

Getting started with your SwitchGear promoter assays

Overview

SwitchGear Genomics offers a genome-wide library of transfection-ready, optimized promoter-luciferase reporter constructs. The cassettes utilize luc2P, a destabilized form of luciferase developed by Promega for its reporter gene assays. The 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. Below is a detailed workflow outline for performing the SwitchGear assays:

- I. Choose a cell line
- II. Understand control vector options.
- III. Choose control and experimental promoters and request a quote
- IV. Order recommended reagents and supplies
- V. Perform transfection and measure luciferase activity - use our recommended protocol and helpful tips

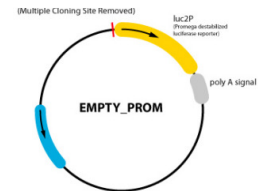
I. Choose a cell line

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 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 protocol for optimal cell seeding recommendations.

II. Understand control vector options

SwitchGear offers a number of control constructs to optimize experimental results. Use the Promoter Construct Protocol 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	Coordinates
RPL10_PROM	Ribosomal protein R10	chrX+:153146645 - 153147658
ACTB_PROM	Beta-actin	chr7-:5343428 - 5344428
GAPDH_PROM	Glyceraldehyde-3-phosphate dehydrogenase	chr12+: 6513163 - 6514226
LDHA_PROM	Lactose dehydrogenase A	chr11+: 18372059 - 18372891

- 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	Coordinates
R01_PROM	chr11+:119015626-119016592
R02_PROM	chr1+:226079717-226080649
R03_PROM	chr6+:15604416-15605350
R04_PROM	chr16+:1645930-1646850

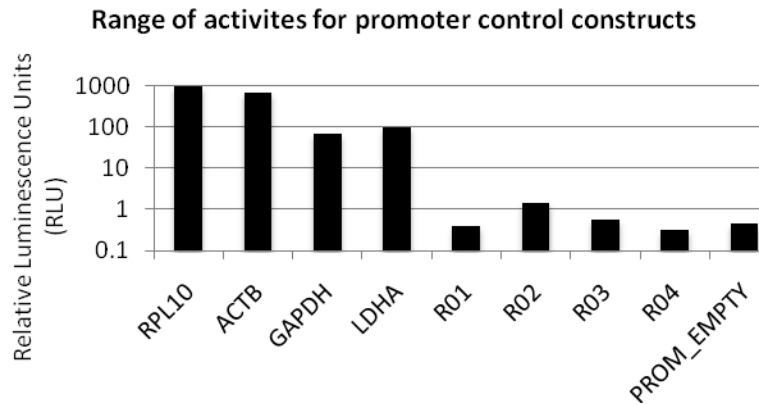


Figure 1. Expected data and analysis of housekeeping and random promoter vectors: Random promoter constructs (R1, R2, R3, R4) serve as negative/background controls and establish baseline luciferase activity. The housekeeping promoter controls (R1, R2, R3, R4) serve as positive controls and give relative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

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

SwitchGear also offers a set of eight technical control constructs for larger-scale studies to normalize for plate-to-plate variation. Studies involving multiple plates per condition should include these 8 controls. These plate controls represent a combination of housekeeping and random sequence controls.

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

- a) **Create a SwitchDB account or log in to 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.
- b) **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 (using the official RefGene symbols will result in the most accurate search), accession numbers, Gene Ontology terms using ID, and/or gene descriptions. <Link>
- c) **Choose your promoters, controls, reagents, and request a quote.**

Additional promoter construct information

- What quantities of plasmid are recommended for promoter vector studies?
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?
Please visit <http://switchgeargenomics.com/resources/vector-maps/> for a map.

- How did SwitchGear select the promoters?
SwitchGear has developed sophisticated algorithms for gene model construction and transcription start site prediction. See the [technical note](#) for more information. 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. SwitchDB catalogs many potential alternative TSSs for genes and ranks them based on experimental evidence.
- Which promoter should I choose when there are alternatives?
Choosing the correct TSS is important to obtain relevant results from a promoter assay. The search results in SwitchDB display the coordinates for each clone, and the primary promoter for each gene is the promoter that has the strongest experimental evidence for containing a transcription start site for that gene. In the absence of any other information, you are best off starting with the primary promoter displayed in the search results for a given gene. When alternative promoters for a gene exist, you can view these in the search result table by clicking on the “view alternatives” link. Clicking on the coordinates in the search result table opens a window in the UCSC genome browser of the exact sequence that is cloned in the vector. This allows you to see the cloned fragment in the context of other genomic features. Using this information, you can decide which of the alternative promoters to choose if you are looking for a promoter that corresponds to certain genomic features of interest.

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
Fugene-6 Transfection Reagent	Roche	11814443001
TransIT-LT1 Transfection Reagent	Mirus	MIR2310s
Arrest-In Transfection Reagent	Open Biosystems	ATR1740
Opti-MEM	Invitrogen	31985-070
Steady-Glo Luciferase Assay Reagent	Promega	E2510, E2520
Foil Plate Sealing Tape	E&K Scientific	T592100
Breathable Plate Sealing Tape	E&K Scientific	T896100-S
Plate Luminometer	Molecular Devices	LMaxII-384

In some cell lines, some transcription factors such as androgen receptors and estrogen receptors are either not expressed or are naturally expressed in low levels. In such cases, we recommend using a different cell line or conducting co-transfection experiments with of cDNA overexpression constructs.

V. Recommended experimental set-up and protocols

SwitchGear offers sample protocols for promoter constructs for general as well as specific test conditions. Please visit our Resources section on our website to find detailed sample transfection protocols and other help guides.