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

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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 BNA²). 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.

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-V600E 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.

Methods

- BNA clamp and probe-based real-time PCR

Results

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

<table>
<thead>
<tr>
<th>Name</th>
<th>Length</th>
<th>No. of BNA</th>
<th>Tm WT</th>
<th>Tm 600E</th>
<th>ΔTm</th>
</tr>
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<tbody>
<tr>
<td>1408F</td>
<td>14</td>
<td>8</td>
<td>68.5</td>
<td>54.5</td>
<td>14</td>
</tr>
<tr>
<td>1411F</td>
<td>14</td>
<td>11</td>
<td>80.1</td>
<td>68.2</td>
<td>11.9</td>
</tr>
<tr>
<td>1412F</td>
<td>14</td>
<td>12</td>
<td>80.7</td>
<td>65.1</td>
<td>15.6</td>
</tr>
<tr>
<td>DNA-F control</td>
<td>14</td>
<td>0</td>
<td>48.5</td>
<td>37.5</td>
<td>10</td>
</tr>
<tr>
<td>1412R</td>
<td>14</td>
<td>12</td>
<td>83.6</td>
<td>66</td>
<td>17.4</td>
</tr>
<tr>
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<td>0</td>
<td>46.5</td>
<td>37.5</td>
<td>9.0</td>
</tr>
</tbody>
</table>

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.

Figure 3. Real-time PCR amplification of BRAF genes in the absence or presence of the BNA clamps. A total 10³ copies of plasmids were used in each assay.

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 measured probes

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

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