

RNA Functional Analysis – enhanced by LNA

- RNA Silencing
- miRNA Inhibitors
- miRNA Mimics
- miRNA Target Site Blockers
- in vitro and in vivo
- QIAGEN LNA®-enhanced research tools

Study RNA function with LNA-enhanced tools

Understanding the diverse RNA world and its network of intricate interactions is one of the most important challenges facing biology today. QIAGEN offers tools to investigate the roles of coding and non-coding RNAs using a variety of approaches. With the high potency and specificity of Locked Nucleic Acids (LNA), you can truly elucidate RNA function – both in vitro and in vivo.



Antisense LNA GapmeRs

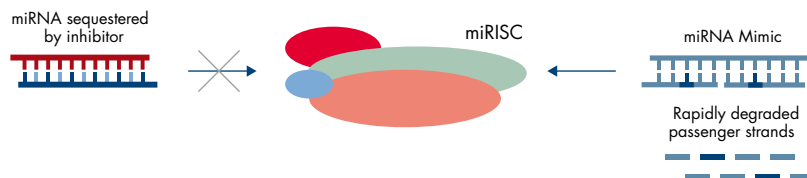
- Potent silencing of any mRNA or lncRNA
- RNase H-dependent degradation of complementary RNA targets
- Effective in vitro and in vivo

www.qiagen.com/gapmers

miRCURY® LNA miRNA Target Site Blockers

- Mask specific miRNA target sites or ribonucleoprotein binding sites on mRNA and lncRNA
- Identify miRNA targets in vitro and in vivo

www.qiagen.com/mirna-TSB



miRCURY LNA miRNA Inhibitors

- Potent miRNA inhibition
- Sequester miRNA in a stable, inactive complex and prevent loading into RISC
- Effective in vitro and in vivo

www.qiagen.com/mirna-inhibitors

miRCURY LNA miRNA Mimics

- Unique, triple-RNA strand design ensures specific miRNA mimicry
- LNA-enhanced, segmented passenger strand
- Only unmodified, active miRNA guide strand is loaded into RISC

www.qiagen.com/mirna-mimics

Functional Analysis Products

RNA Silencing

Antisense LNA GapmeRs

(page 4)

- Efficient silencing of any mRNA or lncRNA
- Specific knockdown with no miRNA-like, off-target effects
- Taken up without transfection reagents
- Target both cytoplasmic and nuclear-retained RNAs

Antisense LNA GapmeR in vivo Ready

- Gene silencing in live animals
- Potent and long-lasting RNA silencing in a broad range of tissues
- Active in vivo without formulation

RNA Function

LNA Oligos for lncRNA Functional Analysis

- Custom designed LNA Oligos
- Study interactions between RNA, DNA and proteins
- lncRNA pull down

RNA

Inhibitors

miRCURY LNA miRNA Inhibitors

(page 9)

- Potent, specific miRNA inhibition
- Taken up without transfection reagents
- Inhibitor libraries for cost-effective screening

Mimics

miRCURY LNA miRNA Mimics

(page 12)

- Unique, triple RNA strand design
- Specific mimicry without off-target miRNA activity
- Biotinylated mimics for identification of miRNA targets in pull-down studies

Target Site Blockers

miRCURY LNA miRNA Power Target Site Blockers

(page 15)

- Study individual miRNA target sites in vitro and identify important target genes

In vivo miRCURY LNA miRNA Power Target Site Blockers, in vivo Large Scale

- Identify important miRNA targets in animal models

Custom miRCURY LNA miRNA

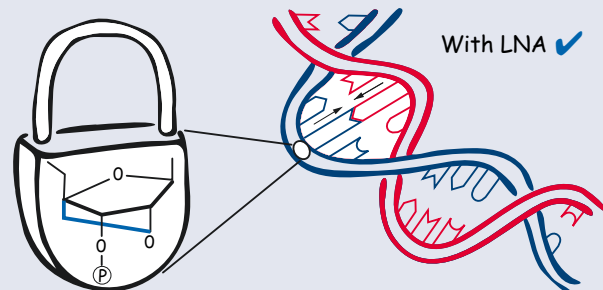
Inhibitors *in vivo* Large Scale

(page 17)

- Potent and long-lasting miRNA inhibition in a broad range of tissues
- Active in vivo without formulation

Learn more:

www.qiagen.com/miRNA-functional-analysis



Antisense LNA GapmeRs

Potent antisense oligonucleotides for highly efficient knockdown of mRNA and lncRNA. Designed using an advanced algorithm to ensure superior performance and high success rates.

At a glance

- Highly potent, single-stranded, antisense oligonucleotides (ASO) for silencing of lncRNA and mRNA
- Efficient, RNase H-dependent degradation of complementary RNA targets
- Active in vitro and in vivo – enabling the analysis of RNA function in a wide range of model systems
- Excellent alternative to siRNA for knockdown of mRNA and lncRNA
- Taken up by cells by transfection or unassisted delivery
- Designed with a sophisticated and empirically developed algorithm for potent and specific knockdown of target RNAs

Product coverage

Antisense LNA GapmeRs can be designed for any mRNA or lncRNA target to suit your application:

- Antisense LNA GapmeR Standard
Cost effective for initial screening of multiple designs using standard cell lines.
- Antisense LNA GapmeR Premium
HPLC- purified LNA GapmeRs with guaranteed purity suitable for most cell assays. Also available with 5' or 3' fluorescent labels.
- Antisense LNA GapmeR in vivo Ready
High purity GapmeRs for careful phenotypic characterization. Also recommended for unassisted delivery.
- Antisense LNA GapmeR in vivo Large Scale
High purity, large-scale GapmeRs for use in animal models.

Validated positive and negative controls are also available.

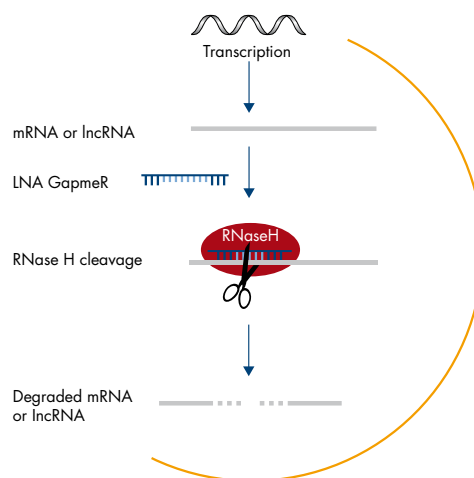


Figure 1. Mode of action of LNA GapmeRs. LNA GapmeRs hybridize with complementary RNA in the nucleus. The central DNA part of the GapmeR catalyzes endonucleolytic cleavage of RNA in the center of the target sequence by recruitment of RNase H. The two generated fragments are then degraded rapidly by exonucleases. The LNA GapmeR is released and will continue catalyzing degradation of further RNA molecules.

Efficient silencing and fewer off-target effects

LNA GapmeRs are ASOs, 15-16 nucleotides in length. They contain a central stretch ("gap") of DNA monomers flanked by LNA modified nucleotides (Figure 2). The central DNA "gap" activates RNase H cleavage of the target RNA upon binding (Figure 1). LNA GapmeRs have fully phosphorothioated backbones, which ensure exceptional resistance to enzymatic degradation. Smart positioning of LNA ensures optimal target affinity regardless of the GapmeR GC content.

Being single stranded, LNA GapmeRs allow strand-specific knockdown of RNAs. LNA GapmeRs act independently of RISC, minimizing off-target effects traditionally associated with siRNA, such as, miRNA-like effects, saturation of RISC and activity from the passenger strand.

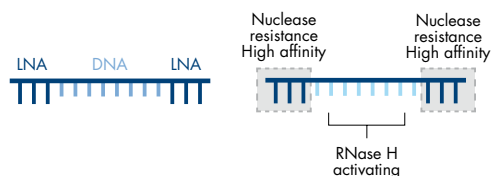


Figure 2. Unique short, single-stranded ASO design. LNA GapmeRs contain a DNA part flanked by LNA. The LNA parts increase the affinity for the target and confer nuclease resistance. RNase H is activated by the DNA part of the ASO.

Efficient knockdowns of both nuclear and cytoplasmic RNA targets

RNase H is primarily located in the nucleus where transcription occurs. Thus, LNA GapmeRs enable extremely efficient knockdowns of both nuclear-retained RNAs (e.g. lncRNAs) and RNAs destined for the cytoplasm (Figure 3).

The method of choice for lncRNA knockdowns

Loss-of-function analysis of lncRNAs is particularly challenging. Many lncRNAs are involved in transcriptional regulation. Confined to the nuclear compartment, these lncRNAs are inefficiently targeted by siRNA, but particularly sensitive to LNA GapmeRs, (Figure 4) exactly because they share the nuclear compartment with RNase H, the endonuclease responsible for LNA GapmeR activity (Figures 2 - 5).

In addition, lncRNAs often derive from transcriptionally complex loci with overlapping sense- and antisense transcripts. Strand-specific knockdown is therefore of crucial importance, and is guaranteed with LNA GapmeRs because they are single stranded.

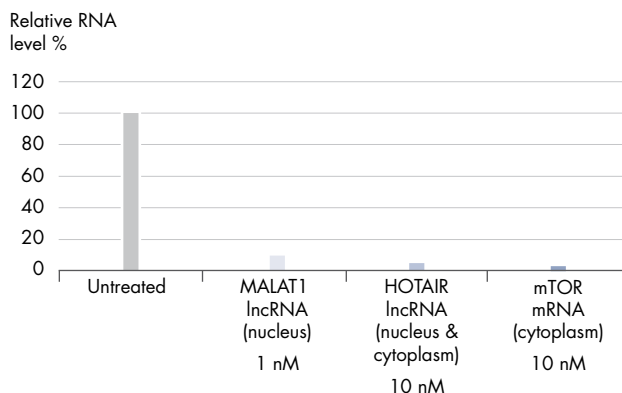


Figure 3. Efficient knockdowns irrespective of type of RNA target and subcellular localization. Antisense LNA GapmeRs provide highly potent knockdown of mRNA and lncRNA targets in both the nucleus and the cytoplasm. LNA GapmeRs shown are available as positive controls. LNA GapmeRs were transfected into HeLa cells, and RNA knockdowns were measured after 48 hrs.

Antisense LNA GapmeRs offer efficient knockdown of mRNA and lncRNA.

Design an LNA GapmeR for your favorite RNA at www.qiagen.com/gapmers

Sophisticated and fast online design software tool

LNA GapmeRs are designed using an empirically derived design tool that incorporates our more than 20 years of experience with LNA design. For each RNA target, the tool evaluates thousands of possible GapmeR designs against over 30 design parameters, identifying the LNA GapmeR most likely to give potent and specific target knockdown. On average, the success rate of our design tool is 2 out of 5 GapmeRs, so screening of 3-5 GapmeRs per design is recommended.

Potent knockdown of mRNA

LNA GapmeR efficacy rivals that of siRNA, making them an excellent alternative for researchers looking for a method that works independently of RISC, with no miRNA-like, off-target effects.

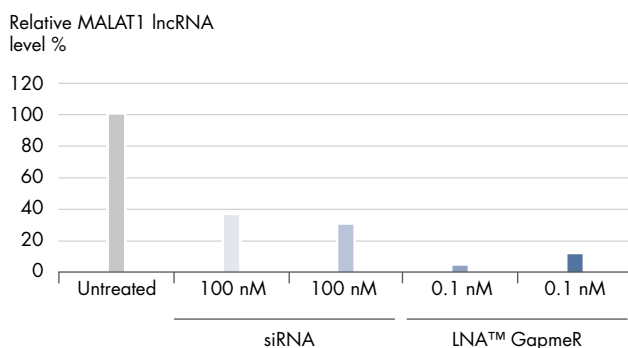


Figure 4. Far superior to siRNA for knockdown of nuclear lncRNA. siRNA and LNA GapmeRs targeting the highly abundant, nuclear retained, lncRNA, MALAT1 were transfected into HeLa cells. Knockdown was incomplete using 100 nM Silencer® Select siRNA, leaving approximately 35% MALAT1 RNA intact. In contrast, nearly complete knockdown of MALAT1 was achieved using just 0.1 nM LNA GapmeR.

Design your LNA GapmeRs at
www.qiagen.com/gapmers

You can also submit your RNA sequence and let us handle the design for you: www.qiagen.com/gapmers

No transfection reagent needed

Due to their small size and stability, LNA GapmeRs are taken up efficiently by cells either by transfection, or directly from the culture medium without transfection reagent. With many cell lines, potent knockdown of target RNA is achievable with unassisted delivery (Figure 5), avoiding potential confounding cytotoxic effects sometimes associated with transfection reagents.

Positive and Negative Controls

Experimentally validated and highly potent LNA GapmeRs are available for several mRNA and lncRNA targets, expressed in a broad range of cell types. Two negative control LNA GapmeRs are available with no homology to any known human, mouse or rat miRNA, lncRNA or mRNA sequence. These are ideal controls for optimization of delivery conditions.

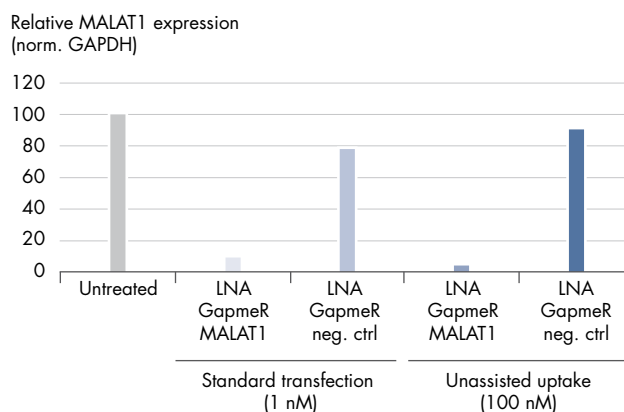


Figure 5. Efficient uptake without transfection reagents. LNA GapmeRs can be delivered to cell lines by adding them directly to the culture medium by unassisted delivery without transfection reagents – gymnosis. This can be useful with hard-to-transfect cell lines and for avoiding experimental artifacts introduced by transfection reagents.

Selected publications: Li et al. Nature 2013, 498(7455): 516-520; Lin, Nat Cell Biol. 2016; 18(2):213-24; Chu, Cell, 2017, 170, 86-101; Heilmann, Oncogene. 2017, 36(46):6446-6461; Miao, Nat Commun. 2018; 9(1):292; Ali, Nat Commun. 2018; 9(1):883

In vivo antisense LNA GapmeRs

In vivo Antisense LNA GapmeRs are powerful tools for RNA silencing in animal models and offer a promising platform for development of antisense drugs.

At a glance

- Enables gene silencing in live animal models
- Potent and long-lasting RNA silencing in a broad range of tissues
- Superior serum stability and broad biodistribution
- Efficient delivery in vivo without formulation
- Large-scale syntheses of highly purified antisense oligos

Gene silencing in animal models

Antisense LNA GapmeRs, enable the discovery of exciting RNA functions in live animals that could never have been uncovered through cell culture experiments. Effective knockdown of mRNA with LNA GapmeRs in organs that accumulate large amounts of oligonucleotide (such as liver and kidney) is well documented. Long-lasting and highly-potent knockdowns of abundant, nuclear-retained lncRNAs can be achieved in a broad range of tissues using LNA GapmeRs (Figure 6).

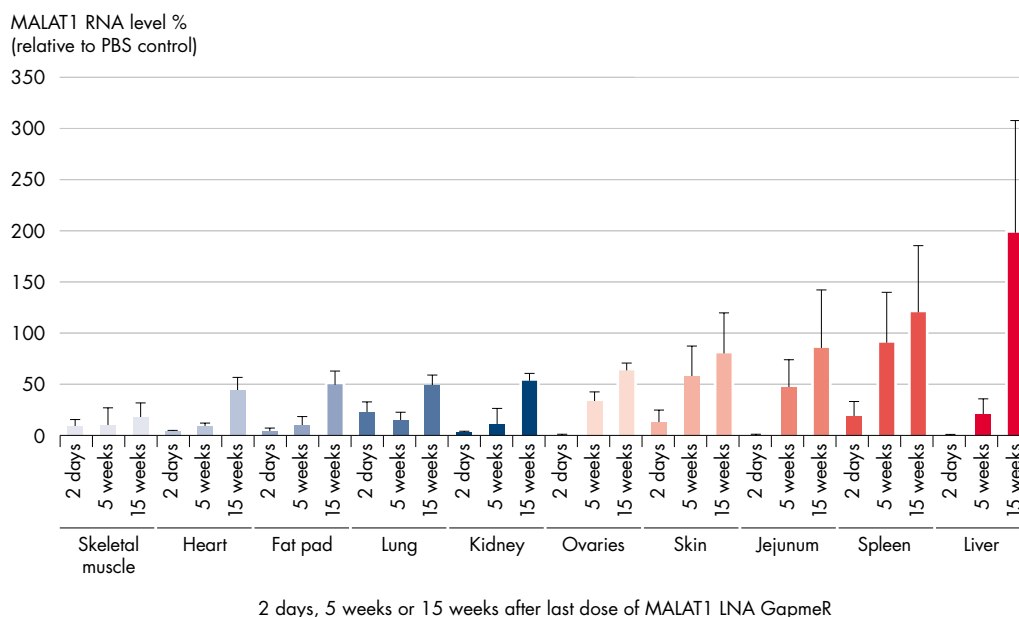


Figure 6. Efficient and long-lasting gene silencing in animals with LNA GapmeRs. Malat1 LNA GapmeRs were injected subcutaneously in mice over a period of 5 weeks. Biopsies were collected from treated animals, 2 days, 5 weeks and 15 weeks after the last GapmeR injection, and analyzed for Malat1 content. Almost complete knockdown was observed in a broad range of tissues two days after the last dose, remaining almost unchanged in most tissues 5 weeks later. Malat1 levels had recovered significantly in most tissues after 15 weeks.

Excellent pharmacokinetic and pharmacodynamic properties

In effective doses, potent Antisense LNA GapmeRs are generally well tolerated by animals with relatively few toxicity problems. LNA technology permits the design of short gapmers (15 -16 nt), which facilitate cell membrane penetration and efficient uptake in vivo while providing the necessary target affinity. Due to their phosphorothioate modified backbone, these highly stable oligonucleotides bind to albumin in the blood stream, ensuring broad tissue distribution. These are the secrets behind the excellent pharmacokinetic and pharmacodynamic properties of LNA GapmeRs.

Active in vivo without formulation

Multiple routes of administration are possible with gapmers. We recommend subcutaneous administration for simple delivery with minimal discomfort to animals. Simple reconstitution in PBS is sufficient, with no need for formulation in expensive nanoparticles, cholesterol conjugation, or use of lipid transfection reagents.

Product coverage

Use our intuitive online QIAGEN LNA design tool to design your LNA GapmeRs for human or mouse studies or let QIAGEN's LNA experts design custom GapmeRs ideally suited for any special project or requirements. RNA silencing is significantly more challenging to achieve in animals than in cell culture. Identification of highly potent GapmeRs is crucial to the successful outcome of animal experiments. Screening of 10 GapmeRs by unassisted uptake in cell cultures representative of the target tissue is recommended when progressing from cell culture experiments to animal models. Antisense LNA GapmeRs in vivo Large Scale are highly purified for safe use in animals. They are available in amounts ranging from 5 mg to kg scale and when required, we can provide gapmeRs in the quality required for pre-clinical studies.

The high potency and excellent PK/PD properties of LNA oligonucleotides in vivo are well demonstrated by the large number of high impact publications using LNA oligonucleotides for in vivo miRNA inhibition.

See page 18

Selected publications: Xing et al. Cell 2014, 159: 1110-1125. Viereck et al. Sci Transl Med. 2016, 8(326):326ra22. Adriens et al. Nat Med. 2016, 22(8):861-868. Lin et al. Nat Cell Biol. 2017. Mar;19(3):238-251

To learn more about Antisense LNA GapmeRs, please visit:
www.qiagen.com/gapmers

miRNA inhibitors and power inhibitors

Highly potent, LNA-enhanced, miRNA antisense inhibitors that work by transfection or unassisted delivery.

At a glance

- Fast, easy and cost-effective solution for miRNA inhibition
- T_m normalized inhibitors with unmatched potency against all miRNAs, regardless of GC content
- Power inhibitors so potent, they work by unassisted delivery, without the need for transfection reagents
- Superior specificity and biological stability for long-lasting, antisense activity
- 1 nmol, 5 nmol and 15 nmol quantities
- Fluorescent labels available for convenient monitoring of transfection efficiency
- Libraries for high-throughput screening
- Specially designed family inhibitors

Potent miRNA inhibitors and power inhibitors

QIAGEN's miRCURY LNA miRNA Inhibitors are ideal specific suppressors of miRNA activity – use them to determine the role of miRNAs in cellular processes and pathological pathways or for identification and validation of miRNA targets.

All miRNA inhibitors were developed using an advanced design algorithm that identifies the optimal combination of length, sequence and LNA positioning. We have exploited the high affinity properties of LNA chemistry to create T_m normalized inhibitors, that ensure high, uniform potency towards all miRNAs regardless of GC content, (Figure 7) combined with excellent specificity and biological stability.

An added benefit of the inhibitor design is that LNA bases are distributed throughout the entire length, ensuring LNA inhibitor / RNA duplexes are not recognized as substrates for RNase H; this results in minimal off-target effects on unrelated, longer RNAs that share the same target sequence.

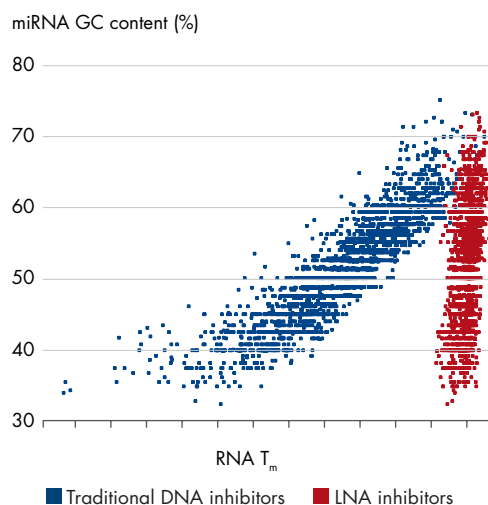


Figure 7. LNA miRNA inhibitors with uniform high potency. T_m normalized LNA miRNA inhibitors are effective with all miRNAs regardless of GC content. Each dot represents an individual inhibitor in which the T_m is shown as a function of the GC content of the miRNA target. Blue dots correspond to full length inhibitors with classical nucleotide chemistry. Red dots correspond to our new LNA miRNA inhibitors. The affinity of traditional, full-length miRNA inhibitors is highly influenced by the GC content and their T_m values span $>40^{\circ}\text{C}$. In contrast, the T_m of miRCURY LNA miRNA inhibitors are all focused within a 10°C interval around an optimal high temperature, ensuring uniform high potency.

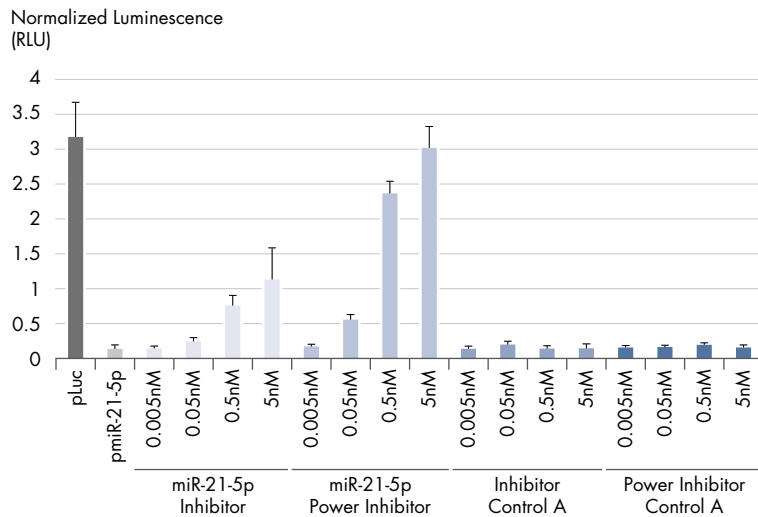


Figure 8. Enhanced potency of miRCURY LNA miRNA Power Inhibitors. Cells were transfected with a plasmid containing a firefly luciferase reporter gene with a miRNA target sequence in the 3'UTR. Firefly luciferase is suppressed by endogenous miRNA. Cells were subsequently transfected with regular and Power miRNA inhibitors and negative controls. miRNA inhibition results in increased firefly luciferase signal. Results illustrate the higher potency of Power inhibitors.

Product coverage

We offer pre-designed inhibitors according to their annotation in miRBase, and custom designed inhibitors.

- **miRCURY LNA miRNA inhibitors**
Efficient inhibitors with normal phosphodiester backbone and subnanomolar potency for in vitro transfection experiments (Figure 8).
- **miRCURY LNA miRNA Power inhibitors**
Fully phosphorothioate (PS) modified backbones dramatically improve stability against enzymatic degradation. The efficacy of these inhibitors is significantly higher than our regular inhibitors (Figure 8). Increased stability and potency allows direct addition to the

cell culture medium without the need for transfection reagents (Figure 9). Power inhibitors are especially useful for difficult applications, i.e. hard-to-transfect cells, highly expressed miRNA targets, long duration experiments and when normal transfection procedures have unacceptable phenotypic consequences.

- **Custom miRCURY LNA Inhibitors and Power Inhibitors**
If your choice of miRNA inhibitor is not available among the pre-designed products, QIAGEN will design it for you. We also provide inhibitors with a range of chemical modifications and different types of purification.
- **miRCURY LNA miRNA Inhibitor Libraries**

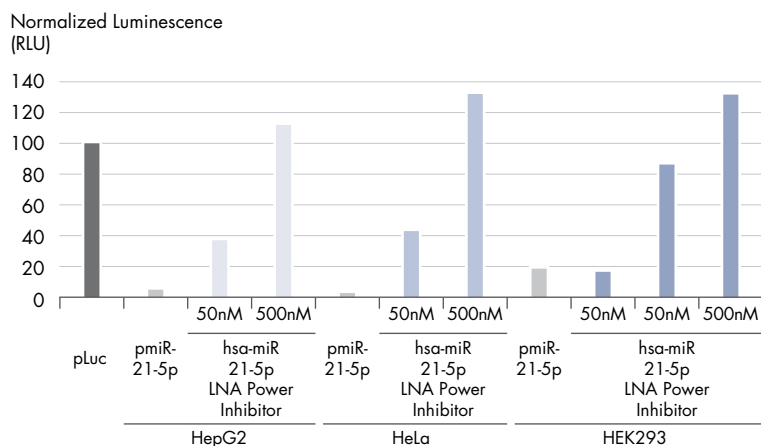


Figure 9. Efficient, unassisted delivery of power inhibitors without transfection reagents. miRCURY LNA Power Inhibitors were added directly to cells in serum containing culture medium, with no transfection reagent used. After 48 hours, efficient miRNA inhibition was observed in three different, commonly used cell types.

miRNA inhibitor libraries

miRCURY LNA miRNA Inhibitor Libraries enable convenient high-throughput screening of mouse and human miRNA function. The libraries are based on our renowned T_m -normalized miRCURY LNA miRNA Inhibitors with phosphodiester backbone.

The inhibitor libraries offer coverage of key miRNAs listed in miRBase v. 20. A number of miRNAs have been excluded for which there is either no or very limited direct experimental evidence. This significantly reduces the cost of screening, and time wasted on potentially false-positive results with very little impact on the true coverage of the screen.

Inhibitor coverage of the libraries:

Human library: 1,972 inhibitors of human miRNAs **Mouse library:** 1,624 inhibitors of mouse miRNAs

Plate layout

Inhibitor libraries are provided in 96-well plates. The plates are organized with empty outer rows and columns, facilitating pipetting and avoiding edge effects due to culture medium evaporation (Figure 10).

The inhibitors are positioned in the plates according to the amount of supporting scientific data; this enables smarter screening workflows with a subset of the plates containing inhibitors of the best validated miRNAs without the need for laborious pipetting and reformatting of the library.

	1	2	3	4	5	6	7	8	9	10	11	12
A	Empty	Empty	Empty	Empty	Empty	Empty	Empty	Empty	Empty	Empty	Empty	Empty
B	Empty	Empty	Positive control	hsa-miR-1233-3p	hsa-miR-641	hsa-miR-1204	hsa-miR-1468-5p	hsa-miR-647	hsa-miR-200b-5p	hsa-miR-302c-5p	hsa-miR-1185-5p	Empty
C	Empty	hsa-miR-944	hsa-miR-659-3p	hsa-miR-520f-3p	hsa-miR-1225-3p	hsa-miR-524-5p	hsa-miR-518a-3p	hsa-miR-1286	hsa-miR-3065-3p	hsa-miR-92b-5p	hsa-miR-380-3p	Empty
D	Empty	hsa-miR-569	hsa-miR-20b-3p	hsa-miR-488-5p	hsa-miR-2110	hsa-miR-487a-3p	hsa-miR-1293	hsa-miR-614	hsa-miR-628-3p	hsa-miR-508-5p	hsa-miR-513b-5p	Empty
E	Empty	hsa-miR-412-3p	hsa-miR-938	hsa-miR-501-3p	hsa-miR-548b-3p	hsa-miR-514a-3p	hsa-miR-101-5p	hsa-miR-365a-5p	hsa-miR-1247-5p	hsa-miR-1226-3p	hsa-miR-633	Empty
F	Empty	hsa-miR-575	hsa-miR-890	hsa-miR-1471	hsa-miR-526b-5p	hsa-miR-1255b-5p	hsa-miR-452-5p	hsa-miR-1226-5p	hsa-miR-548d-3p	hsa-miR-1284	hsa-miR-520d-3p	Empty
G	Empty	hsa-miR-1184	hsa-miR-613	hsa-miR-198	hsa-miR-933	hsa-miR-431-3p	hsa-miR-138-1-3p	hsa-miR-617	hsa-miR-523-3p	hsa-miR-1273c	hsa-miR-548p	Empty
H	Empty	Empty	Empty	Empty	Empty	Empty	Empty	Empty	Empty	Empty	Empty	Empty

Figure 10. Example of a miRCURY LNA miRNA Inhibitor Library plate. This is the layout of plate 10 of the human miRNA inhibitor library. Well B2 is left empty for a control oligonucleotide. A positive transfection control is provided in well B3.

Selected publications: 8: 1614, Poleskaya et al. PLoS One 2013, 8:e71927; Edelstein et al. Nat Med. 2013, 19:1609-16; McFarland et al. Nat Immunol. 2014, 15:72-9; Cardenas et al. PLoS Genet 2013, 9:e1003291; El Helou, Cell Reports, 2017, 18, 2256–2268

For additional product information, please visit: www.qiagen.com/mirna-inhibitors

miRNA mimics

Sophisticated, high-quality mimics designed to simulate naturally-occurring, mature miRNAs in functional analysis studies. A unique, LNA-enhanced, triple-RNA strand design ensures excellent specificity with no off-target effects from the passenger strand.

At a glance

- Third generation, highly potent, mature miRNA mimics with unique, triple-RNA strand design
- 100% strand specific. No off-target miRNA activity from the segmented, LNA-enhanced passenger strand.
- No chemical modification of the miRNA (guide) strand
- miRNA strand sequence according to the annotation in miRBase
- Available in 5 and 20 nmol quantities
- Comprehensive product offering – fluorescently labeled and biotinylated mimics are also available

Product coverage

miRCURY LNA miRNA Mimics have been pre-designed for most human, mouse and rat miRNAs listed in miRBase. Since many miRNAs are phylogenetically conserved, our miRNA mimics cover a large proportion of vertebrate and invertebrate miRNAs.

Fluorescently and biotin labeled mimics, as well as, negative control mimics are available.

miRCURY LNA miRNA Mimics are delivered desalted and desiccated. Once dissolved, the mimics are ready for standard transfection or electroporation.

A unique, triple-RNA strand design

miRCURY LNA miRNA Mimics are uniquely based on 3, rather than 2 RNA strands (Figure 11). The miRNA (guide) strand is an unmodified RNA strand with a sequence corresponding exactly to the annotation in miRBase.

The passenger strand is divided into two, LNA-enhanced, RNA strands, ensuring that only the miRNA strand gets loaded into the RNA-induced silencing complex (RISC), (Figure 12). Off-target effects from incorporation of the passenger strand in RISC can be a serious problem with traditional miRNA mimics.

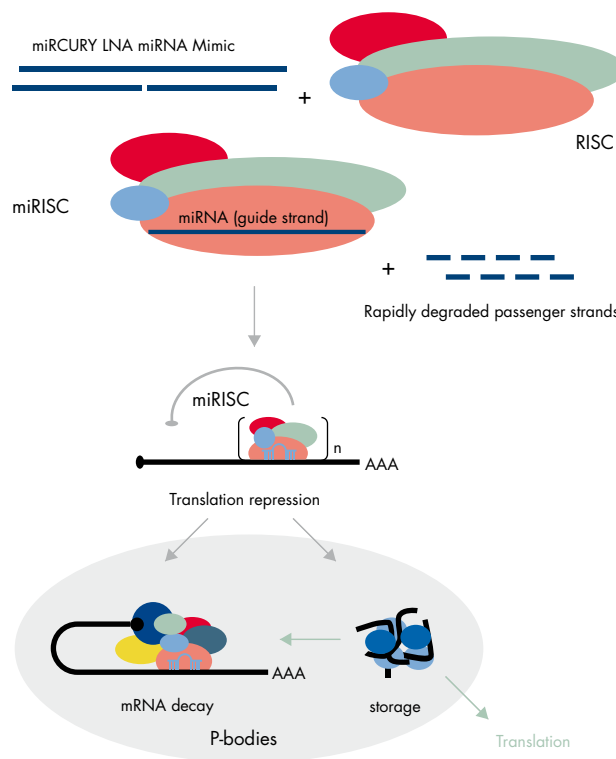


Figure 11. Unique, triple-RNA strand design ensures complete specificity. Only the miRNA strand is incorporated by RISC. The two passenger strands are too short to act as miRNAs and are rapidly degraded after displacement from the miRNA strand. Off-target effects from the passenger strands are therefore minimal with miRCURY LNA miRNA Mimics.

Applications

miRNA mimics simulate the natural functions of endogenous miRNAs and are primarily used in gain-of-function studies, to assess the biological consequences of increasing miRNA activity. The effect of increasing the cellular content of a miRNA (using miRNA mimics) can be studied using cellular assays to monitor cell proliferation, cell differentiation, or apoptosis; gene expression can also be measured at the mRNA or protein level of putative miRNA targets.

miRNA mimics are also frequently used for validating miRNA targets in combination with miRNA inhibitors and target site blockers (Figure 16).

Excellent potency

To achieve as accurate miRNA mimicry as possible, the miRNA strand of miRCURY LNA miRNA Mimics is unmodified RNA. The sequence and LNA spiking pattern of the two complementary passenger strands have been carefully designed to optimize efficient incorporation of the miRNA (guide) strand into RISC.

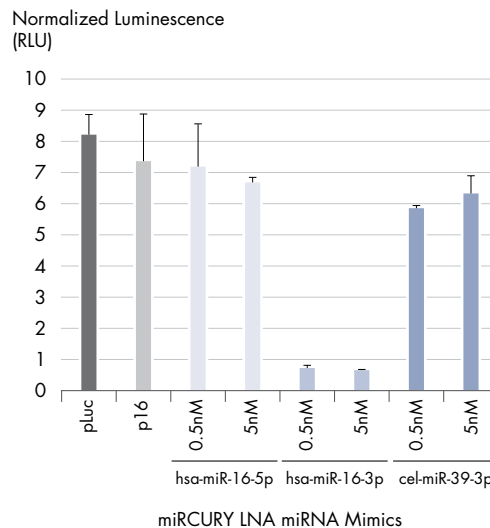


Figure 12. Perfect, miRNA strand-specific activity. Cells harboring hsa-miR-16-3p (above) and hsa-miR-16-5p (not displayed) luciferase reporter plasmids respectively were transfected with hsa-miR-16-3p and hsa-miR-16-5p, miRCURY LNA miRNA Mimics and a negative mimic control. The results demonstrate that suppression of luciferase activity is only achieved with the miRCURY LNA miRNA Mimic corresponding to the reporter assay.

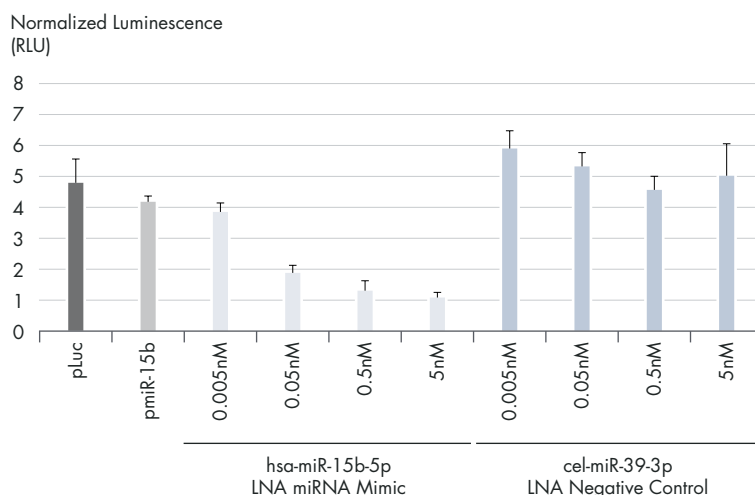


Figure 13. miRCURY LNA miRNA Mimics display sub-nanomolar potency. Using a firefly luciferase reporter assay, HeLa cells were transfected with different concentrations of LNA miRNA mimic or a cel-miR-39-3p negative control mimic. The results illustrate that LNA miRNA Mimics display sub-nanomolar potency under optimal transfection conditions.

Biotinylated mimics for pull-down experiments

Biotinylated LNA miRNA mimics are highly effective tools for identifying miRNA targets in RNA pull-down experiments (Figure 14). Recent advances with this experimental approach have revealed that non-canonical miRNA–mRNA interactions (ignored by target prediction tools) are frequent and lead to target repression.

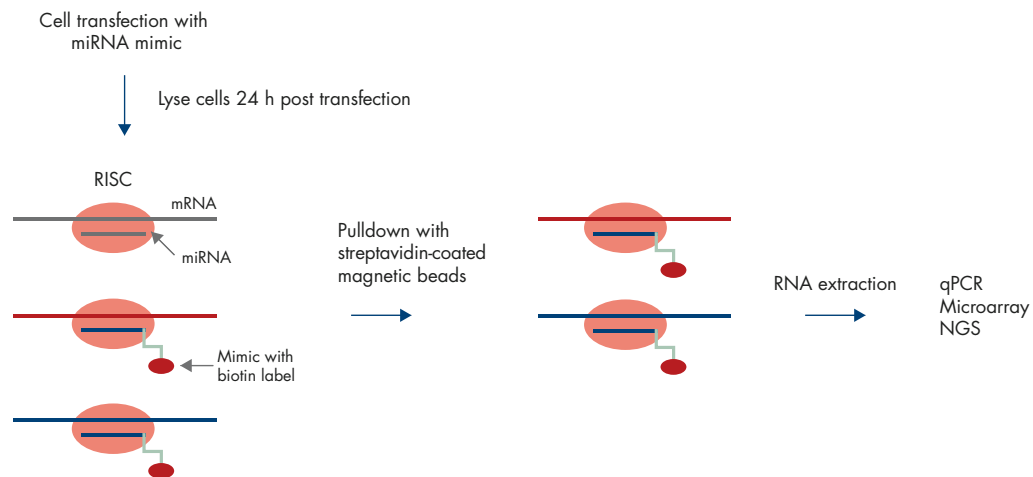
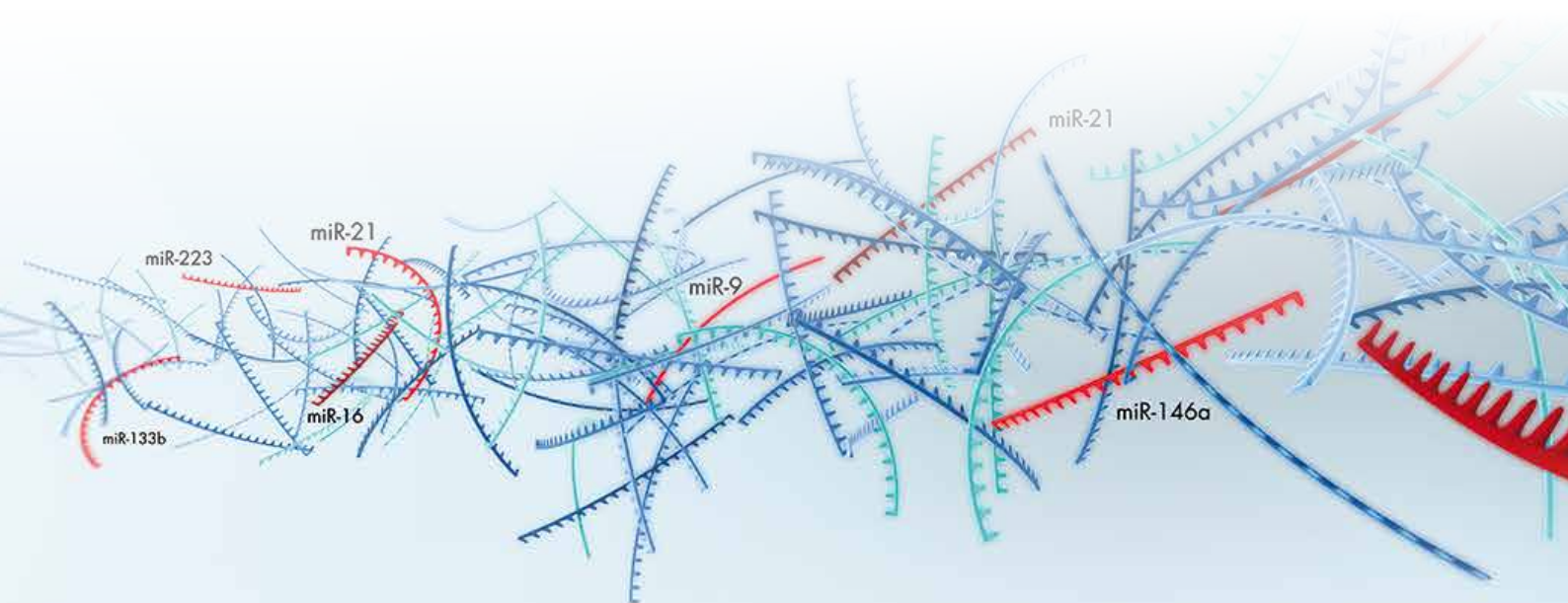


Figure 14. miRNA target identification with biotinylated miRCURY LNA miRNA Mimics.

miRCURY LNA miRNA Mimics

Uniquely specific miRNA mimicry: www.qiagen.com/mirna-mimics



miRNA target site blockers

Target site blockers (TSB) effectively block the interaction of a miRNA with a specific target site. TSBs are valuable tools for investigating the contribution of an individual target to an observed phenotype.

At a glance

- Target site blockers enable detailed study of which miRNA-mRNA interactions are important for miRNA function
- A target site blocker stimulates translation of a specific mRNA by masking a miRNA binding site
- A target site blocker hybridizes with a miRNA binding site on a specific mRNA (or non-coding RNA) preventing the miRNA from interacting with the site, without inhibiting the miRNA itself
- Sophisticated and innovative custom design
- Unmatched efficiency in vivo and in vitro

Target site blockers (TSB)

Cellular responses to manipulation with miRNA inhibitors and mimics can be challenging to interpret, as miRNAs typically regulate expression of many genes. Identifying the targets that contribute most significantly to an observed phenotype is important for understanding miRNA function.

The contribution of an individual target to the obtained phenotype can be investigated with a target site blocker (TSB) – an antisense oligonucleotide that specifically prevents interaction of a miRNA with one of its RNA targets. A TSB allows researchers to study the effects of a miRNA on a single target.

The TSB is designed to mask the miRNA target site in the RNA target of interest, without affecting the activity of the miRNA. As a result, the TSB will achieve specific de-repression of a single intended target only, enabling simple phenotypic interpretation (Figure 15).

The TSB mode of action is illustrated in Figure 15. TSBs can be used effectively in combination with miRNA inhibitors and mimics (see Figure 16).

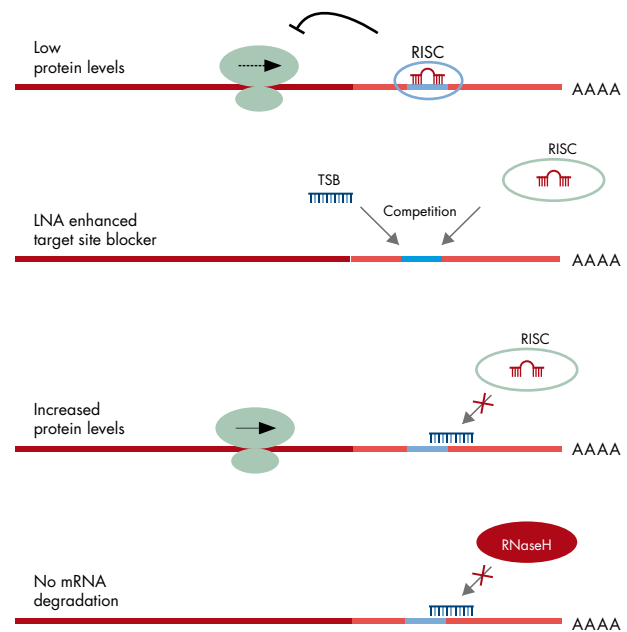


Figure 15. Target site blocker (TSB) mode of action. A target site blocker (TSB) stimulates translation of a specific mRNA by masking the miRNA binding site. The TSB will compete effectively with miRNA-RISC for the miRNA target site. In addition, LNA distribution throughout the LNA-DNA mixmer ensures that the antisense oligonucleotide does not catalyze RNase H-dependent degradation of the mRNA. As a result, the TSB will cause increased production of the protein encoded by the targeted mRNA by preventing miRNA mediated translational attenuation.

Product coverage

miRCURY LNA miRNA Target Site Blockers are available in different purity grades depending on application. They can be used for in vitro experiments, or for in vivo models (see page 17). miRCURY LNA miRNA Target Site Blockers are LNA-enhanced and have a phosphorothioate modified backbone for maximum potency.

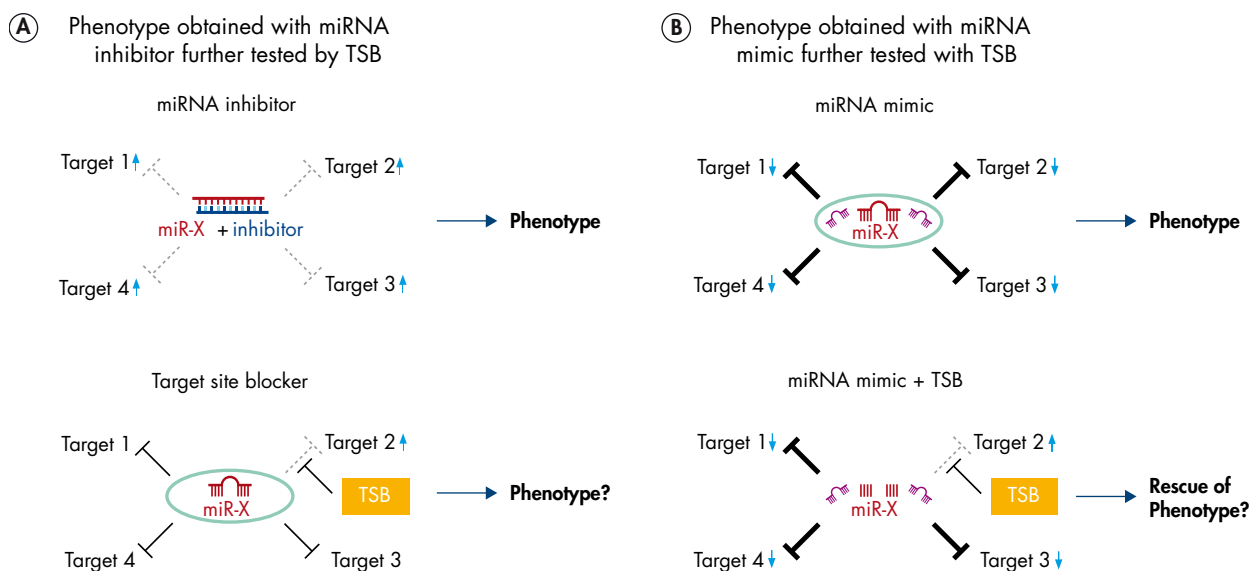


Figure 16. Unravel miRNA function with LNA Tools. Combined use of TSB and miRNA inhibitors and mimics: (A) An interesting phenotype is observed with a miRNA inhibitor. miRNA inhibition leads to increased translation of multiple mRNAs. Question: Which upregulated genes are responsible for the phenotype? This question can be answered by testing a TSB for one mRNA suspect at a time. TSBs that phenocopy the miRNA inhibitor identify important miRNA targets. (B) An interesting phenotype is observed with a miRNA mimic. The increased miRNA activity suppresses translation of multiple mRNAs. Question: Which downregulated genes are responsible for the phenotype? This question can be answered by co-transfecting TSBs of mRNA suspects with the miRNA mimic. TSBs that rescue the phenotype identify important miRNA targets.

Selected publications: Dajas-Bailador et al., Nat Neurosci 2012, 15: 697-69; Cardenas et al., PLoS Genet 2013, 9: e1003291; Viart et al., Eur Respir J 2015, 45: 116-28; Ortega et al., Leukemia 2014, 29: 968-976

miRCURY LNA miRNA Target Site Blockers identify important miRNA targets
www.qiagen.com/mirna-TSB

In vivo miRNA function

Custom miRCURY LNA In vivo miRNA inhibitors and Target Site Blockers are quietly revolutionizing the miRNA field by enabling functional analysis in animal models.

At a glance

- Potent inhibition of miRNAs in a broad range of tissues
- Identification of miRNA targets in vivo
- Enables the discovery of surprising miRNA functions in live animals
- Custom designed and highly purified
- Superior serum stability and nuclease resistance

Well-documented inhibition of miRNAs in vivo

Effective miRNA inhibition has been achieved in multiple organs and tissues by systemic and local administration of custom designed LNA in vivo miRNA inhibitors (see Figure 17). As a result, surprising discoveries about miRNA function have been made that could not have been achieved by cell culture experiments. Successful phase 2, human trials with an LNA miR-122 inhibitor for treatment of HCV infections, is a testimony to the unique, drug-like properties of these short, antisense molecules (Janssen et al, N Engl J Med. 2013, 368(18):1685-94).

In vivo identification of miRNA targets

miRNAs are typically involved in the regulation of large numbers of genes. miRCURY LNA miRNA Power Target Site Blockers, in vivo Ready, mask the miRNA binding site in a particular RNA target of interest (Figure 15), allowing researchers to inhibit the interaction of a miRNA on a single target, and thereby identify the most important miRNA targets in vivo.

Product description

LNA In vivo miRNA Inhibitors and Target Site Blockers (TSBs) are highly purified, custom designed, and optimized for in vivo use. We harness LNA technology to design short (14-16mer) oligonucleotides with high binding affinity, ensuring high potency. The short length and the fully modified phosphorothioate (PS) backbone, serve to optimize the pharmacokinetic and pharmacodynamics properties, enhance serum and nuclease stability, and minimize toxicity.

The LNA in vivo inhibitors and TSBs are available in amounts ranging from 5 mg to kg scales; they can also be delivered in the quantity and quality required for preclinical toxicity studies.

Figure 17. In vivo inhibition of miRNA in a variety of tissues using in vivo LNA miRNA inhibitors

miRNA	Tissue	Organism	Process/Disease	Reference
miR-802	Liver	Mouse	Obesity induced impaired insulin signaling	Kornfeld <i>et al.</i> Nature 2013
miR-142-3p	Spleen dendritic cells	Mouse	Endotoxemia	Sun <i>et al.</i> Blood 2011
miR-192	Kidney	Mouse	Diabetic nephropathy	Putta <i>et al.</i> J Am Soc Nephrol 2012
miR-212	Brain	Rat	Cocaine addiction	Hollander <i>et al.</i> Nature 2010
miR-21-5p	Lung	Mouse	Lung fibrosis	Liu <i>et al.</i> J Exp Med 2010
miR-34a	Heart	Mouse	Cardiac aging	Boon <i>et al.</i> Nature 2013
miR-21-5p	Bone marrow – hemapoietic stem cells	Mouse	Myelodysplastic syndromes	Bhagat <i>et al.</i> Blood 2013
miR-199a, miR-1908	Human melanoma	Mouse	Melanoma metastasis	Pencheva <i>et al.</i> Cell 2012
miR-712	Endothelium	Mouse	Artherosclerosis	Son <i>et al.</i> Nature Commun 2013
miR-21-5p	White adipose tissue – heart	Mouse	Obesity	Seeger <i>et al.</i> Obesity 2014
miR-33	Choroidal macrophages of the eye	Mouse	Age related macular degeneration	Sene <i>et al.</i> Cell Metabolism 2013
miR-142-3p	T-cells	Mouse	Graft-versus-host disease	Sun <i>et al.</i> JCI 2015
miR-29	Liver (skeletal muscle)	Mouse	Cholesterol and fatty acid synthesis	Kurtz <i>et al.</i> Sci Rep 2015
miR-21-5p	Pancreas	Mouse	Pancreatitis	Ma <i>et al.</i> Nature Commun 2015

miRNAs are potential therapeutic targets

Publications using in vivo inhibition of miRNAs indicate that miRNAs have great potential as therapeutic targets for important diseases such as cancer, fibrosis, cardiovascular and heart disease and metabolic disorders (see Figure 17).

Learn more: www.qiagen.com/mirna-inhibitors

Selected publications with miRCURY LNA In vivo miRNA inhibitors: Kornfeld *et al.* Nature 2013, 494: 111-5; Boon *et al.* Nature 2013, 495: 107-10; Sene *et al.* Cell Metab. 2013, 17:549-61; Son *et al.* Nature Communications 2013, 4:3000; Seeger *et al.* Obesity 2014, 22:2352-60; Sassi, Nat Commun. 2017;

Selected publications with miRCURY LNA in vivo Power Target Site Blockers: Messina, Nat Neurosci. 2016, 19(6):835-44; Lippi, Neuron 2016, 92, 1337–1351; Gilot, Nat Cell Biol.; 2017, 19(11):1348-1357; Sonnevile, Nat Commun. 2017; 8(1):710

Ordering Information

Product	Contents	Cat. no.
Antisense LNA GapmeR Standard (5 nmol; 15 nmol)	5 nmol / 15 nmol Custom Antisense LNA GapmeR, Standard in vitro screening grade, provided in tube format	339511; 339512
Antisense LNA GapmeR Premium (5 nmol; 15 nmol)	5 nmol / 15 nmol Custom Antisense LNA GapmeR, Premium cell-culture grade, provided in tube format	339517; 339518
Antisense LNA GapmeR in vivo Ready (5 nmol; 15 nmol)	5 nmol / 15 nmol Custom Antisense LNA GapmeR, in vivo Ready, provided in tube format	339523; 339524
Antisense LNA GapmeR in vivo Large Scale	Custom Antisense LNA GapmeR in vivo Large Scale, purified by HPLC and Na ⁺ salt exchange, available in amounts between 5 mg – 1 kg	339532
Antisense LNA GapmeR Controls (Positive and Negative)	Custom purification, modification and quantity of any of the following controls: Negative control A, Negative control B, MALAT1 (human), GADPH (human), PTEN (human), MALAT1 (mouse), MTOR (mouse)	339515; 339516
miRCURY LNA miRNA Inhibitors (1 nmol; 5 nmol; 15 nmol)	1 nmol / 5 nmol / 15 nmol miRCURY LNA miRNA Inhibitors with normal phosphodiester nucleotide bonds and option of FAM label, provided in tube format	339120; 339121; 339122
miRCURY LNA miRNA Power Inhibitors (1 nmol; 5 nmol; 15 nmol)	1 nmol / 5 nmol / 15 nmol miRCURY LNA miRNA Power Inhibitors with a fully phosphorothioate-modified backbone and option of FAM label, provided in tube format	339130; 339131; 339132
Custom miRCURY LNA miRNA Inhibitors (1 nmol; 5 nmol; 15 nmol)	1 nmol / 5 nmol / 15 nmol Custom miRCURY LNA miRNA Inhibitors with normal phosphodiester nucleotide bonds; option of different purification grades and label modifications; provided in tube format	339140; 339141; 339142
Custom miRCURY LNA miRNA Power Inhibitors (1 nmol; 5 nmol; 15 nmol)	1 nmol / 5 nmol / 15 nmol Custom miRCURY LNA miRNA Power Inhibitors with a fully phosphorothioate-modified backbone; option of different purification grades and label modifications; provided in tube format	339145; 339146; 339147
Custom miRCURY LNA miRNA Inhibitors in vivo Large Scale	Custom miRCURY LNA miRNA Inhibitors in vivo Large Scale, purified by HPLC and Na ⁺ salt exchange, available in amounts between 5 mg – 1 kg	339203
Negative control A	miRCURY LNA miRNA Inhibitor Control	YI00199006
Negative control B	miRCURY LNA miRNA Inhibitor Control	YI00199007
miRCURY LNA miRNA Power Family Inhibitors	Predesigned sets of miRCURY LNA miRNA Inhibitors for miRNA families	339160
Human miRCURY LNA miRNA Inhibitor Library (0.125 nmol; 0.25 nmol)	0.125 nmol / 0.25 nmol miRCURY LNA miRNA Inhibitor Libraries, provided in 96-well plate format	339168; 339169

Ordering Information

Product	Contents	Cat. no.
Mouse miRCURY LNA miRNA Inhibitor Library (0.125 nmol; 0.25 nmol)	0.125 nmol / 0.25 nmol miRCURY LNA miRNA Inhibitor Libraries, provided in 96-well plate format	339168; 339169
miRCURY LNA miRNA Family Power Inhibitor Library (0.125 nmol; 0.25 nmol)	0.125 nmol / 0.25 nmol miRCURY LNA miRNA Family Power Inhibitor Libraries, provided in 96-well plate format	339165; 339166
miRCURY LNA miRNA Mimic (5 nmol; 20 nmol)	5 nmol / 20 nmol miRCURY LNA miRNA Mimics with option of FAM label, provided in tubes	339173; 339174
miRCURY LNA Premium miRNA Mimic (5 nmol; 20 nmol)	5 nmol / 20 nmol miRCURY LNA Premium miRNA Mimics, HPLC purified, with biotin label, provided in tubes	339178; 339179
Negative Control miRCURY LNA miRNA Mimic	miRNA strand: UCACCGGGUGUAAAUCAGCUUG	YM00479902
Negative Control 4 miRCURY LNA miRNA Mimic	miRNA strand: GAUGGCAUUCGAUCAGUUCUA	YM00479903
Negative Control 5 miRCURY LNA miRNA Mimic	miRNA strand: GAUGCUACGGUCA AUGUCUAAG	YM00479904
miRCURY LNA miRNA Power Target Site Blockers (5 nmol; 15 nmol)	5 nmol / 15 nmol miRCURY LNA miRNA Power Target Site Blockers with option of different labels and purifications, provided in tube format	339194; 339195
miRCURY LNA miRNA Power Target Site Blockers, in vivo Ready (5 nmol; 15 nmol)	5 nmol / 15 nmol miRCURY LNA miRNA Power Target Site Blockers, in vivo Ready, with option of different labels and purifications, provided in tube format	339199; 339200
miRCURY LNA miRNA Power Target Site Blockers, in vivo Large Scale	miRCURY LNA miRNA Power Target Site Blockers in vivo Large Scale, purified by HPLC and Na ⁺ salt exchange, available in amounts between 5 mg – 1 kg	339201
HiPerFect Transfection Reagent (0.5 ml; 1 ml; 4 x 1 ml)	HiPerFect Transfection Reagent for up to 166 / 333 / 1332 transfections in 24-well plates or up to 666 / 1333 / 5333 transfections in 96-well plates	301704; 301705; 301707
HiPerFect Transfection Reagent (100 ml)	HiPerFect Transfection Reagent for transfections in up to 1388 96-well plates	301709

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