Cognate putative nuclear localization signal effects strong nuclear localization of a GFP reporter and facilitates gene expression studies in *Caenorhabditis elegans*

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Targeting a gene expression reporter, usually the green fluorescent protein (GFP), to the nucleus via a translationally fused nuclear localization signal (NLS) greatly facilitates recognition and identification of the reporter-expressing cells in Caenorhabditis elegans. Presently circulating nematode transcriptional gene expression vectors use the viral NLS from simian virus 40 (SV40) large T antigen. This NLS, however, fails to ensure sufficient localization of the GFP peptide to the nucleus. We modified the common transcriptional reporter SV40 NLS-GFP by adding to its C terminus a cognate putative NLS from the transcription factor egl-13. The EGL-13 NLS effected clear contrast in fluorescence intensity between the nucleus and the cytoplasm in cells with strong reporter signal and efficiently highlighted the nucleus in tissues with weak reporter expression in a wide range of tested tissues. The SV40 NLS-GFP-EGL-13 NLS vector should become a valuable tool for gene expression studies in *C. elegans.*

Expression of a reporter sequence under the control of cis-acting regulatory elements of a gene of interest is the most widely used method for studying tissue specificity of gene transcription and translation in *Caenorhabditis elegans* (1). The most convenient and commonly used reporter is the green fluorescent protein (GFP) from *Aequorea* (1,2). A GFP-coding sequence can be fused with a chromosomal DNA fragment carrying cis-acting regulatory elements of the gene under investigation to derive either a translational or transcriptional expression cassette (3). In the latter case, subcellular localization of the GFP reporter is open to manipulation by adding a DNA segment encoding a nuclear localization signal (NLS) to the GFP-coding sequence. Concentrating GFP in the nucleus greatly facilitates detection of weak reporter signals and identification of reporter-expressing cells (3,4).

The Fire Lab vector tool kit currently in circulation in the *C. elegans* community offers a set of promoterless NLS-GFP vectors for construction of transcriptional reporter fusions (Reference 5 and documentation available at www.addgene.org/labs/Fire/Andrew/Nec95.pdf). Unfortunately, the single copy of the monopartite NLS from the simian virus 40 (SV40) large T antigen (NLS$^{SV40}$) used in these vectors effects only a minimal over-accumulation of the reporter in the nucleus relative to the cytoplasm (5). This is probably due to several factors, including a likely weak affinity of a mammalian virus NLS for the nematode nuclear import factors (6,7).

A later supplement to the Fire Lab vector kit offers a set of promoterless vectors carrying 4×NLS$^{SV40}$-GFP-coding sequences (documentation available at www.addgene.org/labs/Fire/Andrew/Vec99.pdf). The four consecutive copies of NLS$^{SV40}$ improve nuclear localization of the reporter in at least some tissues (8). However, Fire and colleagues also found that the 4×NLS$^{SV40}$-GFP peptide may be toxic at high levels of expression and that the 4×NLS$^{SV40}$-GFP vectors exhibit significantly lower transformation efficiency in comparison with single NLS$^{SV40}$ constructs (see vector documentation at www.addgene.org/labs/Fire/Andrew/Vec99.pdf).

We sought to assemble a strongly nuclear reporter without the limitations of 4×NLS$^{SV40}$-GFP by adding a DNA segment encoding a cognate *C. elegans* NLS to the GFP-coding sequence in an NLS$^{SV40}$-GFP vector. A literature search indicated that *C. elegans* NLS motifs have not been characterized for the ability to function outside the native protein context. After a further review of the nematode nuclear proteins, a putative bipartite NLS (NLS$^{EGL-13}$) from the transcription factor EGL-13 was selected (9). The single predicted NLS$^{EGL-13}$ consists of the first 25 amino acids at the N terminus of the protein (see Supplementary Figure S1A, available online at www.BioTechniques.com) and resides at a distance (in the primary structure) from the C-terminal DNA binding Sox domain.

A 19-amino acid fragment of NLS$^{EGL-13}$ under the control of an egl-13 promoter region was fused with a GFP sequence (in L2464; see Fire Lab vector kit) to derive pWH18. Transgenic animals isolated after coinjection of pWH18 with a selection vector exhibit reporter signals in tissues in which *egl-13* is normally expressed (9): in the π, intestinal and body wall muscle cells, and most head and tail neurons (data not shown). In all instances, reporter fluorescence is strongly nuclear.

A full-length NLS$^{EGL-13}$ sequence was inserted into L2460 (an NLS$^{SV40}$-GFP construct) immediately before the GFP stop codon to derive pNL74.4 (the NLS$^{SV40}$-GFP-NLS$^{EGL-13}$ vector) (see the supplementary material). To test whether the NLS$^{SV40}$-GFP-NLS$^{EGL-13}$ reporter provided advantages over NLS$^{SV40}$-GFP and 4×NLS$^{SV40}$-GFP, a region of the transbilayer amphiphath transporter (tat-3) gene promoter (10,11) was ligated into L2460 and pNL74.4 to derive tat-3::nls$^{SV40}$-gfp and tat-3::nls$^{SV40}$-gfp-nls$^{EGL-13}$ expression cassettes, respectively. Plasmids carrying the two cassettes were co-precipitated with a selection marker vector onto gold particles (12) and then bombarded into the nematode gonad (4). Transformation efficiencies of the tat-3::nls$^{SV40}$-gfp and tat-3::nls$^{SV40}$-gfp-nls$^{EGL-13}$ vectors were equivalent (one
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REFERENCES


Integrated line per four bombarded plates, and several independent integrated lines were isolated.

The tat-3::nlsSV40-gfp and tat-3::nlsSV40-gfp-nlsEGL-13 transgenic nematodes exhibit essentially the same tissue-specific pattern of reporter expression. A strong GFP signal is detectable in these animals in a subset of pharyngeal cells (Figure 1A). The C. elegans pharynx is a complex organ composed of tightly packed cells of several types (13). In the tat-3::nlsSV40-gfp nematodes, the NLSV40-GFP reporter fails to significantly accumulate in the nucleus and, under the UV illumination, the nuclei of the fluorescent cells are indistinguishable from the cytoplasm. As a result, identification of cells expressing the reporter is difficult. In contrast, in the same pharyngeal cells with expression of the NLSV40-GFP-NLS EGL-13 reporter, the difference in intensity between the cytoplasmic and nuclear fluorescence is dramatic, and the fluorescent nuclei are clearly discernable. The NLSV40-GFP-NLS EGL-13 reporter seems to be more nuclear and to provide a much better contrast in the fluorescence intensity between the nucleus and the cytoplasm than even 4×NLSV40-GFP (8). The C. elegans cell nuclei normally assume fairly invariant locations relative to anatomical landmarks and other nuclei and can be readily identified when clearly seen (13). The locations of brightly fluorescent nuclei in the NLSV40-GFP-NLS EGL-13 animals indicate that the tat-3 cassettes are expressed in the pharyngeal muscle cells.

The tat-3::nlsSV40-gfp and tat-3::nlsSV40-gfp-nlsEGL-13 transgenic nematodes also express the reporters in the hypodermis (Figure 1B). Neither reporter is detectable in the hypodermal cytoplasm. The intensity of nuclear fluorescence in the tat-3::nlsSV40-gfp animals is similarly barely above the threshold of detection. In contrast, NLSV40-GFP-NLS EGL-13 strongly accumulates in the hypodermal nuclei and unambiguously reveals itself in this tissue. In addition to the pharynx and hypodermis, expression of the two reporters is also evident in the seam, anchor, distal tip, and adult vulval cells, and at lower levels, in the progeny of the vulval precursor cells and some unidentified cells in the head and tail regions (Figure 1 and data not shown). In all cases, the NLSV40-GFP-NLS EGL-13 reporter clearly highlights the nucleus.

Thus, NLS EGL-13 functions properly in all tested tissues: those that normally express egl-13 and those that do not. The new NLSV40-GFP-NLS EGL-13 reporter offers significant advantages over both NLSV40-GFP and 4×NLSV40-GFP with respect to facilitating reporter signal detection and fluorescent cell identification. The NLSV40-GFP-NLS EGL-13 peptide ensures an exceptionally clear fluorescence intensity contrast between the nucleus and the cytoplasm when strongly expressed and concentrates in the nucleus when expressed weakly. Furthermore, unlike 4×NLSV40-GFP vectors, constructs carrying the new reporter retain high transformation efficiency.

Figure 1. NLS EGL-13 enhances the nuclear localization of a GFP gene expression reporter and facilitates recognition of reporter expression sites and identification of reporter-expressing cells. (A) Expression of the NLSV40-GFP and NLSV40-GFP-NLS EGL-13 reporters in the pharynx muscle cells. (B) Expression of the NLSV40-GFP and NLSV40-GFP-NLS EGL-13 reporters in the hypodermis and seam cells. Arrows, hypodermis nuclei; arrowheads, seam cell nuclei. (C) Expression of the NLSV40-GFP-NLS EGL-13 reporter in the progeny of the vulval precursor cells and the anchor cell (AC) (left) and in unidentified cells in the head region (right). NLS, nuclear localization signal; EGL-13, egg-laying abnormal-13; GFP, green fluorescent protein; SV40, simian virus 40.


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