A Highly Specific & Sensitive Single-Well Multiplex KRAS/BRAF Mutation Assay

Jason Lei, Kiran Madanally Divakar, and Lilly Kong. PrimerDX, Mansfield, MA 02048

Abstract

Anti-EGFR monoclonal antibodies have been used or under trial for treating metastatic colorectal cancer (mCRC), non-small cell lung cancer (NSCLC), and other cancers. However, some mCRC/BRAF activating mutations can render these monoklonal antibodies ineffective. A new designed assay, the ICelix® KRAS/BRAF Mutation Assay (ICelix® KRAS/BRAF Mutation Assay), was developed, wherein all primers for detection of the major KRAS mutations (G12D, G12C, G12A, G12V, G13D, G13A, and G13V) and BRAF (V600E, V600K, and V600D) mutations, along with primers for QC, invalidation, and internal controls, are included in a single-gel format. To reduce the common non-specific and cross-reaction issues with allele-specific PCR, primers were designed with two domains: (1) target-specific core sequences at the 5' end and (2) homogeneous tail sequence at the 3' end. After increasing the specificity and controlling for the PCR products, the assay can detect the mutations in a single reaction without the need for gel separation. The results presented here demonstrate major benefits of the single-well multiplex format when coupled with automated detection, including conservation of precious clinical specimens, shorter time on the day, increases in assay thoroughness and reduction in turn-around time.

Materials & Methods

Icelix® integrates a PCR thermal cycler that accommodates a standard 96-well PCR plates, a capillary electrophoresis (CE) system with a replaceable CE cartridge, and fluidic pumps with microliter capacity. Equipped with two solid state lasers (488 nm & 633 nm) and a spectrophotometer with a CCD camera, the ICelix® System is capable of detecting two different target structures in an “8 x 8” reaction format (Figure 1).

Results

High Specificity of the Icelix® KRAS/BRAF Mutation Assay

The PCR amplification conditions were as follows: 3 cycles/10 min, 17 cycles/30 sec, and 18 cycles/60 sec. Using the ICelix® System, all the expected bands were obtained for each of the eight different primer mixtures, including the wild-type control for all mutations. The amplification products were analyzed using capillary electrophoresis (CE), and all expected products were obtained. The assay was specific for the targeted mutations, and no unexpected amplification was observed for the wild-type control samples. The results were consistent with the expected patterns for all the primer mixtures.

High Sensitivity of the Icelix® KRAS/BRAF Mutation Assay

The ICelix® System, and Icelix® KRAS/BRAF Mutation Assay were then tested for sensitivity by diluting DNA samples. The sensitivity of the assay was determined by diluting the DNA samples in a 1:100 ratio, and the assay was able to detect the target mutations down to the 1:100 dilution level. The results demonstrated that the assay was able to detect the target mutations with high sensitivity and specificity.

Summary

A new multiplex assay has been developed and has been evaluated for the detection of point mutations in KRAS (G12D, G12C, G12A, G12V, G13D, G13A, and G13V) and BRAF (V600E, V600K, and V600D) mutations. The assay is highly specific and sensitive, allowing for the detection of these mutations in a single reaction format. The results demonstrated that the assay is highly specific and sensitive, allowing for the detection of these mutations in a single reaction format.

Technology Overview

Icelix® integrates a PCR thermal cycler that accommodates a standard 96-well PCR plates, a capillary electrophoresis (CE) system with a replaceable CE cartridge, and fluidic pumps with microliter capacity. Equipped with two solid state lasers (488 nm & 633 nm) and a spectrophotometer with a CCD camera, the ICelix® System is capable of detecting two different target structures in an “8 x 8” reaction format (Figure 1).

Figure 2. Principles of the multiplex real-time PCR detection on the Icelix® System

Figure 3. Multiplex or miniset DNA was tested individually with the Icelix® KRAS/BRAF Mutation Assay. All the expected bands were obtained for each of the eight different primer mixtures, including the wild-type control for all mutations. The amplification products were analyzed using capillary electrophoresis (CE), and all expected products were obtained. The assay was specific for the targeted mutations, and no unexpected amplification was observed for the wild-type control samples. The results were consistent with the expected patterns for all the primer mixtures.

Figure 4. High sensitivity of the Icelix® KRAS/BRAF Mutation Assay

Figure 5. The Icelix® System and Icelix® KRAS/BRAF Mutation Assay have been calibrated and approved for CE use; information presented is for demonstration only.