Multiplex Assays for Cancer Management Using πCode™ MicroDisc Technology

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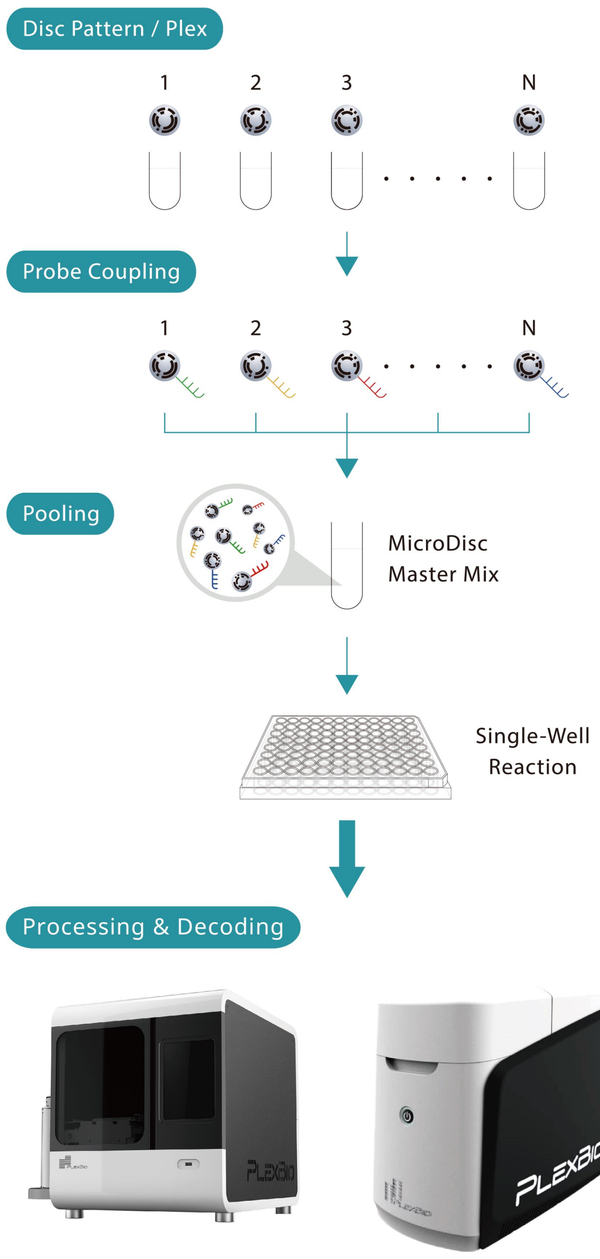
Introduction

The path to a successful cancer management rarely relies on a single independent selection of therapeutic choices, in fact, it often requires a collection of meaningful information in order to offer a holistic approach for the patient, beginning from diagnosis, therapeutic selection, treatment implement all the way to prognosis, monitor recurrence stages. For example, besides the recognizable factors that affect the types of treatment are given, like the type of cancer the patient has or how advanced the disease is, whether or not certain somatic mutations existed in patient's genes will also significantly impact the treatment selection. The determination of somatic mutations in various oncogenes to direct the therapeutic strategies in individual patients has greatly enhanced cancer management. But as useful as this approach is, it can be hampered by the invasive and lengthy procedures needed to obtain tissue samples from the patient and cumbersome assay workflows needed to determine multiple mutation sites. 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 sampling is also gaining broader utility owing to ability to enable clinicians to determine a patient's cancer status with a simple blood or fluids draw and to then quickly implement a treatment plan. PlexBio has developed a multiplex detection platform based on the Precision Image Code (πCode) Technology which is readily applicable to high complexity mutation analysis in FFPE and clinical liquid biopsy specimens. Each πCode MicroDisc carries a distinct circular image pattern and enables capturing and detection of multiple, specific analytes simultaneously in a single reaction well from a complex sample. PlexBio's IntelliPlex™ Lung Cancer Panel, has been shown the ability to assess 55 gene mutations and gene rearrangements (36 DNA single gene mutation sets and 19 RNA gene rearrangement sets) from either from FFPE or clinical liquid biopsy specimens associated with NSCLC. This two-reaction panel was developed using the existing reagent sets of PlexBio's stand-alone CDx products for EGFR, KRAS, BRAF, NRAS, PIK3CA, ALK and ROS1 gene mutations and gene rearrangement assays. In combination with SelectAmp, a mutation-enriching PCR amplification technology to selectively amplify specific mutant sequences while blocking wild-type amplification, a significant increase in mutation detection sensitivity, often to less than 0.1%, makes this analytic approach well-suited for liquid biopsy samples. When using the IntelliPlex™ Lung Cancer Panel to determine the mutation status of clinical liquid biopsy specimens, the results were highly consistent with those from the third-party evaluations and NGS. The PlexBio system offers highly sensitive and specific results and can serve first-line diagnostic requirements as well as subsequent testing needs. This places the system's multiplexing advantages in the complexity space bounded at the low end by traditional single test methods and large NGS panels which may not be practical for recurrent monitoring applications. Also, with the development of a real-time assessment of tumor status using liquid biopsy method through PlexBio multiplex system, it further empowers physicians to monitor treatment selections and responses, hence, optimizing the chance of having a successful outcome.

Methods

Figure 1. Workflow of Multiplexing Assays Using πCode™ Technology - πCode™ is acronym for "Precision Image Code", it is a circular disc manufactured by semi-conductor processes and able to generate over 16,000 distinct image patterns for multiplexing applications. To be used in a broad spectrum of applications, each circular microdisc is encapsulated in a highly stable matter with an added paramagnetic property, which allows it to be suitable for conjugation, washing, and automation procedures.



Sample Hybridization, Washing & SAPE Labeling

πCode Decoding & Fluorescence Detection

Results

Table 1. **Example IntelliPlex™ Kits Developed by PlexBio for FFPE-** IntelliPlex™ kits include CDx assay kits for both the detection of somatic mutations and gene rearrangement in oncogenes.

Product Line	Assay	Sample Type	Target	Sensitivity
Companion Diagnostics* (CDx)	KRAS Mutation	FFPE	12 / 25 Mutations	0.05% - 1.8%
	EGFR Mutation	FFPE	40 Mutations	0.5% - 2.9%
	BRAF Mutation	FFPE	7 Mutations	0.29% - 1.6%
	NRAS Mutation	FFPE	33 Mutations	0.14% - 6.5%
	PIK3CA Mutation	FFPE	17 Mutations	0.14% - 4.0%
	ALK Rearrangement	FFPE	24 Variants	5 ~ 1209 copies
	ROS1 Rearrangement	FFPE	14 Variants	≤ 5 ~ 120 copies
	RET/NTRK1 Rearrangement	FFPE	14 Variants	12 ~ 486 copies

*Products Not Currently Approved in USA

Table 3. **IntelliPlex™ Lung Cancer Panel for FFPE-** NSCLC mutations profiling in FFPE and tissue determined by IntelliPlex™ Lung Cancer Panel is consistent with results tested by third party single gene testing.

Sample ID	Lung Cancer Panel – FFPE (DNA)					External 3rd Party Single Gene Testing**
	EGFR	KRAS	NRAS	PIK3CA	BRAF	
1	WT	G13D	WT	WT	WT	EGFR WT
2	p.L747_T751del	WT	WT	WT	WT	EGFR L747_T751del
3	WT	G12C	WT	WT	WT	EGFR WT
4	p.L858R	WT	WT	WT	WT	EGFR L858R
5	p.E746_A750del	WT	WT	WT	WT	EGFR E746_A750del
6	p.L858R	WT	WT	WT	WT	EGFR L858R
7	p.E746_A750del	WT	WT	WT	WT	EGFR E746_A750del
8	WT	G12A	WT	WT	WT	EGFR WT
9	WT	G13C	WT	WT	WT	EGFR WT
10	WT	WT	WT	WT	V600E1	BRAF
11	WT	WT	WT	WT	V600E1	BRAF
12	WT	G12V	Q61K	WT	WT	KRAS
13	WT	WT	WT	WT	WT	EGFR ex19
14	WT	WT	WT	WT	WT	KRAS 146
15	p.L747_P753>S	WT	WT	H1047R	WT	EGFR ex19
16	WT	Q61K	WT	WT	WT	KRAS 61
17	WT	WT	WT	WT	WT	KRAS 146
18	L858R	WT	WT	WT	WT	EGFR L858R

*Product under development

**Results #1~#9 were verified by ddPCR for EGFR only; results #10~#18 were verified by approved RT-PCR Methods for EGFR, BRAF and KRAS

Table 5. IntelliPlex™ Lung Cancer Panel for FFPE and mock samples - NSCLC mutations profiling determined by IntelliPlex™ Lung Cancer Panel is consistent with results tested by NGS from third party site.

Sample ID	Sample Type	Lung Cancer Panel (RNA)					External 3rd Party NGS Testing**
		ALK	ROS1	RET	NTRK1	cMET	
1	FFPE	v1	WT	WT	WT	WT	V1
2		v1	WT	WT	WT	WT	V1
3		WT	CD74-ROS1	WT	WT	WT	-
4		v1	WT	WT	WT	WT	V1
5		v1	WT	WT	WT	WT	v1
6		WT	WT	WT	WT	WT	ROS1 overexpression
7	Cell Line	WT	WT	CCDC6-RET	WT	WT	CCDC6-RET
8		v3b	WT	WT	WT	WT	EML4-ALK
9		WT	SLC34A2 -ROS1	WT	WT	WT	SLC34A2-ROS1
10		WT	WT	WT	WT	WT	TPM3-TRKA

*Product under development **Sample 3 under further evaluation

Table 2. Clinical Sensitivity and Specificity of IntelliPlex™ KRAS G12/13 Mutation Kit – The clinical data shows that the sensitivity and specificity of IntelliPlex™ KRAS G12/13 Mutation Kit falls within the range of 96.19-100 and 85.12-94.91 respectively. The results include initial sanger sequencing and resolution by pyro sequencing.

		Sequencing (N=248)*	
		Mutation Detected	Mutation Not Detected
IntelliPlex™ KRAS G12/13 Mutation Kit	Mutation Detected	95	14
	Mutation Not Detected	0	139
	Total	95	153

	IntelliPlex KRAS G12/13 Mutation Kit
Clinical Sensitivity (95% CI)	100% (96.19 – 100)
Clinical Specificity (95% CI)	90.8% (85.12 – 94.91)

Clinical Sensitivity = [True Positives / (True Positives + False Negatives)] * 100%
Clinical Specificity = [True Negatives / (True Negatives + False Positives)] * 100%

Table 4. IntelliPlex™ Lung Cancer Panel for Cytology and Liquid Biopsy- NSCLC mutations profiling determined by IntelliPlex™ Lung Cancer Panel is consistent with results tested by varies methods (RT-PCR, ddPCR) from third party site.

Sample ID	Sample Type	Lung Cancer Panel (DNA)					External 3rd Party Single Gene Testing**
		EGFR	KRAS	NRAS	PIK3CA	BRAF	
1	Cytology	T790M	WT	WT	E545Q	WT	EGFR L747_751>P & T790M
2		WT	G12D	WT	WT	WT	EGFR WT
3		WT	G12C	WT	E542K	WT	EGFR WT
4	cfDNA	p.L747_P753>S, T790M	WT	WT	WT	WT	EGFR L747_P753>S & T790M
5		p.E746_A750del	WT	WT	WT	WT	EGFR E746_A750del & T790M
6		p.L747_T751del	WT	WT	WT	WT	EGFR L747_T751del
7		WT	WT	WT	WT	WT	EGFR WT
8		WT	WT	WT	WT	WT	EGFR WT

*Results verified by ddPCR for EGFR only

Summary

- PlexBio has developed a series of individual CDx kits for gene mutation and rearrangement assays, and has further expanded its application by pooling together these single kits to develop oncology panels such as lung cancer.
- IntelliPlex™ Lung Cancer Panel is highly sensitive assays for detecting somatic mutations and gene rearrangements in FFPE as well as in liquid biopsies.
- Mutation profiling results from IntelliPlex™ Lung Cancer Panel is consistent with the results from various detection methods.

References

1. Nature Reviews Clinical Oncology (2017) ; doi:10.1038/nrclinonc.2017.14
2. Myron G. Best, NiK Sol, et al. Cancer Cell 28, 666-676, November 9
3. Lan-Ying Gou and Yi-Long Wu, Lung Cancer: Targets and Therapy 2014:5

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