

## Supplementary material:

**Table 1:** Algorithms or rules for rational design of siRNA molecules

| Ui-Tei Rules  | Amarzguioui Rules  | Reynolds Rules   |
|---|--|--|
| A/U at the 5' terminus of the sense strand                      | Duplex End A/U differential > 0.<br>Strong binding of 5'sense strand | Each rule is assigned a score which is summed up to a total duplex score to improve the efficacy of siRNA. |
| G/C at the 5' terminus of the antisense strand                  | No U at position 1. Presence of A at position 6.                     |  |
| At least 4 A/U residues in the 5' terminal 7 bp of sense strand | Weak binding of 3'sense strand. No G at position 19                  |  |
| No GC stretch longer than 9nt                                   |  |  |

**Table 2:** Predicted siRNA target for T antigen gene of MCV

| Accession No | Target    | Location of target within gene | siRNA target sequence within gene |
|--------------|-----------|--------------------------------|-----------------------------------|
| FJ173805.1   | Target-1  | 253-275                        | TGGGAAGAATATGGAACCTTAAA           |
|              | Target-2  | 2198-2220                      | TTGGTTTAAAGGGCCTATTAACA           |
|              | Target-3  | 2648-2670                      | AACCACTTTATTGCTTTGTCTTA           |
| JN383833.1   | Target-4  | 253-275                        | TGGGAAGAATATGGAACCTTAAA           |
|              | Target-5  | 1564-1586                      | AGCATAGAGTATCTGCTATTAAG           |
|              | Target-6  | 2198-2220                      | TTGGTTTAAAGGGCCTATTAACA           |
| FJ173813.1   | Target-7  | 2648-2670                      | AACCACTTTATTGCTTTGTCTTA           |
|              | Target-8  | 253-275                        | TGGGAAGAATATGGAACCTTAAA           |
|              | Target-9  | 1564-1586                      | AGCATAGAGTATCTGCTATTAAG           |
| FJ173804.1   | Target-10 | 2198-2220                      | TTGGTTTAAAGGGCCTATTAACA           |
|              | Target-11 | 2648-2670                      | AACCACTTTATTGCTTTGTCTTA           |
|              | Target-12 | 253-275                        | TGGGAAGAATATGGAACCTTAAA           |
| FJ173803.1   | Target-13 | 1564-1586                      | AGCATAGAGTATCTGCTATTAAG           |
|              | Target-14 | 2198-2220                      | TTGGTTTAAAGGGCCTATTAACA           |
|              | Target-15 | 2648-2670                      | AACCACTTTATTGCTTTGTCTTA           |
| JN383837.1   | Target-16 | 253-275                        | TGGGAAGAATATGGAACCTTAAA           |
|              | Target-17 | 1564-1586                      | AGCATAGAGTATCTGCTATTAAG           |
|              | Target-18 | 2198-2220                      | TTGGTTTAAAGGGCCTATTAACA           |
| JN383823.1   | Target-19 | 2648-2670                      | AACCACTTTATTGCTTTGTCTTA           |
|              | Target-20 | 253-275                        | TGGGAAGAATATGGAACCTTAAA           |
|              | Target-21 | 1564-1586                      | AGCATAGAGTATCTGCTATTAAG           |
| FM955590.1   | Target-22 | 2198-2220                      | TTGGTTTAAAGGGCCTATTAACA           |
|              | Target-23 | 2648-2670                      | AACCACTTTATTGCTTTGTCTTA           |
|              | Target-24 | 253-275                        | TGGGAAGAATATGGAACCTTAAA           |
| FM955587.1   | Target-25 | 1564-1586                      | AGCATAGAGTATCTGCTATTAAG           |
|              | Target-26 | 2198-2220                      | TTGGTTTAAAGGGCCTATTAACA           |
|              | Target-27 | 2648-2670                      | AACCACTTTATTGCTTTGTCTTA           |
| JN383825.1   | Target-28 | 253-275                        | TGGGAAGAATATGGAACCTTAAA           |
|              | Target-29 | 1564-1586                      | AGCATAGAGTATCTGCTATTAAG           |
|              | Target-30 | 2198-2220                      | TTGGTTTAAAGGGCCTATTAACA           |
| FJ173814.1   | Target-31 | 2648-2670                      | AACCACTTTATTGCTTTGTCTTA           |
|              | Target-32 | 253-275                        | TGGGAAGAATATGGAACCTTAAA           |
|              | Target-33 | 1564-1586                      | AGCATAGAGTATCTGCTATTAAG           |
| FM955588.1   | Target-34 | 2198-2220                      | TTGGTTTAAAGGGCCTATTAACA           |
|              | Target-35 | 2648-2670                      | AACCACTTTATTGCTTTGTCTTA           |
|              | Target-36 | 253-275                        | TGGGAAGAATATGGAACCTTAAA           |
| FM955588.1   | Target-37 | 1564-1586                      | AGCATAGAGTATCTGCTATTAAG           |
|              | Target-38 | 2198-2220                      | TTGGTTTAAAGGGCCTATTAACA           |
|              | Target-39 | 2648-2670                      | AACCACTTTATTGCTTTGTCTTA           |
| FM955588.1   | Target-40 | 253-275                        | TGGGAAGAATATGGAACCTTAAA           |
|              | Target-41 | 1564-1586                      | AGCATAGAGTATCTGCTATTAAG           |
|              | Target-42 | 2198-2220                      | TTGGTTTAAAGGGCCTATTAACA           |
| FM955588.1   | Target-43 | 2648-2670                      | AACCACTTTATTGCTTTGTCTTA           |
|              | Target-44 | 253-275                        | TGGGAAGAATATGGAACCTTAAA           |
|              | Target-45 | 1564-1586                      | AGCATAGAGTATCTGCTATTAAG           |

|            |            |           |                         |
|------------|------------|-----------|-------------------------|
|            | Target-46  | 2198-2220 | TTGGTTTAAAGGGCCTATTAACA |
|            | Target-47  | 2648-2670 | AACCACTTTATTGCTTTGTCTTA |
| FJ173812.1 | Target-48  | 253-275   | TGGGAAGAATATGGAACCTTAAA |
|            | Target-49  | 1564-1586 | AGCATAGAGTATCTGCTATTAAG |
|            | Target-50  | 2198-2220 | TTGGTTTAAAGGGCCTATTAACA |
|            | Target-51  | 2648-2670 | AACCACTTTATTGCTTTGTCTTA |
| JN383834.1 | Target-52  | 253-275   | TGGGAAGAATATGGAACCTTAAA |
|            | Target-53  | 1564-1586 | AGCATAGAGTATCTGCTATTAAG |
|            | Target-54  | 2198-2220 | TTGGTTTAAAGGGCCTATTAACA |
|            | Target-55  | 2648-2670 | AACCACTTTATTGCTTTGTCTTA |
| FM955589.1 | Target-56  | 253-275   | TGGGAAGAATATGGAACCTTAAA |
|            | Target-57  | 1564-1586 | AGCATAGAGTATCTGCTATTAAG |
|            | Target-58  | 2198-2220 | TTGGTTTAAAGGGCCTATTAACA |
|            | Target-59  | 2648-2670 | AACCACTTTATTGCTTTGTCTTA |
| JN383832.1 | Target-60  | 253-275   | TGGGAAGAATATGGAACCTTAAA |
|            | Target-61  | 1564-1586 | AGCATAGAGTATCTGCTATTAAG |
|            | Target-62  | 2198-2220 | TTGGTTTAAAGGGCCTATTAACA |
|            | Target-63  | 2648-2670 | AACCACTTTATTGCTTTGTCTTA |
| JN383831.1 | Target-64  | 253-275   | TGGGAAGAATATGGAACCTTAAA |
|            | Target-65  | 1564-1586 | AGCATAGAGTATCTGCTATTAAG |
|            | Target-66  | 2198-2220 | TTGGTTTAAAGGGCCTATTAACA |
|            | Target-67  | 2648-2670 | AACCACTTTATTGCTTTGTCTTA |
| FM955593.1 | Target-68  | 253-275   | TGGGAAGAATATGGAACCTTAAA |
|            | Target-69  | 1564-1586 | AGCATAGAGTATCTGCTATTAAG |
|            | Target-70  | 2198-2220 | TTGGTTTAAAGGGCCTATTAACA |
|            | Target-71  | 2648-2670 | AACCACTTTATTGCTTTGTCTTA |
| FM955592.1 | Target-72  | 253-275   | TGGGAAGAATATGGAACCTTAAA |
|            | Target-73  | 1564-1586 | AGCATAGAGTATCTGCTATTAAG |
|            | Target-74  | 2198-2220 | TTGGTTTAAAGGGCCTATTAACA |
|            | Target-75  | 2648-2670 | AACCACTTTATTGCTTTGTCTTA |
| FM955586.1 | Target-76  | 253-275   | TGGGAAGAATATGGAACCTTAAA |
|            | Target-77  | 1564-1586 | AGCATAGAGTATCTGCTATTAAG |
|            | Target-78  | 2198-2220 | TTGGTTTAAAGGGCCTATTAACA |
|            | Target-79  | 2648-2670 | AACCACTTTATTGCTTTGTCTTA |
| JN383836.1 | Target-80  | 253-275   | TGGGAAGAATATGGAACCTTAAA |
|            | Target-81  | 1558-1580 | AGCATAGAGTATCTGCTATTAAG |
|            | Target-82  | 2192-2214 | TTGGTTTAAAGGGCCTATTAACA |
|            | Target-83  | 2642-2664 | AACCACTTTATTGCTTTGTCTTA |
| JN383826.1 | Target-84  | 253-275   | TGGGAAGAATATGGAACCTTAAA |
|            | Target-85  | 1559-1581 | AGCATAGAGTATCTGCTATTAAG |
|            | Target-86  | 2193-2215 | TTGGTTTAAAGGGCCTATTAACA |
|            | Target-87  | 2643-2665 | AACCACTTTATTGCTTTGTCTTA |
| JN383824.1 | Target-88  | 252-274   | TGGGAAGAATATGGAACCTTAAA |
|            | Target-89  | 1556-1578 | AGCATAGAGTATCTGCTATTAAG |
|            | Target-90  | 2190-2212 | TTGGTTTAAAGGGCCTATTAACA |
|            | Target-91  | 2640-2662 | AACCACTTTATTGCTTTGTCTTA |
| FM955591.1 | Target-92  | 253-275   | TGGGAAGAATATGGAACCTTAAA |
|            | Target-93  | 1564-1586 | AGCATAGAGTATCTGCTATTAAG |
|            | Target-94  | 2198-2220 | TTGGTTTAAAGGGCCTATTAACA |
|            | Target-95  | 2648-2670 | AACCACTTTATTGCTTTGTCTTA |
| FJ173807.1 | Target-96  | 253-275   | TGGGAAGAATATGGAACCTTAAA |
|            | Target-97  | 1564-1586 | AGCATAGAGTATCTGCTATTAAG |
|            | Target-98  | 2198-2220 | TTGGTTTAAAGGGCCTATTAACA |
|            | Target-99  | 2648-2670 | AACCACTTTATTGCTTTGTCTTA |
| JN383829.1 | Target-100 | 253-275   | TGGGAAGAATATGGAACCTTAAA |
|            | Target-101 | 1564-1586 | AGCATAGAGTATCTGCTATTAAG |
|            | Target-102 | 2198-2220 | TTGGTTTAAAGGGCCTATTAACA |
|            | Target-103 | 2648-2670 | AACCACTTTATTGCTTTGTCTTA |
| JN383828.1 | Target-104 | 253-275   | TGGGAAGAATATGGAACCTTAAA |
|            | Target-105 | 1564-1586 | AGCATAGAGTATCTGCTATTAAG |
|            | Target-106 | 2198-2220 | TTGGTTTAAAGGGCCTATTAACA |
|            | Target-107 | 2648-2670 | AACCACTTTATTGCTTTGTCTTA |

|            |            |           |                          |
|------------|------------|-----------|--------------------------|
| JN383828.1 | Target-108 | 253-275   | TGGGAAGAATATGGAACCTTTAAA |
|            | Target-109 | 1564-1586 | AGCATAGAGTATCTGCTATTAAG  |
|            | Target-110 | 2198-2220 | TTGGTTTAAAGGGCCTATTAACA  |
|            | Target-111 | 2648-2670 | AACCACTTTATTGCTTTGTCTTA  |
| FJ173811.1 | Target-112 | 253-275   | TGGGAAGAATATGGAACCTTTAAA |
|            | Target-113 | 1564-1586 | AGCATAGAGTATCTGCTATTAAG  |
|            | Target-114 | 2198-2220 | TTGGTTTAAAGGGCCTATTAACA  |
|            | Target-115 | 2648-2670 | AACCACTTTATTGCTTTGTCTTA  |
| JN383827.1 | Target-116 | 253-275   | TGGGAAGAATATGGAACCTTTAAA |
|            | Target-117 | 1564-1586 | AGCATAGAGTATCTGCTATTAAG  |
|            | Target-118 | 2199-2221 | TTGGTTTAAAGGGCCTATTAACA  |
|            | Target-119 | 2649-2671 | AACCACTTTATTGCTTTGTCTTA  |
| FJ173810.1 | Target-120 | 253-275   | TGGGAAGAATATGGAACCTTTAAA |
|            | Target-121 | 1564-1586 | AGCATAGAGTATCTGCTATTAAG  |
| FJ173809.1 | Target-122 | 253-275   | TGGGAAGAATATGGAACCTTTAAA |
|            | Target-123 | 1564-1586 | AGCATAGAGTATCTGCTATTAAG  |
| FJ173806.1 | Target-124 | 253-275   | TGGGAAGAATATGGAACCTTTAAA |
|            | Target-125 | 1564-1586 | AGCATAGAGTATCTGCTATTAAG  |
|            | Target-126 | 2007-2029 | TTGGTTTAAAGGGCCTATTAACA  |
|            | Target-127 | 2457-2479 | AACCACTTTATTGCTTTGTCTTA  |
| JN383830.1 | Target-128 | 253-275   | TGGGAAGAATATGGAACCTTTAAA |
|            | Target-129 | 1491-1513 | AGCATAGAGTATCTGCTATTAAG  |
|            | Target-130 | 2575-2597 | AACCACTTTATTGCTTTGTCTTA  |
| JN383835.1 | Target-131 | 253-275   | TGGGAAGAATATGGAACCTTTAAA |
|            | Target-132 | 1497-1519 | AGCATAGAGTATCTGCTATTAAG  |
|            | Target-133 | 2131-2153 | TTGGTTTAAAGGGCCTATTAACA  |
|            | Target-134 | 2581-2603 | AACCACTTTATTGCTTTGTCTTA  |
| FJ173808.1 | Target-135 | 253-275   | TGGGAAGAATATGGAACCTTTAAA |

**Table 3:** Four effective siRNA molecule with GC%, free energy of folding and free energy of binding with target

| Target             | Location of target within mRNA | siRNA target within in consensus target | Predicted duplex                                       | siRNA | GC%   | Free energy of folding of siRNA candidate at 37°C | Free energy of Binding with target |
|--------------------|--------------------------------|---|--|-------|-------|---|------------------------------------|
| Consensus Target-1 | 253-275                        | TGGGAAGAATATGGAACCTTTAAA                | UAAAGUUCCAUUA<br>UCUCCCCA<br>GGAAGAAUAUGGA<br>ACUUUAAA |       | 53.8% | 0.9   | 25.1                               |
| Consensus Target-2 | 1564-1586                      | AGCATAGAGTATCTGCTATTAAG                 | UAAUAGCAGAUAC<br>UCUAUGCU<br>CAUAGAGUAUCUG<br>CUAUUAAG |       | 50%   | 0.0   | 23.3                               |
| Consensus Target-3 | 2198-2220                      | TTGGTTTAAAGGGCCTATTAACA                 | UAAAUAGGCCCUU<br>UAAACCAA<br>GGUUUAAAGGGCC<br>UAUUAACA |       | 46.7% | 1.0   | 23.1                               |
| Consensus Target-4 | 2648-2670                      | AACCACTTTATTGCTTTGTCTTA                 | AGACAAAGCAAUA<br>AAGUGGUU<br>CCACUUUAUUGCUU<br>UGUCUUA |       | 41.2% | 0.6   | 26.2                               |