Expression of a gene for a modified green-fluorescent protein

Abstract

A pre-coelenterazine peptide comprising a modified A. victoria GFP having an amino acid sequence in which Ser.sup.65 is replaced with Tyr. There is further provided a polynucleotide encoding the pre-coelenterazine peptide, allowing synthesis of large, pure amounts of coelenterazine.
This application is a division of application Ser. No. 08/192,158, filed Feb. 4, 1994 now abandoned.

We claim:
1. A method of synthesizing coelenterazine comprising:
a) synthesizing a pre-coelenterazine peptide comprising a modified amino acid sequence of a green-fluorescent protein of A. victoria in which R.sup.65 is Tyr, said method comprising incubating a polynucleotide in the presence of means for effecting expression of said polynucleotide under conditions favorable to expression of said polynucleotide, said polynucleotide comprising one or more sequences of nucleotide bases collectively encoding said pre-coelenterazine peptide, wherein said one or more sequences of bases collectively encoding said amino acid sequence of said pre-coelenterazine peptide are uninterrupted by non-coding sequences; and
b) isolating coelenterazine from said means.

EXAMPLE I
In vitro MUTAGENESIS
TU#58 (described in U.S. patent application Ser. No 08/119,678) is treated with Ncol and EcoRI to generate a fragment of the GFP gene. This fragment is replicated by PCR with an oligomeric primer to insert the C197A mutation. The fragment is also treated with primers (Ncol at 5', T3 at 3') to incorporate restriction sites. The primer which incorporates the C197A mutation has the sequence (SEQ. ID No. 4):
CCT GTT CCA TGG CCA ACA CTT GTC ACT ACT TTC TAT TAT G
The "A" base six bases upstream of the 3' end is the nucleotide which effects the C197A mutation.

---------- Forwarded message ----------
Date: Mon, 21 Jul 2003 07:41:35 -0400
From: William Ward <crebb@rci.rutgers.edu>
To: George Pieczenik <piecze@rci.rutgers.edu>
Cc: "crebb@rci.rutgers.edu" <crebb@rci.rutgers.edu>
Subject: Re: Eveleigh's Control of the Fenton and HEIFA funds

George,

The only conversation you had with me on the coelenterazine idea was when I gave a seminar in room 325. You were sitting in the back of the room under the air conditioner. I remember the scene well and I remember your comment. After I had introduced the idea in the seminar, you jumped in with a comment about site-directed mutagenesis of the GFP gene to produce coelenterazine. I told you that Diane Davis and I had already been discussing that very idea. To my knowledge, this was your sole contribution to my research program and your contribution was after the fact. All other ideas you may have floated were your usual one-liners on the fly with no development and no follow through. Generally, when you pull this stunt, I have no idea what you are talking about and take no note of what you have said.
You continue to think I had something to do with GFP cloning. I did not. You continue to think that I have taken credit for GFP cloning. I have not. You continue to think that I worked with Chalfie and that he took credit for something I did. This is simply false. My sole involvement in the Chalfie work was to verify that the Columbia-cloned GFP is spectrally identical to the native jellyfish GFP, for which I got a "minor co-authorship" on the Science paper, a phrase I used to honestly describe my contribution to the project and for which I got nailed in promotion review. I have been cheated of nothing and you surely have not either.

I had the idea that coelenterazine synthesis was closely related to GFP synthesis years before 1993. I published on the idea in three abstracts before Chalfie announced GFP cloning and before I even know who he was or what he was doing. In fact, I said so much about the idea in print that it was hard to get the patent examiner to agree that parts of the idea (mine, not yours) were still patentable. You had nothing to do with this. Absolutely nothing except to be a respondent in my seminar and confirm what I already know and what I had already published.

United States Patent 5,491,084
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Uses of green-fluorescent protein

Abstract

This invention provides a cell comprising a DNA molecule having a regulatory element from a gene, other than a gene encoding a green-fluorescent protein operatively linked to a DNA sequence encoding the green-fluorescent protein. This invention also provides a method for selecting cells expressing a protein of interest which comprises: a. introducing into the cells a DNAI molecule having DNA sequence encoding the protein of interest and DNAII molecule having DNA sequence encoding a green-fluorescent protein; b. culturing the introduced cells in conditions permitting expression of the green-fluorescent protein and the
protein of interest; and c. selecting the cultured cells which express green-fluorescent protein, thereby selecting cells expressing the protein of interest. Finally, this invention provides various uses of a green-fluorescent protein.

Inventors: Chalfie; Martin (New York, NY); Prasher; Douglas (East Falmouth, MA)
Assignee: The Trustees of Columbia University in the City of New York (New York, NY); Woods Hole Oceanographic Institution (Woods Hole, MA)
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Intern'l Class: C12N 009/02; C12N 005/00; C12P 021/06; C07H 019/00
Field of Search: 435/69.1,69.7,189,240.2,252.3,320.1 536/22.1,23.1,23.4,23.5

References Cited [Referenced By]

Other References

Prasher et al. "Primary structure of the Dequorea victoria . . . "
What is claimed is:
1. A host cell comprising a DNA molecule having a regulatory element from a gene, other than a gene encoding an Aequorea victoria green-fluorescent protein operatively linked to a DNA sequence encoding the fluorescent Aequorea victoria green-fluorescent protein.

In an embodiment, the living organism is C. elegans. In another embodiment, the living organism is Drosophila, zebra fish or bacteriophage.
A bacteriophage carrying the green-fluorescent protein gene can infect a particular type of bacteria. The infection may be easily detected via the expression of the green-fluorescent protein. Therefore, by using appropriate bacteriophages, the presence of that particular type of bacteria may be detected.