Docket #: S16-453

Efficient homologous recombination of large transgenes using AAV donor vectors

Researchers at Stanford have developed methods to overcome the limited packaging capacity of adeno-associated virus (AAV) vectors and enable their use in integration of large transgenes. AAV vectors can transduce dividing and non-dividing cells and have been used as vectors for homologous recombination *in vitro* and *in vivo*. However, AAV vectors have a maximum packaging capacity of approximately 4.5kb. With the required homology arms this leaves approximately 3.7kb for a transgene expression cassette, which limits the use of AAV donor vectors in genome editing applications. To overcome this limitation the inventors have developed a method to integrate large transgenes by using two donor AAV vectors. These vectors undergo sequential homologous recombination stimulated by CRISPR/Cas9 to integrate large DNA that exceeds the packing capacity of a single AAV vector. This technology provides an effective solution to enable more widespread use of AAV vectors in genome editing, including in therapeutic applications.

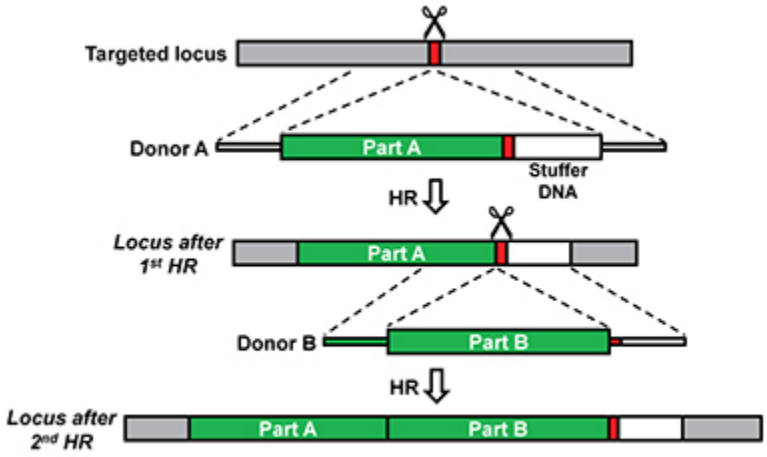


Figure 1: Donor A integrates 'Part A' of the transgene. A key feature of Donor A is that it contains the same sgRNA target site that mediated its integration so that the site is reconstituted in the genome after integration.

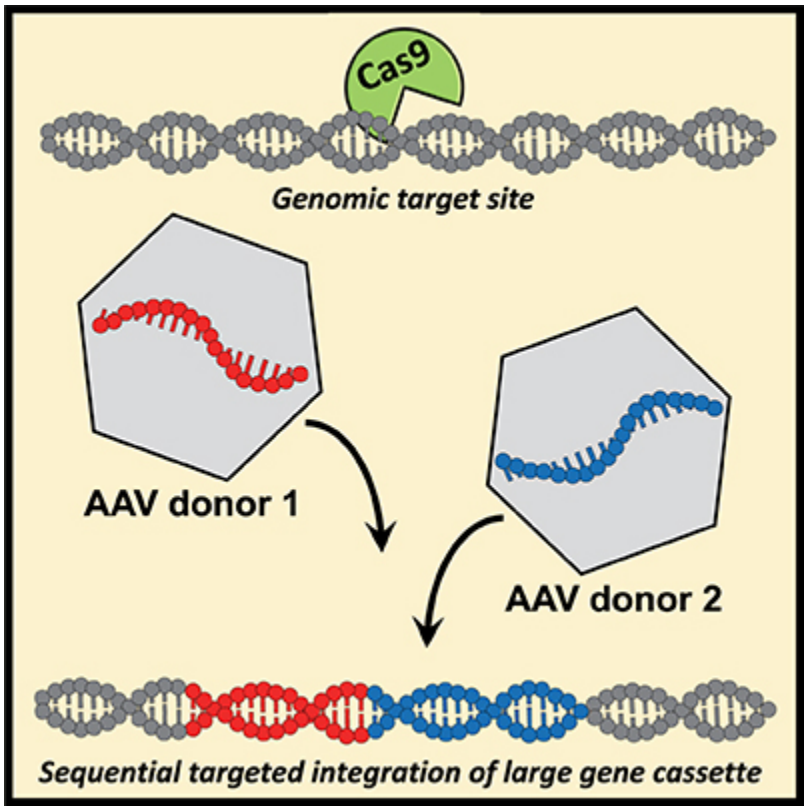


Figure 2: Graphical abstract.

Stage of research
This method has been used in primary human T cells and CD34+ hematopoietic stem and progenitor cells to successfully, site-specifically integrate a 5.7kb transgene expression cassette.

Figure adapted from: Bak, R. O., and Matthew H. Porteus. "CRISPR-Mediated Integration of Large Gene Cassettes Using AAV Donor Vectors." *Cell Reports* (2017). http://dx.doi.org/10.1016/j.celrep.2017.06.064 .4297

Applications

- Genome editing
    - Gene therapy - treat genetic diseases involving mutations in large genes such as:

        - Duchenne Muscular Dystrophy (dystrophin: 11kb)
        - Hemophilia A (Factor VIII: 7kb)
        - Cystic Fibrosis (CFTR: 4.4kb)
    - Cell engineering - use multicistronic expression cassettes to express multiple proteins from the same cassette

Advantages

- Solves an unmet need - enables targeted integration of large transgenes (bigger than 4kb) using AAV vectors
- First successful use of homologous recombination and 2 donor vectors to integrate large DNA
- Single step procedure - does not require serial transfection and transduction of cells

Publications

- Bak, R. O., and Matthew H. Porteus. "CRISPR-Mediated Integration of Large Gene Cassettes Using AAV Donor Vectors." *Cell Reports, 20,* 750-756. 18 July 2017.

Patents

- Published Application: WO2018195555
- Published Application: 20200131539
- Issued: 11,773,409 (USA)

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