

XL1-Blue[pKPY197] from

Cell-Specific Proteomic Analysis in *Caenorhabditis elegans*

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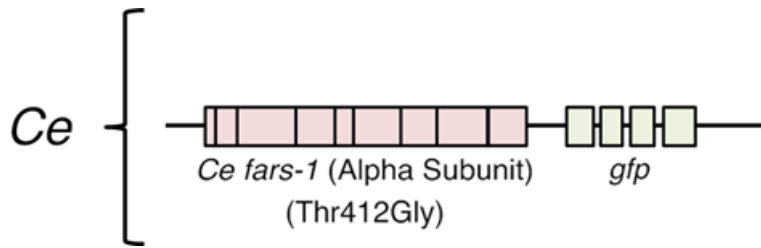
pKPY66: The genes encoding wild-type *C. elegans* PheRS were isolated by RNA extraction (RNeasy Mini Kit, Qiagen) from wild-type (N2) *C. elegans*, reverse transcription (SuperScript III Reverse Transcriptase, Life Technologies) and PCR amplification (PfuUltra II Fusion HS DNA Polymerase, Agilent Technologies). The purified fragments were ligated into pQE-80L (Qiagen) to generate pKPY66.

pKPY100: The genes encoding wild-type *C. elegans* PheRS were isolated by PCR amplification (PfuUltra II Fusion HS DNA Polymerase, Agilent Technologies) of pKPY66. The purified fragments were ligated into pBAD33.

pKPY102: pKPY100 was subjected to site-directed mutagenesis (PfuUltra II Fusion HS DNA Polymerase, Agilent Technologies; DpnI, New England Biolabs) to generate the Thr412Gly¹ mutation.

pKPY197 (*frs-1(Thr412Gly)::fib-1/rps-16::gfp(S65C, SynIVS)::unc-54*): The gene encoding the mutant alpha subunit of *C. elegans* PheRS was isolated by PCR amplification (PfuUltra II Fusion HS DNA Polymerase, Agilent Technologies) of pKPY102. The intercistronic region of the *C. elegans* operon CEOP5428 as described by Lee *et al.* (Lee, L.W., Lo, H.W. and Lo, S.J. *Gene* **455**, 16–21 (2010)) was isolated by genomic DNA extraction (DNeasy Blood & Tissue Kit, Qiagen) from wild-type (N2) *C. elegans* and PCR amplification (PfuUltra II Fusion HS DNA Polymerase, Agilent Technologies). The purified fragments were ligated into pPD95.75 (Fire Lab Vector Kit, Addgene) to generate pKPY197.

¹The first methionine in the alpha subunit of *C. elegans* PheRS, Isoform A, Exon 3 is designated as residue 1 (“Met1”).



pKPY197;

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C. elegans fars-1 (Exon 1-2);

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C. elegans fars-1 (Exon 3-9; Thr412Gly);

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C. elegans fib-1/rps-16 Intercistronic Region;

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M-HA-*tc*;

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