**DNA and RNA sequences**

**PCR primers for Vif mutant library construction**

|  |  |  |
| --- | --- | --- |
| **Mutant** | **Primer** | **Sequence1** |
| R15A | 15A\_Fwd | TGGCAGGTGGACGCCATGCGCATTAACACC |
| 15A\_Rvs | GTTAATGCGCATGGCGTCCACCTGCCACAC |
| K26A | 26A\_Fwd | AAGCGCCTGGTGGCCCACCACATGTACATT |
| 26A\_Rvs | GTACATGTGGTGGGCCACCAGGCGCTTCCA |
| Y30A | 30A\_Fwd | AAGCACCACATGGCCATTAGCCGCAAAGCT |
| 30A\_Rvs | TTTGCGGCTAATGGCCATGTGGTGCTTCAC |
| I31A | 31A\_Fwd | CACCACATGTACGCCAGCCGCAAAGCTAAG |
| 31A\_Rvs | AGCTTTGCGGCTGGCGTACATGTGGTGCTT |
| I31D | 31D\_Fwd | CACCACATGTACGACAGCCGCAAAGCTAAG |
| 31D\_Rvs | AGCTTTGCGGCTGTCGTACATGTGGTGCTT |
| RK33/34A | 33/34A\_Fwd | ATGTACATTAGCGCCGCCGCTAAGGACTGGTTCTAC |
| 33/34A\_Rvs | GAACCAGTCCTTAGCGGCGGCGCTAATGTACATGTG |
| RK33/34EE | 33/34EE\_Fwd | ATGTACATTAGCGAGGAGGCTAAGGACTGGTTC |
| 33/34EE\_Rvs | CCAGTCCTTAGCCTCCTCGCTAATGTACATGTG |
| K36A | 36A\_Fwd | AGCCGCAAAGCTGCCGACTGGTTCTACCGC |
| 36A\_Rvs | GTAGAACCAGTCGGCAGCTTTGCGGCTAAT |
| F39A | 39A\_Fwd | GCTAAGGACTGGGCCTACCGCCACCACTAC |
| 39A\_Rvs | GTGGTGGCGGTAGGCCCAGTCCTTAGCTTT |
| Y44A | 44A\_Fwd | TACCGCCACCACGCCGAGAGCACCAACCCC |
| 44A\_Rvs | GTTGGTGCTCTCGGCGTGGTGGCGGTAGAA |
| D61A | 61A\_Fwd | ATTCCCCTGGGCGCCGCCAAGCTGGTGATT |
| 61A\_Rvs | CACCAGCTTGGCGGCGCCCAGGGGAATGTG |
| K63A | 63A\_Fwd | CTGGGCGACGCCGCCCTGGTGATTACGACC |
| 63A\_Rvs | GGTCGTAATCACCAGGGCGGCGTCGCCCAG |
| W70R | 70R\_Fwd | ATTACGACCTACCGGGGCCTGCACACCGGC |
| 70R\_Rvs | GGTGTGCAGGCCCCGGTAGGTCGTAATCAC |
| E76R | 76R\_Fwd | CTGCACACCGGCCGGCGCGACTGGCACCTG |
| 76R\_Rvs | GTGCCAGTCGCGCCGGCCGGTGTGCAGGCC |
| W79A | 79A\_Fwd | GGCGAGCGCGACGCCCACCTGGGCCAGGGC |
| 79A\_Rvs | CTGGCCCAGGTGGGCGTCGCGCTCGCCGGT |
| R93A | 93A\_Fwd | TGGAGGAAAAAGGCCTATAGCACACAAGTA |
| 93A\_Rvs | TTGTGTGCTATAGGCCTTTTTCCTCCATTC |
| S116A | 116A\_Fwd | TTTGATTGTTTTGCCGAATCTGCTATAAGA |
| 116A\_Rvs | TATAGCAGATTCGGCAAAACAATCAAAATA |

1. Mutagenised codons shaded in grey.

 **PCR primers for Vif mutant library construction (cont.)**

|  |  |  |
| --- | --- | --- |
| **Mutant** | **Primer** | **Sequence1** |
| E117A | 117A\_Fwd | GATTGTTTTTCAGCCTCTGCTATAAGAAAT |
| 117A\_Rvs | TCTTATAGCAGAGGCTGAAAAACAATCAAA |
| S118A | 118A\_Fwd | TGTTTTTCAGAAGCCGCTATAAGAAATACC |
| 118A\_Rvs | ATTTCTTATAGCGGCTTCTGAAAAACAATC |
| R121A | 121A\_Fwd | GAATCTGCTATAGCCAATACCATATTAGGA |
| 121A\_Rvs | TAATATGGTATTGGCTATAGCAGATTCTGA |
| N122A | 122A\_Fwd | TCTGCTATAAGAGCCACCATATTAGGACGT |
| 122A\_Rvs | TCCTAATATGGTGGCTCTTATAGCAGATTC |
| R127A | 127A\_Fwd | ACCATATTAGGAGCCATAGTTAGTCCTAGG |
| 127A\_Rvs | AGGACTAACTATGGCTCCTAATATGGTATT |
| R127E | 127E\_Fwd | ACCATATTAGGAGAGATAGTTAGTCCTAGG |
| 127E\_Rvs | AGGACTAACTATCTCTCCTAATATGGTATT |
| I128A | 128A\_Fwd | ATATTAGGACGTGCCGTTAGTCCTAGGTGT |
| 128A\_Rvs | CCTAGGACTAACGGCACGTCCTAATATGGT |
| I128D | 128D\_Fwd | ATATTAGGACGTGACGTTAGTCCTAGGTGT |
| 128D\_Rvs | CCTAGGACTAACGTCACGTCCTAATATGGT |
| S130A | 130A\_Fwd | GGACGTATAGTTGCCCCTAGGTGTGAATAT |
| 130A\_Rvs | TTCACACCTAGGGGCAACTATACGTCCTAA |
| S130E | 130E\_Fwd | GGACGTATAGTTGAGCCTAGGTGTGAATAT |
| 130E\_Rvs | TTCACACCTAGGCTCAACTATACGTCCTAA |
| P131A | 131A\_Fwd | CGTATAGTTAGTGCCAGGTGTGAATATCAA |
| 131A\_Rvs | ATATTCACACCTGGCACTAACTATACGTCC |
| R132D | 132D\_Fwd | ATAGTTAGTCCTGACTGTGAATATCAAGCA |
| 132D\_Rvs | TTGATATTCACAGTCAGGACTAACTATACG |
| L153A | 153A\_Fwd | GCACTAGCAGCAGCCATAAAACCAAAACAG |
| 153A\_Rvs | TTTTGGTTTTATGGCTGCTGCTAGTGCCAA |
| I154A | 154A\_Fwd | CTAGCAGCATTAGCCAAACCAAAACAGATA |
| 154A\_Rvs | CTGTTTTGGTTTGGCTAATGCTGCTAGTGC |
| K155A | 155A\_Fwd | GCAGCATTAATAGCCCCAAAACAGATAAAG |
| 155A\_Rvs | TATCTGTTTTGGGGCTATTAATGCTGCTAG |
| K157E | 157E\_Fwd | TTAATAAAACCAGAGCAGATAAAGCCACCT |
| 157E\_Rvs | TGGCTTTATCTGCTCTGGTTTTATTAATGC |
| K160E | 160E\_Fwd | CCAAAACAGATAGAGCCACCTTTGCCTAGT |
| 160E\_Rvs | AGGCAAAGGTGGCTCTATCTGTTTTGGTTT |
| N/A | seq | gaagcaggctggagacgtggag |
| Vif\_Fwd | CattcacaggtgcagctcgaggggtcagggATGGAGAACCGG |
| Vif\_Rvs | AattttgtaatccagaggttgattggtaccCTAGTGTCCATTCAT |

1. Mutagenised codons shaded in grey.

**Codon-optimised Vif variants synthesised as gBlocks**

|  |  |
| --- | --- |
| **Vif variant** | **Sequence1** |
| NL4-3 | cattcacaggtgcagctcgaggggtcagggATGGAGAACCGGTGGCAGGTGATGATTGTGTGGCAGGTGGACCGCATGCGCATTAACACCTGGAAGCGCCTGGTGAAGCACCACATGTACATTAGCCGCAAAGCTAAGGACTGGTTCTACCGCCACCACTACGAGAGCACCAACCCCAAGATTAGCAGCGAGGTGCACATTCCCCTGGGCGACGCCAAGCTGGTGATTACGACCTACTGGGGCCTGCACACCGGCGAGCGCGACTGGCACCTGGGCCAGGGCGTCTCCATAGAATGGAGGAAAAAGAGATATAGCACACAAGTAGACCCTGACCTAGCAGACCAACTAATTCATCTGCACTATTTTGATTGTTTTTCAGAATCTGCTATAAGAAATACCATATTAGGACGTATAGTTAGTCCTAGGTGTGAATATCAAGCAGGACATAACAAGGTAGGATCTCTACAGTACTTGGCACTAGCAGCATTAATAAAACCAAAACAGATAAAGCCACCTTTGCCTAGTGTTAGGAAACTGACAGAGGACAGATGGAACAAGCCCCAGAAGACCAAGGGCCACAGAGGGAGCCATACAATGAATGGACACTAGggtaccaatcaacctctggattacaaaatt |
| HXB2 | cattcacaggtgcagctcgaggggtcagggATGGAAAACAGATGGCAGGTTATGATCGTGTGGCAAGTAGATCGCATGAGAATTAGAACTTGGAAGTCATTGGTGAAGCATCACATGTACGTGAGCGGAAAGGCGCGAGGATGGTTCTATAGACATCATTACGAGTCCCCGCATCCACGGATCAGCTCAGAGGTCCATATCCCACTTGGCGATGCACGGCTTGTGATTACAACTTATTGGGGTCTTCACACTGGGGAACGGGACTGGCATCTGGGCCAGGGAGTGAGTATTGAGTGGAGAAAAAAAAGGTATAGCACTCAGGTAGACCCCGAACTCGCGGATCAACTGATACACTTGTATTACTTCGACTGCTTCTCTGACAGCGCGATCCGAAAGGCATTGCTCGGCCATATCGTTAGTCCGCGCTGTGAGTATCAAGCCGGGCATAACAAGGTCGGTTCTCTTCAATATCTGGCGCTCGCAGCGCTTATCACCCCGAAAAAGATTAAACCTCCTTTGCCATCAGTTACCAAGCTGACCGAGGACAGGTGGAACAAACCCCAGAAAACGAAAGGACACAGAGGAAGTCATACGATGAATGGACATTAGggtaccaatcaacctctggattacaaaatt |
| YU-2 | cattcacaggtgcagctcgaggggtcagggATGGAGAATAGATGGCAGGTTATGATAGTTTGGCAGGTAGATCGCATGAGAATTAGAGCTTGGAAGTCATTGGTGAAGCATCACATGTACATATCCGGGAAAGCGCGGGGTTGGTTTTATCGGCATCACTACGAATCACCACATCCTCGCATCAGCTCCGAAGTTCACATTCCTCTTGGGGATGCCAAACTTGTTATTACGACCTATTGGGGGCTGCATACTGGCGAGAGAGACTGGCATTTGGGTCAGGGTGTAAGTATAGAATGGCGCAAAAAGAGGTACTCAACCCAAGTCGATCCCGATTTGGCAGACCAACTGATCCATCTTTATTACTTTGACTGCTTCAGTGAGAGTGCGATACGGAAGGCGATTCTGGGATATCGAGTAAGTCCCCGCTGTGAATATCAAGCTGGTCATAATAAAGTGGGTTCTCTCCAATACCTGGCCCTTACGGCGTTGATAACTCCTAAAAAGACGAAACCTCCTCTTCCCTCCGTCAAGAAACTCACCGAAGACAGATGGAACAAGCCTCAGAAAACAAAGGGGCATAGAGGTTCCAGAACGATGAACGGACACTAGggtaccaatcaacctctggattacaaaatt |
| CH470 | cattcacaggtgcagctcgaggggtcagggATGGAGAACCGATGGCAGGTTATGATAGTCTGGCAAGTGGACAGGATGCGAATCAAAACGTGGAAGAGCTTGGTGAAACACCACATGCACATTTCCAAGAAGGCCCGCGGTTGGTTCTATCGCCACCATTACGAATCAACACACCCAAAGATCTCCTCAGAGGTCCATATCCCACTTCGAGAAGCACGGTTGGTAATAACAACGTACTGGGGGTTGCATACCGGCGAGAGGGATTGGCACTTGGGCCAGGGAGTATCCATTGAATGGAGAAAACGGAAGTACAGTACACAGGTGGACCCAGACCTCGCCGACCAATTGATTCACCTCTATTATTTCGACTGCTTCTCTGAGAGCGCGATTAGGAATGCGCTTCTGGGCCACATCGTGAGTCCGAGATGTGAGTACCAGGCCGGACATAACAAAGTGGGGAGCCTCCAGTATCTTGCCTTGACCGCTCTCGTTGCTCCAAAAAAGACGAAGCCTCCCCTGCCTAGCGTTAAAAAGCTGACCGAGGATAGGTGGAATAAGCCGCAAAAAACGAAGGGACACCGCGGGTCTCATACCATGTCTGGACACTAGggtaccaatcaacctctggattacaaaatt |

1. Vif open reading frames (ORFs) shaded in grey.

**Vif coding sequences in HIV-AFMACS viruses**

|  |  |
| --- | --- |
| **Virus** | **Sequence1** |
| ΔVpr-Vif\_WT | atggaaaacagatggcaggtgatgattgtgtggcaagtagacaggatgaggattaacacatggaaaagattagtaaaacaccatatgtatATTtcaaggaaagctaaggactggttttatagacatcactatgaaagtactaatccaaaaataagttcagaagtacacatcccactaggggatgctaaattagtaataacaacatattggggtctgcatacaggagaaagagactggcatttgggtcagggagtctccatagaatggaggaaaaagagatatagcacacaagtagaccctgacctagcagaccaactaattcatctgcactattttgattgtttttcagaatctgctataagaaataccatattaggaTATAGAgttagtcctaggtgtgaatatcaagcaggacataacaaggtaggatctctacagtacttggcactagcagcattaataaaaccaaaacagataaagccacctttgcctagtgttaggaaactgacagaggac**agG**tggaacaagccccagaagaccaagggccacagagggagccatacaatgaatggacactag |
| ΔVpr-Vif\_AYR | atggaaaacagatggcaggtgatgattgtgtggcaagtagacaggatgaggattaacacatggaaaagattagtaaaacaccatatgtatGCTtcaaggaaagctaaggactggttttatagacatcactatgaaagtactaatccaaaaataagttcagaagtacacatcccactaggggatgctaaattagtaataacaacatattggggtctgcatacaggagaaagagactggcatttgggtcagggagtctccatagaatggaggaaaaagagatatagcacacaagtagaccctgacctagcagaccaactaattcatctgcactattttgattgtttttcagaatctgctataagaaataccatattaggaGCTATAgttagtcctaggtgtgaatatcaagcaggacataacaaggtaggatctctacagtacttggcactagcagcattaataaaaccaaaacagataaagccacctttgcctagtgttaggaaactgacagaggac**agG**tggaacaagccccagaagaccaagggccacagagggagccatacaatgaatggacactag |
| ΔVpr-ΔVif | atggaaaacagatggcaggtgatgattgtgtggcaagtagacaggatgaggattaacacatggaaaagattagtaaaacaccatatgTAATAAtcaaggaaagctaaggactggttttatagacatcactatgaaagtactaatccaaaaataagttcagaagtacacatcccactaggggatgctaaattagtaataacaacatattggggtctgcatacaggagaaagagactggcatttgggtcagggagtctccatagaatggaggaaaaagagatatagcacacaagtagaccctgacctagcagaccaactaattcatctgcactattttgattgtttttcagaatctgctataagaaataccatattaggaGCTATAgttagtcctaggtgtgaatatcaagcaggacataacaaggtaggatctctacagtacttggcactagcagcattaataaaaccaaaacagataaagccacctttgcctagtgttaggaaactgacagaggac**agG**tggaacaagccccagaagaccaagggccacagagggagccatacaatgaatggacactag |

1. Based on native NL4-3 Vif sequence. Mutagenised codons shaded in grey. The mutation in codon 173 (highlighted in bold) is silent in Vif (AGA>AGG; both encoding Arg) but abrogates the Vpr start codon in the +2 reading frame (underlined).

**C-terminal 4xHA-tagged APOBEC3F coding sequence in pHRSIN-S-W-pGK puro**

|  |  |
| --- | --- |
| **Gene** | **Sequence1** |
| APOBEC3F | ATGAAGCCTCACTTCAGAAACACAGTGGAGCGAATGTATCGAGACACATTCTCCTACAACTTTTATAATAGACCCATCCTTTCTCGTCGGAATACCGTCTGGCTGTGCTACGAAGTGAAAACAAAGGGTCCCTCAAGGCCCCGTTTGGACGCAAAGATCTTTCGAGGCCAGGTGTATTCCCAGCCTGAGCACCACGCAGAAATGTGCTTCCTCTCTTGGTTCTGTGGCAACCAGCTGCCTGCTTACAAGTGTTTCCAGATCACCTGGTTTGTATCCTGGACCCCCTGCCCGGACTGTGTGGCGAAGCTGGCCGAATTCCTGTCTGAGCACCCCAATGTCACCCTGACCATCTCCGCCGCCCGCCTCTACTACTACTGGGAAAGAGATTACCGAAGGGCGCTCTGCAGGCTGAGTCAGGCAGGGGCCCGTGTGAAGATTATGGACGATGAAGAATTTGCATACTGCTGGGAAAACTTTGTGTACAGTGAAGGTCAGCCATTCATGCCTTGGTACAAATTCGATGACAATTATGCATTCCTGCACCGCACGCTAAAGGAGATTCTCAGAAACCCGATGGAGGCAATGTATCCACACATATTCTACTTCCACTTTAAAAACCTACGCAAAGCCTATGGTCGGAACGAAAGCTGGCTGTGCTTCACCATGGAAGTTGTAAAGCACCACTCACCTATCTCCTGGAAGAGGGGCGTCTTCCGAAACCAGGTGGATCCTGAGACCCATTGTCATGCAGAAAGGTGCTTCCTCTCTTGGTTCTGTGACGACATACTGTCTCCTAACACAAACTACGAGGTCACCTGGTACACATCTTGGAGCCCTTGCCCAGAGTGTGCAGGGGAGGTGGCCGAGTTCCTGGCCAGGCACAGCAACGTGAATCTCACCATCTTCACCGCCCGCCTCTACTACTTCTGGGATACAGATTACCAGGAGGGGCTCCGCAGCCTGAGTCAGGAAGGGGCCTCCGTGGAGATCATGGGCTACAAAGATTTTAAATATTGTTGGGAAAACTTTGTGTACAATGATGATGAGCCATTCAAGCCTTGGAAAGGACTAAAATACAACTTTCTATTCCTGGACAGCAAGCTGCAGGAGATTCTCGAGGCGGCCGCGGGTTCTGGTTACCCCTACGATGTGCCAGACTACGCTGGTTCTGGTTACCCATACGATGTTCCTGACTATGCGGGCTATCCCTATGACGTCCCGGACTATGCAGGTTCCTATCCATATGACGTTCCAGATTACGCTTAA |

1. 4xHA tag shaded in grey.

**Target sequences for RNAi**

|  |  |  |
| --- | --- | --- |
| **Panel** | **Target** | **Sequence1** |
| 1 | PPP2R5A | UGAAUGAACUGGUUGAGUA |
| PPP2R5B | GAACAAUGAGUAUAUCCUA |
| PPP2R5C | GGAAGAUGAACCAACGUUA |
| PPP2R5D | GGAAGAUGAACCAACGUUA |
| PPP2R5E | GCACAGCUGGCAUAUUGUA |
| DPH7 | CCTGGATTGCTGCTTTCAATT |
| FMR1 | GAGAGTTCAAGGCAGCTTGCC |
| 2 | PPP2R5A | CTGAAGACTGTTCTGCACCGA |
| PPP2R5B | AGTGTGTGGGGAGCACCCGGG |
| PPP2R5C | GAATGTGATCACAGAGCCTAT |
| PPP2R5D | AGCTGCTTGGCCACATCTCCA |
| PPP2R5E | CAATAAGCAGAGGCTGTTTGA |
| DPH7 | CACCCTGTCATGAATGCAGAG |
| FMR1 | CATGAACAGTTTATCGTAAGA |

**PCR primers for real time PCR**

|  |  |  |
| --- | --- | --- |
| **Target** | **Primer** | **Sequence** |
| PPP2R5A | PPP2R5A\_Fwd | TTGGCCTCACATACAGTTGG |
| PPP2R5A\_Rvs | CACGTTCTCTGGGATCTTCAC |
| PPP2R5B | PPP2R5B\_Fwd | ACCAAACCATCGTATCACTGATC |
| PPP2R5B\_Rvs | GACCTTGCCATAACTCCTGAC |
| PPP2R5C | PPP2R5C\_Fwd | GTGAAGATCATGGAACCCCTC |
| PPP2R5C\_Rvs | GCTGCGTTGTCACTGATTAAAC |
| PPP2R5D | PPP2R5D\_Fwd | CCTTGAGTCTCCTGATTTCCAG |
| PPP2R5D\_Rvs | AAAACTTGCCATAGATGCGATG |
| PPP2R5E | PPP2R5E\_Fwd | TCACAGTTTAGGTCTCAAGGC |
| PPP2R5E\_Rvs | ATCAGATAGCGTGTCCATGAAG |
| TBP | TBP\_Fwd | GAGAAGATGGATGTTGAGTTG |
| TBP\_Rvs | GATAGCAGCACGGTATGA |