LightSwitch Luciferase Assay System for Promoter GoClone Constructs

Overview

SwitchGear Genomics' LightSwitch Luciferase Assay System is a fully optimized reporter system that includes a transfection reagent (FuGeneHD), a genome-wide library of transfection-ready promoter-luciferase GoClone reporter constructs, and LightSwitch Luciferase Assay Reagents. The vectors utilize RenSP, a novel synthetic luciferase developed by SwitchGear Genomics with increased enzymatic activity (light output). The reporter gene is fused to a protein destabilization sequence (PEST) to decrease the half-life of the RenSP protein, enabling a detailed analysis of kinetic responses with a highly robust, bright signal. Below is a detailed workflow for performing SwitchGear assays with the LightSwitch Luciferase Assay System. Use all components of the LightSwitch system as recommended to obtain optimal reporter assay results.

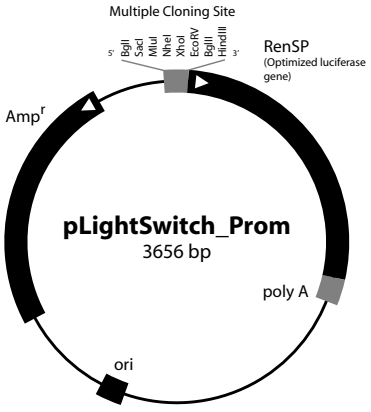
I. Choose a cell line
II. Understand control vector options
III. Choose control and GoClone experimental promoters from our online catalog
IV. Order recommended LightSwitch reagents and supplies. You may also order the Transfection Optimization Kit if you are just getting started
V. Perform transfections and measure luciferase activity - use our recommended protocol and helpful tips

I. Choose a cell line

Choosing a cell line is a critical component of successful transfection of reporter constructs. SwitchGear has optimized transfections with HT1080 cells and recommends that transfections be performed with this cell line when possible because of its transfection efficiency and reproducibility. The three main variables for a successful assay are seeding the appropriate number of cells, transfecting the optimal amount of the SwitchGear construct (plasmid), and timing the post-transfection assay period. For HT1080, Hela, and HCT116 cells, we recommend using 50ng of GoClone plasmid DNA per transfection and assaying luciferase activity ~24 hours post-transfection. For HepG2, we recommend using 100ng of GoClone plasmid DNA per transfection and assaying luciferase activity ~24 hours post-transfection. Please see our protocols for optimal cell seeding recommendations.

II. Optimize experimental conditions to control for non-specific effects

SwitchGear offers a number of control constructs to control for non-specific effects associated with your experimental treatment or condition. Start with one of our GoClone protocols for transfection of these vectors.



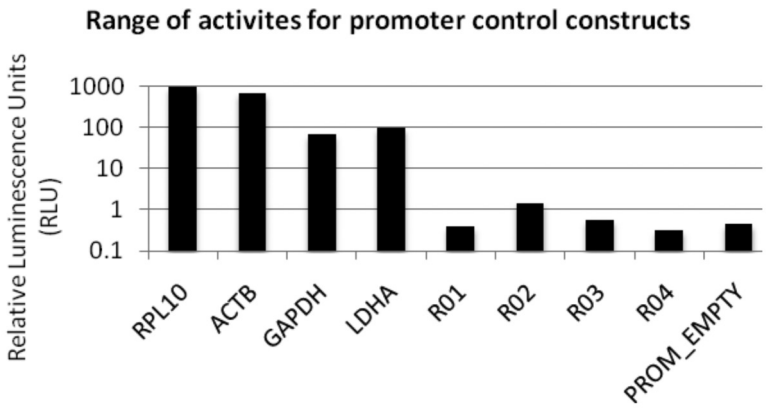
a. **Empty promoter vector:** The empty promoter vector contains the luciferase gene without a promoter. This construct serves as a one measure of background signal in the experiment.

b. **Housekeeping gene promoter vectors:** Housekeeping control constructs contain promoters for common housekeeping genes driving the luciferase reporter. These constructs serve as positive transfection controls and may also serve as controls for comparing signals between conditions if they are known to be unresponsive to the test condition.

Control name	Description	Catalog no.
RPL10_PROM	Ribosomal protein R10	S708908
ACTB_PROM	Beta-actin	S717678
GAPDH_PROM	Glyceraldehyde-3-phosphate dehydrogenase	S721624
LDHA_PROM	Lactose dehydrogenase A	S721613

c. Random control vectors: Random control constructs contain 1 kb non-conserved, non-genic, and non-repetitive fragments from the human genome cloned upstream of the luciferase reporter. These vectors produce slightly higher signals than the empty vectors and are considered optimal negative or background controls:

Control name	Catalog no.
R01_PROM	S790001
R02_PROM	S790002
R03_PROM	S790003
R04_PROM	S790004



Y-axis values shown in log scale. Control vector activity may vary depending on cell line, treatment condition, and protocol changes.

Figure 1. Example data and analysis of housekeeping and random promoter vectors: Random promoter constructs (R1, R2, R3, R4) serve as negative/background controls and establish baseline luciferase activity. The housekeeping promoter controls (R1, R2, R3, R4) serve as positive controls and give relative luciferase activity.

d. The LightSwitch Luciferase Assay System with SwitchGear's unique RenSP luciferase technology eliminates the need for co-transfection of a normalizing control in most cases.

III. Choose your LightSwitch Assay System GoClone experimental promoters and controls and buy online

a. Create an online account or log in to existing account.
The online product catalog of human gene regulatory sequences may be used to identify promoters and 3'UTRs corresponding to a single or multiple genes. You can order the corresponding cloned elements in transfection-ready luciferase reporter vectors as well as our optimized luciferase reagents.

b. Search for your GoClone promoters of interest by typing into the online catalog search box.
You can search for genes and their regulatory elements based on a variety of types of annotation such as gene IDs, symbols or aliases (using the official RefGene symbols will result in the most accurate search), accession numbers, and Gene Ontology terms.

c. Choose your LightSwitch System products: GoClone promoters, controls, transfection and luciferase reagents, and buy online.
You can also order the Transfection Optimization Kit if you are just getting started with reporter assays. Note that it is important that you order the LightSwitch Luciferase Assay Reagent as this system has been specifically optimized for our reporter plasmids with the RenSP reporter gene.

Frequently Asked Questions

- What quantities of plasmid are recommended for promoter vector studies?
  Quantities of plasmid needed typically depend on the cell line used for transfection and your chosen well format. See "Choosing a Cell Line" for guidelines.

- Where can I find a vector map and sequence?
  Please visit http://switchgeargenomics.com/resources/vector-maps/ for more vector information.

- How did SwitchGear select the promoters?
  SwitchGear has developed sophisticated algorithms for gene model construction and transcription start site prediction (see technical note on our website in the "Resources" section). For pathway sets, regulatory elements were assigned based on a combination of literature searches, expression studies, ChIP-chip and other binding data, and sequence motif analysis.

- Why does SwitchGear offer alternative promoters for some genes?
  Approximately 25% of all human genes have more than one transcription start site (TSS). A given transcript may initiate from different start sites in different cell types or under different cellular conditions or stimuli. Several TSSs may also be utilized in a single cell type. Our online catalog references many potential alternative TSSs for genes and ranks them based on experimental evidence.

IV. Recommended reagents and supplies

Item	Vendor	Catalog Number
White Tissue Culture Plates (96-well solid bottom)	VWR	82050-736
Clear Tissue Culture Plates (96-well)	VWR	353072
White Tissue Culture Plates (384-well solid bottom)	VWR	82051-278
Clear Tissue Culture Plates (384-well)	VWR	781186
FugeneHD Transfection Reagent	SwitchGear	F500
Opti-MEM	Invitrogen	31985-070
LightSwitch Luciferase Assay Reagent	SwitchGear	LS010, LS100
Foil Plate Sealing Tape	E&K Scientific	T592100
Breathable Plate Sealing Tape	E&K Scientific	T896100-S
Plate Luminometer	Molecular Devices	SpectraMax L

V. Recommended experimental set-up and protocols

SwitchGear offers sample protocols for the LightSwitch Luciferase Assay System's promoter GoClone constructs. Please visit the Resources section on our website to find detailed sample transfection protocols and other help guides.