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TITLE:

FORWARD AND UNBIASED RNA-INTERFERENCE SCREEN.

ABSTRACT

RNA-interference (Fire et al. 1998) is a popular ‘reverse-genetics’ screening strategy applied in *Caenorhabditis elegans*. Genome-wide RNAi screens are presently carried using RNAi feeding libraries. Here, we report on a complementary resource facilitating an approach to RNAi screen relying on an unbiased ‘forward-genetics’ strategy. We conclude the forward RNA interference screening is useful and feasible, with the strong expectation the presented screening mode will complement and extend on the existing, currently available, genome-wide RNAi resources.

INTRODUCTION

RNA-interference (Fire et al. 1998) is a popular ‘reverse-genetics’ screening strategy applied in *Caenorhabditis elegans*. The particular ingested variant of RNAi (Timmons et al. 1999) gained popularity for genome-wide screens, where bacterially expressed dsRNA is administrated *per os* utilizing modified plasmids and *E. coli* as a feeding vector (Timmons et al. 2001). Genome-wide RNAi screens are presently carried using RNAi feeding libraries.

Two types of genome-wide RNAi ‘feeding libraries’ entailing PCR-amplified target regions are presently available: library of predicted gene-overlapping segments (Kamath et al. 2003) and amplified cDNA library (Rual et al. 2004). However, available genome-wide PCR-based recombinant RNAi libraries - resources consisting of dsRNA producing plasmids - depend heavily on gene predictions, hence the bias toward certain exon-rich gene regions or certain cDNAs.

RESULTS AND DISCUSSION

Here, we report on a complementary resource facilitating an approach to RNAi screen relying on an unbiased ‘forward-genetics’ strategy. The experiments based on this approach started with the construction of the library of genomic segments incorporated into convergent T7 polymerase binding sites plasmid (PBS) vector (L4440 plasmid), I ligated with standard, small scale liquid worm DNA prep digested with EcoRI and HindIII* and transformed into HT115(DE3) *E. coli* (Timmons et al. 2001). The resulting convergent T7 PBS library was estimated as ~95% recombinant. Convergent T7 library clones were fed individually with a standard protocol (as described previously, Kapulkin et al. 2005) into N2-derived worms, to confirm the apparent lethal/sterile phenotype occurs at a frequency of 1-2 per 24 clones tested.

Based on the above experiment, we conclude the forward RNA interference screening is useful and feasible, with the strong expectation the presented screening mode will complement and extend on the existing, currently available, genome-wide RNAi resources.

**Other variants of the experiment explored elsewhere, involve the fragmented DNA ligated with other enzymes and/or linkers.*

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