

**Supplementary Table S1. Oligonucleotides used in this study**

| Name        | Sequence  | Use  |
|-------------|---|--|
| 1K-LGC-F    | AAATAATACGACTCACTATAGCGACCCATCGTCTTTCTG         | template for 1KL RNA                         |
| 1K-LGC-R    | AGTGCCTAGCAAACCTCGGAAG                          | template for 1KL RNA                         |
| 6K-LGC-F    | AAATAATACGACTCACTATAGCGGAAGAAACAAAAAAGC         | template for 6KL RNA                         |
| 6K-LGC-R    | TTAGGCAGAGACAGGCGAAT                            | template for 6KL RNA                         |
| 1K-HGC-F    | AAATAATACGACTCACTATAGCGTTACAGCATGGATGTGG        | template for 1KH RNA                         |
| 1K-HGC-R    | TTACCACCTTAACCGCCTTTG                           | template for 1KH RNA                         |
| 6K-HGC-R    | CGCAATATGCTCACTGGCTA                            | template for 6KH RNA                         |
| 6K-HGC-F    | AAATAATACGACTCACTATAGGCATACAGCAACAACATGG        | template for 6KH RNA                         |
| 6KM13-f2    | AAATAATACGACTCACTATAGCGTTGATGCCACCTTTTCAGC      | template for M13 RNA                         |
| 6KM13-r     | GAGGCGGTTTGCCTATTG                              | template for M13 RNA                         |
| 85-f        | AAATAATACGACTCACTATAACGAAGGAAGAAACCTCG          | template for 1K-1 and 416 RNA                |
| 1109-r      | TGGGTCGCTAATACGCTA                              | template for 1K-1 RNA                        |
| 1241-f2     | AAATAATACGACTCACTATAGGCAATGTAAGATGAAATAAGAGTAGC | template for 1K-2 RNA                        |
| 2389-r      | GCTAACAGGTATCGTTTGG                             | template for 1K-2 RNA                        |
| 2502-f      | AAATAATACGACTCACTATA GCATATACCCATTGCTCAC        | template for 1K-3 RNA                        |
| 3599-r      | CACAGATTCAAGTGGACG                              | template for 1K-3 RNA                        |
| 3749-f2     | AAATAATACGACTCACTATAGGGAATTCTAAGCGGAGATCG       | template for 1K-4 RNA                        |
| 4600-r      | TATTGAAAGAGGCGGGTA                              | template for 1K-4 RNA                        |
| 5148-f2     | AAATAATACGACTCACTATAGCGTTGTGCTTCTCTGGAGTG       | template for 1K-5 RNA                        |
| 6256-r      | GGAATCAAGCAGAAGACAC                             | template for 1K-5 RNA                        |
| 500-r       | GATTCAAATAATAATCAAAAATG                         | template for 130 and 416 RNA                 |
| 371-f       | AAATAATACGACTCACTATA AGACTGAACGCATGAAAT         | template for 130 RNA                         |
| λDNA-up-f   | AAATAATACGACTCACTATAGACAGGTGCTGAAAGCGA          | template for updown RNA                      |
| λDNA-up-r   | TATCTCGAGAAATCTAGAGTATTGTTCCGGTTGCTGATG         | template for updown RNA                      |
| λDNA-down-f | AAATCTAGACACAGTAGCCTGGATTTGTTCTAT               | template for updown RNA                      |
| λDNA-down-r | TATCTCGAGGAATACGGGGCAACCTCA                     | template for updown RNA                      |
| RNA-40      | AAGUCGGUUUUUUUCUUCGUUUUCUCUAACUAUUUUCCA         | Shortest RNA for length-dependent experiment |

**Supplementary Table S2.** Nucleic acid triple helices target sites (polypurine/polypyrimidine tract)

predicted by Triplexator <sup>1</sup>. The threshold score is 10.

| #ID         | Start | End   | Length | Strand | Guanine-rate | Duplicates | TTS                    |
|-------------|-------|-------|--------|--------|--------------|------------|------------------------|
| Length≥20bp |       |       |        |        |              |            |                        |
| λDNA        | 40465 | 40487 | 21     | +      | 0.45         | -1         | AGGGAAGAACGGGAAGGAAAGA |
| λDNA        | 22793 | 22814 | 20     | -      | 0.19         | -1         | AGAGAAAACGAAGAAAAAAA   |
| Length≥10bp |       |       |        |        |              |            |                        |
| λDNA        | 104   | 118   | 14     | +      | 0.29         | -1         | GAAAAGAAAGGAAA         |
| λDNA        | 2426  | 2436  | 10     | +      | 0.2          | -1         | AGGAAAAAAA             |
| λDNA        | 2559  | 2569  | 10     | +      | 0.5          | -1         | AGGAAGAGGA             |
| λDNA        | 3132  | 3145  | 13     | +      | 0.62         | -1         | GGGGAGGAAGAAG          |
| λDNA        | 4403  | 4418  | 15     | +      | 0.53         | -1         | AGAGGAGGAGAAGAG        |
| λDNA        | 7278  | 7288  | 10     | +      | 0.3          | -1         | GAAAGAAGAA             |
| λDNA        | 7311  | 7321  | 10     | +      | 0.5          | -1         | GAAAGAGGAG             |
| λDNA        | 7963  | 7973  | 10     | +      | 0.6          | -1         | GAAAGGGGA              |
| λDNA        | 8140  | 8151  | 11     | +      | 0.45         | -1         | AAAGGAAAGGG            |
| λDNA        | 12459 | 12469 | 10     | +      | 0.6          | -1         | GGGAAGAGAG             |
| λDNA        | 14215 | 14228 | 13     | +      | 0.62         | -1         | GGAGGGGGAAAGA          |
| λDNA        | 17282 | 17293 | 11     | +      | 0.45         | -1         | GGAAAAAGAGG            |
| λDNA        | 20725 | 20736 | 11     | +      | 0.55         | -1         | GAGAAAGGGGA            |
| λDNA        | 22277 | 22287 | 10     | +      | 0.7          | -1         | GGGAGGGGAA             |
| λDNA        | 22442 | 22453 | 11     | +      | 0.36         | -1         | GAAGGAAGAAA            |
| λDNA        | 25024 | 25034 | 10     | +      | 0.3          | -1         | AGGAAGAAAA             |
| λDNA        | 26304 | 26314 | 10     | +      | 0.2          | -1         | AGAGAAAAAAA            |
| λDNA        | 26719 | 26730 | 11     | +      | 0.18         | -1         | AAGGAAAAAAA            |
| λDNA        | 27397 | 27410 | 13     | +      | 0.31         | -1         | GAAAGAAAAGAAG          |
| λDNA        | 27982 | 27992 | 10     | +      | 0.4          | -1         | GAAGGAAAGA             |
| λDNA        | 30668 | 30678 | 10     | +      | 0.3          | -1         | AAAAAAGGAG             |
| λDNA        | 33175 | 33186 | 11     | +      | 0.64         | -1         | AGGAAAGGGGG            |
| λDNA        | 36152 | 36162 | 10     | +      | 0.2          | -1         | GAAAAAGAAA             |
| λDNA        | 36180 | 36191 | 11     | +      | 0.45         | -1         | GGAAAAGGAGA            |
| λDNA        | 36946 | 36957 | 11     | +      | 0.27         | -1         | AAAGAGAAAAG            |
| λDNA        | 38486 | 38496 | 10     | +      | 0.6          | -1         | GGAAGAGGGA             |
| λDNA        | 39135 | 39155 | 19     | +      | 0.15         | -1         | ACAAAAGAAAAAGAAAAGA    |
| λDNA        | 40465 | 40487 | 21     | +      | 0.45         | -1         | AGGGAAGAACGGGAAGGAAAGA |
| λDNA        | 40627 | 40641 | 14     | +      | 0.71         | -1         | GGGGGAGAGGGAAG         |
| λDNA        | 41566 | 41577 | 11     | +      | 0.45         | -1         | GAGAAGGAAGA            |
| λDNA        | 41935 | 41945 | 10     | +      | 0.7          | -1         | GGGAGGGAAG             |
| λDNA        | 42073 | 42086 | 13     | +      | 0.46         | -1         | AGGAAAAGGAGGA          |
| λDNA        | 42418 | 42429 | 11     | +      | 0.45         | -1         | GAGGAAGAAGA            |
| λDNA        | 42586 | 42596 | 10     | +      | 0.3          | -1         | AGAGAAGAAA             |
| λDNA        | 42707 | 42718 | 11     | +      | 0.36         | -1         | AGAGAAAGAGA            |
| λDNA        | 43872 | 43882 | 10     | +      | 0.6          | -1         | AGGGAGAAGG             |
| λDNA        | 48087 | 48098 | 11     | +      | 0.55         | -1         | GGAGGGAAGAA            |
| λDNA        | 156   | 168   | 12     | -      | 0.33         | -1         | AGAGAAAGGAAA           |
| λDNA        | 3084  | 3094  | 10     | -      | 0.3          | -1         | GGAAAAAAGA             |
| λDNA        | 6208  | 6219  | 11     | -      | 0.36         | -1         | GGAAAAAGAGA            |
| λDNA        | 11173 | 11183 | 10     | -      | 0.4          | -1         | AGAGGAGAAA             |
| λDNA        | 14096 | 14106 | 10     | -      | 0.5          | -1         | GGAAAGGAAG             |
| λDNA        | 18340 | 18350 | 10     | -      | 0.5          | -1         | AGGGAAAAGG             |
| λDNA        | 22793 | 22814 | 20     | -      | 0.19         | -1         | AGAGAAAACGAAGAAAAAAA   |
| λDNA        | 23891 | 23902 | 11     | -      | 0.45         | -1         | GGGAAAGAAGA            |
| λDNA        | 24944 | 24956 | 12     | -      | 0.58         | -1         | GGGAGAGAAAGG           |
| λDNA        | 26780 | 26792 | 12     | -      | 0.25         | -1         | AAAAAGAAGAAG           |

|             |       |       |    |   |      |    |                       |
|-------------|-------|-------|----|---|------|----|-----------------------|
| λDNA        | 27029 | 27039 | 10 | - | 0.3  | -1 | AAAAGAGAGA            |
| λDNA        | 28870 | 28880 | 10 | - | 0.6  | -1 | GGGAAGAAGG            |
| λDNA        | 30255 | 30265 | 10 | - | 0.4  | -1 | GGGAAAGAAA            |
| λDNA        | 33027 | 33040 | 13 | - | 0.46 | -1 | GAAGAGAAAGAGG         |
| λDNA        | 33814 | 33824 | 10 | - | 0.3  | -1 | AGAGGAAAAA            |
| λDNA        | 34345 | 34355 | 10 | - | 0.4  | -1 | GGAAGAAGAA            |
| λDNA        | 36744 | 36754 | 10 | - | 0.5  | -1 | GGGAAAGGAA            |
| λDNA        | 37064 | 37075 | 11 | - | 0.36 | -1 | AAAGGGAAAGA           |
| λDNA        | 37857 | 37871 | 14 | - | 0.14 | -1 | GAAAAAAAGAAAA         |
| λDNA        | 37922 | 37932 | 10 | - | 0.1  | -1 | AAAAAAGAAA            |
| λDNA        | 43965 | 43975 | 10 | - | 0.5  | -1 | GGAAAGAGAG            |
| λDNA        | 45696 | 45706 | 10 | - | 0.5  | -1 | GGGAAAGAAG            |
| λDNA        | 48140 | 48151 | 11 | - | 0.36 | -1 | AGAAAAAGAGAG          |
| Length≥10nt |       |       |    |   |      |    |                       |
| RNA-6L      | 86    | 97    | 11 | + | 0.36 | -1 | GUUGGUUGUUU           |
| RNA-6L      | 2668  | 2678  | 10 | + | 0.3  | -1 | UGGUUGUUUU            |
| RNA-6L      | 3948  | 3958  | 10 | + | 0.2  | -1 | UGUGUUUUUU            |
| RNA-6L      | 4363  | 4374  | 11 | + | 0.18 | -1 | UUGGUUUUUUU           |
| RNA-6L      | 5041  | 5054  | 13 | + | 0.31 | -1 | GUUUGUUUUGUUG         |
| RNA-6L      | 5626  | 5636  | 10 | + | 0.4  | -1 | GUUGGUUUGU            |
| RNA-6L      | 437   | 458   | 20 | - | 0.19 | -1 | UGUGUUUUCGUUGUUUUUUUU |
| RNA-6L      | 1535  | 1546  | 11 | - | 0.45 | -1 | GGGUUUGUUGU           |
| RNA-6L      | 2588  | 2600  | 12 | - | 0.58 | -1 | GGGUGUGUUUGG          |
| RNA-6L      | 4424  | 4436  | 12 | - | 0.25 | -1 | UUUUUGUUGUUG          |
| RNA-6L      | 4673  | 4683  | 10 | - | 0.3  | -1 | UUUUGUGUGU            |
| Length≥10nt |       |       |    |   |      |    |                       |
| RNA-6H      | 981   | 992   | 11 | + | 0.45 | -1 | GGUUUUUGUGG           |
| RNA-6H      | 4424  | 4435  | 11 | + | 0.55 | -1 | GUGUUUGGGGU           |
| RNA-6H      | 5976  | 5986  | 10 | + | 0.7  | -1 | GGGUGGGGUU            |
| RNA-6H      | 2039  | 2049  | 10 | - | 0.5  | -1 | UGGGUUUUGG            |

- 1 Buske, F. A., Bauer, D. C., Mattick, J. S. & Bailey, T. L. Triplexator: detecting nucleic acid triple helices in genomic and transcriptomic data. *Genome Res* **22**, 1372-1381, doi:10.1101/gr.130237.111 (2012).