1762–1763 Nucleic Acids Research, 1994, Vol. 22, No. 9 

# *pha-1*, a selectable marker for gene transfer in *C.elegans*

Michael Granato+, Heinke Schnabel and Ralf Schnabel*
Max-Planck-Institut für Biochemie, Am Klopferspitz 18 A, 82152 Martinsried, Germany

Received March 7, 1994; Accepted March 25, 1994 EMBL accession number X74228

The nematode *C.elegans* is a powerful genetic system to study a large spectrum of biological problems. Once mutants with specific defects are identified and phenotypically characterised, a molecular analysis of gene functions is desirable. A standard method for cloning genes in *C.elegans* is to complement mutants with cloned genomic DNA introduced into the mutant strain by germline transformation (1–3). Furthermore, germline transformation of regulatory gene sequences fused to a reporter gene provides a rapid method to study the temporal and spatial expression of cloned genes, without preparing specific antisera (4–7). Germline transformation has also been used to assay the biological activity of *in vitro* manipulated nucleic acid sequences (7–10) or to screen systematically for regulatory promoter sequences using a promoter trap approach (10).

DNA transformation of *C.elegans* is accomplished by microinjecting DNA into the gonadal syncytium of hermaphrodites (2). Upon successful transformation the foreign DNA is usually propagated as free extrachromosomal arrays, which are lost at a high frequency (11). Three main problems arise during establishment and analysis of transgenic *C.elegans* lines: (i) identification of transformed animals, (ii) maintenance of transformed lines, and (iii) obtaining large numbers of transgenic worms for analyses. So far, a coinjected dominant visible marker has been routinely used to identify and maintain transgenic worms (2). Although this procedure works satisfactorily, the high frequency loss of the free duplication (i.e. the transgene) requires that the worms be handled individually and it is thus laborious to produce and to maintain many different transgenic strains or to grow large amounts of transgenic worms.

We describe here a novel system which allows one to select and maintain the transgenic strains easily. The system is based on the temperature sensitive embryonic lethal mutation *pha-1* (*e2123*) (12), which causes worms to grow normally at 15°C, however at 25°C, the *pha-1* mutation is 100% embryonic lethal. A second mutation, *lon-2*(*e678*), which results in longer worms, is included because long worms are more easily injected. Transgenic animals can be selected by coinjecting the small plasmids pC1 or pBX (Fig. 1) carrying a wild type copy of the *pha-1* gene (6). At 25°C these plasmids complement the embryonic lethality of the *pha-1* mutation. Thus transgenic worms

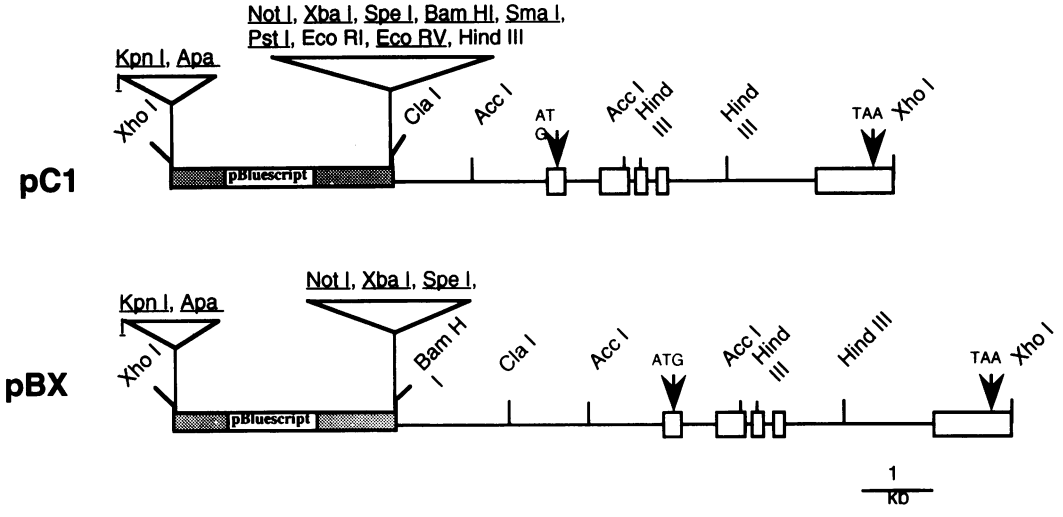


**Figure 1.** The plasmids pBX and pC1 encoding the *pha-1* gene. An 8 kb BamHI/XhoI genomic fragment was subcloned into the bluescript KS+ vector (Stratagene) to produce the pBX vector. pC1 is derived from pBX by removing a 1.5 kb BamHI/ClaI fragment upstream of the *pha-1* gene. The 6.6 kb pC1 insert was sequenced on both strands using T7 polymerase (Pharmacia). The sequence of the 1.5 kb BamHI/ClaI fragment was not determined. Exon intron boundaries and the open reading frame of the *pha-1* gene are indicated. Unique restriction sites are underlined, these can be used to insert DNA fragments to be expressed directly into the selection vector, if it appears convenient not to rely on cotransfection.

+Present address: Max-Planck-Institut für Entwicklungsbiologie, Spemannstrasse 35, 72076 Tübingen, Germany

**Table 1.** Selection of transformed *C.elegans* strains using the *pha-1* selection system

| | Genotype of injected hermaphrodites | Injected DNA | Number of injected hermaphrodites | Number of transformed strains (F2) | Strains expressing cotransformed DNA (F2) |
|---|---|---|---|---|---|
| *pha-1* | *pha-1 III; lon-2 X* | pha-1:lacZ, pBX | 122 | 13 | 12 |
| selection | *vab-7 sud-1/mnC1* II; *pha-1 III; lon-2 X* | *sud-1* cosmid, pC1 | 26 | 4 | 3 |
| *rol-6* | *pha-1 III; lon-2 X* | pBX, pRF4 | 107 | 10 | 8 |
| marker | *pha-1 III; lon-2 X* | pC1, pRF4 | 24 | 5 | 2 |

The *pha-1* selection was used for two purposes: to study the embryonic expression pattern of the gene *pha-1* itself and to clone the maternal effect lethal gene *sud-1*. A cosmid containing the gene *sud-1* was identified by its ability to rescue the progeny of hermaphrodites mutant for the maternal effect gene *sud-1* (K.Kubo, F.Bayle, H.S. and R.S., unpublished). The plasmids pC1 and pBX contain a genomic fragment encoding the *pha-1* gene (see text, Fig. 1) and were used to select for successfully transformed strains. Two different pha-1: lacZ constructs (pBS-DlacZ and pH1-DlacZ, Ref. 6) were used to study the spatial and temporal expression pattern of the *pha-1* gene. Expression of these pha-1: lacZ constructs was scored by staining whole mount embryos for β-galactosidase activity (4). For comparison two transformation experiments using the visible marker *rol-6* are shown. The plasmid pRF4 codes for a dominant mutation in the gene *rol-6*. The expression of this gene causes rolling of worms dominantly, therefore it is possible to identify transformed animals in a background of animals snaking normally over the plate (2). Rolling worms are then tested for the expression of the coinjected DNA. In this case rolling hermaphrodites were shifted to 25°C to screen for complementation of the temperature sensitive mutation *e2123* of *pha-1*. Hermaphrodites were injected using a mixture of about 0.1 to 1 μg/ml of each DNA according to a standard procedure described by Mello *et al.* (2).

can be selected by shifting F1 (or even F2) larvae of injected hermaphrodites from 15°C to 25°C. Only transformed progeny of these worms will survive this selection. To maintain the strain it is simply grown at 25°C. The consecutive selection avoids overgrowth of the population with worms that have lost the injected DNA, which occurs when using only a visible marker. The selective system can also be used to select for stable transformed strains after integration of the extrachromosomal array into the genome (5,6).

We successfully used the selection system to analyse the embryonic expression pattern of the *pha-1* gene itself (6) and to clone an unrelated maternal effect gene, *sud-1* (Table 1). Almost all lines (12/13, 3/4) transformed with the *pha-1* encoding plasmids (pBX, pC1 respectively) also expressed the coinjected DNA. The transformation frequency using these *pha-1* plasmids was similar to the frequency observed with the visible marker *rol-6* (Table 1). We expect that the system can be generally used to facilitate the selection of transformed strains with any DNA to be expressed in *C.elegans*. Methods are described in the Legend to Table 1.

The *pha-1 (e2123) III; lon-2 (e678) X* strain and the vectors can be obtained from the Caenorhabdtis Genetics Center, 250 Biological Sciences Center, 1445 Gortner Avenue, St Paul, MN 55108–1095, USA.

## REFERENCES


1. Fire,A. (1986) *EMBO J.* **5**, 2673–2680.
2. Mello,C.C., Kramer,J.M., Stinchcomb,D. and Ambros,V. (1991) *EMBO J.* **10**, 3959–3970.
3. Bowerman,B., Eaton,B.A. and Priess,J.R. (1992) *Cell* **68**, 1061–1075.
4. Fire,A., White-Harrison,S. and Dixon,D. (1990) *Gene* **93**, 189–198.
5. Krause,M., Fire,A., White-Harrison,S., Priess,J. and Weintraub,H. (1990) *Cell* **63**, 907–919.
6. Granato,M., Schnabel,H. and Schnabel,R. (1994) in preparation.
7. Huang,M. and Chalfie,M. (1994) *Nature* **367**, 467–470.
8. Hong,K. and Driscoll,M. (1994) *Nature* **367**, 470–473.
9. Aamodt,E.J., Chung,M.A. and McGhee,J.D. (1991) *Science* **252**, 579–582.
10. Hope,I. (1991) *Development* **113**, 399–408.
11. Stinchcomb,D., Shaw,J., Carr,S. and Hirsh,D. (1985) *Mol. Cell. Biol.* **10**, 1516–1524.
12. Schnabel,H. and Schnabel,R. (1990) *Science* **250**, 686–688.