miR-express™ Lentiviral microRNA

For over-expression of human pri-microRNA

The use of microRNA mimetics in gain-of-function experiments is a powerful approach for studies of biological function of specific microRNAs as well as for identification of target mRNAs.

The miR-express™ lentiviral microRNA library is a genome-wide collection of individual microRNA cloned into a lentiviral vector allowing efficient over-expression of human primary microRNAs (pri-miRNA).

Highlights include:

- miRNA transcripts are in their native context to ensure endogenous RNAi processing into mature miRNAs
- miRNAs can be expressed through transfection or transduction
- Lentiviral delivery for miRNA expression in a wide range of cell lines including primary and non-dividing cells
- TurboRFP marks miRNA expression
- Puromycin selectable marker
- Transient, stable and *in vivo* studies are possible

The human miRNAs included in the miR-express collection were selected from the Sanger miRbase database version 9.0 and the genome coordinates were used as a reference to design primers to PCR amplify pri-miRNA sequences with 5' and 3' flank sequences. The primers for amplification of all human primary miRNAs were designed to retain as much flanking sequences as possible for efficient Drosha processing and retain any putative regulatory elements in the sequence.

The first release of this collection includes 175 individual microRNA and will be expanded to target all known human microRNAs.

Efficient processing of pri-miRNA precursors into mature miRNAs

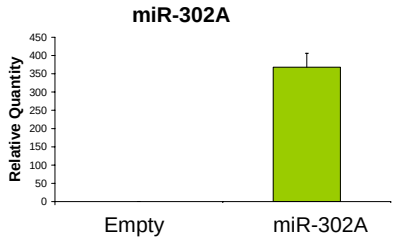
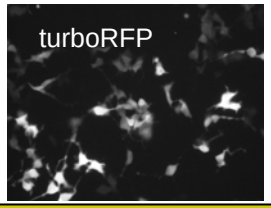
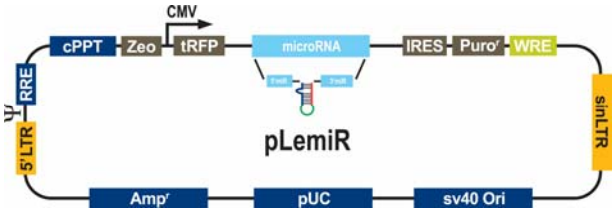


Figure 1. Lentivirus expressed native pri-miRNA precursors are processed into mature miRNA duplexes by the native RNAi machinery in transduced HEK293 cells. Total RNA was isolated 72 hr. post-transduction using miRvana RNA isolation kit (Ambion) and analyzed by qRT-PCR using miRNA specific Taqman probes (Applied Biosystems)

Note: 293 cells do not express miR-302a. RQ values are determined by setting the Ct value of pLemiR-EMPTY to 40 cycles. The data averages two biological replicates assayed in triplicate.



pLemiR Vector Details



Vector Element	Utility
CMV	RNA Polymerase II promoter
cPPT	Central Polypurine tract -helps translocation into the nucleus of non-dividing cells
WRE	Enhances the stability and translation of transcripts
TurboRFP	Marker to track inducible shRNAmir expression
Puro^r	Mammalian selectable marker
RRE	Rev Response element
5'LTR	Wildtype 5' long terminal repeat
SIN-LTR	3' Self-inactivating long terminal repeat
ZEO^r	Bacterial selectable marker

miR-XXX induced GFP expression in GFPu-1 reporter cell line

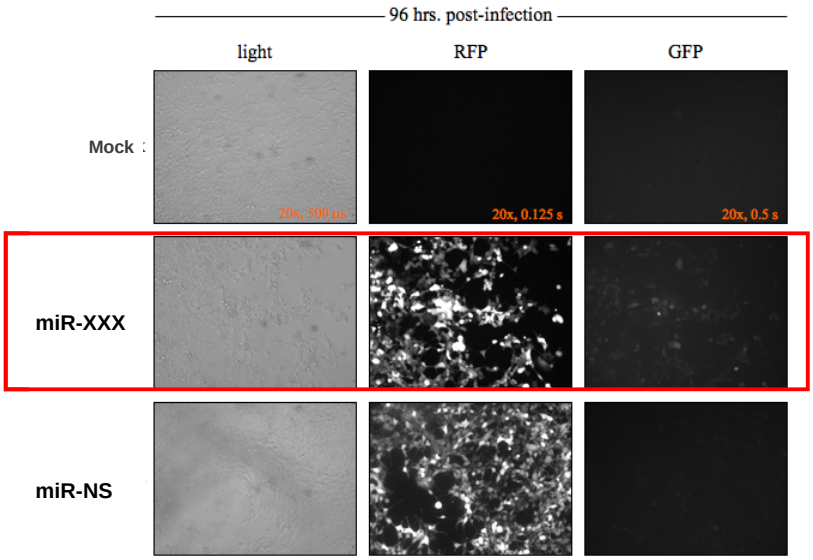


Figure 2. The reporter cell line stably expresses a CL-1 degron (substrate of 26S proteasome) which is fused to GFP, such that in cells with normally functioning ubiquitin-proteasome system, GFP is OFF. GFP signal represents inhibition of the ubiquitin-proteasome system by miRNA-XXX. The results show that over-expression of miR-XXX inhibits proteasome function (GFP is ON) while an irrelevant miRNA (miR-NS) showed no effect.

Finding your microRNA of interest

1. Enter microRNA ID, accession, symbol(s), or catalog numbers into the gene search. Results will be returned in a tabbed format. Click on shRNA under the shRNAs/RNAi tab.
2. Click on the oligo ID link for additional cloning and sequence information
3. Order directly online. Constructs are shipped in 48 hours.

Catalog	Description	Price
HMR4842	miR-express™ human lentiviral microRNA individual clone	**$450**
HMR4843	pLemiR empty vector	**$300**
HMR4867	Non-silencing lentiviral microRNA construct	**$400**

OPEN BIOSYSTEMS
Toll Free 888-412-2225
International +1-256-704-4848
info@openbiosystems.com
www.openbiosystems.com
Fax 256-704-4849