Thermo Scientific Open Biosystems GIPZ Lentiviral shRNAmir Library

- microRNA-adapted for increased and specific knockdown
- Whole genome human and mouse coverage
- Guaranteed Knockdown
expression arrest gipz lentiviral shRNAmir libraries

perform more specific, stable and efficient RNAi with thermo scientific open biosystems gipz lentiviral shRNAmir libraries. human and mouse genomes are targeted with multiple shRNAmir constructs that have been pre-cloned into the pGIPZ lentiviral vector. the result is a powerful shRNAmir resource offering these unique features:

- Efficient low copy knockdown
- TurboGFP marks shRNAmir expressing cells
- Transient, stable, and in vivo RNAi applications
- Lentiviral delivery extends RNAi to primary and non-dividing cells
- Guaranteed knockdown

shRNAmir design allows processing via the endogenous RNAi pathway resulting in more specific and guaranteed silencing.

A.) Endogenous miR-30 primary transcript.
B.) shRNAmir expressed from a miR-30 context. The mature miR-30 sequence has been replaced with a gene specific duplex. This design allows specific processing by Drosha and Dicer and active loading of the RISC complex.

shRNAmir design is based on the primary microRNA-30 (miR-30) transcript.

shRNAmir-GFP: Visualize knockdown

A: Phase image of HEK293 cells 48 hours post-transduction. B: the corresponding fluorescent image. 100% transduction efficiency is seen in these cells.

TurboGFP expression from the pGIPZ lentiviral vector effectively marks shRNAmir expression in target cells.

vector element utility

<table>
<thead>
<tr>
<th>Vector Element</th>
<th>Utility</th>
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<tbody>
<tr>
<td>CMV Promoter</td>
<td>RNA Polymerase II promoter</td>
</tr>
<tr>
<td>cPPT</td>
<td>Central polyuridine tract helps translocation into the nucleus of non-dividing cells</td>
</tr>
<tr>
<td>WRE</td>
<td>Enhances the stability and translation of transcripts</td>
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<tr>
<td>TurboGFP</td>
<td>Marker to track shRNAmir expression</td>
</tr>
<tr>
<td>Pro r</td>
<td>Mammalian selectable marker</td>
</tr>
<tr>
<td>Amp r</td>
<td>Ampicillin (carbenicillin) bacterial selectable marker</td>
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<tr>
<td>5’ LTR</td>
<td>5’ long terminal repeat</td>
</tr>
<tr>
<td>pUC ori</td>
<td>High copy replication and maintenance of plasmid in E. coli</td>
</tr>
<tr>
<td>SIN–LTR</td>
<td>3’ self-inactivating long terminal repeat (Shimada, et al. 1995)</td>
</tr>
<tr>
<td>Zeo resistance</td>
<td>Bacterial selectable marker</td>
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GIPZ Lentiviral shRNAmir: for specific and effective knockdown

The performance of GIPZ lentiviral shRNAmir was validated for knockdown efficiency and ability to produce known cellular phenotypes. For all data presented, GIPZ shRNAmir viral constructs were transduced into cell lines at low multiplicity of infection (MOI = 0.4 - 0.2). Knockdown was validated at both mRNA and protein level. Further, knockdown of the targeted genes with GIPZ shRNAmir produced known cellular phenotypes induced by small molecule inhibitors.

**Knockdown efficiency of GIPZ lentiviral shRNAmir**
On average, two out of three GIPZ lentiviral shRNAmir produced greater than 70% knockdown of mRNA expression when tested at low MOI.

**Phenotypic validation**
GIPZ lentiviral shRNAmir targeted genes whose knockdown produced documented phenotypes consistent with silencing of target genes associated with cell cycle progression and cancer.

Knockdown of AURKB, EG5 and ACTA1 genes with GIPZ lentiviral shRNAmir results in distinct phenotypes resulting from disrupting the cell cycle.

AURKB knockdown produced an endoreplication phenotype.

EG5 knockdown produced the expected preprophase 'stellate' phenotype and endoreplication.

ACTA1 knockdown produced the stereotypical multinucleated phenotype.

The phenotypes generated using various GIPZ shRNA matched very well with previous data using siRNA or small molecule described in the literature.
High-throughput screening with GIPZ lentiviral shRNAmir

GIPZ lentiviral shRNAmir targeting the entire human and mouse genome are available in individually arrayed and pooled library formats. Both formats can be used in high-throughput screening for discovery biology or to dissect components of a signaling pathway.

- Thermo Scientific Open Biosystems Decode RNAi viral screening libraries are pooled viral formats of Human GIPZ lentiviral shRNAmir, offers a convenient and cost-effective strategy for genome-wide RNAi screening.

Decode RNAi viral screening libraries

Perform whole genome or more focused screens using Decode RNAi viral screening libraries. Save time and money and reduce the labor associated with individually arrayed RNAi screens.

- Target the entire human genome or just highly characterized genes
- Ready-to-use high titer viral pool format
- Efficient single copy knockdown, essential for pooled screens
- Lentiviral delivery extends RNAi screening to primary, non-dividing and difficult to transfect cells
- Easily identify hits with barcode microarrays or shRNA sequencing

Decode RNAi viral screening libraries are available for the whole genome (7 pools of 10,000 constructs each) or for highly characterized annotated genes (3 pools of 10,000 constructs each). Each pool contains ~10,000 shRNAmir as viral particles at titers > 5x10^8 TU/ml. Positive and negative selection screening kits contain enough virus to perform at least two screens in triplicate depending on your target cell line.

Multiplexed Screening with Decode RNAi Viral Pools - From Screen to Hits in Weeks

Decode RNAi viral screening libraries can be used in positive and negative selection screening. A positive selection screen allows identification of a shRNA whose expression allows a cell to be isolated from a complex population of cells e.g. survival or selectable phenotype. Negative selection (dropout) screening identifies shRNA whose expression modulates the growth of cells and captures both positive and negative effects on the growth of a complex population of cells.
GIPZ lentiviral shRNAmir Product Overview

Thermo Scientific Open Biosystems GIPZ Lentiviral shRNAmir is available in a variety of formats for silencing single genes or the entire Human and Mouse genomes.

GIPZ lentiviral shRNAmir product formats

**Individual constructs & target gene sets**
- Glycerol stock format
- Viral particle format (1 x 10⁸ TU/ml)
- RNAintro shRNA Starter Kits

**Gene family/pathway sub-libraries (catalog & custom)**
- Arrayed Glycerol stock format
- Arrayed lentiviral particle format (1 x 10⁸ TU/ml)

**Whole genome library**
- Arrayed glycerol stock format
- Pooled high-titer lentiviral particle format (5x10⁸ TU/ml)

RNAintro Starter Kits

Thermo Scientific Open Biosystems RNAintro starter kits gene silencing are simple, complete, and guaranteed to work. All the tools necessary for efficient and guaranteed* knockdown are provided in one convenient package:

- Choice of five shRNAmir constructs
- Validated positive and negative controls
- Optimized shRNAmir delivery with Thermo Scientific Open Biosystems Arrest-In transfection reagent
- Thermo Scientific Open Biosystems Trans-Lentiviral packaging mix
- Transfection or transduction kits
- Guaranteed* knockdown

Save time, labor and cost of packaging lentiviral particles with ready-to-use, high-titer GIPZ lentiviral particles. High-quality, purified virus reduces toxicity and increases your chance of success. GIPZ lentiviral particles are provided as 2 x 25-µl aliquots of >10⁸ transducing units per ml. Purchase three constructs per gene to save over 50% and receive free positive and negative controls and guaranteed** knockdown.

* When used in GIPZ RNAintro kits according to kit instructions.
** One out of three constructs in lentiviral particle format is guaranteed to knockdown at greater than 70% at the mRNA level. Knockdown should be compared to the non-silencing control and the experimental workflow controlled using the GAPDH positive control (provided).

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<tr>
<th>Individual Constructs</th>
<th>Catalog</th>
<th>Gene family and pathway sub-libraries (not a complete list)</th>
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<td>RHS 4430</td>
<td>Human GIPZ cancer genes shRNAmir focused sub-library—Glycerol</td>
<td>RHS5141</td>
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<tr>
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<td>Human Angiogenesis GIPZ lentiviral shRNAmir gene family—Glycerol</td>
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<td>RNAintro GIPZ lentiviral shRNAmir transfection kit</td>
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<td>RNAintro GIPZ lentiviral shRNAmir transduction kit</td>
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<td>Human phosphatase GIPZ lentiviral shRNAmir gene family—Glycerol</td>
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Thermo Scientific Open Biosystems GIPZ
Lentiviral shRNAmir Library

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Euro Literature # PB 2009 47
US Literature # 005223-09-I-01-U

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