Handy Helper

Application Note 02

Blue Converter Screen
For Molecular and Cellular Biology Applications

Fluorescent dyes and proteins are basic analytical tools used in many molecular and cellular biology applications. Fluorescent dyes such as ethidium bromide, SYBR® Green, SYBR® Gold and SYPRO® Orange are used to detect nanogram quantities of DNA, RNA and protein separated by gel electrophoresis techniques.

Auto-fluorescent proteins such as green fluorescent protein (GFP) and its blue and yellow variants have been used as reporter molecules for protein localisation, gene expression, insertional cloning, and other applications. Ultraviolet (UV) transilluminators, commonly used to excite these molecules, emit ultraviolet light in a broad band (approximately 100nm wide) centred around 300nm. The fluorescent yield of these dyes, in complex with their respective binding partners, is considerable when exposed to ultraviolet radiation. However, most of the fluorescent dyes and proteins in common use today have a bimodal excitation spectrum, with one excitation peak in the UV and another in the visible range. For example, enhanced GFP has excitation peaks at 395 and 475nm. The new blue converter screen will convert broad band UV to an excitation range of 410-510nm, coinciding with the second excitation peak of many fluorescent dyes and proteins. The growing use of SYBR Gold and SYBR Green as fluorescent stains requires an excitation wavelength in the blue range and the screen is therefore suitable for these applications.

This blue wavelength also has many other advantages over normal UV:

- UV radiation is mutagenic, carcinogenic and an eye damaging agent requiring special safety precautions for the operator.
- UV radiation rapidly damages molecular and cellular reagents.
- The detection sensitivity is similar between the two light sources. However, another advantage is that DNA visualised using blue illumination exhibits none of the damage associated with ultraviolet radiation. This damage impairs transformation, amplification and transcription by 2-3 orders of magnitude for downstream applications.

Exciting with blue light takes advantage of the increased sensitivity of the SYBR dyes, compared to UV illumination of ethidium bromide. This may be important when identifying very low copy plasmids and artificial bacterial chromosomes.

The blue converter, in conjunction with any of the Syngene image capture systems, can extend the range of lab applications to include the non-destructive detection and purification of DNA and the non-invasive assay of gene expression.

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