AVIDD ASAP: MERS mPRO aka 3CL protease

PAGE22-01340

Author: **Fairhead, Michael**
Date Started: **2022-Jul-21**
Experiment Started:
Projects: **Cloning**;**ASAP**
Related Pages:
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Tags:

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GenBank: AHC74086.1

DEFINITION orf1a [Middle East respiratory syndrome-related coronavirus].

ORF1a, partial [Middle East respiratory syndrome-related coronavirus]

GenBank: AHI48615.1

GenPept Identical Proteins Graphics

>AHI48615.1 ORF1a, partial [Middle East respiratory syndrome-related coronavirus]
LSVASTYFLVRLLQDKTGDFMSTIITSCQTAVSKLLDTCFEATEATFNFLLDLAGLFRIFLRNAYVYTSQ
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GSCIKSVVRHLGVTKGILTGCFSFVKMLFMLPLAYFSDSKLGTTEVKVSALKTAGVVTGNVVKQCCTAAV
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VLNSLCICFVASIPLCIVPYTALYYYATFYFTNEPAFIMHVSWYIMFGPIVPIWMTCVYTVAMCFRHFFW
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YREAAACHLAKALQTYSETGSDLLYQPPNCSITSGVLQSGLVKMSHPSGDVEACMVQVTCGSMTLNGLWL
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AYTFTTVKPGAAFSVLACYNGRPTGTFTVVMRPNYTIKGSFLCGSCGSVGYTKEGSVINFCYMHQMELAN
GTHTGSAFDGTMYGAFMDKQVHQVQLTDKYCSVNVVAWLYAAILNGCAWFVKPNRTSVVSFNEWALANQF
TEFVGTQSVDMLAVKTGVAIEQLLYAIQQLYTGFQGKQILGSTMLEDEFTPEDVNMQIMGVVMQSGVRKV
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CLTYANIVYEPTTPISSALIAVANWLAPTNAYMRTTHTDIGVYISMSLVLVIVVKRLYNPSLSNFALALC
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NVDLDALASDIFDTPSVLQATLSEFSHLATFAELEAAQKAYQEAMDSGDTSPQVLKALQKAVNIAKNAYE
KDKAVARKLERMADQAMTSMYKQARAEDKKAKIVSAMQTMLFGMIKKLDNDVLNGIISNARNGCIPLSVI
PLCASNKLRVVIPDFTVWNQVVTYPSLNYAGALWDITVINNVDNEIVKSSDVVDSNENLTWPLVLECTRA
STSAVKLQNNEIKPSGLKTMVVSAGQEQTNCNTSSLAYYEPVQGRKMLMALLSDNAYLKWARVEGKDGFV
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PQKAYLDFVNAGGAPLTNCVKMLTPKTGTGIAISVKPESTADQETYGGASVCLYCRAHIEHPDVSGVCKY
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3251..3547

region_name="betaCoV_Nsp5_Mpro"

note="betacoronavirus non-structural protein 5, also called Main protease (Mpro); cd21666"

db_xref="CDD:394887"

YAALRNSLTNDAYSRFLGLFNKYKYFSGAMETAAYREAAACHLAKALQTYSETGSDLLYQPPNCSITSGVLQSGLVKMSHPSGDVEACMV
QVTCGSMTLNGLWLDNTVWCPRHVMCPADQLSDPNYDALLISMTNHSFSVQKHIGAPANLRVVGHAMQGTLLKLTVDVANPSTPAYTFTT
VKPGAAFSVLACYNGRPTGTFTVVMRPNYTIKGSFLCGSCGSVGYTKEGSVINFCYMHQMELANGTHTGSAFDGTMYGAFMDKQVHQVQL
TDKYCSVNVVAWLYAAILNGCAWFVKPNRTSVVSFNEWALANQFTEFVGTQSVDMLAVKTGVAIEQLLYAIQQLYTGFQGKQILGSTMLE
DEFTPEDVNMQIMGVVMQSGVRKVTYGTAHWLFATLVSTYVIILQATKFTLWNYLFETIPTQLFPLLFVTMAFVMLLVKHKHTF

Mature protease

SGLVKMSHPSGDVEACMVQVTCGSMTLNGLWLDNTVWCPRHVMCPADQLSDPNYDALLISMTNHSFSVQKHIGAPANLRVVGHAMQGTLL
KLTVDVANPSTPAYTFTTVKPGAAFSVLACYNGRPTGTFTVVMRPNYTIKGSFLCGSCGSVGYTKEGSVINFCYMHQMELANGTHTGSAF
DGTMYGAFMDKQVHQVQLTDKYCSVNVVAWLYAAILNGCAWFVKPNRTSVVSFNEWALANQFTEFVGTQSVDMLAVKTGVAIEQLLYAIQ
QLYTGFQGKQILGSTMLEDEFTPEDVNMQIMGVVMQ

7D3C 2.2 P 21 21 21

4WMF 2.0 P 21 21 21

4WME 1.6 C 1 2 1

4WMD 2.6 C 2 2 21

7DR9 2.8 P 21 21 21

7DR8 2.3 P 21 21 21

7DRA 2.8 P 21 21 21

7ENE 3.0 P 1 21 1

4YLU 2.1 P 1 21 1

5C3N 3.0 C 2 2 21

4RSP 1.6 C 1 2 1

these all have the sequence

SGLVKMSHPSGDVEACMVQVTCGSMTLNGLWLDNTVWCPRHVMCPADQLSDPNYDALLISMTNHSFSVQKHIGAPANLRVVGHAMQGTLL
KLTVDVANPSTPAYTFTTVKPGAAFSVLACYNGRPTGTFTVVMRPNYTIKGSFLCGSCGSVGYTKEGSVINFCYMHQMELANGTHTGSAF
DGTMYGAFMDKQVHQVQLTDKYCSVNVVAWLYAAILNGCAWFVKPNRTSVVSFNEWALANQFTEFVGTQSVDMLAVKTGVAIEQLLYAIQ
QLYTGFQGKQILGSTMLEDEFTPEDVNMQIMGVVMQ

Since the protein is self cleaving it is purified without any tags but using HIC,IEX,SEC

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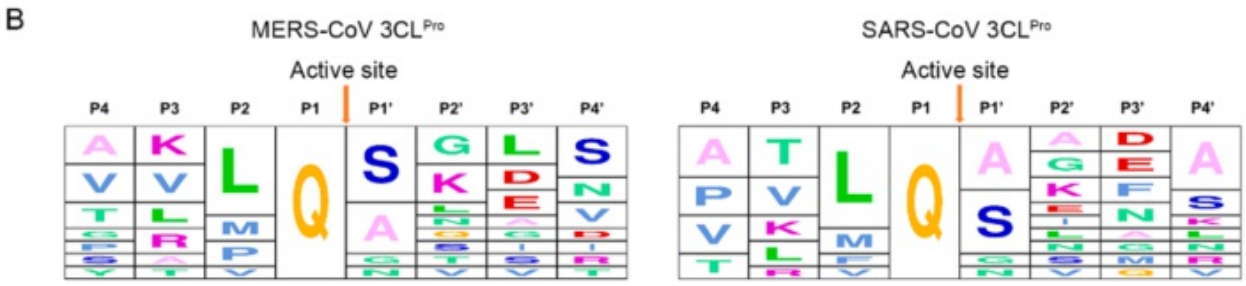
https://dx.doi.org/10.1021/acscatal.0c00110 ACS Catal. 2020, 10, 5871−5890



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6VH2	2.26	P 21 21 21
6VH3	2.2	P 21 21 21
6VH0	1.95	P 21 21 21
6VH1	2.3	P 21 21 21
6VGY	2.05	P 21 21 21
6VGZ	2.25	P 21 21 21
5WKM	2.25	C 1 2 1
5WKK	1.55	C 1 2 1
5WKL	1.85	C 1 2 1
5WKJ	2.05	C 1 2 1
8E6E	1.5	C 1 2 1
8E6B	1.55	P 1 21 1
8E6D	2.7	C 1 2 1
8E6C	2.7	C 1 2 1
7TQ8	1.65	C 1 2 1
7TQ7	1.7	C 1 2 1
8DGY	1.65	C 1 2 1

8CZT 2.1 C 1 2 1

8CZV 1.95 C 1 2 1

8CZU 2.7 C 1 2 1

7T3Z 1.95 P 1 21 1

7T3Y 1.9 P 21 21 21

7T40 1.7 C 1 2 1

7T41 2.1 C 1 2 1

these all have the sequence

MHHHHHHSGLVKMSHPSGDVEACMVQVTCGSMTLNGLWLDNTVWCPRHVMCPADQLSDPNYDALLISMTNHSFSVQKHIGAPANLRVVGH
AMQGTLLKLTVDVANPSTPAYTFTTVKPGAAFSVLACYNGRPTGTFTVVMRPNYTIKGSFLCGSCGSVGYTKEGSVINFCYMHQMELANG
THTGSAFDGTMYGAFMDKQVHQVQLTDKYCSVNVVAWLYAAILNGCAWFVKPNRTSVVSFNEWALANQFTEFVGTQSVDMLAVKTGVAIE
QLLYAIQQLYTGFQGKQILGSTMLEDEFTPEDVNMQIMGVVMQ

Purified using IMAC,SEC

Possible mutants

As well as the obvious active site mutant C148A

SGLVKMSHPSGDVEACMVQVTCGSMTLNGLWLDNTVWCPRHVMCPADQLSDPNYDALLISMTNHSFSVQKHIGAPANLRVVGHAMQGTLL
KLTVDVANPSTPAYTFTTVKPGAAFSVLACYNGRPTGTFTVVMRPNYTIKGSFLCGSAGSVGYTKEGSVINFCYMHQMELANGTHTGSAF
DGTMYGAFMDKQVHQVQLTDKYCSVNVVAWLYAAILNGCAWFVKPNRTSVVSFNEWALANQFTEFVGTQSVDMLAVKTGVAIEQLLYAIQ
QLYTGFQGKQILGSTMLEDEFTPEDVNMQIMGVVMQ

One apparent issue with MERS MPRO is its interconversion from monomer to dimer upon substrate binding according to the below PLOS article the dissociation constant of the MERS dimer, as measured by AUC, in the absence of substrate is 7.7 uM while that of the SARS is more that 10 fold lower at 0.7 uM. In the presence of substrate the Dissociation constants are much more similar 0.7 and 1.7 for MERS and SARS respectively. The MERS monomer to dimer dissociation constant can be lowered by mutating M298 to R (1.1 uM).

SGLVKMSHPSGDVEACMVQVTCGSMTLNGLWLDNTVWCPRHVMCPADQLSDPNYDALLISMTNHSFSVQKHIGAPANLRVVGHAMQGTLL
KLTVDVANPSTPAYTFTTVKPGAAFSVLACYNGRPTGTFTVVMRPNYTIKGSFLCGSCGSVGYTKEGSVINFCYMHQMELANGTHTGSAF
DGTMYGAFMDKQVHQVQLTDKYCSVNVVAWLYAAILNGCAWFVKPNRTSVVSFNEWALANQFTEFVGTQSVDMLAVKTGVAIEQLLYAIQ
QLYTGFQGKQILGSTMLEDEFTPEDVNRQIMGVVMQ

Making this mutation also removes any co-operativity observed in kinetics, Hill coefficient of 0

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MERS ...DEFTPEDVNMQIMGVVMQ
OC43 COV ...DELTPSDVYQQLAGIKLQ
SARS COV ...DEFTPFDVVRQCSGVTFQ
HKu9 ...DEHTPYDIGRQMLGVKLQ
SARS COV2 ...DEFTPFDVVRQCSGVTFQ
```

MERS gene as from Claire at DLS

tcctgcatcttttaatcaaatcccaagatgtgtataaacgcgccggtatgtacaggaagaggtttatactaaactgttacattgcaaacgtggtttcgtgtgccaagtgtgaa
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NGRPTGTFTVVMRPNYTIKGSFLCGSCGSVGYTKEGSVINFCYMHQMELANGTHTGSAFDGTMYGAFMDKQVHQVQLTDKYCSVNVVAWLYAAILNGCAWFVKPNRTSVVSFN
EWALANQFTEFVGTQSVDMLAVKTGVAIEQLLYAIQQLYTGFQGKQILGSTMLEDEFTPEDVNMQIMGVVMQ

MERS
SGLVKMSHPSGDVEACMVQVTCGSMTLNGLWLDNTVWCPRHVMCPADQLSDPNYDALLISMTNHSFSVQKHIGAPANLRVVGHAMQGTLLKLTVDVANPSTPAYTFTTVKPGA
AFSVLACYNGRPTGTFTVVMRPNYTIKGSFLCGSCGSVGYTKEGSVINFCYMHQMELANGTHTGSAFDGTMYGAFMDKQVHQVQLTDKYCSVNVVAWLYAAILNGCAWFVKPN
RTSVVSFNEWALANQFTEFVGTQSVDMLAVKTGVAIEQLLYAIQQLYTGFQGKQILGSTMLEDEFTPEDVNMQIMGVVMQ

```
cta tac tgt aaa tta cat ttt att tac aaa aga agg aga tat acc atg ggt agc agc cat
  M   G   S   S   H
cac cat cat cat cac ggg agc gat agc gaa gtg aac cag gaa gcg aaa ccg gaa gtt aaa
 H   H   H   H   H   G   S   D   S   E   V   N   Q   E   A   K   P   E   V   K
ccg gaa gtg aaa ccg gaa acc cat att aat ctg aaa gtt agc gac ggc agc agc gaa atc
 P   E   V   K   P   E   T   H   I   N   L   K   V   S   D   G   S   S   E   I
ttt ttt aaa att aaa aaa acc acc ccg ctg cgt cgc ctg atg gaa gcc ttt gcg aaa cgt
 F   F   K   I   K   K   T   T   P   L   R   R   L   M   E   A   F   A   K   R
cag ggt aaa gaa atg gat agc ctg cgc ttt ctg tat gac ggc atc cgt att cag gcc gat
 Q   G   K   E   M   D   S   L   R   F   L   Y   D   G   I   R   I   Q   A   D
cag acc ccg gaa gac ctg gat atg gaa gac aac gat att att gaa gcg cat cgc gaa cag
 Q   T   P   E   D   L   D   M   E   D   N   D   I   I   E   A   H   R   E   Q
atc ggt ggt tcg ggc ctg gta aag atg tca cat cca agc ggt gat gta gaa gcg tgc atg
 I   G   G   S   G   L   V   K   M   S   H   P   S   G   D   V   E   A   C   M
```

```
gtg cag gtt acg tgc ggg tct atg acg ctt aat ggt ctt tgg ctt gat aac aca gtg tgg
 V   Q   V   T   C   G   S   M   T   L   N   G   L   W   L   D   N   T   V   W
tgc ccg cgc cat gtt atg tgc cca gcg gat cag ttg agt gac cct aat tat gac gcg ctg
 C   P   R   H   V   M   C   P   A   D   Q   L   S   D   P   N   Y   D   A   L
tta att agt atg act aat cac tcc ttc tcc gtt cag aaa cat atc ggc gca ccg gcg aac
 L   I   S   M   T   N   H   S   F   S   V   Q   K   H   I   G   A   P   A   N
ctt cgc gtc gtt gga cac gct atg caa ggg aca ctt ctt aag ttg acg gtt gat gtt gca
 L   R   V   V   G   H   A   M   Q   G   T   L   L   K   L   T   V   D   V   A
aat cct agc acc cca gca tac act ttc acg acc gtt aag ccc ggt gcc gca ttt agt gtc
 N   P   S   T   P   A   Y   T   F   T   T   V   K   P   G   A   A   F   S   V
ctt gct tgc tac aat gga cgt ccg acg gga aca ttc act gtt gtt atg cgc cct aat tat
 L   A   C   Y   N   G   R   P   T   G   T   F   T   V   V   M   R   P   N   Y
acg att aaa ggt agt ttc ctg tgt gga tca tgt ggc agt gta ggg tat acc aaa gaa ggg
 T   I   K   G   S   F   L   C   G   S   C   G   S   V   G   Y   T   K   E   G
tcc gtt att aac ttc tgt tac atg cac caa atg gaa ttg gcc aac gga act cac acc ggt
 S   V   I   N   F   C   Y   M   H   Q   M   E   L   A   N   G   T   H   T   G
tca gct ttc gac gga act atg tac gga gcg ttt atg gat aag cag gta cac cag gta caa
 S   A   F   D   G   T   M   Y   G   A   F   M   D   K   Q   V   H   Q   V   Q
ctt aca gat aag tat tgt tca gtg aac gtg gtt gcc tgg ctt tac gcg gct att ttg aat
 L   T   D   K   Y   C   S   V   N   V   V   A   W   L   Y   A   A   I   L   N
ggg tgt gca tgg ttt gtc aaa ccc aac cgt aca agt gta gtg tca ttc aat gag tgg gcg
 G   C   A   W   F   V   K   P   N   R   T   S   V   V   S   F   N   E   W   A
tta gct aat caa ttc acc gaa ttt gtt ggc acc cag tca gtg gat atg ctg gca gtg aaa
 L   A   N   Q   F   T   E   F   V   G   T   Q   S   V   D   M   L   A   V   K
act gga gtc gct att gag caa tta tta tat gcc atc cag cag tta tat acc ggc ttc cag
 T   G   V   A   I   E   Q   L   L   Y   A   I   Q   Q   L   Y   T   G   F   Q
ggt aag caa att tta ggt agc act atg ctg gaa gac gag ttc act ccc gaa gac gtt aat
 G   K   Q   I   L   G   S   T   M   L   E   D   E   F   T   P   E   D   V   N
atg cag atc atg gga gtg gtg atg caa tga agc ttt cta gac cat ttt gtg att aac ctc
 M   Q   I   M   G   V   V   M   Q   -
```

```
cta tac tgt aaa tta cat ttt att tac aaa aga agg aga tat acc atg ggt agc agc cat
cac cat cat cat cac ggg agc gat agc gaa gtg aac cag gaa gcg aaa ccg gaa gtt aaa
ccg gaa gtg aaa ccg gaa acc cat att aat ctg aaa gtt agc gac ggc agc agc gaa atc
ttt ttt aaa att aaa aaa acc acc ccg ctg cgt cgc ctg atg gaa gcc ttt gcg aaa cgt
cag ggt aaa gaa atg gat agc ctg cgc ttt ctg tat gac ggc atc cgt att cag gcc gat
cag acc ccg gaa gac ctg gat atg gaa gac aac gat att att gaa gcg cat cgc gaa cag
atc ggt ggt tcg ggc ctg gta aag atg tca cat cca agc ggt gat gta gaa gcg tgc atg
gtg cag gtt acg tgc ggg tct atg acg ctt aat ggt ctt tgg ctt gat aac aca gtg tgg
tgc ccg cgc cat gtt atg tgc cca gcg gat cag ttg agt gac cct aat tat gac gcg ctg
tta att agt atg act aat cac tcc ttc tcc gtt cag aaa cat atc ggc gca ccg gcg aac
ctt cgc gtc gtt gga cac gct atg caa ggg aca ctt ctt aag ttg acg gtt gat gtt gca
aat cct agc acc cca gca tac act ttc acg acc gtt aag ccc ggt gcc gca ttt agt gtc
ctt gct tgc tac aat gga cgt ccg acg gga aca ttc act gtt gtt atg cgc cct aat tat
acg att aaa ggt agt ttc ctg tgt gga tca tgt ggc agt gta ggg tat acc aaa gaa ggg
tcc gtt att aac ttc tgt tac atg cac caa atg gaa ttg gcc aac gga act cac acc ggt
tca gct ttc gac gga act atg tac gga gcg ttt atg gat aag cag gta cac cag gta caa
ctt aca gat aag tat tgt tca gtg aac gtg gtt gcc tgg ctt tac gcg gct att ttg aat
ggg tgt gca tgg ttt gtc aaa ccc aac cgt aca agt gta gtg tca ttc aat gag tgg gcg
tta gct aat caa ttc acc gaa ttt gtt ggc acc cag tca gtg gat atg ctg gca gtg aaa
act gga gtc gct att gag caa tta tta tat gcc atc cag cag tta tat acc ggc ttc cag
ggt aag caa att tta ggt agc act atg ctg gaa gac gag ttc act ccc gaa gac gtt aat
atg cag atc atg gga gtg gtg atg caa tga agc ttt cta gac cat ttt gtg att aac ctc
```

Andre is concerned that this construct does not allow the processsing of the N-terminus of MERS by itself which prevents it undergoing an induced fit mechanism that generates the native dimer preferably the protein would be made with a native N-terminus and allowed to process itself raises the question of how to purify it

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TWIST GENE ORDER

GENE AS ORDERED BY ELLIE

```
>Mike_F_Mers
AAAAAAGGTCTCACATGGACTTACTCTATCAACCGCCAAATTGTTCTATAACGTCTGGCGTTCTGCAATCTGGTCTCGTTAAGATGTCCC
ATCCTAGCGGAGACGTCGAGGCGTGTATGGTACAAGTTACGTGCGGGAGCATGACGTTAAACGGATTGTGGCTCGACAATACAGTTTGGT
GCCCACGACATGTCATGTGTCCGGCTGATCAATTAAGTGACCCAAATTACGACGCGTTGCTCATATCCATGACGAACCACAGCTTTAGCG
TCCAAAAGCACATTGGTGCCCCTGCTAACCTTCGGGTCGTTGGCCATGCTATGCAGGGCACTCTTTTAAAGCTGACAGTTGACGTTGCTA
ACCCTAGTACGCCTGCCTATACATTCACTACGGTTAAACCCGGCGCCGCGTTCTCCGTTTTGGCATGTTACAACGGTCGTCCGACAGGTA
CGTTCACAGTAGTTATGCGGCCAAATTATACCATCAAAGGTAGCTTCCTTTGCGGTTCATGTGGTAGTGTGGGCTACACCAAAGAGGGCT
CGGTGATAAATTTCTGTTACATGCACCAAATGGAACTTGCAAACGGGACCCACACCGGTAGTGCGTTTGATGGTACCATGTATGGAGCTT
TCATGGACAAGCAAGTTCACCAAGTGCAACTGACCGATAAGTACTGCTCTGTTAATGTCGTGGCTTGGCTGTATGCCGCCATCTTGAACG
GCTGCGCCTGGTTTGTCAAGCCAAATCGCACGTCCGTTGTAAGCTTCAACGAGTGGGCCCTGGCAAACCAATTCACTGAGTTCGTTGGGA
CCCAGTCTGTCGACATGTTGGCCGTTAAGACGGGTGTAGCGATAGAGCAGCTGCTTTACGCAATCCAACAATTATACACAGGTTTCCAGG
GAAAGCAGATTCTCGGTTCTACAATGTTAGAGGACGAATTCACGCCCGAGGACGTGAACATGCAGATCATGGGCGTTGTCATGCAATCCG
GTGTCCGGAAGGTTACTTACGGTACAGCACACTGGCTGTAATGACTCGAGAGACCAAAAAA
```

```
aaaaa agg tct cac atg tct ata acg tct ggc gttctg caa tct gg
```

```
aaaaa agg tct cac atg gac tta ctc tat caa ccg cca aat tgt tct ata acg tct ggc gtt
                  M   D   L   L   Y   Q   P   P   N   C   S   I   T   S   G   V

caa tct ggt ctc gtt aag atg tcc cat cct ag
ctg caa tct ggt ctc gtt aag atg tcc cat cct agc gga gac gtc gag gcg tgt atg gta
 L   Q   S   G   L   V   K   M   S   H   P   S   G   D   V   E   A   C   M   V
caa gtt acg tgc ggg agc atg acg tta aac gga ttg tgg ctc gac aat aca gtt tgg tgc
 Q   V   T   C   G   S   M   T   L   N   G   L   W   L   D   N   T   V   W   C
cca cga cat gtc atg tgt ccg gct gat caa tta agt gac cca aat tac gac gcg ttg ctc
 P   R   H   V   M   C   P   A   D   Q   L   S   D   P   N   Y   D   A   L   L
ata tcc atg acg aac cac agc ttt agc gtc caa aag cac att ggt gcc cct gct aac ctt
 I   S   M   T   N   H   S   F   S   V   Q   K   H   I   G   A   P   A   N   L
cgg gtc gtt ggc cat gct atg cag ggc act ctt tta aag ctg aca gtt gac gtt gct aac
 R   V   V   G   H   A   M   Q   G   T   L   L   K   L   T   V   D   V   A   N
cct agt acg cct gcc tat aca ttc act acg gtt aaa ccc ggc gcc gcg ttc tcc gtt ttg
 P   S   T   P   A   Y   T   F   T   T   V   K   P   G   A   A   F   S   V   L
gca tgt tac aac ggt cgt ccg aca ggt acg ttc aca gta gtt atg cgg cca aat tat acc
 A   C   Y   N   G   R   P   T   G   T   F   T   V   V   M   R   P   N   Y   T
atc aaa ggt agc ttc ctt tgc ggt tca tgt ggt agt gtg ggc tac acc aaa gag ggc tcg
 I   K   G   S   F   L   C   G   S   C   G   S   V   G   Y   T   K   E   G   S
gtg ata aat ttc tgt tac atg cac caa atg gaa ctt gca aac ggg acc cac acc ggt agt
 V   I   N   F   C   Y   M   H   Q   M   E   L   A   N   G   T   H   T   G   S
gcg ttt gat ggt acc atg tat gga gct ttc atg gac aag caa gtt cac caa gtg caa ctg
 A   F   D   G   T   M   Y   G   A   F   M   D   K   Q   V   H   Q   V   Q   L
acc gat aag tac tgc tct gtt aat gtc gtg gct tgg ctg tat gcc gcc atc ttg aac ggc
 T   D   K   Y   C   S   V   N   V   V   A   W   L   Y   A   A   I   L   N   G
tgc gcc tgg ttt gtc aag cca aat cgc acg tcc gtt gta agc ttc aac gag tgg gcc ctg
 C   A   W   F   V   K   P   N   R   T   S   V   V   S   F   N   E   W   A   L
gca aac caa ttc act gag ttc gtt ggg acc cag tct gtc gac atg ttg gcc gtt aag acg
 A   N   Q   F   T   E   F   V   G   T   Q   S   V   D   M   L   A   V   K   T
ggt gta gcg ata gag cag ctg ctt tac gca atc caa caa tta tac aca ggt ttc cag gga
 G   V   A   I   E   Q   L   L   Y   A   I   Q   Q   L   Y   T   G   F   Q   G
aag cag att ctc ggt tct aca atg tta gag gac gaa ttc acg ccc gag gac gtg aac atg
 K   Q   I   L   G   S   T   M   L   E   D   E   F   T   P   E   D   V   N   M

g cag atc atg ggc gtt gtc atg caa taa tga ctc gag aga cca aaa
gtc atg caa tcc ggt gtc cgg aag taa tga ctc gag aga cca aaa
cag atc atg ggc gtt gtc atg caa tcc ggt gtc cgg aag gtt act tac ggt aca gca cac
  Q   I   M   G   V   V   M   Q   S   G   V   R   K   V   T   Y   G   T   A   H
tgg ctg taa tga ctc gag aga cca aaa
 W   L   -   -
```

```
MDLLYQPPNCSITSGVLQSGLVKMSHPSGDVEACMVQVTCGSMTLNGLWLDNTVWCPRHVMCPADQLSDPNYDALLISMTNHSFSVQKHI
GAPANLRVVGHAMQGTLLKLTVDVANPSTPAYTFTTVKPGAAFSVLACYNGRPTGTFTVVMRPNYTIKGSFLCGSCGSVGYTKEGSVINF
CYMHQMELANGTHTGSAFDGTMYGAFMDKQVHQVQLTDKYCSVNVVAWLYAAILNGCAWFVKPNRTSVVSFNEWALANQFTEFVGTQSVD
MLAVKTGVAIEQLLYAIQQLYTGFQGKQILGSTMLEDEFTPEDVNMQIMGVVMQSGVRKVTYGTAHWL
```

Versions to make

```
aaaaa agg tct cac atg tct ata acg tct ggc gttctg caa tct gg MERS FP1-GG

SITSGVLQSGLVKMSHPSGDVEACMVQVTCGSMTLNGLWLDNTVWCPRHVMCPADQLSDPNYDALLISMTNHSFSVQKHIGAPANLRVVG
HAMQGTLLKLTVDVANPSTPAYTFTTVKPGAAFSVLACYNGRPTGTFTVVMRPNYTIKGSFLCGSCGSVGYTKEGSVINFCYMHQMELAN
GTHTGSAFDGTMYGAFMDKQVHQVQLTDKYCSVNVVAWLYAAILNGCAWFVKPNRTSVVSFNEWALANQFTEFVGTQSVDMLAVKTGVAI
EQLLYAIQQLYTGFQGKQILGSTMLEDEFTPEDVNMQIMGVVMQSGVRK

gtc atg caa tcc ggt gtc cgg aag taa tga ctc gag aga cca aaa

TTT TGG TCT CTC GAG TCA TTA CTT CCG GAC ACC GGA TTG CAT GAC MERS RP1-GG



aaaaa agg tct cac atg tct ata acg tct ggc gttctg caa tct gg MERS FP1-GG

SITSGVLQSGLVKMSHPSGDVEACMVQVTCGSMTLNGLWLDNTVWCPRHVMCPADQLSDPNYDALLISMTNHSFSVQKHIGAPANLRVVG
HAMQGTLLKLTVDVANPSTPAYTFTTVKPGAAFSVLACYNGRPTGTFTVVMRPNYTIKGSFLCGSCGSVGYTKEGSVINFCYMHQMELAN
GTHTGSAFDGTMYGAFMDKQVHQVQLTDKYCSVNVVAWLYAAILNGCAWFVKPNRTSVVSFNEWALANQFTEFVGTQSVDMLAVKTGVAI
EQLLYAIQQLYTGFQGKQILGSTMLEDEFTPEDVNMQIMGVVMQ

g cag atc atg ggc gtt gtc atg caa taa tga ctc gag aga cca aaa

TTT TGG TCT CTC GAG TCA TTA TTG CAT GAC AAC GCC CAT GAT CTG C mers-rp2-gg
```

Clone into pTXB1 via NdeI and SpeI

aaaaa cat atg tct ata acg tct ggc gttctg caa tct gg MERS FP PTXB1

SITSGVLQSGLVKMSHPSGDVEACMVQVTCGSMTLNGLWLDNTVWCPRHVMCPADQLSDPNYDALLISMTNHSFSVQKHIGAPANLRVVG
HAMQGTLLKLTVDVANPSTPAYTFTTVKPGAAFSVLACYNGRPTGTFTVVMRPNYTIKGSFLCGSCGSVGYTKEGSVINFCYMHQMELAN
GTHTGSAFDGTMYGAFMDKQVHQVQLTDKYCSVNVVAWLYAAILNGCAWFVKPNRTSVVSFNEWALANQFTEFVGTQSVDMLAVKTGVAI
EQLLYAIQQLYTGFQGKQILGSTMLEDEFTPEDVNMQIMGVVMQ-INTEIN

g cag atc atg ggc gtt gtc atg caa TGCATCACGGGAGATGCACTAGTTGCCCT

AGG GCA ACT AGT GCA TCT CCC GTG ATG CAT TGC ATG ACA ACG CCC ATG ATC TGC MERS RP PTXB1

Andres Designs to improve N-terminal processing

aaaaa agg tct cac atg tcc gct gtc ctg caa tct ggt ctc gtt aag atg tcc cat cct ag MERS FP2-GG

MSAVLQSGLVKMSHPSGDVEACMVQVTCGSMTLNGLWLDNTVWCPRHVMCPADQLSDPNYDALLISMTNHSFSVQKHIGAPANLRVVGHA
MQGTLLKLTVDVANPSTPAYTFTTVKPGAAFSVLACYNGRPTGTFTVVMRPNYTIKGSFLCGSCGSVGYTKEGSVINFCYMHQMELANGT
HTGSAFDGTMYGAFMDKQVHQVQLTDKYCSVNVVAWLYAAILNGCAWFVKPNRTSVVSFNEWALANQFTEFVGTQSVDMLAVKTGVAIEQ
LLYAIQQLYTGFQGKQILGSTMLEDEFTPEDVNMQIMGVVMQ

g cag atc atg ggc gtt gtc atg caa taa tga ctc gag aga cca aaa

TTT TGG TCT CTC GAG TCA TTA TTG CAT GAC AAC GCC CAT GAT CTG C mers-rp2-gg

aaaaa agg tct cac atg tcc gct gtc atg caa tct ggt ctc gtt aag atg tcc cat cct ag MERS FP3-GG

MSAVMQSGLVKMSHPSGDVEACMVQVTCGSMTLNGLWLDNTVWCPRHVMCPADQLSDPNYDALLISMTNHSFSVQKHIGAPANLRVVGHA
MQGTLLKLTVDVANPSTPAYTFTTVKPGAAFSVLACYNGRPTGTFTVVMRPNYTIKGSFLCGSCGSVGYTKEGSVINFCYMHQMELANGT
HTGSAFDGTMYGAFMDKQVHQVQLTDKYCSVNVVAWLYAAILNGCAWFVKPNRTSVVSFNEWALANQFTEFVGTQSVDMLAVKTGVAIEQ
LLYAIQQLYTGFQGKQILGSTMLEDEFTPEDVNMQIMGVVMQ

g cag atc atg ggc gtt gtc atg caa taa tga ctc gag aga cca aaa

TTT TGG TCT CTC GAG TCA TTA TTG CAT GAC AAC GCC CAT GAT CTG C mers-rp2-gg

aaaaa agg tct cac atg tcc gct gtc CCT caa tct ggt ctc gtt aag atg tcc cat cct ag MERS-FP4-GG

MSAVPQSGLVKMSHPSGDVEACMVQVTCGSMTLNGLWLDNTVWCPRHVMCPADQLSDPNYDALLISMTNHSFSVQKHIGAPANLRVVGHA
MQGTLLKLTVDVANPSTPAYTFTTVKPGAAFSVLACYNGRPTGTFTVVMRPNYTIKGSFLCGSCGSVGYTKEGSVINFCYMHQMELANGT
HTGSAFDGTMYGAFMDKQVHQVQLTDKYCSVNVVAWLYAAILNGCAWFVKPNRTSVVSFNEWALANQFTEFVGTQSVDMLAVKTGVAIEQ
LLYAIQQLYTGFQGKQILGSTMLEDEFTPEDVNMQIMGVVMQ

g cag atc atg ggc gtt gtc atg caa taa tga ctc gag aga cca aaa

TTT TGG TCT CTC GAG TCA TTA TTG CAT GAC AAC GCC CAT GAT CTG C MERS-RP2-GG

Result

I K G S F L C G S **A** G S V
CATCAAAGGTAGCTTCCTTTGCGGTTCAgccGGTAGTGTGG
GTAGTTTCCATCGAAGGAAACGCCAAGTCGGCCATCACACC

Required Primers

Name (F/R)	Oligo (Uppercase = target-specific primer)	Len	% GC	Tm	Ta *
MERS_CtoA_F	TTGCGGTTCAgccGGTAGTGTGG	23	61	57°C	58°C
MERS_CtoA_R	AGGAAGCTACCTTTGATG	18	44	58°C	

* Ta (recommended annealing temperature)

Result

I K G S F L C G S S G S V
CATCAAAGGTAGCTTCCTTTGCGGTTCAtccGGTAGTGTGG
GTAGTTTCCATCGAAGGAAACGCCAAGTCGGCCATCACACC

Required Primers

Name (F/R)	Oligo (Uppercase = target-specific primer)	Len	% GC	Tm	Ta *
MERS_CtoS_F	TTGCGGTTCAtccGGTAGTGTGG	23	61	57°C	58°C
MERS_CtoS_R	AGGAAGCTACCTTTGATG	18	44	58°C	

* Ta (recommended annealing temperature)

Result

L D N T V W C P R A V M C
GCTCGACAATACAGTTTGGTGCCCACGAgctGTCATGTGTC
CGAGCTGTTATGTCAAACCACGGGTGCTCGACAGTACACAG

Required Primers

Name (F/R)	Oligo (Uppercase = target-specific primer)	Len	% GC	Tm	Ta *
MERS_HtoA_F	GTGCCCACGAgctGTCATGTGTC	23	61	56°C	57°C
MERS_HtoA_R	CAAACTGTATTGTCGAGC	18	44	58°C	

* Ta (recommended annealing temperature)

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COVID-19 is a deadly rampaging infectious disease with over 480 million cases worldwide. Unfortunately, effective therapies remain very limited. Novel antiviral agents are urgently needed to combat this global healthcare crisis. Here, we elucidate the structural basis for replicase polyprotein cleavage and substrate specificity of SARS-CoV-2 main protease (M^{pro}). Through analyzing a series of high-resolution structures of SARS-CoV-2 M^{pro} throughout the proteolytic process, we demonstrate the molecular mechanism of M^{pro} in proteolytic processing that confers substrate specificity. Substrate selectivity is revealed using structures of the H41A mutant in complex with six individual native cleavage substrates. Our study underscores the mechanistic function of M^{pro} in the viral life cycle, which provides structural insights to develop effective inhibitors against this essential target of SARS-CoV-2.

CVMPROA-c002

agtggttttagaaaaatggcattcccatctggtaaagttgagggttgtatggtacaagtaacttgtggtacaactacacttaacggtctt
tggcttgatgacgtagtttactgtccaagacatgtgatctgcacctctgaagacatgcttaaccctaattatgaagatttactcattcgt
aagtctaatcataatttcttggtacaggctggtaatgttcaactcagggttattggacattctatgcaaaattgtgtacttaagcttaag
gttgatacagccaatcctaagacacctaagtataagtttgttcgcattcaaccaggacagacttttcagtgttagcttgttacaatggt
tcaccatctggtgtttaccaatgtgctatgaggcccaatttcactattaagggttcattccttaatggttcatctggtagtgttggtttt
aacatagattatgactgtgtctcttttgttacatgcaccatatggaattaccaactggagttcatgctggcacagacttagaaggtaac
ttttatggacctttgttgacaggcaaacagcacaagcagctggtacggacacaactattacagttaatgttttagcttggttgtacgct
gctgttataaatggagacaggtggtttctcaatcgatttaccacaactcttaatgactttaaccttgtggctatgaagtacaattatgaa
cctctaacacaagaccatgttgacatactaggacctctttctgctcaaactggaattgccgttttagatatgtgtgcttcattaaaagaa
ttactgcaaaatggtatgaatggacgtaccatattgggtagtgctttattagaagatgaatttacacctttgatgttgttagacaatgc
tcaggtgttactttccaataa

SGFRKMAFPSGKVEGCMVQVTCGTTTLNGLWLDDVVYCPRHVICTSEDMLNPNYEDLLIRKSNHNFLVQAGNVQLRVIGHSMQNCVLKLK
VDTANPKTPKYKFVRIQPGQTFSVLACYNGSPSGVYQCAMRPNFTIKGSFLNGSSGSVGFNIDYDCVSFCYMHHMELPTGVHAGTDLEGN
FYGPFVDRQTAQAAGTDTTITVNVLAWLYAAVINGDRWFLNRFTTTLNDFNLVAMKYNYEPLTQDHVDILGPLSAQTGIAVLDMCASLKE
LLQNGMNGRTILGSALLEDEFTPFDVVRQCSGVTFQ-

5'3' Frame 1

agt	ggt	ttt	aga	aaa	atg	gca	ttc	cca	tct	ggt	aaa	gtt	gag	ggt	tgt	atg	gta	caa	gta
S	G	F	R	K	M	A	F	P	S	G	K	V	E	G	C	M	V	Q	V
act	tgt	ggt	aca	act	aca	ctt	aac	ggt	ctt	tgg	ctt	gat	gac	gta	gtt	tac	tgt	cca	aga
T	C	G	T	T	T	L	N	G	L	W	L	D	D	V	V	Y	C	P	R
cat	gtg	atc	tgc	acc	tct	gaa	gac	atg	ctt	aac	cct	aat	tat	gaa	gat	tta	ctc	att	cgt

```
 H   V   I   C   T   S   E   D   M   L   N   P   N   Y   E   D   L   L   I   R
aag tct aat cat aat ttc ttg gta cag gct ggt aat gtt caa ctc agg gtt att gga cat
 K   S   N   H   N   F   L   V   Q   A   G   N   V   Q   L   R   V   I   G   H
tct atg caa aat tgt gta ctt aag ctt aag gtt gat aca gcc aat cct aag aca cct aag
 S   M   Q   N   C   V   L   K   L   K   V   D   T   A   N   P   K   T   P   K
tat aag ttt gtt cgc att caa cca gga cag act ttt tca gtg tta gct tgt tac aat ggt
 Y   K   F   V   R   I   Q   P   G   Q   T   F   S   V   L   A   C   Y   N   G
tca cca tct ggt gtt tac caa tgt gct atg agg ccc aat ttc act att aag ggt tca ttc
 S   P   S   G   V   Y   Q   C   A   M   R   P   N   F   T   I   K   G   S   F
ctt aat ggt tca tct ggt agt gtt ggt ttt aac ata gat tat gac tgt gtc tct ttt tgt
 L   N   G   S   S   G   S   V   G   F   N   I   D   Y   D   C   V   S   F   C
tac atg cac cat atg gaa tta cca act gga gtt cat gct ggc aca gac tta gaa ggt aac
 Y   M   H   H   M   E   L   P   T   G   V   H   A   G   T   D   L   E   G   N
ttt tat gga cct ttt gtt gac agg caa aca gca caa gca gct ggt acg gac aca act att
 F   Y   G   P   F   V   D   R   Q   T   A   Q   A   A   G   T   D   T   T   I
aca gtt aat gtt tta gct tgg ttg tac gct gct gtt ata aat gga gac agg tgg ttt ctc
 T   V   N   V   L   A   W   L   Y   A   A   V   I   N   G   D   R   W   F   L
aat cga ttt acc aca act ctt aat gac ttt aac ctt gtg gct atg aag tac aat tat gaa
 N   R   F   T   T   T   L   N   D   F   N   L   V   A   M   K   Y   N   Y   E
cct cta aca caa gac cat gtt gac ata cta gga cct ctt tct gct caa act gga att gcc
 P   L   T   Q   D   H   V   D   I   L   G   P   L   S   A   Q   T   G   I   A
gtt tta gat atg tgt gct tca tta aaa gaa tta ctg caa aat ggt atg aat gga cgt acc
 V   L   D   M   C   A   S   L   K   E   L   L   Q   N   G   M   N   G   R   T
ata ttg ggt agt gct tta tta gaa gat gaa ttt aca cct ttt gat gtt gtt aga caa tgc
 I   L   G   S   A   L   L   E   D   E   F   T   P   F   D   V   V   R   Q   C
tca ggt gtt act ttc caa taa
 S   G   V   T   F   Q   -
```

Result

I K G S F L N G S C G S V
CTATTAAGGGTTCATTCCTTAATGGTTCAtgtGGTAGTGTTG
GATAATTCCCAAGTAAGGAATTACCAAGTACACCATCACAAC

Required Primers

Name (F/R)	Oligo (Uppercase = target-specific primer)	Len	% GC	Tm	Ta *
SARS_StoC_F	TAATGGTTCAtgtGGTAGTGTTG	23	39	59°C	57°C
SARS_StoC__R	AGGAATGAACCCTTAATAG	19	37	56°C	

* Ta (recommended annealing temperature)

Result

T I K G S F L N G S A G S V
CACTATTAAGGGTTCATTCCTTAATGGTTCAgctGGTAGTGTTG
GTGATAATTCCCAAGTAAGGAATTACCAAGTCGACCATCACAAC

Required Primers

Name (F/R)	Oligo (Uppercase = target-specific primer)	Len	% GC	Tm	Ta *
SARS_StoA_F	TAATGGTTCAgctGGTAGTGTTG	23	43	62°C	60°C
SARS_StoA_R	AGGAATGAACCCTTAATAGTG	21	38	59°C	

* Ta (recommended annealing temperature)

Result

L D D V V Y C P R A V I C T
GCTTGATGACGTAGTTTACTGTCCAAGAgctGTGATCTGCACC
CGAACTACTGCATCAAATGACAGGTTCTCGACACTAGACGTGG

Required Primers

Name (F/R)	Oligo (Uppercase = target-specific primer)	Len	% GC	Tm	Ta *
SARS_HtoA_F	CTGTCCAAGAgctGTGATCTGCACC	25	56	56°C	57°C
SARS_HtoA_R	TAAACTACGTCATCAAGC	18	39	56°C	

* Ta (recommended annealing temperature)

MVMPROA-c002

MERS PTXB1

ATGTCTATAACGTCTGGCGTTCTGCAATCTGGTCTCGTTAAGATGTCCCATCCTAGCGGAGACGTCGAGGCGTGTATGGTACAAGTTACG
TGCGGGAGCATGACGTTAAACGGATTGTGGCTCGACAATACAGTTTGGTGCCCACGACATGTCATGTGTCCGGCTGATCAATTAAGTGAC
CCAAATTACGACGCGTTGCTCATATCCATGACGAACCACAGCTTTAGCGTCCAAAAGCACATTGGTGCCCCTGCTAACCTTCGGGTCGTT
GGCCATGCTATGCAGGGCACTCTTTTAAAGCTGACAGTTGACGTTGCTAACCCTAGTACGCCTGCCTATACATTCACTACGGTTAAACCC
GGCGCCGCGTTCTCCGTTTTGGCATGTTACAACGGTCGTCCGACAGGTACGTTCACAGTAGTTATGCGGCCAAATTATACCATCAAAGGT
AGCTTCCTTTGCGGTTCATGTGGTAGTGTGGGCTACACCAAAGAGGGCTCGGTGATAAATTTCTGTTACATGCACCAAATGGAACTTGCA
AACGGGACCCACACCGGTAGTGCGTTTGATGGTACCATGTATGGAGCTTTCATGGACAAGCAAGTTCACCAAGTGCAACTGACCGATAAG
TACTGCTCTGTTAATGTCGTGGCTTGGCTGTATGCCGCCATCTTGAACGGCTGCGCCTGGTTTGTCAAGCCAAATCGCACGTCCGTTGTA
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ACGCCCGAGGACGTGAACATGCAGATCATGGGCGTTGTCATGCAATGCATCACGGGAGATGCACTAGTTGCCCTACCCGAGGGCGAGTCG
GTACGCATCGCCGACATCGTGCCGGGTGCGCGGCCCAACAGTGACAACGCCATCGACCTGAAAGTCCTTGACCGGCATGGCAATCCCGTG
CTCGCCGACCGGCTGTTCCACTCCGGCGAGCATCCGGTGTACACGGTGCGTACGGTCGAAGGTCTGCGTGTGACGGGCACCGCGAACCAC
CCGTTGTTGTGTTTGGTCGACGTCGCCGGGGTGCCGACCCTGCTGTGGAAGCTGATCGACGAAATCAAGCCGGGCGATTACGCGGTGATT
CAACGCAGCGCATTCAGCGTCGACTGTGCAGGTTTTGCCCGCGGGAAACCCGAATTTGCGCCCACAACCTACACAGTCGGCGTCCCTGGA
CTGGTGCGTTTCTTGGAAGCACACCACCGAGACCCGGACGCCCAAGCTATCGCCGACGAGCTGACCGACGGGCGGTTCTACTACGCGAAA
GTCGCCAGTGTCACCGACGCCGGCGTGCAGCCGGTGTATAGCCTTCGTGTCGACACGGCAGACCACGCGTTTATCACGAACGGGTTCGTC
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GGACAATTGGTCACATATAACGGCAAGACGTATAAATGTTTGCAGCCCCACACCTCCTTGGCAGGATGGGAACCATCCAACGTTCCTGCC
TTGTGGCAGCTTCAATGA

MSITSGVLQSGLVKMSHPSGDVEACMVQVTCGSMTLNGLWLDNTVWCPRHVMCPADQLSDPNYDALLISMTNHSFSVQKHI
GAPANLRVVGHAMQGTLLKLTVDVANPSTPAYTFTTVKPGAAFSVLACYNGRPTGTFTVVMRPNYTIKGSFLCGSCGSVGYTK
EGSVINFCYMHQMELANGTHTGSAFDGTMYGAFMDKQVHQVQLTDKYCSVNVVAWLYAAILNGCAWFVKPNRTSVVSFNE
WALANQFTEFVGTQSVDMLAVKTGVAIEQLLYAIQQLYTGFQGKQILGSTMLEDEFTPEDVNMQIMGVVMQCITGDALVALPE
GESVRIADIVPGARPNSDNAIDLKVLDRHGNPVLADRLFHSGEHPVYTVRTVEGLRVTGTANHPLLCLVDVAGVPTLLWKLIDE
IKPGDYAVIQRSAFSVDCAGFARGKPEFAPTTYTVGVPGLVRFLEAHHRDPDAQAIADELTDGRFYYAKVASVTDAGVQPVYSL
RVDTADHAFITNGFVSHATGLTGLNSGLTTNPGVSAWQVNTAYTAGQLVTYNGKTYKCLQPHTSLAGWEPSNVPALWQLQ-

471681101_MERS_1_T7R_F07.seq

NNNNNNNNTCNTTCGNNNNNTGTTAGCAGCCGGATCCCCTTCCTGCAGTCATTGAAGCTGCCACAAGGCAGGAACGTTGG
ATGGTTCCCATCCTGCCAAGGAGGTGTGGGGCTGCAAACATTTATACGTCTTGCCGTTATATGTGACCAATTGTCCCGCAGTA
TAAGCTGTGTTGACCTGCCAAGCGGATACACCAGGATTTGTCGTGAGGCCTGAGTTCAGACCGGTGAGGCCAGTAGCGTGG
CTGACGAACCCGTTCGTGATAAACGCGTGGTCTGCCGTGTCGACACGAAGGCTATACACCGGCTGCACGCCGGCGTCGGT
GACACTGGCGACTTTCGCGTAGTAGAACCGCCCGTCGGTCAGCTCGTCGGCGATAGCTTGGGCGTCCGGGTCTCGGTGGT
GTGCTTCCAAGAAACGCACCAGTCCAGGGACGCCGACTGTGTAGGTTGTGGGCGCAAATTCGGGTTTCCCGCGGGCAAAA
CCTGCACAGTCGACGCTGAATGCGCTGCGTTGAATCACCGCGTAATCGCCCGGCTTGATTTCGTCGATCAGCTTCCACAGCA
GGGTCGGCACCCCGGCGACGTCGACCAAACACAACAACGGGTGGTTCGCGGTGCCCGTCACACGCAGACCTTCGACCGTA
CGCACCGTGTACACCGGATGCTCGCCGGAGTGGAACAGCCGGTCGGCGAGCACGGGATTGCCATGCCGGTCAAGGACTTT
CAGGTCGATGGCGTTGTCACTGTTGGGCCGCGCACCCGGCACGATGTCGGCGATGCGTACCGACTCGCCCTCGGGTAGGG
CAACTAGTGCATCTCCCGTGATGCATTGCATGACAACGCCCATGATCTGCATGTTCACGTCCTCGGGCGTGAATTCGTCCTCT
AACATTGTAGAACCGAGAATCTGCTTTCCCTGGAAACCTGTGTATAATTGTTGGATTGCGTAAAGCAGCTGCTCTATCGCTAC
ACCCGTCTTAACGGCCAACATGTCGACAGACTGGGTCCCAACGAACTCAGTGAATTGGTTTGCCAGGGCCCACTCGTTGAA
GCTTACAACGGACGTGCGATTTGGCTTGACAAACCAGGCGCAGCCGTTCAAGATGGCGGCANACAGCCAAGCCACGACATT
AACAGAGCAGTACTTATCGGTCAGTTGCCCTTGGTGAACTTGCTTGTCCATGAAANGCTCCATAAATGGNACCATCAANCGC
CCTACCGGTNTGGGTCCCGTTTGCANGTTNCATTTNGGTGCNNNNAAAA

Score	Expect	Identities	Gaps	Strand
2182 bits(1181)	0.0	1198/1209(99%)	2/1209(0%)	Plus/Minus

```
Query  522   GCACC-AAATGGAACTTGCAAACGGGACCCACACCGGTAGTGCGTTTGATGGTACCATGT  580

Sbjct  1257  .....N.....N...N..............N........G...N........N....T.  1198
```

```
Query  581   ATGGAGC-TTTCATGGACAAGCAAGTTCACCAAGTGCAACTGACCGATAAGTACTGCTCT  639
Sbjct  1197  .......N.........................G.........................  1138

Query  640   GTTAATGTCGTGGCTTGGCTGTATGCCGCCATCTTGAACGGCTGCGCCTGGTTTGTCAAG  699
Sbjct  1137  ......................N.....................................  1078

Query  700   CCAAATCGCACGTCCGTTGTAAGCTTCAACGAGTGGGCCCTGGCAAACCAATTCACTGAG  759
Sbjct  1077  ............................................................  1018

Query  760   TTCGTTGGGACCCAGTCTGTCGACATGTTGGCCGTTAAGACGGGTGTAGCGATAGAGCAG  819
Sbjct  1017  ............................................................  958

Query  820   CTGCTTTACGCAATCCAACAATTATACACAGGTTTCCAGGGAAAGCAGATTCTCGGTTCT  879
Sbjct  957   ............................................................  898

Query  880   ACAATGTTAGAGGACGAATTCACGCCCGAGGACGTGAACATGCAGATCATGGGCGTTGTC  939
Sbjct  897   ............................................................  838

Query  940   ATGCAATGCATCACGGGAGATGCACTAGTTGCCCTACCCGAGGGCGAGTCGGTACGCATC  999
Sbjct  837   ............................................................  778

Query  1000  GCCGACATCGTGCCGGGTGCGCGGCCCAACAGTGACAACGCCATCGACCTGAAAGTCCTT  1059
Sbjct  777   ............................................................  718

Query  1060  GACCGGCATGGCAATCCCGTGCTCGCCGACCGGCTGTTCCACTCCGGCGAGCATCCGGTG  1119
Sbjct  717   ............................................................  658

Query  1120  TACACGGTGCGTACGGTCGAAGGTCTGCGTGTGACGGGCACCGCGAACCACCCGTTGTTG  1179
Sbjct  657   ............................................................  598

Query  1180  TGTTTGGTCGACGTCGCCGGGGTGCCGACCCTGCTGTGGAAGCTGATCGACGAAATCAAG  1239
Sbjct  597   ............................................................  538

Query  1240  CCGGGCGATTACGCGGTGATTCAACGCAGCGCATTCAGCGTCGACTGTGCAGGTTTTGCC  1299
```

```
Sbjct  537   ..........................................................  478

Query  1300  CGCGGGAAACCCGAATTTGCGCCCACAACCTACACAGTCGGCGTCCCTGGACTGGTGCGT  1359
Sbjct  477   ..........................................................  418

Query  1360  TTCTTGGAAGCACACCACCGAGACCCGGACGCCCAAGCTATCGCCGACGAGCTGACCGAC  1419
Sbjct  417   ..........................................................  358

Query  1420  GGGCGGTTCTACTACGCGAAAGTCGCCAGTGTCACCGACGCCGGCGTGCAGCCGGTGTAT  1479
Sbjct  357   ..........................................................  298

Query  1480  AGCCTTCGTGTCGACACGGCAGACCACGCGTTTATCACGAACGGGTTCGTCAGCCACGCT  1539
Sbjct  297   ..........................................................  238

Query  1540  ACTGGCCTCACCGGTCTGAACTCAGGCCTCACGACAAATCCTGGTGTATCCGCTTGGCAG  1599
Sbjct  237   ..........................................................  178

Query  1600  GTCAACACAGCTTATACTGCGGGACAATTGGTCACATATAACGGCAAGACGTATAAATGT  1659
Sbjct  177   ..........................................................  118

Query  1660  TTGCAGCCCCACACCTCCTTGGCAGGATGGGAACCATCCAACGTTCCTGCCTTGTGGCAG  1719
Sbjct  117   ..........................................................  58

Query  1720  CTTCAATGA  1728
Sbjct  57    .........  49
```

MXPFMEXFMDKQVHQGQLTDKYCSVNVVAWLXAAILNGCAWFVKPNRTSVVSFNEWALANQFTEFVGTQSVDMLAVKTGVA
IEQLLYAIQQLYTGFQGKQILGSTMLEDEFTPEDVNMQIMGVVMQCITGDALVALPEGESVRIADIVPGARPNSDNAIDLKVLD
RHGNPVLADRLFHSGEHPVYTVRTVEGLRVTGTANHPLLCLVDVAGVPTLLWKLIDEIKPGDYAVIQRSAFSVDCAGFARGKPE
FAPTTYTVGVPGLVRFLEAHHRDPDAQAIADELTDGRFYYAKVASVTDAGVQPVYSLRVDTADHAFITNGFVSHATGLTGLNSG
LTTNPGVSAWQVNTAYTAGQLVTYNGKTYKCLQPHTSLAGWEPSNVPALWQLQ

Score	Expect	Method	Identities	Positives	Gaps
780 bits(2014)	0.0	Compositional matrix adjust.	377/379(99%)	377/379(99%)	0/379(0%)

```
Query  197  FMDKQVHQVQLTDKYCSVNVVAWLYAAILNGCAWFVKPNRTSVVSFNEWALANQFTEFVG  256
Sbjct  8    ........G..............X.................................  67

Query  257  TQSVDMLAVKTGVAIEQLLYAIQQLYTGFQGKQILGSTMLEDEFTPEDVNMQIMGVVMQC  316
Sbjct  68   ............................................................  127

Query  317  ITGDALVALPEGESVRIADIVPGARPNSDNAIDLKVLDRHGNPVLADRLFHSGEHPVYTV  376
Sbjct  128  ............................................................  187

Query  377  RTVEGLRVTGTANHPLLCLVDVAGVPTLLWKLIDEIKPGDYAVIQRSAFSVDCAGFARGK  436
Sbjct  188  ............................................................  247

Query  437  PEFAPTTYTVGVPGLVRFLEAHHRDPDAQAIADELTDGRFYYAKVASVTDAGVQPVYSLR  496
Sbjct  248  ............................................................  307

Query  497  VDTADHAFITNGFVSHATGLTGLNSGLTTNPGVSAWQVNTAYTAGQLVTYNGKTYKCLQP  556
Sbjct  308  ............................................................  367

Query  557  HTSLAGWEPSNVPALWQLQ  575
Sbjct  368  ..................  386
```

Get T7F done but seems correct

471701601_MERS_1_T7F_A10.seq

NNNNNNNNNNNNNCCCTCTNNNNNNNNTTTGTTTAACTTTAAGAAGGAGATATACATATGTCTATAACGTCTGGCGTTCTG
CAATCTGGTCTCGTTAAGATGTCCCATCCTAGCGGAGACGTCGAGGCGTGTATGGTACAAGTTACGTGCGGGAGCATGACG
TTAAACGGATTGTGGCTCGACAATACAGTTTGGTGCCCACGACATGTCATGTGTCCGGCTGATCAATTAAGTGACCCAAATTA
CGACGCGTTGCTCATATCCATGACGAACCACAGCTTTAGCGTCCAAAAGCACATTGGTGCCCCTGCTAACCTTCGGGTCGTT
GGCCATGCTATGCAGGGCACTCTTTTAAAGCTGACAGTTGACGTTGCTAACCCTAGTACGCCTGCCTATACATTCACTACGGT
TAAACCCGGCGCCGCGTTCTCCGTTTTGGCATGTTACAACGGTCGTCCGACAGGTACGTTCACAGTAGTTATGCGGCCAAAT
TATACCATCAAAGGTAGCTTCCTTTGCGGTTCATGTGGTAGTGTGGGCTACACCAAAGAGGGCTCGGTGATAAATTTCTGTTA
CATGCACCAAATGGAACTTGCAAACGGGACCCACACCGGTAGTGCGTTTGATGGTACCATGTATGGAGCTTTCATGGACAAG
CAAGTTCACCAAGTGCAACTGACCGATAAGTACTGCTCTGTTAATGTCGTGGCTTGGCTGTATGCCGCCATCTTGAACGGCT
GCGCCTGGTTTGTCAAGCCAAATCGCACGTCCGTTGTAAGCTTCAACGAGTGGGCCCTGGCAAACCAATTCACTGAGTTCGT
TGGGACCCAGTCTGTCGACATGTTGGCCGTTAAGACGGGTGTAGCGATAGAGCAGCTGCTTTACGCAATCCAACAATTATAC
ACAGGTTTCCAGGGAAAGCAGATTCTCGGTTCTACAATGTTAGAGGACGAATTCACGCCCGAGGACGTGAACATGCAGATCA
TGGGCGTTGTCATGCAATGCATCACGGGAGATGCACTAGTTGCCCTACCCGAGGGCGAGTCGGTACGCATCGCCGACATCG
TGCCGGGNGGCGCGGCCNAACAGTGACAACGCCATCGACCTGAAAGTCCTTGACCGGCATGGCAATCCCGTGNNNCCCGA
ACCGCTTGTTCCACTCCGGGNNNNNNTCCGGGGGTNNNCGGTGNNTTACGGTCCAAAGGTTTTGNNNGNNAAGGGNNNC
CGNAAAACCCCCC

MSITSGVLQSGLVKMSHPSGDVEACMVQVTCGSMTLNGLWLDNTVWCPRHVMCPADQLSDPNYDALLISMTNHSFSVQKHI
GAPANLRVVGHAMQGTLLKLTVDVANPSTPAYTFTTVKPGAAFSVLACYNGRPTGTFTVVMRPNYTIKGSFLCGSCGSVGYTK
EGSVINFCYMHQMELANGTHTGSAFDGTMYGAFMDKQVHQVQLTDKYCSVNVVAWLYAAILNGCAWFVKPNRTSVVSFNE
WALANQFTEFVGTQSVDMLAVKTGVAIEQLLYAIQQLYTGFQGKQILGSTMLEDEFTPEDVNMQIMGVVMQCITGDALVALPE
GESVRIADIVPXGAAXQ

Score	Expect	Identities	Gaps	Strand
1999 bits(1082)	0.0	1098/1108(99%)	2/1108(0%)	Plus/Plus

```
Query 1 ATGTCTATAACGTCTGGCGTTCTGCAATCTGGTCTCGTTAAGATGTCCCATCCTAGCGGA 60
Sbjct 58 ..................................................... 117

Query 61 GACGTCGAGGCGTGTATGGTACAAGTTACGTGCGGGAGCATGACGTTAAACGGATTGTGG 120
Sbjct 118 ..................................................... 177

Query 121 CTCGACAATACAGTTTGGTGCCCACGACATGTCATGTGTCCGGCTGATCAATTAAGTGAC 180
Sbjct 178 ..................................................... 237

Query 181 CCAAATTACGACGCGTTGCTCATATCCATGACGAACCACAGCTTTAGCGTCCAAAAGCAC 240
Sbjct 238 ..................................................... 297

Query 241 ATTGGTGCCCCTGCTAACCTTCGGGTCGTTGGCCATGCTATGCAGGGCACTCTTTTAAAG 300
Sbjct 298 ..................................................... 357

Query 301 CTGACAGTTGACGTTGCTAACCCTAGTACGCCTGCCTATACATTCACTACGGTTAAACCC 360
Sbjct 358 ..................................................... 417

Query 361 GGCGCCGCGTTCTCCGTTTTGGCATGTTACAACGGTCGTCCGACAGGTACGTTCACAGTA 420
Sbjct 418 ..................................................... 477

Query 421 GTTATGCGGCCAAATTATACCATCAAAGGTAGCTTCCTTTGCGGTTCATGTGGTAGTGTG 480
Sbjct 478 ..................................................... 537

Query 481 GGCTACACCAAAGAGGGCTCGGTGATAAATTTCTGTTACATGCACCAAATGGAACTTGCA 540
Sbjct 538 ..................................................... 597

Query 541 AACGGGACCCACACCGGTAGTGCGTTTGATGGTACCATGTATGGAGCTTTCATGGACAAG 600
Sbjct 598 ..................................................... 657

Query 601 CAAGTTCACCAAGTGCAACTGACCGATAAGTACTGCTCTGTTAATGTCGTGGCTTGGCTG 660
Sbjct 658 ..................................................... 717

Query 661 TATGCCGCCATCTTGAACGGCTGCGCCTGGTTTGTCAAGCCAAATCGCACGTCCGTTGTA 720
Sbjct 718 ..................................................... 777

Query 721 AGCTTCAACGAGTGGGCCCTGGCAAACCAATTCACTGAGTTCGTTGGGACCCAGTCTGTC 780
Sbjct 778 ..................................................... 837

Query 781 GACATGTTGGCCGTTAAGACGGGTGTAGCGATAGAGCAGCTGCTTTACGCAATCCAACAA 840
Sbjct 838 ..................................................... 897

Query 841 TTATACACAGGTTTCCAGGGAAAGCAGATTCTCGGTTCTACAATGTTAGAGGACGAATTC 900
Sbjct 898 ..................................................... 957

Query 901 ACGCCCGAGGACGTGAACATGCAGATCATGGGCGTTGTCATGCAATGCATCACGGGAGAT 960
Sbjct 958 ..................................................... 1017

Query 961 GCACTAGTTGCCCTACCCGAGGGCGAGTCGGTACGCATCGCCGACATCGTGCCGGGT-GC 1019
Sbjct 1018 .....................................................NG.. 1077

Query 1020 GCGGCCCAACAGTGACAACGCCATCGACCTGAAAGTCCTTGACCGGCATGGCAATCCCGT 1079
Sbjct 1078 ......N..... 1137

Query 1080 GCTCGCCGACCGGC-TGTTCCACTCCGG 1106
```

```
Sbjct 1138 .NNNC....A.C..T..............  1165
```

Score	Expect	Method	Identities	Positives	Gaps
704 bits(1818)	0.0	Compositional matrix adjust.	338/338(100%)	338/338(100%)	0/338(0%)

```
Query  1    MSITSGVLQSGLVKMSHPSGDVEACMVQVTCGSMTLNGLWLDNTVWCPRHVMCPADQLSD  60
Sbjct  1    ............................................................  60

Query  61   PNYDALLISMTNHSFSVQKHIGAPANLRVVGHAMQGTLLKLTVDVANPSTPAYTFTTVKP  120
Sbjct  61   ............................................................  120

Query  121  GAAFSVLACYNGRPTGTFTVVMRPNYTIKGSFLCGSCGSVGYTKEGSVINFCYMHQMELA  180
Sbjct  121  ............................................................  180

Query  181  NGTHTGSAFDGTMYGAFMDKQVHQVQLTDKYCSVNVVAWLYAAILNGCAWFVKPNRTSVV  240
Sbjct  181  ............................................................  240

Query  241  SFNEWALANQFTEFVGTQSVDMLAVKTGVAIEQLLYAIQQLYTGFQGKQILGSTMLEDEF  300
Sbjct  241  ............................................................  300

Query  301  TPEDVNMQIMGVVMQCITGDALVALPEGESVRIADIVP  338
Sbjct  301  ....................................  338
```

All good

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pNIC-SUMO-MERS Full length TWIST MVMPROA-c001

```
ATGGACTTACTCTATCAACCGCCAAATTGTTCTATAACGTCTGGCGTTCTGCAATCTGGTCTCGTTAAGATGTCCCATCCTAGCGGAGACGTCGAGGCGTGTATGGTACAAGT
TACGTGCGGGAGCATGACGTTAAACGGATTGTGGCTCGACAATACAGTTTGGTGCCCACGACATGTCATGTGTCCGGCTGATCAATTAAGTGACCCAAATTACGACGCGTTGC
TCATATCCATGACGAACCACAGCTTTAGCGTCCAAAAGCACATTGGTGCCCCTGCTAACCTTCGGGTCGTTGGCCATGCTATGCAGGGCACTCTTTTAAAGCTGACAGTTGAC
GTTGCTAACCCTAGTACGCCTGCCTATACATTCACTACGGTTAAACCCGGCGCCGCGTTCTCCGTTTTGGCATGTTACAACGGTCGTCCGACAGGTACGTTCACAGTAGTTAT
GCGGCCAAATTATACCATCAAAGGTAGCTTCCTTTGCGGTTCATGTGGTAGTGTGGGCTACACCAAAGAGGGCTCGGTGATAAATTTCTGTTACATGCACCAAATGGAACTTG
CAAACGGGACCCACACCGGTAGTGCGTTTGATGGTACCATGTATGGAGCTTTCATGGACAAGCAAGTTCACCAAGTGCAACTGACCGATAAGTACTGCTCTGTTAATGTCGTG
GCTTGGCTGTATGCCGCCATCTTGAACGGCTGCGCCTGGTTTGTCAAGCCAAATCGCACGTCCGTTGTAAGCTTCAACGAGTGGGCCCTGGCAAACCAATTCACTGAGTTCGT
TGGGACCCAGTCTGTCGACATGTTGGCCGTTAAGACGGGTGTAGCGATAGAGCAGCTGCTTTACGCAATCCAACAATTATACACAGGTTTCCAGGGAAAGCAGATTCTCGGTT
CTACAATGTTAGAGGACGAATTCACGCCCGAGGACGTGAACATGCAGATCATGGGCGTTGTCATGCAATCCGGTGTCCGGAAGGTTACTTACGGTACAGCACACTGGCTGTAA
TGA
```

```
MDLLYQPPNCSITSGVLQSGLVKMSHPSGDVEACMVQVTCGSMTLNGLWLDNTVWCPRHVMCPADQLSDPNYDALLISMTNHSFSVQKHIGAPANLRVVGHAMQGTLLKLTVD
VANPSTPAYTFTTVKPGAAFSVLACYNGRPTGTFTVVMRPNYTIKGSFLCGSCGSVGYTKEGSVINFCYMHQMELANGTHTGSAFDGTMYGAFMDKQVHQVQLTDKYCSVNVV
AWLYAAILNGCAWFVKPNRTSVVSFNEWALANQFTEFVGTQSVDMLAVKTGVAIEQLLYAIQQLYTGFQGKQILGSTMLEDEFTPEDVNMQIMGVVMQSGVRKVTYGTAHWL—
```

```
471681101_MERS_2_T7R_F08.seq

NNNNNNNNNNNTTCGNNTTGTTAGCAGCCGGATCTCAGTGGTGGTGGTGGTGGTGCTCGAGTGCGGCCGCAAGCTTGTCGACGGGTGCTCGAGTCATTACAGCCAGTGTGCTG
TACCGTAAGTAACCTTCCGGACACCGGATTGCATGACAACGCCCATGATCTGCATGTTCACGTCCTCGGGCGTGAATTCGTCCTCTAACATTGTAGAACCGAGAATCTGCTTT
CCCTGGAAACCTGTGTATAATTGTTGGATTGCGTAAAGCAGCTGCTCTATCGCTACACCCGTCTTAACGGCCAACATGTCGACAGACTGGGTCCCAACGAACTCAGTGAATTG
GTTTGCCAGGGCCCACTCGTTGAAGCTTACAACGGACGTGCGATTTGGCTTGACAAACCAGGCGCAGCCGTTCAAGATGGCGGCATACAGCCAAGCCACGACATTAACAGAGC
AGTACTTATCGGTCAGTTGCACTTGGTGAACTTGCTTGTCCATGAAAGCTCCATACATGGTACCATCAAACGCACTACCGGTGTGGGTCCCGTTTGCAAGTTCCATTTGGTGC
ATGTAACAGAAATTTATCACCGAGCCCTCTTTGGTGTAGCCCACACTACCACATGAACCGCAAAGGAAGCTACCTTTGATGGTATAATTTGGCCGCATAACTACTGTGAACGT
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AAAGAGTGCCCTGCATAGCATGGCCAACGACCCGAAGGTTAGCAGGGGCACCAATGTGCTTTTGGACGCTAAAGCTGTGGTTCGTCATGGATATGAGCAACGCGTCGTAATTT
GGGTCACTTAATTGATCAGCCGGACACATGACATGTCGTGGGCACCAAACTGTATTGTCGAGCCACAATCCGTTTAACGTCATGCTCCCGCACGTAACTTGTACCATACACGC
CTCGACGTCTCCGCTAGGATGGGACATCTTAACGATACCAGATTGCAGAACGCCAGACGTTATAGAACAATTTGGCGGTTGAAAAGAGTAAGTCCATGCCGCCTGTCTGCTCC
NGGNACACCTCAATGAAGTCTTCCNNNTTCCATACCAAGTTCCTTAGGGGGNTGGGTTNNNCCGCAATTNCGCTNGCCCCTNGAANNGGGAAGNGAAANNNNGTTNNNNAGGA
ANCCCCN

GTWYGXXKTSLRCXXSRQAAWTYSFQPPNCSITSGVLQSGIVKMSHPSGDVEACMVQVTCGSMTLNGLWLDNTVWCPRHVMCPADQLSDPNYDALLISMTNHSFSVQKHIGAP
ANLRVVGHAMQGTLLKLTVDVANPSTPAYTFTTVKPGAAFSVLACYNGRPTGTFTVVMRPNYTIKGSFLCGSCGSVGYTKEGSVINFCYMHQMELANGTHTGSAFDGTMYGAF
MDKQVHQVQLTDKYCSVNVVAWLYAAILNGCAWFVKPNRTSVVSFNEWALANQFTEFVGTQSVDMLAVKTGVAIEQLLYAIQQLYTGFQGKQILGSTMLEDEFTPEDVNMQIM
GVVMQSGVRKVTYGTAHWL--
```

Score	Expect	Identities	Gaps	Strand
1868 bits(1011)	0.0	1018/1021(99%)	1/1021(0%)	Plus/Minus

```
Query  1     ATGGACTTACTCT-ATCAACCGCCAAATTGTTCTATAACGTCTGGCGTTCTGCAATCTGG  59
```

```
Sbjct  1114  ............TT.........................................  1055

Query  60    TCTCGTTAAGATGTCCCATCCTAGCGGAGACGTCGAGGCGTGTATGGTACAAGTTACGTG  119

Sbjct  1054  .A..........................................................  995

Query  120   CGGGAGCATGACGTTAAACGGATTGTGGCTCGACAATACAGTTTGGTGCCCACGACATGT  179

Sbjct  994   ..........................................................  935

Query  180   CATGTGTCCGGCTGATCAATTAAGTGACCCAAATTACGACGCGTTGCTCATATCCATGAC  239

Sbjct  934   ..........................................................  875

Query  240   GAACCACAGCTTTAGCGTCCAAAAGCACATTGGTGCCCCTGCTAACCTTCGGGTCGTTGG  299

Sbjct  874   ..........................................................  815

Query  300   CCATGCTATGCAGGGCACTCTTTTAAAGCTGACAGTTGACGTTGCTAACCCTAGTACGCC  359

Sbjct  814   ..........................................................  755

Query  360   TGCCTATACATTCACTACGGTTAAACCCGGCGCCGCGTTCTCCGTTTTGGCATGTTACAA  419

Sbjct  754   ..........................................................  695

Query  420   CGGTCGTCCGACAGGTACGTTCACAGTAGTTATGCGGCCAAATTATACCATCAAAGGTAG  479

Sbjct  694   ..........................................................  635

Query  480   CTTCCTTTGCGGTTCATGTGGTAGTGTGGGCTACACCAAAGAGGGCTCGGTGATAAATTT  539

Sbjct  634   ..........................................................  575

Query  540   CTGTTACATGCACCAAATGGAACTTGCAAACGGGACCCACACCGGTAGTGCGTTTGATGG  599

Sbjct  574   ..........................................................  515

Query  600   TACCATGTATGGAGCTTTCATGGACAAGCAAGTTCACCAAGTGCAACTGACCGATAAGTA  659

Sbjct  514   ..........................................................  455

Query  660   CTGCTCTGTTAATGTCGTGGCTTGGCTGTATGCCGCCATCTTGAACGGCTGCGCCTGGTT  719

Sbjct  454   ..........................................................  395
```

```
Query  720   TGTCAAGCCAAATCGCACGTCCGTTGTAAGCTTCAACGAGTGGGCCCTGGCAAACCAATT  779

Sbjct  394   ............................................................  335


Query  780   CACTGAGTTCGTTGGGACCCAGTCTGTCGACATGTTGGCCGTTAAGACGGGTGTAGCGAT  839

Sbjct  334   ............................................................  275


Query  840   AGAGCAGCTGCTTTACGCAATCCAACAATTATACACAGGTTTCCAGGGAAAGCAGATTCT  899

Sbjct  274   ............................................................  215


Query  900   CGGTTCTACAATGTTAGAGGACGAATTCACGCCCGAGGACGTGAACATGCAGATCATGGG  959

Sbjct  214   ............................................................  155


Query  960   CGTTGTCATGCAATCCGGTGTCCGGAAGGTTACTTACGGTACAGCACACTGGCTGTAATG  1019

Sbjct  154   ............................................................  95


Query  1020  A  1020

Sbjct  94    .  94
```

Score	Expect	Method	Identities	Positives	Gaps
694 bits(1792)	0.0	Compositional matrix adjust.	332/334(99%)	334/334(100%)	0/334(0%)

```
Query 5 YQPPNCSITSGVLQSGLVKMSHPSGDVEACMVQVTCGSMTLNGLWLDNTVWCPRHVMCPA 64
Sbjct 25 F..............I.......................................... 84

Query 65 DQLSDPNYDALLISMTNHSFSVQKHIGAPANLRVVGHAMQGTLLKLTVDVANPSTPAYTF 124
Sbjct 85 ............................................................ 144

Query 125 TTVKPGAAFSVLACYNGRPTGTFTVVMRPNYTIKGSFLCGSCGSVGYTKEGSVINFCYMH 184
Sbjct 145 ............................................................ 204

Query 185 QMELANGTHTGSAFDGTMYGAFMDKQVHQVQLTDKYCSVNVVAWLYAAILNGCAWFVKPN 244
Sbjct 205 ............................................................ 264

Query 245 RTSVVSFNEWALANQFTEFVGTQSVDMLAVKTGVAIEQLLYAIQQLYTGFQGKQILGSTM 304
Sbjct 265 ............................................................ 324

Query 305 LEDEFTPEDVNMQIMGVVMQSGVRKVTYGTAHWL 338
Sbjct 325 ................................... 358
```

Genuine L to I mutation?

Get T7F done but seems correct

471701601_MERS_2_T7F_B10.seq

NNNNNNNNNNNNNNNNNTCTNNNNNNNTTTGTTTAACTTTAAGAAGGAGNTATACATATGCACCACCATCATCACCATGGC
TCAGGAGATCAGGAAGCGAAACCTTCGACCGAGGACCTGGGCGACAAGAAAGAAGGGGAGTATATTAAGTTAAAGGTGATC
GGCCAGGACTCAAGCGAGATCCACTTCAAAGTCAAAATGACCACGCACCTCAAGAAGTTGAAGGAGAGTTACTGTCAACGG

CAAGGGGTTCCTATGAACTCGCTTCGCTTCCTGTTCGAGGGCCAGCGAATTGCGGACAACCACACACCTAAGGAACTTGGTA
TGGAAGAGGAAGACTTCATTGAGGTGTACCAGGAGCAGACAGGCGGCATGGACTTACTCTATCAACCGCCAAATTGTTCTAT
AACGTCTGGCGTTCTGCAATCTGGTATCGTTAAGATGTCCCATCCTAGCGGAGACGTCGAGGCGTGTATGGTACAAGTTACG
TGCGGGAGCATGACGTTAAACGGATTGTGGCTCGACAATACAGTTTGGTGCCCACGACATGTCATGTGTCCGGCTGATCAAT
TAAGTGACCCAAATTACGACGCGTTGCTCATATCCATGACGAACCACAGCTTTAGCGTCCAAAAGCACATTGGTGCCCCTGCT
AACCTTCGGGTCGTTGGCCATGCTATGCAGGGCACTCTTTTAAAGCTGACAGTTGACGTTGCTAACCCTAGTACGCCTGCCT
ATACATTCACTACGGTTAAACCCGGCGCCGCGTTCTCCGTTTTGGCATGTTACAACGGTCGTCCGACAGGTACGTTCACAGT
AGTTATGCGGCCAAATTATACCATCAAAGGTAGCTTCCTTTGCGGTTCATGTGGTAGTGTGGGCTACACCAAAGAGGGCTCG
GTGATAAATTTCTGTTACATGCACCAAATGGAACTTGCAAACGGGACCCACACCGGTAGTGCGTTTGATGGTACCATGTATG
GAGCTTTCATGGACAAGCAAGTTCACCAAGTGCAACTGACCGATAAGTACTGCTCTGTTAATGTCGTGGCTTGGCTGTATGC
CGCCATCTTGAACGGCTGNNCCTGGTTTGTCAAGCCAAATCGCACGTCCGTTGTAAGCTTCAACGAGTGGGCCCTGGCAAA
CCAATTCCCTGAGTTCGTTGGGACCCAGTCTGTCGANNTGTTGGCCCGTTAAAAACGGGGGTAGNNNAAAAANNNCNNNN
N

MHHHHHHGSGDQEAKPSTEDLGDKKEGEYIKLKVIGQDSSEIHFKVKMTTHLKKLKESYCQRQGVPMNSLRFLFEGQRIADN
HTPKELGMEEEDFIEVYQEQTGGMDLLYQPPNCSITSGVLQSGIVKMSHPSGDVEACMVQVTCGSMTLNGLWLDNTVWCPR
HVMCPADQLSDPNYDALLISMTNHSFSVQKHIGAPANLRVVGHAMQGTLLKLTVDVANPSTPAYTFTTVKPGAAFSVLACYNG
RPTGTFTVVMRPNYTIKGSFLCGSCGSVGYTKEGSVINFCYMHQMELANGTHTGSAFDGTMYGAFMDKQVHQVQLTDKYCS
VNVVAWLYAAILNGXXWFVKPNRTSVVSFNEWALANQFPEFVGTQSVXXLAR

Score	Expect	Identities	Gaps	Strand
1496 bits(810)	0.0	827/837(99%)	2/837(0%)	Plus/Plus

```
Query 1 ATGGACTTACTCTATCAACCGCCAAATTGTTCTATAACGTCTGGCGTTCTGCAATCTGGT 60
Sbjct 373 ............................................................ 432

Query 61 CTCGTTAAGATGTCCCATCCTAGCGGAGACGTCGAGGCGTGTATGGTACAAGTTACGTGC 120
Sbjct 433 A............................................................ 492

Query 121 GGGAGCATGACGTTAAACGGATTGTGGCTCGACAATACAGTTTGGTGCCCACGACATGTC 180
Sbjct 493 ............................................................ 552

Query 181 ATGTGTCCGGCTGATCAATTAAGTGACCCAAATTACGACGCGTTGCTCATATCCATGACG 240
Sbjct 553 ............................................................ 612

Query 241 AACCACAGCTTTAGCGTCCAAAAGCACATTGGTGCCCCTGCTAACCTTCGGGTCGTTGGC 300
Sbjct 613 ............................................................ 672

Query 301 CATGCTATGCAGGGCACTCTTTTAAAGCTGACAGTTGACGTTGCTAACCCTAGTACGCCT 360
Sbjct 673 ............................................................ 732

Query 361 GCCTATACATTCACTACGGTTAAACCCGGCGCCGCGTTCTCCGTTTTGGCATGTTACAAC 420
Sbjct 733 ............................................................ 792

Query 421 GGTCGTCCGACAGGTACGTTCACAGTAGTTATGCGGCCAAATTATACCATCAAAGGTAGC 480
Sbjct 793 ............................................................ 852

Query 481 TTCCTTTGCGGTTCATGTGGTAGTGTGGGCTACACCAAAGAGGGCTCGGTGATAAATTTC 540
Sbjct 853 ............................................................ 912

Query 541 TGTTACATGCACCAAATGGAACTTGCAAACGGGACCCACACCGGTAGTGCGTTTGATGGT 600
Sbjct 913 ............................................................ 972

Query 601 ACCATGTATGGAGCTTTCATGGACAAGCAAGTTCACCAAGTGCAACTGACCGATAAGTAC 660
Sbjct 973 ............................................................ 1032

Query 661 TGCTCTGTTAATGTCGTGGCTTGGCTGTATGCCGCCATCTTGAACGGCTGCGCCTGGTTT 720
Sbjct 1033 ..........................................................NN........ 1092

Query 721 GTCAAGCCAAATCGCACGTCCGTTGTAAGCTTCAACGAGTGGGCCCTGGCAAACCAATTC 780
Sbjct 1093 ............................................................ 1152
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Query 781 ACTGAGTTCGTTGGGACCCAGTCTGTCGACATGTTGGCC-GTTAAGA-CGGGTGTAG 835
Sbjct 1153 C.........................NN........C.....A.A....G.... 1209
```

Score	Expect	Method	Identities	Positives	Gaps
563 bits(1450)	0.0	Compositional matrix adjust.	267/273(98%)	268/273(98%)	0/273(0%)

```
Query 1 MDLLYQPPNCSITSGVLQSGLVKMSHPSGDVEACMVQVTCGSMTLNGLWLDNTVWCPRHV 60
Sbjct 106 ..................I..................................... 165

Query 61 MCPADQLSDPNYDALLISMTNHSFSVQKHIGAPANLRVVGHAMQGTLLKLTVDVANPSTP 120
Sbjct 166 ..................................................... 225

Query 121 AYTFTTVKPGAAFSVLACYNGRPTGTFTVVMRPNYTIKGSFLCGSCGSVGYTKEGSVINF 180
Sbjct 226 ..................................................... 285

Query 181 CYMHQMELANGTHTGSAFDGTMYGAFMDKQVHQVQLTDKYCSVNVVAWLYAAILNGCAWF 240
Sbjct 286 .....................................................XX.. 345

Query 241 VKPNRTSVVSFNEWALANQFTEFVGTQSVDMLA 273
Sbjct 346 ..................P........XX.. 378
```

Seems I to L mutation genuine

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pNIC-SUMO-MERS-FP-1/RP-1 MVMPROA-c003

FP-1/RP-1

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ATGTCTATAACGTCTGGCGTTCTGCAATCTGGTCTCGTTAAGATGTCCCATCCTAGCGGAGACGTCGAGGCGTGTATGGTACAAGTTACGTGCGGGAGCATGACGTTAAACGG
ATTGTGGCTCGACAATACAGTTTGGTGCCCACGACATGTCATGTGTCCGGCTGATCAATTAAGTGACCCAAATTACGACGCGTTGCTCATATCCATGACGAACCACAGCTTTA
GCGTCCAAAAGCACATTGGTGCCCCTGCTAACCTTCGGGTCGTTGGCCATGCTATGCAGGGCACTCTTTTAAAGCTGACAGTTGACGTTGCTAACCCTAGTACGCCTGCCTAT
ACATTCACTACGGTTAAACCCGGCGCCGCGTTCTCCGTTTTGGCATGTTACAACGGTCGTCCGACAGGTACGTTCACAGTAGTTATGCGGCCAAATTATACCATCAAAGGTAG
CTTCCTTTGCGGTTCATGTGGTAGTGTGGGCTACACCAAAGAGGGCTCGGTGATAAATTTCTGTTACATGCACCAAATGGAACTTGCAAACGGGACCCACACCGGTAGTGCGT
TTGATGGTACCATGTATGGAGCTTTCATGGACAAGCAAGTTCACCAAGTGCAACTGACCGATAAGTACTGCTCTGTTAATGTCGTGGCTTGGCTGTATGCCGCCATCTTGAAC
GGCTGCGCCTGGTTTGTCAAGCCAAATCGCACGTCCGTTGTAAGCTTCAACGAGTGGGCCCTGGCAAACCAATTCACTGAGTTCGTTGGGACCCAGTCTGTCGACATGTTGGC
CGTTAAGACGGGTGTAGCGATAGAGCAGCTGCTTTACGCAATCCAACAATTATACACAGGTTTCCAGGGAAAGCAGATTCTCGGTTCTACAATGTTAGAGGACGAATTCACGC
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CCGAGGACGTGAACATGCAGATCATGGGCGTTGTCATGCAATCCGGTGTCCGGAAGTAATGA
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MSITSGVLQSGLVKMSHPSGDVEACMVQVTCGSMTLNGLWLDNTVWCPRHVMCPADQLSDPNYDALLISMTNHSFSVQKHIGAPANLRVVGHAMQGTLLKLTVDVANPSTPAY
TFTTVKPGAAFSVLACYNGRPTGTFTVVMRPNYTIKGSFLCGSCGSVGYTKEGSVINFCYMHQMELANGTHTGSAFDGTMYGAFMDKQVHQVQLTDKYCSVNVVAWLYAAILN
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GCAWFVKPNRTSVVSFNEWALANQFTEFVGTQSVDMLAVKTGVAIEQLLYAIQQLYTGFQGKQILGSTMLEDEFTPEDVNMQIMGVVMQSGVRK--
```

471681101_MERS_3_T7R_F09.seq

```
NNNNNNNNNNNNTCNGNCTTGTTAGCAGCCGGATCTCAGTGGTGGTGGTGGTGGTGCTCGAGTGCGGCCGCAAGCTTGTCGACGGGTGCTCGAGTCATTACTTCCGGACACCG
GATTGCATGACAACGCCCATGATCTGCATGTTCACGTCCTCGGGCGTGAATTCGTCCTCTAACATTGTAGAACCGAGAATCTGCTTTCCCTGGAAACCTGTGTATAATTGTTG
GATTGCGTAAAGCAGCTGCTCTATCGCTACACCCGTCTTAACGGCCAACATGTCGACAGACTGGGTCCCAACGAACTCAGTGAATTGGTTTGCCAGGGCCCACTCGTTGAAGC
TTACAACGGACGTGCGATTTGGCTTGACAAACCAGGCGCAGCCGTTCAAGATGGCGGCATACAGCCAAGCCACGACATTAACAGAGCAGTACTTATCGGTCAGTTGCACTTGG
TGAACTTGCTTGTCCATGAAAGCTCCATACATGGTACCATCAAACGCACTACCGGTGTGGGTCCCGTTTGCAAGTTCCATTTGGTGCATGTAACAGAAATTTATCACCGAGCC
CTCTTTGGTGTAGCCCACACTACCACATGAACCGCAAAGGAAGCTACCTTTGATGGTATAATTTGGCCGCATAACTACTGTGAACGTACCTGTCGGACGACCGTTGTAACATG
CCAAAACGGAGAACGCGGCGCCGGGTTTAACCGTAGTGAATGTATAGGCAGGCGTACTAGGGTTAGCAACGTCAACTGTCAGCTTTAAAAGAGTGCCCTGCATAGCATGGCCA
ACGACCCGAAGGTTAGCAGGGGCACCAATGTGCTTTTGGACGCTAAAGCTGTGGTTCGTCATGGATATGAGCAACGCGTCGTAATTTGGGTCACTTAATTGATCAGCCGGACA
CATGACATGTCGTGGGCACCAAACTGTATTGTCGAGCCACAATCCGTTTAACGTCATGCTCCCGCACGTAACTTGTACCATACACGCCTCGACGTCTCCGCTAGGATGGGACA
TCTTAACGAGACCAGATTGCAGAACGCCAGACGTTATAGACATGCCGCCTGTCTGCTCCTGGTACACCTCAATGACGTCTTCCTCTTCCATACCAAGTTCCTTAGGTNNNTGG
TTGTCCGCAATTCGCTGGCCCTCGAANCAGGAAAGCGAAGCNNNTTCAAAGGAACCCCTTGCCGTTGAAAGTAACTTTCCTTCAACTTCTTGAGGGNNNTNGGCCATTTTGAC
```

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TTTGAANNGGGATCTCNNCTTGANNNNCCNGGCCGATNCNCT
```

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MAXXPQEVEGKLLSTARGSFEXASLSXFEGQRIADNXXPKELGMEEEDVIEVYQEQTGGMSITSGVLQSGLVKMSHPSGDVEACMVQVTCGSMTLNGLWLDNTVWCPRHVMCP
ADQLSDPNYDALLISMTNHSFSVQKHIGAPANLRVVGHAMQGTLLKLTVDVANPSTPAYTFTTVKPGAAFSVLACYNGRPTGTFTVVMRPNYTIKGSFLCGSCGSVGYTKEGS
VINFCYMHQMELANGTHTGSAFDGTMYGAFMDKQVHQVQLTDKYCSVNVVAWLYAAILNGCAWFVKPNRTSVVSFNEWALANQFTEFVGTQSVDMLAVKTGVAIEQLLYAIQQ
```

```
LYTGFQGKQILGSTMLEDEFTPEDVNMQIMGVVMQSGVRK
```

Score	Expect	Identities	Gaps	Strand
1784 bits(966)	0.0	966/966(100%)	0/966(0%)	Plus/Minus

```
Query  1     ATGTCTATAACGTCTGGCGTTCTGCAATCTGGTCTCGTTAAGATGTCCCATCCTAGCGGA  60

Sbjct  1060  ............................................................  1001


Query  61    GACGTCGAGGCGTGTATGGTACAAGTTACGTGCGGGAGCATGACGTTAAACGGATTGTGG  120

Sbjct  1000  ............................................................  941


Query  121   CTCGACAATACAGTTTGGTGCCCACGACATGTCATGTGTCCGGCTGATCAATTAAGTGAC  180

Sbjct  940   ............................................................  881


Query  181   CCAAATTACGACGCGTTGCTCATATCCATGACGAACCACAGCTTTAGCGTCCAAAAGCAC  240

Sbjct  880   ............................................................  821


Query  241   ATTGGTGCCCCTGCTAACCTTCGGGTCGTTGGCCATGCTATGCAGGGCACTCTTTTAAAG  300

Sbjct  820   ............................................................  761


Query  301   CTGACAGTTGACGTTGCTAACCCTAGTACGCCTGCCTATACATTCACTACGGTTAAACCC  360

Sbjct  760   ............................................................  701


Query  361   GGCGCCGCGTTCTCCGTTTTGGCATGTTACAACGGTCGTCCGACAGGTACGTTCACAGTA  420

Sbjct  700   ............................................................  641


Query  421   GTTATGCGGCCAAATTATACCATCAAAGGTAGCTTCCTTTGCGGTTCATGTGGTAGTGTG  480

Sbjct  640   ............................................................  581


Query  481   GGCTACACCAAAGAGGGCTCGGTGATAAATTTCTGTTACATGCACCAAATGGAACTTGCA  540

Sbjct  580   ............................................................  521


Query  541   AACGGGACCCACACCGGTAGTGCGTTTGATGGTACCATGTATGGAGCTTTCATGGACAAG  600

Sbjct  520   ............................................................  461


Query  601   CAAGTTCACCAAGTGCAACTGACCGATAAGTACTGCTCTGTTAATGTCGTGGCTTGGCTG  660

Sbjct  460   ............................................................  401


Query  661   TATGCCGCCATCTTGAACGGCTGCGCCTGGTTTGTCAAGCCAAATCGCACGTCCGTTGTA  720

Sbjct  400   ............................................................  341
```

```
Query  721   AGCTTCAACGAGTGGGCCCTGGCAAACCAATTCACTGAGTTCGTTGGGACCCAGTCTGTC  780

Sbjct  340   ............................................................  281


Query  781   GACATGTTGGCCGTTAAGACGGGTGTAGCGATAGAGCAGCTGCTTTACGCAATCCAACAA  840

Sbjct  280   ............................................................  221


Query  841   TTATACACAGGTTTCCAGGGAAAGCAGATTCTCGGTTCTACAATGTTAGAGGACGAATTC  900

Sbjct  220   ............................................................  161


Query  901   ACGCCCGAGGACGTGAACATGCAGATCATGGGCGTTGTCATGCAATCCGGTGTCCGGAAG  960

Sbjct  160   ............................................................  101


Query  961   TAATGA  966

Sbjct  100   ......  95
```

Score	Expect	Method	Identities	Positives	Gaps
671 bits(1730)	0.0	Compositional matrix adjust.	320/320(100%)	320/320(100%)	0/320(0%)

```
Query  1     MSITSGVLQSGLVKMSHPSGDVEACMVQVTCGSMTLNGLWLDNTVWCPRHVMCPADQLSD  60

Sbjct  60    ............................................................  119


Query  61    PNYDALLISMTNHSFSVQKHIGAPANLRVVGHAMQGTLLKLTVDVANPSTPAYTFTTVKP  120

Sbjct  120   ............................................................  179


Query  121   GAAFSVLACYNGRPTGTFTVVMRPNYTIKGSFLCGSCGSVGYTKEGSVINFCYMHQMELA  180

Sbjct  180   ............................................................  239


Query  181   NGTHTGSAFDGTMYGAFMDKQVHQVQLTDKYCSVNVVAWLYAAILNGCAWFVKPNRTSVV  240

Sbjct  240   ............................................................  299


Query  241   SFNEWALANQFTEFVGTQSVDMLAVKTGVAIEQLLYAIQQLYTGFQGKQILGSTMLEDEF  300

Sbjct  300   ............................................................  359


Query  301   TPEDVNMQIMGVVMQSGVRK  320
```

```
Sbjct  360  .................  379
```

Seems good

471701601_MERS_3_T7F_C10.seq

NNNNNNNNNNNNNNTNNCCTCTNNNNNNNTTTTGTTTAACTTTAAGAAGGNGNNNNNCATATGCACCACCATCATCACCA
TGGCTCAGGAGATCAGGAAGCGAAACCTTCGACCGAGGACCTGGGCGACAAGAAAGAAGGGGAGTATATTAAGTTAAAGGT
GATCGGCCAGGACTCAAGCGAGATCCACTTCAAAGTCAAAATGACCACGCACCTCAAGAAGTTGAAGGAGAGTTACTGTCA
ACGGCAAGGGGTTCCTATGAACTCGCTTCGCTTCCTGTTCGAGGGCCAGCGAATTGCGGACAACCACACACCTAAGGAACT
TGGTATGGAAGAGGAAGACGTCATTGAGGTGTACCAGGAGCAGACAGGCGGCATGTCTATAACGTCTGGCGTTCTGCAATC
TGGTCTCGTTAAGATGTCCCATCCTAGCGGAGACGTCGAGGCGTGTATGGTACAAGTTACGTGCGGGAGCATGACGTTAAA
CGGATTGTGGCTCGACAATACAGTTTGGTGCCCACGACATGTCATGTGTCCGGCTGATCAATTAAGTGACCCAAATTACGAC
GCGTTGCTCATATCCATGACGAACCACAGCTTTAGCGTCCAAAAGCACATTGGTGCCCCTGCTAACCTTCGGGTCGTTGGCC
ATGCTATGCAGGGCACTCTTTTAAAGCTGACAGTTGACGTTGCTAACCCTAGTACGCCTGCCTATACATTCACTACGGTTAAA
CCCGGCGCCGCGTTCTCCGTTTTGGCATGTTACAACGGTCGTCCGACAGGTACGTTCACAGTAGTTATGCGGCCAAATTATA
CCATCAAAGGTAGCTTCCTTTGCGGTTCATGTGGTAGTGTGGGCTACACCAAAGAGGGCTCGGTGATAAATTTCTGTTACAT
GCACCAAATGGAACTTGCAAACGGGACCCACACCGGTAGTGCGTTTGATGGTACCATGTATGGAGCTTTCATGGACAAGCAA
GTTCACCAAGTGCAACTGACCGATAAGTACTGCTCTGTTAATGTCGTGGCTTGGCTGTATGCCGCCATCTTGAACGGCTGNG
CCCTGGNTTTGTCAAGCCCAAATCGCNCGTCCNTTGTAAGCTTCAACGAATGGGGCCCNTGGCAAAACCAATTTCACTGAAG
TTCGTTGGGAACCNAATNCTNNTCGNNNTTGTTGGCNNNNTAAANNNNGGNGNNNNNNAAAAAANNNNNNNNNTTTN
NNCANNNNCAAAAATTNNANNNCNNNGGTTTTCCG

MHHHHHHGSGDQEAKPSTEDLGDKKEGEYIKLKVIGQDSSEIHFKVKMTTHLKKLKESYCQRQGVPMNSLRFLFEGQRIADN
HTPKELGMEEEDVIEVYQEQTGGMSITSGVLQSGLVKMSHPSGDVEACMVQVTCGSMTLNGLWLDNTVWCPRHVMCPADQ
LSDPNYDALLISMTNHSFSVQKHIGAPANLRVVGHAMQGTLLKLTVDVANPSTPAYTFTTVKPGAAFSVLACYNGRPTGTFTVV
MRPNYTIKGSFLCGSCGSVGYTKEGSVINFCYMHQMELANGTHTGSAFDGTMYGAFMDKQVHQVQLTDKYCSVNVVAWLYA
AILNGXALXLSSPNRXSXVSFNEWGXWQNQFH

Score	Expect	Identities	Gaps	Strand
1367 bits(740)	0.0	765/777(98%)	8/777(1%)	Plus/Plus

```
Query 1 ATGTCTATAACGTCTGGCGTTCTGCAATCTGGTCTCGTTAAGATGTCCCATCCTAGCGGA 60
Sbjct 376 ..................................................................... 435

Query 61 GACGTCGAGGCGTGTATGGTACAAGTTACGTGCGGGAGCATGACGTTAAACGGATTGTGG 120
Sbjct 436 ..................................................................... 495

Query 121 CTCGACAATACAGTTTGGTGCCCACGACATGTCATGTGTCCGGCTGATCAATTAAGTGAC 180
Sbjct 496 ..................................................................... 555

Query 181 CCAAATTACGACGCGTTGCTCATATCCATGACGAACCACAGCTTTAGCGTCCAAAAGCAC 240
Sbjct 556 ..................................................................... 615

Query 241 ATTGGTGCCCCTGCTAACCTTCGGGTCGTTGGCCATGCTATGCAGGGCACTCTTTTAAAG 300
Sbjct 616 ..................................................................... 675

Query 301 CTGACAGTTGACGTTGCTAACCCTAGTACGCCTGCCTATACATTCACTACGGTTAAACCC 360
Sbjct 676 ..................................................................... 735

Query 361 GGCGCCGCGTTCTCCGTTTTGGCATGTTACAACGGTCGTCCGACAGGTACGTTCACAGTA 420
Sbjct 736 ..................................................................... 795

Query 421 GTTATGCGGCCAAATTATACCATCAAAGGTAGCTTCCTTTGCGGTTCATGTGGTAGTGTG 480
Sbjct 796 ..................................................................... 855

Query 481 GGCTACACCAAAGAGGGCTCGGTGATAAATTTCTGTTACATGCACCAAATGGAACTTGCA 540
Sbjct 856 ..................................................................... 915

Query 541 AACGGGACCCACACCGGTAGTGCGTTTGATGGTACCATGTATGGAGCTTTCATGGACAAG 600
Sbjct 916 ..................................................................... 975
```

```
Query 601 CAAGTTCACCAAGTGCAACTGACCGATAAGTACTGCTCTGTTAATGTCGTGGCTTGGCTG 660
Sbjct 976 ............................................................ 1035

Query 661 TATGCCGCCATCTTGAACGGCTGCGCC-TGG-TTTGTCAAG-CCAAATCGCACGTCCGTT 717
Sbjct 1036 .......................N...C...N.........C.........N.....N.. 1095

Query 718 GTAAGCTTCAACGAGTGGG-CCC-TGGCAAA-CCAATT-CACTG-AGTTCGTTGGGA 769
Sbjct 1096 ..............A....G...N........A.......T.....A............ 1152
```

Score	Expect	Method	Identities	Positives	Gaps
499 bits(1286)	0.0	Compositional matrix adjust.	241/253(95%)	242/253(95%)	2/253(0%)

```
Query 1 MSITSGVLQSGLVKMSHPSGDVEACMVQVTCGSMTLNGLWLDNTVWCPRHVMCPADQLSD 60
Sbjct 106 ............................................................ 165

Query 61 PNYDALLISMTNHSFSVQKHIGAPANLRVVGHAMQGTLLKLTVDVANPSTPAYTFTTVKP 120
Sbjct 166 ............................................................ 225

Query 121 GAAFSVLACYNGRPTGTFTVVMRPNYTIKGSFLCGSCGSVGYTKEGSVINFCYMHQMELA 180
Sbjct 226 ............................................................ 285

Query 181 NGTHTGSAFDGTMYGAFMDKQVHQVQLTDKYCSVNVVAWLYAAILNGCAWFV-KPNRTSV 239
Sbjct 286 ..............................................X.LXLSS...X.X 345

Query 240 VSFNEWAL-ANQF 251
Sbjct 346 ......GXWQ... 358

all good
```

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pNIC-SUMO-MERS-FP-2/RP-2 MVMPROA-c004

FP-2/RP-2

atgtccgctgtcctgCAATCTGGTCTCGTTAAGATGTCCCATCCTAGCGGAGACGTCGAGGCGTGTATGGTACAAGTTACGTGCGGGAGC
ATGACGTTAAACGGATTGTGGCTCGACAATACAGTTTGGTGCCCACGACATGTCATGTGTCCGGCTGATCAATTAAGTGACCCAAATTAC
GACGCGTTGCTCATATCCATGACGAACCACAGCTTTAGCGTCCAAAAGCACATTGGTGCCCCTGCTAACCTTCGGGTCGTTGGCCATGCT
ATGCAGGGCACTCTTTTAAAGCTGACAGTTGACGTTGCTAACCCTAGTACGCCTGCCTATACATTCACTACGGTTAAACCCGGCGCCGCG
TTCTCCGTTTTGGCATGTTACAACGGTCGTCCGACAGGTACGTTCACAGTAGTTATGCGGCCAAATTATACCATCAAAGGTAGCTTCCTT
TGCGGTTCATGTGGTAGTGTGGGCTACACCAAAGAGGGCTCGGTGATAAATTTCTGTTACATGCACCAAATGGAACTTGCAAACGGGACC
CACACCGGTAGTGCGTTTGATGGTACCATGTATGGAGCTTTCATGGACAAGCAAGTTCACCAAGTGCAACTGACCGATAAGTACTGCTCT
GTTAATGTCGTGGCTTGGCTGTATGCCGCCATCTTGAACGGCTGCGCCTGGTTTGTCAAGCCAAATCGCACGTCCGTTGTAAGCTTCAAC
GAGTGGGCCCTGGCAAACCAATTCACTGAGTTCGTTGGGACCCAGTCTGTCGACATGTTGGCCGTTAAGACGGGTGTAGCGATAGAGCAG
CTGCTTTACGCAATCCAACAATTATACACAGGTTTCCAGGGAAAGCAGATTCTCGGTTCTACAATGTTAGAGGACGAATTCACGCCCGAG
GACGTGAACATGCAGATCATGGGCGTTGTCATGCAATAATGA

MSAVLQSGLVKMSHPSGDVEACMVQVTCGSMTLNGLWLDNTVWCPRHVMCPADQLSDPNYDALLISMTNHSFSVQKHIGA
PANLRVVGHAMQGTLLKLTVDVANPSTPAYTFTTVKPGAAFSVLACYNGRPTGTFTVVMRPNYTIKGSFLCGSCGSVGYTKEG
SVINFCYMHQMELANGTHTGSAFDGTMYGAFMDKQVHQVQLTDKYCSVNVVAWLYAAILNGCAWFVKPNRTSVVSFNEWAL
ANQFTEFVGTQSVDMLAVKTGVAIEQLLYAIQQLYTGFQGKQILGSTMLEDEFTPEDVNMQIMGVVMQ—

471681101_MERS_4_T7R_F10.seq

NNNNNNNNNNNTNCGNNNNNNTTAGCAGCCGGATCTCAGTGGTGGTGGTGGTGGTGCTCGAGTGCGNNCCGCAAGCTTGTCGACGGGTGCTCGAGTCATTATTGCATGACAAC
GCCCATGATCTGCATGTTCACGTCCTCGGGCGTGAATTCGTCCTCTAACATTGTAGAACCGAGAATCTGCTTTCCCTGGAAACCTGTGTATAATTGTTGGATTGCGTAAAGCA
GCTGCTCTATCGCTACACCCGTCTTAACGGCCAACATGTCGACAGACTGGGTCCCAACGAACTCAGTGAATTGGTTTGCCAGGGCCCACTCGTTGAAGCTTACAACGGACGTG
CGATTTGGCTTGACAAACCAGGCGCAGCCGTTCAAGATGGCGGCATACAGCCAAGCCACGACATTAACAGAGCAGTACTTATCGGTCAGTTGCACTTGGTGAACTTGCTTGTC
CATGAAAGCTCCATACATGGTACCATCAAACGCACTACCGGTGTGGGTCCCGTTTGCAAGTTCCATTTGGTGCATGTAACAGAAATTTATCACCGAGCCCTCTTTGGTGTAGC
CCACACTACCACATGAACCGCAAAGGAAGCTACCTTTGATGGTATAATTTGGCCGCATAACTACTGTGAACGTACCTGTCGGACGACCGTTGTAACATGCCAAAACGGAGAAC
GCGGCGCCGGGTTTAACCGTAGTGAATGTATAGGCAGGCGTACTAGGGTTAGCAACGTCAACTGTCAGCTTTAAAAGAGTGCCCTGCATAGCATGGCCAACGACCCGAAGGTT
AGCAGGGGCACCAATGTGCTTTTGGACGCTAAAGCTGTGGTTCGTCATGGATATGAGCAACGCGTCGTAATTTGGGTCACTTAATTGATCAGCCGGACACATGACATGTCGTG
GGCACCAAACTGTATTGTCGAGCCACAATCCGTTTAACGTCATGCTCCCGCACGTAACTTGTACCATACACGCCTCGACGTCTCCGCTAGGATGGGAACATCTTAACGAGACC

AGATTGCAGGAAAGCGGACATGCCGCCTGTCTGCTCCTGGTAACCCTCAATGACGTCTTCCTCTTCCATACCAAGTTCCTTAGGGTTGNTGGTTNNNCCCCAATTNCGCTTGG
CCCTCGAAANNGGAAGNCAAAGNNNGTTNNTTAGGAACCCCTTTGCCGTTGANNNNAAACNCTTCCCTTCAACTTTCTTGANGGGGNNGGGGCCATTTTNGANNTTNNGAAGG
GNAATTNCCGGTTG

SHPSGDVEACMVQVTCGSMTLNGLWLDNTVWCPRHVMCPADQLSDPNYDALLISMTNHSFSVQKHIGAPANLRVVGHAMQ
GTLLKLTVDVANPSTPAYTFTTVKPGAAFSVLACYNGRPTGTFTVVMRPNYTIKGSFLCGSCGSVGYTKEGSVINFCYMHQMEL
ANGTHTGSAFDGTMYGAFMDKQVHQVQLTDKYCSVNVVAWLYAAILNGCAWFVKPNRTSVVSFNEWALANQFTEFVGTQSV
DMLAVKTGVAIEQLLYAIQQLYTGFQGKQILGSTMLEDEFTPEDVNMQIMGVVMQ

Score	Expect	Identities	Gaps	Strand
1729 bits(936)	0.0	941/943(99%)	1/943(0%)	Plus/Minus

```
Query 1 ATGTCCGCTGTCCTGCAATCTGGTCTCGTTAAGATGT-CCCATCCTAGCGGAGACGTCGA 59
Sbjct 1038 .........T............................T..................... 979

Query 60 GGCGTGTATGGTACAAGTTACGTGCGGGAGCATGACGTTAAACGGATTGTGGCTCGACAA 119
Sbjct 978 ..............................................................  919

Query 120 TACAGTTTGGTGCCCACGACATGTCATGTGTCCGGCTGATCAATTAAGTGACCCAAATTA 179
Sbjct 918 ..............................................................  859

Query 180 CGACGCGTTGCTCATATCCATGACGAACCACAGCTTTAGCGTCCAAAAGCACATTGGTGC 239
Sbjct 858 ..............................................................  799

Query 240 CCCTGCTAACCTTCGGGTCGTTGGCCATGCTATGCAGGGCACTCTTTTAAAGCTGACAGT 299
Sbjct 798 ..............................................................  739

Query 300 TGACGTTGCTAACCCTAGTACGCCTGCCTATACATTCACTACGGTTAAACCCGGCGCCGC 359
Sbjct 738 ..............................................................  679

Query 360 GTTCTCCGTTTTGGCATGTTACAACGGTCGTCCGACAGGTACGTTCACAGTAGTTATGCG 419
Sbjct 678 ..............................................................  619

Query 420 GCCAAATTATACCATCAAAGGTAGCTTCCTTTGCGGTTCATGTGGTAGTGTGGGCTACAC 479
Sbjct 618 ..............................................................  559

Query 480 CAAAGAGGGCTCGGTGATAAATTTCTGTTACATGCACCAAATGGAACTTGCAAACGGGAC 539
Sbjct 558 ..............................................................  499

Query 540 CCACACCGGTAGTGCGTTTGATGGTACCATGTATGGAGCTTTCATGGACAAGCAAGTTCA 599
Sbjct 498 ..............................................................  439

Query 600 CCAAGTGCAACTGACCGATAAGTACTGCTCTGTTAATGTCGTGGCTTGGCTGTATGCCGC 659
Sbjct 438 ..............................................................  379

Query 660 CATCTTGAACGGCTGCGCCTGGTTTGTCAAGCCAAATCGCACGTCCGTTGTAAGCTTCAA 719
Sbjct 378 ..............................................................  319

Query 720 CGAGTGGGCCCTGGCAAACCAATTCACTGAGTTCGTTGGGACCCAGTCTGTCGACATGTT 779
Sbjct 318 ..............................................................  259

Query 780 GGCCGTTAAGACGGGTGTAGCGATAGAGCAGCTGCTTTACGCAATCCAACAATTATACAC 839
Sbjct 258 ..............................................................  199

Query 840 AGGTTTCCAGGGAAAGCAGATTCTCGGTTCTACAATGTTAGAGGACGAATTCACGCCCGA 899
Sbjct 198 ..............................................................  139

Query 900 GGACGTGAACATGCAGATCATGGGCGTTGTCATGCAATAATGA 942
Sbjct 138 ..........................................  96
```

Score	Expect	Method	Identities	Positives	Gaps

629 bits(1623) 0.0 Compositional matrix adjust. 300/300(100%) 300/300(100%) 0/300(0%)

```
Query 13 SHPSGDVEACMVQVTCGSMTLNGLWLDNTVWCPRHVMCPADQLSDPNYDALLISMTNHSF 72
Sbjct 1 ..................................................................... 60

Query 73 SVQKHIGAPANLRVVGHAMQGTLLKLTVDVANPSTPAYTFTTVKPGAAFSVLACYNGRPT 132
Sbjct 61 ..................................................................... 120

Query 133 GTFTVVMRPNYTIKGSFLCGSCGSVGYTKEGSVINFCYMHQMELANGTHTGSAFDGTMYG 192
Sbjct 121 ..................................................................... 180

Query 193 AFMDKQVHQVQLTDKYCSVNVVAWLYAAILNGCAWFVKPNRTSVVSFNEWALANQFTEFV 252
Sbjct 181 ..................................................................... 240

Query 253 GTQSVDMLAVKTGVAIEQLLYAIQQLYTGFQGKQILGSTMLEDEFTPEDVNMQIMGVVMQ 312
Sbjct 241 ..................................................................... 300
```

Need T7F to confirm

471701601_MERS_4_T7F_D10.seq

NNNNNNNNNNNNNNNNNNCTAGNANNNTTTGTTTAACTTTAAGAAGGAGANATACATATGCACCACCATCANTCACCATGG
CTCAGGAGATCAGGAAGCGAAACCTTCGACCGAGGACCTGGGCGACAAGAAAGAAGGGGAGTATATTAAGTTAAAGGTGAT
CGGCCAGGACTCAAGCGAGATCCACTTCAAAGTCAAAATGACCACGCACCTCAAGAAGTTGAAGGAGAGTTACTGTCAACG
GCAAGGGGTTCCTATGAACTCGCTTCGCTTCCTGTTCGAGGGCCAGCGAATTGCGGACAACCACACACCTAAGGAACTTGG
TATGGAAGAGGAAGACGTCATTGAGGTGTACCAGGAGCAGACAGGCGGCATGTCCGCTGTCCTGCAATCTGGTCTCGTTAA
GATGTCCCATCCTAGCGGAGACGTCGAGGCGTGTATGGTACAAGTTACGTGCGGGAGCATGACGTTAAACGGATTGTGGCT
CGACAATACAGTTTGGTGCCCACGACATGTCATGTGTCCGGCTGATCAATTAAGTGACCCAAATTACGACGCGTTGCTCATAT
CCATGACGAACCACAGCTTTAGCGTCCAAAAGCACATTGGTGCCCCTGCTAACCTTCGGGTCGTTGGCCATGCTATGCAGGG
CACTCTTTTAAAGCTGACAGTTGACGTTGCTAACCCTAGTACGCCTGCCTATACATTCACTACGGTTAAACCCGGCGCCGCGT
TCTCCGTTTTGGCATGTTACAACGGTCGTCCGACAGGTACGTTCACAGTAGTTATGCGGCCAAATTATACCATCAAAGGTAGC
TTCCTTTGCGGTTCATGTGGTAGTGTGGGCTACACCAAAGAGGGCTCGGTGATAAATTTCTGTTACATGCACCAAATGGAAC
TTGCAAACGGGACCCACACCGGTAGTGCGTTTGATGGTNCCATGTATGGAGCTTTCATGGACAAGCAAGTTCACCAAGTGC
AACTGACCGATAAGTACTGCTCTGTTAATGTCGTGGCTTGCTGTATGCNCNATCTTGAACGGCTGCCCCTGGTT

XXLFNFKKEXYICTTIXHHGSGDQEAKPSTEDLGDKKEGEYIKLKVIGQDSSEIHFKVKMTTHLKKLKESYCQRQGVPMNSLRF
LFEGQRIADNHTPKELGMEEEDVIEVYQEQTGGMSAVLQSGLVKMSHPSGDVEACMVQVTCGSMTLNGLWLDNTVWCPRH
VMCPADQLSDPNYDALLISMTNHSFSVQKHIGAPANLRVVGHAMQGTLLKLTVDVANPSTPAYTFTTVKPGAAFSVLACYNGR
PTGTFTVVMRPNYTIKGSFLCGSCGSVGYTKEGSVINFCYMHQMELANGTHTGSAFDGXMYGAFMDKQVHQVQLTDKYCSV
NVVACCMXXLERLPLV

Score	Expect	Identities	Gaps	Strand
1232 bits(667)	0.0	677/683(99%)	2/683(0%)	Plus/Plus

```
Query 1 ATGTCCGCTGTCCTGCAATCTGGTCTCGTTAAGATGTCCCATCCTAGCGGAGACGTCGAG 60
Sbjct 374 ..................................................................... 433

Query 61 GCGTGTATGGTACAAGTTACGTGCGGGAGCATGACGTTAAACGGATTGTGGCTCGACAAT 120
Sbjct 434 ..................................................................... 493

Query 121 ACAGTTTGGTGCCCACGACATGTCATGTGTCCGGCTGATCAATTAAGTGACCCAAATTAC 180
Sbjct 494 ..................................................................... 553

Query 181 GACGCGTTGCTCATATCCATGACGAACCACAGCTTTAGCGTCCAAAAGCACATTGGTGCC 240
Sbjct 554 ..................................................................... 613

Query 241 CCTGCTAACCTTCGGGTCGTTGGCCATGCTATGCAGGGCACTCTTTTAAAGCTGACAGTT 300
Sbjct 614 ..................................................................... 673

Query 301 GACGTTGCTAACCCTAGTACGCCTGCCTATACATTCACTACGGTTAAACCCGGCGCCGCG 360
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Sbjct 674 ..................................................... 733

Query 361 TTCTCCGTTTTGGCATGTTACAACGGTCGTCCGACAGGTACGTTCACAGTAGTTATGCGG 420
Sbjct 734 ..................................................... 793

Query 421 CCAAATTATACCATCAAAGGTAGCTTCCTTTGCGGTTCATGTGGTAGTGTGGGCTACACC 480
Sbjct 794 ..................................................... 853

Query 481 AAAGAGGGCTCGGTGATAAATTTCTGTTACATGCACCAAATGGAACTTGCAAACGGGACC 540
Sbjct 854 ..................................................... 913

Query 541 CACACCGGTAGTGCGTTTGATGGTACCATGTATGGAGCTTTCATGGACAAGCAAGTTCAC 600
Sbjct 914 .....................N............................... 973

Query 601 CAAGTGCAACTGACCGATAAGTACTGCTCTGTTAATGTCGTGGCTTGGCTGTATGCCGCC 660
Sbjct 974 ...............................................-.........N-.N 1031

Query 661 ATCTTGAACGGCTGCGCCTGGTT 683
Sbjct 1032 ..............C....... 1054
```

Score	Expect	Method	Identities	Positives	Gaps
453 bits(1165)	7e-166	Compositional matrix adjust.	214/215(99%)	214/215(99%)	0/215(0%)

```
Query 1 MSAVLQSGLVKMSHPSGDVEACMVQVTCGSMTLNGLWLDNTVWCPRHVMCPADQLSDPNY 60
Sbjct 118 ..................................................... 177

Query 61 DALLISMTNHSFSVQKHIGAPANLRVVGHAMQGTLLKLTVDVANPSTPAYTFTTVKPGAA 120
Sbjct 178 ..................................................... 237

Query 121 FSVLACYNGRPTGTFTVVMRPNYTIKGSFLCGSCGSVGYTKEGSVINFCYMHQMELANGT 180
Sbjct 238 ..................................................... 297

Query 181 HTGSAFDGTMYGAFMDKQVHQVQLTDKYCSVNVVA 215
Sbjct 298 ........X........................... 332

All good
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pNIC-SUMO-MERS-FP-3/RP-2 MVMPROA-c005

FP-3/RP-2

```
atgtccgctgtcatgCAATCTGGTCTCGTTAAGATGTCCCATCCTAGCGGAGACGTCGAGGCGTGTATGGTACAAGTTACGTGCGGGAGC
ATGACGTTAAACGGATTGTGGCTCGACAATACAGTTTGGTGCCCACGACATGTCATGTGTCCGGCTGATCAATTAAGTGACCCAAATTAC
GACGCGTTGCTCATATCCATGACGAACCACAGCTTTAGCGTCCAAAAGCACATTGGTGCCCCTGCTAACCTTCGGGTCGTTGGCCATGCT
ATGCAGGGCACTCTTTTAAAGCTGACAGTTGACGTTGCTAACCCTAGTACGCCTGCCTATACATTCACTACGGTTAAACCCGGCGCCGCG
TTCTCCGTTTTGGCATGTTACAACGGTCGTCCGACAGGTACGTTCACAGTAGTTATGCGGCCAAATTATACCATCAAAGGTAGCTTCCTT
TGCGGTTCATGTGGTAGTGTGGGCTACACCAAAGAGGGCTCGGTGATAAATTTCTGTTACATGCACCAAATGGAACTTGCAAACGGGACC
CACACCGGTAGTGCGTTTGATGGTACCATGTATGGAGCTTTCATGGACAAGCAAGTTCACCAAGTGCAACTGACCGATAAGTACTGCTCT
GTTAATGTCGTGGCTTGGCTGTATGCCGCCATCTTGAACGGCTGCGCCTGGTTTGTCAAGCCAAATCGCACGTCCGTTGTAAGCTTCAAC
GAGTGGGCCCTGGCAAACCAATTCACTGAGTTCGTTGGGACCCAGTCTGTCGACATGTTGGCCGTTAAGACGGGTGTAGCGATAGAGCAG
CTGCTTTACGCAATCCAACAATTATACACAGGTTTCCAGGGAAAGCAGATTCTCGGTTCTACAATGTTAGAGGACGAATTCACGCCCGAG
GACGTGAACATGCAGATCATGGGCGTTGTCATGCAATAATGA
```

MSAVMQSGLVKMSHPSGDVEACMVQVTCGSMTLNGLWLDNTVWCPRHVMCPADQLSDPNYDALLISMTNHSFSVQKHIGA
PANLRVVGHAMQGTLLKLTVDVANPSTPAYTFTTVKPGAAFSVLACYNGRPTGTFTVVMRPNYTIKGSFLCGSCGSVGYTKEG
SVINFCYMHQMELANGTHTGSAFDGTMYGAFMDKQVHQVQLTDKYCSVNVVAWLYAAILNGCAWFVKPNRTSVVSFNEWAL
ANQFTEFVGTQSVDMLAVKTGVAIEQLLYAIQQLYTGFQGKQILGSTMLEDEFTPEDVNMQIMGVVMQ—

471681101_MERS_5_T7R_F11.seq

```
NNNNNNNNNNNTTTCGNNNNNGTTAGCAGCCGGATCTCAGTGGTGGTGGTGGTGGTGCTCGAGTGCGGCCGCAAGCTTGTCGACGGGTGCTCGAGTCATTATTGCATGACAAC
```

GCCCATGATCTGCATGTTCACGTCCTCGGGCGTGAATTCGTCCTCTAACATTGTAGAACCGAGAATCTGCTTTCCCTGGAAACCTGTGTATAATTGTTGGATTGCGTAAAGCA
GCTGCTCTATCGCTACACCCGTCTTAACGGCCAACATGTCGACAGACTGGGTCCCAACGAACTCAGTGAATTGGTTTGCCAGGGCCCACTCGTTGAAGCTTACAACGGACGTG
CGATTTGGCTTGACAAACCAGGCGCAGCCGTTCAAGATGGCGGCATACAGCCAAGCCACGACATTAACAGAGCAGTACTTATCGGTCAGTTGCACTTGGTGAACTTGCTTGTC
CATGAAAGCTCCATACATGGTACCATCAAACGCACTACCGGTGTGGGTCCCGTTTGCAAGTTCCATTTGGTGCATGTAACAGAAATTTATCACCGAGCCCTCTTTGGTGTAGC
CCACACTACCACATGAACCGCAAAGGAAGCTACCTTTGATGGTATAATTTGGCCGCATAACTACTGTGAACGTACCTGTCGGACGACCGTTGTAACATGCCAAAACGGAGAAC
GCGGCGCCGGGTTTAACCGTAGTGAATGTATAGGCAGGCGTACTAGGGTTAGCAACGTCAACTGTCAGCTTTAAAAGAGTGCCCTGCATAGCATGGCCAACGACCCGAAGGTT
AGCAGGGGCACCAATGTGCTTTTGGACGCTAAAGCTGTGGTTCGTCATGGATATGAGCAACGCGTCGTAATTTGGGTCACTTAATTGATCAGCCGGACACATGACATGTCGTG
GGCACCAAACTGTATTGTCGAGCCACAATCCGTTTAACGTCATGCTCCCGCACGTAACTTGTACCATACACGCCTCGACGTCTCCGCTAGGATGGGACATCTTAACGAGACCA
GATTGCATGACAGCGGACATGCCGCCTGTCTGCTCCTGGTACACCTCAATGACGTCTTCCTCTTCCATACCAAGTTCCTTAGGTNGTGTGGTTGTCCGCAATTCGCTGGCCCN
CNAAACAGGAAAGCGAAAGCGGGTTCAAAGGAACCCCTTGCCGTTGACAGTAACTTTCCCTTCAACTTTCTTGGAGGGNNNTGGGCCATTTTTGACTTTGAANNGGATNNNNN

NTTGNNNNCCNGNNNANNCCCCTTNAAATTAAANAANNNCCCCCTTTTTTTT

MSAVMQSGLVKMSHPSGDVEACMVQVTCGSMTLNGLWLDNTVWCPRHVMCPADQLSDPNYDALLISMTNHSFSVQKHIGA
PANLRVVGHAMQGTLLKLTVDVANPSTPAYTFTTVKPGAAFSVLACYNGRPTGTFTVVMRPNYTIKGSFLCGSCGSVGYTKEG
SVINFCYMHQMELANGTHTGSAFDGTMYGAFMDKQVHQVQLTDKYCSVNVVAWLYAAILNGCAWFVKPNRTSVVSFNEWAL
ANQFTEFVGTQSVDMLAVKTGVAIEQLLYAIQQLYTGFQGKQILGSTMLEDEFTPEDVNMQIMGVVMQ

Score	Expect	Identities	Gaps	Strand
1740 bits(942)	0.0	942/942(100%)	0/942(0%)	Plus/Minus

```
Query  1     ATGTCCGCTGTCATGCAATCTGGTCTCGTTAAGATGTCCCATCCTAGCGGAGACGTCGAG  60

Sbjct  1037  ............................................................  978


Query  61    GCGTGTATGGTACAAGTTACGTGCGGGAGCATGACGTTAAACGGATTGTGGCTCGACAAT  120

Sbjct  977   ............................................................  918


Query  121   ACAGTTTGGTGCCCACGACATGTCATGTGTCCGGCTGATCAATTAAGTGACCCAAATTAC  180

Sbjct  917   ............................................................  858


Query  181   GACGCGTTGCTCATATCCATGACGAACCACAGCTTTAGCGTCCAAAAGCACATTGGTGCC  240

Sbjct  857   ............................................................  798


Query  241   CCTGCTAACCTTCGGGTCGTTGGCCATGCTATGCAGGGCACTCTTTTAAAGCTGACAGTT  300

Sbjct  797   ............................................................  738


Query  301   GACGTTGCTAACCCTAGTACGCCTGCCTATACATTCACTACGGTTAAACCCGGCGCCGCG  360

Sbjct  737   ............................................................  678


Query  361   TTCTCCGTTTTGGCATGTTACAACGGTCGTCCGACAGGTACGTTCACAGTAGTTATGCGG  420

Sbjct  677   ............................................................  618


Query  421   CCAAATTATACCATCAAAGGTAGCTTCCTTTGCGGTTCATGTGGTAGTGTGGGCTACACC  480

Sbjct  617   ............................................................  558
```

```
Query  481   AAAGAGGGCTCGGTGATAAATTTCTGTTACATGCACCAAATGGAACTTGCAAACGGGACC  540
Sbjct  557   ............................................................  498

Query  541   CACACCGGTAGTGCGTTTGATGGTACCATGTATGGAGCTTTCATGGACAAGCAAGTTCAC  600
Sbjct  497   ............................................................  438

Query  601   CAAGTGCAACTGACCGATAAGTACTGCTCTGTTAATGTCGTGGCTTGGCTGTATGCCGCC  660
Sbjct  437   ............................................................  378

Query  661   ATCTTGAACGGCTGCGCCTGGTTTGTCAAGCCAAATCGCACGTCCGTTGTAAGCTTCAAC  720
Sbjct  377   ............................................................  318

Query  721   GAGTGGGCCCTGGCAAACCAATTCACTGAGTTCGTTGGGACCCAGTCTGTCGACATGTTG  780
Sbjct  317   ............................................................  258

Query  781   GCCGTTAAGACGGGTGTAGCGATAGAGCAGCTGCTTTACGCAATCCAACAATTATACACA  840
Sbjct  257   ............................................................  198

Query  841   GGTTTCCAGGGAAAGCAGATTCTCGGTTCTACAATGTTAGAGGACGAATTCACGCCCGAG  900
Sbjct  197   ............................................................  138

Query  901   GACGTGAACATGCAGATCATGGGCGTTGTCATGCAATAATGA  942
Sbjct  137   ........................................  96
```

Score	Expect	Method	Identities	Positives	Gaps
652 bits(1682)	0.0	Compositional matrix adjust.	312/312(100%)	312/312(100%)	0/312(0%)

```
Query  1     MSAVMQSGLVKMSHPSGDVEACMVQVTCGSMTLNGLWLDNTVWCPRHVMCPADQLSDPNY  60
Sbjct  1     ............................................................  60

Query  61    DALLISMTNHSFSVQKHIGAPANLRVVGHAMQGTLLKLTVDVANPSTPAYTFTTVKPGAA  120
Sbjct  61    ............................................................  120

Query  121   FSVLACYNGRPTGTFTVVMRPNYTIKGSFLCGSCGSVGYTKEGSVINFCYMHQMELANGT  180
```

```
Sbjct  121  ..........................................................  180


Query  181  HTGSAFDGTMYGAFMDKQVHQVQLTDKYCSVNVVAWLYAAILNGCAWFVKPNRTSVVSFN  240

Sbjct  181  ..........................................................  240


Query  241  EWALANQFTEFVGTQSVDMLAVKTGVAIEQLLYAIQQLYTGFQGKQILGSTMLEDEFTPE  300

Sbjct  241  ..........................................................  300


Query  301  DVNMQIMGVVMQ  312

Sbjct  301  ............  312
```

seems all good

```
471701601_MERS_5_T7F_E10.seq
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CGGCCAGGACTCAAGCGAGATCCACTTCAAAGTCAAAATGACCACGCACCTCAAGAAGTTGAAGGAGAGTTACTGTCAACGG
CAAGGGGTTCCTATGAACTCGCTTCGCTTCCTGTTCGAGGGCCAGCGAATTGCGGACAACCACACACCTAAGGAACTTGGTA
TGGAAGAGGAAGACGTCATTGAGGTGTACCAGGAGCAGACAGGCGGCATGTCCGCTGTCATGCAATCTGGTCTCGTTAAGAT
GTCCCATCCTAGCGGAGACGTCGAGGCGTGTATGGTACAAGTTACGTGCGGGAGCATGACGTTAAACGGATTGTGGCTCGAC
AATACAGTTTGGTGCCCACGACATGTCATGTGTCCGGCTGATCAATTAAGTGACCCAAATTACGACGCGTTGCTCATATCCA
TGACGAACCACAGCTTTAGCGTCCAAAAGCACATTGGTGCCCCTGCTAACCTTCGGGTCGTTGGCCATGCTATGCAGGGCAC
TCTTTTAAAGCTGACAGTTGACGTTGCTAACCCTAGTACGCCTGCCTATACATTCACTACGGTTAAACCCGGCGCCGCGTTC
TCCGTTTTGGCATGTTACAACGGTCGTCCGACAGGTTACTTNCACAGTAGNATCCGCCAAATTATACCANCAANNTAGCTTC
CTTGNGTTCATGTGGTGTNTGGGCTAACCNCANANCCTCGCCNACANCTNNCGTTCNTGCCNCNANGC
```

XXXXXXXXXILFNFKKEXXICTTIXHHGSGDQEAKPSTEDLGDKKEGEYIKLKVIGQDSSEIHFKVKMTTHLKKLKESYCQRQGV
PMNSLRFLFEGQRIADNHTPKELGMEEEDVIEVYQEQTGGMSAVMQSGLVKMSHPSGDVEACMVQVTCGSMTLNGLWLDN
TVWCPRHVMCPADQLSDPNYDALLISMTNHSFSVQKHIGAPANLRVVGHAMQGTLLKLTVDVANPSTPAYTFTTVKPGAAFS
VLACYNGRPTGYXHSXIRQIIPXX

Score	Expect	Identities	Gaps	Strand
800 bits(433)	0.0	464/481(96%)	8/481(1%)	Plus/Plus

```
Query 1 ATGTCCGCTGTCATGCAATCTGGTCTCGTTAAGATGTCCCATCCTAGCGGAGACGTCGAG 60
Sbjct 376 ..................................................................... 435

Query 61 GCGTGTATGGTACAAGTTACGTGCGGGAGCATGACGTTAAACGGATTGTGGCTCGACAAT 120
Sbjct 436 ..................................................................... 495

Query 121 ACAGTTTGGTGCCCACGACATGTCATGTGTCCGGCTGATCAATTAAGTGACCCAAATTAC 180
Sbjct 496 ..................................................................... 555

Query 181 GACGCGTTGCTCATATCCATGACGAACCACAGCTTTAGCGTCCAAAAGCACATTGGTGCC 240
Sbjct 556 ..................................................................... 615

Query 241 CCTGCTAACCTTCGGGTCGTTGGCCATGCTATGCAGGGCACTCTTTTAAAGCTGACAGTT 300
Sbjct 616 ..................................................................... 675

Query 301 GACGTTGCTAACCCTAGTACGCCTGCCTATACATTCACTACGGTTAAACCCGGCGCCGCG 360
Sbjct 676 ..................................................................... 735

Query 361 TTCTCCGTTTTGGCATGTTACAACGGTCGTCCGACAGGT-ACGTTCACAGTAGTTATGCG 419
Sbjct 736 .....................................T..T.N........N-..C.- 793

Query 420 GCCAAATTATACCATCAAAGGTAGCTTCCTTTGCGGTTCATGTGGTAGTGTGGGCTACAC 479
```

```
Sbjct 794 ..............N...NN-..........-.N.-..........-..N.......-.. 848

Query 480 C 480
Sbjct 849 . 849
```

Score	Expect	Method	Identities	Positives	Gaps
285 bits(728)	8e-101	Compositional matrix adjust.	134/140(96%)	135/140(96%)	0/140(0%)

```
Query 1 MSAVMQSGLVKMSHPSGDVEACMVQVTCGSMTLNGLWLDNTVWCPRHVMCPADQLSDPNY 60
Sbjct 126 ..................................................................... 185

Query 61 DALLISMTNHSFSVQKHIGAPANLRVVGHAMQGTLLKLTVDVANPSTPAYTFTTVKPGAA 120
Sbjct 186 ..................................................................... 245

Query 121 FSVLACYNGRPTGTFTVVMR 140
Sbjct 246 .............YXHSXI. 265
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pNIC-SUMO-MERS-FP-4/RP-2 MVMPROA-c006

FP-4/RP-2

```
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CGGTTCATGTGGTAGTGTGGGCTACACCAAAGAGGGCTCGGTGATAAATTTCTGTTACATGCACCAAATGGAACTTGCAAACGGGACCCACACCGGTAGTGCGTTTGATGGTA
CCATGTATGGAGCTTTCATGGACAAGCAAGTTCACCAAGTGCAACTGACCGATAAGTACTGCTCTGTTAATGTCGTGGCTTGGCTGTATGCCGCCATCTTGAACGGCTGCGCC
TGGTTTGTCAAGCCAAATCGCACGTCCGTTGTAAGCTTCAACGAGTGGGCCCTGGCAAACCAATTCACTGAGTTCGTTGGGACCCAGTCTGTCGACATGTTGGCCGTTAAGAC
GGGTGTAGCGATAGAGCAGCTGCTTTACGCAATCCAACAATTATACACAGGTTTCCAGGGAAAGCAGATTCTCGGTTCTACAATGTTAGAGGACGAATTCACGCCCGAGGACG
```

TGAACATGCAGATCATGGGCGTTGTCATGCAATAATGA

```
MSAVPQSGLVKMSHPSGDVEACMVQVTCGSMTLNGLWLDNTVWCPRHVMCPADQLSDPNYDALLISMTNHSFSVQKHIGAPANLRVVGHAMQGTLLKLTVDVANPSTPAYTFT
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```

WFVKPNRTSVVSFNEWALANQFTEFVGTQSVDMLAVKTGVAIEQLLYAIQQLYTGFQGKQILGSTMLEDEFTPEDVNMQIMGVVMQ--

471681101_MERS_6_T7R_F12.seq

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GAACAGGAAGCGAAGCGGGTTCATAGGAACCCCTTGCCGTTGAAANGAAACTCTCCCTTCAACTTCTTGGGGGGGNNNGGGCNNNTTTT

```
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```

WFVKPNRTSVVSFNEWALANQFTEFVGTQSVDMLAVKTGVAIEQLLYAIQQLYTGFQGKQILGSTMLEDEFTPEDVNMQIMGVVMQ—

Score	Expect	Identities	Gaps	Strand
1740 bits(942)	0.0	942/942(100%)	0/942(0%)	Plus/Minus

```
Query 1 ATGTCCGCTGTCCCTCAATCTGGTCTCGTTAAGATGTCCCATCCTAGCGGAGACGTCGAG 60
Sbjct 1037 ..........................................................  978

Query 61 GCGTGTATGGTACAAGTTACGTGCGGGAGCATGACGTTAAACGGATTGTGGCTCGACAAT 120
```

```
Sbjct  977   ..........................................................  918

Query  121   ACAGTTTGGTGCCCACGACATGTCATGTGTCCGGCTGATCAATTAAGTGACCCAAATTAC  180

Sbjct  917   ..........................................................  858

Query  181   GACGCGTTGCTCATATCCATGACGAACCACAGCTTTAGCGTCCAAAAGCACATTGGTGCC  240

Sbjct  857   ..........................................................  798

Query  241   CCTGCTAACCTTCGGGTCGTTGGCCATGCTATGCAGGGCACTCTTTTAAAGCTGACAGTT  300

Sbjct  797   ..........................................................  738

Query  301   GACGTTGCTAACCCTAGTACGCCTGCCTATACATTCACTACGGTTAAACCCGGCGCCGCG  360

Sbjct  737   ..........................................................  678

Query  361   TTCTCCGTTTTGGCATGTTACAACGGTCGTCCGACAGGTACGTTCACAGTAGTTATGCGG  420

Sbjct  677   ..........................................................  618

Query  421   CCAAATTATACCATCAAAGGTAGCTTCCTTTGCGGTTCATGTGGTAGTGTGGGCTACACC  480

Sbjct  617   ..........................................................  558

Query  481   AAAGAGGGCTCGGTGATAAATTTCTGTTACATGCACCAAATGGAACTTGCAAACGGGACC  540

Sbjct  557   ..........................................................  498

Query  541   CACACCGGTAGTGCGTTTGATGGTACCATGTATGGAGCTTTCATGGACAAGCAAGTTCAC  600

Sbjct  497   ..........................................................  438

Query  601   CAAGTGCAACTGACCGATAAGTACTGCTCTGTTAATGTCGTGGCTTGGCTGTATGCCGCC  660

Sbjct  437   ..........................................................  378

Query  661   ATCTTGAACGGCTGCGCCTGGTTTGTCAAGCCAAATCGCACGTCCGTTGTAAGCTTCAAC  720

Sbjct  377   ..........................................................  318

Query  721   GAGTGGGCCCTGGCAAACCAATTCACTGAGTTCGTTGGGACCCAGTCTGTCGACATGTTG  780

Sbjct  317   ..........................................................  258
```

```
Query  781   GCCGTTAAGACGGGTGTAGCGATAGAGCAGCTGCTTTACGCAATCCAACAATTATACACA  840

Sbjct  257   ............................................................  198


Query  841   GGTTTCCAGGGAAAGCAGATTCTCGGTTCTACAATGTTAGAGGACGAATTCACGCCCGAG  900

Sbjct  197   ............................................................  138


Query  901   GACGTGAACATGCAGATCATGGGCGTTGTCATGCAATAATGA  942

Sbjct  137   ........................................  96
```

Score	Expect	Method	Identities	Positives	Gaps
652 bits(1682)	0.0	Compositional matrix adjust.	312/312(100%)	312/312(100%)	0/312(0%)

```
Query  1    MSAVPQSGLVKMSHPSGDVEACMVQVTCGSMTLNGLWLDNTVWCPRHVMCPADQLSDPNY  60

Sbjct  1    ............................................................  60


Query  61   DALLISMTNHSFSVQKHIGAPANLRVVGHAMQGTLLKLTVDVANPSTPAYTFTTVKPGAA  120

Sbjct  61   ............................................................  120


Query  121  FSVLACYNGRPTGTFTVVMRPNYTIKGSFLCGSCGSVGYTKEGSVINFCYMHQMELANGT  180

Sbjct  121  ............................................................  180


Query  181  HTGSAFDGTMYGAFMDKQVHQVQLTDKYCSVNVVAWLYAAILNGCAWFVKPNRTSVVSFN  240

Sbjct  181  ............................................................  240


Query  241  EWALANQFTEFVGTQSVDMLAVKTGVAIEQLLYAIQQLYTGFQGKQILGSTMLEDEFTPE  300

Sbjct  241  ............................................................  300


Query  301  DVNMQIMGVVMQ  312

Sbjct  301  ............  312
```

seems all good

471701601_MERS_6_T7F_F10.seq

NNNNNNNNNNNTNNNNNCTNNNNNNATTTTGTTTAACTTTAAGAAGGAGATATACATATGCACCACCATCATCACCATGGC
TCAGGAGATCAGGAAGCGAAACCTTCGACCGAGGACCTGGGCGACAAGAAAGAAGGGGAGTATATTAAGTTAAAGGTGATC
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NCTTGTTGGCCGTTAAAAACGGGNNNTANNGAATANNNNANGNTGCTTTNANNNNANNCNNAANTTTTTNACCANNGTTNN
NGGNNAAAANNNANNTTTCCGNNTNNTNNATTNTAAAAGAAAAN

MHHHHHHGSGDQEAKPSTEDLGDKKEGEYIKLKVIGQDSSEIHFKVKMTTHLKKLKESYCQRQGVPMNSLRFLFEGQRIADN
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PNYTIKGSFLCGSCGSVGYTKEGSVINFCYMHQMELANGTHTGSAFDGTMYGAFMDKQVHQVQLTDKYCSVNVVAWLYAAIL
NGXXWFVKPNRPSVVSFNEWALANQFTEFVGTQSVXLVGR

Score	Expect	Method	Identities	Positives	Gaps
537 bits(1383)	0.0	Compositional matrix adjust.	254/261(97%)	256/261(98%)	0/261(0%)

```
Query 106 MSAVPQSGLVKMSHPSGDVEACMVQVTCGSMTLNGLWLDNTVWCPRHVMCPADQLSDPNY 165
Sbjct 1   ............................................................ 60

Query 166 DALLISMTNHSFSVQKHIGAPANLRVVGHAMQGTLLKLTVDVANPSTPAYTFTTVKPGAA 225
Sbjct 61  ............................................................ 120

Query 226 FSVLACYNGRPTGTFTVVMRPNYTIKGSFLCGSCGSVGYTKEGSVINFCYMHQMELANGT 285
Sbjct 121 ............................................................ 180

Query 286 HTGSAFDGTMYGAFMDKQVHQVQLTDKYCSVNVVAWLYAAILNGXXWFVKPNRPSVVSFN 345
Sbjct 181 ..............................................CA.......T...... 240

Query 346 EWALANQFTEFVGTQSVXLVG 366
Sbjct 241 ................DMLA 261
```

PLOS ONE | DOI:10.1371/journal.pone.0144865 December 14, 2015

Table 2. The kinetic parameters and dissociation constants of MERS-CoV M^{pro}.

Proteins	Kinetic Parameters[a]			Dissociation Constant[b]	
	K_m (μM)	k_{cat} (s^{-1})	*h*	No substrate(μM)	With 600 μM substrate (μM)
MERS-CoV M^{pro}					
Wild-type	-	2.33 ± 0.13	1.8 ± 0.04	7.7 ± 0.3	0.7 ± 0.04
V4R	-	0.96 ± 0.05	2.7 ± 0.2	23.0 ± 0.4	15.2 ± 0.3
T126S	-	0.56 ± 0.04	2.0 ± 0.2	33.7 ± 0.9	13.9 ± 0.1
E169A	-	0.41 ± 0.02	2.1 ± 0.1	14.3 ± 0.2	14.1 ± 0.5
M298R	181.0 ± 24.0	7.91 ± 0.49	-	1.1 ± 0.1	0.7 ± 0.01
T126S/M298R	419.4 ± 63.9	4.63 ± 0.37	-	2.8 ± 0.1	0.9 ± 0.01
SARS-CoV Mpro	890 ± 130	2.11 ± 0.15	-	0.7 ± 0.02	1.7 ± 0.03[c]

[a] Kinetic data of SARS-CoV M^{pro} and MERS-CoV M298R and T126S/M298R mutants were fitted to the Michaelis-Menten equation (Eq 1), while those of the others were fitted to the Hill equation (Eq 2). The R_{sqr} were from 0.985 to 0.999, respectively. All the assays were repeated twice and the average values were used for the fitting.

[b] The values were derived from a global fit of the AUC data to a monomer-dimer self-association model by SEDPHAT [32]. The experiments for the assay were obtained at protein concentration of 1.5 to 30 μM.

[c] The value was from our previous studies for comparison [28].

doi:10.1371/journal.pone.0144865.t002

Restrictions on Gene ordering

Apparently some issues whant trying to order this gene from companies in the states (some US government restriction)

Try ordering as gBLOCK from IDT (made in Belgium so avoid US restrictions?)
nope rejected due to below

Unfortunately the sequence for reference number 232813136 "AVID_1" includes a partial or full coding region from MERS. Unfortunately this item qualifies as an ECCN 1C353 item under U.S. Dept. of Commerce Export Administration Regulations and therefore requires an export license for shipment outside the United States.

I regret to inform you that IDT's current policy prohibits us from shipping items that require this license, therefore we must cancel this item and issue a full refund. I apologize for any inconveniences this may have caused.

NBS biologicals UK based said also not possible, didn't cite legislation but said "high risk"

GeneArt simply cancelled order

Suspect will run into the same issue with all suppliers until relevant paperwork etc. in place

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Title missing - double click to edit

according to the below at pH 8 and 30% glycerol activity is much reduced if we combine the two during purifcation and then after imac dialyze o/n against pH7 0% glycerol buffer can we get MERS 3CL to celave off its own tag

If one of the `MSAVLQ mutants is slower processing this may work.`

Title missing - double click to edit

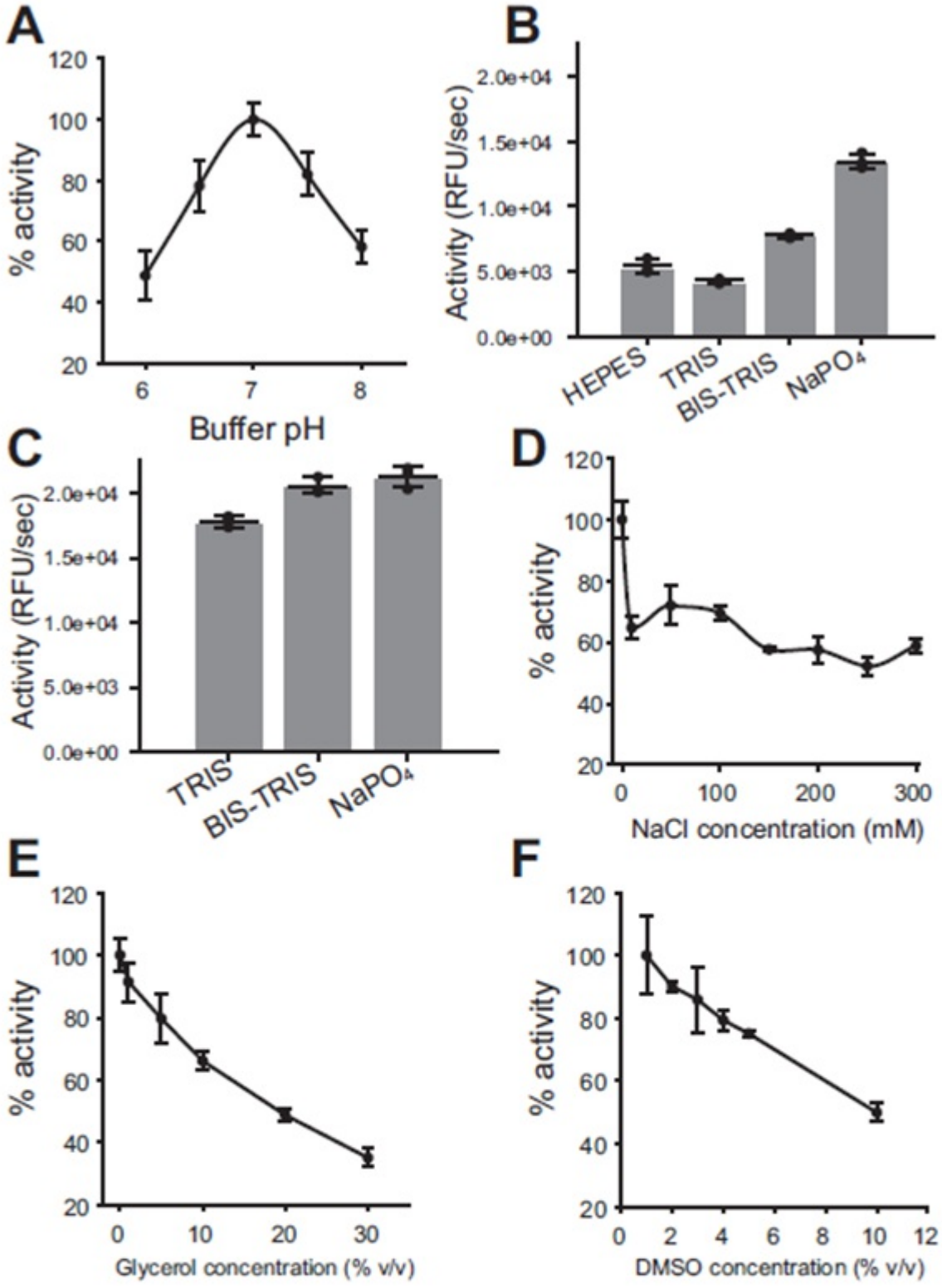

Figure 3. Assessing the effects of common buffer conditions on M^{pro} initial rate. All tests are done in 100 µl buffer containing 100 µM VKLQ–AMC substrate and 200 nM enzyme. *A*, pH optimization in 20 mM $NaPO_4$ (pH 6.0–8.0) with 150 mM NaCl. *B*, preference of M^{pro} for Hepes, Tris, Bis–Tris, or $NaPO_4$, in 20 mM buffering agent, pH 7.0, with 150 mM NaCl. *C*, preference of M^{pro} for Tris, Bis–Tris, and $NaPO_4$ in 20 mM buffering agent, pH 7.0. *D*, effect of 0 to 300 mM NaCl in 20 mM $NaPO_4$ (pH 7.0). *E*, effect of 0 to 30% v/v glycerol in 20 mM $NaPO_4$ (pH 7.0) with 150 mM NaCl. *F*, effect of 1 to 10% v/v DMSO in 20 mM $NaPO_4$ (pH 7.0) with 150 mM NaCl. Each measurement is reported as the mean with error bars showing ±1 standard deviation, $n = 3$. AMC, 7-amino-4-methylcoumarin; DMSO, dimethyl

sulfoxide; M^{pro}, main protease.

Title missing - double click to edit

https://doi.org/10.1074/jbc.M115.651463

>4RSP_1|Chain A|Orf1a protein|Middle East respiratory syndrome coronavirus (1335626)
SGLVKMSHPSGDVEACMVQVTCGSMTLNGLWLDNTVWCPRHVMCPADQLSDPNYDALLISMTNHSFSVQKHIGAPANLRVVGHAMQGTLLKLTVDVANPSTPAYTFTTVKPGA
AFSVLACYNGRPTGTFTVVMRPNYTIKGSFLCGSCGSVGYTKEGSVINFCYMHQMELANGTHTGSAFDGTMYGAFMDKQVHQVQLTDKYCSVNVVAWLYAAILNGCAWFVKPN
RTSVVSFNEWALANQFTEFVGTQSVDMLAVKTGVAIEQLLYAIQQLYTGFQGKQILGSTMLEDEFTPEDVNMQIMGVVMQ
>4RSP_2|Chain B|Peptide inhibitor|null
XSVLX
>4YLU_1|Chains A, B, C, D|ORF1a protein|Middle East respiratory syndrome coronavirus (1335626)
SGLVKMSHPSGDVEACMVQVTCGSMTLNGLWLDNTVWCPRHVMCPADQLSDPNYDALLISMTNHSFSVQKHIGAPANLRVVGHAMQGTLLKLTVDVANPSTPAYTFTTVKPGA
AFSVLACYNGRPTGTFTVVMRPNYTIKGSFLCGSCGSVGYTKEGSVINFCYMHQMELANGTHTGSAFDGTMYGAFMDKQVHQVQLTDKYCSVNVVAWLYAAILNGCAWFVKPN
RTSVVSFNEWALANQFTEFVGTQSVDMLAVKTGVAIEQLLYAIQQLYTGFQGKQILGSTMLEDEFTPEDVNMQIMGVVMQ

Construct Design and Expression of MERS-CoV $3CL^{pro}$

The gene encoding $3CL^{pro}$ protease of MERS-CoV (amino acid residues 3248–3553 in the replicase polyprotein, GenBank™ accession number AHC74086.1) was codon-optimized for optimal expression in *E. coli* (BioBasic Inc). The gene was subcloned into pET-11a expression vector with an N-terminal His_6 tag followed by the nsp4↓nsp5 auto-cleavage site using the forward primer 5′-ATATACATATGCACCACCACCACCACCACAGCGGTGTTCTGCAGTCTGGTC-3′ and the reverse primer 5′-GACGGATCCTTACTGCATCACAACACCCATGATCTGC-3′. The construct was verified by DNA sequencing at the Purdue University Genomics Core Facility. This construct results in the expression of MERS-CoV $3CL^{pro}$ without any N- or C-terminal extensions. MERS-CoV $3CL^{pro}$ was expressed through auto-induction in *Escherichia coli* BL21-DE3 cells in the presence of 100 μg/ml carbenicillin as described previously (41). Cells were harvested by centrifugation at 5000 × *g* for 20 min at 4 °C, and the pellets were stored at −80 °C until further use.

MERS-CoV $3CL^{pro}$ Purification

Frozen pellets from 4 liters of bacterial cell culture were thawed on ice and resuspended in 250 ml of Buffer A (20 mm Tris, pH 7.5, 0.05 mm EDTA, 10% glycerol, and 5 mm β-mercaptoethanol (BME)), containing 500 μg of lysozyme and a small amount of DNase. Cells were then lysed using a single pass through a French press at 1200 p.s.i., and cell debris was removed from the cleared lysate by centrifuging at 29,000 × *g* for 30 min. Solid ammonium sulfate was added to the cleared lysate to a final concentration of 1 m through gradual mixing on ice.

Hydrophobic Interaction Chromatography

The cleared lysate, mixed with ammonium sulfate, was loaded at a flow rate of 3 ml/min onto a 60-ml phenyl-Sepharose 6 fast-flow high-sub column (Amersham Biosciences) equilibrated with Buffer B (50 mm Tris, pH 7.5, 1 m ammonium sulfate, 0.05 mm EDTA, 10% glycerol, and 5 mm BME). The column was then washed with 5× column volume (300 ml) of Buffer B at a flow rate of 4 ml/min. Protein was eluted using a 5× column volume (300 ml) linear gradient to 100% Buffer A. Fractions (12 ml) were collected, and those containing MERS-CoV $3CL^{pro}$, as judged through SDS-PAGE analysis and specific activity measurements, were pooled (120 ml) and exchanged into 2 liters of Buffer A via overnight dialysis in a 10,000 molecular weight cutoff SnakeSkin® dialysis tubing (Thermo Scientific).

DEAE Anion-exchange Chromatography

Dialyzed sample from the previous step was loaded at a flow rate of 3 ml/min onto a 120- ml DEAE anion-exchange column (Amersham Biosciences) equilibrated with Buffer A. The column was then washed with 2× column volume (240 ml) of Buffer A at a flow rate of 4 ml/min. A linear gradient (total volume 480 ml) to 40% Buffer C (50 mm Tris, pH 7.5, 1 m NaCl, 0.05 mm EDTA, 10% glycerol, and 5 mm BME) was used to elute the protein. Fractions (6 ml) were collected, and those containing MERS-CoV $3CL^{pro}$ were pooled (66 ml) and dialyzed for 4 h in 4 liters of Buffer D (20 mm MES, pH 5.5, 0.05 mm EDTA, 10% glycerol, and 5 mm BME).

Mono S Cation-exchange Chromatography

Following dialysis, the pH of the sample was manually adjusted to 5.5 using 1 m solution of MES, pH 5.5, and any precipitated protein was removed by filtering through a 0.22-μm pore size Millex-GP filter (Millipore). The filtered sample was then loaded at a flow rate of 2 ml/min onto an 8-ml Mono S 10/100 column (Amersham Biosciences) equilibrated in Buffer D. The column was then washed with 5× column volume (40 ml) of Buffer D at a flow rate of 2 ml/min. Protein was eluted using a 25× column volume (200 ml) and a linear gradient to 50% Buffer E (50 mm MES, pH 5.5, 1 m NaCl, 0.05 mm EDTA, 10% glycerol, and 5 mm BME). Fractions (2 ml) were collected, and those containing MERS-CoV $3CL^{pro}$ were pooled (22 ml) and concentrate
to ~5 mg/ml.

Gel Filtration Chromatography

As the final purification step, the concentrated protein sample was loaded onto the preparation grade Superdex 75 26/60 gel filtration column (Amersham Biosciences) equilibrated with Buffer F (25 mm HEPES, pH 7.5, 10% glycerol, 2.5 mm dithiothreitol (DTT)). Protein was eluted isocratically at a flow rate of 1 ml/min with Buffer F. Fractions (2 ml) containing MERS-CoV $3CL^{pro}$ were pooled (total volume of 34 ml) and concentrated to ~5 mg/ml. For final storage of the purified MERS-CoV $3CL^{pro}$ enzyme, 300-μl protein aliquots were placed into 1-ml screw-cap vials, flash-frozen under liquid nitrogen, and then stored at −80 °C until further use.

MERS-CoV 3CLpro Crystallization, X-ray Data Collection,
and Structure Determination—Purified MERS-CoV 3CLpro was concentrated to 1.6 mg/ml in 25 mM HEPES, pH 7.5, and 2.5 mM DTT. Inhibitor complexes of MERS-CoV 3CLpro with compounds 6 and 11 were formed by incubating MERS-CoV 3CLpro with the compounds in a 1:3 stoichiometric ratio at 4 °C overnight. After iterative rounds of optimization of the crystallization conditions based on the initial hits obtained from high throughput screening of Qiagen Nextel Screens, crystals of MERS-CoV 3CLpro inhibitor complexes suitable for x-ray diffraction were grown by the hanging-drop, vapor diffusion method at 20 °C in 0.2 M sodium acetate, 0.1 M BisTris, pH 7.0,and 20% PEG-3350 for the MERS-CoV 3CLpro and 6 complex, and 0.2 M ammonium acetate, 0.1 M BisTris, pH 5.5, 12% PEG-3350 for the MERS-CoV 3CLpro and 11 complex. For x-ray data collection, crystals were flash-cooled in liquid nitrogen after dragging the crystals through a cryo-solution that contained the crystallization solution supplemented with 15% 2-methyl-2,4-pentanediol.

>5C3N_1|Chains A, B|ORF1a protein|Middle East respiratory syndrome coronavirus (1335626)
SGLVKMSHPSGDVEACMVQVTCGSMTLNGLWLDNTVWCPRHVMCPADQLSDPNYDALLISMTNHSFSVQKHIGAPANLRVVGHAMQGTLLKLTVDVANPSTPAYTFTTVKPGA
AFSVLACYNGRPTGTFTVVMRPNYTIKGSFLCGSCGSVGYTKEGSVINFCYMHQMELANGTHTGSAFDGTMYGAFMDKQVHQVQLTDKYCSVNVVAWLYAAILNGCAWFVKPN
RTSVVSFNEWALANQFTEFVGTQSVDMLAVKTGVAIEQLLYAIQQLYTGFQGKQILGSTMLEDEFTPEDVNMQIMGVVMQ

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Materials and Methods

Expression Plasmid Construction

The sequence of the MERS-CoV Mpro (GenBank accession number AHC74086; polyprotein residues 3248–3553) was synthesized (MDBio Inc.), digested by NdeI-XhoI and then inserted into the vector pET-28a(+) (Novagen). In this construct, the 6 x His tag is retained at the N-terminus. To remove the fusion tag and generate an authentic N-terminus for protein purification, the codons of the thrombin cutting recognition sequence and a NdeI cutting site were removed and then inserted the codons of Leu-Arg-Leu-Lys-Gly-Gly into the above vector. The forward primer sequence for site-directed mutagenesis was 5′-CATCACAGCAGCGGCCTGCGTCTGAAAGGCGGCAGCGGTTTGGTGAAAATG-3′ and the reverse primer was 5′-CATTTTCACCAAACCGCTGCCGCCTTTC AGACGCAGGCCGCTGCTGTGATG-3′. The reading frame of the final plasmid was confirmed by sequencing.

Expression and Purification of MERS-CoV Mpro
The expression vector was transformed into E. coli BL21 (DE3) cells (Novagen). Cultures were grown in 0.8 liters of LB medium at 37°C for 4 h, induced with 0.4 mM isopropyl-β-D thiogalactopyranoside, and then incubated overnight at 20°C. After centrifuging at 6,000 x g at 4°C for 15 min, the cell pellets were resuspended in lysis buffer (20 mM Tris, pH 8.5, 250 Mm NaCl, 5% glycerol, 0.2% Triton X-100, and 2 mM β-mercaptoethanol) and then lysed by sonication. The crude extract was then centrifuged at 12,000 x g at 4°C for 25 min to remove the insoluble pellet. Next the supernatant was incubated with 1-ml Ni-NTA beads at 4°C for 1 h and then loaded onto an empty column. After allowing the supernatant to flow through, the beads were washed with washing buffer (20 mM Tris, pH 8.5, 250 mM NaCl, 8 mMimidazole, and 2 mMβ-mercaptoethanol). The SARS-CoV papain-like protease [12] (1 mg in 100 mM phosphate buffer (pH 6.5)) was then added and incubated for 3 h. The SARS-CoV papain-like protease digestion, which removed the 6 x His tag and Leu-Arg-Leu-Lys-Gly-Gly fragment, resulted in a native protein product with an authentic N-terminus. The digest was allowed to flow through and then loaded onto a S-100 gel-filtration column (GE Healthcare) equilibrated with running buffer (20 mM Tris, pH 8.5, 100 mM NaCl, and 2 mMdithiothreitol). The purity of the fractions collected was analyzed by SDS-PAGE and the protein was concentrated to 30 mg/ml by Amicon Ultra-4 10-kDa centrifugal filter (Millipore).

Protein Crystallography
Crystals of the MERS-CoV Mpro were obtained at 295 K by the sitting-drop vapor-diffusion method. The protein solution was set up at 5 mg/ml and the reservoir solution consisted of 0.1M Tris, pH 8.4, 15% (w/v) PEG 4000 and 0.2 Msodium acetate. Clusters of needle crystals appeared in 2 days and were used for micro-seeding. Single cystals of rectangle shape and with dimensions of 0.3–0.5 mm were obtained in less than a week. All crystals were cryoprotected in the reservoir solution with 15% glycerol and were flash-cooled in liquid nitrogen.

>7VTC_1|Chains A, B|3C-like proteinase|Middle East respiratory syndrome-related coronavirus (1335626)
SGLVKMSHPSGDVEACMVQVTCGSMTLNGLWLDNTVWCPRHVMCPADQLSDPNYDALLISMTNHSFSVQKHIGAPANLRVVGHAMQGTLLKLTVDVANPSTPAYTFTTVKPGA
AFSVLACYNGRPTGTFTVVMRPNYTIKGSFLCGSCGSVGYTKEGSVINFCYMHQMELANGTHTGSAFDGTMYGAFMDKQVHQVQLTDKYCSVNVVAWLYAAILNGCAWFVKPN
RTSVVSFNEWALANQFTEFVGTQSVDMLAVKTGVAIEQLLYAIQQLYTGFQGKQILGSTMLEDEFTPEDVNMQIMILGSTMLEDEFTPEDVNMQIM

5WKM

Space Group: C 1 2 1
Unit Cell:

Length (Å)	Angle (°)
a = 100.626	α = 90
b = 58.114	β = 112.07
c = 49.862	γ = 90

4.5. X-ray crystallographic studies. Crystallization and data collection
Purified MERS-CoV 3CLpro, in 100 mM NaCl, 20 mM Tris pH 8.0, was concentrated to 8 mg/mL (0.5 mM). Stock solutions of 100 mM GC376, GC813, compound ***10c*** or compound ***10e*** were prepared in DMSO and the complex with MERS 3CLpro was prepared by mixing the concentrated protein supplemented with 3 mM compound and incubating overnight at 4 °C. All crystallization experiments were conducted using Compact 300 (Rigaku Reagents) sitting drop vapor diffusion plates at 20 °C using equal volumes of protein and crystallization solution equilibrated against 75 µL of the latter. Crystals of MERS 3CLpro in complex with GC813, compound ***10c*** and compound ***10e*** that displayed a prismatic morphology were obtained from the Index HT screen (Hampton Research) condition G10 (25% (w/v) PEG 3350, 100 mM Bis-Tris pH 5.5, 200 mM $MgCl_2$) in 1–2 days. Crystals of the GC376 complex were obtained from the Index HT screen (Hampton Research) condition E6 (30% (v/v) PEG 550 MME, 100 mM Bis-Tris pH 6.5, 50 mM $CaCl_2$). Samples were transferred to a fresh drop containing 80% crystallant and 20% (v/v) PEG 200 before storing in liquid nitrogen. X-ray diffraction data were collected at the Advanced Photon Source beamline 17-ID using a Dectris Pilatus 6 M pixel array detector.