Comprehensive Coverage of Lung Cancer Somatic Mutations for Liquid Biopsy Testing by πCode™ Technology

Shengfong Huang[1], Shian-Pin Hu[1], Aidan Coe[1], H-Eugene Liu[2], Yen-Lin Chen[3], Stuart M. Palmer[1], Chin-shiou Huang[1], Dean Tsao[1]

[1]PlexBio Co., Ltd., Taipei, Taiwan; [2]Department of Hematology and Oncology, Municipal Wanfang Hospital, Taipei, Taiwan;
[3]Department of Pathology, Fu-Jen Catholic University, New Taipei, Taiwan

Introduction

Somatic mutations of several oncogenes are known to play critical roles in the progression to metastatic stages in non-small cell lung cancer (NSCLC). It is arguably the most well-known example of using molecular diagnostics to guide cancer treatment. As powerful as this approach has been proven, it is hampered by the tedious assay workflows needed to determine multiple mutation sites and by the invasive and laborious procedures needed to obtain tissue samples. Two methodologies have been developed to overcome these obstacles. By using multiplex assays, multiple reactions to determine a series of related mutations can be reduced to single reactions, saving cost, sample, and time. Liquid biopsy is also gaining broader utility owing to its non-invasiveness, compatibility with higher throughput platforms and is seen as a method to replace tissue sample analysis. Utilizing both these approaches, PlexBio has developed a multiplex detection platform based on the Precision Image Code (πCode) Technology which is readily applicable to high complexity mutation analysis. One of PlexBio's products, IntelliPlex™ EGFR Mutation Kit, is designed to assess the status of 40 somatic mutations of the EGFR gene in tissue or liquid biopsy samples. 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 making this analytic approach ideally suited for liquid biopsy assays. In addition to EGFR, PlexBio has developed a series of CDx products for KRAS, BRAF, NRAS, PIK3CA, ALK, NTRK1, RET, MET, and ROS1 genes for lung cancer mutation or variant detection with similar capabilities. The combined analysis of the products of all 10 genes now make it feasible to determine the status of ~160 individual mutations in just a few multiplex reactions and offers comprehensive coverage of somatic mutation analysis related to lung cancer.

Methods

Figure 1. Workflow of Multiplexing Assays Using πCode™ Technology

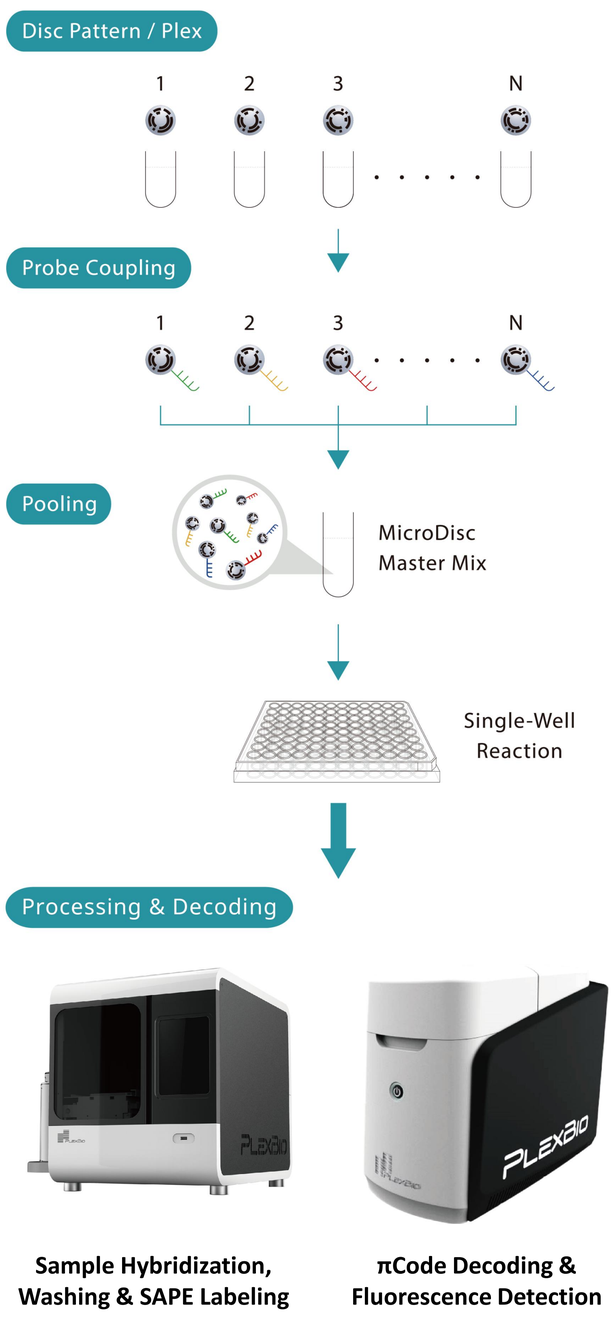
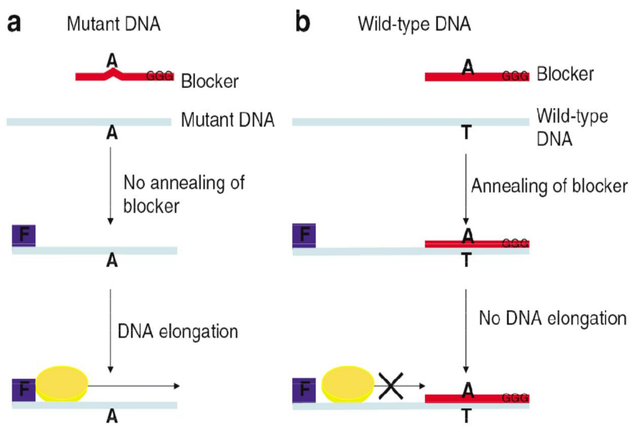


Figure 2. Mutation-enriching PCR Amplification via SelectAmp Technology LNA allows amplification of mutant DNA but not the wild-type.



Results

Table 1. Cell-free DNA (cfDNA) Detection for EGFR Mutation Assay Sensitivities of EGFR Mutation Assay using cell-free DNA (cfDNA) were evaluated by testing mutant plasmid blended with a total of 20ng cfDNA from wild-type EGFR.

Mutation Gene	Exon	A.A Mutation	Sensitivity
EGFR	Exon12	S492R	0.05%
	Exon18	G719C	0.05%
	Exon19	E746_A750del	0.1%
	Exon20	T790M	0.5%
		H773_V774insH	0.5%
	Exon21	L861Q	0.05%
		L858R	0.1%

Figure 3. Specificity of EGFR Mutation Assay The results showed that the πCode MicroDisc fluoresces only when the mutation carried by the capture probe is identical to that is the sample DNA, except for the mutant E746_T751>IP, which exhibits cross reactivity for E746_A750>IP.

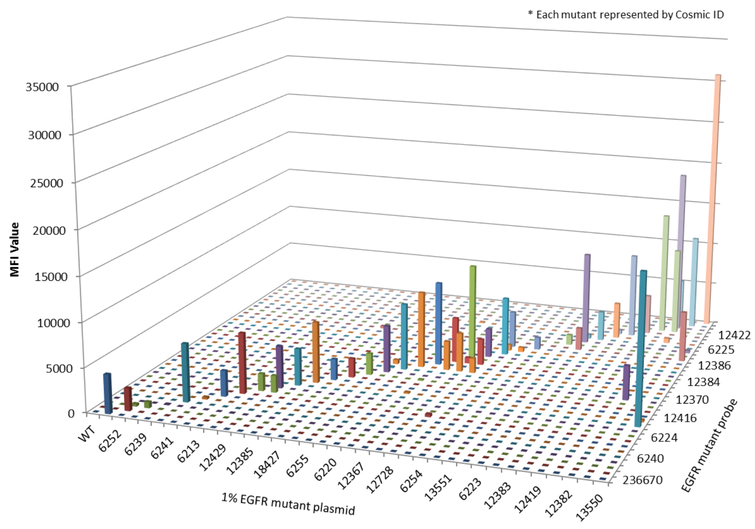


Table 2. EGFR Mutation Profile in cfDNA as Determined by IntelliPlex™ EGFR Kit is Highly Consistent with that in FFPE by a Roche Kit.

Patient No.	Pathology Result	Roche's cobas EGFR Mutation Kit using FFPE specimens	IntelliPlex EGFR Mutation Kit using pleural effusion specimens
1	Lung adenocarcinoma	WT	WT
2	Lung adenocarcinoma	WT	WT
3	Carcinoma	WT	WT
4	Lung adenocarcinoma	WT	WT
5	Lung adenocarcinoma	Exon 20 insertion	WT
6	Inflammation	Not Tested¥	Invalid (Low sample concentration)
7	Lung adenocarcinoma	Exon 20 insertion	Exon 20 insertion (Cosmic ID: 12376)
8	Neuroendocrine carcinoma	Not Tested¥	WT
9	Lung adenocarcinoma	WT	WT
10	Lung adenocarcinoma	WT	WT
11	Inflammation	Not Tested¥	Exon19 deletion (Cosmic ID: 6223)
12	Lung adenocarcinoma	L858R	L858R
13	Lung adenocarcinoma	G719X	G719S
14	Lung adenocarcinoma	L858R	L858R
15	Lung adenocarcinoma	L858R	L858R

¥ FFPE sample is not available.

Figure 4. Comparison of Mutation-Specific Signal between Healthy and Lung Cancer Patients The signals for EGFR mutations are significantly higher in the cfDNA of lung cancer patients than those in healthy counterpart.

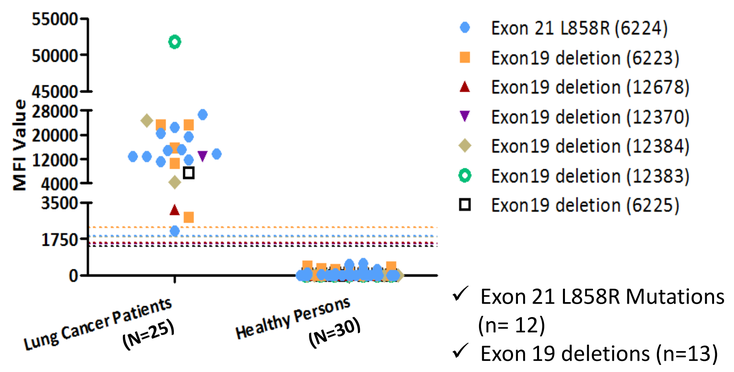


Table 3. Summary of IntelliPlex™ EGFR Assay for cfDNA in Plasma EGFR mutations were detected in 27 lung cancer patients among the total of 66 patients tested (41%). Among the 39 lung cancer patients that tested negative for EGFR mutations, 10 of them were detected with one of or combination of KRAS, NRAS, PIK3CA gene mutations and ALK Rearrangements.

Lung Cancer Patients		
No. of Patients		66
EGFR Mutation Detected	Exon12 S492R	1
	Exon19 deletion	13
	Exon 21 L858R	12
	Exon 21 L861Q	1
WT		39
Detection Rate (%)		41%
Healthy Persons		
No. of Tested		30
WT		30

Summary

- Highly sensitive assays for detecting somatic mutations in liquid biopsies is achieved by the combination of SelectAmp and πCode™ Technology.
- The EGFR mutation profile found in liquid biopsies is consistent with that found in tissue samples.

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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