

Short Technical Reports

Polyubiquitin-Regulated DsRed Marker for Transgenic Insects

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ABSTRACT

Genetic transformation of most insect systems requires dominant-acting markers that do not depend on reverting a mutant phenotype in a host strain, and for this purpose GFP has proven to be useful in several insect orders. However, detection of multiple transgenes and reporters for gene expression requires the development of new visible markers that can be unambiguously detected when co-expressed with GFP. The DsRed fluorescent protein has spectral characteristics that are most distinct from GFP and GFP variants, and we have explored the use of DsRed as a selectable marker for piggyBac transformation in *Drosophila melanogaster* and its use as a reporter when co-expressed with GFP. Transformants marked with polyubiquitin-regulated DsRed1 were detected throughout development at a relatively high frequency, and they exhibited brighter fluorescence than transformants marked with EGFP. The use of a Texas Red® filter set eliminated detection of EGFP fluorescence and autofluorescence, and DsRed expressed from a reporter construct could be unambiguously detected when co-expressed with EGFP. DsRed should prove to be a highly efficient marker system for the selection of transformant insects and as a reporter in gene expression studies.

INTRODUCTION

Germ-line transformation typically requires efficient marker systems for the identification and selection of transformed organisms. For some insects, and for *Drosophila melanogaster* in particular, this has been achieved by the complementation or "mutant-rescue" of eye-color mutants with their respective wild-type allele (19). The primary drawbacks of using a mutant-rescue approach is that only mutant strains can be used as recipient hosts, transformants must be individually selected, and, for some markers, position-effect variegation/suppression of the wild-type phenotype can make detection difficult (9). Efforts to overcome these drawbacks first utilized dominant-acting genes that could confer resistance to chemicals or drugs (2,20), though these are cumbersome, pose some safety risks, and are often unreliable, owing to selection of insects having natural resistance.

The development of transformation vector systems for non-drosophilid insects has made the need for non-mutant-based selections a high priority. Though mutant-rescue is possible for some species (often using the *Drosophila* wild-type gene ortholog as a marker), most species have neither mutant strains nor the cloned wild allele available (19). Several groups have addressed this problem by developing green fluorescent protein (GFP)-based markers (6,18). Using a variety of regulatory promoters with several GFP variants, markers have been created for Diptera (9,10,12,17), Lepidoptera (16,21), and Coleoptera (3). Detection of enhanced GFP (EGFP) is less susceptible to position effect suppression than eye-color markers, and it appears to be highly stable, though one drawback is that it shares spectral characteristics with autofluorescing molecules in insects that are not easily filtered. As gene transfer technology advances in insects, additional markers will also become a necessity for tracking multiple transgenes and for use as reporters for gene expression. Some of these may be provided by the GFP variants that emit blue, yellow, and cyan fluorescence; however, overlapping emission spectra cause difficulty in making clear distinctions between some of them. This may be alleviated by a new fluorescent protein, DsRed, discovered in the coral, *Discosoma striata* (15). This emits red fluorescence that is spectrally distinct from GFP and GFP variants.

To determine if DsRed could be an effective transformation marker in insects and to compare its use to EGFP, we created a vector having DsRed under polyubiquitin promoter regulation placed into the piggyBac transposon. To determine if DsRed could act as an efficient reporter in an EGFP background, we tested it in the GAL4 transcriptional activation system. In *D. melanogaster* transformants, DsRed

expression appears to be effective as a primary transformation selection and as a reporter gene.

MATERIALS AND METHODS

Insect Strains and Rearing

The *D. melanogaster white* strain *w[m]* and transformant progeny were maintained at 23°C–25°C on standard cornmeal-yeast-molasses media.

Plasmids

The pB[PUBDsRed1] piggyBac vector marked with polyubiquitin-regulated DsRed1 (15) was created by isolating the polyubiquitin promoter (14) as an *EcoRI-BamHI* fragment from PUBnls-GFP (8) and ligating it into the the *EcoRI-BglIII* N-terminal cloning site of pDs-Red1-N1 (Clontech Laboratories, Palo Alto, CA, USA), creating pPUB-DsRed1. The polyubiquitin-DsRed1 gene was isolated as a *BglIII-NotI* fragment that was used to replace the PUBnlsEGFP-1 marker cassette within the *BglIII-NotI* site of pB[PUBnlsEGFP] (9). Construction of the *hsp70*-regulated transposase helper, pshpBac, was de-

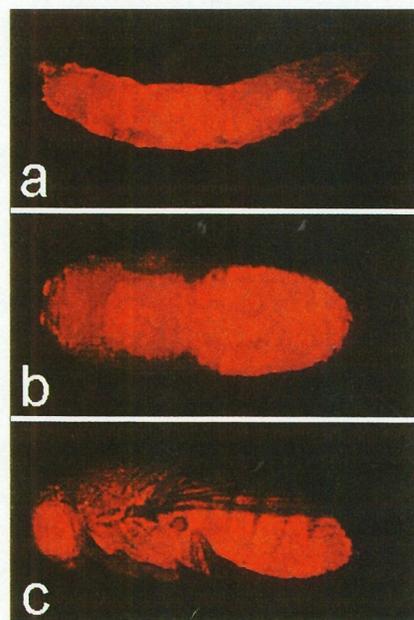


Figure 1. Expression of DsRed during development in *D. melanogaster* individuals transformed with the pB[PUBDsRed1] vector. Developmental stages include (a) third instar larva, (b) late stage pupa, and (c) adulthood.

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scribed previously (9).

The pB[hsp-GAL4, EGFP] vector was created by ligating the hsp70-GAL4 cassette, from pF89 (5) as a *Bg*III-*Stu*I fragment, into the *Bg*III and blunted *Bst*BI site of pB[PUBnlsEGFP]. The pB[UAS-DsRed1, EGFP] vector was created by isolating DsRed1 from pDsRed1-N1 as a *Bam*HI/*Not*I fragment and ligating it into the *Bg*III/*Not*I sites of pUAST (4) to create pUAS-DsRed1. The UAS-DsRed1 fragment from pUAS-DsRed1 was then isolated as a *Bam*HI fragment and ligated into the *Bg*III site of pB[PUBnlsEGFP].

Insect Transformation

Embryo injections used standard procedures (9) with G₀ adults backcrossed to *w*[m] flies in small groups of four G₀s to four to eight *w*[m] flies of the opposite sex. G₁ eggs were collected for two

weeks and reared under standard conditions. Transformants were initially detected by DsRed fluorescence in G₁ larvae and pupae and verified by Southern DNA hybridization. Vector DNA was detected in transformant lines in independent hybridizations using either *piggyBac* or DsRed DNA as probe.

GAL4-Dependent Expression

D. melanogaster transformed with the pB[hsp-Gal4, EGFP] and pB[UAS-DsRed1, EGFP] vectors were inbred as single pair matings for successive generations until all progeny expressed the EGFP marker and were considered to be homozygous. Adult flies from the hsp-Gal4 and UAS-DsRed1 lines were intermated, and their progeny subjected to heat shock at 37°C for 1 h at indicated times. DsRed expression was monitored at daily intervals.

Fluorescent Protein Expression

DsRed and EGFP were observed at various developmental stages from transgenic insects having single integrations as determined by Southern analysis. Fluorescence was observed under a Leica MZ FLIII stereozoom fluorescent microscope using a mercury lamp and appropriate filter sets (Chroma Technology, Brattleboro, VT, USA). For DsRed detection the HQ Texas Red® set no. 41004 was used, having the following filters: exciter HQ560/55x, dichroic Q595LP, and emission HQ645/75m. For EGFP detection, the FITC/RSGFP LP emission set no. HQ 41012 was used, having the following filters: exciter HQ480/40, dichroic Q505LP, and emission HQ510LP. Digital images were obtained with a SPOT-1 cooled CCD camera (Diagnostic Instruments, Sterling Heights, MI, USA) and captured with Adobe® Photoshop® 4.0 software. For comparison of fluorescent protein expression, automatic exposures were calculated, and images were taken for each protein from a transformed adult using the appropriate filter set. These settings were also used as a user-defined exposure for the other protein.

RESULTS

pB[PUBDsRed1] Transformation

The DsRed1 gene was tested as a marker in transgenic insects by placing it under polyubiquitin promoter regulation and integrating it into the *D. melanogaster* genome by *piggyBac* transformation. Transformants were detected in 26 independent group matings of G₀ adults, yielding an approximate transformation frequency of 25%. Southern hybridization of nine of the transformant lines indicated two vector integrations in one line, with single integrations in the eight others.

Transformed G₁ *Drosophila* were initially detected by red fluorescence as larvae, pupae, and adults within glass vials. Fluorescence throughout development and in most tissues was verified by examination of individual transgenic flies (Figure 1), which is consistent with normal polyubiquitin promoter

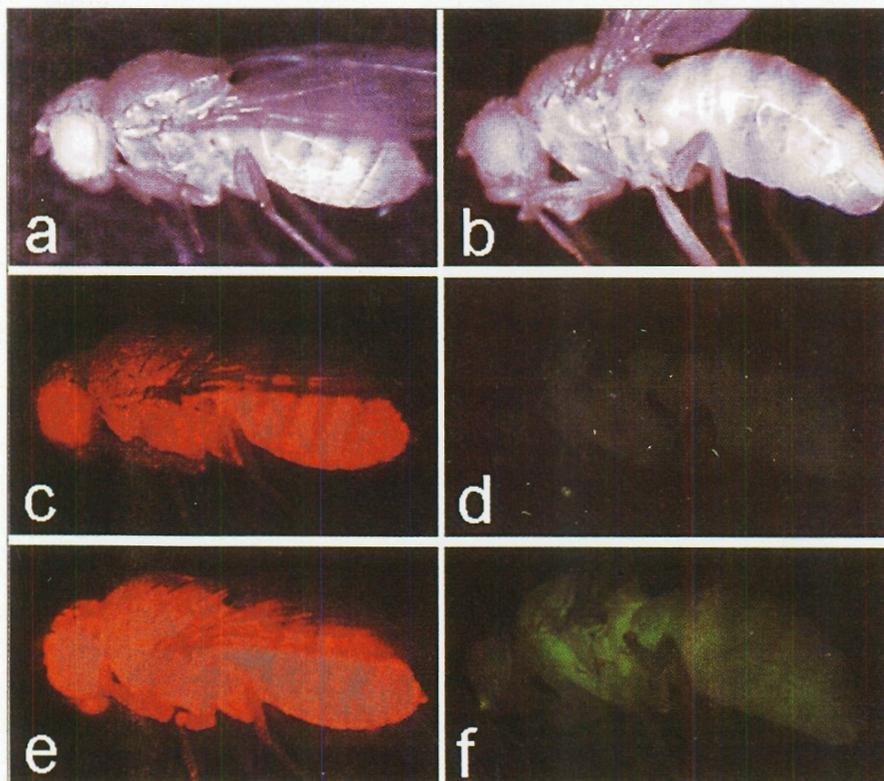


Figure 2. Comparison of fluorescent protein expression from digital images of *D. melanogaster* adults transformed with the DsRed1 or EGFP marker. An adult transformed with pB[PUBDsRed1] under (a) brightfield, (c) fluorescence (Texas Red filter set) with an automatic exposure setting (red, 1.071 s; green, 1.602 s; and blue, 17.085 s), and (e) fluorescence (Texas Red filter set) with a user-defined exposure (auto-setting for EGFP transformant; see Figure 2f). An adult transformed with pB[PUBnlsEGFP] under (b) brightfield, (d) fluorescence (FITC/GFP filter set) with a user-defined exposure (auto-setting for DsRed1 transformant; see Figure 2c), and (f) fluorescence (FITC/GFP filter set) with an automatic exposure setting (red, 2.53 s; green, 3.998 s; and blue, 40.365 s).

function (14), and GFP fluorescence in *Drosophila* transformed with GFP markers regulated by the same promoter (8,9). To compare the efficacy of the EGFP and DsRed markers in transgenic flies, we compared fluorescence from adult flies having a single integration of either the pB[PubDsRed1] vector or the pB[PubnlsEGFP] vector from a previous experiment (9). Subjective comparison by visual inspection of transformed adult flies from several transgenic lines indicated that the DsRed fluorescence was consistently brighter than EGFP and especially at lower magnifications where EGFP could be barely detected. For an objective observation, digital images were taken for both markers using the automatic exposure setting for each fluorescent protein, which was then used as a user-defined exposure for the other protein. The automatic exposure setting for DsRed (Figure 2c) barely detected EGFP (Figure 2d), while the automatic exposure setting for EGFP (Figure 2f) resulted in an overexposure of DsRed (Figure 2e). This is reflected in a more than doubling of the automatic red, green, and blue exposures for GFP relative to DsRed. Thus, for the transgenic *Drosophila* examined, fluorescence from DsRed appears to be significantly more intense than for EGFP.

DsRed was detected using a Texas Red filter, while Clontech Laboratories (7) advises using filters normally used for rhodamine or propidium iodide. As noted by Clontech Laboratories (7), detection of DsRed should be less susceptible to autofluorescence because it emits outside the range of most types of natural fluorescence. We verified the difference in detection of interfering autofluorescence and other fluorescent proteins having overlapping emission spectra by comparing *Drosophila* transformed with DsRed, EGFP, and non-transformed pupae under the FITC/GFP and Texas Red filter sets (Figure 3A). Under the FITC/GFP filter, the EGFP transformed pupa appeared bright green, the DsRed pupa was pink-orange, and the non-transformed pupa emitted detectable yellow autofluorescence from its pupal case. In contrast, under the Texas Red filter, the DsRed pupa was bright red, with only barely detectable fluorescence from the

EGFP and non-transformed pupae. Red autofluorescence was more apparent only at higher magnification.

DsRed Reporter Gene Expression

The use of DsRed as a reporter in an EGFP background was tested in the yeast GAL4/UAS transcriptional activation system (5) with DsRed1 linked to the UAS enhancer and Gal4 under *hsp70* promoter regulation. The pB[UAS-DsRed1, EGFP] and pB[hsp70-Gal4, EGFP] vector constructs were transformed independently into the *D. melanogaster* *w*[m] strain with transformants selected by GFP expression (Figure 3B-a). Flies homozygous for the respective transgenes were intermated, with their progeny subjected to heat shock as larvae or pupae. DsRed expression was first detected in pharate adult pupae one day after two daily heat shocks (two days after the first heat shock; Figure 3B-b).

DISCUSSION

The DsRed fluorescent protein was shown to be an effective marker for the selection of transformants in *D. melanogaster* and as a reporter for gene expression in the same species. Transformants having DsRed1 under polyubiquitin promoter regulation within a *piggyBac* transformation vector were detected at a relatively high frequency and could be observed as G₁ larval progeny within vials. The DsRed1 gene is optimized for mammalian codon usage, but as with the similarly optimized EGFP gene (22), expression in insect tissue apparently was not compromised. Under the same exposure settings, fluorescence from a DsRed1 transformant was visibly more intense than that from an EGFP transformant, and this generally held true for all the lines compared. Our general observations indicate that DsRed is a more effective marker than EGFP, which is consistent with the suggestion by Clontech

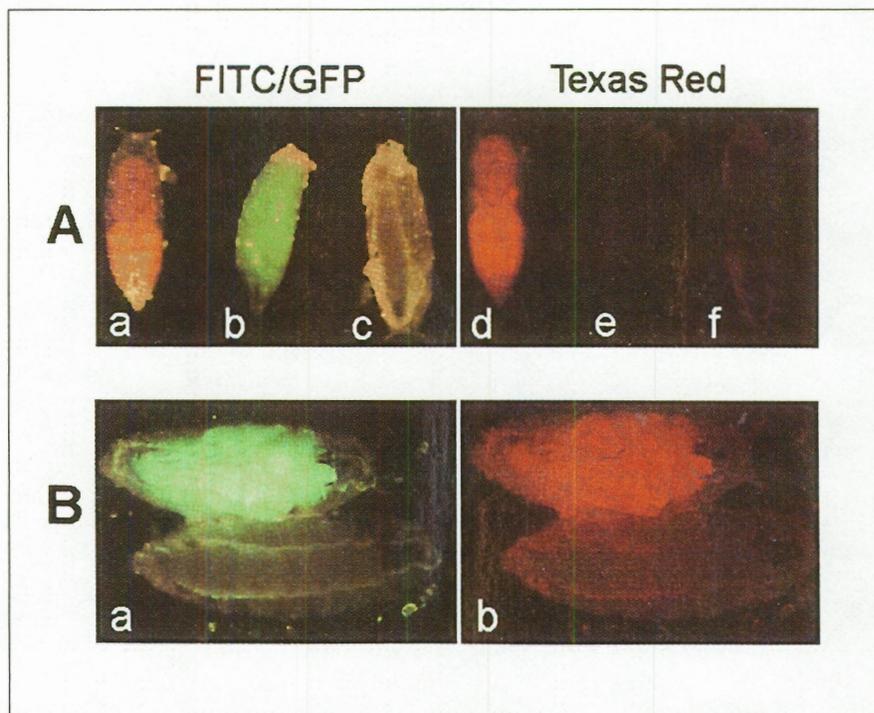


Figure 3. Fluorescent protein expression from *D. melanogaster* pupae. (A) Comparison of fluorescent protein expression and autofluorescence in *D. melanogaster* pupae transformed with the pB[PubDsRed1] vector (a and d), the pB[PubnlsEGFP] vector (b and e), and from the non-transformed *w*[m] strain (c and f). Digital images of fluorescent pupae were taken with a FITC/GFP (a-c) or Texas Red (d-f) filter set. (B) Expression of fluorescent proteins in pupal progeny from the parental mating of flies transformed with pB[UAS-DsRed1, EGFP] and pB[hsp-Gal4, EGFP] after two daily heat shocks at 37°C. The EGFP transformation marker is detected with a FITC/GFP filter set (a), and expression of the DsRed reporter gene in response to heat shock is detected with a Texas Red filter set (b). The transformed pupa is on the top, and a non-transformed pupa is on the bottom.

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Laboratories (7) that DsRed fluorescence may be more easily observable than for other fluorescent proteins because tissues absorb less energy at longer wavelengths and the recent observation by Baird et al. (1) that DsRed has a higher extinction coefficient and fluorescence quantum yield than originally thought. Definitive comparisons of markers in transgenic insects are difficult because their expression may vary with transgene integration site, owing to position effect variegation, and here the EGFP marker was also subject to nuclear localization. Thus, the relative efficacy of DsRed as a transformation marker will be subject to how it is regulated in a specific host insect, but our results support the conclusion that it will be at least equal to if not an improvement over GFP. Recently, we have used the pB[PUBDsRed1] vector to transform the Caribbean fruit fly (unpublished results), which had previously been trans-

formed with *piggyBac* marked with EGFP (10). Thus far, the DsRed marker characteristics described for *Drosophila* also extend to this non-drosophilid.

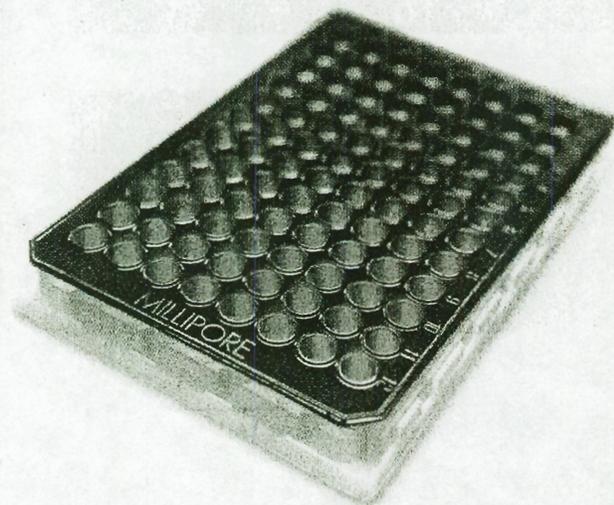
The Texas Red filter set used for DsRed1 detection almost completely blocked EGFP fluorescence and autofluorescence from pupal cuticle. In contrast, a FITC/GFP filter set with a long-pass filter allowed the detection of DsRed1 transgene expression and autofluorescence from non-transformed pupae. The lack of stringency of GFP filters is a significant problem for the use of GFP as a marker system, and especially so for insects. Fluorescence that is difficult to visibly distinguish from GFP or EGFP is often observed in insect gut tissue and from food within the gut, urate crystals in the Malpighian tubules, yolk proteins, cuticle, necrotic tissue, and other biological molecules causing ambiguity during the selection of transformants (11).

Though the extent of autofluorescence varies for different species, it is quite common, and the availability of a fluorescent marker that is unambiguously detected should greatly improve the efficiency of transformant selection. Fluorescence detection is also difficult in many insect species because of quenching by highly pigmented or melanized cuticle in adults. DsRed intensity and its more selective detection may prove highly advantageous for the unambiguous identification of transgenic insects in field applications.

Beyond the selection of transgenic insects, fluorescent proteins are widely used as reporter genes. However, overlapping emission spectra is problematic when attempting to distinguish fluorescence from independent markers expressed in the same tissue, such as when one fluorescent protein is used for transgenic selection and another as a gene expression reporter, or in co-localiza-

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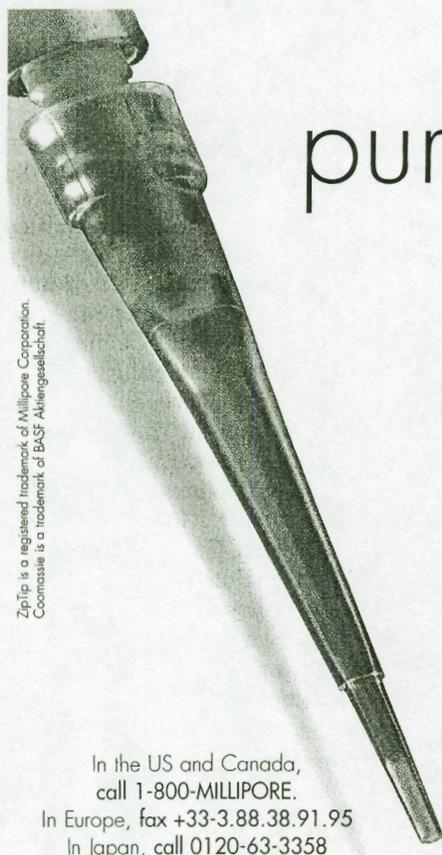
tion studies. The ability of filter systems for DsRed to effectively block EGFP fluorescence indicates that DsRed can be used as an unambiguous reporter in tissue where EGFP is co-expressed. This was suggested by co-expression studies in *E. coli* (13) and demonstrated here for EGFP-marked transgenic flies having DsRed ectopically expressed under GAL4 regulation. Thus, DsRed should prove useful for analysis of multiple transgenes and gene expression studies such as enhancer-trapping, though the maturation time for DsRed will prove problematic for genes under tight temporal regulation.

In summary, the DsRed fluorescent protein provides a new genetic marking system for a number of organisms that will be especially important to the genetic analysis and manipulation of insects. The relatively high intensity of polyubiquitin-regulated DsRed in transformant *Drosophila* is especially encourag-

ing for its use in insects where GFP and its variants are more easily obscured by quenching and autofluorescence. DsRed should be especially useful as a reporter in studies requiring dual-markers or colocalization, and, in particular, for some enhancer-trap studies where non-lethal detection is necessary.

REFERENCES

1. Baird, G.S., D.A. Zacharias, and R.Y. Tsien. 2000. Biochemistry, mutagenesis, and oligomerization of DsRed, a red fluorescent protein from coral. *Proc. Natl. Acad. Sci. USA* 97:11984-11989.
2. Benedict, M.Q., C.E. Salazar, and F.H. Collins. 1995. A new dominant selectable marker for genetic transformation: Hsp70-opd. *Insect Biochem. Mol. Biol.* 25:1061-1065.
3. Berghammer, A.J., M. Klingler, and E.A. Wimmer. 1999. A universal marker for transgenic insects. *Nature* 402:370-371.
4. Brand, A.H. and N. Perrimon. 1993. Targeted gene expression as a means of altering cell
5. Brand, A.H., A.S. Manoukian, and N. Perrimon. 1994. Ectopic expression in *Drosophila*. *Methods Cell Biol.* 44:635-654.
6. Chalfie, M., Y. Tu, G. Euskirchen, W.W. Ward, and D.C. Prasher. 1994. Green fluorescent protein as a marker for gene expression. *Science* 263:802-805.
7. Clontech Laboratories. 1999. Living Colors® Red Fluorescent Protein. Clontech-niques October:2-6.
8. Davis, I., C.H. Girdham, and P.H. O'Farrell. 1995. A nuclear GFP that marks nuclei in living *Drosophila* embryos; maternal supply overcomes a delay in the appearance of zygotic fluorescence. *Dev. Biol.* 170:726-729.
9. Handler, A.M. and R.A. Harrell. 1999. Germline transformation of *Drosophila melanogaster* with the piggyBac transposon vector. *Insect Mol. Biol.* 8:449-458.
10. Handler, A.M. and R.A. Harrell. 2001. Transformation of the Caribbean fruit fly with a piggyBac transposon vector marked with polyubiquitin-regulated GFP. *Insect Biochem. Mol. Biol.* 31:199-205.
11. Higgs, S. and D.L. Lewis. 2000. Green fluorescent protein (GFP) as a marker in transgenic insects, p. 93-108. *In* A.M. Handler and



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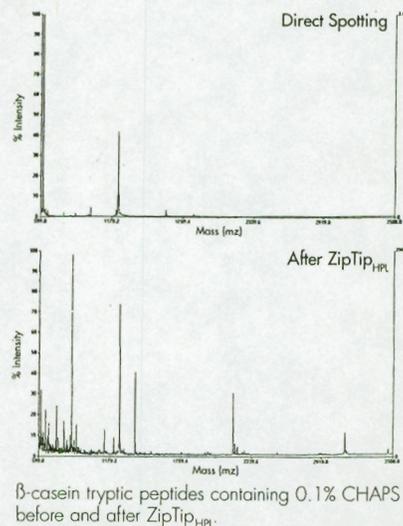
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- A.A. James (Eds.). *Insect Transgenesis: Methods and Applications*: CRC Press, Boca Raton, FL.
- Higgs, S., D. Traul, B.S. Davis, K.I. Kamrud, C.L. Wilcox, and B.J. Beaty. 1996. Green fluorescent protein expressed in living mosquitoes—without the requirement of transformation. *BioTechniques* 21:660-664.
 - Jakobs, S., V. Subramaniam, A. Schonle, T.M. Jovin, and S.W. Hell. 2000. EF-GP and DsRed expressing cultures of *Escherichia coli* imaged by confocal, two-photon and fluorescence lifetime microscopy. *EGFP Lett.* 479:131-135.
 - Lee, H., J.A. Simon, and J.T. Lis. 1988. Structure and expression of ubiquitin genes of *Drosophila melanogaster*. *Mol. Cell. Biol.* 8:4727-4735.
 - Matz, M.V., A.F. Fradkov, Y.A. Labas, A.P. Savitsky, A.G. Zaraisky, M.L. Markelov, and S.A. Lukyanov. 1999. Fluorescent proteins from nonbioluminescent Anthozoa species. *Nat. Biotechnol.* 17:969-973.
 - Peloquin, J.J., S.T. Thibault, R. Staten, and T.A. Miller. 2000. Germ-line transformation of pink bollworm (Lepidoptera: Gelechiidae) mediated by the *piggyBac* transposable element. *Insect Mol. Biol.* 9:323-333.
 - Pinkerton, A.C., K. Michel, D.A. O'Brochta, and P.W. Atkinson. 2000. Green fluorescent protein as a genetic marker in transgenic *Aedes aegypti*. *Insect Mol. Biol.* 9:1-10.
 - Prasher, D.C., V.K. Eckenrode, W.W. Ward, F.G. Prendergast, and M.J. Cormier. 1992. Primary structure of the *Aequorea victoria* green fluorescent protein. *Gene* 111:229-233.
 - Sarkar, A. and F.H. Collins. 2000. Eye color genes for selection of transgenic insects, p. 79-91. In A.M. Handler and A.A. James (Eds.). *Insect Transgenesis: Methods and Applications*. CRC Press, Boca Raton, FL.
 - Steller, H. and V. Pirrotta. 1985. A transposable P vector that confers selectable G418 resistance to *Drosophila* larvae. *EMBO J.* 4:167-171.
 - Tamura, T., T. Thibert, C. Royer, T. Kanda, A. Eappen, M. Kamba, N. Kōmoto, J.-L. Thomas et al. 2000. A *piggyBac* element-derived vector efficiently promotes germ-line transformation in the silkworm *Bombyx mori* L. *Nat. Biotechnol.* 18:81-84.
 - Yang, T.T., L. Cheng, and S.R. Kain. 1996. Optimized codon usage and chromophore mutations provide enhanced sensitivity with the green fluorescent protein. *Nucleic Acids Res.* 24:4592-4593.

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SSCP Screening of Individual Aptamers

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ABSTRACT

Aptamers are specific binding nucleic acids that emerge from *in vitro* selection. During the systematic evolution of ligands by exponential enrichment (SELEX) procedure, analysis of the sequences of the numerous selected individual molecules becomes an important step in the final stage of aptamer selection. The sequencing of cloned aptamers from the selected pool generally reveals groups of identical sequences and rarely occurring individual aptamers. This study demonstrates an approach similar to the single strand conformation polymorphism (SSCP) method used for mutation testing in genes. Human angiotensin I-specific aptamers have been used to show the efficiency of the SSCP method to classify selected individual sequences into identity groups, which minimizes sequencing efforts. Additionally, this approach allows the rapid isolation and identification of aptamers from a mixture.

INTRODUCTION

Initially, when aptamer selection [systematic evolution of ligands by exponential enrichment (SELEX)] (6) is

carried out, a randomized DNA oligonucleotide library containing more than 10^{14} individual sequences is used (1,4,5,9,13). After 10–15 rounds of selection, this diversity decreases to approximately 10^3 , giving rise to a mixture of sequences. Sequencing of randomly selected clones chosen from the selected pool usually reveals separate groups of identical sequences that account for over 30% of the pool. Other groups are less numerous, and some unique sequences are also present (3).

Here, we describe a method of sorting aptamers from an affinity-enriched library into groups based on the distinct migration profiles of individual sequences on non-denaturing polyacrylamide gels. This method is similar to mutation detection by the single strand conformation polymorphism (SSCP) (11) method and allows complex analysis within the affinity-enriched pool without the knowledge of individual aptamer sequences.

MATERIALS AND METHODS

Peptide and Affinity Resin

Peptides corresponding to the sequences of angiotensin I (Asp-Arg-Val-Tyr-Ile-His-Pro-Phe-His-Leu) and angiotensin II (Asp-Arg-Val-Tyr-Ile-His-Pro-Phe) were synthesized using 9-fluorenylmethoxycarbonyl amino acid-based, solid-phase peptide synthesis on a Rink® resin (Calbiochem-Novabiochem AG, Laufelfingen, Switzerland). After synthesis, the peptides were cleaved from the resin and purified by reversed-phase HPLC.

The oligonucleotides were selected on a Tenta-gel® (Rapp Polymere GmbH, Tübingen, Germany) resin with immobilized angiotensin I, synthesized as previously described. The peptide concentration on the resin was 22 nmol/g. To remove Tenta-gel-specific sequences during the selection process, a sample in which angiotensin was replaced by amino groups was used.

Library

Oligonucleotides used for selection were made in an ASM-102V® DNA synthesizer (Biosset, Novosibirsk, Rus-