Lentiviral miRNA Collection

96-well Lentiviral Transduction for Functional Screening

Catalog number: mir-96well
Amount: 150 µl / well
Storage Temperature: -70°C

February 2012

BIOSETTIA
A Better Solution in Life Science
www.biosettia.com
info@biosettia.com

Product Description

Human microRNA (hsa-miRNA) precursors and approximately 100bp upstream and downstream flanking genomic sequences were PCR amplified and cloned into a self-inactivated (SIN) lentiviral vector to generate a lenti-miRNA collection (Fig. 1). The cloning site of pre-miRNA genomic fragments is within the intron of human EF1α promoter region. The miRNA lentiviral stock was prepared by cotransfecting HEK 293T cells with the lenti-miRNA vector and plasmids expressing Gag-Pol gene products and the vesicular stomatitis virus envelope G (VSV-G). The lentiviral supernatants were collected at 48 hours post transfection and stored at -70°C. The titer of the virus is generally above 1×10^7 infection units per ml (IU/ml).

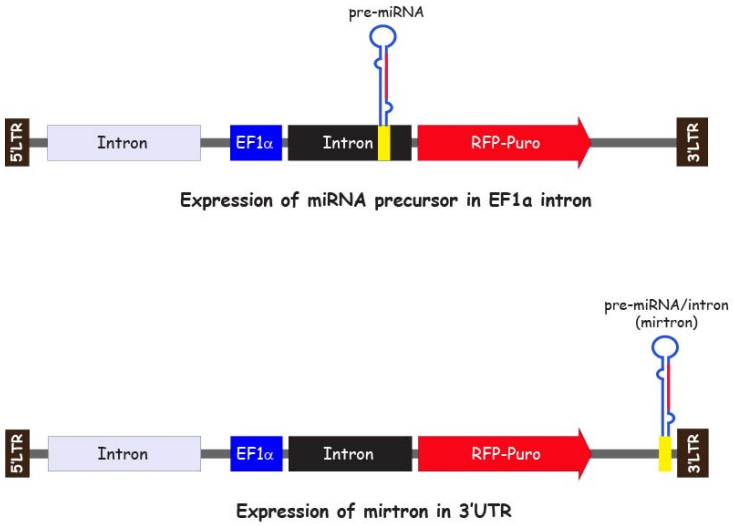


Figure 1. Lentiviral microRNA expression system.

Advantages of the Product

1. The lenti-miRNA is a ready-to-use lentiviral stock

2. Lentiviral transduction is one of the most effective delivery systems to express miRNA. Unlike the retroviral system, the lentiviral integration is cell cycle independent. The genetic materials encoded by the lentivirus can be efficiently delivered into both dividing and non-dividing cells.

3. The lentiviral genome is integrated into the host chromosome, thus the miRNA is stably expressed in transduced cell lines.

4. The human EF1α promoter it is unlikely to be silenced in cells. It has been reported that the activities of some viral promoters such as CMV and SV40 are potentially silenced by DNA methylation after a period of time. The human EF1α promoter, used to express miRNA precursors and the puromycin selection marker, is a house-keeping gene promoter. Therefore, it is unlikely to be silenced by methylation *in vitro* and *in vivo*.

5. The RFP-Puro gene product, expressed from the EF1α promoter, is the red fluorescent puromycin-N-acetyl-transferase. The model cells transduced by lenti-miRNA can not only be selected in the presence of puromycin in the medium, but also display red fluorescence at excitation/emission wavelengths of 587/610 nm (Fig. 2).

6. Each individual miRNA in Biosettia's human lentiviral collection was cloned from its native context, including the stem-loop precursor sequence and approximately 100bp upstream and downstream flanking sequences. This ensured that the miRNA was properly expressed and processed, and would function similarly to its endogenous form.

7. Over 600 miRNA lentiviruses are arrayed in 96-well plates for through-put miRNA functional screens.

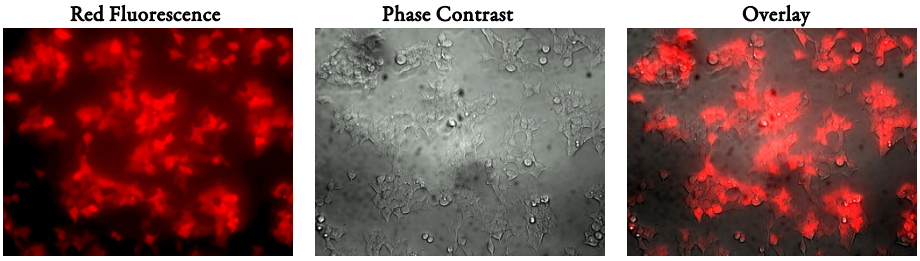


Figure 2. Microscopy images of 293T cells transduced with hsa-mir-24-1 lentivirus.

Product Components

Each 96-well lentiviral miRNA collection includes:

5 vials of mir-control lentivirus for pilot experiments, 1 ml/vial
604 miRNA lentiviruses in seven 96-well plates, 150 µl/well

Protocol

Determining the Optimal Conditions for miRNA Transduction

Day 0: In a 96-well plate, seed different amount of cells ranging from 1 x 10^3 to 1 x 10^5 cells per 100 µl per well.

Suggestion: seed 1 x 10^3, 3 x 10^3, 5 x 10^3, 1 x 10^4, 3 x 10^4, 5 x 10^4, and 1 x 10^5 cells/100 µl/well for at least 3 wells at each density.

Day 1: Transduction for pilot test. Thaw a tube of mir-control lentivirus and gently mix the virus with a pipette tip. Add different amounts (e.g. 10, 30, and 50 µl) of lentivirus and polybrene to each well to a final concentration of 4-8 µg/ml in total 200 µl.

Suggestion: Set up the following transduction mix in each well. The final concentration of polybrene can be between 4-8 µg/ml.

	Vol. (µl)	Vol. (µl)	Vol. (µl)
Cells	**100**	**100**	**100**
Lentivirus	**10**	**30**	**50**
Polybrene (0.8 mg/ml)	**2**	**2**	**2**
Medium	**88**	**68**	**48**
Final Vol.	**200**	**200**	**200**

Spin transduction in a desktop centrifuge (e.g. Sorvall RT6000) at 1,000 g for 60 min at room temperature to help increase transduction efficiency.

Note: Polybrene may be toxic to some cell lines. If so, remove the medium by aspiration and replace it with fresh complete medium immediately after centrifugation. If your model cells are tolerant to

polybrene, we recommend leaving the 96-well plates for overnight incubation before changing the medium.

Day 2: Replace the transduction medium with fresh complete medium to remove lentivirus and polybrene if not already done.

Day 3-5: Observe under a fluorescence microscope to determine the optimal cell number and lentivirus for transduction.

Suggestion: We recommend to go through your experiments with this set up and experimental time frame at least once to ensure the cells will not be over confluent.

Lentiviral Transduction in 96-well Plates for Functional Analysis.

Day 0: Seed cells at appropriate density as determined in the pilot test.

Day 1: Transduction. Gently mix lentivirus with a multichannel pipette, and add the appropriate amount of virus and polybrene to each well.

Note: To prevent the 96-well lentiviruses from cross-contamination, please peel off the plate sealer in the cell culture hood immediately after taking the plates out from the -70°C freezer while the viruses are still frozen.

Spin transduction in a desktop centrifuge (e.g. Sorvall RT6000) at 1,000 g for 60 min at room temperature to help increase transduction efficiency. Replace the transduction mixture with fresh complete medium immediately after centrifugation if polybrene is toxic to the cells, otherwise leave the 96-well plates for overnight incubation.

Day 2: For overnight transduction, replace the transduction medium with fresh complete medium to remove lentivirus and polybrene.

Day 3+: Start your functional assay.

Safety Guidelines for Working with Lenti-miRNA.

The recombinant lentiviruses have been designated as Level 2 organisms by NIH and CDC. A Biosafety Level 2 (BSL-2) facility is required in order to work with lentiviruses. The information of Biosafety in Microbiological and Biomedical Laboratories (BMBL) can be downloaded from the following link:
http://www.cdc.gov/biosafety/publications/bmbl5/BMBL.pdf

Please be aware that you are working with media containing lentiviral particles which could transduce human cells.

Examples of miRNA Expression

To validate the expression of miRNAs from the precursor and flanking sequences inserted in the EF1α intron in the Lenti-miRNA expression system, a variety of analyses such as RT-PCR, Northern Blot, functional analysis and reporter assays have been carried out. The results are shown below:

RT-PCR

HEK 293 cells were transduced with Lenti-miRNA viruses expressing let-7a-1, let-7a-2, and let-7a-3 (catalog# mir-LV001-003; Fig. 3A), or LV-[hsa-mir-ctrl] (catalog# mir-LV000; Fig. 3B) in which no DNA sequence was inserted into the EF1α intron. The total RNAs were extracted 72 hours post-transduction, followed by RT-PCR to detect the transcripts of let-7a precursors. The forward primers used in PCR are let-7a-specific (let-7a-1, gccttcctgtggtgctcaactgtg; let-7a-2, taacttgtaatttccctgcttaag; let-7a-3, gtccccaggaggtgcctctggaag), while the reverse primers are specific for the EF1α intron (cgtactccaaaagctcgagctagc and ctcagtgtggggaaactccatcgc for primer sets 1 and 2, respectively) (Fig. 3).

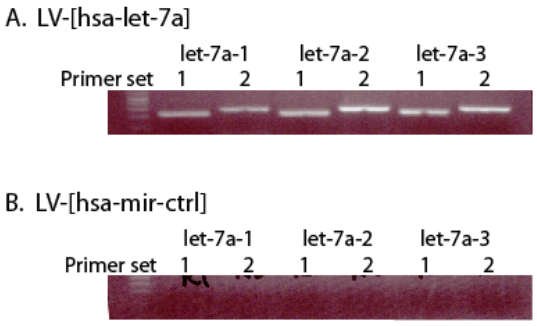


Figure 3. Detection of the expression of let-7a transcripts from the Biosettia LV-[hsa-let-7a] lentiviral transductions by RT-PCR.

Real-Time PCR

The human foreskin (BJ) cells were transduced with LV-[hsa-mir-ctrl] (catalog# mir-LV000) or LV-[hsa-mir-17_18a _19a_20a_19b-1 _92a-1] (catalog# mir-LVc02) viruses. The total RNA were extracted 72 hours post-transduction, followed by TaqMan miRNA assays (Applied Biosystems). The relative level of individual miRNA expression was determined by comparing with the negative control, LV-[hsa-mir-ctrl] (Fig. 4).

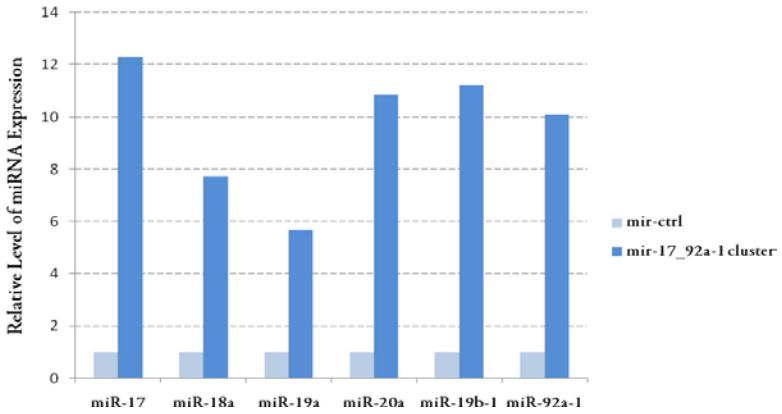


Figure 4. Detection of Lenti-miRNA by real-time PCR.

Northern Blot

HEK 293 cells were transduced with LV-[hsa-mir-ctrl] (catalog# mir-LV000) or LV-[hsa-let-7g] (catalog# mir-LV010) viruses. The total RNA were extracted 72 hours post-transduction and subjected to Northern Blotting analysis as described [1]. The total RNA from Hela cells with high expression levels of let-7g was used as positive control (Fig. 5, lane 1). Co-transduction of Lin28 with LV-[hsa-let-7g] reduced the expression of let-7g (Fig. 5, lane 5), confirming a reported finding that the let-7g biogenesis is suppressed by Lin28 [2, 3]

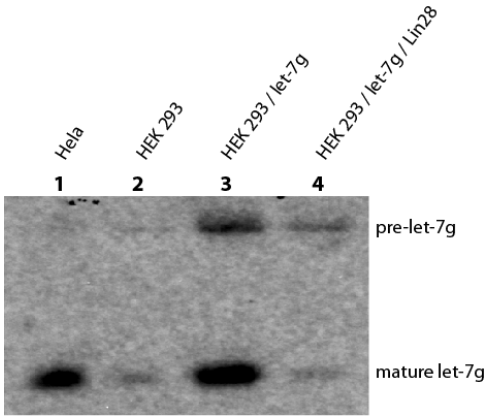
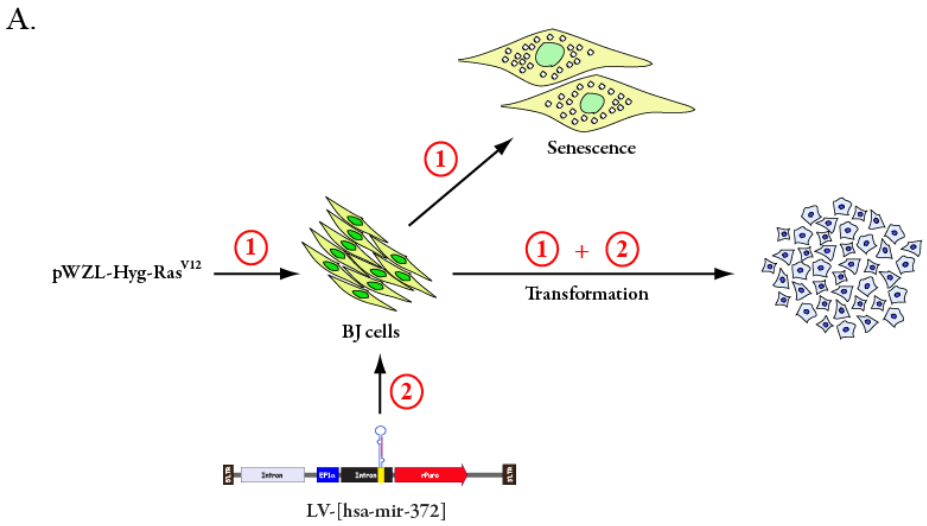


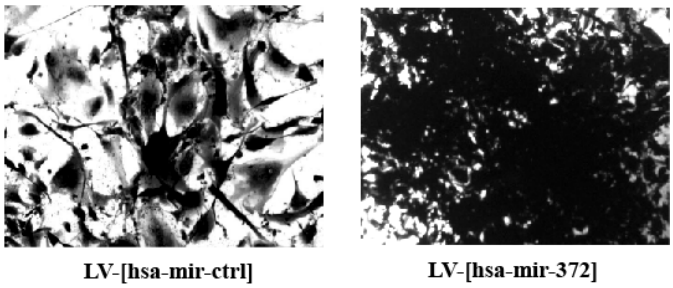
Figure 5. Detection of pre-let-7g and mature let-7g expressed from the Biosettia LV-[hsa-let-7g] lentiviral transductions by Northern Blotting.

Functional Analysis

It has been shown that the presence of microRNAs mir-372 and mir-373 in human primary fibroblast cells allows the cells to bypass H-Ras^{V12} mediated growth arrest [4]. We have transduced BJ cells with retrovirus pWZL-Hyg-Ras^{V12}. After selection with hygromycin, these cells were transduced with LV-[hsa-mir-ctrl], LV-[hsa-mir-372], or LV-[hsa-mir-373] (catalog# mir-LV000, 219, and 220) 4 days after pWZL-Hyg-Ras^{V12} transduction. The BJ cells were grown for another 14 days before microscopic analysis and crystal violet staining (Fig. 6B). Our data showed that mir-372 and mir-373 abolished H-Ras^{V12}-dependent senescence, consistent with the results reported by Voorhoeve *et al.* [4].



B.



C.

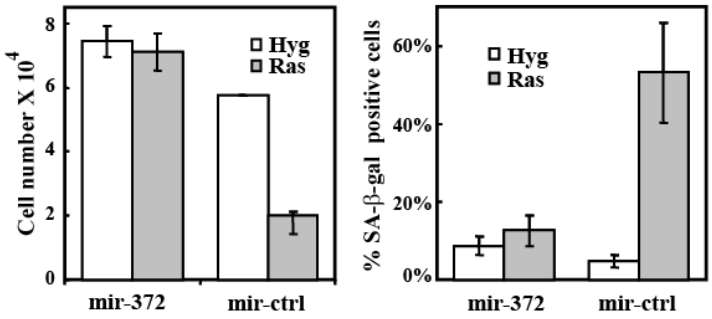
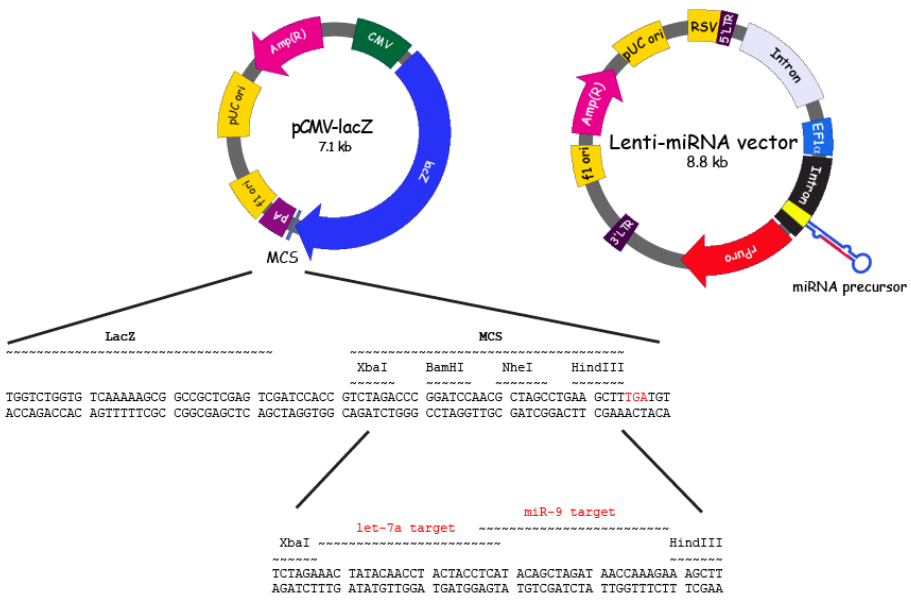


Figure 6. Functional analysis of mir-372 expressed from the Biosettia LV-[hsa-mir-372] lentiviral transductions. (A) Collaboration between oncogenic RAS and mir-372 in the transformation of primary cells. (B) Expression of mir-372 suppresses RAS-mediated senescence and transforms BJ cells. Early passage BJ cells were transduced with pWZL-Hyg-RasV12 (Ras), and then with LV-[hsa-miR-372] or control LV-[hsa-mir-ctrl] from the Biosettia Lenti-miRNA collection. Cells were stained with crystal violet for microscopic analysis at day 14 after RAS transduction. (C) Cell proliferation analysis and senescence SA-beta-gal assay. Early passage BJ cells were transduced with pWZL-Hyg-RasV12 (Ras) or pWZL-Hyg control (Hyg), and then with LV-[hsa-miR-372] or control LV-[hsa-mir-ctrl] from the Biosettia Lenti-miRNA collection. Cells were counted (left panel) and stained for SA-beta-gal (right panel) 8 days after RAS transduction.

Reporter Assay

It has been reported that miRNA is able to cleave mRNA if the mature miRNA sequence is fully complementary to the mRNA target [5]. A 42-bp DNA oligonucleotide (aactatacaacctactacctcatacagctagataaccaaaga) containing sequences complementary to both mature hsa-let-7a (tgaggtagtaggttgtatagtt) and hsa-mir-9 (tctttggttatctagctgtatga) were inserted into the 3' end of the β-galactosidase gene to serve as miRNA targets (Fig. 7A). The β-galactosidase activity is suppressed when either mature let-7a or mir-9 miRNAs is present and targeted to the β-galactosidase fusion mRNA. The β-galactosidase activity is not suppressed by the other let-7 miRNAs that do not perfectly match the inserted target sequence (Fig. 7B). The mir-ctrl, a lentiviral vector without miRNA insertion, is used as negative control.

A.



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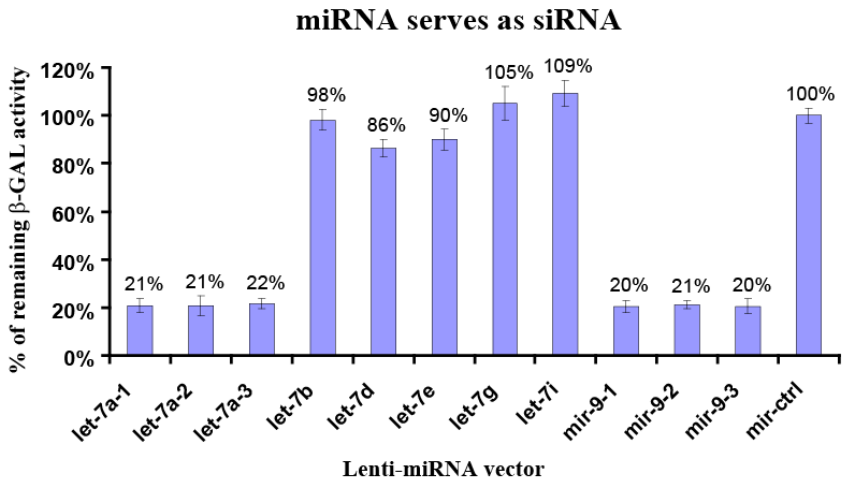

Figure 7. The lacZ reporter assay. The pCMV-lacZ and Lenti-miRNA vectors in a molar ratio of 1:10 were co-transfected into 293T cell. The β–galactosidase activity was determined 24 hours post-transfection.

References

1. Bagga, S., et al., *Regulation by let-7 and lin-4 miRNAs results in target mRNA degradation.* Cell, 2005. **122**(4): p. 553-63.
2. Heo, I., et al., *Lin28 mediates the terminal uridylation of let-7 precursor MicroRNA.* Mol Cell, 2008. **32**(2): p. 276-84.
3. Piskounova, E., et al., *Determinants of microRNA processing inhibition by the developmentally regulated RNA-binding protein Lin28.* J Biol Chem, 2008. **283**(31): p. 21310-4.
4. Voorhoeve, P.M., et al., *A genetic screen implicates miRNA-372 and miRNA-373 as oncogenes in testicular germ cell tumors.* Cell, 2006. **124**(6): p. 1169-81.
5. Zeng, Y., R. Yi, and B.R. Cullen, *MicroRNAs and small interfering RNAs can inhibit mRNA expression by similar mechanisms.* Proc Natl Acad Sci U S A, 2003. **100**(17): p. 9779-84.

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