Vector-free DNA constructs improve transgene expression in *C. elegans*

To the editor: One advantage of the *Caenorhabditis elegans* model system is the ease and speed with which transgenic worms can be generated[1]. This feature has been used to rescue mutant phenotypes, to express genes heterologously and to analyze gene expression patterns and *cis*-regulatory regions with reporter genes[2]. With transgenic approaches, one often relies in part on the interpretation of negative results, such as the failure to observe reporter expression after deletion of putative regulatory elements or the failure to observe a biological effect by expressing a gene in one but not in another cell type.

The conventional strategy to generate expression constructs in *C. elegans* relies on subcloning the piece of DNA under investigation, usually into a set of standard vectors that contain a common backbone[2,3]. While dissecting regulatory regions of several *C. elegans* promoters, we found that subcloned promoter fragments consistently failed to yield detectable expression in expected cell types (**Fig. 1**) whereas the same reporter constructs, when injected in linear form without adjacent vector sequences (generated either by PCR or by restriction digest), yielded consistent, highly penetrant expression in expected cell types (6 different reporter constructs from the promoters of 5 different genes; **Fig. 1, Supplementary Table 1** and **Supplementary Methods** online).

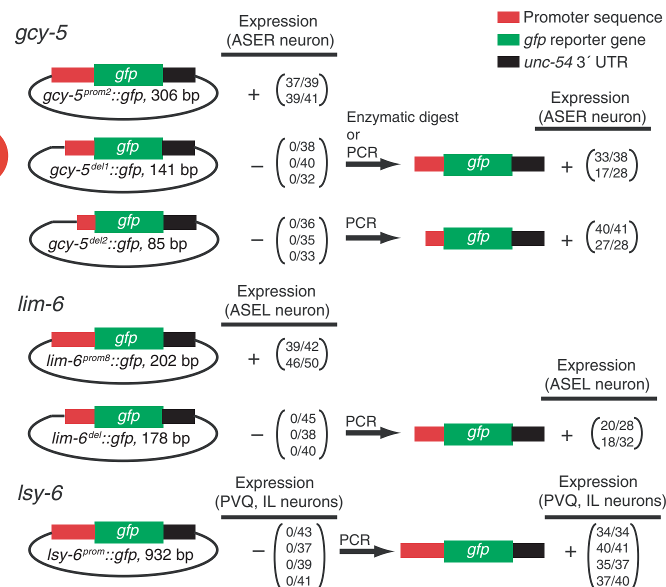

Figure 1 | Expression of reporter gene constructs derived from the *gcy-5, lim-6* and *lsy-6* genes. Multiple independent transgenic lines were scored; the number of worms showing expression in the indicated cell type is listed in parentheses. Linear, vector-free expression constructs were generated by PCR or double restriction digest. For comparison, we also show the previously described *gcy-5^{prom2}* and *lim-6^{prom8}* subcloned expression constructs[5]. For more information on all genes analyzed, see **Supplementary Methods**.

We consider the observed expression of the linear, non-vector-containing constructs to be more authentic than the absence of expression of the subcloned constructs because the observed expression (i) matches the presumed site of function of the gene (for example, *lim-6* acts in the ASEL neuron to repress ASER neuron fate[4]), (ii) matches the expression of larger, subcloned reporter gene constructs derived from the same promoter (**Fig. 1**) and (iii) occurs in the expected cell type based on the presence of well-characterized *cis*-regulatory motifs in the promoter[5].

The lack of expression in the vector-containing construct is unlikely to be due to known transgene silencing effects because we did not observe *gfp* expression in mutant backgrounds in which RNA interference–mediated transgene silencing effects are disrupted (**Supplementary Table 2** online). To test whether expression of the linear reporters is due to the linear structure or the absence of the vector backbone, we linearized one of the subcloned constructs (*gcy-5^{del1}::gfp*) by single restriction digest. None of the resulting transgenic lines (6 in total) showed the reproducible *gfp* expression observed with the linear, vector-free construct, indicating that the absence of the vector is critical for observing reporter gene expression. **Supplementary Table 2** summarizes all experimental conditions tested.

One potential explanation for our observations is that the vector backbone may dictate the packaging of the DNA into higher-order chromatin structure, making regulatory elements in the promoter less accessible. Our findings indicate that negative results obtained with subcloned DNA must be interpreted with caution and should motivate, if feasible, the use of linear, vector-free expression constructs in *C. elegans* that are generated, for example, by PCR fusion[6]. If expression constructs were generated by subcloning, constructs may be PCR-amplified without the vector sequence and injected directly into the gonad.

Note: Supplementary information is available on the Nature Methods website.

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