APPLICATION NOTE

Multiplex Detection of SARS-CoV-2 Variants of Concern using ARMS-PCR on the LabChip® GX Touch™ Nucleic Acid Analyzer

Labchip® GX Touch™ Nucleic Acid Analyzer

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Introduction

Several SARS-CoV-2 variants are now circulating globally. PerkinElmer offers a range of SARS-CoV-2 second-tier assay options for identifying variants associated with VOCs. This gives labs the flexibility to implement the best solution for their needs based on the level of information being sought and facility, resources and workflow considerations. The NGS-based NEXTFLEX® Variant-Seq™ Kit delivers information about all genomic variants contained in a SARS-CoV-2 positive sample, including previously undescribed ones. While NGS is the only solution which can deliver information about all mutations in a sample, it is also slower, more expensive, and more complex to analyze than PCR-based solutions.

If a lab is aiming to identify specific VOCs, PerkinElmer's PKamp™ VariantDetect™ Kit or our new ARMS-PCR assay described herein may be the preferred solutions. ARMS-PCR assay* is able to rapidly analyze a sample for a specific set of up to six or more mutations associated with known VOCs. However, SARS-CoV-2 hotspots are evolving rapidly. The number of mutations which can be detected by a single RT-PCR reaction is limited by the fluorescence detection capabilities of real-time PCR instruments, with an upper limit of three (3-plex) mutations in most assays (one fluorescence channel is reserved for internal control).

Here, we demonstrate a method combining multiplex (amplification refractory mutation system) ARMS-PCR with the LabChip® GX Touch™ Nucleic Acid Analyzer for secondary mutation identification. ARMS-PCR is carried out to amplify mutations followed by automated visualization and analysis of the specific amplicons corresponding to different mutations using a LabChip® GX Touch™ instrument[2,3]. This workflow allows researchers to simultaneously detect multiple mutations for each run of 96 samples with a turn-around time of 6-8 hours (~ 30 minutes hands-on time) from sample to result (Fig.1). ARMS-PCR only requires conventional primers, which can be manufactured relatively quickly compared to fluorogenic primers and does not require sophisticated instruments for assay readout.

* Assay in development

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Materials and Methods

PerkinElmer's chemagic™ 360 instrument is used for automated nucleic acid isolation. Samples which test positive for COVID-19 can be analyzed for SARS-CoV-2 VOCs with mutation identification using ARMS-PCR analyzed on the LabChip® GX Touch™ instrument (Fig.1). To test the specificity of the ARMS-PCR technology, a synthetic plasmid encoding SARS-CoV-2 S gene mutations and Twist® Bioscience SARS-CoV-2 wild type RNA control 2 (Twist Bio part#102024) were used. ARMS-PCR employs the same enzyme cocktail (nCoV enzyme mixture) as the PerkinElmer® SARS-CoV-2 Nucleic Acid Detection Kit[4], which has already demonstrated high multiplexing capability with real time RT-PCR experiments. Primers that generate amplicons of different sizes were designed to amplify multiple S gene mutations using the ARMS-PCR approach. Amplification reactions were set up according to the standard PerkinElmer® SARS-CoV-2 Nucleic Acid Detection Kit's recommendations. Except where otherwise noted, the ARMS-PCR is conducted at 37°C /2 min, 50°C/15 min, 94°C/10 min, 36-40 cycles (94°C/10 s, 60°C/10 s, 65°C/2 min). The resulting PCR products were directly evaluated on the LabChip® GX Touch™ instrument using the DNA 1K assay[5] (PerkinElmer, part# CL5760673, 760517).

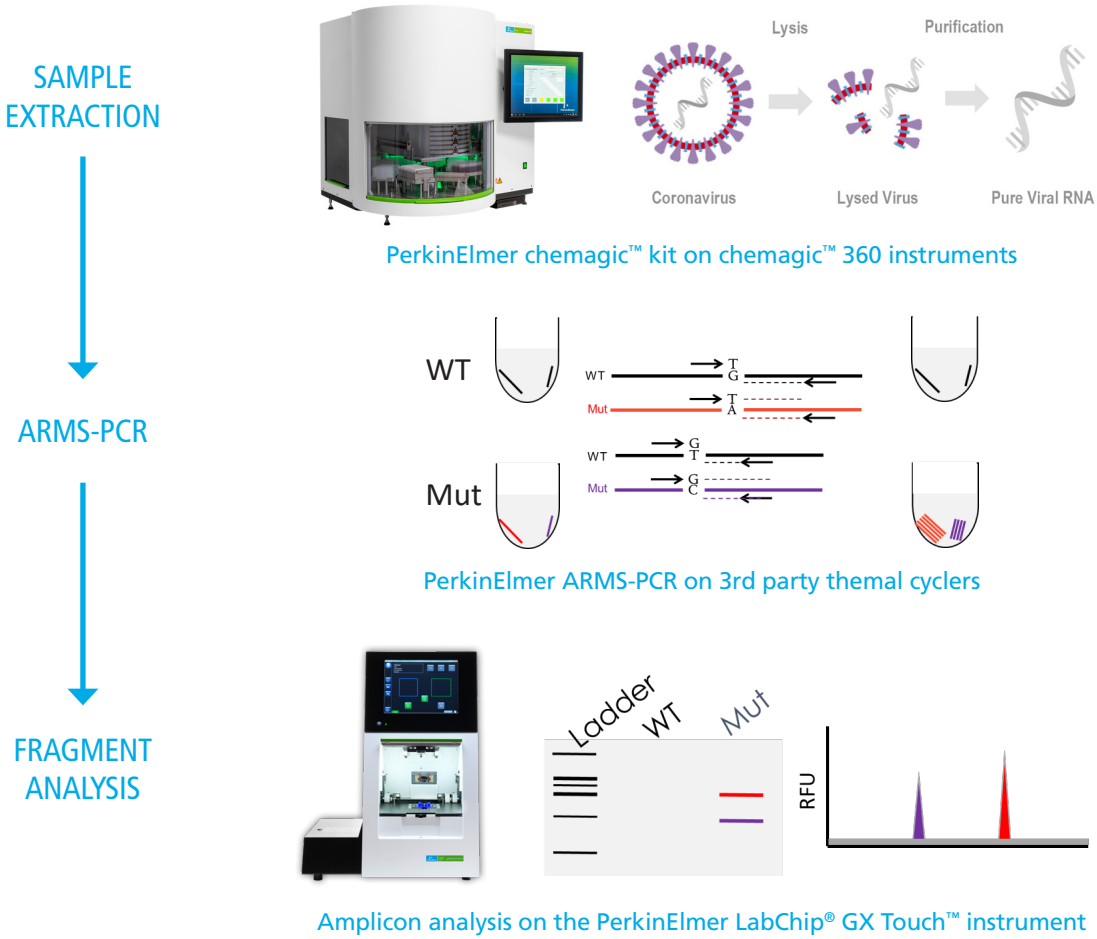

Figure 1. ***Workflow of ARMS-PCR analyzed on the LabChip® GX Touch™ system for SARS-CoV-2 variant detection.*** *Nucleic acid was extracted from samples using the PerkinElmer chemagic™ 360 instrument. ARMS-PCR is carried out to amplify mutations. Amplicons of different lengths are detected on yhe LabChip® GX Touch™ instrument. WT: wild type, Mut: Mutant.*

Results

LabChip® GX Touch™ DNA 1k Assay identifies non-diluted, purification-free ARMS-PCR products

We evaluated the sensitivity and specificity of each primer pair in ARMS-PCR. We also tested whether nonspecific products such as primer dimers or PCR buffer components such as salts could interfere with the LabChip® GX Touch™ analysis. As shown in Figure 2A, sharp bands with correct size confirmed that the PCR formulation of the PerkinElmer SARS-CoV-2 Nucleic Acid Detection Kit is compatible with the LabChip® GX Touch™ DNA 1k Assay. A PCR cleanup procedure can be bypassed as nonspecific amplification of our primers is negligible and can be differentiated by size if they are detected. We also tested the specificity of each ARMS primer pair. As shown in Figure 2B using the N501Y mutation as an example: no amplificon was detected in the wild type RNA sample. The sensitivity of N501Y mutation detection is 200 copies per reaction.

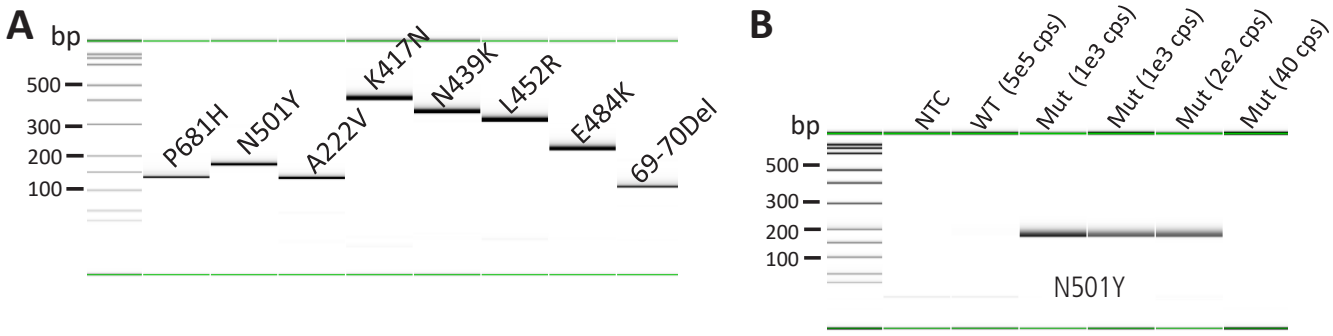

Figure 2. ***ARMS-PCR primer screening and specificity test on LabChip® GX Touch™ instrument.*** *(A) Eight primer pairs for eight S-gene mutation loci were tested in single-plex ARMS-PCR. Different SARS-CoV-2 S gene mutations are labeled above bands as P681, N501Y etc. (B) ARMS primers specifically amplified virus genome with certain mutations, N501Y mutation is shown as an example. NTC: negative control, WT: wild type, Mut: mutant, 1e3 cps: 1,000 copies. A fluorescent dye and 1,000 bp dsDNA serve as the lower and upper marker (green in color), respectively.*

LabChip® GX Touch™ DNA 1k Assay has < 15 bp resolution for multiplex ARMS-PCR Amplicons

Multiplexing PCR allows laboratories to conduct multiple genotyping tests on one pathogen, or multiple pathogen detection in one reaction resulting in considerable savings in reagent cost, time, and workload. To balance each primer pair's amplification efficiency, the target size of ARMS-PCR was designed in a 100 - 500 bp range as shown in Figure 2. To minimize primer cross-hybridization, we screened primer combinations in 3-plex, 4-plex or 6-plex multiplexed formats using the standard PerkinElmer SARS-CoV-2 Nucleic Acid Detection PCR reagents. As seen in Figure 3, none of the 3-plex, 4-plex and 6-plex formats show nonspecific amplification >100 bp in negative control reactions; all expected bands were observed in positive samples. In addition, the LabChip® GX Touch™ instrument distinguished these bands with high sizing resolution and precision (CV ≤3% see table 1), ensuring the peak size calls correspond to defined mutation products.

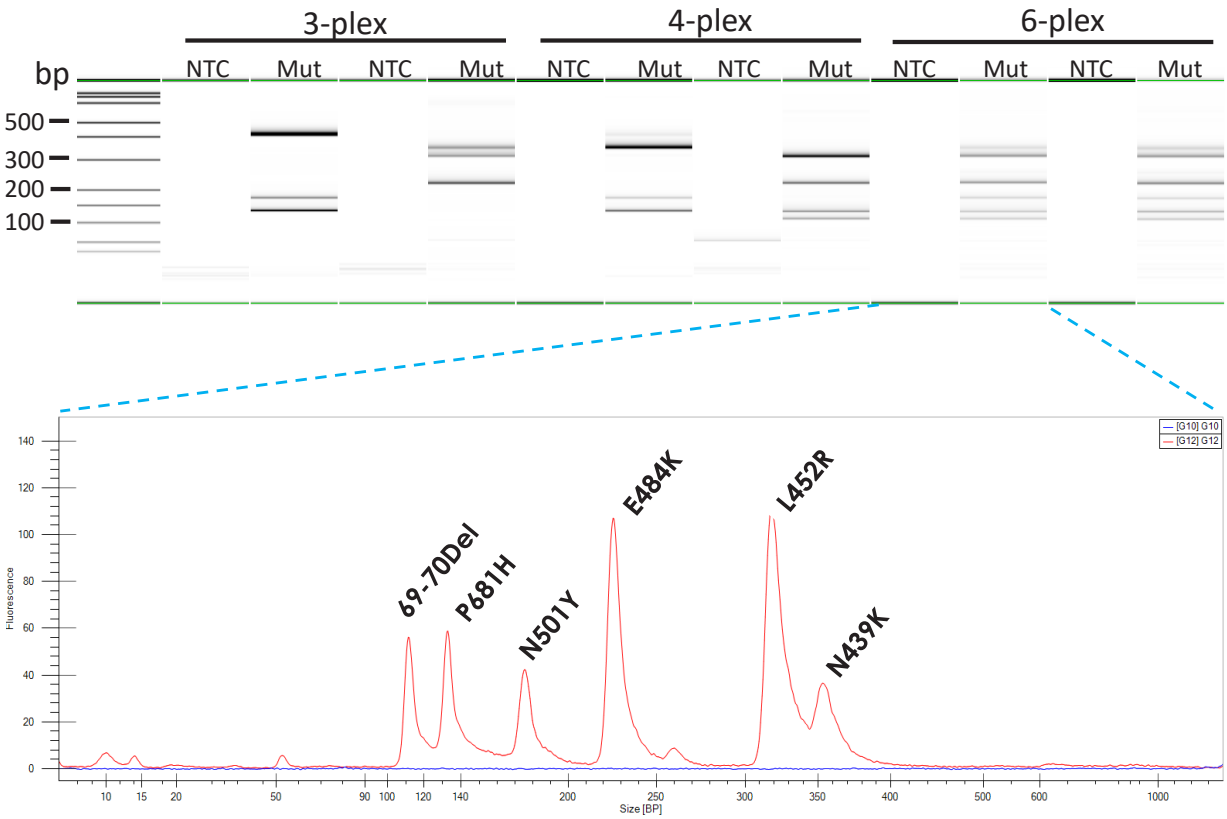

Figure 3. ***Multiplexing capability of ARMS-PCR and high sizing resolution by LabChip® GX Touch™ instrument.*** *Upper panel: Gel image of different multiplexing formats. Lower panel: electrophoretic trace of 6-plex ARMS-PCR for mutation detection; mutations are labeled for individual peaks. NTC: negative control, Mut: Mutant.*

Table 1. Size of ARMS-PCR products using LabChip® GX Touch™ DNA 1K assay.

SARS-CoV-2 variants	Mean ± SD (bp)	Range (bp)	CV (%)	Observations
69-70Del	113 ± 3.4	110-118	3.0	7
A222V	134 ± 0.8	133-135	0.6	5
P681H	135 ± 2.5	131-137	1.9	11
N501Y	178 ± 2.8	174-183	1.6	7
E484K	229 ± 3.0	223-233	1.3	10
L452R	327 ± 4.3	317-331	1.3	11
N439K	361 ± 4.6	353-369	1.3	10
K417N	416 ± 6.2	410-428	1.5	10

Summary

We have demonstrated in a single well a 6-plex detection of SARS-CoV-2 mutations (69-70Del, P681H, N501Y, E484K, L452R, N429K) using the PerkinElmer SARS-CoV-2 Nucleic Acid Detection Kit enzyme cocktail and the LabChip® GX Touch™ instrument. This multiplexed ARMS-PCR amplification of SARS-CoV-2 variants was performed without PCR product dilution or cleanup, and there was no compromise of data quality when compared to a lower plex ARMS-PCR format. Scalability up to 384 samples per plate and accurate sizing (normalized to internal markers and external ladder) using LabChip® GX Touch™ system enables a flexible and convenient microfluidic gel electrophoresis-based assay to identify SARS-CoV-2 mutations. We expect to extend the detection of SARS-CoV-2 mutations to additional targets as new VOCs are discovered, enabling fast, dynamic, and efficient identification of SARS-CoV-2 variants.

References

1. *CDC SARS-CoV-2 Variant Classifications and Definitions*
2. *PerkinElmer® LabChip® GX Touch™ HT Nucleic Acid Analyzer*
3. *S Little (2001) Amplification-refractory mutation system (ARMS) analysis of point mutations. Curr Protoc Hum Genet May;Chapter 9:Unit 9.8*
4. *PerkinElmer® SARS-CoV-2 Real-time RT-PCR assay*
5. *PerkinElmer LabChip® DNA 1K Assay User Guide*

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