exon4,7,22-23
150	*POLE*	NM_006231	SNV, InDel	Exon3-14,19,30-49	Exon19,43
151	*PPP2R1A*	NM_014225	SNV, InDel	Exon1-15	/
152	*PRKACA*	NM_002730	SNV, InDel	Exon5,7	/
153	*PTCH1*	NM_000264	SNV, InDel	Exon1-23	/
154	*PTCH2*	NM_003738	SNV, InDel	Exon2-22	Exon9,18
155	*PTEN*	NM_000314	SNV, InDel, HD	Exon1-9	/
156	*QKI*	NM_006775	Fusion	/	/
157	*RAD50*	NM_005732	SNV, InDel	Exon1-25	/
158	*RAD51B*	NM_133509	SNV, InDel, HD	Exon2-11	/
159	*RAD51C*	NM_058216	SNV, InDel, HD	Exon1-9	/
160	*RAD51D*	NM_002878	SNV, InDel, HD	Exon1-10	/
161	*RAD54L*	NM_001142548	SNV, InDel, HD	Exon2-19	/
162	*RAF1*	NM_002880	SNV, InDel, CNA	Exon2-17	/
163	*RASA1*	NM_002890	SNV, InDel, HD	Exon1-25	Exon18
164	*RASGRF1*	NM_001145648	Fusion	/	/
165	*RB1*	NM_000321	SNV, InDel	Exon1-27	/
166	*RBM10*	NM_005676	SNV, InDel	Exon2-24	/
167	*RET*	NM_020975	SNV, InDel, Fusion	Exon5-6,8,10-11,13-16	Exon8,10-11
168	*RICTOR*	NM_152756	CNA	/	/
169	*RIT1*	NM_006912	SNV, InDel	Exon4-5	/
170	*RNF43*	NM_017763	SNV, InDel	Exon2-10	/
171	*ROS1*	NM_002944	SNV, InDel, Fusion	Exon36-41	Exon36

No.	Gene	Transcript ID	Type of Mutations	Coverage Regions	Partially Covered Exons*
172	*SETD2*	NM_014159	SNV, InDel, HD	Exon1-21	/
173	*SF3B1*	NM_012433	SNV, InDel	Exon14-16,18	/
174	*SMAD4*	NM_005359	SNV, InDel	Exon2-12	/
175	*SMARCA2*	NM_003070	SNV, InDel	Exon2-34	/
176	*SMARCA4*	NM_003072	SNV, InDel	Exon2-35	Exon6
177	*SMARCB1*	NM_003073	SNV, InDel	Exon1-9	/
178	*SMO*	NM_005631	SNV, InDel	Exon1-12	/
179	*SPOP*	NM_001007228	SNV, InDel	Exon2-10	/
180	*STAT3*	NM_139276	SNV, InDel	Exon7,13-14,20-21	/
181	*STK11*	NM_000455	SNV, InDel	Exon1-9	/
182	*SUFU*	NM_016169	SNV, InDel	Exon1-12	/
183	*TERT*	NM_198253	SNV	c.-124C>T (C228T), c.-146C>T	/
184	*TET2*	NM_001127208	SNV, InDel	Exon3-11	/
185	*TGFBR2*	NM_003242	SNV, InDel	Exon2-7	/
186	*TP53*	NM_000546	SNV, InDel, HD	Exon2-11	/
187	*TSC1*	NM_000368	SNV, InDel	Exon3-23	/
188	*TSC2*	NM_000548	SNV, InDel	Exon2-42	Exon39
189	*UGT1A1*	NM_000463	SNPs	rs10929302, rs8175347, rs4148323	/
190	*VEGFA*	NM_001171623	SNV, InDel, CNA	Exon1-8	Exon1,6
191	*VHL*	NM_000551	SNV, InDel	Exon1-3	Exon1
192	*YAP1*	NM_001130145	Fusion	/	/
193	*ZFTA*	NM_001144936	Fusion	/	/
194	*ZNF532*	NM_001375912	Fusion	/	/
195	*ZNF592*	NM_014630	Fusion	/	/
/	MSI	/	MSI	55 micro satellite sites	/
/	GSS	/	GSS	/	/

Note:

* : Due to the product's intended use of detecting only specific regions of exons, or due to poor coverage in certain areas, some exon segments are not covered and are therefore not within the detection range of the kit.

AR-V7: AR:exon3-AR:exon4, EGFRvIII: EGFR:exon1-EGFR:exon8, **MET Exon14 Skipping**: MET:exon13-MET:exon15.

BRAF Splicing includes: BRAF:exon1-BRAF:exon9, BRAF:exon2-BRAF:exon9, BRAF:exon3-BRAF:exon9, BRAF:exon1-BRAF:exon11, BRAF:exon2-BRAF:exon11, BRAF:exon3-BRAF:exon11.

Table S2. Fusions Detectable (RNA Level)

No.	Fusion	Gene	No.	Fusion	Gene
1	EML4:exon15-del131-ALK:exon20	*ALK*	56	AGTRAP:exon5-del7-BRAF:exon8	*BRAF*
2	EML4:exon14-del14-ALK:exon20		57	AKAP9:exon7-BRAF:exon11	
3	KIF5B:exon15-del14-ALK:exon20		58	AKAP9:exon8-BRAF:exon9	
4	BCL11A:exon4-del1849ins14-ALK:exon20		59	AKAP9:exon21-BRAF:exon10	
5	EML4:exon14-del38-ALK:exon20		60	ARMC10:exon4-BRAF:exon11	
6	EML4:exon17-del46ins23-ALK:exon20		61	ATG7:exon20-BRAF:exon9	
7	EML4:exon17-del46ins6-ALK:exon20		62	AP3B1:exon22-BRAF:exon9	
8	EML4:exon14-del49ins11-ALK:exon20		63	BCL2L11:exon2-BRAF:exon10	
9	EML4:exon2-ins117-ALK:exon20		64	BTF3L4:exon3-BRAF:exon11	
10	EML4:exon20-ins18-ALK:exon20		65	CCDC6:exon1-BRAF:exon9	
11	EML4:exon6-ins18-ALK:exon20		66	CCNY:exon1-BRAF:exon10	
12	EML4:exon17-ins30-ALK:exon20		67	CDC27:exon16-BRAF:exon9	
13	EML4:exon6-ins33-ALK:exon20		68	CEP89:exon16-BRAF:exon9	
14	EML4:exon3-ins53-ALK:exon20		69	CLCN6:exon2-BRAF:exon11	
15	EML4:exon13-ins69-ALK:exon20		70	CUX1:exon8-BRAF:exon8	
16	BIRC6:exon10-ALK:exon20		71	CUX1:exon10-BRAF:exon9	
17	CEBPZ:exon2-ALK:exon20		72	DYNC1I2:exon7-BRAF:exon10	
18	CLIP1:exon23-ALK:exon20		73	DGKI:exon6-BRAF:exon10	
19	COL25A1:exon3-ALK:exon20		74	DSTYK:exon1-BRAF:exon10	
20	DCTN1:exon26-ALK:exon20		75	EPS15:exon22-BRAF:exon10	
21	EIF2AK3:exon2-ALK:exon20		76	FAM131B:exon2-BRAF:exon9	
22	EML4:exon13-ALK:exon20		77	FAM131B:exon1-BRAF:exon10	
23	EML4:exon17-ALK:exon20		78	FAM131B:exon3-BRAF:exon9	
24	EML4:exon18-ALK:exon20		79	FAM114A2:exon9-BRAF:exon11	
25	EML4:exon2-ALK:exon20		80	FCHSD1:exon13-BRAF:exon9	
26	EML4:exon20-ALK:exon20		81	GATM:exon2-BRAF:exon11	
27	EML4:exon6-ALK:exon20		82	GHR:UTR5-BRAF:exon10	
28	EML4:exon6-ALK:exon19		83	GNAI1:exon1-BRAF:exon10	
29	GCC2:exon13-ALK:exon20		84	GTF2I:exon4-BRAF:exon10	
30	GCC2:exon19-ALK:exon20		85	HERPUD1:exon4-BRAF:exon7	
31	HIP1:exon19-ALK:exon20		86	KDM7A:exon11-BRAF:exon11	
32	HIP1:exon21-ALK:exon20		87	KIAA1549:exon10-BRAF:exon9	
33	HIP1:exon28-ALK:exon20		88	KIAA1549:exon13-BRAF:exon9	
34	HIP1:exon30-ALK:exon20		89	KIAA1549:exon13-BRAF:exon11	
35	KIF5B:exon17-ALK:exon20		90	KIAA1549:exon14-BRAF:exon9	
36	KIF5B:exon24-ALK:exon20		91	KIAA1549:exon15-BRAF:exon9	
37	KLC1:exon9-ALK:exon20		92	KIAA1549:exon15-BRAF:exon11	
38	LMO7:exon16-ALK:exon20		93	KIAA1549:exon16-BRAF:exon9	
39	MPRIP:exon22-ALK:exon20		94	KIAA1549:exon16-BRAF:exon11	
40	NBAS:exon35-ALK:exon20		95	KIAA1549:exon17-BRAF:exon10	
41	PHACTR1:exon6-ALK:exon20		96	KIAA1549:exon18-BRAF:exon10	
42	PICALM:exon19-ALK:exon20		97	KIAA1549:exon19-BRAF:exon9	
43	PPM1B:UTR5-ALK:exon20		98	KLHL7:exon5-BRAF:exon9	
44	PRKAR1A:exon10-ALK:exon20		99	LSM14A:exon9-BRAF:exon9	
45	PRKAR1A:exon5-ALK:exon20		100	MACF1:exon66-BRAF:exon9	
46	SQSTM1:exon5-ALK:exon20		101	MKRN1:exon3-BRAF:exon10	
47	STRN:exon3-ALK:exon20		102	MKRN1:exon4-BRAF:exon9	
48	TFG:exon4-ALK:exon20		103	MKRN1:exon4-BRAF:exon10	
49	TFG:exon6-ALK:exon20		104	MKRN1:exon4-BRAF:exon11	
50	TNIP2:exon5-ALK:exon20		105	MYRIP:exon16-BRAF:exon9	
51	TPR:exon15-ALK:exon20		106	MZT1:exon2-BRAF:exon11	
52	AR:exon3-AR:exon4	*AR*	107	NFIC:exon6-BRAF:exon10	
53	AGAP3:exon9-BRAF:exon9	*BRAF*	108	NUB1:exon3-BRAF:exon9	
54	AGAP3:exon11-BRAF:exon10		109	NUP214:exon21-BRAF:exon10	
55	AGK:exon2-BRAF:exon8		110	PAPSS1:exon5-BRAF:exon9	

No.	Fusion	Gene	No.	Fusion	Gene
111	PRKAR1B:exon9-BRAF:exon9	*BRAF*	166	TMPRSS2:exon2-ERG:exon3	*ERG*
112	RAD18:exon7-BRAF:exon10		167	TMPRSS2:UTR5-ERG:exon2	
113	RBMS3:exon11-BRAF:exon11		168	TMPRSS2:UTR5-ERG:exon3	
114	RNF130:exon3-BRAF:exon9		169	TMPRSS2:UTR5-ERG:exon4	
115	SLC12A7:exon17-BRAF:exon11		170	FUS:exon5-del12-ERG:exon7	
116	SLC45A3:UTR5-BRAF:exon8		171	FUS:exon6-ERG:exon10	
117	SND1:exon9-BRAF:exon9		172	FUS:exon7-ERG:exon7	
118	SND1:exon10-BRAF:exon9		173	FUS:exon7-ERG:exon9	
119	SND1:exon14-BRAF:exon9		174	FUS:exon7-ERG:exon10	
120	SND1:exon10-BRAF:exon11		175	FUS:exon8-ERG:exon8	
121	SND1:exon11-BRAF:exon11		176	TMPRSS2:UTR5-ERG:UTR5	
122	SND1:exon14-BRAF:exon11		177	EWSR1:exon7-ETV1:exon12	*ETV1*
123	SND1:exon16-BRAF:exon9		178	KLK2:exon2-ETV1:exon9	
124	SND1:exon18-BRAF:exon10		179	KLK2:exon1-ETV1:exon10	
125	STMP1:exon2-BRAF:exon9		180	SLC45A3:UTR5-ETV1:exon7	
126	STRN3:exon3-BRAF:exon10		181	TMPRSS2:exon3-ETV1:exon10	
127	TANK:exon4-BRAF:exon9		182	TMPRSS2:exon1-ETV1:exon7	
128	TAX1BP1:exon8-BRAF:exon11		183	TMPRSS2:exon2-ETV4:exon3	*ETV4*
129	TMEM178B:exon1-BRAF:exon10		184	EWSR1:exon7-ETV4:exon11	
130	TRIM24:exon3-BRAF:exon10		185	TMPRSS2:UTR5-ETV5:UTR5	*ETV5*
131	TRIM24:exon5-BRAF:exon8		186	TMPRSS2:exon3-ETV5:UTR5	
132	TRIM24:exon8-BRAF:exon11		187	BAG4:exon1-FGFR1:exon8	*FGFR1*
133	TRIM24:exon9-del102-BRAF:exon2		188	BAG4:exon1-FGFR1:exon2	
134	TRIM24:exon9-del102-BRAF:exon9		189	BAG4:exon2-FGFR1:exon6	
135	TRIM24:exon10-BRAF:exon9		190	ERLIN2:exon8-FGFR1:exon2	
136	TRIM24:exon11-BRAF:exon2		191	FGFR1:exon17-TACC1:exon7	
137	TRIM4:exon6-del3-BRAF:exon10		192	FN1:exon22-FGFR1:exon4	
138	ZC3HAV1:exon3-BRAF:exon10		193	FN1:exon22-FGFR1:exon3	
139	ZC3HAV1:exon7-BRAF:exon11		194	FN1:exon23-FGFR1:exon4	
140	ZKSCAN1:exon6-del3-BRAF:exon10		195	FN1:exon23-FGFR1:exon3	
141	ZSCAN30:exon3-BRAF:exon10		196	FN1:exon28-FGFR1:exon5	
142	BRAF:exon1-BRAF:exon9		197	NSD3:UTR5-FGFR1:exon2	
143	BRAF:exon2-BRAF:exon9		198	FGFR2:exon18-ins2-BICC1:exon2	*FGFR2*
144	BRAF:exon3-BRAF:exon9		199	FGFR2:exon18-ins2-LAMC1:exon27	
145	BRAF:exon1-BRAF:exon11		200	FGFR2:exon18-ins2-RABGAP1L:exon20	
146	BRAF:exon2-BRAF:exon11		201	APIP:exon1-FGFR2:exon6	
147	BRAF:exon3-BRAF:exon11		202	APIP:exon1-FGFR2:exon10	
148	BRD3:exon9-NUTM1:exon5	*BRD3*	203	FGFR2:exon17-DDX21:exon2	
149	BRD3:exon10-NUTM1:exon3		204	FGFR2:exon17-STK26:exon3	
150	BRD4:exon11-NUTM1:exon3	*BRD4*	205	FGFR2:exon17-POC1B:exon11	
151	BRD4:exon13-NUTM1:exon3		206	FGFR2:exon17-KIAA1217:exon3	
152	BRD4:exon14-del585-NUTM1:exon3		207	FGFR2:exon17-OFD1:exon3	
153	CLDN18:exon5-del36-ARHGAP6:exon2	*CLDN18*	208	FGFR2:exon17-TBC1D1:exon9	
154	CLDN18:exon5-del36-ARHGAP10:exon8		209	FGFR2:exon17-CTNNA3:exon14	
155	CLDN18:exon5-del36-ARHGAP26:exon10		210	FGFR2:exon17-ATP6V1D:exon3	
156	CLDN18:exon4-ARHGAP26:exon11		211	FGFR2:exon17-TACC2:exon17	
157	CLDN18:exon5-del36-ARHGAP26:exon12		212	FGFR2:exon17-TP73:exon2	
158	CLDN18:exon5-del36-ARHGAP42:exon7		213	FGFR2:exon17-CREB5:exon8	
159	DNAJB1:exon1-PRKACA:exon2	*DNAJB1*	214	FGFR2:exon17-BICC1:exon3	
160	DNAJB1:exon2-PRKACA:exon2		215	FGFR2:exon17-CASP7:exon2	
161	EGFR:exon1-EGFR:exon8	*EGFR*	216	FGFR2:exon17-SHTN1:exon9	
162	SLC45A3:UTR5-ins9-ELK4:exon2	*ELK4*	217	FGFR2:exon17-AHCYL1:exon2	
163	SLC45A3:exon2-ins9-ELK4:exon2		218	FGFR2:exon17-CCDC6:exon2	
164	TMPRSS2:exon3-ERG:exon2	*ERG*	219	FGFR2:exon17-PPP1R21:exon16	
165	TMPRSS2:exon2-ERG:exon2		220	FGFR2:exon17-WAC:exon5	

No.	Fusion	Gene	No.	Fusion	Gene
221	FGFR2:exon17-OGA:exon12	*FGFR2*	276	FGFR3:exon17-TACC3:exon6	*FGFR3*
222	FGFR2:exon17-COL14A1:exon34		277	FGFR3:exon17-PHLDB3:exon10	
223	FGFR2:exon17-BICC1:exon2		278	FGFR3:exon17-TLE5:exon2	
224	FGFR2:exon17-TXLNA:exon5		279	FGFR3:exon17-TACC3:exon11	
225	FGFR2:exon17-EIF4A2:exon8		280	FGFR3:exon17-TACC3:exon3	
226	FGFR2:exon17-AFF3:exon8		281	FGFR3:exon17-FBXO28:exon4	
227	FGFR2:exon17-ROCK1:exon2		282	FGFR3:exon17-TACC3:exon10	
228	FGFR2:exon17-PPHLN1:exon3		283	FGFR3:exon17-TACC3:exon7	
229	FGFR2:exon17-SORBS1:exon10		284	FGFR3:exon17-TACC3:exon8	
230	FGFR2:exon17-CIT:exon24		285	CLIP1:exon17-del116-LTK:exon11	*LTK*
231	FGFR2:exon17-BICC1:exon16		286	PTPRZ1:exon1-MET:exon2	*MET*
232	FGFR2:exon17-PCM1:exon7		287	PTPRZ1:exon2-MET:exon2	
233	FGFR2:exon17-GAB2:exon2		288	PTPRZ1:exon3-MET:exon2	
234	FGFR2:exon17-LZTFL1:exon7		289	PTPRZ1:exon8-MET:exon2	
235	FGFR2:exon17-NRBF2:exon4		290	MET:exon13-MET:exon15	
236	FGFR2:exon17-BICC1:exon18		291	MYB:exon13-NFIB:exon11	*MYB*
237	FGFR2:exon17-KIAA1217:exon4		292	MYB:exon9-NFIB:exon10	
238	FGFR2:exon17-CCAR2:exon4		293	MYB:exon8-NFIB:exon10	
239	FGFR2:exon17-BICC1:exon9		294	MYB:exon8-del9-NFIB:exon10	
240	FGFR2:exon17-SEPTIN10:exon6		295	CD74:exon6-ins63-NRG1:exon6	*NRG1*
241	FGFR2:exon17-TACC3:exon11		296	ADAM9:exon18-NRG1:exon2	
242	FGFR2:exon17-SHTN1:exon7		297	AKAP13:exon5-NRG1:exon2	
243	FGFR2:exon17-NRAP:exon24		298	ATP1B1:exon2-NRG1:exon2	
244	FGFR2:exon17-AMOT:exon6		299	CD44:exon5-NRG1:exon2	
245	FGFR2:exon17-ERC1:exon8		300	CD74:exon6-NRG1:exon6	
246	FGFR2:exon17-DZANK1:exon11		301	CD74:exon6-NRG1:exon3	
247	FGFR2:exon17-NOL4:exon7		302	CD74:exon7-NRG1:exon2	
248	FGFR2:exon17-BICC1:exon10		303	CD74:exon7-NRG1:exon6	
249	FGFR2:exon17-PAWR:exon3		304	CD74:exon8-NRG1:exon6	
250	FGFR2:exon17-CCDC186:exon4		305	CDH1:exon2-NRG1:exon2	
251	FGFR2:exon17-SLMAP:exon3		306	CLU:exon2-NRG1:exon6	
252	KLK2:exon1-FGFR2:exon5		307	COX10_AS1:exon1-NRG1:exon2	
253	SLC45A3:UTR5-FGFR2:exon2		308	DIP2B:exon1-NRG1:exon2	
254	FGFR3:exon17-TACC3:exon4	*FGFR3*	309	DPYSL2:exon7-NRG1:exon6	
255	FGFR3:exon17-TACC3:exon5		310	GDF15:exon1-NRG1:exon2	
256	FGFR3:exon17-TACC3:exon15		311	HMBOX1:UTR5-NRG1:exon6	
257	FGFR3:exon17-TACC3:exon16		312	KIF13B:exon2-NRG1:exon2	
258	FGFR3:exon18-TACC3:exon7		313	MCPH1:exon13-NRG1:exon2	
259	FGFR3:exon18-del341-TACC3:exon4		314	MDK:exon4-NRG1:exon6	
260	FGFR3:exon18-del112-TACC3:exon10		315	MRPL13:exon2-NRG1:exon2	
261	FGFR3:exon18-del117-TACC3:exon8		316	MTSS1:exon3-NRG1:exon2	
262	FGFR3:exon18-del124-TACC3:exon9		317	NOTCH2:exon4-NRG1:exon6	
263	FGFR3:exon18-del125-TACC3:exon11		318	NSD3:UTR5-NRG1:exon2	
264	FGFR3:exon18-del147-TACC3:exon7		319	PARP8:exon2-NRG1:exon2	
265	FGFR3:exon18-del27-TACC3:exon11		320	PCM1:UTR5-NRG1:exon6	
266	FGFR3:exon18-del37ins15-TACC3:exon9		321	PDE7A:exon3-NRG1:exon6	
267	FGFR3:exon17-del49-TACC3:exon4		322	POMK:UTR5-NRG1:exon2	
268	FGFR3:exon17-TACC3:exon14		323	RAB3IL1:exon9-NRG1:exon6	
269	FGFR3:exon17-TACC3:exon2		324	RALGAPA1:exon21-NRG1:exon6	
270	FGFR3:exon17-BAIAP2L1:exon2		325	RBPMS:exon5-NRG1:exon2	
271	FGFR3:exon17-AMBRA1:exon15		326	RBPMS:exon5-NRG1:exon6	
272	FGFR3:exon17-TACC3:exon13		327	ROCK1:exon1-NRG1:exon2	
273	FGFR3:exon17-JAKMIP1:exon4		328	SDC4:exon2-NRG1:exon4	
274	FGFR3:exon17-ELAVL3:exon2		329	SDC4:exon2-NRG1:exon6	
275	FGFR3:exon17-TACC3:exon12		330	SDC4:exon4-NRG1:exon6	

No.	Fusion	Gene	No.	Fusion	Gene
331	SETD4:exon2-NRG1:exon2	*NRG1*	386	LMNA:exon11-NTRK1:exon12	*NTRK1*
332	SLC3A2:exon1-NRG1:exon6		387	LMNA:exon2-NTRK1:exon10	
333	SLC3A2:exon2-NRG1:exon6		388	LMNA:exon2-NTRK1:exon12	
334	SLC4A4:exon14-NRG1:exon2		389	LMNA:exon2-NTRK1:exon11	
335	SMAD4:UTR5-NRG1:exon6		390	LMNA:exon2-NTRK1:exon16	
336	TENM4:exon12-NRG1:exon2		391	LMNA:exon3-NTRK1:exon11	
337	THAP7:exon3-NRG1:exon2		392	LMNA:exon4-NTRK1:exon10	
338	THBS1:exon6-NRG1:exon6		393	LMNA:exon4-NTRK1:exon12	
339	TNC:exon10-NRG1:exon6		394	LMNA:exon5-NTRK1:exon11	
340	TNKS:exon3-NRG1:exon2		395	LMNA:exon8-NTRK1:exon12	
341	TSHZ2:exon1-NRG1:exon6		396	LMNA:exon9-NTRK1:exon10	
342	VAMP2:exon4-NRG1:exon4		397	LMNA:exon9-NTRK1:exon12	
343	VTCN1:exon2-NRG1:exon4		398	LMNA:UTR3-ins8-NTRK1:exon12	
344	WRN:exon30-NRG1:exon2		399	LRRC71:exon1-NTRK1:exon10	
345	ZMYM2:UTR5-NRG1:exon2		400	LRRC71:exon1-NTRK1:exon8	
346	NSD3:exon7-NUTM1:exon3	*NSD3*	401	MEF2D:exon9-NTRK1:exon12	
347	TPM3:exon10-del1-NTRK1:exon9	*NTRK1*	402	MPRIP:exon14-NTRK1:exon12	
348	TPM3:exon1-del132-NTRK1:exon12		403	MPRIP:exon19-NTRK1:exon12	
349	LMNA:exon11-del150-NTRK1:exon11		404	MPRIP:exon22-NTRK1:exon14	
350	LMNA:exon6-del172-NTRK1:exon12		405	MPRIP:exon22-NTRK1:exon12	
351	PPL:exon22-del2470-NTRK1:exon11		406	MTMR6:exon1-NTRK1:exon8	
352	TFG:exon5-del258-NTRK1:exon8		407	NFASC:exon20-NTRK1:exon10	
353	GON4L:exon21-del331del289-NTRK1:exon8		408	NFASC:exon21-NTRK1:exon10	
354	TPM3:exon7-del39-NTRK1:exon10		409	PEAR1:exon15-NTRK1:exon10	
355	IRF2BP2:exon1-del48-NTRK1:exon10		410	PLEKHA6:exon14-NTRK1:exon10	
356	TPR:exon16-del54ins13-NTRK1:exon10		411	PLEKHA6:exon21-NTRK1:exon10	
357	LMNA:exon12-ins8-NTRK1:exon12		412	PLEKHA6:exon21-NTRK1:exon9	
358	AFAP1:exon4-NTRK1:exon9		413	PPL:exon11-NTRK1:exon13	
359	AMOTL2:exon6-NTRK1:exon12		414	PPL:exon21-NTRK1:exon10	
360	ARHGEF11:exon40-NTRK1:exon12		415	PPL:exon21-NTRK1:exon11	
361	ARHGEF2:exon21-NTRK1:exon10		416	PRDX1:exon5-NTRK1:exon12	
362	ATP1B1:exon2-NTRK1:exon8		417	RPL7A:exon2-NTRK1:exon10	
363	BCAN:exon12-NTRK1:exon10		418	SCYL3:exon11-NTRK1:exon12	
364	BCAN:exon13-NTRK1:exon11		419	SQSTM1:exon2-NTRK1:exon10	
365	CD74:exon8-NTRK1:exon10		420	SQSTM1:exon5-NTRK1:exon10	
366	CD74:exon8-NTRK1:exon12		421	SQSTM1:exon6-NTRK1:exon10	
367	CEL:exon7-NTRK1:exon8		422	SSBP2:exon12-NTRK1:exon12	
368	CHTOP:exon5-NTRK1:exon10		423	TFG:exon4-NTRK1:exon9	
369	CHTOP:exon5-NTRK1:exon11		424	TFG:exon5-NTRK1:exon10	
370	CTRC:exon1-NTRK1:exon8		425	TFG:exon5-NTRK1:exon9	
371	CTRC:exon2-NTRK1:exon8		426	TFG:exon6-NTRK1:exon10	
372	DIAPH1:exon26-NTRK1:exon10		427	TNFSF15:exon1-NTRK1:exon11	
373	EPHB2:exon3-NTRK1:exon8		428	TPM3:exon10-NTRK1:exon8	
374	EPS15:exon21-NTRK1:exon10		429	TPM3:exon10-NTRK1:exon9	
375	F11:exon4-NTRK1:exon10		430	TPM3:exon5-NTRK1:exon11	
376	F11R:exon4-NTRK1:exon10		431	TPM3:exon5-NTRK1:exon12	
377	GRIPAP1:exon22-NTRK1:exon10		432	TPM3:exon7-NTRK1:exon10	
378	GRIPAP1:exon22-NTRK1:exon12		433	TPM3:exon8-NTRK1:exon10	
379	GRIPAP1:exon22-NTRK1:exon11		434	TPM3:exon8-NTRK1:exon12	
380	IRF2BP2:exon1-NTRK1:exon10		435	TPM3:exon9-NTRK1:exon10	
381	IRF2BP2:exon1-NTRK1:exon8		436	TPR:exon10-NTRK1:exon10	
382	KIF21B:exon14-NTRK1:exon10		437	TPR:exon21-NTRK1:exon10	
383	LMNA:exon10-NTRK1:exon10		438	TPR:exon21-NTRK1:exon9	
384	LMNA:exon10-NTRK1:exon12		439	TPR:exon22-NTRK1:exon10	
385	LMNA:exon10-NTRK1:exon11		440	TPR:exon6-NTRK1:exon12	

No.	Fusion	Gene	No.	Fusion	Gene
441	TRIM33:exon12-NTRK1:exon12	*NTRK1*	496	PHACTR1:exon7-NTRK3:exon14	*NTRK3*
442	TRIM63:exon8-NTRK1:exon9		497	PHACTR1:exon8-NTRK3:exon14	
443	VANGL2:UTR5-NTRK1:exon12		498	RBPMS:exon5-NTRK3:exon14	
444	ZBTB7B:exon2-NTRK1:exon12		499	SNHG26:exon3-NTRK3:exon16	
445	AFAP1:exon14-NTRK2:exon10	*NTRK2*	500	SPECC1L:exon6-NTRK3:exon14	
446	AGBL4:exon5-NTRK2:exon14		501	SPECC1L:exon9-NTRK3:exon13	
447	AGBL4:exon6-NTRK2:exon14		502	SQSTM1:exon4-NTRK3:exon14	
448	AGBL4:exon7-NTRK2:exon14		503	SQSTM1:exon5-NTRK3:exon14	
449	BCR:exon1-NTRK2:exon15		504	SQSTM1:exon6-NTRK3:exon15	
450	ETV6:exon5-NTRK2:exon13		505	SQSTM1:exon7-NTRK3:exon14	
451	GKAP1:exon10-NTRK2:exon14		506	STRN:exon3-NTRK3:exon14	
452	KANK1:exon11-NTRK2:exon12		507	STRN3:exon3-NTRK3:exon14	
453	KCTD8:exon1-NTRK2:exon14		508	TFG:exon6-NTRK3:exon14	
454	NACC2:exon5-NTRK2:exon11		509	TMTC2:exon9-NTRK3:exon15	
455	NOS1AP:exon9-NTRK2:exon11		510	VIM:exon8-NTRK3:exon14	
456	PAN3:exon1-NTRK2:exon15		511	WDR72:exon18-NTRK3:exon15	
457	PRKAR2A:exon2-NTRK2:exon14		512	ACIN1:exon3-NUTM1:exon3	*NUTM1*
458	QKI:exon6-NTRK2:exon14		513	ATXN1:exon8-del8-NUTM1:exon6	
459	SPECC1L:exon11-NTRK2:exon13		514	AVEN:exon2-NUTM1:exon3	
460	SPECC1L:exon12-NTRK2:exon13		515	BCORL1:exon2-NUTM1:exon3	
461	SQSTM1:exon4-NTRK2:exon13		516	MXD1:exon5-NUTM1:exon3	
462	SQSTM1:exon5-NTRK2:exon14		517	CIC:exon20-del3-NUTM1:exon3	
463	SQSTM1:exon5-NTRK2:exon15		518	CIC:exon20-del3-NUTM1:exon5	
464	STRN:exon3-NTRK2:exon14		519	MGA:exon22-NUTM1:exon3	
465	STRN3:exon7-NTRK2:exon14		520	MGA:exon22-NUTM1:exon2	
466	TBC1D2:exon6-NTRK2:exon12		521	MXD4:exon5-NUTM1:exon2	
467	TLE4:exon8-NTRK2:exon13		522	MXD4:exon5-NUTM1:exon3	
468	TRAF2:exon9-NTRK2:exon13		523	MXI1:exon5-NUTM1:exon3	
469	TRIM24:exon12-NTRK2:exon13		524	WWTR1:exon3-NUTM1:exon3	
470	TRIM24:exon12-NTRK2:exon14		525	YAP1:exon3-NUTM1:exon3	
471	VCAN:exon6-NTRK2:exon10		526	YAP1:exon2-NUTM1:exon5	
472	VCL:exon16-NTRK2:exon10		527	YAP1:exon2-NUTM1:exon3	
473	WNK2:exon24-NTRK2:exon14		528	PAX8:exon7-PPARG:exon2	*PAX8*
474	AKAP13:exon14-NTRK3:exon14	*NTRK3*	529	PAX8:exon8-PPARG:exon2	
475	AKAP13:exon16-NTRK3:exon14		530	PAX8:exon9-PPARG:exon2	
476	AKAP13:exon3-NTRK3:exon14		531	PAX8:exon10-PPARG:exon2	
477	BTBD1:exon4-NTRK3:exon14		532	MYB:exon8-QKI:exon5	*QKI*
478	EEF1A1:exon8-NTRK3:exon14		533	MYBL1:exon9-QKI:exon4	
479	EML4:exon2-NTRK3:exon14		534	QKI:exon3-RAF1:exon8	
480	EML4:exon6-NTRK3:exon14		535	OCLN:exon5-RASGRF1:exon14	*RASGRF1*
481	ETV6:exon4-NTRK3:exon15		536	SLC4A4:exon23-RASGRF1:exon11	
482	ETV6:exon4-NTRK3:exon13		537	IQGAP1:exon2-RASGRF1:exon14	
483	ETV6:exon4-NTRK3:exon14		538	TMEM87A:exon15-RASGRF1:exon9	
484	ETV6:exon4-NTRK3:exon12		539	CCDC6:exon1-del107-RET:exon11	*RET*
485	ETV6:exon5-NTRK3:exon15		540	KIF5B:exon15-del107-RET:exon11	
486	ETV6:exon5-NTRK3:exon16		541	NCOA4:exon7-del18-RET:exon12	
487	ETV6:exon5-NTRK3:exon13		542	CCDC6:exon1-del199-RET:exon11	
488	ETV6:exon5-NTRK3:exon14		543	NCOA4:exon7-del199-RET:exon11	
489	ETV6:exon6-NTRK3:exon15		544	CCDC6:exon1-ins132del125-RET:exon11	
490	ETV6:exon6-NTRK3:exon13		545	AKAP13:exon35-RET:exon12	
491	KANK1:exon2-NTRK3:exon14		546	CCDC186:exon10-RET:exon12	
492	LYN:exon8-NTRK3:exon14		547	CCDC186:exon7-RET:exon12	
493	MYO5A:exon23-NTRK3:exon11		548	CCDC186:exon9-RET:exon12	
494	MYO5A:exon23-NTRK3:exon12		549	CCDC6:exon1-RET:exon12	
495	MYO5A:exon34-NTRK3:exon13		550	CCDC6:exon1-RET:exon10	

No.	Fusion	Gene	No.	Fusion	Gene
551	CCDC6:exon1-RET:exon2	*RET*	597	CCDC6:exon5-ROS1:exon35	*ROS1*
552	CCDC6:exon2-RET:exon11		598	CD74:exon6-ROS1:exon32	
553	CCDC6:exon2-RET:exon12		599	CD74:exon6-ROS1:exon34	
554	CCDC6:exon8-RET:exon12		600	CD74:exon6-ROS1:exon35	
555	CUX1:exon10-RET:exon12		601	CD74:exon8-ROS1:exon34	
556	DLG5:exon13-RET:exon12		602	CLTC:exon31-ROS1:exon35	
557	ERC1:exon11-RET:exon12		603	EZR:exon10-ROS1:exon34	
558	ERC1:exon13-RET:exon12		604	EZR:exon10-ROS1:exon35	
559	ERC1:exon18-RET:exon12		605	GOPC:exon4-ROS1:exon36	
560	ERC1:exon8-RET:exon12		606	GOPC:exon8-ROS1:exon35	
561	FKBP15:exon25-RET:exon12		607	LRIG3:exon16-ROS1:exon35	
562	GOLGA5:exon7-RET:exon12		608	MYO5A:exon23-ROS1:exon35	
563	HOOK3:exon11-RET:exon12		609	PPFIBP1:exon9-ROS1:exon35	
564	KIF13A:exon18-RET:exon12		610	SDC4:exon2-ROS1:exon32	
565	KIF5B:exon15-RET:exon12		611	SDC4:exon4-ROS1:exon34	
566	KIF5B:exon16-RET:exon12		612	SDC4:exon4-ROS1:exon32	
567	KIF5B:exon18-RET:exon12		613	SDC4:exon4-ROS1:exon35	
568	KIF5B:exon22-RET:exon12		614	SLC34A2:exon4-ROS1:exon34	
569	KIF5B:exon23-RET:exon12		615	SLC34A2:exon4-ROS1:exon32	
570	KIF5B:exon24-RET:exon11		616	SLC34A2:exon4-ROS1:exon36	
571	KIF5B:exon24-RET:exon8		617	SLC34A2:exon4-ROS1:exon35	
572	KTN1:exon29-RET:exon12		618	TFG:exon4-ROS1:exon35	
573	MPRIP:exon20-RET:exon12		619	TMEM106B:exon2-ROS1:exon35	
574	MYO5C:exon25-RET:exon12		620	TPM3:exon7-ROS1:exon35	
575	NCOA4:exon6-RET:exon12		621	TPM3:exon8-ROS1:exon35	
576	NCOA4:exon7-RET:exon12		622	YAP1:exon5-MAMLD1:exon3	*YAP1*
577	NCOA4:exon8-RET:exon12		623	YAP1:exon5-MAMLD1:exon4	
578	PCM1:exon29-RET:exon12		624	YAP1:exon5-MAML2:exon2	
579	PICALM:exon19-RET:exon12		625	YAP1:exon6-MAML2:exon2	
580	PRKAR1A:exon7-RET:exon12		626	YAP1:exon6-MAMLD1:exon3	
581	RELCH:exon10-RET:exon12		627	YAP1:exon7-ins6-FAM118B:exon3	
582	RUFY2:exon9-RET:exon12		628	YAP1:exon5-KMT2A:exon4	
583	RUFY3:exon12-RET:exon12		629	YAP1:exon4-KMT2A:exon5	
584	SPECC1L:exon10-RET:exon12		630	YAP1:exon6-KMT2A:exon9	
585	TBL1XR1:exon9-RET:exon12		631	YAP1:exon1-TFE3:exon4	
586	TNIP2:exon5-RET:exon12		632	YAP1:exon1-SS18:UTR5	
587	TRIM24:exon9-RET:exon12		633	YAP1:exon1-SS18:exon1	
588	TRIM27:exon3-RET:exon12		634	YAP1:exon1-SS18:exon2	
589	TRIM33:exon11-RET:exon12		635	ZFTA:exon2-RELA:exon2	*ZFTA*
590	TRIM33:exon14-RET:exon12		636	ZFTA:exon3-RELA:exon2	
591	TRIM33:exon16-RET:exon12		637	ZFTA:exon3-RELA:exon3	
592	WAC:exon3-RET:exon12		638	ZFTA:exon2-RELA:exon3	
593	CLIP1:exon21-ROS1:exon36	*ROS1*	639	ZNF532:exon5-NUTM1:exon5	*ZNF532*
594	SLC34A2:exon13-del47-ROS1:exon32		640	ZNF532:exon9-NUTM1:exon2	
595	SLC34A2:exon13-del47-ROS1:exon34		641	ZNF532:exon6-NUTM1:exon3	
596	KDELR2:exon5-ins8-ROS1:exon35		642	ZNF592:exon10-NUTM1:exon3	*ZNF592*

Table S3. Index Sequence Information for Primers

Index Name	Index Sequence	Illumina No.
HOP-N707	GTAGAGAG	N707
HOP-N710	CAGCCTCG	N710
HOP-N711	TGCCTCTT	N711
HOP-N712	TCCTCTAC	N712
HOP-N714	TCATGAGC	N714
HOP-N715	CCTGAGAT	N715
HOP-N723	GAGCGCTA	N723
HOP-N724	CGCTCAGT	N724
HOP-N726	GTCTTAGG	N726
HOP-N727	ACTGATCG	N727
HOP-N728	TAGCTGCA	N728
HOP-N729	GACGTCGA	N729

Index Name	Index Sequence	Illumina No.
HOP-S502	CTCTCTAT	S502
HOP-S503	TATCCTCT	S503
HOP-S505	GTAAGGAG	S505
HOP-S506	ACTGCATA	S506
HOP-S507	AAGGAGTA	S507
HOP-S508	CTAAGCCT	S508
HOP-S510	CGTCTAAT	S510
HOP-S511	TCTCTCCG	S511
HOP-S516	CCTAGAGT	S516
HOP-S517	GCGTAAGA	S517

Table S4. Representative HotSpot SNVs/InDels in Core Regions with 1% LOD (DNA Level)

No.	Gene	Exon	Representative HotSpot Mutations
01	*BRAF*	Exon15	V600E
02	*EGFR*	Exon18	G719A, G719S, G719C
03	*EGFR*	Exon19	Exon19 deletion
04	*EGFR*	Exon20	S768I, T790M, Exon20 insertion
05	*EGFR*	Exon21	L858R, L861Q
06	*ERBB2*	Exon20	Exon20 insertion
07	*KRAS*	Exon2	G12A, G12C, G12S, G12D, G12V
08	*KRAS*	Exon3	Q61H
09	*NRAS*	Exon2	G12D
10	*NRAS*	Exon3	Q61K, Q61R
11	*PIK3CA*	Exon10	E545K
12	*PIK3CA*	Exon21	H1047R

Table S5. Positive Variants (SNVs, Fusions, and HD) in HOP-Positive Control (DNA&RNA)

No.	Gene	Mutation Type	Mutation
01	*PTEN*	SNV	NM_000314: Intron8:c.1026+1G>T:p.?
02	*TERT*	SNV	NM_198253: c.-124C>T:p.?
03	*TP53*	SNV	NM_000546: Exon6:c.638G>A:p.(R213Q)
04	*CDKN2A*	HD	WholeGene HD
05	*CDKN2B*	HD	WholeGene HD
06	*MTAP*	HD	WholeGene HD
07	*ROS1*	Fusion	GOPC:NM_020399:exon8-ROS1:NM_002944:exon35

Note：

- *For quality control of the positive control (PC), all variants listed in the table above must be detected. Failure to detect any of these variants will render the experiment unqualified.*
- *Please be aware that additional positive variants may be present in both the PC and negative control (NC); however, these are not required for quality control. Some variants—such as ERCC2 NM_000400: Intron10:c.949+1_950-1del:p.? observed in the PC—are generally detectable but may occasionally be missed when their frequencies are close to the LOD.*
- *Only pathogenic and likely pathogenic variants are listed above, classified according to ACMG guidelines using the latest evidence. Classifications may change as new evidence emerges. Please refer to the specific ANDAS analysis module version for the corresponding PC variant list used in data QC.*
- *The positive control contains copy number variations in multiple genes at levels near the LoD, which may cause variability in CNA results across different runs. Therefore, the PC is not intended for CNA result evaluation.*