SBINSIGHTS

Choosing a Vector for Gene Delivery

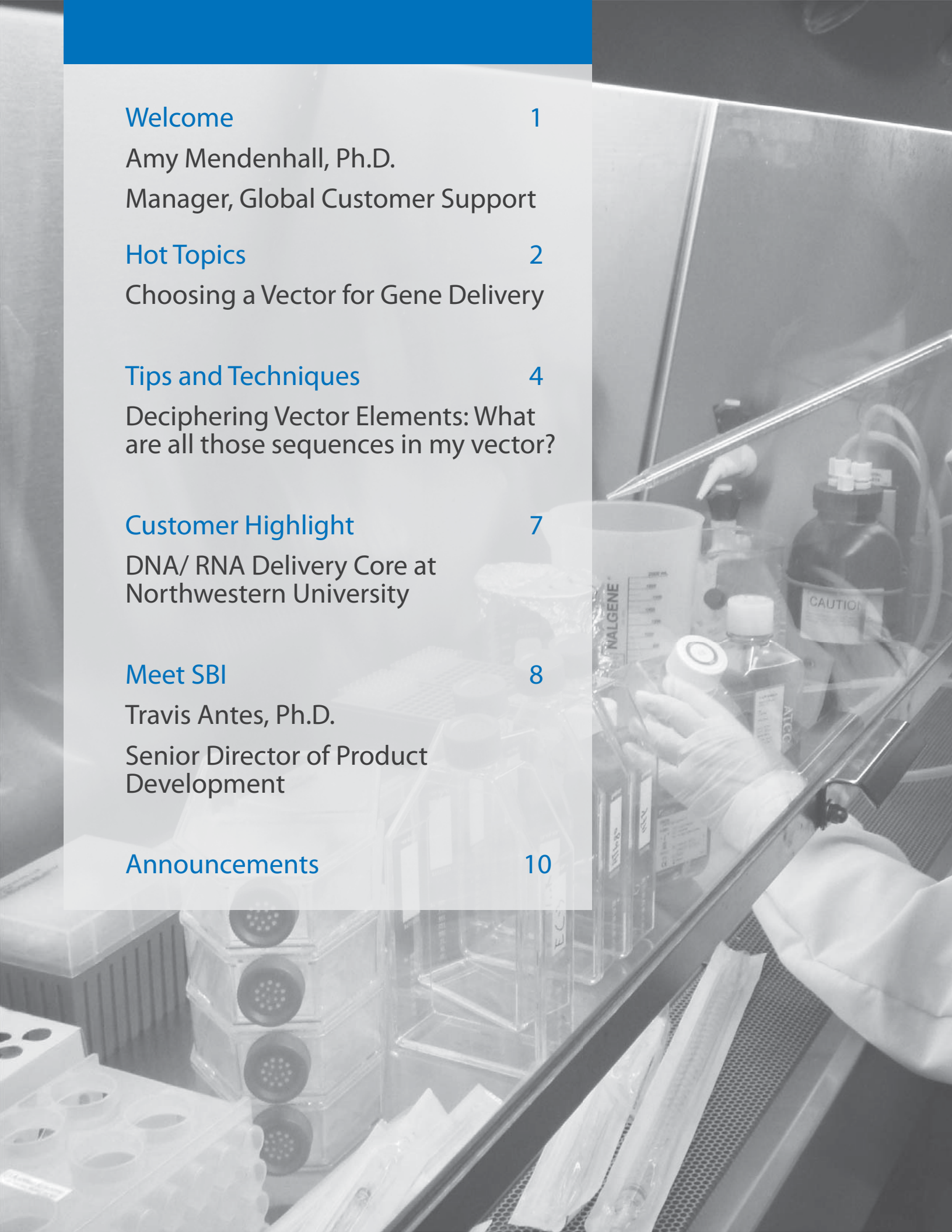
Deciphering Vector Elements

DNA/ RNA Delivery Core at Northwestern University

SBI's Non-coding RNA Department

Issue.
03

ACCELERATING DISCOVERIES THROUGH INNOVATIONS



Dear SBI Customer,

Welcome to the 3rd volume of SBI's quarterly e-newsletter, **SBI**NSIGHTS. The focus of this issue is vectors. We have a series of articles highlighting the different vector systems available from SBI, what the different vector elements are and how they work.

SBI has also recently launched a new free webinar program. Our webinars cover many different topics and are usually held on Thursday mornings. Take a look at the back page of this newsletter for the upcoming schedule, or go to the SBI website to reserve your spot. http://www.systembio.com/webinars

If you have anything that you would like to contribute to **SBI**NSIGHTS, please contact tech@systembio.com. We would enjoy hearing your comments and questions. If you have published a paper or presented your work at a meeting or conference using products or services from SBI, let us know! We would love to hear your feedback and may even feature your work in a future volume.

We hope you enjoy reading **SBI**NSIGHTS.

Amy Mendenhall

Amy Mendenhall, Ph.D.
Manager, Global Customer Support
Editor, **SBI**NSIGHTS

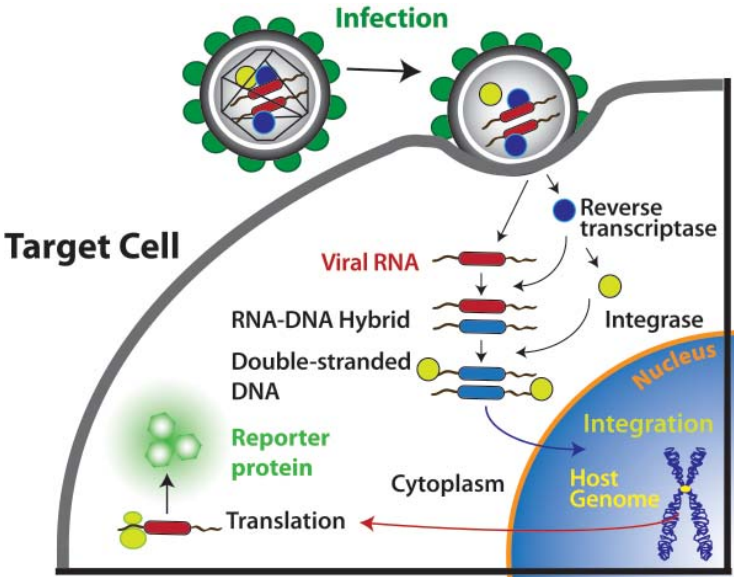
Choosing a Vector for Gene Delivery

Choosing the best gene delivery system for your research is an important part of starting a new project. This guide will describe some of the vector choices available from SBI and help you to choose the most appropriate vector for your work. SBI has three main systems for gene delivery: Lentiviral vectors, Minicircle Technology, and the PiggyBac Transposon system. These systems can all be used to deliver genes, microRNAs, shRNA, or reporter constructs into target cells, but the characteristics of each system are quite different. Here we provide an overview of what each system is and how it works.

Lentiviral Vectors

Lentiviruses are a type of retrovirus that are able to infect non-dividing or dividing cells. They are especially useful for gene delivery into difficult-to-transfect cells, like primary cells, but they can also be used to deliver genes *in vivo* or in cell lines in culture. Through the viral coat protein, lentiviruses infect a target cell. Transgenes contained within the lentivirus are reverse transcribed and then integrate into the host cell genome, thus providing a mechanism of creating stable expression of the transgene. Lentiviruses can be used to delivery genes, microRNAs, shRNAs, or reporter constructs. Since the lentiviral construct integrates into the host cell genome, as the cells divide, they pass on the transgene to the daughter cells, without a dilution in the intensity of expression of the transgene.

While lentivector plasmids can also be transfected into target cells, packaging them into viral particles and transducing target cells enables permanent expression of the transgene. The viral packaging protocol takes approximately 2-3 weeks to complete, including titering of the virus. Lentiviruses from SBI are third generation of biosafety, which means that they are non-replicative and self-inactivating. This means that the viruses can only infect a cell one time, and that the target cells cannot go on to produce more viral particles. *

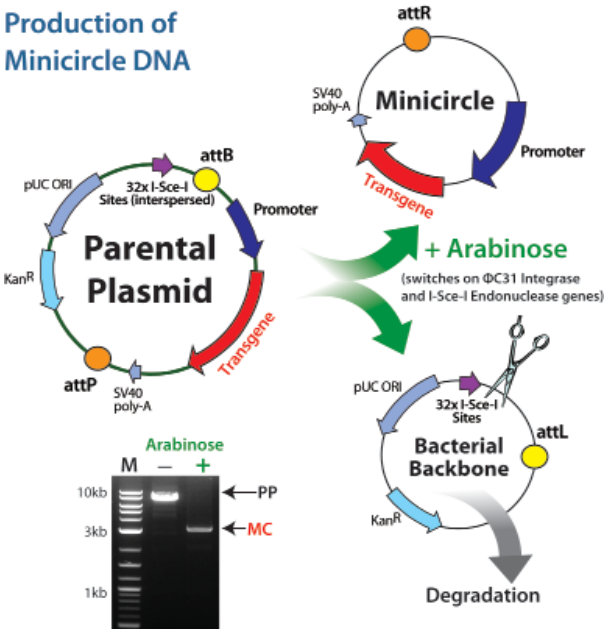


SBI's HIV-based lentivectors and packaging system typically result in viral titers up to 1x10^9 infectious units/ ml. Infectious units measure actual active viral particles as opposed to transduction units or viral particles, which could include empty viruses. SBI has many different options available for choosing your lentivector including promoter choice, constitutive or inducible expression, dual or single promoter, marker gene expression, and IRES or T2A ribosomal skip sites for bi-cistronic expression. Lentiviral vectors have a limit on the size of the transgene that can be included. This limit is based upon the size of the HIV genome. Most of SBI's lentivectors can fit a transgene up to about 4.5 kb. Exceeding the size limitation will decrease the viral packaging efficiency, because the large construct does not fit inside of the viral particles. It is important to check, however, the exact limit of the specific lentiviral vector you are choosing.

*For using lentiviral particles, SBI follows the NIH guidelines for BSL2, and recommends wearing a lab coat and gloves. The viruses are self-inactivating and non-replicative, so that after the target cells have been transduced, the cells are not infectious.

Minicircle Technology

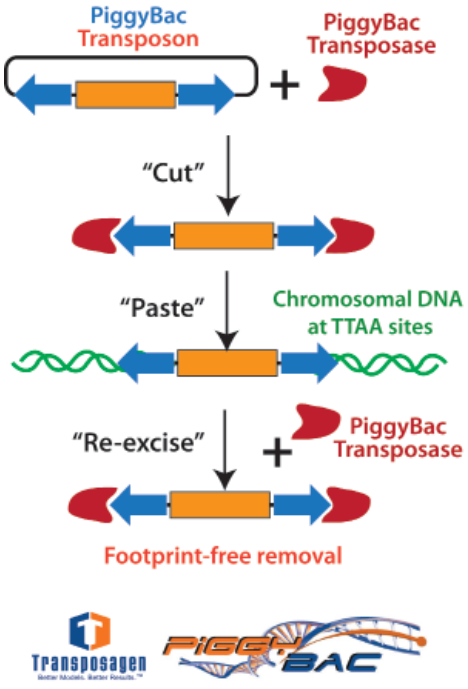
Minicircles are episomal DNA vectors that are produced as circular expression cassettes, and are devoid of the general bacterial backbone elements such as antibiotic resistance genes and the origin of replication. Their smaller molecular size enables more efficient transfection than typical plasmids, and the lack of the bacterial backbone allows the minicircles to remain within the target cell for a long period of time before being degraded. Typically minicircles remain intact within a cell for up to 4 weeks. Since minicircles lack an origin of replication, they cannot replicate within the target cells, and they do not integrate into the host cell genome. Expression of a transgene from a minicircle can be thought of a "long-term transient" expression since the expression lasts for more than the typical 3 days in a transient transfection, but does not integrate into the host cell genome. Minicircles can be used *in vivo* through tail vein injections, as well as *in vitro*.



Minicircles are produced from parental plasmids using an engineered *E. coli* host strain which express an I-SceI endonuclease and ΦC31 integrase, and induction media containing arabinoase. First, the gene, microRNA, shRNA, or reporter construct is cloned into the parental plasmid. The parental plasmid has all of the necessary elements for bacterial propagation. After propagation, the *E. coli* host strain is induced with arabinose. This activates the I-SceI endonuclease and ΦC31 integrase. The integrase causes the parental plasmid to release the minicircle. The endonuclease cleaves the bacterial backbone of the parental plasmid, which has 32 interspersed I-Sce-I sites engineered in it. The minicircles are then purified using a Maxi or Mega plasmid preparation protocol. The minicircle production protocol usually takes about 3 days to complete. Produced minicircles can be transfected into target cells using whatever method is normally used in your lab.

PiggyBac Transposon

The PiggyBac (PB) transposon is a mobile genetic element that efficiently transposes between vectors and chromosomes via a "cut and paste" mechanism. During transposition, the PB transposase recognizes transposon-specific inverted terminal repeat sequences (ITRs) located on both ends of the transposon vector and efficiently moves the contents from the transposon and efficiently integrates them into TTAA chromosomal sites in the target cell. Multiple, simultaneous integrations are possible. The powerful activity of the PiggyBac transposon system enables genes of interest between the two ITRs in the PB vector to be easily mobilized into target genomes.



The unique features about PiggyBac are that there is no limit on the size of the transgene that can be cloned into the vector. Additionally, the integration of the PiggyBac transposon is reversible by transfecting the target cells a second time with the transposase. Therefore, a stable cell line can be created, and then reverted to its original state, providing a convenient control for experiments. PiggyBac can also be used for generation of transgenic animals.

Deciphering Vector Elements
What are all those sequences in my vector?

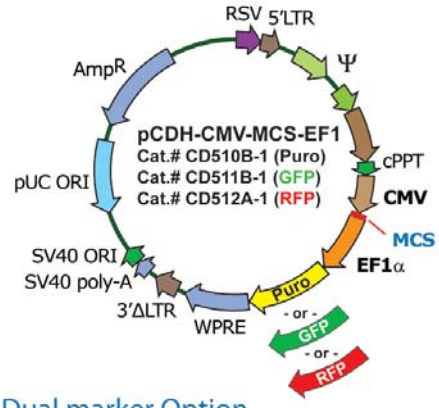
No matter which vector system you choose, there are a number of vector elements that you will need to choose depending on your scientific question. The guide below describes the different promoters, marker genes, bicistronic expression choices, and other vector elements present in SBI vectors.

Promoters

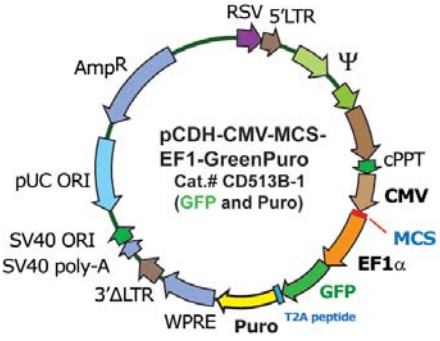
Promoters drive expression of a gene-of-interest, microRNA, marker gene or shRNA. The promoter that you choose is dependent upon the target cells that will be expressing the construct. Some vectors have a dual promoter format, where one promoter drives expression of your insert, and a second promoter drives expression of a marker gene.

Promoters	
CMV	The CMV promoter comes from the cytomegalovirus and is a strong promoter in **cell lines in culture.** The CMV promoter often becomes methylated in primary cells and *in vivo* so it would not be a good choice if you are planning these types of experiments. The CMV promoter is a constitutive promoter.
EF1	The EF1 promoter comes from the human eukaryotic translation elongation factor 1 alpha. The EF1 promoter is a strong promoter in **cell lines in culture, in primary cells, and *in vivo*** and is a good choice for most experiments. It will also work in **hematopoietic stem cells**. The EF1 promoter is a constitutive promoter.
MSCV	The MSCV promoter comes from the murine stem cell virus. It is a strong promoter in **hematopoetic cells, stem cells, and induced pluripotent stem cells**. The MSCV promoter is a constitutive promoter.
H1	The H1 promoter is specific for driving expression of **shRNAs** because it employs RNA polymerase III, which is specific for short hairpin sequences. The H1 promoter is a constitutive promoter.
CuO	The Cumate Operator switch is an inducible promoter that is based on a CMV promoter. When used in conjunction with the CymR repressor, it confers sensitivity to cumate. In the presence of cumate, the cumate operator drives expression of downstream genes.

Single marker Options



Dual marker Option



Bicistronic Expression in a Single Promoter Format

Several vectors have the option of a single promoter (usually EF1) driving expression of your insert as well as a marker gene. The two genes can be separated either by a T2A ribosomal skip site, or by an internal ribosomal entry site (IRES).

Separator	Characteristics
T2A	The T2A is a peptide sequence that introduces a ribosomal skip site. It is derived from the insect virus *Thosea asigna*. The mRNA is transcribed as one transcript with the gene-of-interest and the marker gene. As the ribosome gets to the last residue in the T2A peptide, the peptide bond is not formed. The ribosome remains attached to the mRNA and continues translating the marker gene. **Advantages:** The T2A site enables equal expression of the gene-of-interest and the marker gene. **Possible Disadvantages:** The T2A site adds 18 amino acids to the C-term end of the upstream gene (usually the gene-of-interest). These additional amino acids could affect the function of some proteins, especially those that have a sensitive C-term involved in signaling or binding.
IRES	The IRES is an internal ribosomal entry site. Separate proteins are produced from the gene-of-interest and marker gene, therefore there is no correlation between gene-of-interest expression and marker gene expression. **Advantages:** No extra amino acids are added to the protein-of-interest. **Possible Disadvantages:** The sequence 3' of the IRES is often expressed at a lower level than the upstream sequence. Therefore if the marker gene is in the second position, its expression may be lower than expected.

Marker Genes

Marker genes allow you to detect when the construct has successfully been introduced into the target cell. Typically marker genes are fluorescent markers or antibiotic resistance genes. Some vectors contain more than one marker gene.

Marker Gene	Characteristics
GFP	Green fluorescent protein. SBI uses a GFP from a copepod, so it is sometimes annotated as copGFP. copGFP is a monomeric GFP that is non-toxic and non-aggregating. It fluoresces brighter than EGFP. Some SBI vectors express a destabilized GFP, which has ubiquitination sites added to the C-terminal region to decrease the half-life from 4 hrs to 1 hr. The excitation for GFP is 482 nm; emission is 502 nm.
RFP	Red fluorescent protein. SBI uses mRuby which is a humanized version of the monomeric mars RFP. The excitation for RFP is 558 nm; emission is 605 nm.
Puro	Puromycin resistance. Allows cells to grow in the presence of puromycin.
Neo	Neomycin resistance. Allows cells to grow in the presence of neomycin.
Hygro	Hygromycin resistance. Allows cells to grow in the presence of hygromycin.
Luciferase	Some reporter constructs from SBI contain a firefly luciferase gene as a marker. Luciferase activity can be assayed using a luciferase assay system.

Bacterial Backbone Elements

Some vector elements are common among all plasmid backbones, especially genes relating to propagation in bacterial cells.

Element	Description
SV40 polyA	Enables efficient termination of transcription and processing of recombinant transcripts.
pUC Origin	For propagation of vectors in bacterial culture.
AmpR	Ampicillin resistance, for propagation of plasmids in bacterial culture.
KanR	Kanamycin resistance, for propagation of plasmids in bacterial culture.

Lentivector Elements

Lentivectors have some other elements that are specific for viral packaging and expression.

Element	Description
RSV-5'LTR and 3'Δ-LTR	The RSV-5'LTR is a hybrid long terminal repeat and viral promoter that is derived from the HIV genome. The 3'Δ-LTR indicates the end of the lentiviral sequence. The purpose of these sequences are for viral integration and self-inactivation within the target cell.
ψ (Psi element, or Gag, RRE, Env, and cPPT)	These partial sequences from the gag, RRE and Env genes from the HIV genome are viral packaging signals. They are required for efficient lentiviral packaging. They do not encode for proteins.
WPRE	Enhances stability and translation of transcripts. Can also be used for titering of viral particles or calculation of integrated copy number in target cells.
SV40 Origin	Provides stable propagation of lentivectors in mammalian cells.

Minicircle and Minicircle Parental Plasmid Elements

Minicircle parental plasmids have a few vector elements that are unique as well. These are generally relating to formation of minicircles.

Element	Description
attB and attP sites	These sites are where the minicircle re-circularizes after being excised from the parental plasmid. Mutation of these sites would prevent circularization of the minicircle.
WPRE	Enhances stability and translation of transcripts. Only present in some of the minicircle backbones.
32x I-Sce-I Sites	32 I-Sce-I restriction sites throughout the parental plasmid will degrade the parental plasmid backbone when induced with arabinose.

PiggyBac Elements

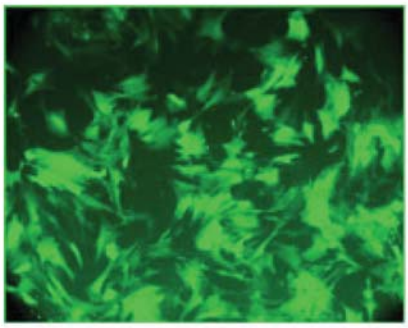
PiggyBac transposons have some vector elements that are unique to integration of the constructs into the host cell genome.

5' and 3' PB Terminal Repeats	These sequences modulate integration of the PiggyBac construct into mammalian cells in the presence of the Super PiggyBac Transposase and TTAA sites.
Core Insulator Sequences	Core insulator sequences provide a buffer region so that elements from nearby genomic regions do not influence expression of genes within the PiggyBac transposon and vice versa.

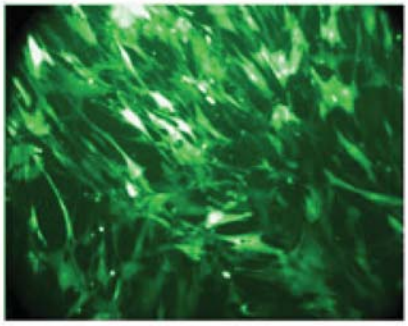
DNA/ RNA Delivery Core Facility at Northwestern University Prefers SBI's Lentiviral Vectors

Alex Yemelyanov, M.D., Ph.D., Co-Director of the DNA/ RNAi Delivery Core facility at Northwestern University's Skin Disease Research Center is a long-time customer of SBI. He and his colleagues regularly use SBI's lentiviral vectors for delivery of genes into human and mouse cell types. Their group studies the role of inflammation-related transcription factors in skin and prostate. Specifically they are looking at the role of glucocorticoid receptors involvement in proliferation and differentiation in epidermal cells and keratinocytes in search of treatments for hyperproliferative skin diseases and cancer.

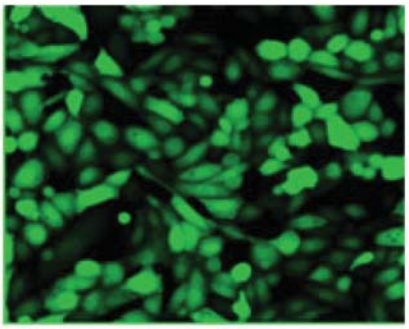
Here, Dr. Yemelyanov's team has infected 150,000 to 250,000 cells with lentiviral particles containing their genes-of-interest in SBI's pCDH-CMV-MCS-EF1-GreenPuro (Cat # CD513B-1) lentivector.



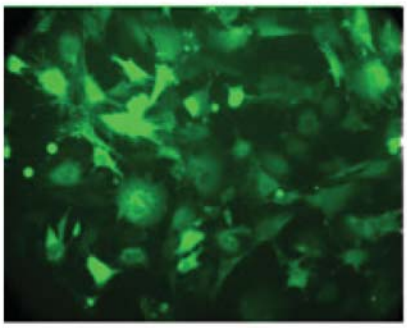
human primary renal mesangial cells



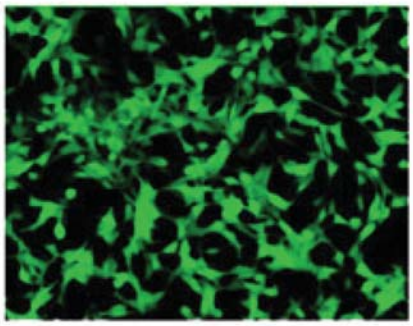
human primary smooth muscle cells



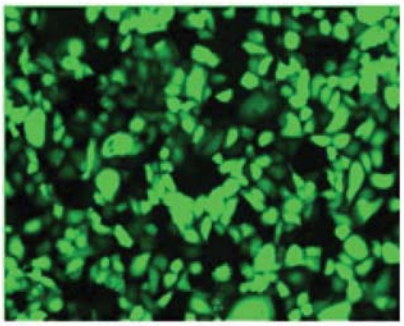
human transformed prostate carcinoma cell line PC3



mouse primary embryonic fibroblasts



human transformed prostate carcinoma cell line LNCaP



human transformed normal urothelium cells

"Our Core facility uses various lentiviral expressing vectors from such companies as Clontech, GeneCopeia, Cell Biolabs, Open Biosystems, System Biosciences and some of the homemade vectors from Addgene. So far SBI vectors have been the best and the CD513B-1 vector has been one of the strongest vectors in our hands." --Alex Yemelyanov.

Meet Travis Antes, Ph.D.

Dr. Antes is the Senior Director of Product Development and has been working here since November 2006.

Where did you do your training?

I completed my Ph.D. at the University of Texas at Dallas in RNA processing and editing. My post-doctoral work was at Stanford in chromatin structure and transcription regulation.

How did you get interested in science and research?

In the seventh grade we had a project involving microscopes and we were to bring in something from our yard to look at. I sampled my neighbor's yucky, swampy fish pond. When I looked at it under the microscope there were thousands of tiny squirming life forms! I was hooked and became a biologist that same day.

What do you like about working at SBI and what do you do there?

I enjoy that every day is different and appreciate the ability to conduct creative science and explore innovative methods and technologies. Being a "gadget guy", this is fun to me. I started at SBI at the bench developing RT-PCR techniques to measure microRNAs by qPCR. Now I head a group of young Ph.D.s who are pioneering the next wave of powerful new products for SBI. I encourage scientists to present unique product ideas at our research and development meetings and discuss how these technologies may be developed into successful products. I also supervise new products from idea to product concept and eventually into a commercialized entity.

The winners of SBI's "Most Creative Halloween Costume" contest, 2011. Ranjita Sengupta, Nandita Sarkar, and Chandreyee Mukherjee dressed as the "Central Dogma".

How can SBI's Non-coding RNA Department help you?

Custom microRNA precursor clones or anti-microRNA MZIP clones

SBI's non-coding RNA team can build custom microRNA precursor clones or anti-microRNA clones for you in the vector backbone of your choice, including lentiviral, minicircle, or PiggyBac vectors. You choose the promoter, the marker gene, and whether the construct is inducible or constitutive.

MicroRNA or Long Non-coding RNA profiling from any biological fluid

Let our non-coding RNA specialists profile your samples for microRNA, long non-coding RNA (lncRNA), or mRNA. You provide us with the total RNA from your samples and we will do the amplification and qPCR profiling for you. We provide you with a list of affected microRNAs or long non-coding RNAs with the fold-change over control samples. Data is delivered to you by e-mail within 2 weeks of receipt of the samples. Profiling available for human, mouse, rat, and pathway-specific microRNAs, or human or mouse Lnc RNAs.

Exosome Precipitation and Characterization of MicroRNAs or LncRNAs

We can precipitate exosomes from your samples using ExoQuick or ExoQuick-TC and then profile microRNAs or lncRNAs. Profiling available for human and mouse samples.

Other miRNA Products	Cat. #	Used For
MicroRNA Target Selection System	MS410A-1 MS040A-1	The miR-Target Selection system is a lentiviral vector that has a firefly luciferase gene and cytotoxic sensor, with MCS to clone in a 3'UTR. When a microRNA binds to the 3'UTR, luciferase activity decreases. If also treated with the cytotoxic drug, the cells only survive if the miRNA is bound.
LentiMir Pooled Virus Library	PMIRH-PLVA-1	This pooled lentiviral microRNA precursor library can be transduced into target cells and then screened for a specific phenotype. It can also be used in conjunction with the MicroRNA Target Selection System to characterize a specific 3'UTR and identify microRNAs that bind. The library contains 585 microRNA precursors.
OncoMir Pooled Virus Library	PMIRHO-PLVA-1	This pooled lentiviral microRNA precursor library contains 140 pooled clones that have been published to be involved in cancer. It can be used in conjunction with the MicroRNA Target Selection System, or by itself to characterize a specific 3'UTR to identify microRNAs that bind.
miRZip Pooled anti-MicroRNA Library	MZIPPLVA-1	Contains 172 anti-microRNA MZIP clones, pre-packaged into lentiviral particles so you can screen anti-microRNAs in a high-throughput way. Can be used in conjunction with the MicroRNA Target Select system or by itself.

We want to hear from you!

Would you like to be featured in SBI's next newsletter? Send us a description of your work , what products or services are your favorite from SBI, and some photos. We will review your submission and contact you when we are ready to feature you and your lab!

LIVE WEBINARS

All webinars are free and open to the public. Space is limited, so register on-line at:
http://www.systembio.com/webinars

Monitoring Differentiation and Transcriptional Activity with SBI's Lentivector Reporter Constructs

Thursday, November 10, 2011, 8:00 AM Pacific Time

This webinar will talk about the different reporter systems available from SBI. We will talk about the benefits of lentiviral constructs, construct design and validation, transduction into target cells, experimental best-practices, and creation of reporter cell lines.

Powerful Tools for MicroRNA Functional Analysis

Thursday, November 17, 2011, 8:00 AM Pacific Time

Once your microRNA profiling experiments are done – what's next? In this webinar, you will learn how to perform microRNA overexpression, knockdown and target identification experiments to nail down what the microRNAs of interest that you identified from your profiling are actually doing in your model system. Having the complete picture from profiling to functional analysis and UTR binding data will make your microRNA studies solid for publication.

Pioneers in Lentivectors: Why Use Lentivirus?

Thursday, December 1, 2011, 10:00 AM Pacific Time

Learn the basics of lentivector and lentiviral technology. Includes topics on biosafety, choosing a lentivector, lentiviral packaging, transduction of target cells, and applications of lentiviral technology.

Genome-Wide shRNA Screening with the GeneNet Lentiviral Libraries

Thursday, December 8, 2011, 11:00 AM Pacific Time

Learn how to use the GeneNet shRNA Lentiviral Libraries to look for genes that are involved in a specific function, pathway, or drug responsiveness. This free one-hour webinar will go through the design of the GeneNet libraries, transducing target cells with the library, designing an appropriate screen, and analyzing the output data.

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