The Next Generation of Promoter Reporters

Obtain excellent fold induction with the DD-Fluorescent Protein Reporter Systems

- **High signal-to-noise ratio**
- **Broad dynamic range**
- **Easy-to-monitor fluorescence readout**

Promoter reporter assays generally struggle with the fact that most promoters are not very "tight." As a result, your promoter of interest may drive reporter expression even without being activated—for example, during the time between transfection and the start of your experiment. If the reporter molecule has a long half-life, it will accumulate during this time before the experiment actually starts. These preexisting reporter molecules (the background) are the main cause of a low signal-to-noise ratio after promoter induction during the actual experiment.

The Challenges: Overcoming High Background & Low Signal Intensity

A previous approach to this problem was to modify reporters for very quick, constitutive degradation ($T_{1/2}$ ≤2–3 hours). This did in fact decrease the background signal that accumulated before the start of the experiment, because the vast majority of pre-existing reporter molecules were degraded.

However, constitutively destabilized reporters have another problem: because they are degraded quickly, it is impossible to accumulate a large quantity of reporter molecules inside the cell upon promoter activation. As a result, only a fraction of the reporter molecules produced in response to promoter activation are present long enough to be measured.

Thus, although constitutively destabilized reporter systems provide a low background, the trade-off is low signal intensity due to quick degradation of the reporter—even when the promoter is activated. That means that these assays have an extremely limited dynamic range.

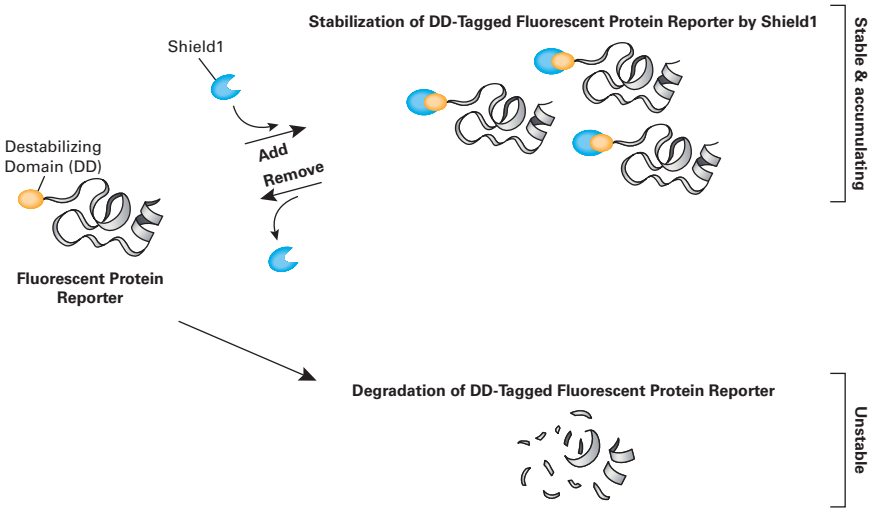

Figure 1. Ligand-dependent, targeted, and reversible fluorescent protein reporter stabilization. A small destabilization domain (DD) is fused to the fluorescent protein reporter. The small membrane-permeant ligand Shield1 binds to the DD and protects the entire fusion protein from proteasomal degradation. Removal of Shield1, however, causes rapid degradation of the entire DD-fluorescent protein reporter. The default pathway for the DD-Fluorescent Protein Reporter Systems is degradation of the DD-fluorescent protein reporter, assuring low background, unless Shield1 is present.

The Solution: Reporters on Demand

Clontech's new **DD-Fluorescent Protein Reporter Systems** meet the challenge by providing both a low background *and* a broad dynamic range. This versatility is possible because they use a combination of technologies: each system includes a bright fluorescent protein reporter (AmCyan1, tdTomato, or ZsGreen1) for high signal intensity, coupled with ligand-dependent ProteoTuner™ protein stabilization/destabilization technology to eliminate background.

In these systems, the fluorescent protein reporter is expressed as a fusion protein tagged on its N-terminus with a ligand-dependent destabilization domain (DD). The DD causes the reporter protein to be targeted to and rapidly degraded by proteasomes, guaranteeing a low reporter background signal at the start of your experiment. However, when the small, membrane-permeant ligand **Shield1** is added to the sample, it binds to the DD and protects the DD-tagged fluorescent protein reporter from proteasomal degradation, so that it can accumulate (Figure 1; 1).

This means that by adding Shield1 simultaneously with your candidate inducer, you can effectively stabilize the reporter protein when it is synthesized in response to promoter activation. The majority of the fluorescent protein reporter molecules expressed during promoter activation will contribute to your readout, allowing for a considerably higher dynamic range and drastically improved signal-to-noise ratio compared to other types of reporter systems.

High Signal, Low Background

In order to demonstrate the high signal-to-noise ratio and wide dynamic range of the DD-Fluorescent Protein Reporter Systems, we compared the fold induction achieved using the DD-fluorescent protein reporters with that achieved using regular (non-destabilized) fluorescent proteins. The inducible CRE promoter was used to drive the expression of each fluorescent protein reporter. After transfection, the

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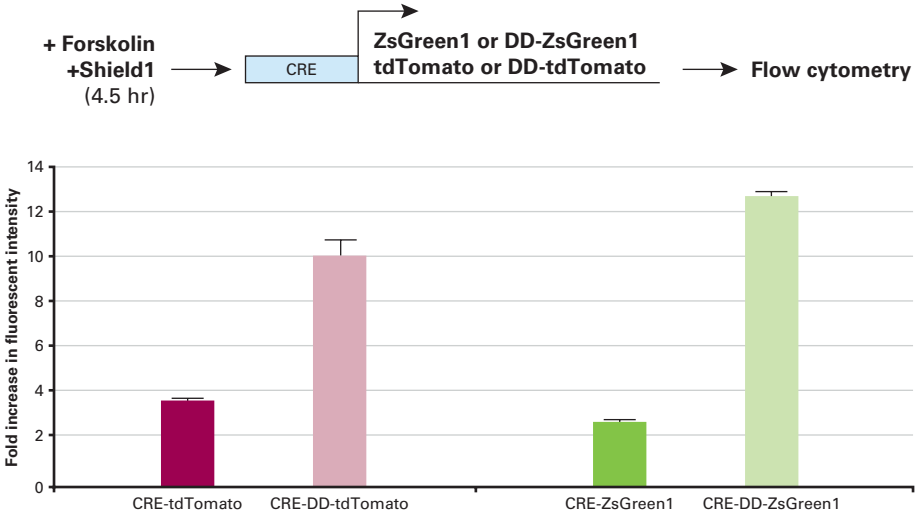

Figure 2. DD-Fluorescent Protein promoter reporters provide a much greater fold increase in signal intensity than traditional fluorescent protein reporters, which do not contain the DD. HEK 293 cells were transfected with plasmids encoding the following reporters: CRE-tdTomato, CRE-DD-tdTomato, CRE-ZsGreen1, and CRE-DD-ZsGreen1. 24 hr later, the cells were stimulated with 10 µM forskolin and simultaneously treated with 1 µM Shield1. After 4.5 hr, fluorescence intensity was measured via flow cytometry, and fold induction was calculated. The tdTomato and ZsGreen1 reporters containing the DD had three- and six-fold greater fluorescence intensity respectively, than the versions without the DD, due to the latter's increased background levels.

cells were induced using forskolin. At the same time, Shield1 was added to stabilize the DD-tagged reporters. The cells were collected 4.5 hours after induction, analyzed via flow cytometry, and the fold increase over background was calculated.

The DD-tagged reporters stabilized by Shield1 had a much wider dynamic range, and therefore a much larger fold increase in the signal than the untagged versions of the same reporters (Figure 2). For the untagged versions, we observed high background fluorescence from reporter molecules that accumulated prior to induction, which drastically reduced the fold increase in signal intensity.

It is notable that we obtained these results after just 4.5 hours of induction. The short induction time was possible because the DD-Fluorescent Protein Reporter Systems kept the background extremely low prior to activating the CRE element, so the reporters quickly accumulated to detectable levels. A longer induction time would increase the fold induction even further, due to the wide dynamic range of the DD-Fluorescent Reporter Systems.

With the DD-Fluorescent Protein Reporter Systems, you can compensate effectively for reporter background without compromising your assay's signal intensity. Low background and a wide dynamic range are no longer mutually exclusive.

Reference

1. Quick & Reversible Control of Your Protein of Interest (April 2008) *Clontechniques* **XXIII**(2):1–2.

Product	Size	Cat. No.
DD-tdTomato Reporter System	each	632190
DD-AmCyan1 Reporter System	each	632191
DD-ZsGreen1 Reporter System	each	632192
Shield1*	60 µl	631037
	200 µl	631038
	500 µl	632189

* The number of reactions depends on the concentration of Shield1 used. At the maximum suggested concentration (1,000 nM), 60 µl = 30-plus reactions in a six-well plate.

DD-tdTomato Reporter System Components

- DD-tdTomato Reporter
- Shield1

DD-AmCyan1 Reporter System Components

- DD-AmCyan1 Reporter
- Shield1

DD-ZsGreen1 Reporter System Components

- DD-ZsGreen1 Reporter
- Shield1

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