PrimeraDx
The Multiplex PCR Company

ONCOLOGY   INFECTIOUS DISEASE   TRANSLATIONAL MEDICINE

SNP Detection | **Copy Number Variation** | Chromosomal Abnormalities | **Gene Expression** | miRNA | Pathogen Detection | Pathogen Quantitation | Methylation | **Multimodal**

Application Brief

cMET/EGFR Copy Number Variation and cMET Gene Expression Panel

Unique All-In-One-Well Copy Number Variation and Gene Expression Multimodal Assay

INTRODUCTION

cMET is a proto-oncogene that encodes for Hepatocyte Growth Factor Receptor (HGFR), a receptor tyrosine kinase, which plays an essential role in normal cellular function and oncogenesis. Recent studies have identified cMET as a biomarker for various cancers as well as for drug resistance in case of anti-EGFR therapy. cMET can be shown to highly over-express and show significant copy number variations under such situations. Additionally, copy number variation in EGFR can occur by a complex interaction between cMET and EGFR. With so many biomarkers in so many complex pathways targeted by so many drugs, there is a need for all-in-one-well, multiplex and multimodal tests. Using the ICEPlex® system, we created an 18-target, multimodal quantitative cMET/EGFR Copy Number Variation and cMET Gene Expression Panel designed to detect:

- cMET/EGFR gene copy number variation (CNV)
- Chromosomal copy number variation
- Over-expression of cMET mRNA

SUMMARY

- Delivers accurate and precise quantification of copy number variation and gene expression.
- Provides built-in controls such as calibration and process controls to assure quality of the materials and samples.
- Enables amplification from FFPE tissue materials because of the smaller amplicon (<100 nucleotides) and requires minimum nucleic acid input.
- Tests versatile sample types: FFPE blocks, fresh-frozen tissues, or cell lines.
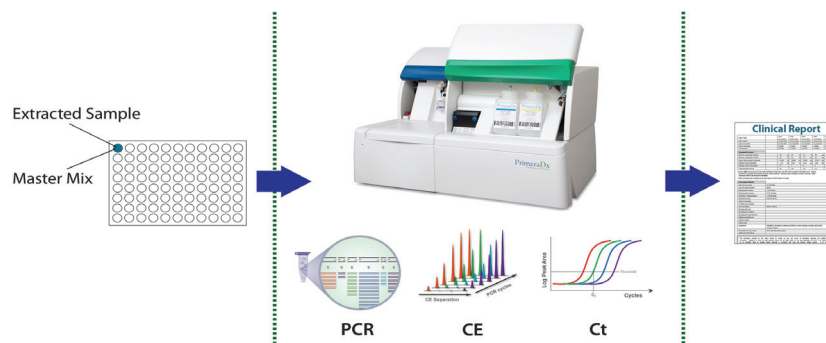
UNIQUE MULTIMODAL CAPABILITY

TYPICAL DATA

A real-time, all-in-one-well 18-target assay was developed to determine cMET/EGFR CNV and cMET expression on the ICEPlex system. Performance testing using total nucleic acids from cell lines with known cMET CNV and overexpression as well as chromosome 7 polysomy matched published results. Resulting data were similar for fresh-frozen or FFPE-derived material. Low amounts of FFPE-extracted material were sufficient to accurately determine cMET/EGFR CNV and cMET expression status.

NOTE: Data were normalized to reference genes Ref2 and Ref3 and fold-change calculated.
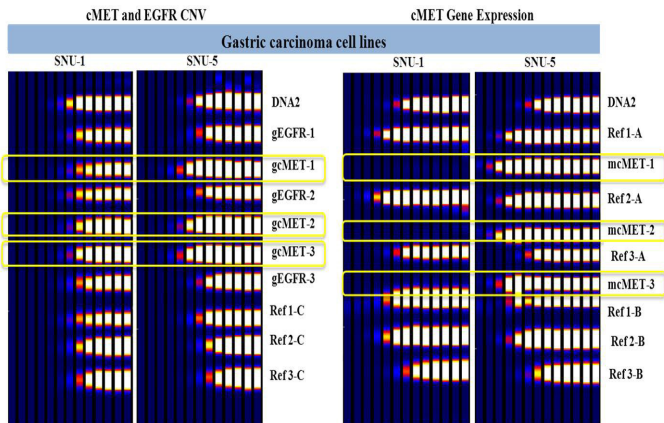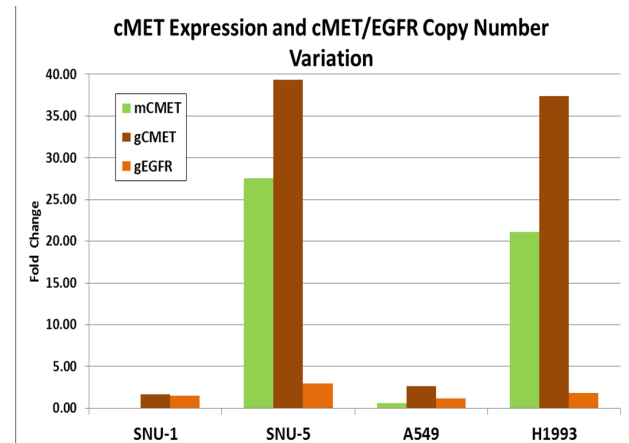
Figure 1

Figure 2

Figure 1 Computer-generated gel view showing simultaneous detection of cMET/EGFR and cMET expression targets.
Figure 2. cMET/EGFR copy number variation, expression and polysomy, all-in-one-well.

METHOD HIGHLIGHTS

- This 18-target panel was constructed to quantify cMET/EGFR Gene Copy Number and cMET Gene Expression.
- For cMET expression profiling, three primer pairs amplifying three different regions of cMET mRNA were designed.
- For cMET/EGFR CNV three primer pairs each amplifying three different genomic regions of cMET and EGFR were designed.
- For normalization and relative quantification, two reference genes were used; two primer pairs for each reference gene mRNA were designed for cMET mRNA quantification and one primer pair for each reference gene was designed for cMET/EGFR copy number variation.
- Multiplexed reactions were optimized on the ICEPlex system using total nucleic acids from various cell lines.
- The fluorescently labeled amplicons for the different cMET/EGFR CNV and cMET gene expression profile targets were injected, separated and detected in the capillary electrophoresis module of the ICEPlex system.
- Amplification curves for all targets and controls were generated automatically by the ICEPlex software and cycle thresholds (Cts) were calculated.

FOR MORE INFORMATION

For a list of publications and to find out more about how PrimeraDx can help your lab, please contact us at 508.618.2300 or visit www.primeradx.com.