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Identification of blue balancers and mutant collections compatible with *hobo* element transgenes.

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Introduction

The establishment of transposable elements as genetic tools has had an enormous impact on our understanding of organismal biology. Our laboratory has contributed to the development of a genetic system based upon the *hobo* element in *Drosophila melanogaster*. Relying upon the well-characterized molecular genetics of the *decapentaplegic* (*dpp*) locus, we recently reported that *hobo*, like the better known *P* element, is capable of local transposition (Newfeld and Takaesu 1999). Two strains generated in this study are new alleles of *dpp.* The other interest of our laboratory is intercellular signaling during embryonic development. We wanted to exploit these new *dpp* alleles to learn more about this complex signaling molecule. However, a major difference between *hobo* and *P* elements is that many laboratory strains contain endogenous *hobo* elements. Before we could use these new alleles in developmental genetics studies of *dpp* we needed to identify *hobo*-free strains of various types (*e.g.*, Dpp pathway mutants, blue balancers, UAS and Gal4 strains). Here we report the results of a survey of 78 strains for the presence of endogenous *hobo* elements. The survey identified a number of useful strains. This information is important to the growing number of investigators interested in utilizing the *hobo* system, as an alternative to *P*, to address developmental questions.

Methods

Standard methods of DNA isolation, restriction enzyme digestion, Southern blot preparation and hybridization were utilized (Newfeld and Takaesu 1999). To identify endogenous *hobo* elements, genomic DNA from each strain was digested with *Xho*I and hybridized with a wild type *hobo* element probe (pRG2.6X; Blackman *et al*., 1987). On the autoradiographs, 2.6kb hybridizing fragments represent full-length (autonomously transposable) *hobo* elements. Shorter fragments, typically 1.5kb, represent internally deleted (non-autonomously transposable) *hobo* elements (Blackman *et al*., 1987). Strains containing any full-length *hobo* elements are designated as H strains. Strains containing only internally deleted *hobo* elements are designated as E' strains. Strains devoid of any *hobo* elements are designated as E strains.

Table 1. Hobo status of 78 strains.

Strain	Hobo	Strain	Hobo
y^1 w^{67c23}	E	w nej^1 FRT101/ FM7	H
y^1 w^{67c23}; In(2LR)Gla/ SM6a	E	w nej^3/ FM7c	H
y^1 w^{67c23}; In(2LR)Gla/ CyO23	H	w nej^3/ FM7c P[w^+;eve.βgal]	H
y^1 w^{67c23}; In(2LR)Gla/ CyO P[ry^+; HSH2]	E	w nej^3 $zw3^{M11-1}$/ FM7c P[w^+; eve.βgal]	H
In(2LR)Gla Bc Elp/ CyO P[ry^+; wg.βgal]	H	w $zw3^{M11-1}$ FRT101/ FM7 (line 1)	H
z w^{11E4}; In(2LR)Gla/ CyO	E	w; P[w^+; Gal4]69B	H
Bc Elp/ CyO23; ry	H	w arm^{XM19} FRT101/ FM7	H
y^1 w^{67c23}; D gl^3/ TM3 Sb Ser	E	w $zw3^{sgg\ D127}$ FRT101/ FM7	H
w^{1118}; Df(3R)Hu, Antp[Hu-rv^1]/ TM3 Sb P[w^+; 6.8Xba.βgal]	E'	w nej^3 arm^{XM19}/ FM7c P[w^+; eve.βgal]	H
z w^{11E4}; $dpp^{d\text{-}ho}$ Mad^1/ CyO	H	y arm^4 w/ FM7c P[ry^+; 7.2ftz.βgal]	H
Mad^2 dp cn bw/ In(2LR)Gla Bc Elp	H	w ovo^{D1} FRT101/ Y; FLP38 (II) males X c(1)DX y f/ Y; FLP38 (II) females	H
Mad^3 dp cn bw/ In(2LR)Gla Bc Elp	H	dp cn *gbb*^{1} bw/ SM6a	H
Mad^4 dp cn bw/ SM6a	H	y^1 w^{67c23}; dp cn *gbb*^{1} bw/ SM6a	H
Mad^5 b pr/ In(2LR)Gla Bc Elp	H	wg^{IL114} cn bw/ CyO	H
Mad^6 b pr/ CyO	H	w; FRT 2πM43D mam^{10}/ CyO	H
Mad^6 b pr/ In(2LR)Gla Bc Elp	H	w; tin^{EC40}/ TM3 Sb P[w^+; βgal]	H
Mad^7 b pr/ In(2LR)Gla Bc Elp	H	Df(2R)bw^{s46}/ SM6a	E
Mad^8 b pr/ In(2LR)Gla Bc Elp	H	y^1 w^{67c23}; Df(2R)bw^{s46}/ SM6a	E
y^1 w^{67c23}; $Mad^{8.2}$/ CyO	H	b scw^{s12} pr bw sp/ CyO	E
Mad^{10} b pr/ CyO	H	dp scw^{E1} cn bw/ SM6a	E
Mad^{10} b pr/ In(2LR)Gla Bc Elp	H	y^1 w^{67c23}; dp scw^{E1} cn bw/ SM6a	E
Mad^{11} b pr/ In(2LR)Gla Bc Elp	H	w; P[w^+; Gal4]24B/ TM3 Sb	H
y^1 w^{67c23}; Mad^{12} b pr/ CyO P[ry^+; wg.βgal]	E'	w $zw3^{M11-1}$ FRT101/ FM7 (line 2)	H
y^1 w^{67c23}; $Mad^{1.2}$/ CyO	H	w; P[w^+; ptc.Gal4]	H
Mad^{12} b pr/ CyO	H	y^1 w^{67c23}; P[w^+; UAS.HA.tkv*]	H
$Mad^{B65.3}$/ CyO	H	w; P[w^+; UAS.HA.sax*]	H
$Mad^{B65.3}$/ In(2LR)Gla Bc Elp	H	y^1 w^{67c23}; P[w^+; UAS.Dad]$^{416+4}$	H
net Df(2L)C28 bw/ CyO P[ry^+; wg.βgal]	H	y^1 w^{67c23}; P[w^+; βgal] l(2)k05807/ CyO	H
$dpp^{d\text{-}ho}$ Df(2L)JS17 dp cn/ CyO P[ry^+; wg.βgal]	H	y^1 w^{67c23}; net dpp^{hr4} dp Sp Dp(2;2)DTD48 $dpp^{d\text{-}ho}$ / SM6a	H
y^1 w^{67c23}; Sp Bl Dp(2;2)DTD48 $dpp^{d\text{-}ho}$/ SM6a	E	y^1 w^{67c23}; net dpp^{hr4} Sp Bl Dp(2;2)DTD48 $dpp^{d\text{-}ho}$ / SM6a	H
Sp Bl Dp(2;2)DTD48 $dpp^{d\text{-}ho}$ / CyO	E	net dpp^{hr4}/ CyO P[ry^+; wg.βgal]	H
Df(2L)JS17 dp cn/ In(2LR)Gla Bc Elp	H	y^1 w^{67c23}; net dpp^{hr4} dp Sp cn sca bw / CyO	H
Df(2L)DTD48/ CyO23	H	z w^{11E4}; net dpp^{hr27} ed/ CyO	E
P[walter]23D/ SM6a	H	y^1 w^{67c23}; dpp^{H46} Sp cn/ CyO23	H
dpp^{H61}/ CyO23	E	dpp^{H47}/ CyO23	H
y^1 w^{67c23}; th st cu sr Med^3/ TM3 Sb Ser	H	y^1 w^{67c23}; mwh red e Med^1/ TM3 Sb Ser	E
th st cu sr Med^3/ TM6B	H	cn; ry^{42}	E
y^1 w^{67c23}; sr e^3 ca Med^4/ TM3 Sb Ser	H	cn l(2)IA109 bw sp/ CyO	E
y^1 w^{67c23}; SE.hs.c-jun^{5xasp}/ CyO P[ry^+; wg.βgal]	H	ru h th st cu sr e^5 kay^1 ca/ TM3 Sb Ser	E

Results and Discussion

Figure 1 shows a representative autoradiograph of a genomic Southern blot hybridized with a *hobo* probe. Of the twenty-two strains shown, twenty are H strains, one is an E' strain and one is an E strain. Other strains that appear to be E' strains have faint 2.6kb hybridizing fragments visible on the actual autoradiograph. Table 1 shows the results of our survey of 78 strains. In the survey we found that 58 are H strains (74%), 18 are E strains (23%) and 2 are E' strains (3%). Despite the high frequency of H strains we were able to identify a number of useful strains including blue balancer strains. In addition, we identified two large mutant collections as possible sources of *hobo*-free strains for developmental genetic analyses.

We identified an E' strain carrying a second chromosome blue balancer - *CyO* P[ry^+ ; *wg*.βgal]. This chromosome expresses β-galactosidase in the *wingless* (*wg*) pattern of 14 segment polarity stripes and the transgene insertion creates a *wg* null allele (Kassis *et al.*, 1992). We identified an E' strain carrying a third chromosome blue balancer - *TM3 Sb* P[w^+; 6.8Xba. βgal]. This chromosome expresses β-galactosidase under the control of a *Sex combs reduced* hindgut enhancer (Gindhart *et al.*, 1995). Expression from both blue balancers begins at cellular blastoderm and continues throughout development in easily identifiable patterns. We have used these balancers with our new *hobo* induced *dpp* alleles for several years and not detected any transposition events (*e.g.*, reversion to dpp^+).

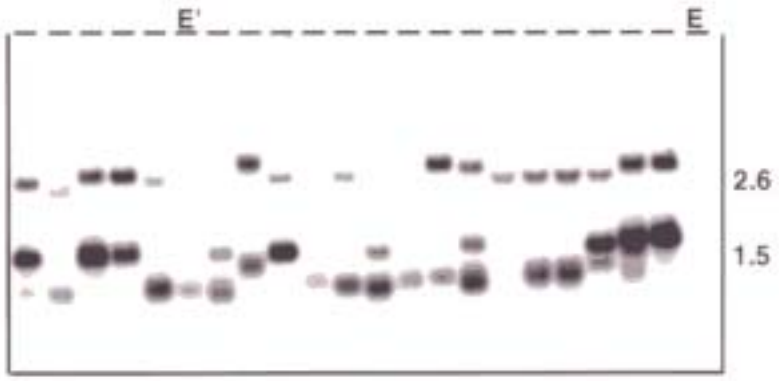

Figure 1. Identification of endogenous *hobo* elements in various strains. A representative autoradiograph is shown of a Southern blot containing genomic DNA digested with *Xho*I and hybridized with a *hobo* probe. The approximate size (in kb) of hybridizing fragments are indicated. One E and one E' strain are identified.

In our survey, we tested three mutant strains (*cn l(2)IA109 bw sp/ CyO* or dJun[1], *b* scw^{s12} *pr bw sp/ CyO* and *ru h th st cu sr* e^5 kay^1 *ca/ TM3 Sb Ser*) derived from the Nusslein-Volhard and Weischaus (1980) screen for embryonic lethal mutations. Each was designated an E strain suggesting that this mutant collection is a valuable source of *hobo*-free strains. The only strain (y^1 w^{67c23}*; mwh red e* Med^1*/ TM3 Sb Ser* or *l(3)SG70*) from the Shearn and Garen (1974) screen for larval/pupal lethal mutations in our survey was designated as an E strain. This suggests, albeit with less confidence, that this mutant collection may also be a source of *hobo*-free strains. Thus, a large number of embryonic and larval/pupal mutants suitable for use with *hobo* transgenes may be available for developmental genetics studies.

In summary, we have identified a number of strains that significantly expand the scope of developmental genetic analyses that can be conducted using *hobo*-mediated transgenes. Investigators interested in further information or in obtaining these strains may contact us.

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