UTILIZING *DE NOVO* GENOME SYNTHESIS PLATFORMS TO GENERATE INFECTIOUS HIV-1 AND BK VIRUS

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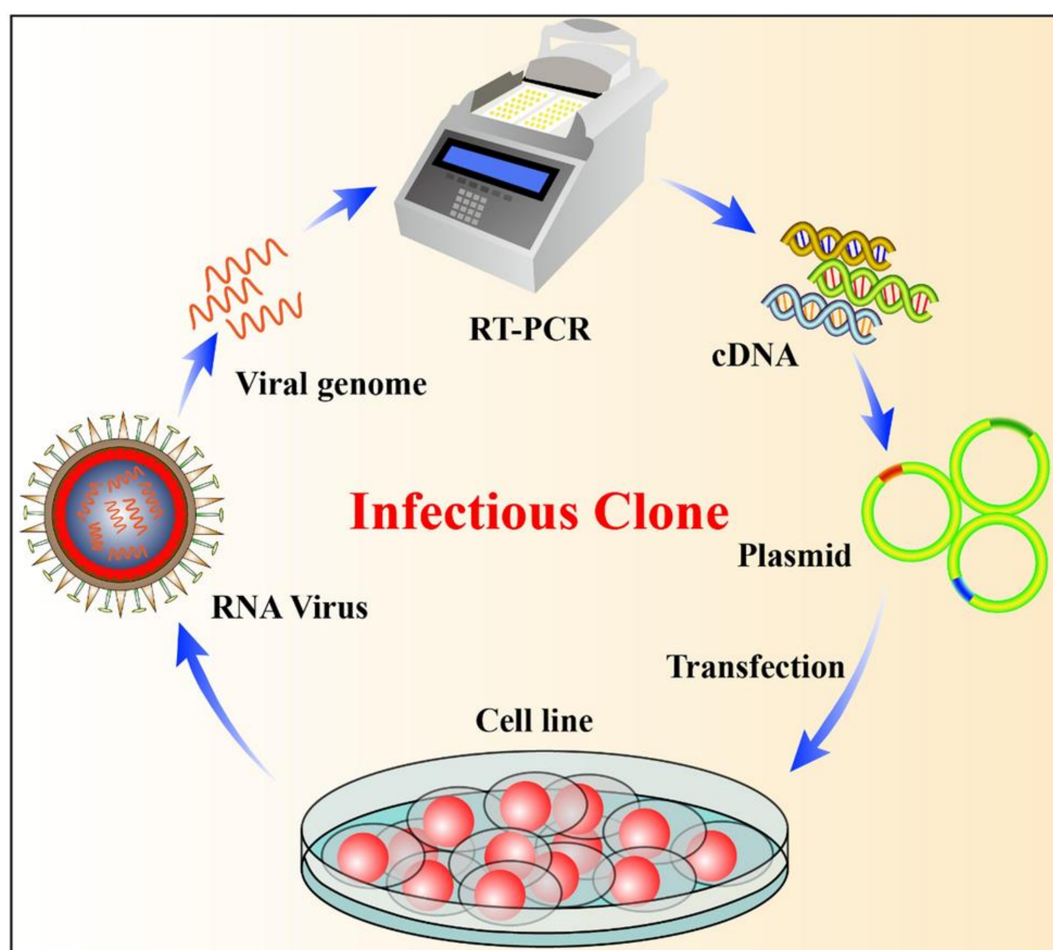
1. INTRODUCTION

- Synthetic virology is a groundbreaking approach to regenerate hard-to-procure and hard-to-culture viruses utilizing the reverse genetics techniques.
- It enables researchers to reconstruct and manipulate viral genomes in the lab and subsequently rescue viruses in host cells via transfection.
- Synthetic virology has multiple applications in vaccine development, gene therapy, antiviral drug testing, studying viral host-cell interactions, biotechnology, biosecurity, and enhancing viral diagnostics.
- Traditional reverse genetics utilize limited techniques for constructing infectious clones of the virus genome (e.g. Clone-based approach) which are time consuming, low throughput, and have low precision. In addition, it requires isolation of infectious virus from clinical samples and obtaining viral genomic materials to work with.



Process of RNA viral rescue with a traditional reverse genetics approach
Source: Li, et al. (2021). Virus Genes, 57, 151-163.

- Modern reverse genetic approaches rely more on *de novo* synthesis of infectious clones for full-length viral genomes (i.e. Synthetic genome). However, these techniques used to be expensive and require sophisticated instrumentation.
- Recent advancements in the *de novo* DNA synthesis chemistry, instrumental technologies, and enhanced gene assembly techniques for larger DNA constructs, along with enzymatic error-correction methods, have dramatically lowered the cost of synthesizing large DNA clones in vitro with high- throughput and error-free.
- More advanced bioinformatics software/tools and open-source databases of extensively annotated genomic sequences across all virus families have become available recently.
- All these recent advancements made the modern reverse genetics and *de novo* DNA synthesis more feasible for synthetic virology application, which is more cost efficient, less time consuming, and eliminates the need for virus isolation from clinical samples.
- However, there are limited number of reports to showcase the powerful applications of the *de novo* DNA synthesis in generating synthetic viruses.
- Isolating active BK virus (BKV) from patients is difficult since virus reactivation is relatively rare.
- HIV-1 has a rapid mutation rate over passages using classical cell culture propagation methods. This makes it challenging to maintain consistent viral strains for research.

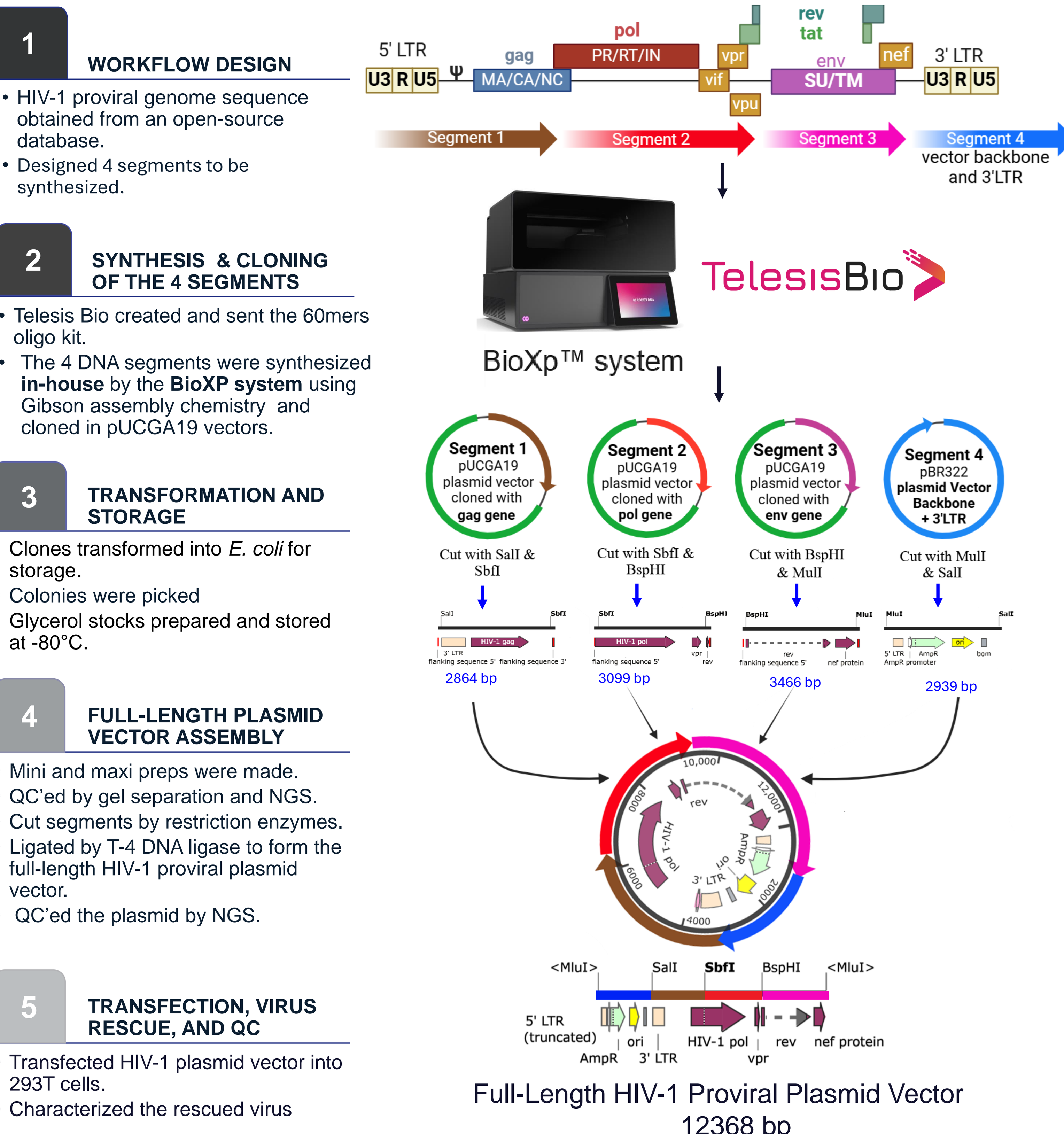
2. AIM OF THE STUDY

This study was aiming to:

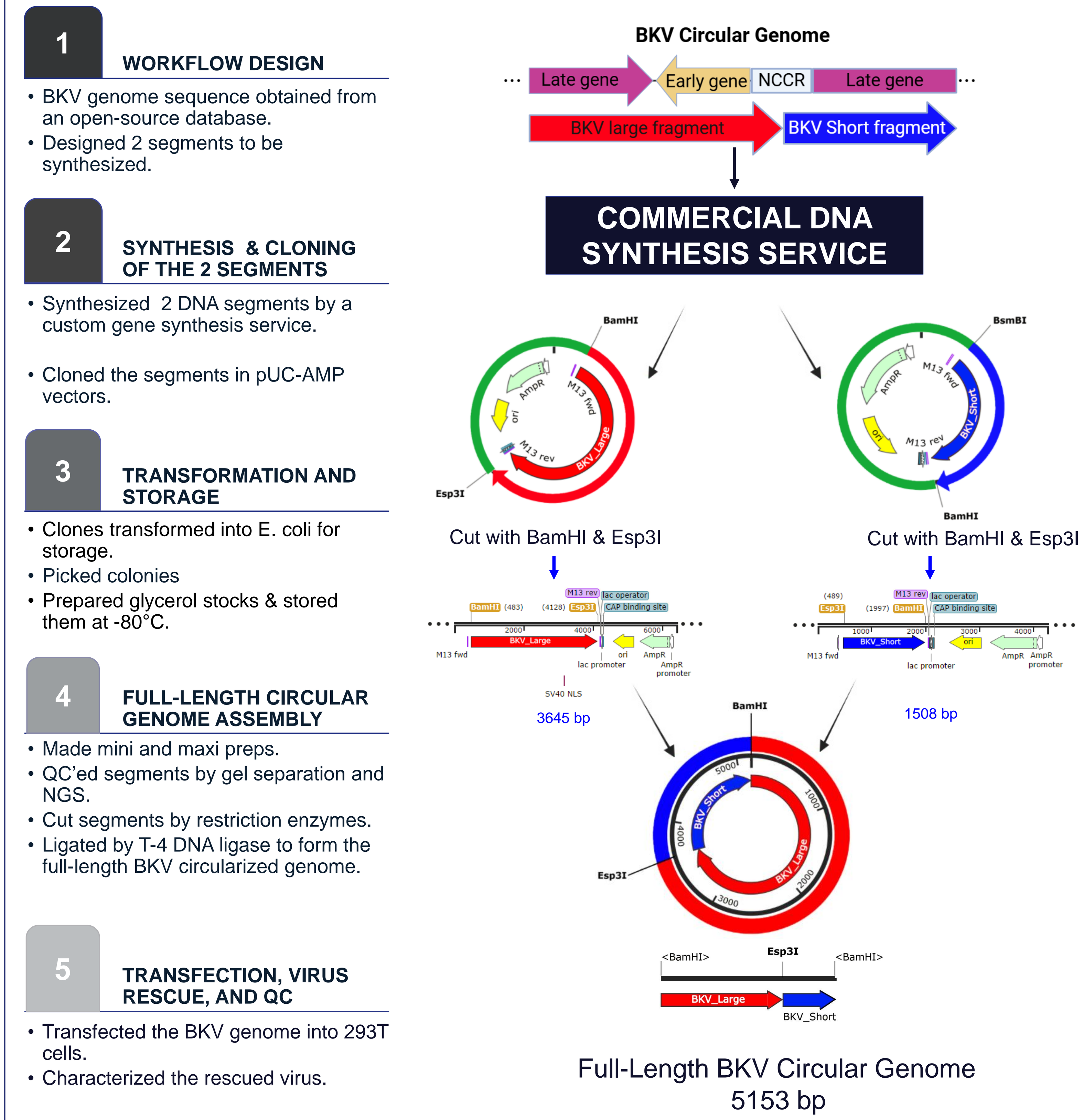
1. Get access to infectious and propagatable HIV-1 and BK virus without a need to isolate them from clinical sample .
2. Utilize two high-throughput *de novo* DNA synthesis platforms, specifically Telesis Bio's BioXp systems, to construct infectious clones of full-length HIV-1 and BKV genomes as a proof-of-the concept.
3. Demonstrate the infectivity and propagability of the rescued viruses after transfection in host cells and the fidelity of the synthetic viral genome sequence.

3. METHODS

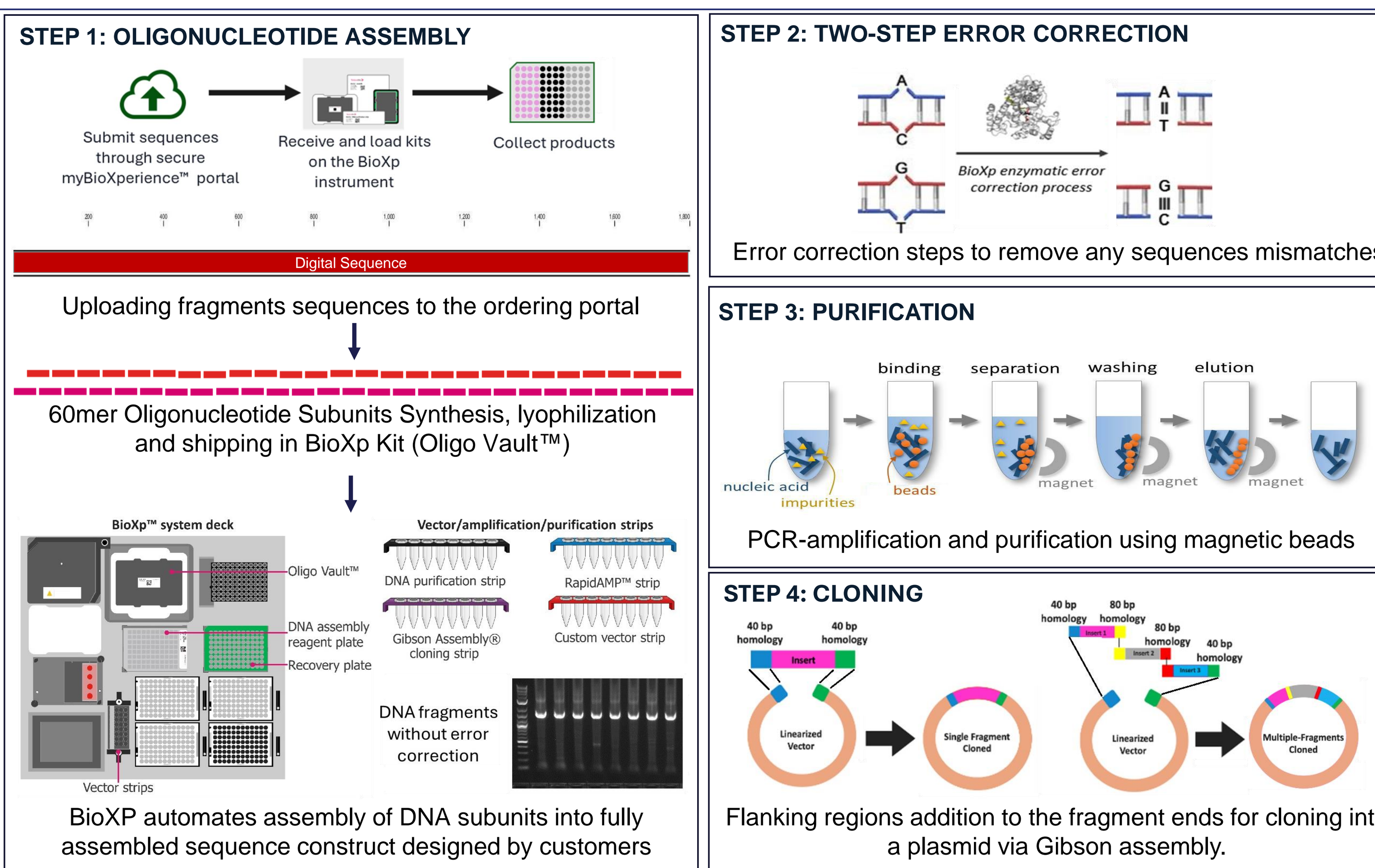
Workflow of the *de novo* synthesis of HIV-1 plasmid vector by BioXP ™ system



Workflow of the *de novo* synthesis of BKV genome by IDT DNA synthesis service



BioXP ™ System Workflow



4. RESULTS

Characterization of HIV-1 plasmid and synthetic BK virus genome

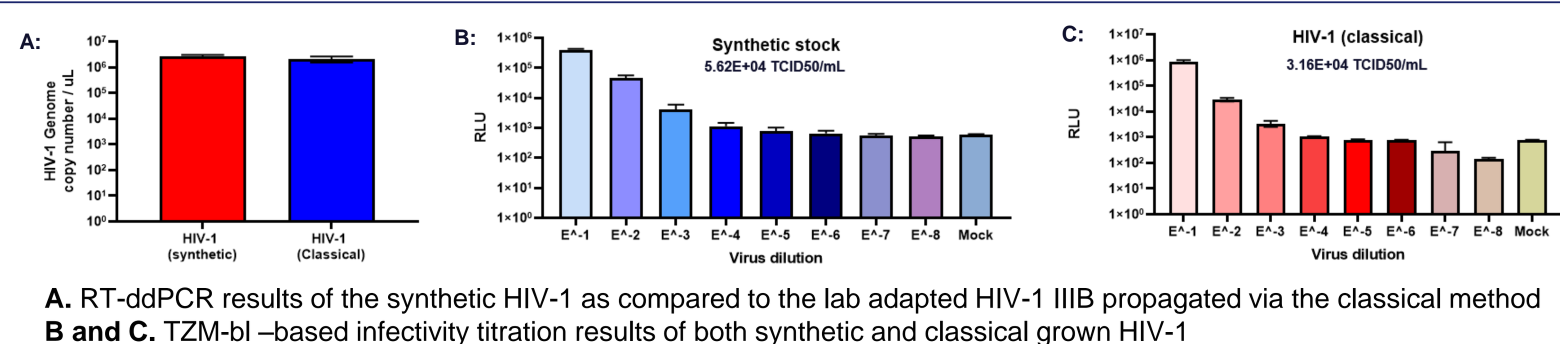
Sequence File name	Identity to reference gene
Full-length HIV-1 proviral plasmid vector (glycerol stock 1)	99.99%
Full-length HIV-1 proviral plasmid vector (glycerol stock 2)	100%
Full-length HIV-1 proviral plasmid vector (glycerol stock 3)	99.93%
Full-length HIV-1 proviral plasmid vector (glycerol stock 4)	100%

Sequence fidelity of the full-length HIV-1 proviral plasmid assessed by Illumina NGS

Sequence File name	Identity to reference gene
Synthetic BKV from transfection #1 28dpt (Supernatant)	100%
Synthetic BKV from transfection #2 28dpt (Supernatant)	100%
Synthetic BKV from transfection #3 21dpt (Supernatant)	100%
Serial propagated BKV from synthetic stock (Supernatant)	100%

Sequence fidelity of the synthetic BK virus genome as assessed by Illumina NGS

Characterization of the synthetic HIV-1 stocks



A. RT-ddPCR results of the synthetic HIV-1 as compared to the lab adapted HIV-1 IIIB propagated via the classical method
B and C. TZM-bl –based infectivity titration results of both synthetic and classical grown HIV-1

4. RESULTS (continued)

Characterization of the synthetic BK virus stocks

Diagram the rescued BKV infectivity characterization approach.

synthetic BK virus
IFA assay for T antigen expression
serial propagation in LLC-MK2 cells
growth media
14 Dpi 10mL supernatant
qPCR titer viral genome

Serial propagated BK virus	BK virus total Genome copy number (GCN/mL)	
	media change 0h baseline	14 dpi supernatant collection
Lot 1	2.35E+05	6.91E+08
Lot 2	6.95E+05	3.50E+09
Lot 3	5.07E+04	1.19E+08
Lot 4	4.78E+06	3.10E+09
Lot 5	2.34E+05	4.56E+08

BKV genome concentration on day 14 post infection of LLC-MK2 cell culture with the synthetic virus, compared to the baseline genome copies of virus inoculum

T-antigen-based IFA assay in LLC-MK2 cells confirming the infectivity of the synthetic BK virus stock.

Mock

Synthetic stock

5. CONCLUSIONS

- Constructed full-length HIV-1 and BKV genomes using advanced *de novo* DNA synthesis platforms, including the on-bench BioXP system from Telesis Bio Inc.
- Demonstrated infectivity and propagability of the synthetic viruses.
- Confirmed the fidelity of the synthetic viral genomes.
- Validated the concept of using *de novo* DNA synthesis to regenerate infectious viruses through a reverse genetics approach, effectively bypassing the need for clinical sample isolation.
- This strategy can be extended to generate other polyomaviruses and retroviruses rapidly and cost-efficiently.
- This synthetic virology approach holds great promise for accelerating research and development in virology and biotechnology for hard-to-access viruses important for public health.

6. REFERENCES

1. Li, et al (2021). Virus Genes, 57, 151-163.
2. Broekema and Imperiale (2012). Virology. Jan 20;422(2):235-41.
3. Montefiori Lab. 2007. Online Link: https://shorturl.at/QwUEk