ANNALS OF LABORATORY MEDICINE

SUPPLEMENTAL MATERIALS AND METHODS

Uracil-DNA-glycosylase (UDG) treatment

Extracted genomic DNA (gDNA) was incubated with 2 U (1 U/100 ng of DNA) of UDG in a final volume of 50 µL at 37°C for 30 minutes. The UDG-treated gDNA was purified using AMPure XP beads (Beckman Coulter Inc., Brea, CA, USA).

Droplet digital PCR (ddPCR)

The ddPCR assays were performed using the QX200 Droplet Digital PCR System (Bio-Rad, Hercules, CA, USA) and Prime-PCR ddPCR Mutation Detection Assay Kit (Bio-Rad). PCRs were run in a 20-µL volume containing 10 µL of 1× Bio-Rad ddPCR Supermix for Probes (no dUTP), 1 µL of primers, 1 µL of probes to detect *RAS* mutations, 5 µL of 100 ng of template, and 2 µL of distilled water. Reference materials with known gene copy numbers was analyzed for *NRAS* c.182A>G and *KRAS* c.35G>A. After droplet generation, end-point PCR was conducted using the following thermal cycling program: enzyme activation at 95°C for 10 minutes, 40 cycles of denaturation at 94°C for 30 seconds, and annealing and extension at 55°C for 1 minute, and enzyme deactivation at 98°C for 10 minutes. Droplets were read using the Bio-Rad QX200 Droplet Reader (Bio-Rad, Hercules, CA, USA) set to the absolute quantification experiment setting. QuantaSoft Analysis Pro v1.7.4.0917 (Bio-Rad) was used for droplet cluster classification and Poisson function application. Samples containing > 1,200 droplets in the 6-carboxyfluorescein (FAM)-positive area were considered positive.

NGS library preparation

gDNA was quantified using the Qubit BR dsDNA Kit (Thermo Fisher Scientific, Waltham, MA, USA). Fragmentation was conducted at 32°C for 18 minutes, followed by enzyme inactivation at 65°C for 30 minutes.