Quantitative analysis of gene expression by fluorescence images using green fluorescence protein

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ABSTRACTS

We have analyzed the fluorescence image obtaining from green fluorescence protein (GFP). In order to monitor the fluorescence of specific gene, we used the amyloid precursor protein promoter which has been known to act as a major role in the development of Alzheimer's disease. The promoter from - 3.0 kb to + 100 base pair was inserted into the gene expression monitoring GFP vector purchased from Clontech. This construct was transfected into the PC12 and fibroblast cells and the fluorescence image was captured by two kinds of methods. One is using cheaper CCD camera and other is SIT-CCD camera. For the higher sensitivity of the fluorescence image, we developed the multiple image grabbing program. As a results, the fluorescence image by conventional CCD camera have the similar sensitivity compared with that of the SIT-camera by applying the multiple image grabbing programs. By this system. it will be possible to construct the fluorescence monitoring system with lower cost. And gene expression in real time by fluorescence image will be possible without changing the fluorescence images.

INTRODUCTION

Green Fluorescence Protein (GFP) has been used as a monitoring tools for the speicfic genes because of its special characteristics (Chalfie et al). Unlike other gene expression monitoring system including chloramphenicol transferase, luciferase assay systems, GFP does not require the extracellular substrates. So, it is possible to monitor the gene expression in real time.

In order to monitor the gene expression by fluorescence intensity, the high quality image grabbing system must be prepared and image processing system was also constructed.

In this study, amyloid precursor protein(APP) was selected for the monitoring expression. The amyloid precursor protein has the mojor role in the development of Alzheimer's disease (Quischke and Goldgaber). The fluorescence image which reflects the expression level of APP was captured by two methods. The one is the CCD camera, the other is SIT-CCD camera. Image processing system was developed to get the high quality of the fluorescence images(Shaw et al). The results shows the possibility of real time gene expression using GFP reporter vectors.

MATERIALS AND METHODS

Amyloid precursor protein promoter was kindly supported from Goldgaber. This promoter (-3.0 - +0.1 kb) was inserted into the GFP expression monitoring vector(purchased from Clontech. Co.). This construct was transfected into the PC12 cells and L929 fibroblast cells using calcium phosphate methods. After the transfection, the cells was harvested and transferred into the cell culture device which is adapted for the grabbing the fluorescence images. The fluorescence image of the transfected cells was grabbed by CCD camera (purchased from Sanyo Co.). The image from this camera was transferred into the PC by image boards (Matrox Co.). The silicone enhanced Transmission (SIT) CCD camera was also used for comparing the fluorescence image from cheaper, conventional CCD camera. The image grabbing software was programmed using visual C++ programs which is optimized in the condition of Windows 95 (Microsoft Co.). The comparison of the image and image processing was performed using PV-WAVE image processing programs.

RESULTS AND DISCUSSION

The schematic diagram of the amyloid precursor protein promoter sequences was shown in Fig.1. The sequence of trans-acting element for the basal level expression of the APP was included in this sequence. The DNA fragment of APP promoter was inserted into the pEGFP-1 promoter reporter vector(Fig.1). This construct was transfected into the PC12 cells and L929 fibroblast cells to monitor the expression of fluorescence. The transfected cells were harvested and transferred into the chamber device which is optimized for the culturing the cells under the fluorescence microscope. The process for capturing the fluorescence image from the cells was illustrated in Fig.2.

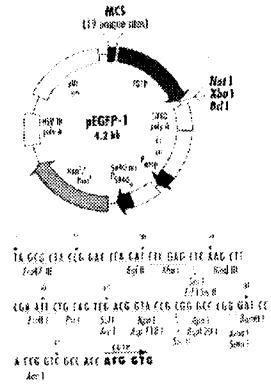

Figure 1. Construct of pEGFP

Two kinds of image capturing devices were used for comparison of the fluorescence images. The conventional CCD-camera was used for the lower sensitivity, and SIT-CCD camera was used for the higher sensitivity system. The image was transferred to the personal computer and stored in it. The fluorescence image from the conventional CCD-camera is shown in Fig. 3. (a) shows the cell image captured by light microscopy and (b and (c) show the fluorescence image captured at the same conditions. The intensity of fluorescence image captured by conventional CCD-camera was so low that it can not be recognized without image processing (Fig.3. b). Figure 3. c. shows the results after processing of the multiple image programs. This image increased its intensity compared to that of single cut image. Figure 4. shows the images taken from the CCD-camera and SIT-CCD camera. Comparing with these images, the multi-sum image (n=3, Fig.4.c) has higher intensity of the fluorescence compared with that of the single cut image(Fig. 4.b). But, the sharpness of the multi sum image was lower than that of the SIT-CCD camera's. In order to measure the fluorescence intensity more quantitatively, the intensity of the cell image was processed by PV-WAVE programs. Figure 5. shows the quantified images of the fibroblast cells. The intensity of the multi sum image, n=3 (b) was 3 times higher than that of single cut image(a). This results reflected the correlation between the images and quantified values. The image intensity of the SIT-CCD camera's is lower than that of multi-sum images. This data reflected the lower background level of the images. Figure 5.b. shows the relative ratio of the fluorescence images (cell/ background). This data shows the little difference of the relative intensities in spite of the difference of the image acquiring methods..

With image processing tools, the monitoring of gene expression of a specific gene. could be quantified with cheaper devices such as conventional CCD-camera. In the near future, monitoring of real time gene expression will be used using GFP reporter systems. .

REFERENCES

Quischke, W. W. and Goldgaber, D., 1989, *J. Bio. Chem.* **267**, 17362-17368.

Chalfie, M., Yuan, T., Euskirchen, G., Ward, W. W., and Pracher, D. C. 1994, *Science*, **263**, 802-805.

Shaw, S. L., Salmon, E. D., and Quatrano, R. S., 1995, *Biotechniques*, **19**, 946-955.

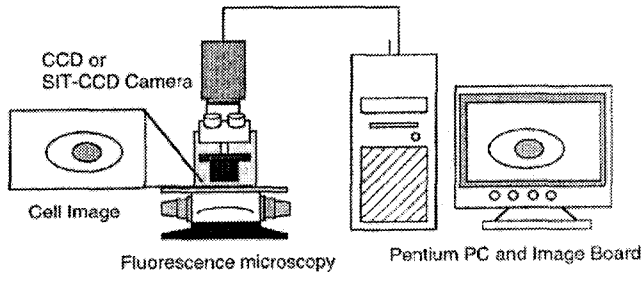

Figure 2. Schematic diagrams of image capturing systems

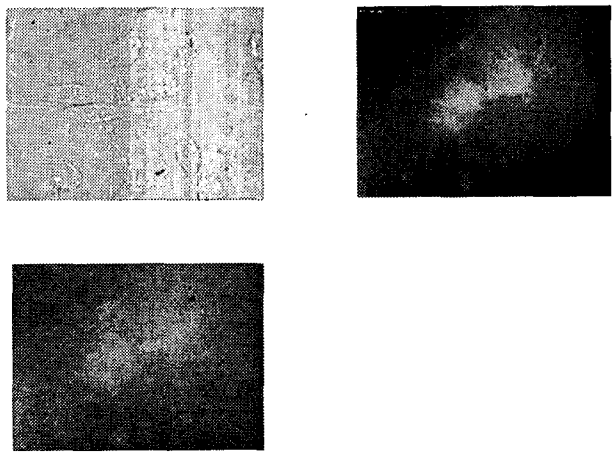

Figure 3. Cell and fluorescence image of GFP captured by CCD camera.

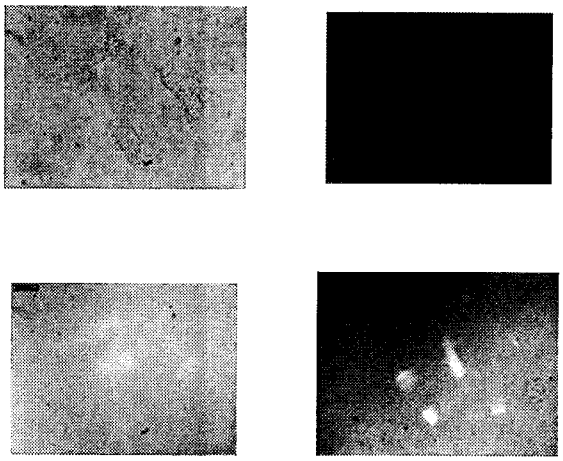

Figure 4. Cell and fluorescence image of GFP captured by CCD and SIT-CCD camera.

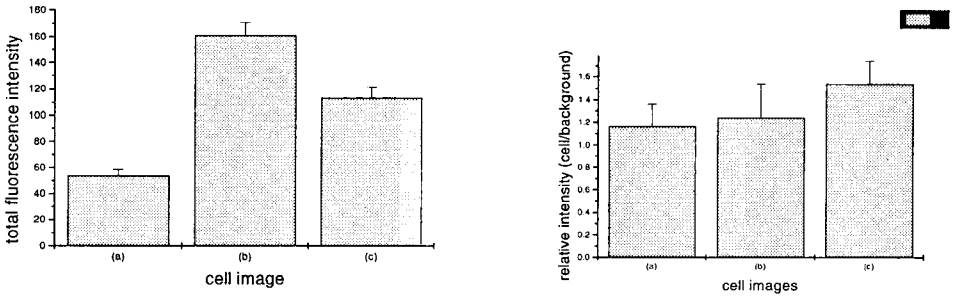

Figure 5. Quantitative analysis of APP expression by fluorescence image using PV-WAVE