Supplemental Materials

MUTATED ALLELE SPECIFIC PCR AMPLIFICATION (MASA)
This technique allows detection of the mutation when present in less than 5% of the alleles. Two different forward primers for the exon 15 of the BRAF gene were designed in order to specifically amplify the mutated or the wild type allele. The two primers differed in a single nucleotide in the 3’end region.

Specific primer for the mutated allele: MBRAF-F TGATTTTGGTCTAGCTACAGA
Specific primer for the wild type allele: WTBRAF-F TGATTTTGGTCTAGCTACAGT
Common reverse primer: BRAF-comR– CACTGATTTTTGTGAATACTG

The amplification reaction was conducted using the Taq Gotaq Hot Start Polymerase (Promega, Italy), with the following protocol:2’ 95°C; [20” 95°C, 20’ 58°C mutated allele/56°C wild type allele, 20” 72°C] x 40 cycles; 3’ 72°C
After amplification PCR products were analyzed by electrophoresis on agarose gel.
To calculate method sensitivity we have mixed genomic DNA from B-CPAP cells that are homozygous for BRAFV600E mutation with WT genomic DNA in variable proportions in order to have 50%, 25%, 10% 5% and 1% of mutated alleles in the entire genomic population.

CELL CULTURES
B-CPAP papillary thyroid tumor cell line were grown at 37°C, 5% CO2 in DMEM supplemented with 10% FBS. Genomic DNA was collected using the Qiagen DNA extraction kit according to manufacturer instructions. Genomic DNA was quantified using the Nanodrop Spectrophotometer.

Supplemental Figure 1 Legend

BRAFV600E mutation analysis using Direct Sequencing and MASA technique
A) MASA amplification of exon 15 of the BRAF gene in 6 PTC samples. Specific forward primers for wild type (WT) or mutated (V600E) allele were used. For each PTC sample analyzed WT allele amplification was loaded on left lane while mutated allele amplification was loaded on the right lane (mut). PTC1 and PTC2 were heterozygous for V600E mutation based on the presence of both amplification products (WT and Mut). PTC3, PTC4, PTC5 and PTC6 were homozygous for the wild type allele since no amplification was obtained for the mutated allele. B) Chromatogram of PTC1 obtained by direct sequence. A double peak A/T
was evident confirming the heterozygous mutation of exon 15 in the BRAF gene. C) Chromatogram of PTC6 obtained by direct sequence. A single peak T was evident confirming the wild type genotype of the sample. D) Evaluation of the sensitivity of the MASA amplification in the detection of the BRAFV600E mutation. WT (lower panel) and V600E mutant allele (upper panel) were amplified using specific primers in a mixed population of genomic DNA from the V600E homozygous mutant B-CPAP cells and a WT genomic DNA. The percentage is relative to the mutated allele in the entire genomic population. As it is clearly evident a PCR band for the mutated allele was detected at all concentrations even when the mutated allele was present in only 1% of the alleles. This demonstrates the high sensitivity of the method.