lambdaMGU2

77367™

Description

This is a vector useful for constructing cDNA libraries. It permits positive selection for inserts using the Spi- phenotype, and excision of phagemid by cre/lox site-specific recombination. To enable the positive selection of inserts, the library should be plated on a P2 lysogen such as *E. coli* Q359 (ATCC 47019). To prepare phagemid from lambdaMGU2, grow recombinants on a RecA- host expressing the Cre protein (*E. coli* 1046[pCRE1], ATCC 77368) and select for ampicillin resisitance. 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 teh cloning region of teh 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.

Clone type: Clone

Shipping information: bacteria-free phage lysate

Storage Conditions

Product format: Freeze-dried

Storage conditions: 2°C to 8°C

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
Type of vector: phage
Cloning sites: BamHI
Markers: ampR
Replicon: lambda, pMB1, m13

Handling Procedures

Starting and Amplifying ATCC Bacteriophage Lambda Clones and Vectors

1. Make 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.
3. 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.

4. Open vial according to instructions. Asceptically add 0.3 to 0.4 mL of liquid medium (SM or 10 mM $MgSO_4$) to the freeze-dried pellet and mix well.
5. Spot a loopful or two 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°C overnight.
8. Add 100 µL 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 (see below) containing 0.2% maltose and mix gently. Pour onto plates. Incubate overnight at 37°C. Fresh plates give larger plaques.
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 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

For Cre-lox conversion proecedures, please refer to the following reference: Gene (Amst.) 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 (ATCC 77367)

References

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

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