System Biosciences
Accelerating discoveries through innovations

Genome Engineering

PrecisionX™ HR Targeting Vectors

Genome engineering with homology-directed recombination vectors to knock-in/out or tag genes

HR Targeting Vectors for Site-specific Genome Modifications

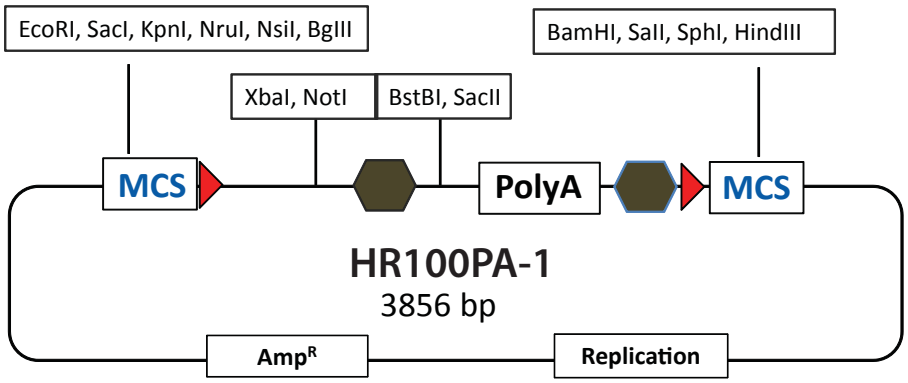
Recent advances in tools for precise genome engineering of target cells have revolutionized the field of biology. Transcription activator-like effector nucleases (TALENs), first described in the plant pathogen *Xanthomonas sp.*, have shown that researchers can efficiently target any genomic DNA sequence using a pair of custom TALEN proteins whose DNA-binding modules can recognize individual DNA nucleotides based on an elegant amino acid cipher. The Cas9/CRISPR technology uses a unique series of short RNAs (collectively termed as "guide RNA") to specifically target a complementary DNA sequence, and upon binding, leads to recruitment of an endonuclease called Cas9 to specifically induce a double-stranded break (DSB) in the DNA sequence targeted by the guide RNA. Similar to TALENs, the introduction of DSBs in the target DNA leads to recruitment of the cellular repair machinery which can drive homologous recombination (HR) at the cleavage site in the presence of complementary sequence arms that are cloned into PrecisionX targeting vectors.

Highlights

- Compatible with ZFNs, TALENs and CRISPR/Cas9 systems
- Dual marker options included
- Built-in transcription insulators
- LoxP sites for genomic removal
- HR-arm multiple cloning sites for simple vector construction

PrecisionX HR Targeting Vector for Gene Knock-in

For targeted insertion of any expression cassette into a specific location in the genome, such as a "safe harbor" locus for stable expression with minimal context-dependent effects (e.g. human AAVS1 or mouse Rosa26 loci), an HR vector containing homology arms at 5' and 3' ends with an expression cassette between the homology arms is required. This strategy involves co-transfection of ZFN, TALEN or Cas9 plasmids with a knock-in targeting vector to direct site-specific integration at the targeted locus. SBI's gene knock-in targeting vector (Cat#HR100PA-1) is designed to be the vector of choice for constructing HR vectors with custom expression cassettes for gene knock-in applications.



MCS Multiple Cloning Sites

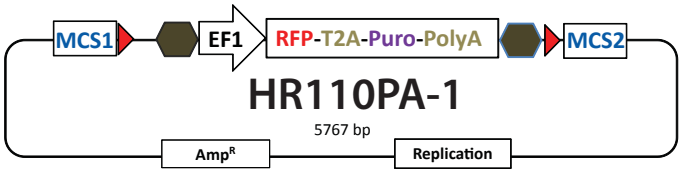
LoxP Sites

Insulator Sequences

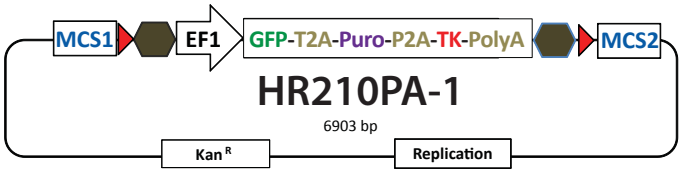
www.systembio.com/hr-vectors

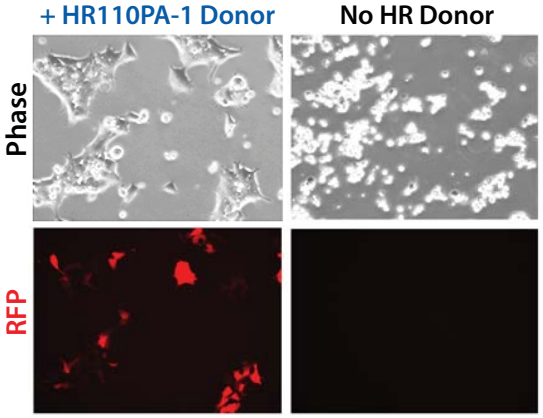
Genomic Knock-out HR Vectors

For HR-based applications needing the disruption of a particular protein-coding or non-coding gene of interest, a gene-knockout targeting vector is desirable. This vector should include homology arms (0.5kb-1kb in length) at the 5' and 3' regions immediately flanking the DNA sequence of interest (e.g. exonic sequence) to specifically direct the targeting vector to insert an expression cassette where the targeted sequence lies, leading to the 'knock-out' of the endogenous sequence by gene addition. SBI's gene knockout targeting vectors (Cat #HR110PA-1 and #HR210PA-1) are designed to be the vectors of choice for this application. Gene-specific homology arms can be cloned into MCS1 and MCS2 to direct HR-based gene disruption in combination with ZFN, TALEN or Cas9 systems. Correctly disrupted cell lines are selected as RFP-positive (GFP for HR210PA-1) and puromycin-resistant colonies. The selection cassettes are flanked by LoxP sites and thus can be removed using a transient expression of Cre recombinase (not included).



MCS Multiple Cloning Sites LoxP Sites Insulator Sequences

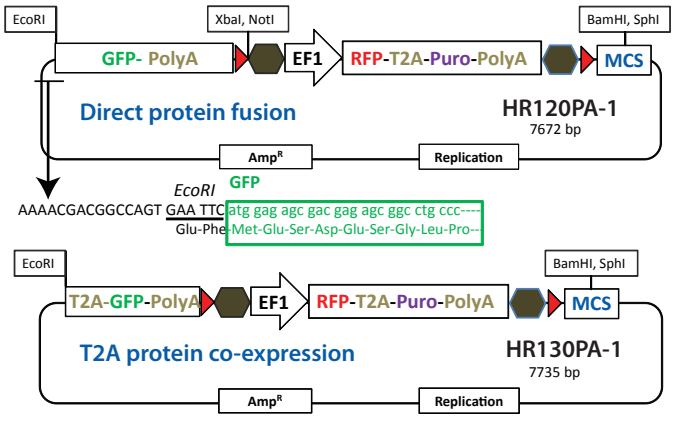


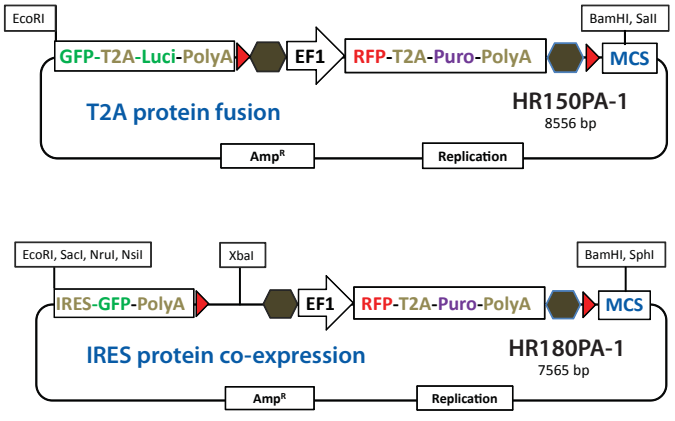


HEK293 Cell Knock-out with TALENs plus puromycin selection

Gene-Tagging HR Vectors

Tag an endogenous sequence of interest with a marker to create a fusion or to co-express a marker tag with the endogenous sequence for tracking spatial dynamics of a protein, lineage tracking, or transcription reporting using HR-tagging vectors.





We Also Offer Custom Services - *Have SBI Design and Build a Custom HR Vector for you.*

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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