Certificate of Analysis for NR-36431

Plasmid pMRLB.7 Containing Gene Rv3875 (Protein Esat6) from *Mycobacterium tuberculosis*

Catalog No. NR-36431
This reagent is the tangible property of the U.S. Government.

Product Description: NR-36431 is a recombinant expression vector containing *Mycobacterium tuberculosis* gene Rv3875, which encodes the early secretory antigenic target Esat6, also known as esxA. Gene Rv3875 was amplified by PCR and cloned into pET23b for expression in *Escherichia coli.* The gene was cloned without a signal sequence and with nucleotides coding for the amino acids phenylalanine, alanine, leucine and glutamic acid (FALE) prior to the histidine tag. These nucleotides increase plasmid stability and promote solubility upon transformation and expression. The expressed protein has an observed molecular weight of 10 kDa. The expected purified protein yield from a one liter culture is approximately 5 mg. Plasmid pMRLB7 contains the gene required for ampicillin (Ap) resistance. The recommended concentration of Ap in culture is 100 µg/mL.

Lot: 09.EC.2.11 **Manufacturing Date: 11FEB2009**

QC testing was performed by Colorado State University under the TB Vaccine Testing and Research Materials Contract (NIH). The Colorado State University documentation for bulk lot 09.EC.2.11 is attached. A plasmid map for pMRLB7 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® ق knowledge.

Support Provided by NIAID

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Recombinant Plasmid Quality Control Record

Plasmid designation pMRLB7.Rv3875 (ESAT-6 in pET23b)

Lot Number 09.EC.2.11.pMRLB.7.Rv3875

Notebook/Pgs BDT notebook 2: pp 35-36
mmcontract1/pp 125-127

Notebook detail Plasmid prep pp mmcontract1: pp 125-126
QC gel BDT notebook 2: pp 36

Media used LB broth + 100 µg/ml ampicillin

Culture size 2 x 150 milliter

Growth conditions: Temp 37 deg Time 24 hr Shaker speed 130 rpm

Plasmid prep type (mini/maxi, kit name or protocol) Qiagen Midi Prep protocol

Plasmid prep detail: Lysate prep – Qiagen Midi prep buffer volumes
Lysate clearing by filtration
Purification (wash and elution) – Qiagen HiSpeed tips

Strain used to produce plasmid TOP10

E. coli ori? Y/N yes

Contains Mycobacterial ori? Y/N no

Final concentration 0.035 µg/µl

Buffer 10mM Tris/1mM EDTA, pH 8.0

Method used for quantifying Nanodrop

QC gel

Restriction enzymes used in QC analysis:

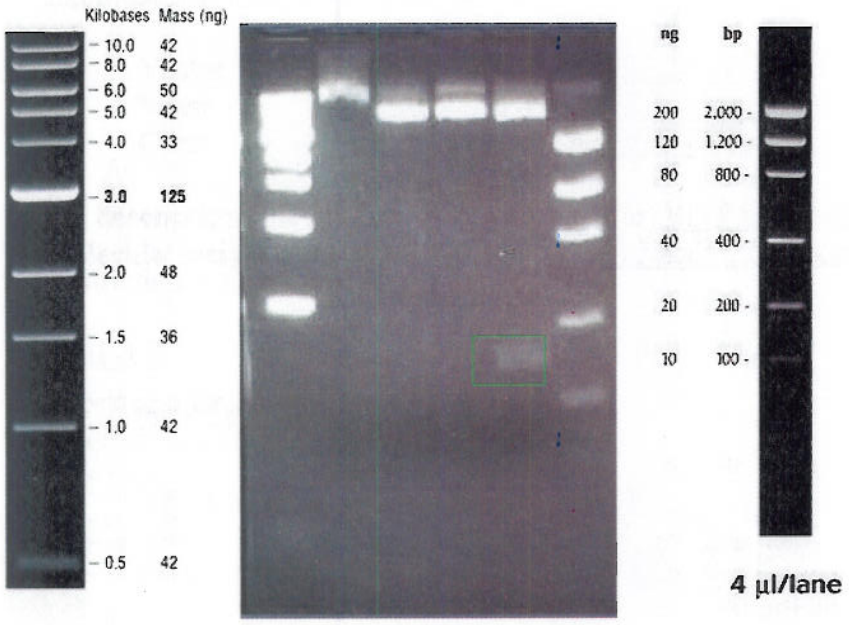
NdeI & XhoI

Expected size of restriction fragments

Vector	3.66 kb pET23b + NdeI + XhoI
Insert	0.29 kb ESAT-6 + NdeI + XhoI
Other	3.94 kb linearized plasmid – NdeI, XhoI double digest

Gel description (file number, % agarose, buffer) BDT notebook 2 pp36, 2%, 1X TAE
Molecular weight markers NEB 1 kb DNA Ladder, Invitrogen Low DNA Mass Ladder

Digest and Gel:

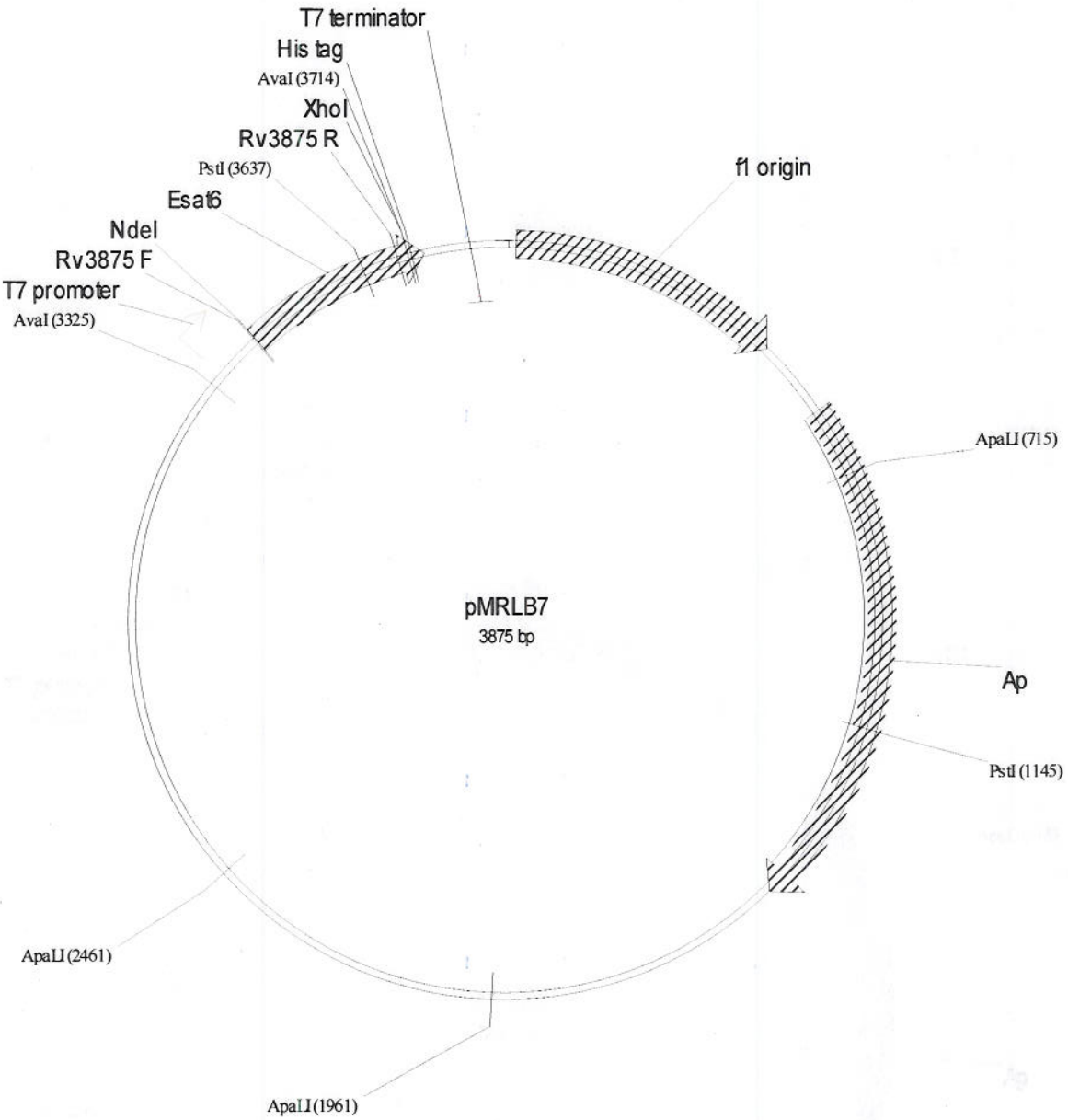


Left → Right:

1. NEB 1kb DNA Ladder
2. pMRLB7 uncut
3. pMRLB7/NdeI
4. pMRLB7/XhoI
5. pMRLB7/NdeI + XhoI
6. Low DNA Mass Ladder

Generated by _____ Date 02/20/13

Supervisor _____ Date 2/20/13


T7 terminator
His tag
AvaI (3714)
XhoI
Rv3875 R
PstI (3637)
Esat6
NdeI
Rv3875 F
T7 promoter
AvaI (3325)
f1 origin
ApaLI (715)
pMRLB7
3875 bp
Ap
PstI (1145)
ApaLI (2461)
ApaLI (1961)