Video Article

Molecular Evolution of the Tre Recombinase

Frank Buchholz[1]

[1]Max Plank Institute for Molecular Cell Biology and Genetics, Dresden

URL: http://www.jove.com/video/791
DOI: doi:10.3791/791

Keywords: Cell Biology, Issue 15, HIV-1, Tre recombinase, Site-specific recombination, molecular evolution

Date Published: 5/29/2008

Citation: Buchholz, F. Molecular Evolution of the Tre Recombinase. *J. Vis. Exp.* (15), e791, doi:10.3791/791 (2008).

Abstract

Here we report the generation of Tre recombinase through directed, molecular evolution. Tre recombinase recognizes a pre-defined target sequence within the LTR sequences of the HIV-1 provirus, resulting in the excision and eradication of the provirus from infected human cells.

We started with Cre, a 38-kDa recombinase, that recognizes a 34-bp double-stranded DNA sequence known as loxP. Because Cre can effectively eliminate genomic sequences, we set out to tailor a recombinase that could remove the sequence between the 5'-LTR and 3'-LTR of an integrated HIV-1 provirus. As a first step we identified sequences within the LTR sites that were similar to loxP and tested for recombination activity. Initially Cre and mutagenized Cre libraries failed to recombine the chosen loxLTR sites of the HIV-1 provirus. As the start of any directed molecular evolution process requires at least residual activity, the original asymmetric loxLTR sequences were split into subsets and tested again for recombination activity. Acting as intermediates, recombination activity was shown with the subsets. Next, recombinase libraries were enriched through reiterative evolution cycles. Subsequently, enriched libraries were shuffled and recombined. The combination of different mutations proved synergistic and recombinases were created that were able to recombine loxLTR1 and loxLTR2. This was evidence that an evolutionary strategy through intermediates can be successful. After a total of 126 evolution cycles individual recombinases were functionally and structurally analyzed. The most active recombinase -- Tre -- had 19 amino acid changes as compared to Cre. Tre recombinase was able to excise the HIV-1 provirus from the genome HIV-1 infected HeLa cells (see "HIV-1 Proviral DNA Excision Using an Evolved Recombinase", Hauber J., Heinrich-Pette-Institute for Experimental Virology and Immunology, Hamburg, Germany). While still in its infancy, directed molecular evolution will allow the creation of custom enzymes that will serve as tools of "molecular surgery" and molecular medicine.

Video Link

The video component of this article can be found at http://www.jove.com/video/791/

Disclosures

The authors have nothing to disclose.

