Supplementary methods

Genomic DNA and clinical tissue and circulating-DNA samples
The DNAeasy™ Blood & Tissue Kit (Qiagen) was used to isolate genomic DNA from these tumor samples per manufacturer’s protocol. The extracted DNA was quantified with a Qubit 3.0 fluorometer and dsDNA HS assay (Thermo Fisher Scientific) and stored at -20°C. For LM-PCR (ligation-mediated PCR) product, 30ng cfDNA from patient or healthy donor (WT) cfDNA were subjected to end-repair, dA-tailing and adaptor ligation respectively, followed by 10 cycle PCR amplification using adaptor primers per manufacturer instruction using the NEBNext™ Ultra II DNA Library Prep Kit (New England Biolabs). After purification with QIAquick™ PCR Purification Kit (Qiagen), a 10-fold dilution of patient LM-PCR product into WT LM-PCR product was generated and used for pre-PCR UVME or UVME-PCR.

Setup for uniform UV irradiation of samples during open-lid PCR
Open-lid PCR was applied on an Eppendorf Mastercycler™ Nexus GX2 Thermal cycler. The lid of this cycler can be opened anytime during PCR without interrupting the PCR program. A UV lamp assembly with an average wavelength ~365 nm wavelength (realUV™ LED Flood Light, Waveform Lighting Inc) was placed in a pre-defined, reproducible position in contact with a 64 well plate (USA Scientific) to provide consistent UV irradiation of PCR wells between experiments. During irradiation, the UV lamp was covered to prevent UV exposure to operators. In this geometry the average distance between the actual light source and PCR samples was ~ 4cm, providing an approximate irradiance of 36.5 mW/cm² to the samples as estimated from the manufacturer-provided specification.

To define the fraction of the 64-well plate receiving uniform UV irradiation among wells, control experiments were performed using cross-linking of DNA with the UV-sensitive intercalator psoralen, Supplementary Figure 2 and Supplementary methods.

To define the fraction of the 64-well plate receiving uniform UV irradiation among wells, control experiments were performed using cross-linking of DNA with the intercalator psoralen that cross-links DNA strands upon irradiation with 365 nm UV light. Five-ul of 50% KRAS-G12V ssDNA was applied in a 25 ul crosslinking reaction containing 2 mM MgCl2, 1X GoTaq Buffer, and 2 ng psoralen (Sigma). Sixty-four tubes containing 25 ul master mix that mentioned above were put
into the 64 blocks of Eppendorf Mastercycler™ Nexus GX2 Thermal cycler. The DNA was first denaturation at 95°C for 2 min, then re-hybridized at 61.2°C for 2 min. UV was applied for 2 min during the re-hybridization at 61.3°C. One-μl of cross-linked DNA was transfer to a 50 μl LCgreen PCR (1X GoTaq buffer, 2 mM MgCl₂, 200 mM KRAS F2 and R2 primers, 0.8 mM dNTP, 0.8X LC green and 0.2 μl GoTaq polymerase) and a 12.5 μl Taqman assay (see supplementary methods).

Upon cross-linking, DNA is rendered un-amplifiable, thereby the delay in real-time PCR amplification following UV irradiation is a measure of DNA crosslinking and UV exposure. Real time PCR was used to quantify the amount of DNA cross-linking and wells that demonstrated less than 1 threshold cycle difference of each other during a subsequent real time PCR reaction were considered as receiving uniform UV irradiation (Supplementary Figure 1B). In the absence of UV irradiation, real-time PCR thresholds were approximately ~5 cycles earlier than those receiving UV irradiation. Using this approach, it was determined that under the geometry applied, 12 wells (rows D-G, columns 1-3) were receiving uniform UV irradiation and were used for subsequent UVME-PCR experiments (Supplementary Figure 1C).

### Primers and CNVK probes used for pre-PCR-UVME and UVME-PCR

Primers and CNVK-modified oligonucleotide probes were purchased from Integrated DNA Technologies and GeneLink™, respectively, and their sequences are listed on Table 1. The CNVK probes were designed against WT sequence with CNVK modification replacing one of the DNA bases and such that a T at position -1 is available in the opposite strand for crosslinking when the probe is hybridized to the targeted DNA. The IDT oligo tools publicly available software was used to predict the Tm and calculate the maximum mismatch temperature for WT DNA vs mutated target DNA, depending on oligonucleotide length where CNVK is placed. The Tm of probes designed were in the range 55 - 63°C when calculated on the IDT oligo-analyzer with 50 mM Na⁺, 2 mM Mg²⁺ and 0.25 μM probe concentration and by accounting for a single base mismatch at the CNVK position. When LCGREEN dye and PCR mastermix is included in the buffer an increase of about 5°C was assumed for the probe Tm. The incubation temperatures used for pre-PCR UVME and for UVME-PCR were approximately 5–10 °C below their calculated Tm. While all these temperatures result to mutation enrichment, fine-tuning to the optimal temperature can be done by testing a gradient of temperatures in that region and assessing the mutation enrichment obtained via ddPCR (not shown).

### Pre-PCR UV-mediated Cross-linking Minor-allele Enrichment (pre-PCR UVME)
Intact genomic DNA from A549, SW480, H2009, LOVO and HMC was fragmented with dsDNA Shearase Plus (Zymo Research) per manufacturer’s instructions and quantified. 0.1% and 1% Kras-mutation-bearing sample was generated by mixing mutated fragmented DNA into fragmented HMC. For MIER3 mutation enrichment from breast cancer patient #295, a similar reaction was set up with 30ng cfDNA and the same UV irradiation protocol was applied with the difference that incubation and UV irradiation was performed at 50 °C instead of 52 °C. After UV irradiation, half (5 µl) of the UV-treated sample or NO-UV control were added into a PCR reaction for a final volume of 25 ul containing 0.8X LC green (BioFire Diagnostics), 1X AmpliTaqGold™ buffer, 0.8 mM dNTP, 0.2 mM forward and reverse primers, 2 mM MgCl2, 2 µl GC enhancer and 0.125 µl AmpiTaq™ polymerase (Thermo Fisher Scientific). PCR amplification was performed with an initial denaturation at 95°C for 2min, followed by 45 cycles of 30 sec denaturation at 95 °C, 30 sec annealing at 57°C and 10s elongation at 72°C.

Comparison of enrichment obtained via pre-PCR UVME and UVME-PCR, and sequential enrichment using pre-PCR UVME plus UVME-PCR.

30 ng fragmented DNA containing 0.1% or 1% KRAS G12V mutation from SW480 were used for these experiments. To combine pre-PCR and UVME PCR, DNA samples first went through pre-PCR UVME treatment with or without UV irradiation for 5 cycles in 10 µl pre-PCR UVME reaction with 100 nM Kras CNVK target-specific probes and CNVK common probes. The whole 10 µl reactions were then used as input and added into 15 µl UVME-PCR reaction to reach a 25 µl UVME PCR reaction with same final composition as above. These experiments were reproduced two independent times.

Direct Sanger Sequencing

The UVME-PCR products were submitted to Genewiz, Inc for Sanger sequencing using target specific sequencing primers (Supplementary Table 1). BioEdit Sequence Alignment Editor (Bioedit Ltd) was applied to illustrate and capture the signaling peaks.

Taqman real time genotyping assays

UVME-PCR and UVME-TT-fast-COLD-PCR reactions were followed by regular Taqman genotyping (1) for detection and quantification of mutations. Sequences of primers and probes used for Taqman assays are listed on Supplementary Table 2. The UVME-PCR or UVME-TT-fast-COLD-PCR products were diluted (1:20,000) in water. One-µl diluted DNA was applied into 12.5 ul volume Taqman genotyping reaction containing 1X TaqMan genotyping master mix, 260
nM probes and 900 nM forward and reverse primers. The Taqman master mix were contained in 96-well plate (Bio-rad, HSP9665) and reaction was conducted on a CFX ConnectTM real-time PCR (Bio-Rad) with 10 min initial activation at 95°C following by 50 cycles of 30 sec denaturation at 95°C and 1 min annealing/extension at 60°C. Amplification curve was analyzed on CFX Maestro Software (Bio-Rad) and the Cq value for FAM (blue curve for WT detection) and HEX (red curve for mutant detection) was determined where the amplification curve was met at the threshold at 800 Relative fluorescence unit (RFUs). No FAM and HEX signal was detected for NTC at 50 cycles. The mutation allelic frequency (mutation abundance) was calculated by correlating a standard curve created from serial dilutions of mutated genomic DNA, as we reported previously (1). All standard curves were shown on Supplementary Figure 3.

Droplet digital PCR (ddPCR)

Droplet digital PCR was used for detection and quantification of mutations after PCR in UV irradiated samples and no-UV control samples. Sequence of primers and probes for p53 and MIER3 mutations are listed on Supplementary Table 3. Droplet amplifications were performed in a 20 µl volume containing 1X ddPCR supermix for probes (Bio-rad), 250 µM probes (for PFSK and MIER3), 900 nM forward and reverse primers (for PFSK and MIER3), and 1µl of diluted target PCR post pre-PCR UVME (1:10⁷ in water) or UVME-PCR product (1:10⁶ dilution in water). For KRAS mutations, 1µl of diluted target PCR post pre-PCR UVME (1:10⁷ in water), UVME-PCR product (1:10⁸ dilution in water), pre-PCR UVME in combine with UVME-PCR (1: 20000 in water) or UVME-TT-fast-COLD-PCR product (1:20000 dilution in water) was employed in a 20 µl volume reaction containing 1 µl of 20X ddPCR gene expression assays (Bio-rad) and 1X ddPCR supermix for probes (Bio-rad). The master mix was then applied on DG8TM droplet generator cartridges (Bio-rad) containing 70 µl of droplet generation oil (Bio-rad) for droplet generation. The Droplets were then transferred to a 96-well plate and sealed with PX1 PCR plate sealer (Bio-rad) for 5 sec at 180°C. The thermal cycling was performed on an Eppendorf Mastercycler (Eppendorf) with an initial denaturation step at 95°C for 10 min following by 40 cycles of 30 sec denaturation at 94°C, 60 sec annealing at 55°C (p53 or KRAS) or 58°C(MIER3) and a final step at 98°C for 10 min. The plate was then transferred to QX200 droplet reader (Bio-rad) for droplets reading. Quantasoft (Bio-rad) was applied to analyze the positive droplets from FAM and HEX channels. Poisson distribution model with 95% confidence level was applied to calculate the percentage of fractional abundance.

High resolution melting (HRM) analysis
Eight-µl of UVME-PCR products were transferred to a 96 well plate (Bio-Rad, HSP9665). Twenty-five-µl of oil were then overlaid to prevent evaporation. The 96 well plate was sealed with PX1 PCR plate sealer (Bio-rad) for 3 sec at 180°C and briefly centrifuged. The plate was run on LightScanner R system (IdahoTechnology) and the Lightscanner software was applied for analysis.

1. Li, J., Wang, L., Janne, P.A. and Makrigiorgos, G.M. (2009) Coamplification at lower denaturation temperature-PCR increases mutation-detection selectivity of TaqMan-based real-time PCR. Clin Chem, 55, 748-756.
Supplementary Figure 1. Illustration of open lid PCR with UV lamp

UVME-PCR was performed on an Eppendorf™ Mastercycler™ Nexus GX2 Thermal cycler that enables opening of the machine lid without interrupting the PCR program. Once the lid was opened, open-lid PCR was performed, as described. A) for the PCR stage involving UV irradiation the PCR lid was opened and a UV lamp (average wavelength ~365nm) was placed in a standard position on-top of the PCR tubes.
Supplementary Figure 2. Verification of UV irradiation uniformity on the 64 blocks of Eppendorf Mastercycler™ Nexus GX2 Thermal cycler via LCgreen and Taqman assay

Sixty-four tubes containing KRAS PCR products plus 2 ng psoralen were placed in an Eppendorf Mastercycler™ Nexus GX2 Thermal cycler. DNA was denatured at 95°C followed by re-hybridization at 61.2°C. UV (365nm) was then applied for 2 min for psoralen crosslinking to DNA. 1 ul cross-linked DNA was then used for (A) LCgreen PCR and (B) Taqman assay. The PCR threshold difference, delta-Cq, value from the Cq of sample E1 was recorded. Wells that revealed approximately equal or less than 1 cycle delta-Cq highlighted with yellow. C). In conclusion, rows D-G and columns 1-3 were used for UV irradiation experiments.
Supplementary Figure 3. Standard real time PCR (Taqman) curves demonstrating the correlation between mutation abundance and △Cq of HEX and FAM on Taqman assay. Serial dilution of PFSK-p53-C275G, A549-KRAS-G12S, SW480-KRAS-G12V, H2009-KRAS-G12A and LOVO-KRAS-G13D into HMC DNA were used to generate gDNA with KRAS mutation allelic frequencies of 0.1%-100%. The Cq difference between HEX and FAM were plotted on Y axis and the mutation abundance (Log$_{10}$) were plotted on X axis. Equation for mutation calculation are shown on each.
Supplementary Figure 4. Application of pre-PCR UVME directly on gDNA, followed by regular PCR and ddPCR to enrich KRAS mutations (G12S, G12V, G12A and G13D). Fragmented gDNA containing 100% WT or KRAS mutations at 0.1% or 1% mutational allelic frequency were subjected to UVME using a single pair of KRAS target specific CNVK-modified probes and KRAS common CNVK-modified probes. Following selective WT-DNA crosslinking and regular PCR, ddPCR was used to evaluate mutation abundance. 25-80-fold enrichment was evident for all mutations tested. Experiments were repeated two independent times.
Supplementary Figure 5. UVME-PCR followed by Taqman genotyping assays for four KRAS mutations reveal significant shifts for 1% mutation frequency. UVME-PCR was applied on 100% WT and 1% KRAS mutation-containing DNA, followed by Taqman genotyping assays. FAM (Blue curve) indicated the detection from WT while HEX (red curve) indicated the detection from mutant. The growth curves and PCR thresholds are consistent with mutation enrichment in the presence of UV irradiation.
Supplementary Figure 6. UVME-PCR followed by Taqman genotyping assays: comparison of one versus two CNVK probes per reaction. UVME-PCR was applied on 100% WT and 1% mutation-containing DNA in the presence of one CNVK probe directed to the sense strand, vs. two CNVK probes directed to both sense and anti-sense strands. Both approaches lead to mutation enrichment as indicated by the Taqman-derived mutation abundance in presence or absence of UV irradiation. However, including both CNVK-modified probes shows higher enrichment than using a single probe directed to the sense strand, for all four KRAS mutations.
Supplementary Figure 7. UVME-PCR followed by direct Sanger Sequencing shows detectable mutant signal down to 0.3% for four KRAS mutations.

UVME-PCR was applied on genomic DNA containing dilutions (MAF 5%-0.01%) of KRAS mutated genomic DNA followed by direct Sanger sequencing, without secondary amplification. The mutations were detectable down to 0.1%-0.3% when UV was applied. No mutation is evident in the absence of UV irradiation or for WT DNA.
Supplementary Figure 8. UVME-PCR followed by direct HRM can detect mutations down to 0.1% for all four KRAS mutations.

UVME-PCR was applied on genomic DNA containing dilutions (MAF 1%-0.01%) of KRAS mutated genomic DNA followed by direct HRM, without secondary amplification. All four KRAS genomic DNA show detectable mutations down to 0.1% mutation input when UV was applied. No mutation is evident in the absence of UV irradiation or for WT DNA.
Supplementary Figure 9. UVME-PCR followed by Taqman real time PCR genotyping shows detectable MAF ≤0.1% for four KRAS mutations.

UVME-PCR was applied on genomic DNA containing dilutions (MAF 5%-0.01%) of KRAS mutated genomic DNA followed by direct Taqman genotyping assays reveal significant enrichment and mutation identification down to 0.03% MAF for G12S/G12V/G12A and 0.1% for G13D respectively.
Supplementary Figure 10. Plot of mutation abundance following UVME-PCR against the original mutation abundance formed by serial dilution of mutated and WT genomic DNA. The data indicate that mutation abundance of genomic DNA after UVEM-PCR correlates with mutation abundance in the starting genomic DNA for all four KRAS mutations.
Supplementary Figure 11. Applying UVME-TT-fast-COLD-PCR boosts the mutation enrichment for KRAS G12S, G12V and G13D

UVME-TT-fast-COLD-PCR was applied to boost further the mutation enrichment obtained by UVME-PCR. A) Taqman genotyping assays and B) ddPCR both show higher enrichment for 0.01% G12S, G12V and G13D via UVME-TT-fast-COLD-PCR as compared to UVME-PCR. The significance (*) was calculated by comparing UV to NO UV samples.
Supplementary Figure 12. Application of pre-PCR UVME or UVME-PCR to MIER3 mutation using plasma-circulating-DNA (cfDNA).

Pre-PCR UVME or UVME-PCR was performed on cfDNA extracted from the plasma of a breast cancer patient, targeting a previously identified MIER3 mutation (p.H548D). cfDNA from a cancer-free donor was also included as a control. (A) Workflow for selective crosslinking of WT-DNA, using unamplified cfDNA or cfDNA LM-PCR product. Either pre-PCR UVME or UVME-PCR were used, followed by amplification and mutation detection via digital PCR (ddPCR). LM-PCR product was created by subjecting cfDNA to end repair, dA-tailing, adaptor ligation and 10 cycles PCR amplification, followed by PCR product purification. (B) Pre-PCR UVME against MIER3 directly on unamplified cfDNA (C) Pre-PCR UVME against MIER3 on LM-PCR product and (D) UVME-PCR against MIER3 on LM-PCR product. A 30-50-fold mutation enrichment of MIER3 mutation was evident when UV irradiation was applied. Experiments were repeated two independent times.
## Supplementary Table 1. PCR target information

| Primer ID | Primer location | Gene ID | Amplicon size | Amplicon sequences |
|-----------|-----------------|---------|---------------|--------------------|
| **TP53 F1** | Exon 8          | 7157    | 155 bp        | TTACCTCGCTTAGCTCCCTGGGGGCAGCTCGTGTTGAGGCTC CCCTTTCTTGCGGAGATTCTTCTTCTCGTGCGGCCGTCTCTCCC AGGACAGGCCAACACACGACCTCCTAAAACGTGTCTCCCGGCTCCTCAGTTAGG |
| **TP53 R1** | Exon 8          |         |               |                    |
| **KRAS F1** | Exon 2          | 3845    | 114 bp        | CAAAATGATTCTGAAATTAGCTGTATCGTGTAAGGCCACTCTTCCCTAA |
| **KRAS R1** | Exon 2          |         |               |                    |
| **MIER3 F1** | Exon 12        | 166968  | 123 bp        | GACCAATGGTTTCATCAGTGCCCATGCTCTGACATAGCACGCACGCC |
| **MIER3 R1** | Exon 12        |         |               |                    |
| **TP53 F2** | Exon 8          | 7157    | 87 bp         | CGGAGATTCTTCTTCTCTGTGCGCCCGGTCTCTCCCCAGGACAGGC ACAAACACGCACCTCAAAGCTGTCCCGTCCCACTAGATTACA |
| **TP53 R2** | Exon 8          |         |               |                    |
| **KRAS F2** | Exon 2          | 3845    | 80 bp         | CTGAATTAGCTGTATCGTGCAAGGCCACTCTTCTGCTACGACCACGC |
| **KRAS R2** | Exon 2          |         |               |                    |
Supplementary Table 2. sequences of primers for Sanger sequencing

| Primer ID               | Sequences                                                                 |
|------------------------|---------------------------------------------------------------------------|
| *TP53* sequencing primer | 5’-CGGAGATTCTCTCTCTCTCCTCTCT-3’                                           |
| *KRAS* sequencing primer | 5’-TTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTT
### Supplementary Table 3. Sequences of primers and probes for Taqman assays

| Primer ID | Sequences               |
|-----------|-------------------------|
| TP53 F2   | 5’-TGGTAATCTACTGGGACG-3’|
| TP53 R2   | 5’-CGGAGATTCTCTTCCTCTCT-3’|
| KRAS F2   | 5’-TGAAAATGACTGAATATAAACTTTTG-3’|
| KRAS R2   | 5’-CTGAATTAGCTGTATCGTCAAG-3’|

| Probe ID     | Sequences                             |
|--------------|---------------------------------------|
| TP53-WT      | 5’-HEX-TTTGAGGTGCGTGTTTGTGCC-BHQ1-3’  |
| TP53-Mut     | 5’-FAM-TGCGTGGTTGCTGCTGTC-BHQ1-3’     |
| KRAS-WT      | 5’-FAM-TGGAGCTGCTGGCGTAG-BHQ1-3’      |
| KRAS-G12A-Mut| 5’-HEX-TGGAGCTGCTGGCGTAG-BHQ1-3’      |
| KRAS-G12S-Mut| 5’-HEX-TGGAGCTAGTGGCGTAGG-BHQ1-3’     |
| KRAS-G12V-Mut| 5’-HEX-TGGAGCTGCTGGCGTAGG-BHQ1-3’     |
| KRAS-G13D-Mut| 5’-HEX-TGGAGCTGGTGACGTAGG-BHQ1-3’     |
| KRAS-G12D-Mut| 5’-HEX-AGCTGATGGCGTAGGCA-BHQ1-3’      |
| Primer ID | Sequences               |
|-----------|-------------------------|
| TP53 F2   | 5’-TGGTAATCTACTGGGACG-3’|
| TP53 R2   | 5’-CGGAGATTCTCTTCCTCT-3’|
| MIER3 F2  | 5’-CCTCAAGTTTACTGGTGC-3’|
| MIER3 R2  | 5’-TTCATCAGTGCCCATG-3’  |

| Probe ID    | Sequences                                      |
|-------------|------------------------------------------------|
| TP53-WT     | 5’-HEX-TTTGAGGTGCGTTTGTGCC-BHQ1-3’             |
| TP53-Mut    | 5’-FAM-TGCGTGTTGCTGCTGTC-BHQ1-3’               |
| MIER3-H548D-WT | 5’-FAM-CGGCCCTACACTCTGAGTGA-BHQ1-3’             |
| MIER3-H548D-Mut | 5’-HEX-CGGCCCTAGACTCTGAGTGA-BHQ1-3’             |