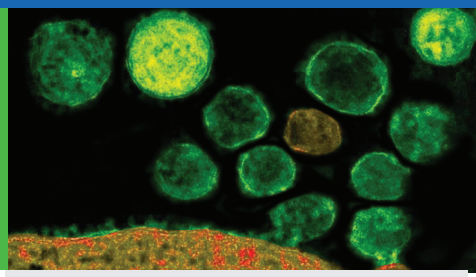
System Biosciences
Accelerating discoveries through innovations

Exosome Research

Package miRNAs into Exosomes

The XMIR, AXMIR and XMIRXpress exosome RNA packaging systems

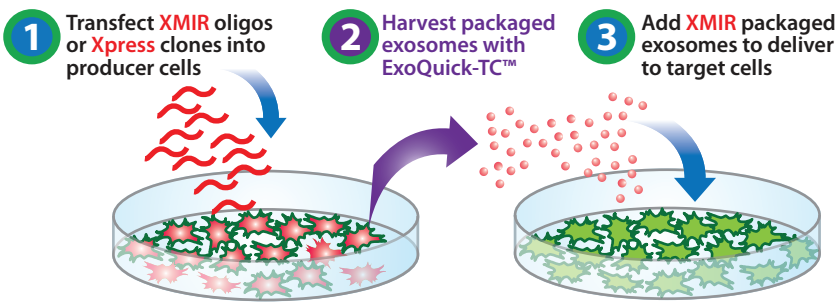


The XMotif RNA Sequence Packages RNAs into Exosomes

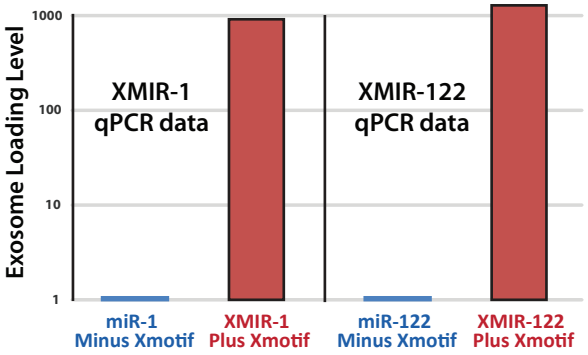
Exosomes contain distinct subsets of RNAs and proteins depending upon the cell type from which they are secreted, making them useful for biomarker discovery. Additionally, their natural function as cell to cell communication vehicles makes them attractive for use as therapeutic shuttles to deliver biological molecules or drugs to target disease cells. The RNA content of exosomes varies depending upon the cell type from which they are secreted. The mechanism of how specific RNA sequences are selectively packaged into exosomes is an intensive area of investigation. SBI has identified a specific RNA sequence tag that targets a small RNA to be packaged into exosomes for secretion. The "XMotif" RNA sequence tag has been incorporated into the miRNA and anti-miRNA oligos for the XMIR/AXMIR products and has been built into the XMIRXpress cloning and expression lentivectors.

Highlights

- XMIR miRNAs delivered by exosomes
- AXMIR anti-miRNAs into exosomes
- Pre-made XMIR lentivectors
- XMIRXpress cloning lentivector
- All tagged with XMotif

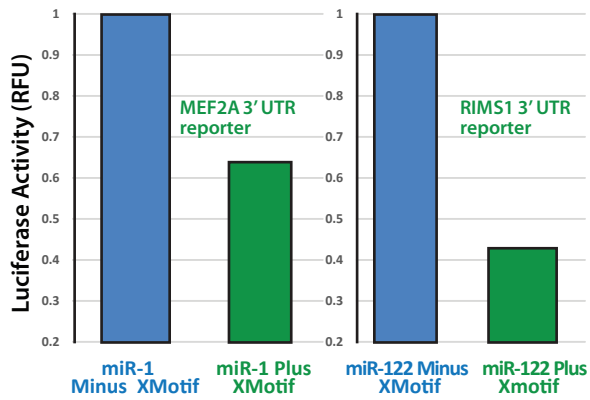


XMIR-1 and XMIR-122 Packaging



The small RNA packaging system works by simply transfecting the XMIR/AXMIR RNA oligo of choice into cells that you want to produce the engineered exosomes. The result is a burst of miRNA loading into secreted exosomes that can be isolated and then used to treat other target cells to test for phenotypic effects. The XMIR-1 and XMIR-122 loaded exosomes (top panel) and added to reporter HEK-293 cells previously transfected with a luciferase gene linked to the 3' UTR for MEF2A, a known miR-1 target, or RIMS1, a known miR-122 target, respectively (lower panel). After 24 hours, luciferase assays were performed to determine bioactivity of the XMIR miRNAs delivered to target cells via exosomes. The degree of knockdown XMIR-1 and XMIR-122 loaded exosomes displayed on the MEF2A and RIMS1 luciferase reporters are similar to that seen for transfections of miRNA oligos using a similar reporter assays, indicating that exosome mediated delivery of miRNAs occurs at maximal efficiency.

XMIRs with 3' UTR Reporter Cells

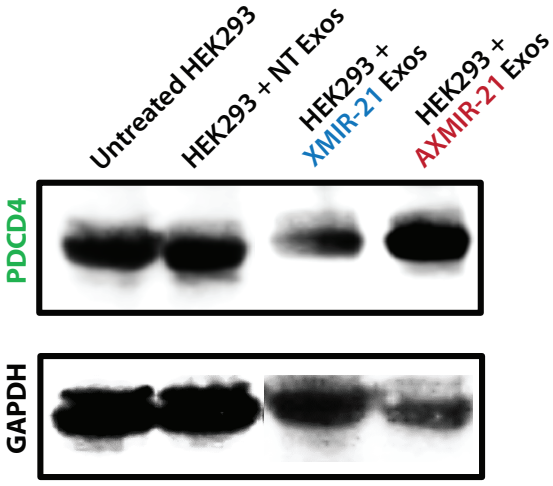


www.systembio.com/xmirs

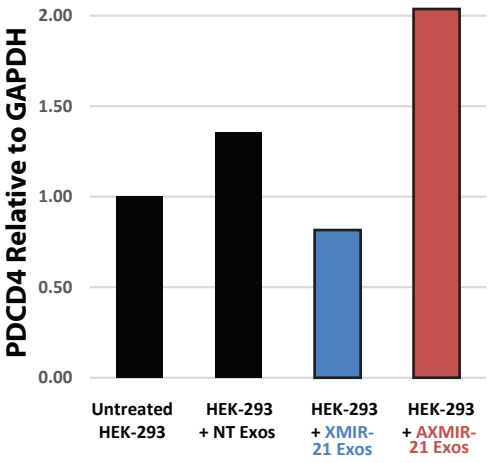
Package miRNAs into Exosomes using XMIR

XMIR-21 Knockdown of Endogenous PDCD4 Proteins Levels

A. Western blot analysis



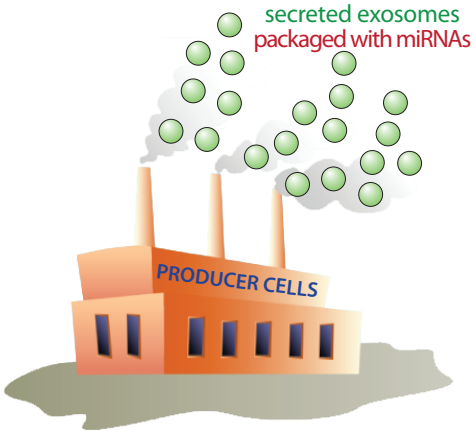
B. Quantitation analysis



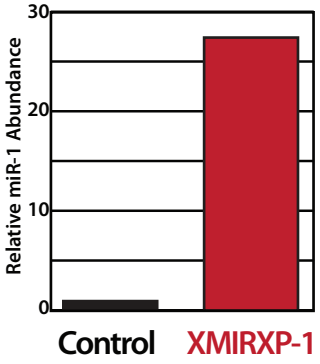
A) Exosomes from HEK-293 cells transfected with a miRNA-21 XMIR oligo (XMIR-21) or an anti-miRNA-21 AXMIR oligo (AXMIR-21) were added to naïve HEK-293 cells in culture. After 24 hours, total cell lysates were taken and Western blots for PDCD4, a known miR-21 target, were performed. GAPDH protein levels detected in the Westerns were used as a loading control and reference signal for band intensity quantitation analysis. B) Quantitative analysis of band intensities from the Western blot shown in Panel A.

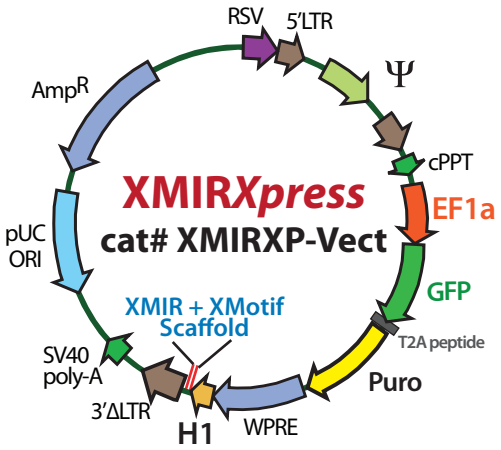
XMIRXpress Lentivector System

The XMIRXpress lentivectors are based on the same XMotif exosomal targeting RNA tag utilized in the XMIR/AXMIR synthetic oligos. There are a number of pre-made XMIR-Express miRNA expression constructs available, and SBI will design and build a custom XMIRXpress lentivector construct for any particular miRNA or anti-miRNA of your choice for the same list price as the pre-made constructs. The lentivectors all feature an EF1a-GFP-Puro selection cassette and a downstream H1 promoter expressing the XMIR + XMotif cassette. Pre-made miRNA containing XMIRExpress lentivectors are offered as well as a cloning MIRXpress lentivector (cat# XMIRXP-VECT), allowing you to clone a fusion of the XMotif to any miRNA, anti-miRN,A or siRNA you choose to make stable exosome cellular "factories".



XMIRXpress XMIRXP-1 Packaged into Exosomes





General oligo cloning design

XMIR-top: 5' - gatccNNNNNNNNNNNNNNNNNNNc - 3'
XMIR-bot: 5' - ctaggNNNNNNNNNNNNNNNNNNNg - 3'

How to make XMIRXpress clones

1. Design olgos with overhangs
2. Anneal top/bottom strand oligos
3. Ligate directly into XMIRXpress vector
4. Transform into competent cells
5. Sequence verify clone
6. Package into virus for stable cell lines

We Also Offer Custom Services - *have SBI build your XMIR clones and cell lines !*

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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