

## A transposable P vector that confers selectable G418 resistance to *Drosophila* larvae

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***Drosophila* larvae are rapidly killed by food containing the antibiotic G418. The bacterial gene for neomycin resistance introduced in the genome by P-mediated transformation renders larvae resistant to G418 and able to grow to fertile adults. The *neo* gene transcribed from the herpes thymidine kinase promoter gives low levels of resistance but high levels can be obtained using the *hsp70* heat-shock promoter. We have constructed a vector for P-mediated transformation which uses this finding to allow dominant selection of transformed progeny. Features of this vector also facilitate cloning and allow the rapid recovery of the inserted transposon from transformed flies. We have also constructed a cosmid vector for P-mediated transformation that incorporates the *hsp70-neo* gene.**

**Key words:** cosmid transposon/G418 resistance/P-transformation vectors

### Introduction

The ability of P elements to transpose into sites in the genome of *Drosophila* has made them valuable as a means of reintroducing cloned sequences into the organism (Rubin and Spradling, 1982; Spradling and Rubin, 1982). P element DNA, injected into the early embryo integrates into the genome of germ line cells of the injected individual, (G0). Transformed flies are obtained from these germ cells in the following generation (G1) with a frequency that varies, in our hands, between 5 and 20%. That is, 5–20% of the survivor G0 flies give rise to at least one transformed fly in their progeny.

P elements can act as carriers of DNA sequences to be introduced in the *Drosophila* genome. The detection of transformed individuals is possible if the DNA introduced contains a gene producing an easily visible phenotype. In the case of many genes of interest, this visible selection is not available and large numbers of flies need to be individually tested by genetic or biochemical means. To solve this problem, Rubin and Spradling constructed a P-vector containing the *rosy* gene which serves as a visible marker when the injected flies carry a *rosy*<sup>-</sup> mutation (Rubin and Spradling, 1983). The *adh* gene has also been used as a marker to select transformants (Goldberg *et al.*, 1983). In this case also, mutant flies need to be used and the selection can only be imposed on the adult G1 flies.

In the present work we show that the bacterial neomycin resistance gene (*neo*) can be used to render *Drosophila* larvae

resistant to the antibiotic G418. We describe P element vectors which carry the *neo* gene and include several features to facilitate their use. The *neo* gene is driven by the *hsp70* heat-shock promoter which has been shown to function in a variety of organisms from *Xenopus* to man, while G418 is toxic to yeast, fungi, algae, plant and animal cells (Jimenez and Davis, 1980; Colbere-Garapin *et al.*, 1981; Southern and Berg, 1982). We suppose therefore that the *hsp70-neo* gene would provide a good selective marker for a wide variety of organisms.

P elements introduced by microinjection have been shown to function in *Drosophila hawaiiensis* (Brennan *et al.*, 1984), a species that does not normally contain them. This suggests the possibility that they may also function in the germ line of other insects, or even less related animals such as nematodes or vertebrates. The availability of a dominant selection makes this an easily testable possibility.

### Results and Discussion

#### Sensitivity to G418

The aminoglycoside G418 is an antibiotic related to gentamycin, neomycin and kanamycin (Davies and Jimenez, 1980). In contrast to these, G418 seems to be universally toxic: prokaryotic as well as eukaryotic cells are rapidly killed by its ability to block protein synthesis.

To test the sensitivity of *Drosophila* to G418, equal numbers of Canton S flies were allowed to lay eggs on standard *Drosophila* food supplemented with various concentrations of the drug. The results, summarized in Table I show that G418 kills larvae. At low concentrations some larvae survive to give adult flies. The survivors require nearly twice the normal developmental time and remain small, with reduced pigmentation but undiminished fertility.

In contrast, adult flies are relatively insensitive and begin to be affected only after 6–10 days of subsistence on food containing 1 mg/ml G418. This relative resistance allows parents to lay eggs unaffected for several days.

#### Transformation to G418 resistance

G418 is inactivated by the phosphotransferase encoded by the gene for neomycin resistance, *neo*, from the bacterial transposon Tn5 (Davies and Smith, 1978). In an initial construction, we isolated from plasmid pAG50 (Colbere-Garapin *et al.*, 1979) a fragment containing the *neo* gene lacking the

**Table I.** Survival of larvae on G418

G418 (µg/ml)	50	100	200	500	1000
survival (imagines)	10–30%	5–10%	0.1–1%	0%	0%
larval growth	++++	++++	++	+	–

Standard *Drosophila* food was melted in a microwave oven, cooled to 40°C and supplemented with G418 at different concentrations. Survival at 25°C is expressed in terms of eclosing imagines while average larval growth is indicated qualitatively.

bacterial promoter but driven by the herpes thymidine kinase (*tk*) promoter. We inserted this fragment into a P-transformation vector and injected this construction into *Drosophila* embryos. The progeny of the injected individuals was tested for ability to survive on G418. In contrast to unicellular organisms or cell cultures, antibiotic resistance of a whole metazoan organism may be more difficult to achieve since insufficiency in one essential tissue, for example the nervous system, might still be lethal. To ensure survival we would need the expression of the *neo* gene in all the cells of the developing larvae or at least in the gut cells that process the ingested food.

Using the *tk* promoter we obtained individuals resistant to 0.5 mg/ml G418 but the *neo* gene is apparently poorly expressed. The surviving larvae take several days more than control larvae to reach pupal stages and are frequently small and weak. Even at relatively low concentrations of G418 (0.5 mg/ml), transformed larvae have a high incidence of mortality and may fail to survive altogether when heterozygous for certain genetic markers or balancer chromosomes. We conclude that the *tk* promoter is not sufficiently active in *Drosophila* or at least not in all cells of the larva.

To increase the level of expression and, in particular to guarantee expression in all tissues, we replaced the thymidine kinase promoter with the *Drosophila hsp70* heat-shock promoter. We used a 456-bp *Xba-Xmn* fragment from the *hsp70* gene, containing the promoter and 206 nucleotides of untranslated leader sequence (Ingolia *et al.*, 1980) which we had previously shown to be heat inducible and expressed in all embryonic cells upon induction (Steller and Pirrotta, 1984).

To select transformed progeny, flies were allowed to lay eggs on food containing 1 mg/ml G418. The flies were then removed and the eggs were given a brief heat shock (30 min, 37°C) shortly before hatching and every third day until they climbed the walls to pupariate.

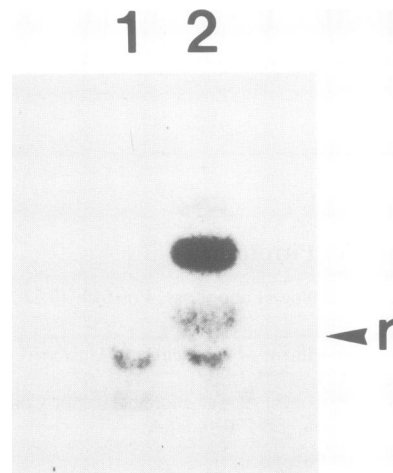
With this regimen we find that 10–20% of the injected adults give transformed progeny. The transformed larvae survive and pupariate as fast as control larvae. They appear healthy, have a low incidence of mortality even in combination with balancer chromosomes and, in general, give rise to normally fertile adults. Uninjected larvae subjected to the same heat-shock regimen die on 1 mg/ml G418 during the first instar stage.

At this concentration of G418, non-transformed larvae die very early, allowing the screening of large numbers of embryos without crowding. However, even large numbers of resistant larvae do not consume or inactivate the G418 in the food, as tested by its ability to kill bacteria or untransformed larvae.

#### Heat shock-induced resistance

Resistant lines were established by crossing transformed flies individually with flies carrying various balancer chromosomes and again selecting G418-resistant offspring. Genomic Southern blot hybridization of five independent lines showed that each contained a single *hs-neo* transposon integrated at a different chromosomal site. Northern blot hybridization of one of these lines showed that the transcription of the *neo* gene was undetectable at 25°C but was massively induced by treatment at 37°C for 1 h (Figure 1).

We tested several transformed lines carrying the *hs-neo* transposon in different chromosomal sites for survival of larvae on 1 mg/ml G418 with or without heat shock, compared with survival on normal food. In most cases, heat shock



**Fig. 1.** Heat shock-induced transcription of the *neo* gene. Flies carrying a precursor of the pUCHsneo transposon, containing a 1.6-kb *neo* fragment were homogenised without or immediately after heat shock for 1 h at 37°C. The total RNA was extracted as described by Steller and Pirrotta (1984) and one fly equivalent of RNA was electrophoresed in a formaldehyde-agarose gel, blotted onto a nitrocellulose filter and hybridised with a probe including only the *neo* gene. **Lane 1:** not heat shocked, **lane 2:** heat shocked. The size of the heat shock-induced RNA species is ~2.4 kb, consistent with termination near the 3' P terminal repeat [200 bp *hsp70* leader + 1600 bp *neo* + 400 bp P'3 and P'3 end + poly(A)<sup>+</sup> tail]. The lower band seen in both lanes co-migrates with the rRNA and is probably artifactual.

**Table II.** Survival of transformed lines on G418

line	survivors (% flies)		
	+ hs – G418	+ hs + G418	– hs + G418
F1	100%	70-90%	70-90%
F2	100%	50-70%	50-70%
M1	100%	50-70%	40-60%
M6	100%	20-30%	1-5%

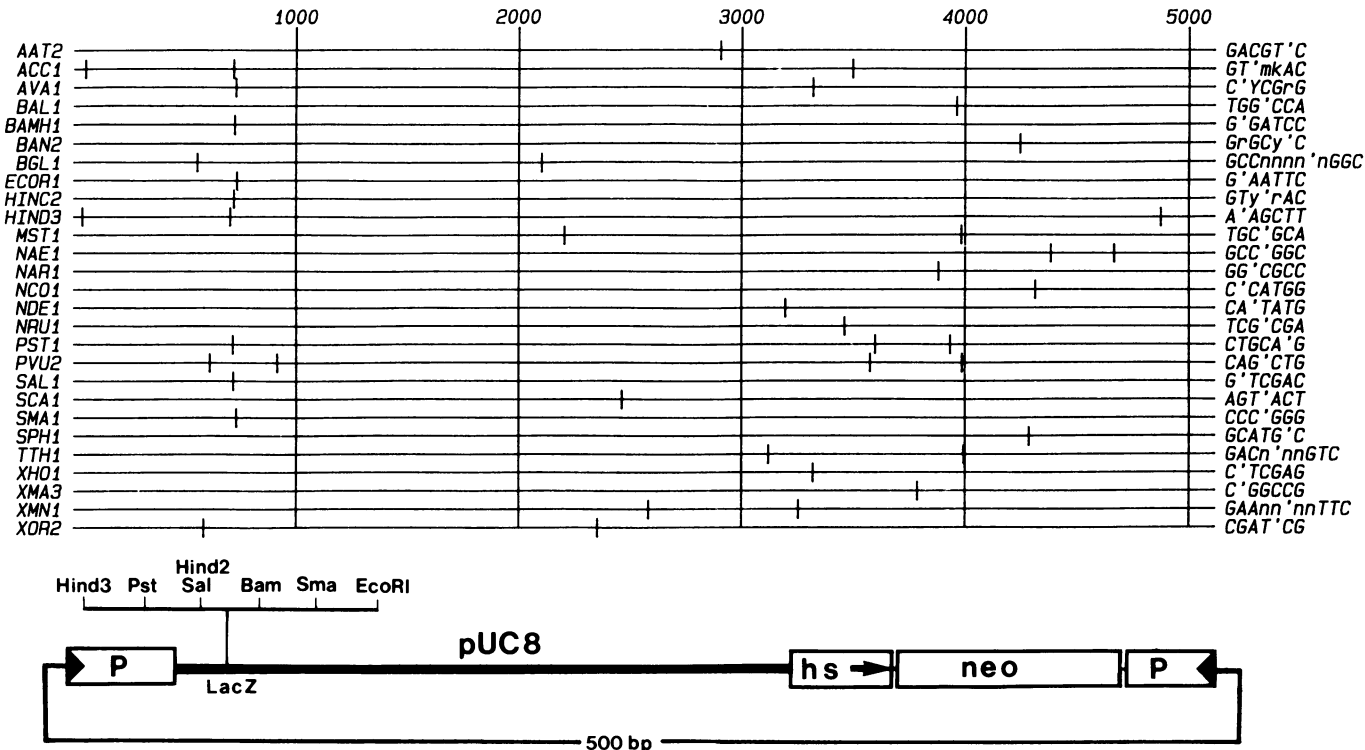
Approximately equal numbers of flies from four different transformed lines were allowed to lay eggs for 2 days on normal food and on food containing 1 mg/ml G418. The developing embryos were then given a heat-shock treatment for 1 h at 37°C every other day until pupariation. The yield of adult flies is given as percentage of the yield without selection.

results in survival of up to 90% of the progeny but, depending on the site of integration, equally good survival is obtained even without heat shock. We know from other experiments using the heat-shock promoter that, while induction can result in a several hundred fold increase in transcription, the basal level of the promoter is significant (Steller and Pirrotta, in preparation). Table II summarises the results obtained with four transformed lines which represent the range of G418 resistance observed.

In some of the transformants, the *hs-neo* transposon was apparently inserted in chromosomal sites less favourable to expression. In these cases, *neo* RNA was barely detectable even after heat induction and the larvae required heat-shock treatment for good survival on G418. Since the heat-shock regimen causes no detectable developmental abnormalities, we recommend its use to avoid possible loss of these borderline cases.

#### The pUCHsneo transformation vector

We have incorporated the *hs-neo* construction in a transformation vector called pUCHsneo. Figure 2 describes its restric-



**Fig. 2.** The pUChsneo vector. The composition is indicated below the map showing restriction sites in the transposon. The P sequences on the left come from the 5' end of the p6.1 P element. They are joined to the *Nar* site of plasmid pUC8. The other *Nar* end of pUC8 is attached to a 456-bp *Xba-Xmn* fragment containing the *hsp70* heat-shock promoter. The *neo* gene is in a *BglII-Sma* fragment which lacks the bacterial promoter but includes the entire coding region of the phosphotransferase. The P sequences on the right come from the 3' end of the p6.1 P element. The two ends of the P element are connected by ~500 bp of DNA derived from the *white* locus and present in the p6.1 clone. The polylinker contains unique *EcoRI*, *Sma*, *Bam*, *HindIII* and *Sal* cloning sites. The *HindIII* and *Pst* sites cannot be used for cloning since they cut elsewhere in the transposon.

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10      20      30      40      50      60      70      80      90     100
CATGATAAA TAACATAAGG TGGTCCCGTC GAAGAGCGAA GCTTACCAGG ATATACACTT AAATTCAGTG CAGCTTGGCT TGTTCGAGAG AAAAGGTGTG
5' P
110     120     130     140     150     160     170     180     190     200
TCCGGACGAA TTTTFTTTTT AAAACATTTA CCTCCATCGT GAATAAAAAA AAATGAAATA TTGCAAAATF TCGTCCGAAG CTGTCGACTGG AGTAAATTTA
210     220     230     240     250     260     270     280     290     300
ATTCACCTGG CCAAGCTGTG TATTAGAGA AATTGTGGG AGCAGAGCTT TGGGTCCGCG CTCTGTGAAA ACTCCCAAAT TTGTGATACC CACTTTAATG
310     320     330     340     350     360     370     380     390     400
ATTCCGAGTG GAAGGCTGCA CCTGCAAAAG GTCAGACATT TAAAAGAGGG CGACTCAACG CAGATGCGCT ACCTAGTAAA GTGATAGAGC CTGAACCAGA
410     420     430     440     450     460     470     480     490     500
AAGATATAAA GAAGGCTATA CCACTGGGAG TACACAACAA CAGTAACTTT GAATGATAAA AAAATCAATT TATGATAAAA ATAACGTGAC TGTGGCTTAG
510     520     530     540     550     560     570     580     590     600
GTCCTGTCCA TTGTTTAAAT AAATTAAGAG CTTCAGGAAA AGCCCATATC GCCATTACGG CTACCACTACT GTTGGAGAGG GOGATCGGTC GCGGGCTCTT
610     620     630     640     650     660     670     680     690     700
CUCTATTACG CCAGCTGGGC AAGGGGGGAT GTCTGCAAGC GGGATTAAGT TGGTATACGC CAGGGTFTTC CCACTCACTA CTTTTATAAA CACCGGCGGG
710     720     730     740     750     760     770     780     790     800
TCCCAAGCCT CGGTCGAGCT CGACGATATC CGGGGAATTC TTAATCATGC CTATACAGTG TCCCTGGTGT AAATTTGATF CCGCTCCACA TTCCACACAA
810     820     830     840     850     860     870     880     890     900
CATACGAGCC GGAAGCATAA AGTGTAAAGC CTGGGCTGCC TAATGATGTA GGTAACTAGC ATTAATGSSG TTGGCTCTGC TTCCCGCTTT CCACTGGGGA
910     920     930     940     950     960     970     980     990     1000
AACCCTGTGT GCCAGCTGGA TTAATGAATC GGGCCAGCCC CGGGGAGAGG GGGTTCGCTT ATTGGUGGCT CTTCGCCTCT GCCTCGCTGC
1010    1020    1030    1040    1050    1060    1070    1080    1090    1100
GCTCGCTGCT TCCGCTGGGG CGAGCGTATC CAGCTCACTC AAAGCGGTTA ATACGGTTAT CCACAGAATC AGGGAGTAAC GCACGAAGA AATCTCGACC
1110    1120    1130    1140    1150    1160    1170    1180    1190    1200
AAAAGGCGAC CAAAGAGGCCA GGAAGCGTAA AAAGCGGCCC TTTCGCGGCT TTTCCATAG GCTCCGCCCC CCTGACGAGC ATCCACAAAA TCGACCTCCA
1210    1220    1230    1240    1250    1260    1270    1280    1290    1300
AGTCAGAGT GGCAGAACC GACAGGACTA TAAGATACC AGGCGTTCCT CCGTGTAAAG TCCCTGTGTC GCTCTCGTGT TCGCACAGCT CCGCTTACC
1310    1320    1330    1340    1350    1360    1370    1380    1390    1400
GATACTGTCT CCGCTTCTCT CCTTCGGAAA GCGTGGCGCT TTCTCAATGC TCAGCGGTTA GGTATCTCAG TTGGGTAGT GTGCTCTGCT CCAAGCTGGG
1410    1420    1430    1440    1450    1460    1470    1480    1490    1500
CTGTGTGACG GAACCCCGCC TTCAGGCCGA CCGTCTGGCC TTATCCGTTA ACTATGCTCT TGAATCAAC CCGTATGAA AGCACTTATC GCCACTGGCA
1510    1520    1530    1540    1550    1560    1570    1580    1590    1600
GCAGCCACTG GTAACAGGAT TAGCAGAGCC AGGTATGATC GGGTGTCAAG AAGTTCCTG CTAACATGCG CTACACTAGA AGGCAGATAT
1610    1620    1630    1640    1650    1660    1670    1680    1690    1700
TTGTATCTGC CUCTCTGTC AAGCCAGTTA CCTTGGAAA AAGATTTGCT AGCTCTGAT CCGGCAACCA AACCCAGCTG GDTAGCGGTG GTTTTTTTT
1710    1720    1730    1740    1750    1760    1770    1780    1790    1800
TTGCACAGC CAGATTAGCC GCAGAAAAGC AAGATCTCA AAGATCTGCT TGATCTTTC TACGGGCTT GAGCTCTACT GGAACAAA CTAACGTAG
1810    1820    1830    1840    1850    1860    1870    1880    1890    1900
GGATTTTGGT CATGAGATA TCAAAAAGCA TCTTCACTA GATCTTTTFA AATTAANAAT GAAGTTTFA ATCAATCTAA AGTATATATG AGTAAACTAG
1910    1920    1930    1940    1950    1960    1970    1980    1990    2000
GTCTGAGCT TACCAATGCT TAATCAGTA GGCACCATT TCAGCGATCT GCTATTTTGG TTCACTCATA GTTGGCTCCT TCCGGCTGTT GTAGATAACT
2010    2020    2030    2040    2050    2060    2070    2080    2090    2100
ACGATACGCG AGGGCTTACC ATCTGCCCC AGTGTCCGA TATACCTGG AGACCCAGCG TCACCCTGCT CAGATTTTCT AGCATAAAAC CAGCCAGCCG
2110    2120    2130    2140    2150    2160    2170    2180    2190    2200
GAAGGCGCCA GCGCAGAGAT GGTCTGCGCA CTTTATCGCC CCTCCATCCG TCTATTAAIT GTTCCGGGGA AGCTAGATA AGTATGTCGG CAGTAAATAG
2210    2220    2230    2240    2250    2260    2270    2280    2290    2300
TTTGGCCAAC GTTGTGCCA TTGTACAGC CATCTGCTGC TCACGCTGCT GCTTGGTAT GGGTCTATCC AGCTCCGTT CCTCACGATCT AAGCGAGTT
2310    2320    2330    2340    2350    2360    2370    2380    2390    2400
ACATATGCC CCATGTGTG CAAAAAGGG GTTAAGCTCT TCCGCTTCTG GATGCTTCTC AGAAAGTAT TGGCCGAGT GFTATCACTC ATGTGTTATG
2410    2420    2430    2440    2450    2460    2470    2480    2490    2500
CAGCAGTCA TAACTTCTT ACTGTCTGC CATCTGTAG ATGTGTTCT GTACCTGGT AGTACTAAC CAACTATTC TGAGATAACT GTATGCGCG
2510    2520    2530    2540    2550    2560    2570    2580    2590    2600
ACCGATTCG TTTTGGCGGG CCTCAATAGC GGATATACC GCGCAATA GAGAATTT AAAATGTGTC ATCATGGAA ACCGTCTCTC GGGCGAAA
2610    2620    2630    2640    2650    2660    2670    2680    2690    2700
CTCAAGAA TCTTACCCT GTTAGATCC AGTTCGATGT AACCCCAAC TGATCTTAC TATCTTTC TTTCCACCAG CATCTTTTAC TTTCCACCAG GTTTCTGGT
2710    2720    2730    2740    2750    2760    2770    2780    2790    2800
GAGCAAAA AGGAAAGCAA AATGCCCAA AAAAGGAAT AAGGGCGACA CGGAATGTT GAATCACTAT ACTCTTCTCT TTTCAATT ATTAAGACT
2810    2820    2830    2840    2850    2860    2870    2880    2890    2900
TTATCAGGTT TATTGCTCA TGAGCGGATA CATATTTGAA TGTATTTGAA AAAAATAACA AATAGGGTT CCGCCACAT TTTCCCGAAA AGTCCACCT
2910    2920    2930    2940    2950    2960    2970    2980    2990    3000
GAGTCTAAG AACCCATTAT TATCATCCA TTAACCTATA AAAATAGGCG TATCACAGG CCCCITGCT TCGGCGCTT CGGTATGAC GGTGAAAACC
3010    3020    3030    3040    3050    3060    3070    3080    3090    3100
TCTGACACT CGACTCCCG GAGACGCTA CAGTCTTCT GTAACGGTAT CCGCGGACCA GACAAGCCCC TCAGGGCGCC TCAGCGGTG TTGGCGGTG
3110    3120    3130    3140    3150    3160    3170    3180    3190    3200
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3210    3220    3230    3240    3250    3260    3270    3280    3290    3300
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3310    3320    3330    3340    3350    3360    3370    3380    3390    3400
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3410    3420    3430    3440    3450    3460    3470    3480    3490    3500
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3510    3520    3530    3540    3550    3560    3570    3580    3590    3600
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3610    3620    3630    3640    3650    3660    3670    3680    3690    3700
CAACTAAAT AACCTCACT ACTGAATCT GCCAAGAAT AATTATGAA TACAGAAGA GAACCTGTA TAGGGATCT GATCAGACA CAGGATGAG
3710    3720    3730    3740    3750    3760    3770    3780    3790    3800
ATGTCTTTC ATGATTAAC AAGATGATT GCACGAGCT TCTCGCCG CTTGGGTGGA GAGGCTATT GCGTATGACT GGGCAACA GACAATCGG
3810    3820    3830    3840    3850    3860    3870    3880    3890    3900
TGCTGTGAT GCGCTGCTT CCGCTGTCA GCGGAGGGG GCGCGTCT TTTTCTCAG ACCGACTGT CCGGTGCCCT GAATCACTC CAGGACGAG
3910    3920    3930    3940    3950    3960    3970    3980    3990    4000
CAGCGCGCT ATCTGGCTC GCAACAGCG GCGTCTCTG CCGACTGTG TCTGCTACG GGAAGAAGC TGGCTCTAT TGGGGAGGT
4010    4020    4030    4040    4050    4060    4070    4080    4090    4100
GCGGGGGC GATCTCTGT CATCTCACT TGCTCTGCC GAGAAGAT CCATCTAGC TGATGCAAG CCGCGGCTC ATACGCTGA TCCGCTACC
4110    4120    4130    4140    4150    4160    4170    4180    4190    4200
TGCCATTG ACCACAGCG GAACAACGG ATCGAGGAC CAGTACTG GATGGAAGG GGTCTGTG ATCAGATGA TCTGGAGCA GAGCATGCG
4210    4220    4230    4240    4250    4260    4270    4280    4290    4300
GGCTCGCC GAGCCAACTC TTGCGAGG TCAAGCCCG CATGCCACC GGCAGGATC TGCTGTGAC CAGTGGGAT GCTCTCTG CGAATATC
4310    4320    4330    4340    4350    4360    4370    4380    4390    4400
GGTGAAAT GCGCCGTTT CTGATATCAT CACTGTGG GCGGCTGTG TTGGCGACC GTATCAGAC ATAGGGTGG CTAACCGTGA TATTCTGA
4410    4420    4430    4440    4450    4460    4470    4480    4490    4500
GAGCTGTGC GGGATGGGC TGACCGCTC CTGCTGCTT ACGGTATCG GCTCCGCGG TCCGAGCGCA TCGCTTCTA TCGCTTCT GAGACTCT
4510    4520    4530    4540    4550    4560    4570    4580    4590    4600
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4610    4620    4630    4640    4650    4660    4670    4680    4690    4700
TTGGAAAT TTTTCCCGA CCGGCGCTG ATGACTCC AGGGGGGGA TCTCATGCT GAGTCTTC CCCCAGGCA TCCCTGGAC GATAAACAT
4710    4720    4730    4740    4750    4760    4770    4780    4790    4800
CTGTAAAA AAGGATTTCT TTTCCAGT CTGACACT TTGACAGAT GTTACAGAT GTGCATAA AAAAGAGGAT TTGAGTGTG GTCATAGCC
4810    4820    4830    4840    4850    4860    4870    4880    4890    4900
TAATGACAG TGATGGATG GATGACGCC ACAAGCTTT GCATCTCTT CAAATTTA AAAATAAAC TTTAAATA ATTTCTCTA ATTAATATA
4910    4920    4930    4940    4950    4960    4970    4980    4990    5000
TGAGTAAT CAATGCCAC GGCATGCTA AGGGTAACT AACCACTA TCGCTGCTT ACTCACTC AATACGAC AATCACTACT ATTCCTTCA
5010    5020    5030    5040    5050    5060    5070
CTCGACTTA TTGCAAGAT ACGTAAAGT GATGTCTCT GCGGACGG CCACTTATG TTTAATCCT ATC

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**Fig. 3.** Sequence of the pUChsneo transposon. The sequence starts from the 5' end of the P element and does not include the 500 nucleotides joining the two P ends. The double arrowheads indicate beginnings and ends of each constituent block and the terminal repeats of the P element are underlined. The P element sequences were deduced from O'Hare and Rubin (1983), the *hsp70* sequence from Ingolia *et al.* (1980), the *neo* sequence from Beck *et al.* (1982) and the pUC8 sequence from Vieira and Messing (1982).

tion map and its constitution while Figure 3 gives the predicted nucleotide sequence of the transposon deduced from its components. Note that the *neo* gene in this construction renders bacteria carrying pUCHsneo resistant to 10 µg/ml kanamycin. This is not due to transcription starting from the *hsp70* promoter, which is inactive in bacteria, but presumably to read-through from a plasmid promoter.

In contrast to the Carnegie vectors (Rubin and Spradling, 1983), pUCHsneo includes the plasmid replicon and ampicillin resistance gene within the P terminal repeats. As a consequence of this design, these sequences also become integrated in the genome of a transformed fly, allowing the recovery of the inserted DNA by the 'plasmid rescue' method (Perucho *et al.*, 1980). Genomic DNA of the transformed fly is digested with an enzyme that does not cut in the transposon, diluted and ligated under conditions that allow circularisation of the fragments. The ligation mixture is then used to transform *Escherichia coli* hosts. This technique, described in more detail by Steller and Pirrotta (in preparation), is useful to isolate the sequences flanking the insertion site, to analyse the inserted transposon for rearrangements, mutations etc.

The plasmid replicon, which consists of the pUC8 vector (Vieira and Messing, 1982) in its entirety, includes also the *lacZ* fragment and polylinker cloning sites which facilitate the identification of insertions on plates containing X-gal (5-bromo-4-chloro-3-indolyl-β-galactopyranoside). Because of the presence of *Hind*III sites in the P sequences, and of *Pst* sites in the *hs-neo* sequence, these two enzymes cannot be used for cloning although their sites are present in the polylinker.

The G418 selection is well suited for mass screening. By eliminating untransformed individuals at a very early stage, it allows the transformed ones to survive in uncrowded conditions. Because G418 is a powerful antibiotic, it also protects the food from contamination with mold or bacterial growths. Most importantly, it does not require the use of mutant strains for the selection of transformants. As a result, it could be applied to other *Drosophila* species for which little or no genetics may be available, to other insects and, in principle, to any other organism in which P-mediated transposition can be made to occur.

#### A cosmid transposition vector carrying *hs-neo*

P-mediated transposition allows the testing of a cloned DNA fragment for its biological activity. If large genomic regions could be tested by this method, chromosomal walks in search of a gene of interest could be undertaken even in the absence of any detailed molecular information or any means to identify the gene other than its biological function. We have constructed a cosmid vector whose cloning site is flanked by the P terminal sequences. Genomic cosmid clones constructed with this vector can be used directly for P mediated transformation.

As a starting point we took cos4, a cosmid vector previously used by Pirrotta *et al.* (1983). Cos4 contains three λcos sites for historical reasons, although only two are essential to its design. To this vector, by a series of steps detailed in Materials and methods, we added the 3' P end from Carnegie-1 and the 5' P end from p6.1 (Rubin and Spradling, 1983). This yielded cos-P, a cosmid vector with a unique *Bam*HI site for cloning fragments generated by partial *Sau*3A digestion, bracketed by the P ends. As reported elsewhere (Haenlin *et al.*, in preparation), we have used a cosmid clone isolated in this vector to introduce a 43-kb DNA fragment

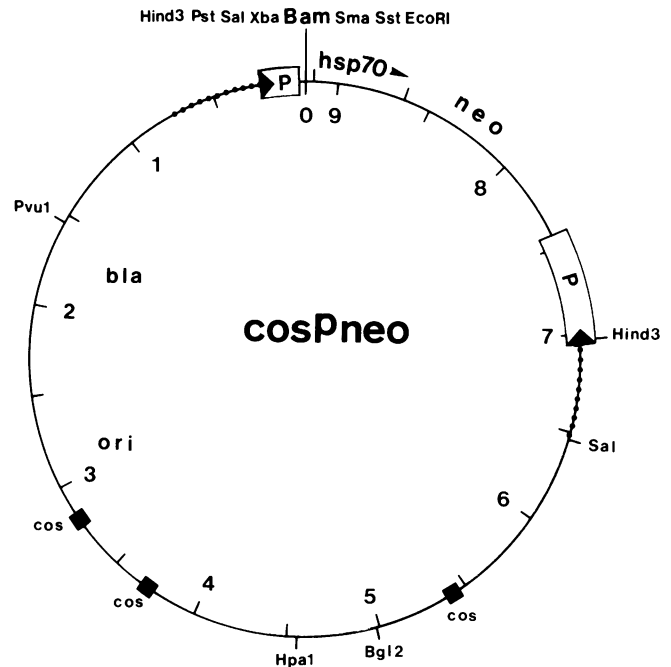


Fig. 4. The cosPneo cosmid vector. The λcos sites are indicated by small black boxes. The dotted region represents sequences from the *white* locus flanking the insertion site of the P element in clone p6.1. The P sequences are shown boxed in and *bla* indicates the β-lactamase gene. The *hsp70* promoter is directed away from the polylinker site and transcribes the *neo* gene. The *Hind*III, *Pst* and *Sal* sites cannot be used for cloning but the *Xba*, *Bam*, *Sma*, *Sst* and *Eco*RI sites are unique.

containing the K10 female sterility locus into the genome of homozygous K10 flies, thus restoring fertility.

We then introduced the *hsp70-neo* gene into cos-P to obtain cosPneo. A map of cosPneo indicating the constituent elements is shown in Figure 4. Note that the *Hind*III, *Pst* and *Sal* sites in the polylinker cannot be used for cloning because they are not unique. However the *Xba*I, *Bam*HI, *Sma*I, *Sst*I and *Eco*RI sites are unique. To avoid possible interference with the expression of cloned genes, the *hsp70* promoter is directed away from these cloning sites. Like pUCHsneo, cosPneo also renders bacteria resistant to kanamycin, presumably due to transcription read through from the plasmid region.

Embryos injected with cosPneo yield progeny transformed to G418 resistance. Although we have not yet attempted transformation with cosmid clones obtained with this vector, we conclude that cosPneo is fully active both as a transposon and as a selectable marker.

#### Materials and methods

The G418 used in these experiments was from a preproduction batch of the Plough-Schering Co. and kindly supplied to us by A. Falaschi and G. Della Valle. It can be obtained commercially from Gibco. Standard *Drosophila* food was supplemented with G418 by melting it in a microwave oven, cooling to 40°C and adding G418 at a final concentration of 50–1000 µg/ml. The G418 in the food remains active for at least 2 weeks when stored at 5°C.

Microinjection procedures were slightly modified from those described by Steller and Pirrotta (1984). The principal difference was that the DNA solution was injected in the posterior quarter of the embryo. Plasmid transposon DNA was co-precipitated with helper plasmid and resuspended in injection buffer (5 mM KCl, 0.1 mM sodium phosphate, pH 6.8) at a final concentration of 500 µg/ml of transposon and 100 µg/ml helper DNA. As helper we used pπ25wc (kindly supplied by G. Rubin) or phsπ, a construction containing the P coding region transcribed by the *hsp70* heat-shock promoter (Steller and Pirrotta, in preparation). Both helpers lack an intact P terminal repeat and are unable to integrate.

### Construction of vectors

The construction of the pUCsneo plasmid went through a number of intermediates used for other purposes. The essential steps in the construction are briefly summarised as follows. The 5' P end comes from clone p6.1, up to the *Xmn* site at position 538 in the P sequence (O'Hare and Rubin, 1983). The 3' P end comes from the same clone, starting from the *Xho* site. The sequences flanking the P element in p6.1 come from the *white* locus. They were joined together by ligating the two *Sal* sites present in p6.1 and then partially deleted by treatment with *Ba31*. This eliminated the *Sall* site and the *EcoRV* site and reduced the *white* sequences to ~500 bp.

The entire pUC8 sequence (Vieira and Messing, 1982), starting from the *NarI* site was attached to the *Xmn* site of the 5' P end. The *hsp70-neo* gene was constructed from a 456-bp *Xba-Xmn* fragment containing the *hsp70* promoter and 206 bp of the transcribed leader (Ingolia *et al.*, 1980), ligated through a *Bam* linker to a *BglII-Bam* fragment containing the coding region but not the promoter of the *neo* gene from the bacterial Tn5 transposon (Jorgensen *et al.*, 1979). The *neo* gene was subsequently reduced by excising the *Sma-Bam* fragment at its 3' end which does not contain *neo* coding sequences.

To construct the cosmid vector, the *Xho-Sal* fragment containing the 3' P end from p6.1 was cloned in the *Sal* site of pUC8 and then excised with *Bam* + *Sal*. This cosmid vector was assembled by a three-way ligation between this fragment, a 4.7-kb *PvuI-Sal* fragment from *cos4* containing the *cos* sites, the plasmid replicon and part of the  $\beta$ -lactamase gene and a 1.5-kb *PvuI-Bam* fragment from Carnegie-1 (Rubin and Spradling, 1983) containing the rest of the  $\beta$ -lactamase gene, the 5' P end and part of the polylinker. The resulting plasmid was converted to *cosP* by partial *PvuII* digestion and mild *Ba31* treatment to remove the *PvuII* site in the 5' P fragment.

To generate *cosPneo*, we used the *hsp70-neo* gene which had been introduced into Carnegie-4 (Rubin and Spradling, 1983) as follows. An *EcoRI-Sma* fragment containing the *hsp70-neo* gene was ligated to the *EcoRI* site of Carnegie-4. The ligation was interrupted to fill in the remaining *EcoRI* ends with DNA polymerase and then resumed to join the filled in *EcoRI* site to the *Sma* end of *hsp70-neo*. The resulting clone, Ca4hsneo provided a *HindIII* fragment containing part of the 5' P end, the *hsp70-neo* gene and the polylinker for ligation to *cosP* cut with *HindIII*. The resulting plasmid, with the fragment inserted in the correct orientation to regenerate a 5' P end, is *cosPneo*.

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