



Jul 30, 2025

Harnessing Microbial Tools: Escherichia coli as a Vehicle for Neuropeptide Functional Analysis in Caenorhabditis elegans

DOI

<https://dx.doi.org/10.17504/protocols.io.5qpvwzzbl4o/v1>

Elizabeth M DiLoreto¹, Shruti Shastry¹, Emily J Leptich², Douglas K Reilly^{3,1}, Rachel N Arey², Jagan Srinivasan^{1,4}

¹Department of Biology and Biotechnology, Worcester Polytechnic Institute, 100 Institute Drive, Worcester, MA, USA, 01609;

²Department of Molecular and Cellular Biology, Baylor College of Medicine, 6450 E Cullen 10 St, Houston, TX, USA 77030;

³Department of Biology, Tufts University, Medford, MA, USA;

⁴Neuroscience Program, Worcester Polytechnic Institute, Worcester, MA, USA



Shruti Shastry

Worcester Polytechnic Institute

Create & collaborate more with a free account

Edit and publish protocols, collaborate in communities, share insights through comments, and track progress with run records.

Create free account

OPEN  ACCESS



DOI: <https://dx.doi.org/10.17504/protocols.io.5qpvwzzbl4o/v1>



Protocol Citation: Elizabeth M DiLoreto, Shruti Shastry, Emily J Leptich, Douglas K Reilly, Rachel N Arey, Jagan Srinivasan 2025. Harnessing Microbial Tools: Escherichia coli as a Vehicle for Neuropeptide Functional Analysis in Caenorhabditis elegans. protocols.io <https://dx.doi.org/10.17504/protocols.io.5qpvowzzbl4o/v1>

License: This is an open access protocol distributed under the terms of the **Creative Commons Attribution License**, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited

Protocol status: Working

We use this protocol and it's working

Created: June 02, 2025

Last Modified: July 30, 2025

Protocol Integer ID: 219364

Keywords: designing peptide plasmid, peptide plasmid, harnessing microbial tool, vehicle for neuropeptide functional analysis, neuropeptide rescue, microbial tool, assembling plasmid, bacterial delivery, neuropeptide functional analysis, competent cells for expression

Abstract

This protocol describes the generation of vectors for *C. elegans* neuropeptide rescue by bacterial delivery, as detailed in several publications and patents. The process involves designing peptide plasmids, assembling plasmids, and transforming them into competent cells for expression.

Guidelines

Neuropeptide Transport: Synthesized and packaged in neural soma. Moves in dense core vesicles through axon. Released at synaptic terminal. Offers dynamic control of behaviors to modulate the functional connectome. **Gut-Brain Connection:** Living in the human gut are trillions of bacteria, fungi, and viruses. Bidirectional communication between the gut and the brain. **Caenorhabditis elegans:** *C. elegans* have a simple nervous system of <400 neurons. 113 neuropeptide genes make over 300 individual peptides.

Materials

DNA sequences encoding individual peptides, Gateway Cloning sites attB1 and attB2, IDT DNA Oligo and Ultramer DNA Oligo services, Duplex Buffer (100 mM Potassium Acetate; 30 mM HEPES, pH 7.5), Thermocycler, pDONR p1-2 donor vector, pENTRY clones, pDEST-527, DH5 α cells, SOC medium, LB+KAN plates, LB+AMP plates, Macherey-Nagel Nucleospin Plasmid Kit, Macherey-Nagel NucleoBond Xtra Midi, Universal T7 promoter primer.

Peptide Plasmid Design and Generation

- 1 DNA sequences encoding individual peptides were identified via www.wormbase.org. Sequences were flanked with the endogenous cleavage sites for the EGL-3 processing enzyme, which cleaves dibasic residues.
- 2 Sequences encoding MRFGKR and KRK-STOP codons were placed prior to, and following the peptide codon sequences, respectively. Gateway Cloning sites attB1 and attB2 were attached to the ends of the sequences.
- 3 These final sequences were ordered from IDT using their DNA Oligo and Ultramer DNA Oligo services, depending on the size of the oligo ordered. Both forward and reverse sequences were ordered. Alternatively, an annealed version of this oligo can also be purchased.

Assembling Plasmid

- 4 Lyophilized oligos were prepared following IDT Annealing Oligonucleotides Protocol. They were resuspended in Duplex Buffer, preheated to 94 °C to a final concentration of 40 µM. Complimentary oligo sequences were then mixed in equimolar ratios, and placed in a thermocycler at 94 °C for prior to a stepwise cooling to room temperature.
 - 4.1 Heat 100 µL Duplex Buffer to 94°C.
 - 4.2 Suspend each oligo in 40 µL heated Duplex Buffer.
 - 4.3 Mix 20 µL of each oligo into a new PCR tube.
 - 4.4 Run annealing procedure: (94°C 30 seconds) x1, (94°C 30 seconds) x70, (24°C 30s, 23°C 30s, 22°C 30s, 21°C 30s, 20°C 10 minutes, 15°C 5 minutes, 10°C 5 minutes) x1, 10°C hold.
- 5 Annealed oligos were used to perform a BP reaction with pDONR p1-2 donor vector to generate pENTRY clones.

Assembly of a Neuropeptide Expression Vector

- 6 Dilute annealed oligo to 15-150 ng/µL.



- 7 Combine 150 ng of the oligo, with 150 ng pDONRp1-p2, and TE buffer to 8 μ L and mix.
- 8 Add 2 μ L Gateway BP Clonase II Enzyme Mix to PCR tube and mix thoroughly before adding.
- 9 Incubate at 25°C for 1 hour.
- 10 Add 1 μ L proteinase K.
- 11 Incubate at 37°C for 10 minutes.
- 12 The BP reaction is then transformed into DH5 α cells. From there individual colonies are grown and mini prepped to confirm BP reaction worked.
 - 12.1 Add 2 μ L of BP reaction to 25 μ L cells.
 - 12.2 Incubate on ice for 30 minutes.
 - 12.3 Heat shock 42°C for 20 seconds.
 - 12.4 Incubate on ice for 3 minutes.
 - 12.5 Add 250 μ L SOC medium to the cells.
 - 12.6 Incubate at 37°C for 1.5 hours.
 - 12.7 On one LB+ 50 μ g/mL KAN plate, plate 75 μ L of cells, on another plate 150 μ L.
 - 12.8 Grow at 37°C 16 hours.

- 12.9 Grow 5 individual colonies in 5 mL LB + 50 µg/mL KAN at 37°C 16 hours.
- 12.10 Mini Prep culture: Macherey-Nagel Nucleospin Plasmid Kit.
- 12.11 Perform restriction enzyme digest on Entry clone to confirm appropriate band sizes on gel.

Entry Clones Recombination

- 13 Entry clones were then recombined with pDEST-527 in LR reactions generating expression clones.
 - 13.1 Combine 100–300 ng of the oligo, with equal mass of destination vector, and TE buffer to 8 µL and mix.
 - 13.2 Add 2 µL Gateway LR Clonase II Enzyme Mix to PCR tube and mix thoroughly before adding.
 - 13.3 Incubate at 25°C for 1 hour.
 - 13.4 Add 1 µL proteinase K.
 - 13.5 Incubate at 37°C for 10 minutes.
- 14 The LR reaction is then transformed into DH5α cells. From there individual colonies are grown, restriction enzyme digest, and sequenced to confirm LR reaction worked.
 - 14.1 Add 2 µL of LR reaction to 25 µL cells.
 - 14.2 Incubate on ice for 30 minutes.



- 14.3 Heat shock 42°C for 20 seconds.
- 14.4 Incubate on ice for 3 minutes.
- 14.5 Add 250 µL SOC medium to the cells.
- 14.6 Incubate at 37°C for 1.5 hours.
- 14.7 On one LB+ 50 µg/mL AMP plate, plate 75 µL of cells, on another plate 150 µL.
- 14.8 Grow at 37°C 16 hours.
- 14.9 Grow 5 individual colonies in 5 mL LB + 50 µg/mL AMP at 37°C 16 hours.
- 14.10 Reserve 1 mL of growth.
- 14.11 With rest of culture Mini Prep culture: Macherey-Nagel Nucleospin Plasmid Kit.
- 14.12 Perform restriction enzyme digest on Entry clone to confirm appropriate band sizes on gel.
- 14.13 Once identify appropriate band sizes in a sample, grow reserved 1 mL of culture in 100 mL LB+AMP for 16 hours shaking at 37°C.
- 14.14 Reserve 1 mL of culture, and Midi prep the rest: Macherey-Nagel NucleoBond Xtra Midi.
- 14.15 Send LR reaction for sequencing, using Universal T7 promoter primer.

Peptide Plate Preparation

- 15 The expression clone for both the peptide of interest and the control was transformed into competent DH5 α cells. Peptide-expressing cultures on LB agar plates containing 100 μ g/ μ L ampicillin were stored at 4 °C for up to 2 months. From this plate, single colonies were selected for growth in 5 mL of LB media containing 100 μ g/ μ L ampicillin at 37 °C for 16 hours.
- 16 The optical density of the grown cultures (OD600) was adjusted to 1.0. This growth can be stored at 4°C and used for up to a week.
- 17 Plate 75 μ L of 1.0 OD600 peptide-expressing culture onto 60 mm NGM agar plates containing 100 μ g/ μ L ampicillin and 1 mM isopropyl- β -D-thiogalactoside (IPTG) at room temperature for at least 8 hours prior to use. Use the peptide plates within a week.

Peptide Feeding

- 18 All animals were maintained on bacterial lawns of OP50 *E. coli*, at 20 °C, on NGM agar plates until the start of experiments. L4-staged mutant or control animals were picked to lawns of DH5 α *E. coli* expressing either scramble peptide or the rescue peptide-of-interest. Animals were left to lay eggs on the peptide-expressing lawns, and progeny were assayed for rescue of mutant phenotype at the appropriate developmental stage.