The CEE-Selector™ Assay: A tool for the identification of rare allele variants

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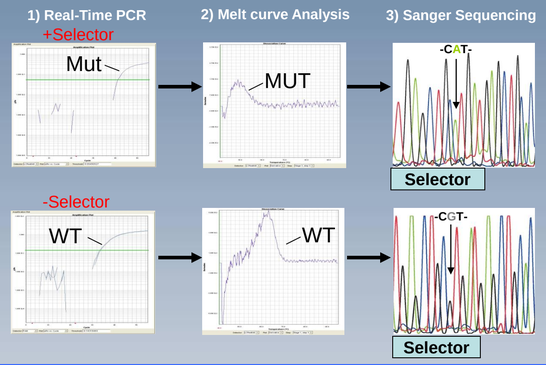
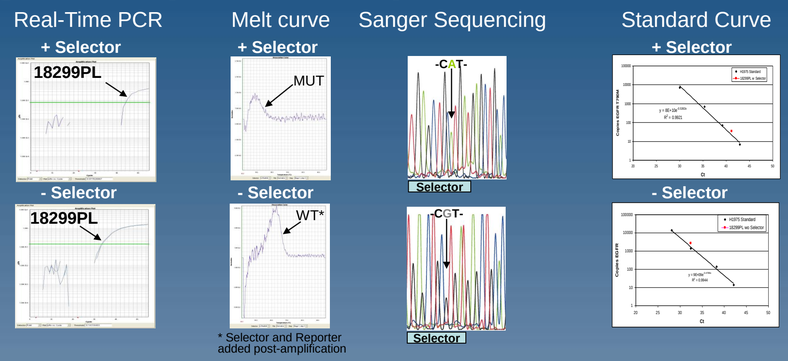
Abstract

Molecular assays for the identification of rare allele occurrences are important tools for proper cancer classification and treatment. A prime example is the T790M mutation in EGFR which leads to resistance to the tyrosine kinase inhibitors gefitinib (Iressa®) and erlotinib (Tarceva®) used in the treatment of non-small cell lung cancer (NSCLC). Identification of the T790M mutation in cancer-shed particles in blood (either as whole cells or subcellular vesicles) calls out the need for an alternative cancer treatment. We have developed a highly sensitive PCR-based assay which allows the identification of the T790M mutation in blood plasma (either when present in mRNA or genomic DNA). The assay combines Real-Time PCR as well as melt curve analysis of the mutant PCR product and is followed by sequencing to verify the presence of the mutation. The Selector™ Assay is based on a wild-type specific PCR blocker and allows the mutant template to be amplified in a high background of wild-type template. A few copies of T790M mutant can be detected in greater than a 1000-fold excess of wild-type. Data using the Selector™ Assay with clinical lung cancer samples as well as H1975 cells spiked and recovered from whole blood using Biocept's microchannel technology are presented. The Selector™ Assay can be applied to other mutations relevant to cancer and is a valuable tool for clinical diagnostics.

Methods



Lung cancer plasma samples

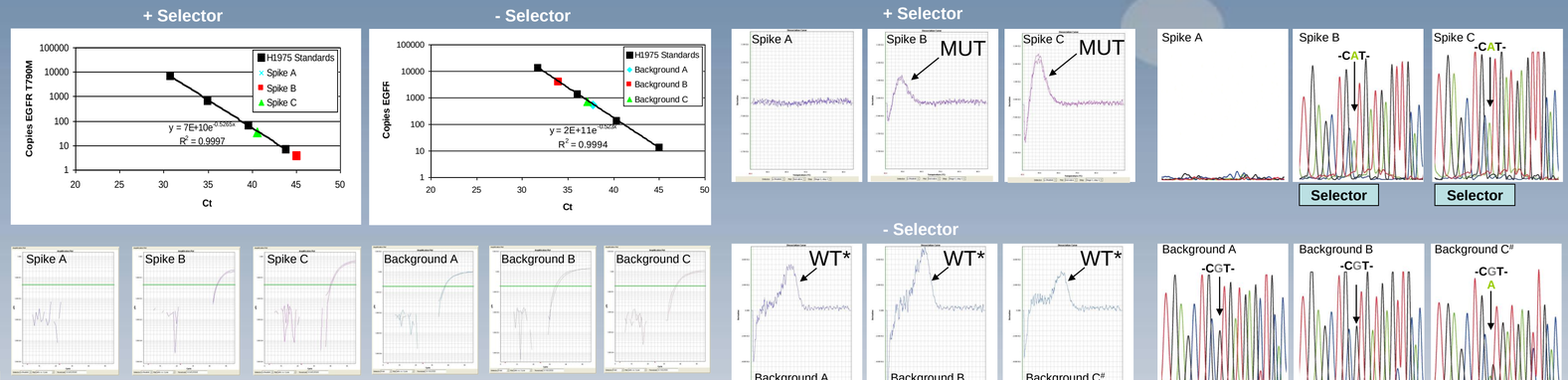


NSCLC Patient Results: T790M# Selector™ Assay

Sample number	Copies of EGFR*	Copies of mutant*	Percent Mutation	T790M**	Mutation found
18280PL	61000	17	0.014/0.014	-	L792F/silent mut
18298PL	61000	9	0.014	+	T790M
18299PL	46300	577	1.24	+	T790M
18312PL	240000	3800	1.56	+	T790M
18321PL	1900	0	0	-	None
18329PL	490000	9	0.002	-	L792F
18335PL	7600	0	0	-	None
18354PL	240000	9	0.004	+	T790M
18363PL	30000	9	0.03	+	T790M
18406PL	2975	0	0	-	None
18409PL	81500	0	0	-	None
18417PL	12100	0	0	-	None
18530PL	6260	0	0	-	None

#When present T790M makes the tyrosine kinase inhibitors Tarceva (erlotinib) and Iressa (gefitinib) ineffective and requires a change in therapy.
*In 3ml plasma, **Confirmed by sequencing

Biocept Microchannel: Spike and Recovery of H1975 from whole blood

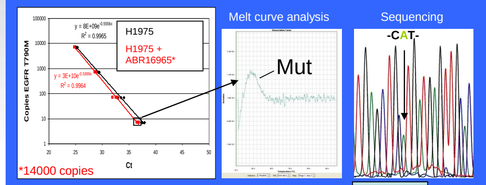


*Selector and Reporter added post-amplification
#Background C contains about 10% mutant so it is detected in melt and sequencing analysis in the absence of Selector

Results

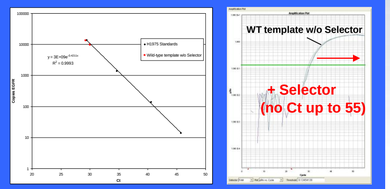
Selector™ Assay Performance

The Selector™ Assay is Quantitative and A Large Excess of Wild-Type Genomic DNA Minimally Effects Selector™ Assay Performance



WT: C**C**T
T790M: C**A**T

Suppression of Wild-type Amplification by Selector™



In the presence of the Selector™ Probe the wild-type target is completely suppressed

Selector™ Assay T790M Matches Closely the Microchannel Results

Spike	H1975 cells in Assay eluted from microchannel	
	Captured on microchannel (Assay equivalent)	Number of Detected cells after elution (Selector™ Assay w/ Selector)
A	0	0
B	3	3
C	16	36

Conclusions

- **Selector™ Assay suppresses wild-type amplification by >100,000 fold.**
- **Has little to no suppressive effect on the amplification of mutant alleles.**
- **Mutations are detected in a wild-type background at better than 1:10,000.**
- **The presence of a wild-type allele at >2,000 fold excess, in a complex genomic background has no adverse effect on mutant allele amplification, detection or quantification.**
- **Works with both DNA and RNA targets from clinical samples.**
- **Demonstrated the utility of the T790M Selector™ assay in NSCLC patient samples.**
- **Works in real-time, end-point, and melt-curve analysis. Seamlessly interfaces to sequencing, and other confirmatory methods of analysis, once mutant alleles are selectively amplified.**

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