To define a structurally variable regions between *in vivo* and *in vitro*, we used a sliding-window based method to search significantly difference regions. The algorithm summarized as below.

|  |
| --- |
| **Input**  Virus genome length  *in vivo* icSHAPE score rep1:  *in vivo* icSHAPE score rep2:  *in vitro* icSHAPE score rep1:  *in vitro* icSHAPE score rep2:  *in vivo* icSHAPE score (combined):  *in vitro* icSHAPE score (combined):  Window size:  Window step:  **Output**  Significant difference windows  **Process**  **Step 1: Estimate the background noise**  Define as an empty list          Estimate the background noise as  **Step 2: Sliding window search significant difference windows**  Define  as an empty list  Calculate the score difference      add  to      **Step 3: Define top difference windows**  Define  as an empty list    add to    Sort by with decreasing order.  Define  Sort  by window start position  **Step 4: Combine overlapped windows**  Combine overlapped windows in |

**Algorithm 1** The algorithm we used to search structural significantly difference region between *in vivo* and *in vitro*. is the indicator function. is defined as the number of elements in .

We also compared the results to our previous method (Sun et al., 2019). The algorithm is described as below:

|  |
| --- |
| **Input**  Virus genome length  *in vivo* icSHAPE score rep1:  *in vivo* icSHAPE score rep2:  *in vitro* icSHAPE score rep1:  *in vitro* icSHAPE score rep2:  *in vivo* icSHAPE score (combined):  *in vitro* icSHAPE score (combined):  Window size:  Window step:  **Output**  Significant difference windows  **Process**  **Step 1: Calculate the** **significantly difference bases**  Define  and as empty lists            **Step 2: Define significantly difference windows**  Define as empty list      add to      **Step 3: Combine overlapped windows**  Combine overlapped windows in |

**Algorithm 2** The algorithm used to define structurally difference regions in (Sun et al., 2019).