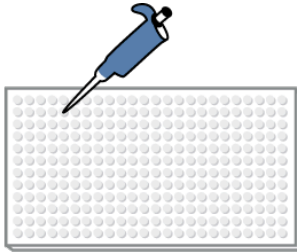
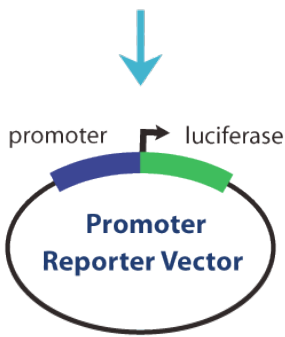


PROMOTER CONSTRUCTS: SAMPLE PROTOCOL FOR ADHERENT CELLS

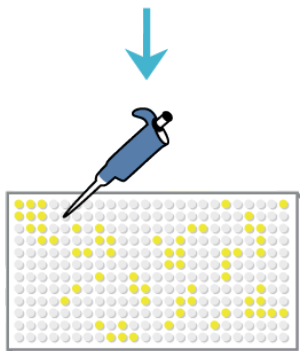
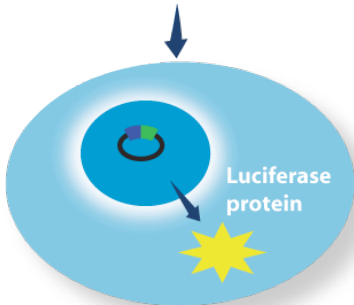
SwitchGear promoter reporter assay workflow



**Step 1:** Seed cells in plate format



**Step 2:** Transfect SwitchGear reporter constructs into the cells. Apply stimulus of interest.



**Step 3:** Add luciferase reagent and read on luminometer.

## DAY 1

**Goal:** Seed cells to yield 40-80% confluence after 24 hours

- Seed the appropriate number of cells for the assay (see Table 1).
- Seed the appropriate number of cells for assessing desired confluence in parallel (see Table 1).
  - Note: Use a 96- or 384-well white plate for the assay plate. Use a clear plate to assess confluency.

Table 1

Number of wells in plate	Total volume for cell seeding*	Number of cells/well that yields target confluency of 40-80%*
96	100 $\mu$ l	2,000-10,000
384	30 $\mu$ L	1,000-3,000

\* The recommendations in Table 1 were optimized using Fugene-6 (Roche) transfection reagents. Consult other manufacturer recommendations for optimal cell seeding conditions for target confluence.

## DAY 2

**Goal:** Prepare reporter constructs and transfect into the seeded cells

### Prepare constructs

- Thaw SwitchGear constructs (plasmid DNA) at room temperature. Mix well.
- Centrifuge the tubes or plates to remove condensation from lid.

### Transfections

- Combine the reagents in Table 2 for each transfection. Conduct at least three replicate transfections per construct.
- We recommend at least 50ng of plasmid DNA per well for 96-well experiments and 30ng of plasmid DNA per well for 384-well experiments.

**Tip:** First, make a master mix of transfection reagent + OptiMEM and add to each construct (add transfection reagent to OptiMEM without touching sides of tube). Then, for each construct, make a master mix of DNA, transfection reagent, OptiMEM for desired number of replicates (make at least 1.5 extra aliquots to account for pipetting error and evaporation). See Appendix for the Experimental Design Example.

Table 2

Component	Per well (96-well format)	Per well (384-well format)
Transfection Reagent*	0.30 $\mu$ L	0.12 $\mu$ L
Opti-MEM (serum free media)	3.03 $\mu$ L	1.88 $\mu$ L
SwitchGear plasmid DNA construct (30ng/ $\mu$ L)	1.67 $\mu$ L	1.00 $\mu$ L
<b>TOTAL</b>	<b>5.00<math>\mu</math>L</b>	<b>3.00<math>\mu</math>L</b>

\* Transfection reagents known to work with this protocol include Fugene-6 (Roche), TransIT-LT1 (Mirus), and Arrest-In (Open Biosystems)

- Mix DNA, transfection reagent, OptiMEM combination well. Let sit at room temperature for 30-60 minutes.
- Gently drip 5 $\mu$ L or 3 $\mu$ L (96- or 384-well formats) onto seeded cells
- Shake plate gently, cover with lid or breathable sealing tape
- Put back in incubator for 24-48 hours (+/- stimulus of interest)

**Note:** *Cells that transfect poorly may require the use of more DNA and/or longer incubation times. We recommend optimizing conditions for each cell line before beginning large-scale experiments.*

### DAY 3

**Goal:** Measure luciferase activity

- Remove plate from incubator and bring to room temperature
- Add 100 $\mu$ L (96-well format) or 30 $\mu$ L (384-well format) Promega Steady-Glo Luciferase Assay Reagent, cover with lid or foil tape, and incubate for 15-30 minutes in a dark area.
- Read in a plate luminometer.

## APPENDIX

Experimental Design Example (for a 96-well plate with triplicate transfections for each construct):

**Step 1:** Make master mix 1.

Master Mix 1: Opti-MEM and Transfection Reagent – includes extra amounts for pipetting error

Number of constructs	Transfection Reagent	Opti-MEM (serum free media)	Total volume of Master Mix 1
For 1 construct in triplicate	1.35 $\mu\text{L}$	13.65 $\mu\text{L}$	15 $\mu\text{L}$
For 10 constructs in triplicate	13.5 $\mu\text{L}$	136.5 $\mu\text{L}$	150 $\mu\text{L}$

**Step 2:** Make master mix 2.

Master Mix 2: Make for each Switchgear construct (plasmid DNA) + Master Mix 1

	Plasmid DNA	Master Mix 1	Total volume of Master Mix 2
For each construct in triplicate (recommended)	6.68 $\mu\text{L}^*$	13.32 $\mu\text{L}$	20 $\mu\text{L}$

- 1.67  $\mu\text{L}$  \* 4 replicates (extra replicate for pipetting error)

**Step 3:** Aliquot 5  $\mu\text{L}$  of Master Mix 2 onto seeded cells per transfection as directed to achieve triplicate transfections for each construct.

## EXAMPLE CATALOG NUMBERS

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
	VWR	47749-926
Plate Luminometer	Molecular Devices	LMaxII-384