# BRAF(V600E) Mutation Allele Specific PCR Assay in Circulating Tumor Cells

*Vladislava O. Melnikova, Margaret Pace, Kerry Brandl, Yuan Wang, Jacky Woo, Darren W. Davis*
*ApoCell Inc., Houston, TX 77054*

## Abstract

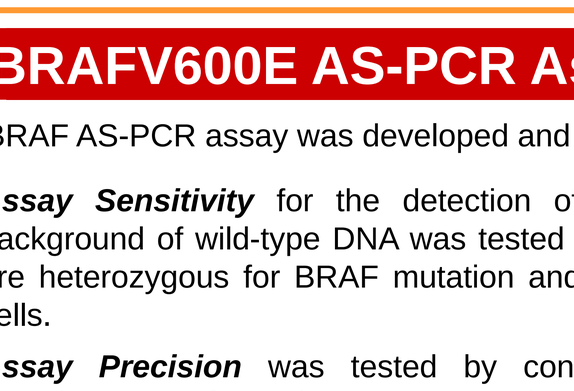
**Background:** ApoCell specializes in molecular characterization of Circulating Tumor Cells (CTCs). CTCs represent a powerful tool for molecular characterization of tumors for which biopsies are not available. An activating mutation in the serine/threonine kinase, BRAFV600E, is present in up to 70% of melanomas and 10% of colon carcinomas. Because of the low frequency of CTCs, assay sensitivity for mutational analysis needs to be below 1% of BRAFV600E copies in a background of wild-type DNA. We have developed and validated a highly-sensitive allele-specific PCR (AS-PCR) assay to detect the presence of the BRAFV600E mutation in CTCs. **Methods:** A CTC BRAFV600E AS-PCR assay was developed using HT-29 colon cancer cells that are heterozygous for BRAFV600E, and BRAF wild-type MDA-MB-231 breast cancer cells. Assay performance, limit of detection, assay precision and assay specificity were further defined by spiking HT-29 BRAFV600E cells into wild-type cells. The specificity of the complete assay, combining CTC isolation using CellSearch® Profile kit and the BRAFV600E AS-PCR was tested using HT-29 cells spiked into normal donor blood. The feasibility of the CTC BRAFV600E AS-PCR assay was demonstrated using melanoma and colon cancer patient blood. **Results:** Specificity of the BRAFV600E AS-PCR assay was successfully tested in healthy donor blood spiked with HT-29 BRAFV600E cells and MDA-MB-231 BRAF wild-type cells. The limit of detection of the assay was found to be 0.01% or 5 BRAFV600E mutant copies in a wild-type background. Assay precision was demonstrated by repeating the assay at the limit of detection on three different days. **Conclusions:** We have developed and validated an AS-PCR assay for detection of the BRAFV600E mutation in CTCs. Testing melanoma and colon cancer patient blood specimens showed feasibility of the BRAFV600E mutation assay in liquid biopsy. Further concordance testing between tumor biopsies and blood specimens is ongoing and will establish whether liquid biopsies are a suitable alternative for use in identifying patients for whom a particular targeted therapy may or may not be effective based on genetic status.

## BRAFV600E AS-PCR Assay Development

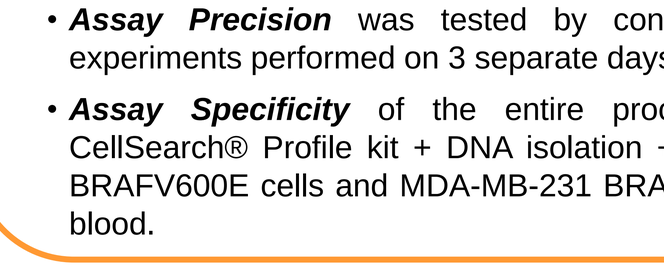
The BRAF AS-PCR assay was developed and tested as follows:

- ***Assay Sensitivity*** for the detection of BRAFV600E DNA copies on a background of wild-type DNA was tested using HT-29 BRAFV600E cells that are heterozygous for BRAF mutation and the MDA-MB-231 BRAF wild-type cells.
- ***Assay Precision*** was tested by conducting a series of cell spiking experiments performed on 3 separate days.
- ***Assay Specificity*** of the entire procedure of CTC enrichment with CellSearch® Profile kit + DNA isolation + AS-PCR was tested using HT-29 BRAFV600E cells and MDA-MB-231 BRAF wild-type cells spiked into normal blood.

## BRAFV600E AS-PCR Assay Sensitivity

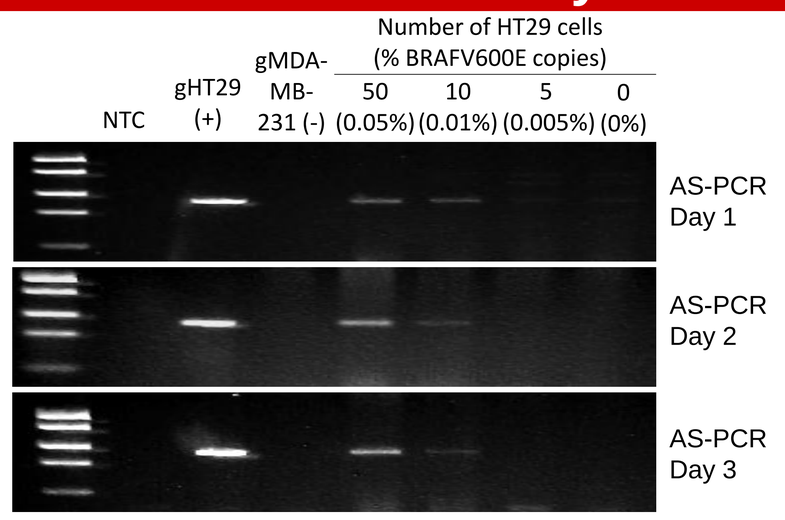



5000, 500, 50, 10, and 5 HT-29 cells were spiked into 50,000 MDA-MB-231 cells. Genomic (g) DNA was extracted and BRAFV600E AS-PCR was performed on gDNA template.



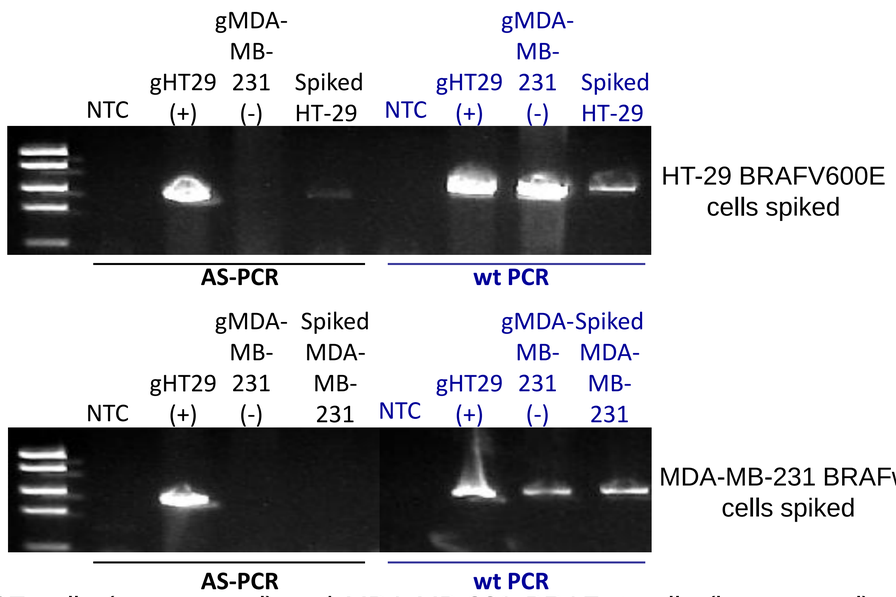

***Sensitivity of the AS-PCR for BRAFV600E is at least 0.01% of mutant cells in a background of wild-type BRAF cells.***

## BRAFV600E AS-PCR Assay Precision



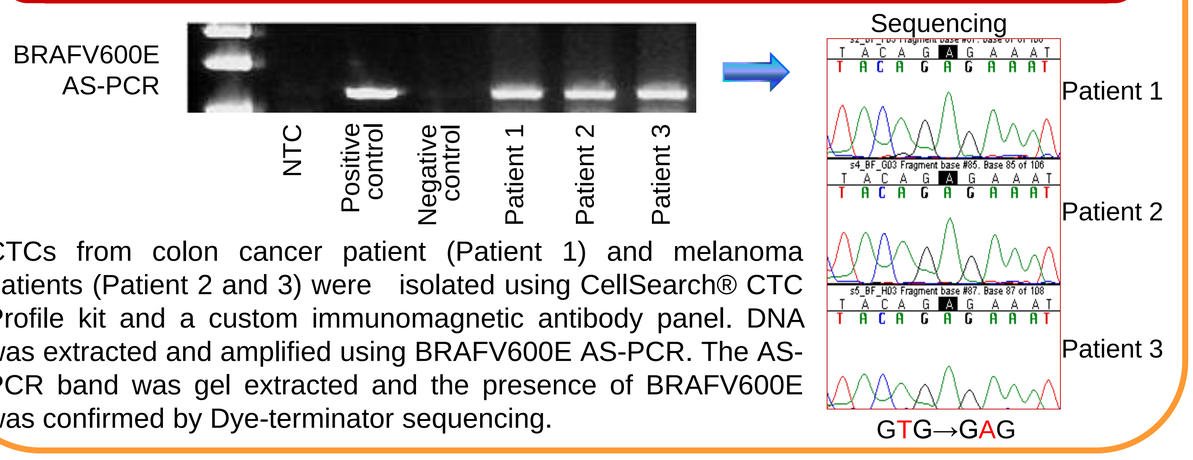

50, 10, 5 and 0 HT-29 cells were spiked into 50,000 MDA-MB-231 cells on day 1 (see assay sensitivity) to day 3. Genomic DNA was extracted and BRAFV600E AS-PCR was performed on gDNA template without whole genome amplification. Sensitivity of AS-PCR for the detection of BRAFV600E mutant cells on a background of wild-type BRAF cells was reproducibly shown to be 0.01%.

## CTC BRAFV600E Assay Specificity




HT-29 BRAFV600E cells (upper panel) and MDA-MB-231 BRAFwt cells (lower panel) were spiked into normal donor blood and isolated using CellSearch® CTC Profile kit. DNA was extracted and amplified using BRAFV600E AS-PCR and BRAFwt PCR. The AS-PCR assay did amplify DNA from spiked HT-29 BRAFV600E cells, but did not amplify DNA from spiked MDA-MB-231 BRAFwt cells.

## CTC BRAFV600E Assay Performance in Patient Blood




CTCs from colon cancer patient (Patient 1) and melanoma patients (Patient 2 and 3) were isolated using CellSearch® CTC Profile kit and a custom immunomagnetic antibody panel. DNA was extracted and amplified using BRAFV600E AS-PCR. The AS-PCR band was gel extracted and the presence of BRAFV600E was confirmed by Dye-terminator sequencing.

## Conclusions

- We have developed, validated, and successfully utilized AS-PCR assay for detection of BRAFV600E mutation in circulating tumor cells isolated from patient blood.
- The assay sensitivity was shown to be 0.01% of BRAFV600E copies in a wild-type background. The assay limit of detection is 5 BRAFV600E mutant copies. When tested with cells spiked into donor blood, the specificity of the complete assay, AS-PCR in CTCs, was confirmed by demonstrating that the assay amplified DNA from spiked HT-29 BRAFV600Ecells, but not from spiked MDA-MB-231 BRAFwt cells.
- The approach presented here will be used to develop additional PCR assays such as Locked Nucleic Acid or Scorpion to detect mutations in other cancer relevant genes.