USING NEW FLUORESCENT ACTIVATION FOR BETTER RESULTS

IN VIVO FLUORESCENT PROTEIN DETECTION

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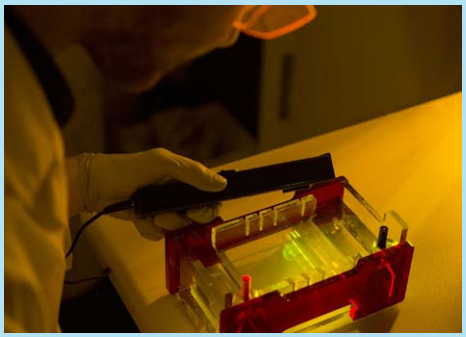
Applications

- Detection of GFP, etc.
- Very intense signal
- Gel documentation
- No DNA degradation
- Detection of nucleic acids
- No cellular stress



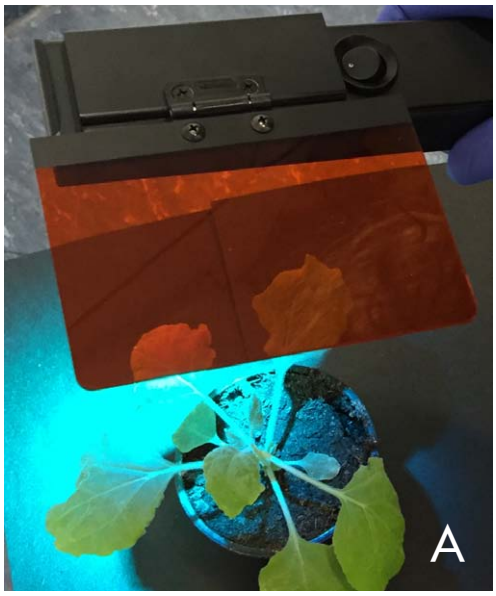
FastGene® BG-LED Flashlight: Originally developed for the detection of fluorescent DNA dyes.

Introduction

Fluorescent proteins are frequently used in plant molecular biology as markers for transgenic events. These events can be the consequence of stable or transient transformation of whole plants or plant organs. In this context it is desirable to monitor the success of the transformation in an effortless and non-invasive approach. It was show previously that illumination with blue light can visualize GFP expression in plants (Baker et al. 2012). Here we demonstrate that the FastGene® Blue/Green LED Flashlight originally designed to visualize DNA in gels can be used to detect strong fluorescent signals originating from green and non-green plant tissues expressing yellow and green fluorescent protein.

Methods

Vectors used for creating transgenic plants either harbored 702GFP protein with introns (Dadami et al. 2013) driven by the cauliflower mosaic virus promotor (35S) or contained a protein fusion with enhanced yellow fluorescent protein also under control of a 35S promotor (GenBank: KF499077.1, Dahncke, Witte 2013). The procedure for transient infiltration of Nicotiana benthamiana leaves was described previously (Witte et al. 2004; Werner et al. 2008) as well as the 'hairy root' transformation procedure for soybean (Kereszt et al. 2007). The 'hairy root' transformation of Brassica carinata was modified from Vieweg et al. 2004, in short seeds of Brassica carinata were vernalized for three days in the dark at 4°C and then germinated for three days (photoperiod, 16/8 h light/dark; temperature, 20/15 °C day/night; relative humidity, 100 %; light intensity, 220 µmol m-2 s-1). Agrobacterium tumefaciens C58, containing a root inducing (RI) plasmid (Arqua1), was grown for two days on a YEB plate, scratched off the plate and resuspended in 6 mL PS buffer (0.7% Na2HPO4 (w/v), 0.3% KH2PO4 (w/v), 0.5% NaCl (w/v), 150 µM acetosyringone, pH 7). After an incubation time of at least 1.5 h the B. carinata seedlings were dampened with the bacterial solution and wounded along the hypocotyl with an insulin injection (U-40 Insulin, 0.3 mm x 12 mm). Additionally, the bacterial solution was simultaneously injected into the plants. Then the seedlings were further cultivated in clay granulate under the same conditions as during the germination except for an incubation in the dark for 20 h directly after the transformation. After one week, the plants were fertilized with a nutrient solution containing (mM) 2.25 Ca(NO3)2 x 4 H2O, 2.5 K2SO4, 1 MgSO4 x 7 H2O, 0.25 KCl and (µM) 25 H3BO3, 1.5 MnSO4, 1.5 ZnSO4, 0.5 CuSO4, 0.025 (NH4)6Mo7O24 and 35.8 Fe (FeIII-EDTA). Two weeks after the transformation, clay granulate was removed from grown hairy roots. One additional week later the plants were screened for transgenic roots using GFP as fluorescent marker. The stable transformation of Arabidopsis was accomplished with the 'flower dip'



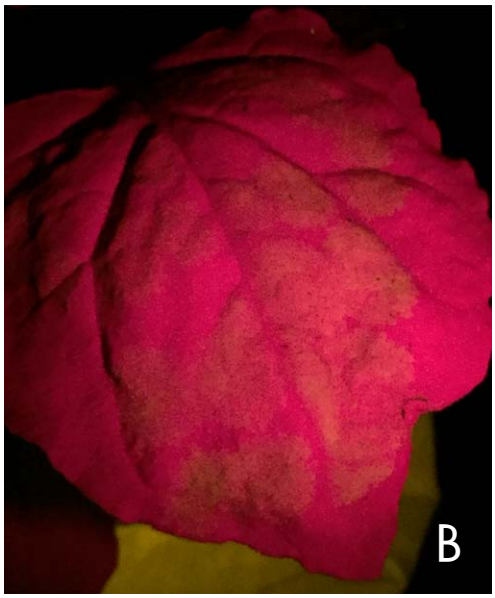


Figure 1. (A) Demonstration of the flashlight use on a Nicotiana benthamiana plant. (B) YFP expression in transiently infiltrated N. benthamiana leaves. The infiltrated area can be seen as yellowish area clearly different from red parts of the leaf without infiltration (C) Close-up of a soybean root (red) with hairy roots expressing GFP (green) (D) whole Brassica carinata plant with hairy roots expressing GFP (visible in green) (E) stably transformed Arabidopsis thaliana seedlings expressing either YFP (yellow) or no fluorescent protein (red).

method previously described (Clough, Bent 1998). Photos of plants and plant organs were taken during illumination with the FastGene® Blue/Green LED flashlight through the supplied filter with a cell phone camera in a dark room.

Results

Photosynthetically active plant tissue appears red with the FastGene® Blue/Green LED flashlight whereas plant roots can be light red or white. However, the presence of fluorescent protein expression is clearly visible upon illumination with the FastGene® Blue/Green LED flashlight as a green/yellow signal. In the hairy root transformation and the leaf infiltration method the observed yellow/green signal was consistent with other methods determining fluorescence (confocal laser microscopy, fluorescent binocular). In stably transformed Arabidopsis seedlings all seedlings predicted to be fluorescent could be verified with other methods, however many additional transgenic seedlings were identified that did not give any signal with the FastGene® Blue/Green LED flashlight suggesting that the expression of fluorescent protein needs to be strong enough for detection with the FastGene® Blue/Green LED flashlight.

Discussion

The FastGene® Blue/Green LED flashlight is a convenient device for detection of green and yellow fluorescent protein in different plant tissues. Except for an electrical plug and relative darkness (in a plant chamber this can be accomplished with a dark cloth) no additional equipment is required allowing for an easy detection of fluorescence e.g. to access the success of a transformation procedure. In some instances the FastGene® Blue/Green LED flashlight may not have the sufficient sensitivity for the detection of weak fluorescent plant tissue, however this can also be seen as an advantage enabling the selection of e.g. seedlings within a population that exhibits especially strong protein expression without using laborious methods. This is especially interesting since a correlation between GFP marker gene expression and the expression strength of a target gene was demonstrated (Harper et al. 1999).

Publication bibliography

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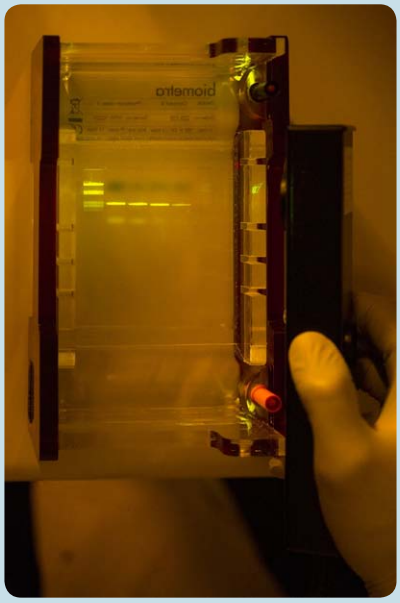

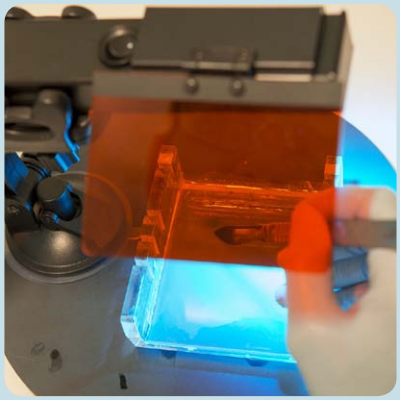

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Imprint

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SUMMARY

Detection of green and yellow fluorescent protein was possible in plant tissue using the FastGene Flashlight with blue/ green LEDs.

The relative expression levels of GFP could also be estimated by the brightness of the fluorescence. Hence, the flashlight was also used for the selection of strongly expressing organisms.

Ordering Information

Cat.No.	Description
FG-11	**FastGene® B/G LED Flashlight**
MG04	**MIDORI[GREEN] Advance**
MG06	**MIDORI[GREEN] Direct**
FG-08	**FastGene® B/G LED Transilluminator**
GP04LED	**B/G LED GelPicBox**
GP05LED	**FAS Digi**
GP06LED	**FAS Nano**
GP-FAS-V	**FAS-V**