AmoyDx® Myeloid Blood Cancer Panel

Instructions for Use

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

REF 8.06.0082 24 tests/kit For Illumina MiSeq, MiSeqDx (RUO Mode), NextSeq 500, NextSeq 550, NovaSeq 6000

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Background

Myeloid Leukemia is a malignant disease of myeloid hematopoietic stem cells. It is mainly characterized by abnormal proliferation of primitive and juvenile myeloid cells in bone marrow and peripheral blood. The clinical manifestations are anemia, hemorrhage, infection and fever, infiltration of viscera, abnormal metabolism, etc. The treatment of acute myeloid leukemia (AML) is still challenging with high relapse rates and poor survival. Before 2017, there were almost no targeted drugs for AML, chemotherapy, high-dose chemotherapy together with hematopoietic SCT (HSCT) were the main treatment strategies to improve the outcome. While after 2017, several new targeted drugs have been approved by the US Food and Drug Administration (FDA) for AML treatment, such as DNA methyltransferase (DNMT) inhibitor decitabine, isocitrate dehydrogenase-1 (IDH1) inhibitor ivosidenib, isocitrate dehydrogenase-2 (IDH2) inhibitor enasidenib, FMS-like tyrosine kinase 3 tyrosine kinase domain (FLT3-TKD) inhibitor gilteritinib, polo-like kinase 1 (PLK1) inhibitor volasertib, etc. In addition to AML, at 2001 Imatinib was approved by the FDA for the treatment of BCR-ABL1 positive chronic myelogenous leukemia (CML) patients. Ponatinib was approved by the FDA in 2012 for CML patients with ABL1(T315I) gene mutation. The approval of various targeted drugs indicates the increasing demand for multi-target detection in clinical diagnostics of myeloid leukemia. [1-7]

Intended Use

AmoyDx® Myeloid Blood Cancer Panel is a next-generation sequencing (NGS) based assay intended for qualitatively detect and identify single nucleotide variants (SNVs), insertions and deletions (InDels) and gene fusions in 55 myeloid leukemia associated genes (see Table S1) using DNA and RNA isolated from bone marrow aspirite specimens from acute myeloid leukemia patients and chronic myeloid leukemia patients.

The kit is intended to be used by trained professionals in a laboratory environment.

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 index (UMI) at both ends, which allows high sensitivity in variant detection by eliminating any library amplification and sequencing bias. The test kit uses DNA and RNA extracted from bone marrow aspirite specimens, and it offers a time-efficient protocol that can be completed within 5 hours, and requires just about 1 hour of hands-on time.

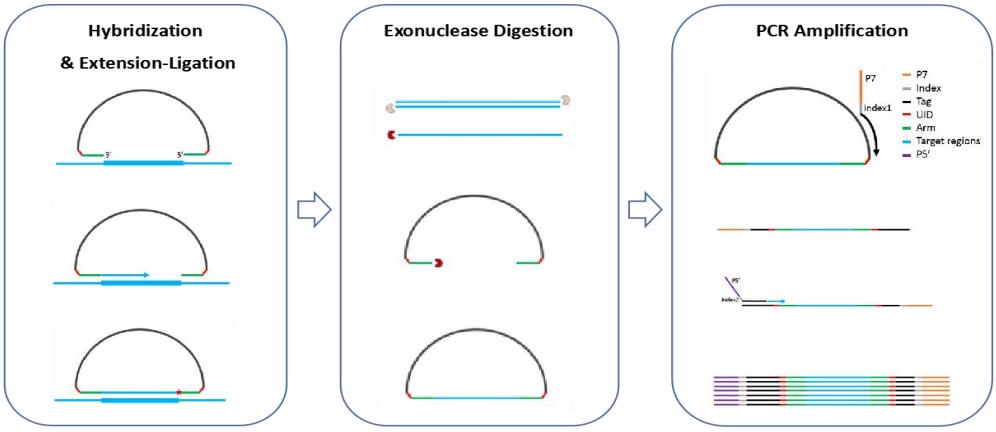

Figure 1. Principle of Library Construction (HANDLE System)

The probe contains an extension arm and a ligation arm which are complementary to the target gene region. Firstly, the probe anneals onto the DNA template of the target region. Secondly, the DNA is extended from the extension arm to the ligation arm with the help of DNA polymerase, then the nicks are repaired to generate the circular products with the help of DNA ligase. Next, the excess linear probes, single-strand and double-strand DNA are digested with the help of enzyme exonuclease, only the synthesized circular DNA molecules contains targeted region will be remained for PCR amplification. Finally, the universal PCR amplification is performed to enrich the target DNA, 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 identify the variants in the target region.

Kit Contents

This kit contains the following components in Table 1.

Table 1. Kit Contents

No.	Content	Main Ingredient	Quantity
1-RT	**MBCP-RT Primers**	Oligonucleotides	56 µL/tube ×1
2-RT	**MBCP-RT Reaction Mix**	Tris-HCl, K^+, Mg^{2+}, dNTPs	35 µL/tube ×1
3-RT	**MBCP- Reverse Transcriptase**	Reverse Transcriptase	7 µL/tube ×1
4-Hyb	**MBCP-Probe**	Oligonucleotides	56 µL/tube ×1
5-Hyb	**MBCP-Hybridization Buffer**	Tris-HCl, Mg^{2+}	28 µL/tube ×1
6-EL	**MBCP-Extension Ligation Master Mix**	DNA polymerase, dNTPs, DNA Ligase, Ligation buffer	28 µL/tube ×1
7-ED	**MBCP-Exonuclease A**	DNA Exonuclease	14 µL/tube ×1
8-ED	**MBCP-Exonuclease B**	DNA Exonuclease	14 µL/tube ×1
9-Amp	**MBCP-PCR Master Mix**	Tris, Mg^{2+}, dNTPs, DNA polymerase	650 µL/tube ×1
716，718~724，726~729	**MBCP-N7 Primer ***	Oligonucleotides	5 µL/tube ×12
513，515~518，520~522	**MBCP-S5 Primer ***	Oligonucleotides	5 µL/tube ×8
PC-D	**MBCP-Positive Control-DNA**	DNA	10 µL/tube ×1
PC-R	**MBCP-Positive Control-RNA**	RNA	16 µL/tube ×1
NC-D	**MBCP-Negative Control-DNA**	DNA	10 µL/tube ×1
NC-R	**MBCP-Negative Control-RNA**	RNA	16 µL/tube ×1

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

* *The kit contains enough reagents for 6 reactions of Positive Control and Negative Control (both DNA and RNA).*

Storage and Stability

The kit needs to be shipped in cold chain at -20℃±5℃, and the shipping time should be less than one week. All contents of the kit should be stored immediately upon receipt at -20℃±5℃.

The shelf-life of the kit is eight months. The maximum number of freeze-thaw cycles is five.

Materials Required but Not Supplied

1) PCR instrument: Applied Biosystems™ 2720 Thermal Cycler, MiniAmp™ Thermal Cycler or Bio-Rad T100™ Thermal Cycler is recommended.

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® 2.0/3.0/4 Fluorometer (Thermo Fisher Scientific) is recommended.
5) DNA extraction kit: AmoyDx Blood/Bone Marrow DNA Kit (Amoy Diagnostics) is recommended for DNA extraction from bone marrow aspirite specimens.
6) RNA extraction kit: AmoyDx Tissue RNA Kit (Amoy Diagnostics) is recommended for RNA extraction from bone marrow aspirite specimens.
7) DNA purification kit: Agencourt AMPure XP Kit (Beckman Coulter) or CleanNGS magnetic beads (Vdobiotech) is recommended.
8) 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.
9) Sequencing Instrument: Illumina MiSeq, MiSeqDx (RUO Mode), NovaSeq 6000 or NextSeq 500/550 is recommended.
10) Sequencing reagent: Illumina 300 cycles (Paired-End Reads, 2×150 cycles) is recommended.
11) Illumina PhiX Control V3.
12) Magnetic stand: DynaMag™-2 Magnet (Thermo Fisher Scientific) is recommended.
13) Mini centrifuge.
14) Vortex mixer.
15) Ice box for 0.2 mL and 1.5 mL tubes.
16) Nuclease-free 1.5 mL centrifuge tubes.
17) Nuclease-free 0.2 mL PCR tubes.
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) or 10 mM Tris solution (pH 8.0).

Precautions and Handling Requirements

- Any serious incident that has occurred in relation to the device shall be reported to the manufacturer and the competent authority of the Member State in which the user and/or the patient is established.

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.

- 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.
- 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.
- 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 nucleic acid contamination to the reagents.
- All disposable materials are for single use. DO NOT reuse.
- The unused reagents, used kit, and waste must be disposed properly. Waste disposal shall follow local regulation.

Cleaning

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

Specimen Preparation

- Sample DNA and RNA should be extracted from bone marrow aspirite sample of acute myeloid leukemia patients and chronic myeloid leukemia patients.
- The bone marrow aspirite should be more than 2 mL. The EDTA anticoagulant is recommended during the blood collection, avoid using heparin anticoagulant. The blood sample should be extracted immediately or being transported at 2℃ to 8℃, if not applicable, store the blood sample at -80℃±5℃ for no more than 3 years.
- It is recommended to use a commercialized DNA or RNA extraction kit to perform the DNA or RNA extraction, and use RNaseA to degrade RNA during the DNA extraction. After extraction, measure the concentration of extracted DNA and RNA using Quantus™ or Qubit® Fluorometer. The DNA concentration should be no less than 7.2 ng/μL, and total DNA should be no less than 50 ng; The RNA concentration should be no less than 30.8 ng/μL, and total RNA should be no less than 200 ng. For unqualified samples, re-collection or re-extraction are required.
- The qualified extracted DNA and RNA should be used for library preparation immediately. If not applicabe, the DNA should be stored at -20℃±5℃ and the RNA should be stored at -80℃±5℃ for no more than 12 months, avoid repeated freezing and thawing.

Assay Procedure

Note:

- *It is recommended to include positive controls (PCs, including (PC-D) MBCP-Positive Control-DNA and (PC-R) MBCP-Positive Control-RNA) and negative controls (NCs, includes (NC-D) MBCP-Negative Control-DNA and (NC-R) MBCP-Negative Control-RNA) 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) to verify the absence of contamination. The NTC can be used for the quality control of the library construction process, while for sequencing or data analysis process is not needed.*
- *During the following DNA library preparation process, please prepare each reaction mixture on ice to avoid non-specificity, and use the corresponding adaptor in the thermocycler to avoid the PCR products evaporation.*
- *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) MBCP-RT Primers and thaw the reagents at room temperature. When the reagents are completely thawed, mix well by vortexing and spin the vials briefly, then keep the tube on ice.

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

Table 2. Pre-reverse Transcription Reaction

Reagent	Volume
Nuclease-free water	6.5-χ μL
RNA	χ μL
(1-RT) MBCP-RT Primers	2 μL
Total	**8.5 μL**

Note:

- *For bone marrow aspirite samples, "χ" stands for the volume of 200~400 ng RNA (400 ng is recommended).*
- *For the MBCP-Positive Control-RNA (PC-R) or the MBCP-Negative Control-RNA (NC-R), take 2 μL for library construction (χ=2).*

1.3 Mix the solution thoroughly by vortexing or pipetting and spin briefly to collect the liquid, then place the sample in a thermocycler. Set the reaction volume as 9 μL and perform the following program: 65℃ for 5 min, then put the tubes on ice for at least 1 min. Then proceed immediately to step 1.4.

1.4 Take out the (2-RT) MBCP-RT Reaction Mix and thaw the reagents at the room temperature. When the reagent is completely thawed, mix well by vortexing and spin briefly, then keep the tube on ice. Prepare the reverse transcription reaction mix on ice by adding the following components according to Table 3.

Table 3. Reverse Transcription Reaction

Reagent	Volume
(2-RT) MBCP-RT Reaction Mix	1.25 μL
(3-RT) MBCP-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 (2-RT) MBCP-RT Reaction Mix and (3-RT) MBCP-Reverse Transcriptase for uniformity and precision of the final reaction mix when prepare three or more samples simultaneously.*

1.5 Mix the solution thoroughly by vortexing or pipetting, and spin briefly, then place the sample in a thermocycler. Set the reaction volume as 10 μL and perform the following program: 50℃ for 50 min, 98℃ for 10 min, 4℃ hold.

Note: *The* reverse transcription products should be stored at 2℃ to 8℃ for no more than 20 hours if not to proceed to the next step immediately.

2. Hybridization

2.1 Take out the (4-Hyb) **MBCP-Probe** and (5-Hyb) **MBCP-Hybridization Buffer** and thaw the reagents at room temperature. When the reagents are completely thawed, mix well by vortexing and spin briefly, then keep the tube on ice.

2.2 Take out the aforementioned product from previous step (Step 1.5) from the thermocycler and keep the tube on ice. Prepare the hybridization reaction mix on ice by adding the following components according to Table 4.

Table 4. Hybridization Reaction

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

Note*:*

- *For bone marrow aspirite samples, "χ" stands for the volume of 50~100 ng DNA (100 ng is recommended).*
- *For the MBCP-Positive Control-DNA (PC-D) or the MBCP-Negative Control-DNA (NC-D), take 1 μL for library construction (χ=1).*
- *It is recommended to prepare **freshly ready-to-use premix** of (4-Hyb) MBCP-Probe and (5-Hyb) MBCP-Hybridization Buffer for uniformity and precision of the final reaction mix when prepare three or more samples simultaneously.*

2.3 Mix the solution thoroughly by vortexing or pipetting and spin 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*:*

- *Keep the tubes at low temperature after hybridization 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 it is finished, take out the reaction*

tube and put it in ice box immediately.

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

3. Extension-Ligation

3.1 Take out the (6-EL) **MBCP-Extension Ligation Master Mix** and thaw the reagent at room temperature. When the reagent is completely thawed, mix well by vortexing and spin briefly, then keep the tube on ice.

3.2 Take out the aforementioned hybridization product from the thermocycler and keep the tube on ice. Add 1 μL (6-EL) **MBCP -Extension Ligation Master Mix** into the PCR tubes, mix the solution thoroughly by vortexing or pipetting, and spin briefly, then place the sample in a thermocycler, set the reaction volume as 21 μL and perform the following program: 60°C for 10 min, 4°C hold.

***Note**:*

- *It is recommended to start the PCR program first and place the reaction tube in while the thermocycler has been heated to >50 ℃.*
- *Perform the subsequent exonuclease digestion step immediately when the extension-ligation step is finished.*

4. Exonuclease Digestion

4.1 Take out the (7-ED) **MBCP -Exonuclease A** and (8-ED) **MBCP -Exonuclease B**, mix well by vortexing and spin briefly, then keep the tube on ice.

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

Table 5. Exonuclease Digestion Reaction

Reagent	Volume
Extension-Ligation product (from step 3.2)	21 μL
(7-ED) MBCP-Exonuclease A	0.5 μL
(8-ED) MBCP-Exonuclease B	0.5 μL
Total	**22 μL**

4.3 Mix the solution thoroughly by vortexing or pipetting, and spin briefly, then place the sample in a thermocycler, set the reaction volume as 22 μL and perform the following program: 37°C for 30 min, 95°C for 10 min, 4°C hold.

***Note**: The products of exonuclease digestion should be stored at 2 ℃~8 ℃ for no more than 20 hours if not to proceed to the next step immediately.*

5. PCR Amplification

5.1 Take out the **MBCP-N7 Primer, MBCP-S5 Primer** and (9-Amp) **MBCP-PCR Master Mix** and thaw the reagents at room temperature. When the reagents are completely thawed, mix well by vortexing and spin briefly, then keep the tube on ice.

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

Table 6. PCR Amplification Reaction

Reagent	Volume
Exonuclease digestion product (from step 4.3)	22 μL
(9-Amp) MBCP -PCR Master Mix	25 μL
(716, 718-724, 726-729) MBCP-N7 Primer	1.5 μL
(513, 515-518, 520-522) MBCP-S5 Primer	1.5 μL
Total	**50 μL**

***Note**:*

- *Each of the (716, 718-724, 726-729) MBCP-N7 Primer or (513, 515-518, 520-522) MBCP-S5 Primer has a different index sequence. Use a different combination of MBCP-S5 Primer and MBCP-N7 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.*
- *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 or pipetting, and spin 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℃	1 min	1
98℃	20 s	25
61℃	30 s	
72℃	20 s	
72℃	5 min	1
4℃	∞	1

***Note**:*

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

6. Purification

6.1 Take out the AMPure XP beads (or CleanNGS magnetic beads) and verify them have been kept at room temperature for at least 30 min, and vortex the bottle of the beads to resuspend any magnetic particles that may have settled.

6.2 Add **40 μL homogeneous suspended beads** and **40 μL PCR products** into a clean 1.5 mL centrifuge tube, mix thoroughly by vortexing or pipetting, then incubate for 5 min at room temperature.

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 touch 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 target DNA.*

6.7 Remove the tube from the magnet. Elute target DNA from the beads by adding 30 μL TE-low solution (pH 8.0) or 10 mM Tris solution (pH 8.0), mix thoroughly by vortexing or pipetting, and incubate for 3 min at room temperature.

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**: The purified library should be stored at -20℃±5℃ for no more than one week if not to proceed directly to sequencing.*

7. Library Quality Control (QC)

7.1 Library concentration QC: Quantify the DNA library concentration using Quantus™ or Qubit® Fluorometer, the DNA concentration should be no less than 10 ng/μL.

7.2 Library fragment size QC: Assess the library quality with a recommended capillary electrophoresis analyzer and related kit, the main peak size of the DNA library fragment should be at 260~400 bp, as shown in Figure 2.

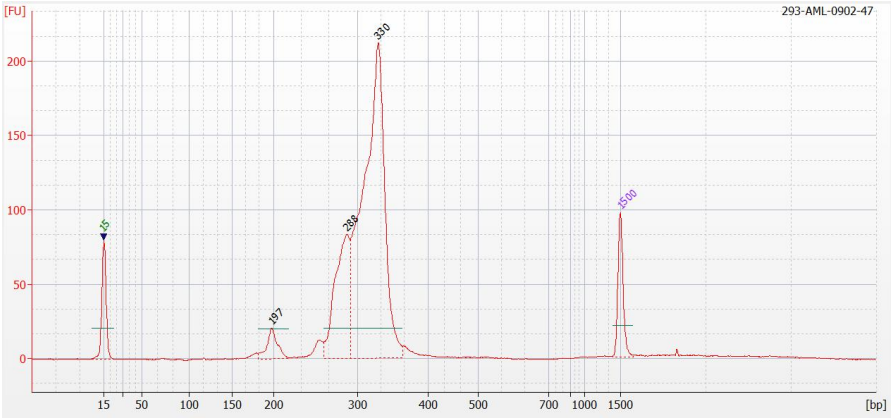

Figure 2. Example of the qualified library distribution on Agilent 2100 Bioanalyzer (Agilent DNA 1000 Reagents)

Note:

- *If the library QC pass, then move to the sequencing. If not, the library should be reconstructed.*
- *If the library concentration is less than 10 ng/μL, the library is unqualified. Please firstly check whether it is caused by operating errors and try to repeat the library construction. If the operating error has been ruled out, it may be caused by the poor quality of the sample DNA, the DNA/RNA concentration should be re-measured and the libraries should be re-constructed, and it is recommended to increase the hybridization time (up to 16 hours at 60℃) to rebuild the library.*

8. Sequencing:

Illumina 300 cycles (Paired-End Reads, 2×150 cycles) and the matched reagents and instrument are recommended for the sequencing. The recommended percentage of Illumina PhiX Control v3 is 1%. The data output per sample should be no less than 2.5 Gb. The suggested sample quantity per run is listed in Table 8.

Table 8. Recommended Sequencer and Sample Quantity per Run

Illumina Sequencer	Flow Cell	Read Length	Sample Quantity/Run
MiSeq / MiSeqDx (RUO Mode)	V3	2× 150 bp	3
NextSeq 500/550	Mid Output	2×150 bp	16
	High Output	2×150 bp	48
NovaSeq 6000	SP	2×150 bp	80
	S1	2×150 bp	Up to 96*

Note:

** Maximum 96 indexes available.*

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.

Table 9. Recommended Final Concentration of Sequencing Library

Illumina Sequencer	Final Concentration
MiSeq / MiSeqDx (RUO Mode)	6~8 pM
NextSeq 500/550	0.6~0.8 pM
NovaSeq 6000	1~1.4 nM

Note: *The concentration converting formula:*

$$\text{Library Concentration [nM]} = \frac{\text{Library Concentration[ng/μL]} \times 10^6}{660 \times 300}$$

9. Data Analysis:

When the sequencing is finished, adopt AmoyDx NGS Data Analysis System (ANDAS) to analyze the sequencing data and detect the variants of the 55 genes (Table S1). Select the module ADXHS-AML for data analysis.

Check Q30 value for the sequencing data:

If Q30 value of the sequencing data is ≥ 75%, the run data is passed/qualified. If not, the sequencing data is failed.

If Q30 value of each library is ≥ 75%, the library sequencing data is passed/qualified. If not, the library sequencing data is failed.

Data Quality Control (QC):

The passed/qualified criteria for data QC is shown in Table 10. If the minimum requirements of Table 10 are not met, the annotation fails to be reported.

Table 10. Qualified and Risky Criteria for Data QC

QC Parameter	Qualified	Risky
Clean Q30	≥ 75%	N/A
Depth	≥ 1000×	500× ~ 1000×
RNA-Control	≥ 20	N/A

Note:

Q30: One base call in 1,000 is predicted to be incorrect meaning a base call accuracy of 99.9%.

Depth: The average depth of the target region after UMI calibration.

Result Interpretation:

The mutations are detected if meeting the following requirements:

The cut-off metrics are list in Table 11.

Table 11. Cut-off Metrics

Sample	Item	Description	Cut-off	
			Normal sites (Non-Polymer/non-STR sites)	Polymer/STR sites
DNA	Depth	Total read depth	≥ 50	≥ 50
	AltDepth	Allele depth	≥ 4	≥ 6
	Freq	Allele frequency	≥ 1.5%	≥ 3%
RNA	Hotspot fusion*	Fusion genes with known breakpoint which have clear clinical significance.	Copy Number ≥ 10	
	Non-hotspot fusion	Fusion gene with unknown breakpoint.	Copy Number ≥ 20	

***Note**:*

Polymer means the regions with 5 or more consecutive identical nucleotides.

Short tandem repeats (STRs) means short tandemly repeated DNA sequences that involve a repetitive unit of 2 or more base pairs with 5 or more repeats

** Hotspot fusion: Please refer to Appendix Table.S2.*

The PC should be detected as positive result for the corresponding variants as shown in Table S4, and the NC should be detected as negative in the detection range of this kit. Otherwise, the testing is unqualified, it is necessary to check if there is any operational error and the sample should be re-tested.

Performance

Limit of Detection (LoD)

The LoD for SNVs/InDels was at 3% allele frequency, and the LoD for gene fusions was at 600 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 human bone marrow aspirite samples.
3) Reliable results are dependent on proper sample processing, transport, and storage.
4) Negative results can not completely exclude the existence of gene variants. Low tumor cell content, severe DNA/RNA degradation or the frequency under the limit of detection may also cause a false negative result.
5) InDels ≤ 26 bp in length can be detected by this assay. For InDels with the length longer than 26 bp, the detection ability may decrease as the length increases.
6) Different parts of the tumor tissue or different sampling times may cause different mutation results due to tumor heterogeneity.

7) When SNVs/InDels occur on the primers of the probes, it may cause a false negative result.

8) A few exon regions in the list are not fully covered.

References

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4. DiNardo, Courtney D et al. “10-day decitabine with venetoclax for newly diagnosed intensive chemotherapy ineligible, and relapsed or refractory acute myeloid leukaemia: a single-centre, phase 2 trial.” The Lancet. Haematology vol. 7,10 (2020): e724-e736.
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6. Miura, Masatomo.“Therapeutic drug monitoring of imatinib, nilotinib, and dasatinib for patients with chronic myeloid leukemia.” Biological & pharmaceutical bulletin vol. 38,5 (2015): 645-54.
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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. Target Regions

Number	Gene	Transcript	Alteration Type	Target Region
1	*ABL1*	NM_005157	SNV, InDel, Fusion	Exon1,3-11
2	*ASXL1*	NM_015338	SNV, InDel	Exon2,4-13
3	*BCOR*	NM_001123385	SNV, InDel	Exon2-15
4	*BRAF*	NM_004333	SNV, InDel	Exon1-4,6-8,11,12,15,17,18
5	*CALR*	NM_004343	SNV, InDel	Exon1-9
6	*CBFB*	NM_022845	SNV, InDel, Fusion	Exon3
7	*CBL*	NM_005188	SNV, InDel	Exon1-5,7-9,11,14-16
8	*CEBPA*	NM_004364	SNV, InDel	Exon1
9	*CREBBP*	NM_004380	SNV, InDel, Fusion	Exon2,25,30
10	*CSF3R*	NM_000760	SNV, InDel	Exon10,14-16,17
11	*CTCF*	NM_006565	SNV, InDel	Exon5,7,8
12	*DIS3*	NM_014953	SNV, InDel	Exon2,6,10,11,16,17,20
13	*DNMT3A*	NM_022552	SNV, InDel	Exon3-23
14	*ETV6*	NM_001987	SNV, InDel, Fusion	Exon1-3,5-8
15	*EZH2*	NM_004456	SNV, InDel	Exon2-20
16	*FBXW7*	NM_033632	SNV, InDel	Exon9,10
17	*FLT3*	NM_004119	SNV, InDel	Exon1,5,6,8-24
18	*GATA1*	NM_002049	SNV, InDel	Exon2
19	*GATA2*	NM_032638	SNV, InDel	Exon2-6
20	*IDH1*	NM_005896	SNV, InDel	Exon3,4,7
21	*IDH2*	NM_002168	SNV, InDel	Exon1,3,4,6,7,10
22	*IKZF1*	NM_006060	SNV, InDel	Exon2-8
23	*JAK2*	NM_004972	SNV, InDel, Fusion	Exon10,12-17
24	*JAK3*	NM_000215	SNV, InDel	Exon11,13,15,16,18,19
25	*KIT*	NM_000222	SNV, InDel	Exon1,2,3,5,7-18
26	*KMT2A*	NM_001197104	SNV, InDel, Fusion	Exon2,4-36
27	*KRAS*	NM_033360	SNV, InDel	Exon2-5
28	*MAX*	NM_002382	SNV, InDel	Exon4
29	*MPL*	NM_005373	SNV, InDel	Exon1,3,4,10,12
30	*MYC*	NM_002467	SNV, InDel	Exon2
31	*MYD88*	NM_002468	SNV, InDel	Exon2-5
32	*MLLT10*	NM_004641	Fusion	/
33	*NF1*	NM_001042492	SNV, InDel	Exon2-58
34	*NPM1*	NM_002520	SNV, InDel, Fusion	Exon7,10,11
35	*NRAS*	NM_002524	SNV, InDel	Exon2-4
36	*NUP98*	NM_016320	SNV, InDel, Fusion	Exon2,5,16,18,23
37	*NUP214*	NM_005085	Fusion	/
38	*PDGFRA*	NM_006206	SNV, InDel, Fusion	Exon9,10,12,14,18,22
39	*PHF6*	NM_032458	SNV, InDel	Exon2-10
40	*PTPN11*	NM_002834	SNV, InDel	Exon1-4,7,8,12,13
41	*RB1*	NM_000321	SNV, InDel	Exon1-14,16-27
42	*RIT1*	NM_006912	SNV, InDel	Exon5
43	*RUNX1*	NM_001754	SNV, InDel, Fusion	Exon2-8
44	*RARA*	NM_000964	Fusion	/
45	*SETBP1*	NM_015559	SNV, InDel	Exon2,4,6

46	*SF3B1*	NM_012433	SNV, InDel	Exon8,13-21
47	*SRSF2*	NM_003016	SNV, InDel	Exon1,2
48	*STAG2*	NM_001042749	SNV, InDel	Exon3-35
49	*STIL*	NM_001048166	Fusion	/
50	*TET2*	NM_001127208	SNV, InDel	Exon3-11
51	*TP53*	NM_000546	SNV, InDel	Exon2-11
52	*TCF3*	NM_003200	Fusion	/
53	*U2AF1*	NM_006758	SNV, InDel	Exon2,5,6
54	*WT1*	NM_024426	SNV, InDel	Exon2-9
55	*ZRSR2*	NM_005089	SNV, InDel	Exon1-11

Table S2. Targeted Gene Fusions

No.	Fusion	Gene
1	ABL1:exon1-BCR:exon15	*ABL1*
2	ABL1:exon1-BCR:exon2	
3	ABL1:exon1-NUP214:exon6	
4	ABL1:exon6-NUP214:exon10	
5	BCR:exon13-ABL1:exon2	
6	BCR:exon13-ABL1:exon3	
7	BCR:exon14-ABL1:exon2	
8	BCR:exon14-ABL1:exon3	
9	BCR:exon1-ABL1:exon3	
10	BCR:exon3-ABL1:exon2	
11	BCR:exon6-ABL1:exon2	
12	ETV6:exon4-ABL1:exon2	
13	NUP214:exon17-ABL1:exon5	
14	PML:exon3-RARA:exon3	*RARA*
15	PML:exon4-RARA:exon3	
16	PML:exon6-del158-RARA:exon3	
17	PML:exon6-ins8-RARA:exon3	
18	PML:exon6-RARA:exon3	
19	PML:exon7-del50ins647-RARA:exon3	
20	RARA:exon2-del126-STAT5B:exon14	
21	RARA:exon2-ZBTB16:exon4	
22	RARA:exon2-ZBTB16:exon5	
23	RARA:exon2-PML:exon4	
24	RARA:exon2-PML:exon7	
25	STAT5B:exon15-RARA:exon3	
26	ZBTB16:exon3-RARA:exon3	
27	AFF1:exon4-KMT2A:exon9	*KMT2A*
28	CENPK:exon6-KMT2A:exon9	
29	CREBBP:exon2-KMT2A:exon11	
30	CREBBP:exon2-KMT2A:exon9	
31	DAB2IP:exon4-del150-KMT2A:exon9	
32	EPS15:exon1-KMT2A:exon9	
33	KMT2A:exon10-CREBBP:exon3	
34	KMT2A:exon10-ELL:exon2	
35	KMT2A:exon10-EP300:exon13	
36	KMT2A:exon10-EP300:exon15	
37	KMT2A:exon10-KMT2A:exon2	
38	KMT2A:exon10-KMT2A:exon3	
39	KMT2A:exon10-LPP:exon9	
40	KMT2A:exon10-MAML2:exon2	
41	KMT2A:exon10-MLLT1:exon2	
42	KMT2A:exon10-MLLT1:exon6	
43	KMT2A:exon10-MLLT1:exon7	
44	KMT2A:exon10-MLLT10:exon9	
45	KMT2A:exon10-MLLT3:exon5	
46	KMT2A:exon10-MLLT3:exon6	
47	KMT2A:exon10-MLLT3:exon9	
48	KMT2A:exon10-SEPTIN5:exon3	
49	KMT2A:exon10-SEPTIN6:exon2	
50	KMT2A:exon11-AFDN:exon2	
51	KMT2A:exon11-ELL:exon2	
52	KMT2A:exon11-EP300:exon15	
53	KMT2A:exon11-ins272ins242-EP300:exon14	
54	KMT2A:exon11-KMT2A:exon3	
55	KMT2A:exon11-KMT2A:exon5	
56	KMT2A:exon11-MAML2:exon2	
57	KMT2A:exon11-MLLT1:exon2	
58	KMT2A:exon11-MLLT3:exon5	
59	KMT2A:exon11-MLLT3:exon6	
60	KMT2A:exon11-SEPTIN6:exon2	
61	KMT2A:exon12-KMT2A:exon5	
62	KMT2A:exon1-KMT2A:exon9	
63	KMT2A:exon20-USP2:exon3	
64	KMT2A:exon21-USP2:exon3	
65	KMT2A:exon22-AFDN:exon2	
66	KMT2A:exon22-USP2:exon3	
67	KMT2A:exon23-AFDN:exon2	
68	KMT2A:exon5-SEPTIN6:exon2	
69	KMT2A:exon6-AFDN:exon3	

70	KMT2A:exon6-MLLT10:exon14	
71	KMT2A:exon6-SEPTIN5:exon4	
72	KMT2A:exon6-SEPTIN6:exon2	
73	KMT2A:exon7-ABI2:exon4	
74	KMT2A:exon7-ABI2:exon5	
75	KMT2A:exon7-AFDN:exon2	
76	KMT2A:exon7-del8del36-ZFYVE19:exon4	
77	KMT2A:exon7-MLLT10:exon15	
78	KMT2A:exon7-MLLT10:exon9	
79	KMT2A:exon7-MLLT3:exon7	
80	KMT2A:exon7-MLLT6:exon11	
81	KMT2A:exon7-SEPTIN5:exon3	
82	KMT2A:exon7-SEPTIN6:exon2	
83	KMT2A:exon7-SEPTIN9:exon2	
84	KMT2A:exon7-SEPTIN9:exon3	
85	KMT2A:exon7-TET1:exon10	
86	KMT2A:exon8-ABI1:exon3	
87	KMT2A:exon8-ABI2:exon4	
88	KMT2A:exon8-AFDN:exon2	
89	KMT2A:exon8-ARHGAP26:exon12	
90	KMT2A:exon8-ARHGEF12:exon12	
91	KMT2A:exon8-CBL:exon10	
92	KMT2A:exon8-CEP170B:exon4	
93	KMT2A:exon8-CREBBP:exon16	
94	KMT2A:exon8-CREBBP:exon3	
95	KMT2A:exon8-DAB2IP:exon5	
96	KMT2A:exon8-del480-KNL1:exon11	
97	KMT2A:exon8-del59-DAB2IP:exon4	
98	KMT2A:exon8-EPS15:exon2	
99	KMT2A:exon8-ins208del36-CREBBP:exon15	
100	KMT2A:exon8-ins77-MLLT11:exon2	
101	KMT2A:exon8-KMT2A:exon2	
102	KMT2A:exon8-KMT2A:exon3	
103	KMT2A:exon8-KNL1:exon12	
104	KMT2A:exon8-MAML2:exon2	
105	KMT2A:exon8-MLLT10:exon10	
106	KMT2A:exon8-MLLT10:exon15	
107	KMT2A:exon8-MLLT10:exon16	
108	KMT2A:exon8-MLLT10:exon8	
109	KMT2A:exon8-MLLT10:exon9	
110	KMT2A:exon8-MLLT11:exon2	
111	KMT2A:exon8-MLLT3:exon10	
112	KMT2A:exon8-MLLT3:exon6	
113	KMT2A:exon8-MLLT3:exon9	
114	KMT2A:exon8-MLLT6:exon9	
115	KMT2A:exon8-PDS5A:exon16	
116	KMT2A:exon8-SARNP:exon2	
117	KMT2A:exon8-SEPTIN2:exon3	
118	KMT2A:exon8-SEPTIN5:exon2	
119	KMT2A:exon8-SEPTIN5:exon3	
120	KMT2A:exon8-SEPTIN6:exon2	
121	KMT2A:exon8-SEPTIN9:exon3	
122	KMT2A:exon8-SH3GL1:exon2	
123	KMT2A:exon8-TET1:exon9	
124	KMT2A:exon8-ZFYVE19:exon4	
125	KMT2A:exon9-ABI1:exon3	
126	KMT2A:exon9-AFDN:exon2	
127	KMT2A:exon9-AFF1:exon4	
128	KMT2A:exon9-AFF4:exon6	
129	KMT2A:exon9-ARHGAP26:exon19	
130	KMT2A:exon9-CIP2A:exon17	
131	KMT2A:exon9-CREBBP:exon3	
132	KMT2A:exon9-ELL:exon2	
133	KMT2A:exon9-EP300:exon13	
134	KMT2A:exon9-EP300:exon15	
135	KMT2A:exon9-ins188ins44-SEPTIN5:exon3	
136	KMT2A:exon9-ins447-AFF4:exon6	
137	KMT2A:exon9-ins478del97-TOP3A:exon1	
138	KMT2A:exon9-KMT2A:exon3	
139	KMT2A:exon9-LASP1:exon7	
140	KMT2A:exon9-MAML2:exon2	
141	KMT2A:exon9-MAML2:exon3	
142	KMT2A:exon9-MLLT1:exon2	
143	KMT2A:exon9-MLLT1:exon4	
144	KMT2A:exon9-MLLT1:exon6	
145	KMT2A:exon9-MLLT10:exon10	
146	KMT2A:exon9-MLLT10:exon15	
147	KMT2A:exon9-MLLT10:exon7	
148	KMT2A:exon9-MLLT10:exon9	
149	KMT2A:exon9-MLLT11:exon2	
150	KMT2A:exon9-MLLT3:exon6	
151	KMT2A:exon9-MLLT3:exon9	
152	KMT2A:exon9-MYO1F:exon2	
153	KMT2A:exon9-PICALM:exon8	
154	KMT2A:exon9-SEPTIN2:exon3	
155	KMT2A:exon9-SEPTIN5:exon3	
156	KMT2A:exon9-SEPTIN6:exon2	
157	KMT2A:exon9-SEPTIN6:exon3	
158	KMT2A:exon9-SORBS2:exon21	
159	LASP1:exon6-KMT2A:exon10	
160	LPP:exon8-KMT2A:exon11	
161	MLLT1:exon3-KMT2A:exon11	
162	TOP3A:exon1-del302ins68-KMT2A:exon10	
163	ETV6:exon3-NTRK3:exon16	*ETV6*
164	ETV6:exon4-NTRK3:exon15	
165	ETV6:exon4-PDGFRB:exon11	
166	ETV6:exon4-PDGFRB:exon12	
167	ETV6:exon5-NTRK3:exon15	

168	ETV6:exon5-RUNX1:exon3	
169	ETV6:exon5-RUNX1:exon4	
170	ETV6:exon7-PDGFRB:exon10	
171	MN1:exon1-ETV6:exon3	
172	MN1:exon1-ETV6:exon4	
173	BCR:exon13-JAK2:exon11	*JAK2*
174	PCM1:exon24-JAK2:exon17	
175	DEK:exon9-NUP214:exon18	*NUP214*
176	GPSM1:exon13-NUP214:exon2	
177	GPSM1:exon14-NUP214:exon2	
178	NACC2:exon2-NUP214:exon36	
179	NOTCH1:exon2-NUP214:exon25	
180	NUP214:exon21-CACNA1B:exon40	
181	NUP214:exon27-del174-IGF2:exon5	
182	NUP214:exon27-NELFB:exon11	
183	NUP214:exon29-del1180-ARHGAP26:exon12	
184	NUP214:exon29-del1599-TOM1L2:exon2	
185	NUP214:exon29-XKR3:exon2	
186	NUP214:exon33-del100del164-AGGF1:exon14	
187	NUP214:exon5-AIF1L:exon4	
188	NUP214:exon8-del18-SEC14L2:exon12	
189	POMT1:exon1-NUP214:exon31	
190	SET:exon7-NUP214:exon17	
191	SET:exon7-NUP214:exon18	
192	SET:exon8-del8ins753-NUP214:exon18	
193	SET:exon8-NUP214:exon18	
194	SQSTM1:exon5-NUP214:exon33	
195	NSD1:exon5-NUP98:exon13	*NUP98*
196	NUP98:exon11-del570-HOXA9:exon1	
197	NUP98:exon11-HOXA9:exon2	
198	NUP98:exon11-NSD1:exon6	
199	NUP98:exon12-del570-HOXA9:exon1	
200	NUP98:exon12-HOXA9:exon2	
201	NUP98:exon12-HOXD13:exon2	
202	NUP98:exon12-NSD1:exon6	
203	NUP98:exon13-del201-PHF23:exon4	
204	NUP98:exon13-KDM5A:exon27	
205	NUP98:exon13-PHF23:exon4	
206	NUP98:exon13-TOP1:exon8	
207	MLLT10:exon3-PICALM:exon20	*MLLT10*
208	MLLT10:exon8-PICALM:exon20	
209	PICALM:exon17-MLLT10:exon9	
210	PICALM:exon19-MLLT10:exon9	

211	CBFB:exon4-del105-MYH11:exon28	*CBFB*
212	CBFB:exon4-MYH11:exon29	
213	CBFB:exon4-MYH11:exon33	
214	CBFB:exon4-MYH11:exon34	
215	CBFB:exon5-del69-MYH11:exon31	
216	CBFB:exon5-ins7del88-MYH11:exon32	
217	CBFB:exon5-MYH11:exon28	
218	CBFB:exon5-MYH11:exon29	
219	CBFB:exon5-MYH11:exon30	
220	CBFB:exon5-MYH11:exon32	
221	CBFB:exon5-MYH11:exon33	
222	RUNX1:exon2-RUNX1T1:exon12	*RUNX1*
223	RUNX1:exon2-RUNX1T1:exon3	
224	RUNX1:exon2-RUNX1T1:exon7	
225	RUNX1:exon2-RUNX1T1:exon8	
226	RUNX1:exon3-del72-RUNX1T1:exon6	
227	RUNX1:exon3-RUNX1T1:exon10	
228	RUNX1:exon3-RUNX1T1:exon7	
229	RUNX1:exon4-del169-RUNX1T1:exon3	
230	RUNX1:exon4-del198-RUNX1T1:exon6	
231	RUNX1:exon4-del203del63-RUNX1T1:exon6	
232	RUNX1:exon6-CBFA2T3:exon2	
233	RUNX1:exon6-CBFA2T3:exon3	
234	RUNX1:exon6-CBFA2T3:exon4	
235	RUNX1:exon6-MECOM:exon2	
236	RUNX1:exon6-RUNX1T1:exon3	
237	RUNX1:exon7-CBFA2T3:exon4	
238	RUNX1:exon7-MECOM:exon2	
239	KAT6A:exon16-CREBBP:exon2	*CREBBP*
240	TCF3:exon15-HLF:exon4	*TCF3*
241	TCF3:exon16-HLF:exon4	
242	TCF3:exon16-PBX1:exon3	
243	NPM1:exon5-MLF1:exon2	*NPM1*
244	FIP1L1:exon10-del68-PDGFRA:exon12	*PDGFRA*
245	FIP1L1:exon11-PDGFRA:exon12	
246	FIP1L1:exon12-del82-PDGFRA:exon12	
247	FIP1L1:exon12-PDGFRA:exon12	
248	FIP1L1:exon13-PDGFRA:exon12	
249	FIP1L1:exon17-del105-PDGFRA:exon9	
250	FIP1L1:exon9-PDGFRA:exon12	
251	STIL:exon1-TAL1:exon3	*STIL*
252	STIL:exon1-TAL1:exon4	

Table S3. Index Sequence Information for Primers

Primer Name	Primer Index	Illumina Nextera
MBCP-N716	TAGCGAGT	N716
MBCP -N718	GTAGCTCC	N718
MBCP-N719	TACTACGC	N719
MBCP-N720	AGGCTCCG	N720
MBCP-N721	GCAGCGTA	N721
MBCP-N722	CTGCGCAT	N722
MBCP-N723	GAGCGCTA	N723
MBCP-N724	CGCTCAGT	N724
MBCP-N726	GTCTTAGG	N726
MBCP-N727	ACTGATCG	N727
MBCP-N728	TAGCTGCA	N728
MBCP-N729	GACGTCGA	N729

Primer Name	Primer Index	Illumina Nextera
MBCP-S513	TCGACTAG	S513
MBCP-S515	TTCTAGCT	S515
MBCP-S516	CCTAGAGT	S516
MBCP-S517	GCGTAAGA	S517
MBCP-S518	CTATTAAG	S518
MBCP-S520	AAGGCTAT	S520
MBCP-S521	GAGCCTTA	S521
MBCP-S522	TTATGCGA	S522

Table S4. Positive Variants in MBCP -Positive Control (PC-DNA/RNA)

No	Gene	CDS Change
1	*BRAF*	NM_004333:exon3:c.356C>G:p.(T119S)
2	*KRAS*	NM_033360:exon2:c.35G>A:p.(G12D)
3	*SETBP1*	NM_015559:exon4:c.3801C>T:p.(G1267=)
4	*ABL1*	*BCR:NM_004327:exon6-ABL1:NM_005157:exon2*

- *For the quality control of PC, all the variants listed in the above table must be detected, otherwise, the experiment is unqualified.*
- *Please note that there are additional positive variants in PC and NC, but these variants are not necessary for quality control. Some of the variants will be detected under normal circumstances, but occasionally they may be missed.*