TransLucent™ Reporter Vectors

Cat. #LR0001-LR00090
Product User Manual
Released 05/04/04

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1. INTRODUCTION

Eukaryotic gene expression is regulated by a wide variety of developmental and environmental stimuli. First, an extracellular signaling molecule binds to a specific receptor. The signal is then transmitted through a series of molecular cascades, which activate or deactivate specific transcription factors (TFs) that regulate gene expression. The expression of any given gene is controlled by multiple transcription factors, which in turn are modulated by multiple signal transduction pathways. These pathways are also interconnected by molecular "crosstalk" (1–2).

In order to fully understand the mechanisms underlying gene expression, we must tease apart these multiple layers of regulation. An important step toward this goal is studying the activation of transcription factors. A popular method for this is the use of transcription reporter vectors, which contain a *cis*-acting DNA response element upstream of a reporter gene, such as luciferase. By transfecting such a vector into cells, you can examine the effects of various stimuli on a given signaling pathway *in vivo*.

TransLucent Reporter Vectors

Panomics' TransLucent Reporter Vectors are designed to monitor transcription factor binding activity *in vivo* through the use of the standard luciferase assay. Each TransLucent Reporter Vector contains multiple repeats of a specific transcription factor binding element. Binding at this recognition site by the corresponding transcription factor results in the expression of luciferase, which initiates a powerful bioluminescent reaction when exposed to its substrate, luciferin. Light emitted from the chemical reaction is directly proportional to the amount of enzyme and thus the binding activity of the targeted transcription factor.

Currently, TransLucent Reporter Vectors are available for almost 90 transcription factors, all of which are also covered by our TranSignal Protein/DNA Arrays. See www.panomics.com for an up-to-date list of all of the TransLucent Reporter Vectors.

Principle of TransLucent Reporter Vectors

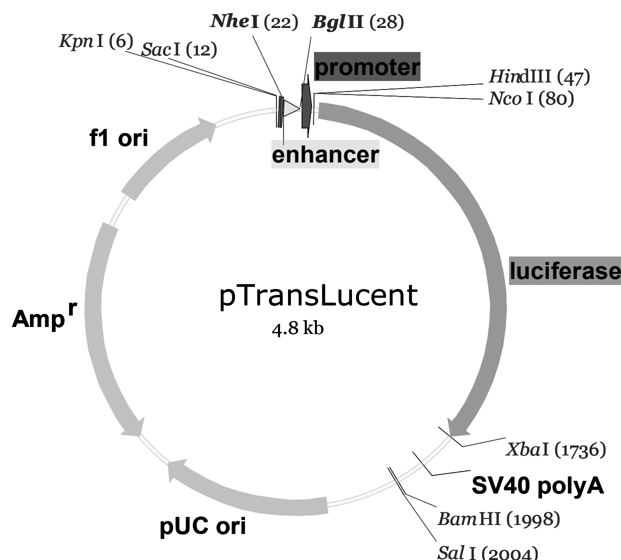
The TransLucent Reporter Vectors have been specially constructed to report the binding activity of an individual TF. Multiple copies of the cis-acting enhancer element have been inserted into each vector upstream of a minimal TA promoter, the TATA box from the Herpes simplex virus thymidine kinase promoter. This promoter sequence drives expression of the luciferase gene (luc). The backbone of the vector contains an antibiotic resistance gene for cloning purposes, an origin of replication, and an f1 origin for single-stranded DNA production.

To assess *in vivo* TF binding activity, the TransLucent Vector is first transfected into cells. If desired, an antibiotic resistant vector can be cotransfected to establish a stable cell line. After a set amount of time, the cells are lysed and subjected to the standard luciferase assay. Luminescence is detected and measured by a luminometer or scintillation counter. The resulting data can be used to quantify TF activity.

A Valuable Tool for Monitoring Transactivation

TransLucent Reporter Vectors facilitate the study of signal transduction. With the aid of the reporter vectors, *in vivo* transcription factor activity in cell lines of various origins or those treated with a stimulus of interest can easily be compared. Cotransfect a vector expressing a gene of interest along with a TransLucent Reporter Vector to observe the effects of the gene of interest on signaling pathway activity.

TransLucent Reporter Vectors are also perfectly suited to be used in conjunction with Panomics' own TranSignal™ Arrays as well. Like our popular EMSA gel-shift kits, TransLucent Reporter Vectors can be used to confirm and validate binding data from the TranSignal Protein/DNA Arrays. The most popular transcription factors from the three version of the TranSignal Protein/DNA Arrays can be monitored by our reporter vectors.



The cis-acting enhancer element sequence resides between the NheI and BglII restriction sites, upstream of the TATA box promoter, which drives expression of the firefly luciferase reporter gene upon transcription factor binding. Unique restriction sites are listed on the map. The length of the enhancer element differs for each TransLucent Reporter Vector, therefore the positions indicated on the vector map should be adjusted accordingly. All of the TransLucent Vectors contain the Panomics signature sequence. These vectors are intended for research use only and should not be used commercially.

2. MATERIALS PROVIDED

- TransLucent Reporter Vector (20 µl of 500 ng/µl; 10 µg)
- TransLucent Control Reporter Vector (20 µl of 500 ng/µl; 10 µg)*

* Sequence information is available on our website.

3. ADDITIONAL MATERIALS REQUIRED

Reagents & Solutions

- FuGENE™ 6 Transfection Reagent (Roche, Cat. # 1 815 019)
- Opti-MEM® I Reduced Serum Medium (Invitrogen, Cat. # 31985-062) or serum-free culture media
- Standard cell culture supplies
- Luciferase Assay System (Promega, cat.# E150C)

4. TRANSFECTION

The procedure is modified for using FuGENE™ 6 (Roche) in a 12-well culture plate. If you decide to use another transfection method, follow the manufacturer's instructions.

Guidelines for transfecting TransLucent Reporter Vector with FuGENE 6

Culture Vessel	Volume of plating medium	Vector DNA (µg) and dilution volume	FuGENE 6 Reagent
96-well	100 µl	0.05 µg in 5 µl	0.1 - 0.3 µl
24-well	500 µl	0.2 µg in 20 µl	0.6 - 1.8 µl
12-well	1 ml	1.0 µg in 50 µl	1.2 - 3.6 µl
35-mm	2 ml	2.0 µg in 100 µl	3.0 - 9.0 µl
6-well	2 ml	2.0 µg in 100 µl	3.0 - 9.0 µl
60-mm	5 ml	5 µg in 200 µl	6.0 -20.0 µl

1 The day before transfection, plate 1-3x10^5 cells in 1 ml of their normal growth medium containing serum **without antibiotics.** Always plate cells in duplicate. This amount of cells should yield 50–80% confluence on the day of transfection. ***Note**: Lower confluence is required to allow enough surface area for growth during the experiment period.*

2 For each well of cells to be transfected, dilute 0.5 µg (1 µl) of the TransLucent Reporter Vector or the TransLucent Control Vector with 50 µl of Opti-MEM® I Reduced Serum Medium or serum-free culture media. ***Note:** Each vector should be transfected in duplicate; one group of cells is for treatment and the other group is for control.*

3 For each well, dilute 3 µl FuGENE 6 Reagent with 50 µl of Opti-MEM I Reduced Serum Medium or serum-free culture media, mix, and incubate for 5 min at room temperature, but no longer. This dilution can be prepared in bulk for multiple wells. ***Note**: Do not allow undiluted FuGENE 6 Reagent to come into contact with plastic surfaces other than pipette tips. Once the FuGENE 6 Reagent is diluted, combine it with the diluted DNA (from Step 3.2) within 45 min.*

4 Combine the diluted TransLucent Vector with the diluted FuGENE 6 Reagent and mix gently. Incubate for 15-45 min at room temperature to allow DNA/transfection reagent complexes to form.

5 Add 200 µl of the DNA/transfection reagent complexes directly into each well containing cells and medium and mix gently by rocking the plate back and forth.

6 Incubate the cells at 37°C in a CO_2 incubator.

7 **Note:** After 3-8 hrs of incubation, the medium can be removed. For an untreated sample, replace old medium with new medium; for a treated sample, replace old medium with new medium that has been treated with the stimulus. When removing and replacing medium, use caution to ensure that the cells remain attached to the bottom of the plate. Keep the transfected cells at 37°C in a CO_2 incubator for the remainder of the incubation period.

5. COLLECTING CELLS

1 After incubation, aspirate to completely remove the media from the culture plates. Be careful not to disturb the cells in the process.

2 Lyse the attached cells by adding lysis buffer (Promega, Luciferase Assay System) to each well. Use approximately 50 µl per well for a 12-well plate; 100 µl per well for a 6-well plate.

3 To detach cells from the plate, immediately pipet the mixture up and down.

4. Transfer the cell lysate/buffer solution to a clean 1.5-ml microcentrifuge tube. Keep on ice or store at -20°C.

Assay for luciferase activity following the instructions given by the supplier (Promega, Luciferase Assay System, cat.#* E150C*).

6. REFERENCES

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