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Applying antibiotic selection markers for nematode genetics

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ABSTRACT

Antibiotic selection markers have been recently developed in the multicellular model organism *Caenorhabditis elegans* and other related nematode species, opening great opportunities in the field of nematode transgenesis. Here we describe how these antibiotic selection systems can be easily combined with many well-established genetic approaches to study gene function, improving time- and cost-effectiveness of the nematode genetic toolbox.

1. Introduction

For more than forty years, antibiotic resistance genes have been used in combination with antibiotics as selectable markers for the efficient monitoring of DNA transformation in bacteria [1,2]. Due to its versatility and numerous advantages, this system has been rapidly adapted to other research models, like yeast and cultured eukaryotic cells [3]. However, applications in multicellular organisms such as *Cænorhabditis elegans* and related nematodes have not been developed until very recently [4–6].

DNA transformation of *C. elegans* was successfully developed in the 80s [7,8], and since then it has become an invaluable tool, widely applied to functional studies in nematodes. The many applications of *C. elegans* transgenesis to the study of gene function have been extensively reviewed elsewhere [9].

1.1. C. elegans transgenesis

The principle of DNA transformation in *C. elegans* relies on the introduction of exogenous DNA (plasmid or PCR product) directly into the syncytial gonad of the self-fertilizing hermaphrodite, either by DNA microinjection or by particle bombardment.

DNA microinjection is a relatively easy technique, which results in the formation of extrachromosomal arrays, consisting of multiple copies (80–300) of the exogenous DNA arranged as concatemers [7,10]. These arrays behave as artificial chromosomes, as they are efficiently replicated and segregated to the progeny, producing stable transgenic lines [7,10]. However, extrachromosomal arrays can be lost during cell divisions, leading to a variable transmission rate (depending on the strain, the array is transmitted from 10% to 90% of the progeny) [10].

During gene bombardment, DNA-coated beads are used as vectors to introduce DNA into the animals [11]. This method also produces extrachromosomal arrays, but in addition, random integration of several copies of the transgene into the genome is observed in 1/4 to 1/8 of the obtained strains [12,13]. Nowadays, the most established bombardment protocol uses *unc-119(ed3)* mutant animals as a recipient strain. These animals display an easy observable locomotor defect and are unable to enter the diapause state *Dauer* upon starvation [14]. During bombardment, the *unc-119* gene is used as a co-transformation marker, allowing the selection of transformed animals based on their wild-type locomotion and their ability to survive starvation [14].

The majority of transformation markers used for *C. elegans* transgenesis are easily scorable under a dissecting scope. They are based on the rescue of nonlethal mutations [12,15], and the use of dominant [8,10] or fluorescent markers [16,17], allowing visual identification of specific traits (Table 1). In most cases, these

Abbreviations: PCR, polymerase chain reaction; GFP, green fluorescent protein; RNAi, RNA interference; MosSCI, Mos mediated single copy insertion; NGM, nematode growth medium; NeoR, neomycin resistance gene; CRISPR, Clustered Regularly Interspaced Short Palindromic Repeats; TALENs, Transcription Activator-Like Effector Nucleases; *Dpy*, dumpy; *Unc*, uncoordinated; *Lin*, cell lineage variant.

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Table 1
Available markers for *C. elegans* transgenesis. Mutant rescue: the introduction of the wild type gene rescues a mutant phenotype. *sup-7(st-5)* rescues temperature sensitive sterility of *tra-3(e1107)* mutants [8], *lin-15(+)* rescues temperature sensitive multivulva phenotype of *lin-15(n765ts)* [36], *dpy-20(+)* rescues *dpy-20(e1282ts)* dumpy phenotype [37], *unc-119(+)* rescues *unc-119(ed3)* locomotor and *Dauer* larva formation defects [14], *pha-1(+)* rescues embryonic lethality of *pha-1(e2123)* at 25 °C [15]. Dominant phenotypes: *rol-6(su1006)* gives a roller phenotype [38], *unc-22* antisense gives a twitcher phenotype [39]. Fluorescent reporters: fluorescent proteins such as GFP (green fluorescent protein), mCherry or dsRed (red fluorescent proteins) expressed under the control of a strong promoter. Antibiotic selection: a resistance cassette gives a selective advantage to transgenic individuals in the presence of antibiotics: NeoR: G418 [5]; PuroR: puromycin [4]; HygR: hygromycin B [6]. Compatible with any genotype: the marker does not require a specific genetic background to be efficient and can be used directly with any recipient strain. Hands off selection: the marker confers a selective advantage for transformed versus non-transformed animals (*only in specific environmental conditions). Compatible with other nematode species: fluorescent and antibiotic markers can be directly expressed in other nematode species under the control of *C. elegans* regulatory sequences.

	Compatible with any genotype	Hands-off selection	Compatible with other nematodes
Mutant rescue			
dpy-20(+)	✗	✗	✗
sup-7(st5)	✗	✗	✗
lin-15(+)	✗	✓*	✗
pha-1(+)	✗	✓*	✗
unc-119(+)	✗	✓*	✗
Dominant phenotypes			
unc-22 antisense	✓	✗	✗
rol-6(su1006)	✓	✗	✗
Fluorescent markers	✓	✗	✓
Antibiotic selection			
NeoR	✓	✓	✓
PuroR	✓	✓	✓
HygR	✓	✓	✓

markers do not provide a selective advantage to transformed animals compared to the non-transformed siblings, and when they do, it is only in specific non-permissive conditions (i.e., starvation for *unc-119(ed3)* mutants). Moreover, these markers require the use of specific mutant animals as recipient strains, which are generally more difficult to grow than the wild type, and may complicate genetic interaction studies.

With some transformation markers the selection and manual maintenance of non-integrated transgenic strains (extrachromosomal array lines) is a time consuming task. Based on visual marker scoring, transgenic worms need to be selected and transferred from one plate to another every few generations. This process has to be performed repeatedly if transgenic populations have to be enriched for biological analysis or maintained for long periods of time.

1.2. Antibiotic markers for nematode transgenesis

Antibiotic selection in nematodes is based on the transformation of animals with vectors carrying a bacterial antibiotic resistance cassette, expressed under the control of a nematode ubiquitous promoter. Three distinct markers have been published to date, based on neomycin [5], puromycin [4] and hygromycin B [6,18] resistance, which offer the possibility to generate antibiotic resistant animals both by microinjection and microparticle bombardment.

Antibiotic resistance cassettes are universal markers that can be used in many different nematode species and in any genetic background, facilitating comparative evolutionary and genetic studies in nematode models. Successfully transformed animals can develop and reproduce normally in solid or liquid antibiotic containing media, whereas non-transformed siblings arrest at early larval stages. Antibiotic resistance markers thus allow both hands-off obtention and maintenance of transgenic populations [4,5]. Since almost 100% of the animals growing on selective medium are transgenic, independently of the array transmission rate, non-integrated strains can be maintained without human intervention [4,5]. Thus basic routine techniques such as strain freezing or synchronization, as well as biological analyses where a large number of animals are needed can be performed without manual selection of transgenic animals.

A common concern among the community is the expensiveness of the use of antibiotics. Based on the online catalog of Fisher Scientific 2013, we calculated that the cost for one 9 cm plate of NGM is around 0.067$ and is increased to 0.27$ by G418, and to 0.11$ by hygromycin-B. In other words, G418 and hygromycin B increase the cost by less than 15$/L of NGM (Puromycin is 10 times more expensive). However, these costs may vary depending on supplier and country. In our experience, the time saved expressed in person.hour largely outweighs the extra cost of the antibiotics themselves.