Cancer Biomarker Research using castPCR™ Technology

Yun Bao, Bonnie Ching, Mokang Mouanoutoua, Yu Wang, David Keys, Toinette Harshorne, Sejal Desai and Junko Stevens

Life Technologies, 850 Lincoln Centre Dr. Foster City, CA94404, USA

ABSTRACT

Cancer biomarkers have applications in the diagnosis, staging, prognosis and monitoring of disease progression, as well as in the predication and monitoring of drug response. Profiling and validation research tools are needed that exhibit the combined features of high sensitivity and high specificity for cancers. However, the sensitivity of molecular methods such as DNA sequencing and conventional genotyping in tumor samples is limited, typically ranging from 5-20%. We have recently developed TaqMan® Mutation Detection Assays using our competitive allele specific TaqMan® PCR (castPCR™) technology for cancer biomarker research. castPCR™ assays were tested with >300 tumor research samples (either fresh/frozen or formalin-fixed, paraffin-embedded samples) and cell lines to assess mutation status at multiple independent laboratories. The results showed that castPCR™ technology can robustly detect mutations as low as 0.1% and has >99% concordance to other technologies including PCR-based technology and sequencing. In this study, a large panel of TaqMan® Mutation Detection Assays for AKT1, BRAF, CTNNB1, HRAS, KRAS, NRAS, PIK3CA, PTEN and TP53 genes were used for investigating somatic mutations in breast tumor research samples. Initially, 4 model FFPE cell lines were used to validate the assays. Mutant DNAs were titrated in the wild type DNAs from 50% to 0.1%. Mutations were identified down to 0.1% titration with high reproducibility. No false positives were found in non-tumor samples. The results obtained by TaqMan® Mutation Detection Assays for 20 breast tumor samples (FFPE/fresh frozen) were concordant to those reported by other methods. Our data showed that castPCR™ technology provides an excellent tool for identifying cancer biomarkers or confirming potential cancer markers such as those obtained by next-generation sequencing and other technologies.

INTRODUCTION

An important but challenging part of cancer research is the identification of key biomarkers from the heterogeneous tumor samples. castPCR™ technology has provided a platform for cancer biomarker profiling, mutation detection and screening. The aim of this study was to evaluate its performance as a sensitive and accurate tool for cancer biomarker profiling in research samples.

RESULTS

1. Assay Specificity and Sensitivity

Mutant allele detection is based on an allele-specific primer, while the wild type allele background is suppressed by the proprietary MGB blocker oligonucleotide. Assays can detect down to 0.1% mutant allele in the presence of wild type allele background. For each assay developed, 0.1% mutant allele samples were generated by spiking 10 copies of mutant allele synthetic templates into 10,000 copies (30 ng) of cell line wild type DNA.

Example amplification plots for KIT 1314 mu assay and PIK3CA_776_mu assay on 0.1% mutant allele sample and wild type gDNA.

There is a significant difference in amplification Ct values between the 0.1% mutant allele sample and wild type gDNA (p-value < 0.05 for 46 out of 48 assays in the example graph).

2. FFPE Cell Line Titration Experiment

DNA extracted from 4 FFPE mutant cell lines and 1 wild type cell line were used to validate the assays. Mutated DNA was diluted in 30 ng wild type gDNA from 50% to 0.1%. Each sample was run with the corresponding mutant allele assay and gene reference assay in four replicates.

<table>
<thead>
<tr>
<th>Mutant FFPE gDNA</th>
<th>Zygosity</th>
<th>Wild type FFPE gDNA</th>
<th>Mutation</th>
<th>Mutant Assay</th>
<th>Reference Assay</th>
</tr>
</thead>
<tbody>
<tr>
<td>PDH-1</td>
<td>Heterozygous</td>
<td>Kras G12R</td>
<td>Kras_518_mu</td>
<td>Kras_rf</td>
<td></td>
</tr>
<tr>
<td>SW600</td>
<td>Homozygous</td>
<td>Kras G12V</td>
<td>Kras_520_mu</td>
<td>Kras_rf</td>
<td></td>
</tr>
<tr>
<td>PANC-1</td>
<td>Homozygous</td>
<td>Kras G12D</td>
<td>Kras_521_mu</td>
<td>Kras_rf</td>
<td></td>
</tr>
<tr>
<td>NCI-HED309</td>
<td>Heterozygous</td>
<td>Kras G12A</td>
<td>Kras_522_mu</td>
<td>Kras_rf</td>
<td></td>
</tr>
</tbody>
</table>

Notes

Copyright© 2012 Life Technologies Corporation. All rights reserved. TaqMan is a registered trademark of Roche Molecular Systems, Inc. For Research Use Only. Not for human or animal therapeutic or diagnostic use.

3. Mutation Profiling of Heterogeneous FFPE/Fresh Frozen Breast Tumor Samples

A panel of 62 TaqMan® Mutation Detection Assays for AKT1, BRAF, CTNNB1, HRAS, KRAS, NRAS, PIK3CA, PTEN and TP53 genes were used for mutation profiling of FFPE or fresh frozen breast tumor research samples. 20 breast tumor samples and 10 normal breast tissue samples were used in this study. Those samples were previously uncharacterized. No false positives were found in normal breast tissue samples. The results obtained by these castPCR™ technology-based assays for 20 breast tumor samples (10 FFPE and 10 paired fresh frozen samples) were concordant to those analyzed by ARMS-castPCR™ technology and by Ion AmpliSeq™ Cancer Panel sequencing technology.

CONCLUSIONS

- TaqMan® Mutation Detection Assays demonstrated high specificity and sensitivity/selectivity for mutation detection, capable of detecting 0.1% mutations in 10,000 copies of wild type gDNA background.
- castPCR™ technology provides a sensitive and robust method for mutation profiling in heterogeneous cancer samples.
- Results from castPCR™ technology are highly concordant to results from other technologies including sequencing using Ion AmpliSeq™ Cancer Panel.