Supplementary Methods. Step-down protocol used for the Sequenom.

PCR reactions were carried out in 5 µL in standard 384-well plates. All chemicals were obtained from Sequenom Inc. (San Diego, CA, US) if not mentioned otherwise. PCR reactions were performed using 5 ng of genomic DNA in a 5 µL reaction volume containing 0.5 U Taq DNA Polymerase (Roche, Woerden, Netherlands), 500 µM dNTP mix, 1X Sequenom PCR buffer, 2 mM $MgCl_2$ and 100 nM of each PCR primer (Metabion GmbH, Martinsried, Germany). Primer sequences are shown in the Supplementary Table 2. A 'step-down' PCR thermal cycling was carried out in a GeneAmp® PCR System 9700 machine (LifeTechnologies™, Bleiswijk, Netherlands) for 4 min at 95°C, followed by 4 cycles of 20 s at 95°C, 30 s at 65°C and 1 min at 72°C, then followed by 4 cycles of 20 s at 95°C, 30 s at 58°C and 1 min at 72°C, and subsequently followed by 38 cycles of 20 s at 95°C, 30 s at 53°C and 1 min at 72°C. Final extension was carried out at 72°C for 3 min and the sample was cooled to 15°C. To the completed PCR reaction, 2 µL containing 0.5 U of Shrimp Alkaline Phosphatase (SAP) and 0.17 µL 10X SAP buffer were added, and the reaction was incubated for 40 min at 37°C followed by inactivation for 5 min at 85°C. After adjusting the concentrations of extension primers to equilibrate signal-to-noise ratios, the post-PCR primer extension reaction of the iPLEX™ Gold assay was performed in a final volume of 9 µL. Additionally, 2 µL extension reaction containing 0.2 µL iPLEX termination mix, 0.04 µL iPLEX enzyme, 0.2 µL 10X iPLEX buffer and 1 µL of 625–1250 nM extension primer (Metabion GmbH) mix were added to the remaining 7 µL. A two-step 200 short cycles program was used for the iPLEX™Gold reaction: initial denaturation was for 30 s at 94°C, followed by 5 s at 94°C and five cycles of 5 s at 52°C and 5 s at 80°C. An additional 40 annealing and extension cycles were then looped back to 5 s at 94°C, 5 s at 52°C and 5 s at 80°C. The final extension was carried out at 72°C for 3 min and the sample was cooled to 15°C. The iPLEX™Gold reaction products were desalted by diluting samples with 25 µL of water and adding 6 mg of resin, then centrifuged to spin down the resin. The products were spotted on a SpectroCHIP (Sequenom Inc.) processed and analyzed with the MassARRAY® Compact Mass Spectrometer and MassARRAY® Workstation (version 3.4) software (Sequenom Inc.).