**LY104**

**FULL SEQUENCE--6940**

GACGAAAGGGCCTCGTGATACGCCTATTTTTATAGGTTAATGTCATGATAATAATGGTTTCTTAGGACGGATCGCTTGCCTGTAACTTACACGCGCCTCGTATCTTTTAATGATGGAATAATTTGGGAATTTACTCTGTGTTTATTTATTTTTATGTTTTGTATTTGGATTTTAGAAAGTAAATAAAGAAGGTAGAAGAGTTACGGAATGAAGAAAAAAAAATAAACAAAGGTTTAAAAAATTTCAACAAAAAGCGTACTTTACATATATATTTATTAGACAAGAAAAGCAGATTAAATAGATATACATTCGATTAACGATAAGTAAAATGTAAAATCACAGGATTTTCGTGTGTGGTCTTCTACACAGACAAGATGAAACAATTCGGCATTAATACCTGAGAGCAGGAAGAGCAAGATAAAAGGTAGTATTTGTTGGCGATCCCCCTAGAGTCTTTTACATCTTCGGAAAACAAAAACTATTTTTTCTTTAATTTCTTTTTTTACTTTCTATTTTTAATTTATATATTTATATTAAAAAATTTAAATTATAATTATTTTTATAGCACGTGATGAAAAGGACCCAGGTGGCACTTTTCGGGGAAATGTGCGCGGAACCCCTATTTGTTTATTTTTCTAAATACATTCAAATATGTATCCGCTCATGAGACAATAACCCTGATAAATGCTTCAATCCTGTTTTTGCTCACCCAGAAACGCTGGTGAAAGTAAAAGATGCTGAAGATCAGTTGGGTGCACGAGTGGGTTACATCGAACTGGATCTCAACAGCGGTAAGATCCTTGAGAGTTTTCGCCCCGAAGAACGTTTTCCAATGATGAGCACTTTTAAAGTTCTGCTATGTGGCGCGGTATTATCCCGTATTGACGCCGGGCAAGAGCAACTCGGTCGCCGCATACACTATTCTCAGAATGACTTGGTTGAGTACTCACCAGTCACAGAAAAGCATCTTACGGATGGCATGACAGTAAGAGAATTATGCAGTGCTGCCATAACCATGAGTGATAACACTGCGGCCAACTTACTTCTGACAACGATCGGAGGACCGAAGGAGCTAACCGCTTTTTTTCACAACATGGGGGATCATGTAACTCGCCTTGATCGTTGGGAACCGGAGCTGAATGAAGCCATACCAAACGACGAGCGTGACACCACGATGCCTGTAGCAATGGCAACAACGTTGCGCAAACTATTAACTGGCGAACTACTTACTCTAGCTTCCCGGCAACAATTAATAGACTGGATGGAGGCGGATAAAGTTGCAGGACCACTTCTGCGCTCGGCCCTTCCGGCTGGCTGGTTTATTGCTGATAAATCTGGAGCCGGTGAGCGTGGGTCTCGCGGTATCATTGCAGCACTGGGGCCAGATGGTAAGCCCTCCCGTATCGTAGTTATCTACACGACGGGCAGTCAGGCAACTATGGATGAACGAAATAGACAGATCGCTGAGATAGGTGCCTCACTGATTAAGCATTGGTAACTGTCAGACCAAGTTTACTCATATATACTTTAGATTGATTTAAAACTTCATTTTTAATTTAAAAGGATCTAGGTGAAGATCCTTTTTGATAATCTCATGACCAAAATCCCTTAACGTGAGTTTTCGTTCCACTGAGCGTCAGACCCCGTAGAAAAGATCAAAGGATCTTCTTGAGATCCTTTTTTTCTGCGCGTAATCTGCTGCTTGCAAACAAAAAAACCACCGCTACCAGCGGTGGTTTGTTTGCCGGATCAAGAGCTACCAACTCTTTTTCCGAAGGTAACTGGCTTCAGCAGAGCGCAGATACCAAATACTGTCCTTCTAGTGTAGCCGTAGTTAGGCCACCACTTCAAGAACTCTGTAGCACCGCCTACATACCTCGCTCTGCTAATCCTGTTACCAGTGGCTGCTGCCAGTGGCGATAAGTCGTGTCTTACCGGGTTGGACTCAAGACGATAGTTACCGGATAAGGCGCAGCGGTCGGGCTGAACGGGGGGTTCGTGCACACAGCCCAGCTTGGAGCGAACGACCTACACCGAACTGAGATACCTACAGCGTGAGCATTGAGAAAGCGCCACGCTTCCCGAAGGGAGAAAGGCGGACAGGTATCCGGTAAGCGGCAGGGTCGGAACAGGAGAGCGCACGAGGGAGCTTCCAGGGGGGAACGCCTGGTATCTTTATAGTCCTGTCGGGTTTCGCCACCTCTGACTTGAGCGTCGATTTTTGTGATGCTCGTCAGGGGGGCCGAGCCTATGGAAAAACGCCAGCAACGCGGCCTTTTTACGGTTCCTGGCCTTTTGCTGGCCTTTTGCTCACATGTTCTTTCCTGCGTTATCCCCTGATTCTGTGGATAACCGTATTACCGCCTTTGAGTGAGCTGATACCGCTCGCCGCAGCCGAACGACCGAGCGCAGCGAGTCAGTGAGCGAGGAAGCGGAAGAGCGCCCAATACGCAAACCGCCTCTCCCCGCGCGTTGGCCGATTCATTAATGCAGCTGGCACGACAGGTTTCCCGACTGGAAAGCGGGCAGTGAGCGCAACGCAATTAATGTGAGTTACCTCACTCATTAGGCACCCCAGGCTTTACACTTTATGCTTCCGGCTCCTATGTTGTGTGGAATTGTGAGCGGATAACAATTTCACACAGGAAACAGCTATGACCATGATTACGCCAAGCTCGGAATTAACCCTCACTAAAGGGAACAAAAGCTGGGTACCCGACAGGTTATCAGCAACAACACAGTCATATCCATTCTCAATTAGCTCTACCACAGTGTGTGAACCAATGTATCCAGCACCACCTGTAACCAAAACAATTTTAGAAGTACTTTCACTTTGTAACTGAGCTGTCATTTATATTGAATTTTCAAAAATTCTTACTTTTTTTTTGGATGGACGCAAAGAAGTTTAATAATCATATTACATGGCATTACCACCATATACATATCCATATACATATCCATATCTAATCTTACTTATATGTTGTGGAAATGTAAAGAGCCCCATTATCTTAGCCTAAAAAAACCTTCTCTTTGGAACTTTCAGTAATACGCTTAACTGCTCATTGCTATATTGAAGTACGGATTAGAAGCCGCCGAGCGGGTGACAGCCCTCCGAAGGAAGACTCTCCTCCGTGCGTCCTCGTCTTCACCGGTCGCGTTCCTGAAACGCAGATGTGCCTCGCGCCGCACTGCTCCGAACAATAAAGATTCTACAATACTAGCTTTTATGGTTATGAAGAGGAAAAATTGGCAGTAACCTGGCCCCACAAACCTTCAAATGAACGAATCAAATTAACAACCATAGGATGATAATGCGATTAGTTTTTTAGCCTTATTTCTGGGGTAATTAATCAGCGAAGCGATGATTTTTGATCTATTAACAGATATATAAATGCAAAAACTGCATAACCACTTTAACTAATACTTTCAACATTTTCGGTTTGTATTACTTCTTATTCAAATGTAATAAAAGTATCAACAAAAAATTGTTAATATACCTCTATACTTTAACGTCAAGGAGAAAAAACCCCGGATCGAATTCCCTACTTCATACATTTTCAATTAAGATGCAGTTACTTCGCTGTTTTTCAATATTTTCTGTTATTGCTTCAGTTTTAGCACAGGAACTGACAACTATATGCGAGCAAATCCCCTCACCAACTTTAGAATCGACGCCGTACTCTTTGTCAACGACTACTATTTTGGCCAACGGGAAGGCAATGCAAGGAGTTTTTGAATATTACAAATCAGTAACGTTTGTCAGTAATTGCGGTTCTCACCCCTCAACAACTAGCAAAGGCAGCCCCATAAACACACAGTATGTTTTTAAGGACAATAGCTCGACGATTGAAGGTAGATACCCATACGACGTTCCAGACTACGCTCTGCAGGCTAGTGGTGGAGGAGGCTCTGGTGGAGGCG**GTAGCGGAGGCGGAGGGTCG**GATGAAATGGAAGAA**TGTGCTTCTCATTTGGGCAGTGATTATAAAGATGATG**ATGATAAAGGCAGTGAAAATTTGTATTTCCAATCTGGCAGTCATCATCATCATCATCATGGTGGGGGAGGCTCCGCTAGCATGGCGCCTATCGGCTCAGTAGTAATCGTAGGCAGAATCATCCTGTCCGGCCGTGGTGGCCCCATCACGGCGTACGCCCAGCAGACAAGGGGCCTCCTAGGGTGCATAATCACCAGCCTAACTGGCCGGGACAAAAACCAAGTGGAGGGTGAGGTCCAGATTGTGTCAACTGCTGCCCAAACCTTCCTGGCAACGTGCATCAATGGGGTGTGCTGGACTGTCTACCACGGGGCCGGAACGAGGACCATCGCGTCACCCAAGGGTCCTGTCATCCAGATGTATACCAATGTAGACCAAGACCTTGTGGGCTGGCCCGCTTCGCAAGGTACCCGCTCATTGACACCCTGCACTTGCGGCTCCTCGGACCTTTACCTGGTCACGAGGCACGCCGATGTCATTCCCGT***GCGCCGGCGGGGTGATAGC***AGGGGCAGCCTGCTGTCGCCCCGGCCCATTTCCTACTTGAAAGGCTCCTCGGGGGGTCCGCTGTTGTGCCCCGCGGGGCACGCCGTGGGCATATTTAGGGCCGCGGTGTGCACCCGTGGAGTGGCTAAGGCGGTGGAC***TTTATCCCTGTGGAGAACCTAGAGACAACCATGAGGTCGG***GATCCGAACAAAAGCTTATTTCTGAAGAGGACTTGTTCGAACACGACGAATTGTAATAGCTCGAGATCTGATAACAACAGTGTAGATGTAACAAAATCGACTTTGTTCCCACTGTACTTTTAGCTCGTACAAAATACAATATACTTTTCATTTCTCCGTAAACAACATGTTTTCCCATGTAATATCCTTTTCTATTTTTCGTTCCGTTACCAACTTTACACATACTTTATATAGCTATTCACTTCTATACACTAAAAAACTAAGACAATTTTAATTTTGCTGCCTGCCATATTTCAATTTGTTATAAATTCCTATAATTTATCCTATTAGTAGCTAAAAAAAGATGAATGTGAATCGAATCCTAAGAGAATTGAGCTCCAATTCGCCCTATAGTGAGTCGTATTACAATTCACTGGCCGTCGTTTTACAACGTCGTGACTGGGAAAACCCTGGCGTTACCCAACTTAATCGCCTTGCAGCACATCCCCCCTTCGCCAGCTGGCGTAATAGCGAAGAGGCCCGCACCGATCGCCCTTCCCAACAGTTGCGCAGCCTGAATGGCGAATGGCGCGACGCGCCCTGTAGCGGCGCATTAAGCGCGGCGGGTGTGGTGGTTACGCGCAGCGTGACCGCTACACTTGCCAGCGCCCTAGCGCCCGCTCCTTTCGCTTTCTTCCCTTCCTTTCTCGCCACGTTCGCCGGCTTTCCCCGTCAAGCTCTAAATCGGGGGCTCCCTTTAGGGTTCCGATTTAGTGCTTTACGGCACCTCGACCCCAAAAAACTTGATTAGGGTGATGGTTCACGTAGTGGGCCATCGCCCTGATAGACGGTTTTTCGCCCTTTGACGTTGGAGTCCACGTTCTTTAATAGTGGACTCTTGTTCCAAACTGGAACAACACTCAACCCTATCTCGGTCTATTCTTTTGATTTATAAGGGATTTTGCCGATTTCGGCCTATTGGTTAAAAAATGAGCTGATTTAACAAAAATTTAACGCGAATTTTAACAAAATATTAACGTTTACAATTTCCTGATGCGGTATTTTCTCCTTACGCATCTGTGCGGTATTTCACACCGCAGGCAAGTGCACAAACAATACTTAAATAAATACTACTCAGTAATAACCTATTTCTTAGCATTTTTGACGAAATTTGCTATTTTGTTAGAGTCTTTTACACCATTTGTCTCCACACCTCCGCTTACATCAACACCAATAACGCCATTTAATCTAAGCGCATCACCAACATTTTCTGGCGTCAGTCCACCAGCTAACATAAAATGTAAGCTTTCGGGGCTCTCTTGCCTTCCAACCCAGTCAGAAATCGAGTTCCAATCCAAAAGTTCACCTGTCCCACCTGCTTCTGAATCAAACAAGGGAATAAACGAATGAGGTTTCTGTGAAGCTGCACTGAGTAGTATGTTGCAGTCTTTTGGAAATACGAGTCTTTTAATAACTGGCAAACCGAGGAACTCTTGGTATTCTTGCCACGACTCATCTCCATGCAGTTGGACGATATCAATGCCGTAATCATTGACCAGAGCCAAAACATCCTCCTTAGGTTGATTACGAAACACGCCAACCAAGTATTTCGGAGTGCCTGAACTATTTTTATATGCTTTTACAAGACTTGAAATTTTCCTTGCAATAACCGGGTCAATTGTTCTCTTTCTATTGGGCACACATATAATACCCAGCAAGTCAGCATCGGAATCTAGAGCACATTCTGCGGCCTCTGTGCTCTGCAAGCCGCAAACTTTCACCAATGGACCAGAACTACCTGTGAAATTAATAACAGACATACTCCAAGCTGCCTTTGTGTGCTTAATCACGTATACTCACGTGCTCAATAGTCACCAATGCCCTCCCTCTTGGCCCTCTCCTTTTCTTTTTTCGACCGAATTAATTCTTAATCGGCAAAAAAAGAAAAGCTCCGGATCAAGATTGTACGTAAGGTGACAAGCTATTTTTCAATAAAGAATATCTTCCACTACTGCCATCTGGCGTCATAACTGCAAAGTACACATATATTACGATGCTGTCTATTAAATGCTTCCTATATTATATATATAGTAATGTCGTTTATGGTGCACTCTCAGTACAATCTGCTCTGATGCCGCATAGTTAAGCCAGCCCCGACACCCGCCAACACCCGCTGACGCGCCCTGACGGGCTTGTCTGCTCCCGGCATCCGCTTACAGACAAGCTGTGACCGTCTCCGGGAGCTGCATGTGTCAGAGGTTTTCACCGTCATCACCGAAACGCGCGA

**Parts**

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TCCTGTTTTTGCTCACCCAGAAACGCTGGTGAAAGTAAAAGATGCTGAAGATCAGTTGGGTGCACGAGTGGGTTACATCGAACTGGATCTCAACAGCGGTAAGATCCTTGAGAGTTTTCGCCCCGAAGAACGTTTTCCAATGATGAGCACTTTTAAAGTTCTGCTATGTGGCGCGGTATTATCCCGTATTGACGCCGGGCAAGAGCAACTCGGTCGCCGCATACACTATTCTCAGAATGACTTGGTTGAGTACTCACCAGTCACAGAAAAGCATCTTACGGATGGCATGACAGTAAGAGAATTATGCAGTGCTGCCATAACCATGAGTGATAACACTGCGGCCAACTTACTTCTGACAACGATCGGAGGACCGAAGGAGCTAACCGCTTTTTTTCACAACATGGGGGATCATGTAACTCGCCTTGATCGTTGGGAACCGGAGCTGAATGAAGCCATACCAAACGACGAGCGTGACACCACGATGCCTGTAGCAATGGCAACAACGTTGCGCAAACTATTAACTGGCGAACTACTTACTCTAGCTTCCCGGCAACAATTAATAGACTGGATGGAGGCGGATAAAGTTGCAGGACCACTTCTGCGCTCGGCCCTTCCGGCTGGCTGGTTTATTGCTGATAAATCTGGAGCCGGTGAGCGTGGGTCTCGCGGTATCATTGCAGCACTGGGGCCAGATGGTAAGCCCTCCCGTATCGTAGTTATCTACACGACGGGCAGTCAGGCAACTATGGATGAACGAAATAGACAGATCGCTGAGATAGGTGCCTCACTGATTAAGCATTGGTAACTGTCAGACCAAGTTTACTCATATATACTTTAGATTGATTTAAAACTTCATTTTTAATTTAAAAGGATCTAGGTGAAGATCCTTTTTGATAATCTCATGACCAAAATCCCTTAACGTGAGTTTTCGTTCCACTGAGCGTCAGACCCCGTAGAAAAGATCAAAGGATCTTCTTGAGATCCTTTTTTTCTGCGCGTAATCTGCTGCTTGCAAACAAAAAAACCACCGCTACCAGCGGTGGTTTGTTTGCCGGATCAAGAGCTACCAACTCTTTTTCCGAAGGTAACTGGCTTCAGCAGAGCGCAGATACCAAATACTGTCCTTCTAGTGTAGCCGTAGTTAGGCCACCACTTCAAGAACTCTGTAGCACCGCCTACATACCTCGCTCTGCTAATCCTGTTACCAGTGGCTGCTGCCAGTGGCGATAAGTCGTGTCTTACCGGGTTGGACTCAAGACGATAGTTACCGGATAAGGCGCAGCGGTCGGGCTGAACGGGGGGTTCGTGCACACAGCCCAGCTTGGAGCGAACGACCTACACCGAACTGAGATACCTACAGCGTGAGCATTGAGAAAGCGCCACGCTTCCCGAAGGGAGAAAGGCGGACAGGTATCCGGTAAGCGGCAGGGTCGGAACAGGAGAGCGCACGAGGGAGCTTCCAGGGGGGAACGCCTGGTATCTTTATAGTCCTGTCGGGTTTCGCCACCTCTGACTTGAGCGTCGATTTTTGTGATGCTCGTCAGGGGGGCCGAGCCTATGGAAAAACGCCAGCAACGCGGCCTTTTTACGGTTCCTGGCCTTTTGCTGGCCTTTTGCTCACATGTTCTTTCCTGCGTTATCCCCTGATTCTGTGGATAACCGTATTACCGCCTTTGAGTGAGCTGATACCGCTCGCCGCAGCCGAACGACCGAGCGCAGCGAGTCAGTGAGCGAGGAAGCGGAAGAGCGCCCAATACGCAAACCGCCTCTCCCCGCGCGTTGGCCGATTCATTAATGCAGCTGGCACGACAGGTTTCCCGACTGGAAAGCGGGCAGTGAGCGCAACGCAATTAATGTGAGTTACCTCACTCATTAGGCACCCCAGGCTTTACACTTTATGCTTCCGGCTCCTATGTTGTGTGGAATTGTGAGCGGATAACAATTTCACACAGGAAACAGCTATGACCATGATTACGCCAAGCTCGGAATTAACCCTCACTAAAGGGAACAAAAGCTGGGTACCCGACAGGTTATCAGCAACAACACAGTCATATCCATTCTCAATTAGCTCTACCACAGTGTGTGAACCAATGTATCCAGCACCACCTGTAACCAAAACAATTTTAGAAGTACTTTCACTTTGTAACTGAGCTGTCATTTATATTGAATTTTCAAAAATTCTTACTTTTTTTTTGGATGGACGCAAAGAAGTTTAATAATCATATTACATGGCATTACCACCATATACATATCCATATACATATCCATATCTAATCTTACTTATATGTTGTGGAAATGTAAAGAGCCCCATTATCTTAGCCTAAAAAAACCTTCTCTTTGGAACTTTCAGTAATACGCTTAACTGCTCATTGCTATATTGAAGTACGGATTAGAAGCCGCCGAGCGGGTGACAGCCCTCCGAAGGAAGACTCTCCTCCGTGCGTCCTCGTCTTCACCGGTCGCGTTCCTGAAACGCAGATGTGCCTCGCGCCGCACTGCTCCGAACAATAAAGATTCTACAATACTAGCTTTTATGGTTATGAAGAGGAAAAATTGGCAGTAACCTGGCCCCACAAACCTTCAAATGAACGAATCAAATTAACAACCATAGGATGATAATGCGATTAGTTTTTTAGCCTTATTTCTGGGGTAATTAATCAGCGAAGCGATGATTTTTGATCTATTAACAGATATATAAATGCAAAAACTGCATAACCACTTTAACTAATACTTTCAACATTTTCGGTTTGTATTACTTCTTATTCAAATGTAATAAAAGTATCAACAAAAAATTGTTAATATACCTCTATACTTTAACGTCAAGGAGAAAAAACCCCGGATCGAATTCCCTACTTCATACATTTTCAATTAAG

# AGA2

ATGCAGTTACTTCGCTGTTTTTCAATATTTTCTGTTATTGCTTCAGTTTTAGCACAGGAACTGACAACTATATGCGAGCAAATCCCCTCACCAACTTTAGAATCGACGCCGTACTCTTTGTCAACGACTACTATTTTGGCCAACGGGAAGGCAATGCAAGGAGTTTTTGAATATTACAAATCAGTAACGTTTGTCAGTAATTGCGGTTCTCACCCCTCAACAACTAGCAAAGGCAGCCCCATAAACACACAGTATGTTTTT

# Linker

AAGGACAATAGCTCGACG

# Factor

ATTGAAGGTAGATAC

# HA

CCATACGACGTTCCAGACTACGCTCTGCAGGCTAGT

**(Gly4Ser)3**

GGTGGAGGAGGCTCTGGTGGAGGCGGTAGCGGAGGCGGAGGGTCG

**HCV substrate-FLAG-TEV substrate – 6xHis-HCV protease**

GATGAAATGGAAGAATGTGCTTCTCATTTGGGCAGTGATTATAAAGATGATGATGATAAAGGCAGTGAAAATTTGTATTTCCAATCTGGCAGTCATCATCATCATCATCATGGTGGGGGAGGCTCCGCTAGCATGGCGCCTATC

**HCV**

GGCTCAGTAGTAATCGTAGGCAGAATCATCCTGTCCGGCCGTGGTGGCCCCATCACGGCGTACGCCCAGCAGACAAGGGGCCTCCTAGGGTGCATAATCACCAGCCTAACTGGCCGGGACAAAAACCAAGTGGAGGGTGAGGTCCAGATTGTGTCAACTGCTGCCCAAACCTTCCTGGCAACGTGCATCAATGGGGTGTGCTGGACTGTCTACCACGGGGCCGGAACGAGGACCATCGCGTCACCCAAGGGTCCTGTCATCCAGATGTATACCAATGTAGACCAAGACCTTGTGGGCTGGCCCGCTTCGCAAGGTACCCGCTCATTGACACCCTGCACTTGCGGCTCCTCGGACCTTTACCTGGTCACGAGGCACGCCGATGTCATTCCCGTGCGCCGGCGGGGTGATAGCAGGGGCAGCCTGCTGTCGCCCCGGCCCATTTCCTACTTGAAAGGCTCCTCGGGGGGTCCGCTGTTGTGCCCCGCGGGGCACGCCGTGGGCATATTTAGGGCCGCGGTGTGCACCCGTGGAGTGGCTAAGGCGGTGGACTTTATCCCTGTGGAGAACCTAGAGACAACCATGAGGTCGGGATCC

[note

134 138 147

R G D S R G S L L S P R P I S Y L K

CGGGGTGATAGCAGGGGCAGCCTGCTGTCGCCCCGGCCCATTTCCTACTTGAAA

170 175 180 185

R A A V C T R G V A K A V D F I P V E

AGGGCCGCGGTGTGCACCCGTGGAGTGGCTAAGGCGGTGGACTTTATCCCTGTGGAG

]

**c-myc**

GAACAAAAGCTTATTTCTGAAGAGGACTTG

**FEHDEL**

TTCGAACACGACGAATTG

**STOP**

TAATAG

CTCGAGATCTGATAACAACAGTGTAGATGTAACAAAATCGACTTTGTTCCCACTGTACTTTTAGCTCGTACAAAATACAATATACTTTTCATTTCTCCGTAAACAACATGTTTTCCCATGTAATATCCTTTTCTATTTTTCGTTCCGTTACCAACTTTACACATACTTTATATAGCTATTCACTTCTATACACTAAAAAACTAAGACAATTTTAATTTTGCTGCCTGCCATATTTCAATTTGTTATAAATTCCTATAATTTATCCTATTAGTAGCTAAAAAAAGATGAATGTGAATCGAATCCTAAGAGAATTGAGCTCCAATTCGCCCTATAGTGAGTCGTATTACAATTCACTGGCCGTCGTTTTACAACGTCGTGACTGGGAAAACCCTGGCGTTACCCAACTTAATCGCCTTGCAGCACATCCCCCCTTCGCCAGCTGGCGTAATAGCGAAGAGGCCCGCACCGATCGCCCTTCCCAACAGTTGCGCAGCCTGAATGGCGAATGGCGCGACGCGCCCTGTAGCGGCGCATTAAGCGCGGCGGGTGTGGTGGTTACGCGCAGCGTGACCGCTACACTTGCCAGCGCCCTAGCGCCCGCTCCTTTCGCTTTCTTCCCTTCCTTTCTCGCCACGTTCGCCGGCTTTCCCCGTCAAGCTCTAAATCGGGGGCTCCCTTTAGGGTTCCGATTTAGTGCTTTACGGCACCTCGACCCCAAAAAACTTGATTAGGGTGATGGTTCACGTAGTGGGCCATCGCCCTGATAGACGGTTTTTCGCCCTTTGACGTTGGAGTCCACGTTCTTTAATAGTGGACTCTTGTTCCAAACTGGAACAACACTCAACCCTATCTCGGTCTATTCTTTTGATTTATAAGGGATTTTGCCGATTTCGGCCTATTGGTTAAAAAATGAGCTGATTTAACAAAAATTTAACGCGAATTTTAACAAAATATTAACGTTTACAATTTCCTGATGCGGTATTTTCTCCTTACGCATCTGTGCGGTATTTCACACCGCAGGCAAGTGCACAAACAATACTTAAATAAATACTACTCAGTAATAACCTATTTCTTAGCATTTTTGACGAAATTTGCTATTTTGTTAGAGTCTTTTACACCATTTGTCTCCACACCTCCGCTTACATCAACACCAATAACGCCATTTAATCTAAGCGCATCACCAACATTTTCTGGCGTCAGTCCACCAGCTAACATAAAATGTAAGCTTTCGGGGCTCTCTTGCCTTCCAACCCAGTCAGAAATCGAGTTCCAATCCAAAAGTTCACCTGTCCCACCTGCTTCTGAATCAAACAAGGGAATAAACGAATGAGGTTTCTGTGAAGCTGCACTGAGTAGTATGTTGCAGTCTTTTGGAAATACGAGTCTTTTAATAACTGGCAAACCGAGGAACTCTTGGTATTCTTGCCACGACTCATCTCCATGCAGTTGGACGATATCAATGCCGTAATCATTGACCAGAGCCAAAACATCCTCCTTAGGTTGATTACGAAACACGCCAACCAAGTATTTCGGAGTGCCTGAACTATTTTTATATGCTTTTACAAGACTTGAAATTTTCCTTGCAATAACCGGGTCAATTGTTCTCTTTCTATTGGGCACACATATAATACCCAGCAAGTCAGCATCGGAATCTAGAGCACATTCTGCGGCCTCTGTGCTCTGCAAGCCGCAAACTTTCACCAATGGACCAGAACTACCTGTGAAATTAATAACAGACATACTCCAAGCTGCCTTTGTGTGCTTAATCACGTATACTCACGTGCTCAATAGTCACCAATGCCCTCCCTCTTGGCCCTCTCCTTTTCTTTTTTCGACCGAATTAATTCTTAATCGGCAAAAAAAGAAAAGCTCCGGATCAAGATTGTACGTAAGGTGACAAGCTATTTTTCAATAAAGAATATCTTCCACTACTGCCATCTGGCGTCATAACTGCAAAGTACACATATATTACGATGCTGTCTATTAAATGCTTCCTATATTATATATATAGTAATGTCGTTTATGGTGCACTCTCAGTACAATCTGCTCTGATGCCGCATAGTTAAGCCAGCCCCGACACCCGCCAACACCCGCTGACGCGCCCTGACGGGCTTGTCTGCTCCCGGCATCCGCTTACAGACAAGCTGTGACCGTCTCCGGGAGCTGCATGTGTCAGAGGTTTTCACCGTCATCACCGAAACGCGCGA

**Blue: F1 origin sequence**

HCV protease

R138

GGCTCAGTAGTAATCGTAGGCAGAATCATCCTGTCCGGCCGTGGTGGCCCCATCACGGCGTACGCCCAGCAGACAAGGGGCCTCCTAGGGTGCATAATCACCAGCCTAACTGGCCGGGACAAAAACCAAGTGGAGGGTGAGGTCCAGATTGTGTCAACTGCTGCCCAAACCTTCCTGGCAACGTGCATCAATGGGGTGTGCTGGACTGTCTACCACGGGGCCGGAACGAGGACCATCGCGTCACCCAAGGGTCCTGTCATCCAGATGTATACCAATGTAGACCAAGACCTTGTGGGCTGGCCCGCTTCGCAAGGTACCCGCTCATTGACACCCTGCACTTGCGGCTCCTCGGACCTTTACCTGGTCACGAGGCACGCCGATGTCATTCCCGTGCGCCGGCGGGGTGATAGCAGGGGCAGCCTGCTGTCGCCCCGGCCCATTTCCTACTTGAAAGGCTCCTCGGGGGGTCCGCTGTTGTGCCCCGCGGGGCACGCCGTGGGCATATTTAGGGCCGCGGTGTGCACCCGTGGAGTGGCTAAGGCGGTGGACTTTATCCCTGTGGAGAACCTAGAGACAACCATGAGGTCG

170 primer D183 primer 151 primer 147 primer 138 primer

GGCTCAGTAGTAATCGTAGGCAGAATCATCCTGTCCGGCCGTGGTGGCCCCATCACGGCGTACGCCCAGCAGACAAGGGGCCTCCTAGGGTGCATAATCACCAGCCTAACTGGCCGGGACAAAAACCAAGTGGAGGGTGAGGTCCAGATTGTGTCAACTGCTGCCCAAACCTTCCTGGCAACGTGCATCAATGGGGTGTGCTGGACTGTCTACCACGGGGCCGGAACGAGGACCATCGCGTCACCCAAGGGTCCTGTCATCCAGATGTATACCAATGTAGACCAAGACCTTGTGGGCTGGCCCGCTTCGCAAGGTACCCGCTCATTGACACCCTGCACTTGCGGCTCCTCGGACCTTTACCTGGTCACGAGGCACGCCGATGTCATTCCCGTGCGCCGGCGGGGTGATAGCAGGGGCAGCCTGCTGTCGCCCCGGCCCATTTCCTACTTGAAAGGCTCCTCGGGGGGTCCGCTGTTGTGCCCCGCGGGGCACGCCGTGGGCATATTTAGGGCCGCGGTGTGCACCCGTGGAGTGGCTAAGGCGGTGGACTTTATCCCTGTGGAGAACCTAGAGACAACCATGAGGTCG

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G R Y P Y D V P D Y A L Q A S G G G G S

HA

ggtggaggcggtagcggaggcggagggtcggatgaaatggaagaatgtgcttctcatttg

G G G G S G G G G S D E M E E C A S H L

Substrate

ggcagtgattataaagatgatgatgataaaggcagtgaaaatttgtatttccaatctggc

G S D Y K D D D D K G S E N L Y F Q S G

FLAG TEV substrate

agtcatcatcatcatcatcatggtgggggaggctccgctagcatggcgcctatcggctca

S H H H H H H G G G G S A S M A P I G S

His tag 1

gtagtaatcgtaggcagaatcatcctgtccggccgtggtggccccatcacggcgtacgcc

V V I V G R I I L S G R G G P I T A Y A

5 10 15 20

cagcagacaaggggcctcctagggtgcataatcaccagcctaactggccgggacaaaaac

Q Q T R G L L G C I I T S L T G R D K N

25 30 35 40

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Q V E G E V Q I V S T A A Q T F L A T C

45 50 55 60

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I N G V C W T V Y H G A G T R T I A S P

65 70 75 80

aagggtcctgtcatccagatgtataccaatgtagaccaagaccttgtgggctggcccgct

K G P V I Q M Y T N V D Q D L V G W P A

85 90 95 100

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S Q G T R S L T P C T C G S S D L Y L V

105 110 115 120

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T R H A D V I P V R R R G D S R G S L L

125 130 135 138 140

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S P R P I S Y L K G S S G G P L L C P A

145 147 150 155 160

gggcacgccgtgggcatatttagggccgcggtgtgcacccgtggagtggctaaggcggtg

G H A V G I F R A A V C T R G V A K A V

165 170 173 175 180

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D F I P V E N L E T T M R S G S E Q K L

183 185 190 195 GS C-MYC tag

atttctgaagaggacttgttcgaacacgacgaattgtaatagctcgagatctgataacaa

I S E E D L F E H D E L - - L E I - - Q

ER Retention

cagtgtagatgtaacaaaatcgactttgttcccactgtacttttagctcgtacaaaatac

Q C R C N K I D F V P T V L L A R T K Y

aatatacttttcatt

N I L F I

F1 (R138F I147A K151W)

R1 (R170Q D183R)

F2 (R138I)

R2 (R170Q A171S V173I T175K D183R)

ASyn 82-97:

gtagagggagctggatcaatcgctgctgctacgggatttgtaaaaaaa

V E G A G S I A A A T G F V K K

82 85 90 95 97

gggcaggaagatcccaacagtttgcgccataaatataactttatcgcggacgtggtggag

G Q E D P N S L R H K Y N F I A D V V E

HTRA1-PROTEASE-158 165 170 175

aagatcgcccctgccgtggttcatatcgaattgtttcgcaagcttccgttttctaaacga

K I A P A V V H I E L F R K L P F S K R

180 185 190 195

gaggtgccggtggctagtgggtctgggtttattgtgtcggaagatggactgatcgtgaca

E V P V A S G S G F I V S E D G L I V T

200 205 210 215

aatgcccacgtggtgaccaacaagcaccgggtcaaagttgagctgaagaacggtgccact

N A H V V T N K H R V K V E L K N G A T

220 225 230 235

tacgaagccaaaatcaaggatgtggatgagaaagcagacatcgcactcatcaaaattgac

Y E A K I K D V D E K A D I A L I K I D

240 245 250 255

caccagggcaagctgcctgtcctgctgcttggccgctcctcagagctgcggccgggagag

H Q G K L P V L L L G R S S E L R P G E

260 265 270 275

ttcgtggtcgccatcggaagcccgttttcccttcaaaacacagtcaccaccgggatcgtg

F V V A I G S P F S L Q N T V T T G I V

280 285 290 295

agcaccacccagcgaggcggcaaagagctggggctccgcaactcagacatggactacatc

S T T Q R G G K E L G L R N S D M D Y I

300 305 310 315

cagaccgacgccatcatcaactatggaaactcgggaggcccgttagtaaacctggacggt

Q T D A I I N Y G N S G G P L V N L D G

320 325 328 330 335

gaagtgattggaattaacactttgaaagtgacagctggaatctcctttgcaatcccatct

E V I G I N T L K V T A G I S F A I P S

340 345 350 355

gataagattaaaaagttcctcacggagtcccatgaccgacaggccaaaggaaaagccatc

D K I K K F L T E S H D R Q A K G K A I

360 365 END-PROTEASE-370

accaagaagaagtatattggtatccgaatgatgtcactcacgtccagcaaagccaaagag

T K K K Y I G I R M M S L T S S K A K E

HTRA1-PDZ-380 385 390 395

ctgaaggaccggcaccgggacttcccagacgtgatctcaggagcgtatataattgaagta

L K D R H R D F P D V I S G A Y I I E V

400 405 410 415

attcctgataccccagcagaagctggtggtctcaaggaaaacgacgtcataatcagcatc

I P D T P A E A G G L K E N D V I I S I

420 425 430 435

aatggacagtccgtggtctccgccaatgatgtcagcgacgtcattaaaagggaaagcacc

N G Q S V V S A N D V S D V I K R E S T

440 445 450 455

ctgaacatggtggtccgcaggggtaatgaagatatcatgatcacagtgattcccgaagaa

L N M V V R R G N E D I M I T V I P E E

460 465 470 475

attgaccca

I D P END-PDZ-480

Primers

**Sequencing:**

F: GGACAATAGCTCGACGATTG

R: GGATATTACATGGGAAAACATGTTGTTTACGGAG

**HCV substrates:**

Substrate Cutting:

F: TGTGCTTCTCATTTGGGCAGTGATTATAAAGATGATG

R: CGACCCTCCGCCTCCGCTAC

Rcomp: GTAGCGGAGGCGGAGGGTCG

CEDYF

F: GTAGCGGAGGCGGAGGGTCGTGCGAGGACTACTTTTGTGCTTCTCATTTGGGCAG

R: CTGCCCAAATGAGAAGCACAAAAGTAGTCCTCGCACGACCCTCCGCCTCCGCTAC

DVDAR

F: GTAGCGGAGGCGGAGGGTCGGACGTAGACGCTCGTTGTGCTTCTCATTTGGGCAG

R: CTGCCCAAATGAGAAGCACAACGAGCGTCTACGTCCGACCCTCCGCCTCCGCTAC

DVDAS

F: GTAGCGGAGGCGGAGGGTCGGACGTAGACGCTTCATGTGCTTCTCATTTGGGCAG

R: CTGCCCAAATGAGAAGCACATGAAGCGTCTACGTCCGACCCTCCGCCTCCGCTAC

DVDDY (not ordered)

F: GTAGCGGAGGCGGAGGGTCGGACGTAGACGACTACTGTGCTTCTCATTTGGGCAG

R: CTGCCCAAATGAGAAGCACAGTAGTCGTCTACGTCCGACCCTCCGCCTCCGCTAC

ENDAR (not ordered)

F: GTAGCGGAGGCGGAGGGTCGGAGAATGACGCTCGTTGTGCTTCTCATTTGGGCAG

R: CTGCCCAAATGAGAAGCACAACGAGCGTCATTCTCCGACCCTCCGCCTCCGCTAC

EQDAY (not ordered)

F: GTAGCGGAGGCGGAGGGTCGGAGCAGGACGCTTACTGTGCTTCTCATTTGGGCAG

R: CTGCCCAAATGAGAAGCACAGTAAGCGTCCTGCTCCGACCCTCCGCCTCCGCTAC

TEDDY (not ordered)

F: GTAGCGGAGGCGGAGGGTCGACGGAGGACGACTACTGTGCTTCTCATTTGGGCAG

R: CTGCCCAAATGAGAAGCACAGTAGTCGTCCTCCGTCGACCCTCCGCCTCCGCTAC

HNVDP (predicted cleaved)

F: GTAGCGGAGGCGGAGGGTCGCACAATGTAGACCCATGTGCTTCTCATTTGGGCAG

R: CTGCCCAAATGAGAAGCACATGGGTCTACATTGTGCGACCCTCCGCCTCCGCTAC

YRVVP (predicted cleaved)

F: GTAGCGGAGGCGGAGGGTCGTACCGTGTAGTACCATGTGCTTCTCATTTGGGCAG

R: CTGCCCAAATGAGAAGCACATGGTACTACACGGTACGACCCTCCGCCTCCGCTAC

HNVDE (predicted cleaved)

F: GTAGCGGAGGCGGAGGGTCGCACAATGTAGACGAGTGTGCTTCTCATTTGGGCAG

R: CTGCCCAAATGAGAAGCACACTCGTCTACATTGTGCGACCCTCCGCCTCCGCTAC

HERCI (predicted middle)

F: GTAGCGGAGGCGGAGGGTCGCACGAGCGTTGCATCTGTGCTTCTCATTTGGGCAG

R: CTGCCCAAATGAGAAGCACAGATGCAACGCTCGTGCGACCCTCCGCCTCCGCTAC

VISMI (predicted uncleaved)

F: GTAGCGGAGGCGGAGGGTCGGTAATCTCAATGATCTGTGCTTCTCATTTGGGCAG

R: CTGCCCAAATGAGAAGCACAGATCATTGAGATTACCGACCCTCCGCCTCCGCTAC

VISMV (predicted uncleaved)

F: GTAGCGGAGGCGGAGGGTCGGTAATCTCAATGGTATGTGCTTCTCATTTGGGCAG

R: CTGCCCAAATGAGAAGCACATACCATTGAGATTACCGACCCTCCGCCTCCGCTAC

VISMS (predicted uncleaved)

F: GTAGCGGAGGCGGAGGGTCGGTAATCTCAATGTCATGTGCTTCTCATTTGGGCAG

R: CTGCCCAAATGAGAAGCACATGACATTGAGATTACCGACCCTCCGCCTCCGCTAC

HERCI (predicted middle)

F: GTAGCGGAGGCGGAGGGTCGCACGAGCGTTGCATCTGTGCTTCTCATTTGGGCAG

R: CTGCCCAAATGAGAAGCACAGATGCAACGCTCGTGCGACCCTCCGCCTCCGCTAC

KERTW (predicted middle)

F: GTAGCGGAGGCGGAGGGTCGAAAGAGCGTACGTGGTGTGCTTCTCATTTGGGCAG

R: CTGCCCAAATGAGAAGCACACCACGTACGCTCTTTCGACCCTCCGCCTCCGCTAC

KERWI (predicted middle)

F: GTAGCGGAGGCGGAGGGTCGAAAGAGCGTTGGATCTGTGCTTCTCATTTGGGCAG

R: CTGCCCAAATGAGAAGCACAGATCCAACGCTCTTTCGACCCTCCGCCTCCGCTAC

Generic substrate R primer:

CTGCCCAAATGAGAAGCACA

CMSTN: GTAGCGGAGGCGGAGGGTCGTGCATGTCAACGAATTGTGCTTCTCATTTGGGCAG

GGGGG: GTAGCGGAGGCGGAGGGTCGGGAGGAGGAGGAGGATGTGCTTCTCATTTGGGCAG

HHIIL: GTAGCGGAGGCGGAGGGTCGCACCACATCATCCTTTGTGCTTCTCATTTGGGCAG

HKRTM: GTAGCGGAGGCGGAGGGTCGCACAAACGTACGATGTGTGCTTCTCATTTGGGCAG

KKWTQ: GTAGCGGAGGCGGAGGGTCGAAAAAATGGACGCAGTGTGCTTCTCATTTGGGCAG

KSSTY: GTAGCGGAGGCGGAGGGTCGAAATCATCAACGTACTGTGCTTCTCATTTGGGCAG

RPDPS: GTAGCGGAGGCGGAGGGTCGCGTCCAGACCCATCATGTGCTTCTCATTTGGGCAG

VPGPG: GTAGCGGAGGCGGAGGGTCGGTACCAGGACCAGGATGTGCTTCTCATTTGGGCAG

YTTIL: GTAGCGGAGGCGGAGGGTCGTACACGACGATCCTTTGTGCTTCTCATTTGGGCAG

YYIIP: GTAGCGGAGGCGGAGGGTCGTACTACATCATCCCATGTGCTTCTCATTTGGGCAG

**HCV mutations**

Mutation cutting:

F: TTTATCCCTGTGGAGAACCTAGAGACAACCATGAGGTCGG

R: GCTATCACCCCGCCGGCGC

Rcomp: GCGCCGGCGGGGTGATAGC

CEDYF:

F1 (138F147A151W):

GCGCCGGCGGGGTGATAGCTTTGGCAGCCTGCTGTCGCCCCGGCCCGCTTCCTACTTGTGGGGCTCCTCGGGGGGTCCGCTGTTGTGCCCCGCGGGGCAC

R1 (170Q183R):

AGGTTCTCCACAGGGATAAAACGCACCGCCTTAGCCACTCCACGGGTGCACACCGCGGCCTGAAATATGCCCACGGCGTGCCCCGCGGGGCACAACAGCG

DVDAR/DVDAS:

F2 (138I):

GCGCCGGCGGGGTGATAGCATCGGCAGCCTGCTGTCGCCCCGGCCCATTTCCTACTTGAAAGGCTCCTCGGGGGGTCCGCTGTTGTGCCCCGCGGGGCAC

R2 (170Q171S173I175K183R):

AGGTTCTCCACAGGGATAAAACGCACCGCCTTAGCCACTCCACGTTTGCAGATCGCTGACTGAAATATGCCCACGGCGTGCCCCGCGGGGCACAACAGCG

**Protease Swaps:**

Protease\_F\_cutting:

GGATCCGAACAAAAGCTTATTTC

Protease\_R\_cutting:

GATAGGCGCCATGCTAG

Htra1\_insert\_F:

CTCCGCTAGCATGGCGCCTATCGGGCAGGAAGATC

Htra1\_full\_insert\_R:

CAGAAATAAGCTTTTGTTCGGATCCTGGGTCAATTTCTTCGGG

Htra1\_protease\_insert\_R:

CAGAAATAAGCTTTTGTTCGGATCCTTTGGCCTGTCGG

Htra1\_PDZ\_insert\_F:

CTCCGCTAGCATGGCGCCTATCATCACCAAGAAGAAG

Htra1\_A328S\_F:

CCGACGCCATCATCAACTATGGAAACTCCGGAGGCC

Htra1\_A328S\_R:

CCAGGTTTACTAACGGGCCTCCGGAGTTTCCA

Total length: 51bp

Htra1\_S328A\_F:

CCGACGCCATCATCAACTATGGAAACGCGGGAGGCC

Htra1\_S328A\_R:

CCAGGTTTACTAACGGGCCTCCCGCGTTTCCA

Htra1\_remove\_PDZ\_F:

CCTCACGGAGTCCCATGACCGAGGATCCGAACA

Htra1\_remove\_PDZ\_R:

GTCCTCTTCAGAAATAAGCTTTTGTTCGGATCCTCGGTCA

Substrates:

ASyn\_82-97\_F

GTAGCGGAGGCGGAGGGTCGGTAGAGGGAGCTGGATCAATCGCTGCTGCTACGGGATTTGTAAAAAAAGGCAGTGATTATAAAGATGATGATGATAAAGG

ASyn\_82-97\_R

CCTTTATCATCATCATCTTTATAATCACTGCCTTTTTTTACAAATCCCGTAGCAGCAGCGATTGATCCAGCTCCCTCTACCGACCCTCCGCCTCCGCTAC

ASyn\_82-90\_F

GTAGCGGAGGCGGAGGGTCGGTAGAGGGAGCTGGATCAATCGCTGCTGGCAGTGATTATAAAGATGATGATGATAAAGG

ASyn\_82-90\_R

CCTTTATCATCATCATCTTTATAATCACTGCCAGCAGCGATTGATCCAGCTCCCTCTACCGACCCTCCGCCTCCGCTAC

ASyn\_85-93\_F

GTAGCGGAGGCGGAGGGTCGGCTGGATCAATCGCTGCTGCTACGGGAGGCAGTGATTATAAAGATGATGATGATAAAGG

ASyn\_85-93\_R

CCTTTATCATCATCATCTTTATAATCACTGCCTCCCGTAGCAGCAGCGATTGATCCAGCCGACCCTCCGCCTCCGCTAC

ASyn\_88-96\_F

GTAGCGGAGGCGGAGGGTCGATCGCTGCTGCTACGGGATTTGTAAAAGGCAGTGATTATAAAGATGATGATGATAAAGG

ASyn\_88-96\_R

CCTTTATCATCATCATCTTTATAATCACTGCCTTTTACAAATCCCGTAGCAGCAGCGATCGACCCTCCGCCTCCGCTAC

**LY104 mods**

Remove\_ER\_retention (elongate first) Insert: 65bp

F: CCGAACAAAAGCTTATTTCTGAAGAGGACTTGTAATAGCTCGAGAT

R: TCTACACTGTTGTTATCAGATCTCGAGCTATTACAAGTCCTCT

Fix\_cmyc (elongate first) Insert 3bp

F: TGACCCAGGATCCGAACAAAAGCTTATTTCTGAAGAGGACTTG

R: TCAGATCTCGAGCTATTACAAGTCCTCTTCAGAAATAAGCTTTTG

**Htra1 protease changes**:

Y325K (elongate first)(Asyn 89-GAGSIAAA)

F: CCAGACCGACGCCATCATCAACAAAGGAAACTCGG

R: CTAACGGGCCTCCCGAGTTTCCTTTGTTGATGAT

L309E\_T344N\_L345Q (react with template first) (90-AGSIAA)

F: GAGGCGGCAAAGAGCTGGGGGAGCGCAACTCAGACATGG  
R: GGGATTGCAAAGGAGATTCCAGCTGTCACTTTCTGATTGTTAATTCCAATCACTTCACCG

L309F (elongate first)(Asyn 91-GSIAAA)

F: GCGAGGCGGCAAAGAGCTGGGGTTTCGCAACT

R: GGATGTAGTCCATGTCTGAGTTGCGAAACCCCAGC

N343V\_K346V\_S352A (elongate first)(Asyn 92-SIAAAT)

Insert: 91bp

F: ACCTGGACGGTGAAGTGATTGGAATTGTAACTTTGGTAGTGACAGCTGGAAT

R: AGGAACTTTTTAATCTTATCAGATGGGATTGCAAAAGCGATTCCAGCTGTCACTACCAAA

**Htra1 PDZ changes**:

D1: N446E (elongate first) Insert: 51bp

F: TGGACAGTCCGTGGTCTCCGCCGAGGATGTCA

R: TCCCTTTTAATGACGTCGCTGACATCCTCGGCGGA

D2: I415K (elongate first) Insert: 59bp

F: CCAGACGTGATCTCAGGAGCGTATATAAAAGAAGTAATTCCT

R: GCTTCTGCTGGGGTATCAGGAATTACTTCTTTTATATACGCTCC

D3: S389D\_S444R\_N446Q (react with template first)

Insert:235bp

F: TCACCAAGAAGAAGTATATTGGTATCCGAATGATGGACCTCACGTCCAGCAAAGCCAAAG

CCTTTTAATGACGTCGCTGACATCCTGGGCCCTGACCACGGACTGTCC

D4: R386V\_M388R\_K394V\_I415K\_E416Y (react with template first)

Insert: 150bp

F: GATCCATCACCAAGAAGAAGTATATTGGTATCGTCATGAGGTCACTCACGTCCAGCGTCGCCAAAGAGCTGAAGG

R:

GCTTCTGCTGGGGTATCAGGAATTACATACTTTATATACGCTCCTGAGATCACGTCTGGGAAGTCCCGGTGC

OLD frame shift:

GCTTCTGCTGGGGTATCAGGAATTACATACTTATATACGCTCCTGAGATCACGTCTGGGAAGTCCCGGTGC

**pETcon**

seq\_pETcon\_Down\_Myc

CAAGTCCTCTTCAGAAATAAGCTTTTGTTC

seq\_pETcon\_Up-GS

GGACAATAGCTCGACGATTGAAGGTAGATACCCATA

RF\_Gibson\_PDZ (react with pET vector first)

Insert: 351 bp 97 ng insert / 84.4 ng plasmid

F: CGGTAGCGGAGGCGGAGGGTCGATGGCTAGCTGGAGCCAC

R: CCTCTTCAGAAATAAGCTTTTGTTCGGATCCTGGGTCAATTTCTTCGGGAA

|  |  |  |
| --- | --- | --- |
| **Plasmid** | **Substrate** | **Protease** |
| Ly104 | DEMEE (WT) | WT |
| C1 9/6/18 | CEDYF | WT |
| R4 9/8/18 | DVDAR | WT |
| D2 2/15/19 | DVDAS | WT |
| 1D 11/2/18 | CEDYF | F1R1 (138F147A151W/170Q183R) |
| A3 2/15/19 | DEMEE (WT) | F2R2 (138I/170Q171S173I175K183R) |
| 2D 11/2/18 | DVDAR | F2R2 (138I/170Q171S173I175K183R) |
| L2 12/4/18 | DVDAS | F2R2 (138I/170Q171S173I175K183R) |
| 1G 11/2/18 | CEDYF | F1R1\* (138F147A151W/170Q183R) |
| 2E 11/2/18 | DVDAR | F2R2\* (138I/170Q171S173I175K183R) |
| B3 2/15/19 | DEMEE (WT) | F1R2 (138F147A151W/170Q171S173I175K183R) |
| F2 9/24/18 | CEDYF | F1R2 (138F147A151W/170Q171S173I175K183R) |
| I4 9/24/18 | DVDAR | F1R1 (138F147A151W/170Q183R) |
| J1 9/24/18 | DVDAR | F1R2 (138F147A151W/170Q171S173I175K183R) |

LY104 with full active HTRA1 and synuclein 82-97 substrate

7339 BP

GACGAAAGGGCCTCGTGATACGCCTATTTTTATAGGTTAATGTCATGATAATAATGGTTTCTTAGGACGGATCGCTTGCCTGTAACTTACACGCGCCTCGTATCTTTTAATGATGGAATAATTTGGGAATTTACTCTGTGTTTATTTATTTTTATGTTTTGTATTTGGATTTTAGAAAGTAAATAAAGAAGGTAGAAGAGTTACGGAATGAAGAAAAAAAAATAAACAAAGGTTTAAAAAATTTCAACAAAAAGCGTACTTTACATATATATTTATTAGACAAGAAAAGCAGATTAAATAGATATACATTCGATTAACGATAAGTAAAATGTAAAATCACAGGATTTTCGTGTGTGGTCTTCTACACAGACAAGATGAAACAATTCGGCATTAATACCTGAGAGCAGGAAGAGCAAGATAAAAGGTAGTATTTGTTGGCGATCCCCCTAGAGTCTTTTACATCTTCGGAAAACAAAAACTATTTTTTCTTTAATTTCTTTTTTTACTTTCTATTTTTAATTTATATATTTATATTAAAAAATTTAAATTATAATTATTTTTATAGCACGTGATGAAAAGGACCCAGGTGGCACTTTTCGGGGAAATGTGCGCGGAACCCCTATTTGTTTATTTTTCTAAATACATTCAAATATGTATCCGCTCATGAGACAATAACCCTGATAAATGCTTCAATCCTGTTTTTGCTCACCCAGAAACGCTGGTGAAAGTAAAAGATGCTGAAGATCAGTTGGGTGCACGAGTGGGTTACATCGAACTGGATCTCAACAGCGGTAAGATCCTTGAGAGTTTTCGCCCCGAAGAACGTTTTCCAATGATGAGCACTTTTAAAGTTCTGCTATGTGGCGCGGTATTATCCCGTATTGACGCCGGGCAAGAGCAACTCGGTCGCCGCATACACTATTCTCAGAATGACTTGGTTGAGTACTCACCAGTCACAGAAAAGCATCTTACGGATGGCATGACAGTAAGAGAATTATGCAGTGCTGCCATAACCATGAGTGATAACACTGCGGCCAACTTACTTCTGACAACGATCGGAGGACCGAAGGAGCTAACCGCTTTTTTTCACAACATGGGGGATCATGTAACTCGCCTTGATCGTTGGGAACCGGAGCTGAATGAAGCCATACCAAACGACGAGCGTGACACCACGATGCCTGTAGCAATGGCAACAACGTTGCGCAAACTATTAACTGGCGAACTACTTACTCTAGCTTCCCGGCAACAATTAATAGACTGGATGGAGGCGGATAAAGTTGCAGGACCACTTCTGCGCTCGGCCCTTCCGGCTGGCTGGTTTATTGCTGATAAATCTGGAGCCGGTGAGCGTGGGTCTCGCGGTATCATTGCAGCACTGGGGCCAGATGGTAAGCCCTCCCGTATCGTAGTTATCTACACGACGGGCAGTCAGGCAACTATGGATGAACGAAATAGACAGATCGCTGAGATAGGTGCCTCACTGATTAAGCATTGGTAACTGTCAGACCAAGTTTACTCATATATACTTTAGATTGATTTAAAACTTCATTTTTAATTTAAAAGGATCTAGGTGAAGATCCTTTTTGATAATCTCATGACCAAAATCCCTTAACGTGAGTTTTCGTTCCACTGAGCGTCAGACCCCGTAGAAAAGATCAAAGGATCTTCTTGAGATCCTTTTTTTCTGCGCGTAATCTGCTGCTTGCAAACAAAAAAACCACCGCTACCAGCGGTGGTTTGTTTGCCGGATCAAGAGCTACCAACTCTTTTTCCGAAGGTAACTGGCTTCAGCAGAGCGCAGATACCAAATACTGTCCTTCTAGTGTAGCCGTAGTTAGGCCACCACTTCAAGAACTCTGTAGCACCGCCTACATACCTCGCTCTGCTAATCCTGTTACCAGTGGCTGCTGCCAGTGGCGATAAGTCGTGTCTTACCGGGTTGGACTCAAGACGATAGTTACCGGATAAGGCGCAGCGGTCGGGCTGAACGGGGGGTTCGTGCACACAGCCCAGCTTGGAGCGAACGACCTACACCGAACTGAGATACCTACAGCGTGAGCATTGAGAAAGCGCCACGCTTCCCGAAGGGAGAAAGGCGGACAGGTATCCGGTAAGCGGCAGGGTCGGAACAGGAGAGCGCACGAGGGAGCTTCCAGGGGGGAACGCCTGGTATCTTTATAGTCCTGTCGGGTTTCGCCACCTCTGACTTGAGCGTCGATTTTTGTGATGCTCGTCAGGGGGGCCGAGCCTATGGAAAAACGCCAGCAACGCGGCCTTTTTACGGTTCCTGGCCTTTTGCTGGCCTTTTGCTCACATGTTCTTTCCTGCGTTATCCCCTGATTCTGTGGATAACCGTATTACCGCCTTTGAGTGAGCTGATACCGCTCGCCGCAGCCGAACGACCGAGCGCAGCGAGTCAGTGAGCGAGGAAGCGGAAGAGCGCCCAATACGCAAACCGCCTCTCCCCGCGCGTTGGCCGATTCATTAATGCAGCTGGCACGACAGGTTTCCCGACTGGAAAGCGGGCAGTGAGCGCAACGCAATTAATGTGAGTTACCTCACTCATTAGGCACCCCAGGCTTTACACTTTATGCTTCCGGCTCCTATGTTGTGTGGAATTGTGAGCGGATAACAATTTCACACAGGAAACAGCTATGACCATGATTACGCCAAGCTCGGAATTAACCCTCACTAAAGGGAACAAAAGCTGGGTACCCGACAGGTTATCAGCAACAACACAGTCATATCCATTCTCAATTAGCTCTACCACAGTGTGTGAACCAATGTATCCAGCACCACCTGTAACCAAAACAATTTTAGAAGTACTTTCACTTTGTAACTGAGCTGTCATTTATATTGAATTTTCAAAAATTCTTACTTTTTTTTTGGATGGACGCAAAGAAGTTTAATAATCATATTACATGGCATTACCACCATATACATATCCATATACATATCCATATCTAATCTTACTTATATGTTGTGGAAATGTAAAGAGCCCCATTATCTTAGCCTAAAAAAACCTTCTCTTTGGAACTTTCAGTAATACGCTTAACTGCTCATTGCTATATTGAAGTACGGATTAGAAGCCGCCGAGCGGGTGACAGCCCTCCGAAGGAAGACTCTCCTCCGTGCGTCCTCGTCTTCACCGGTCGCGTTCCTGAAACGCAGATGTGCCTCGCGCCGCACTGCTCCGAACAATAAAGATTCTACAATACTAGCTTTTATGGTTATGAAGAGGAAAAATTGGCAGTAACCTGGCCCCACAAACCTTCAAATGAACGAATCAAATTAACAACCATAGGATGATAATGCGATTAGTTTTTTAGCCTTATTTCTGGGGTAATTAATCAGCGAAGCGATGATTTTTGATCTATTAACAGATATATAAATGCAAAAACTGCATAACCACTTTAACTAATACTTTCAACATTTTCGGTTTGTATTACTTCTTATTCAAATGTAATAAAAGTATCAACAAAAAATTGTTAATATACCTCTATACTTTAACGTCAAGGAGAAAAAACCCCGGATCGAATTCCCTACTTCATACATTTTCAATTAAGATGCAGTTACTTCGCTGTTTTTCAATATTTTCTGTTATTGCTTCAGTTTTAGCACAGGAACTGACAACTATATGCGAGCAAATCCCCTCACCAACTTTAGAATCGACGCCGTACTCTTTGTCAACGACTACTATTTTGGCCAACGGGAAGGCAATGCAAGGAGTTTTTGAATATTACAAATCAGTAACGTTTGTCAGTAATTGCGGTTCTCACCCCTCAACAACTAGCAAAGGCAGCCCCATAAACACACAGTATGTTTTTAAGGACAATAGCTCGACGATTGAAGGTAGATACCCATACGACGTTCCAGACTACGCTCTGCAGGCTAGTGGTGGAGGAGGCTCTGGTGGAGGCGGTAGCGGAGGCGGAGGGTCG***gtagagggagctggatcaatcgctgctgctacgggatttgtaaaaaaa***GGCAGTGATTATAAAGATGATGATGATAAAGGCAGTGAAAATTTGTATTTCCAATCTGGCAGTCATCATCATCATCATCATGGTGGGGGAGGCTCCGCTAGCATGGCGCCTATCgggcaggaagatcccaacagtttgcgccataaatataactttatcgcggacgtggtggagaagatcgcccctgccgtggttcatatcgaattgtttcgcaagcttccgttttctaaacgagaggtgccggtggctagtgggtctgggtttattgtgtcggaagatggactgatcgtgacaaatgcccacgtggtgaccaacaagcaccgggtcaaagttgagctgaagaacggtgccacttacgaagccaaaatcaaggatgtggatgagaaagcagacatcgcactcatcaaaattgaccaccagggcaagctgcctgtcctgctgcttggccgctcctcagagctgcggccgggagagttcgtggtcgccatcggaagcccgttttcccttcaaaacacagtcaccaccgggatcgtgagcaccacccagcgaggcggcaaagagctggggctccgcaactcagacatggactacatccagaccgacgccatcatcaactatggaaactcgggaggcccgttagtaaacctggacggtgaagtgattggaattaacactttgaaagtgacagctggaatctcctttgcaatcccatctgataagattaaaaagttcctcacggagtcccatgaccgacaggccaaaggaaaagccatcaccaagaagaagtatattggtatccgaatgatgtcactcacgtccagcaaagccaaagagctgaaggaccggcaccgggacttcccagacgtgatctcaggagcgtatataattgaagtaattcctgataccccagcagaagctggtggtctcaaggaaaacgacgtcataatcagcatcaatggacagtccgtggtctccgccaatgatgtcagcgacgtcattaaaagggaaagcaccctgaacatggtggtccgcaggggtaatgaagatatcatgatcacagtgattcccgaagaaattgacccaGGATCCGAACAAAAGCTTATTTCTGAAGAGGACTTGTTCGAACACGACGAATTGTAATAGCTCGAGATCTGATAACAACAGTGTAGATGTAACAAAATCGACTTTGTTCCCACTGTACTTTTAGCTCGTACAAAATACAATATACTTTTCATTTCTCCGTAAACAACATGTTTTCCCATGTAATATCCTTTTCTATTTTTCGTTCCGTTACCAACTTTACACATACTTTATATAGCTATTCACTTCTATACACTAAAAAACTAAGACAATTTTAATTTTGCTGCCTGCCATATTTCAATTTGTTATAAATTCCTATAATTTATCCTATTAGTAGCTAAAAAAAGATGAATGTGAATCGAATCCTAAGAGAATTGAGCTCCAATTCGCCCTATAGTGAGTCGTATTACAATTCACTGGCCGTCGTTTTACAACGTCGTGACTGGGAAAACCCTGGCGTTACCCAACTTAATCGCCTTGCAGCACATCCCCCCTTCGCCAGCTGGCGTAATAGCGAAGAGGCCCGCACCGATCGCCCTTCCCAACAGTTGCGCAGCCTGAATGGCGAATGGCGCGACGCGCCCTGTAGCGGCGCATTAAGCGCGGCGGGTGTGGTGGTTACGCGCAGCGTGACCGCTACACTTGCCAGCGCCCTAGCGCCCGCTCCTTTCGCTTTCTTCCCTTCCTTTCTCGCCACGTTCGCCGGCTTTCCCCGTCAAGCTCTAAATCGGGGGCTCCCTTTAGGGTTCCGATTTAGTGCTTTACGGCACCTCGACCCCAAAAAACTTGATTAGGGTGATGGTTCACGTAGTGGGCCATCGCCCTGATAGACGGTTTTTCGCCCTTTGACGTTGGAGTCCACGTTCTTTAATAGTGGACTCTTGTTCCAAACTGGAACAACACTCAACCCTATCTCGGTCTATTCTTTTGATTTATAAGGGATTTTGCCGATTTCGGCCTATTGGTTAAAAAATGAGCTGATTTAACAAAAATTTAACGCGAATTTTAACAAAATATTAACGTTTACAATTTCCTGATGCGGTATTTTCTCCTTACGCATCTGTGCGGTATTTCACACCGCAGGCAAGTGCACAAACAATACTTAAATAAATACTACTCAGTAATAACCTATTTCTTAGCATTTTTGACGAAATTTGCTATTTTGTTAGAGTCTTTTACACCATTTGTCTCCACACCTCCGCTTACATCAACACCAATAACGCCATTTAATCTAAGCGCATCACCAACATTTTCTGGCGTCAGTCCACCAGCTAACATAAAATGTAAGCTTTCGGGGCTCTCTTGCCTTCCAACCCAGTCAGAAATCGAGTTCCAATCCAAAAGTTCACCTGTCCCACCTGCTTCTGAATCAAACAAGGGAATAAACGAATGAGGTTTCTGTGAAGCTGCACTGAGTAGTATGTTGCAGTCTTTTGGAAATACGAGTCTTTTAATAACTGGCAAACCGAGGAACTCTTGGTATTCTTGCCACGACTCATCTCCATGCAGTTGGACGATATCAATGCCGTAATCATTGACCAGAGCCAAAACATCCTCCTTAGGTTGATTACGAAACACGCCAACCAAGTATTTCGGAGTGCCTGAACTATTTTTATATGCTTTTACAAGACTTGAAATTTTCCTTGCAATAACCGGGTCAATTGTTCTCTTTCTATTGGGCACACATATAATACCCAGCAAGTCAGCATCGGAATCTAGAGCACATTCTGCGGCCTCTGTGCTCTGCAAGCCGCAAACTTTCACCAATGGACCAGAACTACCTGTGAAATTAATAACAGACATACTCCAAGCTGCCTTTGTGTGCTTAATCACGTATACTCACGTGCTCAATAGTCACCAATGCCCTCCCTCTTGGCCCTCTCCTTTTCTTTTTTCGACCGAATTAATTCTTAATCGGCAAAAAAAGAAAAGCTCCGGATCAAGATTGTACGTAAGGTGACAAGCTATTTTTCAATAAAGAATATCTTCCACTACTGCCATCTGGCGTCATAACTGCAAAGTACACATATATTACGATGCTGTCTATTAAATGCTTCCTATATTATATATATAGTAATGTCGTTTATGGTGCACTCTCAGTACAATCTGCTCTGATGCCGCATAGTTAAGCCAGCCCCGACACCCGCCAACACCCGCTGACGCGCCCTGACGGGCTTGTCTGCTCCCGGCATCCGCTTACAGACAAGCTGTGACCGTCTCCGGGAGCTGCATGTGTCAGAGGTTTTCACCGTCATCACCGAAACGCGCGA