Sequence of human dynamin II (spliced variant bb)

```
                                                             bp  aa
atgggcaaccgcgggatggaagagctgatcccgctggtcaacaaactgcaggacgccttc 60
 M  G  N  R  G  M  E  E  L  I  P  L  V  N  K  L  Q  D  A  F      20
agctccatcggccagagctgccacctggacctgccgcagatcgctgtagtgggcggccag 120
 S  S  I  G  Q  S  C  H  L  D  L  P  Q  I  A  V  V  G  G  Q      40
agcgccggcaagagctcggtgctggagaacttcgtgggccgggacttccttccccgcggt 180
 S  A  G  K  S  S  V  L  E  N  F  V  G  R  D  F  L  P  R  G      60
tcaggaatcgtcacccggcggcctctcattctgcagctcatcttctcaaaaacagaacat 240
 S  G  I  V  T  R  R  P  L  I  L  Q  L  I  F  S  K  T  E  H      80
gccgagtttttgcactgcaagtccaaaaagtttacagactttgatgaagtccggcaggag 300
 A  E  F  L  H  C  K  S  K  K  F  T  D  F  D  E  V  R  Q  E      100
attgaagcagagaccgacagggtcacggggaccaacaaaggcatctccccagtgcccatc 360
 I  E  A  E  T  D  R  V  T  G  T  N  K  G  I  S  P  V  P  I      120
aaccttcgagtctactcgccacacgtgttgaacttgaccctcatcgacctcccgggtatc 420
 N  L  R  V  Y  S  P  H  V  L  N  L  T  L  I  D  L  P  G  I      140
accaaggtgcctgtgggcgaccagcctccagacatcgagtaccgagtcaaggacatgatc 480
 T  K  V  P  V  G  D  Q  P  P  D  I  E  Y  R  V  K  D  M  I      160
ctgcagttcatcagccgggagagcagcctcattctggctgtcacgcccgccaacatggac 540
 L  Q  F  I  S  R  E  S  S  L  I  L  A  V  T  P  A  N  M  D      180
ctggccaactccgacgccctcaagctggccaaggaagtcgatccccaaggcctacggacc 600
 L  A  N  S  D  A  L  K  L  A  K  E  V  D  P  Q  G  L  R  T      200
atcggtgtcatcaccaagcttgacctgatggacgagggcaccgacgccagggacgtcttg 660
 I  G  V  I  T  K  L  D  L  M  D  E  G  T  D  A  R  D  V  L      220
gagaacaagttgctcccgttgagaagaggctacattggcgtggtgaaccgcagccagaag 720
 E  N  K  L  L  P  L  R  R  G  Y  I  G  V  V  N  R  S  Q  K      240
gatattgagggcaagaaggacatccgtgcagcactggcagctgagaggaagttcttcctc 780
 D  I  E  G  K  K  D  I  R  A  A  L  A  A  E  R  K  F  F  L      260
tcccacccggcctaccggcacatggccgaccgcatgggcacgccacatctgcagaagacg 840
 S  H  P  A  Y  R  H  M  A  D  R  M  G  T  P  H  L  Q  K  T      280
ctgaatcagcaactgaccaaccacatccgggagtcgctgccggccctacgtagcaaacta 900
 L  N  Q  Q  L  T  N  H  I  R  E  S  L  P  A  L  R  S  K  L      300
cagagccagctgctgtccctggagaaggaggtggaggagtacaagatctttcggcccgac 960
 Q  S  Q  L  L  S  L  E  K  E  V  E  E  Y  K  I  F  R  P  D      320
gaccccacccctaaaaccaaagccctgctgcagatggtccagcagtttggggtggatttt 1020
 D  P  T  P  K  T  K  A  L  L  Q  M  V  Q  Q  F  G  V  D  F      340
gagaagaggatcgagggctcaggagatcaggtggacactctggagctctccgggggcgcc 1080
 E  K  R  I  E  G  S  G  D  Q  V  D  T  L  E  L  S  G  G  A      360
cgaatcaatcgcatcttccacgagcggttcccatttgagctggtgaagatggagtttgac 1140
 R  I  N  R  I  F  H  E  R  F  P  F  E  L  V  K  M  E  F  D      380
gagaaggacttacgacgggagatcagctatgccattaagaacatccatggagtcaggacc 1200
 E  K  D  L  R  R  E  I  S  Y  A  I  K  N  I  H  G  V  R  T      400
gggcttttcaccccggacttggcattcgaggccattgtgaaaaagcaggtcgtcaagctg 1260
 G  L  F  T  P  D  L  A  F  E  A  I  V  K  K  Q  V  V  K  L      420
aaagagccctgtctgaaatgtgtcgacctggttatccaggagctaatcaatacagttagg 1320
 K  E  P  C  L  K  C  V  D  L  V  I  Q  E  L  I  N  T  V  R      440
cagtgtaccagtaagctcagttcctacccccggttgcgagaggagacagagcgaatcgtc 1380
 Q  C  T  S  K  L  S  S  Y  P  R  L  R  E  E  T  E  R  I  V      460
accacttacatccgggaacgggagggagaacgaaggaccagattcttctgctgatcgac 1440
 T  T  Y  I  R  E  R  E  G  R  T  K  D  Q  I  L  L  L  I  D      480
attgagcagtcctacatcaacacgaaccatgaggacttcatcgggtttgccaatgcccag 1500
 I  E  Q  S  Y  I  N  T  N  H  E  D  F  I  G  F  A  N  A  Q      500
cagaggagcacgcagctgaacaagaagagagccatccccaatcaggtgatccgcaggggc 1560
 Q  R  S  T  Q  L  N  K  K  R  A  I  P  N  Q  V  I  R  R  G      520
tggctgaccatcaacaacatcagcctgatgaaaggcggctccaaggagtactggtttgtg 1620
 W  L  T  I  N  N  I  S  L  M  K  G  G  S  K  E  Y  W  F  V      540
ctgactgccgagtcactgtcctggtacaaggatgaggaggagaaagagaagaagtacatg 1680
 L  T  A  E  S  L  S  W  Y  K  D  E  E  E  K  E  K  K  Y  M      560
ctgcctctggacaacctcaagatccgtgatgtggagaagggcttcatgtccaacaagcac 1740

========================================================================
```

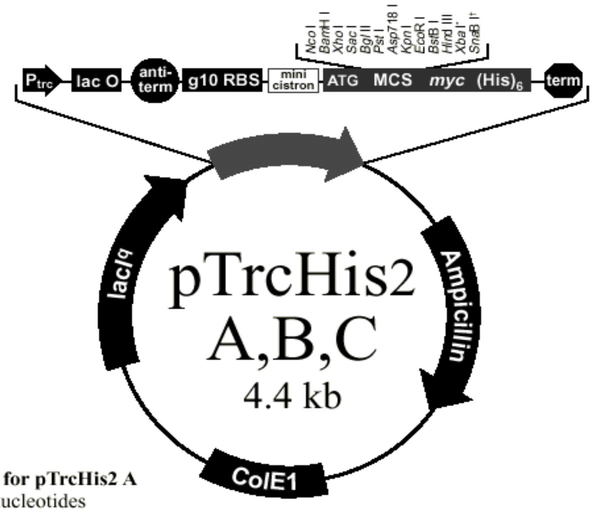
```
 L  P  L  D  N  L  K  I  R  D  V  E  K  G  F  M  S  N  K  H      580
gtcttcgccatcttcaacacggagcagagaaacgtctacaaggacctgcggcagatcgag 1800
 V  F  A  I  F  N  T  E  Q  R  N  V  Y  K  D  L  R  Q  I  E      600
ctggcctgtgactcccaggaagacgtggacagctggaaggcctcgttcctccgagctggc 1860
 L  A  C  D  S  Q  E  D  V  D  S  W  K  A  S  F  L  R  A  G      620
gtctaccccgagaaggaccaggcagaaaacgaggatggggcccaggagaacaccttctcc 1920
 V  Y  P  E  K  D  Q  A  E  N  E  D  G  A  Q  E  N  T  F  S      640
atggaccccaactggagcggcaggtggagaccattcgcaacctggtggactcatacgtg 1980
 M  D  P  Q  L  E  R  Q  V  E  T  I  R  N  L  V  D  S  Y  V      660
gccatcatcaacaagtccatccgcgacctcatgccaaagaccatcatgcacctcatgatc 2040
 A  I  I  N  K  S  I  R  D  L  M  P  K  T  I  M  H  L  M  I      680
aacaatacgaaggccttcatccaccacgagctgctggcctacctatactcctcggcagac 2100
 N  N  T  K  A  F  I  H  H  E  L  L  A  Y  L  Y  S  S  A  D      700
cagagcagcctcatggaggagtcggctgaccaggcacagcggcgggacgacatgctgcgc 2160
 Q  S  S  L  M  E  E  S  A  D  Q  A  Q  R  R  D  D  M  L  R      720
atgtaccatgccctcaaggaggcgctcaacatcatcggtgacatcagcaccagcactgtg 2220
 M  Y  H  A  L  K  E  A  L  N  I  I  G  D  I  S  T  S  T  V      740
tccacgcctgtacccccgcctgtcgatgacacctggctccagagcgccagcagccacagc 2280
 S  T  P  V  P  P  P  V  D  D  T  W  L  Q  S  A  S  S  H  S      760
cccactccacagcgccgaccggtgtccagcatacaccccctggccggcccccagcagtg 2340
 P  T  P  Q  R  R  P  V  S  S  I  H  P  P  G  R  P  P  A  V      780
aggggccccactccagggccccccctgattcctgttcccgtgggggcagcagcctccttc 2400
 R  G  P  T  P  G  P  P  L  I  P  V  P  V  G  A  A  A  S  F      800
tcggcgcccccaatcccatcccggcctggaccccagagcgtgtttgccaacagtgacctc 2460
 S  A  P  P  I  P  S  R  P  G  P  Q  S  V  F  A  N  S  D  L      820
ttcccagccccgcctcagatcccatctcggccagttcggatcccccccagggattcccccca 2520
 F  P  A  P  P  Q  I  P  S  R  P  V  R  I  P  P  G  I  P  P      840
ggagtgcccagcagaagacccccctgctgcgcccagccggcccaccattatccgcccagcc 2580
 G  V  P  S  R  R  P  P  A  A  P  S  R  P  T  I  I  R  P  A      860
gagccatccctgctcgactag
 E  P  S  L  L  D
```

Ref. Diatloff,Z.C., Gordon,A.J., Duchaud,E., and Merlin,G. (1995). Isolation of an ubiquitously expressed cDNA encoding human dynamin II, a member of the large GTP-binding protein family. Gene *163*, 301-306.

==

Map of pTrcHis2

The figure below summarizes the features of the pTrcHis2 vectors. The complete nucleotide sequence of pTrcHis2 A is provided after page 13. Complete sequences for all three pTrcHis2 vectors can be downloaded from our World Wide Web site. See page 12 for information on the Invitrogen Web Resource. Details of each multiple cloning site are shown on pages 5-6.



Comments for pTrcHis2 A
4406 nucleotides

trc promotor region: bases 190-382
- -35 region: bases 193-198
- -10 region: bases 216-221
- lac operator (*lac*O): bases 228-248
- *rrn*B antitermination signal: bases 264-333
- gene 10 region: bases 346-354
- Ribosome binding site: bases 369-373
- pTrcHis forward priming site: bases 370-390
- Minicistron ORF: bases 383-409
- Reinitiation RBS: bases 398-403

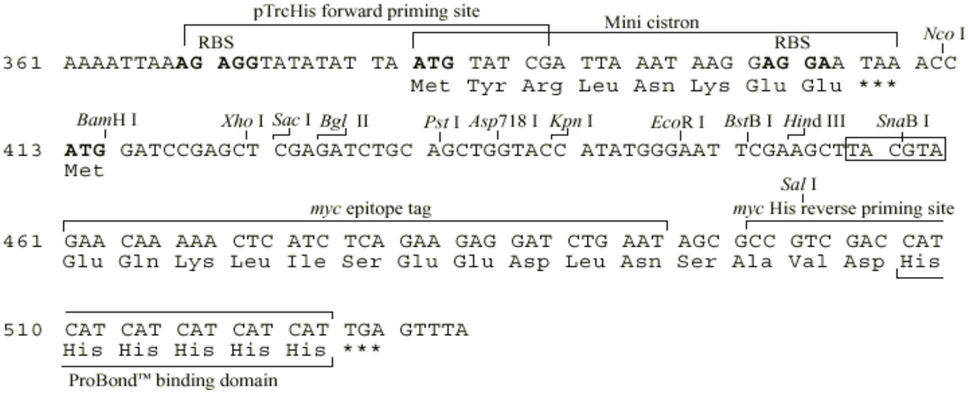
Expression ATG: bases 413-415
Multiple cloning site: bases 411-464
myc epitope: bases 471-503
(His)$_6$ tag: bases 516-533
*myc*His reverse priming site: bases 508-527
*rrn*B T1 and T2 transcriptional terminators: bases 639-796
Ampicillin resistance ORF: bases 1076-1936
ColE1 origin: bases 2081-2754
Lac Repressor (*lac*I^q) ORF: bases 3285-4367

* *Xba* I is only found in pTrcHis2 B

† *Sna*B I is only found in pTrcHis2 C

Multiple Cloning Site of pTrcHis2 C

Below is the multiple cloning site for pTrcHis2 C. Restriction sites are labeled to indicate cleavage site. The boxed sequence is the variable region that facilitates in frame cloning with the C-terminal peptide. This variable region is located between the *Hin*d III site and the *myc* epitope. The multiple cloning site has been confirmed by sequencing and functional testing.



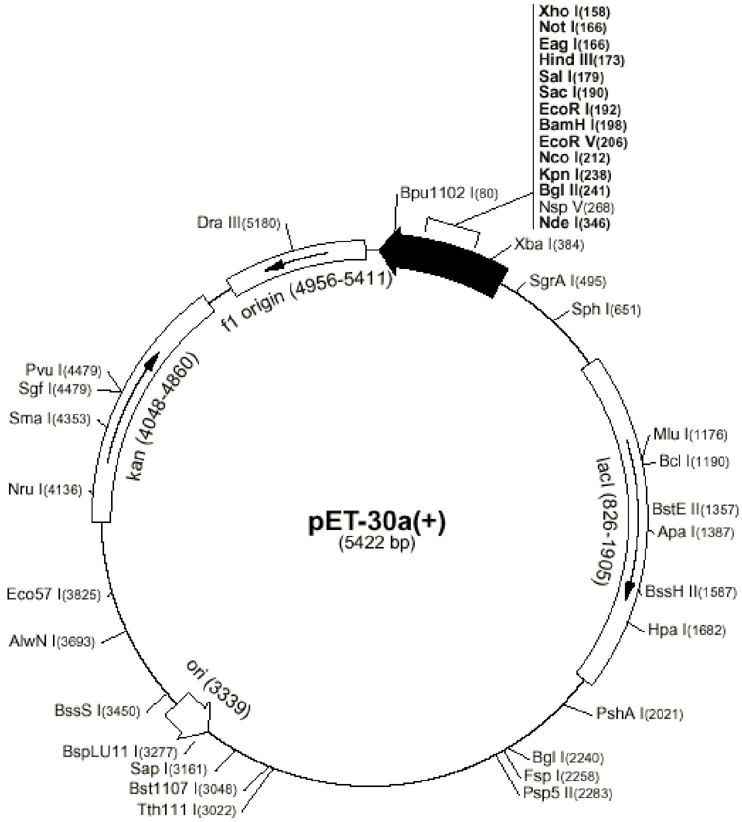
pET-30-a-c(+) Vectors

The pET-30a-c(+) vectors carry an N-terminal His•Tag®/thrombin/S•Tag™/enterokinase configuration plus an optional C-terminal His•Tag sequence. Unique sites are shown on the circle map. Note that the sequence is numbered by the pBR322 convention, so the T7 expression region is reversed on the circular map. The cloning/expression region of the coding strand transcribed by T7 RNA polymerase is shown below. The f1 origin is oriented so that infection with helper phage will produce virions containing single stranded DNA that corresponds to the coding strand. Therefore, single stranded sequencing should be performed using the T7 terminator primer.

pET-30a(+) sequence landmarks

T7 promoter	419-435
T7 transcription start	418
His•Tag coding sequence	327-344
S•Tag coding sequence	249-293
Multiple cloning sites (*Nco* I - *Xho* I)	158-217
His•Tag coding sequence	140-157
T7 terminator	26-72
lacI coding sequence	826-1905
pBR322 origin	3339
Kan coding sequence	4048-4860
f1 origin	4956-5411

The maps for pET-30b(+) and pET-30c(+) are the same as pET-30a(+) (shown) with the following exceptions: pET-30b(+) is a 5421 bp plasmid; subtract 1 bp from each site beyond *Bam*H I at 198. pET-30c(+) is a 5423 bp plasmid; add 1 bp to each site beyond *Bam*H I at 198.



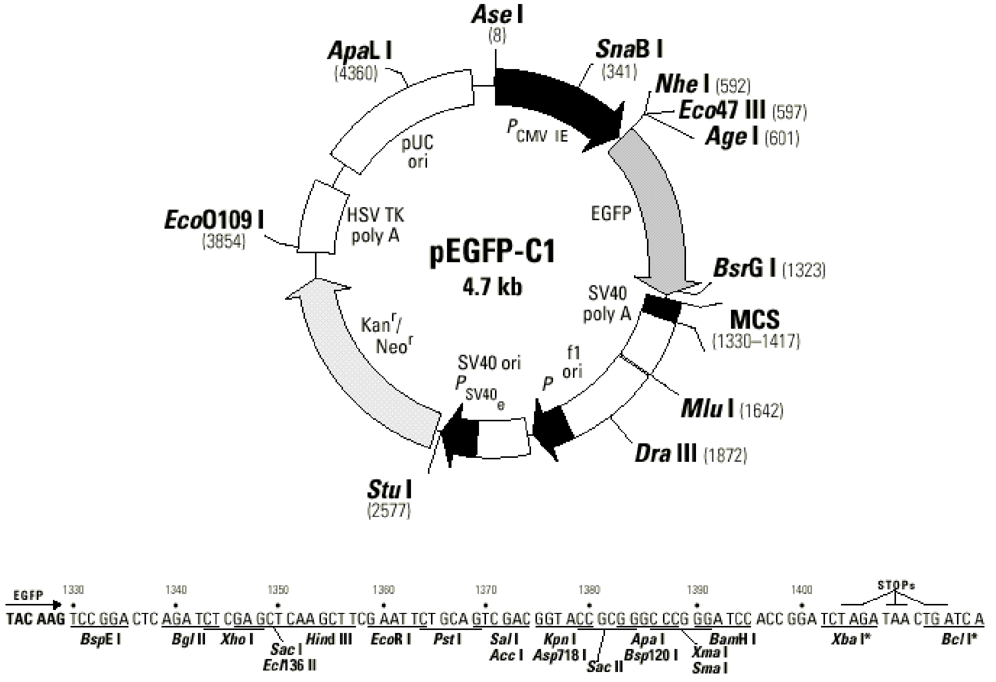
pET-30a-c(+) cloning/expression region

pEGFP-C1 Vector Information

PT3028-5

GenBank Accession #: U55763

Catalog #6084-1



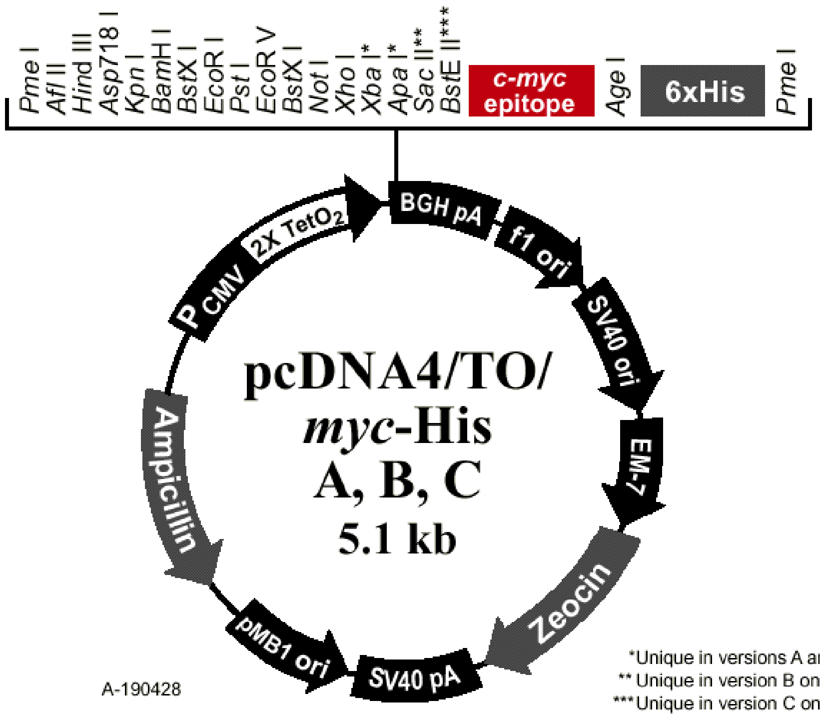
Restriction Map and Multiple Cloning Site (MCS) of pEGFP-C1. (Unique restriction sites are in bold). The *Xba* I and *Bcl* I sites (*) are methylated in the DNA provided by CLONTECH. If you wish to digest the vector with these enzymes, you will need to transform the vector into a *dam*⁻ host and make fresh DNA.

Description:

pEGFP-C1 encodes a red-shifted variant of wild-type GFP (1–3) which has been optimized for brighter fluorescence and higher expression in mammalian cells. (Excitation maximum = 488 nm; emission maximum = 507 nm.) pEGFP-C1 encodes the GFPmut1 variant (4) which contains the double-amino-acid substitution of Phe-64 to Leu and Ser-65 to Thr. The coding sequence of the EGFP gene contains more than 190 silent base changes which correspond to human codon-usage preferences (5). Sequences flanking EGFP have been converted to a Kozak consensus translation initiation site (6) to further increase the translation efficiency in eukaryotic cells. The MCS in pEGFP-C1 is between the EGFP coding sequences and the SV40 poly A. Genes cloned into the MCS will be expressed as fusions to the C-terminus of EGFP if they are in the same reading frame as EGFP and there are no intervening stop codons. SV40 polyadenylation signals downstream of the EGFP gene direct proper processing of the 3' end of the EGFP mRNA. The vector backbone also contains an SV40 origin for replication in mammalian cells expressing the SV40 T-antigen. A neomycin resistance cassette (Neo^r), consisting of the SV40 early promoter, the neomycin/kanamycin resistance gene of Tn5, and polyadenylation signals from the Herpes simplex virus thymidine kinase (HSV TK) gene, allows stably transfected eukaryotic cells to be selected using G418. A bacterial promoter upstream of this cassette expresses kanamycin resistance in *E. coli.* The pEGFP-C1 backbone also provides a pUC origin of replication for propagation in *E. coli* and an f1 origin for single-stranded DNA production.

Comments for pcDNA4/TO/*myc*-His A
5151 nucleotides

CMV promoter: bases 232-958
TATA box: bases 804-810
Tetracycline operator 2 (2X $TetO_2$) sequences: bases 820-859
CMV Forward priming site: bases 769-789
Multiple cloning site: bases 968-1069
c-myc epitope: bases 1073-1102
Polyhistidine (6xHis) tag: bases 1118-1135
pcDNA3.1/BGH reverse priming site: bases 1158-1175
BGH polyadenylation sequence: bases 1164-1388
f1 origin: bases 1434-1862
SV40 promoter and origin: bases 1867-2211
EM-7 promoter: bases 2253-2319
Zeocin™ resistance gene: bases 2320-2694
SV40 early polyadenylation sequence: bases 2824-2954
pMB1 (pUC-derived) origin: bases 3337-4010 (complementary strand)
bla promoter: bases 5016-5114 (complementary strand)
Ampicillin (*bla*) resistance gene: bases 4155-5015 (complementary strand)



*Unique in versions A and B only
** Unique in version B only
***Unique in version C only

The sequences of pcDNA4/TO/*myc*-His A, B, and C have been compiled from information in sequence databases, published sequences, and other sources. These vectors have not been completely sequenced. If you suspect an error in the sequence, please contact Invitrogen's Technical Service Department.

Sequences of dynamin II domains expressed in *E.coli*

PHD-his

MDPSSRS**QQR STQLNKKRAI PNQVIRRGWL TINNISLMKG GSKEYWFVLT AESLSWYKDE**
EEKEKKYMLP LDNLKIRDVE KGFMSNKHVF AIFNTEQRNV YKDLRQIELA CDSQEDVDSW
KASFLRAGEF EAYVEQKLIS EEDLNSAVDH HHHHH
Number of amino acids: 155
The putative WM: 18.3kDa
The Theoretical pI:6.38

PRD-his

MDPSSRS**STV STPVPPPVDD TWLQSASSHS PTPQRRPVSS IHPPGRPPAV RGPTPGPPLI**
PVPVGAAASF SAPPIPSRPG PQSVFANSDL FPAPPQIPSR PVRIPPGIPP GVPSRRPPAA
PSRPTIIRPA EPSLLDEFEA YVEQKLISEE DLNSAVDHHH HHH
Number of amino acids: 163
The putative WM: 17.3kDa
The Theoretical pI:7.11

PCP-his

MDPSSRS**QQR STQLNKKRAI PNQVIRRGWL TINNISLMKG GSKEYWFVLT AESLSWYKDE**
EEKEKKYMLP LDNLKIRDVE SYVAIINKSI RDLMPKTIMH LMINNTKAFI HHELLAYLYS
SADQSSLMEE SADQAQRRDD MLRMYHALKE ALNIIGDIST STVSTPVPPP VDDTWLQSAS
SHSPTPQRRP VSSIHPPGRP PAVRGPTPGP PLIPVPVGAA ASFSAPPIPS RPGPQSVFAN
SDLFPAPPQI PSRPVRIPPG IPPGVPSRRP PAAPSRPTII RPAEPSLLDE FEAYVEQKLI
SEEDLNSAVD HHHHHH
Number of amino acids: 401
The putative WM: 45.1kDa
The Theoretical pI:6.24

his-s-PHD:

MHHHHHHSSG LVPRGSGMKE TAAAKFERQH MDSPDLGTDD DDKAMADIGS **QQRSTQLNKK**
RAIPNQVIRR GWLTINNISL MKGGSKEYWF VLTAESLSWY KDEEEKEKKY MLPLDNLKIR
DVEKGFMSNK HVFAIFNTEQ RNVYKDLRQI ELACDSQEDV DSWKASFLRA GEFEACGRTR
APPPPPLRSG C
Number of amino acids: 191
The putative WM: 21.9kDa
The Theoretical pI:7.77

his-s-PRD:

MHHHHHHSSG LVPRGSGMKE TAAAKFERQH MDSPDLGTDD DDKAMADIGS **STVSTPVPPP**
VDDTWLQSAS SHSPTPQRRP VSSIHPPGRP PAVRGPTPGP PLIPVPVGAA ASFSAPPIPS
RPGPQSVFAN SDLFPAPPQI PSRPVRIPPG IPPGVPSRRP PAAPSRPTII RPAEPSLLDE
FEACGRTRAP PPPPLRSGC
Number of amino acids: 199
The putative WM: 20.8kDa
The Theoretical pI: 9.37

==

Sequences of dynamin II domains expressed in *E.coli* (continued)

his-s-PCP:

MHHHHHHSSG LVPRGSGMKE TAAAKFERQH MDSPDLGTDD DDKAMADIGS **QQRSTQLNKK**
RAIPNQVIRR GWLTINNISL MKGGSKEYWF VLTAESLSWY KDEEEKEKKY MLPLDNLKIR
DVEKGFMSNK HVFAIFNTEQ RNVYKDLRQI ELACDSQEDV DSWKASFLRA GVYPEKDQAE
NEDGAQENTF SMDPQLERQV ETIRNLVDSY VAIINKSIRD LMPKTIMHLM INNTKAFIHH
ELLAYLYSSA DQSSLMEESA DQAQRRDDML RMYHALKEAL NIIGDISTST VSTPVPPPVD
DTWLQSASSH SPTPQRRPVS SIHPPGRPPA VRGPTPGPPL IPVPVGAAAS FSAPPIPSRP
GPQSVFANSD LFPAPPQIPS RPVRIPPGIP PGVPSRRPPA APSRPTIIRP AEPSLLDEFE
ACGRTRAPPP PPLRSGC
Number of amino acids: 437
The putative WM: 48.6kDa
The Theoretical pI: 6.71

Note: Sequence of dynamin II domains were shown in bold font.

==

Predicted secondary structure of his-s-PRD protein

```
           ....,....1....,....2....,....3....,....4....,....5....,....6
AA         MHHHHHHSSGLVPRGSGMKETAAAKFERQHMDSPDLGTDDDDKAMADIGSSTVSTPVPPP
PHD_sec                      HHHHHHHHHHH            HHHHHHH
Rel_sec    985113678645788656255897776524499999999942668851344579888999

P_3_acc    e ebbbebeeeeeeeeeeeebbbeebe ebbeeeeeeeeeeebbbebbeeeebeeeebeb
Rel_acc    101010000101101100400110310000211121111012060111200320321120

           ....,....7....,....8....,....9....,....10..,....11..,....12
AA         VDDTWLQSASSHSPTPQRRPVSSIHPPGRPPAVRGPTPGPPLIPVPVGAAASFSAPPIPS
PHD_sec       EEEEE    E                   EEEE        E
Rel_sec    995687845772212222688999999887618993379996520122677899999999

P_3_acc    bebbbb bebeb bbeb eeeeee bebeeebbbbbbeeee bbbbbebbbeeebeebee
Rel_acc    200220030312061230131104112012407276411100745141521201320002

           ....,....13..,....14..,....15..,....16..,....17..,....18
AA         RPGPQSVFANSDLFPAPPQIPSRPVRIPPGIPPGVPSRRPPAAPSRPTIIRPAEPSLLDE
PHD_sec                                                            HHHH
Rel_sec    999989989999999999999999989999999999999999999999889999986 0557

P_3_acc    eeeeee bbbbeebeb eebbb beebbeeb eebeeeeeeeeee bebe ee bbbbee
Rel_acc    121010031100001000030010001013102100013220110101101011214211

           ....,....19..,....20
AA         FEACGRTRAPPPPPLRSGC
PHD_sec    HHHH
Rel_sec    8871688789999767899

P_3_acc    bebbeeeeeeeeeebeeee
Rel_acc    5240011010122201210
```

Note: Sequence in yellow background color represents PRD of dynamin II

Predicted secondary structure composition:

Helix: %13.1
Extended: %5.5
Loop: %81.4

Discription:

AA :	amino acid sequence
PHD_sec:	PHD predicted secondary structure: H=helix, E=extended (sheet), blank=other (loop) PHD = PHD: Profile network prediction HeiDelberg
Rel_sec:	reliability index for PHDsec prediction (0=low to 9=high)
P_3_acc:	PHD predicted relative solvent accessibility (acc) in 3 states: b =0-9%, i = 9-36%, e = 36-100%
Rel_acc:	reliability index for PHDacc prediction (0=low, to 9=high)