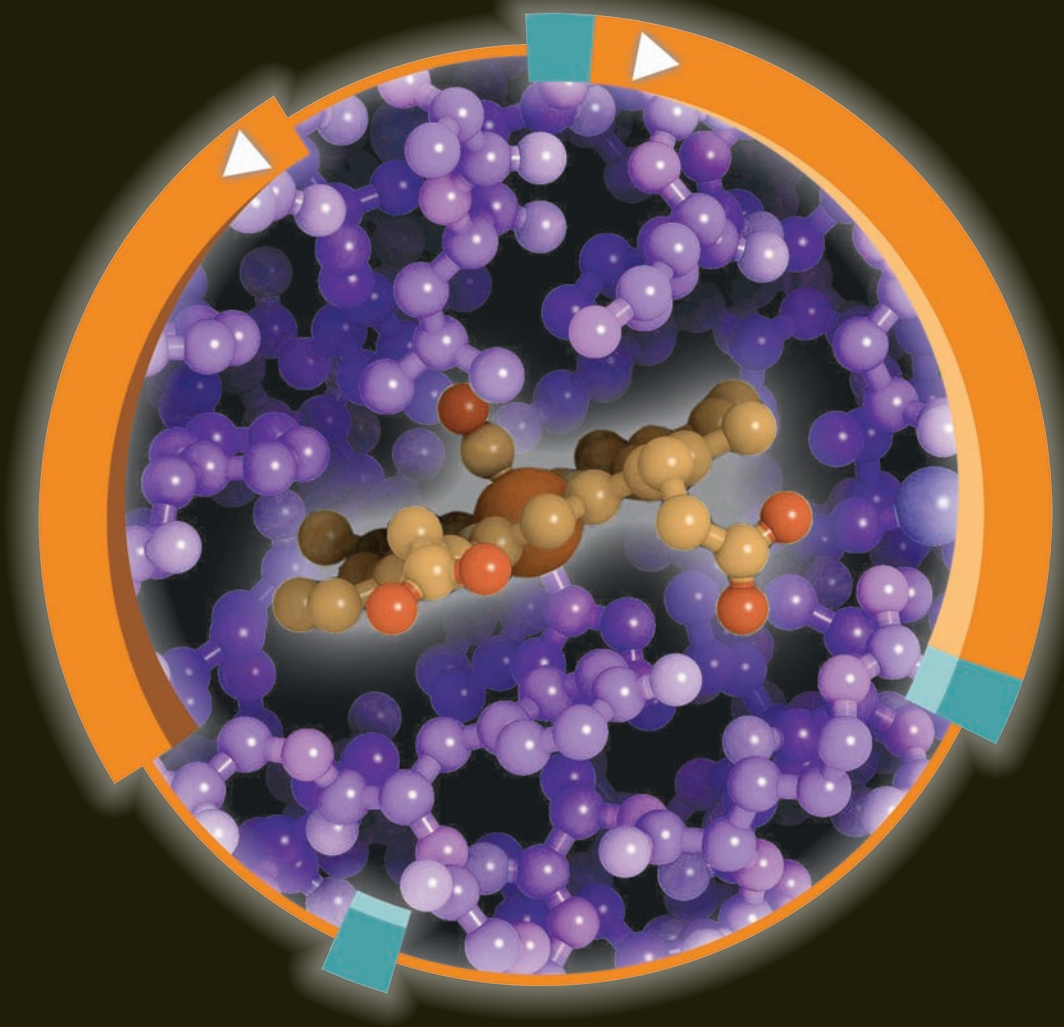


LightSwitch™ Luciferase Assay System

Promoter Reporter GoClones™

Synthetic Response Elements

Pathway Reporter Cell Lines

3´UTR Reporter GoClones™

miRNA Mimics & Inhibitors

Synthetic miRNA Targets

Transfection Reagents

Luciferase Assay Kits

Custom Services

Gene expression is controlled at both the transcriptional and post-transcriptional levels. Transcription factors and other DNA-binding proteins are important regulators of transcription initiation, while microRNAs and the RISC complex interact with the 3´UTR of gene transcripts to control mRNA stability and translation.

The LightSwitch™ Luciferase Assay System is a comprehensive system for studying transcriptional and post-transcriptional regulation. It includes over 30,000 pre-cloned promoter and 3´UTR reporter constructs, optimized companion reagents, and full protocols that support your project from start to finish.

ACTIVE MOTIF®

Enabling Epigenetics Research

LightSwitch™ – comprehensive, optimized luciferase reporter system

The LightSwitch™ Luciferase Assay System is a complete solution for studying transcriptional & post-transcriptional gene regulation in living mammalian cells by directly measuring the functional activity of promoters and 3´UTRs. With GoClone™ Collections of over 18,000 human promoters and 12,000 3´UTRs available as transfection-ready LightSwitch luciferase reporter vectors, you can perform your reporter assay experiments immediately, without the need to clone or prepare DNA.

The LightSwitch System utilizes an engineered luciferase gene (RenSP) and optimized assay & transfection reagents that are designed to provide superior results with easy-to-use protocols. Other products in the system include validated positive and negative control vectors, stable reporter cell lines, miRNA mimics & inhibitors, and collections of synthetic long-range response element constructs and synthetic 3´UTR target validation constructs. We also offer comprehensive services for custom cloning, mutagenesis, pathway screening, miRNA target validation and sequence variant analysis. For more information, please visit **www.activemotif.com/lightswitch**.

Advantages of the LightSwitch Luciferase Assay System

- **Fast & convenient** – LightSwitch GoClones are sequence-verified, transfection-ready human promoter and 3´UTR reporter constructs. No cloning, DNA preparation or assay optimization is needed. To begin, simply search for your regulatory elements of interest using our online search tool (Figure 1).
- **Enhanced sensitivity** – We also offer collections of cloned synthetic regulatory elements that can provide stronger responses than the endogenous promoter and 3´UTR sequences.
- **Quantitative** – All LightSwitch vectors contain RenSP, an optimized luciferase gene that our scientists have engineered for increased brightness and responsiveness (Back Cover). Our LightSwitch Luciferase Assay Substrate was formulated specifically for use with RenSP to provide high sensitivity over a broad dynamic range.
- **Cost-effective** – The LightSwitch Assay scales up easily, making it possible to efficiently screen large numbers of clones for activation and/or repression under a multitude of different conditions.

Search for Promoter & 3´UTR Reporter constructs at www.activemotif.com/goclones.

SEARCH BY GENE IDENTIFIER

In the box below, type or paste in a list of one or more gene identifiers on separate lines. Click on one of the buttons below to search.

DNMT3A
DNMT3B

Search for: Promoter | 3´UTR | Both

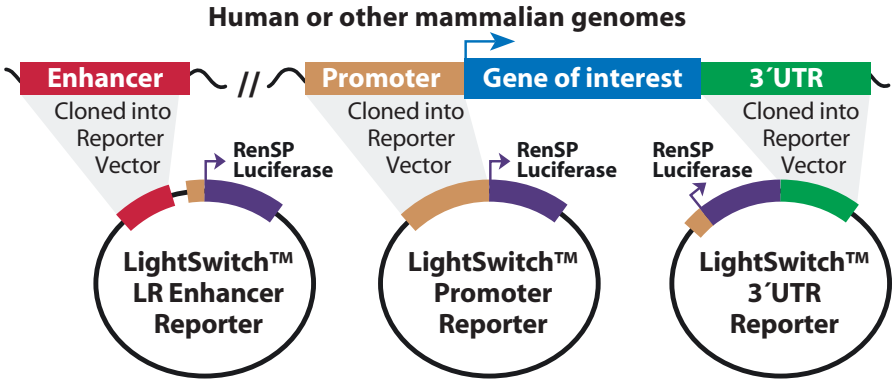
Prod ID	Gene Symbol	Type	Sequence of Cloned Elements	Format
S709833	DNMT3A	PROMOTER	Sequence and Clone Info	5 µg
S709808	DNMT3B	PROMOTER	Sequence and Clone Info	5 µg
S808608	DNMT3A	3´UTR	Sequence and Clone Info	5 µg
S809202	DNMT3B	3´UTR	Sequence and Clone Info	5 µg

Figure 1: Online search tool for finding pre-cloned LightSwitch GoClone Promoter and 3´UTR constructs. Finding cloned regulatory elements for your genes of interest is fast and easy. Simply enter your gene's name, accession number or other identifier in the search box and click a button to search for promoter constructs, 3´UTR constructs, or both. The search results detail which GoClone constructs are available for your elements, including links to view the cloned sequence as well as gene information at NCBI. In this example, a search was performed for both promoter and 3´UTR constructs of DNMT3A and DNMT3B.

Selecting LightSwitch Luciferase Assay System products for your experiments

Step 1: Select or prepare your reporter constructs

Choose from our collection of over 30,000 human promoters and 3´UTRs (Figure 1), TF response elements, synthetic miRNA target reporters, or clone your own promoter, 3´UTR or enhancer elements into the appropriate empty LightSwitch Reporter vector. Alternatively, our Custom Services team can quickly and economically clone any fragment from the human, mouse or rat genome into any LightSwitch vector for you.

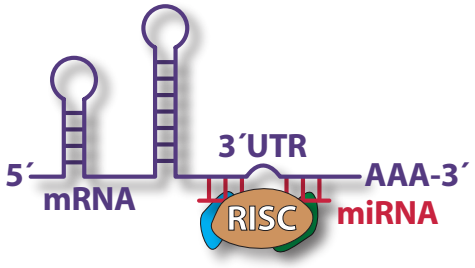


Step 2: Choose appropriate positive & negative control constructs

Because no single promoter can serve as an ideal control for all experimental conditions, we offer a panel of positive and negative controls for use with LightSwitch Promoter & 3´UTR Reporter vectors. These control constructs are used to assess transfection efficiency, measure background, and to normalize for non-specific effects associated with a treatment or change in condition.

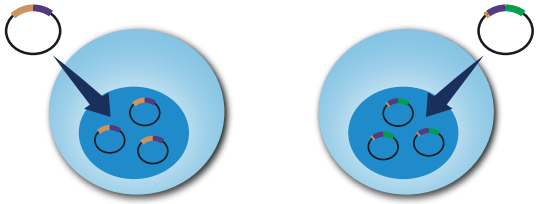
Step 3: Select miRNA mimics and inhibitors (for 3´UTR-miRNA experiments)

When assessing the functional impact of miRNA-3´UTR interactions, you will need to select miRNA mimics and/or inhibitors, which will be co-transfected along with your 3´UTR Reporter or Synthetic miRNA Target Reporter constructs.



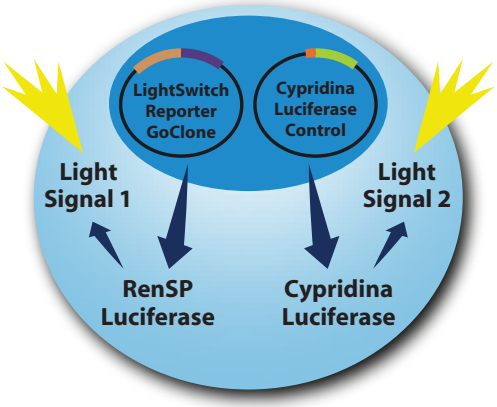
Step 4: Choose transfection reagents

For conducting plasmid transfections and co-transfections, we offer FuGENE® HD Transfection Reagent because of its superior efficiency and low cytotoxicity across a wide variety of cell lines. If you are co-transfecting plasmids and miRNA mimics or inhibitors, we offer DharmaFECT® Duo Transfection Reagent, which we have found to be the most efficient reagent for co-transfecting plasmids and small RNAs.



Step 5: Select a LightSwitch Luciferase Assay or Dual Assay Kit

All LightSwitch reporter constructs contain the optimized RenSP luciferase gene, and LightSwitch Luciferase Assay Reagents MUST be used to obtain optimal results. The kit contains a novel, proprietary substrate that was formulated specifically for use with RenSP. (Other luciferase assay reagents are not compatible with RenSP.) The kit's assay buffer was designed to enable one-step reagent addition, eliminating the need to perform a separate lysis step.



Due to the availability of improved transfection and assay reagents, there is typically no longer a need to co-transfect a control plasmid to normalize luciferase assays (Table 1, Back Cover). However, for difficult-to-transfect cell lines, we offer the LightSwitch Dual Assay Kit, which is used to assay both RenSP and Cypridina luciferases. Note, positive & negative control constructs should still be used to normalize between your experimental conditions.

For more complete information on all LightSwitch products, please visit www.activemotif.com/lightswitch.

LightSwitch™ – comprehensive, optimized luciferase reporter system

Study regulation of promoter activity modulated by transcription factor function

Transcription factors are important regulators of eukaryotic transcription. They interact with DNA-encoded regulatory elements like promoters to modulate when, where and how much of a gene's mRNA is produced. The activity of transcription factors can be affected by the cell's environmental conditions including exposure to signaling molecules like hormones and growth factors.

Reporter assays are valuable for directly measuring promoter activity in living cells. They are a simple and scalable solution for monitoring changes in the transcriptional activity of a promoter. Changes in activity may be induced by over-expression of a cDNA, knockdown by an siRNA or treatment with a small molecule.

Applications for LightSwitch promoter products

- Monitor promoter activity in response to variations in transcription factor function.
- Understand the mechanisms by which transcription is induced or repressed.
- Assess the functional consequences of binding events detected through ChIP.
- Dissect transcription factor motif function using mutagenesis.
- Measure the effects of naturally occurring sequence variants.
- Generate dose response curves to various test compounds or growth conditions.

LightSwitch promoter product portfolio

The LightSwitch Luciferase Assay System makes it fast & easy to measure the functional effects of transcription factor activity. After a promoter of interest is cloned upstream of RenSP in the LightSwitch Promoter Reporter vector, it is transfected into live cells. Any changes in promoter activity cause changes in the reporter signal, which is measured as light output (Figures 2 & 3).

- **LightSwitch Promoter Reporter GoClone Collection** – An algorithm was used to evaluate over 5 million human cDNA sequences to identify transcription start sites (TSS) throughout the human genome. Based on these TSS predictions, over 18,000 human promoter sequences of ~1 kb (–900...+100 bp relative to the TSS) were cloned into the LightSwitch Promoter Reporter vector. Each GoClone is purified and sequence-verified during our extensive quality control process, and is delivered ready for transfection. Simply find and order your reporter clones using our online search tool (Figure 1), and then begin your assays.
- **LightSwitch Synthetic Response Elements** – These constructs include multiple repeats of a transcription factor binding site motif cloned upstream of a minimal promoter and RenSP. In some cases these response elements provide higher sensitivity than the endogenous human promoters found in the Promoter GoClone Collection, making them well suited for use in primary screens to identify compounds or conditions that affect biological pathways.
- **LightSwitch Pathway Reporter Stable Cell Lines** – These cell lines contain a stably integrated, human regulatory element driving expression of RenSP. Each has been validated on several plate-based formats for measuring the activity of a specific biological pathway, making them ideal for both primary and secondary high-throughput screening.
- **Validated Promoter Controls** – Constructs for use as positive & negative controls.
- **Empty Promoter & Long-range Enhancer reporter vectors** – Clone your own sequences.
- **Pathway Screening Services to study disease-related biological pathways** (see other side)

For more complete information, please visit www.activemotif.com/ls-promoter.

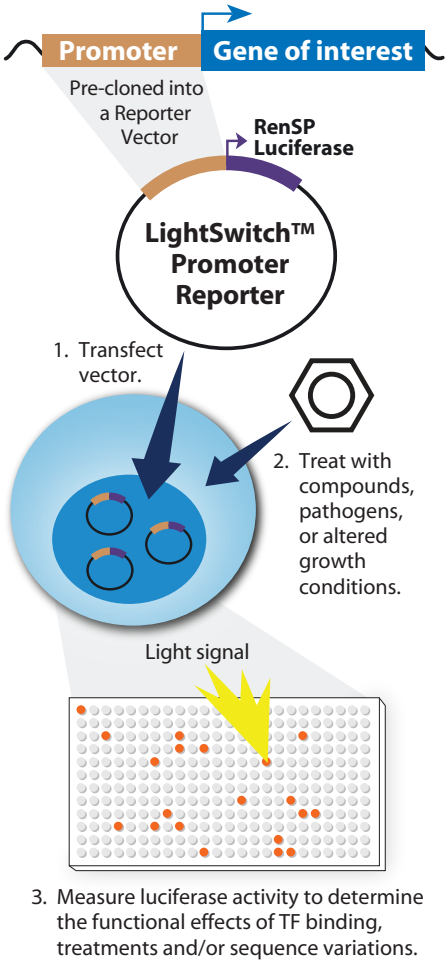


Figure 2: LightSwitch Promoter Reporter Assays. To study a promoter's regulation, it is cloned into a LightSwitch Promoter Reporter vector and transfected. The cells are treated, if desired, and then assayed. The luciferase produced oxidizes the assay substrate in a reaction that also produces light. The amount of light is proportional to the effect of the cloned promoter.

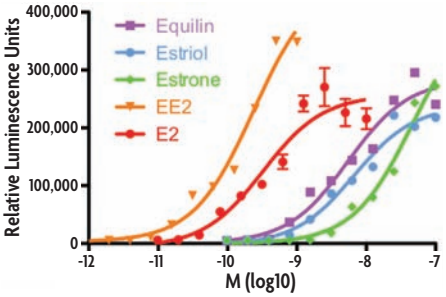


Figure 3: Dose response of LightSwitch SYT8 Promoter Reporter construct to various estrogen compounds. HT1080 cells were co-transfected with a SYT8 Promoter GoClone (Product ID S714388) and an ER cDNA expression plasmid, then treated with five estrogen compounds for 24 hours before the luminescence was measured using the LightSwitch Luciferase Assay Kit.

Study post-transcriptional regulation mediated through miRNA-3´UTR interactions

MicroRNAs (miRNAs) are small, non-coding RNA molecules of ~22 nucleotides that play a large role as post-transcriptional regulators. miRNAs are thought to repress expression by targeting the 3´UTR (untranslated) region of mRNA transcripts. The interaction of a miRNA with a 3´UTR results in either inhibition of translation or increased degradation of the targeted transcript.

While the human genome is thought to encode only about 1000 miRNAs, they are believed to target ~60% of mRNA transcripts. A given miRNA may have multiple 3´UTR targets, and a given 3´UTR may be targeted by multiple miRNAs. Therefore, there is considerable interest in studying how the many possible miRNA-3´UTR interactions impact post-transcriptional regulation.

Applications for LightSwitch miRNA-3´UTR products

- Understand the mechanism by which a gene is induced or repressed.
- Measure the impact of a 3´UTR on post-transcriptional gene regulation.
- Validate the targets of a miRNA or siRNA.
- Measure the effects of sequence variants on 3´UTR or miRNA function.

LightSwitch miRNA-3´UTR product portfolio

The LightSwitch System is ideal for performing miRNA target validation and assessing the functional impact of miRNA-3´UTR interactions. The 3´UTR sequence of interest is cloned downstream of RenSP in the LightSwitch 3´UTR Reporter vector. The vector's constitutive promoter drives expression of a hybrid RenSP luciferase-3´UTR transcript that is the basis of the assay (Figure 4). Total luciferase output depends on the effects that the cloned 3´UTR and miRNAs have on the stability and/or translational efficiency of the hybrid transcript (Figure 5).

- **LightSwitch 3´UTR Reporter GoClone Collection** – Following systematic identification of 3´UTR sequences in the human genome using RefSeq and other cDNA resources, over 12,000 human 3´UTRs ranging from 300 bp to 3,000 bp were cloned into the LightSwitch 3´UTR Reporter vector. Each vector is purified and sequence-verified during our extensive quality control process, and is supplied as a transfection-ready product. Simply find and order your clones using our online search tool (Figure 1), and then begin your experiments.
- **LightSwitch Synthetic miRNA Target Reporter Collection** – These constructs contain an optimized synthetic target consisting of sequence repeats that are fully complementary to a variety of human and viral miRNAs based on miRBase 16 annotations. These products can be used as very sensitive screening assays for miRNA functional activity, and they also serve as strong positive knockdown controls for use in other miRNA experiments.
- **LightSwitch miRNA Mimics and Inhibitors** – Our miRNA Mimics and Inhibitors are chemically optimized synthetic double-stranded and single-stranded small RNAs, respectively. The Mimics are used as functional equivalents to endogenous human miRNAs, while the Inhibitors are used to knock down expression of endogenous human miRNAs in living cells.
- **Validated 3´UTR Controls** – Constructs for use as positive & negative controls.
- **Empty 3´UTR reporter vector** – Clone your own 3´UTR sequences.
- **Complete screening service to validate 3´UTR targets for any miRNA** (see other side)

For more complete information, please visit www.activemotif.com/ls-3utr.

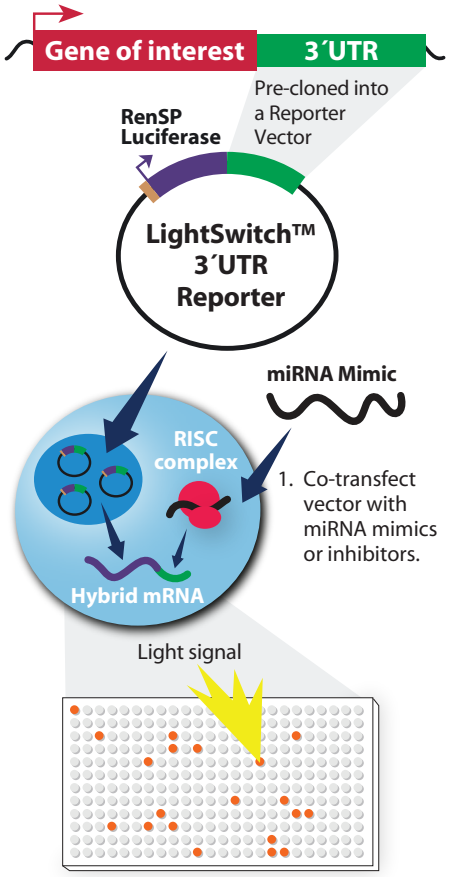


Figure 4: LightSwitch 3´UTR Reporter Assays. A 3´UTR cloned into a LightSwitch 3´UTR Reporter vector is co-transfected with miRNA mimics or inhibitors. The relative amounts of light produced in cells with and without miRNA are used to measure the effect of the miRNA-3´UTR interaction on translation of the hybrid RenSP-3´UTR mRNA transcript.

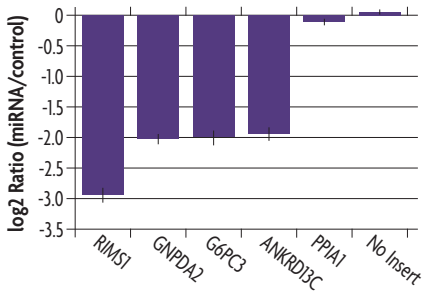


Figure 5: Interaction of miR-122 with 3´UTR targets. 3´UTR targets of miR-122 were cloned into the LightSwitch 3´UTR vector and co-transfected with either 20 nM of miR-122 mimic or a non-targeting control miRNA in K-562 cells. The graph above shows the four strongest responders along with two non-responding controls.

LightSwitch™ Custom Services can help accelerate your research

In addition to the many different products that make up the LightSwitch Luciferase Assay System, we offer a wide variety of custom services that can accelerate your gene regulation research.

Advantages of LightSwitch Custom Services

- Utilize our unique resource of promoter and 3´UTR clones
- Leverage our expertise in cloning and reporter assays
- Receive superior data validated by our control processes
- Focus on data analysis instead of lab work

For more complete information on LightSwitch Custom Services, please visit www.activemotif.com/ls-services.

Custom Cloning & Mutagenesis

In addition to the 30,000 human regulatory elements available as pre-cloned LightSwitch reporter vectors, we offer a custom cloning service to quickly and economically clone any genomic element from human, mouse or rat into any LightSwitch reporter vector. We also offer a custom mutagenesis service to create sequence variants of any element cloned into a LightSwitch vector. Custom mutagenesis allows you to map functional motifs or characterize the effects of sequence changes on gene expression (Figure 6).

Pathway Screening Services

Our in-house experts use our unique collection of validated reporter assays in a cell-based screening service to measure the effects of your compounds on a variety of biological pathways (Figure 7). We offer 48 validated human promoter reporter vectors to measure gene expression changes associated with 11 disease-related pathways. This service is ideal for:

- Secondary screening
- Lead optimization
- Dose response analysis
- Phenotypic screening / deconvolution

Figure 7: Response of pathway reporter constructs to various treatments. The heat map at right shows the inducible activity of 29 different reporter constructs and 5 controls (rows) under 15 different conditions (columns). The red boxes indicate promoters that are up-regulated by the condition while the blue boxes indicate down-regulated promoters. The conditions used in these experiments are treatments with known inducers of different biological pathways. For example, DFO and 1% O_2 are known inducers of the hypoxia pathway. As expected, all 3 hypoxia reporters tested are strongly induced by both of these conditions. The heat map also shows pathways that are activated by multiple conditions. For example, PMA and TNF-α both strongly activate the NFκB reporters. All experiments were performed in HT1080 cells, and the constructs are a combination of endogenous human promoters and synthetic response elements cloned into LightSwitch reporter vectors.

	WT seed seq	Mutant seed seq
RIMS1	ACACTCC	AGTCTCC
GNPDA2	ACACTCCA	AGTCTCCA
ANKRD13C	ACACTCC	ACAGACC
G6PC3	ACACTCCA	ACAGACCA
G3BP2	ACACTCC	GGTCTCC
ALDOA	ACACTCCA	AGGTCTCCA

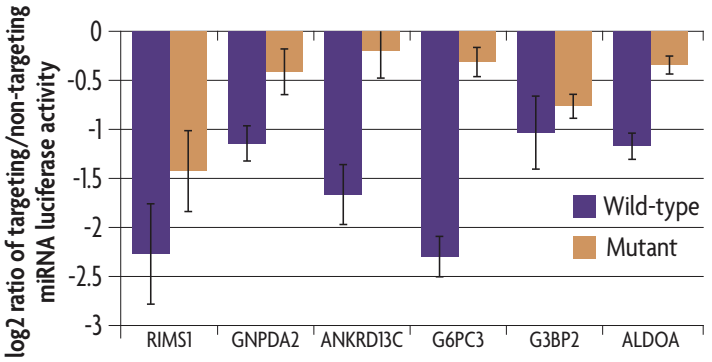
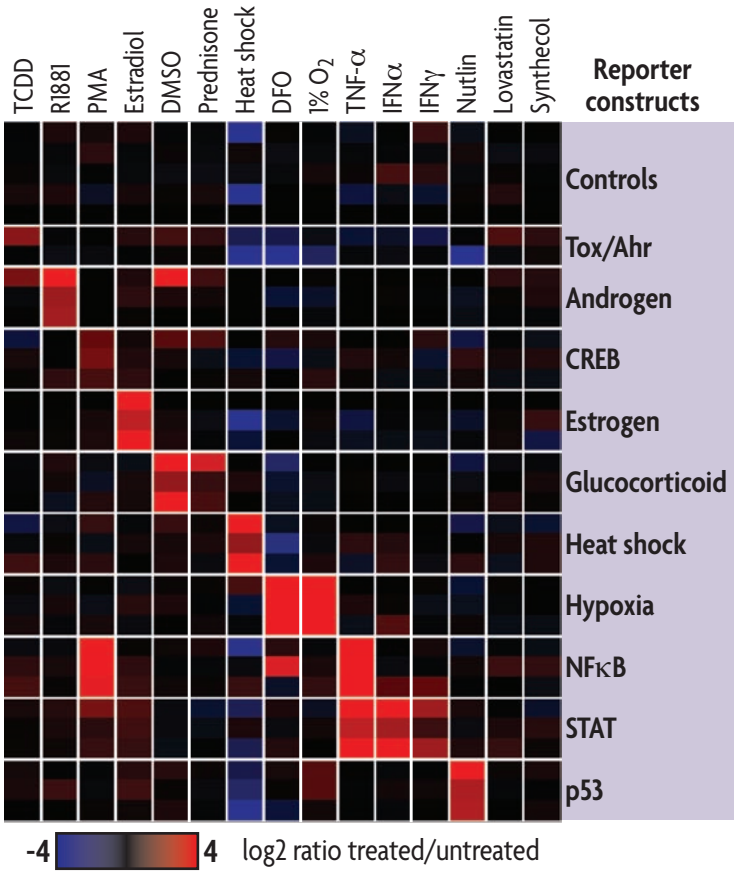


Figure 6: Mutagenesis used to identify seed sequences necessary for miR-122 function. LightSwitch 3´UTR Reporter constructs containing the 3´UTRs of 6 genes known to be miR-122 target sites were subjected to site-directed mutagenesis. The wild-type and mutant constructs were co-transfected in HT1080 cells with a miR-122 miRNA mimic or a non-targeting control in triplicate and then assayed using the LightSwitch Assay Kit.



LightSwitch™ Custom Services (cont.)

Sequence Variant Assay Services

Naturally occurring sequence variation can have large impacts on the function of promoters or 3´UTRs. Once you have identified a regulatory element of interest, our Custom Services team can perform site-directed mutagenesis or custom cloning of haplotypes, then conduct reporter assays in living cells to quantify the functional effects of the sequence differences. Many researchers have used this service to study the effects of naturally occurring sequence variation, while others have created specific mutations in motifs, transcription factor binding sites or putative miRNA targets (Figure 6).

To use the service, all you need to do is to specify your regulatory elements of interest as well as the variants you want us to create for individual SNPs or mutated motifs. We can also analyze larger haplotypes from individual genomic samples (Figure 8). After we perform the service, you will receive both a complete raw data set and a statistical analysis of significant differences between the variants.

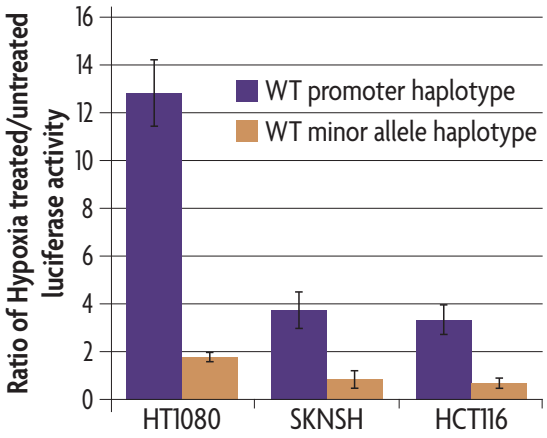


Figure 8: Measuring the functional effects of naturally occurring sequence variants. LightSwitch Reporter Assays were used to measure the functional differences between two promoter haplotypes of the MIF gene (minor allele frequency of 1%). The 1 kb promoter sequences differed by a single SNP located in the predicted HIF-1α binding site located 218 bases upstream of the transcription start of the gene. Each haplotype reporter vector was transfected in three different cells types (HT1080, SKNSH and HCT116) and promoter activity was measured before and after hypoxia induction (1% oxygen treatment for 24 hours). Each vector and condition was measured in triplicate. The purple bars in the figure show the inducible activity of the wild-type promoter sequence (major allele), while the copper bars show a significant reduction in inducible activity in all 3 cell types tested for the minor allele. This example illustrates the use of reporter assays for measuring the functional effects of naturally occurring sequence variants.

miRNA Target Validation Services

Accurately identifying which miRNAs bind to a specific 3´UTR can be a challenge. A given miRNA may interact with multiple 3´UTR targets, while each 3´UTR may be targeted by multiple miRNAs. Furthermore, miRNA target prediction algorithms suffer from low specificity and sensitivity. These factors make it necessary to experimentally validate the targets of your miRNA of interest.

For this reason, we provide a complete miRNA Target Validation Service that researchers can use to validate the 3´UTR targets of specific miRNAs. Using our genome-wide collection of human 3´UTRs cloned into our optimized LightSwitch 3´UTR Reporter vector, we can validate the targets of miRNAs of interest using our high-throughput, cell-based reporter assay.

Simply provide a list of candidate target genes for each of your miRNAs of interest, and we will perform the cell-based reporter assays to determine whether the 3´UTRs of these genes are functional targets of the miRNA. If you do not have a list of candidate targets for your miRNA, we can generate one for you (Figure 9). Our extensive set of validated positive & negative 3´UTR reporter controls enable you to analyze your results with confidence.

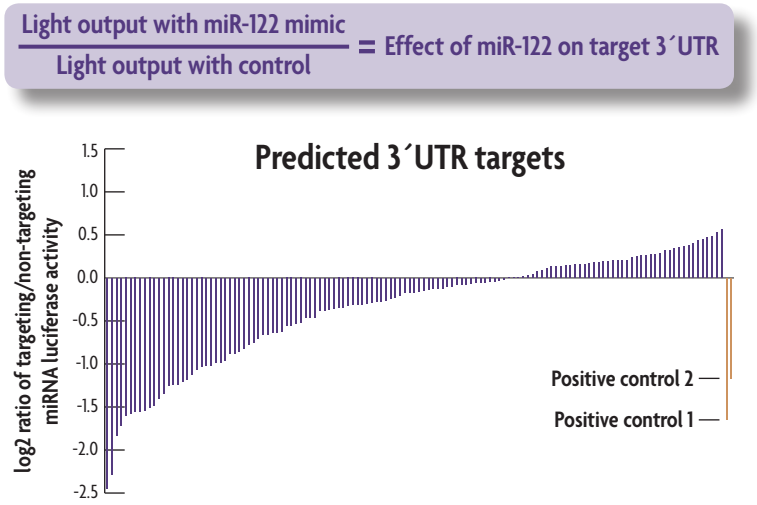


Figure 9: Luciferase-based screen to identify miR-122 targets. LightSwitch 3´UTR Reporter constructs containing the 3´UTRs of 139 genes predicted to be miR-122 target sites were assayed in HT1080 cells for repression in response to miR-122 treatment. This barchart shows the degree of knockdown ranked highest to lowest from left to right. Each 3´UTR reporter vector was co-transfected with a mimic or non-targeting control in triplicate and assayed using the LightSwitch Assay Kit. The degree of knockdown was measured as the ratio of mimic divided by the non-targeting control.

Engineered RenSP luciferase and optimized LightSwitch reagents for the best results possible

With over 30,000 human regulatory elements cloned and available as transfection-ready LightSwitch reporter vectors, Active Motif is the leading provider of regulatory content. In addition to pre-cloned content, our scientists also developed and optimized every component of the LightSwitch System to ensure that it would be easy to use and provide optimal results.

All LightSwitch reporter vectors utilize RenSP, an optimized marine luciferase gene that has been engineered for maximum brightness and short protein half-life. Starting with a base sequence of a native marine luciferase gene, thousands of synthetic gene sequence variants were functionally screened for increased enzymatic activity (light output). Furthermore, a protein destabilization domain was added to decrease the half-life of the RenSP protein (see below), and putative transcription factor binding sites were removed from the sequence, as these might confound experimental results. The result is that the RenSP luciferase we created is 50% brighter than other luciferases (Figure 10).

In parallel to the protein engineering efforts, we also created novel, proprietary companion assay reagents that provide high sensitivity over a broad dynamic range when used with RenSP. As an additional benefit, the reagents were developed to enable one-step reagent addition directly to cultured cells, eliminating the need for a separate lysis step. Collectively, these many improvements ensure that LightSwitch delivers superior results with simple and robust protocols.

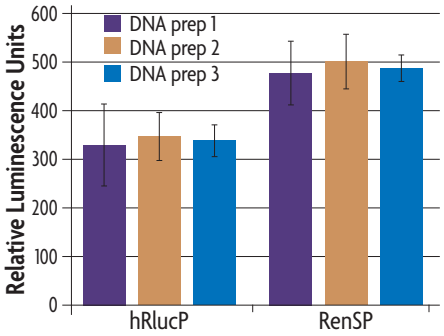


Figure 10: The RenSP signal is brighter than hRlucP.
To determine the relative brightness of RenSP compared to hRlucP, a humanized form of *Renilla* luciferase, the RenSP and hRlucP genes were cloned into separate vectors along with the human RPL10 promoter. Three independent plasmid purifications were conducted for each vector, and 50 ng of each plasmid was transfected with FuGENE HD in triplicate in human HT1080 cells using a 96-well format. After 24 hours of incubation, the cells were assayed. The results show that the optimized RenSP luciferase is significantly brighter than hRlucP.

Enhanced degradation rate of RenSP improves assay responsiveness and accuracy

One limitation of other reporter proteins is that they accumulate in the cell due to a relatively long half-life. Slow protein turnover can decrease the measured response to a stimulation or completely mask the ability to detect repression. To minimize this problem, the RenSP gene has been engineered to contain a PEST protein degradation sequence from mouse Ornithine Decarboxylase (mODC) that increases its rate of turnover. Consequently, the destabilized RenSP luciferase protein has a half-life of approximately one hour compared to the > 3 hour half-life of native luciferase protein. Combined with the increased signal detailed above, the shorter half-life of RenSP provides a more sensitive and accurate measure of the induction or repression of reporter gene activity, especially when studying subtle or fast-acting effects or when generating high-quality dose response curves.

Single assay vs. Dual assay experimental design

Historically, some luciferase experiments used a control plasmid that was co-transfected with the experimental construct to normalize for variations in transfection efficiency and cell lysis between transfection replicates. However, modern transfection reagents such as FuGENE HD and the optimized lysis and assay reagents found in LightSwitch Luciferase Assay Kits often reduce these types of variation to coefficients of variance (%CVs) of less than 10% (Table 1). Because of these improvements, normalization by co-transfection often provides little benefit. Because dual assay reagents cost twice as much money and can reduce assay sensitivity, we recommend that you first try a single assay design. However, if you are using a difficult-to-transfect cell line, the LightSwitch Dual Assay Kit is available, and has been optimized for use with all LightSwitch reporter vectors.

Promoter	TK	ACTB	RPL10	ANKRD37	EGLN1	ENO2	AVG.
%CV Single Tfx	18%	2%	13%	8%	6%	7%	**9%**
%CV Co-Tfx normalized	14%	22%	11%	12%	4%	7%	**12%**

Table 1: Variation between replicate transfections is essentially the same for single transfections and normalized co-transfections.
Six different promoter constructs were cloned into the pLG4 vector (Promega). In the Single Tfx experiment, they were independently transfected into Hep G2 cells in triplicate without a co-transfection control, then assayed with Steady-Glo (Promega) 24 hours after transfection. In the Co-Tfx experiment, the same 6 constructs were co-transfected with a TK-*Renilla* plasmid then assayed with Dual-Glo (Promega). The co-transfected plasmid data were normalized by dividing the Firefly signal by the *Renilla* signal. The table above shows the coefficient of variation (%CV = stdev/mean) of the 3 replicates of each construct. The average %CV for the single transfections is 9%, whereas the average %CV for the normalized co-transfections is 12%. Similar results were seen when LightSwitch GoClones and Assays were used. (Data not shown.)