The PinPoint Integrase System for Generation of Isogenic Cell Lines

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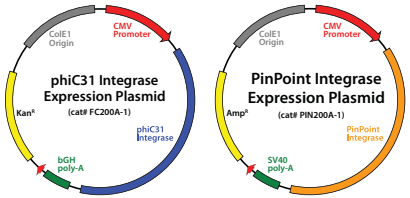
Introduction

PinPoint targeted integration system™

The PinPoint targeted integration system uses the phage integrase ΦC31 to stably place a single copy of a targeting plasmid in a sequence-specific manner within any genome. ΦC31 integrase-mediated integrations, unlike the more commonly used recombinases Cre and Flp, are unidirectional in nature due to the fact that recombination occurs between two dissimilar sites. Once the targeting plasmid is placed, stable cell lines are generated and are used in the PinPoint integrase-mediated site-specific targeting of DNA elements of your choice to a sequence within the placed plasmid. This process allows for the generation of isogenic cell lines from your organism of choice.

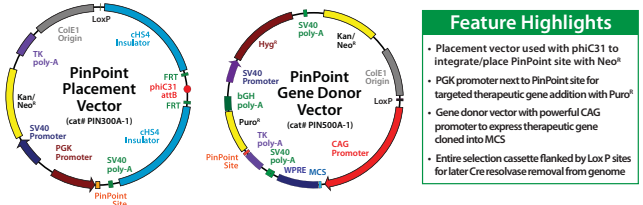
Integrase Expression Plasmids



Feature Highlights

- Potent CMV promoter driving transient expression of Integrases
- Polyadenylation sites to ensure Integrase transcription and expression
- Easy-to-use antibiotic markers for high copy propagation in *E. coli*

Placement and Gene Donor Vectors



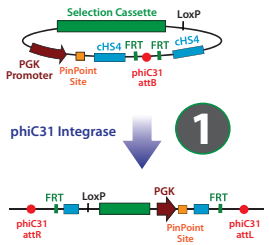
Feature Highlights

- Placement vector used with phiC31 to integrate/place PinPoint site with Neo^R
- PGK promoter next to PinPoint site for targeted therapeutic gene addition with Puro^R
- Gene donor vector with powerful CAG promoter to express therapeutic gene cloned into MCS
- Entire selection cassette flanked by Lox P sites for later Cre resolvase removal from genome

How PinPoint Works

In the first step, PinPoint placement cell lines are generated. This is achieved through use of ΦC31 integrase to place a single copy of the targeting plasmid within the genome in a sequence-specific manner. Stable cell lines are subsequently isolated through neomycin selection. The placement plasmid also contains the PinPoint integrase recombination site for retargeting as well a PGK promoter for drug selection of retargeted cell lines in the following step.

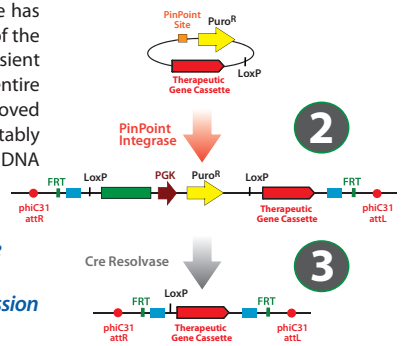
PinPoint Placement Vector



In the second step, a PinPoint donor plasmid containing DNA elements of your choice is retargeted to the initially placed plasmid through PinPoint integrase mediated-recombination. The PinPoint donor vector contains a promoterless puromycin resistance gene. Following retargeting, this puromycin gene is place immediately downstream of the previously placed PGK promoter resulting in cells that are puromycin resistant.

Finally, to remove the majority of the unwanted bacterial plasmid sequences, a single LoxP site has been engineered into each of the two vectors. Following transient Cre transfection the entire selection cassette is removed leaving behind your stably integrated and insulated DNA elements in the third step.

PinPoint Therapeutic Gene Donor Plasmid



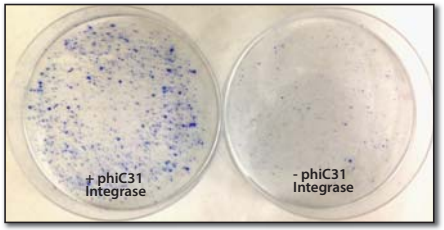
Therapeutic Gene Cassette Cleanly placed into Target Genomes for Stable Expression

Benefits of PinPoint

Unlike the R4 integrase used in a similar system, the PinPoint integrase does not recognize pseudo-sites and will only integrate at its placed recognition sequence. This feature results in the increased efficiency of correctly retargeted cell lines compared to the R4 integrase system. The majority of the unwanted plasmid sequences can be excised using the LoxP site following transient Cre transfection. The entire therapeutic gene expression cassette is active and fully insulated with HS4 DNA elements.

Neomycin resistant HEK293 cells with placed PinPoint site

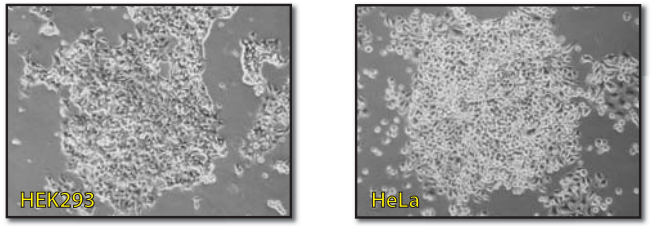
Insulated PinPoint Placement Vector colony count assays



Approximately 8x10^5 HEK293 cells or HeLa cells were transfected with 40 ng PinPoint placement vector and 1,960 ng phiC31 integrase (1:50 ratio). Positive cells were selected with 400 µg/ml G418 for 14 days. Six separate lines of HEK293 and HeLa cells were picked and expanded. These lines were then transfected with a PinPoint Gene Donor vector for precise integration.

Puromycin resistant HEK293 and HeLa targeted cells

Insulated PinPoint Gene Donor Vector Placed and colony visualization



About 8x10^5 HEK293 or HeLa PinPoint placed cells were transfected with 40 ng PinPoint gene donor vector and 1,960 ng PinPoint integrase (1:50 ratio). Positive cells were selected with 0.5-1.0 µg/ml puromycin for 7 days. The PinPoint system enables site-specific gene addition with single copy integration to deliver therapeutic expression levels of genes of interest.

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