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**Supplementary Data**

**Construction and characterization of a synthesized herpes simplex virus H129-Syn-G2**

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**Supplementary Table S1** Primers used in this research.

|  |  |  |
| --- | --- | --- |
| Name | Sequence (5′–3′) a | Description |
| pGF-1F | gggcaaacacttggggactgtaggtttctgtggtgccgaccctaggcgctGTTTAAACTAGAGTCGACCTGCAGGCATGCAAGCTT | To amplify pGF vector for H1 |
| pGF-1R | cggccccagttgggtcgggcgacccagatgtttacttaaaaggcgtgccgGTTTAAACGAGGATCCCGGGTACCGAGCTCGAATTC | To amplify pGF vector for H1 |
| pGF-2F | gccgggtgaacgctgattctcacgacagcgcgtgccgcgcgcacgggttgGTTTAAACTAGAGTCGACCTGCAGGCATGCAAGCTT | To amplify pGF vector for H2 |
| pGF-2R | agtaacacaaccacgacgtcgtgccacacaagccccgtatccccgttcccGTTTAAACGAGGATCCCGGGTACCGAGCTCGAATTC | To amplify pGF vector for H2 |
| pGF-3F | accaccggggcctgaacaaacaaaccacagacacggttacagtttattcgGTTTAAACTAGAGTCGACCTGCAGGCATGCAAGCTT | To amplify pGF vector for H3 |
| pGF-3R | ggcgcgctcaaccaccgctccccccacgtcgtctcggaaatggagtccacGTTTAAACGAGGATCCCGGGTACCGAGCTCGAATTC | To amplify pGF vector for H3 |
| pGF-4F | ttccggtattgtctccttccgtgttTAGAGTCGACCTGCAGGCATGCAAGCTT | To amplify pGF vector for H4 |
| pGF-4R | actgacacacattccacagctggttGAGGATCCCGGGTACCGAGCTCGAATTC | To amplify pGF vector for H4 |
| pGF-5F | atagagcctttgatactctagcatgagccccccgtcgaagctgatgtcccGTTTAAACTAGAGTCGACCTGCAGGCATGCAAGCTT | To amplify pGF vector for H5 |
| pGF-5R | caagatggggggtgggatgagggggcgcgatggcacagcggaccggcgtcGTTTAAACGAGGATCCCGGGTACCGAGCTCGAATTC | To amplify pGF vector for H5 |
| pGF-6F | cccggcatcccgttcgcggacccaggcccgggggattgtccggatgtgcgGTTTAAACTAGAGTCGACCTGCAGGCATGCAAGCTT | To amplify pGF vector for H6 |
| pGF-6R | gcaacattcgacgagtttcctccgccgtagcgccggcacccaccgccccgGTTTAAACGAGGATCCCGGGTACCGAGCTCGAATTC | To amplify pGF vector for H6 |
| pGF-7F | gggcgctttttatcgggaggagcttatgggcgtggcgggcctcccagcccGTTTAAACTAGAGTCGACCTGCAGGCATGCAAGCTT | To amplify pGF vector for H7 |
| pGF-7R | acttaagtggccgtataacaccccgcgaagacgcggggtactcgcaacgcGTTTAAACGAGGATCCCGGGTACCGAGCTCGAATTC | To amplify pGF vector for H7 |
| pGF-8F | ttgggctggtacgcgtcgcccaaccgagggccgcgtccacgggacgcgccGTTTAAACTAGAGTCGACCTGCAGGCATGCAAGCTT | To amplify pGF vector for H8 |
| pGF-8R | cgatggccctggacgcggcataactccgaccggcgggtcccgaccgaacgGTTTAAACGAGGATCCCGGGTACCGAGCTCGAATTC | To amplify pGF vector for H8 |
| pGF-9F | gatagcagatgtgaggaagtctgggccgttcgccgcgaacggcgatcagaGTTTAAACTAGAGTCGACCTGCAGGCATGCAAGCTT | To amplify pGF vector for H9 |
| pGF-9R | gctggtgtgcatggccgtttcggcctgccaggtggcgttgaggcagtaagGTTTAAACGAGGATCCCGGGTACCGAGCTCGAATTC | To amplify pGF vector for H9 |
| pGF-10F | ggggttgggtctggctcatctcgagagccacgggggggaaccaccctccgGTTTAAACTAGAGTCGACCTGCAGGCATGCAAGCTT | To amplify pGF vector for H10 |
| pGF-10R | ggaccacagtggcttcccccccccgacgagcaggaagcggtccacgcaacGTTTAAACGAGGATCCCGGGTACCGAGCTCGAATTC | To amplify pGF vector for H10 |
| H4-1F | gacaacgaccgcagttctcgGTTTAAACgacgcctctccgggggggaagggaa | To amplify H4 |
| H4-1R | CTTGCATGCCTGCAGGTCGACTCTAaacacggaaggagacaataccggaaggaac | To amplify H4 |
| H4-2F | GAATTCGAGCTCGGTACCCGGGATCCTCtcagttagcctcccccatctcccgggcaaac | To amplify H4 |
| H4-2R | ttcccccccggagaggcgtcGTTTAAACcgagaactgcggtcgttgtcctaactaccagacc | To amplify H4 |
| H10-1F | ggaagccactgtggtcctccgggacgttttc | To amplify H10 |
| H10-1R | ggagacgttggtgggaacccccgatacacc | To amplify H10 |
| H10-2F | accggcctggacccgctactacaagat | To amplify H10 |
| H10-2R | ttcggcgatggccgtcagggagggcatt | To amplify H10 |
| H10-3F | atggctcctgcgacccgaaact | To amplify H10 |
| H10-3R | gatcggcacgtaagaggttccggcggggcgtaaggatccttacttg | To amplify H10 |
| H10-4F | caagtaaggatccttacgccccgccggaacctcttacgtgccgatc | To amplify H10 |
| H10-4R | tggctctcgagatgagccagacccaac | To amplify H10 |

a Among the sequence of the primers, uppercase letters present sequence located at pGF vector, lowercase letters present sequence located at H129-G4 genome, the underline sequence present *Pme*I recognition site.

**Supplementary Table S2** Primers used for PCR identification of the junctions.

|  |  |  |
| --- | --- | --- |
| **Name** | **Sequence** (5′–3′) | **Description** |
| **J1-F** | gtcttgccgcaagtgcgt | To identify the junction between H10 and H1 |
| **J1-R** | ttcccgcaggaggaacgc |
| **J2-F** | ggcgctggagataacggc | To identify the junction between H1 and H2 |
| **J2-R** | gcgcctggcgtatctct |
| **J3-F** | aacacagataatccaggcggg | To identify the junction between H2 and H3 |
| **J3-R** | cgaatttaccaaccaccgcc |
| **J4-F** | tcgtaggccggtacacaca | To identify the junction between H3 and H4 |
| **J4-R** | tccacccgggatatcgagt |
| **J5-F** | attgagcctaggacccagc | To identify the junction between H4 and H5 |
| **J5-R** | gcacgatctggacctccat |
| **J6-F** | gtacaccaacaagcgcctgg | To identify the junction between H5 and H6 |
| **J6-R** | attcgagcatcgcgcct |
| **J7-F** | tgacctccatctcccggaag | To identify the junction between H6 and H7 |
| **J7-R** | ggatcttgtccagggcctg |
| **J8-F** | cagcagcacgtgaaggct | To identify the junction between H7 and H8 |
| **J8-R** | ctttgccgacaccgtggt |
| **J9-F** | tggtcgtcgggtgccaaag | To identify the junction between H8 and H9 |
| **J9-R** | gttgtcgccgtcggtct |
| **J10-F** | tccggcgcacataaaggc | To identify the junction between H9 and H10 |
| **J10-R** | gcgccgtagcattgcatttg |

**Supplementary Table S3** Detailed information of H fragments.

|  |  |  |  |
| --- | --- | --- | --- |
| **Fragment** | **Nucleotide position a** | **Open reading frame** | **Overlapping size (bp) b** |
| **H1** | 1–15984 | US12–RL2 | 100 |
| **H2** | 15885–33619 | UL1–UL12 | 100 |
| **H3** | 33520–50470 | UL13–UL21 | 100 |
| **H4** | 50371–65530 | UL22–UL24; pGF vector | 100 |
| **H5** | 65431–83143 | UL25–UL30 | 100 |
| **H6** | 83044–100783 | UL31–UL37 | 100 |
| **H7** | 100684–117533 | UL38–UL46 | 100 |
| **H8** | 117434–133585 | UL47–UL56 | 1548 |
| **H9** | 132036–150124 | UL55–US1 | 1542 |
| **H10** | 148583–100 | US1–US11 | 100 |

a Nucleotide position showed the sites of the start and end nuclotides of each fragment referring to the genome sequence of H129-Syn-G2.

b Overlapping size refers to the overlapping sequence between the fragment and the following fragment in the table.



**Supplementary Fig S1.** The restriction enzyme analysis of 10 individual H fragments (**A**) and 3 intermediate fragments (**B**). Lane 1 represented computational prediction of the restriction enzyme profiles and lane 2 represented experimental results of the restriction enzyme profiles.