Plasmid & Fluorescent Protein Replication for Cell Imaging

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WHERE DISCOVERIES BEGIN

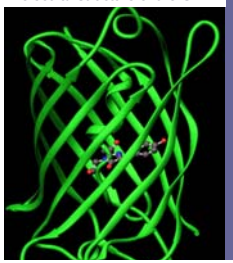
Abstract:

In order to persist and be stably maintained in the cell, a plasmid DNA molecule must contain an origin of replication, which allows it to be replicated in the cell independently of the chromosome. In this trial, the plasmid used (mTagRFP) was approximately 4.7kb. To begin the process of amplification of the six fluorescent proteins, three solutions of plasmid were digested using the restriction enzymes EcoRI and NotI and three solutions of plasmid were digested using restriction enzymes BamHI and NotI. Through the process of ligation, stock vectors of mito, Golgi, and zyxin were added to the plasmids digested with BamHI and NotI and stock vectors of alpha-actinin, keratin, and and paxillin were added to the plamids digested with EcoRI and NotI.

These vectors were then amplified in e.coli before transfection. Applying molecular cloning methods we successfully fused the following targeting proteins: alpha-actinin, keratin, paxillin, Golgi, mitochondria, and zyxin.

Introduction:

Molecular structure of the GFP



The green fluorescent protein (GFP) was first discovered from *A. victoria* in the 1960s by Osamu Shimomura. However, only after molecular biologists managed to clone GFP did scientists begin to realize enormous potential as a biological marker and its uses in microscopy. The S65T mutant by the Tsien and Remington groups in *Science* in 1996 was the first reported crystal structure of a GFP. This discovery made the GFP a much more valuable fluorescent protein.

All fluorescent proteins (FPs) feature fluorescence emission when they are excited with certain wavelengths. FPs can be fused with most proteins in living cells using plasmids, which are double-stranded, circular, extra-chromosomal DNA.

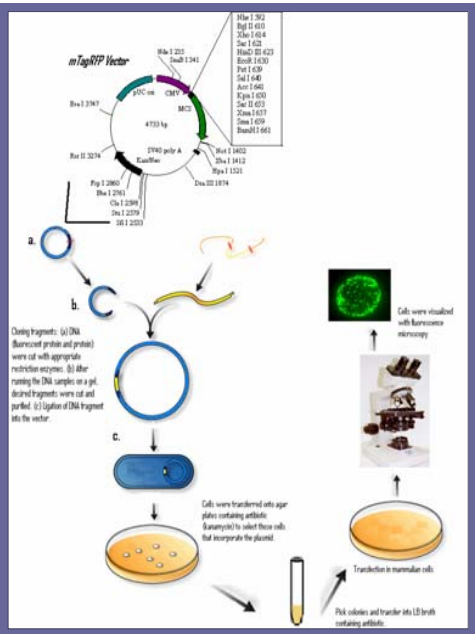
The brightness of FPs can vary due to a number of factors: intrinsic brightness of the protein, illumination wavelength and intensity, camera or eye sensitivity, and the presence or absence of molecular oxygen. The brightness of the GFP is a product of the molar extinction coefficient and the quantum yield.

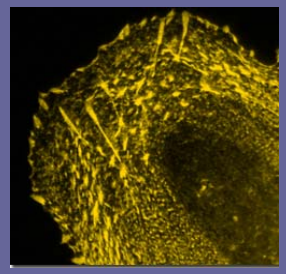
Cell toxicity can occur a variety of ways. Excessive concentrations, over expression, and cell health all pay a role in cell stability. Phototoxicity, light induced damage, also occurs due to repeated fluorescence exposure. Thus the photostability of the FP is important when deciding which to use. The GFP is fairly stable and commonly employed.

The use of FPs is minimally invasive for living cells. Since FPs can be modified to attach to specific cell parts, and the brightness and photostability continues to be advanced, FPs are highly effective for cell imaging.

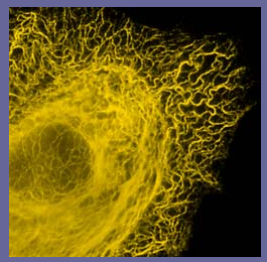
The FPs were ligated with six different vectors of mTagRFP to express alpha-actinin, keratin, paxillin, Golgi, mitochondria, and zyxin via cloning. Cloning is the process of moving a gene from a chromosome in which it naturally occurs to a vector that replicates it. This process involves isolating a large amount of vector and chromosomal DNA and then cutting it with restriction enzymes. The restriction enzymes Not I, Bam HI, and EcoRI were used to cut the DNA in this trial. The DNA must then be ligased. A gel can be run to purify the DNA afterwards.

The methods used to replicate and express fluorescent proteins.

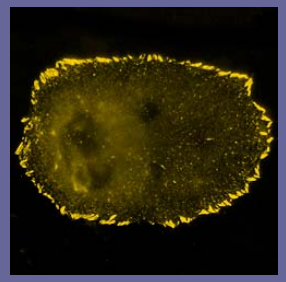




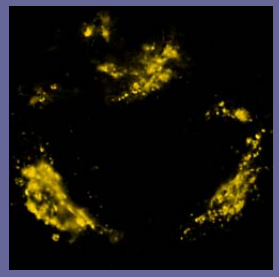
Microscopy of mTagRFP-alpa-actinin



Microscopy of mTagRFP-keratin

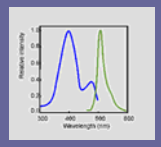


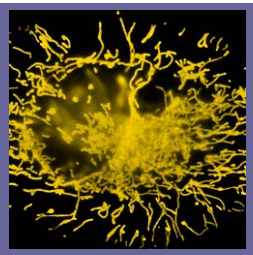
Microscopy of mTagRFP-paxillin



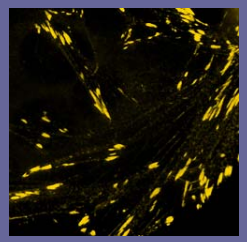
Microscopy of mTagRFP-Golgi

The excitation spectrum of native GFP from *A. victoria* (blue) has two excitation maxima at 395 nm and at 470 nm. The GFP excites at 488 nm, emits at 507 nm, and has a shoulder at 540 nm.





Microscopy of mTagRFP-mito



Microscopy of mTagRFP-zyxin

Conclusion:

More than 250 fluorescent proteins have been isolated from a variety of marine organisms. This allows scientists a varied color palette FPs to use. These FPs are extremely worthwhile for visualizing, monitoring, and tracking individual molecules, proteins and cells with fluorescence. The green fluorescent protein has proven especially useful in cell imaging.

Applying molecular cloning methods we successfully fused and imaged the following: alpha-actinin, keratin, paxillin, Golgi, mitochondria, and zyxin .

Resources:

Merzlyak, E.M, et al. “Bright monomeric red fluorescent protein with an extended fluorescence lifetime.” *Nature Mathods* (2007): 1-3.

Olenych, S.G., Claxton, N.S., Ottenberg, G.K., & Davidson, M.W. “The Fluorescent Protein Color Palette.” *Current Protocols in Cell Biology* (2006): 21.5.1-21.5.34.

Sambrook, J., Fritsch, E.F., and Maniatis, T. 1989. *MolecularCloning: A laboratory Manual.* Cold SpringHarbor, NY: Cold Spring Harbor Laboratory.

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