Certificate of Analysis for NR-19394

Plasmid pMRLB78 Containing Gene ML0380 (Protein GroES) from *Mycobacterium leprae*

Catalog No. NR-19394
This reagent is the tangible property of the U.S. Government.

Product Description: NR-19394 is a recombinant expression vector containing *Mycobacterium leprae* gene ML0380, which encodes chaperonin protein GroES [also known as major cytoplasmic protein (MCP)]. Gene ML0380 was amplified by PCR and cloned into pET28 for expression in *Escherichia coli*, strain TOP10. The expressed protein is histidine-tagged and has an observed molecular weight of 10 kDa.

Lot: 61002004 **Manufacturing Date: 12MAY2011**

Production and QC testing were performed by Colorado State University (CSU). The CSU documentation for the bulk lot pMRLB78 12.20.2010B is attached. This lot was received as dry pellets from CSU and rehydrated in TE buffer (10 mM Tris-HCl, 1 mM EDTA buffer, pH 8) at ATCC®. Approximately 100 µL of a 0.01 µg/µL solution was dispensed per vial yielding a final amount of 1 µg per vial. A plasmid map for pET28 is attached.

ATCC®, on behalf of BEI Resources, hereby represents and warrants that the material provided under this certificate has been subjected by the contractor to the tests and procedures specified and that the results described, along with any other data provided in this certificate, are true and accurate to the best of ATCC®'s knowledge.

Support Provided by NIAID

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Recombinant Plasmid Quality Control Record

Plasmid designation: pMRLB78 (pET28-GroES ML0380)
Lot Number: pMRLB78 12.20.2010B

Notebook/Pgs: JNKBook#1/pp.114-116,124
JNKBook#2/pp10
Notebook detail: Plasmid Prep pp. 111,114
A260/280 and stock dilution pp. 116
QC Gel pp 10

Media used: LB Broth 50 µg/ml Kanamycin
Culture size: 1 x 1L

Growth conditions: Temp 37°C Time 16 hr. Shaker speed 130 rpm

Plasmid prep type (mini/maxi, kit name or protocol) Qiagen Maxi Prep protocol
Plasmid prep detail: Maxi prep lysate and tip washing conditions
Purification (Wash & elution)- Qia500 tips-

Strain used to produce plasmid: TOP-10

E. coli ori? Y/N: Y

Contains Mycobacterial ori? Y/N: N

Final concentration: Dried, 20 µg/tube
Number of batches: 2 x 20 µg

Buffer: TE Buffer

Method used for quantifying: A260

QC gel

Restriction enzymes used in QC analysis: NdeI, HindIII, & BamHI

Expected size of restriction fragments
Vector: 5.369kb pET28 NdeI + HindIII
Insert: 0.239kb GroES NdeI + HindIII
Other: 5.608kb linearized plasmid NdeI, HindIII and BamHI single digest

Gel results: All predicted bands visible JNK Book#2/ pp. 10

Gel description file number, % agarose, buffer: ED GroES12011-05-06, 1.5% 1 X TAE
Sequence file: SequenceGroES.ppt

Plasmid Map: Attached

Generated by Jade Smith Date 5/12/11

Supervisor John D Spencer Date 5/12/11

BEI Resources
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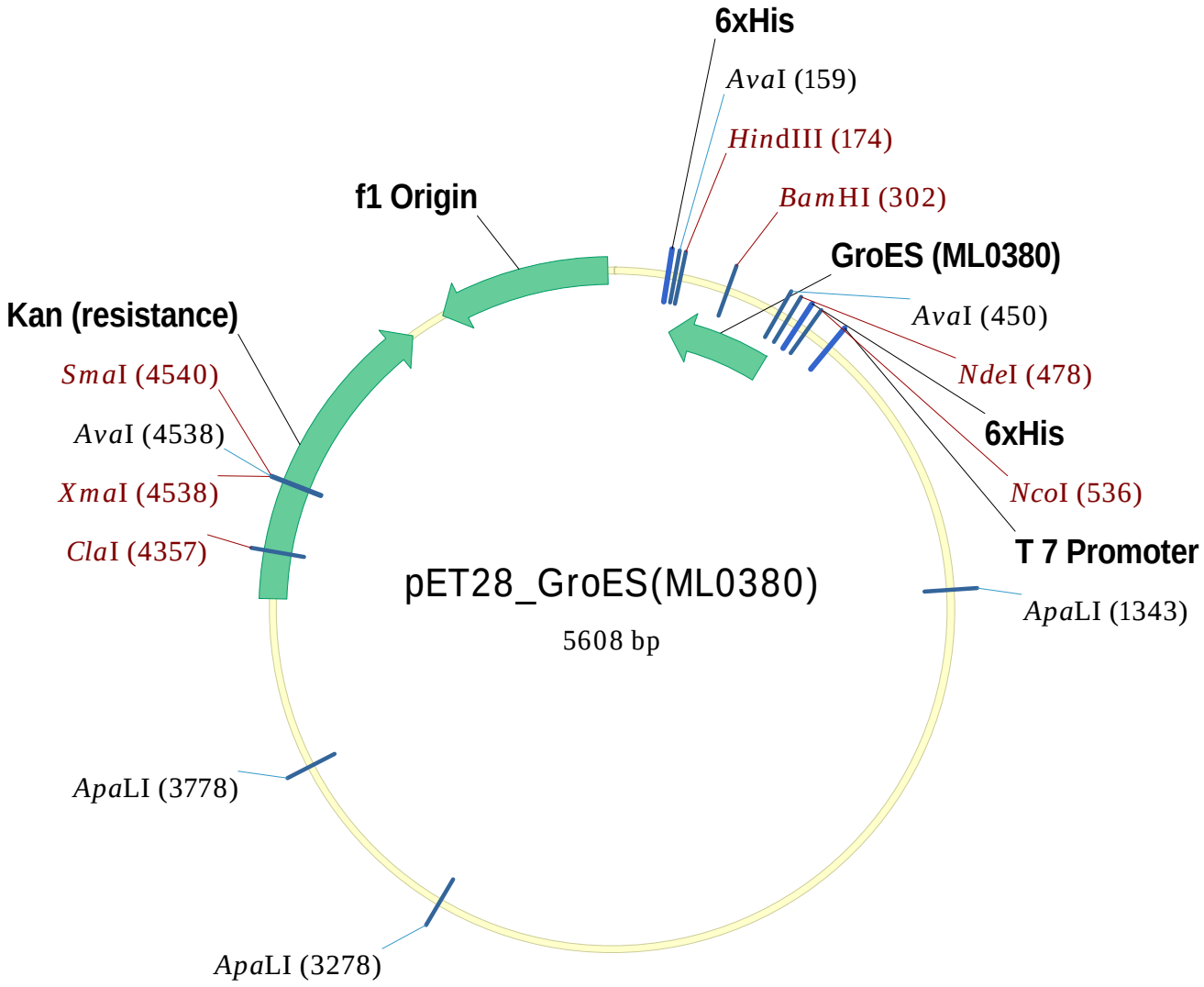
pET28a(+)


6xHis
AvaI (159)
HindIII (174)
BamHI (302)
f1 Origin
GroES (ML0380)
AvaI (450)
Kan (resistance)
NdeI (478)
SmaI (4540)
6xHis
AvaI (4538)
NcoI (536)
XmaI (4538)
T 7 Promoter
ClaI (4357)
pET28_GroES(ML0380)
ApaLI (1343)
5608 bp
ApaLI (3778)
ApaLI (3278)