

TECHNICAL NOTE:

Co-transfection controls with reporter assays

Do I need a co-transfection control?

In most cases you do not need a co-transfection control. This technical bulletin offers guidelines and experimental data that will help you evaluate the needs of your reporter assay experiment.

What does a co-transfection control do?

Historically, an experimental plasmid was co-transfected with a reference or control plasmid to help normalize two main sources of variation between transfection replicates:

1. **Low and variable transfection efficiency**
2. **Variable cell lysis and variable lysate stability**

In the days of calcium phosphate transfections and home brew assay reagents, co-transfections were essential. However, modern ultra high-efficiency transfection reagents such as FuGENE HD and optimized lysis buffer and assay reagents such as the LightSwitch Assay Reagent often reduce these types of variation to coefficients of variance (CVs) of less than 10% (Table 1).

Co-transfection controls were originally designed to remove variability due to transfection efficiency alone (1). Such controls were not designed nor are they adequate for comparing between different conditions or cell lines.

Promoter (pGL4 vector)	TK	ACTB	RPL10	ANKRD37	EGLN1	ENO2	AVG
%CV Single Tfx (3 reps)	18%	2%	13%	8%	6%	7%	9%
%CV Co-Tfx normalized (3 reps)	14%	22%	11%	12%	4%	7%	12%

Table 1: Variation between replicate transfections is the same for single transfections and normalized co-transfections

In the single transfection (tfx) experiment, 6 different promoter-luc2P reporter constructs were independently transfected into HepG2 cells without a co-transfection control in triplicate and assayed with Steady-Glo (Promega) 24 hrs after transfection. Additionally, the same 6 promoter-luc2P vectors were co-transfected with a TK-renilla plasmid and assayed with Dual-Glo (Promega). The co-transfected plasmid data were normalized by dividing the firefly signal by the renilla signal. The table above shows the coefficient of variation (%CV= stdev/mean) of the 3 replicates of each construct. The average %CV for the single transfection is 9%, whereas the average %CV for the normalized co-transfection is 12%.

Are there any down-sides to doing a co-transfection control?

Co-transfecting two plasmids is significantly different than transfecting a single plasmid. The effect of a co-transfection in your experimental design should be carefully considered. The most important considerations should be the following:

1. Time, Cost, and Signal: Dual-reporter assay reagents are more than twice as expensive and take more than 2 times as long as a single transfection. A single transfection design can double the scope of a project that can be completed in half of the time. Furthermore, dual-reporter assay reagents yield lower raw signal than reagents that have been optimized specifically for a single reporter protein (Figure 1).

2. Interaction between vectors: Co-transfected plasmids have long been known to interact with each other. Strong promoters can sequester basal factors and artificially repress the signal of the control. Conversely, promoters with inducible response elements can trans-activate the promoter on the control vector. Sorting out these confounding effects can be extremely challenging and give results that are difficult to interpret.

3. Reliance on a single control promoter: Dual-reporter assay reagents are more than twice as expensive and take more than 2 times as long as a single transfection. A single transfection design can double the scope of a project that can be completed in half of the time. Furthermore, dual-reporter assay reagents yield lower raw signal than reagents that have been optimized specifically for a single reporter protein (Figure 1).

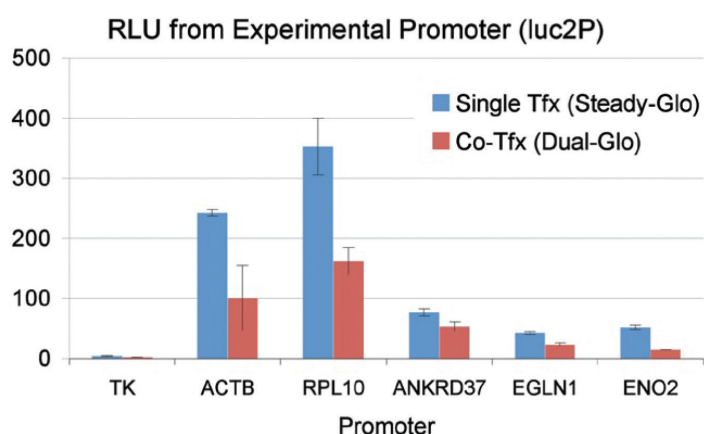


Figure 1: Relative luminescence from single and co-transfected promoter reporter vectors

In the single transfection (tfx) experiment, 6 different promoter-luc2P reporter constructs were independently transfected into HepG2 cells without a co-transfection control in triplicate and assayed with Steady-Glo (Promega) 24 hrs after transfection. Additionally, the same 6 promoter-luc2P vectors were co-transfected with a TK-renilla plasmid and assayed with Dual-Glo (Promega). The data from both experiments represent raw (un-normalized) firefly luminescence. The average RLU and standard deviation of each construct is shown in the bar chart above.

If I don't use a co-transfection control, which controls should I use?

One of the big advantages of using a single transfection design is that you can include many different control vectors in your experiment, such as various housekeeping promoters, to allow comparisons across a variety of cell lines or treatment conditions. In this experimental setup, instead of one control construct being co-transfected with every experimental plasmid, many different control promoters are included in the experiment, each in its own well, in addition to the experimental promoters to be tested. Because no single promoter will serve as an ideal control, LightSwitch offers a panel of positive and negative control vectors to measure background and non-specific responses with the highest degree of confidence.

How do I decide whether I need a co-transfection control?

The easiest thing to do is to try your experiment without co-transfecting the control plasmid and measure the variation between replicate single transfections. More often than not, your %CV will be within an acceptable limit (Table 1). With few exceptions, your experimental conclusions will be the same as if you used a co-transfection control (Figure 2). If you feel like your experiment does require a co-transfection control, the LightSwitch Dual Assay System is fully compatible and optimized for use with LightSwitch GoClones.

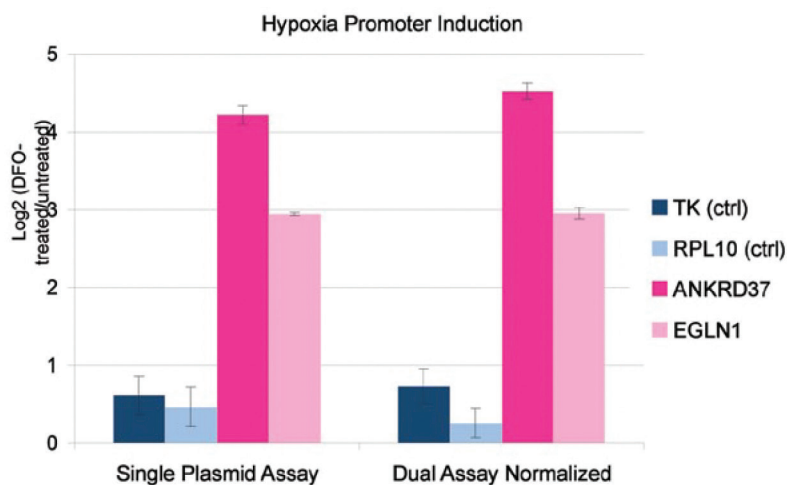


Figure 2: Hypoxia promoter induction ratios are the same with and without a co-transfection control

In this experiment, two housekeeping control promoters and two hypoxia-inducible promoters were transfected in triplicate with and without a co-transfection control. The 4 promoters were exposed to either 100uM DFO or normal media for 24 hrs and luciferase activity was measured with Steady-Glo (single transfection) or Dual-Glo (co-transfection). The graph above shows the log2 ratio of the DFO treated to untreated activity for each of the 4 promoters. The fold induction for each promoter is not significantly different between the single and co-transfected vectors.

References

1. Schagat, T., Paguio, A., and Kopish, K. 1996. *Normalizing genetic reporter assays: approaches and considerations for increasing consistency and statistical significance*. Cell Notes 17:9-12.
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3. Ho, C., and Strauss, J. 2004. *Activation of the control reporter plasmids pRL-TK and pRL-SV40 by multiple GATA transcription factors can lead to aberrant normalization of transfection efficiency*. BMC Biotechnology. 4:10.
4. Matuszyk, J., Ziolo, E., Malgorzata, C., Kochel, I., and Strzadala, L. 2002. *Nurr1 affects pRL-TK but not phRG-B internal control plasmid in genetic reporter system*. Biochem. Biophys. Res. Comm. 294: 1036-1039.