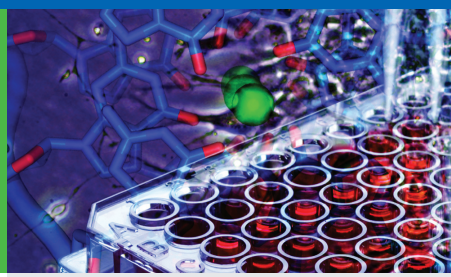
System Biosciences
Accelerating Discoveries in Functional Genomics

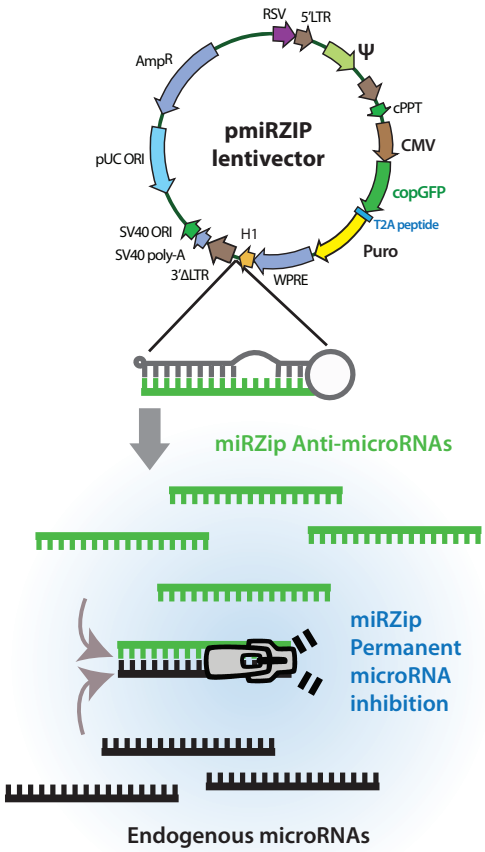
microRNA Research

miRZip™ Anti-microRNAs

Permanent Lentiviral-based MicroRNA Knockdown

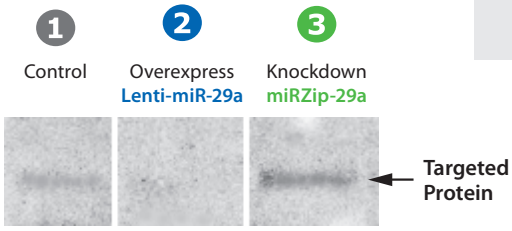


miRZip™ anti-sense microRNAs are stably expressed RNAi hairpins that have anti-microRNA activity. The miRZip shRNAs produce short, single-stranded anti-microRNAs that competitively bind their endogenous microRNA target and inhibit its function. The result is the derepression and elevation of the protein levels of the transcripts targeted by the microRNA being "zipped".



miRZip lentivector constructs can be used for both GFP sorting and Puromycin selection for stable cell lines.

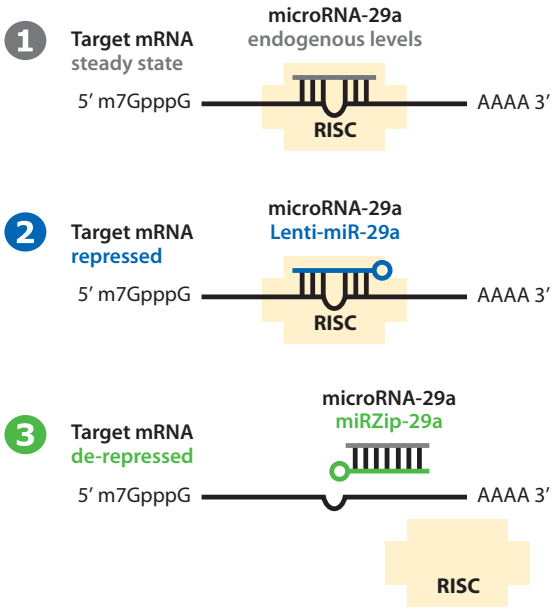
The miRZip short hairpin RNAs are cloned into SBI's pGreenPuro™ shRNA expression lentivector. The miRZip hairpins are rationally designed for asymmetry such that the upper strand of the hairpin (in gray) does not contain the endogenous microRNA sequence and the lower strand is preferred for producing anti-sense microRNAs (in green) that are fully complementary to a specific microRNA target.



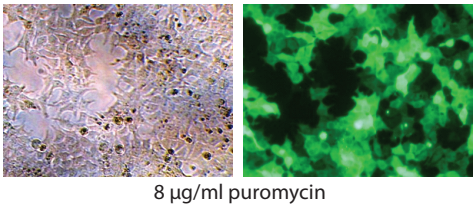
Western blot probed with α-Target protein antibodies

Modulation of miR-29a 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.



miRZip Lentivector Performance Data



8 µg/ml puromycin

Highlights

- Stable & permanent anti-microRNA expresssion from constitutive H1 promoter
- Anti-sense microRNAs sequester specific endogenous microRNAs
- Select for positive expressing cells with GFP or Puromycin
- Uncover phenotypes using microRNA inhibition
- Perform phenotypic screens using pooled miRZip libraries

www.systembio.com/mirzips

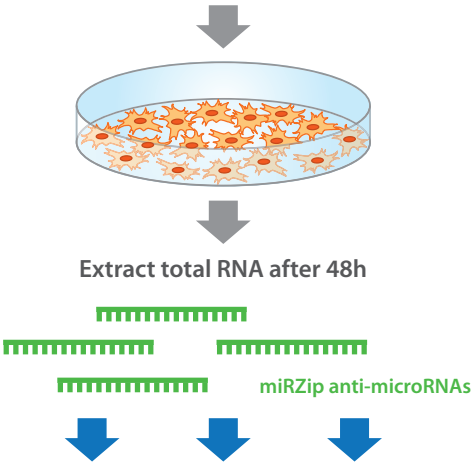
miRZip™ Anti-microRNAs

Thoroughly Understand microRNA Function

SBI's miRZip system provides a convenient and effective approach to permanently inhibit microRNAs—create stable cell lines or transgenic animal models. miRZip phenotypic screens using individual constructs or pooled libraries is a powerful approach to identify and characterize microRNA signaling networks.

miRZip Expression Validation Tests

Transfect miRZip plasmid constructs into HEK293 cells

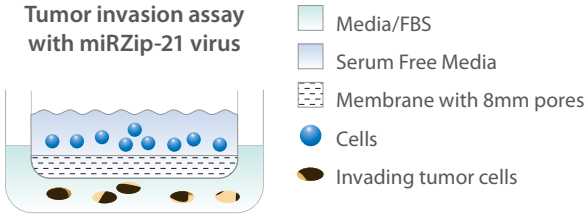


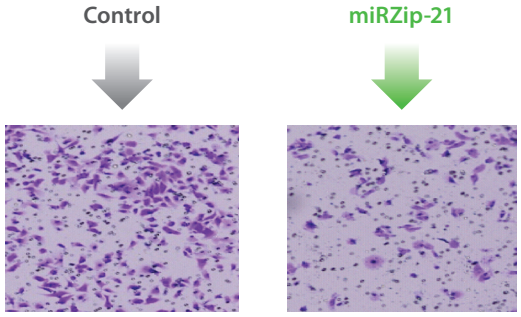
Quantitate anti-microRNAs using QuantiMir and qPCR

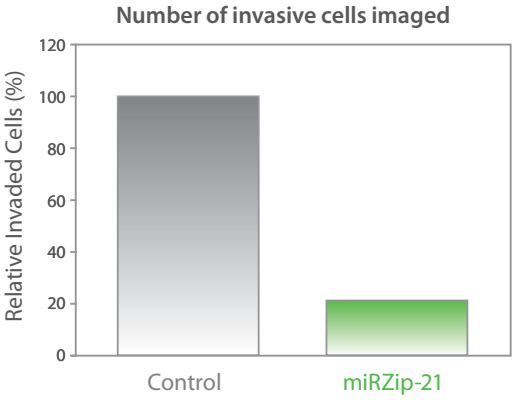
MiRZip constructs are tested for anti-microRNA expression individually by transfecting HEK293 cells with miRZip plasmid DNA. The RNA samples are converted to cDNA using SBI's QuantiMir RT system for qPCR analysis. The levels of anti-microRNA expression are measured using QuantiMir primer assays designed for the specific miRZip anti-microRNA and then compared to untransfected controls. The miRZip anti-microRNA constructs produce high levels of anti-sense microRNAs to competitively bind endogenous microRNAs for efficient functional suppression.

Suppression of cell invasion and tumor metastasis by miRZip-21

MDA-MB-231 breast cancer cells transduced with miRZip-21 lentivirus were tested for changes in metastatic phenotype. Cell invasion assays of control and miRZip-21 transduced cells were subjected to matrigel chamber assays (~40,000 cells per chamber) and the number of invasive cells were counted. Dramatic metastatic phenotypic changes were observed in the miRZip-21 transduced cells.







Invasive cells reduced by 80% permanently by miRZip-21 lentivirus

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