

pMCSG 81 construct

pMCSG53 was amplified with the following primers:

F-AAAGATAATGCATCATCACCATCACCATTGAGATCC

R-GGTGATGATGCATTATCTTTACTCCTTCTTAAAGTTAAACAAAATTATTTTC

The sequencing data gave the following results:

1. Fragments in green come from unaltered pMCSG53
2. Red capital letters cover upstream LIC site
3. Black capital letters cover downstream LIC site.
4. Vector T4 treatment in presence of dCTP
5. Insert T4 treatment in presence of dGTP

attccccctctagaaataatTTTTGTTTAACTTTAAGAAAGGAGTAAAGATAATG-insert-CATCATCAC

F P S R N N F V - L - E G V K I M -insert H H H
CATCACcatTgagatccggctgctaacaagccccgaaaggaagctgagttggctgctgccc
H H H - D P A A N K A R K E A E L A A A
accgctgagcaataactagcataacccttggggcctctaaacgggtcttgagggggtttt
T A E Q - L A - P L G A S K R V L R G F
ttgctgaaaggaggaactatatccggattggcgaatgggacgcgccctgtagcggcgcat
L L K G G T I S G L A N G T R P V A A H
taagcgcggcggtgtggtggttacgcgcagcgtgaccgctacacttgccagcgccttag
- A R R V W W L R A A - P L H L P A P -
cgccccgtccttttgccttttcccttctccttctcgccacggttcgcccggctttccccgctc
R P L L S L S S L P F S P R S P A F P V
aagctctaaatcgggggctcccttttaggggtccgatttagtgctttacggcacctcgacc
K L - I G G S L - G S D L V L Y G T S T
ccaaaaaacttgattggtgatggttcacattaacgcttacaattaggtggcactttt
P K N L I R V M V H I N A Y N L G G T F
cggggaaatgtgcgcggaaccctatttgtttatttttctaaatacattcaaataatgtat
R G N V R G T P I C L F F - I H S N M Y
ccgctcatgagacaataaccctgataaatgcttcaataatggaaaaggaagagatga
P L M R Q - P - - M L Q - F E K G R V -
gtattcaacatttccgtgtcgccttattcccttttttgcggcattttgccttccctgttt
V F N I S V S P L F P F L R H F A F L F
ttgctcaccagaaacgctggtgaaagtaaaagatgctgaagatcagttgggtgcacgag
L L T Q K R W - K - K M L K I S W V H E
tgggttacatcgaactggatctcaacagcggtaagatccttgagagttttcgccccgaag
W V T S N W I S T A V R S L R V F A P K
aacgttttccaatgatgagcacttttaagttctgctatgtggcgcggtattatccccgta
N V F Q - - A L L K F C Y V A R Y Y P V
ttgacgccccggaagagcaactcggctcgccgcatacactatttctcagaatgacttggttg
L T P G K S N S V A A Y T I L R M T W L
agtactcaccagtcacagaaaagcatntttacggatggcatgacagtaagagaattatgc
S T H Q S Q K S X F T D G M T V R E L C
agtgtgcataacatgagtgatacncctgcccgaacttacttctgac
S A A I T M S D X L R P T Y F -

The experimental sequence for pMCSG81

**ATTCCCCTCTAGAAATAATTTTTGTTTAACTTTAAGAAGGAGTAAAGATAATGCATCATCACCATCACCATTGAGATC
CGGCTGCTAACAAGCCCCGAAAGGAAGCTGAGTTGGCTGCTGCCACCGCTGAGCAATAACTAGCATAACCCCTTGGG
GCCTCTAAACGGGTCTTGAGGGGTTTTTTGCTGAAAGGAGGAAGTATATCCGGATTGGCGAATGGGACGCGCCCTGT
AGCGGCGCATTAAAGCGCGGGGTGTGGTGGTTACGCGCAGCGTGACCGCTACACTTGCCAGCGCCCTAGCGCCCGC
TCCTTTTCGCTTTCTTCCCTTCTTCTCGCCACGTTCCGGGCTTTCCCGTCAAGCTCTAAATCGGGGGCTCCCTT**

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TAGGGTTCCGATTTAGTGCTTTACGGCACCTCGACCCCAAAAACTTGATTAGGGTGATGGTTCACATTAACGCTTA
CAATTTAGGTGGCACTTTTCGGGAAATGTGCGCGGAACCCCTATTTGTTATTTTTCTAAATACATTCAAATATGT
ATCCGCTCATGAGACAATAACCCTGATAAATGCTTCAATAATTTGAAAAAGGAAGAGTATGAGTATTCAACATTTCC
GTGTCGCCCTTATTCCCTTTTTTTCGGCATTTCCTTCTGTTTTTCTCACCCAGAAACGCTGGTGAAAGTAAAA
GATGCTGAAGATCAGTTGGGTGCACGAGTGGGTACATCGAACTGGATCTCAACAGCGGTAAGATCCTTGAGAGTTT
TCGCCCCGAAGAACGTTTTCCAATGATGAGCACTTTAAAGTTCTGCTATGTGGCGCGGTATTATCCCGTATTGACG
CCGGGCAAGAGCAACTCGGTCGCCGCATACACTATTCTCAGAATGACTTGGTTGAGTACTCACCAGTCACAGAAAAG
CATNTTACGGATGGCATGACAGTAAGAGAATTATGCAGTGCTGCCATAACCATGAGTGATACNCTGCGGCCAACTT
ACTTCTGAC
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Modified C-terminal vector (pMCSG81)

The C-terminal vector utilizes modified rbs of GroEL gene and 2nd Hisx6 that is not in frame in our pMCSG7 series vector. Histag is not cleavable, there are not any extra aminoacids beside 6 histidines.

LIC site is in green background

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pMCSG53-TTTTGTTTAACTTTAAGAAAGGAGTAAAGATAATG-INSERT-CATCATCACCATCACCATTGAG
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The blunt linearized vector is created by amplifying pMCSG53 with the following primers:

F- CATCATCACCATCACCATTGAGATCCG

R- CATTATCTTTACTCCTTCTTAAAGTTAAACAAAATTATTTCTAGAG

The primers for insert amplification are as follow:

F-GGAGTAAAGATAATG-insert (ATG must be excluded from insert)

R-GTGATGGTGATGATG-insert (swapped)