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**Supplementary Data**

**Integration of HiBiT into enteroviruses: A universal tool for advancing enterovirus virology research**

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**Supplementary data**

**Table S1** The primers for constructing the EV-A71 infectious clones.

|  |  |
| --- | --- |
| Name | Sequence |
| EV-A71-F1 | Forward: CACTATAGGGTTAAAACAGCCTGTGGGTTGCACC |
| Reverse: TGAGTGAGGGCTCTGCTCACGCTATCTCCTATAGAAC |
| EV-A71-F2 | Forward: TGAGCAGAGCCCTCACTCACGCTCTACCAG |
| Reverse: GCCCTGCATCCAGTCTACCTAGATCTGTTTTGTACG |
| EV-A71-F3 | Forward: AGGTAGACTGGATGCAGGGCGAGCCGCTAAAC |
| Reverse: CTAAAATAACTCGAGCCAATTGCGTCTAAG |
| PL451-Vector | Forward:GGCTCGAGTTATTTTAGAAAAAAAAAAAAAAAAAAAAAAAAAAGGTTATACACACCTCAACCCCACC |
| Reverse: GCTGTTTTAACCCTATAGTGAGTCGTATTAGCGGCCG |

**Table S2** The primers for constructing the EVAs-NLuc infectious clones.

|  |  |
| --- | --- |
| Name | Sequence |
| EV-A71-NLuc | Forward:TGGCGGCCATTACTACCCTTATGGGTTCGCAAGTGTCTACACAGCG |
| Reverse:GAACACCTGGGATCCCATGCGAACCCATGTTTAGCTGTGT |
| CVA10-NLuc | Forward:TGGCGGCCATTACTACCCTTGGAGCACAAGTTTCATCTCAGAGG |
| Reverse:GAACACCTGGGATCCCATCATCTTCTGATATTTAACACCAAGATG |
| CVA7-NLuc | Forward:TGGCGGCCATTACTACCCTTGGCGCTCAAATATCAACACAAAAATC |
| Reverse:GAACACCTGGGATCCCATCATTTTGCTTTGTCCAAACTCAGTATC |
| CVA16-NLuc | Forward:GCCATTACTACCCTTGGGTCACAAGTCTCCACCCA |
| Reverse:CACCTGGGATCCCATTTCTTACAGTTGAGGAGCAA |
| NLuc | Forward:AAATGGGATCCCAGGTGTTCACACTCGAAGATTTCGTTGG |
| Reverse: AAGGGTAGTAATGGCCGCCA |

**Table S3** The primers for constructing the four HiBiT-tagged infectious clones.

|  |  |
| --- | --- |
| Name | Sequence |
| EV-A71-HiBiT | Forward:GCTGTTCAAGAAGATTAGCGCCATTACTACCCTTGGTTCGCAAGTGTCTACACA |
| Reverse:ATCTTCTTGAACAGCCGCCAGCCGCTCACCTGGATCCCATGTTTAGCTGTGTTAAGGG |
| CVA10-HiBiT | Forward:CGGCTGTTCAAGAAGATTAGCGCCATTACTACCCTTGGAGCACAAGTTTCATCTCAGAG |
| Reverse:CTTCTTGAACAGCCGCCAGCCGCTCACCACCTGAGCACCCATCTTCTGATATTTAACACCAAGA |
| CVA7-HiBiT | Forward:GGCTGTTCAAGAAGATTAGCGCCATTACTACCCTTGGCGCTCAAATATCAACACAA |
| Reverse:TCTTCTTGAACAGCCGCCAGCCGCTCACCTGTGCTCCCATTTTGCTTTGTCCAAACT |
| CVA16-HiBiT | Forward:GCGGCTGTTCAAGAAGATTAGCGCCATTACTACCCTTGGGTCACAAGTCTCCACCC |
| Reverse:CTTCTTGAACAGCCGCCAGCCGCTCACCTGGGATCCCATTTCTTACAGTTGAGGAGC |

**Table S4** The primers for real-time quantification of viral genomes.

|  |  |
| --- | --- |
| Virus | Sequence |
| EV-A71/EV-A71-HiBiT | Forward: GCAGCCCAAAACAACTTCAC |
| Reverse: AATTTCAGCAGCTTGGAGTGC |
| CVA10/CVA10-HiBiT | Forward: AGTGTGGGACATTGACATCATGGG |
| Reverse: TACTGTAACATAAATGGCCTTGCCTCG |
| CVA7/CVA7-HiBiT | Forward: GGTGCTAACACTCAAGCTAGCC |
| Reverse: ACGTAAACTCGGCGGAGAATC |
| CVA16/CVA16-HiBiT | Forward: GCTAGTCCCCCAATTACTCCAG |
| Reverse: GTCATTTGCTTGGAGGTGCTCAC |
| CVA16-NLuc  (Competitionexperiment) | Forward: GCTGGCGGCTGTGCGAACG |
| Reverse: CTTGGAGACTTTAGCGGTGGGGCC |
| CVA16-HiBiT  (Competition experiment) | Forward: GAGCGGCTGGCGGCTGTTC |
| Reverse: CTTGGAGACTTTAGCGGTGGGGCC |



**Figure S1**. Amino acid sequence alignment of EV-A71, CVA10, CVA16 and CVA7 near VP1 145 (Suramin binding hotspot).