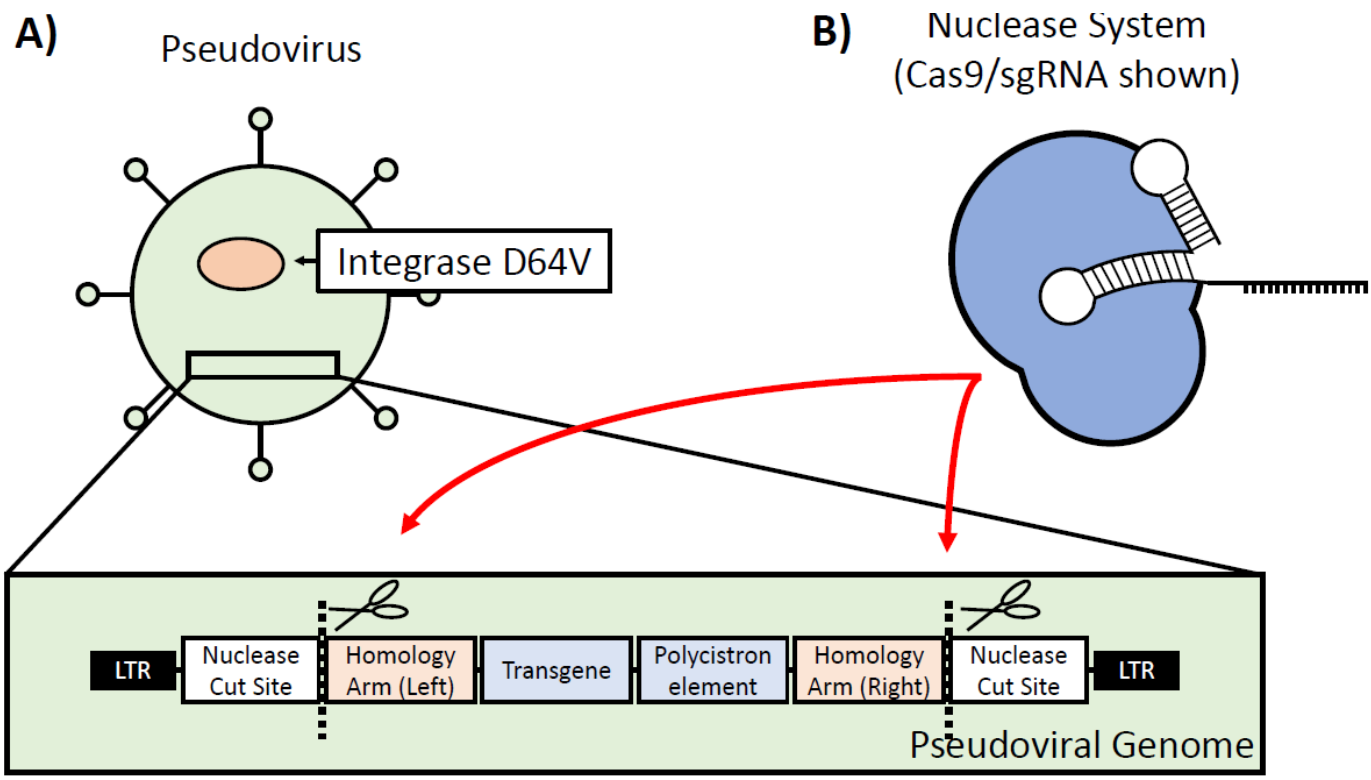
Docket #: S20-259

Knock-In of Large DNA for Stable and Long-Term High Genomic Expression

Advances in CRISPR-Cas technology have sparked a multitude of novel gene editing possibilities for therapeutic applications. However, synthetically engineered cells using lentiviral systems or adeno-associated virus (AAV) often do not express transgenes to a high enough concentration and cannot persist for a long period of time. In addition, knock-in (KI) efficiency of gene fragments into a precise genomic locus requires improvement, while maintaining long-term, stable, and high expression of the desired KI fragments.

The Qi lab invented a novel KI system and methodology that increases efficient KI of DNA fragments with stable, long-term genomic expressions. The two-part KI system includes a nuclease system and an engineered pseudovirus system from a lentivirus to enhance KI and gene translocation. This technology enables incorporation of large genes (7-8kB), exceeding the limitations from currently available technology. The technology also allows simultaneous KI of two genes at different genomic loci in a one-pot reaction. After KI, the cargo can be expressed even after months, showing superior expression stability. By increasing the efficiency and efficacy of gene incorporation, followed by stable gene expression, the invention can overcome the current limitations of existing methods and be a valuable tool in advancing cell-based therapies.



Design of KI system: A) the pseudovirus KI template design; B) the nuclease system
Image courtesy the Stanley Qi lab @ Stanford Bioengineering

Stage of Development – Proof of Concept In Vitro

Applications

- Cellular engineering and manufacturing
- T cell therapy (i.e., CAR-T)
- iPSC engineering
- Stem cell therapy
- Cell-based vaccines

Advantages

- More efficient than traditional KI methods
- Can handle large payloads (over 4kbs, high efficient even for payload 7~8kb)
- Long-term, stable, and high gene expression
- Precise insertion of single and multiple gene fragments

Publications

- Chavez, M., Rane, D. A., Chen, X., & Qi, L. S. (2023). Stable expression of large transgenes via the knock-in of an integrase-deficient lentivirus. *Nature Biomedical Engineering*, 1-11.

Patents

- Published Application: WO2022104344
- Published Application: 20240018493

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