# Sensitive Detection of BRAF V600E Mutation by BNA Clamping Real-time PCR

Xiaoyun Liu[1], Houquan Dai[1], Aaron Castro[1], Leticia Loredo[1], Yuewei Zhao[1], Sung-Kun Kim[2], Austin Dinkel[2], Miguel Castro[1]

[1]Biosynthesis Inc, 612 E. Main St, Lewisville, TX 75057, USA

[2]Department of Natural Sciences, Northeastern State University, 611 N. Grand Ave, Tahlequah, OK 74464, USA

## Purpose

We report a sensitive method for the rapid detection of the 1799T>A (V600E) conversion on BRAF gene, using 2'-O,4'-aminoethylene bridged nucleic acid (BNA or BNA$^{NC}$). We have designed, synthesized, and investigated several BNA clamps for their superior binding ability to perfectly matched DNA templates while discriminating the BRAF mutant from the wild-type gene. We have also produced a fluorescence-labelled BNA probe for specific detection of the BRAF-V600E mutation. We have found that the BNA clamp-probe combination is able to detect mutants at abundance levels as low as 0.1%, indicating an improved sensitivity for the diagnosis of gene mutations.

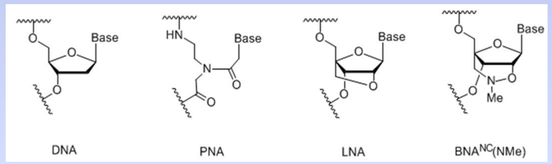
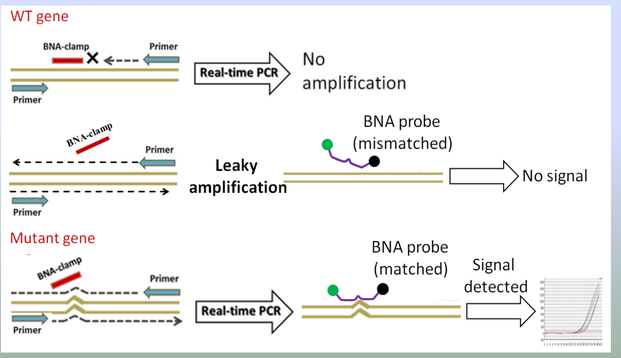



Figure 1. The structures of DNA and synthetic analogues.


Figure 2. Illustration of the B-raf protein kinase and the location of the V600E mutation.

## Methods

• BNA clamp and probe-based real-time PCR




## Key Findings

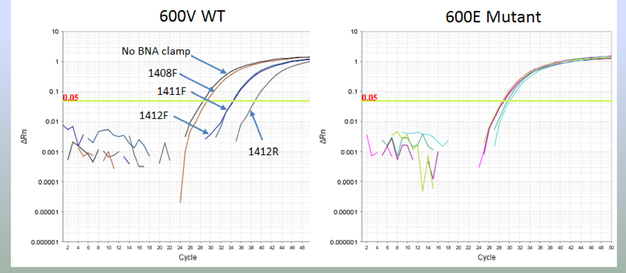
- Incorporation of BNA bases significantly increases melting temperatures of the oligonucleotides.
- The inhibitory effect (ΔCt) of the BNA clamps on the amplification of the WT gene, directly correlates to their Tm values, while BNA clamp-1412R shows the highest efficacy (ΔCt > 12).
- The BNA probe is able to specifically detect the BRAF-600E mutant. It is able to detect levels as low as 10 cps of the mutant gene.
- **In combination with BNA Clamp-1412R, the BNA probe is able to detect a mutation level as low as 0.1% in a background of the wild-type gene.**
- BNA clamp-1412R and BNA probe is able to specifically detect the BRAF-V600E mutation present in genomic DNA.

## Results

Table 1. BNA-clamping oligonucleotides and Tm values for synthetic ssDNA 14-mer templates of the BRAF 600V WT and 600E mutant genes

| Name | Length | No. of BNA | $T_m$ | | |
|---|---|---|---|---|---|
| | | | WT | 600E | ΔTm |
| 1408F | 14 | 8 | 68.5 | 54.5 | 14.0 |
| 1411F | 14 | 11 | 80.1 | 68.2 | 11.9 |
| 1412F | 14 | 12 | 80.7 | 65.1 | 15.6 |
| DNA-F control | 14 | 0 | 48.5 | 37.5 | 11.0 |
| | | | | | |
| **1412R** | 14 | 12 | 83.6 | 66 | 17.4 |
| DNA-R control | 14 | 0 | 46.5 | 37.5 | 9.0 |

**Figure 3. Real-time PCR amplification of BRAF genes in the absence or presence of the BNA clamps. A total $10^3$ copies of plasmids were used in each assay.**




## Results

**Figure 4. Direct correlations of the melting temperatures of BNA clamps with the inhibitory effect on the PCR amplification of the WT gene**

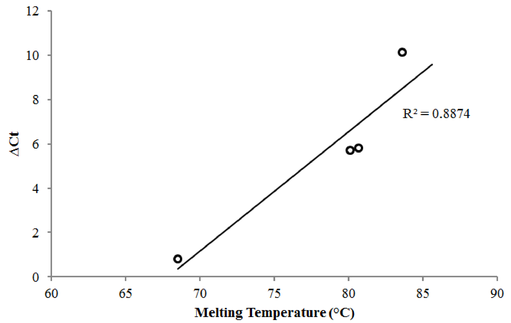



**Figure 5. Real-time PCR detection of the BRAF 600E mutant by the BNA and universal probes**

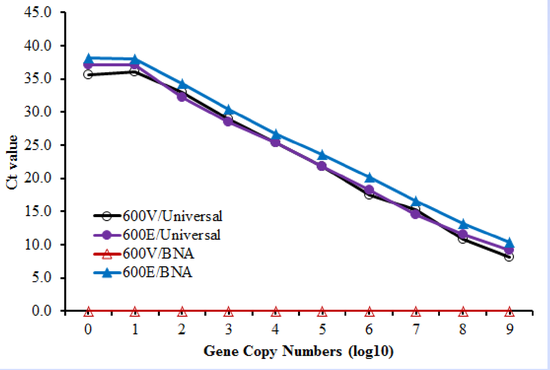
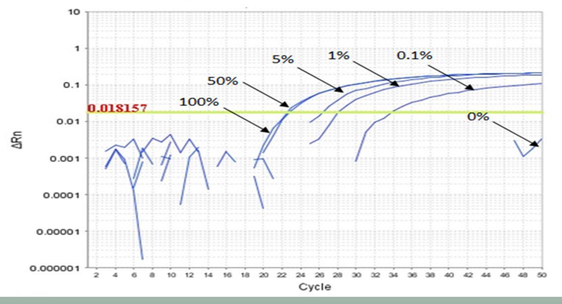



**Figure 6. Ultra sensitive detection of the BRAF-V600E mutant by BNA clamp-1412R and probe.**




## Results

Figure 7. Real-time PCR detection of the BRAF-V600E mutation in genomic DNA (gDNA) samples by BRAF BNA clamp-1412R and BNA probe.

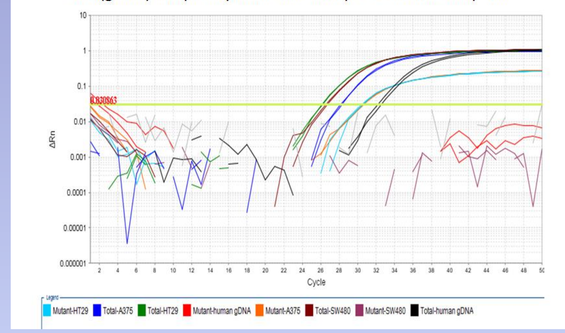



Table 2. Real-time PCR detection by the BNA clamp-1412R and BNA probe, of the BRAF-V600E mutant present in the genomic DNA samples

| Samples | BRAF Status | Assay | Ct |
|---|---|---|---|
| **Human gDNA** | 600V | Total | 32.4 ± 0.3 |
| | (WT) | Mutant | **> 50** |
| **SW480** | 600V | Total | 26.7 ± 0.1 |
| | (WT) | Mutant | **> 50** |
| **HT29** | 600E | Total | 26.2 ± 0.0 |
| | (heterozygous) | Mutant | 30.4 ± 0.1 |
| **A375** | 600E | Total | 28.2 ± 0.2 |
| | (homozygous) | Mutant | 30.6 ± 0.1 |
| **NTC** | N/A | Total | >50 |
| | | Mutant | >50 |

## Future Directions

The BNA clamp-1412R and probe assay will be tested on DNA extracted from formalin-fixed, paraffin-embedded (FFPE) human tissues and freshly isolated tumor samples.

## References

1. Hirama T, Shiono A, Egashira H, et al. PCR-Based Rapid Identification System Using Bridged Nucleic Acids for Detection of Clarithromycin-Resistant Mycobacterium avium-M. intracellulare Complex Isolates. J Clin Microbiol. 2016;54(3):699-704.

2. Morishita S, Takahashi K, Araki M, et al. Melting Curve Analysis after T Allele Enrichment (MelcaTle) as a Highly Sensitive and Reliable Method for Detecting the JAK2V617F Mutation. PLoS One. 2015;10(3):e0122003.