AVIDD ASAP: MVMPROA c907 (c900 Arg mutant) Sequencing Expression and Purification

PAGE23-00183

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Date Started: **2023-Jan-24**
Experiment Started:
Projects: **Cloning;Expression;Purification;ASAP**
Related Pages:
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Title missing - double click to edit

Received two plasmids from MArco at DLS F1 and F3 both should be Arginine mutant

`MVMPROA-c900` (icludes corrections in cloning regions)

gctcctgggcaacgtgctggttattgtgctgtctcatcattttggcaaagaattggatcggaccgaaattaatacgactcactatagggggaattgtgagcggataa
caattccccggagttaatccgggacctttaattcaacccaacacaatatattatagttaaataagaattattatcaaatcatttgtatattaattaaaatactatactgt
aaattacattttatttacaatcaaaggagatataccatgggtagcagccatcaccatcatcatcacgggagcgatagcgaagtgaaccaggaagcgaaaccgg
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GAAGCGTGCATGGTGCAGGTTACGTGCGGGTCTATGACGCTTAATGGTCTTTGGCTTGATAACACAGTGTGGTGCCCGCGC
CATGTTATGTGCCCAGCGGATCAGTTGAGTGACCCTAATTATGACGCGCTGTTAATTAGTATGACTAATCACTCCTTCTCCGTT
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MSHPSGDVEACMVQVTCGSMTLNGLWLDNTVWCPRHVMCPADQLSDPNYDALLISMTNHSFSVQKHIGAPANLRVVGHAMQGTLLKLTVDVANPSTPAYTFTTVKPGAAFSVL
ACYNGRPTGTFTVVMRPNYTIKGSFLCGSCGSVGYTKEGSVINFCYMHQMELANGTHTGSAFDGTMYGAFMDKQVHQVQLTDKYCSVNVVAWLYAAILNGCAWFVKPNRTSVV

SFNEWALANQFTEFVGTQSVDMLAVKTGVAIEQLLYAIQQLYTGFQGKQILGSTMLEDEFTPEDVN
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MQIMGVVMQ-

`Arg mutant F1 clone` sequencing

`472782801_1_T7F_A08.seq`

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CGCATCGCGAACAGATCGGTGGTTCGGGCCTGGTAAAGATGTCACATCCAAGCGGTGATGTAGAAGCGTGCATGGTGCAGGTTACGTGCGGGTCTATGACGCTTAATGGTCTT
TGGCTTGATAACACAGTGTGGTGCCCGCGCCATGTTATGTGCCCAGCGGATCAGTTGAGTGACCCTAATTATGACGCGCTGTTAATTAGTATGACTAATCACTCCTTCTCCGTT
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TCACGACCGTTAAGCCCGGTGCCGCATTTAGTGTCCTTGCTTGCTACAATGGACGTCCGACGGGAACATTCACTGTTGTTATGCGCCCTAATTATACGATTAAAGGTAGTTTC
CTGTGTGGATCATGTGGCAGTGTAGGGTATACCAAAGAAGGGTCCGTTATTAACTTCTGTTACATGCACCAAATGGAATTGGCCAACGGAACTCACACCGGTTCAGCTTTCGA
CGGAACTATGTACGGAGCGTTTATGGATAAGCAGGTACACCAGGTACAACTTACAGATAAGTATTGTTCAGTGAACGTGGTTGCCTGGCTTTACCCGGCTATTTTGAATGGGT
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GTGCATGGTTTGTCAAACCCAACCGTACAAGTGTAGTGTCATTCAATGAGTGGGCGTTAGCTAATCAATTCACCGAATTTGTTGGCACCCAGTCAGTGGATATGCTGGCAGTG
AAAACTGGAGTCGCTATTGANCAATTNTTAATATGCCATCCAGCAGTTTATTNCNGGCTTCAGGGNANNCAAATTTNNGGTAG

	Score	Expect	Identities	Gaps	Strand
	2108 bits(1141)	0.0	1163/1177(99%)	4/1177(0%)	Plus/Plus

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Query  1    ATGGGTAGCAGCCATCACCATCATCATCACGGGAGCGATAGCGAAGTGAACCAGGAAGCG  60

Sbjct  152  ...........................................................  211


Query  61   AAACCGGAAGTTAAACCGGAAGTGAAACCGGAAACCCATATTAATCTGAAAGTTAGCGAC  120

Sbjct  212  ...........................................................  271


Query  121  GGCAGCAGCGAAATCTTTTTTAAAATTaaaaaaaaCCACCCCGCTGCGTCGCCTGATGGAA  180

Sbjct  272  ...........................................................  331


Query  181  GCCTTTGCGAAACGTCAGGGTAAAGAAATGGATAGCCTGCGCTTTCTGTATGACGGCATC  240

Sbjct  332  ...........................................................  391


Query  241  CGTATTCAGGCCGATCAGACCCCGGAAGACCTGGATATGGAAGACAACGATATTATTGAA  300

Sbjct  392  ...........................................................  451


Query  301  GCGCATCGCGAACAGATCGGTGGTTCGGGCCTGGTAAAGATGTCACATCCAAGCGGTGAT  360

Sbjct  452  ...........................................................  511


Query  361  GTAGAAGCGTGCATGGTGCAGGTTACGTGCGGGTCTATGACGCTTAATGGTCTTTGGCTT  420

Sbjct  512  ...........................................................  571


Query  421  GATAACACAGTGTGGTGCCCGCGCCATGTTATGTGCCCAGCGGATCAGTTGAGTGACCCT  480

Sbjct  572  ...........................................................  631


Query  481  AATTATGACGCGCTGTTAATTAGTATGACTAATCACTCCTTCTCCGTTCAGAAACATATC  540

Sbjct  632  ...........................................................  691


Query  541  GGCGCACCGGCGAACCTTCGCGTCGTTGGACACGCTATGCAAGGGACACTTCTTAAGTTG  600

Sbjct  692  ...........................................................  751


Query  601  ACGGTTGATGTTGCAAATCCTAGCACCCCAGCATACACTTTCACGACCGTTAAGCCCGGT  660

Sbjct  752  ...........................................................  811
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Query  661   GCCGCATTTAGTGTCCTTGCTTGCTACAATGGACGTCCGACGGGAACATTCACTGTTGTT  720

Sbjct  812   ............................................................  871


Query  721   ATGCGCCCTAATTATACGATTAAAGGTAGTTTCCTGTGTGGATCATGTGGCAGTGTAGGG  780

Sbjct  872   ............................................................  931


Query  781   TATACCAAAGAAGGGTCCGTTATTAACTTCTGTTACATGCACCAAATGGAATTGGCCAAC  840

Sbjct  932   ............................................................  991


Query  841   GGAACTCACACCGGTTCAGCTTTCGACGGAACTATGTACGGAGCGTTTATGGATAAGCAG  900

Sbjct  992   ............................................................  1051


Query  901   GTACACCAGGTACAACTTACAGATAAGTATTGTTCAGTGAACGTGGTTGCCTGGCTTTAC  960

Sbjct  1052  ............................................................  1111


Query  961   GCGGCTATTTTGAATGGGTGTGCATGGTTTGTCAAACCCAACCGTACAAGTGTAGTGTCA  1020

**Sbjct  1112  C...........................................................  1171**


Query  1021  TTCAATGAGTGGGCGTTAGCTAATCAATTCACCGAATTTGTTGGCACCCAGTCAGTGGAT  1080

Sbjct  1172  ............................................................  1231


Query  1081  ATGCTGGCAGTGAAAACTGGAGTCGCTATTGAGCAATTATT-ATATGCCATCCAGCAGTT  1139

**Sbjct  1232  ...............................N.....N..A...................  1291**


Query  1140  ATATACC-GGCTTCCAGGGTAAGCAAATTTTAGGTAG  1175

**Sbjct  1292  -...TN.N.....-.....N.NN.......NN.....  1326**
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MGSSHHHHHHGSDSEVNQEAKPEVKPEVKPETHINLKVSDGSSEIFFKIKKTTPLRRLMEAFAKRQGKEMDSLRFLYDGIRIQADQTPEDLDMEDNDIIEAHREQIGGSGLVK
MSHPSGDVEACMVQVTCGSMTLNGLWLDNTVWCPRHVMCPADQLSDPNYDALLISMTNHSFSVQKHIGAPANLRVVGHAMQGTLLKLTVDVANPSTPAYTFTTVKPGAAFSVL
ACYNGRPTGTFTVVMRPNYTIKGSFLCGSCGSVGYTKEGSVINFCYMHQMELANGTHTGSAFDGTMYGAFMDKQVHQVQLTDKYCSVNVVAWLYPAILNGCAWFVKPNRTSVV

SFNEWALANQFTEFVGTQSVDMLAVKTGVAIXQXLICHPAVYXXLQXXQIXG

Score	Expect	Method	Identities	Positives	Gaps
763 bits(1969)	0.0	Compositional matrix adjust.	364/379(96%)	365/379(96%)	0/379(0%)

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Query  13   DSEVNQEAKPEVKPEVKPETHINLKVSDGSSEIFFKIKKTTPLRRLMEAFAKRQGKEMDS  72

Sbjct  13   ...........................................................  72
```

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Query  73    LRFLYDGIRIQADQTPEDLDMEDNDIIEAHREQIGGSGLVKMSHPSGDVEACMVQVTCGS  132

Sbjct  73    .........................................................  132


Query  133   MTLNGLWLDNTVWCPRHVMCPADQLSDPNYDALLISMTNHSFSVQKHIGAPANLRVVGHA  192

Sbjct  133   .........................................................  192


Query  193   MQGTLLKLTVDVANPSTPAYTFTTVKPGAAFSVLACYNGRPTGTFTVVMRPNYTIKGSFL  252

Sbjct  193   .........................................................  252


Query  253   CGSCGSVGYTKEGSVINFCYMHQMELANGTHTGSAFDGTMYGAFMDKQVHQVQLTDKYCS  312

Sbjct  253   .........................................................  312


Query  313   VNVVAWLYAAILNGCAWFVKPNRTSVVSFNEWALANQFTEFVGTQSVDMLAVKTGVAIEQ  372

```
**Sbjct**  313   `........`**P**`...................................................`**X**`.`  372
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Query 373   LLYAIQQLYTGFQGKQILG 391

```
**Sbjct**  373   **X**`.`**ICHPAV**`.`**XXL**`.`**XX**`..`**X**`.`  391
```


Need reverse

Arg mutant F3 clone

472782801_2_T7F_B08.seq

NNNNNNNNNNNNNNNGNNNNNNCCGGGACCTTTAATTCAACCCAACACAATATATTATAGTTAAATAAGAATTATTATCAAATCATTTGTATATTAATTAAAATACTATACTG
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CGGAAGTGAAACCGGAAACCCATATTAATCTGAAAGTTAGCGACGGCAGCAGCGAAATCTTTTTTAAAATTAAAAAAACCACCCCGCTGCGTCGCCTGATGGAAGCCTTTGCG
AAACGTCAGGGTAAAGAAATGGATAGCCTGCGCTTTCTGTATGACGGCATCCGTATTCAGGCCGATCAGACCCCGGAAGACCTGGATATGGAAGACAACGATATTATTGAAGC
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GGCTTGATAACACAGTGTGGTGCCCGCGCCATGTTATGTGCCCAGCGGATCAGTTGAGTGACCCTAATTATGACGCGCTGTTAATTAGTATGACTAATCACTCCTTCTCCGTT
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GGAACTATGTACGGAGCGTTTATGGATAAGCAGGTACACCAGGTACAACTTACAGATAAGTATTGTTCAGTGAACGTGGTTGCCTGGCTTTACGCGGCTATTTTGAATGGGTG
TGCATGGTTTGTCAAACCCAACCGTACAAGTGTAGTGTCATTCAATGAGTGGGCGTTAGCTAATCAATTCACCGAATTTGTTGGCACCCAGTCAGTGGANTTGCTGGCAGTGA
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MSHPSGDVEACMVQVTCGSMTLNGLWLDNTVWCPRHVMCPADQLSDPNYDALLISMTNHSFSVQKHIGAPANLRVVGHAMQGTLLKLTVDVANPSTPAYTFTTVKPGAAFSVL
ACYNGRPTGTFTVVMRPNYTIKGSFLCGSCGSVGYTKEGSVINFCYMHQMELANGTHTGSAFDGTMYGAFMDKQVHQVQLTDKYCSVNVVAWLYAAILNGCAWFVKPNRTSVV

SFNEWALANQFTEFVGTQSVXLLAVKTGVPIXQXFXCHPAVXYRLPG

      Score   Expect   Identities   Gaps   Strand
  2111 bits(1143) 0.0   1160/1171(99%) 1/1171(0%) Plus/Plus

Query 1   ATGGGTAGCAGCCATCACCATCATCATCACGGGAGCGATAGCGAAGTGAACCAGGAAGCG 60

Sbjct 151   210
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4

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Query  61   AAACCGGAAGTTAAACCGGAAGTGAAACCGGAAACCCATATTAATCTGAAAGTTAGCGAC  120

Sbjct  211  ............................................................  270


Query  121  GGCAGCAGCGAAATCTTTTTTAAAATTaaaaaaaCCACCCCGCTGCGTCGCCTGATGGAA  180

Sbjct  271  ............................................................  330


Query  181  GCCTTTGCGAAACGTCAGGGTAAAGAAATGGATAGCCTGCGCTTTCTGTATGACGGCATC  240

Sbjct  331  ............................................................  390


Query  241  CGTATTCAGGCCGATCAGACCCCGGAAGACCTGGATATGGAAGACAACGATATTATTGAA  300

Sbjct  391  ............................................................  450


Query  301  GCGCATCGCGAACAGATCGGTGGTTCGGGCCTGGTAAAGATGTCACATCCAAGCGGTGAT  360

Sbjct  451  ............................................................  510


Query  361  GTAGAAGCGTGCATGGTGCAGGTTACGTGCGGGTCTATGACGCTTAATGGTCTTTGGCTT  420

Sbjct  511  ............................................................  570


Query  421  GATAACACAGTGTGGTGCCCGCGCCATGTTATGTGCCCAGCGGATCAGTTGAGTGACCCT  480

Sbjct  571  ............................................................  630


Query  481  AATTATGACGCGCTGTTAATTAGTATGACTAATCACTCCTTCTCCGTTCAGAAACATATC  540

Sbjct  631  ............................................................  690


Query  541  GGCGCACCGGCGAACCTTCGCGTCGTTGGACACGCTATGCAAGGGACACTTCTTAAGTTG  600

Sbjct  691  ............................................................  750


Query  601  ACGGTTGATGTTGCAAATCCTAGCACCCCAGCATACACTTTCACGACCGTTAAGCCCGGT  660

Sbjct  751  ............................................................  810


Query  661  GCCGCATTTAGTGTCCTTGCTTGCTACAATGGACGTCCGACGGGAACATTCACTGTTGTT  720

Sbjct  811  ............................................................  870


Query  721  ATGCGCCCTAATTATACGATTAAAGGTAGTTTCCTGTGTGGATCATGTGGCAGTGTAGGG  780
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5

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Sbjct  871    ............................................................  930


Query  781    TATACCAAAGAAGGGTCCGTTATTAACTTCTGTTACATGCACCAAATGGAATTGGCCAAC  840

Sbjct  931    ............................................................  990


Query  841    GGAACTCACACCGGTTCAGCTTTCGACGGAACTATGTACGGAGCGTTTATGGATAAGCAG  900

Sbjct  991    ............................................................  1050


Query  901    GTACACCAGGTACAACTTACAGATAAGTATTGTTCAGTGAACGTGGTTGCCTGGCTTTAC  960

Sbjct  1051   ............................................................  1110


Query  961    GCGGCTATTTTGAATGGGTGTGCATGGTTTGTCAAACCCAACCGTACAAGTGTAGTGTCA  1020

Sbjct  1111   ............................................................  1170


Query  1021   TTCAATGAGTGGGCGTTAGCTAATCAATTCACCGAATTTGTTGGCACCCAGTCAGTGGAT  1080

Sbjct  1171   .........................................................N  1230


Query  1081   ATGCTGGCAGTGAAAACTGGAGTCGCTATTGAGCAATTATTATA-TGCCATCCAGCAGTT  1139

Sbjct  1231   T.....................C......N......N..TN.T..............  1290


Query  1140   ATATACCGGCTTCCAGGGTAAGCAAATTTTA  1170

Sbjct  1291   NNT...........................  1321
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Score	Expect	Method	Identities	Positives	Gaps
777 bits(2006)	0.0	Compositional matrix adjust.	368/372(99%)	369/372(99%)	0/372(0%)

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Query  1    MGSSHHHHHHGSDSEVNQEAKPEVKPEVKPETHINLKVSDGSSEIFFKIKKTTPLRRLME  60

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


Query  61   AFAKRQGKEMDSLRFLYDGIRIQADQTPEDLDMEDNDIIEAHREQIGGSGLVKMSHPSGD  120

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


Query  121  VEACMVQVTCGSMTLNGLWLDNTVWCPRHVMCPADQLSDPNYDALLISMTNHSFSVQKHI  180

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


Query  181  GAPANLRVVGHAMQGTLLKLTVDVANPSTPAYTFTTVKPGAAFSVLACYNGRPTGTFTVV  240
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Sbjct  181  ........................................................  240


Query  241  MRPNYTIKGSFLCGSCGSVGYTKEGSVINFCYMHQMELANGTHTGSAFDGTMYGAFMDKQ  300

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


Query  301  VHQVQLTDKYCSVNVVAWLYAAILNGCAWFVKPNRTSVVSFNEWALANQFTEFVGTQSVD  360

Sbjct  301  ....................................................X  360


Query  361  MLAVKTGVAIEQ  372

Sbjct  361  L.......P.X.  372

Need reverse
```

Assuming sequencing is OK will do expression and purification

T7termalt

caaggggttatgctagttac

472800501_F1_T7term_T7term_C11.seq

```
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AAAAAATTCCAACACACTATTGCAATGAAAATAAATTTCCTTTATTAGCCAGAAGTCAGATGCTCAAGGGGCTTCATGATGTCC
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TGATAGGCAGCCTGCACCTGAGGTTAATCACAAACTGGTCTAGAAAGCTTCATTGCATCACCACTCCCATGATCTGCCGATTA
ACGTCTTCGGGAGTGAACTCGTCTTCCAGCATAGTGCTACCTAAAATTTGCTTACCCTGGAAGCCGGTATATAACTGCTGGAT
GGCATATAATAATTGCTCAATAGCGACTCCAGTTTTCACTGCCAGCATATCCACTGACTGGGTGCCAACAAATTCGGTGAATT
GATTAGCTAACGCCCACTCATTGAATGACACTACACTTGTACGGTTGGGTTTGACAAACCATGCACACCCATTCAAAATAGCC
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AAAGNNNAGGCTNTCNTTTNNTTNACCCTGANGTTNCN
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MXGSVFRPIRPRKTWXWKPTDIIEXXXEQIGGSGLEKXXHPSGDVEACMVQVTCGSMTLNGLWLDNTVWCPRHVMCPADQL
SDPNYDALLISMTNHSFSVQKHIGAPANLRVVGHAMQGTLLKLTVDVANPSTPAYTFTTVKPGAAFSVLACYNGRPTGTFTVV
MRPNYTIKGSFLCGSCGSVGYTKEGSVINFCYMHQMELANGTHTGSAFDGTMYGAFMDKQVHQVQLTDKYCSVNVVAWLYA
AILNGCAWFVKPNRTSVVSFNEWALANQFTEFVGTQSVDMLAVKTGVAIEQLLYAIQQLYTGFQGKQILGSTMLEDEFTPEDV
NRQIMGVVMQ
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Score	Expect	Identities	Gaps	Strand
2313 bits(1252)	0.0	1278/1295(99%)	1/1295(0%)	Plus/Minus

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Query  481  TATGACGGCATCCGTATTCAGGCCGATCAGACCCCGGAAGACCTGGATATGGAAGACAAC  540
Sbjct 1315  ......N.G.................................N.......C....  1256


Query  541  G-ATATTATTGAAGCGCATCGCGAACAGATCGGTGGTTCGGGCCTGGTAAAGATGTCACA  599
```

```
Sbjct  1255  .G.............NN..NTN.........................A.....NT.NN..  1196

Query  600  TCCAAGCGGTGATGTAGAAGCGTGCATGGTGCAGGTTACGTGCGGGTCTATGACGCTTAA  659
Sbjct  1195  ...........................................................  1136

Query  660  TGGTCTTTGGCTTGATAACACAGTGTGGTGCCCGCGCCATGTTATGTGCCCAGCGGATCA  719
Sbjct  1135  ...........................................................  1076

Query  720  GTTGAGTGACCCTAATTATGACGCGCTGTTAATTAGTATGACTAATCACTCCTTCTCCGT  779
Sbjct  1075  ...........................................................  1016

Query  780  TCAGAAACATATCGGCGCACCGGCGAACCTTCGCGTCGTTGGACACGCTATGCAAGGGAC  839
Sbjct  1015  ...........................................................  956

Query  840  ACTTCTTAAGTTGACGGTTGATGTTGCAAATCCTAGCACCCCAGCATACACTTTCACGAC  899
Sbjct  955  ...........................................................  896

Query  900  CGTTAAGCCCGGTGCCGCATTTAGTGTCCTTGCTTGCTACAATGGACGTCCGACGGGAAC  959
Sbjct  895  ...........................................................  836

Query  960  ATTCACTGTTGTTATGCGCCCTAATTATACGATTAAAGGTAGTTTCCTGTGTGGATCATG  1019
Sbjct  835  ...........................................................  776

Query  1020  TGGCAGTGTAGGGTATACCAAAGAAGGGTCCGTTATTAACTTCTGTTACATGCACCAAAT  1079
Sbjct  775  ...........................................................  716

Query  1080  GGAATTGGCCAACGGAACTCACACCGGTTCAGCTTTCGACGGAACTATGTACGGAGCGTT  1139
Sbjct  715  ...........................................................  656

Query  1140  TATGGATAAGCAGGTACACCAGGTACAACTTACAGATAAGTATTGTTCAGTGAACGTGGT  1199
Sbjct  655  ...........................................................  596

Query  1200  TGCCTGGCTTTACGCGGCTATTTTGAATGGGTGTGCATGGTTTGTCAAACCCAACCGTAC  1259
Sbjct  595  ...........................................................  536

Query  1260  AAGTGTAGTGTCATTCAATGAGTGGGCGTTAGCTAATCAATTCACCGAATTTGTTGGCAC  1319
Sbjct  535  ...........................................................  476

Query  1320  CCAGTCAGTGGATATGCTGGCAGTGAAAACTGGAGTCGCTATTGAGCAATTATTATATGC  1379
Sbjct  475  ...........................................................  416

Query  1380  CATCCAGCAGTTATATACCGGCTTCCAGGGTAAGCAAATTTTAGGTAGCACTATGCTGGA  1439
Sbjct  415  ...........................................................  356

Query  1440  AGACGAGTTCACTCCCGAAGACGTTAATATGCAGATCATGGGAGTGGTGATGCAATGAAG  1499
Sbjct  355  ...............................CG..........................  296

Query  1500  CTTTCTAGACCAGTTTGTGATTAACCTCAGGTGCAGGCTGCCTATCAGAAGGTGGTGGCT  1559
Sbjct  295  ...........................................................  236

Query  1560  GGTGTGGCCAATGCCCTGGCTCACAAATACCACTGAGATCGATCTTTTTCCCTCTGCCAA  1619
Sbjct  235  ...........................................................  176

Query  1620  AAATTATGGGGACATCATGAAGCCCCTTGAGCATCTGACTTCTGGCTAATAAAGGAAATT  1679
Sbjct  175  ...........................................................  116

Query  1680  TATTTTCATTGCAATAGTGTGTTGGAATTTTTTGTGTCTCTCACTCGGAAGGACATATGG  1739
Sbjct  115  ...........................................................  56

Query  1740  GAGGGCAAATCATTTAAAACATCAGAATGAGTATT  1774
Sbjct  55  ...................................  21
```

Score	Expect	Method	Identities	Positives	Gaps
650 bits(1677)	0.0	Compositional matrix adjust.	311/318(98%)	311/318(97%)	0/318(0%)

```
Query  97  DIIEAHREQIGGSGLVKMSHPSGDVEACMVQVTCGSMTLNGLWLDNTVWCPRHVMCPADQ  156
```

```
Sbjct   21 ....XXX.......E.XX........................................ 80

Query  157 LSDPNYDALLISMTNHSFSVQKHIGAPANLRVVGHAMQGTLLKLTVDVANPSTPAYTFTT 216
Sbjct   81 ......................................................... 140

Query  217 VKPGAAFSVLACYNGRPTGTFTVVMRPNYTIKGSFLCGSCGSVGYTKEGSVINFCYMHQM 276
Sbjct  141 ......................................................... 200

Query  277 ELANGTHTGSAFDGTMYGAFMDKQVHQVQLTDKYCSVNVVAWLYAAILNGCAWFVKPNRT 336
Sbjct  201 ......................................................... 260

Query  337 SVVSFNEWALANQFTEFVGTQSVDMLAVKTGVAIEQLLYAIQQLYTGFQGKQILGSTMLE 396
Sbjct  261 ......................................................... 320

Query  397 DEFTPEDVNMQIMGVVMQ 414
Sbjct  321 ........R....... 338
```

has expected Arg mutation

472800501_F3_T7term_T7term_B11.seq

```
NNNNNNNGNCAAACTCTNNNNNNNNACTCATTCTGATGTTTTAAATGATTTGCCCTCCCATATGTCCTTCCGAGTGAGAGACA
CAAAAAATTCCAACACACTATTGCAATGAAAATAAATTTCCTTTATTAGCCAGAAGTCAGATGCTCAAGGGGCTTCATGATG
TCCCCATAATTTTTGGCAGAGGGAAAAAGATCGATCTCAGTGGTATTTGTGAGCCAGGGCATTGGCCACACCAGCCACCACC
TTCTGATAGGCAGCCTGCACCTGAGGTTAATCACAAAATGGTCTAGAAAGCTTCATTGCATCACCACTCCCATGATCTGCCG
ATTAACGTCTTCGGGAGTGAACTCGTCTTCCAGCATAGTGCTACCTAAAATTTGCTTACCCTGGAAGCCGGTATATAACTGC
TGGATGGCATATAATAATTGCTCAATAGCGACTCCAGTTTTCACTGCCAGCATATCCACTGACTGGGTGCCAACAAATTCGG
TGAATTGATTAGCTAACGCCCACTCATTGAATGACACTACACTTGTACGGTTGGGTTTGACAAACCATGCACACCCATTCAA
AATAGCCGCGTAAAGCCAGGCAACCACGTTCACTGAACAATACTTATCTGTAAGTTGTACCTGGTGTACCTGCTTATCCATA
AACGCTCCGTACATAGTTCCGTCGAAAGCTGAACCGGTGTGAGTTCCGTTGGCCAATTCCATTTGGTGCATGTAACAGAAGT
TAATAACGGACCCTTCTTTGGTATACCCTACACTGCCACATGATCCACACAGGAAACTACCTTTAATCGTATAATTAGGGCG
CATAACAACAGTGAATGTTCCCGTCGGACGTCCATTGTAGCAAGCAAGGACACTAAATGCGGCACCGGGCTTAACGGTCGTG
AAAGTGTATGCTGGGGTGCTAGGATTTGCAACATCAACCGTCAACTTAAGAAGTGTCCCTTGCATAGCGTGTCCAACGACGC
GAAGGTTCGCCGGTGCGCCGATATGTTTCTGAACGGAGAAGGAGTGATTAGTCATACTAATTAACAGCGCGTCATAATTAGG
GTCACTCAACTGATCCGCTGGGCACATAACATGGCGCGGGCACCACACTGTGTTATCAAGCCAAAGACCATTAAGCGTCATA
GACCCGCACGTAACCTGCACCATGCACGCTTCTACATCACCGCTTGGATGTGACATCTTTACCAGGCCCGAACCACCGATCT
GTTCGCGATGNGCTTCAATAATNTCGTTGTCTTCCATATCCAGGTCTTCCGGGGNCTGATCGGCCTGAATACGGATGCCGTC
ATANNGAAAGGCCAGGCNATCCATTTCTTTACCCTGACGTTTCNNAAAGGCTTCCATCAGGNNACCAACGGGG
```

```
EAFXKRQGKEMDXLAFXYDGIRIQADQXPEDLDMEDNXIIEXHREQIGGSGLVKMSHPSGDVEACMVQVTCGSMTLNGLWLD
NTVWCPRHVMCPADQLSDPNYDALLISMTNHSFSVQKHIGAPANLRVVGHAMQGTLLKLTVDVANPSTPAYTFTTVKPGAAF
SVLACYNGRPTGTFTVVMRPNYTIKGSFLCGSCGSVGYTKEGSVINFCYMHQMELANGTHTGSAFDGTMYGAFMDKQVHQVQ
LTDKYCSVNVVAWLYAAILNGCAWFVKPNRTSVVSFNEWALANQFTEFVGTQSVDMLAVKTGVAIEQLLYAIQQLYTGFQGK
QILGSTMLEDEFTPEDVNRQIMGVVMQ
```

Score	Expect	Identities	Gaps	Strand
2438 bits(1320)	0.0	1345/1362(99%)	1/1362(0%)	Plus/Minus

```
Query  410 CCCCGCTGCGTCGCCTGATGGAAGCCTTTGCGAAACGTCAGGGTAAAGAAATGGATAGCC 469
Sbjct 1385 .....T..-..NN.................NN.........................N... 1327

Query  470 TGCGCTTTCTGTATGACGGCATCCGTATTCAGGCCGATCAGACCCCGGAAGACCTGGATA 529
Sbjct 1326 ..GC.....NN............................N................... 1267

Query  530 TGGAAGACAACGATATTATTGAAGCGCATCGCGAACAGATCGGTGGTTCGGGCCTGGTAA 589
Sbjct 1266 .............N.........N............................... 1207

Query  590 AGATGTCACATCCAAGCGGTGATGTAGAAGCGTGCATGGTGCAGGTTACGTGCGGGTCTA 649
Sbjct 1206 ......................................................... 1147

Query  650 TGACGCTTAATGGTCTTTGGCTTGATAACACAGTGTGGTGCCCGCGCCATGTTATGTGCC 709
Sbjct 1146 ......................................................... 1087

Query  710 CAGCGGATCAGTTGAGTGACCCTAATTATGACGCGCTGTTAATTAGTATGACTAATCACT 769
Sbjct 1086 ......................................................... 1027
```

```
Query  770   CCTTCTCCGTTCAGAAACATATCGGCGCACCGGCGAACCTTCGCGTCGTTGGACACGCTA 829
Sbjct  1026  ........................................................... 967

Query  830   TGCAAGGGACACTTCTTAAGTTGACGGTTGATGTTGCAAATCCTAGCACCCCAGCATACA 889
Sbjct  966   ........................................................... 907

Query  890   CTTTCACGACCGTTAAGCCCGGTGCCGCATTTAGTGTCCTTGCTTGCTACAATGGACGTC 949
Sbjct  906   ........................................................... 847

Query  950   CGACGGGAACATTCACTGTTGTTATGCGCCCTAATTATACGATTAAAGGTAGTTTCCTGT 1009
Sbjct  846   ........................................................... 787

Query  1010  GTGGATCATGTGGCAGTGTAGGGTATACCAAAGAAGGGTCCGTTATTAACTTCTGTTACA 1069
Sbjct  786   ........................................................... 727

Query  1070  TGCACCAAATGGAATTGGCCAACGGAACTCACACCGGTTCAGCTTTCGACGGAACTATGT 1129
Sbjct  726   ........................................................... 667

Query  1130  ACGGAGCGTTTATGGATAAGCAGGTACACCAGGTACAACTTACAGATAAGTATTGTTCAG 1189
Sbjct  666   ........................................................... 607

Query  1190  TGAACGTGGTTGCCTGGCTTTACGCGGCTATTTTGAATGGGTGTGCATGGTTTGTCAAAC 1249
Sbjct  606   ........................................................... 547

Query  1250  CCAACCGTACAAGTGTAGTGTCATTCAATGAGTGGGCGTTAGCTAATCAATTCACCGAAT 1309
Sbjct  546   ........................................................... 487

Query  1310  TTGTTGGCACCCAGTCAGTGGATATGCTGGCAGTGAAAACTGGAGTCGCTATTGAGCAAT 1369
Sbjct  486   ........................................................... 427

Query  1370  TATTATATGCCATCCAGCAGTTATATACCGGCTTCCAGGGTAAGCAAATTTTAGGTAGCA 1429
Sbjct  426   ........................................................... 367

Query  1430  CTATGCTGGAAGACGAGTTCACTCCCGAAGACGTTAATATGCAGATCATGGGAGTGGTGA 1489
Sbjct  366   ...............................CG.................... 307

Query  1490  TGCAATGAAGCTTTCTAGACCAGTTTGTGATTAACCTCAGGTGCAGGCTGCCTATCAGAA 1549
Sbjct  306   ...................T....................................... 247

Query  1550  GGTGGTGGCTGGTGTGGCCAATGCCCTGGCTCACAAATACCACTGAGATCGATCTTTTTC 1609
Sbjct  246   ........................................................... 187

Query  1610  CCTCTGCCAAAAATTATGGGGACATCATGAAGCCCCTTGAGCATCTGACTTCTGGCTAAT 1669
Sbjct  186   ........................................................... 127

Query  1670  AAAGGAAATTTATTTTCATTGCAATAGTGTGTTGGAATTTTTTGTGTCTCTCACTCGGAA 1729
Sbjct  126   ........................................................... 67

Query  1730  GGACATATGGGAGGGCAAATCATTTAAAACATCAGAATGAGT 1771
Sbjct  66    .......................................... 25
```

Score	Expect	Method	Identities	Positives	Gaps
730 bits(1884)	0.0	Compositional matrix adjust.	347/355(98%)	347/355(97%)	0/355(0%)

```
Query  60    EAFAKRQGKEMDSLRFLYDGIRIQADQTPEDLDMEDNDIIEAHREQIGGSGLVKMSHPSG 119
Sbjct  1     ...X........X.A.X.........X........X...X.................... 60

Query  120   DVEACMVQVTCGSMTLNGLWLDNTVWCPRHVMCPADQLSDPNYDALLISMTNHSFSVQKH 179
Sbjct  61    ........................................................... 120

Query  180   IGAPANLRVVGHAMQGTLLKLTVDVANPSTPAYTFTTVKPGAAFSVLACYNGRPTGTFTV 239
Sbjct  121   ........................................................... 180

Query  240   VMRPNYTIKGSFLCGSCGSVGYTKEGSVINFCYMHQMELANGTHTGSAFDGTMYGAFMDK 299
Sbjct  181   ........................................................... 240
```

```
Query  300  QVHQVQLTDKYCSVNVVAWLYAAILNGCAWFVKPNRTSVVSFNEWALANQFTEFVGTQSV  359
Sbjct  241  ............................................................  300

Query  360  DMLAVKTGVAIEQLLYAIQQLYTGFQGKQILGSTMLEDEFTPEDVNMQIMGVVMQ  414
```

Sbjct 301 `...............................................R........` 355

```
Also has expected Arg mutation
```

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agttaaataagaattattatcaaatcatttgtatattaattaaaatactatactgtaaattacattttatttacaatcaaaggagatataccatgggtagcagccatca
ccatcatcatcacgggagcgatagcgaagtgaaccaggaagcgaaaccggaagttaaaccggaagtgaaaccggaaacccatattaatctgaaagttagcg
acggcagcagcgaaatctttttaaaattaaaaaaaccaccccgctgcgtcgcctgatggaagcctttgcgaaacgtcagggtaaagaaatggatagcctgcgc
tttctgtatgacggcatccgtattcaggccgatcagaccccggaagacctggatatggaagacaacgatattattgaagcgcatcgCGAACAGATCGGTG
GTTCGGGCCTGGTAAAGATGTCACATCCAAGCGGTGATGTAGAAGCGTGCATGGTGCAGGTTACGTGCGGGTCTATGACGC
TTAATGGTCTTTGGCTTGATAACACAGTGTGGTGCCCGCGCCATGTTATGTGCCCAGCGGATCAGTTGAGTGACCCTAATTAT
GACGCGCTGTTAATTAGTATGACTAATCACTCCTTCTCCGTTCAGAAACATATCGGCGCACCGGCGAACCTTCGCGTCGTTG
GACACGCTATGCAAGGGACACTTCTTAAGTTGACGGTTGATGTTGCAAATCCTAGCACCCCAGCATACACTTTCACGACCGTT
AAGCCCGGTGCCGCATTTAGTGTCCTTGCTTGCTACAATGGACGTCCGACGGGAACATTCACTGTTGTTATGCGCCCTAATTA
TACGATTAAAGGTAGTTTCCTGTGTGGATCATGTGGCAGTGTAGGGTATACCAAAGAAGGGTCCGTTATTAACTTCTGTTACA
TGCACCAAATGGAATTGGCCAACGGAACTCACACCGGTTCAGCTTTCGACGGAACTATGTACGGAGCGTTTATGGATAAGCA
GGTACACCAGGTACAACTTACAGATAAGTATTGTTCAGTGAACGTGGTTGCCTGGCTTTACGCGGCTATTTTGAATGGGTGT
GCATGGTTTGTCAAACCCAACCGTACAAGTGTAGTGTCATTCAATGAGTGGGCGTTAGCTAATCAATTCACCGAATTTGTTGG
CACCCAGTCAGTGGATATGCTGGCAGTGAAAACTGGAGTCGCTATTGAGCAATTATTATATGCCATCCAGCAGTTATATACCG
GCTTCCAGGGTAAGCAAATTTTAGGTAGCACTATGCTGGAAGACGAGTTCACTCCCGAAGACGTTAATCGGCAGATCATGGG
AGTGGTGATGCAATGAAGCTTTCTAGACCAGtttgtgattaacctcaggtgcaggctgcctatcagaaggtggtggctggtgtggccaatgccctgg
ctcacaaataccactgagatcg

MGSSHHHHHHGSDSEVNQEAKPEVKPEVKPETHINLKVSDGSSEIFFKIKKTTPLRRLMEAFAKRQGKEMDSLRFLYDGIRIQA
DQTPEDLDMEDNDIIEAHREQIGGSGLVKMSHPSGDVEACMVQVTCGSMTLNGLWLDNTVWCPRHVMCPADQLSDPNYDA
LLISMTNHSFSVQKHIGAPANLRVVGHAMQGTLLKLTVDVANPSTPAYTFTTVKPGAAFSVLACYNGRPTGTFTVVMRPNYTIK
GSFLCGSCGSVGYTKEGSVINFCYMHQMELANGTHTGSAFDGTMYGAFMDKQVHQVQLTDKYCSVNVVAWLYAAILNGCAW
FVKPNRTSVVSFNEWALANQFTEFVGTQSVDMLAVKTGVAIEQLLYAIQQLYTGFQGKQILGSTMLEDEFTPEDVNRQIMGVV
MQ

Number of amino acids: 414

Molecular weight: 45710.05

Theoretical pI: 5.87

Extinction coefficients:

Extinction coefficients are in units of M^{-1} cm^{-1}, at 280 nm measured in water.

Ext. coefficient 46005
Abs 0.1% (=1 g/l) 1.006, assuming all pairs of Cys residues form cystines

SGLVKMSHPSGDVEACMVQVTCGSMTLNGLWLDNTVWCPRHVMCPADQLSDPNYDALLISMTNHSFSVQKHIGAPANLRVV
GHAMQGTLLKLTVDVANPSTPAYTFTTVKPGAAFSVLACYNGRPTGTFTVVMRPNYTIKGSFLCGSCGSVGYTKEGSVINFCY
MHQMELANGTHTGSAFDGTMYGAFMDKQVHQVQLTDKYCSVNVVAWLYAAILNGCAWFVKPNRTSVVSFNEWALANQFTE
FVGTQSVDMLAVKTGVAIEQLLYAIQQLYTGFQGKQILGSTMLEDEFTPEDVNRQIMGVVMQ

Number of amino acids: 306

Molecular weight: 33355.28

Theoretical pI: 6.03

Extinction coefficients:

Extinction coefficients are in units of M^{-1} cm^{-1}, at 280 nm measured in water.

Ext. coefficient 44515
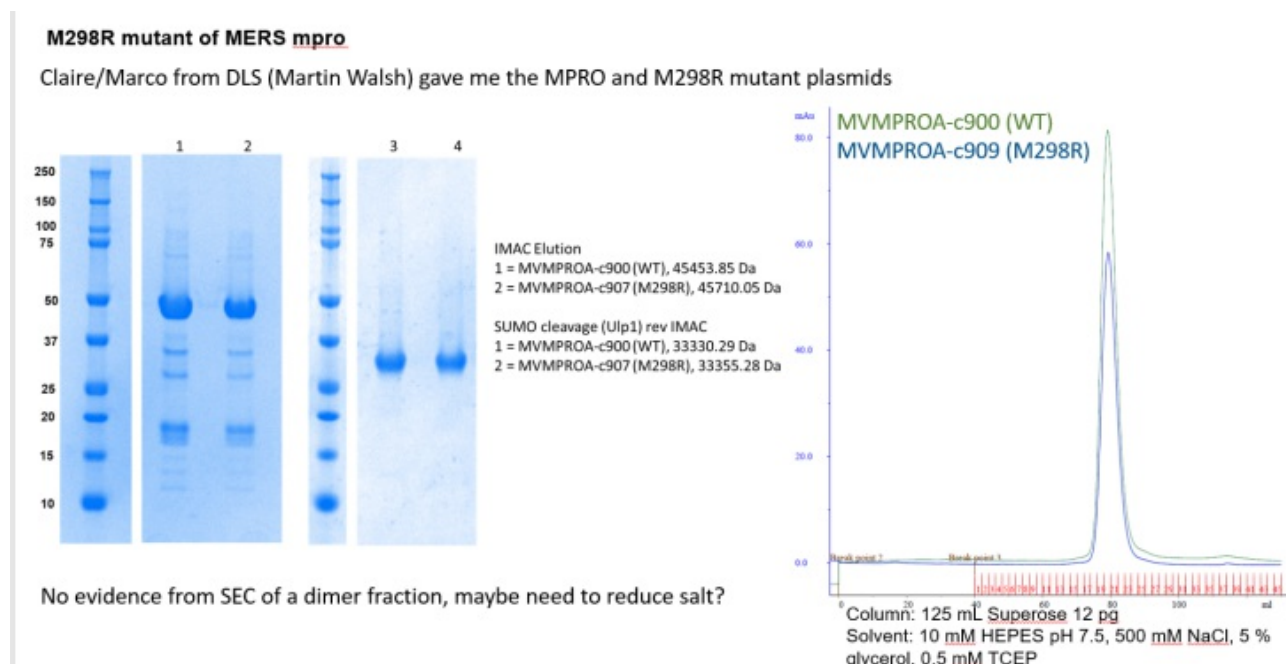Abs 0.1% (=1 g/l) 1.335, assuming all pairs of Cys residues form cystines

---

MagneHis 1mL test purification
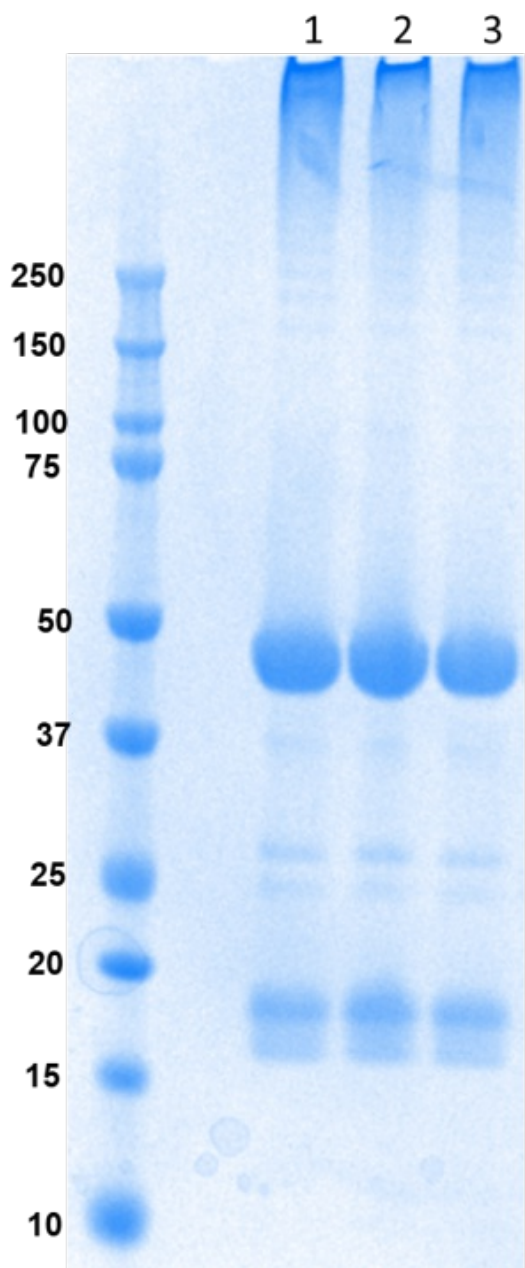
MagneHis 1mL test purification
1 = MVMPROA-c901 (Arg mutant "F3")
2 = MVMPROA-c901 (Arg mutant "F1")
3 = MVMPROA-c900 (WT)

Title missing - double click to edit

Looks fine do scale up, from SEC of 100 mL PREPX seems to be still monomeric though (at loeast in Base Buffer

---

M298R mutant of MERS mpro

Claire/Marco from DLS (Martin Walsh) gave me the MPRO and M298R mutant plasmids

IMAC Elution
1 = MVMPROA-c900 (WT), 45453.85 Da
2 = MVMPROA-c907 (M298R), 45710.05 Da

SUMO cleavage (Ulp1) rev IMAC
1 = MVMPROA-c900 (WT), 33330.29 Da
2 = MVMPROA-c907 (M298R), 33355.28 Da

No evidence from SEC of a dimer fraction, maybe need to reduce salt?

MVMPROA-c900 (WT)
MVMPROA-c909 (M298R)

Column: 125 mL Superose 12 pg
Solvent: 10 mM HEPES pH 7.5, 500 mM NaCl, 5 % glycerol, 0.5 mM TCEP

IDs and Expression & Purification

MVMPROA-c907

MHHHHHHGSDSEVNQEAKPEVKPEVKPETHINLKVSDGSSEIFFKIKKTTPLRRLMEAFAKRQGKEMDSLRFLYDGIRIQADQTPEDLDMEDNDIIEAHREQIGGSGLVKMSHPSGDVEACMVQVTCGSMTLNGLWLDNTVWCPRHVMCPADQLSDPNYDALLISMTNHSFSVQKHIGAPANLRVVGHAMQGTLLKLTVDVANPSTPAYTFTTVKPGAAFSVLACYNGRPTGTFTVVMRPNYTIKGSFLCGSCGSVGYTKEGSVINFCYMHQMELANGTHTGSAFDGTMYGAFMDKQVHQVQLTDKYCSVNVVAWLYAAILNGCAWFVKPNRTSVVSFNEWALANQFTEFVGTQSVDMLAVKTGVAIEQLLYAIQQLYTGFQGKQILGSTMLEDEFTPEDVNRQIMGVVMQ

Number of amino acids: 411

Molecular weight: 45478.85

Theoretical pI: 5.87

Extinction coefficients are in units of M⁻¹ cm⁻¹, at 280 nm measured in water.

Ext. coefficient 46005
Abs 0.1% (=1 g/l) 1.012, assuming all pairs of Cys residues form cystines

MVMPROA-c907 cut

SGLVKMSHPSGDVEACMVQVTCGSMTLNGLWLDNTVWCPRHVMCPADQLSDPNYDALLISMTNHSFSVQKHIGAPANLRVVGHAMQGTLLKLTVDVANPSTPAYTFTTVKPGAAFSVLACYNGRPTGTFTVVMRPNYTIKGSFLCGSCGSVGYTKEGSVINFCYMHQMELANGTHTGSAFDGTMYGAFMDKQVHQVQLTDKYCSVNVVAWLYAAILNGCAWFVKPNRTSVVSFNEWALANQFTEFVGTQSVDMLAVKTGVAIEQLLYAIQQLYTGFQGKQILGSTMLEDEFTPEDVNRQIMGVVMQ

Number of amino acids: 306

Molecular weight: 33355.28

Theoretical pI: 6.03

Extinction coefficients are in units of M^{-1} cm^{-1}, at 280 nm measured in water.

Ext. coefficient 44515
Abs 0.1% (=1 g/l) 1.335, assuming all pairs of Cys residues form cystines

MVMPROA-k002/MVMPROA-e002/MVMPROA-p002

Expression

Transformed BL21[DE3]RR with MVMPROA-c901 (AmpR)

Grew 100 mL o/n in SOC + Amp

Used 10 mL to inoculate 1 L FORM-TB + Amp (6 L total)

Grew 4h 37C 180 rpm shaking (OD600 = 1)

Grew 18C 180 rpm shaking 1h

Added 0.5 mM IPTG final conc.

Grew o/n 18C 180 rpm shaking

Harvested 4000g 12C 20 minutes

Froze pellet -80C (final wcw = g/L g total)

Formedium TB custom

12 g/L tryptone

24 g/L yeast extract

3.3 g/L Ammonium Sulphate (NH4)2SO4

6.8g /L Potassium Dihydrogen Phosphate KH2PO4

7.1 g/L DiSodium Hydrogen Phosphate Na2HPO4 7.1

0.15 g/L Magnesium Sulphate MgSO4

0.03 g/L Trace Elements

55.85 g/L in MilliQ water added 20 mL 50 % glycerol

Autoclave

Add 1 mL 10% Antifoam 204 and Antibiotic

Purification

Purification

Dissolve pellet in 4 mL per g using Lysis buffer (10 mM HEPES pH 7.5, 500 mM NaCl, 5 % glycerol, 0.5 mM TCEP, 1 % TX-100, 30 mM Imidazole, 0.5 mg/mL lysozyme, 0.01 mg/mL benzonase)

After dissolveing (1h RT stirring) incubate on ice 1h and then centrifuge 30000 g 1h 4C

Pass over 20 mL Ni-Sepharose-FF

Wash 3 x 100 mL Wash buffer (10 mM HEPES pH 7.5, 500 mM NaCl, 5 % glycerol, 0.5 mM TCEP, 30 mM Imidazole)

Elute 3 x 25 mL Elution Buffer (10 mM HEPES pH 7.5, 500 mM NaCl, 5 % glycerol, 0.5 mM TCEP, 500 mM Imidazole)

Pool peak fractions

50 mL at A280 of 15 (750 mg)

Add 1:100 sumo protease (Ulp1) and dialyse o/n against 1 L Wash Buffer

Pass over 20 mL Ni-Sepharose-FF (collect flow through)

Wash with 2 x 25 mL Wash Buffer (collect flow through)

Pool peak fractions

Calculate yield and check on gel, looks good

Concentrate to 40 mg/mL using 10,000 MWCO concentrator

Yield at this stage is approx 12 mL with A280 of 39, about 470 mg (One of the concnetrators leaked)

Do SEC using 5 mL aliquots (approx. 20 mg) on 125 mL superose 12 pg using 10 mM HEPES pH 7.5, 500 mM NaCl, 5 % glycerol, 0.5 mM TCEP as mobile phase

Still elutes as monomer even loading at this high concentration

Concentrate peak fractions to 0.42 mM (13.9 mg/mL, A280 of 18.5)

Do MS to confirm MW and flash freeze in LN2 and store in 100 µL single use aliquots (11.2 mL 155 mg total)

Scale up purification
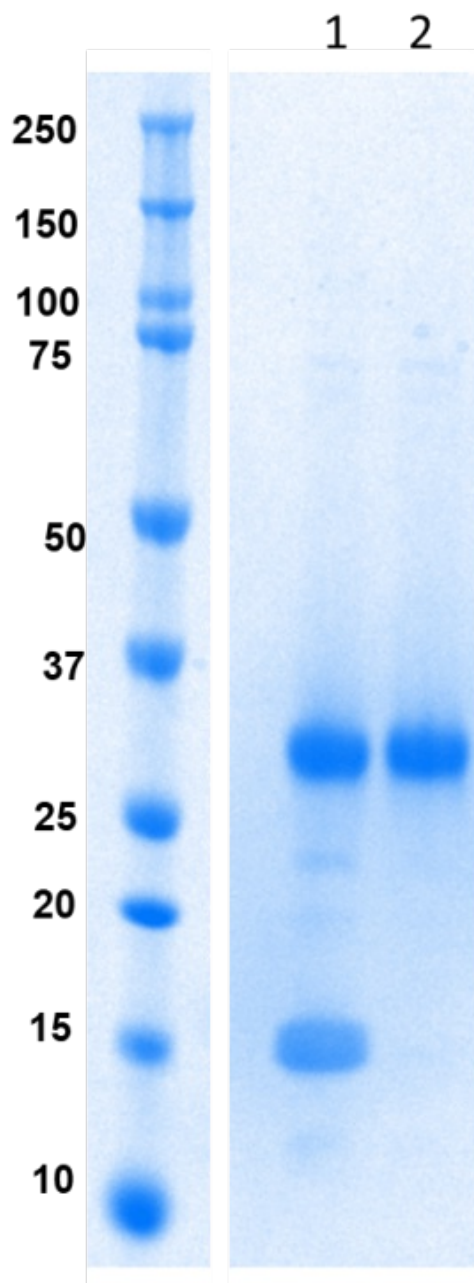
SUMO cleavage (Ulp1)
1 = MVMPROA-c907, 33798.76 Da
2 = rev IMAC

MVMPROA_c907_125mL_SUPEROSE_12_PG_SEC_PROFILE_Large_Scale