Improvements to Luciferase Reporter Assays for Nuclear Receptor Function

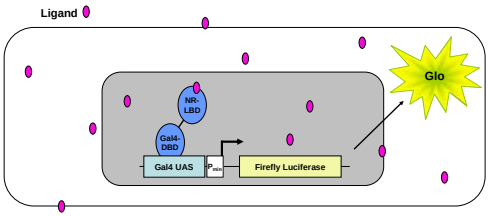
Aileen Paguio, Pete Stecha, Frank Fan, and Keith Wood
2800 Woods Hollow Rd., Madison, WI, USA 53704

Promega

Introduction

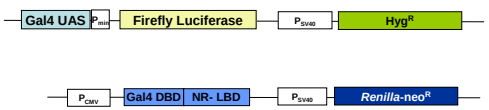
Luciferase reporter assays are frequently used to study nuclear receptor function because of their wide dynamic range, low endogenous activity, and ease of use. Recent improvements to pGL4 luciferase reporter vectors have further enhanced its utility for research and drug discovery. We used an assay containing the ligand binding domain (LBD) of a nuclear receptor fused to the Gal4 DNA binding domain (DBD) on one vector and multiple Gal4 Upstream Activator Sequences (UAS) upstream of luciferase reporter on another vector to monitor ligand binding and transactivation. We showed that codon optimization of luciferase reporter genes increased expression levels. Incorporation of protein destabilizing sequences into luciferase leads to a larger assay dynamic range up to several hundred fold. The optimum number of UAS to generate the best response was also evaluated. The expression vector for nuclear receptor LBD/GAL4 DBD fusion also constitutively expresses a *Renilla* luciferase-neo^R fusion, which can be used for selection (neo^R) as well as an internal control (Renilla luciferase) to detect compound cytotoxicity in a dual luciferase assay format for high throughput screening.

Nuclear Receptor Transactivation Assay



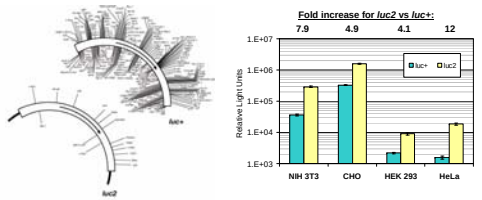
The DNA binding domain (DBD) of the nuclear receptor of interest is replaced by the DBD from the yeast Gal4 transcription factor. The Gal4 UAS is placed upstream of firefly luciferase. The activation of the nuclear receptor by the binding of a ligand is monitored through measuring the expression of the luciferase reporter gene.

Dual Luciferase Reporter Assay for Nuclear Receptor Binding and Transactivation



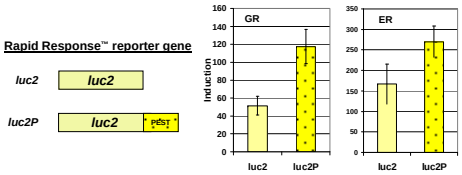
The ligand binding domain (LBD) of Estrogen Receptor alpha (ERα) or Glucocorticoid Receptor (GR) fused to the Gal4 DBD on one vector and multiple Gal4 UAS upstream of luciferase reporter on another vector to monitor ligand binding and transactivation. Each vector contains a drug resistant marker, Neo^R and Hyg^R respectively, for selecting stable cell lines. The Neo^R gene is fused to *Renilla* luciferase, so that it may be used as a secondary constitutive reporter for the detection of possible off-target responses.

Codon Optimization Increases Expression of Luciferase Reporter Gene (luc2)



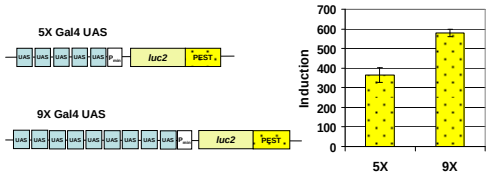
Improved performance of the *luc2* reporter gene was achieved through codon optimization to enhance luciferase expression and elimination of potential transcription factor binding sites within the reporter gene sequence. A comparison of luciferase expression from cells transfected with reporter vectors using either *luc+* or *luc2* in the identical vector backbone demonstrate higher luciferase expression from the *luc2* gene.

Rapid Response™ Luciferase Enhances Reporter Dynamics



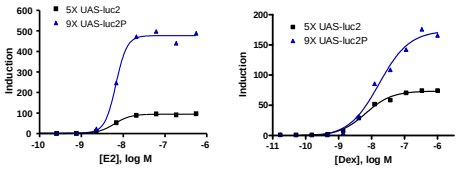
A version of the *luc2* gene containing a protein degradation sequence was used to enhance reporter dynamics. HEK293 cells were transiently transfected with 5X Gal4 UAS- *luc2* or *luc2*P in a 96-well plate. Each reporter was co-transfected with either ERα-LBD or GR-LBD. Cells were induced with either 10 nM 17β-Estradiol (ERα) or 1.0 µM dexamethasone (GR) Firefly luciferase activity was quantified 24 hours after induction using the Dual-Glo™ Assay System. Induction = induced Firefly RLU/uninduced Firefly RLU.

Multiple Gal4 UAS Further Improves Reporter Dynamics



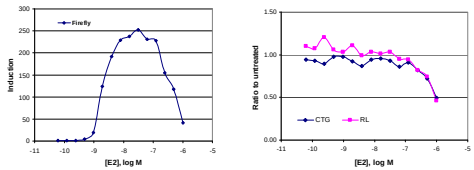
HEK293 cells were transiently transfected with 5X Gal4 UAS-*luc2P* or 9X Gal4 UAS-*luc2P* in 96-well plates. Each reporter was co-transfected with ERα-LBD fused to the Gal4 DBD. Cells were induced with 10 nM 17β-Estradiol (E2). Firefly luciferase activity was quantified 24 hours after treatment using the Dual-Glo™ Assay System.

Improved Reporter Allows Larger Dynamic Range without Changing EC_{50}



HEK293 cells were transiently co-transfected in 96-well plates 5X UAS-*luc2* or the 9XUAS-*luc2*P vectors and either GR or ERα-LBD vectors. 24 hours after transfection, 1:3 serial dilutions of each compound were added to the wells. 24 hours post treatment, firefly luciferase activity was measured using the Dual-Glo™ Assay System.

Internal Control Reporter *Renilla* Luciferase Allows Identification of Cytotoxic Compounds



High concentrations of E2 have an effect on cell viability. HEK293 cells were transiently co-transfected in 96-well plates with ERα-LBD/ 5X *luc2*P vectors. 24 hours post transfection, 1:2 serial dilutions of E2 was added to the respective wells. A another plate was prepared in parallel and measured with CellTiter-Glo Luminescent Viability Assay. Firefly and *Renilla* luciferase activities were measured using the Dual-Glo™ Assay System.

Summary

Together these elements provide better tools for nuclear receptor research and drug discovery programs:

- The next generation synthetic luciferase gene (*luc2*) is brighter than native luciferase.
- A brighter gene enables the use of a Rapid Response™ *luc2P* gene for nuclear receptor activation with improved reporter dynamics.
- Nine copies of the Gal4 UAS upstream of *luc2P* further improves the dynamic response of the Estrogen Receptor alpha assay.
- Multiplexing with the Dual Luciferase System improves data quality by providing an internal control reporter to simultaneously monitor for non-receptor specific responses such as cytotoxicity.