

Getting started with your SwitchGear UTR assays

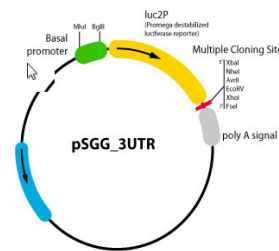
Overview:

- I. Introduction
- II. Choosing a cell line
- III. Understanding control vector options.
- IV. Choose your control and experimental UTR constructs and request a quote
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- VI. Set up the control and reporter assay experimental design
- VII. Perform transfection and measure luciferase activity - use our recommended protocol and helpful tips

Introduction

SwitchGear Genomics offers a genome-wide library of transfection-ready, optimized 3'UTR luciferase-based reporter constructs. The cassettes utilize luc2P, a destabilized form of luciferase developed by Promega for its reporter gene assays. This optimized luciferase protein has a half-life of ~1 hour enabling a detailed analysis of kinetic responses with a highly robust signal. Each clone has been purified and sequence-verified during our extensive quality control process.

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



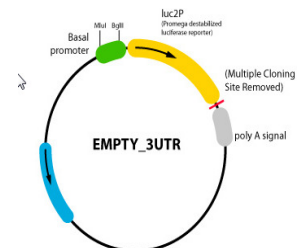
I. Choosing a cell line

Choosing a cell line is a critical component of successful transfection of the reporter constructs. SwitchGear has optimized transfections 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 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 plasmid DNA per transfection and assaying luciferase activity ~24 hours post-transfection. Please see the transfection protocol at <http://switchgeargenomics.com/resources/protocols/> for optimal cell seeding recommendations.

II. Understanding control vectors

SwitchGear offers a number of control constructs to optimize experimental conditions and to normalize for non-specific events that may cause signal variation. **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 (Luc2P) and its constitutive promoter with the multiple cloning site removed. 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	Coordinates
ACTB_3UTR	Beta-actin	chr7-: 5340002 - 5340681
LDHA_3UTR	Lactose dehydrogenase A	chr11+: 18385382 - 18386064
PPIA_3UTR	Peptidylprolyl isomerase A	chr7+: 44614226 - 44616012
GAPDH_3UTR	Glyceraldehyde-3-phosphate dehydrogenase	chr12+: 6517534 - 6517833

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

Control name	Coordinates
R01_3UTR	chr13+: 82190847 - 82193385
R02_3UTR	chr2+: 122701379 - 122703948
R03_3UTR	chr13+: 82120892 - 82121673
R04_3UTR	chr22+: 26144830 - 26145608

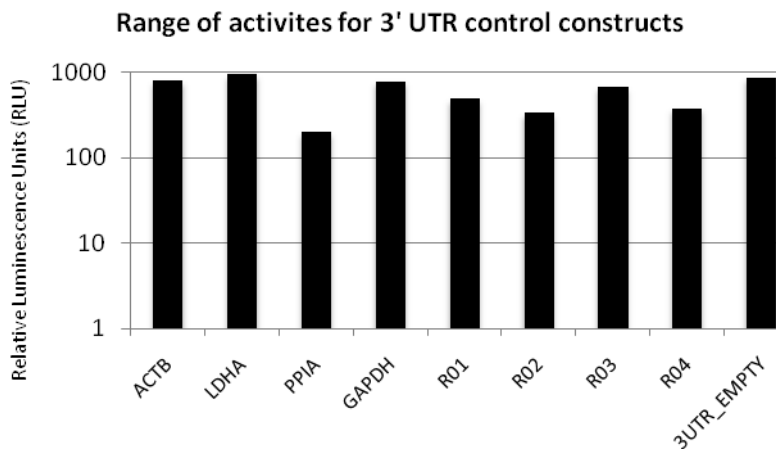


Figure 1. Example data for SwitchGear 3'UTR control vectors. Here 50ng of each vector was transfected with FuGene6 (Roche) into HT1080 cells. Absolute and relative activity will vary depending on cell line, experimental conditions, and transfection reagents.

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

III. Choose your control and experimental promoters and request a quote

- Create a SwitchDB account or log into existing account.** SwitchDB is SwitchGear's database and online product catalog of human gene regulatory sequences. Use SwitchDB 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.
- Search for your promoters of interest by typing into the SwitchDB search box.** SwitchDB search allows you to search for genes and their regulatory elements based on a variety of different types of annotation.

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, Gene Ontology terms using ID, and/or gene descriptions. <Link>

c) **Choose your 3'UTR constructs, controls, reagents, and request a quote.**

Additional 3'UTR construct information

- What quantities are recommended for 3'UTR vectors?
Quantities of plasmid typically depend on the cell line used for transfection. See “Choosing a Cell Line” for guidelines.
- Where can I find a vector map?
See <http://switchgeargenomics.com/resources/vector-maps/>

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
Steady-Glo Luciferase Assay Reagent	Promega	E2510, E2520
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	LMaxII-384

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 our recommended UTR construct transfection protocol at <http://switchgeargenomics.com/resources/protocols/>