
Supplementary Materials*Article*

Effects of Lower Temperature on Expression and Biochemical Characteristics of HCV NS3 Antigen Recombinant Protein

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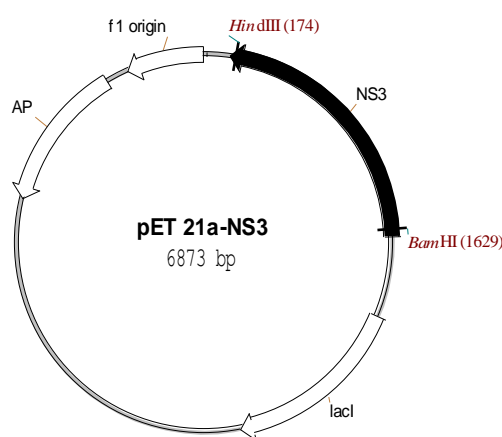
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(A)

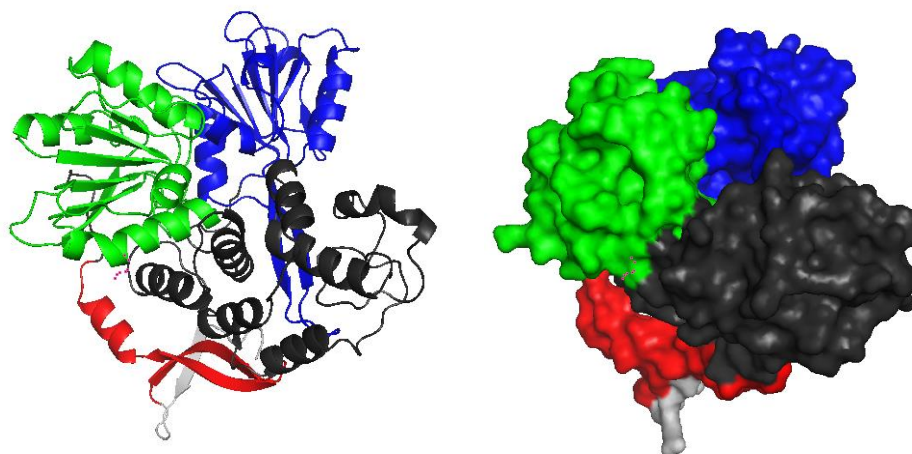
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1  catatgggctagcatgactgggtggacagcaaatgggtcgcggtatcccatgttgtgggcattcttccgggctgct
   M A S M T G G Q Q M G R G S H V V G I F R A A
70  gtgtgcacccgggggttgcaaggcgggtgactttgtgcccgttgagtctatggaaactaccatgcgggtcc
   V C T R G V A K A V D F V P V E S M E T T M R S
142  ccggtcttcacggacaattcgtctccccggccgtaccgcaggcgttccaagtggcccatctacacgtctccc
   P V F T D N S S P P A V P Q A F Q V A H L H A P
214  actggcagcggcaagagcactaaggtgccggctgcatatgcagcccaagggtacaagggtcgttctttaa
   T G S G K S T K V P A A Y A A Q G Y K V L V L N
286  ccattctgttgccgccaccttaggttttgggcgatgatgtctaaggcacatgggtgcgaccctaaccattaga
   P S V A A T L G F G A Y M S K A H G V D P N I R
358  actggggtaaggaccatcaccacgggcgtccccatcacgtactccacattatggcaagttccttgcggacggt
   T G V R T I T T G A P I T Y S T Y G K F L A D G
430  ggctgctctggggggcctatgacatcataatgtgtgatgagtgccactcaactgactcgactaccattcttg
   G C S G G A Y D I I M C D E C H S T D S T T I L
502  ggcacatcgccacagtcctagaccaagcggagacggctggagcgcggctcgtcgtgctcgccaccgctacgcct
   G I G T V L D Q A E T A G A R L V V L A T A T P
574  ccgggatcggtcacccgtgccgcacctaaccattgaggaaatagccctgtccaacactggagagatcccccttc
   P G S V T V P H P N I E E I A L S N T G E I P F
646  tatggcaaagccatccccatcgagaccatcaaggggggagggcatctcatcttctgccattccaagaagaaa
   Y G K A I P I E T I K G G R H L I F C H S K K K
718  tgtgacgagctcgtgctgaagctgtcgccctcggaattcatgctgtagcatattaccgggggtcttgatgtg
   C D E L A A K L S A L G I H A V A Y Y R G L D V
790  tccgtcataccggctagcgggaatgtcgttgtcgtggcaacggacgctctaataacacggctttaccggcgac
   S V I P A S G N V V V V A T D A L M T G F T G D
862  tttgactcgggtgatcgactgtaaacagtggtgtcaccacagacagtcgacttcagcttggaccctaccttcacc
   F D S V I D C N T C V T Q T V D F S L D P T F T
934  attgagacgacgaccatgccccaaagacgcggtgtcacgctcgcagcggcgaggcaggactagtaggggcagg
   I E T T T M P Q D A V S R S Q R R G R T A T P
1006  agaggcatctacaggttcgtgactccaggagaacggccctcgggcatgttcgattcttccggtcctgtgcgag
   R G I Y R F V T P G E R P S G M F D S S V L C E
1078  tgcctatgacgcggtgtgtgcttggtacgagctcacaccccgccgagacctcgggttaggttgcgggcttaccta
   C Y D A G C A W Y E L T P A E T S V R L R A Y L
1150  aatacaccagggttgcccggttgccaggaccatctggagttctgggagagcgtcttcacaggccttaccac
   N T P G L P V C Q D H L E F W E S V F T G L T H
1222  atagatgcccatttcttgtcccagactaagcaggcaggagacaacttcccctacctggtagcataccaagca
   I D A H F L S Q T K Q A G D N F P Y L V A Y Q A
1294  acggtgtgtgccagggtcaggctccgcctccatcatgggaccaaagtgtggaagtgtctcacacggctaaaa
   T V C A R A Q A P P P S W D Q M W K C L T R L K
1366  cccacactacacgggccaacacccctgttgtagactgggagccgtccaaaatgaggtcacccctcacgcac
   P T L H G P T P L L Y R L G A V Q N E V T L T H
1438  ccataaccaaatacatcatggcatgctggtcgccgacctcgaggtcgtcacgaagcgttgcggccgcaactc
   P I T K Y I M A C M S A D L E V V T K L A A A L
1510 gagcaccaccaccaccaccactga
     E H H H H H H *

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(b)

Figure S1. Map of plasmid pET 21a-NS3 (A) and the sequence of recombinant product (B). The original truncated NS3 gene was between *Bam*HI (1629 in A, underline in B) and *Hind*III (174 in A, double underline in B) of pET 21a. The extra gene shown in Fig. 1B from pET 21a includes a T7 tag (as boxed) and a His-tag (shown in bold).



MASMTGGQQMGRGSHVVGIFRAAVCTRGVAKAVDFVPVESMETTMRSPVFTDNSSPPAVPQAFQVAHLHAPTGS GK
 STKVPAAYAAQGYKVLVLNPSVAATLGFGAYMSKAHGVDPNIRTGVRTITTTGAPITYSTY GKFLADGGCSGGAYD
 IMCDECHSTDSTILGIGTVLDQAETAGARLVVLATATPPGSVTVPHPNIEEIALSNTGEIPFYGKAIPETIKGGRHLI
 FCHSKKCCDELAALKLSALGIHAVAYYRGLDVSVIPASGNVVVVATDALMTGFTGDFDSVIDCNTCVTQTVD FSLDPTFTI
 ETTTTPQDAVSRSQRRGRTSRGRRGIYRFVTPGERPSGMFDSSVLCECYDAGCAWYELTPAETSVRLRAYLNTPGLPVC
 QDHLEFWESVFTGLTHIDAHFLSQTQAGDNFPYLVAYQATVCARAQAPPSWDQMWKCLTRLKPTLHGPTPLLYRLG
 AVQNEVTLTHPITKYIMACMSADLEVVTKLAAALEHHHHHHH

[Gray: from plasmid; Red: partial sequence of protease; Green: Helicase ATP-binding domain (TI=0.82)(Tm prediction=55~65); Blue: DEAD-like helicase C domain (TI=1.5)(Tm prediction higher than 65); Whole recombinant protein: TI=0.75 (Tm prediction=55~65)]

Figure S2. The recombinant protein sequence was modeled for a theoretical structure using SWISS-MODEL (Guex and Peitsch, 1997; Schwede et al., 2000, 2003).

Method: Tm Index (TI) is predicted by a free software platform (<http://tm.life.nthu.edu.tw/>).
<https://doi.org/10.1016/j.combiolchem.2009.10.002>

Structure modeling: The recombinant protein sequence was modeled for a theoretical structure using SWISS-MODEL (Guex and Peitsch, 1997; Schwede et al., 2000, 2003).