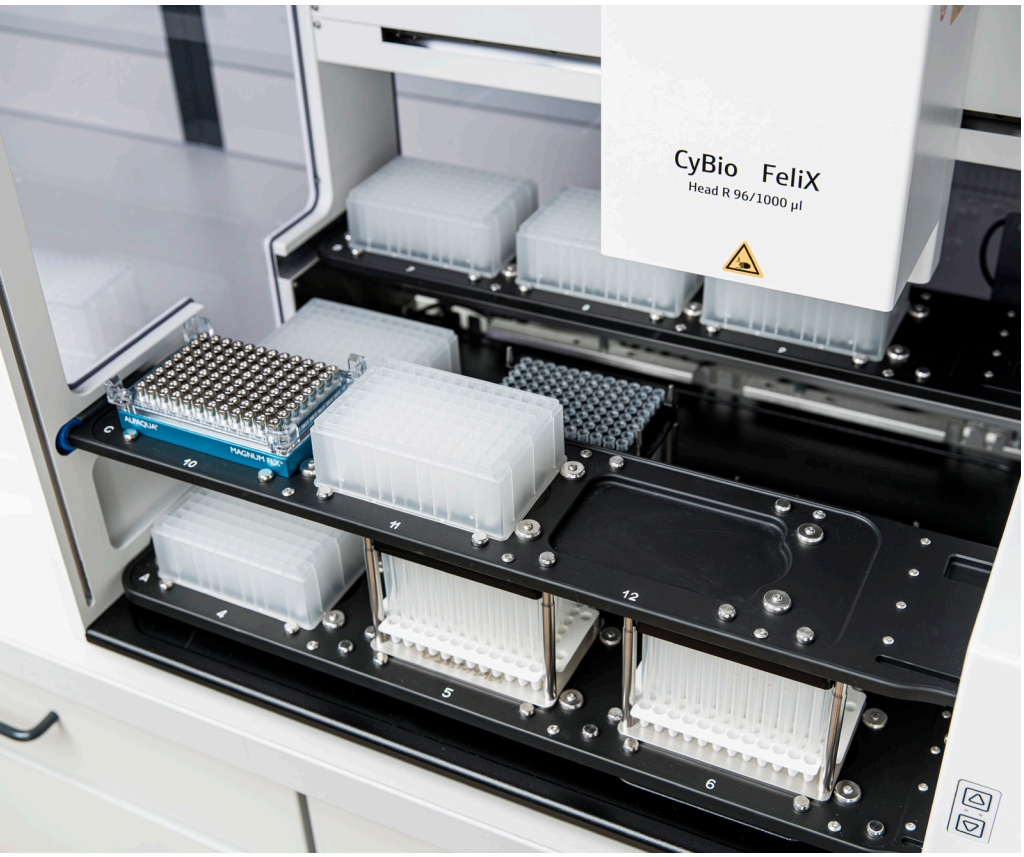
Application Note · CyBio FeliX



Challenge
High-throughput purification of viral DNA and RNA from a variety of sample types. High-quality DNA/RNA ready for downstream applications like RT/qPCR, NGS or hybridization

Solution
Efficient automation of bead-based nucleic acid (NA) extraction with CyBio FeliX for precise and repeatable results as well as use of an established hardware configuration optimized for NA extraction

Intended audience
Life scientists who are interested in a liquid handling platform that is open for a multitude of applications

Automated Purification of Viral DNA and RNA from Biological Samples using Zymo Research Quick-DNA/RNA Viral MagBead

Introduction

The Quick DNA/RNA Viral MagBead Kit from Zymo has been automated with the Cybio FeliX pipetting robot from Analytik Jena. By using the CyBio FeliX configuration optimised for nucleic acid extraction, a convenient and user-friendly method of sample processing is created for downstream applications such as PCR analysis.
Zymo's Quick DNA/RNA Viral MagBead Kit offers high flexibility and is compatible with the nucleic acid-preserving DNA/RNA Shield, which enables sample storage. By automating liquid processing, the automated solution with CyBio FeliX efficiently saves laboratory time by automating the pre-filling of components and the extraction process. The user-friendliness of the solution is achieved through the easy-to-use software interface, the CyBio FeliX Application Studio.

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Materials and Methods

Samples

- Human saliva samples and swabs in DNA/RNA Shield collection
- Swabs in transport medium
- PBS as extraction control

Instrumentation

- CyBio FeliX eXtract hardware configuration, consisting of:
  - CyBio FeliX Basic Unit with Enclosure (Cat.# OL5015-24-100, Analytik Jena)
  - CyBio FeliX Extraction Set (Cat.# OL5015-25-120, Analytik Jena)
- LightCycler® 480 Instrument II, 384-well (Cat.# 05 015 243 001, Roche)

Consumables an chemical products

- Quick-DNA/RNA Viral MagBead (Cat.# R2141-E, Zymo Research)
- DNA/RNA Shield Collection Tubes w/Swab - DX (Cat.# R1107-E, Zymo Research)
- DNA/RNA Shield (2X Concentrate, Cat.# R1200-25, Zymo Research)
- 2-Mercaptoethanol (Cat.# M3148, Millipore Sigma)
- Deep Well Plate 96 square well, 2.0 mL (Cat.# 845-FX-8500025, Analytik Jena)
- Reservoir 2-column
- Reservoir 4-column

Sample Preparation

- Addition of 500 µL 2-mercaptoethanol per 100 mL Viral DNA/RNA Buffer (final 0.5 % (v/v)).
- Addition of 80 mL isopropanol (99.5-100%) to the MagBead DNA/RNA Wash 1 concentrate.
- Addition of 120 mL isopropanol (99.5-100%) to the MagBead DNA/RNA Wash 2 concentrate.
- Reconstitution of lyophilized Proteinase K to a concentration of 7.7 mg/mL in Proteinase K Storage Buffer by vortexing. Material not used immediately was stored as frozen aliquots.

In general, samples were either processed directly after sampling or stored inactivated at room temperature for later analysis. For samples using DNA/RNA Shield as medium, no further treatment was necessary prior to storage. Other samples were inactivated by adding an equal volume of 2x concentrated DNA/RNA Shield.

- Samples in DNA/RNA Shield collection devices (swabs, saliva, etc.) and Swabs (UTM/VTM, PBS, saline, etc.) and inactivated samples:
  - Transfer of 400 µL sample to Process Plate followed by immediate purification followed by immediate purification
  - 200 µL Probe + 200 µL PBS followed by immediate purification
- Liquids (plasma, serum, cerebrospinal fluid (CSF), blood, saliva, urine, cell suspension, cell culture media)
  - Addition of an equal volume of 2x DNA/RNA Shield followed by mixing
  - 100µl supernatant / Swab + (100µL Medium + 200 µL Shield + 20 µL Proteinase K)
  - Transfer of 400 µL sample to Process Plate. Proceed with purification.

Method

The Analytik Jena CyBio FeliX eXtract configuration was used to perform nucleic acid extraction. The configuration is equipped with the required accessories (i.e., a 96-well plate shaker) allowing seamless implementation of the Zymo kit.

CyBio FeliX Setup

The kit reagents can either be prefilled manually, or via a scripted process allowing a column-wise assay preparation. This enables the user to extract 8 to 96 samples in 8-sample steps. For manual preparation, the required number of wells per plate are filled with the kit reagents described in Table 1. Complete prefilling is not necessary, the user can prepare as many reactions as required.

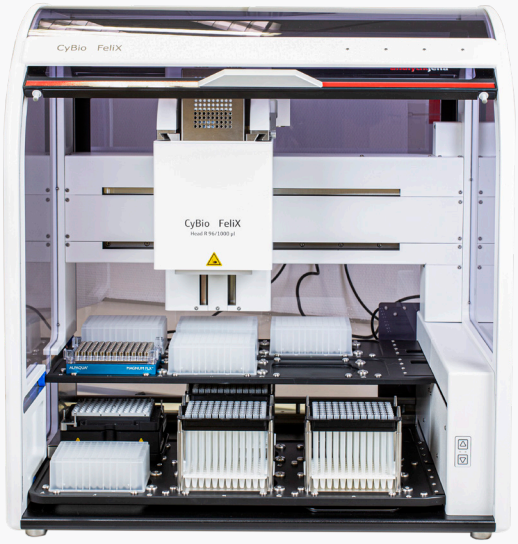

Figure 1: Analytik Jena CyBio FeliX hardware configuration and decks equipped for extraction applications.

Table 1: Manual plate preparation. The indicated volumes of liquid are calculated volumes per well/extraction.

Plate	Label	Content per well
Plate 1	Process	400 µL sample + 20 µL Proteinase K
Plate 2	Viral DNA/RNA Buffer + Mag Beads	800 µL Viral DNA/RNA Buffer + 20 µL MagBinding Beads* optional: PCR IC as recommended by the detection assay
Plate 3	MagBead DNA/RNA Wash 1	600 µL MagBead DNA/RNA Wash 1
Plate 4	MagBead DNA/RNA Wash 2	600 µL MagBead DNA/RNA Wash 2
Plate 5	Ethanol	1100 µL Ethanol, 95-100%
Plate 6	DNase/RNase-free Water	250 µL RNase-free Water (for Elution)
Plate 7	Elution	Empty

*Vortex MagBinding Beads thoroughly prior to dispensing. MagBinding Beads settle quickly, ensure that beads are kept in suspension while dispensing.

For the automated prefilling, empty plates are positioned on the CyBio FeliX deck (Figure 2, left), the liquid is transferred from reservoir plates. The script/ protocol is embedded in the Application Studio of CyBio FeliX eXtract and prompts the user to enter the number of samples. As an additional checkpoint after loading deck positions according to the software instructions, the program displays the calculated minimum volumes of reagents in the reservoirs that are required for the procedure. The process execution time depends on the number of samples and ranges from 7 minutes (8 samples) to 38 minutes (96 samples).

Based on the CyBio FeliX configuration used for automated prefilling, the deck layout is only slightly modified for the main extraction process (Figure 2, right). This enables the user to quickly proceed to the main extraction protocol. As for the automated prefilling, a message box displays the deck loading instructions to set up the CyBio FeliX for extraction. The process run time for an entire plate (96 samples) is 52 minutes.

In detail, the total process time amounts to:

Reservoir filling with kit solutions and deck loading:	4 minutes
Automated prefilling (full plate):	38 minutes
Deck preparation for extraction:	2 minutes
Automated extraction (full plate):	52 minutes
	Total: 96 minutes (1 minute/sample)*

*This calculation does not include the time required to transfer samples from primary tubes into the process plate, which can be carried out during the automated plate prefilling.

Figure 2: Deck layouts for automated prefilling (left) and the main extraction protocol (right).

Between protocols, the majority of the deck layout is maintained, allowing for a seamless workflow. Altered deck positions are marked in red. Prefilled plates remain in position, two reservoirs (5, 12), a TipRack (2) and the 8-channel adapter (6) are replaced by RoboTipTrays (2, 5, 6) and the final elution plate (12). After placing the process plate with samples directly on the BioShake (1), extraction can start immediately.

Due to the limited evaporation possibilities of the automated setup, a higher elution volume (100 µL) than recommended by the manual was selected to limit the potential inhibitory effects that residual ethanol on the magnetic beads may exert on PCR efficiency by dilution. For comparison, a sample subset was also eluted with 50 µL to assess PCR inhibition versus potentially increased concentration obtained by using less elution volume. Extracted samples can be detected immediately or stored frozen until further processing.

Polymerase Chain Reaction (PCR)

A custom PCR setup according to the Drosten protocol published January 2020[1] was used to amplify viral target sequences. The assay consisted of three SARS-CoV-2 targets (RdRP gene, E gene, 2019-nCoV specific RdRP gene) plus two controls: a) RPP30 (human sampling control), and b) extraction control. From the extraction procedure described above, 2.5 µL of extracted nucleic acid were used as template. PCR samples were prepared as duplicates in 384-well PCR plates. Primers and probes used in accordance with a protocol published by the WHO[2] are given in Table 2.

Table 2: Primers targeting the E gene and a generic as well as a 2019-nCoV (SARS-CoV-2)-specific RdRP gene were used.

Assay/use	Oligonucleotide	Sequence 5'-3'[a]	Final concentration [nM]
RdRP gene	RdRp_SARSr-F2	GTGARATGGTCATGTGTGGCGG	600
	RdRP_SARSr-P1	FAM-CCAGGTGGWACRTCATCMGGTGATGC-BBQ	200
	RdRp_SARSr-R1	CARATGTTAAASACACTATTAGCATA	800
E gene	E_Sarbeco_F1	ACAGGTACGTTAATAGTTAATAGCGT	400
	E_Sarbeco_P1	FAM-ACACTAGCCATCCTTACTGCGCTTCG-BBQ	200
	E_Sarbeco_R2	ATATTGCAGCAGTACGCACACA	400
RdRP gene specific for 2019-nCoV	RdRp_SARSr-F2	GTGARATGGTCATGTGTGGCGG	600
	RdRP_SARSr-P2	FAM-CAGGTGGAACCTCATCAGGAGATGC-BBQ	200
	RdRp_SARSr-R1	CARATGTTAAASACACTATTAGCATA	500

[a] W is A/T; R is G/A; M is A/C; S is G/C. FAM: 6-carboxyfluorescein; BBQ: blackberry quencher

Results and Discussion

Seven patient-derived samples were extracted using the Zymo Quick-DNA/RNA Viral MagBead Kit using an elution volume of 100 µL, three of them were additionally prepared with a reduced elution volume of 50 µL.

Table 3 shows the Ct values obtained for the three viral targets (RdRP gene, E gene, 2019-nCoV), the extraction control, and the human control target RPP30, respectively. Within a sample, Ct values varied slightly between the viral SARS-CoV-2 targets. In average, E gene and 2019-nCoV had the same Ct range (average intra-sample difference 0.94 ± 2.36 cycles), whereas the average Ct of RdRP was systematically higher than that of E gene (intra-sample difference 1.17 ± 0.09 cycles).

The consistent amplification of the human RPP30 control verified the correct sampling. The extraction control results were stable, with only slight variation in Ct between samples (28.07 ± 0.19 cycles), including the PBS no template control (Sample D), verifying a stable and reproducible extraction of nucleic acids using the Zymo kit in combination with automated liquid handling on the CyBio FeliX.

All samples were positive for SARS-CoV-2, except for sample H. Sample H was positive for corona virus sequences, but not the novel SARS-CoV-2 virus.For sample E, the 2019-nCoV target amplified only in 1 of the two replicates. Sample D represents a no template control in which PBS was extracted and which is PCR-negative except for the extraction control.

Table 3: PCR results of human samples extracted with the Zymo Quick-DNA/RNA Viral MagBead Kit and analyzed for the viral targets E, RdRP and 2019-nCoV, the human control target RPP30, and the extraction control. Samples were analyzed by PCR in duplicate, except for sample H.

Sample	E gene	RdRP gene	2019-nCoV	RPP30	Ext. control
A	23.41 ± 0.17	24.60 ± 0.13	26.89 ± 0.15	34.01 ± 0.24	27.90 ± 0.00
B	26.04 ± 0.09	27.28 ± 0.17	29.21 ± 0.03	35.94 ± 0.48	28.08 ± 0.05
C	29.92 ± 0.22	31.01 ± 0.28	29.49 ± 0.00	31.26 ± 0.08	27.88 ± 0.08
E	32.94 ± 0.2	34.03 ± 0.82	29.45	31.34 ± 0.22	27.94 ± 0.04
F	28.81 ± 0.2	30.12 ± 0.17	30.01 ± 0.15	31.63 ± 0.09	28.27 ± 0.23
G	28.18 ± 0.05	29.44 ± 0.08	29.87 ± 0.13	35.16 ± 0.00	28.19 ± 0.02
H	33.53	34.59	no Ct	29.11	27.85
D (PBS)	no Ct	no Ct	no Ct	no Ct	28.42 ± 0.21

As an alternative to the standard elution volume of 100 µL, the automated extraction was also carried out using a smaller elution volume of 50 µL. Three of the seven samples (B, F, G) were tested; the average Ct was slightly but systematically higher when using the reduced elution volume (plus 1.17 ± 0.84 cycles). Differences tended to be target specific, with the SARS-CoV-2 specific 2019-nCoV being most affected with >2 cycles difference (Table 3). Concerning the nucleic acid concentration in the eluates, these data suggest that the elution inhibition derived from residual washing ethanol surpassed the effect of concentration by less elution volume. As consequence, 100 µL were set as standard elution volume.

Shown are the Ct values of three samples that were eluted with two different elution volumes (50 µL and 100 µL) and then analyzed with PCR. Except for the extraction control, where results were inconsistent, all target amplifications using the lower elution volumes were higher. As consequence, 100 µL was used as the standard protocol.

Table 4: Effect of elution volume on PCR results.

Sample / elution volume	PCR target				
	E gene	RdRP gene	2019-nCoV	RPP30	Ext. control
B (50 µL)	26.60	27.80	31.75	37.66	27.53
B (100 µL)	26.04	27.28	29.21	35.94	28.08
F (50 µL)	29.77	30.80	32.10	33.70	27.49
F (100 µL)	28.81	30.12	30.01	31.63	28.27
G (50 µL)	29.16	30.00	31.88	no Ct	30.44
G (100 µL)	28.18	29.44	29.87	35.16	28.19

Overall results were well comparable with a comparison commercial magnetic beads-based kit dedicated to extract viral nucleic acids previously automated on the CyBio FeliX. Using the same sample input and elution volume for the extraction, subsequent PCR from samples from Zymo Quick-DNA/RNA Viral MagBead extraction led to Ct values that were in average 1.6±0.6 cycles lower than those obtained with the comparison method (data not shown).

Summary

This Application Note describes the automation of nucleic acid extraction using Analytik Jena's CyBio FeliX and Zymo's Quick-DNA/RNA Viral MagBead kit for downstream PCR analysis. The setup combines the advantages of Zymo's kit, including flexibility and compatibility with DNA/RNA Shield for sample storage, with automated liquid handling provided by CyBio FeliX. The automated process involves prefilling components and executing the extraction process, streamlined through the CyBio FeliX Application Studio software.

The CyBio FeliX system automates the nucleic acid extraction process using the Zymo kit, significantly reducing hands-on time. The automated extraction process takes around 52 minutes for a plate of 96 samples.

Polymerase Chain Reaction (PCR) is performed with custom setups to amplify viral target sequences. Results show consistent amplification of viral targets, human samples, and extraction controls. Comparison with another kit indicates similar performance with lower Ct values for the Zymo Quick-DNA/RNA Viral MagBead extraction.

Overall, the automated workflow offers efficient and user-friendly nucleic acid extraction from various sample types, supported by the combination of Analytik Jena´s hard- and software as well as Zymo's kit.

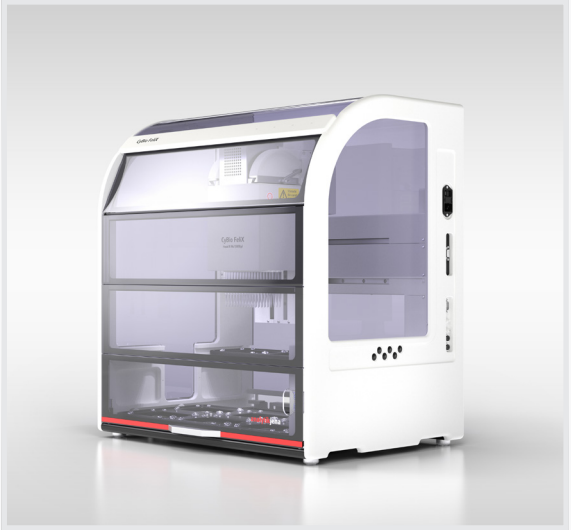

Figure 3: CyBio FeliX.

Recommended device configuration

Table 2: Device and accessories.

Article	Article number
CyBio FeliX Basic Unit with Enclosure	OL5015-24-100
CyBio FeliX Extraction Set	OL5015-25-120
CyBio TipRack 96/1000 µL	OL3811-25-939-F

Acknowledgements

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References

[1] Corman, Victor M et al. "Detection of 2019 novel coronavirus (2019-nCoV) by real-time RT-PCR." Euro surveillance : bulletin Europeen sur les maladies transmissibles = European communicable disease bulletin vol. 25,3 (2020): 2000045. doi:10.2807/1560-7917.ES.2020.25.3.2000045

[2] https://www.who.int/docs/default-source/coronaviruse/protocol-v2-1.pdf

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