**Table 1 Oligonucleotides for PCR reactions and DNA sequencing**

|  |  |  |
| --- | --- | --- |
| **Name** | **Sequence (5’-3’)** | **Note** |
| sFvVP16F | 5′-TTGTTTCTTTTTCTGCACAAT-3′ | Hybridizes 93 bps upstream of the start codon (ATG) of VP16AD and is used to amplify part or all of the single domain fragments contained in pVP16\* |
| sFvVP16R | 5′-CAACATGTCCAGATCGAA-3′ | Hybridizes to the VP16AD region and is used to amplify and sequence part or all of the single domain fragments in pVP16\* and pEFVP16 |
| BTM116F | 5′-CAGAGCTTCACCATTGAA-3′ | Hybridizes to the LexA region of pBTM116 and is used to sequence the bait antigen |
| BTM116R | 5′-TCAATAAGAGCGACCTCATG-3′ | Hybridizes to ADH1 in the terminator region of pBTM116 and pBD-Gal4 and is used to sequence the bait antigen |
| rdmVHCDR2 Rev | 5′-CAGAGTCTGCATAGTATATMNNMNNMNNMNN MNNACTAATGTATGAAACCCAC-3′ M indicates A or C | Used to randomize complementarity determining region (CDR)2 residues of single variable heavy (VH) chain domains and, together with sFvVP16, to amplify the N-terminal regions of the VH domain |
| VHCDR2Fw | 5′-ATATACTATGCAGACTCTG-3′ | Complementary to a part of rdmVHCDR2Rev and, with sFvVP16R, used to amplify C-terminal regions of the VH domains |
| rdmVLCDR2 Rev | 5′-AACCTTGATGGGACCCCACTMNNMNNMNNGG  ATGCMNNATAGATCAGGAGCTTAGGGG-3′ | Used to randomize CDR2 residues of single variable light (VL) chain domains and, with sFvVP16R, to amplify the N-terminal regions of VL domains |
| VLCDR2Fw | 5′-AGTGGGGTCCCATCAAGGTTCAG-3′ | Complementary to part of rdmVLCDR2Rev and used to amplify C-terminal regions of VL domain together with sFvVP16R |
| rdmHCDR1 Rev | 5′-CCTGGAGCCTGGCGGACCCAMNNCATMNNMN NMNNACTGAAGCTGAATCCAGAGG-3′ | Used to randomize CDR1 residues of single VH domains and with sFvVP16R, to amplify N-terminal regions of VH domains |
| rdmVHCDR1 Rev2 | 5′-CCTGGAGCCTGGCGGACCCAGTTCATMNNMN NMNNMNNMNNMNNMNNTCCAGAGGCTGCACA GGAGAG-3′ | Complementary to part of rdmVHCDR1Rev or rdmVHCDR1Rev2 and with sFvVP16R, used to amplify C-terminal regions of VH domains |
| VHCDR1Fw | 5′-TGGGTCCGCCAGGCTCCAGG-3′ | Complementary to part of rdmVHCDR1Rev or rdmVHCDR1Rev2 and with sFvVP16R, used to amplify C-terminal regions of VH domains |
| rdmVLCDR1 Rev | 5′-CCTGGTTTCTGCTGATACCAMNNTAAMNNGCT  GCTAATMNNCTGACTTGCCCGGCAAGTGATG-3′ | Used to randomize CDR2 residues of single VL domains and with sFvVP16R to amplify N-terminal regions of VL domains |
| VLCDR1Fw | 5′-TGGTATCAGCAGAAACCAGG-3′ | Complementary to part of rdmVLCDR1Rev and, with sFvVP16R, used to amplify C-terminal regions of VL domains |