applied biosystems

A high-throughput approach for multi-omic testing for prostate cancer research

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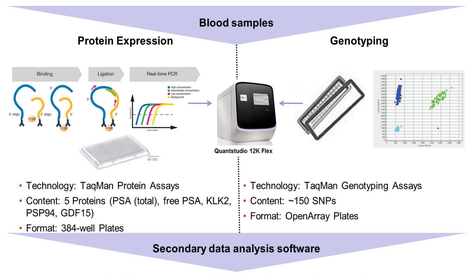
INTRODUCTION

The proliferation of genetic testing technologies and genome-scale studies has increased our understanding of the genetic basis of complex diseases. However, this information alone tells an incomplete story of the underlying biology. Integrative approaches that combine data from multiple sources, such as the genome, transcriptome and/or proteome, can provide a more comprehensive and multi-dimensional model of complex diseases. Similarly, the integration of multiple data types in disease screening can improve our understanding of disease in populations. In a series of groundbreaking multi-omic, population-based studies of prostate cancer, researchers at the Karolinska Institutet in Stockholm, Sweden identified sets of genetic and protein biomarkers that when evaluated together with other clinical research data performed significantly better in predicting cancer risk (1,2) than the most-widely used single protein biomarker, the prostate-specific antigen (PSA). These studies are the basis of what is referred to as the Stockholm3 model which can potentially increase the detection of aggressive cancers by 20% while reducing the number of unnecessary biopsies by 50% when compared to PSA testing alone. Like many multi-omic approaches, these studies used different platforms for nucleic acid and protein analysis, which adds a layer of complexity to data collection and interpretation. In the current study, we have simplified and streamlined these processes by measuring both genetic and protein markers on the same platform, the Applied Biosystems QuantStudio™ 12K Flex Real-time PCR System. As in the previous studies, our workflow uses TaqMan® Genotyping Assays in OpenArray™ plates; however, we replaced the ISAC immunoassay technology with TaqMan® Protein Assays in 384 well plates. Both TaqMan® chemistries are optimized to maximize sensitivity and specificity and requires only a blood draw research sample. In addition, we automated the liquid handling steps for the protein assays which further simplifies the workflow. This approach could enable prostate cancer researchers to perform both large-scale studies and routine testing of genetic and protein markers on a single system.

MATERIALS AND METHODS

149 SNPs from the Stockholm3 studies were used in the current study, including 15 assays redesigns. Genotyping was performed in OpenArray plates on the QuantStudio 12k Flex Real-time PCR system using standard methods (Thermo Fisher Scientific, USA). Initial analysis of real-time PCR data was performed with TaqMan Genotyping Data Analysis Software (Thermo Fisher Scientific). Further analysis was performed with GAPP Software (Ridgeview AB, Sweden). 69 DNA control samples (Coriell, USA) were used for validating genotyping assays. A set of five Taqman Protein Assays for prostate cancer (Thermo Fisher Scientific) were used to measure protein concentrations in EDTA plasma samples. Test samples were provided by the Karolinska Institutet Biobank. CCM2 Software (Thermo Fisher Scientific) was used to determine protein concentrations. Protein concentrations obtained with the ISAC platform were provided by OncoAlgorithms AB (Sweden) for comparison.

Figure 1. Multi-omic Workflow Overview



Individualized genetic and protein marker profiles

RESULTS

Figure 2. Genotyping Workflow for OpenArray™ Plates

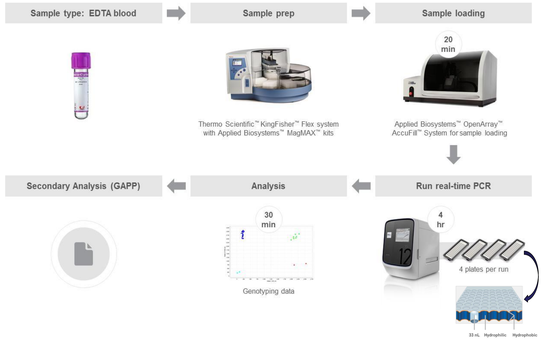


Figure 3. Genotyping Panel Content and Performance

- Genotyping panel consists of 149 SNPs with <0.2% minor variant frequencies under TaqMan primers/probes
- Control Coriell DNA samples were used to validate SNP assay performance on Applied Biosystems TaqMan OpenArray Plates and analyzed with TaqMan Genotyping Data Analysis Software.
  - GAPP Software was used for quality control and data management purposes
- Overall call rate ≥99% call rate, Overall accuracy >99.9%

Cluster plots of selected assays

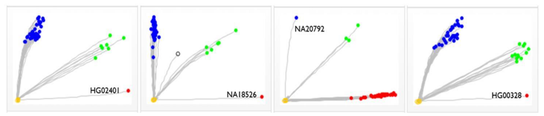
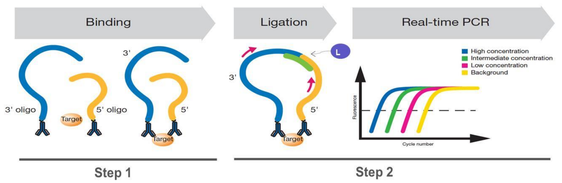


Figure 4. TaqMan Protein Assays Use Proximity Ligation Assay (PLA™) Technology



- Uses qPCR to detect and quantify proteins
- Assay based on two antibodies, each conjugated to a different oligonucleotide (one 3' oligo, one 5' oligo)
- When the two conjugated antibodies bind and are in close proximity, the oligonucleotides can be ligated, serving as the template for real-time PCR amplification and quantification

Table 1. Protein Assay Content and Assay Ranges

Assay Name	Assay Range (nU/mL) Maximum	Minumum
Free PSA (FPSA)	20	0.00128
Total PSA (PSA)	20	0.00128
KLK2	25	0.0016
PSP94	0.8	0.000256
GDF15	10	0.00064

Figure 5. Protein Assay Workflow for 384-well Plates

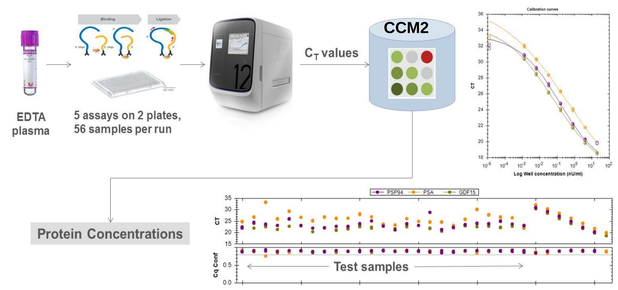


Figure 6. Real-time PCR Results For FPSA Assay Calibration Curve and plasma Sample Set

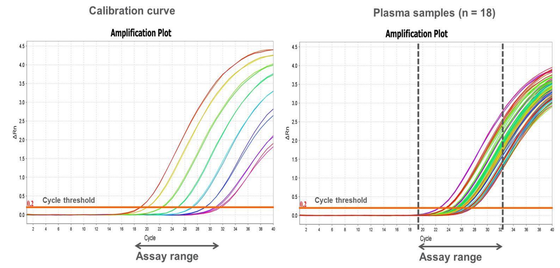
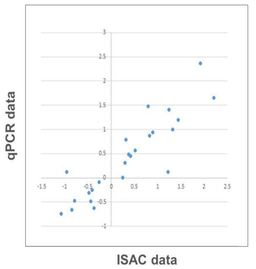


Table 2. Protein Assay Uniformity (Intra-assay) and Precision (Inter-assay)

Assay Name	Uniformity (Intra-assay) Concentration (nU/mL)	% CV	# of replicate wells	Precision (Inter-assay) Concentration (nU/mL)	% CV	# of runs
Free PSA (FPSA)	7.0	10.6	112	7.9	15.6	4
Total PSA (PSA)	9.6	9.8	112	10.1	7.4	4
KLK2	0.06	25.7	218	0.06	9.8	4
PSP94	0.8	18.5	110	1.2	20.1	4
GDF15	3.0	14.1	110	3.5	19.4	4

Figure 7. Stockholm3 Model Comparison with ISAC or TaqMan Protein Assay Data for 24 Samples



CONCLUSIONS

In this study we present the development of an integrated, multi-omic workflow that enables genetic and protein biomarkers for prostate cancer to be measured on the sample analytical platform, the QuantStudio 12k Flex Reali-timePCR System. Proteins are measured with TaqMan Protein Assay technology which has several advantages over traditional ELISA methods including, homogeneous (no wash) design, low sample requirement (2μL), and broad dynamic range (~4-5 logs). Fully automated liquid handling further simplifies the protein assay workflow and reduces hands-on time. When applied to a set of 24 high value samples, our workflow performed well in the Stockholm3 model, demonstrating good concordance with previously published results. While this workflow has been used specifically for prostate cancer research, it can be easily adapted for research of other diseases and model systems.

REFERENCES

1. Gronberg H, et al. Prostate cancer screening in men aged 50-69 years (STHLM3): a prospective population-based diagnostic study. Lancet Oncol (2015)16:1667–76.

2. Ström P, et al. The Stockholm-3 Model for Prostate Cancer Detection: Algorithm Update,
Biomarker Contribution, and Reflex Test Potential. Eur Urol (2018).

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