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Supplemental Appendix for:
From somatic variants towards precision oncology: an investigation of reporting practice for NGS-based ctDNA analysis
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Supplemental Methods

SAMPLE VALIDATION BY ARMS

ARMS kit for *EGFR* mutations detection (AmoyDx, Xiamen, China) was used to validate our study samples according to the manufacturer's instructions. Briefly, the ARMS PCR reaction mix were blended DNA templates with primers, probes, dNTPs, buffer, Mg^{2+} and Taq DNA polymerases. PCR reactions were carried out on ABI 7500 PCR system using the cycling conditions as follows: 1 cycle at 95°C for 5 min, 15 cycles of [95°C for 25 s, 64°C for 20 s, 72°C for 20 s], and 31 cycles of [93°C for 25 s, 60°C for 35 s, 72°C for 30 s]. The raw data were analyzed using the software installed with the instrument.

SAMPLE VALIDATION BY DIGTAL PCR

Digital PCR reaction mix had a final volume of 15 μL and included 7.5 μL of 2×QuantStudio 3D Digital PCR Master Mix (Thermo Fisher Scientific, California, USA), 0.375 μL of 40×Custom TaqMan SNP Genotyping Assay (Thermo Fisher Scientific, California, USA), and 7.125 μL of mixed DNA. The reaction mix was loaded onto QuantStudio v2 dPCR chips using QuantStudio 3D Digital PCR Chip Loader. Thermal cycling was performed using standard conditions. Subsequently,

chips were read on a QuantStudio 3D instrument to detect the number of wells positive for VIC or FAM channel. Chip quality assessment and data analysis were finally performed using QuantStudio 3D Analysis Suite.

SAMPLE VALIDATION BY TARGETED NEXT-GENERATION SEQUENCING

80 ng of our synthetic reference material was used as input for the sequencing library preparation. Sequencing library was prepared using a combination of KAPA Biosystems Hyper Prep Kit (Kapa Biosystems, Massachusetts, USA) and xGen Lockdown Probes (Integrated DNA Technologies, Iowa, USA) according to the corresponding manufacturer's instruction. In brief, DNA fragments were incubated with an end-repair and A-tailing enzyme for 30 min at 20 °C followed by 30 min at 65 °C, which generated end-repaired, 5′-phosphorylated, 3′-dA-tailed dsDNA fragments. For adaptor ligation, the ligation mixture was incubated at 16 °C for 12-16 h and purified using Agencourt AMPure XP beads (Beckman Coulter, California, USA) according to the manufacturer's instructions. To distinguish authentic variations in the samples from errors introduced by sample preparation and sequencing steps, all input DNA molecules were tagged with 6-bp molecular barcodes. Pre-capture library amplification reaction mixtures were subjected to the following thermal cycling program: one cycle of 98 °C for 45 s, eight cycles of [98 °C for 15 s, 65 °C for 30 s, and 72 °C for 30 s], and one cycle of 72 °C for 5 min. DNA library was purified by Agencourt AMPure XP beads. Hybridization-based enrichment of target region was

performed using a custom targeted panel for 176 genes according to the manufacturer's instructions (Integrated DNA Technologies, Iowa, USA). After quality control, captured libraries were barcoded and pooled for sequencing. Sequencing was performed using 2 × 75 paired-end reads with an 8-bases indexing read on NextSeq500 (Illumina, California, USA) according to the standard cluster generation and sequencing protocols.

Data analysis was performed using a custom standard sequencing processing pipeline. In brief, after removal of adapter sequences, all reads were aligned to the reference genome (hg19) using BWA mem (version 0.7.12-r1044) with default parameters. Local realignment, duplicate removal, and base quality recalibration were performed using the Genome Analysis Toolkit (version 3.4) and Picard (version 2.9.0). SNVs and indels were called by in-house software. All candidate mutations were reviewed manually using Integrative Genomics Viewer (version 2.3).