**Supplementary File 1**

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| **Oligomer** | **Sequence (5’ to 3’)** | **Relevant figures** |
| Proto-spacer | GAGTTACTACTCGTTCTGGCTCTGTCgagccagaacgagtagtaactctgtc | Figures 1, 2, 3, 4, S1, S3, S4, S5, S7, S8 |
| Cy5-PS | Cy5-GAGTTACTACTCGTTCTGGCTCTGTCCy5-gagccagaacgagtagtaactctgtc | Figure S1 |
| Pre-spacer mimics | GAGTTACTACTCGTTCTGGCTCTGTCGGGcccTTTTgagccagaacgagtagtaactctgtcgagccagaacgagtagtaactctgtc | Figure S7 |
| Target site | CGATCGATTAGTCTACGAGGTTTTAGAGCTATGCTGTTTTGAATGGTCCCAAAACTGCGCTGGTTGATTTACATGTCTCTCTagagagacatgtaaatcaaccagcgcagttttgggaccattcaaaacagcatagctctaaaacctcgtagactaatcgatcg | Figures 2, 3, 4, S4, S5, S7 and S8 |
| Target site (nicked) | CGATCGATTAGTCTACGAGGTTTTAGAGCTATGCTGTTTTGAATGGTCCCAAAACTGCGCTGGTTGATTTACATGTCTCTCTAGAGAGACATGTAAATCAACCAGCGCAGTTTTGGGACCATTCAAAACAGCATAGCTCTAAAACCTCGTAGACTAATCGATCG | Figures S4 |
| Proto-spacers with (T)n insertions | GAGTTACTACTtttttCGTTCTGGCTCTGTCgagccagaacgtttttagtagtaactctgtcGAGTTACTACTttttttttttCGTTCTGGCTCTGTCGAGTTACTACTttttttttttCGTTCTGGCTCTGTC | Figures 3 and S5 |
| Target sites with (T)n insertions | TAGTCTACGAGGTTTTAGAGCtttttGTCCCAAAACTGCGCTGGTTGATTTACATGTCTCTCTagagagacatgtaaatcaaccagcgcagttttgggactttttgctctaaaacctcgtagactaTAGTCTACGAGGTTTTAGAGCttttttttttGTCCCAAAACTGCGCTGGTTGATTTACATGTCTCTCTagagagacatgtaaatcaaccagcgcagttttgggacttttttttttgctctaaaacctcgtagactaTAGTCTACGAGGTTTTAGAGCtttttttttttttttGTCCCAAAACTGCGCTGGTTGATTTACATGTCTCTCTagagagacatgtaaatcaaccagcgcagttttgggactttttttttttttttgctctaaaacctcgtagacta | Figures 3 and S5 |
| Half-target site (L-R). | CGATCGATTAGTCTACGAGGTTTTAGAGCTATGCTGTacagcatagctctaaaacctcgtagactaatcgatcg | Figure 4 |
| Half-target site (R-S) | TTTGAATGGTCCCAAAACTGCGCTGGTTGATTTACATGTCTCTCTagagagacatgtaaatcaaccagcgcagttttgggaccattcaaa | Figure 4 |
| Half-target site (L-R, +4) | CGATCGATTAGTCTACGAGGTTTTAGAGCTATGCTGTtttgcaaaacagcatagctctaaaacctcgtagactaatcgatcg  | Figure 4 |
| Half-target site (R-S, +4) | ctgtTTTGAATGGTCCCAAAACTGCGCTGGTTGATTTACATGTCTCTCTagagagacatgtaaatcaaccagcgcagttttgggaccattcaaaacag | Figure 4 |
| Half-target site (R-S, +6) | tgctgtTTTGAATGGTCCCAAAACTGCGCTGGTTGATTTACATGTCTCTCTagagagacatgtaaatcaaccagcgcagttttgggaccattcaaaacagca | Figure 4 |
| Semi-integration mimics | GAGTTACTACTCGTTCTGGCTCTGTCGTTTTAGAGCTATGCTGTTTTGAATGGTCCCAAAACTGCGCTGGTTGATTTACATGTCTCTCTgagccagaacgagtagtaactctgtcatcgatcgat CGATCGATTAGTCTACGAGagagagacatgtaaatcaaccagcgcagttttgggaccattcaaaacagcatagctctaaaacctcgtagactaatcgatcgatcgatcgatCGATCGATTAGTCTACGAC/3ddCGAGTTACTACTCGTTCTGGCTCTGTCGTTTTAGAGCTATGCTGTTTTGAATGGTCCCAAAACTGCGCTGGTTaaccagcgcagttttgggaccattcaaaacagcatagctctaaaacctcgtagactaatcgatcgGAGTTACTACTCGTTCTGGCTCTGTCGTTTTAGAGCTATGCTGTTTTGAATGGTCCCAAAACTGCGCTGGTTtttttctctcttttttGagccagaacgagtagtaactctgtc | Figures 5, 6 and S8Figures 5 and 6Figure 6 |
| Semi-integration mimics  | GAGTTACTACTCGTTCTGGCTCTGTCgttttgggaccattcaaaacagcatagctctaaaacctcgtagactaatcgatcggagccagaacgagtagtaactctgtcatcgatcgatagagagacatgtaaatcaaccagcgcaCGATCGATTAGTCTACGAGGTTTTAGAGCTATGCTGTTTTGAATGGTCCCAAAACTGCGCTGGTTGATTTACATGTCTCTCT | Figure S8 |

**Supplementary File 1. Synthetic oligonucleotides**. The sequences of the oligonucleotides used for this study are listed. The lower case ‘t’s refer to thymine insertions that remain bulged (with no paired or unpaired bases on the opposite strand) or double-looped (unpaired thymine bases on the opposite strand) in the assembled substrates. The ‘dd’ abbreviation refers to ‘dideoxy’. The assays in which the oligos were used are indicated by the corresponding figure numbers.