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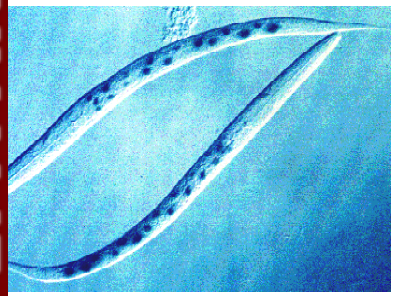
004 EPA STAR Graduate Fellowship Conference *Next Generation Scientists—Next Opportunities*

Raising Transgenic Lines of *Caenorhabditis elegans* for the Study of Stress-Inducible Genes

pic Overview

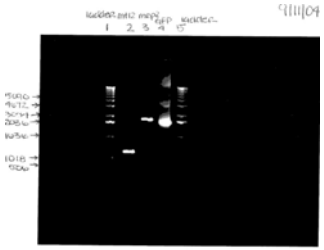
. elegans can be used for the study of ess-inducible proteins and as biomarkers environmental contamination

e overall goal of the research is to raise several different sgenic lines of *Caenorhabditis elegans* in which the moter of a stress-inducible gene is fused to the gene for P. When *GFP* is fused to a promoter of a *C. elegans* gene, resulting transcription and translation product will be ressed in cells as if it were the full gene. Once transgenic s of *C. elegans* containing the promoter-*GFP* fusion ducts are raised, we hope to study the effect of certain cants on gene expression (for example, Cd) and to use the ms as biomonitors for environmental contamination. Work his field has been performed using B-galactosidase (see ure below).



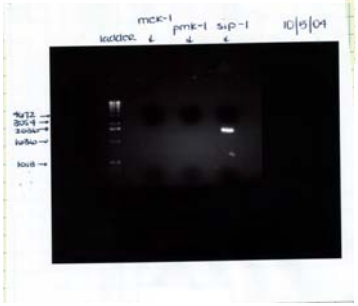
Left: Two *C. elegans* containing B-galactosidase reporter genes respond to cadmium.

Picture from: Freedman, J.H., Slice, L.W., Dixon, D., Fire, A. and Rubin, C.S. The novel metallothionein genes of *Caenorhabditis elegans*. Structural organization and cell-specific transcriptional activation. *J. Biol. Chem.* 268, 2554-2564 (1993)



Left: Amplification of *mrp-2* and *mtl-2* promoters through PCR of genomic DNA. *GFP* was also amplified from the 95.67 plasmid.

Right: Fusion of *mrp-2* and *mtl-2* promoters to *GFP* via PCR.



Left: Amplification of *sip-1* promoter through PCR of genomic DNA.

Right: Fusion of *sip-1* promoter to *GFP* via PCR.

Scientific Approach

- Research Plan

Create promoter-*gfp* transgenes through polymerase chain reaction

-Promoter-*GFP* fusion products were created as described by Hobert[1] for three *C. elegans* genes: *mtl-2* (metallothionein-2), *mrp-2* (multi-drug resistance protein-2) and *sip-1* (stress-induced protein-1). The resulting amplicons from each PCR were elucidated via 1% agarose gel electrophoresis, using ethidium bromide and UV light for visualization (at left). The fusion products were then purified and quantified in preparation for microinjection.

Microinject adult *C. elegans* with promoter-*gfp* fusion product

-Adult *C. elegans* worms will be injected in the gonad prior to egg-laying. This will result in the F1 generation containing the transgenes, as well as the F2 and subsequent generations.

Expose transgenic *C. elegans* to stressors

-The main stressor that will be focused on is cadmium.

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[1]Hobert, O. PCR Fusion-Based Approach to Reporter Gene Constructs for Expression Analysis in Transgenic *C. elegans*. *Biotechniques* 32:728-30 (2002).

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