Automated NGS Fluidic-Based Device For Sample to Library Preparation: A Flexible Solution For Rapid Decentralized Sequencing for Infectious Disease Diagnostics

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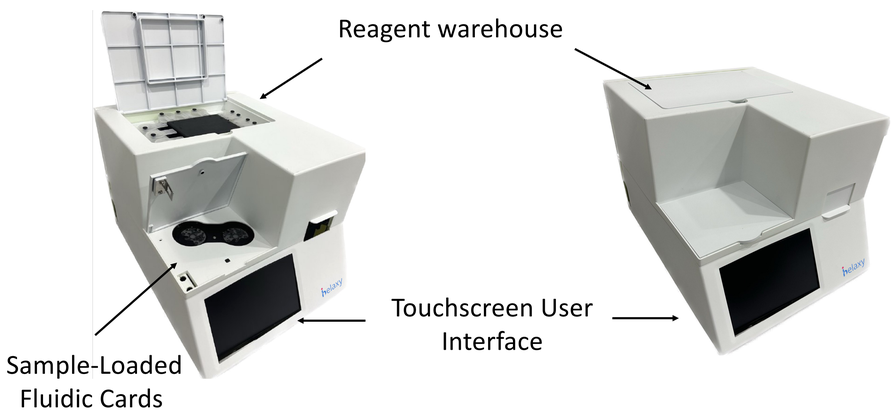
Background

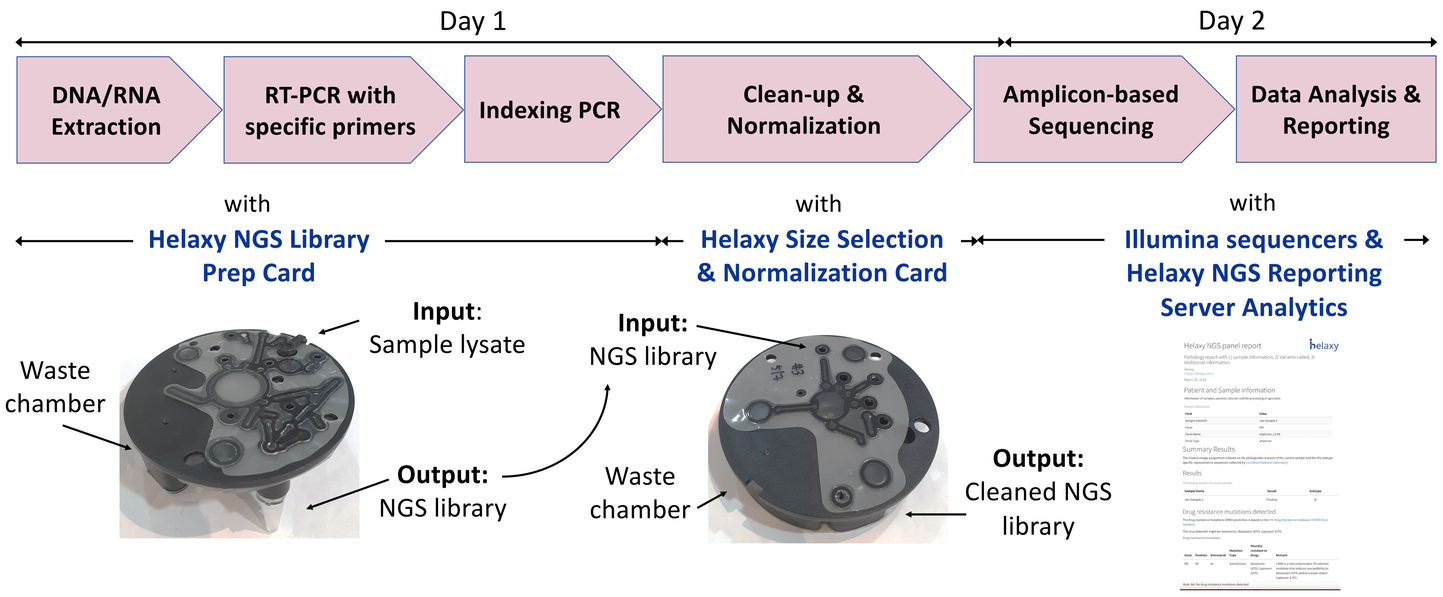
In addition to the established use of Next Generation Sequencing (NGS) such as in drug resistance monitoring in HIV, the recent COVID19 pandemic has shown the crucial need of NGS technologies to identify clinically relevant variants of the SARS-CoV-2 virus. Typically, to be cost-effective in these settings, most NGS analyses were conducted using larger batch sizes, often resulting in long turn-around times of 2-3 weeks, the need for trained personnel, and centralized testing facilities. We have developed an automated fluidic-based NGS device that will bridge these gaps and provide a solution for decentralized testing.

Helaxy NGS Fluidic Device

Automated fluidic-based benchtop device



Helaxy Workflow (TAT: 2 Days; HOT: 30 mins) (not drawn to scale)

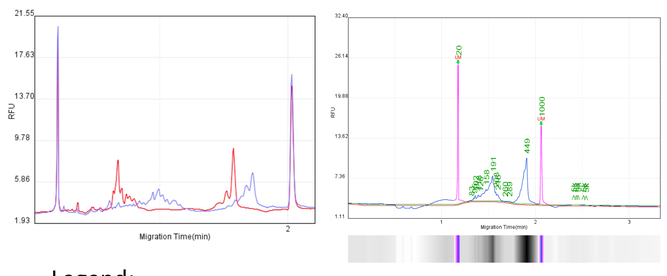


Applications

Sample Input: Inactivated SARS-CoV2 Virus

Sample Type: Nasopharyngeal swabs + commercially available inactivated SARS-CoV2 BA.1

Extraction Output for PCR: SARS-CoV2 RNA



Legend:
Red = PCR product
Blue = Indexed Library

Cleaned SARS-CoV2 amplicon library

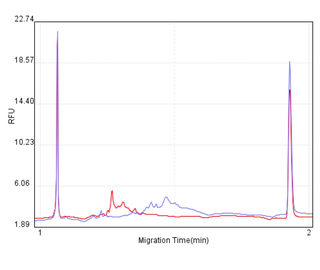
Sequencing Coverage	Mutations Picked Up
>1000x for all amplicons	T95I, G142-, V143-, Y144-, Y145D, **N211-**, **L212I**, N440K, **G446S**, S477N, T478K, E484A, Q493R, **G496S**, Q498R, N501Y, Y505H, D614G, H655Y, N679K, P681H
Conclusion: Sample is positive for Omicron BA.1 strain.	

Mutations highlighted in **bold red** are unique to BA.1.

Sample Input: Inactivated HIV-1 Virus

Sample Type: Commercially available inactivated plasma + inactivated HIV-1

Extraction Output for PCR: HIV-1 RNA



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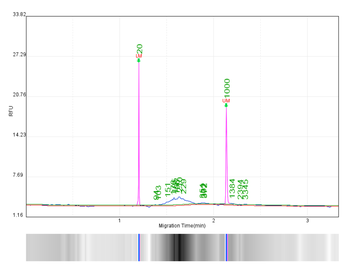


Cleaned HIV-1 amplicon library

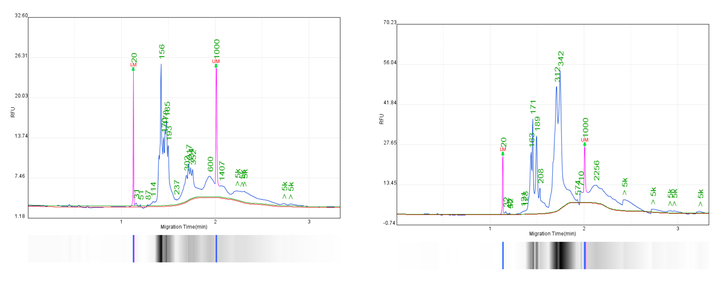
Expected Drug-Resistant Mutations	HIV Drugs
I62K	Atazanavir
L63P	Lopinavir/Ritonavir
L90M	Saquinavir, Nelfinavir, Indinavir, Atazanavir
N232D	Raltegravir

Drug-resistant mutations verified by Sanger sequencing.

Sample Input: FFPE Reference Materials

Sample Type: FFPE Reference Materials

Extraction Output for PCR: Human gDNA



Illumina TruSight Tumor15 Assay – Cleaned Library A & B

Other Use Cases for Application

Sample Type	Application
Nasopharyngeal swabs	Respiratory diseases
	Anti-microbial resistance
Stools	Metagenomics
	Gut microbiome
Peripheral blood	Hepatitis B & C
	Cell-Free DNA
Sputum	Tuberculosis
Urine	Urinary tract infections
Liquid biopsy	Oncology

Conclusion

The Helaxy NGS fluidic device can successfully extract nucleic acid from commercially available sample types: SARS-CoV-2 RNA from nasopharyngeal swabs, HIV-1 RNA from plasma and human gDNA from FFPE reference materials, and generate libraries of target amplicons to reveal expected variants and mutations in the samples. Further developments have been planned for more applications using both in-house and commercially available assays including target enrichment-based library preparation. This fluidic device can thus effectively provide an easy-to-use automated solution for sample library preparation in decentralized facilities to reduce the turn-around-time from sample collection to result reporting for clinical diagnosis and treatment.