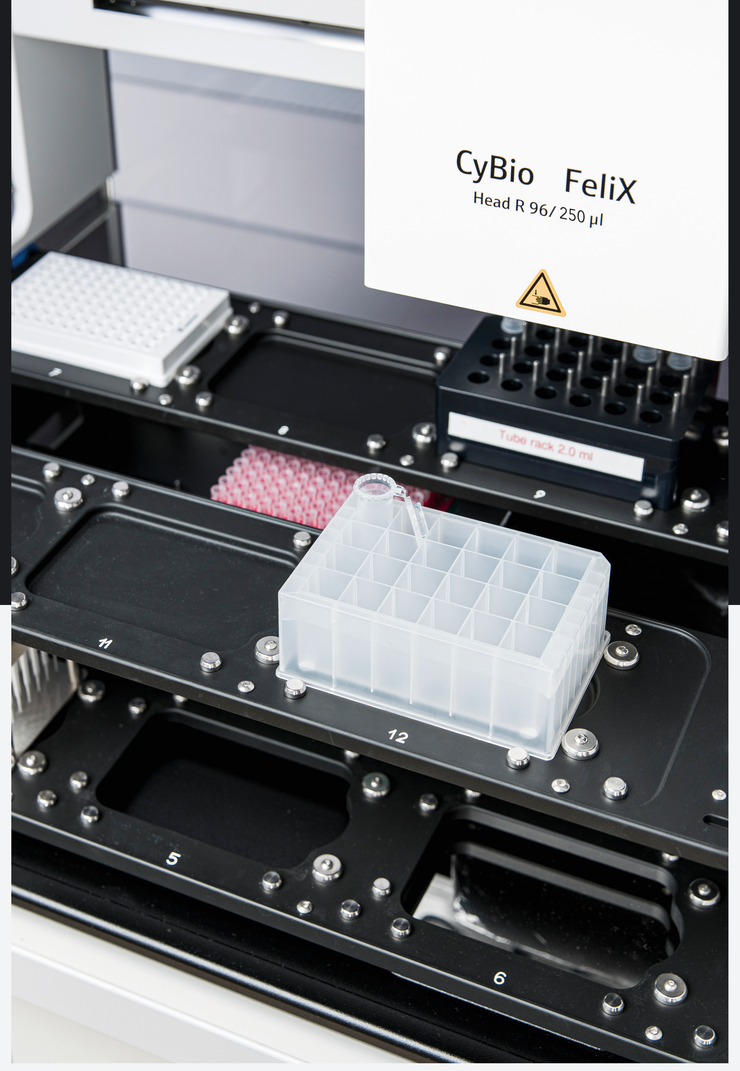

Automated Workflow for rapid SARS-CoV-2 Detection with the LumiraDx RNA STAR Complete Kit

Authors: Christoph Heddergott & Anne Koch,
Analytik Jena GmbH, Konrad-Zuse-Str. 1, 07745 Jena, Germany

ABSTRACT

The detection of nucleic acids can be very time-consuming and traditionally involves a multi-step extraction of nucleic acids from the sample material as well as the amplification and detection of the extracted nucleic acids using real-time PCR. The convenient and flexible extraction and reaction plate preparation with the CyBio FeliX Liquid Handler as well as the target and control signal detection with the qTOWER3 from Analytik Jena ensure fast results for SARS-CoV-2 detection with the LumiraDx SARS-CoV-2 RNA STAR in Complete Kit in less than in hour for 96 samples.

INTRODUCTION

In nucleic acid detection, two main steps must be performed, both of which can be very tedious and time-consuming. First, the extraction of nucleic acids from the sample material which is usually carried out in a multi-step process. Second, the amplification and detection of the extracted nucleic acids which is usually performed by a conventional real-time PCR. With the LumiraDx SARS-CoV-2 RNA STAR Complete Kit, nucleic acid extraction is reduced to one step, in which samples or controls are mixed with an extraction buffer. Compared to conventional qPCR, the qSTAR approach (qualitative selective temperature amplification reaction) dramatically shortens the time to detect a specific sequence in a thermal cycler. It reduces the time to amplification to only 20 minutes by reducing the temperature delta (~57°C–~63°C) during the reaction. An additional nicking enzyme cleaves the newly formed DNA and generates more target DNA template while the polymerase works in parallel. The small temperature delta allows the enzymes to work optimally, with one enzyme being preferred over the other depending on the current temperature. By automating the LumiraDx SARS-CoV-2 RNA STAR Complete Kit on Analytik Jena equipment, extraction and amplification can be performed rapidly with maximum convenience for the customer (Figure 1).

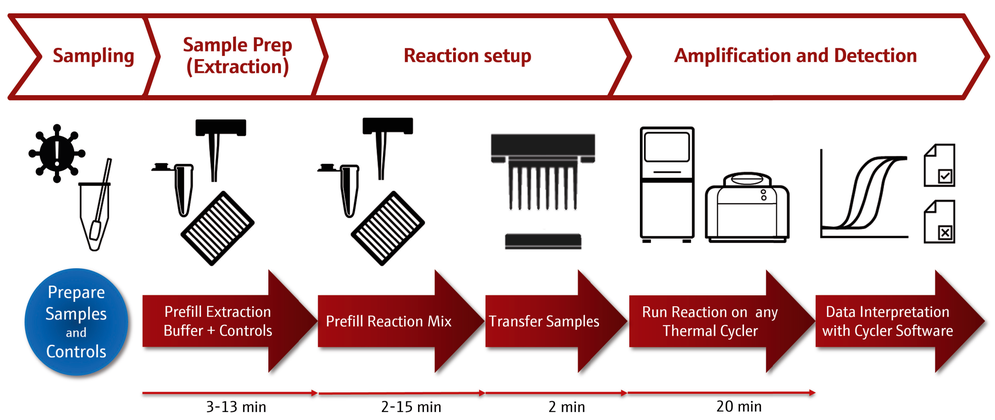

Figure 1 | Workflow automation: Modular system of automated steps (red arrows) of the SARS-CoV-2 RNA STAR Complete Kit with Analytik Jena devices CyBio FeliX for sample preparation and reaction setup, qTOWER^3 family for amplification and detection.

METHODS

The CyBio FeliX automates the LumiraDx assay in sub steps. The user interface guides the user through the software and provides instructions for placing the reagents and plates on the deck. The Analytik Jena CyBio FeliX prepares the extraction and reaction plates and transfers the samples. These sub steps can be combined into a complete run, started in a modular fashion or individually.

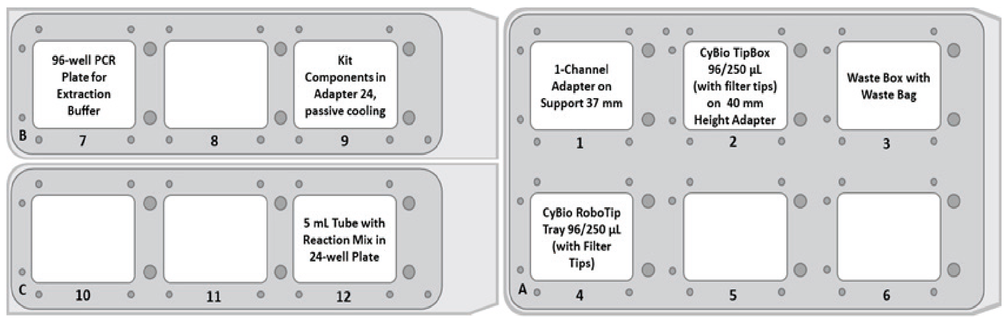

Figure 2 | Exemplary graphical deck layout at the beginning of a complete run in which one plate is to be processed. The CyBio FeliX provides 12 deck positions on 3 moveable decks. The upper decks B and C equipped with the required accessories are shown on the left, the lower deck A is shown on the right.

In this way, extraction plates can be prepared and stored at a cool temperature (2–8 °C) for later use. Pipetting of the reaction mixture and transfer of the samples can then be started as required. After transfer of the prepared and sealed reaction plates into the Analytik Jena qTOWER3 real-time thermal cycler, the target sequence of SARS-CoV-2 (OFR1a) and the internal control sequence are amplified and detected in the FAM and ROX channels, respectively. In a direct comparison, automatically processed samples (n=72 sample positions) were compared with manual procedures (n=6 sample positions) on one plate.

RESULTS

The automated preparation of the plate with Extraction Buffer and Reaction Mix by the Analytik Jena CyBio FeliX yielded results that were very homogeneous with respect to the Ct values for the target (ORF 1a of SARS-CoV-2) and the control sequence (Table 1). These results were within the same range as those of the manually prepared wells, showing only slightly higher Ct values. The target sequence was only detected in wells with positive control medium (PCM) whereas the internal control sequence was detected in all wells. Due to the qSTAR approach, Ct values generated with the SARS-CoV-2 RNA STAR Complete Kit are markedly lower than those obtained with conventional real-time PCR assays.

Table 1 | Average Ct values of amplification curves.

Reaction Setup	Sample Type	ORF1a of SARS-CoV-2		Internal Control	
		Mean Ct	SD	Mean Ct	SD
Automated	PCM	7,16	0,20	7,13	0,05
	NCM	No Ct	-	6,80	0,05
Manual	PCM	6,97	0,05	7,24	0,16
	NCM	No Ct	-	7,06	0,14

Abbreviations: PCM positive control medium, NCM negative control medium, ORF open reading frame

CONCLUSION

The LumiraDx SARS-CoV-2 RNA STAR Complete Kit minimises the time consuming extraction from swab samples to just one step and enables very rapid amplification of target sequences. Process automation on the Analytik Jena CyBio FeliX allows flexible and customised maximisation of throughput with efficient use of times with low sample volumes. The modular design of the Application Studio software allows each automated process step to be executed separately and up to 3 plates to be processed in parallel (288 samples plus positive/negative controls). In addition, the workflow is very flexible in terms of the number of samples to be processed, with any number between 8 and 96 samples per plate possible. Also, amplification with the qTOWER^3 is efficient, highly reproducible and yields homogeneous results over the entire reaction plate.

For more information check out the detailed Application Note.

For research use only. Not for use in diagnostic procedures.

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