lambdaMGU2 *C. elegans* cDNA library

77366™

Description

Organism: *Caenorhabditis elegans* Maupas
Clone type: Library
Host: *Escherichia coli* Q358 (ATCC 47018)
Escherichia coli q358 (ATCC 47018) (for propagation); *Escherichia coli* 1046 [pCRE1] (ATCC 77368) (for conversion to phagemid)
Volume: 0.4 mL
Shipping information: bacteria-free phage lysate

Storage Conditions

Product format: Frozen
Storage conditions: -80°C or colder

Intended Use

This product is intended for laboratory research use only. It is not intended for any animal or human therapeutic use, any human or animal consumption, or any diagnostic use.

BSL 1

ATCC determines the biosafety level of a material based on our risk assessment as guided by the current edition of *Biosafety in Microbiological and Biomedical Laboratories (BMBL)*, U.S. Department of Health and Human Services. It is your responsibility to understand the hazards associated with the material per your organization's policies

and procedures as well as any other applicable regulations as enforced by your local or national agencies.

Certificate of Analysis

For batch-specific test results, refer to the applicable certificate of analysis that can be found at www.atcc.org.

Vector Information

Intact vector size: 41.7
Vector name: lambdaMGU2
Type of vector: phage
Construction: lambda2690, pMGU
Vector end: BamHI
Vector information: Library information:
Genome: *Caenorhabditis elegans*
Strain: N2
Type of insert: cDNA
Vector: lambdaMGU2
Insert size ra

- **Insert detection:** Spi+
- **Markers:** ampR
- **Replicon:** lambda, pMB1, m13

Handling Procedures

Thaw contents of the vial in a 37°C water bath with gentle agitation until no ice crystals remain. Library can be diluted and plated following standard protocols. Recommended growth media is LB. Recommended growth temperature is 37°C.

Starting and Amplifying ATCC Bacteriophage Lambda Clones and Vectors:

1. Prepare fresh plating bacteria. Grow *E. coli* host strain overnight or at least to A $_{600}$ = 0.4 in medium containing 0.2% maltose (to give higher titers).
2. Spin down cells in a low speed centrifuge. Resuspend in 0.4 volumes 10 mM $MgSO_4$ or SM buffer. Store at 4°C until ready to use. These cells are good for up to 2 weeks if stored at 4°C.
3. Open freeze dried vial containing the phage according to instructions.Asceptically add 0.3 to 0.4 mL of liquid medium to the freeze-dried pellet and mix well.
4. Pipette 100 µL of the host suspension to a sterile test tube. Add 3 mL. of warm (50°C) LB lambda top agar (see below) containing 0.2% maltose and mix gently. Pour onto plates. Allow the plates to solidify.
5. Spot a loopful or two of the phage suspension on the lawn of the freshly poured bacteria.
6. Incubate overnight at 37°C. Fresh plates give larger plaques.
7. Cut plaques out of agar and add them to 0.5 mL of 10 mM $MgSO_4$ or SM buffer and store at 4^0 C overnight.
8. Add 100 µL of the overnight phage dilution to 100 µL prepared plating bacteria and mix gently. Incubate in a 37°C water bath for 20 minutes to allow phage to adsorb.
9. Add 3 mL. LB lambda top agar containing 0.2% maltose and mix gently. Pour onto plates. Incubate overnight at 37°C.
10. Invert open plate over a chloroform-saturated adsorbent paper for 10 minutes.
11. Add 7.5 mL of 10 mM $MgSO_4$ or SM buffer to the plate and allow it to stand at room temp for 1 hour or in 4°C overnight.
12. Collect and save the liquid on the plate. This should be a high titer lysate. Add a few drops of chloroform if its going to be stored for more than a few days.

LB Lambda top agar medium:
NaCl, 5 g
Tryptone, 10 g
Yeast extract, 5 g
Distilled water to 1 L

Sterilize at 121°C, 15 minutes. Cool to approximately 50°C and add the following sterile solutions.

1M $CaCl_2$, 5 mL

$MgSO_4$ H_2O to a final concentration of 0.2% w/v
50% maltose, 5 mL

Notes

To prepare phagemid from lambdaMGU2, grow recombinants on a RecA- host expressing the Cre protein such as *E. coli* 1046 [pCRE1] (ATCC77368) and select for ampicillin resistance. The pMGU product is 4.185 kb. Efficiency of phagemid recovery is approximately 20%. Plasmid pCRE1 may be a low level contaminant, but is easily distinguished from pMGU DNA. Inserts can be amplified using the following primers flanking the BamHI cloning site : upstream 5' -AAGAGGCAGAACTGGCAG-3' and downstream 5' -ATCGATGCATAGCGATTC-3'. The order of the major features in the cloning region of the lambda vector is : lambda J - SmaI - SalI - loxP - EcoRI - M13 ori - ampR - pMB1 ori - hindIII - 3'gam/BamHI/5'gam - XhoI - loxP - SalI - lambda N.

- Gene 120 : 135-141, 1992.

Single stranded DNA may be recovered from phagemid constructs using M13KO7 helper phages.

- Gene 120 : 135-141, 1992

Material Citation

If use of this material results in a scientific publication, please cite the material in the following manner: lambdaMGU2 *C. elegans* cDNA library (ATCC 77366)

References

References and other information relating to this material are available at www.atcc.org.

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Revision

This information on this document was last updated on 2025-09-09

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