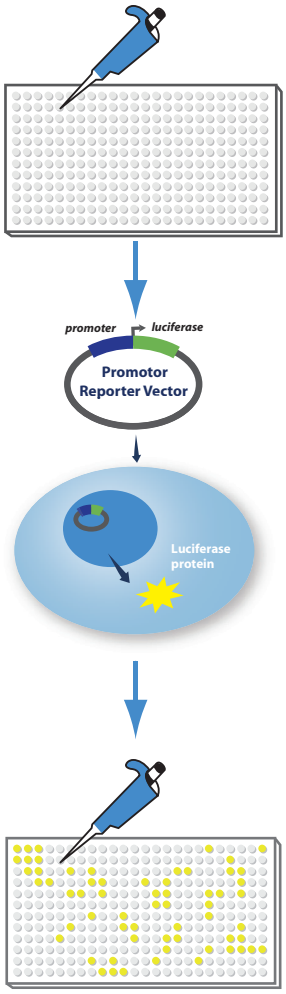
LiGHTSW!TCH
Luciferase Assay System

Protocol

High-throughput Transfection Protocol for GoClone Reporter Assays



LightSwitch Luciferase Assay System

GoClone Reporter Assay Workflow

Step 1: Simultaneously seed and transfect SwitchGear GoClone reporter constructs in plate format. Apply stimulus of interest.

Step 2: Add LightSwitch Luciferase Assay Reagent and read on luminometer.

All SwitchGear reporter constructs utilize RenSP, a destabilized form of a novel luciferase developed by SwitchGear Genomics 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.

Introduction

The LightSwitch Luciferase Assay System is a fully optimized reporter system that includes a transfection reagent (FuGENE HD), GoClone constructs utilizing the RenSP luciferase gene, and LightSwitch Luciferase Assay Reagents. Use all components of the LightSwitch System as recommended to obtain optimal reporter assay results.

This protocol is for simultaneous cell seeding and transfection of adherent cells. This method offers time savings (time to results is reduced from 3 days to 2 days) and achieves lower variability between transfection replicates compared to the standard protocol. Before attempting this protocol, you should optimize transfection based on the standard two-step protocol, especially if you do not have previous transfection experience or are working with a new cell line.

This is the preferred protocol for use in high-throughput screening applications.

Attention! Before attempting this protocol, please make sure that:

- You use the optimized reagents from the LightSwitch Luciferase Assay System including the LightSwitch Luciferase Assay Reagents and FuGENE HD for best results.
- The cell line is transfectable.
- You are comfortable with your previous transfection results with the cell line.

PROTOCOL FOR SIMULTANEOUS SEED AND TRANSFECT

DAY 1

Goal: Seed and transfect cells.

Tip: Table 1 lists the volumes to be added per well. See Appendix for an Experimental Design Example for tips on making master mixes for reagent addition to multiple wells.

Prepare Constructs

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

Transfections

5. Combine the reagents listed in Table 1 for each transfection. Conduct at least three replicate transfections per GoClone 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 GoClone plasmid DNA per well for 96-well experiments and 30ng of GoClone plasmid DNA per well for 384-well experiments.

Table 1

The FuGENE HD to plasmid DNA ratio shown in this example is 3:1 (150nL FuGENE HD to 50ng GoClone plasmid DNA). The most effective ratio may vary by cell line.

	Per well (96-well format)	Per well (384-well format)
FuGENE HD Transfection Reagent	0.15µL	0.09µL
Opti-MEM (serum free media)	3.18µL	1.91µL
GoClone plasmid DNA construct (30ng/µL)	1.67µL	1.00µL
TOTAL	**5.00µL**	**3.00µL**

6. Mix DNA, FuGENE HD, OptiMEM combination well. Add FuGENE to OptiMEM without touching the sides of the tube. Let sit at room temperature for 30 minutes.

Count and resuspend cells

7. Determine the appropriate number of cells for the assay (see Table 2)
   - Note: Use 1.5-2 times the number of cells you would normally use to achieve 50-80% confluency 24 hours after seeding (e.g. if you normally seed 5,000 cells per well, increase this to 7,500-10,000)

Table 2

Number of wells in plate	Number of cells/well
96	7,500-20,000
384	3,500-10,000

8. Resuspend the appropriate number of cells in pre-warmed media for a total volume of 95µL per well multiplied by the total number of wells (96-well format) or 47µL per well (384-well format).
   - Note: Be sure to include extra volume for pipetting error

Mix and aliquot re-suspended cells and transfection mixtures

9. For each construct, aliquot a volume of resuspended cells sufficient for all transfection replicates into an individual well of a deep-well block.
   - Note: Depending on the number of transfection replicates, larger or smaller blocks may be used. Please check with block manufacturers to determine the maximum useful volume in individual wells of a particular block.
10. After the DNA and transfection mixtures have incubated at room temperature for 30 minutes, transfer the mixtures to a deep well block containing re-suspended cells.
    - Note: An alternative to transferring transfection mixtures is to prepare transfection mixtures in a deep well block and then add re-suspended cells.
11. Mix well and aliquot the appropriate volume of the mixture containing the transfection and resuspended cells into replicate wells (Table 3).

Table 3

Number of wells in plate	Volume/well
96	100 µL
384	50 µL

12. Shake plate gently, cover with lid or breathable sealing tape.
13. Incubate cell and transfection mix for 24-48 hours depending on cell type.

DAY 2 or 3

Goal: Measure luciferase activity with LightSwitch Assay Reagents

Note: Luciferase assays may be conducted immediately or the plates may be frozen at -80˚C (freezing generally increases cell lysis and luciferase signal). If using frozen plates, thaw and bring to room temperature before assaying.

1. Remove plate from incubator and bring to room temperature.
2. Prepare LightSwitch Assay Reagents (for LS010 kit, protocols of other kit sizes may be found online):

   A. Reconstitute 100X Substrate by adding 100µL of Substrate Solvent to tube of lyophilized Assay Substrate. *Protect from light and minimize time at room temperature. 100X Substrate may be stored at -20C for 2-3 weeks. For best results, use freshly reconstituted substrate.*

   B. Prepare Assay Solution by thawing 10mL bottle of Assay Buffer in room temperature water bath and add 100µL of reconstituted 100X Substrate prior to use. *For best results, avoid additional freeze-thaw cycles. To thaw re-frozen buffer, incubate in a warm (37C) water bath for at least 1 hour and mix well to ensure that all components go back into solution.*

3. Use a multi-channel pipettor to add 100µL LightSwitch Assay Solution (buffer+substrate) directly to each sample well in a white 96-well plate.
   If cells were grown in another plate or flask format, transfer samples to a white 96-well plate in 100µL total volume (media or PBS).
4. Cover plate, protect from light, and incubate for 30 minutes at room temperature.
   If assaying more than one plate, stagger addition of assay solution so that each plate incubates for 30 minutes before reading.
5. Read each well for 2 seconds in a plate luminometer (SpectraMax L or equivalent).

APPENDIX

Experimental Design Example

(1 GoClone construct X 96 compounds, 96-well format)

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

1 construct X 96 compounds X 3 replicate transfections per compound
= 288 wells to transfect
Make extra transfection and cell mixture to account for pipetting error
= 320 total replicates

Transfection mix calculations:

	Per well (96-well format)	x 320
FuGENE HD	0.15µL	48.0 µL
Opti-MEM (serum free media)	3.18µL	1017.6 µL
GoClone plasmid DNA construct (30ng/µL)	1.67µL	534.4 µL
TOTAL	**5.00µL**	**1600.0 µL**

Step 2: Mix OptiMEM, FuGENE HD, and GoClone DNA combination well. Incubate at room temperature for 30 minutes. Add FuGENE to OptiMEM without touching the sides of the tube.

Step 3: Resuspend a sufficient number cells for all replicate transfections.

	Per well	x 320
HT1080 cells in 95 µL of complete media	10,000 cells in 95µL	$3.2x10^6$ cells in 30.4mL

Step 4: Add the transfection mix (1.6mL) to 30.4mL cells in media and mix well.

Step 5: Use a multi-channel pipettor to aliquot 100µL cell + transfection mix to each well of a 96-well white TC plate. Also add a 100µL of the mix to a few wells of a clear 96-well TC plate to assess confluence and cell viability.

Step 6: Add compounds to each of the 3 replicate wells.

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
Opti-MEM	Invitrogen	31985-070
LightSwitch Luciferase Assay Reagent	SwitchGear	LS010
Foil Plate Sealing Tape	E&K Scientific	T592100
Breathable Plate Sealing Tape	E&K Scientific	T896100-S
Plate Luminometer	Molecular Devices	SpectraMax L
FuGENE HD Transfection Reagent	SwitchGear	F500
ACTB promoter positive control reporter construct	SwitchGear	S717678
R01 negative control reporter construct	SwitchGear	S790001