



Handy Helper

Application Note 06

Visualising Green Fluorescent Protein Using a Syngene Image Capture System

What is GFP?

Green fluorescent protein (GFP) from *Aequorea victoria* (jellyfish) has stimulated a great deal of excitement among molecular, developmental and cell biologists. This is because GFP is a small protein (27 Kd) and the DNA sequences coding for GFP can be manipulated by recombinant DNA technology to create gene fusions between GFP and any protein of interest. Such DNA constructs can then be introduced into living cells to express the GFP fluorescent tag on the protein of interest to the investigator.

The major use of the tag is to localise the protein of interest to a specific cell type and/or subcellular localisation in living cells and organisms. The cloning of the wild-type GFP gene has established GFP as a novel genetic reporter system. When expressed in eukaryotic cells and illuminated by blue or UV light, GFP yields a bright green fluorescence. Light stimulated GFP fluorescence is species independent and does not require any cofactors, substrates, or additional gene products. Additionally, detection of GFP and its variants can be performed with living tissues instead of fixed samples.

Visualisation

The detection process for GFP is typically achieved using laser based microscopy systems and a CCD based camera. There are several variants of GFP and these include wild type (wt) and enhanced GFP (eGFP). Wt GFP has an excitation peak of 395nm and therefore mid (302nm) or long (365nm) wave UV can be used for excitation. In this case the short pass emission filter is utilised. There is an additional excitation peak at 475nm and so blue light can be used as an excitation source, by using the Syngene blue light converter. In this case a SG emission filter is needed, rather than selecting the short pass filter. This is to prevent any overlap between the excitation and emission spectra. Contact Syngene for details of this SG emission filter. EGFP has been shown to be 35 times brighter than wt GFP when excited with blue light. We therefore recommend blue light excitation and the SG emission filter.

Syngene image capture systems uses a CCD based camera, attached to an ergonomic darkroom cabinet. With the option of a motorised filter wheel or filter slider it is possible to fit a GFP optimised emission filter.

In some instances, GFP may be used on opaque membranes or in plates whereby an epi-excitation light source will be required. In this instance, the darkroom can be further modified to provide epi-long wave UV or a blue wavelength, again depending on the variety of GFP used. All the Syngene image capture systems are controlled via a computer and are fully motorised, running GeneSnap imaging acquisition software and GeneTools analysis software.



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