System Biosciences
Accelerating discoveries through innovations

Genome Engineering

phiC31 Integrase Vector System

One-step gene addition technology

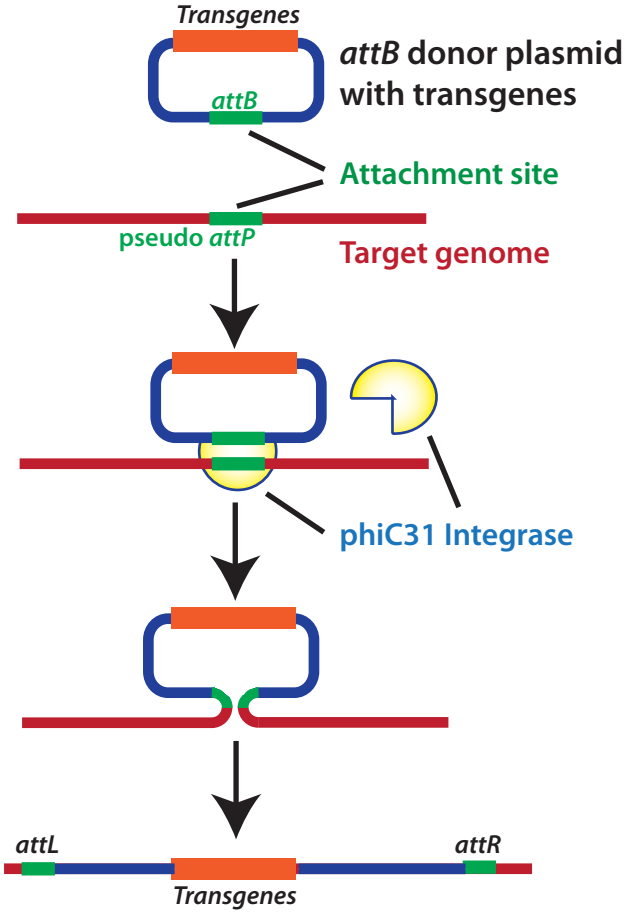
Nonviral transgene delivery

The phiC31 integrase is a sequence-specific recombinase encoded within the genome of the bacteriophage phiC31. The phiC31 integrase mediates recombination between two 34 base pair sequences termed attachment sites (att), one found in the phage and the other in the bacterial host. This serine integrase has been shown to function efficiently in many different cell types, including mammalian cells. In the presence of phiC31 integrase, an attB-containing donor plasmid can be unidirectionally integrated into a target genome through recombination at sites with sequence similarity to the native attP site (termed pseudo-attP sites). phiC31 integrase can integrate a plasmid of any size, as a single copy, and requires no cofactors. The integrated transgenes are stably expressed and heritable.

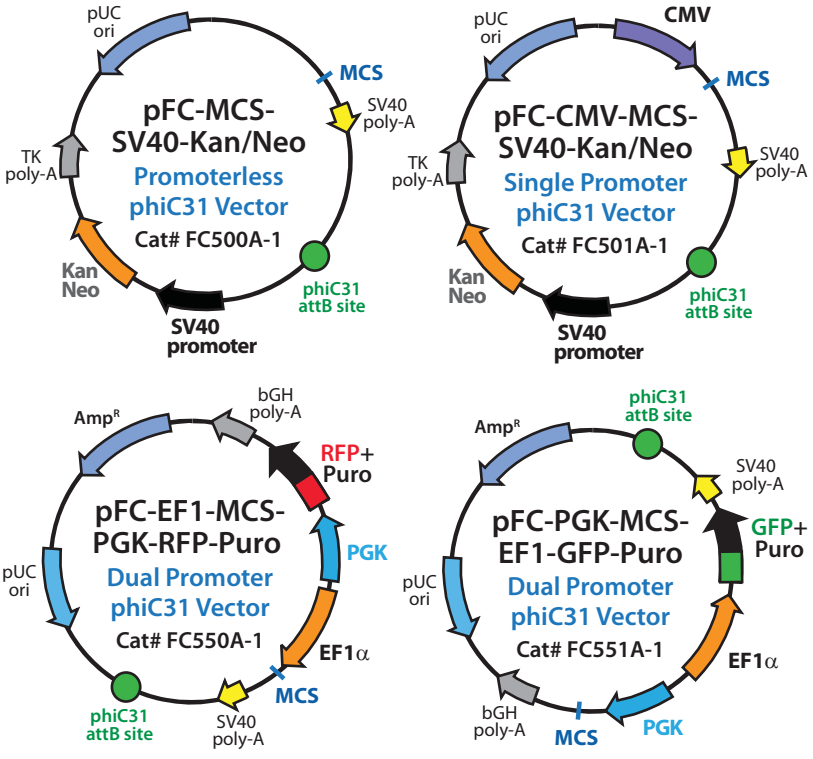
Highlights

- Nonviral transgenesis
- Single-copy integration
- Preferred integration at active transcription sites
- Unlimited cargo capacity
- Easily make stable cell lines using transfection-based methods
- Suitable for engineering Stem Cells and In vivo animal models

How phiC31 integrase works



phiC31 integrase donor vector formats



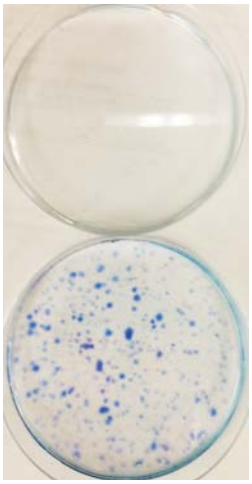
www.systembio.com/phic31

One-step phiC31-mediated gene addition

HEK293 cells seeded onto 6-well plates were transfected the next day in triplicate according to the manufacturer's instructions with Lipofectamine 2000 (Invitrogen) and 40 ng pFC-PGK-MCS-EF1-GFP-Puro Dual Promoter phiC31 Donor Vector (Cat# FC551A-1) and either 2 μg carrier plasmid DNA or 2 μg phiC31 integrase expression plasmid. The cells were trypsinized after 48 hours, resuspended in 1 ml of media, and split 1:20 onto 10 cm plates containing 10 ml of media. After 24 hours, puromycin 0.5 μg/ml was added to the medium to begin selection. After 11 days, the cells were fixed and stained with a solution of 50% methanol plus 1% methylene blue. The plates were washed twice with PBS and allowed to air dry. The number of visible colonies were imaged and colony number counts assessed. There were no visible colonies on the minus phiC31 integrase and over 300 colonies were counted on the plus phiC31 integrase plate (colony assay data shown to the right).

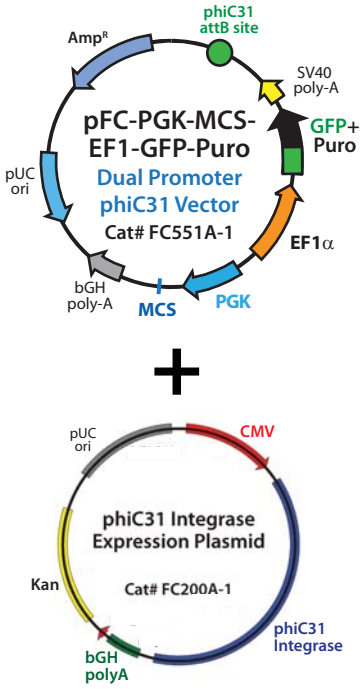
- phiC31 Integrase

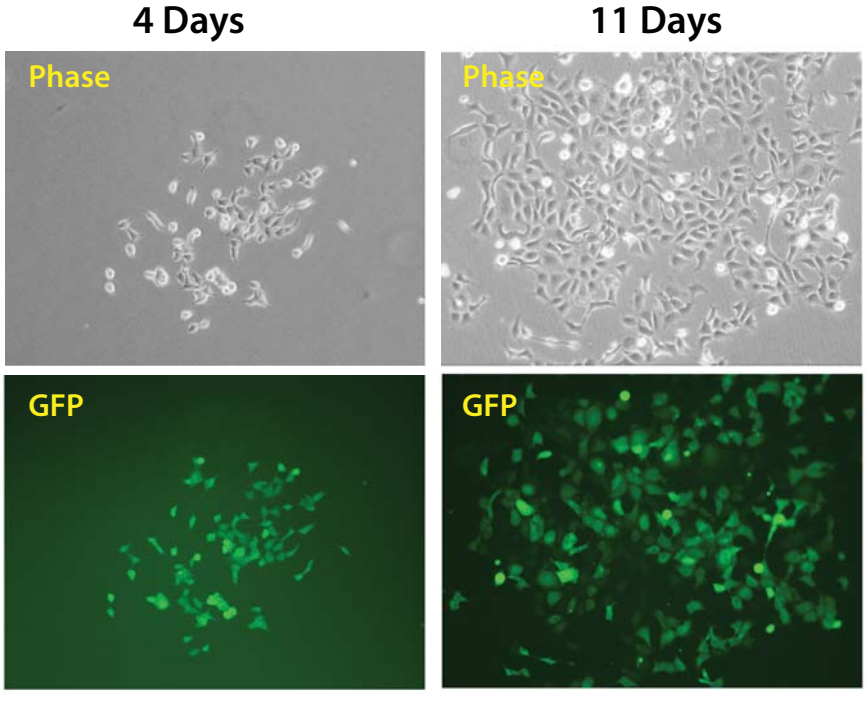
+ phiC31 Integrase



phiC31 donor integration with GFP and Puro marker expression

Approximately 400,000 HEK293 cells were co-transfected with 1.9 μg Integrase expression vector with 40ng pFC-PGK-MCS-EF1-GFP-Puro Dual Promoter phiC31 Donor Vector (Cat# FC551A-1). The cells were split 1:10 after 24 hours post-transfection. Puromycin selection at 1 μg/ml was initiated after another 24 hours with continuous selection for 4 days. Cells were imaged at 4 and 11 days. Representative GFP fluorescence and phase contrast fields are shown below.





We Also Offer Custom Services - *Custom phiC31 donor vector cloning and cell lines*

System Biosciences offers a wide-range of custom services to support your research, allowing you to spend less time making tools, and more time making discoveries. To learn more, visit our website at www.systembio.com/service or call us at 888-266-5066.

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