**Materials and methods**

**Assay method:**

**Q-PCR program**

Our Q-PCR thermal cycling protocol consisted of a polymerase activation step, and three repeated steps: a 3-min Hot-start Polymerase activation denaturation step at 95°C, followed by 40 repeated cycles at 95°C for 10 s, and then at 60°C for 30 s. Melting curves were obtained by increasing the temperature from 55°C to 90°C with a plate reading every 0.2°C.

**ccfDNA quantification system**

The concentration was calculated from Cq detected by Q-PCR and also a control standard curve on DNA of known concentration and copy number (Sigma-Aldrich). Serial dilutions of genomic DNA from human placenta cells (Sigma) were used as a standard for quantification and their concentration and quality were assessed using a Qubitspectrofluorimeter (Invitrogen). Q-PCR amplifications were carried out on a CFX96 instrument (Bio-Rad) using the CFX manager software (Bio-Rad). Intplex runs were analyzed with the CFX Manager Software (Bio-Rad).

**Q-PCR reactions**

The PCR run was assayed at least in duplicate in a 25 µL reaction volume constituted with 12.5 µL of the master mix (Supermix SYBR green, Bio-Rad), 2.5 µL of each primer (3 pmol/µL, final concentration), 2.5 µL of PCR analyzed water and 5 µL of template DNA. Non-template controls were performed in each experiment for the different primer sets. Positive controls for mutation assessment were also added to each PCR run. These controls are genomic DNA from cell lines with known mutations. The respective correspondence between cell lines and the corresponding mutation was further detailed: HCT-116 for the G13D KRAS mutation, SW620 for the G12V KRAS mutation, A549 for the G12S KRAS mutation, LS174T for the G12D KRAS mutation, MiaPaca2 for the G12C mutation, SW1116 for the G12A KRAS mutation, and HT29 for the V600E BRAF mutation. Synthetic DNA bearing the KRAS sequence of interest (Horizon Discovery Ltd) was used as a positive control for KRAS G12R.

**Determination of the sensitivity level**

Evaluation of the sensitivity level of our method was conducted on genomic DNA. A positive control corresponded to each targeted mutation. DNA from the cells harboring targeted mutation was serially diluted six times in high-concentrated WT genomic DNA from human placenta (Sigma Aldrich) up to a dilution of 2 mutated copies in 20,000 WT copies.

**Primer design**

The sequences and characteristics of the selected primers are presented in Supplementary Table 1. The primers were designed using the Primer 3 software and all sequences were checked for self- or inter-molecular annealing with nucleic-acid-folding software (mfold and oligoAnalyzer 1.2). We performed local-alignment analyses with the BLAST program to confirm the specificity of the designed primers. Oligonucleotides were synthesized and puriﬁed on HPLC by Eurofins (Ebersberg, Germany) and quality control of the oligonucleotides was performed by MALDI TOF. Each primer was evaluated and validated before its use for this study.



**Supplementary methods Table 1**