REPORTER CONSTRUCT
TRANSFECTION:
SAMPLE PROTOCOL
FOR ADHERENT CELLS
SWITCHGEAR 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.

All SwitchGear reporter constructs 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.
GETTING STARTED

- Use low passage number cells (highly passaged cells generally yield noisy data)
- Optimize the general transfection protocol for your specific cell line and experimental design
  
  *We recommend optimizing with at least one strong positive control construct and one negative control construct (e.g. ACTB_prom and R01_prom)*
  
  - Test a variety of cell seeding densities
  
  *The ideal confluency for transfection varies by cell line*
  
  - Evaluate at least two FuGENE HD transfection reagent to DNA ratios
    
    3:1 and 6:1 work for many cell types (nL FuGENE HD: ng plasmid DNA)
  
  - Compare overall signal and variability between replicate transfections from cells at 24 and 48 hours post-transfection

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**

<table>
<thead>
<tr>
<th>Number of wells in plate</th>
<th>Total volume for cell seeding</th>
<th>Number of cells/well that yields target confluency of 40-80%</th>
</tr>
</thead>
<tbody>
<tr>
<td>96</td>
<td>100µl</td>
<td>5,000-15,000</td>
</tr>
<tr>
<td>384</td>
<td>30µL</td>
<td>2,500-10,000</td>
</tr>
</tbody>
</table>

**Tip:** HT1080 cells have been optimized with SwitchGear assays and are recommended for transfection. Use standard media recommended by ATCC. Set up enough replicate transfections for control and treatment conditions.

DAY 2

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

**Prepare constructs and reagents**

- Thaw SwitchGear constructs (plasmid DNA) at room temperature. Mix well.
- Centrifuge the tubes or plates of DNA to remove condensation from lid.
- Bring FuGENE HD transfection reagent to room temperature, mix well.
- Pre-warm Opti-MEM media in a 37°C water bath.
Transfections

- Combine the reagents in Table 2 for each transfection. Conduct at least three replicate transfections per construct in each condition (e.g. untreated and treated). Volumes listed in the table are for a single replicate transfection in a single well. 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.

### Table 2

<table>
<thead>
<tr>
<th>Component</th>
<th>Per well (96-well format)</th>
<th>Per well (384-well format)</th>
</tr>
</thead>
<tbody>
<tr>
<td>FuGENE HD* Transfection Reagent</td>
<td>0.15µL</td>
<td>0.09µL</td>
</tr>
<tr>
<td>Opti-MEM (serum free media)</td>
<td>3.18µL</td>
<td>1.91µL</td>
</tr>
<tr>
<td>SwitchGear plasmid DNA construct (30ng/µL)</td>
<td>1.67µL</td>
<td>1.00µL</td>
</tr>
<tr>
<td>TOTAL</td>
<td>5.00µL</td>
<td>3.00µL</td>
</tr>
</tbody>
</table>

* This FuGENE HD to plasmid DNA ratio shown in this example is 3:1 (150nL FuGENE HD to 50ng plasmid DNA)

- Mix DNA, FuGENE HD, OptiMEM combination well. Let sit at room temperature for 30 minutes
- Gently drip 5µL or 3µL (96- or 384-well formats) onto seeded cells
- Shake plate gently, cover with lid or breathable sealing tape and return to incubator
- Incubate for 24 to 48 hours before assaying
- Alternatively, incubate cells with transfection mix for 16 to 24 hours before changing conditions or applying a stimulus

**DAY 3**

**Goal:** Measure luciferase activity

**Note:** Luciferase assays may be conducted immediately or the plates may be stored at -80. If using frozen (stored) plates, thaw and bring to room temperature before assaying.

- Remove plate from incubator and bring to room temperature
- Add 100µL (96-well format) or 30µL (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: transfection set-up for hypoxia inductions in 96-well plate format (triplicate transfections for each construct in each condition)

Step 1: Calculations (including extra for pipetting error and evaporation)

Variables: X replicate transfections per construct and Y constructs (experimental + controls)

Here: 9 total replicates per construct (3 replicates per condition * 3 conditions - untreated, hypoxia, DFO)

24 total constructs (16 experimental + 8 controls)

Transfection mix calculations

<table>
<thead>
<tr>
<th></th>
<th>Per replicate well (96-well format)</th>
<th>Per construct (9 replicates + 1.5 extra) x 10.5</th>
</tr>
</thead>
<tbody>
<tr>
<td>FuGENE HD</td>
<td>0.15µL</td>
<td>1.57µL</td>
</tr>
<tr>
<td>Opti-MEM (serum free media)</td>
<td>3.18µL</td>
<td>33.39µL</td>
</tr>
<tr>
<td>SwitchGear plasmid DNA construct (30ng/µL)</td>
<td>1.67µL</td>
<td>17.54µL</td>
</tr>
<tr>
<td><strong>TOTAL</strong></td>
<td><strong>5.00µL</strong></td>
<td><strong>52.5µL</strong></td>
</tr>
</tbody>
</table>

Step 2: Make Master Mix #1 – OptiMEM + FuGENE HD mix that will be added to each DNA aliquot

<table>
<thead>
<tr>
<th></th>
<th>Per construct (9 replicates + 1.5 extra)</th>
<th>Master Mix #1 (24 constructs + 10% extra) x 26.4</th>
</tr>
</thead>
<tbody>
<tr>
<td>FuGENE HD</td>
<td>1.57µL</td>
<td>41.45µL</td>
</tr>
<tr>
<td>Opti-MEM (serum free media)</td>
<td>33.39µL</td>
<td>881.50µL</td>
</tr>
<tr>
<td>SwitchGear plasmid DNA construct (30ng/µL)</td>
<td>17.54µL</td>
<td>Unique to each</td>
</tr>
<tr>
<td><strong>TOTAL</strong></td>
<td><strong>52.5µL</strong></td>
<td><strong>922.95µL</strong></td>
</tr>
</tbody>
</table>
**Step 3:** Make Master Mix #2 – Master Mix #1 + DNA for all replicates of one construct

<table>
<thead>
<tr>
<th>Item</th>
<th>Vendor</th>
<th>Catalog Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>Master Mix #1</td>
<td></td>
<td>34.96µL</td>
</tr>
<tr>
<td>SwitchGear plasmid DNA construct (30ng/µL)</td>
<td></td>
<td>17.54µL</td>
</tr>
<tr>
<td><strong>TOTAL</strong></td>
<td></td>
<td><strong>52.5µL</strong></td>
</tr>
</tbody>
</table>

**Step 4:** For each construct, gently drip 5µL of Master Mix #2 onto each of the 9 replicate wells

**Example Catalog Numbers**

<table>
<thead>
<tr>
<th>Item</th>
<th>Vendor</th>
<th>Catalog Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>White Tissue Culture Plates (96-well solid bottom)</td>
<td>VWR</td>
<td>82050-736</td>
</tr>
<tr>
<td>Clear Tissue Culture Plates (96-well)</td>
<td>VWR</td>
<td>353072</td>
</tr>
<tr>
<td>White Tissue Culture Plates (384-well solid bottom)</td>
<td>VWR</td>
<td>82051-278</td>
</tr>
<tr>
<td>Clear Tissue Culture Plates (384-well)</td>
<td>VWR</td>
<td>781186</td>
</tr>
<tr>
<td>Opti-MEM</td>
<td>Invitrogen</td>
<td>31985-070</td>
</tr>
<tr>
<td>Steady-Glo Luciferase Assay Reagent</td>
<td>Promega</td>
<td>E2510, E2520</td>
</tr>
<tr>
<td>Foil Plate Sealing Tape</td>
<td>E&amp;K Scientific</td>
<td>T592100</td>
</tr>
<tr>
<td>Breathable Plate Sealing Tape</td>
<td>E&amp;K Scientific</td>
<td>T896100-S</td>
</tr>
<tr>
<td>Plate Luminometer</td>
<td>Molecular Devices</td>
<td>LMaxII-384</td>
</tr>
<tr>
<td>FuGENE HD Transfection Reagent</td>
<td>SwitchGear</td>
<td>F500</td>
</tr>
<tr>
<td>ACTB promoter positive control reporter construct</td>
<td>SwitchGear</td>
<td>S117678</td>
</tr>
<tr>
<td>R01 negative control reporter construct</td>
<td>SwitchGear</td>
<td>S190001</td>
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