Detection of BD Living Colors™ Novel Fluorescent Proteins using the Acumen Explorer™

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Abstract

Proprietary Novel Fluorescent Proteins (NFP) from BD Biosciences Clontech include fluorescent proteins cloned from reef corals and the jellyfish *Aequorea coerulescens*. Fluorescent emissions of distinct proteins from this portfolio were analysed by the Acumen Explorer. Single and mixed populations of cells expressing NFP genes were detected in addition to the measurement of proteins linked to different colored NFP's localized to cell compartments. The combination of these technologies provides an excellent tool for the investigation of functional genomic applications.

Introduction

With the sequencing of many genomes now completed, biologists are faced with the challenge of deciphering the function and association of an immense number of resultant proteins.

Understanding the specific processes and actions of proteins and chemicals that comprise the cells and tissues of an organism, will be a necessary next step to elucidating cellular function in healthy and disease states.

Simultaneous and quantitative measurement of the level of expression, for tens of thousands of genes, aids in the definition of a comprehensive molecular phenotype of cells and cellular processes.

Proprietary Novel Fluorescent Proteins from BD Biosciences Clontech include fluorescent proteins cloned from reef corals and the jellyfish *Aequorea coerulescens*. From this portfolio, five distinct proteins, AcGFP1 (monomer), ZsGreen1, ZsYellow1, AsRed2 and DsRed2 can be excited by a 488 nm argon ion laser.

The Acumen Explorer family of instruments utilize a 488 nm argon ion laser and exploit the power of fluorescence emission to monitor and elucidate subtle changes in inter and intracellular biochemical events.

Fluorescence emissions are directed to four photo multiplier tubes, which are tuned to band-pass ranges in green, yellow, red, and far-red spectrums. Four colors/wavelengths regions can be scanned simultaneously thereby allowing true multiplexing with NFPs.

In the examples detailed here, HEK293 cells expressing genes encoding different fluorescent proteins were analysed in order to demonstrate the application of quantitative functional genomics.

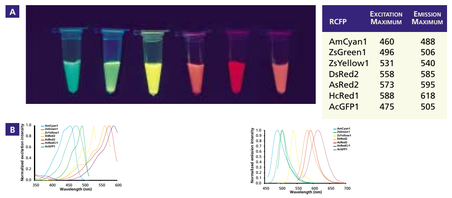
Conclusion

The proprietary software algorithms permitted measurement of:

- Single and mixed populations of cells expressing NFP genes
- Detection of proteins linked to different colored NFPs localized to cell compartments

The versatility of the Acumen Explorer in combination with the growing portfolio of NFPs from BD Biosciences Clontech provides an excellent tool for the investigation of functional genomic applications.

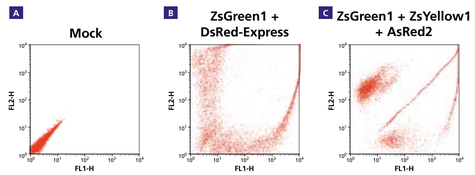
1 Spectral Characteristics of Novel Fluorescent Proteins



RCFP	Excitation Maximum	Emission Maximum
AmCyan1	460	488
ZsGreen1	496	506
ZsYellow1	531	540
DsRed2	558	585
AsRed2	573	595
HcRed1	588	618
AcGFP1	475	505

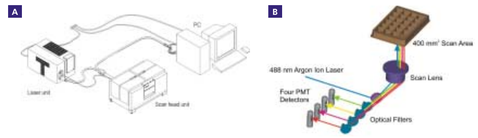
Panel A. Six Novel Fluorescent Proteins (NFPs) were overexpressed in *E. coli* and purified. Their fluorescence emission was visualized under UV light (in order from left to right): AmCyan1, ZsGreen1, ZsYellow1, DsRed2, AsRed2 and HcRed1. **Panel B.** Fluorescent excitation (left) and emission (right) spectra of the seven Novel Fluorescent Proteins. The table shows the wavelengths of excitation and emission peak maxima of each fluorescent protein.

2 Multicolor Detection of Novel Fluorescent Proteins by Flow Cytometry



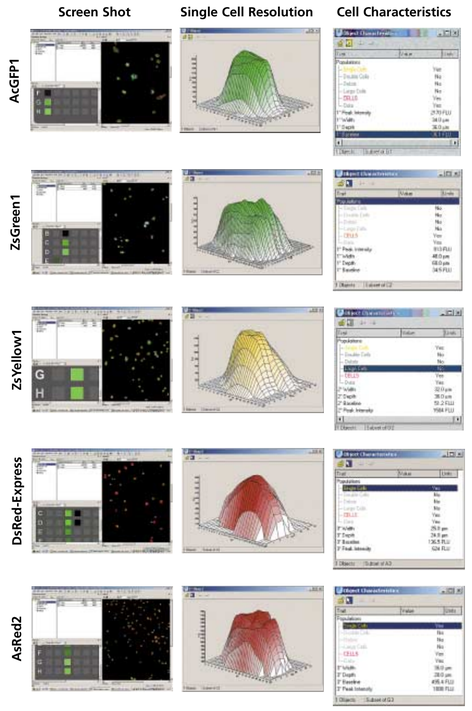
Separation of NFP signals by flow cytometry. HEK 293 cells were analyzed using a BD FACSCalibur (488 nm excitation) and channels FL1 (530/30 band pass) and FL2 (585/42 band pass) for detection. **Panel A.** HEK 293 cells. **Panel B.** Mixed HEK 293 clones expressing ZsGreen1 and DsRed-Express. **Panel C.** Mixed HEK 293 clones expressing ZsGreen1, ZsYellow1 and AsRed2.

3 Acumen Explorer Diagram



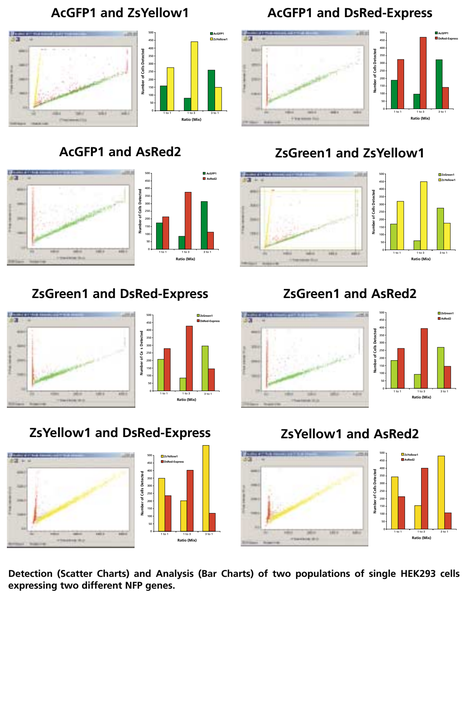
Panel A. The Acumen Explorer scanning system comprises: A laser unit housing an air-cooled laser. The scan head unit incorporating laser optics and plate alignment systems. An external PC. The scan unit is de-coupled from the laser unit by an umbilical cable to enable its accommodation into conventional robotic plate handling systems. **Panel B.** The Acumen Explorer family of instruments are multiple photo-multiplier (PMT) based systems that exploit the power of fluorescence. The laser is focussed by a scan lens into a narrow beam spot and scans the selected area of a plate. Area-based scanning (400 mm) enables the instrument to scan a large number of wells simultaneously. In practical terms this means scan times are independent of plate density; i.e. a 1536 well plate can be scanned in the same time as a 96 well plate. All plate types that have the SBS standard footprint can be used from 24 well up to 1536. The resulting fluorescence emission is directed to four PMT detectors. The collection optics consist of four dichroic beam splitters and four emission filters that are tuned to band-pass ranges that allow green, yellow, red and far-red emissions to be monitored. The Acumen Explorer detects and records all fluorescent objects whose intensities are above the background. Four colors/wavelengths regions are scanned simultaneously thereby measuring multiple emissions permitting different dyes to be used in the same assay. Cell populations with multiple intracellular and extracellular events can be measured.

4 Detection of Single Novel Fluorescent Proteins on the Explorer



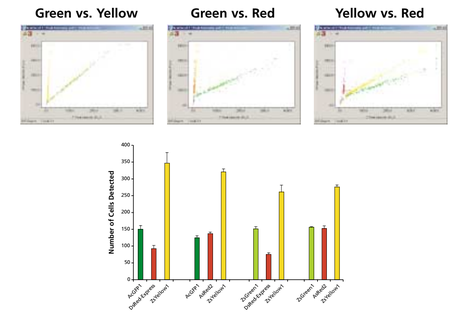
Detection of NFPs following the stable expression of each NFP gene in individual cells.

5 Multiplexing of Two Novel Fluorescent Proteins on the Explorer



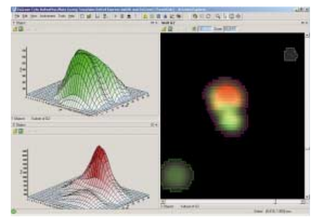
Detection (Scatter Charts) and Analysis (Bar Charts) of two populations of single HEK293 cells expressing two different NFP genes.

6 Multiplexing of Three Novel Fluorescent Proteins on the Explorer



Detection (Scatter Charts) and Analysis (Bar Charts) of three populations of single HEK293 cells expressing three different NFP genes.

7 Subcellular Localization of NFPs: DsRed2-Nuc and AcGFP1-N1



Single HEK293 cell multiplexing and detection of ZsGreen1 fluorescent protein in the cytoplasm and a DsRed2 fluorescent protein linked to a nuclear localization signal. This example demonstrates detection of functional protein compartmentalization.

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