Customized Cancer Mutation Panel Development on the Luminex Platform

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ABSTRACT

Mutation detection panels are designed and used for cancer patient profiling in clinical studies/trials, and companion diagnostics for actionable mutations. Currently, these panels are designed and applied on NGS platforms, which are more costly and time-consuming.

Here, we have described the customized design of QClamp® Plex that can detect up to 100 targets using the Luminex platform. We added our proprietary XNA technology to the design to enrich the mutant alleles of the targets and increase the assay sensitivity to below 0.1% VAF (Variant Allele Frequency) using 10 ng of cell-free DNA. Through amplification, ligation, and hybridization (3-steps) the assay specifically detects designated targets without sequencing.

The whole detection procedure takes one day to complete, significantly reducing the time and cost spent on the workflow. Because of the customized design, one can make any cancer panels of their interest based on their own studies. Pharma companies can use the customized panel design for companion diagnostic tests as well.

INTRODUCTION

Targeted NGS panels have been widely used for both research and clinical practice. For research use, the panels profile patients in both translational studies and clinical trials before and after drug therapy. In clinical practice, NGS panels are often used to identify gene alterations in biopsy after cancer diagnosis. The mutation information can be used to guide target therapies.

However, NGS testing takes 1~2 weeks to get answers and is generally more costly. The Luminex platform, on the other hand, has much shorter turn-around times and is less costly. Adding these mutation targets on the Luminex platform through DNA hybridization, the mutations can be semi-quantified and up to 100 of these targets can be added to a single panel and individually detected based on the spectral signature of the bead.

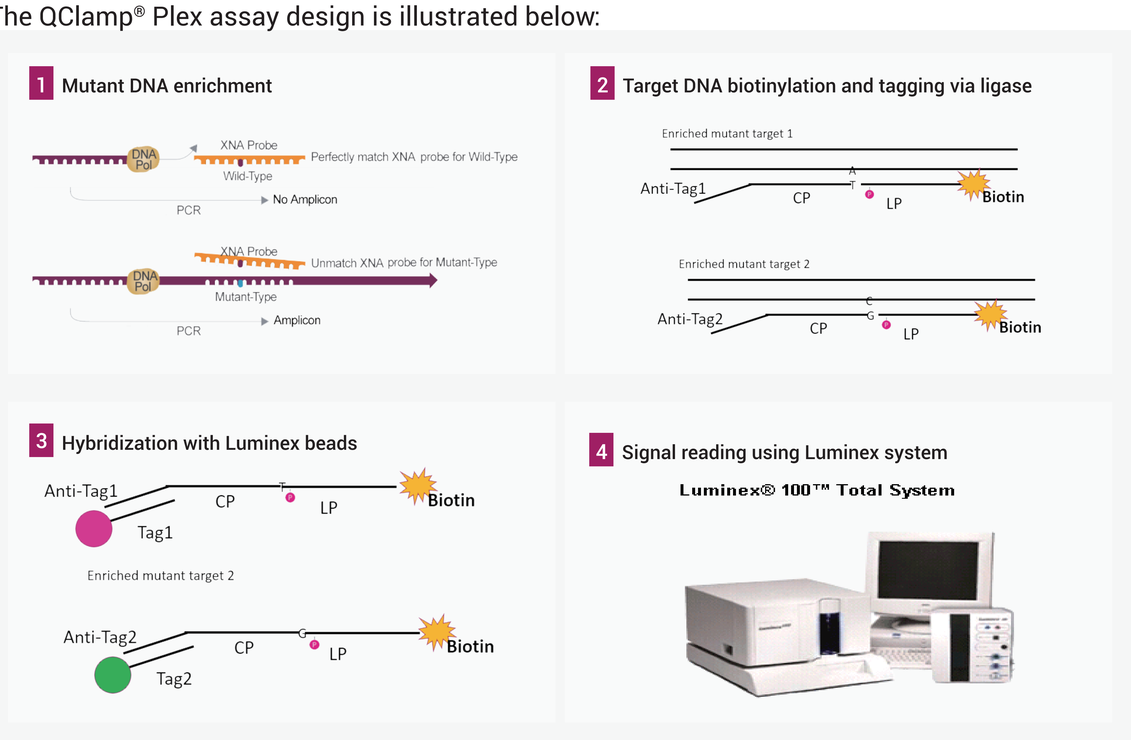
Based on the advantages of Luminex technology and wide availability of the instruments worldwide, we have developed the QClamp® Plex assay to showcase multiple gene mutation detection using the Luminex platforms. Our assay includes three steps: target enrichment, ligation, and hybridization. We add our proprietary XNA technology to the assay to enrich low-frequency mutations in cell-free DNA (cfDNA), which significantly increases assay sensitivity and overcomes the problem of sensitivity limitations that liquid biopsy may have for mutation detection. Ligation is the next step, which also helps to select against the wildtype allele, further reducing the background and increasing specificity. Finally, by hybridizing with probes conjugated on the corresponding beads, as many as 100 targets are detected and relatively quantified.

Here we have shown the data for detection of 9-targets associated with colorectal cancer, with the same targets used in development of our ColoScape test. All of the targets can be detected at 0.1% VAF or better. The assay can be customized for different targets and applications. The high sensitivity capability of the assay provides the platform with great potential for MRD (Molecular Residual Disease) testing in addition to regular detection of mutations in selected targets.

RESULTS

Assay Design

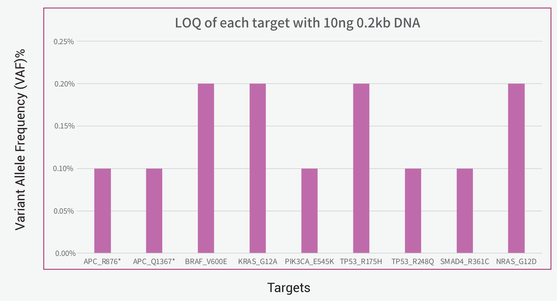
The QClamp® Plex assay design is illustrated below:



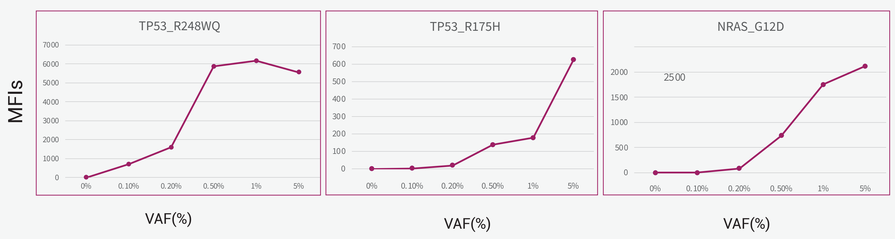
QClamp® Plex Example – Colorectal Cancer Panel

Tags matched on beads	Gene	Target location (codon)
Tag1	Actin	Wildtype, internal control
Tag2	APC	Q1367
Tag3	APC	R876
Tag4	BRAF	V600
Tag5	KRAS	G12 G13
Tag6	NRAS	G12
Tag7	PIK3CA	E542
Tag8	SMAD4	R361
Tag9	TP53	R175
Tag10	TP53	R248

Detection of Mutant Population for 9 Targets

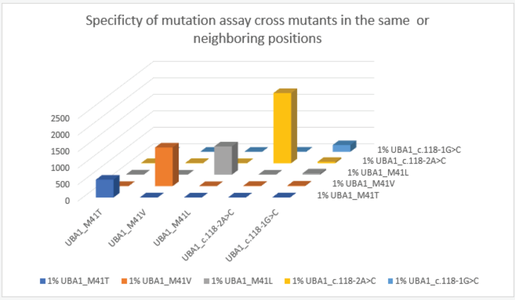


Various Mutant Populations Detection are NOT in the Linear Range



The Mutation Detection by the QClamp® Plex Assay is Specific

We have tested the specificity of mutations by detecting cross-reactions with the other neighboring mutations in the next codon or even the next base in the same codon. We do not find any cross-reactions of the neighboring mutations being detected.



Applications of Luminex Gene Panels

- Profiling of patients of different types of cancers
- Determination of actionable mutations
- Potential MRD applications especially when XNAs are integrated in the assay design

CONCLUSIONS

- We have shown that the Luminex platform can successfully detect low-frequency mutations when integrated with the XNA technology
- The QClamp® Plex assay can be customized for designated gene panels