System Biosciences
Accelerating discoveries through innovations

Genome Engineering

PinPoint™ Targeted Integration System

Site-specific gene addition to create isogenic cell lines

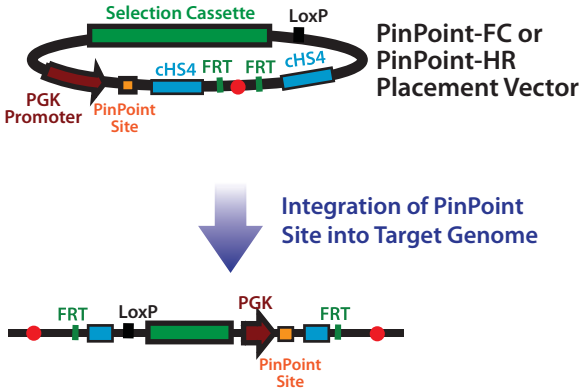
The PinPoint technology

The PinPoint targeted integration system allows users to easily and efficiently create isogenic cell lines in mammalian as well as many other cell types. Multiple genetic features (such as promoter-gene combinations) can be integrated into a genome of choice in a site-specific manner. This technology allows the researcher to engineer platform cell lines to study the effects of knocking-in various transgenes of interest at the same genetic locus in cells with the same genetic background. This level of targeting control allows for studying of phenotypic effects free from context and positional effects, which results in more accurate genotype to phenotype correlation.

Highlights

- Specific construct insertion at a defined genomic locus
- Place PinPoint insertion site at AAVS1 safe harbor or any genomic locus
- Create isogenic cell lines
- Avoid gene integration location site variabilities between cell lines
- Control copy number easily

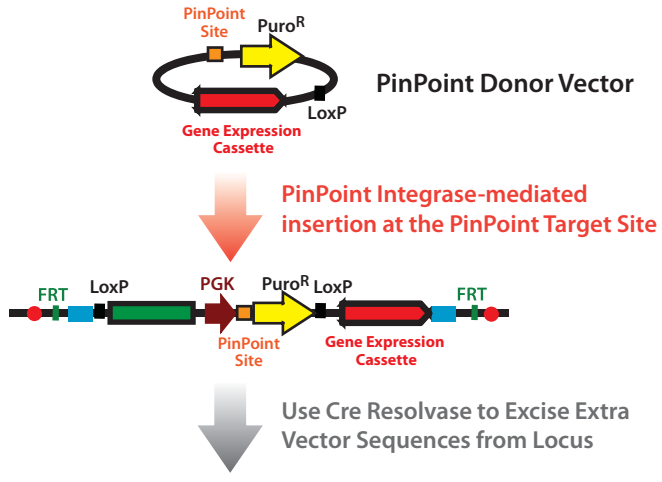
Step 1: PinPoint Target Site Placement



The PinPoint system is a two-step approach for engineering of target cells with an optional third step for selection cassette removal by Cre resolvase. The first step involves insertion of a plasmid bearing the PinPoint placement site via transfection into the target cell genome.

PinPoint targeted integration system™

Step 2: Targeted Integration of PinPoint Donor



Optional Step 3: Cre-mediated Cassette removal

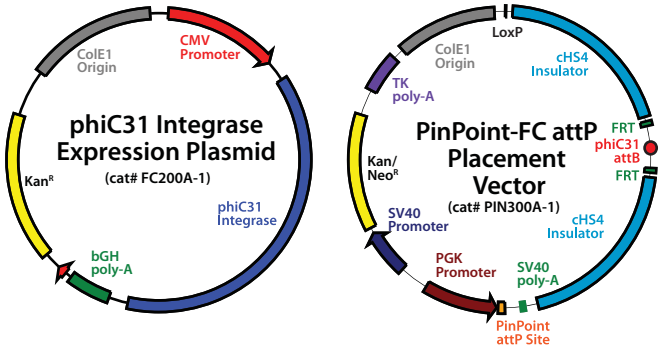
This can be done using two distinct approaches: i) the PinPoint-FC system that involves the well-characterized phiC31 integrase system or ii) the PinPoint-HR system that uses either TALE-Nuclease or Cas9 genome engineering tools to induce a double-stranded DNA break in the genome and insertion of the PinPoint placement site by homology-directed recombination (HDR) in a site-specific manner. The second part of the PinPoint system relies on the introduction of a donor vector containing your desired gene cassette insert, which is integrated into the placed PinPoint site using a hyperspecific and efficient PinPoint integrase. The PinPoint integrase catalyzes the attB x attP reaction between the placed site (attP) and the attB site in the donor vector to insert the donor vector at the placed site each and every targeting event. The third optional step invloves the removal of the entire backbone (excluding the insert and its promoter) using the well-characterized Cre/LoxP reaction leaving only the promoter/insert combination (and a single LoxP site) in the genome.

www.systembio.com/pinpoint

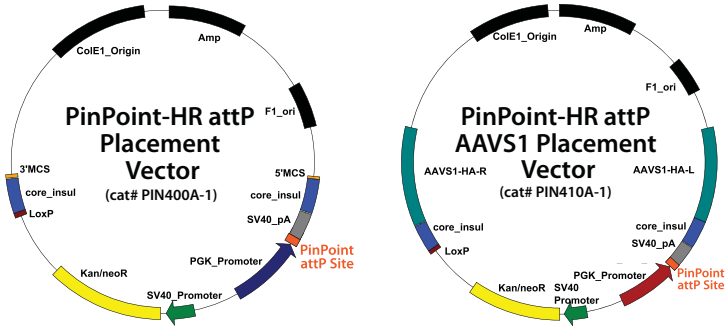
Vectors and features of the PinPoint system

Once the platform cell lines containing the PinPoint attP site placed via phiC31 integrase (Cat# FC200A-1 with PIN300A-1) or HR-based (Cat# PIN400A-1 or PIN410A-1) methods have been established, the cells are ready to be targeted using the PinPoint integrase (Cat# PIN200A-1) and a donor vector construct of choice. This transfection-based reaction is very efficient and limited only by the transfection efficiency of the cell line being targeted. Co-transfection of the PinPoint integrase and the donor vector containing your insert driven by EF1α (Cat# PIN500A-1), CAGs (Cat# PIN510A-1), or a user-defined promoter (Cat# PIN520A-1) into the PinPoint cell line will result in a specific recombination reaction between the PinPoint attP site and the attB site in the donor plasmid. This results in stable integration of the donor plasmid into the PinPoint attP site. As a result of this reaction, correctly targeted donor vectors will have their promoterless puromycin-resistance gene now under control of the PGK promoter present in the PinPoint placement vector, and will be resistant to puromycin when subjected to selection. With expansion of the platform cell lines with the placed PinPoint attP site, each cell line can now be engineered to express different genes of interest at the exact same genomic location for precise determination of underlying phenotypes, a critical feature that distinguishes the PinPoint system from other targeted gene placement systems.

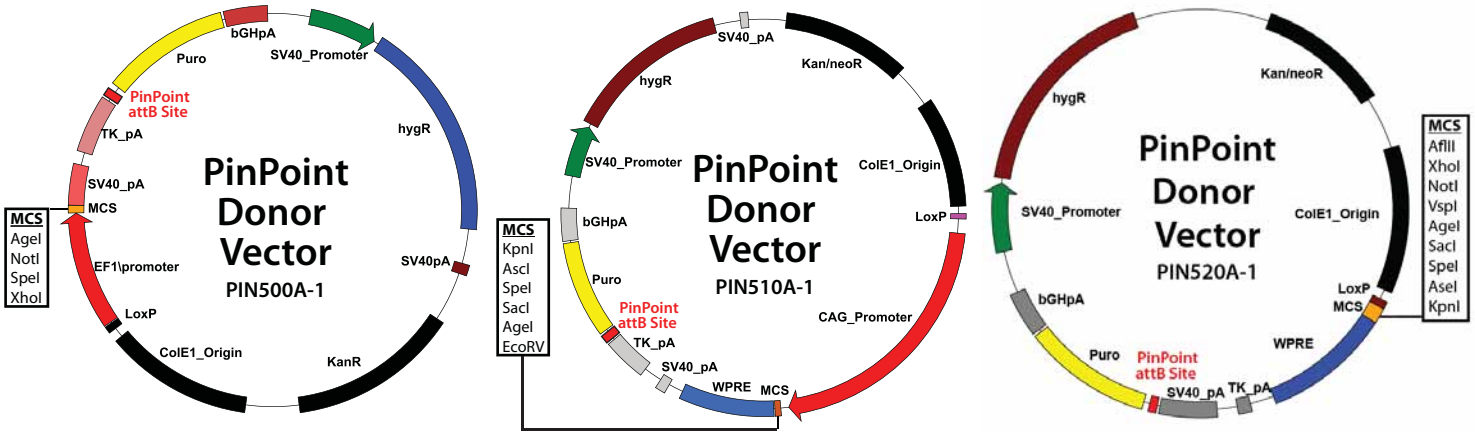
PinPoint attP placement system



PinPoint attP HR placement vectors



Donor plasmids for PinPoint site targeting



We Also Offer Custom Services - *Have SBI Design and Build a Custom PinPoint Vector.*

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.

SBI System Biosciences

System Biosciences, Inc.
265 North Whisman Rd.
Mountain View, CA 94043

Toll Free: 888.266.5066
Fax: 650-968-2277
Email: info@systembio.com
www.systembio.com