

Supplementary data for the article:

Jovanovic, P.; Jeremic, S.; Djokic, L.; Savic, V.; Radivojevic, J.; Maslak, V.; Ivkovic, B.; Vasiljevic, B.; Nikodinovic-Runic, J. Chemoselective Biocatalytic Reduction of Conjugated Nitroalkenes: New Application for an Escherichia Coli BL21(DE3) Expression Strain. *Enzyme Microb. Technol.* **2014**, *60*, 16–23. <https://doi.org/10.1016/j.enzmictec.2014.03.010>

## Supporting Information: N-ethylmaleimide reductases (NemA) sequence analysis and primer design

### E.coli BL21(DE3)

LOCUS YP\_003054249 365 aa linear CON 27-AUG-2013  
DEFINITION N-ethylmaleimide reductase, FMN-linked [Escherichia coli BL21(DE3)].  
ACCESSION YP\_003054249  
VERSION YP\_003054249.1 GI:254288501  
DBLINK Project: [161947](#)  
BioProject: [PRJNA161947](#)  
DBSOURCE REFSEQ: accession [NC\\_012971.2](#)  
KEYWORDS RefSeq.  
SOURCE Escherichia coli BL21(DE3)  
ORGANISM [Escherichia coli BL21\(DE3\)](#)  
Bacteria; Proteobacteria; Gammaproteobacteria; Enterobacteriales; Enterobacteriaceae; Escherichia.  
REFERENCE 1 (residues 1 to 365)  
AUTHORS Jeong,H., Barbe,V., Lee,C.H., Vallenet,D., Yu,D.S., Choi,S.H., Couloux,A., Lee,S.W., Yoon,S.H., Cattolico,L., Hur,C.G., Park,H.S., Segurens,B., Kim,S.C., Oh,T.K., Lenski,R.E., Studier,F.W., Daegelen,P. and Kim,J.F.  
TITLE Genome sequences of Escherichia coli B strains REL606 and BL21(DE3)  
JOURNAL J. Mol. Biol. 394 (4), 644-652 (2009)  
PUBMED [19786035](#)  
REFERENCE 2 (residues 1 to 365)  
CONSRM NCBI Genome Project  
TITLE Direct Submission  
JOURNAL Submitted (11-APR-2012) National Center for Biotechnology Information, NIH, Bethesda, MD 20894, USA Republic of Korea  
COMMENT PROVISIONAL [REFSEQ](#): This record has not yet been subject to final NCBI review. The reference sequence is identical to [ACT43478](#). Method: conceptual translation.  
FEATURES Location/Qualifiers  
source 1..365  
/organism="Escherichia coli BL21(DE3)"  
/strain="BL21(DE3)"  
/db\_xref="taxon:[469008](#)"  
[Protein](#) 1..365  
/product="N-ethylmaleimide reductase, FMN-linked"  
/EC\_number="1.-.-.-"  
/calculated\_mol\_wt=39371  
[Region](#) 1..365  
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/note="NADH:flavin oxidoreductases, Old Yellow Enzyme family [Energy production and conversion]; COG1902"

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Region 5..345
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OYE was the first flavin-dependent enzyme identified,
however its true physiological role remains elusive to
this day. Each monomer of OYE contains FMN as a
non-covalently bound cofactor, uses NADPH as a...;
cd02933"
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Site order(25,27,59,101,234,302,322,324..326)
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Site order(25,27,59,101,103,110,182,185,187,234,241,302,322,
324..325)
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Site 187
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121 vapsalsagt rtslrdengq airvetsmpr aleleeipgi vndfrqaian areagfdlve
181 lhsahgyllh qflspssnhr tdqyggsven rarlvlevvd agieewgadr igirvspigt
241 fqntdngpne eadalylieq lgkrgiaylh msepdwagge pytdafrekv rarfhgpiig
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**P.putida KT2440**

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/protein\_id="NP\_745317.1"  
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AIANWSADRVGIRVFLGGFNGVDNGEDQEAAGLYLIRELAKRNLAYLHLSEPDWAGG  
KPLRDEFRQAIRAAYPGVIIAAGAYTAEKGEDLIGRGLIDAVAFGRAYIANPDLVERL  
RLQAPLNEHRAKFDYANGPEGYTDYPFLKQA"

ORIGIN

1 gggcatgcgt ccacgtcggg cttcaatgac gtcgcgcat atgtgagtgg gcggtcggca  
61 cgcagctgac gcattgggga gccctgagcc gggctcccgg tcccccttt cccaatcac  
121 cccggtatcg tgcgcacat tggttttcca cctctggagc tgcaatgaaa ctcttgcaac  
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421 aaggcatcca tgccgagggc ggccacagcg ccgtgcaggt gtggcacacc gggcgtgtgt  
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## Bacillus thuringiensis Bt407

375 aa

LOCUS NC\_018877 1466 bp DNA linear CON 11-JUN-2013

DEFINITION Bacillus thuringiensis Bt407 chromosome, complete genome.

ACCESSION [NC\\_018877](#) REGION: complement(3630445..3631910)

VERSION NC\_018877.1 GI:409187965

DBLINK Project: [177931](#)  
BioProject: [PRJNA177931](#)

KEYWORDS RefSeq.

SOURCE Bacillus thuringiensis Bt407  
ORGANISM [Bacillus thuringiensis Bt407](#)  
Bacteria; Firmicutes; Bacilli; Bacillales; Bacillaceae; Bacillus; Bacillus cereus group.

REFERENCE 1 (bases 1 to 1466)  
AUTHORS Sheppard,A.E., Poehlein,A., Liesegang,H., Rosenstiel,P. and Schulenburg,H.  
TITLE The complete genome sequence of Bacillus thuringiensis strain 407 Cry-  
JOURNAL Unpublished

REFERENCE 2 (bases 1 to 1466)  
CONSRM NCBI Genome Project  
TITLE Direct Submission  
JOURNAL Submitted (03-NOV-2012) National Center for Biotechnology Information, NIH, Bethesda, MD 20894, USA

REFERENCE 3 (bases 1 to 1466)  
AUTHORS Sheppard,A.E., Poehlein,A., Liesegang,H., Rosenstiel,P. and Schulenburg,H.  
TITLE Direct Submission  
JOURNAL Submitted (09-OCT-2012) Department of Genomic and Applied Microbiology, Goettingen Genomics Laboratory, Georg-August-University-Goettingen, Grisebachstr. 8, Goettingen, Lower Saxony 37079, Germany

COMMENT PROVISIONAL [REFSEQ](#): This record has not yet been subject to final NCBI review. The reference sequence is identical to [CP003889](#).  
Source DNA available from Hinrich Schulenburg, Evolutionary Ecology  
Genetics Zoological Institute, CAU Kiel, Am Botanischen Garten 1-9  
24118 Kiel, Germany.

##Assembly-Data-START##  
Assembly Method :: Newbler v. v2.6  
Sequencing Technology :: Sanger dideoxy sequencing; 454  
##Assembly-Data-END##  
COMPLETENESS: full length.

FEATURES Location/Qualifiers  
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CDS 169..1296
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FRKPFDTPEAMTLEEIQEVIGQYAQAAKNAIEAGFDGVEIHGAHGYLIDQFTYEFANK
RTDKYGGNLKQRLTFMKEVTAAVIEAVGANKTLLRFSAFKGDNPTYMWENPEFAIETF
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GQKVNEKIAFVRNVRKGETINSPNRFSLKNAADTLYFIQQLQESGGKPVGMKIVIGQ  
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ORIGIN

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121 aaagtaagta  tgatgcgatt  atagtaagat  tttttagggg  gaagaaaaat  gacaagttca
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1321 tagtgttctc  cttttattat  ggaatttcat  agggggaagc  gggatgagtg  aaacgctact
1381 cgttattatt  agtatattac  ttttttaaat  gttgctttta  atcgtctttt  ttatcattac
1441 attttttata  aaaaaacgaa  cacatc
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CLUSTAL 2.1 multiple sequence alignment

BL21 MSSEKLYSPLKVGAITAANRIFMAPLTRLSIEPGDIPPLMAEYYRQRASAGLIIEAT 60
KT2440 ---MKLLQPLQIGPLTLPNRFVMAPLTRLSLEPGDVPTALMAEYYRQRASAGLIITEAT 57
\*\* .\*:\*:\*.:\* .\*:\*:\*\*\*\*\*:\*\*\*\*:\*.\*\*\*\*\*:\*\*\*\*\*:\*\*\*

BL21 QISAQAKGYAGAPGIHSPEQIAAWKKITAGVHAENGHMAVQLWHTGRISHASLQPGGQAP 120
KT2440 QISFQAKGYSGPSGIHSAEQIAAWKHINEGIHAEGGHSAVQVWHTGRVSHSTLQPGGKAP 117
\*\*\* \*\*\*\*\*:\*.\*\*\*\*\*.\*\*\*\*\*:\*. \*:\*:\*.\* \*\* \*:\*:\*\*\*\*\*:\*.\*\*\*\*\*:\*\*\*

BL21 VAPSALSAGTRTSLRDENGQAIRVETSMPRALELEEIPGIVNDFRQAIANAREAGFDLVE 180
KT2440 VAPSALPADARTTLRDAQGNLTRVETSAPRALSEAEIAGIVADFGQAAINACEAGDFDIE 177
\*\*\*\*\*.\*.:\*:\*:\* \*:\* \*\*\*\*\* \*\*\*\*. \*\*.\* \*\* \*\* \* \* \*\*\*\*\*:.\*

BL21 LHAHGYLLHQFLSPSSNHRDQYGGSVENRARLVLEVVDAGIEEWGADRIGIRVSPIGT 240
KT2440 LHAHGYLLHQFLTPSANQREDRYGGSVENRARIVLEAVDAAIANWSADRVGIRVFPPLGG 237
\*:\*:\*\*\*\*\*:\*.\*\*\*:\*. \*:\*:\*\*\*\*\*:\*.\*\*\*.\* \* :\*.\*\*\*:\*\*\*\* \*:\*

BL21 FQNTDNGPNEEADALYLIEQLGKRGIAYLHMSEPDWAGGEPYTDAFREKVRARFHGPIIG 300
KT2440 FNGVDNGEDQEAGGLYLIRELAKRNLAYLHLSEPDWAGGKPLRDEFRQAIRAAYPGVIIA 297
\*:.\*\*\* :\*.\* \*\*\*\*\*:\*.\*\*\*:\*\*\*\*\*:\*\*\*\*\*:\* \* \*\* :\* \* : \* \*\*

BL21 AGAYTVEKAETLIGKGLIDAVAFGRDWIANPDLVARLQKADLNPQRAESFYGGGAEGYT 360
KT2440 AGAYTAEKGEDLIGRGLIDAVAFGRAYIANPDLVERLRLQAPLNEHRAKFDYANGPEGYT 357
\*\*\*\*\*.\*.\* \*\*:\*:\*\*\*\*\* \*:\*:\*\*\*\*\* \*\* :\* \*\* :\*:\* \*..\*.\*\*\*\*\*

BL21 DYPTL--- 365
KT2440 DYPFLKQA 365
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CLUSTAL 2.1 multiple sequence alignment

BL21 -----MSSEKLYSPLKVGAITAANRIFMAPLTRLSIEPG 35
KT2440 -----MKLLQPLQIGPLTLPNRFVMAPLTRLSLEPG 32
Bacillus MTSSSNAASIYEGTNVNAGGFFENVEETKLFDP IQIGAWSLRNRIAMAPMTRCFADNET 60
\*\* .\*:\*:\*. : \*\* : \*\*\*\*:\* : :

BL21 DIPTPLMAEYYRQRASA--GLIIEATQISAQAKGYAGAPGIHSPEQIAAWKKITAGVHA 93
KT2440 DVPTALMAEYYRQRASA--GLIITEATQISFQAKGYSGPSGIHSAEQIAAWKHINEGIHA 90
Bacillus GVVGADVVEYYRKRADGIGLITTEGIVISPRAKGNPGVPGIYTQE QIDSWKPVTEAVHK 120
.: . :.\*\*\*\*\*:\*: \*\*\*\*\*.\* \*\* :\*\*\* .\* \*\*\*: : \*\* :\*\* :. :.\*

BL21 ENGHMAVQLWHTGRISHASLQPGGQAPVAPSALSAGTRTSLRDENGQAIRVETSMPRALE 153
KT2440 EGGHSAVQVWHTGRVSHSTLQPGGKAPVAPSALPADARTTLRDAQGNLTRVETSAPRALS 150
Bacillus EGGTIIAQIWHVGRMSHHEII--GGQMPQAPSAIAAEGNVPR-----FRKPFDTPEAMT 172
\*.\* .\*:\*.\*\*\*:\* \* : \*\* : \* \*\*\*\*\*.\* ... \* .\*.\*:

BL21 LEEIPGIVNDFRQAIANAREAGFDLVELHSAHGYLLHQFLSPSSNHRDQYGGSVENRAR 213
KT2440 EAEIAGIVADFGQAAINACEAGDFDIELHAAHGYLLHQFLTPSANQREDRYGGSVENRAR 210
Bacillus LEEIQEVIGQYAQAAKNAIEAGFDGVEIHGAHGYLIDQFTYEFANKRTDKYGGNLKQRLT 232
\*\* : : : \* \*\* \*\*\*\*\* :\*:\*:\*\*\*\*\*:\*\*\* :\*:\* \*:\*:\*\*\*:\*\*\*

BL21 LVLEVVDAGIEEWGADRIGIRVSPIGTFQNTDNGPNEEADALYLIEQLGKRGIAYLHMSE 273
KT2440 IVLEAVDAAIANWSADRVGIRVFPPLGGFNGVDNGEDQEAGGLYLIRELAKRNLAYLHLSE 270
Bacillus FMKEVTAAVIEAVGANKTLLRFSAFKGDNPYMWENPEFAIETFINMFTEVGLTMIHPST 292
: : \*.. \* \* .\*: : \* . : : . : \* : \* . : : : : \* \*

BL21 PDWAGGEPYTDAFREKVRARFHGPIIGAGAYTVEKAETLIGKGLIDAVAFGRDWIANPDL 333

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KT2440      PDWAGGKPLRDEFRQAIRAAYPGVIIAAGAYTAEKGEDLIGRGLIDAVAFGRAYIANPDL 330
Bacillus    MNYTQEIADGKNFHQLVRKYWDGTIVGVGNLNPKEAEEALQEGTIDVAAFGRPLIANPDF 352
           ::: . . *:: :* : * *..* . :..* : .* **..***** *****:

BL21        VARLQRKADLNPQRAESFYGGGAEGYTDYPTL--- 365
KT2440      VERLRLQAPLNEHRAKFDYANGPEGYTDYPFLKQA 365
Bacillus    VHRIKHAESLVEYDAKEHLATLV----- 375
           * *:: * *: .

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CLUSTAL 2.1 multiple sequence alignment

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BL21        -----ATGT----- 4
KT2440      GGGCATGCGTCCACGTCGGGCTCAATGACGTCGCGCATTATGTGAGTGGGCGGCTGGCA 60
Bacillus    -----ATGACAAGTTCAAGTAATAAAGCAGCGAGTATTTATGAAGGA----- 42
                                   * *

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BL21        --CATCTG-AAAACTGTA-----TTCCCA----- 27
KT2440      CGCAGCTG-ACGCATTGGGGAGCCCTGAGCCGGGCTCCCGTCCCCCTTTCCCAATCA 119
Bacillus    -ACAAATGTAAATGCTGGGGGA-----TTTTTTGAAA 74
           ** ** * ** **

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BL21        -----
KT2440      CCCCGGTATCGTGCGCACCATTGGTTTTCCACCTCTGGAGCTGCAATGAAACTCTTGCAA 179
Bacillus    CGTTGAA-----GAAACAAAGTTGTTTGAT 99

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BL21        ---CTGAAAGTGGG--CGCGATCACGGCGGCAAACCGTATTTTATGGCACCCTGACGC 82
KT2440      CCGCTGCAAATCGG--CCCACTCACCCCTGCCCAACCGCGTATTCATGGCCCCCTCACC 237
Bacillus    CCAATTCAAATGGAGCTTGGTCTCTTCG--TAATCGTATAGCGATGGCACCAGTACAA 157
           * ** * * * * * * * * * * * * * * * *

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BL21        G---TCTGCGCAGTATTGAACCGGGTGACATTCCTACCCCGTTGATGGCGGAATACTAT 138
KT2440      G---CCTGCGCAGCCTGGAGCCGGGTGATGTACCCACCGCGTATGGCCGAGTACTAC 293
Bacillus    GATGCTTTGCAGATAATGAACTGGA----GTAGTTGGTGCGGATGTAGTGGAGTACTAC 213
           * ** * * * * * * * * * * * * * * * *

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BL21        CGCCAACGTGCCA-----GTGCCGTTTGTATTATTAGTGAAGCCACGCAAATTTCTGCC 192
KT2440      CGTCAGCGTGCCA-----GTGCTGGCCTGATCATCACTGAAGCCACACAAATCTCCTTC 347
Bacillus    AGAAAACGTGCTGCGGATGGAATGGACTAATATTACAGAAGGAATTGTCATTAGTCCG 273
           * * * * * * * * * * * * * * * *

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BL21        CAGGCAAAGGATATGCAGGTGCGCCTGGCATCCATAGTCCGGAGCAAATGCCGCATGG 252
KT2440      CAGGCAAAGGGCTATTCGGGCTGCCCCGGCATTACAGCGCCGAACAGATCGTGCCTGG 407
Bacillus    AGAGCAAAGGAAACCCAGGAGTACCAGGTATTTATACACAAGAACAATCGATTCTTGG 333
           ** ** * * * * * * * * * * * * * * * *

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BL21        AAAAAATCACCGCTGGCGTTTCATGCTGAAAATGGTCATATGGCCGTGCAGCTGTGGCAC 312
KT2440      AAGCACATCAACGAAGGCATCCATGCCGAGGGCGGCCACAGCGCCGTGCAGGTGTGGCAC 467
Bacillus    AAACCTGTTACAGAGGCAGTACATAAAGAAGGCGGGACGATATTGCTCAAATATGGCAT 393
           ** * * * * * * * * * * * * * * * *

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BL21        ACCGGACGCATTTCTCACGCCAGCTGCAAC--CTGGCGGTCAGGCACCGGTAGCGCCTT 370
KT2440      ACCGGGCGTGTGTCGATACCTCCCTGCAAC--CTGGCGGCAAGGCACCCGTGGCCCTT 525
Bacillus    GTTGGGCGTATGAGTCAT-----CATGAAATAATTGGTGGTCAAATGCCACAAGCACCGT 448
           ** ** * ** * * * * * * * * * * * *

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BL21        CAGCACTTAGCGCGG--GAACACGTAFTTC-TCTGCGCGATGAAAATGGTCAGGCGATCC 427
KT2440      CGGCACTGCCGGCAG--ATGCACGCACCAC-CCTGCGTGACGCGCAAGGCAACCTGACAC 582
Bacillus    CGGCTATCGCTGCAGAGGGAAATGTTCCACGCTTTCGTAA-----ACCGTTTGATAC 500

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KT2440      AGGCGCCCGTTTTTTTGTGTGCGCCAGGCATGGCGCGTTGCGCGTAAGCGCAACCCGCTTG 1343
Bacillus    AG-----TTGAATATGACGCGAAAG----- 1105
            *                               * *          ****  ***

BL21        GCTATA----CCGATT-----ACCCGAC----- 1091
KT2440      GTTGTGGTGGCCAAGTGGGTGACTTGGAGGTGAAAGTCCTCTACACACCCGGCAAGGGGA 1403
Bacillus    -----AGC-----ATCTTGCTA----- 1117
            *                               * *          *

BL21        ---GTT-----GTAA----- 1098
KT2440      AGTGTTAGCCGGAGGCAAGGGTGC 1428
Bacillus    --CGTTA-----GTATGA----- 1128
            ***           * *

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PRIMERS:

1) nemA\_gen (F)

ATGGCACCGCTGACCC

2) nemA\_gen (R)

AGGTCNGGTTNGCAAT

3) nemA\_BL21 (F)

TCATCTGAAAACTGTATTCCCC

4) nemA\_BL21 (R)

AACGTCGGGTAATCGGTATAG

5) nemA\_BL21\_int (F)

CACCGCTGGCGTTCATGCT

6) nemA\_BL21\_int (R)

GCATCGGCTTCTTCATTCG