Quantitative Analysis of GFP in Plant Extracts Using the VersaFluor™ Fluorometer

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Introduction

The Green Fluorescent Protein (GFP) from *Aequorea victoria* is a useful reporter gene in higher organisms, including plants and animals. The fluorescence emitted is easy to detect and requires no substrate or cofactors. Furthermore, improved GFP versions have been created with higher fluorescence coefficients, improved expression characteristics for eukaryotes, or altered spectral properties. One form, GFP-S65T incorporates a threonine amino acid substitution in place of serine at position 65, which results in "red-shifted" fluorescence characteristics.[1] This form is one that has been proven to be very beneficial for use in plants.

Quantification of GFP is unlike widely used enzyme-based reporters such as β-glucuronidase (GUS) and firefly luciferase (LUC). We show here that fluorescence detection using the VersaFluor fluorometer system with appropriate narrow band filters can be used to detect and quantify GFP-S65T protein levels in plant extracts against a standard curve made from purified GFP-S65T protein.

Method

The test samples consisted of sugarcane cv Q117 callus expressing Ubi1-s*gfp*S65T-nos3'. Approximately 500 mg of tissue was ground in extraction buffer (10 mM Tris-EDTA, pH 8.0, 0.02% [w/v] sodium azide) at 4 °C. This extract was centrifuged in a microcentrifuge at 13,000 rpm for 20 min. and the supernatant removed for analysis. Fluorescence microscopy showed that all visible GFP fluorescence was located in the soluble fraction. Protein concentrations of the plant extracts were determined using a Bio-Rad Protein Assay Kit. Plant samples were diluted to 400 µg/ml of extractable protein in buffer. Two milliliters of diluted extract were used for each reading. A dilution series (0.1–0.4 µg/ml) of purified rGFP-S65T protein (Clontech), was made in either extraction buffer or 400 µg/ml untransformed callus extract.

The VersaFluor fluorometer was fitted with a 490 ± 5 nm excitation filter and a 510 ± 5 nm emission filter so that the reflective face of the filters faced towards the incident beam. The VersaFluor fluorometer was used according to the supplied instructions and blanked using 2 ml of extraction buffer. The range (on medium gain) was set using the highest plant sample. All samples and standards were measured by taking the mean of measurements at 20, 40 and 60 seconds.

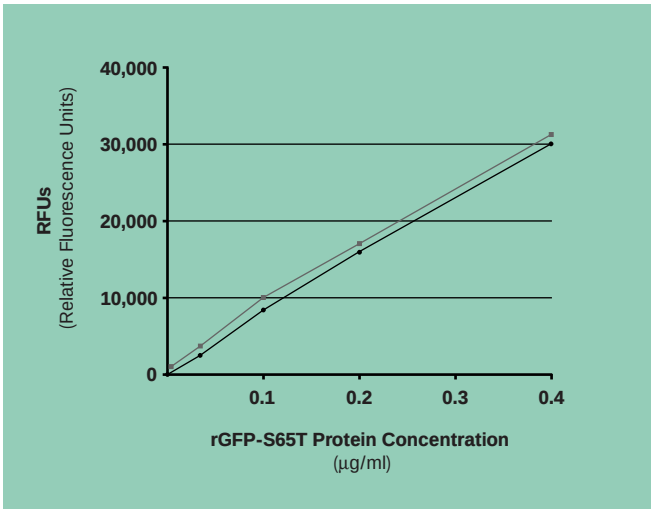

Fig. 1. Calibration of the VersaFluor fluorometer using purified rGFP-S65T protein in extraction buffer (●) or untransformed calli extract (■).

Results and Discussion

Figure 1 shows the RFUs for the GFP dilution series, quantified using the VersaFluor fluorometer. The presence of other fluorescent compounds in the plant extracts did not increase the RFUs significantly, indicating that the 10 nm bandpass emission filter adequately omitted fluorescence at other wavelengths.

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The curve for RFUs against rGFP-S65T concentration was linear over the range of plant GFP concentrations measured. Measurements were made after the optical sensor in the VersaFluor had stabilized. An example of stabilization of the fluorescence readings is shown for a 0.2 µg/ml rGFP-S65T protein standard in Figure 2. For most readings, the signal stability varied less than 10% of the original value over 180 seconds. Plant samples were then read as for the GFP-S65T dilution series. GFP concentration was extrapolated from the standard curve corresponding to the dilution series in untransformed calli extract to account for the background fluorescence. GFP levels in calli expressing Ubi-s*gfp*S65T-nos3' ranged up to 0.525 µg rGFP-S65T per mg of extractable protein.

The ability to quantify GFP in extracts from transgenic organisms facilitates the comparative assessment of expression levels directed and influenced by attached genetic sequences, such as promoters, introns and terminators. The ability to test gene sequences in this way is a prerequisite for the correct deployment of useful gene sequences in transgenic plants.

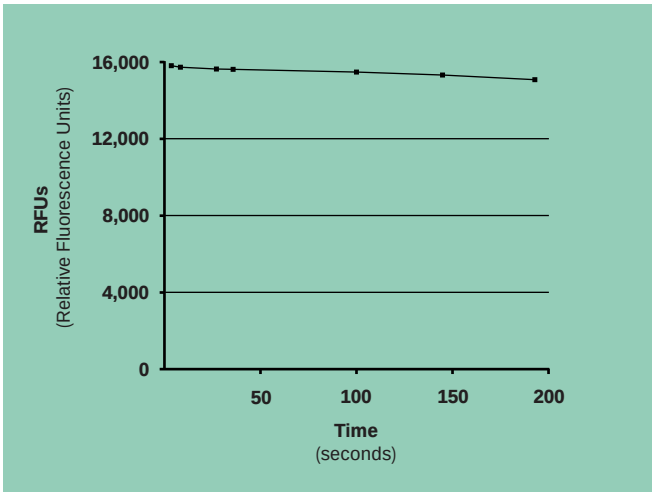

Fig. 2. Stability of a fluorescence reading using the VersaFluor fluorometer.

Reference

1 Heim, R., Cubitt, A. B. and Tsien, R. Y. Nature, *373, 663-664 (1995).*

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