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Note

A New Family of Drosophila Balancer Chromosomes With a w^- *dfd*-GMR Yellow Fluorescent Protein Marker

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ABSTRACT

We report new w^- fluorescent balancers scorable from stage 13 through adulthood that bear a nuclear-localized yellow fluorescent protein marker directly driven by *dfd* and GMR enhancer elements. The utility of this marker is enhanced by identification of an anti-GFP/yellow fluorescent protein (YFP) serum that is compatible with heat fixation.

THE advent of balancer chromosomes expressing the green fluorescent protein (GFP) (Chalfie *et al.* 1994) allowed Drosophila researchers to identify live embryos and larvae of specific genotypes with far more flexibility than was possible with viable genetic markers. In addition, fluorescent balancers enable embryos and larvae to be scored and collected using automated sorters such as the COPAS instrument (Union Biometrica, Somerville, MA). While multiple fluorescent balancers are in wide use, existing balancers have several limitations that are particularly problematic for embryonic experiments when autofluorescence creates significant background and tissues have had little time to express the fluorescent marker. To obtain high expression levels many balancers use the Gal4/UAS expression system of Brand and Perrimon (1993), which precludes using such balancers with toxic UAS transgenes. This problem is avoided with "direct-drive" constructs in which an enhancer directly drives the expression of the marker, but without Gal4-mediated amplification available direct-drive balancers are difficult to score during embryogenesis (*e.g.*, actin GFP in Figure 1). An additional limitation common to both Gal4 and direct-drive balancers is that they have been constructed using w^+ as a transformation marker, which obscures the w^+ markers of transposon-insertion mutations or transgenic constructs and complicates many genetic cross strategies.

To create a fluorescent marker that could be used for scoring mid- to late-stage embryos, we inserted the Hz2.7rev fragment of the *deformed* (*dfd*) enhancer (Bergson and McGinnis 1990) into a modified Pelican transformation vector (Barolo *et al.* 2004) in which we had replaced the GFP-coding region with the eYFP (Clontech Laboratories, Mountain View, CA) and inserted a short intron cassette from the *ftz* gene (Rio 1988). We chose the *dfd* HZ2.7rev enhancer (hereafter referred to as *dfd*) because a TM3 balancer bearing the lacZ gene directly driven by this *dfd* enhancer (D. Bilder, personal communication) was the most strongly expressed balancer marker from mid- through late embryogenesis that we encountered, and the spatially restricted expression of lacZ in the head regions both aids in scoring of the marker and leaves most of the embryo clear of lacZ staining, which allows heterozygous embryos to be used as internal controls (Figure 1 and Paul *et al.* 2003; Wu *et al.* 2004). We based the

Sequence data from this article have been deposited with the EMBL/GenBank Data Libraries under accessions nos. AY391252 (Yellow H-Pelican), AY730637 (Yellow HI-Pelican), DQ835189 (*dfd* in Yellow H-Pelican), DQ835190 (*dfd* in Yellow HI-Pelican), and DQ835188 (*dfd*-GMR-nvYFP in modified pCaSpeR4 backbone). Stocks from this article have been deposited with the Bloomington *Drosophila* Stock Center under stock nos. BL8578 (CyO *dfd*-eYFP w^+), BL8623 [CyO *dfd*-eYFP w^- (made by γ-ray mutagenesis of the BL8578)], and BL8704 (TM6B Sb Tb Hu *dfd*-eYFP w^+).

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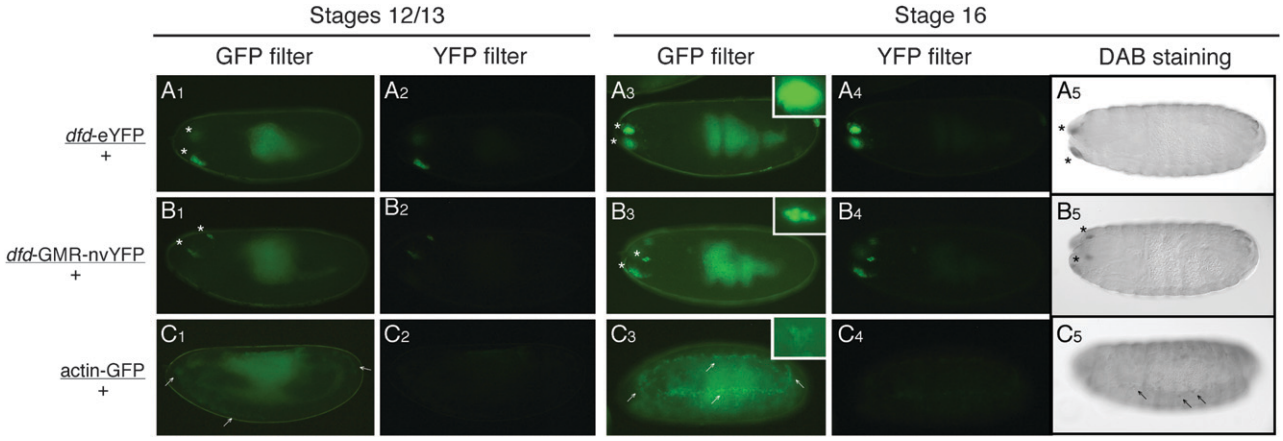

FIGURE 1.—*dfd*-based YFP constructs are effective markers for mid- to late-stage embryos. When viewing embryos by standard fluorescence microscopy, *dfd*-based YFP markers (A1–A4, B1–B4) are more visible than the commonly used actin-based GFP marker (J.-M. REICHHART and D. FERRANDON, personal communication) (C1–C4) using either GFP or YFP filter sets (Chroma Technology, Rockingham, VT). *dfd*-driven YFP fluorescence is readily visible in the head regions (asterisks in A1, A3, B1, and B3) while the more diffuse actin-driven GFP is marginally visible at stage 13 (arrows in C1 and C3) and moderately visible by stage 16 (arrow in C3). Note the nuclear localization of the nvYFP marker compared to the cytoplasmic localization of the eYFP and GFP markers (insets B3 *vs.* A3 and C3). In immunohistochemically stained embryos, the *dfd*-based YFP markers are also much more readily detected than the actin-GFP marker (A5 and B5 *vs.* C5). The shown embryos were heat-fixed (MILLER *et al.* 1989; PEIFER *et al.* 1993), stained with unpreadsorbed ab290 anti-GFP primary antibody at 1:10,000, and briefly developed to simulate use of the markers with antibodies that give moderate background so as to demonstrate the greater scorability of the YFP-based markers. Primary staining was visualized using biotinylated anti-rabbit secondary (1:300; Jackson Immunoresearch, West Grove, PA) and streptavidin–HRP (Vector Labs, Burlingame, CA) and developed using H_2O_2/*d*i*a*mino *b*enzidine (DAB) (Sigma-Aldrich, St. Louis) for 45 sec. Images were acquired on a Zeiss Axioplan2 using an AxioCam at the same settings and subjected to the same linear level adjustments.

marker construct on YFP rather than the more common GFP because the excitation and emission spectra of YFP have less overlap with the embryonic and larval autofluorescence (Figure 1). Importantly, a YFP marker is still readily scorable with the more widely available GFP filters (Figure 1) and not only is compatible with the COPAS embryo sorter, but also the reduced autofluorescence background in the YFP channel should improve discrimination of transgene copy number (B. WANG, personal communication). A small intron was inserted because introns can enhance mRNA production in *Caenorhabditis elegans* and vertebrates (LE HIR *et al.* 2003); however, there was no obvious difference in fluorescence between our constructs that either contained or lacked the intron (data not shown). Balancers bearing the *dfd*-eYFP marker could be scored from stage 12/13 through the end of larval development and in adulthood (Figure 1 and data not shown). For late embryonic use, these balancers proved to be generally superior to existing fluorescent balancers and have been distributed to the Drosophila community. However, as a general marker, the *dfd*-eYFP was suboptimal because it could not be scored in pupae, adult expression was inconvenient to score, and it used the w^+ transformation marker. In addition, tests on multiple independent insertions revealed that this marker construct was extremely difficult to mobilize (~1 in 5000 hops/fly compared to ~1 in 3 hops/fly for the *dfd*-lacZ transgene of BERGSON and MCGINNIS 1990) (data not shown) because of either the insulator elements present in the Pelican vector or the combination of the insulator elements and the *dfd*-eYFP gene.

We sought to improve the marker by adding the eye-specific *g*lass *m*ultimer *r*eporter (GMR) enhancer (HAY *et al.* 1994), replacing the YFP with a Drosophila codon-optimized Venus YFP [a faster-folding, brighter variant of YFP (NAGAI *et al.* 2002)], adding a nuclear localization signal (NLS), and moving the resulting *dfd*-GMR-nvYFP to a modified pCaSpeR4 transformation vector that lacked the insulator elements and w^+ transformation marker of the Pelican vector. In transgenic flies, the *dfd*-GMR-nvYFP marker is scorable from embryonic stage 12/13 onward (Figure 1), with the GMR enhancer driving YFP expression in the eyes and eye discs strongly enough to allow clear scoring of the marker in pupal cases (data not shown). In a wild-type w^+ background, the red eye pigment blocks most of the YFP signal from late pupal-hood onward, but adults bearing the marker can be scored by their fluorescent proboscises (data not shown).

In addition to developing a new fluorescent marker construct, we also report the identification of an antiserum (ab290 rabbit anti-GFP; Abcam, Cambridge, UK) that recognizes GFP and YFP fixed with heat-treatment protocols (MILLER *et al.* 1989; PEIFER *et al.* 1993) (Figure 1, A5, B5, and C5). Although heat fixation is essential for staining with several commonly used antibodies such as the anti-armadillo N2 7A1 monoclonal (PEIFER *et al.* 1994) and often works well for many anti-peptide

antisera that do not work with standard fixation protocols (*e.g.*, anti-sinuous; Wu *et al.* 2004), there apparently have not been sera or monoclonal antibodies that react with either GFP or β-galactosidase after heat fixation. Thus, the ab290 anti-GFP serum dramatically improves identification of nonbalancer embryos when using heat-fixation protocols. The ab290 antisera also recognize GFP and YFP after standard formaldehyde/heptane fixation procedures (data not shown).

In summary, the *w*⁻ *dfd*-GMR-nvYFP balancers and the heat-fixation compatible anti-GFP/YFP serum are each significant improvements over currently available reagents. Together they enable a single robustly scorable balancer chromosome to be used for live imaging, automated sorting, and immunohistochemical staining of animals older than embryonic stage 13.

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