Simultaneous Detection of KRAS and TP53 Mutations in Human Cancer Cell Lines Using Multiplex qPCR

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ABSTRACT

Genetic alterations in KRAS and TP53 genes are common in many cancers and the understanding of their role in progression of cancer is vital for the design of successful treatment approaches. Studies suggest that mutations in *KRAS* and *TP53* genes independently hindered the use of EGFR inhibiting drugs like Erbitux Cetuximab® for the treatment of colorectal cancer (1). The genetic alterations in these genes are checked before starting any expensive treatments. Here, we successfully demonstrated use of duplex multi-color SNP genotyping assays to detect KRAS and TP53 mutation in human colon cancer cell lines. This method can be potentially used to analyze samples with limited quantity and get as much information as possible from a single sample. To study the impact of these mutations, we generated singleplex gene expression profiles of 672 genes related to signaling pathways by using the Human signal transduction OpenArray® Panel. Selected cancer genes like *EGFR, BAX, CFLAR, MYC,* and others were tested using multiplexed single-tube PCR simultaneously detecting four different targets in the same sample. These results demonstrate duplex SNP genotyping and multiplex gene expression profiling makes more efficient use of sample than singleplex PCR.

INTRODUCTION

Life Technologies has introduced a new multiplexing solution with four reporter dyes, a new quencher, and a new multiplex master mix. ABY® and JUN® are new reporter dyes which complement our existing offering of FAM™ and VIC® reporter dyes for multiplexing. These four reporter dyes with distinct spectra are optimized to work together with minimal spectral overlap (Fig.1). We have developed a new non -fluorescent quencher, QSY®, for designing custom TaqMan® probes in order to facilitate multiplex qPCR with the four dyes. Finally, we have developed a new TaqMan® Multiplex Master Mix to be compatible with these four reporter dyes and give accurate and precise results. The master mix uses MUSTANG PURPLE® (MP) as a passive reference dye. This multiplexing solution is compatible with Life Technologies' QuantStudio™ 6, 7, and 12K Flex, ViiA™ 7, and 7500/7500 Fast Real-Time PCR systems.

Figure 1: Fluorescence emission spectra of different dyes used for multiplex PCR.

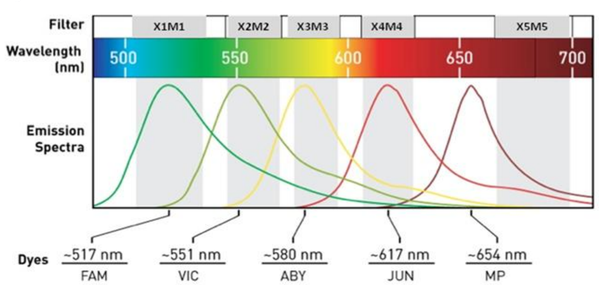
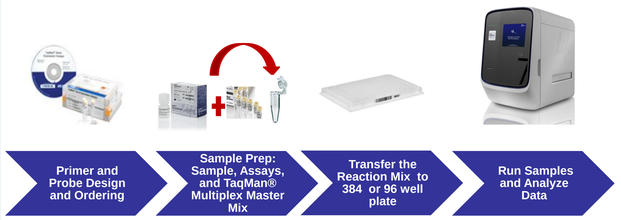


Figure 2: TaqMan® Multiplex Workflow



Multiplex Workflow. Detailed protocol for genotyping, gene expression, and copy number variation (CNV) analysis are available on the Life Technologies website (2).

Figure 3. Experimental Approach for Multiplexing: Genotyping and Gene Expression

Genotyping (GT)

- Obtain genomic DNA from:
  - SW620 colorectal adenocarcinoma cell line
  - Panc 03.27 pancreatic adenocarcinoma cell line
  - MDA-MB-468 breast adenocarcinoma cell line
  - 23 normal individuals for reference
- Design multiplex GT assays
  - Tp53 R273H
  - KRAS G12V
- Multiplex GT assays with validation by singleplex SNP GT on QuantStudio™ 7 system

Gene Expression (GEx)

- Obtain total RNA from:
  - SW620 cells
  - Human Total Colon RNA
- Converted to cDNA using SuperScirpt VILO cDNA Synthesis Kit
- Design multiplex GEx assays (Gene selection was done with profiling the samples using OpenArray® on QuantStudio™ 12K Flex Real-Time PCR System)
- Multiplex GEx assays with validation by single gene expression assay on QuantStudio™ 7 system

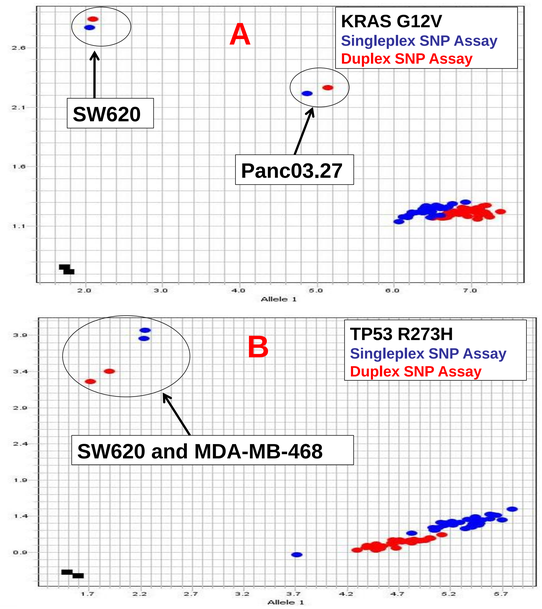
Sample Information

Samples	Source	Mutations *	Zygosity *
SW620	ATCC: CCL-227	KRAS G12V (c.35G>T), TP53 R273H (c.818G>A)	Homozygous
Panc03.27	ATCC: CRL-2549	KRAS G12V (c.35G>T)	Heterozygous
MDA-MB-468	ATCC: HTB-132	TP53 R273H (c.818G>A)	Homozygous
Human Colon Total RNA	LT: AM7986		
Reference Human gDNA	Coriell		

* Courtesy : COSMIC (Catalogue of Somatic Mutations In Cancer)

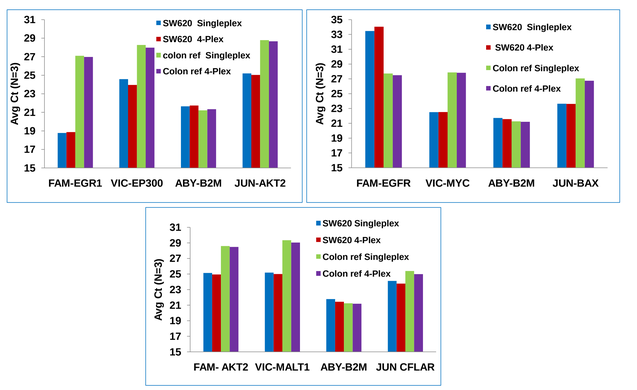
RESULTS

Figure 4: Multiplex Genotyping Application



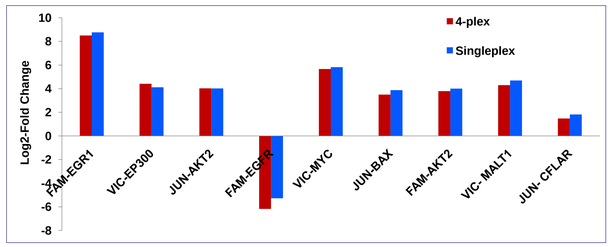
Comparing KRAS G12V and TP53 R273H genotyping in singleplex (Blue) and duplex (Red) reactions across three cancer cell lines and 23 Coriell gDNA samples at 10ng/rxn. The KRAS assays are FAM™ &VIC® labeled with MGB and the TP53 assays are ABY® and JUN® labeled with QSY®. The respective final concentrations of primers and probes was 900nM and 200nM. A: Panc 03.27 and SW620 are shown to be heterozygous and homozygous for the KRAS G12V mutation (G>T), respectively. B: MDA-MB-468 and SW620 are homozygous for a G>A mutation in TP53.

Figure 5: Multiplex Gene Expression Application



Comparison of average Ct (N=3) of 10 loci from SW620 cell line RNA and normal colon RNA in singleplex and multiplex qPCR format. Comparison of 10 ng of total cDNA in a 4-plex reaction vs. 40ng of total cDNA divided into four singleplex reactions.

Figure 6: Fold Change Comparison



Fold change comparison of nine targets normalized to B2M in singleplex and multiplex qPCR format.

CONCLUSIONS

- **We successfully detected KRAS and TP53 mutation in human cancer cell lines using a new TaqMan® Multiplex solution.**
- **The new multiplexing solution is as accurate and sensitive as singleplex qPCR on the same loci.**
- **Multiplex qPCR results comparable to singleplex qPCR can be obtained with less input material, saving time and cost associated with complex project.**

REFERENCES

1. A Oden-Gangloff, F Di Fiore, F Bibeau, A Lamy, G Bougeard, F Charbonnier, F Blanchard, D Tougeron, M Ychou, F Boissière, F Le Pessot, J-C Sabourin, J-J Tuech, P Michel and T Frebourg.*TP53 mutations predict disease control in metastatic colorectal cancer treated with cetuximab-based chemotherapy. British Journal of Cancer* (2009) 100, 1330–1335
2. TaqMan® Multiplex PCR Optimization User Guide, Life Technologies

ACKNOWLEDGEMENTS

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TRADEMARKS/LICENSING

The QuantStudio™ 6,7, and 12K Flex Real-Time PCR System is For Research Use Only. Not for use in diagnostic procedures.