AmoyDx® Master Panel

Instructions for Use

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

REF 8.06.0144 24 reactions/kit For Illumina NovaSeq 6000, NextSeq 500, NextSeq 550

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Background

Lung cancer is one of the most common malignant tumors, and 80-85% of lung cancers are non-small cell lung cancer (NSCLC). There are many driver mutations in NSCLC. The guidelines for NSCLC indicated that gene testing for *EGFR* gene mutation, *MET* gene amplification, *MET* exon 14 skipping, and *ALK, RET, ROS1* and *NTRK* gene rearrangement are required before targeted therapy, and it is strongly recommended to conduct multi-target test for the optimal precision oncology treatment. Colorectal cancer (CRC) is the third most common cancer worldwide with the metastatic disease accounts for 40-50% of newly diagnosed patients. The guidelines for colon cancer indicated that gene testing for *KRAS, NRAS, BRAF* mutation are required before targeted therapy, and universal mismatch repair (MMR) or microsatellite instability (MSI) testing is recommended in all newly diagnostic patients with colon cancer.[1-5]

Homologous recombination repair (HRR) is one of the major mechanisms for repairing DNA double-strand breaks (DSB). Homozygous deletions (HD) refer to deletions on both alleles, and the DNA-repair functions could be impacted when HDs occur on certain HRR genes. In the TOPARP-A trial, prostate cancer patients with homozygous deletions in DNA-repair genes were observed to response favorably to olaparib. Loss-of-function alterations in the genes involved in the HRR pathway could lead to the Homologous Recombination Deficiency (HRD) phenotype in multiple malignant tumors, especially in ovarian cancer, breast cancer, pancreatic ductal cancer and prostate cancer. The prostate cancer guidelines recommend germline and/or somatic HRR gene testing to identify pathogenic mutations for treatment with PARP inhibitor. The ovarian cancer guidelines unified the drug recommendation of people carrying *BRCA1/2* variants, and suggested that tumor HRD status could provide information on the eligibility of the patients for PARP inhibitors. Furthermore, FDA expanded the applicable population of olaparib combined with bevacizumab for first-line maintenance treatment of adult advanced ovarian cancer, from patients carrying pathogenic or likely pathogenic BRCA mutations to a wider range of HRD positive patients.[6-8]

Cancers with a defective DNA mismatch repair (dMMR) system contain thousands of mutations most frequently located in monomorphic microsatellites and are thereby defined as having MSI. MSI/dMMR together with tumour mutational burden (TMB) and PD-1/PD-L1 expression are proved to be predictive biomarkers for immunotherapy. TMB was defined as the number of somatic coding single nucleotide variants and insertions/deletions per megabase of genome examined. TMB is an emerging biomarker of sensitivity to immune checkpoint inhibitors. T cell–inflamed gene expression profile (GEP) and tumor microenvironment (TME) are detected through transcriptome information, and are proved to be predictive biomarkers for pembrolizumab. Recently, studies demonstrated that a T cell–inflamed GEP model contained 18 IFN-γ–responsive genes related to antigen presentation, chemokine expression, cytotoxic activity, and adaptive immune resistance was established and were observed to be positively associated with improved clinical outcomes from PD-1 inhibitor treatment, and some studies further indicated that GEP can be taken as an independent biomarker for immunotherapy to be considered together with TMB. Patients with both TMB high and GEP high features had the highest objective response rates to anti-PD-1 therapies. Based on the functional gene expression signatures that represent the major functional components, immune, stromal, and other cellular populations of the tumor, each tumor sample can be classified into 4 TME subtypes: IE/F, IE, F, and D. Studies demonstrated that the TME subtypes are correlated with patient response to immunotherapy in multiple tumor types. Patients possessing the immune-favorable

subtypes IE/F and IE would benefit more from immunotherapy, compared to patients with F and D TMEs. TME classification before and on treatment led to significantly better prediction of response to immunotherapy compared with TMB alone. EBV is the first virus found to be associated with human tumorigenesis such as nasopharyngeal carcinoma, gastric cancer and lymphoma. EBV-positive patients are associated with higher levels of immune infiltration, which indicates that their immune microenvironment is mostly activated. EBV is also a biomarker for immunotherapy response in gastric cancer. It is one of the 4 molecular subtypes of gastric cancer defined by TCGA. As observed in a study, all EBV-positive metastatic gastric cancer patients are responders to pembrolizumab.[9-12]

Intended Use

The AmoyDx® Master 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 from solid tumors. On the DNA level, the panel covers 571 genes (see Appendix I, Table S1-S4), enabling the detection of somatic single-nucleotide variants (SNV), insertions and deletions (InDel), novel and known gene fusions, copy number variants (CNV), and homozygous deletions (HD). In addition, the panel also enables the detection of tumor microsatellite instability (MSI), tumor mutation burden (TMB), homologous recombination deficiency (HRD), and the genetic polymorphisms of drug-metabolising enzymes in cancer chemotherapy. The panel enables the detection of tumor HRD status via qualitative detection and classification of SNV/InDel in protein coding regions and intron/exon boundaries of the *BRCA1* and *BRCA2* genes and the determination of the Genomic Scare Score (GSS) which is an algorithmic measurement of genomic instability status. On the RNA level, the panel covers a total of 2660 genes (see Appendix I, Table S5), enabling the detection of gene fusions and gene expressions.

The panel is also designed to detect Epstein-Barr virus (EBV) (see Appendix I, Table S6), T cell–inflamed gene expression profile (GEP) and tumor microenvironment (TME), allowing for the comprehensive analysis of cancer-associated genes.

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

Principles of the Procedure

The test kit is based on dual-directional capture (ddCAP) technology which is a targeted next generation sequencing method that employs biotinylated oligonucleotide baits (probes) to hybridize with specific target regions. The kit is designed for use with fragmented genomic DNA or RNA. During the DNA library construction process, each DNA molecule is tagged with an unique molecular index (UMI) at both ends, enabling the elimination of amplification and sequencing biases, thereby enhancing the accuracy of variant detection.

The library construction process involves both DNA and RNA workflows. For the DNA workflow, the extracted genomic DNA is first fragmented to the optimal size. The fragments are then incubated with end repair and A-tailing enzymes/reagents to generate blunt-ended fragments with dA-tails, which are subsequently ligated to adapters with complementary dT-overhangs. Following the beads purification step, PCR amplification is carried out to enrich the libraries and to label each library with unique dual indexes. For the RNA workflow, the extracted total RNA is fragmented to desired size via high temperature and Mg^{2+}, and reverse-transcribed into complementary DNA (cDNA). The cDNA is then ligated to adapters and subjected to PCR amplification for library enrichment and dual index labeling. Both

DNA and RNA libraries undergo target enrichment via hybrid capture. This process includes denaturation of the double-stranded libraries, hybridization with biotinylated probes targeting specific regions, enrichment using streptavidin beads, and elution of the captured DNA from beads. A final PCR amplification with universal primers is performed to amplify the enriched libraries. After quality control (QC) libraries meeting the required criteria are sequenced on Illumina sequencing platforms. The resulting sequencing data can then be analyzed using the AmoyDx NGS data analysis system (ANDAS) to identify variants in the targeted regions and to assess relevant biomarkers.

Kit Contents

This kit contains the following components in Table 1.

Table 1 Kit Contents

Serial No.	Abbreviation	Components	Quantity
1	E1-D	**Master-End Prep Reaction Buffer**	84 μL×1 tube
2	E2-D	**Master-End Prep Enzyme**	36 μL×1 tube
3	L1-D	**Master-Ligation Master Mix**	360 μL×1 tube
4	L2-D	**Master-Ligation Enhancer**	12 μL×1 tube
5	L3-D	**Master-Adapter**	24 μL×1 tube
6	F1-R	**Master-RNA Fragmentation Reagent F1**	24 μL×1 tube
7	F2-R	**Master-RNA Fragmentation Reagent F2**	48 μL×1 tube
8	F3-R	**Master-RNA Fragmentation Reagent F3**	96 μL×1 tube
9	F4-R	**Master-RNA Fragmentation Reagent F4**	48 μL×1 tube
10	RT1-R	**Master-Reverse Transcriptase RT1**	24 μL×1 tube
11	RT2-R	**Master-Reverse Transcriptase RT2**	24 μL×1 tube
12	EI-R	**Master-Exonuclease I**	51 μL×1 tube
13	EB-R	**Master-Exonuclease I Buffer**	70 μL×1 tube
14	A1-R	**Master-Ligation Reagent A1**	48 μL×1 tube
15	A2-R	**Master-Ligation Reagent A2**	48 μL×1 tube
16	A3-R	**Master-Ligation Reagent A3**	30 μL×1 tube
17	A4-R	**Master-Ligation Enzyme A4**	12 μL×1 tube
18	A5-R	**Master-Ligation Enzyme A5**	12 μL×1 tube
19	P1	**Master-Amplification Buffer①**	1200 μL×1 tube
20	D501-D508	**Master-D501-D508**	12 μL×8 tubes
21	D701-D712	**Master-D701-D712**	8 μL×12 tubes
22	H1	**Master-Blocker**	84 μL×1 tube
23	H2	**Master-Hyb Buffer**	120 μL×1 tube
24	B1	**Master-Beads Wash Buffer**	1500 μL×1 tube
25	W1	**Master-5×Wash Buffer①**	1056 μL×1 tube
26	W2	**Master-5×Wash Buffer②**	792 μL×1 tube
27	W3	**Master-5×Wash Buffer③**	528 μL×1 tube
28	W4	**Master-5×Wash Buffer④**	528 μL×1 tube
29	P2	**Master-Amplification Buffer②**	348 μL×1 tube
30	P3	**Master-Polymerase**	12 μL×1 tube
31	TD	**Master-Probe-TD**	30 μL×1 tube
32	TR	**Master-Probe-TR**	30 μL×1 tube

33	PC-D	**Master-DNA-Positive Control**	100 μL×1 tube
34	NC-D	**Master-DNA-Negative Control**	100 μL×1 tube
35	PC-R	**Master-RNA-Positive Control**	20 μL×1 tube
36	NC-R	**Master-RNA-Negative Control**	20 μL×1 tube

Note:

1. For labeling and sequence information of the primers, refer to Appendix II.

2. The positive variants in the DNA/RNA Positive Control are listed in Appendix III.

Storage and Stability

The kit requires shipment in cold chain, and the shipping time should be less than one week. All contents of the kit should be stored immediately upon receipt at -25℃ to -15℃.

The shelf-life of the kit is twelve months. Repeated thawing and freezing should be avoided. The maximal number of freeze-thaw cycles is five.

Materials Required but Not Supplied

1) DNA/RNA Extraction kit: The QIAamp DNA FFPE Tissue Kit (QIAGEN) or AmoyDx® Magnetic FFPE DNA Extraction kit (Amoy Diagnostics) is recommended for DNA extraction; the QIAamp RNA FFPE Tissue Kit (QIAGEN) or AmoyDx® FFPE RNA kit (Amoy Diagnostics) is recommended for RNA extraction.
2) DNA/RNA quantification kit: Qubit™ 2.0/3.0/4 Fluorometer (Thermo Fisher Scientific) and related kits (Qubit dsDNA HS Assay Kit, and Qubit RNA HS Assay Kit), or Quantus™ Fluorometer (Promega) and related kits (QuantiFluor dsDNA System and QuantiFluor RNA System) are recommended.
3) DNA purification kit: Agencourt AMPure XP Kit (Beckman Coulter Genomics) is recommended.
4) Streptavidin coupled magnetic beads: Dynabeads MyOne™ Streptavidin T1 (Thermo Fisher Scientific) is recommended.
5) Capillary electrophoresis analyzer and related kit: Agilent 2100 Bioanalyzer system and the related DNA Kit (Agilent Technologies), Agilent 2200 TapeStation and the related kit (Agilent Technologies) or LabChip GX Touch and the related kit (PerkinElmer) are recommended.
6) Ultrasonicator: Covaris M220 Focused-ultrasonicator (Covaris) and microTUBE-130 AFA Fiber Screwcap (Covaris) are recommended.
7) Vacuum Concentrator: Concentrator Plus™ complete system (Eppendorf) is recommended.
8) Vacuum lyophilizer or other instrument with the same function is recommended.
9) Thermocycler: ABI MiniAmp A37028, Bio-Rad T100, Applied Biosystems™ 2720 Thermal Cycler, or other instruments with the same function is recommended.
10) Sequencing reagent: Illumina 300 cycles (Paired-End Reads, 2×150 cycles) reagents such as Illumina NextSeq 500/550 Mid Output Reagent kit V2 (300 cycles), Illumina NextSeq 500/550 High Output Reagent kit V2 (300 cycles)and Illumina NovaSeq 6000 SP/S1/S2/S4 Reagent kit (300 cycles) are recommended.
11) Sequencer: Illumina NovaSeq 6000, NextSeq 500, NextSeq 550.

12) Illumina PhiX Control v3.

13) Magnetic stand: DynaMag™-2 Magnet (Thermo Fisher Scientific) is recommended.

14) Shaking Thermo Cell (Bioer Technology).

15) Mini centrifuge.

16) Vortex mixer.

17) Ice box for 0.2 mL and 1.5 mL tubes.

18) 1.5 mL nuclease-free centrifuge tubes.

19) 0.2 mL nuclease-free PCR tubes.

20) 0.5 mL PCR tubes: Use only thin-wall, clear 0.5 mL PCR tubes (eg. Axygen) during the DNA/RNA quantification process with Fluorometer.

21) Low-binding centrifuge tube: 0.2 mL and 1.5 mL colorless low-binding tubes (Axygen) are recommended to use in the hybrid capture process.

22) Nuclease-free filtered pipette tips.

23) Absolute ethanol (AR).

24) Nuclease-free water.

25) TE-low solution (10 mM Tris, 0.1 mM EDTA, pH 8.0).

Precautions and Handling Requirements

Precautions

- Please read the instructions carefully and become familiar with all components of the kit prior to use. Please follow the instructions strictly during operation.
- Before use, prepare the required number of reagents according to the sample numbers to avoid unnecessary freezing and thawing of reagents.
- DO NOT use the kit or any kit component after their expiry date.
- DO NOT use any other reagent from different lots in the test.
- DO NOT use any other reagent from another test kit.
- 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 contact of skin, eyes, and mucous membranes with the reagents. In case of contact, flush with water immediately.

Decontamination and Disposal

- The kit contains positive controls; 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.
- Unused reagents, used kit, and waste must be disposed properly. Waste disposal shall follow local regulations.

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/RNA should be extracted from FFPE tumor tissue specimens.
- The FFPE tissue sample should be fixed in 10% neutral buffered formalin for no more than 48 hours (recommend within 6 hours). It is recommended to use the central section of paraffin blocks. The freshly cut sections of FFPE tissue should be used for DNA/RNA extraction at the earliest convenience. Storage period of the FFPE block should be less than 5 years, with an optimal period of less than 18 months.
- Prior to testing, each FFPE tissue specimen should be subjected to independent pathology review to confirm to the presence and percentage of tumor cells. It is recommended that the tumor cell content is no less than 20%. For GSS and CNV detection, the tumor cell content should be no less than 30%; For HD detection, the tumor cell content should be no less than 30% (at least 30% for HD detection at gene level, and at least 40% for HD detection at exon level). For samples with a tumor cell content lower than the above requirements, it is recommended to perform microdissection or macrodissection to enrich the tumor cells, or re-collect samples if necessary.
- It is recommended to use the commercialized extraction kit to perform the DNA/RNA extraction from FFPE samples. It is recommended to use RNase A to digest RNA during the FFPE DNA extraction. After extraction, measure the concentration of extracted DNA and RNA using Quantus™ or Qubit®.
- **Requirements of DNA/RNA input amount for library construction:** The optimal input amount of FFPE DNA is 150 ng or above, with a minimum of 60 ng. After fragmentation, the optimal input amount of the fragmented DNA is 60 ng, with a minimum of 30 ng (30-60 ng is considered as risky); the optimal input amount of FFPE RNA is 200 ng, with a minimum of 5 ng (5-200 ng is considered as risky for gene fusion detection in RNA level).
- For unqualified samples, re-collection or re-extraction are required.
- The quantified 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. During storage, avoid repeated freezing and thawing.

Assay Procedure

Note:

- *It is recommended to include a Master-DNA-Positive Control (PC-D) and a Master-DNA-Negative Control (NC-D) in the process of DNA library preparation, sequencing, and data analysis.*
- *It is recommended to include a Master-RNA-Positive Control (PC-R) and a Master-RNA-Negative Control (NC-R) in the process of RNA library preparation, sequencing, and data analysis.*
- *During library preparation process, please use the corresponding adaptor in the thermocycler to avoid the PCR products evaporation.*
- *The library preparation process consists of three sections: (A) DNA Library Preparation, (B) RNA Library Preparation, and (C) Hybrid Capture.*

A. DNA Library Preparation

1. DNA Fragmentation

For genomic DNA (gDNA) derived from FFPE tissue samples, it is recommended to use ultrasonic fragmentation (Covaris M220) to shear the gDNA into short fragments (150-350 bp). If ultrasonic fragmentation instrument is not available, enzymatic fragmentation (not provided) is an alternative method.

For Master-DNA-Positive Control (PC-D) and Master-DNA-Negative Control (NC-D), skipping DNA fragmentation process (Step 1 and Step 2), since they were derived from fragmented cell line DNA and can be directly used in the End Repair step (Section A, Step 3) for DNA library preparation.

1.1 Ultrasonic Fragmentation:

It is recommended to use Covaris M220 Focused-ultrasonicator (Covaris, Cat. No. 500295) and microTUBE-130 AFA Fiber Screwcap (Covaris, Cat. No. 520216) for DNA fragmentation, and the procedure is as follows.

Add genomic DNA (150 ng or above is recommended, with a minimum of 60 ng, add TE-low solution to a final volume of 130 μL) into the Covaris microtube. Place the tube in the DNA shearing instrument and perform the shearing according to the parameters in Table 2.

Table 2 Covaris M220 Parameters for DNA Shearing

Parameter	Setting value
Duty Factor	20%
Peak Incident Power(W)	50
Cycles Burst	200
Time(s)	180

Note: *The shearing time might be adjusted according to the sample quality.*

1.2 Enzymatic Fragmentation:

If ultrasonic fragmentation instrument is not available, it is recommended to use KAPA Frag Kit (KK8602) for DNA fragmentation.

1.2.1 Add genomic DNA sample (150 ng is recommended) into the PCR tube, then add nuclease-free water to a final volume of 35 μL.

Assemble the enzymatic fragmentation mixture on ice by adding the following components according to Table 3.

Table 3 Enzymatic Fragmentation Reaction Mix

Component	Volume
KAPA Frag Enzyme	10 µL
KAPA Frag Buffer	5 µL
Genomic DNA	X µL
Nuclease-free water	35-X µL
Total volume	50 µL

Note:

- *For FFPE samples, "X" stands for the volume of 60-150 ng DNA (150 ng is recommended).*
- *The enzymatic reaction system is sensitive to EDTA, so it is recommended to use nuclease-free water to elute the genomic DNA during the DNA extraction process, and avoid using TE solution for DNA elution.*

1.2.2 Mix the solution thoroughly by vortexing or pipetting, and centrifuge briefly, then place the sample in a thermocycler with the heated lid off, and perform the following program according to Table 4.

Table 4 Enzymatic Fragmentation Program (Heated lid off !)

Temperature	Time
4℃	1 min
37℃	10 min
4℃	Hold

1.2.3 After the procedure finished, add 5 µL Stop Solution to stop the reaction immediately, and then proceed to next step immediately.

2. Fragment Purification

2.1 Take out the AMPure XP beads and equilibrate them to room temperature for at least 30 min, and vortex the beads to resuspend before use.

2.2 For ultrasonic fragmentation, transfer 125 µL of the fragmented DNA product (from Step 1.1) to a clean nuclease-free 1.5 mL centrifuge tube, add 250 µL resuspended AMPure XP beads and mix well on a vortex mixer; for enzymatic fragmentation, transfer all of the above fragmented DNA product (~55 µL, from Step 1.2.3) into a clean nuclease-free 1.5 mL centrifuge tube, add 110 µL resuspended AMPure XP beads and mix well on a vortex mixer. Then incubate for 10 min at room temperature.

2.3 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 tube is on the magnetic stand. **Do not** touch the beads.

2.4 Keep the tubes on the magnetic stand, add 400 µL of freshly prepared 80% ethanol to the tube while in the magnetic stand. Incubate at room temperature for at least 30 seconds, and then carefully remove and discard the supernatant.

2.5 Repeat the Step 2.4 once.

2.6 Briefly spin the tube and put the tube back in the magnetic stand. 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.*

2.7 Remove the tube from the magnetic stand. Elute DNA from the beads by adding 28 µL of nuclease-free water (not provided), mix well

by pipetting up and down 10 times, or on a vortex mixer. Incubate for at least 2 min at room temperature.

2.8 Place the tube on the magnetic stand until the solution becomes clear (3~5 min). Open the cap carefully without disturbing the bead pellet, transfer 26 µL of the supernatant into a clean 1.5 mL centrifuge tube to obtain the elution product (fragmented DNA).

2.9 Check the concentration of the DNA using Quantus™ or Qubit™ Fluorometer, the optimal DNA amount should be no less than 60 ng, with a minimum of 30 ng (30-60 ng is considered as risky).

Note: *If the DNA is not to be used immediately for the next step, store at -25℃ to -15℃ for no more than 1 week. Repeated thawing and freezing should be avoided.*

3. End Repair

3.1 Assemble the end repair reaction on ice in a clean nuclease-free 0.2 mL PCR tube according to Table 5.

Table 5 End Repair Reaction Mix

Component	Volume
(PC-D/NC-D) Fragmented DNA / PC-D / NC-D	X µL
(E1-D) Master-End Prep Reaction Buffer	3.5 µL
(E2-D) Master-End Prep Enzyme	1.5 µL
Nuclease-free water	25-X µL
Total Volume	30 µL

Note:

- *For FFPE sample, "X" stands for the volume of 30-60 ng fragmented DNA (60 ng is recommended, minimum 30 ng, 30-60 ng is considered as risky).*
- *For Master-DNA-Positive Control or Master-DNA-Negative Control, take 25 µL for library construction (X=25).*

3.2 Mix well by pipetting up and down. Centrifuge the sample tube briefly and place it in a thermocycler. Perform the program according to Table 6. Then proceed immediately to Adapter Ligation.

Table 6 End Repair Program (Heated lid at 105℃)

Temperature	Time
20℃	30 min
65℃	30 min
4℃	Hold

4. DNA Adapter Ligation

4.1 Assemble the DNA adapter ligation reaction on ice according to Table 7.

Table 7 DNA Adapter Ligation Reaction Mix

Operation Notes	Component	Volume
/	End-repaired product (Step 3.2)	30 µL
Master mix can be prepared for L1-D and L2-D	(L1-D) Master-Ligation Master Mix	15 µL
	(L2-D) Master-Ligation Enhancer	0.5 µL
Add the above L1-D and L2-D solution to the end-repaired product, mix well by pipetting up and down.		
Add L3-D separately	(L3-D) Master-Adapter	1 µL
/	Total Volume	46.5 µL

Note:

- *(L1-D) Master-Ligation Master Mix should be stored at -25℃ to -15℃ and should be operated on ice.*
- ***Important!*** *To avoid adapter self-ligation, please add L3-D separately after L1-D and L2-D are mixed well with the end-repaired product.* ***DO NOT*** *add L3-D (Master-Adapter) to a premix with L1-D and L2-D.*

4.2 Mix well by pipetting up and down. Centrifuge the sample tube briefly and place it in a thermocycler. Perform the program according to Table 8. Proceed immediately to the next step after adapter ligation.

Table 8 DNA Adapter Ligation Program (Heated lid off !)

Temperature	Time
20℃	15 min
4℃	Hold

5. Purification after Adapter Ligation

5.1 Take out the AMPure XP beads and equilibrate them to room temperature for at least 30 min, and vortex the beads to resuspend.

5.2 Transfer all of the above ligation product (from Step 4.2) into a clean nuclease-free 1.5 mL centrifuge tube, then add 42 μL resuspended AMPure XP beads, mix thoroughly by vortexing or pipetting, then incubate for 5 min at room temperature. Place the centrifuge tubes onto the magnetic stand for 3~5 min until the solution turns clear.

5.3 Gently remove and discard the supernatant while the tube is on the magnetic stand. **Do not** touch the beads.

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

5.5 Repeat the Step 5.4 once.

5.6 Briefly spin the tube, and put the tube back in the magnetic stand. Completely remove the residual ethanol, and air dry the beads for 3~5 min while the tube is on the magnetic stand with the lid open until the beads show matt surface.

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

5.7 Remove the tube from the magnetic stand. Elute DNA target from the beads by adding 23 μL nuclease-free water (not provided), mix thoroughly by vortexing or pipetting, and incubate for 2 min at room temperature.

5.8 Place the tubes on the magnetic stand until solution becomes clear (3~5 min). Open the cap carefully, without disturbing the bead pellet, transfer 21 μL of the supernatant into a clean nuclease-free 0.2 mL PCR tube.

6. DNA Library Amplification

6.1 Take out the following reagents and thaw at room temperature. When the reagents are completely thawed, shake the tubes to mix well. Assemble the PCR amplification reaction on ice by adding the following components according to Table 9.

Table 9 DNA Library Amplification Reaction Mix

Component	Volume
Purified Ligation Products (Step 5.8)	21 μL
(P1) Master-Amplification Buffer①	25 μL
(D501-D508) Master-D501~D508	2 μL
(D701-D712) Master-D701~D712	2 μL
Total Volume	50 μL

Note: *There are 8 tubes of Master-D5 Primer (Master-D501~D508) and 12 tubes of Master-D7 Primer (Master-D701~D712). Each of the Master-D5 Primer and Master-D7 Primer has a different index sequence. Use a different combination of Master-D5 Primer and Master-D7 for each sample library.* ***Do not*** *use the same combination of indexes for two or more sample libraries in a single sequencing run. The detailed information for the index sequence is shown in Appendix Table S7.*

6.2 Mix well by pipetting up and down. Centrifuge the sample tube briefly and place it in a thermocycler. Perform the program according to Table 10.

Table 10 DNA Library Amplification Program (Heated lid at 105℃)

Temperature	Time	Cycle
98℃	45 sec	1
98℃	15 sec	11
60℃	30 sec	
72℃	30 sec	
72℃	1 min	1
4℃	Hold	1

7. DNA Library Purification

7.1 Take out the AMPure XP beads and equilibrate them to room temperature for at least 30 min, and vortex the beads to resuspend before use.

7.2 Transfer all of the above PCR product (from Step 6.2) into a clean nuclease-free 1.5 mL centrifuge tube, then add 40 μL resuspended AMPure XP beads, mix thoroughly by vortexing or pipetting, then incubate for 5 min at room temperature.

7.3 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 tube is on the magnetic stand. **Do not** touch the beads.

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

7.5 Repeat the Step 7.4 once.

7.6 Briefly spin the tube, and put the tube back in the magnetic stand. 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 until the beads show matt surface.

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

7.7 Remove the tube from the magnetic stand. Elute DNA target from the beads by adding 32 μL TE-low solution (not provided), mix thoroughly by vortexing or pipetting, and incubate for 2 min at room temperature.

7.8 Place the tubes on the magnetic stand until solution becomes clear (3-5 min). Open the cap carefully without disturbing the bead pellet, transfer all of the supernatant into a clean nuclease-free 1.5 mL centrifuge tube.

Note: *If the products are not to be used immediately for the next step, store at -25℃ to -15℃ for no more than 6 months. Repeated thawing and freezing should be avoided.*

8. DNA Library Quality Control (QC)

8.1 Library concentration QC: Check the concentration of the DNA library using Quantus™ or Qubit™ Fluorometer, the total amount of each DNA library should be no less than 500 ng. For unqualified samples, re-collection, re-extraction, or library re-construction are required.

8.2 Library size distribution QC (recommended): Assess the library size distribution with a recommended capillary electrophoresis analyzer and relevant kit. The peak size distribution of the DNA library should be at 300-500 bp, without obvious peaks of small and big fragments out of the range. An example of a qualified DNA library is as shown in Figure 1. For unqualified samples, re-collection, re-extraction, or library re-construction are required.

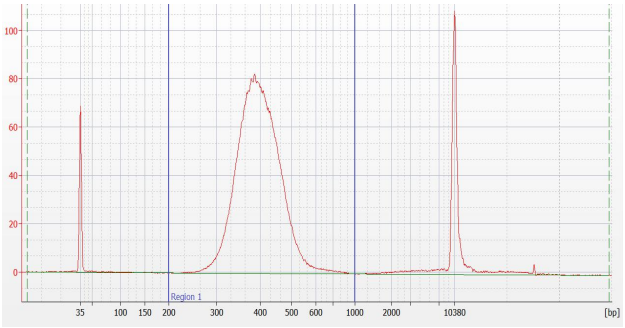

Figure 1 Example of DNA Library Size Distribution on a Bioanalyzer

B. RNA Library Preparation

1. RNA Fragmentation

1.1 Take out the reagents listed in Table 11 to thaw at room temperature, mix well with vortex and spin down. Place them on ice box and prepare the reaction mix according to Table 11.

Table 11 RNA Fragmentation Reaction Mix

Component	Volume
(F1-R) Master-RNA Fragmentation Reagent F1	1 µL
(F2-R) Master-RNA Fragmentation Reagent F2	2 µL
(F3-R) Master-RNA Fragmentation Reagent F3	4 µL
(F4-R) Master-RNA Fragmentation Reagent F4	2 µL
(PC-R/NC-R) Master-RNA Positive/Negative Control or RNA sample	X µL
Nuclease-Free Water (not provided)	13-X µL
Total volume	22 µL

Note:

- *For FFPE sample, "X" stands for the volume of 200 ng RNA amount (optimal), minimum is 5 ng. Please note that less than 200 ng is considered as risky for gene fusion detection in RNA level.*
- *For Master-RNA-Positive Control or Master-RNA-Negative Control, take 5 µL for library construction (X=5).*

1.2 Vortex the reaction tubes to mix thoroughly and spin down, then place the reaction tubes in a thermocycler and perform the program according to Table 12.

Table 12 RNA Fragmentation Program (Heated lid at 105℃)

Sample Type	Temperature	Time
FFPE or fresh frozen tissue-derived RNA	65℃	5 min
Cell line-derived RNA or negative/positive control RNA	94℃	12 min

Note: *It is recommended to set the thermocycler for an incubation period of more than 5 min or 12 min, respectively, to ensure the instrument will not start to cool down before taking the tubes out.*

1.3 When the program is finished, immediately transfer the sample tubes to ice and incubate for 2 min.

2. Reverse Transcription

2.1 Take out the reagents listed in Table 13 to thaw at room temperature, mix well with vortex and spin down. Place them on ice box and prepare the reaction mix according to Table 13.

Table 13 Reverse Transcription Reaction Mix

Component	Volume
(RT1-R) Master-Reverse Transcriptase RT1	1 µL
(RT2-R) Master-Reverse Transcriptase RT2	1 µL
RNA fragmented products (Step 1.3)	22 µL
Total volume	24 µL

2.2 Vortex the reaction tubes to mix thoroughly and spin down, then place the reaction tubes in a thermocycler to perform the following program.

Table 14 Reverse Transcription Program (Heated lid at 105℃)

Temperature	Time
25℃	20 min
42℃	30 min
50℃	10 min
70℃	15 min
4℃	Hold

3. Exo I Digestion

3.1 Take out the reagents listed in Table 15 to thaw at room temperature, mix well with vortex and spin down. Place them on ice box and prepare the reaction mix according to Table 15.

Table 15 Exo I Digestion Reaction Mix

Component	Volume
(EI-R) Master-Exonuclease I	2.1 µL
(EB-R) Master-Exonuclease I Buffer	2.9 µL
Reverse Transcription product (Step 2.2)	24 µL
Total volume	29 µL

3.2 Vortex the reaction tubes to mix thoroughly and spin down, then place the reaction tubes in a thermocycler to perform the following program according to Table 16.

Table 16 Exo I Digestion Program (Heated lid at 105℃)

Temperature	Time
37℃	30 min
4℃	Hold

4. cDNA Purification

4.1 Take out the AMPure XP beads and equilibrate them to room temperature for 30 min, and vortex the bottle of the beads for 1 min to resuspend magnetic particles evenly.

4.2 Add 21 μL TE-low solution (not provided) to the tube with 29 μL product from Step 3.2, vortex to mix well and spin down, then add 90 μL of the the AMPure XP beads, vortex to mix well and spin down, incubate at room temperature for 5 min.

4.3 Place the mix from previous step 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.

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

4.5 Repeat the Step 4.4 once.

4.6 Briefly spin the tube and gently discard the residual liquid, then immediately add 12 μL TE-low solution (not provided) to resuspend the magnetic particles evenly, incubate at room temperature for 3 min.

4.7 Put the tube back on the magnetic stand until the solution turns clear (3~5 min). Carefully take 10 μL of the supernatant for use in the coming steps.

Note: *If the libraries are not to be used immediately for the next step, store at -25℃ to -15℃ for no more than 1 week. Repeated thawing and freezing should be avoided.*

5. RNA Adapter Ligation

5.1 Put the tube containing the 10 μL purification product from Step 4.7 to a preheated thermocycler that has already reached 95℃, incubate for 2 min. Afterwards, immediately transfer the tubes on ice to incubate for 2 min.

Note:

- *It is recommended to set the thermocycler for an incubation period of more than 2 min to ensure the instrument will not start to cool down before taking the tubes out.*
- *The temperature of the PCR tube at this step could be high, please take necessary protection to prevent getting scald. Also please pay more attention when transferring the tubes, it is suggested to press the caps when holding the tubes and carefully remove the caps to prevent contamination caused by their accidental popping off.*

5.2 Take out the reagents listed in Table 17 to thaw at room temperature, mix well with vortex and spin down. Place them on ice box and prepare the reaction mix according to Table 17.

Table 17 RNA Adapter Ligation Reaction Mix

Component	Volume
(A1-R) Master-Ligation Reagent A1	2 μL
(A2-R) Master-Ligation Reagent A2	2 μL
(A3-R) Master-Ligation Reagent A3	1.25 μL
(A4-R) Master-Ligation Enzyme A4	0.5 μL
(A5-R) Master-Ligation Enzyme A5	0.5 μL
TE-low solution (not provided)	4.25 μL
Total volume	10.5 μL

5.3 Add the 10.5 μL adapter ligation reaction mix (from Step 5.2) into the preheated 10 μL purification product (from Step 5.1). Vortex the reaction tubes to mix thoroughly and spin down, then place the reaction tubes in a thermocycler to perform the following program:

Table 18 RNA Adapter Ligation Program (Heated lid at 105℃)

Temperature	Time
37℃	15 min
95℃	2 min
4℃	Hold

6. RNA Library Amplification

6.1 Take out the reagents listed in Table 19 to thaw at room temperature, mix well with vortex and spin down. Place them on ice box and prepare the reaction mix according to Table 19.

Table 19 RNA Library Amplification Reaction Mix

Component	Volume
Adapter-ligated Product (Step 5.3)	20.5 μL
(P1) Master-Amplification Buffer①	25 μL
(D501-D508) Master-D501~D508	2 μL
(D701-D712) Master-D701~D712	2 μL
Total Volume	49.5 μL

Note: *There are 8 tubes of Master-D5 Primer (Master-D501~D508) and 12 tubes of Master-D7 Primer (Master-D701~D712). Each of the Master-D5 Primer and Master-D7 Primer has a different index sequence. Use a different combination of Master-D5 Primer and Master-D7 for each sample library. Do not use the same combination of indexes for two or more sample libraries in a single sequencing run. The detailed information for the index sequence is shown in Appendix Table S7.*

6.2 Vortex the reaction tubes to mix thoroughly and spin down, then place the reaction tubes in a thermocycler to perform the following program:

Table 20 RNA Library Amplification (Heated lid at 105℃)

Temperature	Time	Cycle
98℃	45 sec	1
98℃	15 sec	14
60℃	30 sec	
72℃	30 sec	
72℃	1 min	1
4℃	∞	1

7. RNA Library Purification

7.1 Take out the AMPure XP beads and equilibrate them to room temperature, vortex it with the maximum speed for 1 min to ensure the beads are resuspended evenly.

7.2 After vortexing, take 40 μL resuspended AMPure XP beads to add to the amplification product from Step 6.2, vortex briefly and spin down, incubate at room temperature for 5 min.

7.3 Place the mix from previous step onto the magnetic stand for 3~5 min until the solution turns clear. Gently remove and discard the

supernatant while the tube is on the magnetic stand. Do not touch the beads with pipette tip.

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

7.5 Repeat the Step 7.4 once.

7.6 Briefly spin the tube and gently discard the residual liquid, then air dry the magnetic beads at room temperature till no moist luster can be observed. Do not over-dry the beads. This may result in lower recovery of the RNA libraries.

7.7 Remove the tubes from the magnet stand. Elute the RNA libraries from the beads by adding 33 μL TE-low solution (not provided), mix thoroughly by vortexing or pipetting to resuspend the beads evenly, and incubate for 2 min at room temperature.

7.8 Put the tube on the magnetic stand for 3~5 min until the solution turns clear. Without disturbing the beads, carefully transfer 31 μL supernatant into a clean nuclease-free 1.5 mL centrifuge tube.

Note: *If the libraries are not to be used immediately for the next step, store at -25℃ to -15℃ for no more than 6 months. Repeated thawing and freezing should be avoided.*

8. RNA Library QC

8.1 Library concentration QC: Check the concentration of the RNA library using Quantus™ or Qubit™ Fluorometer, the total amount of each RNA library should be no less than 500 ng. For unqualified samples, re-collection, re-extraction, or library re-construction are required.

8.2 Library size distribution QC (recommended): Assess the library size distribution with a recommended capillary electrophoresis analyzer and relevant kit. The peak size distribution of the RNA library should be at 250-500 bp, without obvious peaks of small and big fragments out of the range. An example of a qualified RNA library is as shown in Figure 2. For unqualified samples, re-collection, re-extraction, or library re-construction are required.

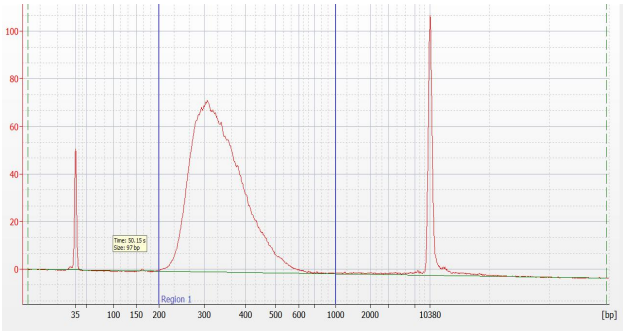

Figure 2 Example of RNA Library Size Distribution on a Bioanalyzer

C. Hybrid Capture

1. Reagent Preparation

1.1 Pool the libraries into a clean nuclease-free 0.2 mL PCR tube according to Table 21. The FFPE DNA library and FFPE RNA library should be pooled separately, and the Positive Control (PC) or Negative Control (NC) library should be pooled separately from the FFPE library.

Table 21 Suggested Library Pooling Amount

Library Pooling Strategy		DNA Library Total Amount	RNA Library Total Amount
Sample Pool	Pooling with 1 sample	750 ng	750 ng
	Pooling with 2 samples	1 μg (500 ng/library*2)	1 μg (500 ng/library*2)
	Pooling with 3 samples	1.5 μg (500 ng/library*3)	1.5 μg (500 ng/library*3)
	Pooling with 4 samples	2 μg (500 ng/library*4)	2 μg (500 ng/library*4)
	Pooling with 5 samples	2.5 μg (500 ng/library*5)	2.5 μg (500 ng/library*5)
	Pooling with 6 samples	2.4 μg (400 ng/library*6)	2.4 μg (400 ng/library*6)
PC/NC Pool	Pooling with PC and NC	1 μg (500 ng/library*2)	1 μg (500 ng/library*2)

Note:

- *It is recommended to mix equal amounts of libraries of the same sample type for hybridization, and each library should have a different index combination (Master-D5 Primer and Master-D7 Primer). Do not use the libraries with the same combination of index in a single hybridization pool. The maximum number of libraries in a single pool is 6.*
- *For FFPE samples, the recommended input amount for each library is as shown in the table above, and the maximum library amount in each pool should be no more than 2.5 μg.*
- *It is recommended to pool the PC DNA library and NC DNA library together, with 500 ng per library, and they should be pooled separately from the FFPE DNA libraries.*
- *It is recommended to pool the PC RNA library and NC RNA library together, with 500 ng per library, and they should be pooled separately from the FFPE RNA libraries.*

1.2 Take out the (H1) Master-Blocker and thaw at room temperature. When the reagents are completely thawed, vortex the tubes to mix well and centrifuge briefly. Add the (H1) Master-Blocker into the 0.2 mL PCR tubes according to Table 22, mix well by pipetting up and down, and centrifuge briefly.

Table 22 Hybridization Preparation

Component	Volume
Pooled Library (1~6)	≤ 120 μL
(H1) Master-Blocker	7 μL
Total	≤ 127 μL

1.3 Place the tube into a Vacuum Concentrator with the tube's lid open, and incubate at 60℃ until the solution in the tubes evaporates completely. Avoid overdrying.

Note: *If Vacuum Concentrator is not available, AMPure XP Beads can also be used for DNA concentration (optional), the procedures are demonstrated briefly here: To the hybridization mix from step 1.2 which contains the libraries and the Master-Blocker, add AMPure XP Beads at twice the volume of the hybridization mix. Use 200 μL 80% freshly prepared ethanol for washing (a total of two washes), and 10 μL of the (H2) Master-Hyb Buffer for DNA elution. Then transfer all DNA eluates to a clean nuclease-free 0.2 mL PCR tube and proceed to Step 2.3 below.*

2. Hybridization

2.1 Take out the (H2) Master-Hyb Buffer, (TD) MasterProbe-TD, and (TR) MasterProbe-TR, thaw the reagents at room temperature.

When the reagents are completely thawed, mix well on a vortex mixer and centrifuge briefly, then keep the tube on ice.

2.2 Carefully remove the sample tubes from the Vacuum Concentrator, add 10 μL of the (H2) Master-Hyb Buffer into each sample tube and cap the tubes, vortex to mix well, then centrifuge briefly.

2.3 Add 5 μL (TD) MasterProbe-TD or 5 μL (TR) MasterProbe-TR into each of the above sample tubes according to the sample type, mix thoroughly by vortexing and centrifuge briefly.

Note： *(1) Use the (TD) MasterProbe-TD probes for FFPE DNA (or PC/NC DNA) library hybridization；*

（2）Use the (TR) MasterProbe-TR probes for FFPE RNA (or PC/NC RNA) library hybridization。

2.4 Place the tubes into a thermocycler, perform the following program :

Table 23 Hybridization Program (Heated lid at 105℃)

Temperature	Time
95℃	10 min
52℃	12~20 hours (optimal 16 hours)

Note: *Do not hybridize for more than 20 hours or less than 12 hours.*

3. Capture

3.1 Take out the Dynabeads MyOne™ Streptavidin T1 Magnetic Beads and equilibrate them to room temperature for at least 30 min, and vortex the beads to resuspend before use.

3.2 Aliquot sufficient Streptavidin T1 Magnetic Beads (not provided) according to the number of capture pools. Add the Streptavidin T1 Magnetic Beads at the ratio of 25 μL per capture pool into a clean nuclease-free 1.5 mL low-binding centrifuge tube (eg. with 2 capture pools, you need to aliquot 50 μL Beads). Then add the same volume of the (B1) Master-Beads Wash Buffer, mix well by pipetting up and down for 10~20 times.

Note: *Low-binding centrifuge tubes are suggested especially during the Capture and Washing steps to prevent sample loss from beads binding to the tubes.*

3.3 Briefly spin the tube, then place the tubes onto a magnetic stand until the solution turns clear (~1 min).

3.4 Gently remove and discard the supernatant while the tube is on the magnetic stand. Do not touch the beads. Then add the (B1) Master-Beads Wash Buffer at twice the volume of the beads added (based on the volume of beads in Step 3.2) to the tube containing beads, mix well by pipetting up and down for 10~20 times.

3.5 Briefly spin the tube, then place the tubes onto a magnetic stand until the solution turns clear (~1 min).

3.6 Repeat the Step 3.4 once.

3.7 Prepare a sufficient number of clean 0.2 mL low-binding tubes according to the number of capture pools (one PCR tube for each capture pool), and aliquot 50 μL of the resuspended beads (Step 3.6) into a new 0.2 mL low-binding tube for each capture reaction, then place the tube onto the magnetic stand (DynaMag™-96 Side Magnet is recommended) until the solution turns clear (~1 min).

3.8 Gently remove and discard the supernatant while the tube is on the magnetic stand. **Do not** touch the beads. Then quickly transfer all

of the hybridization product (from Step 2.4) into the 0.2 mL tube with beads, pipette or shake gently to resuspend the beads quickly (avoid temperature drop during resuspension).

3.9 Place the PCR tubes on a thermocycler and perform the following program (Set the heated lid at 105℃): 52℃ for 45 min, 52℃ hold. Set a timer to 45 min, and every 15 min, quickly remove the tube from the thermocycler and shake gently to resuspend the beads and then place back into the thermocycler, each mixing process must be carried out quickly to prevent a sudden drop in temperature.

4. Washing

Important! It is critical to ensure that the following 3 steps (from Step 4.1 to Step 4.3) has been finished in advance before completing the above Step 3.9.

4.1 Turn on the ThermoCell with shaking function, set the temperature at 52℃.

4.2 Take out the (W1~W4) 5×Wash Buffer ①~④ and thaw the reagents at room temperature. When the reagents are completely thawed, shake the tubes to mix well until the solutions turn clear. Dilute the 5×Wash Buffer to 1× working solution to prepare a sufficient volume of 1× working solution according to the ratio in Table 24.

Table 24 Dilution of Wash Buffer (per capture pool)

5× Wash Buffer	1× Working Solution	Volume of 5× Wash Buffer	Volume of Water	Total Volume
(W1) 5×Wash Buffer ①	1×Wash Buffer ①	88 µL	352 µL	440 µL
(W2) 5×Wash Buffer ②	1×Wash Buffer ②	66 µL	264 µL	330 µL
(W3) 5×Wash Buffer ③	1×Wash Buffer ③	44 µL	176 µL	220 µL
(W4) 5×Wash Buffer ④	1×Wash Buffer ④	44 µL	176 µL	220 µL

4.3 Place 1×Wash Buffer ① and 1×Wash Buffer ② (transferred into a new tube) onto the ThermoCell and incubate at 52℃ for at least 10 min. The 1×Wash Buffer ③ and the 1×Wash Buffer ④ should be kept at room temperature.

4.4 When Step 3.9 is finished, add 100 µL preheated 1×Wash Buffer ② to the PCR tubes with beads (Step 3.9), mix well by pipetting up and down for 10 times, then transfer all of the solution (containing beads) into a clean 1.5 mL low-binding centrifuge tube. Centrifuge briefly and place the tubes onto a magnetic stand until the solution turns clear (~1 min).

4.5 Gently remove and discard the supernatant while the tube is on the magnetic stand. **Do not** touch the beads. Remove the tube from the magnetic stand, add 200 µL preheated 1×Wash Buffer ①, pipette up and down to mix well quickly (avoid temperature drop during resuspension). Incubate the tubes at 52℃ and shake at 500 rpm for 5 min. Then centrifuge briefly and place the tubes onto a magnetic stand until the solution turns clear (~30 seconds).

Note： *If such shaking condition (500 rpm) is not available, please mix manually every 2 minutes (quickly take out the tube, mix well gently by pipetting up and down, and then put it back into the 52°C heating block). Each mixing process must be carried out quickly to prevent a sudden drop in temperature.*

4.6 Repeat the Step 4.5 once.

4.7 Gently remove and discard the supernatant while the tube is on the magnetic stand. **Do not** touch the beads. Remove the tube from the

magnetic stand, add 200 μL preheated 1×Wash Buffer ②, pipette up and down to mix well quickly (avoid temperature drop during resuspension). Incubate the tubes at 52℃ and shake at 500 rpm for 5 min to improve the beads-binding specificity. Then centrifuge briefly and place the tubes onto a magnetic stand until the solution turns clear (~1 min).

Note： *If such shaking condition (500 rpm) is not available, please mix manually every 2 minutes (quickly take out the tube, mix well gently by pipetting up and down, and then put it back into the 52°C heating block). Each mixing process must be carried out quickly to prevent a sudden drop in temperature.*

4.8 Gently remove and discard the supernatant while the tube is on the magnetic stand. **Do not** touch the beads. Remove the tube from the magnetic stand, add 200 μL 1×Wash Buffer ③, shake the tubes at 2000 rpm for 1 min, centrifuge briefly and place the tubes onto a magnetic stand until the solution turns clear (~1 min).

Note： *If such shaking condition (2000 rpm) is not available, one can also vortex the tubes to mix well for 1 min.*

4.9 Gently remove and discard the supernatant while the tube is on the magnetic stand. **Do not** touch the beads. Remove the tube from the magnetic stand, add 200 μL 1×Wash Buffer ④, shake the tubes at 2000 rpm for 30 seconds, centrifuge briefly and place the tubes onto a magnetic stand until the solution turns clear (~1 min).

Note： *If such shaking condition (2000 rpm) is not available, one can also vortex the tubes to mix well for 30 seconds.*

4.10 Gently remove and discard the supernatant while the tube is on the magnetic stand. **Do not** touch the beads. Remove the tube from the magnetic stand, add 20 μL nuclease-free water (not provided), shake the tubes to mix well, then centrifuge briefly.

5. Amplification of the Captured Products

5.1 Shake the captured products containing beads (Step 4.10) to resuspend the beads. Assemble the Hybrid Capture PCR Mixture on ice by adding the following components according to Table 25.

Table 25 Hybrid Capture PCR Reaction Mix

Component	Volume
Captured Library (Step 4.10)	20 μL
(P2) Master-Amplification Buffer②	29 μL
(P3) Master-Polymerase	1 μL
Total volume	50 μL

5.2 Mix well by pipetting up and down, and centrifuge the sample tube briefly. Place the tube in a thermocycler (Set the heated lid at 105℃), and perform the following PCR program according to Table 26.

Table 26 Hybrid Capture PCR Program (Heated lid at 105℃)

Temperature	Time	Cycle
95℃	5 min	1
95℃	30 s	12*
60℃	45 s	
60℃	2 min	1
4℃	∞	1

* *For single sample hybridization (only one sample library in a single hybridization pool), 13 cycles are recommended.*

6. Purification after Amplification

6.1 Take out the AMPure XP beads and equilibrate them to room temperature for at least 30 min, and vortex the beads to resuspend before use.

6.2 Transfer all of the above PCR products (from Step 5.2) into a clean nuclease-free 1.5 mL centrifuge tube, then add 50 µL AMPure XP beads, 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.

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

6.5 Repeat the Step 6.4 once.

6.6 Briefly spin the tube, and put the tube back in the magnetic stand. Completely remove the residual ethanol, and air dry the beads for 3~5 min while the tube is on the magnetic stand with the lid open until the beads show matt surface.

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

6.7 Remove the tube from the magnetic stand. Elute DNA target from the beads by adding 32 µL TE-low solution (not provided), mix thoroughly by vortexing or pipetting, and incubate for 2 min at room temperature.

6.8 Place the tubes on the magnetic stand until solution becomes clear (3~5 min). Open the cap carefully without disturbing the bead pellet, transfer 30 µL of the supernatant into a clean nuclease-free 1.5 mL centrifuge tube.

***Note:** If the products are not to be used immediately for the next step, store at -25℃ to -15℃ for no more than 6 months. Repeated thawing and freezing should be avoided.*

7. QC of the Captured Library

7.1 Library concentration QC: Check the concentration of the captured library using Quantus™ or Qubit™ Fluorometer, the total amount of each captured library should be no less than 75 ng. For unqualified samples, re-collection, re-extraction, or library re-construction are required.

7.2 Library size distribution QC (recommended): Assess the library size distribution with a recommended capillary electrophoresis analyzer and related kit. The peak size distribution of the captured library should be at 250-500 bp, without obvious peaks of small and big fragments, as shown in Figure 3. For unqualified samples, re-collection, re-extraction, or library re-construction are required.

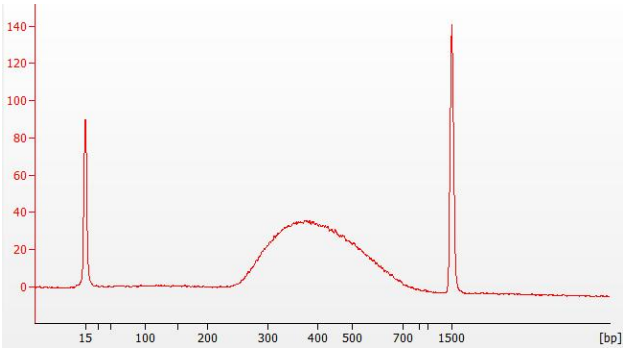

Figure 3. Example of Captured Libraries Size Distribution on a Bioanalyzer

Sequencing

Illumina 300 cycles (Paired-End Reads, 2 × 150 cycles) sequencing reagents and corresponding Illumina instruments are suggested. The recommended spike-in percentage of Illumina PhiX Control v3 is 1%. It is recommended that the sequencing data per sample should be no less than 10 Gb for FFPE tissue DNA (or PC/NC DNA) and no less than 2 Gb for FFPE tissue RNA (or PC/NC RNA). All sequencing operations should follow instrument's standard procedures. The suggested sample quantity per run is listed in Table 27.

Table 27 Recommended Sequencing Instruments and Recommended Samples per Run

Sequencer	Flow Cell	Read Length	Recommended Samples per Run[#]	
			DNA+RNA library (12 Gb/sample)	DNA library only (10 Gb/sample)
NextSeq 500/550	Mid output	2×150 bp	3 DNA + 3 RNA	4 DNA
	High output	2×150 bp	10 DNA + 10 RNA	12 DNA
NovaSeq 6000	SP	2×150 bp	20 DNA + 20 RNA	25 DNA
	S1	2×150 bp	41 DNA + 41 RNA	50 DNA
	S2/S4	2×150 bp	Up to 48 DNA + 48 RNA[*]	Up to 96 DNA[*]

[*] *Maximum 96 indexes available.*

[#] *The recommended number of samples per run mentioned in the above table may include libraries constructed from FFPE tumor tissue samples, PC, or NC. For example, for the high-output flow cells on NextSeq 500/550, it is recommended to load 10 DNA libraries (8 FFPE DNA samples + 1 PC DNA + 1 NC DNA) and 10 RNA libraries (8 FFPE RNA samples + 1 PC RNA + 1 NC RNA).*

Perform the denaturation and dilution of the libraries according to the instrument's instructions. The final concentration of sequencing library is recommended in Table 28.

Table 28 Recommended Final Concentration of Sequencing Library

Illumina Sequencer	Final Concentration
NextSeq 500/550	1.2-1.8 pM
NovaSeq 6000	0.75-1 nM

Note:

- *The concentration converting formula is as follows.*

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

- *It is recommended to perform the concentration conversion based on each library size obtained by quality control (Sectioin C. Hybrid Capture, Step 7.2). If the library size of each library is not available, a fixed value of 380 bp can also be used for concentration conversion. Please note that there may be a risk of affecting the data output (higher or lower data output than expected) when using fixed values.*

Data analysis

When the sequencing is finished, adopt AmoyDx ANDAS Data Analyzer to analyze the sequencing data.

Quality assessment of sequencing files

1. The background monitoring program of the analysis software can automatically monitor the running status of the sequencer connected with the system. When a new sequencing file is detected, the analysis software will start the data processing program, and use the bcl2fastq v2.17 software of Illumina company to convert the .bcl file into the. fastq file.
2. Use the Illumina v0.6 software to read the record information of InterOp directory in the sequencing file and to evaluate the quality of the sequencing results. The Q30 value of the sequencing data should be no less than 75%. If not, the sequencing data is unqualified, re-sequencing or library re-construction is required.
3. Create analysis process. After the quality assessment is completed, click the "Create Analysis" button in the analysis center page of the analysis software, and select the analysis module in the pop-up dialog box according to the sample type. For FFPE tissue DNA (or PC/NC DNA), choose the "ADXMaster-DNA-Int" module; for FFPE tissue RNA (or PC/NC RNA), choose the "ADXMaster-RNA-Int" module.
4. On the sample selection page, select the sequencing batch containing the sample to be analyzed in the "Select RUN" entry, and then check the sample to be analyzed in the sample dialog box and add it to the analysis list.
5. After sample selection, click the "Create Analysis" button to enter the analysis preview page. After confirming that all parameters are selected correctly, click the "START" button to start the automatic analysis process.

QC standard

Criteria of data QC：

The qualified criteria and risky criteria for DNA library data QC are shown in Table 29.

Table 29 DNA Library Data QC Qualified and Risky Criteria (FFPE DNA/PC DNA/NC DNA)

Parameters	Qualified	Risky
cleanQ30	≥ 75%	NA
Coverage	≥ 95%	90% ~ 95%
HotUNIQUni-20%	≥ 90%	80% ~ 90%
NonHotUNIQUni-20%	≥ 80%	70% ~ 80%
HotUNIQDepth	≥ 1000×	800× ~ 1000×
NonHotUNIQDepth	≥ 500×	400× ~ 500×

Note：

- *cleanQ30: The proportion of bases in the cleanData with accuracy reaching more than 99.9%.*
- *Coverage: The percentage of the target region that has a coverage depth of at least 1×.*
- *HotUNIQUni-20%: The percentage of the hotspot region that has a coverage depth (unique depth) of at least 20% of the average unique depth.*
- *NonHotUNIQUni-20%: The percentage of the non-hotspot region that has a coverage depth (unique depth) of at least 20% of the average unique depth.*
- *HotUNIQDepth:The average unique depth of the hotspot region.*
- *NonHotUNIQDepth: The average unique depth of the non-hotspot region.*

- *The hotspot region of master panel includes hotspot mutations, genes for fusion detection and MSI sites; the non-hotspot region of master panel includes non-hotspot mutations, chemotherapy & radiotherapy SNPs, genes for CNV and TMB detection, etc.*

The qualified criteria and risky criteria for RNA library data QC are shown in Table 30.

Table 30 RNA Library Data QC Qualified and Risky Criteria (FFPE RNA/PC RNA/NC RNA)

Tissue RNA	Qualified	Risky
cleanQ30	≥ 75%	NA
Mapping	≥ 80%	70% ~ 80%
End2SenseRate	≥ 90%	80% ~ 90%
effectiveReads	≥ 4 Million	3.5 ~ 4 Million

Note：

- *Mapping: The proportion of the sequencing reads mapped to human genome.*
- *End2SenseRate: the ratio of probe mapped to original template strand.*
- *effectiveReads: The number of QC qualified reads in target regions.*

The above library data QC parameters are sample-level QC criteria that ensure the assay performance meets the expected standards.

- ❖ If the library data QC is qualified, proceed to result interpretation.
- ❖ If the library data QC is at risky level, re-extraction and re-testing is recommended. If the remaining sample is not enough for re-extraction or re-testing, it should be noted that risky level of sample data QC may lead to the risk of missed detection of SNV/InDel/fusion variants and may affect the accuracy of TMB/MSI testing, and the test results of the sample with risky QC should be for reference only.
- ❖ If the library data QC is failed, the test results will be deemed unqualified, and re-extraction and re-testing is recommended.

Result Interpretation

- **For SNV/InDel detection**

The cut-off metrics for SNV/InDel are as follows.

Variant Type	Freq_US	Var_US	Freq_SS	Var_SS	Var_DS
Hot InDel (≥6 bp)	≥ 0.15%	≥ 5	≥ 0.14%	≥ 3	/
HotSpot (Non C/T or G/A)	≥ 0.35%	≥ 7	≥ 0.35%	≥ 3	/
HotSpot (C/T or G/A)	≥ 0.67%	≥ 10	≥ 0.48%	≥ 3	/
Non-HotSpot (Non C/T or G/A)	≥ 3.00%	≥ 15	≥ 3.00%	≥ 4	/
Non-HotSpot (C/T or G/A)	≥ 3.00%	≥ 18	≥ 3.00%	≥ 4	/
19 HRR genes (Non C/T or G/A)	≥ 0.80%	≥ 15	≥ 0.80%	≥ 7	/
19 HRR genes (C/T or G/A)	≥ 0.90%	≥ 18	≥ 0.80%	≥ 7	≥ 2
Polymer or STR	≥ 5.00%	≥ 20	≥ 5.00%	≥ 20	/

Note：

- *The "Hot InDel (≥6 bp)" category includes clinically important InDels (≥6 bp) such as important EGFR 19dels/20ins and MET 14 skipping variants.*
- *19 HRR genes: ATM, ATR, BARD1, BRCA1, BRCA2, BRIP1, CDK12, CHEK1, CHEK2, FANCA, FANCL, MLHI, MRE11, NBN,*

PALB2, RAD51B, RAD51C, RAD51D, RAD54L.

- *The categories "HotSpot (Non C/T or G/A)", "HotSpot (C/T or G/A)", "Non-HotSpot (Non C/T or G/A)", and "Non-HotSpot (C/T or G/A)" include SNV and InDel (<6 bp) variants which are not in the 19 HRR genes. The concept "HotSpot" indicates those variants with relatively clearer clinical significance.*

- **For Fusion detection**

For the fusion detection based on DNA level, the cut-off metrics for fusions are as follows.

Fusion types	ssbcFreq	dsbcAD + rdsbcAD
HotFusion	/	≥ 3
HotGene	/	≥ 4
others	≥ 1%	≥ 8

For the fusion detection based on RNA level, the cut-off metrics for fusions are as follows.

Fusion types	SupportReads	NormSupportReads
Hot gene fusions	≥ 4	≥ 0.3
others	≥ 10	≥ 0.3

Fusion detection interpretation rule: When the detection result of either DNA or RNA is positive for fusion, it is considered as a positive gene fusion.

- **For CNV detection**

Data QC criteria for CNV detection: the qualified criteria is CNVNoise ≤ 0.25. Otherwise the CNV results will be deemed unqualified, and re-sequencing or re-construct library is required.

If the data QC is qualified, the CNV can be defined as positive if meeting the following requirements: CopyNum ≥ Ploidy+2, and CopyNum ≥ 5.

- **For Homozygous Deletion (HD) status of 20 HRR genes**

Data QC criteria for HD detection: the qualified criteria are CDSDepthNoise ≤ 0.4 and CDSBafNoise ≤ 0.06. Otherwise the HD results will be deemed unqualified, and re-sequencing or re-construct library is required.

If the data QC is qualified, the HD can be defined as positive if meeting the following requirements: Tumor content model interpretation (Fitness) ≥ 0.3, the number of CNV events (Cnvs) > 5, and the probability (Prob) ≥ 0.7.

- **For Genomic Scar Score (GSS) status**

Data QC criteria for GSS detection: the qualified criteria are DepthNoise ≤ 0.4 and BAFNoise ≤ 0.06. Otherwise the GSS results will be deemed unqualified, and re-sequencing or re-construct library is required.

If the data QC is qualified, the GSS can be defined as follows: GSS ≥ 45 is defined as GSS positive; GSS < 45 is defined as GSS negative.

- **For HRD status**

A positive HRD status result is defined by either the presence of a pathogenic/likely pathogenic variant in *BRCA1* and *BRCA2* genes or a positive GSS (GSS ≥ 45).

A negative HRD status is defined by negative results in both *BRCA1/2* SNVs/InDels and GSS.

***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

- **For MSI/TMB/EBV/GEP status**

The cut-off metrics for MSI/TMB/EBV/GEP are as follows.

Biomarkers	**Cut-off Metrics**
MSI	MSI_score ≥ 200
TMB	Lung cancer: TMBValue ≥ 9.48; Other tumors: TMBValue ≥ 7.76
EBV	FPKM ≥ 0.5
GEP	Score ≥ 5.14

Note：

- *Freq_US: Frequency of mutant allele after deduplication.*
- *Var_US: Depth of mutant allele after deduplication.*
- *Freq_SS: Frequency of mutant allele after single strand calibration.*
- *Var_SS: Depth of mutant allele after single strand calibration.*
- *Var_DS: Depth of mutant allele after double strand calibration.*
- *Polymer means the regions with 7 or more consecutive identical nucleotides.*
- *Short tandem repeats (STRs) represent the regions with 5 or more consecutive repeat units comprising of 2 to 6 bp.*
- *HotFusion: Hot gene fusions, the breakpoints occur at a typical region.*
- *HotGene: Hot gene fusions, the breakpoints does not occur at a typical region.*
- *ssbcFreq: Frequency of fusion allele based on forward strand after single strand calibration.*
- *dsbcAD: Depth of fusion allele based on forward strand after double strand calibration.*
- *rdsbcAD: Depth of fusion allele based on reverse strand after double strand calibration.*
- *SupportReads: Effective support reads of gene fusion.*
- *NormSupportReads: Normalized support reads of gene fusion.*
- *CNVNoise: The background depth noise of the CNV calling.*
- *CopyNum: gene amplification copy number.*
- *Ploidy: The estimated tumor cell ploidy of the sample.*
- *CDSDepthNoise: The background depth noise of the CDS regions.*
- *CDSBafNoise: The background B-allele frequency (BAF) noise of the CDS regions.*
- *DepthNoise: The background depth noise of the whole genome regions.*
- *BAFNoise: The background BAF noise of the whole genome regions.*
- *GSS: genomic scar score.*
- *MSI: microsatellite instability.*

- *MSI_score: score for MSI status determination.*
- *TMB: tumor mutation burden, described in mutations per Mega base (mut/Mb) unit.*
- *EBV: Epstein-Barr virus.*
- *FPKM: fragments per kilobase of exon per million mapped fragments.*
- *GEP: gene expression profile.*
- *Score (for GEP): gene expression profile score.*
- *The PC should be detected as positive result for the corresponding variants as shown in Appendix III, and the NC should be detected as negative in the detection range of this kit (see Appendix III for more details). Otherwise, the testing is unqualified, and it is necessary to check if there is any operational error and re-test.*
- *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 HRR genes 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 HRRm (including BRCA1/2 genes) positive.*

Performance

Limit of Detection (LoD)

For FFPE tissue sample derived DNA, with 60 ng fragmented DNA input, the LoD for hotspot SNV/Indel/Fusion is 2.5% allele frequency, the LoD for non-hotspot SNV/Indel/Fusion is 5% allele frequency, the LoD of CNV detection is Ploidy+6 at 30% tumor content, the LoD of MSI and TMB is 20% tumor content, the LoD of GSS is 30% tumor content.

For FFPE tissue sample derived RNA, with 200 ng RNA input, the LoD of fusion detection is 500 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. Reliable results are dependent on proper sample processing, transport, and storage. Improper sample processing, transport and storage, as well as improper experimental operation and experimental environment may lead to false negative or false positive results.
4. A Negative result can not completely exclude the existence of mutated genes. Low tumor cell content, severe DNA/RNA degradation, low depth of the variant (Depth < 500×), or the frequency under the limit of detection may also cause a false negative result.
5. The detection of HRD status (GSS) using this kit is only validated in FFPE samples from ovarian cancer and breast cancer.
6. The detection of EBV using this kit is only validated in FFPE samples from gastric cancer.
7. Different parts of the tumor tissue or different sampling times may lead to different mutation results due to tumor heterogeneity.

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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 I

Table S1 Gene list for SNV/Indel detection (571 genes, DNA level)

ABCB1	*ABL1*	*ABL2*	*ABRAXAS1*	*ACVR1B*	*AGO1*	*AKT1*	*AKT2*	*AKT3*	*ALK*
ALOX12B	*AMER1*	*AP3B1*	*APC*	*APC2*	*APEX1*	*AR*	*ARAF*	*ARFRP1*	*ARID1A*
ARID1B	*ARID2*	*ARID5B*	*ASXL1*	*ATM*	*ATR*	*ATRX*	*AURKA*	*AURKB*	*AUTS2*
AXIN1	*AXIN2*	*AXL*	*B2M*	*BAP1*	*BARD1*	*BCL2*	*BCL2L1*	*BCL2L11*	*BCL2L2*
BCL6	*BCOR*	*BCORL1*	*BCR*	*BIRC3*	*BLK*	*BLM*	*BMP2*	*BMP4*	*BMPR1A*
BRAF	*BRCA1*	*BRCA2*	*BRD3*	*BRD4*	*BRIP1*	*BTG1*	*BTK*	*C8orf34*	*CALR*
CARD11	*CASP8*	*CBFB*	*CBL*	*CBLB*	*CCN6*	*CCND1*	*CCND2*	*CCND3*	*CCNE1*
CD274	*CD34*	*CD44*	*CD74*	*CD79A*	*CD79B*	*CD80*	*CD86*	*CDA*	*CDC73*
CDH1	*CDK12*	*CDK4*	*CDK6*	*CDK8*	*CDKN1A*	*CDKN1B*	*CDKN2A*	*CDKN2B*	*CDKN2C*
CEBPA	*CHD2*	*CHD4*	*CHEK1*	*CHEK2*	*CIC*	*CORO2A*	*CREBBP*	*CRKL*	*CRLF2*
CSF1	*CSF1R*	*CSF3R*	*CTCF*	*CTLA4*	*CTNNA1*	*CTNNB1*	*CUL3*	*CXCL8*	*CYLD*
CYP19A1	*CYP2C8*	*CYP2D6*	*DAXX*	*DCUN1D1*	*DDR1*	*DDR2*	*DICER1*	*DIS3*	*DKK3*
DNMT1	*DNMT3A*	*DOT1L*	*DPYD*	*DYNC2H1*	*EED*	*EGFR*	*EIF1AX*	*EIF4A2*	*EMSY*
ENG	*EP300*	*EPAS1*	*EPCAM*	*EPHA3*	*EPHA5*	*EPHA6*	*EPHA7*	*EPHB1*	*ERBB2*
ERBB3	*ERBB4*	*ERCC1*	*ERCC2*	*ERCC3*	*ERG*	*ERRFI1*	*ESR1*	*ETS2*	*ETV1*
ETV4	*ETV5*	*ETV6*	*EWSR1*	*EZH2*	*F2R*	*FANCA*	*FANCC*	*FANCD2*	*FANCE*
FANCF	*FANCG*	*FANCI*	*FANCL*	*FANCM*	*FAS*	*FAT1*	*FBXW7*	*FCGR2B*	*FGF10*
FGF14	*FGF19*	*FGF23*	*FGF3*	*FGF4*	*FGF6*	*FGF7*	*FGFR1*	*FGFR2*	*FGFR3*
FGFR4	*FGR*	*FH*	*FLCN*	*FLT1*	*FLT3*	*FLT4*	*FOXA1*	*FOXL2*	*FOXO1*
FOXP1	*FRS2*	*FUBP1*	*FUS*	*FYN*	*GABRA6*	*GATA1*	*GATA2*	*GATA3*	*GATA4*
GATA6	*GEN1*	*GLI1*	*GNA11*	*GNA13*	*GNAQ*	*GNAS*	*GREM1*	*GRIN2A*	*GRM3*
GSK3B	*GSTP1*	*H1-2*	*H2BC5*	*H3-3A*	*H3-5*	*H3C2*	*HAMP*	*HAVCR2*	*HCK*
HDAC2	*HEY1*	*HGF*	*HIF1A*	*HLA-A*	*HLA-B*	*HLA-C*	*HNF1A*	*HOXB13*	*HRAS*
HSD3B1	*HSP90AA1*	*HSPB1*	*ICOS*	*ICOSLG*	*IDH1*	*IDH2*	*IFNGR1*	*IFNGR2*	*IGF1*
IGF1R	*IGF2*	*IKBKE*	*IKZF1*	*IL13*	*IL1A*	*IL4*	*IL6*	*IL7R*	*INHBA*
INPP4A	*INPP4B*	*INSR*	*IP6K1*	*IRF1*	*IRF2*	*IRF4*	*IRS2*	*ITGB2*	*ITGB6*
JAK1	*JAK2*	*JAK3*	*JUN*	*KDM5A*	*KDM5C*	*KDM6A*	*KDR*	*KEAP1*	*KEL*
KIT	*KLF4*	*KLHL6*	*KMT2A*	*KMT2B*	*KMT2C*	*KMT2D*	*KRAS*	*LAG3*	*LATS1*
LATS2	*LCK*	*LGALS3*	*LIG4*	*LIN28B*	*LMO1*	*LRP1B*	*LYN*	*LZTR1*	*MAGI2*
MAP2K1	*MAP2K2*	*MAP2K4*	*MAP3K1*	*MAP3K13*	*MAP3K14*	*MAPK1*	*MAPK3*	*MAPK4*	*MAX*
MCL1	*MDC1*	*MDM2*	*MDM4*	*MED12*	*MEF2B*	*MEN1*	*MET*	*MGA*	*MGME1*
MGMT	*MIF*	*MITF*	*MKI67*	*MLH1*	*MLH3*	*MMP1*	*MMP7*	*MPL*	*MPO*
MRE11	*MSH2*	*MSH3*	*MSH6*	*MST1R*	*MT2A*	*MTHFR*	*MTOR*	*MTRR*	*MUC16*
MUC5B	*MUTYH*	*MYB*	*MYC*	*MYCL*	*MYCN*	*MYD88*	*MYOD1*	*NAA11*	*NAB2*
NBN	*NCOA2*	*NCOA3*	*NCOR1*	*NEIL1*	*NF1*	*NF2*	*NFE2L2*	*NFKB1*	*NFKBIA*
NKX2-1	*NOS2*	*NOS3*	*NOTCH1*	*NOTCH2*	*NOTCH3*	*NOTCH4*	*NPM1*	*NQO1*	*NR1I2*
NR4A3	*NRAS*	*NRG1*	*NSD1*	*NTRK1*	*NTRK2*	*NTRK3*	*NUP93*	*NUTM1*	*OXSR1*
PAK1	*PAK3*	*PAK5*	*PALB2*	*PAPPA2*	*PARP1*	*PAX3*	*PAX5*	*PAX7*	*PAX8*
PBRM1	*PDCD1*	*PDCD1LG2*	*PDGFB*	*PDGFRA*	*PDGFRB*	*PDK1*	*PDPK1*	*PEG3*	*PGR*
PHF6	*PIK3C2B*	*PIK3C2G*	*PIK3C3*	*PIK3CA*	*PIK3CB*	*PIK3CD*	*PIK3CG*	*PIK3R1*	*PIK3R2*
PIM1	*PLCG2*	*PLK2*	*PMS1*	*PMS2*	*PNRC1*	*POLD1*	*POLE*	*POLE4*	*PPARG*
PPP2R1A	*PPP2R2A*	*PRDM1*	*PRDX1*	*PRDX6*	*PREX2*	*PRKAA1*	*PRKACA*	*PRKAR1A*	*PRKCI*
PRKDC	*PRKN*	*PRSS8*	*PSMD4*	*PTCH1*	*PTEN*	*PTGS2*	*PTPN11*	*PTPRD*	*PTTG1*
PXDNL	*QKI*	*RAC1*	*RAD21*	*RAD50*	*RAD51*	*RAD51B*	*RAD51C*	*RAD51D*	*RAD52*
RAD54L	*RAF1*	*RANBP2*	*RARA*	*RASA1*	*RASAL1*	*RB1*	*RBM10*	*RECQL*	*RECQL4*
REL	*RET*	*REV3L*	*RHEB*	*RHOA*	*RICTOR*	*RIPK4*	*RIT1*	*RNASEL*	*RNF43*
ROBO2	*ROS1*	*RPPH1*	*RPS6KB1*	*RPS6KB2*	*RPTOR*	*RSF1*	*RUNX1*	*RUNX1T1*	*SCN8A*
SDHA	*SDHAF2*	*SDHB*	*SDHC*	*SDHD*	*SEMA3C*	*SERPINB3*	*SERPINB4*	*SERPINE1*	*SETBP1*

SETD2	*SF3B1*	*SIK1*	*SKP2*	*SLC28A3*	*SLC47A1*	*SLCO1B1*	*SLIT2*	*SLX4*	*SMAD2*
SMAD3	*SMAD4*	*SMARCA4*	*SMARCB1*	*SMARCD1*	*SMO*	*SNCAIP*	*SOCS1*	*SOD2*	*SOX10*
SOX17	*SOX2*	*SOX9*	*SPEN*	*SPOP*	*SPTA1*	*SRC*	*SRSF2*	*SS18*	*STAG2*
STAT3	*STAT4*	*STAT6*	*STK11*	*SUFU*	*SUZ12*	*SYK*	*TAF1*	*TAOK1*	*TBX3*
TCF7L1	*TCF7L2*	*TENT5C*	*TERT*	*TET1*	*TET2*	*TFE3*	*TGFB1*	*TGFBR2*	*TIGIT*
TMEM127	*TMPRSS2*	*TNF*	*TNFAIP3*	*TNFRSF14*	*TNFRSF18*	*TNFRSF9*	*TOP1*	*TOP2A*	*TP53*
TPMT	*TRAF3*	*TRAF7*	*TRRAP*	*TSC1*	*TSC2*	*TSHR*	*TXNRD2*	*TYMS*	*U2AF1*
UGT1A1	*UMPS*	*VEGFA*	*VHL*	*WRN*	*WT1*	*XPC*	*XPO1*	*XRCC1*	*XRCC2*
XRCC3	*XRCC4*	*XRCC5*	*YES1*	*YWHAE*	*ZBTB2*	*ZFHX4*	*ZNF217*	*ZNF703*	*ZNRF3*
ZRSR2									

Table S2 Gene list for CNV detection (30 genes, DNA level)

AKT2	*AKT3*	*AURKA*	*CCND1*	*CCNE1*	*CD274*	*CDK4*	*CDK6*	*EGFR*	*ERBB2*
FGF19	*FGF3*	*FGFR1*	*FGFR2*	*FGFR3*	*HGF*	*IGF1R*	*MAPK1*	*MDM2*	*MDM4*
MET	*MYC*	*NTRK3*	*PDGFRA*	*PGR*	*PIK3CA*	*RET*	*RICTOR*	*SMO*	*TOP2A*

Table S3 Gene list for HD detection (20 genes, DNA level)

ATM	*BARD1*	*BRCA1*	*BRCA2*	*BRIP1*	*CDH1*	*CDK12*	*CHEK1*	*CHEK2*	*FANCA*
FANCL	*HDAC2*	*PALB2*	*PPP2R2A*	*PTEN*	*RAD51B*	*RAD51C*	*RAD51D*	*RAD54L*	*TP53*

Table S4 Gene list for fusion and splicing detection (48 genes, DNA and RNA)

ALK	*AR*	*BRAF*	*CD74*	*CLDN18*	*EGFR*	*ERBB2*	*ERBB4*	*ESR1*	*ETV1*
ETV4	*ETV5*	*ETV6*	*EWSR1*	*FGFR1*	*FGFR2*	*FGFR3*	*FGFR4*	*FUS*	*HEY1*
KIT	*MET*	*MYB*	*NAB2*	*NCOA2*	*NOTCH2*	*NR4A3*	*NRG1*	*NRG2*	*NRG3*
NTRK1	*NTRK2*	*NTRK3*	*NUTM1*	*PAX3*	*PAX7*	*PAX8*	*PDGFB*	*PDGFRA*	*PDGFRB*
RAF1	*RET*	*ROS1*	*SS18*	*STAT6*	*TFE3*	*TMPRSS2*	*YWHAE*		

Table S5 Gene list for RNA gene expression detection (2660 genes, RNA level)

A2M	*ABCB1*	*ABCF1*	*ABL1*	*ABR*	*ABTB2*	*ACAD9*	*ACADM*	*ACAN*	*ACOT12*
ACSF3	*ACTA2*	*ACTB*	*ACTG1*	*ACTG2*	*ACTL6A*	*ACTL6B*	*ACTR3B*	*ACVR1B*	*ACVR1C*
ACVR2A	*ACY1*	*ADA*	*ADAM12*	*ADAMTS16*	*ADGRE1*	*ADGRE2*	*ADGRE5*	*ADH1A*	*ADH1B*
ADH1C	*ADH4*	*ADH6*	*ADM*	*ADORA2A*	*AFAP1*	*AFDN*	*AFF3*	*AGAP3*	*AGBL4*
AGGF1	*AGK*	*AGR2*	*AGTRAP*	*AHCYL1*	*AHR*	*AICDA*	*AIF1*	*AIRE*	*AKAP1*
AKAP13	*AKAP9*	*AKR1C3*	*AKR1C4*	*AKT1*	*AKT2*	*AKT3*	*ALAD*	*ALAS1*	*ALCAM*
ALDOA	*ALDOC*	*ALK*	*ALKBH2*	*ALKBH3*	*ALOX15B*	*AMBP*	*AMBRA1*	*AMER1*	*AMH*
AMMECR1L	*AMOT*	*AMOTL2*	*ANGPT1*	*ANGPT2*	*ANGPTL4*	*ANKLE2*	*ANKRD28*	*ANKRD46*	*ANLN*
ANO3	*ANP32B*	*ANXA1*	*AP1M1*	*AP3B1*	*APAF1*	*APBB1*	*APC*	*APC2*	*APH1B*
API5	*APIP*	*APLNR*	*APOA1*	*APOA2*	*APOA4*	*APOB*	*APOBEC3B*	*APOC2*	*APOC3*
APOD	*APOE*	*APOL6*	*APOLD1*	*APOM*	*APP*	*APPL1*	*AQP9*	*AR*	*ARAF*
AREG	*ARF1*	*ARG1*	*ARG2*	*ARHGEF2*	*ARHGEF6*	*ARID1A*	*ARID1B*	*ARID2*	*ARID5A*
ARMC10	*ARMH3*	*ARNT*	*ARNT2*	*ARNTL*	*ASAP2*	*ASCL1*	*ASL*	*ASNS*	*ASPA*
ASPG	*ASPN*	*ASPSCR1*	*ASXL1*	*ATF1*	*ATF2*	*ATF3*	*ATF4*	*ATF7IP*	*ATG10*
ATG12	*ATG16L1*	*ATG5*	*ATG7*	*ATIC*	*ATM*	*ATOX1*	*ATP11C*	*ATP1B1*	*ATP2A2*
ATP5F1D	*ATP5F1E*	*ATP5ME*	*ATP6V1D*	*ATR*	*ATRX*	*AURKA*	*AURKB*	*AXIN1*	*AXIN2*
AXL	*AZGP1*	*B2M*	*B3GAT1*	*B4GALT6*	*BACH2*	*BAD*	*BAG1*	*BAG4*	*BAIAP2L1*
BAIAP3	*BAK1*	*BAMBI*	*BAP1*	*BATF*	*BATF3*	*BAX*	*BBC3*	*BBS1*	*BCAN*
BCAT1	*BCL10*	*BCL11B*	*BCL2*	*BCL2A1*	*BCL2L1*	*BCL2L11*	*BCL2L14*	*BCL3*	*BCL6*
BCL6B	*BCOR*	*BCR*	*BDNF*	*BGN*	*BICC1*	*BID*	*BIRC2*	*BIRC3*	*BIRC5*

BIRC7	*BLK*	*BLM*	*BLNK*	*BLVRA*	*BMI1*	*BMP2*	*BMP4*	*BMP5*	*BMP6*
BMP7	*BMP8A*	*BMPR1B*	*BNIP3*	*BNIP3L*	*BRAF*	*BRCA1*	*BRCA2*	*BRD2*	*BRD3*
BRD4	*BRD7*	*BRIP1*	*BRIX1*	*BST1*	*BST2*	*BTBD1*	*BTF3L4*	*BTK*	*BTLA*
BUB1	*BUB1B-PAK6*	*BYSL*	*C1QA*	*C1QB*	*C1QBP*	*C1R*	*C1S*	*C2*	*C2CD5*
C3	*C3AR1*	*C4B*	*C4BPA*	*C5*	*C5AR1*	*C6*	*C7*	*C8A*	*C8B*
C8G	*C8orf34*	*C9*	*CA12*	*CA2*	*CA4*	*CACNA1C*	*CACNA1D*	*CACNA1E*	*CACNA1G*
CACNA1H	*CACNA2D1*	*CACNA2D2*	*CACNA2D3*	*CACNA2D4*	*CACNB2*	*CACNB3*	*CACNB4*	*CACNG1*	*CACNG4*
CACNG6	*CADPS*	*CALM1*	*CALM2*	*CALM3*	*CALML3*	*CALML4*	*CALML5*	*CALML6*	*CAMK1*
CAMK1D	*CAMK1G*	*CAMK2A*	*CAMK2B*	*CAMK2D*	*CAMK2G*	*CAMK4*	*CAMP*	*CAPN2*	*CAPN6*
CAPZA2	*CARD11*	*CARD9*	*CASP1*	*CASP10*	*CASP12*	*CASP3*	*CASP7*	*CASP8*	*CASP9*
CAV1	*CBL*	*CBLB*	*CBLC*	*CBR4*	*CBX5*	*CC2D1B*	*CCAR2*	*CCDC186*	*CCDC198*
CCDC6	*CCDC91*	*CCL1*	*CCL11*	*CCL13*	*CCL14*	*CCL15*	*CCL16*	*CCL17*	*CCL18*
CCL19	*CCL2*	*CCL20*	*CCL21*	*CCL22*	*CCL23*	*CCL24*	*CCL25*	*CCL26*	*CCL27*
CCL28	*CCL3*	*CCL3L1*	*CCL4*	*CCL5*	*CCL7*	*CCL8*	*CCNA1*	*CCNA2*	*CCNB1*
CCNB2	*CCNB3*	*CCND1*	*CCND2*	*CCND3*	*CCNE1*	*CCNE2*	*CCNO*	*CCR1*	*CCR2*
CCR3	*CCR4*	*CCR5*	*CCR6*	*CCR7*	*CCR9*	*CCRL2*	*CD101*	*CD14*	*CD160*
CD163	*CD164*	*CD180*	*CD19*	*CD1A*	*CD1B*	*CD1C*	*CD1D*	*CD1E*	*CD2*
CD200	*CD207*	*CD209*	*CD22*	*CD226*	*CD24*	*CD244*	*CD247*	*CD27*	*CD274*
CD276	*CD28*	*CD300A*	*CD33*	*CD34*	*CD36*	*CD37*	*CD38*	*CD3D*	*CD3E*
CD3G	*CD4*	*CD40*	*CD40LG*	*CD44*	*CD46*	*CD47*	*CD48*	*CD5*	*CD52*
CD53	*CD55*	*CD58*	*CD59*	*CD6*	*CD63*	*CD68*	*CD69*	*CD7*	*CD70*
CD74	*CD79A*	*CD79B*	*CD80*	*CD81*	*CD83*	*CD84*	*CD86*	*CD8A*	*CD8B*
CD9	*CD96*	*CD99*	*CDC14A*	*CDC14B*	*CDC20*	*CDC25A*	*CDC25B*	*CDC25C*	*CDC27*
CDC42	*CDC42EP1*	*CDC6*	*CDC7*	*CDCA3*	*CDH1*	*CDH11*	*CDH16*	*CDH17*	*CDH2*
CDH3	*CDH5*	*CDK1*	*CDK12*	*CDK2*	*CDK4*	*CDK6*	*CDKN1A*	*CDKN1B*	*CDKN1C*
CDKN2A	*CDKN2B*	*CDKN2C*	*CDKN2D*	*CDKN3*	*CDX2*	*CEACAM1*	*CEACAM3*	*CEACAM5*	*CEACAM6*
CEACAM8	*CEBPA*	*CEBPB*	*CEBPE*	*CELSR2*	*CENPF*	*CEP43*	*CEP55*	*CEP72*	*CEP85L*
CEP89	*CES3*	*CFB*	*CFD*	*CFI*	*CFL1*	*CFP*	*CGAS*	*CHAD*	*CHEK1*
CHEK2	*CHGA*	*CHI3L1*	*CHIT1*	*CHRM3*	*CHSY1*	*CHTOP*	*CHUK*	*CIC*	*CIDEA*
CIITA	*CIT*	*CKLF*	*CKS1B*	*CKS2*	*CLCF1*	*CLCN6*	*CLDN18*	*CLEC10A*	*CLEC14A*
CLEC4A	*CLEC4C*	*CLEC4E*	*CLEC5A*	*CLEC6A*	*CLEC7A*	*CLECL1*	*CLIP1*	*CLTC*	*CLU*
CMA1	*CMKLR1*	*CMTM4*	*CMTM6*	*CNIH4*	*CNOT10*	*CNOT2*	*CNOT4*	*CNTFR*	*CNTRL*
COG7	*COL11A1*	*COL11A2*	*COL14A1*	*COL16A1*	*COL17A1*	*COL1A1*	*COL1A2*	*COL24A1*	*COL27A1*
COL2A1	*COL3A1*	*COL4A1*	*COL4A2*	*COL4A3*	*COL4A4*	*COL4A5*	*COL4A6*	*COL5A1*	*COL5A2*
COL6A3	*COL6A6*	*COLEC12*	*COMP*	*CORO1A*	*COX11*	*COX4I1*	*COX5B*	*COX6A1*	*COX6B1*
CPA3	*CPEB2*	*CPSF7*	*CR1*	*CR2*	*CRABP2*	*CREB1*	*CREB3*	*CREB3L1*	*CREB3L2*
CREB3L3	*CREB3L4*	*CREB5*	*CREBBP*	*CRK*	*CRKL*	*CRLF2*	*CRP*	*CRTAM*	*CSF1*
CSF1R	*CSF2*	*CSF2RB*	*CSF3*	*CSF3R*	*CSNK1A1*	*CSNK1A1L*	*CST2*	*CT45A1*	*CTAG1B*
CTAG2	*CTAGE1*	*CTBP1*	*CTBP2*	*CTCFL*	*CTLA4*	*CTNNA1*	*CTNNA2*	*CTNNA3*	*CTNNB1*
CTRC	*CTSC*	*CTSG*	*CTSH*	*CTSL*	*CTSS*	*CTSV*	*CTSW*	*CTTN*	*CUL1*
CUL2	*CUL3*	*CUX1*	*CWH43*	*CX3CL1*	*CX3CR1*	*CXADR*	*CXCL1*	*CXCL10*	*CXCL11*
CXCL12	*CXCL13*	*CXCL14*	*CXCL16*	*CXCL2*	*CXCL3*	*CXCL5*	*CXCL6*	*CXCL8*	*CXCL9*
CXCR1	*CXCR2*	*CXCR3*	*CXCR4*	*CXCR5*	*CXCR6*	*CXXC4*	*CXXC5*	*CYB561*	*CYBB*
CYCS	*CYFIP2*	*CYLD*	*CYP17A1*	*CYP19A1*	*CYP1B1*	*CYP2D6*	*CYP4A11*	*CYP4A22*	*CYP8B1*
CYSTM1	*DAB2*	*DACH2*	*DAPK1*	*DAPK2*	*DAPK3*	*DAXX*	*DCC*	*DCSTAMP*	*DCTN1*
DDB1	*DDB2*	*DDIT3*	*DDIT4*	*DDX21*	*DDX43*	*DDX50*	*DDX58*	*DEFB1*	*DEFB134*
DEPTOR	*DGAT2*	*DGCR2*	*DGLUCY*	*DHX15*	*DHX16*	*DIAPH1*	*DIO1*	*DIO2*	*DIPK2B*
DKK1	*DKK2*	*DKK4*	*DLK1*	*DLL1*	*DLL3*	*DLL4*	*DLX2*	*DMBT1*	*DNAJC14*
DNMT1	*DNMT3A*	*DOCK9*	*DPF1*	*DPF3*	*DPP4*	*DSC3*	*DSP*	*DST*	*DTX1*
DTX3	*DTX3L*	*DTX4*	*DUOX1*	*DUOX2*	*DUSP1*	*DUSP10*	*DUSP2*	*DUSP4*	*DUSP5*
DUSP6	*DUSP8*	*DVL1*	*DVL2*	*DVL3*	*DYNC1I2*	*DZANK1*	*E2F1*	*E2F2*	*E2F3*

E2F4	*E2F5*	*EBI3*	*ECSIT*	*EDC3*	*EDN1*	*EEF1G*	*EFNA1*	*EFNA2*	*EFNA3*
EFNA4	*EFNA5*	*EGF*	*EGFR*	*EGLN1*	*EGLN2*	*EGLN3*	*EGR1*	*EGR2*	*EGR3*
EHHADH	*EIF1*	*EIF2AK2*	*EIF2AK3*	*EIF2B4*	*EIF3L*	*EIF4A2*	*EIF4EBP1*	*EIF5AL1*	*ELANE*
ELAVL3	*ELAVL4*	*ELK1*	*ELMO1*	*ELOB*	*ELOC*	*ELOVL6*	*EML4*	*EMX2*	*ENDOG*
ENG	*ENO1*	*ENTPD1*	*EOMES*	*EP300*	*EPAS1*	*EPCAM*	*EPHA2*	*EPM2AIP1*	*EPO*
EPOR	*EPS15*	*EPS8L3*	*ERBB2*	*ERBB4*	*ERC1*	*ERCC1*	*ERCC2*	*ERCC3*	*ERCC4*
ERCC5	*ERCC6*	*EREG*	*ERG*	*ERLIN2*	*ERN2*	*ERO1A*	*ERP44*	*ESR1*	*ESR2*
ETHE1	*ETS1*	*ETS2*	*ETV1*	*ETV4*	*ETV5*	*ETV6*	*ETV7*	*EVA1A*	*EWSR1*
EXO1	*EYA1*	*EZH2*	*EZR*	*F11*	*F11R*	*F12*	*F13A1*	*F2RL1*	*FAAP24*
FABP1	*FABP4*	*FADD*	*FAM114A2*	*FAM124B*	*FAM131B*	*FAM13C*	*FAM167A*	*FAM30A*	*FANCA*
FANCB	*FANCC*	*FANCD2*	*FANCE*	*FANCF*	*FANCG*	*FANCL*	*FAP*	*FAS*	*FASLG*
FAU	*FBP1*	*FBXO28*	*FBXW7*	*FCAR*	*FCER1A*	*FCER1G*	*FCER2*	*FCF1*	*FCGR1A*
FCGR2A	*FCGR2B*	*FCGR3A*	*FCGR3B*	*FCGRT*	*FCHO1*	*FCHSD1*	*FCN1*	*FCRL2*	*FCRLA*
FEN1	*FEZ1*	*FGF1*	*FGF10*	*FGF11*	*FGF12*	*FGF13*	*FGF14*	*FGF16*	*FGF17*
FGF18	*FGF19*	*FGF2*	*FGF20*	*FGF21*	*FGF22*	*FGF23*	*FGF3*	*FGF4*	*FGF5*
FGF6	*FGF7*	*FGF8*	*FGF9*	*FGFR1*	*FGFR1OP2*	*FGFR2*	*FGFR3*	*FGFR4*	*FH*
FHIT	*FILIP1*	*FIP1L1*	*FKBP15*	*FLCN*	*FLG*	*FLI1*	*FLNA*	*FLNB*	*FLNC*
FLT1	*FLT3*	*FLT3LG*	*FLT4*	*FN1*	*FOLH1*	*FOS*	*FOSL1*	*FOXA1*	*FOXA2*
FOXC1	*FOXE1*	*FOXG1*	*FOXJ1*	*FOXL2*	*FOXM1*	*FOXO1*	*FOXO3*	*FOXO4*	*FOXP3*
FPR1	*FPR2*	*FPR3*	*FRAT1*	*FRAT2*	*FST*	*FSTL3*	*FUBP1*	*FUT4*	*FUT5*
FUT7	*FUT8*	*FYB1*	*FYN*	*FZD1*	*FZD10*	*FZD2*	*FZD3*	*FZD4*	*FZD5*
FZD6	*FZD7*	*FZD8*	*FZD9*	*G6PD*	*GAB1*	*GAB2*	*GABPA*	*GABRB2*	*GADD45A*
GADD45B	*GADD45G*	*GADD45GIP1*	*GADL1*	*GAGE1*	*GAGE10*	*GAGE12F*	*GAGE12I*	*GAGE12J*	*GAGE13*
GAGE2A	*GAGE2C*	*GAGE2E*	*GAPDH*	*GAS1*	*GATA1*	*GATA2*	*GATA3*	*GBP1*	*GBP2*
GBP4	*GCG*	*GCGR*	*GDF15*	*GDF6*	*GEMIN4*	*GFAP*	*GHITM*	*GHR*	*GIMAP4*
GIMAP6	*GIT2*	*GJA1*	*GJB6*	*GKAP1*	*GLI1*	*GLI2*	*GLI3*	*GLIS3*	*GLOD4*
GLS	*GLUD1*	*GLUL*	*GMIP*	*GNA11*	*GNA14*	*GNAQ*	*GNAS*	*GNG12*	*GNG4*
GNG7	*GNGT1*	*GNL3*	*GNLY*	*GOLGA5*	*GOPC*	*GOT1*	*GOT2*	*GPATCH3*	*GPC4*
GPI	*GPM6B*	*GPR160*	*GPR18*	*GPR3*	*GPS1*	*GPSM3*	*GPT*	*GPX1*	*GPX3*
GPX4	*GRAP2*	*GRB10*	*GRB2*	*GRB7*	*GREM1*	*GRIA3*	*GRIN1*	*GRIN2A*	*GRIN2B*
GRIPAP1	*GSK3B*	*GSN*	*GSTA1*	*GSTA2*	*GSTA3*	*GSTA4*	*GSTA5*	*GSTM1*	*GSTM2*
GSTM3	*GSTM4*	*GSTM5*	*GSTO1*	*GSTO2*	*GSTP1*	*GSTT1*	*GSTT2*	*GSTT2B*	*GTF2H3*
GTF2I	*GTF2IRD1*	*GTF3C1*	*GTPBP4*	*GUSB*	*GYG1*	*GZMA*	*GZMB*	*GZMH*	*GZMK*
GZMM	*H2AX*	*H3-3A*	*H3-5*	*H3C10*	*H3C2*	*H3C8*	*HACD2*	*HAMP*	*HAVCR2*
HBB	*HBEGF*	*HCK*	*HDAC1*	*HDAC10*	*HDAC11*	*HDAC2*	*HDAC3*	*HDAC4*	*HDAC5*
HDAC6	*HDC*	*HELLS*	*HERC6*	*HES1*	*HES5*	*HEY1*	*HEY2*	*HEYL*	*HFM1*
HGD	*HGF*	*HHEX*	*HHIP*	*HIF1A*	*HIP1*	*HK1*	*HK2*	*HLA-A*	*HLA-B*
HLA-C	*HLA-DMA*	*HLA-DMB*	*HLA-DOA*	*HLA-DOB*	*HLA-DPA1*	*HLA-DPB1*	*HLA-DQA1*	*HLA-DQA2*	*HLA-DQB1*
HLA-DQB2	*HLA-DRA*	*HLA-DRB1*	*HLA-DRB3*	*HLA-DRB4*	*HLA-DRB5*	*HLA-E*	*HLA-F*	*HLA-F-AS1*	*HLA-G*
HLF	*HMBS*	*HMGA1*	*HMGA2*	*HMGB1*	*HMGN5*	*HMOX1*	*HNF1A*	*HNRNPA2B1*	*HNRNPL*
HOXA10	*HOXA11*	*HOXA9*	*HOXC10*	*HOXD11*	*HPGD*	*HPRT1*	*HRAS*	*HSD11B1*	*HSD17B8*
HSDL2	*HSF2BP*	*HSP90AA1*	*HSP90AB1*	*HSP90B1*	*HSPA1A*	*HSPA2*	*HSPA6*	*HSPB1*	*HTR3A*
HYDIN	*IBSP*	*ICAM1*	*ICAM2*	*ICAM3*	*ICAM4*	*ICAM5*	*ICOS*	*ICOSLG*	*ID1*
ID2	*ID3*	*ID4*	*IDH1*	*IDH2*	*IDO1*	*IDO2*	*IER3*	*IFI16*	*IFI27*
IFI35	*IFI44L*	*IFI6*	*IFIH1*	*IFIT1*	*IFIT2*	*IFIT3*	*IFITM1*	*IFITM2*	*IFNA1*
IFNA17	*IFNA2*	*IFNA7*	*IFNA8*	*IFNAR1*	*IFNAR2*	*IFNB1*	*IFNG*	*IFNGR1*	*IFNGR2*
IFNL1	*IFNL2*	*IGF1*	*IGF1R*	*IGF2*	*IGF2R*	*IGFBP2*	*IGFBP3*	*IGFBP7*	*IGLL1*
IGSF6	*IHH*	*IKBKB*	*IKBKE*	*IKBKG*	*IKZF1*	*IKZF2*	*IKZF3*	*IKZF4*	*IL10*
IL10RA	*IL11*	*IL11RA*	*IL12A*	*IL12B*	*IL12RB1*	*IL12RB2*	*IL13*	*IL13RA1*	*IL13RA2*
IL15	*IL15RA*	*IL16*	*IL17A*	*IL17B*	*IL17F*	*IL17RA*	*IL17RB*	*IL18*	*IL18R1*
IL18RAP	*IL19*	*IL1A*	*IL1B*	*IL1R1*	*IL1R2*	*IL1RAP*	*IL1RAPL2*	*IL1RL1*	*IL1RL2*

IL1RN	*IL2*	*IL20RA*	*IL20RB*	*IL21*	*IL21R*	*IL22*	*IL22RA1*	*IL22RA2*	*IL23A*
IL23R	*IL24*	*IL25*	*IL26*	*IL27*	*IL2RA*	*IL2RB*	*IL2RG*	*IL3*	*IL32*
IL33	*IL34*	*IL3RA*	*IL4*	*IL4R*	*IL5*	*IL5RA*	*IL6*	*IL6R*	*IL6ST*
IL7	*IL7R*	*IL9*	*ILF3*	*ILK*	*ING4*	*INHBA*	*INHBB*	*INPP5D*	*INS*
INSL4	*INSRR*	*IRAK1*	*IRAK2*	*IRAK3*	*IRAK4*	*IRF1*	*IRF2*	*IRF2BP2*	*IRF3*
IRF4	*IRF5*	*IRF7*	*IRF8*	*IRF9*	*IRGM*	*IRS1*	*ISG15*	*ISG20*	*ISL1*
ITCH	*ITGA1*	*ITGA2*	*ITGA2B*	*ITGA3*	*ITGA4*	*ITGA5*	*ITGA6*	*ITGA7*	*ITGA8*
ITGA9	*ITGAE*	*ITGAL*	*ITGAM*	*ITGAV*	*ITGAX*	*ITGB1*	*ITGB2*	*ITGB3*	*ITGB4*
ITGB6	*ITGB7*	*ITGB8*	*ITK*	*ITPK1*	*JADE1*	*JAG1*	*JAG2*	*JAK1*	*JAK2*
JAK3	*JAKMIP1*	*JAM3*	*JAML*	*JCHAIN*	*JUN*	*JUNB*	*JUP*	*KAT2B*	*KATNAL2*
KBTBD8	*KCNAB1*	*KCNIP3*	*KCNJ11*	*KCNN4*	*KCTD8*	*KDELR2*	*KDM5C*	*KDM6A*	*KDM7A*
KDR	*KEAP1*	*KIAA1217*	*KIAA1549*	*KIAA1598*	*KIF12*	*KIF2C*	*KIF5B*	*KIF7*	*KIR2DL1*
KIR2DL2	*KIR2DL3*	*KIR2DS4*	*KIR3DL1*	*KIR3DL2*	*KIR3DL3*	*KIR3DS1*	*KIT*	*KITLG*	*KLC1*
KLF2	*KLF4*	*KLHL7*	*KLK2*	*KLK3*	*KLRB1*	*KLRC1*	*KLRC2*	*KLRD1*	*KLRF1*
KLRG1	*KLRK1*	*KMT2C*	*KMT2D*	*KRAS*	*KREMEN1*	*KRT1*	*KRT10*	*KRT13*	*KRT14*
KRT15	*KRT17*	*KRT18*	*KRT19*	*KRT20*	*KRT5*	*KRT6A*	*KRT6B*	*KRT6C*	*KRT7*
KYAT1	*L1CAM*	*LAG3*	*LAIR1*	*LAIR2*	*LAMA1*	*LAMA2*	*LAMA3*	*LAMA4*	*LAMA5*
LAMB1	*LAMB2*	*LAMB3*	*LAMB4*	*LAMC1*	*LAMC2*	*LAMC3*	*LAMP1*	*LAMP2*	*LAMP3*
LAPTM5	*LAT*	*LBP*	*LCK*	*LCN2*	*LCOR*	*LCP1*	*LDHA*	*LDHB*	*LEF1*
LEFTY1	*LEFTY2*	*LEP*	*LEPR*	*LEXM*	*LFNG*	*LGALS3*	*LGALS4*	*LGALS9*	*LGR5*
LHX3	*LIF*	*LIFR*	*LIG1*	*LIG3*	*LIG4*	*LILRA1*	*LILRA4*	*LILRA5*	*LILRB1*
LILRB2	*LILRB3*	*LILRB4*	*LIMA1*	*LLGL1*	*LMNA*	*LOH12CR1*	*LOXL2*	*LRG1*	*LRP1*
LRP2	*LRP5*	*LRP6*	*LRRC32*	*LRRC71*	*LRRN3*	*LSM12*	*LSM14A*	*LST1*	*LTA*
LTB	*LTBP1*	*LTBR*	*LTF*	*LTK*	*LUC7L2*	*LUM*	*LY6E*	*LY6K*	*LY86*
LY9	*LY96*	*LYN*	*LYZ*	*LZTFL1*	*M6PR*	*MAD1L1*	*MAD2L1*	*MAD2L2*	*MADCAM1*
MAF	*MAFF*	*MAGEA1*	*MAGEA10*	*MAGEA12*	*MAGEA3*	*MAGEA4*	*MAGEA6*	*MAGEB2*	*MAGEC1*
MAGEC2	*MAGI3*	*MALT1*	*MAML2*	*MAP2K1*	*MAP2K2*	*MAP2K3*	*MAP2K4*	*MAP2K6*	*MAP3K1*
MAP3K12	*MAP3K13*	*MAP3K14*	*MAP3K20*	*MAP3K5*	*MAP3K7*	*MAP3K8*	*MAP4K2*	*MAPK1*	*MAPK10*
MAPK11	*MAPK12*	*MAPK14*	*MAPK3*	*MAPK8*	*MAPK8IP1*	*MAPK8IP2*	*MAPK9*	*MAPKAPK2*	*MAPT*
MARCKS	*MARCO*	*MASP1*	*MASP2*	*MAVS*	*MAX*	*MBL2*	*MBNL1*	*MBNL3*	*MCAM*
MCAT	*MCL1*	*MCM2*	*MCM4*	*MCM5*	*MCM7*	*MDC1*	*MDFIC*	*MDM2*	*MDM4*
ME2	*MECOM*	*MED12*	*MEF2C*	*MEF2D*	*MEFV*	*MEIS1*	*MELK*	*MEN1*	*MERTK*
MET	*MFGE8*	*MFNG*	*MGEA5*	*MGMT*	*MGP*	*MGST1*	*MGST2*	*MGST3*	*MIA*
MIB1	*MICA*	*MICB*	*MIF*	*MITF*	*MKI67*	*MKRN1*	*MLANA*	*MLEC*	*MLF1*
MLH1	*MLLT10*	*MLLT3*	*MLPH*	*MME*	*MMP1*	*MMP11*	*MMP12*	*MMP2*	*MMP3*
MMP7	*MMP9*	*MMRN2*	*MNAT1*	*MNX1*	*MORC3*	*MPL*	*MPO*	*MPPED1*	*MPRIP*
MR1	*MRC1*	*MRE11*	*MRM2*	*MRPL19*	*MRPS5*	*MS4A1*	*MS4A2*	*MS4A4A*	*MS4A6A*
MSH2	*MSH3*	*MSH6*	*MSMB*	*MSN*	*MSR1*	*MSRB2*	*MST1R*	*MTF1*	*MTF2*
MTMR14	*MTOR*	*MTRR*	*MUC1*	*MUC2*	*MUC4*	*MUTYH*	*MX1*	*MXI1*	*MYB*
MYBL2	*MYC*	*MYCN*	*MYCT1*	*MYD88*	*MYH9*	*MYO18A*	*MYO5A*	*MYRIP*	*MZT1*
NAALAD2	*NAB2*	*NACC2*	*NANOG*	*NASP*	*NAT1*	*NAT8L*	*NBN*	*NCAM1*	*NCF1*
NCF4	*NCL*	*NCOA1*	*NCOA2*	*NCOA3*	*NCOA4*	*NCOR1*	*NCOR2*	*NCR1*	*NCR3*
NDC1	*NDC80*	*NDUFA1*	*NDUFA11*	*NDUFA12*	*NDUFA13*	*NDUFA2*	*NDUFA3*	*NDUFA4L2*	*NDUFA6*
NDUFA7	*NDUFB1*	*NDUFB10*	*NDUFB11*	*NDUFB4*	*NDUFB7*	*NDUFB8*	*NDUFS7*	*NDUFS8*	*NECTIN1*
NECTIN2	*NECTIN4*	*NEFL*	*NEIL1*	*NEIL3*	*NF1*	*NF2*	*NFAM1*	*NFASC*	*NFATC1*
NFATC2	*NFATC3*	*NFATC4*	*NFE2L2*	*NFIB*	*NFIL3*	*NFKB1*	*NFKB2*	*NFKBIA*	*NFKBIE*
NFKBIZ	*NGF*	*NGFR*	*NID2*	*NKD1*	*NKG7*	*NKX2-1*	*NKX3-1*	*NLRC5*	*NLRP3*
NOD1	*NOD2*	*NODAL*	*NOG*	*NOL4*	*NOL7*	*NOP16*	*NOS1*	*NOS1AP*	*NOS2*
NOS3	*NOTCH1*	*NOTCH2*	*NOTCH3*	*NOTCH4*	*NOX1*	*NPM1*	*NPM2*	*NPTX2*	*NPY1R*
NQO1	*NR3C1*	*NR4A1*	*NR4A3*	*NRAP*	*NRAS*	*NRBF2*	*NRDE2*	*NRG1*	*NRG2*
NRG3	*NRP1*	*NSD1*	*NSD2*	*NSD3*	*NT5E*	*NTF3*	*NTHL1*	*NTN3*	*NTRK1*

NTRK2	NTRK3	NUB1	NUBP1	NUF2	NUMB	NUMBL	NUP107	NUP214	NUPR1
OAS1	OAS2	OAS3	OASL	OAT	OAZ1	OCIAD1	OFD1	OLFML2B	OLR1
OPN3	ORC6	OSM	OTC	OTOA	OXR1	P2RY13	P4HA1	P4HA2	PAK1
PAK2	PAK3	PAK4	PAK5	PAK6	PALMD	PAN3	PANX3	PAPD7	PAPSS1
PARG	PARP12	PARP2	PARP4	PARP9	PASD1	PAWR	PAX3	PAX5	PAX8
PBK	PBRM1	PBX1	PBX3	PC	PCBP1	PCDH7	PCK1	PCK2	PCLAF
PCM1	PCNA	PCP4	PDCD1	PDCD1LG2	PDE5A	PDE7A	PDE9A	PDGFA	PDGFB
PDGFC	PDGFD	PDGFRA	PDGFRB	PDK1	PDLIM4	PDPK1	PDPN	PDZK1IP1	PEAR1
PEBP1	PECAM1	PEG3	PER2	PF4	PFKFB3	PFKM	PGAP3	PGF	PGK1
PGM2	PGPEP1	PGR	PHC3	PHF10	PHF12	PHF6	PHGDH	PHLDA2	PHLDB3
PI15	PIAS1	PIAS2	PIAS3	PIAS4	PIGR	PIK3CA	PIK3CB	PIK3CD	PIK3CG
PIK3R1	PIK3R2	PIK3R3	PIK3R4	PIK3R5	PIM1	PIM2	PIN1	PITX2	PKM
PKMYT1	PKP3	PLA1A	PLA2G10	PLA2G1B	PLA2G2A	PLA2G3	PLA2G4A	PLA2G4C	PLA2G4E
PLA2G4F	PLA2G5	PLA2G6	PLAT	PLAU	PLAUR	PLCB1	PLCB4	PLCD3	PLCE1
PLCG1	PLCG2	PLD1	PLD2	PLEKHA5	PLEKHG6	PLK1	PLK3	PLOD2	PMAIP1
PMCH	PMEL	PMEPA1	PML	PMS2	PNKP	PNMA1	PNOC	PNPLA5	POC1B
POF1B	POLB	POLD1	POLD2	POLD4	POLE2	POLK	POLR1B	POLR1C	CD3EAP
POLR2A	POLR2D	POLR2H	POLR2J	POLR3G	POSTN	POU2AF1	POU2F2	POU5F1	PPA1
PPAN	PPARD	PPARG	PPARGC1A	PPARGC1B	PPAT	PPBP	PPFIBP1	PPHLN1	PPIA
PPL	PPP1R1B	PPP1R21	PPP2CB	PPP2R1A	PPP2R2B	PPP2R2C	PPP2R3A	PPP3CA	PPP3CB
PPP3CC	PPP3R1	PPP3R2	PPP4R3B	PRAME	PRC1	PRCC	PRDM1	PRDM6	PRDX1
PRDX5	PRF1	PRG2	PRICKLE1	PRKAA2	PRKACA	PRKACB	PRKACG	PRKAR1A	PRKAR1B
PRKAR2A	PRKAR2B	PRKCA	PRKCB	PRKCD	PRKCE	PRKCG	PRKCQ	PRKDC	PRKX
PRL	PRLR	PRM1	PRMT8	PROM1	PROS1	PRPF38A	PRR5	PRRX1	PRSS1
PRUNE1	PSAT1	PSEN1	PSEN2	PSMB10	PSMB2	PSMB3	PSMB5	PSMB7	PSMB8
PSMB9	PSMC4	PSMD7	PSPH	PTCD2	PTCH1	PTCH2	PTCRA	PTEN	PTGDR2
PTGDS	PTGER4	PTGFRN	PTGS2	PTK2	PTK7	PTN	PTPN11	PTPN5	PTPN6
PTPN7	PTPRC	PTPRCAP	PTPRD	PTPRE	PTPRN2	PTPRR	PTPRZ1	PTTG1	PTTG2
PUM1	PURA	PVR	PVRIG	PWWP2A	PYCARD	PYCR1	PYCR2	PYCR3	PYGL
QKI	RAB3IL1	RAB7A	RABGAP1L	RAC1	RAC2	RAC3	RAD18	RAD21	RAD23B
RAD50	RAD51	RAD51C	RAD52	RAD54L	RAF1	RAG1	RALA	RALB	RALBP1
RALGDS	RANBP2	RAP1A	RAP1B	RAPGEF1	RARA	RARB	RASA4	RASAL1	RASGEF1B
RASGRF1	RASGRF2	RASGRP1	RASGRP2	RASSF1	RASSF5	RB1	RBL2	RBM45	RBMS3
RBP4	RBPMS	RBX1	RCC1	REG4	REL	RELA	RELB	RELN	REN
REPS1	RET	REV1	REV3L	RFC3	RFC4	RGMB	RGS17	RHOA	RHOB
RICTOR	RIMKLA	RIMKLB	RIN1	RIPK1	RIPK2	RIPK3	RNF130	RNF213	RNF43
RNF8	RNLS	ROBO4	ROCK1	ROPN1	ROR2	RORA	RORC	ROS1	RPA3
RPL23	RPL3	RPL4	RPL7A	RPLP0	RPS11	RPS14	RPS27A	RPS4Y1	RPS6
RPS6KA5	RPS6KA6	RPS6KB1	RPS6KB2	RPS9	RPTOR	RRAD	RRAS2	RRM2	RRS1
RSAD2	RSPH14	RTN4RL1	RUNX1	RUNX1T1	RUNX2	RUNX3	RXRA	RXRB	RXRG
RYBP	S100A12	S100A2	S100A4	S100A7	S100A8	S100A9	S100B	S100P	SAA1
SAMD9	SAMHD1	SAMSN1	SAP130	SARS	SBNO2	SCGB2A2	SCP2	SCUBE2	SCYL3
SDC1	SDC4	SDHA	SEC22B	SEC31A	SEC61G	SEL1L3	SELE	SELENBP1	SELENOK
SELL	SELP	SELPLG	SEMA6A	SEMG1	SENP1	SEPT10	SEPT14	SEPTIN3	SERINC1
SERINC2	SERINC3	SERINC5	SERPINA1	SERPINA3	SERPINB2	SERPINB3	SERPINB5	SERPINE1	SERPING1
SERPINH1	SETBP1	SETD2	SF3A1	SF3A3	SF3B1	SFN	SFRP1	SFRP2	SFRP4
SFTPB	SFTPC	SFXN1	SGK1	SGK2	SH2B2	SH2B3	SH2D1A	SH2D1B	SHC1
SHC2	SHC3	SHC4	SHH	SHROOM3	SHTN1	SIGIRR	SIGLEC1	SIGLEC5	SIGLEC8
SIL1	SIN3A	SIRPA	SIRPB2	SIRT4	SIT1	SIX1	SKAP2	SKP1	SKP2
SLAMF1	SLAMF6	SLAMF7	SLAMF8	SLC11A1	SLC12A7	SLC16A1	SLC16A2	SLC1A5	SLC23A2
SLC25A1	SLC26A4	SLC2A1	SLC34A2	SLC35F2	SLC35F3	SLC39A6	SLC3A1	SLC3A2	SLC43A1

SLC43A2	*SLC45A3*	*SLC4A1AP*	*SLC4A4*	*SLC4A7*	*SLC5A5*	*SLC5A8*	*SLC6A13*	*SLC7A5*	*SLMAP*
SMAD2	*SMAD3*	*SMAD4*	*SMAD5*	*SMAD9*	*SMAP1*	*SMARCA2*	*SMARCA4*	*SMARCB1*	*SMARCC1*
SMARCC2	*SMARCD1*	*SMARCD2*	*SMARCD3*	*SMARCE1*	*SMC1A*	*SMC1B*	*SMC3*	*SMO*	*SMPD3*
SNAI1	*SNAI2*	*SNCA*	*SND1*	*SOCS1*	*SOCS2*	*SOCS3*	*SOD1*	*SOD2*	*SORBS1*
SORBS2	*SOS1*	*SOS2*	*SOST*	*SOX10*	*SOX11*	*SOX17*	*SOX2*	*SOX4*	*SOX9*
SP1	*SPA17*	*SPACA3*	*SPAG17*	*SPANXB1*	*SPECC1L*	*SPI1*	*SPIB*	*SPINK1*	*SPINK5*
SPINT1	*SPN*	*SPO11*	*SPOCK2*	*SPOP*	*SPP1*	*SPRED1*	*SPRED2*	*SPRY1*	*SPRY2*
SPRY4	*SQSTM1*	*SRC*	*SRD5A2*	*SREBF1*	*SRGN*	*SRP54*	*SRR*	*SRSF2*	*SS18*
SSBP1	*SSBP2*	*SST*	*SSX1*	*SSX2*	*SSX4*	*ST6GAL1*	*ST7*	*STAG2*	*STARD3*
STAT1	*STAT2*	*STAT3*	*STAT4*	*STAT5A*	*STAT5B*	*STAT6*	*STC1*	*STING1*	*STK11*
STK11IP	*STK17B*	*STK26*	*STK4*	*STMN1*	*STMN2*	*STON1-GTF2A*	*STRN*	*STRN3*	*SUFU*
SULF1	*SULT2A1*	*SUMO1*	*SUV39H2*	*SYCP1*	*SYK*	*SYT12*	*SYT17*	*TAB1*	*TACC1*
TACC2	*TACC3*	*TACSTD2*	*TAF3*	*TAGAP*	*TAL1*	*TANK*	*TAP1*	*TAP2*	*TAPBP*
TAPBPL	*TARP*	*TATDN1*	*TAX1BP1*	*TBC1D1*	*TBC1D10B*	*TBC1D2*	*TBK1*	*TBL1XR1*	*TBP*
TBX21	*TBXAS1*	*TCF3*	*TCF7*	*TCF7L1*	*TCF7L2*	*TCIM*	*TCL1A*	*TCL1B*	*TDO2*
TEAD2	*TECR*	*TERC*	*TERF2*	*TERT*	*TET2*	*TFDP1*	*TFE3*	*TFEB*	*TFG*
TFRC	*TG*	*TGFA*	*TGFB1*	*TGFB2*	*TGFB3*	*TGFBR1*	*TGFBR2*	*TH*	*THBD*
THBS1	*THBS4*	*THEM4*	*THRA*	*THRB*	*THY1*	*TIAM1*	*TICAM1*	*TICAM2*	*TIE1*
TIGIT	*TIRAP*	*TLCD2*	*TLE4*	*TLE5*	*TLK2*	*TLR1*	*TLR10*	*TLR2*	*TLR3*
TLR4	*TLR5*	*TLR6*	*TLR7*	*TLR8*	*TLR9*	*TLX1*	*TM4SF4*	*TMEFF2*	*TMEM106B*
TMEM140	*TMEM163*	*TMEM165*	*TMEM43*	*TMEM45B*	*TMPRSS2*	*TMPRSS3*	*TMPRSS4*	*TMUB2*	*TNC*
TNF	*TNFAIP3*	*TNFAIP6*	*TNFAIP8*	*TNFRSF10A*	*TNFRSF10B*	*TNFRSF10C*	*TNFRSF10D*	*TNFRSF11A*	*TNFRSF11B*
TNFRSF12A	*TNFRSF13B*	*TNFRSF13C*	*TNFRSF14*	*TNFRSF17*	*TNFRSF18*	*TNFRSF19*	*TNFRSF1A*	*TNFRSF1B*	*TNFRSF25*
TNFRSF4	*TNFRSF6B*	*TNFRSF8*	*TNFRSF9*	*TNFSF10*	*TNFSF11*	*TNFSF12*	*TNFSF13*	*TNFSF13B*	*TNFSF14*
TNFSF15	*TNFSF18*	*TNFSF4*	*TNFSF8*	*TNFSF9*	*TNKS*	*TNN*	*TNR*	*TOLLIP*	*TOP2A*
TOX	*TP53*	*TP63*	*TP73*	*TPD52L1*	*TPI1*	*TPM1*	*TPM2*	*TPM3*	*TPM4*
TPO	*TPR*	*TPSAB1*	*TPSB2*	*TPTE*	*TPX2*	*TRAF1*	*TRAF2*	*TRAF3*	*TRAF4*
TRAF5	*TRAF6*	*TRAF7*	*TRAK1*	*TRAT1*	*TREM1*	*TREM2*	*TRIM15*	*TRIM21*	*TRIM24*
TRIM27	*TRIM29*	*TRIM33*	*TRIM39*	*TRIM63*	*TSC1*	*TSC2*	*TSHR*	*TSLP*	*TSPAN7*
TSPAN8	*TTC30A*	*TTC31*	*TTK*	*TTPA*	*TTR*	*TUBB*	*TUSC3*	*TWF1*	*TWIST1*
TWIST2	*TXK*	*TXLNA*	*TXLNGY*	*TXN2*	*TXNIP*	*TXNRD1*	*TXNRD2*	*TXNRD3*	*TYK2*
TYMP	*TYMS*	*TYROBP*	*TYRP1*	*U2AF1*	*UBA7*	*UBB*	*UBC*	*UBE2C*	*UBE2T*
ULBP2	*UNC5D*	*UNG*	*UPK1B*	*UPK3A*	*UQCR10*	*UQCR11*	*UQCRQ*	*USP10*	*USP39*
USP8	*USP9Y*	*UST*	*UTY*	*VCAM1*	*VCAN*	*VCL*	*VEGFA*	*VEGFB*	*VEGFC*
VEGFD	*VHL*	*VIM*	*VOPP1*	*VPS33B*	*VSIR*	*VSTM2A*	*VTCN1*	*WAC*	*WDCP*
WDR3	*WDR76*	*WEE1*	*WIF1*	*WIPF1*	*WIPF2*	*WNK2*	*WNT1*	*WNT10A*	*WNT10B*
WNT11	*WNT16*	*WNT2*	*WNT2B*	*WNT3*	*WNT3A*	*WNT4*	*WNT5A*	*WNT5B*	*WNT6*
WNT7A	*WNT7B*	*WNT8A*	*WNT8B*	*WNT9A*	*WNT9B*	*WRN*	*WT1*	*WWC1*	*XAGE1B*
XCL1	*XCL2*	*XCR1*	*XIAP*	*XIST*	*XPA*	*XRCC2*	*XRCC4*	*XRCC5*	*XRCC6*
XXYLT1	*YRDC*	*YTHDF2*	*YWHAE*	*ZAN*	*ZAP70*	*ZBTB16*	*ZBTB17*	*ZBTB20*	*ZBTB32*
ZBTB46	*ZC3H12A*	*ZC3H14*	*ZC3HAV1*	*ZCCHC8*	*ZEB1*	*ZEB2*	*ZIC2*	*ZKSCAN5*	*ZMYM2*
ZMYM4	*ZNF143*	*ZNF205*	*ZNF34*	*ZNF346*	*ZNF365*	*ZNF384*	*ZNF485*	*ZNF703*	*ZSCAN30*

Table S6 RNA expression profiling for 7 EBV-related genes

EBER1	*EBER2*	*EBNA1*	*LMP1*	*LMP2A*	*BZLF1*	*BARF1*			

Note: The final EBV status is determined based on the expression level of EBER1 and EBER2 gene.

Appendix II

Table S7 Index Sequence Information for Primers

Primer Name	Sample Sheet Index Information (NextSeq/NovaSeq)	Corresponding No.in TruSeq HT Sample Prep Kits
Master-D701	ATTACTCG	D701
Master-D702	TCCGGAGA	D702
Master-D703	CGCTCATT	D703
Master-D704	GAGATTCC	D704
Master-D705	ATTCAGAA	D705
Master-D706	GAATTCGT	D706
Master-D707	CTGAAGCT	D707
Master-D708	TAATGCGC	D708
Master-D709	CGGCTATG	D709
Master-D710	TCCGCGAA	D710
Master-D711	TCTCGCGC	D711
Master-D712	AGCGATAG	D712

Primer Name	Sample Sheet Index Information (NextSeq/ NovaSeq V1.5)	Sample Sheet Index Information (NovaSeq V1.0)	Corresponding No.in TruSeq HT Sample Prep Kits
Master-D501	AGGCTATA	TATAGCCT	D501
Master-D502	GCCTCTAT	ATAGAGGC	D502
Master-D503	AGGATAGG	CCTATCCT	D503
Master-D504	TCAGAGCC	GGCTCTGA	D504
Master-D505	CTTCGCCT	AGGCGAAG	D505
Master-D506	TAAGATTA	TAATCTTA	D506
Master-D507	ACGTCCTG	CAGGACGT	D507
Master-D508	GTCAGTAC	GTACTGAC	D508

Appendix III

Table S8 Positive Variants (HotSpot Mutations and Fusions) in Master-DNA-Positive Control

No.	Gene	Alteration Type	Variants
1	*EGFR*	SNV	NM_005228: exon20: c.2369C>T: p.(T790M)
2	*EGFR*	SNV	NM_005228: exon21: c.2573T>G: p.(L858R)
3	*KRAS*	SNV	NM_033360: exon2: c.35G>T: p.(G12V)
4	*MET*	MET exon 14 skipping	NM_000245: intron14: c.3028+1G>T: p.?
5	*SLC34A2-ROS1*	Fusion	SLC34A2: NM_006424_exon4-ROS1: NM_002944_exon32

Note：

- *There might be hotspot mutations in the DNA positive control that are outside the detection scope of this panel.*
- *The Master-DNA-Positive Control is a mixture of multiple tumor cell lines, so there might be positive outputs for non-hotspot mutations and/or CNVs.*
- *The Master-DNA-Negative Control is derived from tumor cell line, so there might be positive outputs for non-hotspot mutations and/or CNVs.*

Table S9 Positive Variants (Hot Fusions) in Master-RNA-Positive Control

No.	Gene	Alteration Type	Variants
1	*SLC34A2-ROS1*	Fusion	SLC34A2: NM_006424_exon4-ROS1: NM_002944_exon32
2	*GOPC-ROS1*	Fusion	GOPC: NM_020399_exon8-ROS1: NM_002944_exon35
3	*EML4-ALK*	Fusion	EML4: NM_019063_exon13-ALK: NM_004304_exon20