LightSwitch Luciferase Assay System for 3'UTR GoClone Constructs

Overview

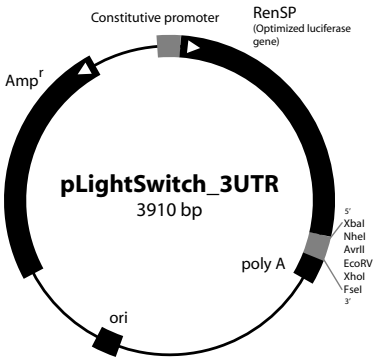
Introduction

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 3'UTR-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 protein is fused to a PEST sequence 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.

200bp-3kb human 3'UTR fragments are cloned into the MCS of the vector shown below to produce a hybrid transcript that contains the luciferase gene fused to the UTR of interest.

I. Choose a cell line

Choosing a cell line is a critical component of successful transfection of the reporter constructs. SwitchGear has optimized tranfections with HT1080 cells and recommends that all transfections be performed with this cell line. The three main variables for a successful assay are seeding the appropriate number of cells, transfecting the optimal amount of the SwitchGear GoClone construct (plasmid), and timing the post-transfection assay period. For HT1080, Hela, and HCT116 cells, we recommend using 50ng of 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 transfection protocols at http://switchgeargenomics.com/resources/protocols/ for optimal cell seeding recommendations.



II. Understanding GoClone control vectors

SwitchGear offers a number of GoClone control constructs to control for non-specific effects associated with your experimental treatment or condition. **We recommend including at least one control of each type listed below to minimize the possibility of experimental conditions interacting with a control.**

a. **Empty 3'UTR vector:** The empty 3'UTR vector contains only the luciferase gene (RenSP) and its constitutive promoter. This construct may serve as a positive control for the transfection.

b. Housekeeping gene 3'UTR vectors: Housekeeping control constructs contain the 3'UTRs for common housekeeping genes cloned downstream of the luciferase reporter.

Control name	Description	Catalog no.
ACTB_3UTR	Beta-actin	S804753
LDHA_3UTR	Lactose dehydrogenase A	S804821
PPIA_3UTR	Peptidylprolyl isomerase A	S810112
GAPDH_3UTR	Glyceraldehyde-3-phosphate dehydrogenase	S801378

c. Random 3'UTR control vectors: Random control constructs contain non-conserved, non-genic, and non-repetitive human genomic fragments.

Control name	Catalog no.
R01_3UTR	S890001
R02_3UTR	S890002
R03_3UTR	S890003
R04_3UTR	S890004

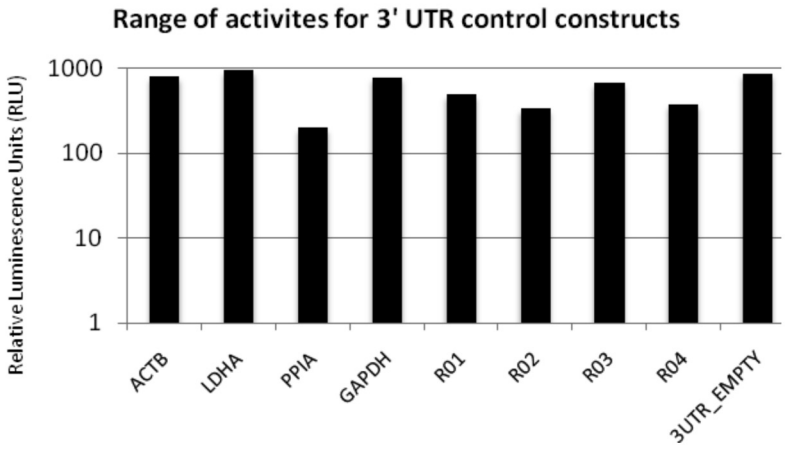


Figure 1. Example data for GoClone 3'UTR control vectors. Here 50ng of each vector was transfected with FuGENE HD into HT1080 cells. Absolute and relative activity will vary depending on cell line, experimental conditions, and transfection reagents.

Values shown in log scale. Relative activities will differ depending on cell type and conditions.

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 into existing account.
The online product catalog of human gene regulatory sequences may be used to identify promoters and 3'UTRs corresponding to a single gene or a large collection of genes that are most relevant to your research. You can then simply order the corresponding cloned elements in transfection-ready luciferase reporter vectors.

b. Search for your 3'UTR GoClones of interest by typing into the online catalog search box.
You can search for the promoters and 3'UTRs of a single gene or many genes at once based on the following types of annotation: gene IDs, symbols (or aliases), accession numbers, and Gene Ontology terms.

c. Choose your LightSwitch System products: GoClone 3'UTRs, 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 are recommended for 3'UTR GoClones?
  Quantities of plasmid needed typically depend on the cell line used for transfection. See "Choosing a Cell Line" for guidelines.

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

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) with lid	VWR	353072
LightSwitch Luciferase Assay Reagent	SwitchGear	LS010, LS100
DharmaFECT® Duo Transfection Reagent (0.75mL)	Dharmacon	T-2010-02
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

- Including the **empty vector control**: A very high luciferase signal results from this "no UTR" vector that contains only the luciferase gene and the constitutive promoter. This vector serves as a positive control for transfection.

- Including the **random controls** and **housekeeping controls**: For best experimental results, we recommend measurement of signal from each construct before and after a given treatment. In addition, we recommend using the SwitchGear housekeeping gene vectors and random control vectors containing random genomic fragments for accurately separating sequence specific vs. non-specific effects. For example, if you observe a 5-fold decrease in the average of the control readings with addition of a miRNA mimic compared to a non-targeting control molecule, you would need to observe a decrease greater than 5-fold from an experimental construct to say you see UTRX+miRNA-specific effects.

- Co-transfection of any additional oligos/plasmids with SwitchGear reporter constructs reduces overall luciferase signals in a non-specific manner. For miRNA experiments, compare the effects if an miRNA mimic or inhibitor with a non-targeting molecule with similar properties (e.g. non-targeting controls from Dharmacon).

- Use optimized reagents for the luciferase assay: use the LightSwitch Luciferase Assay Reagent for optimal results. Use our recommended 3'UTR construct transfection and miRNA co-transfection protocols at http://switchgeargenomics.com/resources/protocols.