Accelerating Discoveries in Functional Genomics


# 2010 Brochure

## Lentiviral Technology

- **Efficient Delivery**
- **Stable, Heritable Expression**
- **Non-disruptive to Cells**
- **Biosafe**

Broad range of cloning and expression lentivectors for cDNA, shRNA, microRNA and transcription reporter constructs, as well as complete viral packaging, concentration and titering systems.

## Stem Cell Research

- **Reprogramming Tools**
- **iPS Cell Lines**
- **Pluripotency Reporters**
- **Differentiation Reporters**

Lentiviral expression systems for iPSC factors and certified iPS cell lines for Human or Mouse. Stem cell-specific transcriptional reporters to confirm pluripotency and track differentiation. Media, growth factors and low passage source cells for reprogramming.

## MicroRNA Research

- **Expression Profiling**
- **AGO2 Capture Systems**
- **MicroRNA Overexpression**
- **MicroRNA Knockdown**
- **Target Identification**

Innovative technologies for qPCR expression profiling, RISC capture and cloning, overexpression and knockdown of microRNAs to enhance your research discoveries. Accurately pinpoint the UTR targets of microRNAs for signaling pathway analysis.

## Gene Analysis and Molecular Tools

- **Access the Transciptome**
- **Complete mRNA Coverage**
- **Ultra-fast Cloning System**
- **Powerful Transfection Tool**

Complete transcriptome amplification of RNA from any sample, including FFPE specimens and rare tissue sources. Revolutionary cloning system for assembling multiple inserts into any vector at any site—all in one step. Nanotechnology-based gene delivery tool with low toxicity.

## Screening Libraries

- **Genome-wide shRNA Pools**
- **Pathway Focused shRNAs**
- **MicroRNA Virus Pools**
- **Multiplexed Anti-microRNAs**

Perform high-throughput genome-wide or pathway-focused gene knockdown studies. Ready-to-infect virus pools enable simultaneous identification of multiple genes that alter specific cellular phenotypes in a single screening experiment.

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## Advantages of SBI's Lentivector Technology

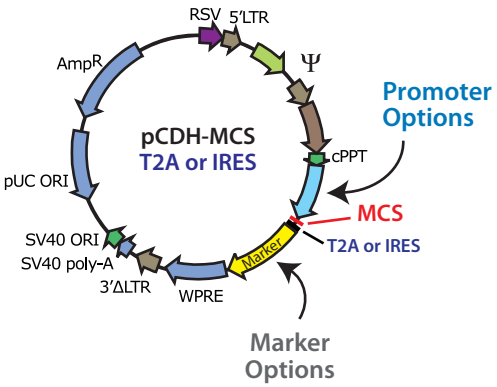
- Third-generation biosafe lentivector backbones provide maximum infection efficiency for most cell types, including non-dividing and hard-to-transfect cells (primary, hematopoietic, stem cells)
- High-titer enabled (>10^9 IFU/ml) for in vivoapplications
- Constructs integrate into genomic DNA through viral transduction for stable, heritable expression. Easily create stable cell lines and transgenic animals
- Replication-incompetent design provides maximum biosafety, inhibiting proliferation of virus for both HIV and FIV lentivectors
- Extensive selection of lentivectors with different fluorescent and antibiotic resistance markers for robust cDNA, shRNA and microRNA expression

## Lentivectors

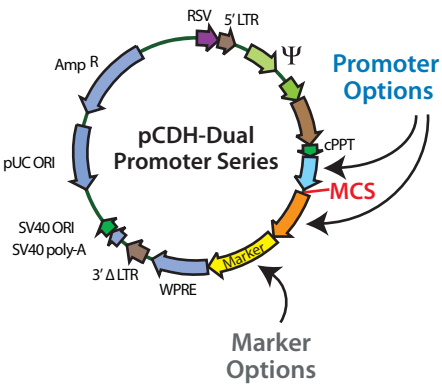
System Biosciences (SBI) has engineered highly effective and versatile lentivector systems for the expression of shRNA, cDNA or microRNA sequences. Choose from a variety of promoter and reporter options, including GFP, RFP, Puromycin, Hygromycin, Neomycin and Zeocin selection, as well as new inducible expression vectors. All SBI lentivectors contain viral stability elements, such as cPPT, WPRE and RRE sequences, for enhanced packaging and infection efficiency. SBI lentiviral preparations have been used to effectively transduce stem cells, hematopoietic and non-dividing primary cells and animal models. Easily test infection efficiency of SBI's packaged lentiviral particles in your cells using ready-to-transduce positive control viruses. Evaluate promoter activities and strengths in your specific model system.

### Stably Express cDNAs

#### Single Promoter Formats




#### Dual Promoter Formats




#### Promoter Options

| Promoter |
|---|
| CMV |
| EF1 alpha |
| PGK |
| UbC |
| MSCV |

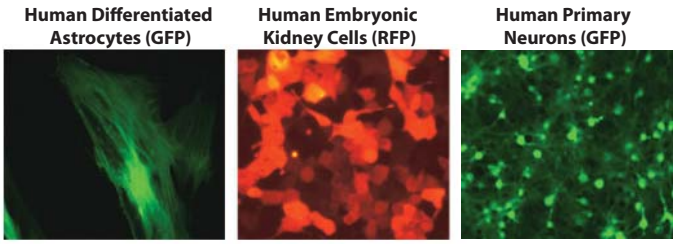
#### Marker Options

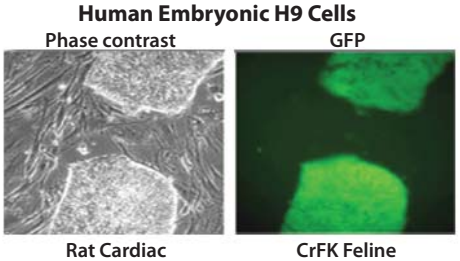
| Selection | Fluorescence |
|---|---|
| Puro | GFP |
| Neo | RFP |
| Hygro | GFP+Puro |
| Zeo | RFP+Puro |

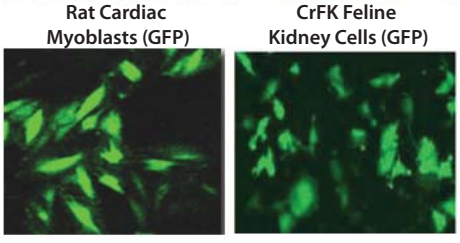
#### Multiple Cloning Sites

| MCS (5' 3') |
|---|
| XbaI |
| NheI |
| EcoRI |
| BstBI |
| SwaI |
| BamHI |
| NotI |

### Broad Lentivirus Tropism

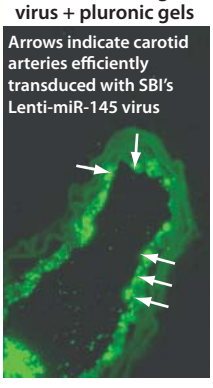


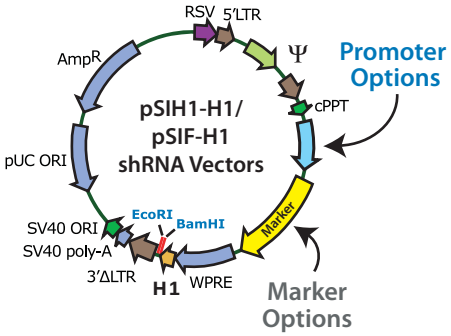



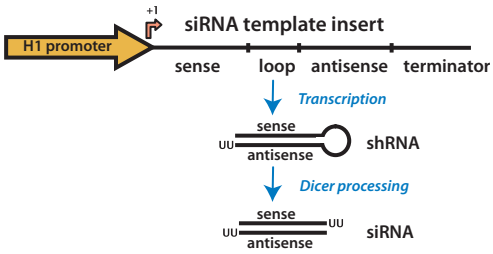

***In vivo* Models**

Mouse FVB/NJ arterial vessel walls transduced using virus + pluronic gels




### Permanent RNA Interference

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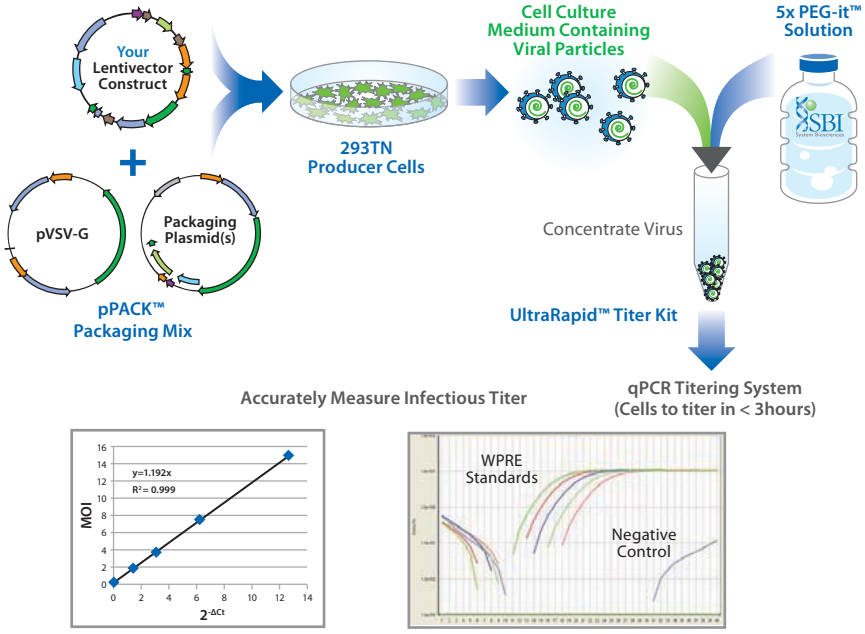
# Lentiviral Technology

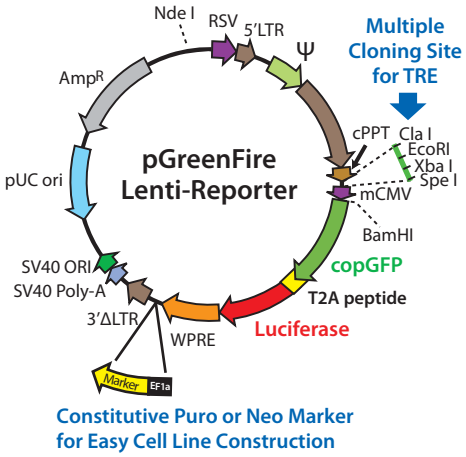
## Safe, Stable and Effective Gene Delivery

To achieve highly efficient delivery, lentiviral constructs can be packaged into VSV-G pseudoviral particles using SBI's lentivirus packaging systems. Lentivector constructs are co-transfected with the pPACK™ Packaging Mix into 293TN Producer Cells for robust virus production. Pseudoviral particles are harvested from the cell culture medium and concentrated using SBI's one-step virus concentration solution, PEG-it™. Viral titer is measured using the qPCR-based UltraRapid™ Titer Kit for accurate and sensitive quantitation of infectious particles. Target cells are then transduced with high-titer viral particles for stable integration and expression.

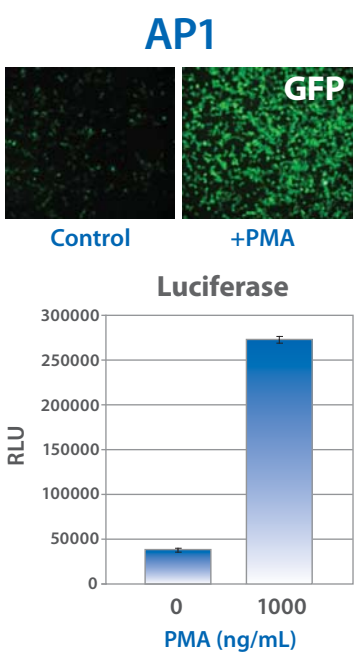
Overview of Lentiviral packaging, concentration, titering and transduction with SBI's complete lentiviral delivery system.




## pGreenFire™ Pathway Reporters




pGreenFire1 (pGF1) is a versatile HIV-based lentivector that co-expresses destabilized copGFP and Firefly Luciferase reporters that enable the detection of both GFP signals for live cell imaging as well as quantitative transcription activation reporter assays using Luciferase.

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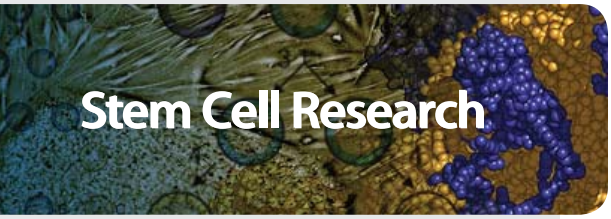
## Complete System for Packaging, Concentration and Titering

- Efficiently package any 2nd or 3rd generation FIV or HIV-based lentivector
- High titer enabled (>10^9 IFU/ml) - suitable for *in vivo* applications
- Concentrate lenti and retro viruses 10-100 fold without ultracentrifugation using PEG-it™
- Convenient all-in-one kit to make transducible lentiviral particles - LentiSuite™
- Rapidly determine accurate titers of virus in less than 3 hours with qPCR
- Stably express lentivector constructs in a wide range of mammalian cells
- Prove it to yourself - try the LentiStarter™ Trial Kit
- SBI's lentiviral systems combine to deliver superior transduction efficiency

***Trust SBI's proven lentiviral technologies to deliver quality data for your next big discovery***

## Study pathway activation using lenti-based transcription reporters

- Extensive selection of pre-built reporters for:
  - DNA Damage
  - Hypoxia
  - Interferon response
  - Inflammation
  - Sterol sensing
  - Stem cell factors Oct4 and Nanog
  - Oncogenic signaling
- Dual reporter vector system to quantitate Firefly Luciferase and GFP for live cell imaging
- Ready-to-use constructs with a wide range of Transcriptional Response Elements (TREs)
- Study transactivation and epigenetic effects more accurately in a native chromosomal context
- Low background with robust transcription activation signals
- Establish stable reporter cell lines




## Induce Pluripotency, iPS Cell Lines and Pluripotency Monitors *Ge*

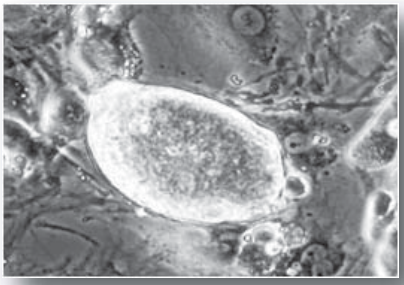
- All six iPSC factors (Oct4, Sox2, Nanog, Klf4, Lin-28 and c-Myc) available as ready-to-transduce lentivirus
- Choose from the "Y" (Oct4, Sox2, Klf4, and c-Myc) or "T" sets (Oct4, Sox2, Nanog, and Lin-28 ) of iPSC factor combinations
- Pre-made iPS Cell Lines reprogrammed with Oct4, Sox2, Klf and c-Myc and certified positive immunostaining for stem cell markers
- High quality source cells, feeder cells and stem cell media to grow and support embryonic and iPS cells
- Choice of iPSC reprogramming from low passage fibroblasts or epidermal keratinocytes
- Confirm and enrich for pluripotent cells with Human and Mouse Oct4 and Nanog promoter reporters (GFP+Zeo)
- Monitor and quantitate Oct4 and Sox2 activity using SBI's CR4 and SRR2 response reporters (GFP+Luciferase)
- Easily create stem cell reporter cell lines

***Gentaur high titer lentiviruses efficiently transduce Stem Cells***

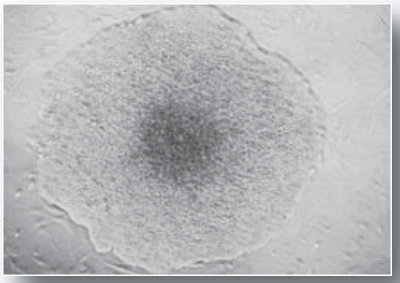
## Induce Pluripotency

Reprogram adult cells into a pluripotent state using SBI's iPSC factor expression lentivectors. Select any combination of master pluripotency regulators to create stable iPS cell lines and new disease models. iPSC lentivectors are available in two formats, with or without an RFP fluorescent marker. All iPSC constructs are available as lentiviral plasmids and pre-packaged lentiviral particles.

**Mouse iPS Colony**



**Human iPS Colony**



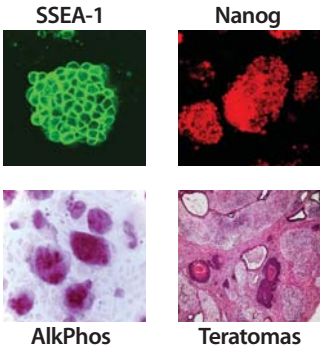
Phase contrast images

## Certified iPS Cell Lines

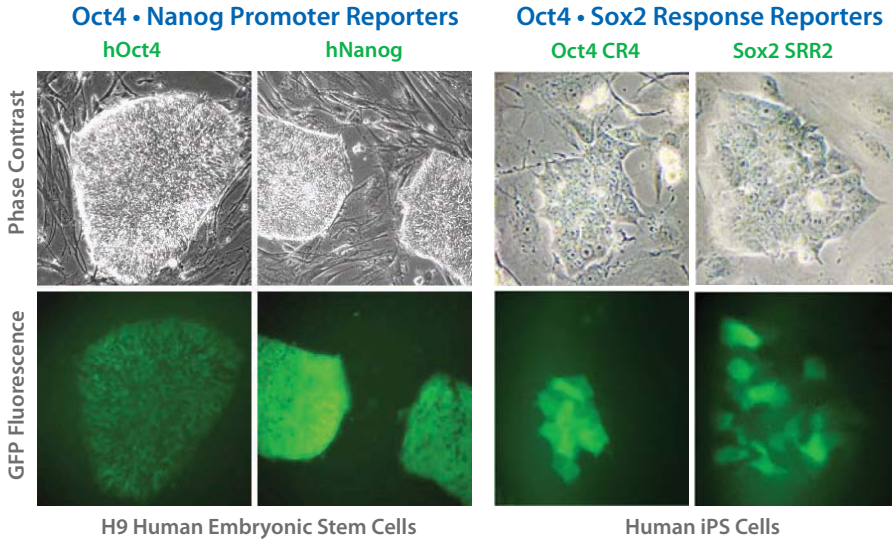
SBI offers Human or Mouse Induced Pluripotent Stem (iPS) Cell Lines with matched source fibroblasts. These cell lines are verified for stem cell pluripotency markers and are pre-made and validated - ready for your iPSC research. The iPS cells can be used to study differentiation - allowing research into the pathways and factors involved in fate specification.

The matched set cell lines enable you to directly compare the differentiated and the induced pluripotent state to discover fundamental stem cell properties. SBI's high quality iPS cells have no fluorescent or drug resistant markers. Media, feeder cells and low passage fibroblasts and epidermal keratinocytes are also available.

**The iPS Cell lines are certified for pluripotency markers SSEA-4/1 and Nanog as well as tested for Alkaline Phosphatase activity.**




Stem Cell-specific Promoter and Response Reporters




## Confirm Pluripotency

Verify the pluripotent state of your stem cells using Oct4 & Nanog promoter reporters or SBI's Oct4 conserved enhancer (CR4) or Sox2 (SRR2) response reporters. These tools allow for the easy and accurate monitoring and enrichment of pluripotent cells. Promoter reporters feature dual markers for live cell imaging, sorting and Zeocin selection of pluripotent cells. The CR4 and SRR2 response reporters work for both Human and Mouse stem cells and enable more specific monitoring of Oct4 and Sox2 transcription factor activities. These response reporters feature both GFP and Luciferase for quantitative transactivation assays.

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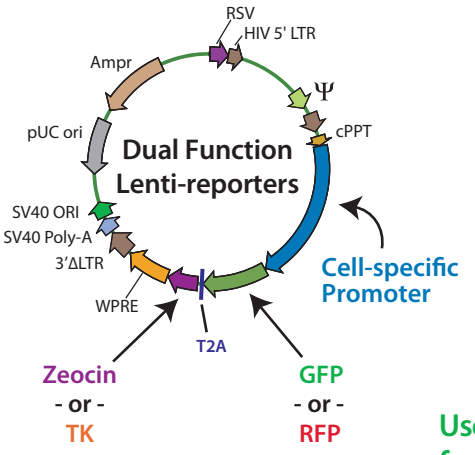
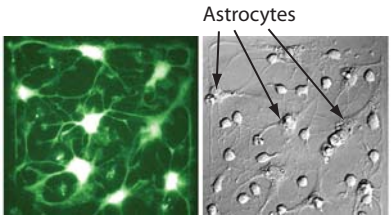
## Track Differentiation

Cell-specific promoters drive GFP/RFP and Zeocin/TK markers in differentiating cells to allow monitoring of specification in real time. Trace differentiation across Neural, Hematopoietic, Myogenic, Structural and Endocrine lineages. These lentiviral reporters can be used to develop new directed differentiation protocols and to study cell fate specification. The dual function lenti-reporters are conveniently available as lentivector plasmids or ready-to-transduce lentiviral particles.

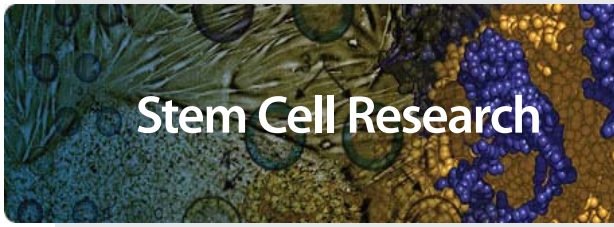



Adapted from: D. Hoeppner and R. McKay. Cell Stem Cell, December 2008.

Astrocyte-specific GFAP promoter reporter data is shown above. Clearly identify specific cells within a mixed population. Only the astrocytes are GFP positive in a neural network including mature neurons and oligodendrocytes .

**Use pGreenZeo and pRedZeo for positive selection and pGreenTK or pRedTK for negative selection.**




### Track Stem Cell Activity using Cell-specific Promoter Reporters

- Rapidly create embryonic and progenitor reporter cell lines to study differentiation
- Optional Puro/Neo selection cassette to easily establish stable cell lines
- Cell specific promoters drive GFP or RFP and Zeocin or TK selection in differentiated cells
- Utilize the fluorescent proteins to trace differentiation in living cells
- Develop directed differentiation protocols across five different lineages
- Choose from pre-packaged virus or plasmid

## Choose from Five Categories of Lineage Reporters

***Track Cell Differentiation in Real time***

### Neural

| Target Cell Type | Species | Promoter |
|---|---|---|
| Macrophage, microglia | Mouse | Cd68 |
| Astrocyte | Human | GFAP |
| Astrocyte | Mouse | Gfap |
| Microglia | Human | CD11b |
| Microglia | Mouse | EMR1 |
| Microglia | Mouse | Iba-1 |
| Muller glia | Mouse | Cd44 |
| Neuron | Human | BM88 |
| Neuron | Mouse | Camk2α |
| Neuron | Mouse | GAD67 |
| Neuron | Rat | NSE |
| Neuron | Mouse | Tα1 α-tubulin |
| Oligodendrocyte | Mouse | MBP |
| Photoreceptor | Human | Opsin |
| Neural Stem Cell | Rat | Nestin |
| Neural Stem Cell | Human | Nestin |
| Neuron | Human | Doublecortin |
| Neuron | Human | MAP2 |
| Neuron | Human | FABP7 |

### Endocrine

| Target Cell Type | Species | Promoter |
|---|---|---|
| Beta cell | Human | Insulin |
| Islet | Human | PDX1 |
| Islet | Mouse | Pdx1 |
| Islet | Human | NGN3 |

### Hematopoietic

| Target Cell Type | Species | Promoter |
|---|---|---|
| B-cell | Human | B29 |
| B-cell | Mouse | B29 |
| CD8 T-cell | Mouse | CD8 |
| Erythroid | Human | HLA-DRα |
| Macrophage,microglia | Mouse | Cd68 |
| PanT-cell | Human | CD2 |
| Lymphocyte | Human | LCK |

### Myogenic

| Target Cell Type | Species | Promoter |
|---|---|---|
| Cardiomyocyte | Mouse | Actc |
| Cardiomyocyte | Human | MLC-2v |
| Cardiomyocyte | Human | TNNT2 |
| Cardiomyocyte | Mouse | Tnnt2 |
| Smooth muscle myocyte | Mouse | SM22α |
| Cardiomyocyte | Human | ACTC |
| Skeletal myocyte | Mouse | Myogenin |

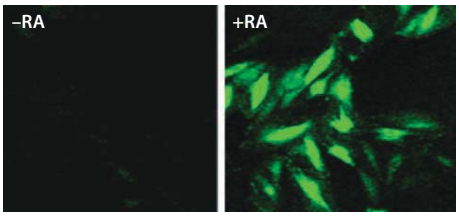
### Structural

| Target Cell Type | Species | Promoter |
|---|---|---|
| Chondrocyte | Mouse | Col2a1 |
| Osteoblast | Human | SPP1 |
| Osteoblast | Human | Osteocalcin |
| Adipocyte | Mouse | ALBP |
| Epithelium | Human | Keratin 14 |

### Differentiation Reporter Data

**Mouse Troponin Reporter**

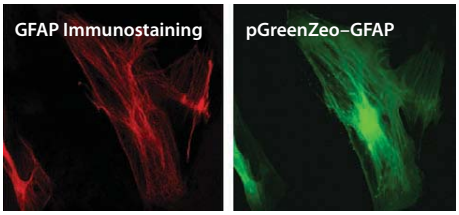
Differentiation with Retinoic Acid




h9c2 rat cardiac myoblasts stably transduced with pGZ-mTnnt2 differentiation reporter and incubated in the presence or absence of retinoic acid for 2 days.

**Human GFAP Reporter**

Visualization of Astrocytes

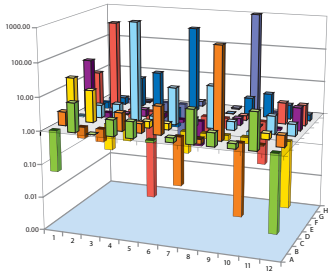



Primary cultures of human brain astrocytes infected with pGZ-GFAP differentiation reporter and immuno-stained with anti-GFAP monoclonal antibody.

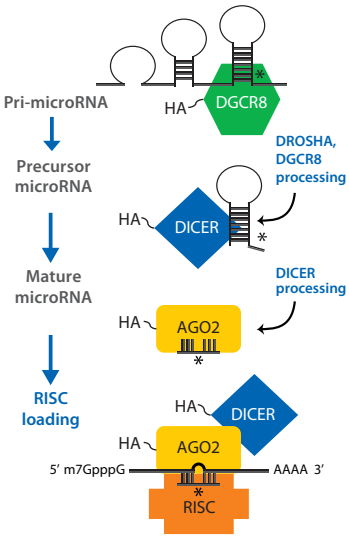

## Essential Tools for MicroRNA Studies

- Profile hundreds of microRNAs from a single cDNA synthesis with QuantiMir™
- Pre-formatted qPCR arrays for Cancer or Stem Cell specific microRNAs
- Genome-wide microRNA profiling with Human, Mouse, Rat, Drosophila and Zebrafish miRNome Profiler qPCR arrays
- One-step exosome enrichment with ExoQuick™ for biomarker discovery
- Immunopurify microRNA and piRNA complexes with miR- and PIWI-SnaREs™
- Globally amplify and discover new small RNAs ready for deep sequencing

## Accurate MicroRNA qPCR Arrays



## Immunopurify Small RNAs using Epitope-tagged Protein Factors



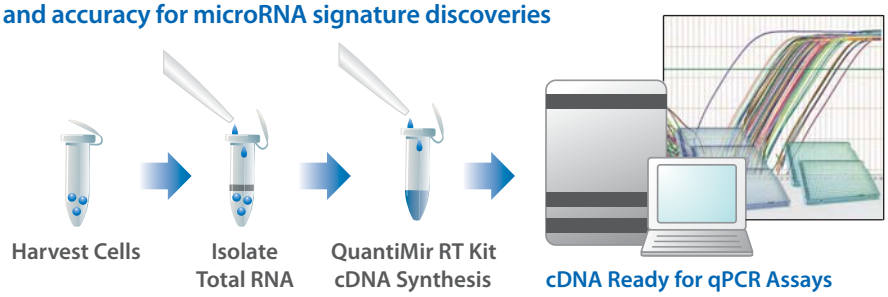

## Accurate and Sensitive microRNA Profiling from Cells, Tissues, Serum and other Bio-fluids

**Measure microRNAs by qPCR with QuantiMir™**

MicroRNA and siRNA expression analysis is made easy with the QuantiMir™ small RNA quantitation system. Generate qPCR-ready cDNA from total RNA for accurate and sensitive expression measurements. Convert enough RNA to cDNA for 5,000 different microRNA qPCR measurements from a single cDNA synthesis reaction. Complete Human, Mouse, Rat, Drosophila and Zebrafish qPCR Arrays - 100% miRBase updated. Characterize microRNA signatures in stem cells, cancer cell lines, FFPE samples and even patient serum and bio-fluids.

**Simple and effective qPCR platform with high sensitivity and accuracy for microRNA signature discoveries**

**miRNome Profilers**

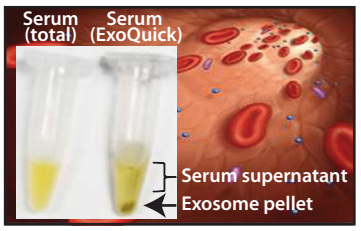



## Enrich and Discover MicroRNA Biomarkers from Patient Serum and Bio-fluids

**Isolate Circulating Exosomes with ExoQuick™ for MicroRNA Biomarkers**

Exosomes are 40 –100 nm membrane vesicles secreted by most cell types *in vivo* and *in vitro*. Exosomes are found in blood, urine, amniotic fluid, malignant ascite fluids and contain distinct subsets of microRNAs depending upon the tumor from which they are secreted. SBI's ExoQuick exosome precipitation reagent makes microRNA biomarker discovery simple, reliable and quantitative. Enrich for circulating exosomal microRNAs with ExoQuick and accurately profile them using QuantiMir qPCR arrays.

**Circulating Exosomes contain MicroRNAs**




## Discovery and Cloning—Tools to Immunopurify RNAs and Efficiently Clone Small RNAs

**Immunopurify RNAs with miR and PIWI-SNaREs™**

Overexpress epitope tagged DGCR8 (Pasha), DICER and Argonautes 1, 2, 3 and 4 for IP pull-downs of RNAs and associated protein factors. SBI also offers PIWI-SnaREs for the affinity isolation of PIWI and piRNA complexes. These lentivector-based constructs of microRNA processing factors or PIWI factors are available with co-expression of either RFP for fluorescent cell sorting or Puromycin for selection of stable cell lines.

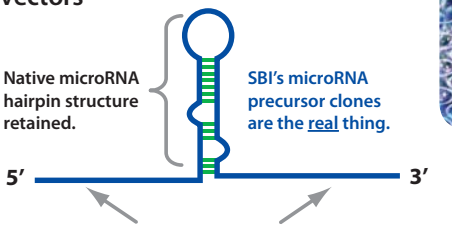
**Global MicroRNA Amplification and Cloning Kit**

Efficiently amplify small RNAs, including microRNAs, for expression studies from limited starting sample RNA. Create comprehensive microRNA and small RNA cDNA libraries adapted for Deep Sequencing platforms.

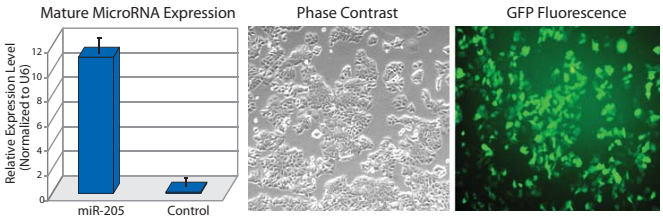

## The Lenti-miR Precursor Clone Collection

**Stably Overexpress MicroRNAs Using Lentivectors**

System Biosciences (SBI) has the largest commercially-available collection of microRNAs that are cloned into lentiviral vectors. Each construct in SBI's collection consists of the native stem loop structure and 200-400 base pairs of upstream and downstream flanking genomic sequence. This unique feature ensures that the microRNAs expressed from SBI's constructs will be stably expressed and correctly processed into mature microRNAs. True precursor clones deliver more accurate data.

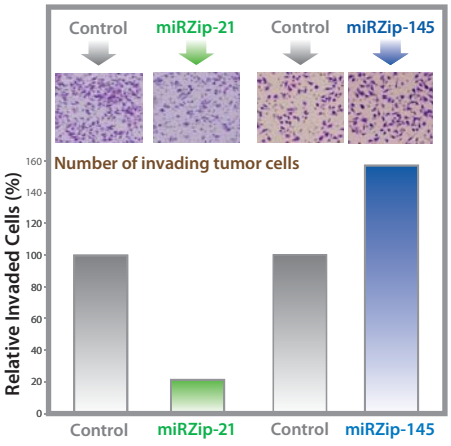



**MCF-7 Breast Cancer Cells Transduced with Lenti-miR-205**




## Innovative anti-miR Technology - miRZips™

**Permanently Knockdown MicroRNAs**




**Tumor Invasion Assays using miRZips**
Inhibition of miR-21 decreases tumor invasion, while inhibition of miR-145 increases tumor cell invasion.

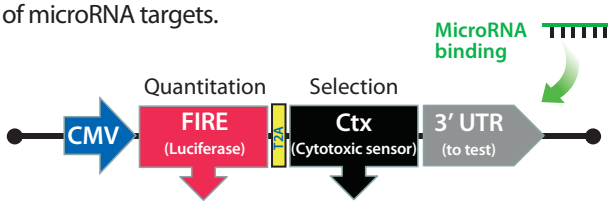
miRZip™ anti-sense microRNAs are stably expressed short hairpins that have anti-microRNA activity. The miRZip hairpins are rationally designed for asymmetry such that the upper strand of the hairpin (in gray) does not contain the endogenous microRNA sequence and the lower strand is preferred for producing anti-sense microRNAs (in green) that are fully complementary to a specific microRNA target. The result is the derepression and elevation of the protein levels of the transcripts targeted by the microRNA being "zipped".

## Detect and Validate microRNA Targets with miR-Select

**Identify Binding Sites through Cellular Selection and Quantitate Target Interactions**

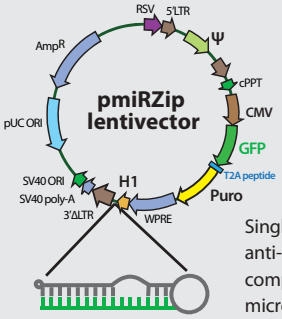
Novel cellular method to detect microRNA binding to its target mRNA using a dual reporter system featuring Luciferase (Fire) and a Cytotoxic Sensor (Ctx). The miR-Select platform captures the 3′ UTR to microRNA binding event using a survival screen by modulating the reduction of the cytotoxic sensor. Validation is made simple using the built-in Luciferase reporter. This powerful and elegant technology finally enables the accurate identification of microRNA targets.

**The miR-Select Fire-Ctx-UTR Lentivector**




## Powerful Tools to Study MicroRNA Functions

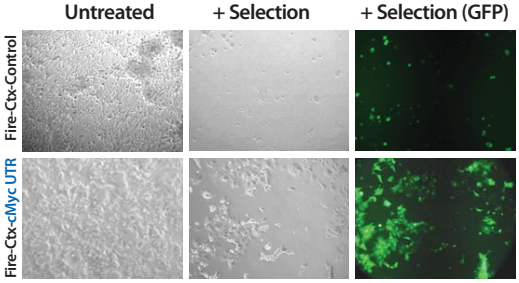
- Largest collection of microRNA precursor clones available in lentivectors
- Native microRNA context ensures accurate and robust mature microRNA production
- Confirm positive expressing cells with GFP for convenient sorting of transfected or transduced cells
- miRZips permanently suppress specific endogenous microRNAs
- Select for positive expressing cells with either GFP or Puromycin




Single-stranded anti-microRNAs that competitively inhibit microRNA function.

- Identify microRNAs that bind to UTRs using the miR-Select UTR system
- Designed for high-throughput screens to rapidly and accurately identify microRNAs that target a specific 3′ UTR of interest
- Validate and quantitate microRNA binding interactions within 3′ UTRs with Luciferase

**miR-Selection for cMyc 3′ UTR + miR-145**

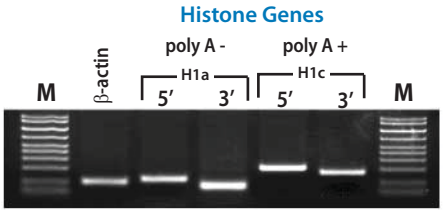



cMyc 3′ UTR Enables Survival with miR-145

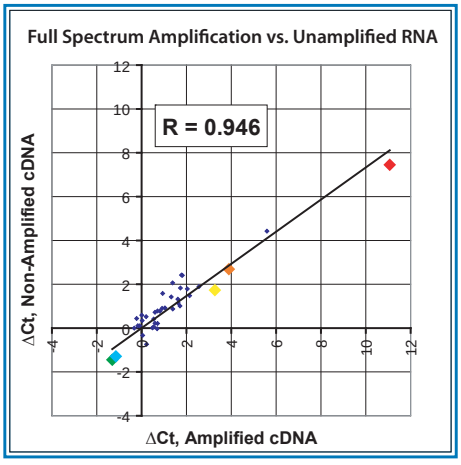

## Complete Transcriptome Analysis

- Uniform amplification across entire transcript without bias towards the 3′ or 5′ ends
- High fidelity maintenance of relative levels of each mRNA species after amplification
- Improve yields of amplified RNA with only nanogram amounts of starting sample
- Robust amplification using either intact or degraded RNA samples
- Single tube approach that yields high quality amplified template in 3 hours
- Balanced sequence representation across the transcriptome
- Flexible Full Spectrum MultiStart Primer Sets suitable for Deep Sequencing platforms

## Amplify Poly-A Minus mRNA Transcripts



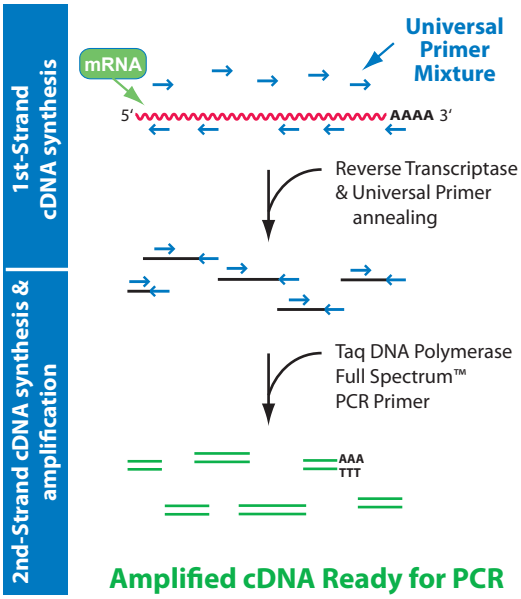

## Maintain Sequence Representation Uniformly after Amplification




# Access the Entire Transcriptome with Full Spectrum™

The Full Spectrum™ Complete Transcriptome RNA Amplification Kit provides a superior approach to amplify RNA for expression analysis. In addition to robustly and reliably amplifying difficult RNA from valuable samples in a way that maintains the relative levels of each transcript, the Full Spectrum approach ensures that all regions of the transcripts are present in the amplified products. The Full Spectrum MultiStart Primer mix initiates first strand cDNA synthesis at multiple points along the mRNA as well as from the poly A tail. This means that the complete mRNA sequence is preserved, even if the mRNA is degraded. Starting with just nanogram quantities of RNA, the system provides enough amplified template for quantitative PCR expression analysis of 100-200 different transcripts. It is now possible to amplify mRNA while maintaining the complete mRNA sequence.

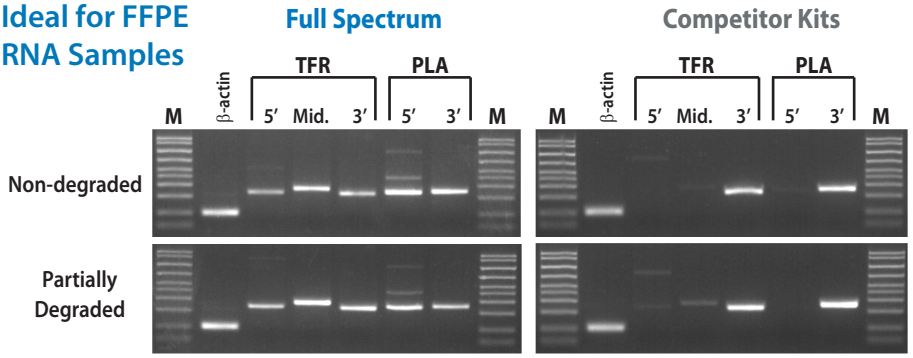
## How does Full Spectrum Work ?




**Amplified cDNA Ready for PCR**

The Full Spectrum RNA Amplification Kit makes use of a specially developed Universal Primer mixture that robustly and uniformly amplifies all regions of gene transcripts using low-cycle PCR. The Universal Primer mixture is composed of a proprietary, non-degenerate set of primers that bind to, and prime synthesis from numerous specific sites found throughout mRNA sequences. The combination of this primer mix and low-cycle PCR (typically < 20 cycles) produces uniform amplification of gene transcripts so the relative levels of each transcript in the starting mRNA sample are maintained—even when using starting amounts of RNA as low as 20 ng. This unique approach is particularly robust for amplifying degraded RNA, making the Full Spectrum method the obvious choice for amplifying RNA from all sources, including FFPE tissues. **Full Spectrum MultiStart primers are also adaptable for Deep Sequencing.**

## Ideal for FFPE RNA Samples



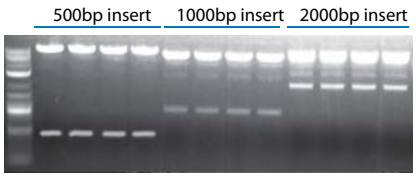

Comparison of the Full Spectrum system versus competitor kits clearly reveals the advantages of SBI's technology. The Full Spectrum method maintains mRNA sequence information all along the human transferrin receptor (TFR) and phospholipase A1 (PLA) mRNAs in both non-degraded and degraded RNA samples. These results illustrate the extreme 3′-end bias of competitor kits, a flaw eliminated by the unique priming method used in SBI's Full Spectrum Complete Transcriptome RNA Amplification Kit.


# Cold Fusion Cloning Kit

**Next Generation Cloning Technology**

The Cold Fusion technology allows you to directly clone any PCR product(s) into any linearized expression vector, at any site. Simply design primers with at least 15 bases of homology at the ends to direct the fusion location of the desired DNA fragment. Convenient one tube reaction, with a 5 minute incubation at room temperature followed by 10 minutes on ice. The Cold Fusion master mix prepares the DNA ends for sequence-directed alignment. The PCR product(s) rapidly and accurately fuse into the linearized vector in the desired location and orientation. Cold Fusion is so robust that multiple DNA fragments can be assembled simultanously and cloned into one construct in a single step. The system is highly efficient, with more than 95% positive cloning rate.

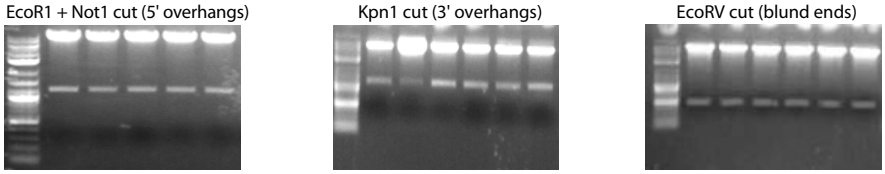
## Broad PCR product cloning range with high efficiency



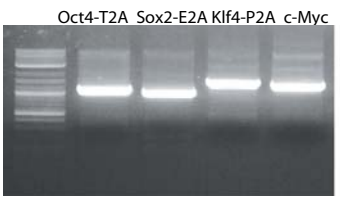

COLD Fusion Cloning technology

***Seamlessly clone any insert, at any site, within any vector***

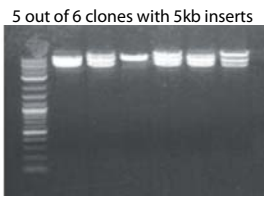
## Use with any vector with 5' overhangs, 3' overhangs and even blunt ends



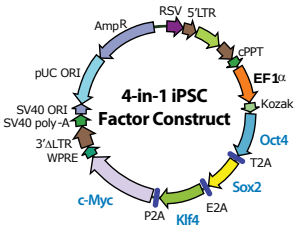

## Assemble and clone multiple DNA fragments simultaneously into one construct




PCR of the four iPSC cDNA fragments

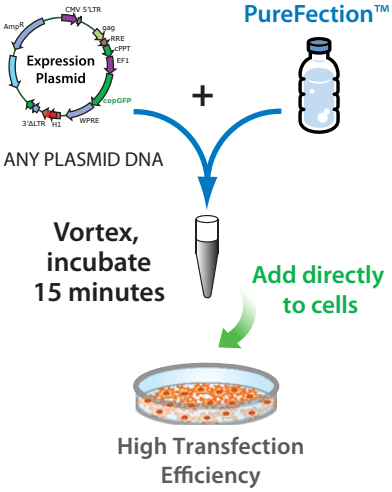



Screen of positive clones



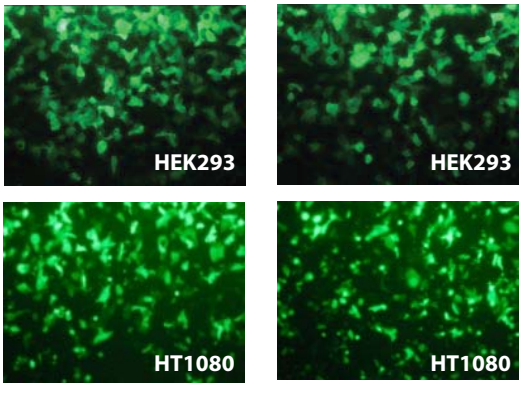

# Purefection™ Transfection Reagent

**Deliver more DNA than lipid-based transfection**




PureFection is a powerful, broadly applicable transfection reagent for effective and reproducible transfections. The PureFection reagent self-assembles nanoparticles in the presence of DNA. These complexes are readily taken up by target cells for efficient gene delivery. No media changes are required as PureFection works in the presence of antibiotics and serum. Easy-to-use protocol with rapid, one-step incubation for 15 minutes before adding directly to target cells makes PureFection well-suited for high-throughput transfection experiments.

## Comparison of transfection efficiency




PureFection | Vendor I

## Efficient and Accurate Cloning

- Clone any insert, at any site, within any vector with Cold Fusion
- Restriction enzyme, phosphatase, recombinase and ligase free system
- Join multiple fragments simultaneously in one reaction
- Fast and efficient cloning system - ideal for high-throughput cloning applications
- Perform gene modifications with ease to introduce or correct mutations

## Transfect Cells using Novel Nanotechnology Reagent

- Highly effective transfection technology
- Works with most cell types
- Cost-effective alternative to lipid-based products
- Use PureFection to package ultra-high titer lentivirus
- Nano-based gene delivery with low toxicity

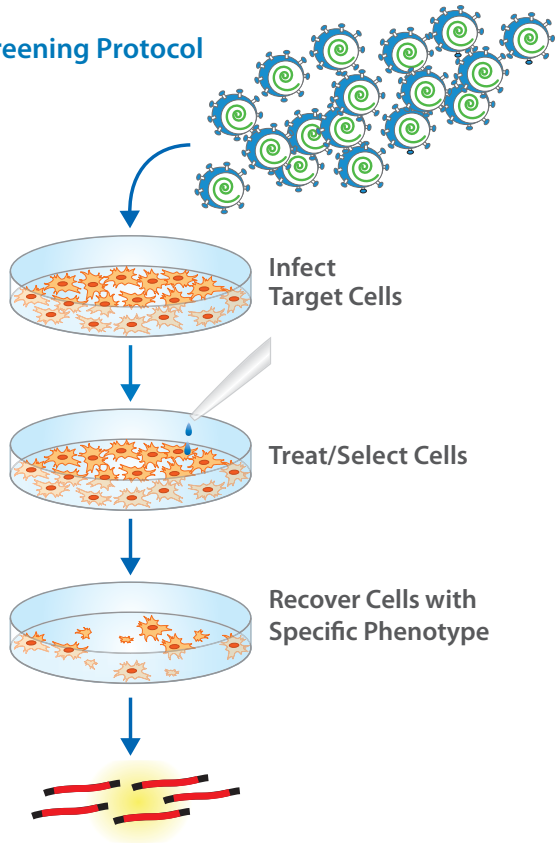

## Pooled Lentiviral Libraries for High-throughput Screening

- Powerful technologies to identify genes involved in your model system
- Affordable, cost-effective approach to dissect signaling pathways
- Identify potential drug targets or diagnostic markers
- Use GeneNet shRNA libraries to screen the messenger RNA transcriptome
- Overexpress microRNAs in phenotypic screens with Lenti-miR precursor libraries
- Apply miRZip anti-miR libraries for microRNA interference screens
- Available as either pooled, pre-packaged virus or plasmid collections

# How to use Pooled Lentiviral Libraries

SBI's pooled lentiviral libraries allow you to perform high-throughput screening studies on a genome-wide or pathway-focused basis. Pooled lentiviral libraries enable simultaneous identification of multiple genes that alter a specific cellular phenotype in a single experiment. Lentiviral libraries are available as prepackaged virus, so you can begin transducing cells the day you receive the library.

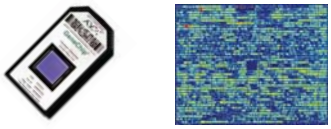
## Overview of Library Screening Protocol



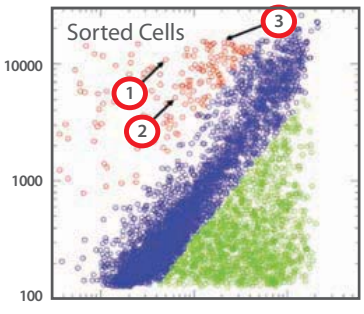

## Messenger RNA Screens

### GeneNet™ RNAi Knockdown Screens

**Hybridize**
to Affymetrix GeneChip®



**Analyze**
with statistical software




○ = New Target Identified

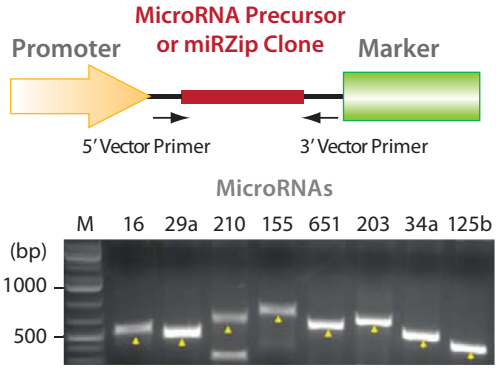
○ = Enriched shRNAs
○ = Unchanged shRNAs
○ = Selected out shRNAs

**mRNA Targets Discovered**

## MicroRNA Screens

### Lenti-miR microRNA Overexpression or miRZip™ microRNA Knockdown Screens

**Simple Genomic PCR to Identify microRNA Effectors**




**Directly Sequence PCR Amplicons**

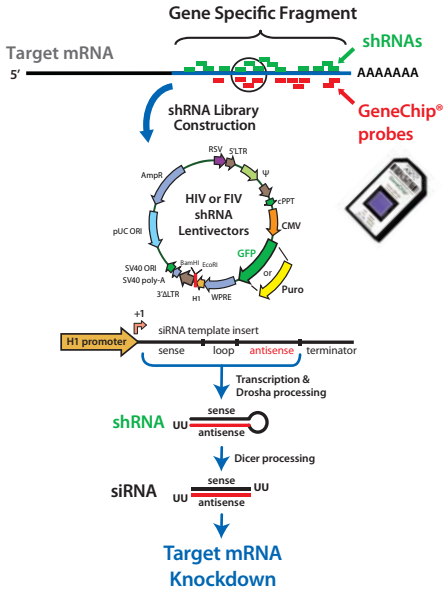
**MicroRNAs Affecting Phenotype Identified**

www.gentaur.com

# GeneNet™ shRNA Pooled Lentiviral Libraries

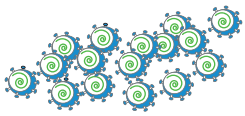
System Biosciences GeneNet™ shRNA Libraries allow you to perform high-throughput gene knockdown studies on a genome-wide or pathway-focused basis. GeneNet Libraries are proven, high performance screening tools for gene knockdown studies. Three to four shRNA sequences target each mRNA transcript and are synthesized and cloned into SBI's shRNA lentivectors that provide superior infection efficiciency across numerous cell types and models.


## GeneNet Library Design




Highly-specific and unique shRNA sequences are designed to be compatible with Affymetrix® GeneChips. This design feature provides the ability to hybridize the shRNA effectors recovered after screens to GeneChips for easy identification of the exact shRNA producing the experimental phenotype. Cost-effective RNAi screens can be performed by any research group.

***Choose Gentaur for high-throughput RNAi***



## Available GeneNet™ Libraries

**GeneNet:** Genome-Wide shRNA Libraries

| HIV-based Libraries | Transcripts Targeted | No. of shRNAs |
|---|---|---|
| Human 50K | 47,400 | 200,000 |
| Mouse 40K | 39,000 | 150,000 |

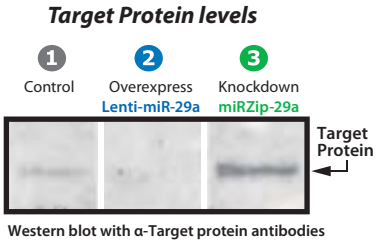
| FIV-based Libraries | Transcripts Targeted | No. of shRNAs |
|---|---|---|
| Human 50K | 47,400 | 200,000 |
| Mouse 40K | 39,000 | 150,000 |

**GeneNet:** Pathway Focused shRNA Libraries

| HIV-based Libraries | Targeted Genes | No. of shRNAs |
|---|---|---|
| Human Apoptosis | 597 | 6,876 |
| Human Kinase | 897 | 10,453 |
| Human Phosphatase | 244 | 2,719 |

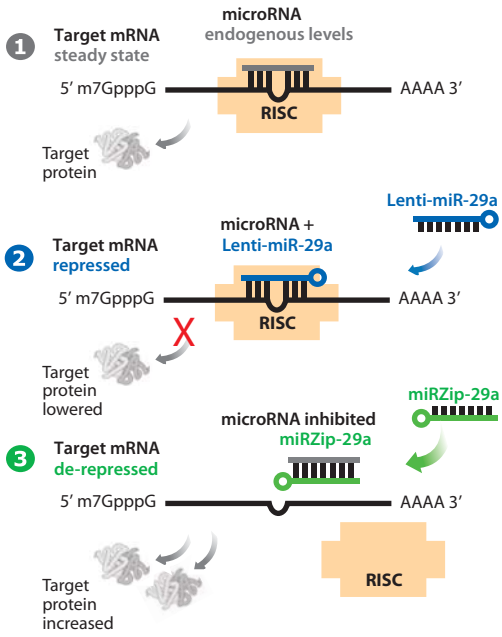
# Lenti-miR and miRZip™ MicroRNA Pooled Lentiviral Libraries

Screen all microRNAs simultaneously for unique phenotypes using SBI's Lenti-miR or miRZip virus libraries. Each virus within the pool will express an individual microRNA precursor or miRZip anti-sense microRNA. Ideal for discovering novel phenotypes based on the modulation of target protein levels affected by microRNAs. Each virus within the pool expresses a microRNA precursor in its native context while preserving hairpin structure. This ensures biologically relevant interactions with endogenous processing and regulatory machinery.




Western blot with α-Target protein antibodies

Modulation of Target Protein levels using SBI's Lenti-miR-29a and miRZip-29a microRNA constructs. The higher the levels of microRNA-29a, the lower the levels of Target Protein and vice versa. Study the phenotypic effects of microRNA overexpression and knockdown.




## Modulate Target Protein levels using MicroRNAs to Uncover Compelling Phenotypes

### Lenti-miR Overexpression Screens

- Pooled virus library contains entire Lenti-miR precursor clone collection (> 600 clones)
- Study phenotypic effects associated with the overexpression of microRNAs
- Recover microRNAs responsible for generating the phenotypes of interest through simple genomic PCR

### miRZip™ microRNA Knockdown Screens

- Perform microRNA interference with the pooled miRZip antagomir virus library
- Discover microRNAs that produce dramatic cellular changes when suppressed
- Identify the miRZip effector using lentivector-specific primers on target cell's genomic DNA

# **Custom Services:** Put our Scientists to Work for You

## Spend less time making tools and more time making discoveries

System Biosciences offers a wide-range of custom services. Utilize SBI's expertise with lentiviral cloning and packaging, pooled shRNA and microRNA screening libraries, and microRNA profiling to accelerate your research.

## Lentiviral Cloning
- cDNAs
- shRNAs
- microRNAs and anti-miRs
- transcription reporters

## Express Lentiviral Packaging

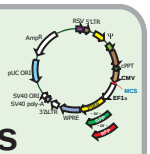
## Custom shRNA Libraries

## iPS Cell Line Generation

## Reporter Cell Line Construction

## MicroRNA qPCR Profiling

### Custom Lentiviral Constructs



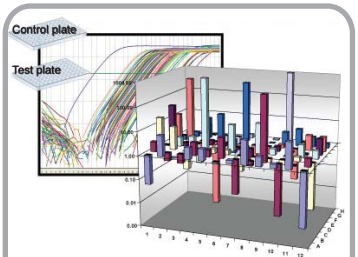
**SBI BUILDS AMAZING CONSTRUCTS**

Let the experts create your next lentivector tool.

- shRNA
- cDNA
- microRNA
- Reporter
- GFP or RFP
- Puro or Zeo
- EF1 or CMV
- Customize


**CUSTOM VIRUS PACKAGING**

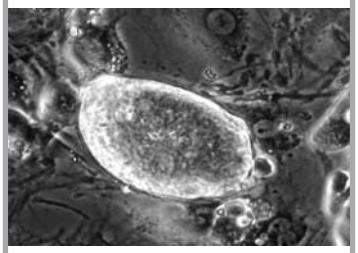
Have SBI package your lentivector into high titer ready-to-transduce virus.




**CUSTOM MICRORNA PROFILING**

Expert qPCR microRNA profiling service for rapid discoveries.

### iPSC CUSTOM REPROGAMMING



Reprogram model system cell lines into the pluripotent state.

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