LiGHTSW!TCH
Luciferase Assay System

A comprehensive platform for
promoter and 3'UTR reporter assays

SYSTEM OVERVIEW

The LightSwitch Luciferase Assay System includes:

- **LightSwitch GoClone Collections**
  Genome-wide collections of human promoters and 3'UTRs pre-cloned into LightSwitch reporter vectors

- **RenSP: a novel luciferase reporter gene and vector**
  Optimized for maximizing brightness and superior performance in induction and repression experiments

- **LightSwitch Luciferase Assay Reagents**
  One-step reagent addition directly to cultured cells, optimized specifically for use with RenSP

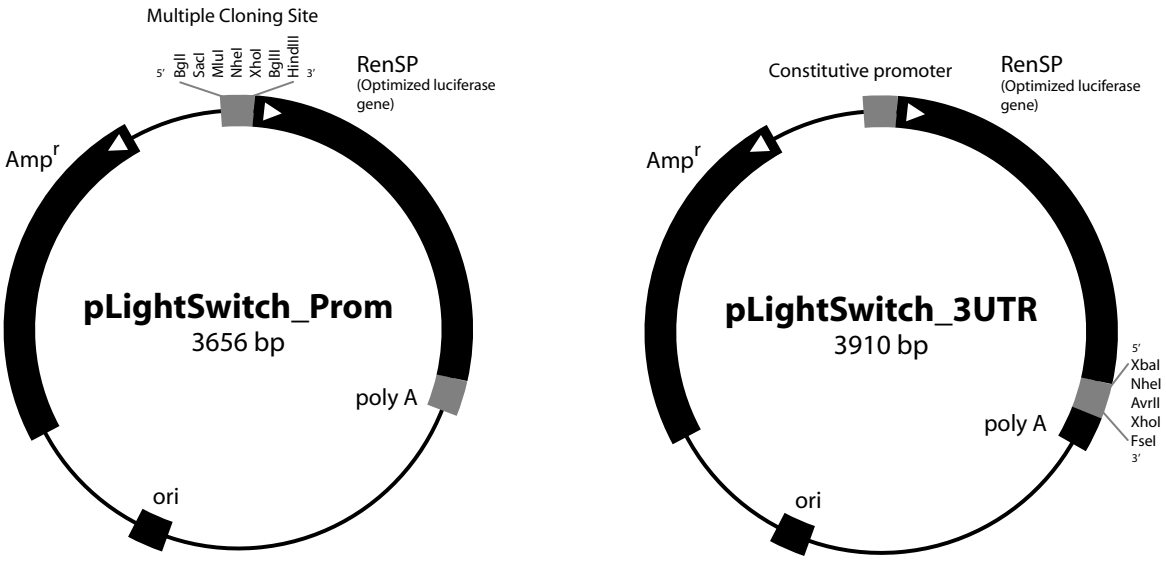
The LightSwitch Luciferase Assay System is a unique and comprehensive collection of cloned regulatory elements from the human genome. The system includes an improved luminescent reporter gene (RenSP), an optimized companion assay reagent, and pre-cloned promoters and 3'UTRs. Utilizing the novel RenSP reporter gene with LightSwitch Assay Reagents, the LightSwitch Luciferase Assay System achieves an ideal balance between signal brightness and optimal kinetics. The LightSwitch Luciferase Assay System provides a seamless start-to-finish solution for your reporter assay needs and may be used in a range of applications from single gene studies to high-throughput screening applications such as:

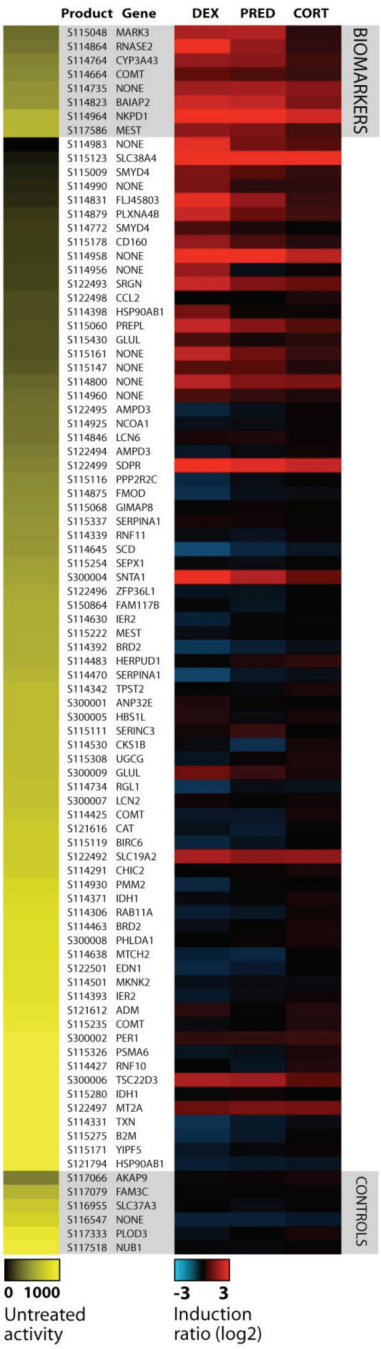
- Studying the mechanisms of transcriptional and post-transcriptional gene regulation
- Measuring the effects of transcription factor binding
- Validating predicted miRNA targets in 3'UTRs
- High-throughput screening: experimentally validated pathway biomarkers for primary screens or comprehensive pathway profiling
- Measuring the effects of naturally occurring sequence variants or site-directed mutagenesis for validating functional elements or motifs

GoClones: Genome-wide Collections of Human Promoters and 3'UTRs

GoClone reporter vectors are transfection-ready vectors with no cloning or DNA preparation required. We have cloned more than 30,000 unique regulatory elements into our RenSP reporter vectors so you can eliminate the cloning effort and immediately start experiments. SwitchGear scientists are recognized experts in genome-wide regulatory element identification and high-throughput experimental verification (1-5). Our GoClone collections include more than 18,000 human promoters and 12,000 3'UTRs cloned into LightSwitch reporter vectors (Figure 1). GoClones are delivered as sequence-validated, transfection-ready plasmids at a standard concentration in a proprietary stabilization buffer ready for experiments. In addition, we provide convenient and easy-to-use protocols to support a wide variety of experiments and plate formats. Visit the SwitchGear website to search our online catalog for your genes of interest.

Figure 1: LightSwitch Vector Maps





In addition to our genome-wide GoClone collections, SwitchGear also offers functionally validated sets of promoter and 3'UTR reporter constructs for measuring a variety of biological responses. As an example, Figure 2 shows experimental results from our validated set of glucocorticoid receptor target promoters in response to three anti-inflammatory drugs. Figure 3 shows the functional knockdown of four human 3'UTRs in response to the presence of the microRNA mir-122. Overall, SwitchGear offers more than 20 validated sets of responsive promoters and 3'UTRs including targets of hypoxia, hormone signaling, inflammation, DNA-damage, cholesterol metabolism, and many more. Please visit our website for more information on SwitchGear's validated pathway products.

Figure 2: Glucocorticoid pathway profiling panel of promoter reporters

The promoter constructs shown in the heatmap represent the SwitchGear Glucocorticoid Receptor Profiling Plate. The heatmap summarizes the untreated activity of each promoter in the left column. On the right side of the figure, the log2 ratios of treated/untreated activity are shown for 3 different compounds according to the color scale shown at the base of the heat map; "DEX"= HT1080 fibrosarcoma cells treated with 100 nM dexamethasone for 4 hours; "PRED"= HT1080 fibrosarcoma cells treated with 1 uM prednisone for 4 hours; "CORT"= HT1080 fibrosarcoma cells treated with 1 uM cortisone for 4 hours.

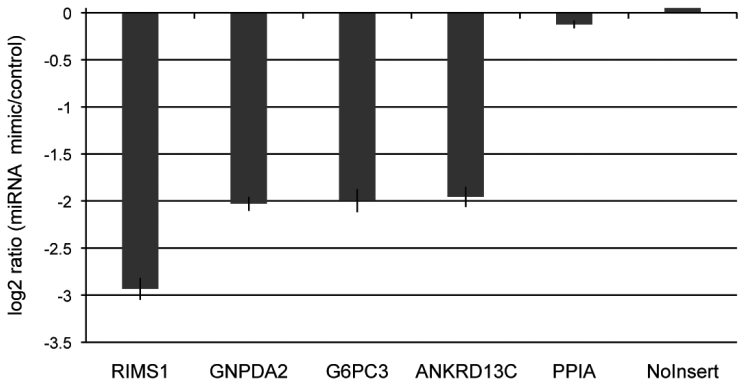


Figure 3: Validated 3'UTR targets of miR-122

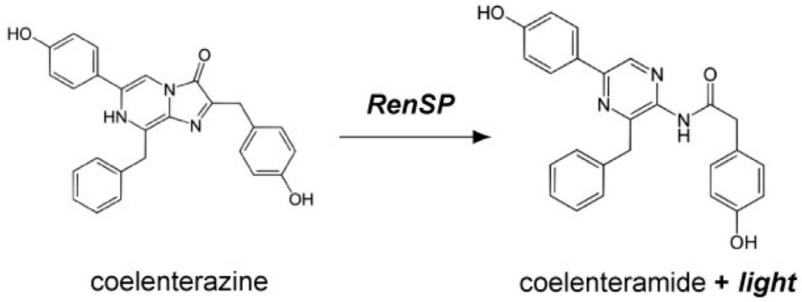
The putative 3' UTR targets of miR-122 were initially predicted by the miRanda algorithm. We co-transfected putative miR-122 targets with either 20nM of miR-122 mimic or a non-targeting control miRNA in K562 cells. We selected the four strongest responders from this experiment along with two non-responding controls.

The Novel RenSP Reporter Gene

In recent years, marine luciferases have become popular alternatives to firefly luciferase as a genetic reporter based on assay simplicity, high sensitivity, and a broad linear range of signal that provides greater sensitivity over firefly luciferases (6,7).

Marine bioluminescence has evolved independently many times, so luciferases that target the same substrate (coelenterazine) bear little resemblance to one another (8). Renilla reniformis is a sea pansy (a small soft coral) that is found in shallow sandy bottoms. It responds to mechanical stimulation by generating a blue-green bioluminescence (8). The small size of its native luciferase gene and protein (936bp and 36kD) and its lack of dependence on ATP provide a distinct advantage over larger ATP-dependent luciferases like those from fireflies (~1.6kb and 62kD) (9-12). The protein catalyzes oxidation of its coelenterazine substrate in the reaction shown below to produce light at 480 nm which is easily read by standard luminometers (13).

SwitchGear has created an optimized Renilla luminescent reporter gene, called RenSP, by increasing its overall enzymatic activity (light output) and adding a protein destabilization domain to decrease the half-life of the RenSP protein.



Starting with a base sequence of the native Renilla gene, we functionally screened thousands of synthetic gene sequence variants that included a variety of predicted improvements. We also removed transcription factor binding sites from the gene sequence that might interfere with or confound expression measurements. As a result, we created a luciferase that is at least 50% brighter than other humanized versions of Renilla luciferase (Figure 4) (7).

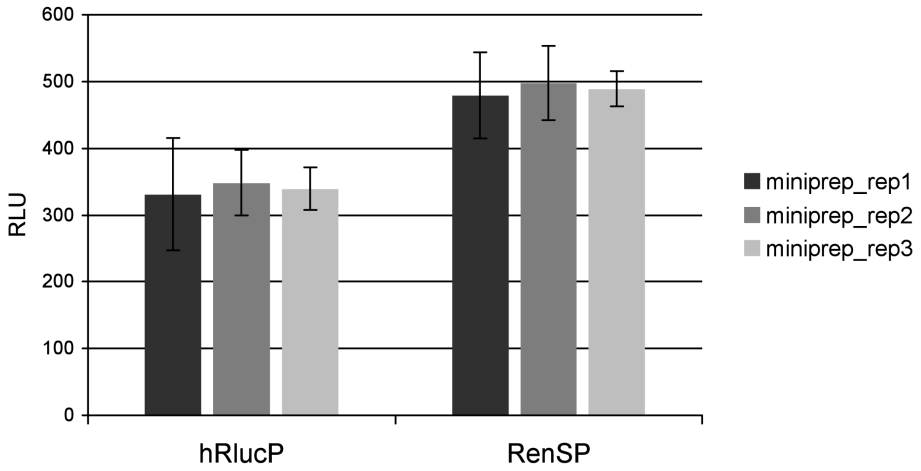


Figure 4: Absolute signal of RenSP is significantly brighter than hRlucP

To determine the relative brightness of RenSP compared to hRlucP, another humanized form of the Renilla luciferase, the RenSP and hRlucP genes were cloned into separate vectors each containing the human RPL10 promoter. Three independent plasmid purifications were conducted for each vector, and 50ng of each plasmid was transfected with FuGENE HD in triplicate in human HT1080 cells in 96-well format. After 24 hours of incubation, 100uL of LightSwitch Reagent was added to each well and incubated for 30 minutes before being read for2 seconds on an LmaxII-384 luminometer. These results show that RenSP is significantly brighter than hRlucP.

We also fused a protein destabilization domain to the RenSP gene to reduce protein accumulation that often interferes with the ability to detect expression changes. The PEST sequence from mouse Ornithine Decarboxylase (mODC) is a well-studied protein domain that has been shown to increase rates of protein turnover (14-17). The RenSP fusion protein therefore combines the benefits of increased signal with a short half-life reporter to provide a sensitive measure of the induction or repression of reporter gene activity. Figure 5 highlights an example in which signal knock-down of a 3'UTR reporter after addition of a miRNA is more easily detected when the target 3'UTR is fused to a PEST-containing reporter gene.

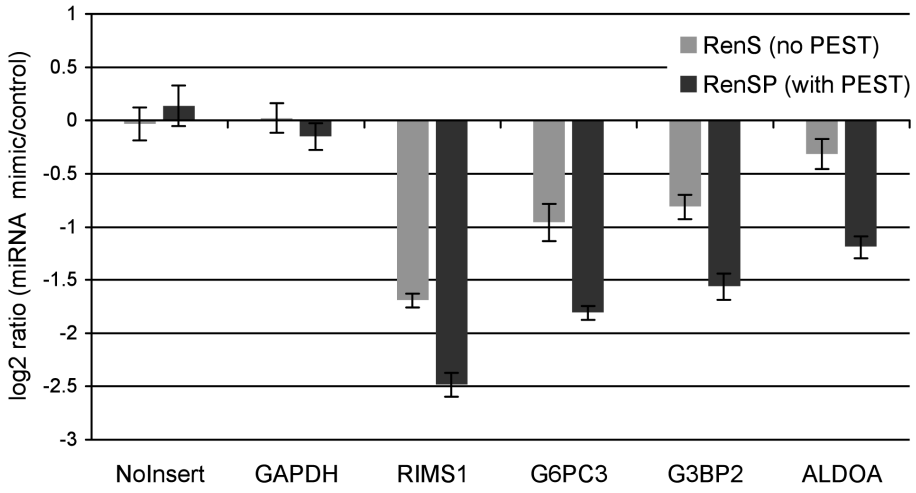


Figure 5: RenSP with PEST increases the knock-down of 3'UTR targets in the presence of mir-122

The knockdown of 3'UTR-luciferase activity in the presence of mir-122 was measured by co-transfecting a 3'UTR GoClone reporter with a synthetic miRNA. DharmaFECT DUO was used to transfect HT1080 cells in triplicate in 96-well format with 100ng of 3'UTR reporter and 20nM of mimic or non-targeting control miRNA. After 24 hours of incubation, 100ul of LightSwitch Assay reagent was added to each well, plates were incubated at room temperature for 30 minutes and read on a LmaxII-384 luminometer. The log2 ratio of the average mimic signal divided by the average signal from the non-targeting control was calculated and shows that the RenSP luciferase with PEST gives a significantly stronger knockdown than RenS without PEST.

LightSwitch Assay Reagent

We formulated novel LightSwitch Assay Reagents in parallel with our gene improvements to create a fully optimized reporter system. Historically, coelenterazine-based luciferase assays faced challenges associated with high background readings. The combination of the LightSwitch Assay Reagents and the RenSP reporter gene overcomes these issues to yield a bright and stable signal over many orders of magnitude with low background levels (Figure 6). The LightSwitch Assay Reagent may be added directly to cultured cells (in FBS-containing or FBS-free media) in a single step and is convenient to use for a small number of samples or thousands of assays in a large screen. By optimizing the RenSP gene specifically for use with the LightSwitch Assay Reagents, the LightSwitch Assay System provides a robust and convenient reporter-based solution for all phases of your experimental workflow.

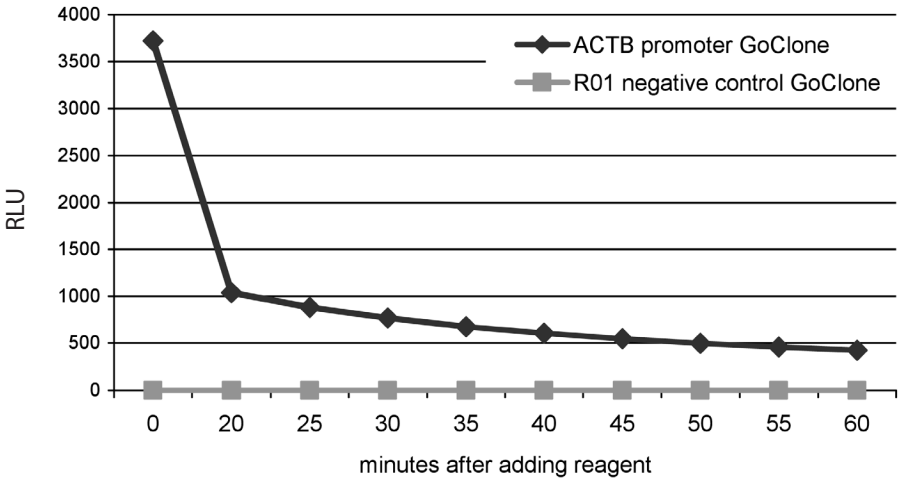


Figure 6: Signal stability of LightSwitch Assay Reagents

To determine the signal stability of LightSwitch Reagents, HT1080 cells were transfected with FuGENE HD in 96-well format with the ACTB promoter GoClone and the R01 negative control GoClone in separate wells. After incubating for 24 hours, 100uL of LightSwitch reagent was added to each well and luminescence was recorded for 2 seconds at 5 minute intervals during a 60 minute time-course on an LmaxII-384 luminometer. The light output stabilizes after the initial luminescent flash common to all coelenterazine-based luciferases.

PRODUCT TABLE

Description	Product ID	Quantity
LightSwitch Assay Reagent™ (100 assays)	**LS010**	**10 mL**
LightSwitch Assay Reagent™ (1000 assays)	**LS100**	**100 mL**

* *For use with all GoClone plasmids*

GoClones™ Promoters	**S7- - - - -**	**5 ug tfx-ready DNA**
GoClones™ Promoter Controls	**S79- - - -**	**5 ug tfx-ready DNA**
GoClones™ 3'UTRs	**S8- - - - -**	**5 ug tfx-ready DNA**
GoClones™ 3'UTR Controls	**S89- - - -**	**5 ug tfx-ready DNA**

* *GoClones are designed for use with LightSwitch Assay Reagents*

LightSwitch Transfection Optimization Kit	**TFXOPT**	**1 kit**

FuGENE HD® Transfection Reagent	**F200**	**200 uL**
FuGENE HD® Transfection Reagent	**F500**	**500 uL**

PRODUCT DESCRIPTIONS

LightSwitch Luciferase Assay Reagent™

- LightSwitch Assay Reagents have been optimized specifically for RenSP GoClones

100 assay kit components:

Qty	Description	Storage
1 tube	Lyophilized 100X Substrate	-20°C for 6 months
150 uL	Substrate Solvent	room temp
10 mL	Assay Buffer	-20°C for 6 months
1	LightSwitch Protocol	

1000 assay kit components:

Qty	Description	Storage
1 tube	Lyophilized 100X Substrate	-20°C for 6 months
1 mL	Substrate Solvent	room temp
100 mL	Assay Buffer	-20°C for 6 months
1	LightSwitch Protocol	

LightSwitch Transfection Optimization Kit

The LightSwitch Transfection Optimization Kit includes the key reagents and protocols needed for optimizing transfection conditions for your cell line or condition of interest:

Kit components:

Qty	Description	Storage
1 tube	GoClone™ positive control	-20°C for 6 months
1 tube	GoClone™ negative control	-20°C for 6 months
200 uL	FuGENE HD tfx reagent	4°C for 6 months
10 mL	LightSwitch Assay Reagent™	-20°C for 6 months
1	Optimization Protocol	

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