Performance of IntelliPlex™ Companion Diagnostics Application in Cancer Management

Abstract Number : 2744
AACR Poster: 4145

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Introduction

Cancer is a group of various diseases with diverse causes which lead to the uncontrolled growth of abnormal cells. Somatic mutations of several oncogenes are known to play crucial roles in the cancer progression. Due to the complexity of cancers, cancer management is shifting to the field of precision and personalized medicine by tailoring the treatment based on the underlying genetic alteration. Hence, molecular profiling has become an indispensable tool for targeted treatment therapies to improve cancer management.

The power and value of molecular diagnostics approach is often hampered by the complexity and diversity of mutational status of genetic alteration, making it a time-consuming and tedious undertaking. However, now these multiplex assays and multiple reactions to determine a series of related mutations can be reduced to single reaction, which saves cost, sample, and time. The IntelliPlex™ system combines familiar PCR workflow with πCode™ (Precision Image Code) technology, providing a powerful multiplexing solution for molecular profiling in just one reaction.

PlexBio has developed several single target gene assays utilizing DNA samples derived from formalin-fixed paraffin-embedded (FFPE) tumor tissues. The IntelliPlex EGFR Mutation kit is intended for detection of 40 mutations in the EGFR gene. The IntelliPlex BRAF V600 Mutation Kit is designed for identification of 7 nucleotide changes. The IntelliPlex KRAS Mutation Plus kit targets 25 single nucleotide changes in the KRAS gene, and the IntelliPlex NRAS Mutation kit qualitatively detects 33 single nucleotide changes in the NRAS gene.

All IntelliPlex assays analyze the mutational status of each target in less than 5 hours (nucleic acid extraction included) and allow high-throughput screening of up to 94 samples in parallel with minimal hands-on time. In combination with SelectAmp, a mutation-enriching PCR amplification that dramatically increases mutation detection sensitivities, achievement of sensitivities up to 0.1% is common in many mutation points. PlexBio's IntelliPlex™ system is readily applicable to high complexity mutation analysis and is suitable to support precision medicine in cancer management. In addition, the system offers a rapid and cost-effective way to analyze patient specimens with high sensitivity and specificity in a clinical environment.

Method

Figure 1. Mutation-enriching PCR Amplification via SelectAmp Technology
Wild-type blockers selectively block wild-type template amplification, which leads to an enrichment of biotinylated mutant amplicons. Subsequently, mutant amplicons are hybridized with mutation-specific probe coupled on πCode (Figure 2).

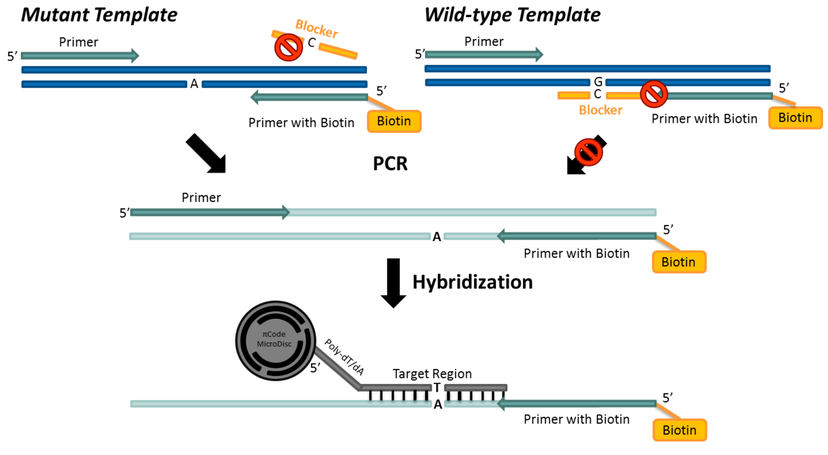


Figure 2. Multiplexing Assays using πCode MicroDisc Technology
πCode MicroDisc is manufactured to generate more than 85,000 distinct circular image patterns for multiplexing applications. Each πCode MicroDisc has a distinct circular image pattern, which corresponds to a specific capture agent conjugated to the surface of the disc. πCode tagged with different capture agents are pooled, enabling specific detection of multiple analytes in a one well reaction.

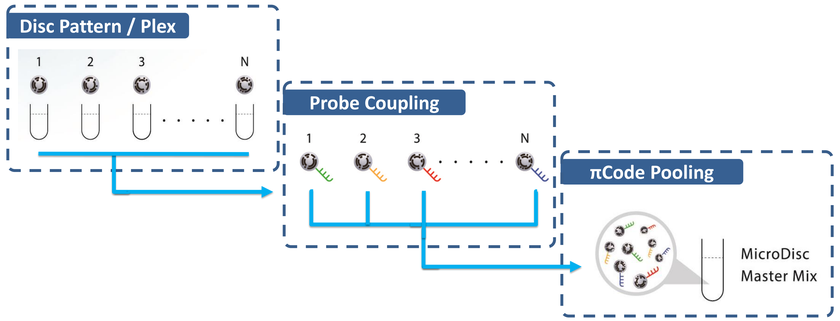
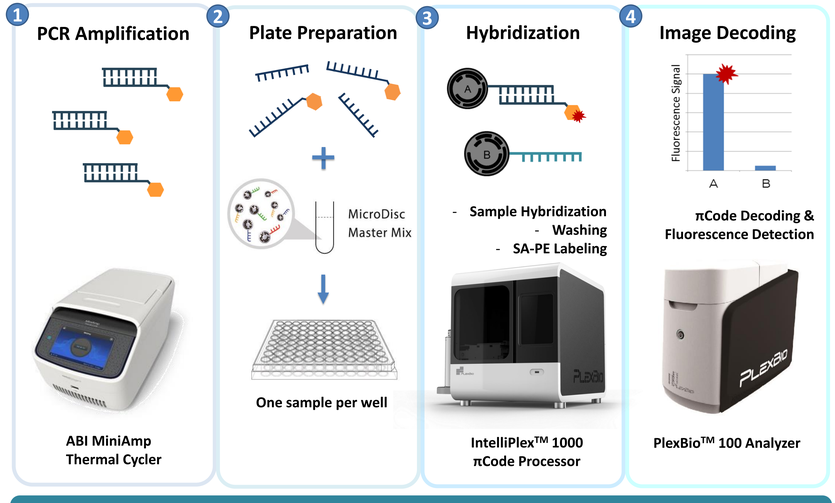


Figure 3. Workflow of IntelliPlex™ System



Results

The performance of IntelliPlex system was evaluated on archived FFPE DNA specimens by comparing the results of the respective IntelliPlex assay with results previously obtained using the commercially available molecular assays (Roche Cobas, EntroGen). All tests were conducted in a hospital lab located in Italy.

EGFR		RT-PCR based Comparison Method			
		Positive	Negative	Concordance	90.0 %
IntelliPlex EGFR Mutation kit	Positive	15	0	Sensitivity :	88.2 %
	Negative	2	3	Specificity :	100 %

KRAS		RT-PCR based Comparison Method			
		Positive	Negative	Concordance	95.8 %
IntelliPlex KRAS Mutation Plus kit	Positive	23	0	Sensitivity :	95.8 %
	Negative	1	0	Specificity :	N/A

NRAS		RT-PCR based Comparison Method			
		Positive	Negative	Concordance	93.8 %
IntelliPlex NRAS Mutation kit	Positive	13	0	Sensitivity :	92.9 %
	Negative	1	2	Specificity :	100 %

BRAF		RT-PCR based Comparison Method			
		Positive	Negative	Concordance	93.8 %
IntelliPlex BRAF Mutation kit	Positive	12	0	Sensitivity :	92.3 %
	Negative	1	3	Specificity :	100 %

Summary

- Among 76 FFPE DNA specimens tested, the overall agreement between IntelliPlex system and the comparison method was found to be 93.4%. The individual concordance for each assay kit is 90.0%, 95.8%, 93.8%, and 93.8% for EGFR, KRAS Plus, NRAS and BRAF, respectively.
- Highly sensitive assays for detecting somatic mutations in FFPE samples are achieved by the combination of SelectAmp and πCode™ Technology.
- All IntelliPlex mutation assays above are CE IVD approved.

Overall Performance		RT-PCR based Comparison Method			
		Positive	Negative	Concordance:	93.4 %
IntelliPlex Assay kits	Positive	63	0	Sensitivity :	92.6 %
	Negative	5	8	Specificity :	100 %

References
1. Nature Reviews Clinical Oncology (2017) ; doi:10.1038/nrclinonc.2017.14
2. Patrick L Dominguez and Michael S Lolodnev 2005. Oncogene 24 6830-6834.

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