Ambion Anti-miR™ miRNA Inhibitors and Pre-miR™ miRNA Precursors

Uncover miRNA Function

With Ambion Pre-miR miRNA Precursors and Anti-miR miRNA Inhibitors, you can:

- Determine the biological function of a particular miRNA
- Validate that a particular miRNA regulates the expression of a putative target gene
- Screen for miRNAs that affect a cellular process or that down-regulate a specific gene

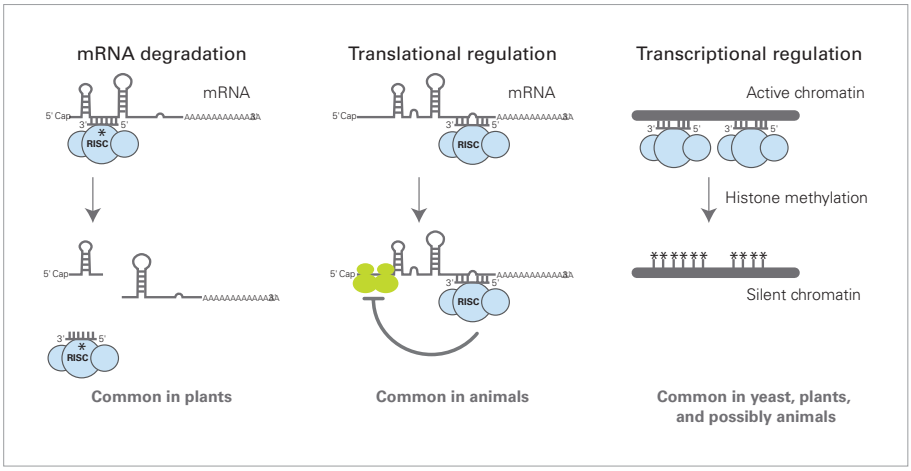


Figure 1. Activity of Ambion Pre-miR™ miRNA Precursor Molecules and Anti-miR™ miRNA Inhibitors.

miRNAs have been implicated as regulators in a variety of biological processes, yet their function is poorly understood. You can now study the actions of these critical biological regulators with miRNA mimics and inhibitors designed for all miRNAs listed in the miRBase miRNA Database. Custom designed Ambion® Pre-miR and Anti-miR molecules are also available. Ambion Pre-miR™ miRNA Precursors and Anti-miR™ miRNA Inhibitors, as well as the pMIR-REPORT™ miRNA Expression Reporter Vector, comprise an integral part of Applied Biosystems complete line of kits and reagents designed to accelerate identification and characterization of miRNAs.

Ambion Pre-miR™ miRNA Precursors

Mimics of Endogenous miRNAs

- Chemically synthesized dsRNAs that, when introduced into cells, efficiently mimic specific endogenous miRNAs
- Optimized design results in correct strand utilization by RISC
- Effective at ≤10 nM
- Available for all miRNAs listed in the miRBase database and for miRNA sequences you provide

Ambion Pre-miR™ miRNA Precursor Molecules are small, chemically modified double-stranded RNA molecules designed to mimic endogenous mature miRNA molecules. These miRNA mimics, which are similar but not identical to siRNAs, can be introduced into cells via transfection or electroporation to perform gain-of-function experiments (Figure 2). Their design has been carefully optimized such that the strand representing the mature miRNA sequence enters into RISC in a manner analogous to the Ambion Pre-miR miRNA Precursor's naturally occurring counterpart (Figure 3).

Find Pre-miR miRNA Precursors for all miRNAs in the miRBase Database by searching our miRNA database at **www.appliedbiosystems.com/miRNAinfo**.

Ambion Pre-miR™ miRNA Precursor Negative Controls

Ambion Pre-miR miRNA Precursor Negative Controls are random sequence molecules ideal for use as negative experimental controls. These controls enter the miRNA pathway in the same manner as described above for the Ambion Pre-miR miRNA Precursors, but have a random sequence rather than a functional targeting sequence. Ambion Pre-miR miRNA Precursor Negative Controls have been extensively validated in human cell lines and tissues and do not produce identifiable effects on known miRNA function.

Ambion Anti-miR™ miRNA Inhibitors

For Down-regulating Specific miRNA Activity

- Single-stranded, modified RNAs that tightly bind to and efficiently inhibit specific endogenous miRNAs
- Effective at ≤10 nM
- Available for all miRNAs listed in the miRBase database and for miRNA sequences you provide

Ambion Anti-miR™ miRNA Inhibitors are chemically modified and optimized

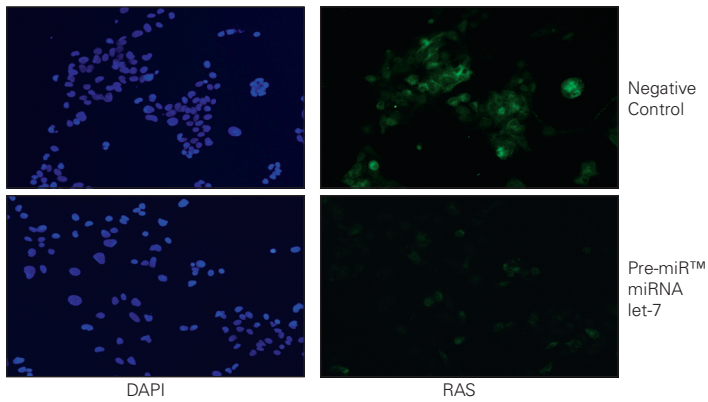


Figure 2. Using Ambion Pre-miR™ miRNA Precursors to Prove that let-7 Influences the Expression of RAS. HepG2 cells were transfected in 24-well plates using siPORT™ *NeoFX*™ Transfection Agent with either 30 nM let-7a Ambion Pre-miR™ miRNA Precursor or a negative control miRNA Precursor. Three days post-transfection, RAS expression was monitored by immunofluorescence. Introduction of the let-7 FFC-miR miRNA resulted in lowered levels of RAS protein. Modified with permission from *Cell* 120: 635–647.

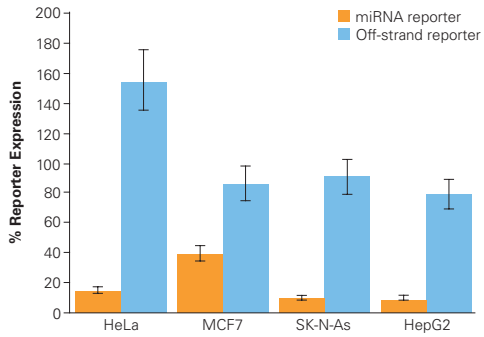


Figure 3. Correct Strand Activation of Ambion Pre-miR™ miRNA Precursor Molecules. HeLa, MCF7, SK-N-As, and HepG2 cells were co-transfected in triplicate with 10 nM miR-33 Ambion Pre-miR miRNA Precursor or Ambion Pre-miR Negative Control #1 (NC#1) and 200 ng mature miR-33 luciferase reporter plasmid (pMIR-REPORT™ Vector) or off-strand miR-33 luciferase reporter plasmid (Off-strand reporter). 24 h post-transfection, the expression of luciferase was measured and normalized to that of cells transfected with NC#1.

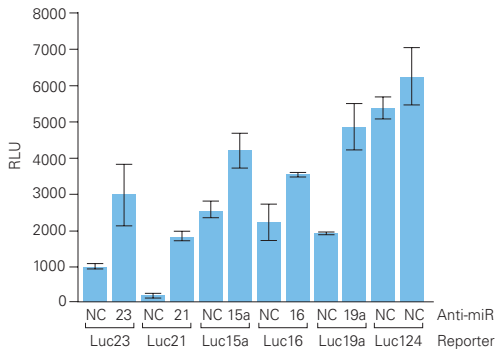


Figure 4. Inhibition of Endogenous miRNAs with Ambion Anti-miR™ miRNA Inhibitors Leads to Enhanced Expression of the Corresponding Target Genes. HeLa cells (5 x 10^4 cells/well; 24-well plates) were transfected in triplicate with three components: pMIR-REPORT™ containing a target site for a specific miRNA (e.g., Luc23=pMIR-REPORT with a miR-23 target site), the corresponding Ambion Anti-miR miRNA Inhibitor (or a negative control inhibitor, NC), and pMIR-REPORT β-Galactosidase Reporter Control Vector. 24 hours post-transfection, cells were assayed for luciferase and β-galactosidase expression using the Dual-Light® Combined Reporter Gene Assay System. The β-gal signals were used to normalize the luciferase signals to control for any variations in transfection efficiency.

single-stranded nucleic acids designed to specifically inhibit naturally occurring mature microRNA (miRNA) molecules in cells (Figure 4). Use of the Ambion Anti-miR miRNA Inhibitors, which are typically introduced into cultured cells via transfection or electroporation, enables miRNA functional analysis by loss-of-function experiments (Figure 5).

Find Ambion Anti-miR miRNA Inhibitors for all miRNAs in the miRBase Database by searching the miRNA database at **www.appliedbiosystems.com/miRNAinfo.**

Anti-miR™ miRNA Inhibitor Negative Control

Ambion Anti-miR miRNA Inhibitor Negative Control #1 is a random sequence Ambion Anti-miR molecule offered for use as a negative experimental control. The Ambion Anti-miR Negative Control has been extensively tested in many human cell lines and tissues and validated to not produce any identifiable effect on known miRNA function.

Ambion Pre-miR™ miRNA Precursor Libraries and Anti-miR™ miRNA Inhibitor Libraries

For Screening of miRNA Function and Target Sites

- Collections of miRNA mimics and inhibitors to all 328 human mature miRNAs in the miRBase database
- Enables the rapid identification of miRNAs involved in a particular pathway or biological process
- Facilitates identification of miRNAs that interact with a target transcript

One way to ascertain miRNA function or to identify miRNAs that interact with a particular target transcript is to transfect miRNA mimics or miRNA inhibitors into cells in a systematic fashion and then analyze the induced phenotype (Figure 6) or the impact of those molecules on a particular target gene product. Ambion® Pre-miR miRNA Precursor Library Human V2 and Anti-miR miRNA Inhibitor Library Human V2 greatly facilitate these types of studies. Each library consists of 328 miRNA mimics or inhibitors, respectively, corresponding to the 328 human mature miRNAs cataloged in miRBase version 8.0. Each Pre-miR miRNA Precursor or Anti-miR miRNA Inhibitor is supplied dried in 96-well plates at 250 pmol, which is sufficient for 400 transfections when delivered at 10 nM concentration in 96-well plates.

Custom Ambion Pre-miR miRNA Precursor and Anti-miR miRNA Inhibitor Libraries are also available. Email us at **aus.libraries@appliedbiosystems.com** for additional details.

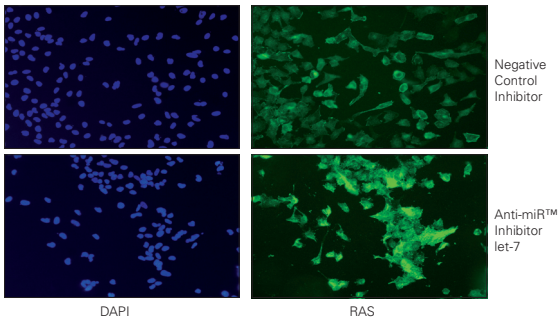


Figure 5. Using Ambion Anti-miR™ miRNA Inhibitors to Prove that let-7 Influences the Expression of RAS. HepG2 cells were transfected in 24-well plates using siPORT™ *NeoFX*™ Transfection Agent (Ambion) with either 30 nM let-7a Ambion Anti-miR™ miRNA Inhibitors or a negative control miRNA inhibitor. Three days post-transfection, RAS expression was monitored by immunofluorescence. Modified with permission from *Cell* (2005) 120: 635–647.

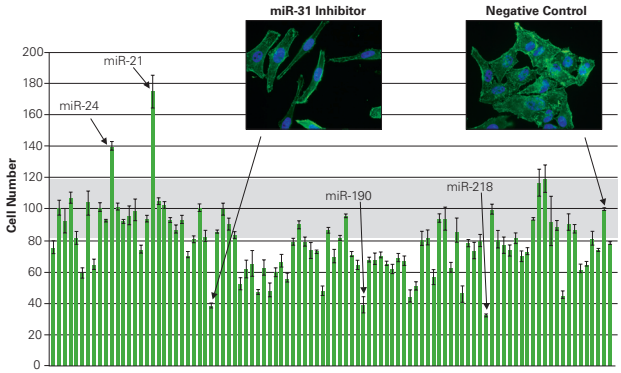


Figure 6. Identification of miRNAs Involved in Cell Proliferation by Screening with Ambion Anti-miR™ miRNA Inhibitors. HeLa cells (5 x 10^3) were transfected in 96-well plates with individual Anti-miR miRNA Inhibitors (30 nM) in triplicate using siPORT™ *NeoFX*™ Transfection Agent. 72 hr post-transfection, cells were fixed and stained with propidium iodide to count total cell number (TTP LabTech Acumen Exporer™). Cells were then stained for β-actin using immunofluorescence. The horizontal shaded area represents the normal range of cell numbers for this cell type, as exemplified by cells transfected with a negative control that does not affect cell proliferation (second bar from the right). The right inset shows the morphology of these control cells. The left inset shows the morphology of cells transfected with an Ambion Anti-miR Inhibitor to miR-31.

Individual Ambion Pre-miR Precursor and Anti-miR Inhibitor Molecules Now Available for All Known miRNAs

The entire collection of Ambion Pre-miR miRNA Precursors and Anti-miR miRNA Inhibitors is cataloged in a searchable database accessible from **www.appliedbiosystems.com/miRNAinfo**. This miRNA database provides a valuable resource for researchers and includes miRNA sequence information, references, and links directly to the miRBase miRNA Database maintained by the Sanger Institute. You can order Ambion Pre-miR and Anti-miR Molecules corresponding to any miRNA in the database or for a sequence you provide.

pMIR-REPORT™ miRNA Expression Reporter Vector System

- Clone miRNA targets and evaluate miRNA regulation
- Screen putative miRNA target sequences
- Includes both reporter and control vectors

The pMIR-REPORT™ miRNA Expression Reporter Vector System provides accurate, quantitative, in-cell measurement of miRNA expression. This validated reporter system contains two mammalian expression vectors (Figure 7).

The pMIR-REPORT™ Luciferase miRNA Expression Reporter Vector contains firefly luciferase under the control of a mammalian promoter/terminator system, with a miRNA target cloning region downstream of the luciferase translation sequence. This vector is optimized for cloning of miRNA targets and evaluation of miRNA regulation. pMIR-REPORT Luciferase can be transfected into mammalian cells to evaluate endogenous miRNA expression, or used to evaluate the up- or down-regulation resulting from the transfection of Ambion Pre-miR™ miRNA Precursors (Figure 3) or Anti-miR™ miRNA Inhibitors, respectively.

A second vector, pMIR-REPORT™ β-galactosidase Reporter Control Vector, is provided for normalization of transfection efficiency.

The two vectors in the pMIR-REPORT System are supplied as separate glycerol stocks of *E. coli* pre-transformed with each of the two supercoiled plasmids.

siPORT™ *NeoFX*™ Transfection Agent

- Efficiently delivers miRNA precursors and inhibitors
- Co-transfects miRNA precursors/inhibitors with pMIR-REPORT™ vectors
- Excellent reproducibility with low toxicity
- Has broad cell line compatibility
- Achieves efficient delivery even at low nucleic acid concentrations

siPORT™ *NeoFX*™ Transfection Agent is a versatile lipid-based reagent that efficiently and reproducibly delivers miRNA precursors and miRNA inhibitors, as well as siRNA, into many different mammalian adherent cell types. The reagent can also be used to co-transfect miRNA precursors or inhibitors with plasmids. The streamlined "reverse transfection" protocol can be adapted to a wide range of cells and experimental designs, including high throughput applications.

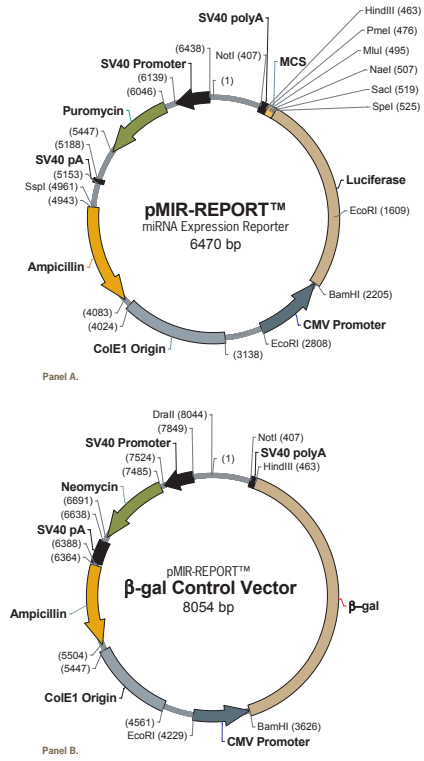


Figure 7. Vectors Comprising the pMIR-REPORT™ miRNA Expression Reporter Vector System.

MicroRNA Information Resources

Applied Biosystems is committed to providing the research community with products and information necessary to advance microRNA (miRNA) research. We develop innovative products specifically for miRNA experimental needs. Applied Biosystems Research and Development scientists:

- Develop novel, market-leading miRNA products
- Have decades of RNA experience, with greater than three years of specific experience in the miRNA field
- Establish critical research collaborations with thought leaders in the miRNA field

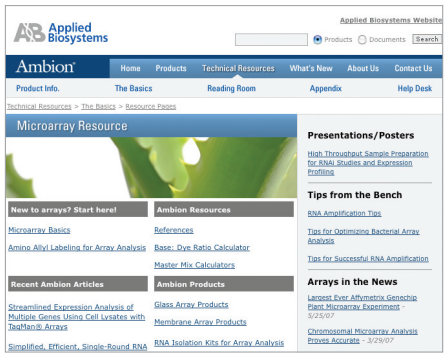
As part of the miRNA research community, Applied Biosystems provides extensive access to our miRNA expertise, by providing free, in-depth experimental design consultation and support. We provide the following information resources:

miRNA Resource
www.appliedbiosystems.com/miRNAresource

A gateway to all types of information about microRNAs. This resource includes links to articles, tools, news, products, other websites, and more.

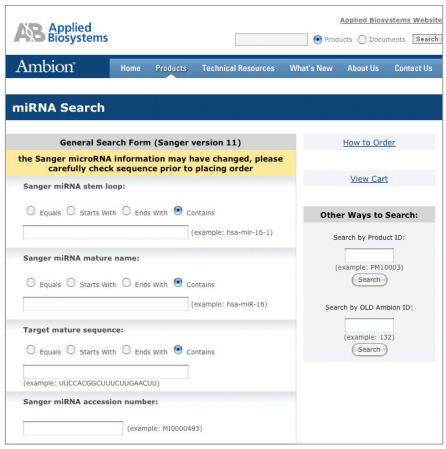
miRNA Array Resource
www.appliedbiosystems.com/microarrays

A resource for those conducting microRNA array experiments that includes tips, troubleshooting advice, frequently updated miRNA annotation files, and data analysis support.



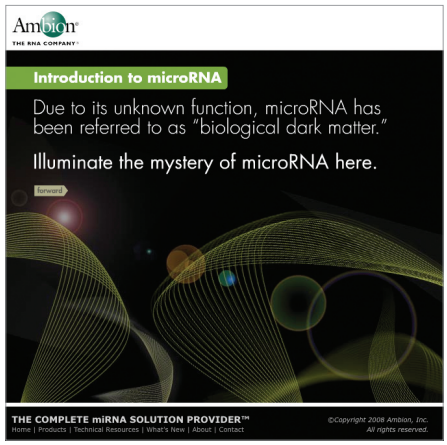
miRNA Database
www.appliedbiosystems.com/miRNAdatabase

A comprehensive database of known microRNAs. Search by stem loop name, mature name, accession number, and/or species. Search results include information about the miRNA, as well as a list of associated Ambion products–simply click to order!



Introduction to microRNAs
www.appliedbiosystems.com/microRNA

This interactive web feature answers the questions "What are miRNAs?", "How do they work?", "How do I study them?", and "Where can I learn more about them?".



ORDERING INFORMATION

Description	Size	P/N
Ambion Pre-miR™ miRNA Precursor Molecule	5 nmol	AM17100
Ambion Pre-miR™ miRNA Precursor Molecule	20 nmol (4 x 5 nmol)	AM17101
Customer-defined Ambion Pre-miR™ miRNA Precursor Molecule	20 nmol	AM17103
Ambion Pre-miR™ miRNA Precursor Molecules– Negative Control #1	5 nmol	AM17110
Ambion Pre-miR™ miRNA Precursor Molecules– Negative Control #2	5 nmol	AM17111
Ambion Anti-miR™ miRNA Inhibitor	5 nmol	AM17000
Ambion Anti-miR™ miRNA Inhibitor	20 nmol (4 x 5 nmol)	AM17001
Customer-defined Ambion Anti-miR™ miRNA Inhibitor	20 nmol	AM17003
Ambion Anti-miR™ miRNA Inhibitors– Negative Control #1	5 nmol	AM17010
Ambion Pre-miR™ miRNA Precursor Library — Human V2	328 x 250 pmol	AM17300V2
Ambion Anti-miR™ miRNA Inhibitor Library — Human V2	328 x 250 pmol	AM17200V2
Custom Ambion Pre-miR™ miRNA Precursor Library	Custom	Inquire
Custom Ambion Anti-miR™ miRNA Inhibitor Library	Custom	Inquire
For pricing and ordering information, email us at **aus.libraries@appliedbiosystems.com**		
pMIR-REPORT™ miRNA Expression Reporter Vector System	1 kit	AM5795
siPORT™ *NeoFX*™ Transfection Agent	0.4 mL	AM4510
siPORT™ *NeoFX*™ Transfection Agent	1 mL	AM4511

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Printed in the USA, 11/2008 Publication 137PB17-02

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