A streamlined workflow for liquid biopsy sample extraction and highplex digital PCR analysis using the Maxwell® CSC system and 6-color Crystal Digital PCR™

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Abstracts
Circulating cell free (c)DNA extracted from liquid biopsy samples have become established sample types for characterizing oncology targets. Currently, there are several extraction protocols and genomic platforms for researchers to select when interrogating genetic information. The focus of this work was to identify a flexible method for sample extraction that seamlessly integrates into a straightforward and sensitive genetic analysis workflow from plasma samples. Here, we present a workflow combining the Maxwell® CSC 48 instrument for automated cDNA extraction and Crystal Digital PCR™ on the naica® system for simultaneous highplex digital PCR and without a simple workflow that can be completed with less than 15 minutes of hands-on-time (Figure 2) that can be completed with less than 15 minutes of hands-on-time.

Introduction
Liquid biopsies, such as blood samples, can harbor a wealth of genetic information from both healthy and unhealthy cells (Figure 1) to inform disease diagnosis and treatment. (c)DNA measurements require a highly sensitive and reliable detection technology to quantify often low-level genetic aberrations within a high background of wild-type sequences. Rigorously qualified pre-analytical protocols are vital to ensure the performance of downstream liquid biopsy workflows to ensure high-quality sample to results workflows (Figure 2). This proof-of-concept study evaluates the performance of the cDNA extraction protocols of the Promega Maxwell® CSC system paired with the detection by 6-color Digital PCR™ on the naica® system of PIK3CA and EGFR mutations described in NSCLC. Furthermore, the following sampling and extraction workflow, we enabled the detection of a set of PIK3CA mutations from cDNA samples. This proof-of-concept workflow creates the foundation for the further development of streamlined sample-to-answer protocols that will better assist cancer researchers across the biomarker testing landscape.

Material and Methods
Plasma-like samples (Sema) reference material ref S/D00002, S/D00016, S/D00089) and human KEDTA plasma samples collected from healthy donors were extracted with the Promega Maxwell® CSC LV (cDNA plasma extraction kit (Promega, ref AS1840), according to supplier recommendations. Before extraction, all samples were spiked with a known quantity of an exogenous Extraction Control DNA (EC), and a portion of the human plasma samples were spiked with known amounts of synthetic mutant DNA. For comparison, plasma was also extracted with QIAamp circulating nucleic acid kit (QIAGEN, ref 51514) according to supplier recommendations.

cDNA were analyzed on the naica® 6-color digital PCR system with two independent detection assays - A custom Ralcal Cancer 6-color assay detecting PIK3CA wild-type (WT), four PIK3CA mutations E342K, E343K, P1047A, and P1047T, and the EC - The EGFR 6-color Digital PCR™ kit (Stilla Technologies, Ref R3006B) detecting EGFR V6 and 32 EGFR mutations (for more information, see poster 75).

Results

- The Maxwell® CSC system paired with the detection by 6-color Crystal Digital PCR™ on the naica® system of PIK3CA and EGFR mutations described in NSCLC.

The results from the complete library of the samples extracted with the Maxwell® RSC LV cDNA Plasma Extraction kit exhibited high-quality sample to results workflows (Figure 3). The complete library of the samples extracted with the Maxwell® CSC system was highly paired with the detection by 6-color Digital PCR™ on the naica® system of EGFR mutations described in NSCLC.

Conclusions
In this study, we show high compatibility between the automated Maxwell® CSC cDNA extraction system and highplex detection from liquid biopsy samples by Crystal Digital PCR™ on the naica® system. This workflow enables the highly sensitive detection and quantification of the main somatic EGFR mutations described in NSCLC and a set of PIK3CA mutations described in breast and rectal cancers. Both technologies have a fast time-to-result with minimum hands-on-time, enabling a complete sample-to-result workflow in less than a day. This proof-of-concept workflow creates the foundation for the further development of streamlined sample-to-answer protocols that will better assist cancer researchers across the biomarker testing landscape.