Identifying GFP-Transgenic Animals by Flashlight

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In recent years, GFP has proven to be a valuable tool in many biological systems. Wild-type GFP has a major absorption in the UV region at 398 nm and a minor absorption in the blue region at 475 nm (3). Recently, several groups have independently carried out amino acid modifications of the GFP protein and have optimized these optical characteristics (6). For a comprehensive review on the photophysical behavior of GFP and GFP mutants, see Reference 7. Two widely used mutant forms of GFP, eGFP (BD Biosciences Clontech, Palo Alto, CA, USA) and mmGFP6 (6), have greatly enhanced absorbance around 475 nm, thus allowing excitation with blue light alone.

Many lines of transgenic mice expressing GFP and GFP fusion proteins have been described. Commonly, such mice are maintained by intercrossing of heterozygotes, a breeding strategy that necessitates the identification of transgenic and non-transgenic progeny. In animals that express GFP widely, this can be done by examining tissue under a suitable fluorescence microscope. Small pieces of tissue removed from animals during ear-notching (for identification purposes) are suitable. However, this approach has several drawbacks. Ear-notching is an invasive procedure that must be performed under appropriate animal husbandry practice, is open to errors of misidentifying from which mice biopsies have come, and is a relatively time-consuming way of screening large numbers of litters. In addition, ears are not sufficiently developed to allow ear-notching before around three weeks of age.

Previously, a genotyping protocol has been described that uses a UV light source and filters to visualize GFP in exposed tissue (1). Using this as a starting point, we investigated the possibility of using blue light excitation of GFP as a means to genotype GFP organisms. As a model, we used the tauGFP expressing transgenic line TgTP6.3 developed in our laboratory (4). These animals express a fusion protein in which GFP is joined to the microtubule-binding protein tau. The transgene is expressed at high levels and in most tissues. We decided not to use UV light, since we wanted to identify the GFP fluorescence in living animals and UV light is a hazard to both the operator and the animals. In addition, there were a number of other criteria for our GFP visualization equipment. We wanted it to be (i) noninvasive, (ii) easily brought in to the animal care facility, (iii) amenable to disinfection, (iv) quick to use (without significant warm-up time), (v) to be readily available, and (vi) inexpensive.

A survey of commercially available macroscopic GFP visualization equipment found that the cheapest was more than $1100 for a system that satisfies points i, ii, iii, and iv, and v. However, these systems are designed to high specifications that far exceed our requirements.

Blue light GFP visualization works by illuminating the tissue with light with a peak intensity at 475 nm and a steep decline in intensity at other wavelengths. Thus, the greatest possible amount of light from extraneous wavelengths is excluded. This is usually achieved by using an appropriate filter. The tissue is then visualized through a second barrier filter that excludes the blue light and passes only the emitted green fluorescent light. This is typically achieved using a filter that cuts out light from wavelengths less than 500 nm. These filters should also help to reduce the background autofluorescence.

A “homemade” system for GFP detection has been previously described (5). This system used a single blue light emitting diode (LED) to visualize GFP in E. coli transformed with a GFP containing plasmid. However, we considered that a single blue LED would be unlikely to generate enough light to excite GFP to detectable levels in the transgenic animals in vivo. In addition, this system used a photomultiplier tube (PMT) to convert photons to an electrical signal for computer analysis. PMTs are sensitive detectors for low-intensity applications such as fluorescence and typically can create millions of electrons for each photoelectron detected. This suggests that without the PMT amplification the GFP signal would not be strong enough from a single blue LED.

We have identified a commercially available blue LED flashlight (Inova™X5™; Emissive Energy, Warwick, RI, USA) with an emission wavelength of 470 nm. However, there are many available, and any blue LED flashlight with a wavelength of 470 nm would be suitable. The flashlight used here has five blue LEDs. Blue LEDs are also available from many semiconductor suppliers and a homemade flashlight could be produced. Several different types of blue LED are available. The compounds used in the LED manufacture govern the wavelength of the light. The majority of gallium nitride and indium gallium nitride on Al2O3 LEDs have a peak wavelength of around 470 nm: the exact wavelength is given in the

![Figure 1. Using a flashlight to visualize GFP in GFP-transgenic animals.](image-url)
manufacturer’s specifications.

On the front of the flashlight we have attached a Kodak® Polymax Filter PC3 (Eastman Kodak, Rochester, NY, USA), and we observe mice through a Kodak Wratten Gelatin Filter 12 (Sigma, Poole, UK). Details of the exact specifications for the filters are available from Eastman Kodak (2). Filter PC3 is no longer available on its own, but an equivalent is available as part of either the Kodak Polymax filter set or the ILFORD Multigrade filter set IV (ILFORD, Cheshire, UK). Any of the higher-contrast filters in the sets will work. Figure 1A illustrates the GFP visualization setup. Filter PC3 restricts the emitted light to around 475 nm, and filter 12 cuts out light below about 500 nm. These filters are not of a high enough quality for high-magnification photomicrographic work; however, for the purpose of identifying GFP expressing animals, they are acceptable. Figure 1B demonstrates the readily visible difference between transgenic and non-transgenic littermates. Using this system, we can identify transgenic animals immediately after birth. Indeed, the optimum time for identification of many transgenic mice is in the first week before hair grows. After the hair has grown, GFP-expressing tissue is still visible (e.g., parts of the nose and feet), but the effect in these areas is not as pronounced.

The system that we have described here fulfills all of our criteria for a GFP visualization system. It is noninvasive. The system is portable and so can be transported and used in an animal care facility with little logistical difficulties. The flashlight identified here is also waterproof and so is easily disinfected. The system requires no warm-up or cool-down period. The components are readily available, and it is relatively inexpensive. The setup described here costs less than $160.

From ethical, time-saving, and financial points of view, it is important to optimize the efficiency of breeding transgenic animals. As more and more laboratories make use of existing GFP transgenic animals, and more GFP transgenics are made, the importance of more effective screening will increase. We feel that the system described here will not be limited to genotyping widely or ubiquitously expressing GFP-transgenic mice but could probably be used for any GFP transgenic organism where the GFP protein is visible. The protein could be expressed either somewhere on the organism’s surface or in superficial internal organs visible through the skin.

REFERENCES


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Preservation of Fluorescent Protein Activity in Tumor Tissue

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It is now routine to use fluorescent protein technology to monitor developmental brain expression and following tumor growth and metastasis in vivo (1-4). We have explored the use of this technology to assess the impact of radiation and gene therapy using adenovirus on human gliomas grown in nude mice. Tumor cells tagged with GFP in vitro can be implanted intra-cerebrally and subsequently infused with an adenovirus that expresses a complementary fluorescent protein such as DsRed to follow tumor growth and virus transduction. This strategy would not only allow for noninvasive imaging but also one should be able to sort cells isolated from tumor tissue by flow cytometry to assess the effects of therapy on gene expression using microarray analysis. Recently, it was demonstrated that RNAlater™ (designated the reagent) (Ambion, Austin, TX, USA) not only preserves tissues for subsequent RNA isolation and microarray analysis but also does an excellent job of preserving intracellular structures, making this reagent suitable for proteomics analyses as well (5,6). To preserve the RNA of treated GFP tumors for future microarray analysis, we collected tumors in RNAlater. Subsequently, upon reexamination of the tissues, we discovered that the activities of EGFP and DsRed (both from BD Biosciences Clontech, Palo Alto, CA, USA) fluorescent proteins were preserved for weeks when stored in the cold, making it feasible to analyze tissues expressing fluorescent proteins days or even weeks after collecting the specimen.

Human glioma U87 cells were expression tagged with EGFP to monitor tumor growth and to assess the effect of radiation on tumor growth. One clone (U87/EGFP-B1) showed little to no difference in growth and radiosurvival compared to the parental U87 cells (Rosenberg et al., in preparation). These cells were then used in subsequent experiments. Female nu/nu mice (Harlan) were injected intra-cerebrally with...