

# Functional Gene Analysis

Effective *RNA-Mediated* Gene Knockdown



Print product with financial  
**climate contribution**

[ClimatePartner.com/12885-2406-1003](https://ClimatePartner.com/12885-2406-1003)



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# Functional Gene Analysis

## Products in Each Section at a Glance

### 1 dsRNA Production in Your Hands

page 1

| Product                         | Quantity             | Cat. # |
|---------------------------------|----------------------|--------|
| T7 RiboMax™ Express RNAi System | 50 x 20 µl reactions | P1700  |

### 2 Gentle and Efficient Transfection of dsRNA

page 2

| Product                         | Quantity | Cat. # |
|---------------------------------|----------|--------|
| FuGENE® SI Transfection Reagent | 1 ml     | E9311  |
|                                 | 5 x 1 ml | E9312  |

### 3 Screen siRNA/miRNA Activity Levels

page 4

| Product                                              | Quantity | Cat. #    |
|------------------------------------------------------|----------|-----------|
| pmirNanoGLO Dual-Luciferase miRNA Target Exp. Vector | 20 µg    | CS194105* |
| pmirGLO Dual-Luciferase miRNA Target Exp. Vector     | 20 µg    | E1330     |
| FuGENE® HD Transfection Reagent                      | 1 ml     | E2311     |
| Dual-Glo® Luciferase Assay System                    | 10 ml    | E2920     |
| Nano-Glo® Dual-Luciferase® Reporter Assay System     | 10 ml    | N1610     |

### 4 Monitor Live Target Gene Knockdown

page 5

| Product                                        | Quantity | Cat. #           |
|------------------------------------------------|----------|------------------|
| Nano-Glo® Endurazine™ Live Cell Substrate      | 0.1 ml   | N2570            |
| Nano-Glo® Vivazine® Live Cell Substrate        | 0.1 ml   | N2580            |
| Nano-Glo® HiBiT Lytic Detection System         | 10 ml    | N3030            |
| Nano-Glo® HiBiT Extracellular Detection System | 10 ml    | N2420            |
| CellTiter-Fluor™ Cell Viability Assay          | 10 ml    | G6080            |
| CRISPR HiBiT Knock In Cell Lines               | 2 vials  | please enquire** |

\* This is an Early Access product.  
Please enquire for more information.

\*\*Download an up-to-date list of available  
CRISPR HiBiT Knock In Cell lines here:  
[www.promega.com/hibit-crispr-knock-in](http://www.promega.com/hibit-crispr-knock-in)



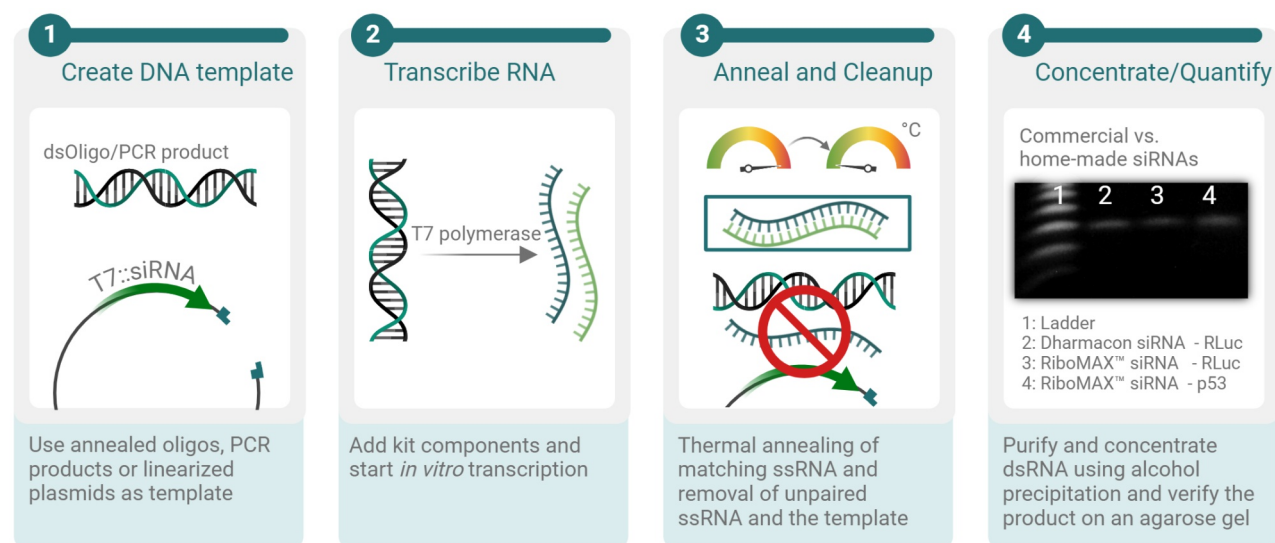
# dsRNA Production in Your Hands

## T7 RiboMAX™ Express RNAi System

The T7 RiboMAX™ Express RNAi System rapidly produces milligram amounts of double-stranded RNA (dsRNA) in just 30 minutes, streamlining RNA interference (RNAi) experiments in both mammalian and non-mammalian systems.

It synthesizes 21 bp short interfering RNAs (siRNAs) for use in mammalian cells and longer dsRNA (up to ~200 bp) for non-mammalian systems, starting from a T7 promoter-containing DNA template (either plasmid, PCR product, or dsOligo), followed by annealing and purification.

The system simplifies setup by combining rNTPs and transcription buffer, reducing pipetting errors and preparation time.



Do you need restriction enzymes, PCR reagents and more?

You'll find them in the **Genomic Essentials Catalog**

[www.promega.com/genomic-essentials-catalog](http://www.promega.com/genomic-essentials-catalog)



# GloMax® Detection Systems

## A Versatile, Reliable, and Intuitive Lab Companion

GloMax® Discover is an advanced multimode plate reader designed to provide optimal performance for Promega reagents with high-performance luminescence, fluorescence, UV-visible absorbance, BRET and FRET, two-color filtered luminescence, and kinetic measurement capabilities. GloMax® Discover can be used as a standalone plate reading instrument or integrated into high-throughput automated workflows. Results are easy to interpret using integrated data analysis software.

### One Instrument. Numerous Applications.

- Reporter Gene Assays
- Cell Viability, Cytotoxicity & Apoptosis Assays
- Kinetic Measurements
- Multiplexing
- Oxidative Stress & Cell Metabolism
- ELISA
- BRET/FRET Analysis
- Lumit® Immunoassays

## GloMax DISCOVER

A high-performance, easy-to-use multimode plate reader for luminescence, fluorescence, absorbance, BRET and FRET applications.

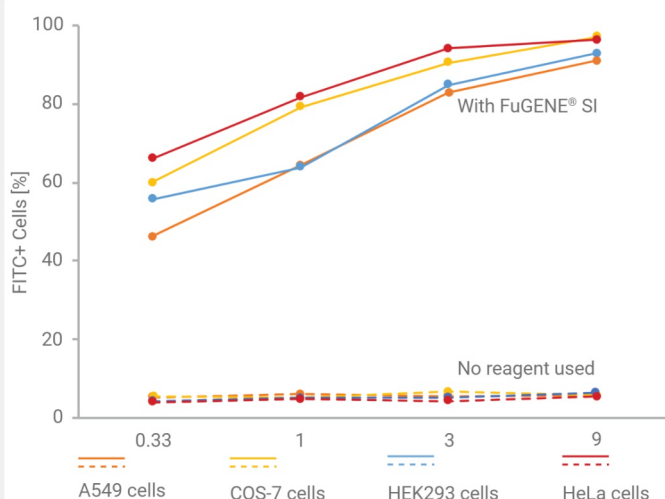


# Gentle and Efficient Transfection of dsRNA

## FuGENE® SI Transfection Reagent

The 100% synthetic FuGENE® SI Transfection Reagent is a high-efficiency, low-toxicity solution for delivering siRNA, miRNA, and other small RNA molecules into a wide range of eukaryotic cell lines.

This is particularly suitable for researchers seeking to achieve maximum knockdown efficiency with minimal cell disruption.



### FuGENE® SI provides efficient transfection of labeled small RNAs

A549, COS-7, HEK293 and HeLa cells plated in 96-well microplates were transfected with 0.4  $\mu$ l of FuGENE® SI or a no-reagent negative control and various amounts of FITC-labeled control siRNA.

24 h after transfection, the cells were analyzed via flow cytometry for FITC-positive signal.

As expected, the FITC signal is near zero when no reagent is used and is high when using FuGENE® SI in diverse cell lines.



**Boost Your Lab Efficiency with FuGENE® SI!**

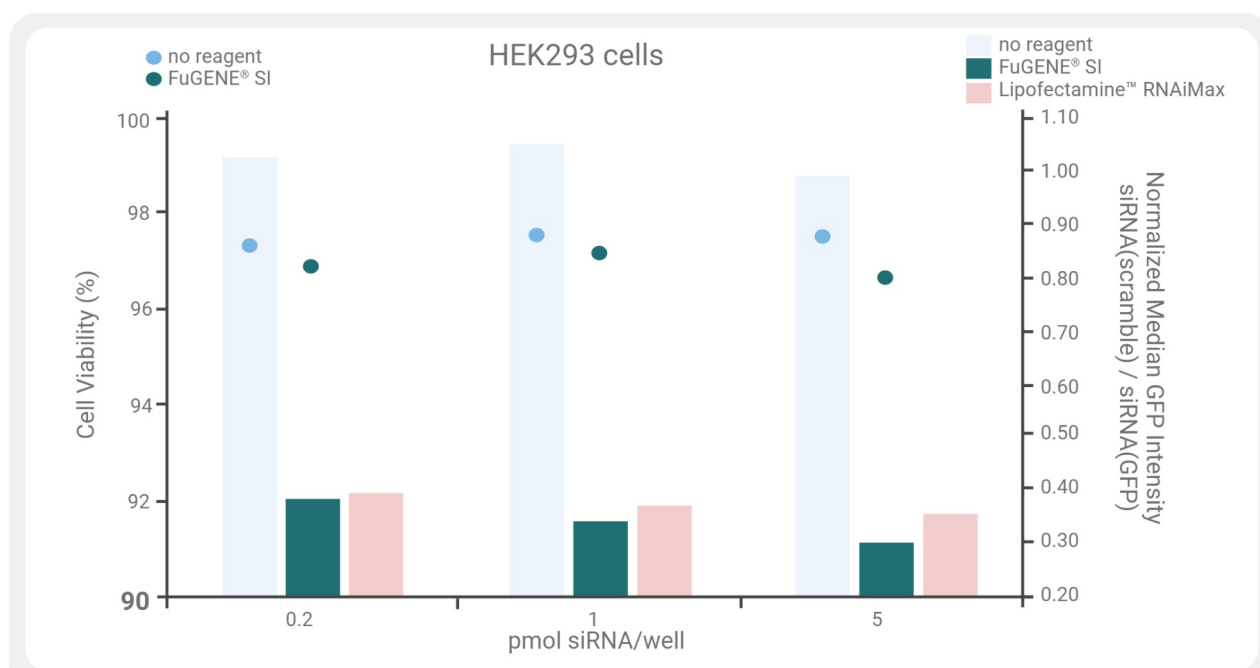
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# Proven Performance

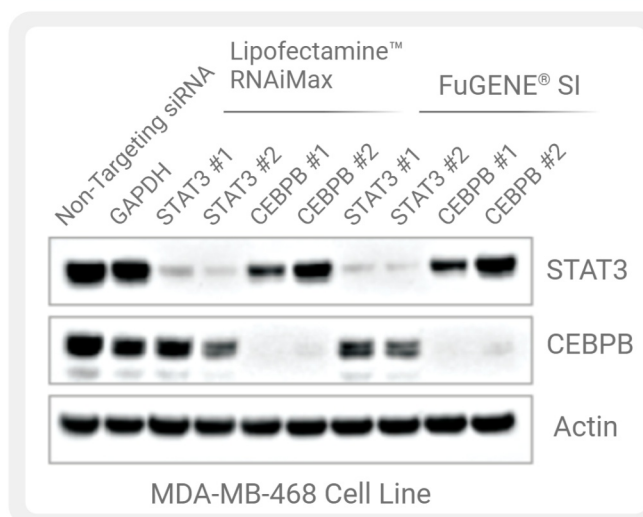
## FuGENE® SI Transfection Reagent



### Effective knockdown of exogenous reporter genes with FuGENE® SI with minimal impact on cell health

HEK293-GFP cells were seeded in a 96-well plate and transfected with siRNA against GFP or a scrambled control with either 0.3  $\mu$ l FuGENE® SI Transfection reagent or 0.3  $\mu$ l Lipofectamine™ RNAiMAX (Life Technologies). Cells were analyzed via flow cytometry 48 h after knockdown for GFP expression levels and cellular viability.

Please note the high knockdown efficiency and the low impact on cell health when using FuGENE® SI.



### Effective knockdown of endogenous target genes with FuGENE® SI

Human MDA-MB-468 cells were reverse-transfected in 6-well plates with 40 pmol of the indicated targeting siRNAs or negative/positive controls with 7.5  $\mu$ l of either FuGENE® SI Transfection Reagent or Lipofectamine™ RNAiMAX per well. The cells were analyzed 72 hours after transfection via Western blot for expression of target genes STAT3 and CEBPB.

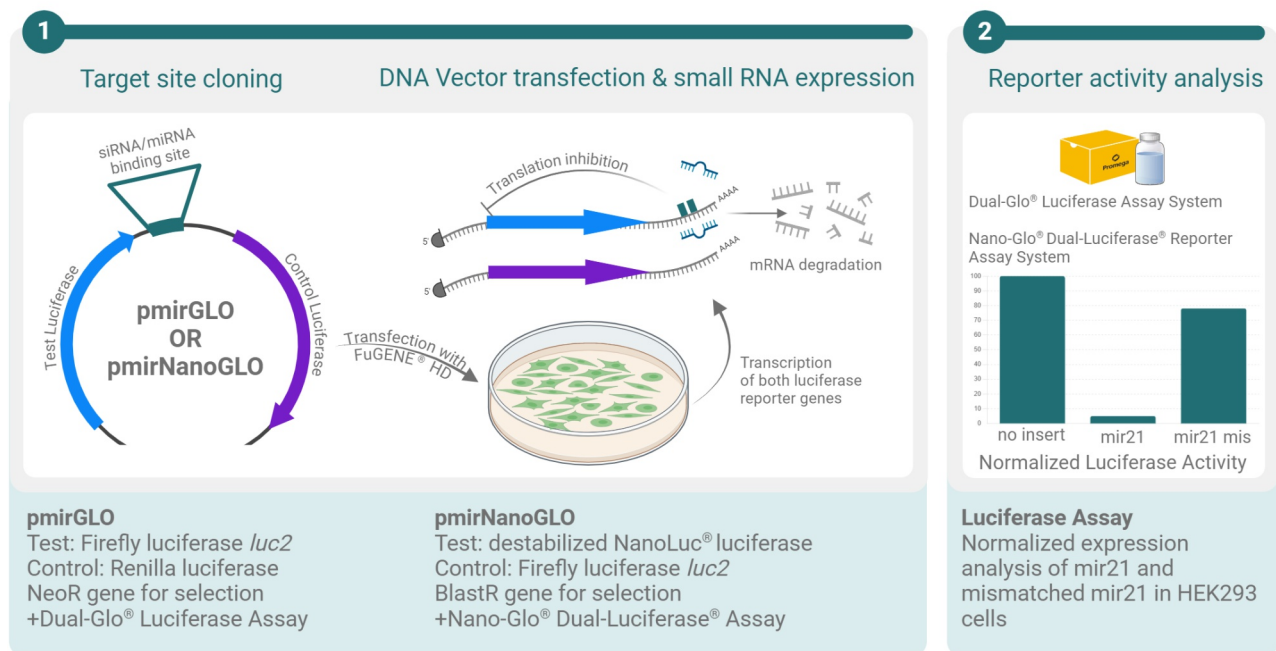
Pay attention to the similar or better knockdown performance by FuGENE® SI.

# Screen siRNA/miRNA Activity Levels

## pmirGLO/pmirNanoGLO Dual-Luciferase miRNA Target Exp. Vector

The pmirGLO vectors are designed to quantitatively evaluate siRNA or miRNA activity by inserting target sites downstream or 3' of the test luciferase gene (optimized firefly luciferase *luc2* or destabilized NanoLuc®).

The test luciferase is the primary reporter gene and reduced activity indicates the binding of endogenous or introduced miRNAs or siRNAs to complementary mRNA. The moderate-strength PGK promoter provides more biologically relevant analysis not possible with strong promoters. A control reporter (either Renilla luciferase or firefly luciferase *luc2*) allows for data normalization and selection for stable cell lines.



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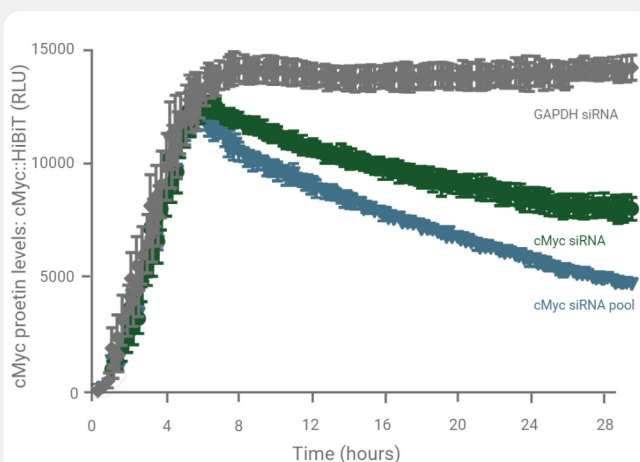
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# Monitor Live Target Gene Knockdown

## Cloning-Free HiBiT Protein Tag Knock In and Analysis

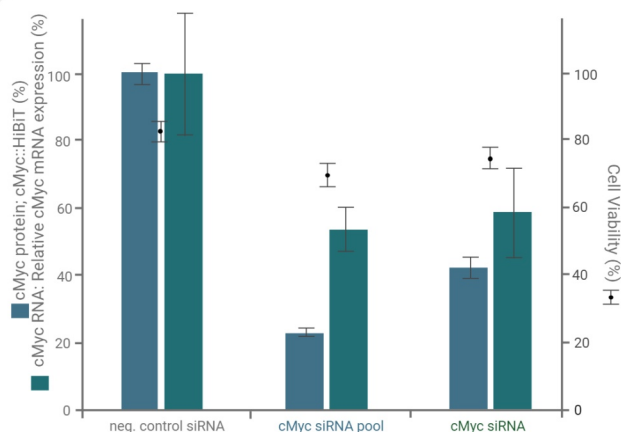
Accurately quantify tagged proteins across varying expression levels over a wide dynamic range. HiBiT (High BiT) is an 11 amino acid long multifunctional protein tag. It enables protein detection using bioluminescent add-and-read reagents. These comprise an inactive luciferase subunit, LgBiT (Large BiT), which spontaneously associates with HiBiT to reconstitute active NanoBiT<sup>®</sup> luciferase.



### Live-cell kinetic monitoring of endogenous cMyc knockdown

Kinetic monitoring of endogenous cMyc was performed in the cMyc-HiBiT Knock-In HEK293 (LgBiT) cells, which stably express LgBiT allowing for kinetic studies. Cells were treated with siRNAs against cMyc or GAPDH. Nano-Glo<sup>®</sup> Endurazine<sup>™</sup> Live Cell Substrate was added at the time of transfection, and luminescence was monitored every 10 minutes on a GloMax<sup>®</sup> Discover Microplate Reader.

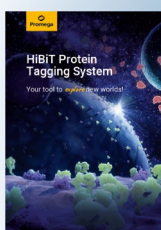
Knockdown dynamics can be visualized with the HiBiT protein tag in cells co-expressing LgBiT.



### Knockdown normalized to cell viability

cMyc expression levels for cells transfected with the negative control siRNA and siRNAs against cMyc were determined by HiBiT quantification using the Nano-Glo<sup>®</sup> HiBiT Lytic Detection System (cMyc protein) and by qRT-PCR using Maxwell<sup>®</sup> RSC miRNA Tissue Kit for RNA extraction and the GoTaq<sup>®</sup> RT-qPCR System (cMyc RNA). Cell viability was measured using the CellTiter-Fluor<sup>™</sup> Cell Viability Assay normalized to untransfected cells.

The cMyc expression levels is normalized to cell viability to ensure comparability of the data.



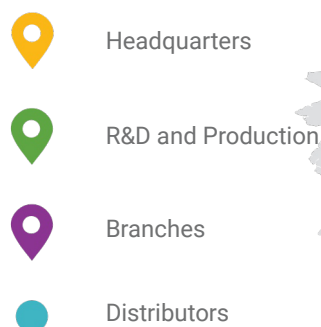
### What else you can do with HiBiT?

You'll find out in the brochure **HiBiT Protein Tagging System**

[www.promega.com/hibit-brochure](http://www.promega.com/hibit-brochure)



# Worldwide Promega Contact Information



## Europe

### Germany/Austria

Tel: +49 6227 6906 0  
Fax: +49 6227 6906 222  
de\_custserv@promega.com

### Switzerland

Tel: +44 878 90 00  
Fax: +44 878 90 10  
ch\_custserv@promega.com

### France

Tel: +33 (0)437 2250 00  
Fax: +33 (0)437 2250 10  
contactfr@promega.com

### Italy

Tel: +39 0254 0501 94  
Fax: +39 0254 0501 45  
customer.service.italia@promega.com

### Poland

Tel: +48 22 531 0667  
Fax: +48 22 531 0669  
pl\_custserv@promega.com

### Central/Eastern Europe

Tel: +49 6227 6906 0  
Fax: +49 6227 6906 222  
de\_ceesales@promega.com

### Spain

Tel: +34 902 538 200  
Fax: +34 902 538 300  
esp\_custserv@promega.com

### Belgium/Luxembourg/Netherlands

Tel: +31 71 532 42 44  
Fax: +31 71 532 49 07  
benelux@promega.com

### Denmark, Estonia, Finland, Iceland, Norway, Sweden

Tel: +46 8 452 2450  
Fax: +46 8 452 2455  
sweorder@promega.com

### United Kingdom

Tel: +44 23 8076 0225  
Fax: +44 20 8076 7014  
ukcustserve@promega.com

## North America

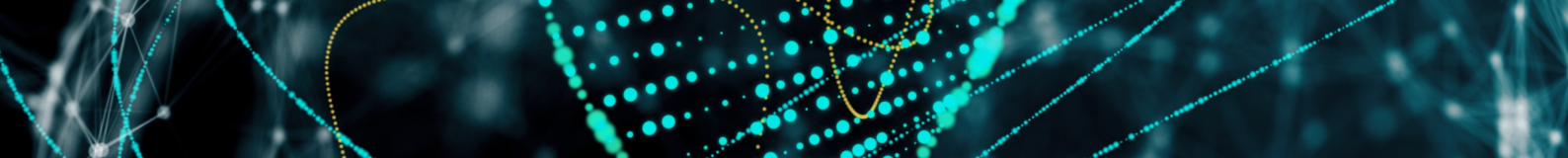
### USA/Canada

Tel: +1 608 274 4330  
Fax: +1 608 277 2516  
custserv@promega.com

## South America

### Brazil

Tel: +55 11 5090 3780  
Fax: +55 11 5096 3780  
promega.brasil@promega.com



## Asia

### China

Tel: +86 10 5825 6268  
Fax: +86 10 5825 6160  
[info@promega.com.cn](mailto:info@promega.com.cn)

### Japan

Tel: +81 3 3669 7981  
Fax: +81 3 3669 7982  
[jpmarketing@jp.promega.com](mailto:jpmarketing@jp.promega.com)

### Korea

Tel: +82 2158 83718  
Fax: +82 2262 85418  
[CustServiceKR@promega.com](mailto:CustServiceKR@promega.com)

### India

Tel: +91 11 43005814/15/16/17  
Fax: +91 11 41035028  
[ind\\_custserv@promega.com](mailto:ind_custserv@promega.com)

### Asia-Pacific region, Singapore

Tel: +65 6513 3450  
Fax: +65 6773 5210  
[sg\\_custserv@promega.com](mailto:sg_custserv@promega.com)

## Australia

### Australia

Tel: +61 2 8838 3800  
Fax: +61 2 8838 3855  
[austcustserv@promega.com](mailto:austcustserv@promega.com)

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