**Human Mitochondrial Malate Dehydrogenase 2 (hMDH2)** (sequence and structure) was purchased from Addgene. <http://www.addgene.org/38792/> Cloned in using LIC method. T7 5’ sequencing primer and 3’ sequencing primer = T7-term

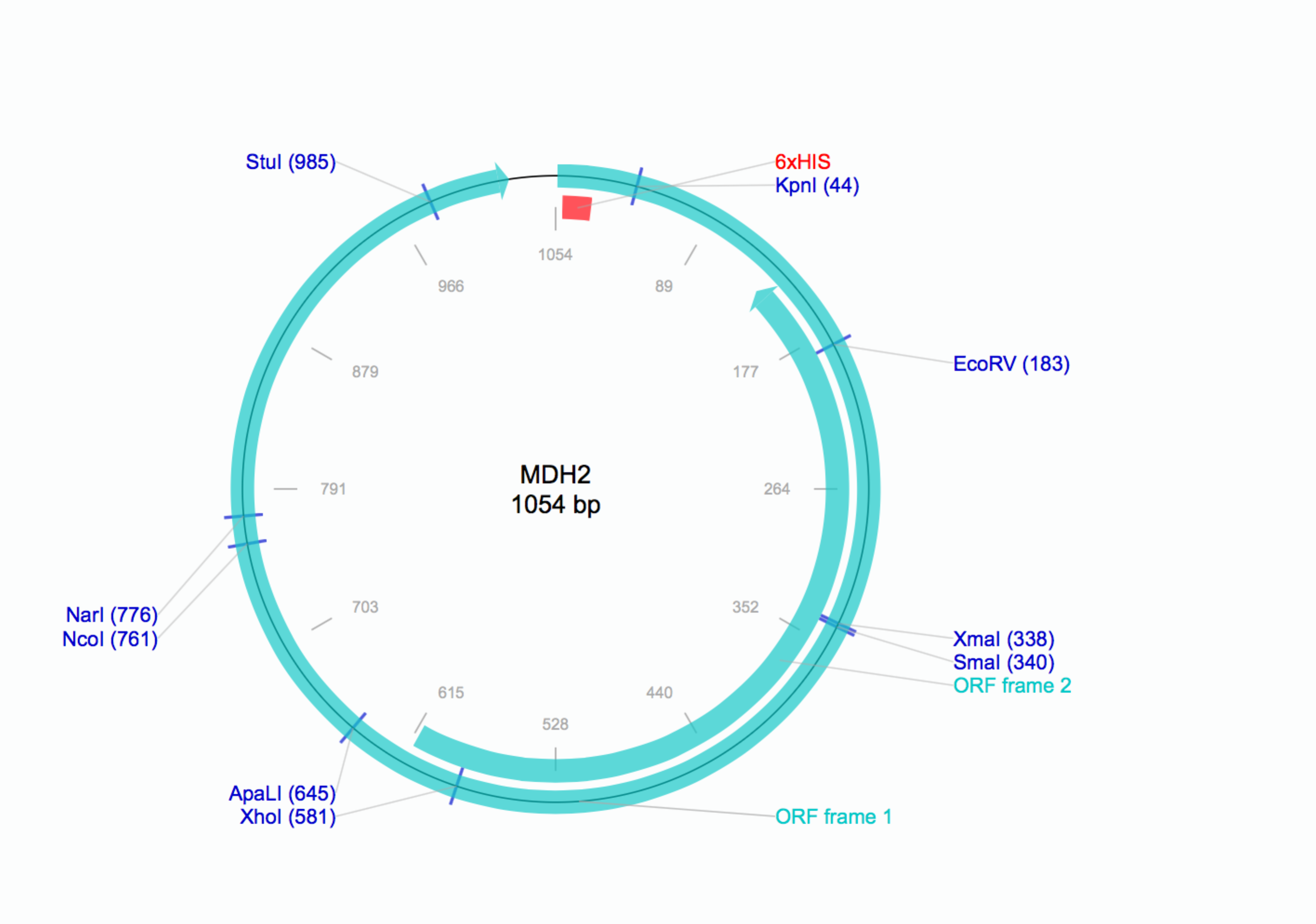
Vector Backbone – pNIC28-Bsa4 (7284 bp) Kan resistance. (this is a customized pET vector). Low Copy Size with insert = 8,388 bp

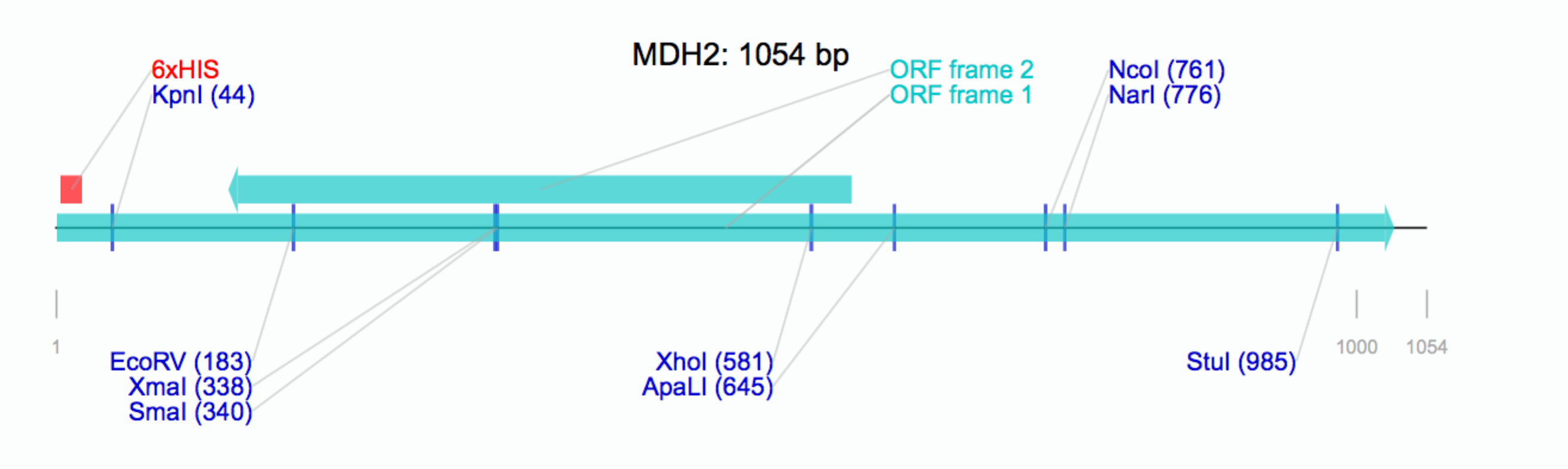
MDH cannot tolerate N term tags – blocks dimerizing and activity. This needs to be cut out and moved into a new vector. The SGC link shows expression and purification but no description of activity. The N term was not resolved in the crystal structure…

- Gene Entrez Report (https://www.ncbi.nlm.nih.gov/gene/4191)

- Insert size (1054 bp) - PDB Code 2DFD Entry Clone MDH2A-s001 ( gi|21735621)

- This construct was used to get a structure of MDH2 (http://www.thesgc.org/structures/2DFD/)





Insert Sequence

>MDH2 sequence 1054 bps

ATGCACCATCATCATCATCATTCTTCTGGTGTAGATCTGGGTACCGAGAACCTGTACTTCCAATCCATGT

CGGCCCAGAACAATGCTAAAGTAGCTGTGCTAGGGGCCTCTGGAGGCATCGGGCAGCCACTTTCACTTCT

CCTGAAGAACAGCCCCTTGGTGAGCCGCCTGACCCTCTATGATATCGCGCACACACCCGGAGTGGCCGCA

GATCTGAGCCACATCGAGACCAAAGCCGCTGTGAAAGGCTACCTCGGACCTGAACAGCTGCCTGACTGCC

TGAAAGGTTGTGATGTGGTAGTTATTCCGGCTGGAGTCCCCAGAAAGCCAGGCATGACCCGGGACGACCT

GTTCAACACCAATGCCACGATTGTGGCCACCCTGACCGCTGCCTGTGCCCAGCACTGCCCGGAAGCCATG

ATCTGCGTCATTGCCAATCCGGTTAATTCCACCATCCCCATCACAGCAGAAGTTTTCAAGAAGCATGGAG

TGTACAACCCCAACAAAATCTTCGGCGTGACGACCCTGGACATCGTCAGAGCCAACACCTTTGTTGCAGA

GCTGAAGGGTTTGGATCCAGCTCGAGTCAACGTCCCTGTCATTGGTGGCCATGCTGGGAAGACCATCATC

CCCCTGATCTCTCAGTGCACCCCCAAGGTGGACTTTCCCCAGGACCAGCTGACAGCACTCACTGGGCGGA

TCCAGGAGGCCGGCACGGAGGTGGTCAAGGCTAAAGCCGGAGCAGGCTCTGCCACCCTCTCCATGGCGTA

TGCCGGCGCCCGCTTTGTCTTCTCCCTTGTGGATGCAATGAATGGAAAGGAAGGTGTTGTGGAATGTTCC

TTCGTTAAGTCACAGGAAACGGAATGTACCTACTTCTCCACACCGCTGCTGCTTGGGAAAAAGGGCATCG

AGAAGAACCTGGGCATCGGCAAAGTCTCCTCTTTTGAGGAGAAGATGATCTCGGATGCCATCCCCGAGCT

GAAGGCCTCCATCAAGAAGGGGGAAGATTTCGTGAAGACCCTGAAGTAACAGTAAAGGTGGATACGGATC

CGAA

ORF

|  |  |  |
| --- | --- | --- |
| 1  51  101  151  201  251  301 | MHHHHHHSSG VDLGTENLYF QSMSAQNNAK VAVLGASGGI GQPLSLLLKN  SPLVSRLTLY DIAHTPGVAA DLSHIETKAA VKGYLGPEQL PDCLKGCDVV  VIPAGVPRKP GMTRDDLFNT NATIVATLTA ACAQHCPEAM ICVIANPVNS  TIPITAEVFK KHGVYNPNKI FGVTTLDIVR ANTFVAELKG LDPARVNVPV  IGGHAGKTII PLISQCTPKV DFPQDQLTAL TGRIQEAGTE VVKAKAGAGS  ATLSMAYAGA RFVFSLVDAM NGKEGVVECS FVKSQETECT YFSTPLLLGK  KGIEKNLGIG KVSSFEEKMI SDAIPELKAS IKKGEDFVKT LK\* | 50  100  150  200  250  300 |

**Human Citrate Synthase hCS (Accession# NM-004077)** Human Citrate synthase gene was PCR amplified from human brain cDNA pool (purchased from Biochain Inc.). Amplified cDNA was re-amplified with added restriction sites (NcoI at 5’ and XhoI at 3’) and sub-cloned into pET28a expression vector.

**Protein Sequence**

10 20 30 40 50 60   
MALLTAAARL LGTKNASCLV LAARHASASS TNLKDILADL IPKEQARIKT FRQQHGKTVV   
  
 70 80 90 100 110 120   
GQITVDMMYG GMRGMKGLVY ETSVLDPDEG IRFRGFSIPE CQKLLPKAKG GEEPLPEGLF   
  
 130 140 150 160 170 180   
WLLVTGHIPT EEQVSWLSKE WAKRAALPSH VVTMLDNFPT NLHPMSQLSA AVTALNSESN   
  
 190 200 210 220 230 240   
FARAYAQGIS RTKYWELIYE DSMDLIAKLP CVAAKIYRNL YREGSGIGAI DSNLDWSHNF   
  
 250 260 270 280 290 300   
TNMLGYTDHQ FTELTRLYLT IHSDHEGGNV SAHTSHLVGS ALSDPYLSFA AAMNGLAGPL   
  
 310 320 330 340 350 360   
HGLANQEVLV WLTQLQKEVG KDVSDEKLRD YIWNTLNSGR VVPGYGHAVL RKTDPRYTCQ   
  
 370 380 390 400 410 420   
REFALKHLPN DPMFKLVAQL YKIVPNVLLE QGKAKNPWPN VDAHSGVLLQ YYGMTEMNYY   
  
 430 440 450 460 470   
TVLFGVSRAL GVLAQLIWSR ALGFPLERPK SMSTEGLMKF VDSKSGLEHH HHHH

Theoretical pI/Mw: 8.18 / 52777.56  Blue letters indicates added amino acids as result of sub-cloning into pET28a vector.

cDNA Sequence:

CCATGGCTTTACTTACTGCGGCCGCCCGGCTCTTGGGAACCAAGAATGCATCTTGTCTTGTTCTTGCAGCCCGGCATGCCAGTGCTTCCTCCACGAATTTGAAAGACATATTGGCTGACCTGATACCTAAGGAGCAGGCCAGAATTAAGACTTTCAGGCAGCAACATGGCAAGACGGTGGTGGGCCAAATCACTGTGGACATGATGTATGGTGGCATGAGAGGCATGAAGGGATTGGTCTATGAAACATCAGTTCTTGATCCTGATGAGGGCATCCGTTTCCGAGGCTTTAGTATCCCTGAATGCCAGAAACTGCTACCCAAGGCTAAGGGTGGGGAAGAACCCCTGCCTGAGGGCTTATTTTGGCTGCTGGTAACTGGACATATCCCAACAGAGGAACAGGTATCTTGGCTCTCAAAAGAGTGGGCAAAGAGGGCAGCTCTGCCTTCCCATGTGGTCACCATGCTGGACAACTTTCCCACCAATCTACACCCCATGTCTCAGCTCAGTGCAGCTGTTACAGCCCTCAACAGTGAAAGTAACTTTGCCCGAGCATATGCACAGGGTATCAGCCGAACCAAGTACTGGGAGTTGATTTATGAAGACTCTATGGATCTAATCGCAAAGCTACCTTGTGTTGCAGCAAAGATCTACCGAAATCTCTACAGAGAAGGCAGCGGTATTGGGGCCATTGACTCTAACCTGGACTGGTCTCACAATTTCACCAACATGTTAGGCTATACTGATCATCAGTTCACTGAGCTCACGCGCCTGTACCTCACCATCCACAGTGACCATGAGGGTGGCAATGTAAGTGCCCATACCAGCCATTTGGTGGGCAGTGCCCTTTCCGACCCTTACCTGTCCTTTGCAGCAGCCATGAACGGGCTGGCAGGGCCTCTCCATGGACTGGCAAATCAGGAAGTGCTTGTCTGGCTAACACAGCTGCAGAAGGAAGTTGGCAAAGATGTGTCAGATGAGAAGTTACGAGACTACATCTGGAACACACTCAACTCAGGACGGGTTGTTCCAGGCTATGGCCATGCAGTACTAAGGAAGACTGATCCGCGATATACCTGTCAGCGAGAGTTTGCTCTGAAACACCTGCCTAATGACCCCATGTTTAAGTTGGTTGCTCAGCTGTACAAGATTGTGCCCAATGTCCTCTTAGAGCAGGGTAAAGCCAAGAATCCTTGGCCCAATGTAGATGCTCACAGTGGGGTGCTGCTCCAGTATTATGGCATGACGGAGATGAATTACTACACGGTCCTGTTTGGGGTGTCACGAGCATTGGGTGTACTGGCACAGCTCATCTGGAGCCGAGCCTTAGGCTTCCCTCTAGAAAGGCCCAAGTCCATGAGCACAGAGGGTCTGATGAAGTTTGTGGACTCTAAGTCAGGGCTCGAGCACCACCACCACCACCACTGA

Blue letters indicates added amino acids as result of sub-cloning into pET28a vector.

Map of Citrate Synthase in pET28a Vector.



PCR amplified gene was cloned into **NcoI,XhoI** digested **pET28a(+)**.

Open reading frame orientation as illustrated. ***Not all unique restriction sites are shown in the map. Extra nucleotides or unique restrictionsites may be found on both ends of your gene for subcloning purpose.***

**Seq:**

LOCUS GS55461-1 pET28a(+)-CS 6631 bp DNA circular SYN 18-FEB-2014

DEFINITION Ligation of inverted CS into pET28a

ACCESSION GS55461-1 pET28a(+)-CS

KEYWORDS .

SOURCE Unknown.

ORGANISM Unknown

Unclassified.

REFERENCE 1 (bases 1 to 6631)

AUTHORS Self

JOURNAL Unpublished.

COMMENT SECID/File created by SciEd Central, Scientific & Educational Software

COMMENT SECNOTES|Vector molecule: pET28a

Fragment ends: NcoI and XhoI

Fragment size: 5231

Insert molecule: CS

Fragment ends: NcoI and XhoI

Fragment size: 1400

FEATURES Location/Qualifiers

CDS complement (158..1563)

/gene="CS"

/SECDrawAs="Gene"

CDS complement (1632..1650)

/gene="T7 promoter"

/SECDrawAs="Gene"

CDS 2035..3114

/gene="LacI"

/SECDrawAs="Gene"

CDS 5257..6069

/gene="KanR"

/SECDrawAs="Gene"

CDS complement (6165..6620)

/gene="f1 origin"

/SECDrawAs="Gene"

ORIGIN

1 atccggatat agttcctcct ttcagcaaaa aacccctcaa gacccgttta gaggccccaa

61 ggggttatgc tagttattgc tcagcggtgg cagcagccaa ctcagcttcc tttcgggctt

121 tgttagcagc cggatctcag tggtggtggt ggtggtgctc gagccctgac ttagagtcca

181 caaacttcat cagaccctct gtgctcatgg acttgggcct ttctagaggg aagcctaagg

241 ctcggctcca gatgagctgt gccagtacac ccaatgctcg tgacacccca aacaggaccg

301 tgtagtaatt catctccgtc atgccataat actggagcag caccccactg tgagcatcta

361 cattgggcca aggattcttg gctttaccct gctctaagag gacattgggc acaatcttgt

421 acagctgagc aaccaactta aacatggggt cattaggcag gtgtttcaga gcaaactctc

481 gctgacaggt atatcgcgga tcagtcttcc ttagtactgc atggccatag cctggaacaa

541 cccgtcctga gttgagtgtg ttccagatgt agtctcgtaa cttctcatct gacacatctt

601 tgccaacttc cttctgcagc tgtgttagcc agacaagcac ttcctgattt gccagtccat

661 ggagaggccc tgccagcccg ttcatggctg ctgcaaagga caggtaaggg tcggaaaggg

721 cactgcccac caaatggctg gtatgggcac ttacattgcc accctcatgg tcactgtgga

781 tggtgaggta caggcgcgtg agctcagtga actgatgatc agtatagcct aacatgttgg

841 tgaaattgtg agaccagtcc aggttagagt caatggcccc aataccgctg ccttctctgt

901 agagatttcg gtagatcttt gctgcaacac aaggtagctt tgcgattaga tccatagagt

961 cttcataaat caactcccag tacttggttc ggctgatacc ctgtgcatat gctcgggcaa

1021 agttactttc actgttgagg gctgtaacag ctgcactgag ctgagacatg gggtgtagat

1081 tggtgggaaa gttgtccagc atggtgacca catgggaagg cagagctgcc ctctttgccc

1141 actcttttga gagccaagat acctgttcct ctgttgggat atgtccagtt accagcagcc

1201 aaaataagcc ctcaggcagg ggttcttccc cacccttagc cttgggtagc agtttctggc

1261 attcagggat actaaagcct cggaaacgga tgccctcatc aggatcaaga actgatgttt

1321 catagaccaa tcccttcatg cctctcatgc caccatacat catgtccaca gtgatttggc

1381 ccaccaccgt cttgccatgt tgctgcctga aagtcttaat tctggcctgc tccttaggta

1441 tcaggtcagc caatatgtct ttcaaattcg tggaggaagc actggcatgc cgggctgcaa

1501 gaacaagaca agatgcattc ttggttccca agagccgggc ggccgcagta agtaaagcca

1561 tggtatatct ccttcttaaa gttaaacaaa attatttcta gaggggaatt gttatccgct

1621 cacaattccc ctatagtgag tcgtattaat ttcgcgggat cgagatctcg atcctctacg

1681 ccggacgcat cgtggccggc atcaccggcg ccacaggtgc ggttgctggc gcctatatcg

1741 ccgacatcac cgatggggaa gatcgggctc gccacttcgg gctcatgagc gcttgtttcg

1801 gcgtgggtat ggtggcaggc cccgtggccg ggggactgtt gggcgccatc tccttgcatg

1861 caccattcct tgcggcggcg gtgctcaacg gcctcaacct actactgggc tgcttcctaa

1921 tgcaggagtc gcataaggga gagcgtcgag atcccggaca ccatcgaatg gcgcaaaacc

1981 tttcgcggta tggcatgata gcgcccggaa gagagtcaat tcagggtggt gaatgtgaaa

2041 ccagtaacgt tatacgatgt cgcagagtat gccggtgtct cttatcagac cgtttcccgc

2101 gtggtgaacc aggccagcca cgtttctgcg aaaacgcggg aaaaagtgga agcggcgatg

2161 gcggagctga attacattcc caaccgcgtg gcacaacaac tggcgggcaa acagtcgttg

2221 ctgattggcg ttgccacctc cagtctggcc ctgcacgcgc cgtcgcaaat tgtcgcggcg

2281 attaaatctc gcgccgatca actgggtgcc agcgtggtgg tgtcgatggt agaacgaagc

2341 ggcgtcgaag cctgtaaagc ggcggtgcac aatcttctcg cgcaacgcgt cagtgggctg

2401 atcattaact atccgctgga tgaccaggat gccattgctg tggaagctgc ctgcactaat

2461 gttccggcgt tatttcttga tgtctctgac cagacaccca tcaacagtat tattttctcc

2521 catgaagacg gtacgcgact gggcgtggag catctggtcg cattgggtca ccagcaaatc

2581 gcgctgttag cgggcccatt aagttctgtc tcggcgcgtc tgcgtctggc tggctggcat

2641 aaatatctca ctcgcaatca aattcagccg atagcggaac gggaaggcga ctggagtgcc

2701 atgtccggtt ttcaacaaac catgcaaatg ctgaatgagg gcatcgttcc cactgcgatg

2761 ctggttgcca acgatcagat ggcgctgggc gcaatgcgcg ccattaccga gtccgggctg

2821 cgcgttggtg cggatatctc ggtagtggga tacgacgata ccgaagacag ctcatgttat

2881 atcccgccgt taaccaccat caaacaggat tttcgcctgc tggggcaaac cagcgtggac

2941 cgcttgctgc aactctctca gggccaggcg gtgaagggca atcagctgtt gcccgtctca

3001 ctggtgaaaa gaaaaaccac cctggcgccc aatacgcaaa ccgcctctcc ccgcgcgttg

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3121 caacgcaatt aatgtaagtt agctcactca ttaggcaccg ggatctcgac cgatgccctt

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3301 cattttcggc gaggaccgct ttcgctggag cgcgacgatg atcggcctgt cgcttgcggt

3361 attcggaatc ttgcacgccc tcgctcaagc cttcgtcact ggtcccgcca ccaaacgttt

3421 cggcgagaag caggccatta tcgccggcat ggcggcccca cgggtgcgca tgatcgtgct

3481 cctgtcgttg aggacccggc taggctggcg gggttgcctt actggttagc agaatgaatc

3541 accgatacgc gagcgaacgt gaagcgactg ctgctgcaaa acgtctgcga cctgagcaac

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3661 ctgcaccatt atgttccgga tctgcatcgc aggatgctgc tggctaccct gtggaacacc

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3901 aaatccccct tacacggagg catcagtgac caaacaggaa aaaaccgccc ttaacatggc

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4141 cacagcttgt ctgtaagcgg atgccgggag cagacaagcc cgtcagggcg cgtcagcggg

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4501 gccagcaaaa ggccaggaac cgtaaaaagg ccgcgttgct ggcgtttttc cataggctcc

4561 gcccccctga cgagcatcac aaaaatcgac gctcaagtca gaggtggcga aacccgacag

4621 gactataaag ataccaggcg tttccccctg gaagctccct cgtgcgctct cctgttccga

4681 ccctgccgct taccggatac ctgtccgcct ttctcccttc gggaagcgtg gcgctttctc

4741 atagctcacg ctgtaggtat ctcagttcgg tgtaggtcgt tcgctccaag ctgggctgtg

4801 tgcacgaacc ccccgttcag cccgaccgct gcgccttatc cggtaactat cgtcttgagt

4861 ccaacccggt aagacacgac ttatcgccac tggcagcagc cactggtaac aggattagca

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4981 ctagaaggac agtatttggt atctgcgctc tgctgaagcc agttaccttc ggaaaaagag

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5761 gagcgtaatg gctggcctgt tgaacaagtc tggaaagaaa tgcataaact tttgccattc

5821 tcaccggatt cagtcgtcac tcatggtgat ttctcacttg ataaccttat ttttgacgag

5881 gggaaattaa taggttgtat tgatgttgga cgagtcggaa tcgcagaccg ataccaggat

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6421 gtcgaggtgc cgtaaagcac taaatcggaa ccctaaaggg agcccccgat ttagagcttg

6481 acggggaaag ccggcgaacg tggcgagaaa ggaagggaag aaagcgaaag gagcgggcgc

6541 tagggcgctg gcaagtgtag cggtcacgct gcgcgtaacc accacacccg ccgcgcttaa

6601 tgcgccgcta cagggcgcgt cccattcgcc a

//

**Human Malate Dehydrogenase1 (Accession# NM-001199111)** Human Malate dehydrogenase 1 gene was PCR amplified from human brain cDNA pool (purchased from Biochain Inc.). Amplified cDNA was re-amplified with added restriction sites (NcoI at 5’ and XhoI at 3’) and sub-cloned into pET28a expression vector.

**Protein Sequence**

10 20 30 40 50 60   
MGRRCSYFPK DVTVFDKDDK SEPIRVLVTG AAGQIAYSLL YSIGNGSVFG KDQPIILVLL   
  
 70 80 90 100 110 120   
DITPMMGVLD GVLMELQDCA LPLLKDVIAT DKEDVAFKDL DVAILVGSMP RREGMERKDL   
  
 130 140 150 160 170 180   
LKANVKIFKS QGAALDKYAK KSVKVIVVGN PANTNCLTAS KSAPSIPKEN FSCLTRLDHN   
  
 190 200 210 220 230 240   
RAKAQIALKL GVTANDVKNV IIWGNHSSTQ YPDVNHAKVK LQGKEVGVYE ALKDDSWLKG   
  
 250 260 270 280 290 300   
EFVTTVQQRG AAVIKARKLS SAMSAAKAIC DHVRDIWFGT PEGEFVSMGV ISDGNSYGVP   
  
 310 320 330 340 350 360   
DDLLYSFPVV IKNKTWKFVE GLPINDFSRE KMDLTAKELT EEKESAFEFL SSALEHHHHH

H

Theoretical pI/Mw: 6.80 / 39992.06

Blue letters indicates added amino acids as result of sub-cloning into pET28a vector.

cDNA Sequence:

CCATGggtCGACGCTGCAGCTATTTTCCAAAGGACGTTACGGTGTTTGATAAGGACGATAAGTCTGAACCAATCAGAGTCCTTGTGACTGGAGCAGCTGGTCAAATTGCATATTCACTGCTGTACAGTATTGGAAATGGATCTGTCTTTGGTAAAGATCAGCCTATAATTCTTGTGCTGTTGGATATCACCCCCATGATGGGTGTCCTGGACGGTGTCCTAATGGAACTGCAAGACTGTGCCCTTCCCCTCCTGAAAGATGTCATCGCAACAGATAAAGAAGACGTTGCCTTCAAAGACCTGGATGTGGCCATTCTTGTGGGCTCCATGCCAAGAAGGGAAGGCATGGAGAGAAAAGATTTACTGAAAGCAAATGTGAAAATCTTCAAATCCCAGGGTGCAGCCTTAGATAAATACGCCAAGAAGTCAGTTAAGGTTATTGTTGTGGGTAATCCAGCCAATACCAACTGCCTGACTGCTTCCAAGTCAGCTCCATCCATCCCCAAGGAGAACTTCAGTTGCTTGACTCGTTTGGATCACAACCGAGCTAAAGCTCAAATTGCTCTTAAACTTGGTGTGACTGCTAATGATGTAAAGAATG

TCATTATCTGGGGAAACCATTCCTCGACTCAGTATCCAGATGTCAACCATGCCAAGGTGAAATTGCAAGGAAAGGAAGTTGGTGTTTATGAAGCTCTGAAAGATGACAGCTGGCTCAAGGGAGAATTTGTCACGACTGTGCAGCAGCGTGGCGCTGCTGTCATCAAGGCTCGAAAACTATCCAGTGCCATGTCTGCTGCAAAAGCCATCTGTGACCACGTCAGGGACATCTGGTTTGGAACCCCAGAGGGAGAGTTTGTGTCCATGGGTGTTATCTCTGATGGCAACTCCTATGGTGTTCCTGATGATCTGCTCTACTCATTCCCTGTTGTAATCAAGAATAAGACCTGGAAGTTTGTTGAAGGTCTCCCTATTAATGATTTCTCACGTGAGAAGATGGATCTTACTGCAAAGGAACTGACAGAAGAAAAAGAAAGTGCTTTTGAATTTCTTTCCTCTGCCCTCGAGCTCGAGCACCACCACCACCACCACTGA

Blue letters indicates added amino acids as result of sub-cloning into pET28a vector.

PCR amplified gene was cloned into **NcoI,XhoI** digested **pET28a(+)**.

Open reading frame orientation as illustrated. ***Not all unique restriction sites are shown in the map. Extra nucleotides or unique restrictionsites may be found on both ends of your gene for subcloning purpose.***

LOCUS GS55461-2 pET28a(+)-MDH1 6292 bp DNA circular SYN 18-FEB-2014

DEFINITION Ligation of inverted MDH1 into pET28a

ACCESSION GS55461-2 pET28a(+)-MDH1

KEYWORDS .

SOURCE Unknown.

ORGANISM Unknown

Unclassified.

REFERENCE 1 (bases 1 to 6292)

AUTHORS Self

JOURNAL Unpublished.

COMMENT SECID/File created by SciEd Central, Scientific & Educational Software

COMMENT SECNOTES|Vector molecule: pET28a

Fragment ends: NcoI and XhoI

Fragment size: 5231

Insert molecule: MDH1

Fragment ends: NcoI and XhoI

Fragment size: 1061

FEATURES Location/Qualifiers

CDS complement (158..1224)

/gene="MDH1"

/SECDrawAs="Gene"

CDS complement (1293..1311)

/gene="T7 promoter"

/SECDrawAs="Gene"

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6241 caccacaccc gccgcgctta atgcgccgct acagggcgcg tcccattcgc ca

Map of MDH1 in pET28a Vector



PCR amplified gene was cloned into **NcoI,XhoI** digested **pET28a(+)**.

Open reading frame orientation as illustrated. ***Not all unique restriction sites are shown in the map. Extra nucleotides or unique restrictionsites may be found on both ends of your gene for subcloning purpose.***

**Seq:**

LOCUS GS55461-1 pET28a(+)-CS 6631 bp DNA circular SYN 18-FEB-2014

DEFINITION Ligation of inverted CS into pET28a

ACCESSION GS55461-1 pET28a(+)-CS

KEYWORDS .

SOURCE Unknown.

ORGANISM Unknown

Unclassified.

REFERENCE 1 (bases 1 to 6631)

AUTHORS Self

JOURNAL Unpublished.

COMMENT SECID/File created by SciEd Central, Scientific & Educational Software

COMMENT SECNOTES|Vector molecule: pET28a

Fragment ends: NcoI and XhoI

Fragment size: 5231

Insert molecule: CS

Fragment ends: NcoI and XhoI

Fragment size: 1400

FEATURES Location/Qualifiers

CDS complement (158..1563)

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CDS complement (1632..1650)

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6421 gtcgaggtgc cgtaaagcac taaatcggaa ccctaaaggg agcccccgat ttagagcttg

6481 acggggaaag ccggcgaacg tggcgagaaa ggaagggaag aaagcgaaag gagcgggcgc

6541 tagggcgctg gcaagtgtag cggtcacgct gcgcgtaacc accacacccg ccgcgcttaa

6601 tgcgccgcta cagggcgcgt cccattcgcc a

//

**Cp-MDH (Cryptosporidium parvum)** Purchased from Addgene (http://www.addgene.org/25584/).

Entrez Gene (<https://www.ncbi.nlm.nih.gov/gene/3371767>) cgd7\_470

Protozoan parasite (cryptosporidium parvum in the malaria family – divergent from toxoplasma and plasmodium parasites in biochemistry). of the intestinal track causing acute watery nonbloody diarrhea.

Purchased from Addgene. Plasmid p15TV-L – modified pET vector. Backbone = 7746 bp, insert 987, plasmid = 8433 bp

N terminal His tag. No information on activity. N term tag leaves MDH unable to dimerize and usually with low activity.

From SGC.org “Cryptosporidiosis is a protozoan disease that is rampant in undeveloped regions of the world.

Outbreaks in North American and Europe are infrequent and usually caused by contaminated water.

While individuals in good health will suffer a short period of diarrhea, immuno-compromised patients face serious risks including death. While the genome of *Cryptosporidium parvum* was sequenced in 2004, the organism responsible for cryptosporidiosis is not a popular topic of research and remains a mysterious member of the phylum of *Apicomplexa*, often divergent from *Plasmodium* and *Toxoplasma* parasites in its biochemical pathways.”

PDB 2HJR Entry clone cgd7-470 has structure with citric acid.

*From SGC.org* Cp-MDH was expressed in E. coli BL21-(DE3)-R3 cells in Terrific Broth (TB) in the presence of kanamycin/chloramphenicol (50 µg/mL and 25 µg/mL respectively). A single colony was inoculated into 10 mL of LB with of kanamycin/chloramphenicol (50 µg/mL and 25 µg/mL respectively) in a 125 mL flask and incubated with shaking at 250 rpm overnight at 37 ºC. The culture was transferred into 100 mL of TB with 50 µg/mL kanamycin in a 250 mL shaking flask and incubated at 37 ºC for 3 hours. The culture was transferred into 2 X 1.8 L TB with 50 µg/mL kanamycin and 0.3 mL of antifoam (Sigma) in 2 L bottles and cultured using the LEX system to an OD600 of 2.5. The culture was cooled to 15 ºC, and isopropyl-1-thio-D-galactopyranoside (IPTG) was added to 0.4 mM, and the culture was incubated overnight at 15 ºC.

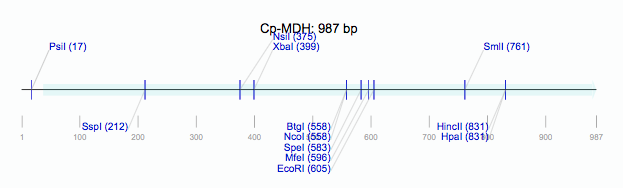
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Insert Sequence

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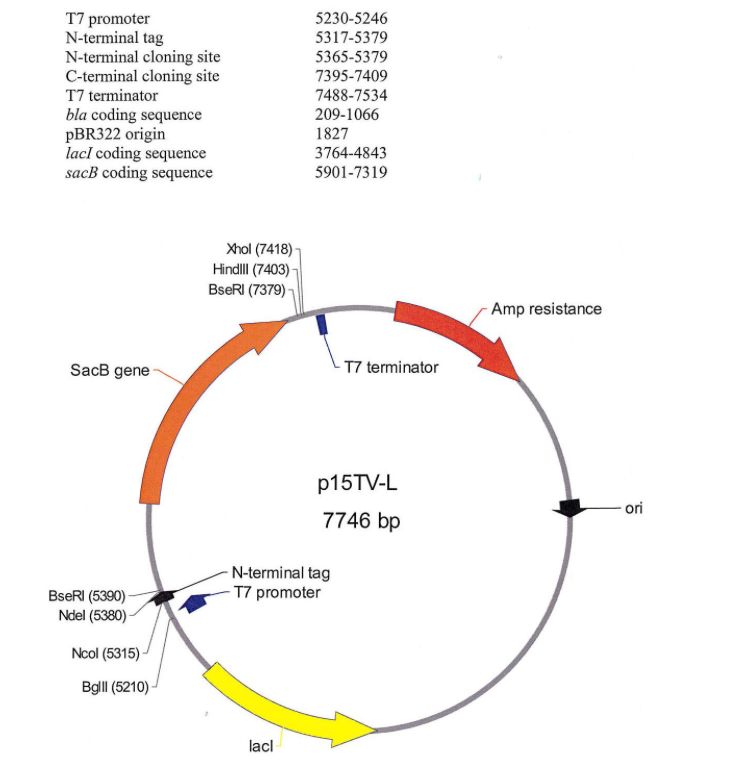
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ACGAAAAATCATTATTTTCTAAATCAGTTGAATCTATTCAAAACCTTGTCCAAGACTTAAAAAGCCTTAA

TCTTTAA



**Vector Information**

**Name**: p15Tv-L

**Source:** Created

**Company:**n/a

**Created by**: Jennifer Guthrie **Date**: Jan, 2005 **Backbone**: pET15b

**Size:**7740bp

**Antibiotic Resistance**: Ampicillin

**Cloning Site**: BseRI

**Screen clones with:** T7P & T7t primers

**His tag**: Yes

**Cleavage by**: TEV

**Promoter for expression**: T7

**Special Notes**:

To be used as a ligation independent cloning vector, 15bp addition to 5’end of primers

Fwd Primer: 5’-ttg tat ttc cag ggc-----3’

Rev Primer: 5’-caa gct tcg tca tca-----3’

Contains 2kb sacB gene between the BseRI cloning site

**Sequence:**

>fwd sequence of cloning site:

5’-tTCCCCTCTAgaaataaTTTtgtttaactTTAAGAAGGAGATaTaCCATGGGCAGCAGCCATCATCATCATCATCACAGCAGCGGCAGAGAAAACTTGTATTTCCAGGGCCATATGAGTTCTCCTCCTGAAAGATCCATAACTTCGTATAGCATACATTATACGAAGTTATGCGGCCGCGACGTCCACATATACCTGCCGTTCACTATTATTTAGTGAAATGAGATATTATGATATTTTCTGAATTGTGATTAAAAAGGCAACTTTATGCCCATGCAACAGAAACTATAAAAAATACAGAGAATGAAAAGAAACAGATAG

ATTTTTTAGTTCTTTAGGCCCGTAGTCTGCAAATCCTTTTATGATTTTCTATCAAACAAAAGAGGAAAATAGACCAGTTGCAATCCAAACGAGAGTCTAATAGAATGAGGTCGAAAAGTAAATCGCGCGGGTTTGTTACTGATAAAGCAGGCAAGACCTAAAATGTGTAAAGGGCAAAGTGTATACTTTGGCGTCACCCCTTACATATTTTAGGTCTTTTTTTATTGTGCGTAACTAACTTGCCATCTTCAAACAGGAGGGCTGGAAGAAGCAgACCGCTAACACAGTACATAAAAAAGGAGACATGAACGATGAACATCAAAAAGTTTGCAAAACAAGCAACAGTATTAACCTTTACTACCGCACTGCTGGCAgGaggCGCAACTCaAGCGTTTGcGAAAG

**Sequencing primer**: T7P

**Sequence:**

>reverse complement of sequence of cloning site

5’-ATCTTTATTTAACAAAGCATAcTATGgCAAAAGCACATCATTCTTCCGTCAAGAAAGTCAAAAACTTcTGCAAAGCGATAAAAAACGCACGGcTGAGTTAGCAAACGGCGCTCTCGGTATGATTGAGCTAAACGATGATTACACACTGAAAAAAGTGATGAAACCGCTGATTGCATCTAACACAGTAACAGATGAAATTGAACGCGCGAACGTCTTTAAAATGAACGGCAAATGGTACCTGTTCACTGACTCCCGCGGATCAAAAATGACGATTGACGGCATTACGTCTAACGATATTTACATGCTTGGTTATGTTTCTAATTCTTTAACTGGCCCATACAAGCCGCTGAACAAAACTGGCCTTGTGTTAAAAATGGATCTTGATCCTAACGATGTAACCTTTACTTACTCACACTTCGCTGTACCTCAAGCGAAAGGAAACAATGTCGTGATTACAAGCTATATGACAAACAGAGGATTCTACGCAGACAAACAATCAACGTTTGCGCCTAGCTTCCTGCTGAACATCAAAGGCAAGAAAACATCTGTTGTCAAAGACAGCATCCTTGAACAAGGACAATTAACAGTTAACAAATAAAAACGCAAAAGAAAATGCCGATATCCTATTGGCATTGACGTCAGGTGGCACTTTTCGAGGAGATCATGCACATGATGACGAAGCTTGCGGccgCACTCGAGGATCCGGcTgctaacAAAGcccgAAAGGAAGCtGA

**Sequencing primer**: T7t

**Proposed full sequence:**

>proposed sequence of p15Tv-L

5’-ttcttgaagacgaaagggcctcgtgatacgcctatttttataggttaatgtcatgataataatggtttcttagacgtcaggtggcacttttcggggaaatgtgcgcggaacccctatttgtttatttttctaaatacattcaaatatgtatccgctcatgagacaataaccctgataaatgcttcaataatattgaaaaaggaagagtatgagtattcaacatttccgtgtcgcccttattcccttttttgcggcattttgccttcctgtttttgctcacccagaaacgctggtgaaagtaaaagatgctgaagatcagttgggtgcacgagtgggttacatcgaactggatctcaacagcggtaagatccttgagagttttcgccccgaagaacgttttccaatgatgagcacttttaaagttctgctatgtggcgcggtattatcccgtgttgacgccgggcaagagcaactcggtcgccgcatacactattctcagaatgacttggttgagtactcaccagtcacagaaaagcatcttacggatggcatgacagtaagagaattatgcagtgctgccataaccatgagtgataacactgcggccaacttacttctgacaacgatcggaggaccgaaggagctaaccgcttttttgcacaacatgggggatcatgtaactcgccttgatcgttgggaaccggagctgaatgaagccataccaaacgacgagcgtgacaccacgatgcctgcagcaatggcaacaacgttgcgcaaactattaactggcgaactacttactctagcttcccggcaacaattaatagactggatggaggcggataaagttgcaggaccacttctgcgctcggcccttccggctggctggtttattgctgataaatctggagccggtgagcgtgggtctcgcggtatcattgcagcactggggccagatggtaagccctcccgtatcgtagttatctacacgacggggagtcaggcaactatggatgaacgaaatagacagatcgctgagataggtgcctcactgattaagcattggtaactgtcagaccaagtttactcatatatactttagattgatttaaaacttcatttttaatttaaaaggatctaggtgaagatcctttttgataatctcatgaccaaaatcccttaacgtgagttttcgttccactgagcgtcagaccccgtagaaaagatcaaaggatcttcttgagatcctttttttctgcgcgtaatctgctgcttgcaaacaaaaaaaccaccgctaccagcggtggtttgtttgccggatcaagagctaccaactctttttccgaaggtaactggcttcagcagagcgcagataccaaatactgtccttctagtgtagccgtagttaggccaccacttcaagaactctgtagcaccgcctacatacctcgctctgctaatcctgttaccagtggctgctgccagtggcgataagtcgtgtcttaccgggttggactcaagacgatagttaccggataaggcgcagcggtcgggctgaacggggggttcgtgcacacagcccagcttggagcgaacgacctacaccgaactgagatacctacagcgtgagctatgagaaagcgccacgcttcccgaagggagaaaggcggacaggtatccggtaagcggcagggtcggaacaggagagcgcacgagggagcttccagggggaaacgcctggtatctttatagtcctgtcgggtttcgccacctctgacttgagcgtcgatttttgtgatgctcgtcaggggggcggagcctatggaaaaacgccagcaacgcggcctttttacggttcctggccttttgctggccttttgctcacatgttctttcctgcgttatcccctgattctgtggataaccgtattaccgcctttgagtgagctgataccgctcgccgcagccgaacgaccgagcgcagcgagtcagtgagcgaggaagcggaagagcgcctgatgcggtattttctccttacgcatctgtgcggtatttcacaccgcatatatggtgcactctcagtacaatctgctctgatgccgcatagttaagccagtatacactccgctatcgctacgtgactgggtcatggctgcgccccgacacccgccaacacccgctgacgcgccctgacgggcttgtctgctcccggcatccgcttacagacaagctgtgaccgtctccgggagctgcatgtgtcagaggttttcaccgtcatcaccgaaacgcgcgaggcagctgcggtaaagctcatcagcgtggtcgtgaagcgattcacagatgtctgcctgttcatccgcgtccagctcgttgagtttctccagaagcgttaatgtctggcttctgataaagcgggccatgttaagggcggttttttcctgtttggtcactgatgcctccgtgtaagggggatttctgttcatgggggtaatgataccgatgaaacgagagaggatgctcacgatacgggttactgatgatgaacatgcccggttactggaacgttgtgagggtaaacaactggcggtatggatgcggcgggaccagagaaaaatcactcagggtcaatgccagcgcttcgttaatacagatgtaggtgttccacagggtagccagcagcatcctgcgatgcagatccggaacataatggtgcagggcgctgacttccgcgtttccagactttacgaaacacggaaaccgaagaccattcatgttgttgctcaggtcgcagacgttttgcagcagcagtcgcttcacgttcgctcgcgtatcggtgattcattctgctaaccagtaaggcaaccccgccagcctagccgggtcctcaacgacaggagcacgatcatgcgcacccgtggccaggacccaacgctgcccgagatgcgccgcgtgcggctgctggagatggcggacgcgatggatatgttctgccaagggttggtttgcgcattcacagttctccgcaagaattgattggctccaattcttggagtggtgaatccgttagcgaggtgccgccggcttccattcaggtcgaggtggcccggctccatgcaccgcgacgcaacgcggggaggcagacaaggtatagggcggcgcctacaatccatgccaacccgttccatgtgctcgccgaggcggcataaatcgccgtgacgatcagcggtccagtgatcgaagttaggctggtaagagccgcgagcgatccttgaagctgtccctgatggtcgtcatctacctgcctggacagcatggcctgcaacgcgggcatcccgatgccgccggaagcgagaagaatcataatggggaaggccatccagcctcgcgtcgcgaacgccagcaagacgtagcccagcgcgtcggccgccatgccggcgataatggcctgcttctcgccgaaacgtttggtggcgggaccagtgacgaaggcttgagcgagggcgtgcaagattccgaataccgcaagcgacaggccgatcatcgtcgcgctccagcgaaagcggtcctcgccgaaaatgacccagagcgctgccggcacctgtcctacgagttgcatgataaagaagacagtcataagtgcggcgacgatagtcatgccccgcgcccaccggaaggagctgactgggttgaaggctctcaagggcatcggtcgagatcccggtgcctaatgagtgagctaacttacattaattgcgttgcgctcactgcccgctttccagtcgggaaacctgtcgtgccagctgcattaatgaatcggccaacgcgcggggagaggcggtttgcgtattgggcgccagggtggtttttcttttcaccagtgagacgggcaacagctgattgcccttcaccgcctggccctgagagagttgcagcaagcggtccacgctggtttgccccagcaggcgaaaatcctgtttgatggtggttaacggcgggatataacatgagctgtcttcggtatcgtcgtatcccactaccgagatatccgcaccaacgcgcagcccggactcggtaatggcgcgcattgcgcccagcgccatctgatcgttggcaaccagcatcgcagtgggaacgatgccctcattcagcatttgcatggtttgttgaaaaccggacatggcactccagtcgccttcccgttccgctatcggctgaatttgattgcgagtgagatatttatgccagccagccagacgcagacgcgccgagacagaacttaatgggcccgctaacagcgcgatttgctggtgacccaatgcgaccagatgctccacgcccagtcgcgtaccgtcttcatgggagaaaataatactgttgatgggtgtctggtcagagacatcaagaaataacgccggaacattagtgcaggcagcttccacagcaatggcatcctggtcatccagcggatagttaatgatcagcccactgacgcgttgcgcgagaagattgtgcaccgccgctttacaggcttcgacgccgcttcgttctaccatcgacaccaccacgctggcacccagttgatcggcgcgagatttaatcgccgcgacaatttgcgacggcgcgtgcagggccagactggaggtggcaacgccaatcagcaacgactgtttgcccgccagttgttgtgccacgcggttgggaatgtaattcagctccgccatcgccgcttccactttttcccgcgttttcgcagaaacgtggctggcctggttcaccacgcgggaaacggtctgataagagacaccggcatactctgcgacatcgtataacgttactggtttcacattcaccaccctgaattgactctcttccgggcgctatcatgccataccgcgaaaggttttgcgccattcgatggtgtccgggatctcgacgctctcccttatgcgactcctgcattaggaagcagcccagtagtaggttgaggccgttgagcac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**Watermellon glycoxosomal MDH (gMDH or wgMDH)**

In pQE60 (Qiagen) vector – some question exists on a leader sequence or not. Working on sequencing the construct now… More coming.

His tag (no cleavage site) on C –term of MDH.

Glyoxysomal in germinating watermelon seedlings (Citrullus uulgaris).

CLONING: pQE60 (Quiagen) between the restriction sites Ncol and BgllI; the Ncol-site also provided the start codon. The necessary restriction sites at the 5'-end and 3'-end of the cDNA sequence were added by PCR. The mature subanits without presequence, gMDH and mMDH, including the Ncol- and Bglll-site were prepared by PCR and cloned into the same vector. Biochemica et Biophysica Acta 1274 (1996) 48-58. And FEBS Journal 272 (2005) 643-654. Structure and kinetics of the

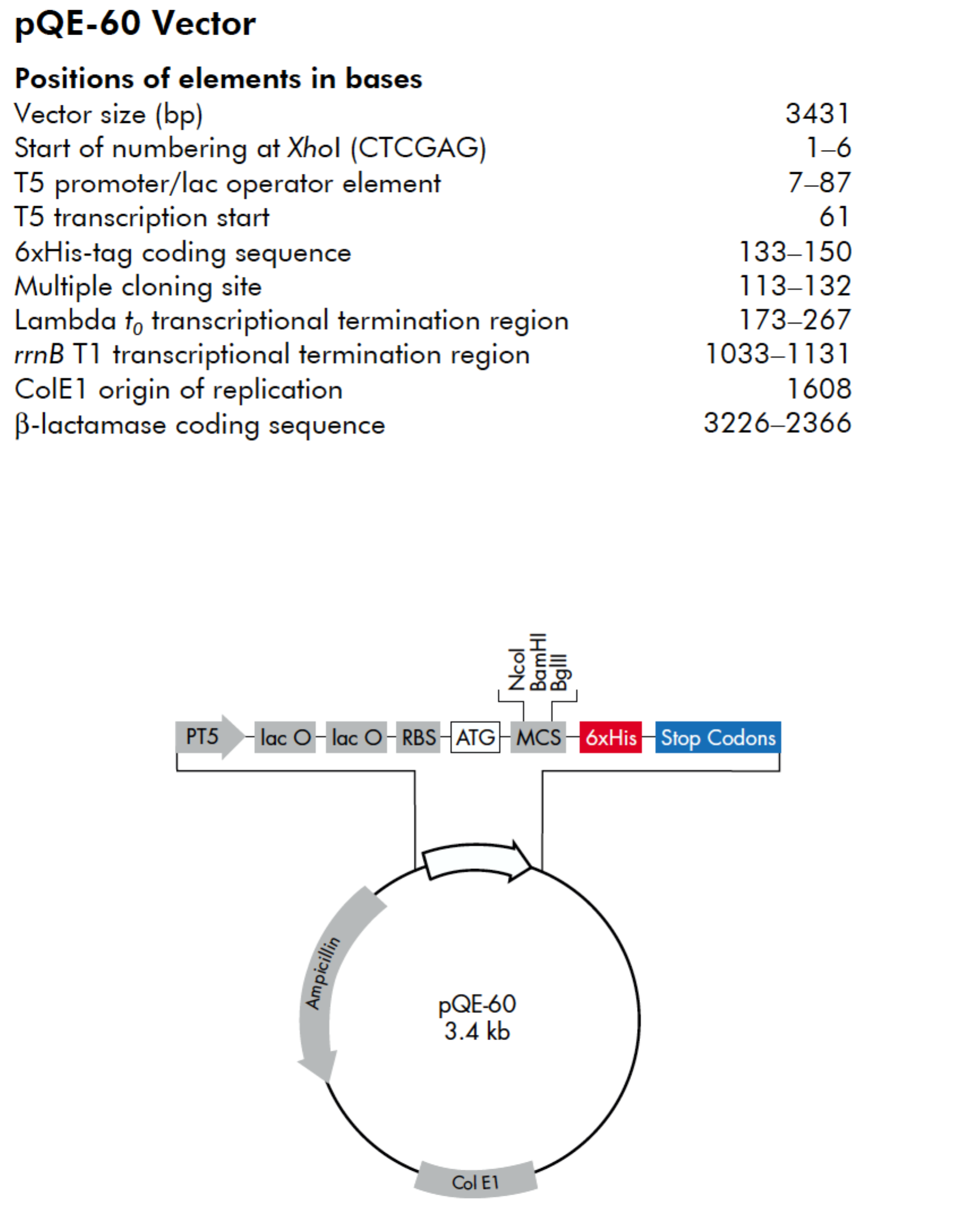
For full length protein (which is cloned into vector)

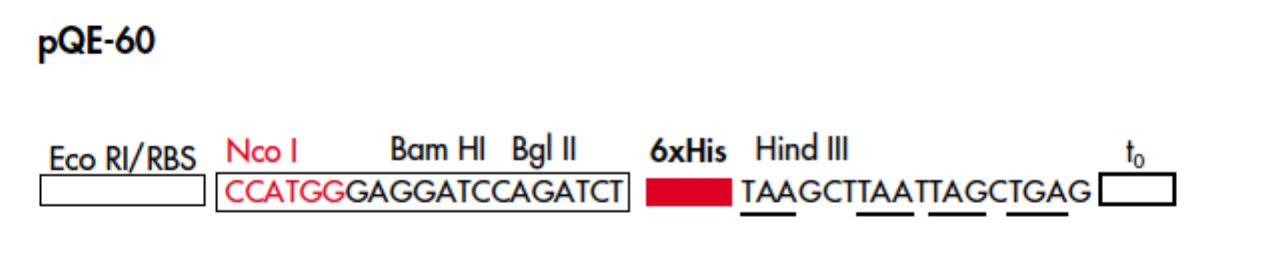
UniProt:  P19446

Gene: MDHG\_CITLA

mRNA: M33148

Structure known 1smk\_f is the sequence that was traced into the x-ray data for chain f.  It is missing N-term sequence & includes the C-term his tag from vector.  You can find all the chains 1smk\_a, b, c, d, e, f, g, h.





**ACCESSION:** 1SMK\_F

**GI:** 60593492.

1 RAKGGAPGFK VAILGAAGGI GQPLAMLMKM NPLVSVLHLY DVVNAPGVTA 50

NADH Binding Dopmain

\*

\*

51 DISHMDTGAV VRGFLGQQQL EAALTGMDLI IVPAGVPRKP GMTRDDLFKI 100

Mobile loop

Active site loop

P Binding Domain

101 NAGIVKTLCE GIAKCCPRAI VNLISNPVNS TVPIAAEVFK KAGTYDPKRL 150

\*

LGVTMLDVVR ANTFVAEVLG LDPRDVDVPV VGGHAGVTIL PLLSQVKPPS

Active site

SFTQEEISYL TDRIQNGGTE VVEAKAGAGS ATLSMAYAAV KFADACLRGL

RGDAGVIECA FVSSQVTELP FFASKVRLGR NGIEEVYSLG PLNEYERIGL

\* = LDH VS MDH Activity

EKAKKELAGS IEKGVSFIRS HHHHHH

Sequence

CATTATTATCATGACATTAACCTATAAAAATAGGCGTATCACGAGGCCCTTTCGTCTTCACCTCGAGAAATCATAAAAAATTTATTTGCTTTGTGAGCGGATAACAATTATAATAGATTCAATTGTGAGCGGATAACAATTTCACACAGAATTCATTAAAGAGGAGAAATTAACTATGGCTAAAGGCGGAGCTCCCGGGTTCAAAGTCGCAATACTTGGCGCTGCCGGTGGCATTGGCCAGCCCCTTGCGATGTTGATGAAGATGAATCCTCTGGTTTCTGTTCTACATCTATATGATGTAGTCAATGCCCCTGGTGTCACCGCTGATATTAGCCACATGGACACGGGTGCTGTGGTGCGTGGATTCTTGGGGCAGCAGCAGCTGGAGGCTGCGCTTACTGGCATGGATCTTATTATAGTCCCTGCAGGTGTTCCTCGAAAACCAGGAATGACGAGGGATGATCTGTTCAAAATAAACGCAGGAATTGTCAAGACTCTGTGTGAAGGGATTGCAAAGTGTTGTCCAAGAGCCATTGTCAACCTGATCAGTAATCCTGTGAACTCCACCGTGCCCATCGCAGCTGAAGTTTTCAAGAAGGCTGGAACTTATGATCCAAAGCGACTTCTGGGAGTTACAATGCTCGACGTAGTCAGAGCCAATACCTTTGTGGCAGAAGTATTGGGTCTTGATCCTCGGGATGTTGATGTTCCAGTTGTTGGCGGTCATGCTGGTGTAACCATTTTGCCCCTTCTATCTCAGGTGAAGCCTCCAAGTTCTTTCACACAAGAAGAGATTAGTTACCTGACTGATAGGATTCAAAATGGTGGAACAGAAGTTGTCGAGGCCAAAGCAGGAGCTGGTTCAGCAACTCTCTCAATGGCTTATGCTGCCGTTAAGTTTGCAGATGCATGCCTCAGGGGCTTAAGAGGAGATGCTGGTGTCATTGAATGCGCGTTTGTGTCTTCTCAGGTGACTGAACTTCCATTCTTTGCATCAAAAGTACGACTTGGTCGCAATGGTATCGAAGAAGTATACTCCCTTGGCCCGCTAAATGAGTATGAGAGGATTGGATTGGAGAAAGCGAAGAAAGAGTTGGCAGGAAGCATTGAGAAGGGAGTTTCCTTCATCAGAAGCAGATCTCATCACCATCACCATCACTAAGCTTAATTAGCTGAGCTTG

**E. coli R153C MDH (eMDH)**

Structure described – JBC 2001 Vol 276, No. 33, pp 31156-31162

Cloning info in J Mol Biol. 1991 Aug 5;220(3):551-3.

Backbone (pBR322 vector) Medium Copy, vector = 4361 bp Amp resistant

Map (https://www.neb.com/~/media/NebUs/Page%20Images/Tools%20and%20Resources/Interactive%20Tools/DNA%20Sequences%20and%20Maps/pBR322\_map.pdf)