Supplementary Methods

Droplet digital polymerase chain reaction (ddPCR) for MET exon 14 skipping mutation

RNA was extracted from FFPE tissue using a Maxwell CSC RNA FFPE Kit (Promega Inc.), quantified using Nanodrop (Thermo Fisher Scientific), and stored at –80℃ deep freezer according to the manufacturer's protocol (Gencruix Inc.). The Droplex cMET Exon14 Skipping Kit is composed to perform a two-step process: reverse transcription and digital PCR. To detect the presence of the cMET Exon14 skipping mutation, 160 ng (10 ng/μL) of RNA extracted from the sample was subjected to reverse transcription, and then ddPCR reaction mixture prepared with the synthesized cDNA and ddPCR reagents (Supermix, Oligo Mix, and DTT). Then droplets were generated using QX200 Droplet Generator (Bio-Rad) by loading 20 μL of PCR reaction mixture and 70 μL of Droplet Generation Oil for Probes (Bio-Rad) onto each well of a DG8 Cartridge (Bio-Rad). Almost 42.5 μL of the droplet-oil mixture were transferred using 8-Channel Electronic Pipette to a semi-skirted 96-Well plate (Bio-Rad or Eppendorf). The plate was sealed with a pierceable foil heat seal using PX1 PCR Plate Sealer (Bio-Rad). Then the 96-Well plate was loaded to T100 Thermal Cycler (Bio-Rad), and run following thermal cycling condition; 95℃ for 10 minutes, followed by 40 cycles of (94℃ for 30 seconds; 58℃ for 1 minute), and 98℃ for 10 minutes. In all PCR steps, the ramp rate was settled as 2℃/sec. After completion of the PCR process, the plate was read using QX200 Droplet Reader (Bio-Rad) with the following setting; channel 1 as FAM and channel 2 as HEX.

After the droplet reading, analysis was conducted with QuantaSoft software (Bio-Rad), manually. The FAM channels of OM1 and OM2 represent the copy number of cMET Exon14 skipping mutation and the total cMET, respectively, which are used to calculate the mutation index (MI%). The MI% is used as the second indicator of mutation calls. The HEX channels of OM1 and OM2 are the number of copies of the internal control and are an indicator of whether the entire process such as the quality of RNA extracted from the specimen and the cDNA synthesis process has been performed normally. Therefore, if the RNA extraction or cDNA synthesis process is not performed normally, the copy number of the internal control is not detected.