# Supplementary Materials

# SPEAR: CRISPR-mediated ultrasensitive, specific and rapid one-pot detection strategy for cancer-related SNPs

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## Materials and Methods

### The sgRNA preparation

Single guide RNA (sgRNA) was synthesized and purified with T7 Transcription Kit. Firstly, the template was amplified via PCR assay. Then, 2 μL of T7 polymerase, 10 μL of NTP buffer and 4 μL of the PCR product were mixed, and DEPC water was added to make up a 30 μL transcription system. This mixture was then incubated at 37 °C for 24 h to transcribe the DNA template into RNA. Next, 50 μL reaction system including DNase I 5 μL ,10 × DNase I buffer 5 μL, RNase inhibitor 20 units was heated at 37 °C for 30 min to ensure complete digestion of the DNA template. Then, the obtained RNA products were purified using HiPure RNA Pure Micro Kit, following the operational instructions. Finally, the RNA was eluted into RNase-free water and preserved at −80 °C refrigerator.

### Cell culture and nucleic acid extraction

Pancreatic cancer cell line PaTu8988 and human fetal hepatocyte line 293T were cultured in petri dishes. The culture medium was 5 mL DMEM containing 100 U/mL penicillin-streptomycin and 10% FBS. Cells were cultured at 37 °C humidified atmosphere containing 5% $CO_2$. Total DNA of cell samples was extracted using the TIANGEN Genomic DNA Kit (input volume 200 μL, elution volume 50 μL).

### Preparation of mutant mock DNA samples

Plasmid Mutant Mock DNA Samples: Mutant and wild-type (WT) templates were mixed at various ratios, with the *FLT*3 D835Y mutation comprising 100%, 50%, 25%, 10%, 1%, 0.1%, and 0.01% (i.e., 100 copies of the D835Y template in a total of 1,000,000 templates) of the total template pool. A total of $1 \times 10^6$ copies of the mixed plasmid DNA was used as input for the SPEAR assay.

Cell Line Genomic DNA Mutation-Mocked Samples: Genomic DNA extracted from the PaTu8988 cell line (containing the G12V mutation) was mixed with wild-type genomic DNA from

blood cells to generate mutant concentrations ranging from 1% to 0.01%. A total of 80 ng of the genomic DNA was used as input for the SPEAR assay.

The cfDNA Mutation-Mocked Samples: ctDNA containing 19.4% G12V mutations from plasma samples of pancreatic cancer patients was mixed with wild-type ctDNA from healthy individuals to prepare ctDNA mutation-doped samples with mutation concentrations ranging from 19.4% to 0.01%. A total of 80 ng of genomic DNA was used as input for the SPEAR assay.

**Polyacrylamide Gel Electrophoresis Analysis**

After the SPEAR reaction, 10 μL product was mixed with 10 × loading buffer. Electrophoresis was carried out on 20% polyacrylamide gel with 5 bp DNA Ladder (Thermo Fisher Scientific, MD, USA). Electrophoresis was conducted at 110 V constant voltage for 2 h. After electrophoresis, the gel was immersed in 0.1 M NaCl solution with 3 × GelRed nucleic acid dye (Biomed, Beijing, China) for 30 min and subsequently imaged using the ChemiDoc$^{TM}$ XRS+ Imager (Bio-Rad, California, USA).

**First-generation sequencing (FGS) and next-generation sequencing (NGS)**

For FGS, the extracted DNA was amplified by PCR. The amplification system contained *TaKaRa Taq* HS (5 U/μL) 0.25 μL, 10 × PCR Buffer ($Mg^{2+}$ plus) 5 μL, dNTP Mixture (each 2.5 mM) 5 μL, Template 3 μL, primer F/R 400 nM (Specific primer sequences were shown in Table S4), and DEPC $H_2O$ to make up the final volume to 50 μL. The PCR procedure was 95 °C 5 min, (95 °C 20s, 55 °C 30s, 72 °C 30s) × 40 cycles, and 72 °C 5 min. 20 μl PCR products were sent for FGS by Sangon Biotech (Shanghai, China). The mutation rate was obtained through quantitative analysis of the FGS sequencing map using the website https://moriaritylab.shinyapps.io/editr_v10/.

For NGS, Firstly, the extracted DNA was amplified by PCR with primers containing barcodes. The amplification system contained 2 × PCR buffer for KOD Fx 25 μL, 2 mM dNTPs 10 μL, 10 μM Primer F/R 1.5 μL (Specific primer sequences were shown in Table S5), Template 5 μL, KOD Fx (1.0 U/μL) 1 μL, and DEPC $H_2O$ to make up the final volume to 50 μL. First round PCR procedure was 94 °C 2 min, (98 °C 10 s, 55 °C 30 s, 68 °C 30 s) × 25 cycles, and 68 °C 7 min. In

the second round of PCR, different barcode primers were used to amplify the KTAS G12 and *FLT*3-D835 region of samples. The amplification system and procedure were as follows, 2 × PCR buffer for KOD Fx 25 μL, 2mM dNTPs 10 μL, 10 μM Primer F/R 1.5 μL (Specific primer sequences were shown in Table S5), 6 μL Amplicons of the first round PCR, KOD Fx (1.0 U/μL) 1 μL, and DEPC $H_2O$ to 50 μL. The second round PCR procedure was 94 °C 2 min, (98 °C 10 s，57 °C 30 s，68 °C 30 s) × 15 cycles, 68 °C 7 min. The PCR products were purified by TIANgel Midi Purification Kit, and sent to Mingma Technologies (Shanghai，China) for NGS.

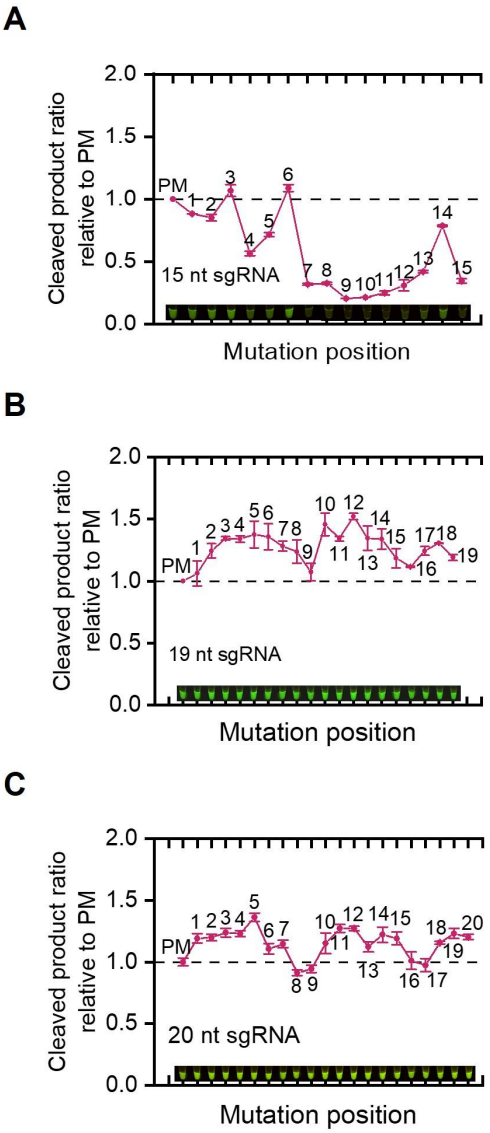



**Figure S1.** Specificity evaluation of AapCas12b cleavage with different length of sgRNAs. (A) 15 nt sgRNA, (B) 19 nt sgRNA, (C) 20 nt sgRNA. Data were the mean ± SD (n = 3).

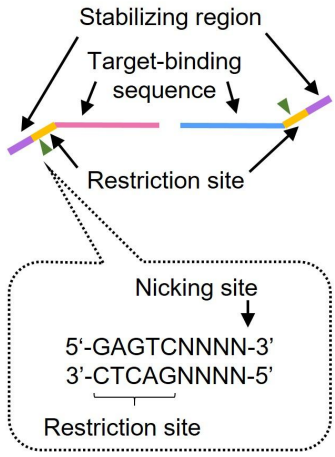



**Figure S2.** Structure of primers in SPEAR assay. Each primer consists of three parts: a stabilizing region, a restriction site, and a target-binding sequence. The function of the stabilizing region is to stabilize the binding of the restriction enzyme with the primer and template during the amplification process. The restriction site is identified by nicking enzyme. The target-binding sequence is the part where the primer complementary pairing with the target. The restriction enzyme cleavage site is located between the $4^{th}$ and $5^{th}$ positions near the restriction site (GAGTC).

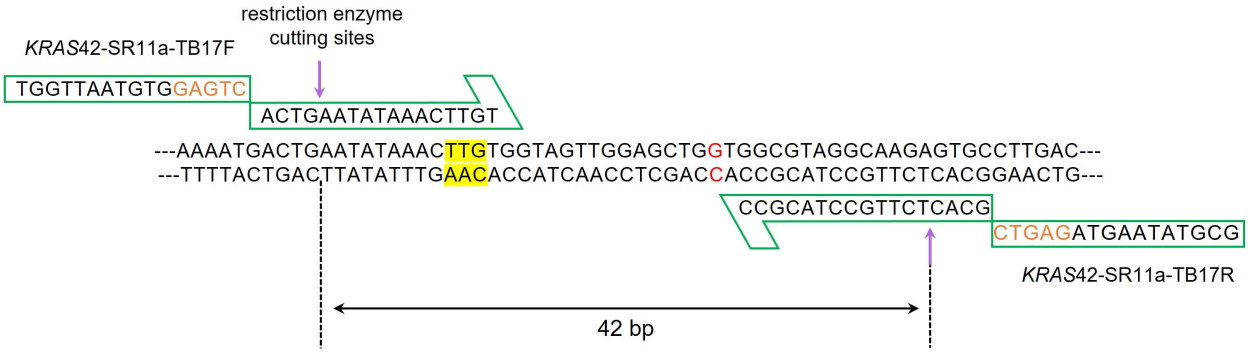



**Figure S3.** Schematic diagram illustrating primer binding and target amplification of the *KRAS* G12V gene. The yellow-filled region represents the PAM sequence, while the red font indicates the mutation site.

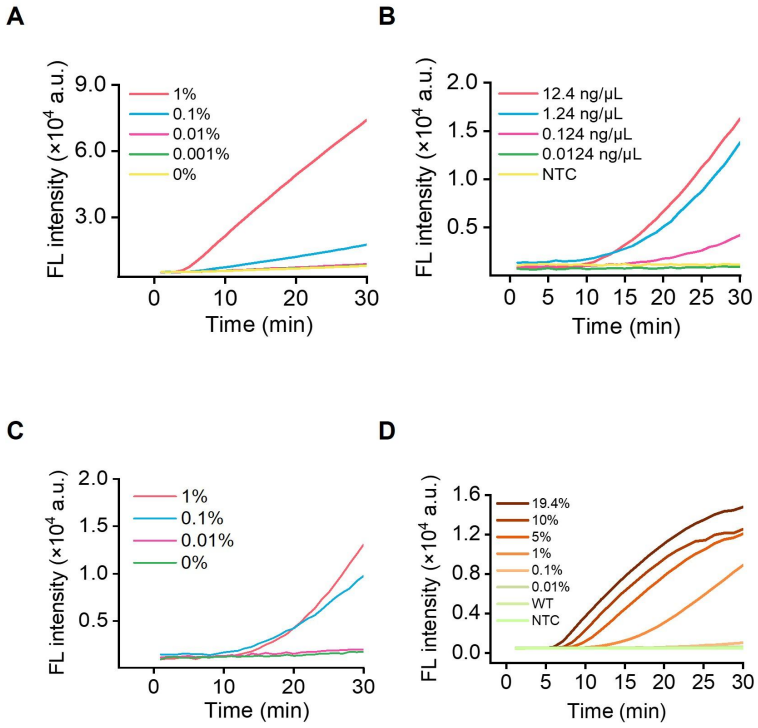



**Figure S4.** Sensitivity evaluation of SPEAR method. (A) Sensitivity of SPEAR method in detecting 1e6 copies of plasmid templates with gradient *KRAS* G12V mutation rates. The real-time fluorescence was presented. (B) Detection sensitivity of genomic DNA of PaTu8988 cell line using SPEAR method. The real-time fluorescence was presented. (C) Sensitivity of mutation rate detection with genomic DNA as template, a mixture of PaTu8988 and 293T genomic DNA, totaling 80 ng and containing gradient *KRAS* G12V mutation rates, was used as templates for SPEAR detection. The real-time fluorescence was presented. (D) Sensitivity of mutation rate detection with cfDNA as template. A mixture of cfDNA extracted from pancreatic cancer patients and healthy individuals, totaling 80 ng and containing gradient *KRAS* G12V mutation rates, was used as templates for SPEAR detection. NTC: no template control. Data were the mean ± SD (n = 3).

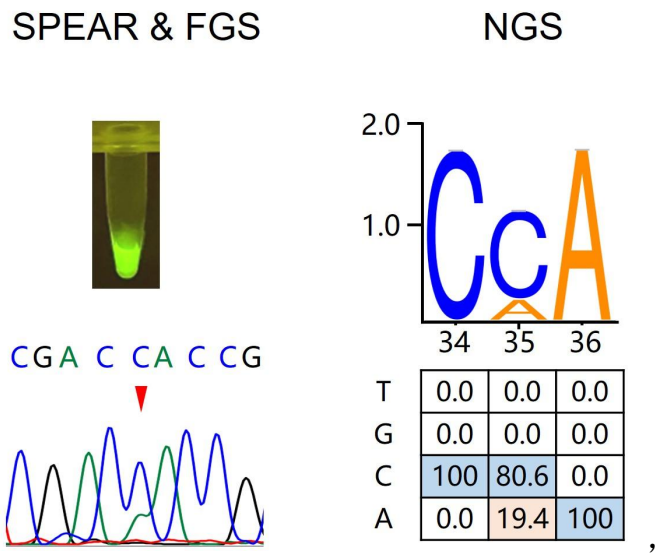



**Figure S5.** Detection results of a PC clinical sample using the NGS, FGS, and SPEAR method. The visualization result of the SPEAR method is displayed on the upper left. The FGS peak diagrams is shown on the lower left, with mutant base marked by red triangle. The genotype and mutation rate detected by NGS are shown on the right, WT and mutated bases were marked with blue and orange, respectively.

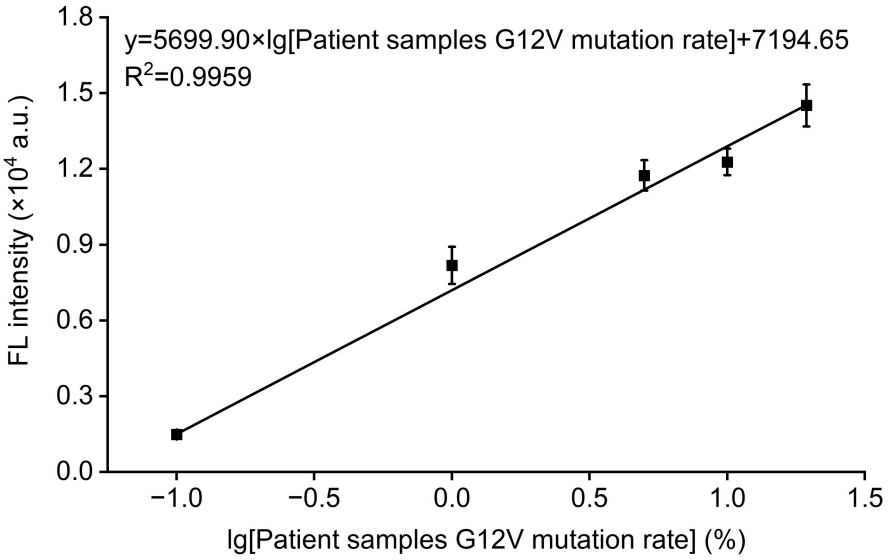



**Figure S6.** The fitted curve between patient sample G12V mutation rate (lg) and fluorescence intensity.

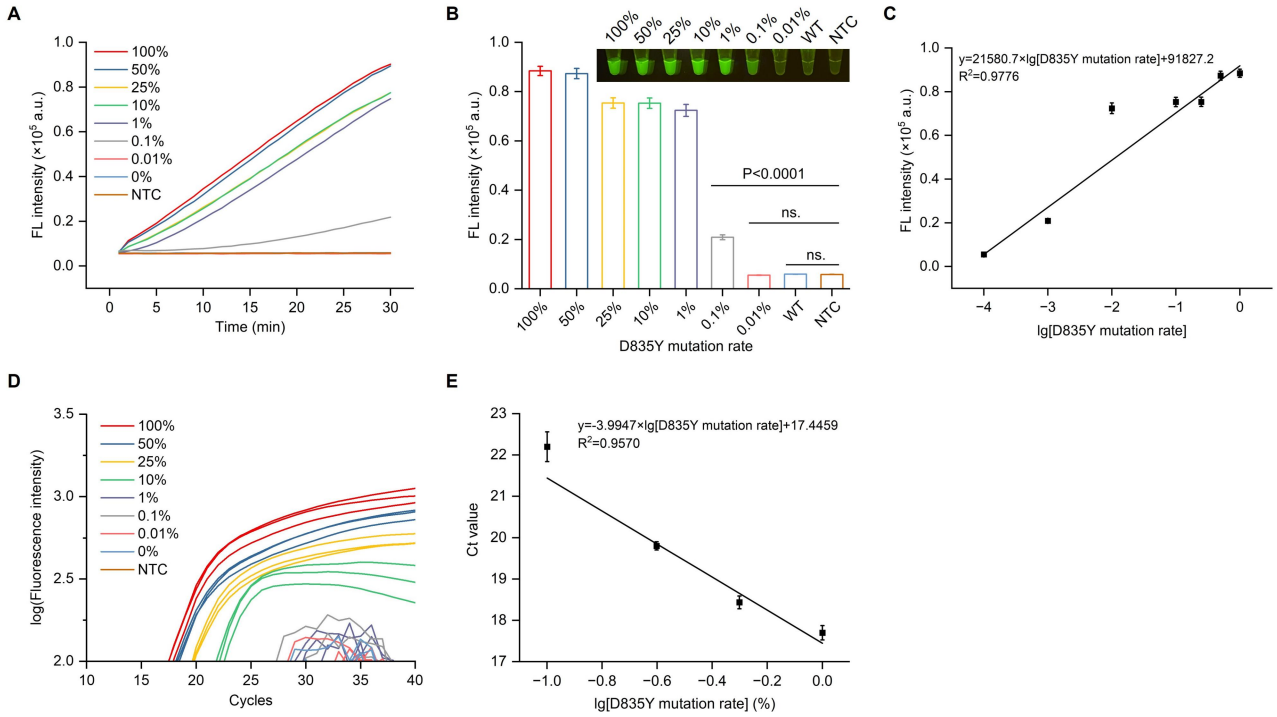



**Figure S7.** SPEAR and qPCR were used to detect the D835Y mutation rates. (A) Sensitivity of SPEAR method in detecting 1e6 copies of plasmid templates with gradient *FLT* 3 D835Y mutation rates. The real-time fluorescence was presented. (B) The 30 min endpoint fluorescence values and visualization results. (C) The fitted curve between 0.01%, 0.1%, 1%, 10%, 25%, 50%, 100% D835Y mutation rates (lg) and the corresponding fluorescence intensity. (D) The qPCR amplification plot of 1e6 copies of plasmid templates with gradient D835Y mutation rates. (E) The fitted curve between 10%, 25%, 50%, 100% *FLT*3 D835Y mutation rates (lg) and the corresponding ct values. Data are the mean ± SD (n = 3).

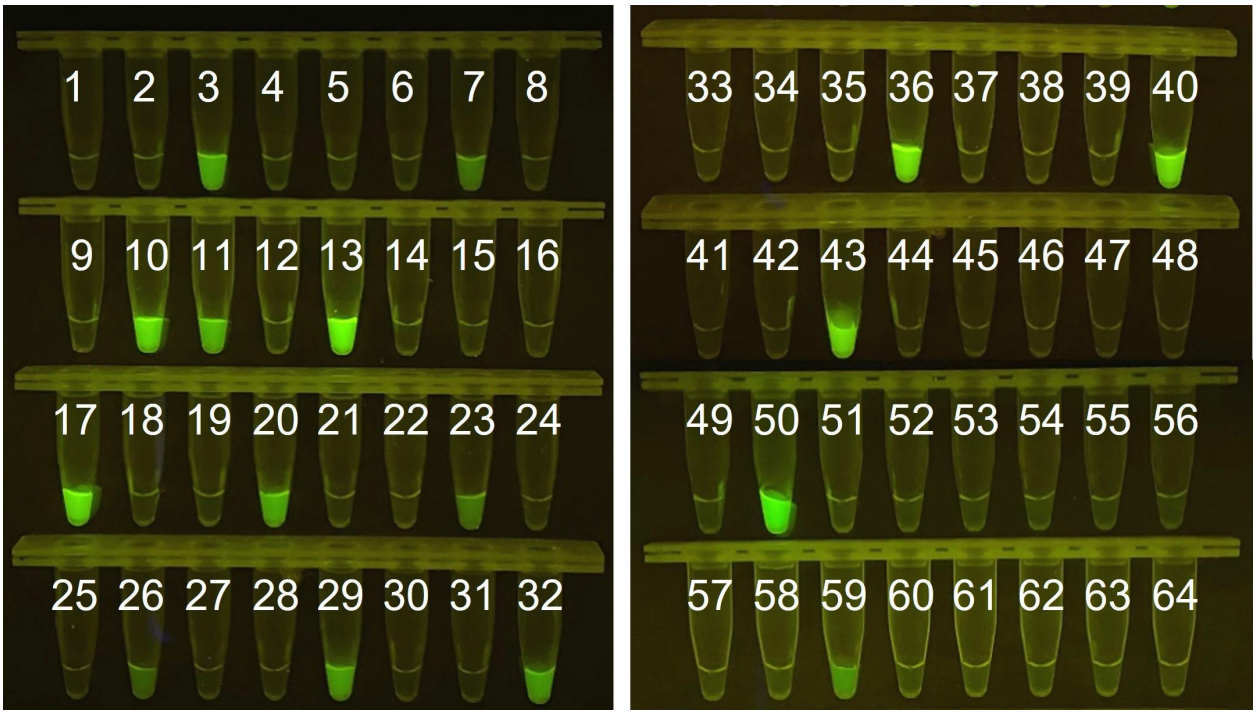
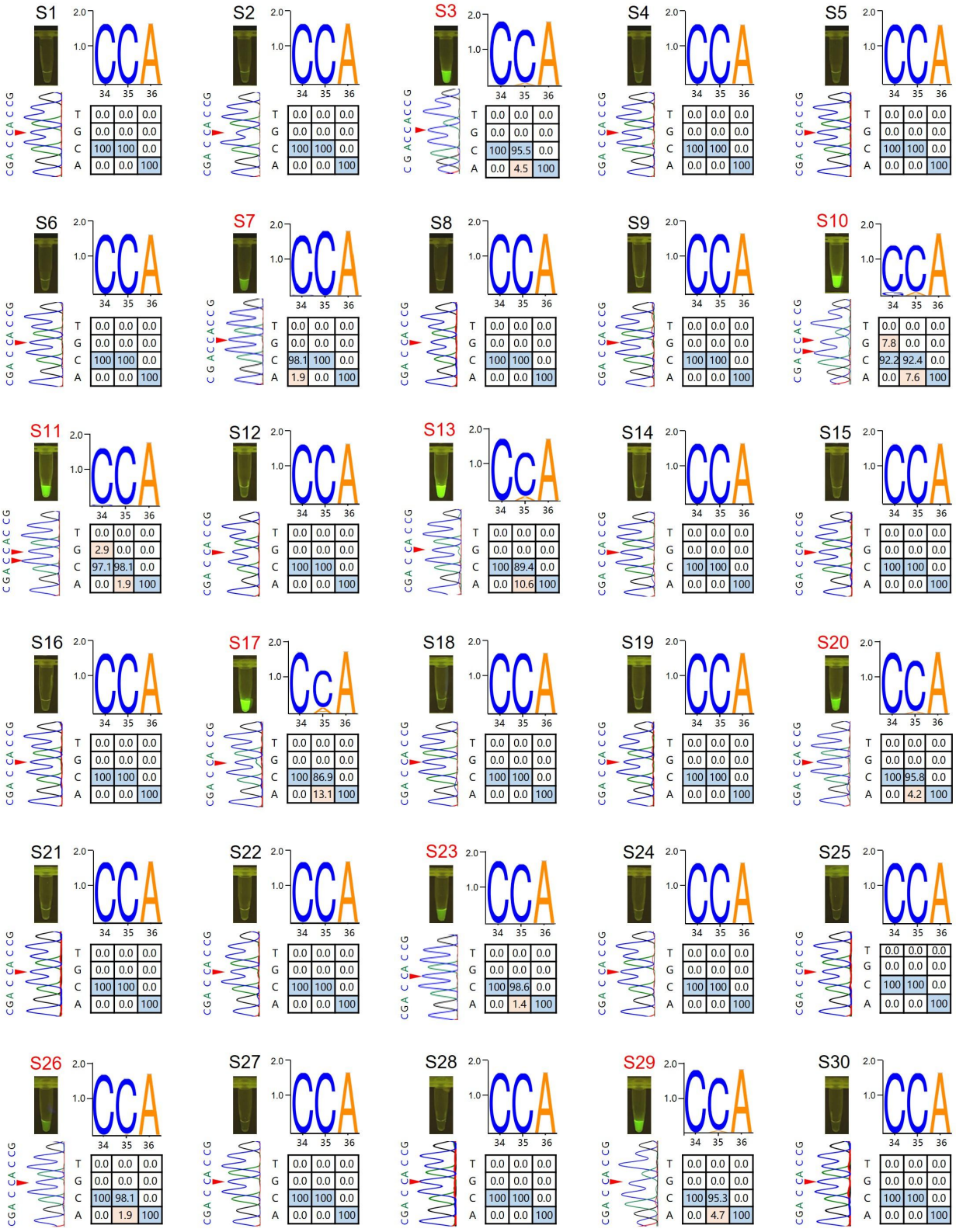
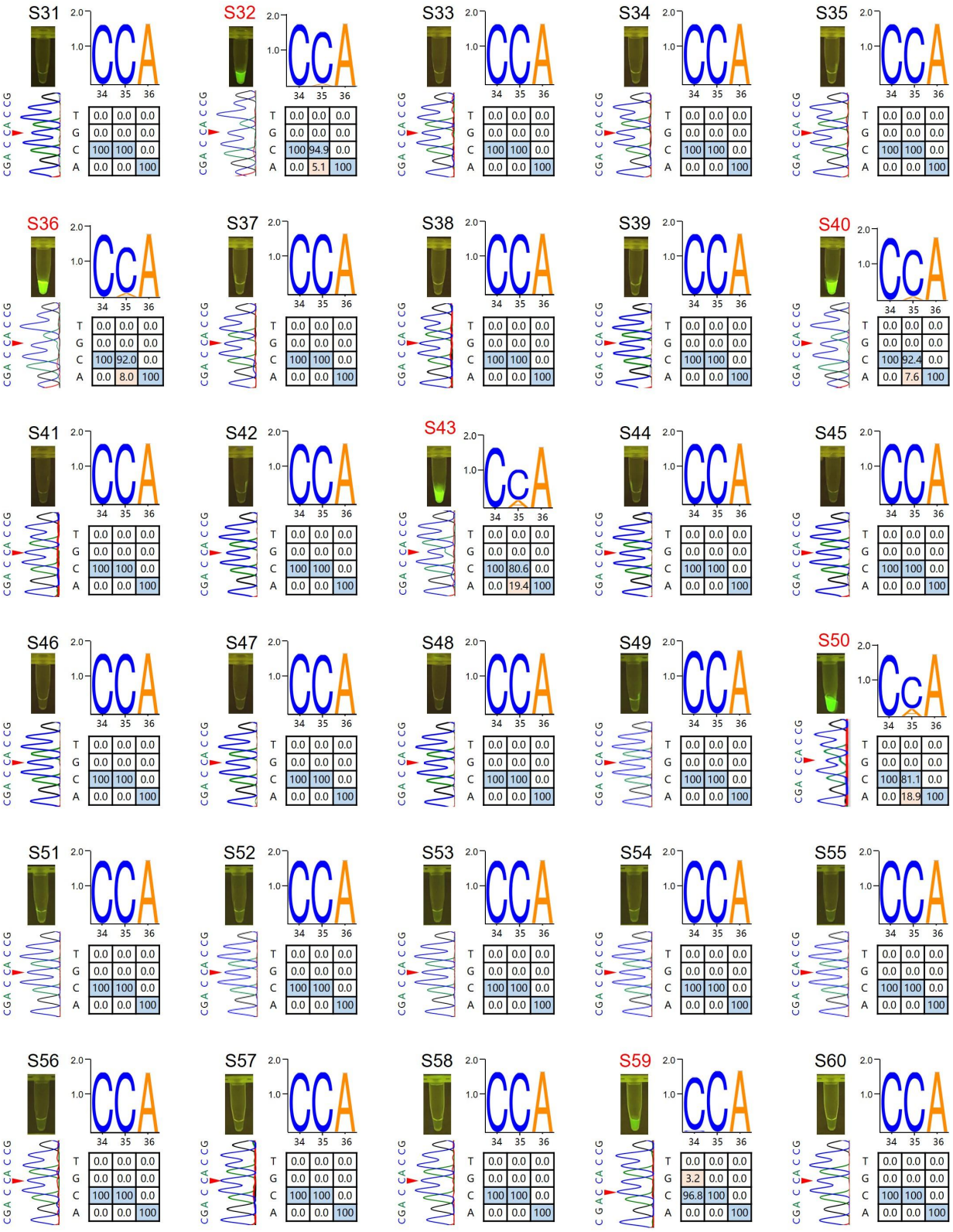


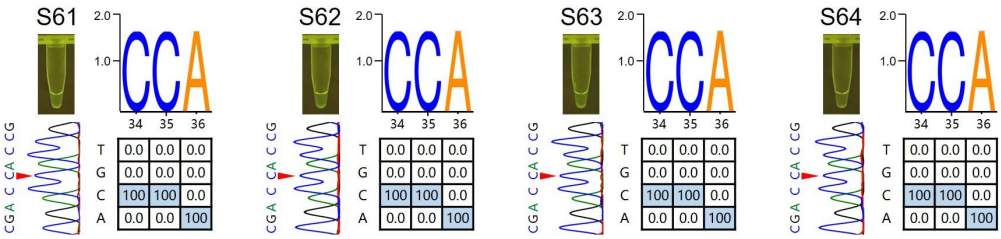

**Figure S8.** SPEAR results of 64 PC patient samples observed by naked eyes under 485 nm blue light.


S1 S2 S3 S4 S5
S6 S7 S8 S9 S10
S11 S12 S13 S14 S15
S16 S17 S18 S19 S20
S21 S22 S23 S24 S25
S26 S27 S28 S29 S30
2.0
1.0
34 35 36
T 0.0 0.0 0.0
G 0.0 0.0 0.0
C 100 100 0.0
A 0.0 0.0 100

142


S31 S32 S33 S34 S35
S36 S37 S38 S39 S40
S41 S42 S43 S44 S45
S46 S47 S48 S49 S50
S51 S52 S53 S54 S55
S56 S57 S58 S59 S60
2.0
1.0
34 35 36
CGA C CA C CG
T 0.0 0.0 0.0
G 0.0 0.0 0.0
C 100 100 0.0
A 0.0 0.0 100
S32 C 100 94.9 0.0 A 0.0 5.1 100
S36 C 100 92.0 0.0 A 0.0 8.0 100
S40 C 100 92.4 0.0 A 0.0 7.6 100
S43 C 100 80.6 0.0 A 0.0 19.4 100
S50 C 100 81.1 0.0 A 0.0 18.9 100
S59 G 3.2 0.0 0.0 C 96.8 100 0.0

143

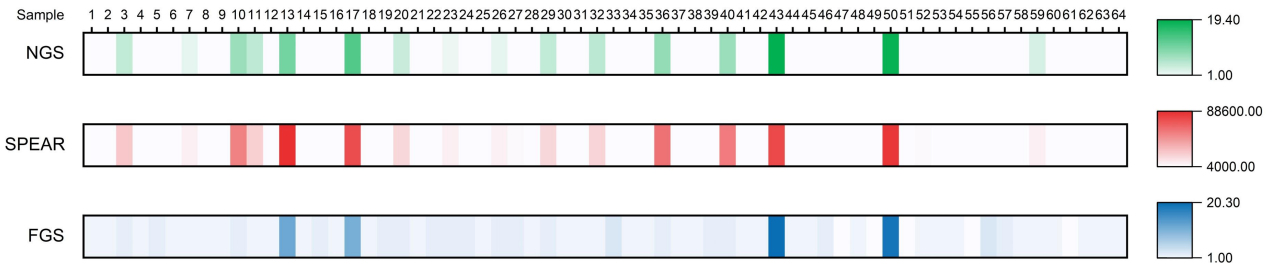



144

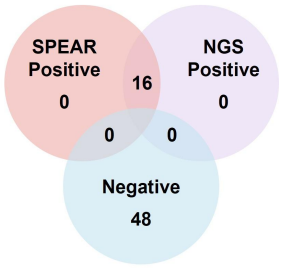
145 **Figure S9.** SPEAR, FGS and NGS results of 64 PC patient blood samples. The visualization results
146 of the SPEAR method are displayed on the upper left. The FGS peak diagrams are shown on the
147 lower left, with mutant bases marked by red triangles. The genotypes and mutation rates detected by
148 NGS are shown on the right, WT and mutated bases are marked with blue and orange, respectively.
149 NGS displays the 3'-5' sequence of the *KRAS* G12 gene, while FGS shows the 3'-5' sequence of the
150 *KRAS* A11, G12, and G13 genes.

151

A




B




C

| Methods | | NGS | | Total |
|---|---|---|---|---|
| | | Positive | Negative | |
| SPEAR | Positive | 16 | 0 | 16 |
| | Negative | 0 | 48 | 48 |
| | Total | 16 | 48 | 64 |

Sensitivity: 100%; Specificity: 100%; Accuracy: 100%; PPV: 100%; NPV: 100%;

**Figure S10.** (A) Results of NGS, SPEAR, and FGS for detecting mutations in blood samples from 64 patients with pancreatic cancer. From top to bottom: heat map analysis of mutation rates detected by NGS, heat map analysis of fluorescence intensity detected by SPEAR, and heat map analysis of mutation rates detected by FGS (FGS sequencing map quantitatively analyzed using the website https://moriaritylab.shinyapps.io/editr_v10/). (B) Venn diagram showing the results of SPEAR and NGS for detecting KRAS G12V/D/R mutations in clinical pancreatic cancer samples. (C) The negative predictive value (NPV), positive predictive value (PPV), sensitivity, and specificity of SPEAR for detecting KRAS G12V/D/R mutations in clinical pancreatic cancer samples.

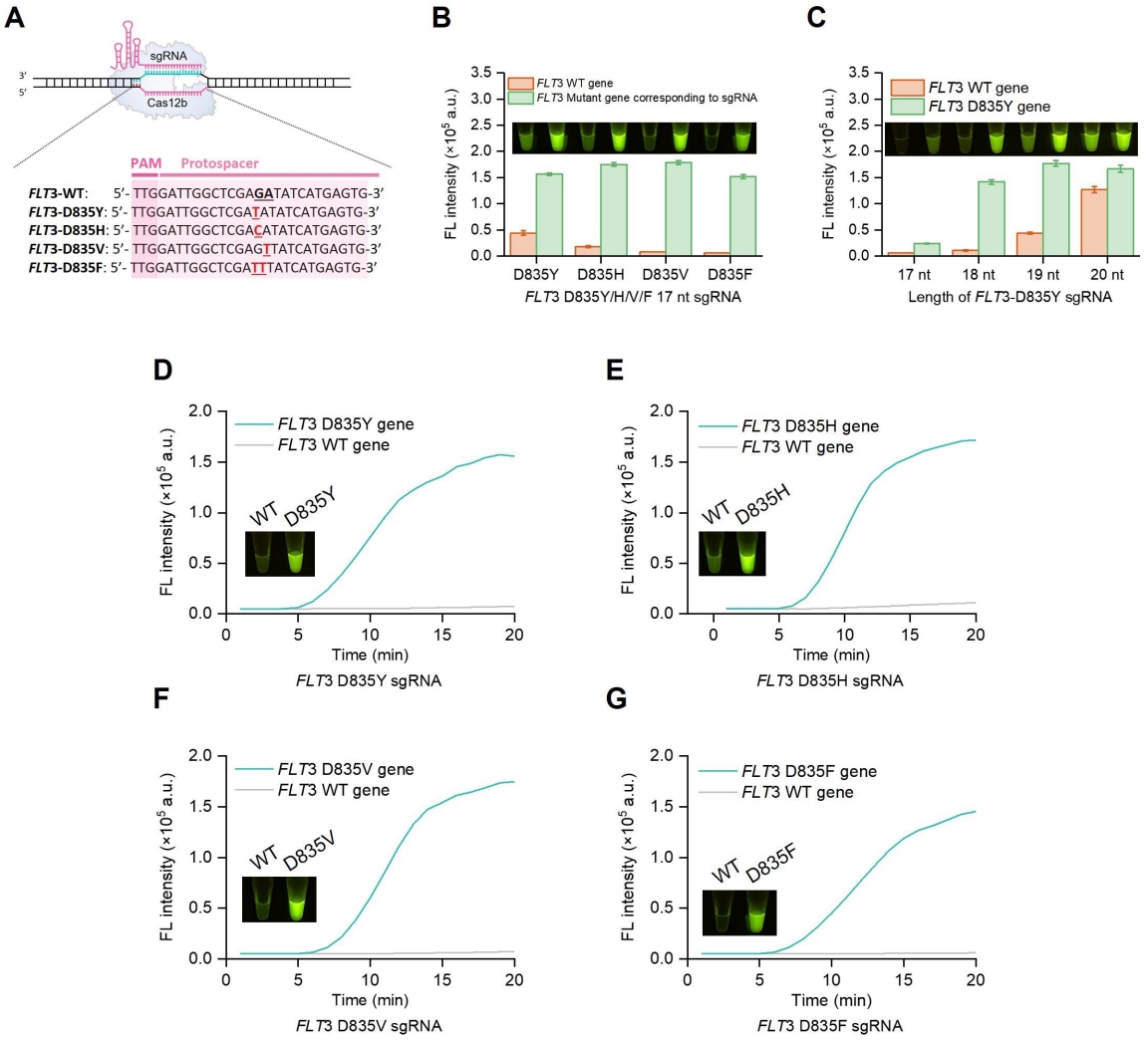



**Figure S11.** The application of SPEAR in identifying AML *FLT3* D835Y/H/V/F mutations. (A) Sequence information of *FLT3* D835Y/H/V/F. (B) Exploring the optimal sgRNAs targeting *FLT3* mutations D835Y/H/V/F individually. The 17 nt sgRNAs were designed to target the D835Y/H/V/F mutations. The 30 min endpoint fluorescence values and visualization results are presented. (C) Specificity of 17, 18, 19, 20 nt *FLT3*-D835Y sgRNA with artificially introduced mutation. The 30 min endpoint fluorescence values and visualization results are provided. d-g the optimal sgRNAs were used to detect *FLT3* mutations D835Y (D), D835H (E), D835F (F), and D835V (G) in the SPEAR reaction. The real-time fluorescence and visualization results are presented. The sequence information of the optimal sgRNAs is shown in Table S3. Data were the mean ± SD (n = 3).

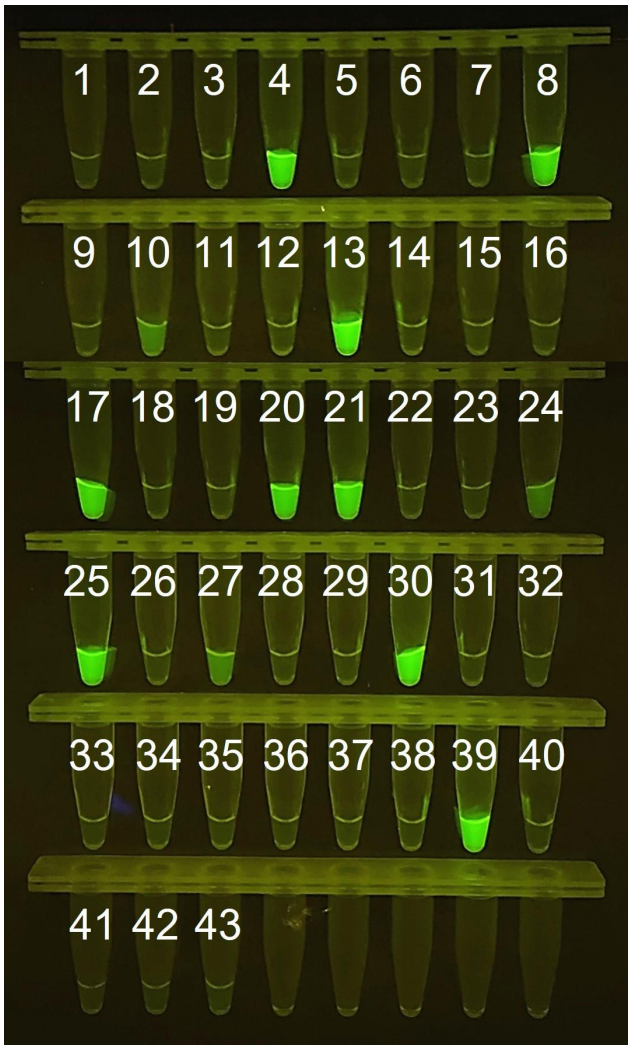



**Figure S12.** SPEAR results of 43 AML patient samples observed by naked eyes under 485 nm blue light.


S1 2.0 1.0 GAT 2503 2504 2505 C G A G A T A T C T 0.0 0.0 100 G 100 0.0 0.0 C 0.0 0.0 0.0 A 0.0 100 0.0
S2 2.0 1.0 GAT 2503 2504 2505 C G A G A T A T C T 0.0 0.0 100 G 100 0.0 0.0 C 0.0 0.0 0.0 A 0.0 100 0.0
S3 2.0 1.0 GAT 2503 2504 2505 C G A G A T A T C T 0.0 0.0 100 G 100 0.0 0.0 C 0.0 0.0 0.0 A 0.0 100 0.0
S4 2.0 1.0 GAT 2503 2504 2505 C G A G A T A T C T 19.9 0.0 100 G 80.1 0.0 0.0 C 0.0 0.0 0.0 A 0.0 100 0.0
S5 2.0 1.0 GAT 2503 2504 2505 C G A G A T A T C T 0.0 0.0 100 G 100 0.0 0.0 C 0.0 0.0 0.0 A 0.0 100 0.0
S6 2.0 1.0 GAT 2503 2504 2505 C G A G A T A T C T 0.0 0.0 100 G 100 0.0 0.0 C 0.0 0.0 0.0 A 0.0 100 0.0
S7 2.0 1.0 GAT 2503 2504 2505 C G A G A T A T C T 0.0 0.0 100 G 100 0.0 0.0 C 0.0 0.0 0.0 A 0.0 100 0.0
S8 2.0 1.0 GAT 2503 2504 2505 C G A G A T A T C T 18.6 0.0 100 G 81.4 0.0 0.0 C 0.0 0.0 0.0 A 0.0 100 0.0
S9 2.0 1.0 GAT 2503 2504 2505 C G A G A T A T C T 0.0 0.0 100 G 100 0.0 0.0 C 0.0 0.0 0.0 A 0.0 100 0.0
S10 2.0 1.0 GAT 2503 2504 2505 C G A G A T A T C T 2.2 0.0 100 G 97.8 0.0 0.0 C 0.0 0.0 0.0 A 0.0 100 0.0
S11 2.0 1.0 GAT 2503 2504 2505 C G A G A T A T C T 0.0 0.0 100 G 100 0.0 0.0 C 0.0 0.0 0.0 A 0.0 100 0.0
S12 2.0 1.0 GAT 2503 2504 2505 C G A G A T A T C T 0.0 0.0 100 G 100 0.0 0.0 C 0.0 0.0 0.0 A 0.0 100 0.0
S13 2.0 1.0 GAT 2503 2504 2505 C G A G A T A T C T 20.2 0.0 100 G 79.8 0.0 0.0 C 0.0 0.0 0.0 A 0.0 100 0.0
S14 2.0 1.0 GAT 2503 2504 2505 C G A G A T A T C T 0.0 0.0 100 G 100 0.0 0.0 C 0.0 0.0 0.0 A 0.0 100 0.0
S15 2.0 1.0 GAT 2503 2504 2505 C G A G A T A T C T 0.0 0.0 100 G 100 0.0 0.0 C 0.0 0.0 0.0 A 0.0 100 0.0
S16 2.0 1.0 GAT 2503 2504 2505 C G A G A T A T C T 0.0 0.0 100 G 100 0.0 0.0 C 0.0 0.0 0.0 A 0.0 100 0.0
S17 2.0 1.0 GAT 2503 2504 2505 C G A G A T A T C T 16.3 0.0 100 G 83.7 0.0 0.0 C 0.0 0.0 0.0 A 0.0 100 0.0
S18 2.0 1.0 GAT 2503 2504 2505 C G A G A T A T C T 0.0 0.0 100 G 100 0.0 0.0 C 0.0 0.0 0.0 A 0.0 100 0.0
S19 2.0 1.0 GAT 2503 2504 2505 C G A G A T A T C T 0.0 0.0 100 G 100 0.0 0.0 C 0.0 0.0 0.0 A 0.0 100 0.0
S20 2.0 1.0 GAT 2503 2504 2505 C G A G A T A T C T 7.1 0.0 100 G 92.9 0.0 0.0 C 0.0 0.0 0.0 A 0.0 100 0.0
S21 2.0 1.0 GAT 2503 2504 2505 C G A G A T A T C T 8.9 0.0 100 G 91.1 0.0 0.0 C 0.0 0.0 0.0 A 0.0 100 0.0
S22 2.0 1.0 GAT 2503 2504 2505 C G A G A T A T C T 0.0 0.0 100 G 100 0.0 0.0 C 0.0 0.0 0.0 A 0.0 100 0.0
S23 2.0 1.0 GAT 2503 2504 2505 C G A G A T A T C T 0.0 0.0 100 G 100 0.0 0.0 C 0.0 0.0 0.0 A 0.0 100 0.0
S24 2.0 1.0 GAT 2503 2504 2505 C G A G A T A T C T 3.5 0.0 100 G 96.5 0.0 0.0 C 0.0 0.0 0.0 A 0.0 100 0.0
S25 2.0 1.0 GAT 2503 2504 2505 C G A G A T A T C T 20.5 0.0 100 G 79.5 0.0 0.0 C 0.0 0.0 0.0 A 0.0 100 0.0
S26 2.0 1.0 GAT 2503 2504 2505 C G A G A T A T C T 0.0 0.0 100 G 100 0.0 0.0 C 0.0 0.0 0.0 A 0.0 100 0.0
S27 2.0 1.0 GAT 2503 2504 2505 C G A G A T A T C T 2.5 0.0 100 G 97.5 0.0 0.0 C 0.0 0.0 0.0 A 0.0 100 0.0
S28 2.0 1.0 GAT 2503 2504 2505 C G A G A T A T C T 0.0 0.0 100 G 100 0.0 0.0 C 0.0 0.0 0.0 A 0.0 100 0.0
S29 2.0 1.0 GAT 2503 2504 2505 C G A G A T A T C T 0.0 0.0 100 G 100 0.0 0.0 C 0.0 0.0 0.0 A 0.0 100 0.0
S30 2.0 1.0 GAT 2503 2504 2505 C G A G A T A T C T 19.1 0.0 100 G 80.9 0.0 0.0 C 0.0 0.0 0.0 A 0.0 100 0.0

177

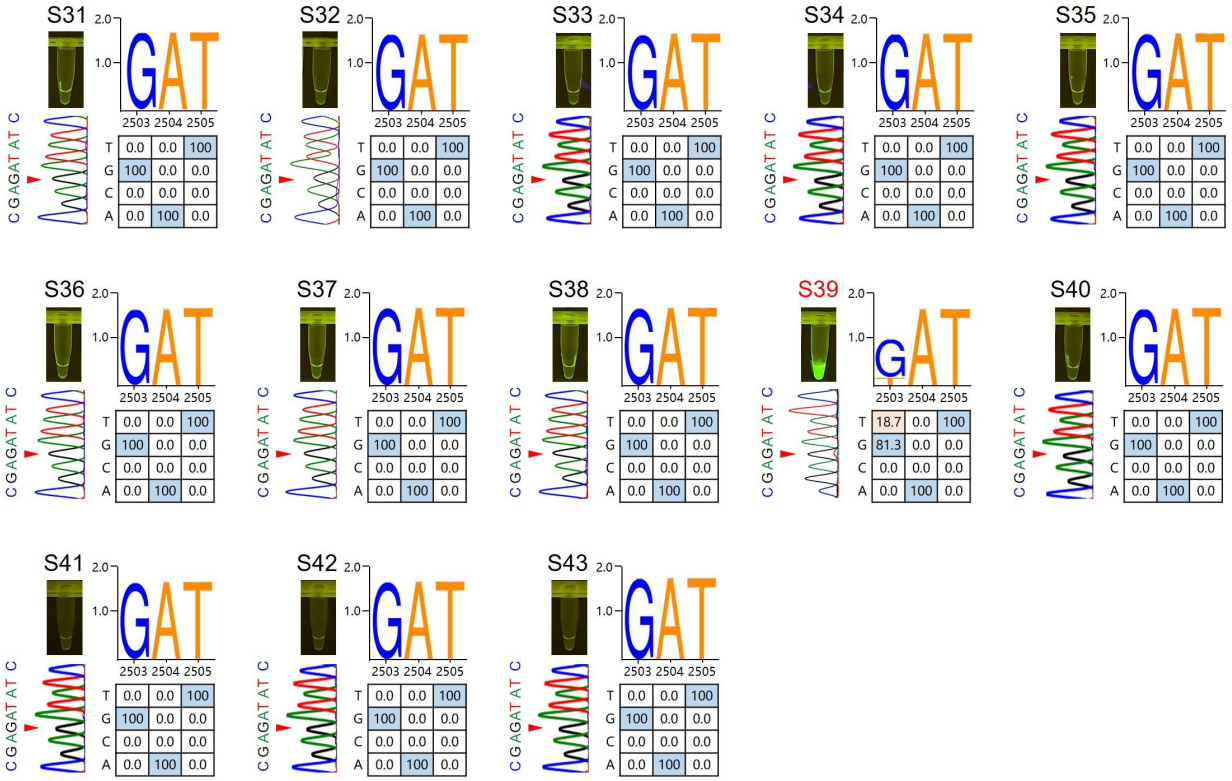
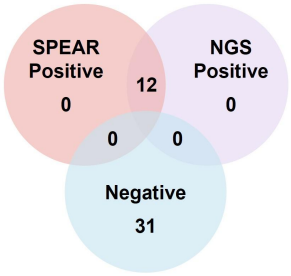



**Figure S13.** SPEAR, FGS and NGS results of 43 AML patient blood samples. The visualization results of the SPEAR method are displayed on the upper left. The FGS peak diagrams are shown on the lower left, with mutant bases marked by red triangles. The genotypes and mutation rates detected by NGS are shown on the right, WT and mutated bases were marked with blue and orange, respectively. NGS displays the sequence of *FLT*3 D835 gene, while FGS shows the sequence of *FLT*3 R834, D835, and I836 genes.

A




B

| Methods | | NGS | | Total |
|---|---|---|---|---|
| | | Positive | Negative | |
| SPEAR | Positive | 12 | 0 | 12 |
| | Negative | 0 | 31 | 31 |
| | Total | 12 | 31 | 43 |
| Sensitivity: 100%; Specificity: 100%; Accuracy: 100%; PPV: 100%; NPV: 100%; | | | | |

185

186 **Figure S14.** (A) Venn diagram showing the results of SPEAR and NGS for *FLT*3 D835Y/V/F/H
187 mutations detection in clinical AML samples. (B) NPV, PPV, sensitivity, and specificity of SPEAR
188 for detecting the *FLT*3 D835Y/V/F/H mutation in clinical AML samples.

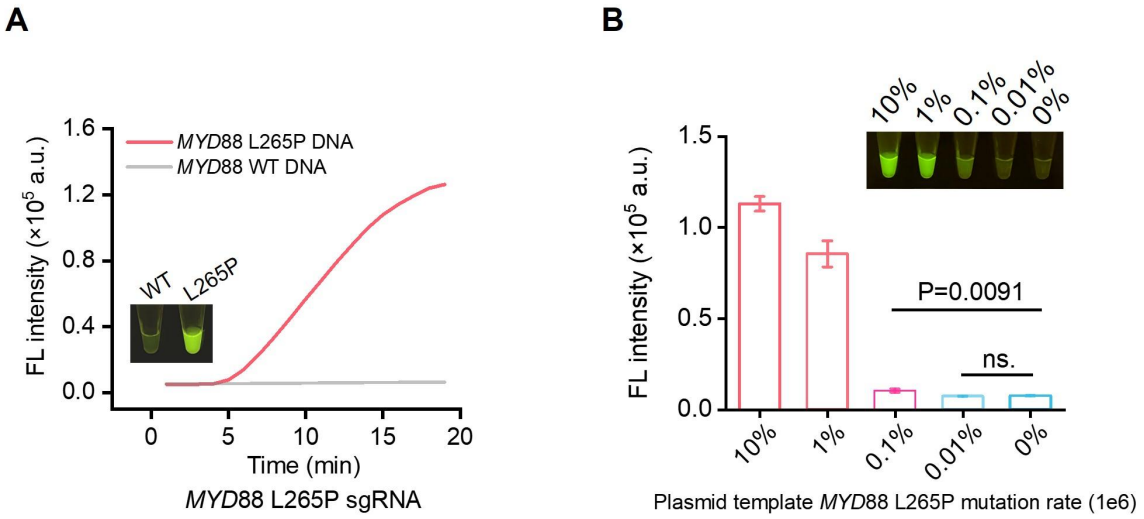



**Figure S15.** The application of SPEAR in identifying DLBCL *MYD*88 L265P mutations. (A) The specific sgRNA was used to detect the *MYD*88 L265P gene in the SPEAR reaction. The real-time fluorescence and visualization results are presented. (B) Sensitivity of SPEAR method in detecting 1e6 copies of plasmid templates with gradient *MYD*88 L265P mutation rates. The 30 min endpoint fluorescence values and visualization results are demonstrated. Data were the mean ± SD (n = 3).

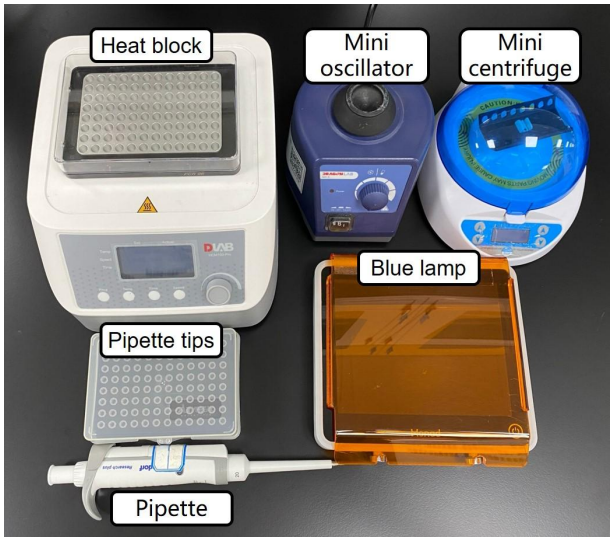



**Figure S16.** Equipment needed in SPEAR assay.

198 **Table S1.**

199 **Sequence information for *in vitro* sgRNA transcription**

| Name | *Sequence (5′-3′) |
|---|---|
| sgRNA-T7-universal | TAATACGACTCACTATAGGG |
| AapCas12b-sgRNA | TAATACGACTCACTATAGGGGTCTAGAGGACAGAATTTTTCAACGGGTGTGCCAATGGCCACTTTCCAGGTGGCAAAGCCCGTTGAGCTTCTCAAATCTGAGAAGTGGCACGTAGCAGCAAGATTAGCAGAAGCT |
| *KRAS*-12b-WT-20gR | GCCACCAGCTCCAACTACCA-GTGCCACTTCTCAGATTTGA |
| *KRAS*-12b-WT-19gR | CCACCAGCTCCAACTACCA-GTGCCACTTCTCAGATTTGA |
| *KRAS*-12b-WT-18gR | CACCAGCTCCAACTACCA-GTGCCACTTCTCAGATTTGA |
| *KRAS*-12b-WT-17gR | ACCAGCTCCAACTACCA-GTGCCACTTCTCAGATTTGA |
| *KRAS*-12b-WT-16gR | CCAGCTCCAACTACCA-GTGCCACTTCTCAGATTTGA |
| *KRAS*-12b-WT-15gR | CAGCTCCAACTACCA-GTGCCACTTCTCAGATTTGA |
| *KRAS*-12b-G12V-18gR | CAACAGCTCCAACTACCA-GTGCCACTTCTCAGATTTGA |
| *KRAS*-12b-G12V-17gR | AACAGCTCCAACTACCA-GTGCCACTTCTCAGATTTGA |
| *KRAS*-12b-G12V-16gR | ACAGCTCCAACTACCA-GTGCCACTTCTCAGATTTGA |
| *KRAS*-12b-G12D-18gR | CATCAGCTCCAACTACCA-GTGCCACTTCTCAGATTTGA |
| *KRAS*-12b-G12D-17gR | ATCAGCTCCAACTACCA-GTGCCACTTCTCAGATTTGA |
| *KRAS*-12b-G12D-16gR | TCAGCTCCAACTACCA-GTGCCACTTCTCAGATTTGA |
| *KRAS*-12b-G12R-18gR | CACGAGCTCCAACTACCA-GTGCCACTTCTCAGATTTGA |
| *KRAS*-12b-G12R-17gR | ACGAGCTCCAACTACCA-GTGCCACTTCTCAGATTTGA |
| *KRAS*-12b-G12R-16gR | CGAGCTCCAACTACCA-GTGCCACTTCTCAGATTTGA |
| *KRAS*-12b-G12R-15gR | GAGCTCCAACTACCA-GTGCCACTTCTCAGATTTGA |
| *FLT*3-12b-D835Y-17gR | GATATATCGAGCCAATC-GTGCCACTTCTCAGATTTGA |
| *FLT*3-12b-D835H-17gR | GATATGTCGAGCCAATC-GTGCCACTTCTCAGATTTGA |
| *FLT*3-12b-D835V-17gR | GATAACTCGAGCCAATC-GTGCCACTTCTCAGATTTGA |
| *FLT*3-12b-D835F-17gR | GATAAATCGAGCCAATC-GTGCCACTTCTCAGATTTGA |
| *FLT*3-12b-D835Y-17MR | GATGTATCGAGCCAATC-GTGCCACTTCTCAGATTTGA |
| *FLT*3-12b-D835Y-18MR | TGATGTATCGAGCCAATC-GTGCCACTTCTCAGATTTGA |
| *FLT*3-12b-D835Y-19MR | ATGATGTATCGAGCCAATC-GTGCCACTTCTCAGATTTGA |
| *FLT*3-12b-D835Y-20MR | CATGATGTATCGAGCCAATC-GTGCCACTTCTCAGATTTG |
| *MYD*88-12b-L265P-17gR | CCGATCCCCATCAAGTA-GTGCCACTTCTCAGATTTGA |
| *MYD*88-12b-L265P-16gR | CGATCCCCATCAAGTA-GTGCCACTTCTCAGATTTGA |

* The T7 promoter sequence is in green. The sequence before the dash is a template for the Cas12b sgRNA spacer sequence. The mutation sites are marked by red color different from spacer sequences, and the artificially introduced mutation sites are marked by yellow fill. The DNA template of the optimal sgRNA corresponding to each target is marked with an underline.

204 **Table S2.**

205 **Oligonucleotide Sequence of dsDNA templates with SNP**

| Name | *Sequence (5′ -3′ ) |
|---|---|
| *KRAS* Target-wtF | ATGTATGACCAGTTGAGATTGTGGTAGTTGGAGCTGGTGGCTGATGAAGACGACTCTGA |
| *KRAS* Target-wtR | TCAGAGTCGTCTTCATCAGCCACCAGCTCCAACTACCACAATCTCAACTGGTCATACAT |
| *KRAS* Target-mm1F | ATGTATGACCAGTTGAGATTGAGGTAGTTGGAGCTGGTGGCTGATGAAGACGACTCTGA |
| *KRAS* Target-mm1R | TCAGAGTCGTCTTCATCAGCCACCAGCTCCAACTACCTCAATCTCAACTGGTCATACAT |
| *KRAS* Target-mm2F | ATGTATGACCAGTTGAGATTGTCGTAGTTGGAGCTGGTGGCTGATGAAGACGACTCTGA |
| *KRAS* Target-mm2R | TCAGAGTCGTCTTCATCAGCCACCAGCTCCAACTACGACAATCTCAACTGGTCATACAT |
| *KRAS* Target-mm3F | ATGTATGACCAGTTGAGATTGTGCTAGTTGGAGCTGGTGGCTGATGAAGACGACTCTGA |
| *KRAS* Target-mm3R | TCAGAGTCGTCTTCATCAGCCACCAGCTCCAACTAGCACAATCTCAACTGGTCATACAT |
| *KRAS* Target-mm4F | ATGTATGACCAGTTGAGATTGTGGAAGTTGGAGCTGGTGGCTGATGAAGACGACTCTGA |
| *KRAS* Target-mm4R | TCAGAGTCGTCTTCATCAGCCACCAGCTCCAACTTCCACAATCTCAACTGGTCATACAT |
| *KRAS* Target-mm5F | ATGTATGACCAGTTGAGATTGTGGTTGTTGGAGCTGGTGGCTGATGAAGACGACTCTGA |
| *KRAS* Target-mm5R | TCAGAGTCGTCTTCATCAGCCACCAGCTCCAACAACCACAATCTCAACTGGTCATACAT |
| *KRAS* Target-mm6F | ATGTATGACCAGTTGAGATTGTGGTACTTGGAGCTGGTGGCTGATGAAGACGACTCTGA |
| *KRAS* Target-mm6R | TCAGAGTCGTCTTCATCAGCCACCAGCTCCAAGTACCACAATCTCAACTGGTCATACAT |
| *KRAS* Target-mm7F | ATGTATGACCAGTTGAGATTGTGGTAGATGGAGCTGGTGGCTGATGAAGACGACTCTGA |
| *KRAS* Target-mm7R | TCAGAGTCGTCTTCATCAGCCACCAGCTCCATCTACCACAATCTCAACTGGTCATACAT |
| *KRAS* Target-mm8F | ATGTATGACCAGTTGAGATTGTGGTAGTAGGAGCTGGTGGCTGATGAAGACGACTCTGA |
| *KRAS* Target-mm8R | TCAGAGTCGTCTTCATCAGCCACCAGCTCCTACTACCACAATCTCAACTGGTCATACAT |
| *KRAS* Target-mm9F | ATGTATGACCAGTTGAGATTGTGGTAGTTCGAGCTGGTGGCTGATGAAGACGACTCTGA |
| *KRAS* Target-mm9R | TCAGAGTCGTCTTCATCAGCCACCAGCTCGAACTACCACAATCTCAACTGGTCATACAT |
| *KRAS* Target-mm10F | ATGTATGACCAGTTGAGATTGTGGTAGTTGCAGCTGGTGGCTGATGAAGACGACTCTGA |
| *KRAS* Target-mm10R | TCAGAGTCGTCTTCATCAGCCACCAGCTGCAACTACCACAATCTCAACTGGTCATACAT |
| *KRAS* Target-mm11F | ATGTATGACCAGTTGAGATTGTGGTAGTTGGTGCTGGTGGCTGATGAAGACGACTCTGA |
| *KRAS* Target-mm11R | TCAGAGTCGTCTTCATCAGCCACCAGCACCAACTACCACAATCTCAACTGGTCATACAT |
| *KRAS* Target-mm12F | ATGTATGACCAGTTGAGATTGTGGTAGTTGGACCTGGTGGCTGATGAAGACGACTCTGA |
| *KRAS* Target-mm12R | TCAGAGTCGTCTTCATCAGCCACCAGGTCCAACTACCACAATCTCAACTGGTCATACAT |
| *KRAS* Target-mm13F | ATGTATGACCAGTTGAGATTGTGGTAGTTGGAGGTGGTGGCTGATGAAGACGACTCTGA |
| *KRAS* Target-mm13R | TCAGAGTCGTCTTCATCAGCCACCACCTCCAACTACCACAATCTCAACTGGTCATACAT |
| *KRAS* Target-mm14F | ATGTATGACCAGTTGAGATTGTGGTAGTTGGAGCAGGTGGCTGATGAAGACGACTCTGA |
| *KRAS* Target-mm14R | TCAGAGTCGTCTTCATCAGCCACCTGCTCCAACTACCACAATCTCAACTGGTCATACAT |
| *KRAS* Target-mm15F | ATGTATGACCAGTTGAGATTGTGGTAGTTGGAGCTCGTGGCTGATGAAGACGACTCTGA |
| *KRAS* Target-mm15R | TCAGAGTCGTCTTCATCAGCCACGAGCTCCAACTACCACAATCTCAACTGGTCATACAT |
| *KRAS* Target-mm16FA | ATGTATGACCAGTTGAGATTGTGGTAGTTGGAGCTGATGGCTGATGAAGACGACTCTGA |
| *KRAS* Target-mm16RA | TCAGAGTCGTCTTCATCAGCCATCAGCTCCAACTACCACAATCTCAACTGGTCATACAT |
| *KRAS* Target-mm16FT | ATGTATGACCAGTTGAGATTGTGGTAGTTGGAGCTGTTGGCTGATGAAGACGACTCTGA |
| *KRAS* Target-mm16RT | TCAGAGTCGTCTTCATCAGCCAACAGCTCCAACTACCACAATCTCAACTGGTCATACAT |
| *KRAS* Target-mm17F | ATGTATGACCAGTTGAGATTGTGGTAGTTGGAGCTGGAGGCTGATGAAGACGACTCTGA |

| | |
|---|---|
| *KRAS* Target-mm17R | TCAGAGTCGTCTTCATCAGCCTCCAGCTCCAACTACCACAATCTCAACTGGTCATACAT |
| *KRAS* Target-mm18F | ATGTATGACCAGTTGAGATTGTGGTAGTTGGAGCTGGTCGCTGATGAAGACGACTCTGA |
| *KRAS* Target-mm18R | TCAGAGTCGTCTTCATCAGCGACCAGCTCCAACTACCACAATCTCAACTGGTCATACAT |
| *KRAS* Target-mm19F | ATGTATGACCAGTTGAGATTGTGGTAGTTGGAGCTGGTGCCTGATGAAGA CGACTCTGA |
| *KRAS* Target-mm19R | TCAGAGTCGTCTTCATCAGGCACCAGCTCCAACTACCACAATCTCAACTGGTCATACAT |
| *KRAS* Target-mm20F | ATGTATGACCAGTTGAGATTGTGGTAGTTGGAGCTGGTGGGGTGATGAAGACGACTCTGA |
| *KRAS* Target-mm20R | TCAGAGTCGTCTTCATCACCCACCAGCTCCAACTACCACAATCTCAACTGGTCATACAT |

* PAM sites are highlighted with yellow background, sgRNA recognition sequences are underlined, and mismatch sites are highlighted in red font. Template F and template R are complementary pairs and annealed to form a double-stranded template.

209 **Table S3.**

210 **Primer sequences used in SPEAR**

| Purpose | Name | *Sequence (5′-3′) |
|---|---|---|
| Optimization of the length of target-binding sequence | *KRAS*50-SR11-TB11F | TGGTTAATGTGGAGTCAAAATGACTGA |
| | *KRAS*50-SR11-TB11R | GCGTATAAGTAGAGTCAGGCACTCTTG |
| | *KRAS*50-SR11-TB13F | TGGTTAATGTGGAGTCAAAATGACTGAAT |
| | *KRAS*50-SR11-TB13R | GCGTATAAGTAGAGTCAGGCACTCTTGCC |
| | *KRAS*50-SR11-TB15F | TGGTTAATGTGGAGTCAAAATGACTGAATAT |
| | *KRAS*50-SR11-TB15R | GCGTATAAGTAGAGTCAGGCACTCTTGCCTA |
| | *KRAS*50-SR11-TB17F | TGGTTAATGTGGAGTCAAAATGACTGAATATAA |
| | *KRAS*50-SR11-TB17R | GCGTATAAGTAGAGTCAGGCACTCTTGCCTACG |
| | *KRAS*50-SR11-TB19F | TGGTTAATGTGGAGTCAAAATGACTGAATATAAAC |
| | *KRAS*50-SR11-TB19R | GCGTATAAGTAGAGTCAGGCACTCTTGCCTACGCC |
| Optimization of the length of stabilizing region | *KRAS*50-SR7-TB17F | TAATGTGGAGTCAAAATGACTGAATATAA |
| | *KRAS*50-SR7-TB17R | ATAAGTAGAGTCAGGCACTCTTGCCTACG |
| | *KRAS*50-SR9-TB17F | GTTAATGTGGAGTCAAAATGACTGAATATAA |
| | *KRAS*50-SR9-TB17R | GTATAAGTAGAGTCAGGCACTCTTGCCTACG |
| | *KRAS*50-SR11-TB17F | TGGTTAATGTGGAGTCAAAATGACTGAATATAA |
| | *KRAS*50-SR11-TB17R | GCGTATAAGTAGAGTCAGGCACTCTTGCCTACG |
| | *KRAS*50-SR13-TB17F | TGTGGTTAATGTGGAGTCAAAATGACTGAATATAA |
| | *KRAS*50-SR13-TB17R | TCGCGTATAAGTAGAGTCAGGCACTCTTGCCTACG |
| | *KRAS*50-SR15-TB17F | AGTGTGGTTAATGTGGAGTCAAAATGACTGAATATAA |
| | *KRAS*50-SR15-TB17R | ACTCGCGTATAAGTAGAGTCAGGCACTCTTGCCTACG |
| | *KRAS*50-SR17-TB17F | ACAGTGTGGTTAATGTGGAGTCAAAATGACTGAATATAA |
| | *KRAS*50-SR17-TB17R | GTACTCGCGTATAAGTAGAGTCAGGCACTCTTGCCTACG |
| Optimization of the length of target DNA | *KRAS*38-SR11a-TB17F | TGGTTAATGTGGAGTCTATAAACTTGTGGTAGT |
| | *KRAS*38-SR11a-TB17R | GCGTATAAGTAGAGTCAGGCACTCTTGCCTACG |
| | *KRAS*42-SR11a-TB17F | TGGTTAATGTGGAGTCACTGAATATAAACTTGT |
| | *KRAS*42-SR11a-TB17R | GCGTATAAGTAGAGTCGCACTCTTGCCTACGCC |
| | *KRAS*45-SR11a-TB17F | TGGTTAATGTGGAGTCCTGAATATAAACTTGTG |
| | *KRAS*45-SR11a-TB17R | GCGTATAAGTAGAGTCCAAGGCACTCTTGCCTA |
| | *KRAS*53-SR11a-TB17F | TGGTTAATGTGGAGTCAATGACTGAATATAAAC |
| | *KRAS*53-SR11a-TB17R | GCGTATAAGTAGAGTCCGTCAAGGCACTCTTGC |
| Optimization of the stabilizing region sequence features | *KRAS*42-SR11a-TB17F | TGGTTAATGTGGAGTCACTGAATATAAACTTGT |
| | *KRAS*42-SR11a-TB17R | GCGTATAAGTAGAGTCGCACTCTTGCCTACGCC |
| | *KRAS*42-SR11b-TB17F | GTGTGCTTCTAGAGTCACTGAATATAAACTTG |
| | *KRAS*42-SR11b-TB17R | GTGTGCTTCTAGAGTCGCACTCTTGCCTACGCC |

| | | |
|---|---|---|
| | *KRAS*42-SR11c-TB17F | ATGGGTAGGTTGAGTCACTGAATATAAACTTGT |
| | *KRAS*42-SR11c-TB17R | TGTATTGTATTGAGTCGCACTCTTGCCTACGCC |
| | *KRAS*42-SR11d-TB17F | GGCCCTGTCTTGAGTCACTGAATATAAACTTGT |
| | *KRAS*42-SR11d-TB17R | GGTCCTGTTCTGAGTCGCACTCTTGCCTACGCC |
| *FLT*3 primer | *FLT*3/42-SR11a-TB17F | TGGTTAATGTGGAGTCAAGATATGTGACTTTGG |
| | *FLT*3/42-SR11a-TB17F | GCGTATAAGTAGAGTCTAGTTGGAATCACTCAT |
| *MYD*88 primer | *MYD*88/40-SR11a-TB17F | TGGTTAATGTGGAGTCGTGCCCATCAGAAGCGA |
| | *MYD*88/40-SR11a-TB17R | GCGTATAAGTAGAGTCTCTTCATTGCCTTGTAC |

* Taking *KRAS*50-SR11-TB11F as an example to introduce the naming of primers: *KRAS* refers to the target gene, 50 indicates the length of the amplification product in the SPEAR system, SR denotes Stabilizing Region, followed by 11 indicating the length of the Stabilizing Region, TB stands for Target-Binding Sequence, followed by 11 indicating the length of the Target-Binding Sequence, and F denotes the forward primer. The naming of other primers follows the same meaning as this example. Different color markings indicate the optimal primer pairs for this gene.

**Table S4.**

**Primer sequences for First generation sequencing**

| Target | Name | *Sequence (5′-3′) |
|---|---|---|
| *KRAS* gene | *KRAS*-FGS-F | ATGACTGAATATAAACTTGT |
| | *KRAS*-NGS-R | CTCTATTGTTGGATCATATT |
| *FLT*3 gene | *FLT*3-FGS-F | TCGTGTGTTCACAGAGACCT |
| | *FLT*3-FGS-R | GGCATTGCCCCTGACAACATAGT |
| *FLT*3 D835Y TaqMan probe | D835Y-TaqMan | FAM-CTCGATATATCATGAGTG-MGB |

* D835Y mutated bases were colored in red.

221 **Table S5.**

222 **Primer sequences for Next-generation sequencing**

| Name | *Sequence (5′-3′) |
|---|---|
| *KRAS*-NGS-F | ACACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNATGACTGAATATAAACTTGT |
| *KRAS*-NGS-R | ACTGGAGTTCAGACGTGTGCTCTTCCGATCTNNNNCTCTATTGTTGGATCATATT |
| *FLT*3-NGS-F | ACACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNTCGTGTGTTCACAGAGACCT |
| *FLT*3-NGS-R | ACTGGAGTTCAGACGTGTGCTCTTCCGATCTNNNNGGCATTGCCCCTGACAACATAGT |
| P5-index1-F | AATGATACGGCGACCACCGAGATCTACACTGAACCTTACACTCTTTCCCTACACGAC |
| P5-index2-F | AATGATACGGCGACCACCGAGATCTACACTGCTAAGTACACTCTTTCCCTACACGAC |
| P5-index3-F | AATGATACGGCGACCACCGAGATCTACACTAAGACACACACTCTTTCCCTACACGAC |
| P5-index4-F | AATGATACGGCGACCACCGAGATCTACACTGTTCTCTACACTCTTTCCCTACACGAC |
| P5-index5-F | AATGATACGGCGACCACCGAGATCTACACCTAATCGAACACTCTTTCCCTACACGAC |
| P5-index6-F | AATGATACGGCGACCACCGAGATCTACACCTAGAACAACACTCTTTCCCTACACGAC |
| P5-index7-F | AATGATACGGCGACCACCGAGATCTACACTAAGTTCCACACTCTTTCCCTACACGAC |
| P5-index8-F | AATGATACGGCGACCACCGAGATCTACACTAGACCTAACACTCTTTCCCTACACGAC |
| P5-index37-F | AATGATACGGCGACCACCGAGATCTACACTATAGCCTACACTCTTTCCCTACACGAC |
| P5-index38-F | AATGATACGGCGACCACCGAGATCTACACATAGAGGCACACTCTTTCCCTACACGAC |
| P5-index39-F | AATGATACGGCGACCACCGAGATCTACACCCTATCCTACACTCTTTCCCTACACGAC |
| P5-index40-F | AATGATACGGCGACCACCGAGATCTACACGGCTCTGAACACTCTTTCCCTACACGAC |
| P5-index41-F | AATGATACGGCGACCACCGAGATCTACACAGGCGAAGACACTCTTTCCCTACACGAC |
| P5-index42-F | AATGATACGGCGACCACCGAGATCTACACTAATCTTAACACTCTTTCCCTACACGAC |
| P5-index43-F | AATGATACGGCGACCACCGAGATCTACACCAGGACGTACACTCTTTCCCTACACGAC |
| P5-index44-F | AATGATACGGCGACCACCGAGATCTACACGTACTGACACACTCTTTCCCTACACGAC |
| P7-adapter25-R | CAAGCAGAAGACGGCATACGAGATCGAGTAATGTGACTGGAGTTCAGACGTGTG |
| P7-adapter26-R | CAAGCAGAAGACGGCATACGAGATTCTCCGGAGTGACTGGAGTTCAGACGTGTG |
| P7-adapter27-R | CAAGCAGAAGACGGCATACGAGATAATGAGCGGTGACTGGAGTTCAGACGTGTG |
| P7-adapter28-R | CAAGCAGAAGACGGCATACGAGATGGAATCTCGTGACTGGAGTTCAGACGTGTG |
| P7-adapter29-R | CAAGCAGAAGACGGCATACGAGATTTCTGAATGTGACTGGAGTTCAGACGTGTG |
| P7-adapter30-R | CAAGCAGAAGACGGCATACGAGATACGAATTCGTGACTGGAGTTCAGACGTGTG |
| P7-adapter31-R | CAAGCAGAAGACGGCATACGAGATAGCTTCAGGTGACTGGAGTTCAGACGTGTG |
| P7-adapter32-R | CAAGCAGAAGACGGCATACGAGATGCGCATTAGTGACTGGAGTTCAGACGTGTG |
| P7-adapter33-R | CAAGCAGAAGACGGCATACGAGATCATAGCCGGTGACTGGAGTTCAGACGTGTG |
| P7-adapter34-R | CAAGCAGAAGACGGCATACGAGATTTCGCGGAGTGACTGGAGTTCAGACGTGTG |
| P7-adapter35-R | CAAGCAGAAGACGGCATACGAGATGCGCGAGAGTGACTGGAGTTCAGACGTGTG |
| P7-adapter36-R | CAAGCAGAAGACGGCATACGAGATCTATCGCTGTGACTGGAGTTCAGACGTGTG |
| P7-adapter37-R | CAAGCAGAAGACGGCATACGAGATGTCGTGATGTGACTGGAGTTCAGACGTGTG |
| P7-adapter38-R | CAAGCAGAAGACGGCATACGAGATACCACTGTGTGACTGGAGTTCAGACGTGTG |
| P7-adapter39-R | CAAGCAGAAGACGGCATACGAGATTGGATCTGGTGACTGGAGTTCAGACGTGTG |
| P7-adapter40-R | CAAGCAGAAGACGGCATACGAGATCCGTTTGTGTGACTGGAGTTCAGACGTGTG |
| P7-adapter41-R | CAAGCAGAAGACGGCATACGAGATTGCTGGGTGTGACTGGAGTTCAGACGTGTG |

| | |
|---|---|
| P7-adapter42-R | CAAGCAGAAGACGGCATACGAGATGAGGGGTTGTGACTGGAGTTCAGACGTGTG |
| P7-adapter43-R | CAAGCAGAAGACGGCATACGAGATAGGTTGGGGTGACTGGAGTTCAGACGTGTG |
| P7-adapter44-R | CAAGCAGAAGACGGCATACGAGATGTGTGGTGGTGACTGGAGTTCAGACGTGTG |
| P7-adapter45-R | CAAGCAGAAGACGGCATACGAGATTGGGTTTCGTGACTGGAGTTCAGACGTGTG |
| P7-adapter46-R | CAAGCAGAAGACGGCATACGAGATTGGTCACAGTGACTGGAGTTCAGACGTGTG |
| P7-adapter47-R | CAAGCAGAAGACGGCATACGAGATTTGACCCTGTGACTGGAGTTCAGACGTGTG |
| P7-adapter48-R | CAAGCAGAAGACGGCATACGAGATCCACTCCTGTGACTGGAGTTCAGACGTGTG |

* *KRAS*-NGS-F/R and *FLT*3-NGS-F/R are the primers for the first PCR of NGS. P5 and P7 are different barcoded primers.

**Table S6.**

**Template and primer sequence of *KRAS* G12D/V/R multiple SPEAR assay**

| purpose | Name | *Sequence (5′ -3′ ) |
|---|---|---|
| The location of *KRAS* G12 mutations and inner control sequence on the *KRAS* gene. | *KRAS*-WT gene | ACTGAATATAAACTTGTGGTAGTTGGAGCTGGTGGCGTAGGC AAGAGTGCCTTGACGATACAGCTAATTCAGAATCATTTTGTG |
| | *KRAS*-G12D gene | ACTGAATATAAACTTGTGGTAGTTGGAGCTGATGGCGTAGGC AAGAGTGCCTTGACGATACAGCTAATTCAGAATCATTTTGTG |
| | *KRAS*-G12V gene | ACTGAATATAAACTTGTGGTAGTTGGAGCTGTTGGCGTAGGC AAGAGTGCCTTGACGATACAGCTAATTCAGAATCATTTTGTG |
| | *KRAS*-G12R gene | ACTGAATATAAACTTGTGGTAGTTGGAGCTCGTGGCGTAGGCA AGAGTGCCTTGACGATACAGCTAATTCAGAATCATTTTGTG |
| Sequence information for in *vitro* sgRNA transcription | sgRNA-T7-universal | TAATACGACTCACTATAGGG |
| | AapCas12b-sgRNA | TAATACGACTCACTATAGGGGTCTAGAGGACAGAATTTTT CAACGGGTGTGCCAATGGCCACTTTCCAGGTGGCAAAGCC CGTTGAGCTTCTCAAATCTGAGAAGTGGCACGTAGCAGCA AGATTAGCAGAAGCT |
| | *KRAS*-12b-WT-17gR | ACCAGCTCCAACTACCA-GTGCCACTTCTCAGATTTGA |
| | *KRAS*-12b-G12V-17gR | AACAGCTCCAACTACCA-GTGCCACTTCTCAGATTTGA |
| | *KRAS*-12b-G12D-17gR | ATCAGCTCCAACTACCA-GTGCCACTTCTCAGATTTGA |
| | *KRAS*-12b-G12R-17gR | ACGAGCTCCAACTACCA-GTGCCACTTCTCAGATTTGA |
| | *KRAS*-12b-IR-17gR | TGAATTAGCTGTATCGT-GTGCCACTTCTCAGATTTGA |
| SPEAR Primer | *KRAS*-mixF | TGGTTAATGTGGAGTCACTGAATATAAACTTGT |
| | *KRAS*-mixR | GCGTATAAGTAGAGTCCACAAAATGATTCTGAA |

* The recognition sequence of the *KRAS* G12 sgRNAs are highlighted in yellow, the PAM site in cyan, and the mutation sites are highlighted in red. The recognition sequence of inner control sgRNA is underlined, the PAM site is indicated by wavy line.

## Supplementary Note

### Note S1. Sequence information of plasmids

The sgRNA recognition sequence is highlighted in yellow, the PAM site in cyan, the target binding sequences of the F and R primers for SPEAR are underlined below, and the mutation site is marked in red.

**> *KRAS*-WT gene (499-nt)**

TCCTAGGCGGCGGCCGCGGCGGCGGAGGCAGCAGCGGCGGCGGCAGTGGCGGCGGCGAAG
GTGGCGGCGGCTCGGCCAGTACTCCCGGCCCCCGCCATTTCGGACTGGGAGCGAGCGCGGCG
CAGGCACTGAAGGCGGCGGCGGGGCCAGAGGCTCAGCGGCTCCCAGGTGCGGGAGAGAGG
CCTGCTGAAAATGACTGAATATAAACTTGTGGTAGTTGGAGCTGGTGGCGTAGGCAAGAGTG
CCTTGACGATACAGCTAATTCAGAATCATTTTGTGGACGAATATGATCCAACAATAGAGGATTC
CTACAGGAAGCAAGTAGTAATTGATGGAGAAACCTGTCTCTTGGATATTCTCGACACAGCAGG
TCAAGAGGAGTACAGTGCAATGAGGGACCAGTACATGAGGACTGGGGAGGGCTTTCTTTGTG
TATTTGCCATAAATAATACTAAATCATTTGAAGATATTCACCATTATAGAGAACAAATTAAAAGA
GT

**> *KRAS*-G12D gene (499-nt)**

TCCTAGGCGGCGGCCGCGGCGGCGGAGGCAGCAGCGGCGGCGGCAGTGGCGGCGGCGAAG
GTGGCGGCGGCTCGGCCAGTACTCCCGGCCCCCGCCATTTCGGACTGGGAGCGAGCGCGGCG
CAGGCACTGAAGGCGGCGGCGGGGCCAGAGGCTCAGCGGCTCCCAGGTGCGGGAGAGAGG
CCTGCTGAAAATGACTGAATATAAACTTGTGGTAGTTGGAGCTGATGGCGTAGGCAAGAGTGC
CTTGACGATACAGCTAATTCAGAATCATTTTGTGGACGAATATGATCCAACAATAGAGGATTCC
TACAGGAAGCAAGTAGTAATTGATGGAGAAACCTGTCTCTTGGATATTCTCGACACAGCAGGT
CAAGAGGAGTACAGTGCAATGAGGGACCAGTACATGAGGACTGGGGAGGGCTTTCTTTGTGT
ATTTGCCATAAATAATACTAAATCATTTGAAGATATTCACCATTATAGAGAACAAATTAAAAGAG
T

**> *KRAS*-G12V gene (499-nt)**

TCCTAGGCGGCGGCCGCGGCGGCGGAGGCAGCAGCGGCGGCGGCAGTGGCGGCGGCGAAG
GTGGCGGCGGCTCGGCCAGTACTCCCGGCCCCCGCCATTTCGGACTGGGAGCGAGCGCGGCG
CAGGCACTGAAGGCGGCGGCGGGGCCAGAGGCTCAGCGGCTCCCAGGTGCGGGAGAGAGG
CCTGCTGAAAATGACTGAATATAAACTTGTGGTAGTTGGAGCTGTTGGCGTAGGCAAGAGTGC

CTTGACGATACAGCTAATTCAGAATCATTTTGTGGACGAATATGATCCAACAATAGAGGATTCC
TACAGGAAGCAAGTAGTAATTGATGGAGAAACCTGTCTCTTGGATATTCTCGACACAGCAGGT
CAAGAGGAGTACAGTGCAATGAGGGACCAGTACATGAGGACTGGGGAGGGCTTTCTTTGTGT
ATTTGCCATAAATAATACTAAATCATTTGAAGATATTCACCATTATAGAGAACAAATTAAAAGAG
T

**> *KRAS*-G12R gene (499-nt)**

TCCTAGGCGGCGGCCGCGGCGGCGGAGGCAGCAGCGGCGGCGGCAGTGGCGGCGGCGAAG
GTGGCGGCGGCTCGGCCAGTACTCCCGGCCCCCGCCATTTCGGACTGGGAGCGAGCGCGGCG
CAGGCACTGAAGGCGGCGGCGGGGCCAGAGGCTCAGCGGCTCCCAGGTGCGGGAGAGAGG
CCTGCTGAAAATGACTGAATATAAACTTGTGGTAGTTGGAGCTCGTGGCGTAGGCAAGAGTGC
CTTGACGATACAGCTAATTCAGAATCATTTTGTGGACGAATATGATCCAACAATAGAGGATTCC
TACAGGAAGCAAGTAGTAATTGATGGAGAAACCTGTCTCTTGGATATTCTCGACACAGCAGGT
CAAGAGGAGTACAGTGCAATGAGGGACCAGTACATGAGGACTGGGGAGGGCTTTCTTTGTGT
ATTTGCCATAAATAATACTAAATCATTTGAAGATATTCACCATTATAGAGAACAAATTAAAAGAG
T

**> *FLT*3-WT gene (499-nt)**

TTTCACTCTGAAGATGAAATTGAATATGAAAACCAAAAAAGGCTGGAAGAAGAGGAGGACTT
GAATGTGCTTACATTTGAAGATCTTCTTTGCTTTGCATATCAAGTTGCCAAAGGAATGGAATTT
CTGGAATTTAAGTCGTGTGTTCACAGAGACCTGGCCGCCAGGAACGTGCTTGTCACCCACGG
GAAAGTGGTGAAGATATGTGACTTTGGATTGGCTCGAGATATCATGAGTGATTCCAACTATGTT
GTCAGGGGCAATGCCCGTCTGCCTGTAAAATGGATGGCCCCCGAAAGCCTGTTTGAAGGCAT
CTACACCATTAAGAGTGATGTCTGGTCATATGGAATATTACTGTGGGAAATCTTCTCACTTGGT
GTGAATCCTTACCCTGGCATTCCGGTTGATGCTAACTTCTACAAACTGATTCAAAATGGATTTA
AAATGGATCAGCCATTTTATGCTACAGAAGAAATATACATTATAATGCAATCCTGCT

**> *FLT*3-D835Y gene (499-nt)**

TTTCACTCTGAAGATGAAATTGAATATGAAAACCAAAAAAGGCTGGAAGAAGAGGAGGACTT
GAATGTGCTTACATTTGAAGATCTTCTTTGCTTTGCATATCAAGTTGCCAAAGGAATGGAATTT
CTGGAATTTAAGTCGTGTGTTCACAGAGACCTGGCCGCCAGGAACGTGCTTGTCACCCACGG
GAAAGTGGTGAAGATATGTGACTTTGGATTGGCTCGATATATCATGAGTGATTCCAACTATGTT
GTCAGGGGCAATGCCCGTCTGCCTGTAAAATGGATGGCCCCCGAAAGCCTGTTTGAAGGCAT
CTACACCATTAAGAGTGATGTCTGGTCATATGGAATATTACTGTGGGAAATCTTCTCACTTGGT

GTGAATCCTTACCCTGGCATTCCGGTTGATGCTAACTTCTACAAACTGATTCAAAATGGATTTA
AAATGGATCAGCCATTTTATGCTACAGAAGAAATATACATTATAATGCAATCCTGCT

**> *FLT*3-D835H gene (499-nt)**

TTTCACTCTGAAGATGAAATTGAATATGAAAACCAAAAAAGGCTGGAAGAAGAGGAGGACTT
GAATGTGCTTACATTTGAAGATCTTCTTTGCTTTGCATATCAAGTTGCCAAAGGAATGGAATTT
CTGGAATTTAAGTCGTGTGTTCACAGAGACCTGGCCGCCAGGAACGTGCTTGTCACCCACGG
GAAAGTGGTGAAGATATGTGACTTTGGATTGGCTCGACATATCATGAGTGATTCCAACTATGTT
GTCAGGGGCAATGCCCGTCTGCCTGTAAAATGGATGGCCCCCGAAAGCCTGTTTGAAGGCAT
CTACACCATTAAGAGTGATGTCTGGTCATATGGAATATTACTGTGGGAAATCTTCTCACTTGGT
GTGAATCCTTACCCTGGCATTCCGGTTGATGCTAACTTCTACAAACTGATTCAAAATGGATTTA
AAATGGATCAGCCATTTTATGCTACAGAAGAAATATACATTATAATGCAATCCTGCT

**> *FLT*3-D835V gene (499-nt)**

TTTCACTCTGAAGATGAAATTGAATATGAAAACCAAAAAAGGCTGGAAGAAGAGGAGGACTT
GAATGTGCTTACATTTGAAGATCTTCTTTGCTTTGCATATCAAGTTGCCAAAGGAATGGAATTT
CTGGAATTTAAGTCGTGTGTTCACAGAGACCTGGCCGCCAGGAACGTGCTTGTCACCCACGG
GAAAGTGGTGAAGATATGTGACTTTGGATTGGCTCGAGTTATCATGAGTGATTCCAACTATGTT
GTCAGGGGCAATGCCCGTCTGCCTGTAAAATGGATGGCCCCCGAAAGCCTGTTTGAAGGCAT
CTACACCATTAAGAGTGATGTCTGGTCATATGGAATATTACTGTGGGAAATCTTCTCACTTGGT
GTGAATCCTTACCCTGGCATTCCGGTTGATGCTAACTTCTACAAACTGATTCAAAATGGATTTA
AAATGGATCAGCCATTTTATGCTACAGAAGAAATATACATTATAATGCAATCCTGCT

**> *FLT*3-D835F gene (499-nt)**

TTTCACTCTGAAGATGAAATTGAATATGAAAACCAAAAAAGGCTGGAAGAAGAGGAGGACTT
GAATGTGCTTACATTTGAAGATCTTCTTTGCTTTGCATATCAAGTTGCCAAAGGAATGGAATTT
CTGGAATTTAAGTCGTGTGTTCACAGAGACCTGGCCGCCAGGAACGTGCTTGTCACCCACGG
GAAAGTGGTGAAGATATGTGACTTTGGATTGGCTCGATTTATCATGAGTGATTCCAACTATGTT
GTCAGGGGCAATGCCCGTCTGCCTGTAAAATGGATGGCCCCCGAAAGCCTGTTTGAAGGCAT
CTACACCATTAAGAGTGATGTCTGGTCATATGGAATATTACTGTGGGAAATCTTCTCACTTGGT
GTGAATCCTTACCCTGGCATTCCGGTTGATGCTAACTTCTACAAACTGATTCAAAATGGATTTA
AAATGGATCAGCCATTTTATGCTACAGAAGAAATATACATTATAATGCAATCCTGCT

**> *MYD*88-WT gene (499-nt)**

TGAGGGAATGTGTAGGTGGGGCCTCTGGATTGTCAGCCTTCCCTCCCCAAGGACTGTGGATGC
AGTACCAAAGAACTGCTGAAGATCTCTGCACACCTGAGCATGTGTGCATGTGTGTGCCTTTTT
GTGTGAGTGAATGTGTGCCAGGGGTACTTAGATGGGGGATGGCTGTTGTTAACCCTGGGGTTG
AAGACTGGGCTTGTCCCACCATGGGGCAAGGGCCTGATGCCAGCATGGCACCCCTTGGCTTG
CAGGTGCCCATCAGAAGCGACTGATCCCCATCAAGTACAAGGCAATGAAGAAAGAGTTCCCC
AGCATCCTGAGGTTCATCACTGTCTGCGACTACACCAACCCCTGCACCAAATCTTGGTTCTGG
ACTCGCCTTGCCAAGGCCTTGTCCCTGCCCTGAAGACTGTTCTGAGGCCCTGGGTGTGTGTGT
ATCTGTCTGCCTGTCCATGTACTTCTGCCCTGCCTCCTCCTTTCGTTGTAGGAGGAATCT

**> *MYD*88-L265P gene (499-nt)**

TGAGGGAATGTGTAGGTGGGGCCTCTGGATTGTCAGCCTTCCCTCCCCAAGGACTGTGGATGC
AGTACCAAAGAACTGCTGAAGATCTCTGCACACCTGAGCATGTGTGCATGTGTGTGCCTTTTT
GTGTGAGTGAATGTGTGCCAGGGGTACTTAGATGGGGGATGGCTGTTGTTAACCCTGGGGTTG
AAGACTGGGCTTGTCCCACCATGGGGCAAGGGCCTGATGCCAGCATGGCACCCCTTGGCTTG
CAGGTGCCCATCAGAAGCGACCCGATCCCCATCAAGTACAAGGCAATGAAGAAAGAGTTCCCC
AGCATCCTGAGGTTCATCACTGTCTGCGACTACACCAACCCCTGCACCAAATCTTGGTTCTGG
ACTCGCCTTGCCAAGGCCTTGTCCCTGCCCTGAAGACTGTTCTGAGGCCCTGGGTGTGTGTGT
ATCTGTCTGCCTGTCCATGTACTTCTGCCCTGCCTCCTCCTTTCGTTGTAGGAGGAATCT