Supplemental Figure S1: Experiment Mapping. This figure shows the effect of perturbing each single experiment based on the experimental error. A) Mean base pair probability RMSD, averaged between +/- 3 σ perturbations of the experimental values. The x-axis, which is shared with subplot B, shows the experiment number. This shows how perturbing a single experiment and then propagating the perturbation through the nearest neighbor parameters values alters the predicted base pairing probabilities. B) The impact of changing an individual experimental value by - 3 σ on the parameter values. Independent parameters are along the y-axis, organized by motif type, and the experiment number is shown on the x-axis. This shows how perturbing each experiment perturbs the nearest neighbor parameters. C) Mean base pair probability RMSD, averaged between +/- 3 σ perturbations of the parameter values. Parameter indices are along the y-axis, grouped by parameter type and shared with subplot B. An interactive version of this figure is available at http://rna.urmc.rochester.edu/publications.html.
Supplemental Figure S2: Average parameter values and observed parameter standard deviations. Randomly perturbed experiment values were used to generate 100,000 parameter sets. A) The difference between the average values of the parameters across all 100,000 parameter sets and the values calculated using unmodified experiment values is plotted against parameter index. B) The difference between the errors calculated by the propagation of uncertainties and the observed standard deviations for each free parameter is plotted against parameter index.
Supplemental Figure S3: Score distributions for perturbed parameter sets. The average sensitivity and positive predictive value was calculated for each perturbed parameter set compared against predictions made with an unperturbed parameter set. Parameter sets were generated by either randomly perturbing every free parameter value independently or by randomly perturbing every experiment value within experimental uncertainty. 1,000 parameter sets were evaluated for both classes.
Supplemental Figure S4: Base pair probability RMSD distributions for perturbed parameter sets. Parameter sets were generated by either randomly perturbing every free parameter value independently or by randomly perturbing every experimental value within experimental uncertainty. A) The mean base pair probability RMSD across 1650 sequences was calculated for each parameter set compared against predictions made with an unperturbed parameter set. B) The mean base pair probability length-corrected RMSD across 1650 sequences was calculated for each parameter set compared against predictions made with an unperturbed parameter set. 1,000 parameter sets were evaluated for both classes.
Supplemental Figure S5: Convergence of mean RMSD calculations. The cumulative mean average RMSD is plotted as the number of parameter sets is increased. The convergence of the values for both data tables generated by simultaneously perturbing all free nearest neighbor parameter values and data tables generated by perturbing all experiment values within experimental uncertainty demonstrates that sufficient numbers of data table sets have been sampled for both types.
Supplemental Figure S6: Accuracy distributions for randomly perturbed parameter sets. 1000 parameter sets were generated by randomly perturbing optical melting values within experimental uncertainty. The parameter sets were then benchmarked by predicting secondary structures for 1,450 sequences of known structure. The average positive predictive value (PPV) and sensitivity scores across the RNA families are plotted (A and B respectively). The red line indicates the score for the unperturbed parameter set and the blue line indicates the score of the “average” parameter set.
Supplemental Figure S7: Accuracy scores for randomly perturbed parameter sets. 1000 parameter sets were generated by randomly perturbing optical melting values within experimental uncertainty. The parameter sets were then benchmarked by predicting secondary structures for 1,450 sequences of known structure. The sensitivities are plotted against PPVs, where the predicted structures are scored against the known structures. Each data point represents the scores for a single parameter set, with the red data point illustrating the performance of the unperturbed parameter set and the blue dot representing the scores for the “average” parameter set.
Supplemental Figure S8: RMSD difference between positive and negative ΔG perturbations. Experiment indices are along the x-axis, organized by structure type. Difference in Mean base pair probability RMSD for the entire sequence archive except randomized sequences for +3 and -3 σ perturbations. The details of each experiment are available in the Supplemental Tables S1-S8.
Supplementary Table 1: Helical Duplex Experiment List
Note: The top strand is shown 5’ to 3’. The bottom strand is shown 3’ to 5’.

| Index | ID# | Duplex | Folding Free Energy Change (kcal/mol) | Reference |
|-------|-----|--------|---------------------------------------|-----------|
| 1     | R1  | UCAUGA GUACU | -4.30 (1) |           |
| 2     | R3  | CAAAAAAAG GUUUUUUC | -4.10 (2) |           |
| 3     | R4  | CAAAAAAAG GUUUUUUUC | -5.00 (2) |           |
| 4     | R5  | CGGG GGCC | -4.55 (3) |           |
| 5     | R6  | ACCGGU UGGCCA | -8.48 (3) |           |
| 6     | R8  | GGCC CGGG | -5.37 (4) |           |
| 7     | R9  | GGCC CGGG | -5.90 (4) |           |
| 8     | R12 | GGCC CGGG | -5.32 (5) |           |
| 9     | R14 | AGGCCU UCCGGA | -8.50 (5) |           |
| 10    | R15 | CGGCCG GCCGGC | -9.90 (5) |           |
| 11    | R18 | GCCGGC CGGGCG | -11.22 (5) |           |
| 12    | R19 | UCCGGA AGGCCU | -7.86 (5) |           |
| 13    | R20 | GCCG CGCG | -4.63 (6) |           |
| 14    | R21 | GCGCGC CGCGCG | -10.60 (6) |           |
| 15    | R22 | CGCGCG CGCGCG | -9.06 (6) |           |
| 16    | R24 | AUGCGU UGCGUA | -4.22 (7) |           |
| 17    | R25 | AUGCGCGU UGCGCGUA | -9.31 (7) |           |
|    |   |    |    |    |
|----|---|----|----|----|
| 18 | R26 | AUGCGUAU | -5.27 | (7) |
| 19 | R27 | AUGUGCAU | -6.17 | (7) |
| 20 | R28 | GCUGGC | -6.47 | (7) |
| 21 | R29 | GGGCU | -4.67 | (7) |
| 22 | R30 | CUGCGG | -4.31 | (7) |
| 23 | R31 | CGGCUG | -5.55 | (7) |
| 24 | R32 | AUGCAU | -4.73 | (7) |
| 25 | R33 | AUGCGCAU | -10.19 | (7) |
| 26 | R34 | AUACGUAU | -6.57 | (7) |
| 27 | R35 | AUGUACAU | -6.49 | (7) |
| 28 | R36 | GCUAGC | -7.89 | (7) |
| 29 | R37 | GACGUC | -7.24 | (7) |
| 30 | R38 | CUGCAG | -7.11 | (7) |
| 31 | R39 | CAGCUG | -6.72 | (7) |
| 32 | R40 | UGGCGG | -8.56 | (8) |
| 33 | R41 | UCCGGG | -7.44 | (8) |
| 34 | R42 | GCCGGU | -9.17 | (8) |
| 35 | R43 | GGGCU | -8.42 | (8) |
| 36 | R44 | GCAUGC | -7.34 | (9) |
| 37 | R45 | GUGCAC | -7.65 | (9) |
| 38 | R46 | GUCUAGAC | -10.09 | (9) |
|   |   |   |   |   |
|---|---|---|---|---|
| 39 | R47 | GAUAUAUC | CUUAUAUAG | -6.05 | (9) |
| 40 | R48 | GUAUAUAC | CAUAUAUG | -5.99 | (9) |
| 41 | R49 | GAGAGA | CUACU | -6.86 | (9) |
| 42 | R50 | AGAGAGAG | UCUCUCUC | -11.14 | (9) |
| 43 | R51 | AAUGCAUU | UUACGUAA | -7.17 | (9) |
| 44 | R52 | UAUGCAUA | UAACGUAA | -7.22 | (9) |
| 45 | R53 | GAUGCAUC | CUACGUAG | -10.18 | (9) |
| 46 | R54 | CAUGCAUG | GUACGUAC | -9.72 | (9) |
| 47 | R55 | AGAUAUCU | UCUAUAGA | -6.50 | (10) |
| 48 | R56 | AUCUAGAU | UAGAUCUA | -7.17 | (10) |
| 49 | R57 | AACUAGUU | UUGAUCAA | -7.15 | (10) |
| 50 | R58 | AGUUAACU | UCAAUUGA | -6.19 | (10) |
| 51 | R59 | ACUUAAGU | UGAAUUCA | -6.26 | (10) |
| 52 | R60 | GAACGUUC | CUUGCAAG | -9.39 | (10) |
| 53 | R61 | GUUCGAAC | CAAGCUUG | -8.76 | (10) |
| 54 | R62 | UCUAUAGA | AGUUAACU | -6.89 | (10) |
| 55 | R63 | UAGAUCUA | AUCUAGAU | -7.06 | (10) |
| 56 | R64 | GUACGUAC | UGUCAUG | -7.08 | (10) |
| 57 | R65 | ACUAUAGU | UGUAUACU | -7.09 | (10) |
| 58 | R66 | UGAUCA | ACUAU | -5.00 | (10) |
| 59 | R68 | UCAUGA | AGUACU | -3.82 | (10) |
|   |   |   |   |   |
|---|---|---|---|---|
| 60 | R70 | UGCGCA ACGCGU | -8.17 | (11) |
| 61 | R71 | AGCGCU UCGCGA | -8.01 | (11) |
| 62 | R72 | GGCGCC CGGCGG | -11.31 | (11) |
| 63 | R73 | UGCgCA ACGCGU | -8.12 | (12) |
| 64 | R74 | GAUGCAUU UUACGUGA | -6.82 | (13) |
| 65 | R75 | UAUGCAUG GUACGUAAU | -6.44 | (13) |
| 66 | R76 | GCGGCC CGGCCG | -10.43 | (14) |
| 67 | R78 | AUGGUCAU UACUGGGU | -5.42 | (15) |
| 68 | R79 | CCUGUAGG GGAUGUCC | -6.81 | (15) |
| 69 | R80 | CGGUGCGG GCUCGGGG | -11.18 | (15) |
| 70 | R81 | CGUUGACG GCAGUUGC | -6.94 | (15) |
| 71 | R82 | CUGGUCAG GACUGGUC | -7.11 | (15) |
| 72 | R83 | GGAUGUCC CCUUGAGG | -6.45 | (15) |
| 73 | R84 | GGAUGUCC CCUUGAGG | -8.36 | (15) |
| 74 | R85 | GCGGUGCC CGGUCGGG | -9.23 | (15) |
| 75 | R86 | GUGGUGAC CAGUGGUG | -6.05 | (15) |
| 76 | R87 | GAGUUGAG CUCGGCUC | -8.21 | (15) |
| 77 | R88 | AGGCUU UUCGGA | -4.04 | (15) |
| 78 | R89 | AGUCGAUU UUAGCUGA | -5.97 | (15) |
| 79 | R90 | CGGAUUUC GCUUAGGC | -6.54 | (15) |
| 80 | R91 | CCUAGG GGAUCC | -7.86 | (15) |
|   |   |   |   |   |
|---|---|---|---|---|
| 81 | R93 | GGAUCC CCUAGG | -7.46 | (15) |
| 82 | R94 | CACAG GUGUC | -4.41 | (16) |
| 83 | R95 | UGACCUCA ACUGGAGU | -12.34 | (17) |
| 84 | R96 | GAGCUC CUCGAG | -7.73 | (18) |
| 85 | R97 | GCCUGGC CGUGCG | -5.11 | (18) |
| 86 | R98 | GAGGUCUC CUCUGGAG | -8.77 | (19) |
| 87 | R99 | GAGCUC CUCGAG | -7.93 | (19) |
| 88 | R100 | GGUACC CCAUGG | -7.32 | (19) |
| 89 | R101 | GAGUGCUC CUCUGGAG | -9.17 | (20) |
| 90 | R102 | GGUUGACC CCAUGG | -8.30 | (20) |
| 91 | R103 | GCCUGGCC CCGUCGG | -13.26 | (20) |
| 92 | R106 | CCAUGUGG GGUJUACC | -7.82 | (21) |
| 93 | R107 | CCAGUUGG GGUUGACC | -5.71 | (21) |
| 94 | R108 | CCAUGG GGUJUACC | -7.39 | (21) |
| 95 | R109 | GAGGAG CUCUCUC | -8.50 | (22) |
| 96 | R110 | GAGUGGAG CUCUGUC | -9.69 | (22) |
| 97 | R112 | GAGUGGAG CUCUCUC | -7.62 | (22) |
| 98 | R113 | CGCG GCUC | -3.66 | (1) |
| 99 | R114 | ACGCA UGCGU | -4.96 | (1) |
| 100 | R115 | AGCGA UGCGU | -5.06 | (1) |
| 101 | R116 | GCACG CUGGC | -6.17 | (1) |
|   |   |   |   |   |
|---|---|---|---|---|
| 102 | R117 | GCUCG  
|     |     | CGAGC |   | -6.13 | (1) |
| 103 | R118 | CACGUG  
|     |     | GUGCAC |   | -6.58 | (1) |
| 104 | R119 | CCGCGG  
|     |     | GGCGCC |   | -9.83 | (1) |
| 105 | R120 | GCAACG  
|     |     | CGUUGC |   | -6.99 | (1) |
| 106 | R121 | GCAUUG  
|     |     | CGUAGC |   | -7.26 | (1) |
| 107 | R122 | GCCGCG  
|     |     | CGCGGC |   | -10.87 | (1) |
| 108 | R123 | GCCGCCG  
|     |     | CGCGGC |   | -10.92 | (1) |
| 109 | R124 | GCGCGG  
|     |     | CGCGCC |   | -11.39 | (1) |
| 110 | R125 | GCGUCG  
|     |     | CGCAGC |   | -8.77 | (1) |
| 111 | R126 | GCUACG  
|     |     | CGAUGC |   | -7.56 | (1) |
| 112 | R127 | GCCGCG  
|     |     | CCGCCG |   | -10.77 | (1) |
| 113 | R128 | GUGGUG  
|     |     | CACCAC |   | -7.68 | (1) |
| 114 | R129 | GUGUCG  
|     |     | CACAGC |   | -7.18 | (1) |
| 115 | R130 | UCGCGA  
|     |     | AGGCUC |   | -6.85 | (1) |
| 116 | R131 | UCUAGA  
|     |     | AGAUCU |   | -4.96 | (1) |
| 117 | R132 | AAGGAGG  
|     |     | UCCUCC |   | -9.53 | (1) |
| 118 | R133 | ACUGUCA  
|     |     | UGACAGU |   | -7.92 | (1) |
| 119 | R134 | AGUCUGA  
|     |     | UACAGAC |   | -7.50 | (1) |
| 120 | R135 | GACUCAG  
|     |     | CUGAGUC |   | -9.06 | (1) |
| 121 | R136 | GAGUGAG  
|     |     | CUCACUC |   | -9.70 | (1) |
| 122 | R137 | GUCACUG  
|     |     | CAGUGAC |   | -8.62 | (1) |
| \#  | R     | Sequence                      | Score  | ID |
|-----|-------|------------------------------|--------|----|
| 123 | R138  | ACCUUUGC UGGAACG             | -10.64 | (1)|
| 124 | R139  | CGACGCAUG GCUGCGUC           | -12.32 | (1)|
| 125 | R140  | CUCGCACA GAGCGGUGU           | -12.10 | (1)|
| 126 | R141  | GGCUUCAA CGGAGUU             | -10.20 | (1)|
| 127 | R142  | UCCUUGCA AGGAACGU            | -11.09 | (1)|
| 128 | R143  | UUCCGGAA AAGGCUUU            | -10.80 | (1)|
| 129 | R144  | UUGCACGAA AACGCGU            | -10.19 | (1)|
| 130 | R145  | UUGGCCCAA AACCGGCU           | -11.02 | (1)|
| 131 | R146  | UUGUACAA AACAUGUU            | -6.71  | (1)|
| 132 | R147  | AAGGUUGGAA UUCCACCUU         | -12.69 | (1)|
| 133 | R148  | CAUGC GUCACG                 | -7.01  | (1)|
| 134 | R150  | GCUGGAG CGACUC               | -7.71  | (1)|
| 135 | R151  | GUGCAG CAGCUC                | -7.68  | (1)|
| 136 | R156  | UCCGCGCA AGGCGCGU            | -14.59 | (1)|
| 137 | R157  | GCGGCG CGCCCCG               | -10.91 | (23)|
| 138 | R158  | GCGGCG CGCGCG                | -10.82 | (23)|
| 139 | R159  | AAGGCCGCAA UUCCGGCCUU        | -18.15 | (24)|
| 140 | R160  | CCUCUGGUGA GGAGACCGCU        | -15.31 | (24)|
| 141 | R161  | AGGCGGCA UCCGGCCU            | -15.26 | (24)|
| 142 | R162  | GAGCCGAC CUCGGGCU            | -13.76 | (24)|
| 143 | R163  | UCACCUGA AGUGGACU            | -10.84 | (24)|
|   |   |   |   |   |
|---|---|---|---|---|
| 144 | R166 | CUGGUC GACCAG | -8.05 | (25) |
| 145 | R168 | GAGUUGAC CUCAACUG | -10.60 | (26) |
| 146 | R169 | GAGUGAG CUCGCUC | -7.81 | (23) |
| 147 | R170 | AUCUAGGU UGGAUCUA | -5.90 | Unpublished |
| 148 | R171 | GUCUAGAU UAGAUUCUG | -7.66 | Unpublished |
Supplementary Table 2: Dangling End Duplex Experiment List

Note: The top strand is shown 5’ to 3’. The bottom strand is shown 3’ to 5’. Unpaired nucleotides are in lower case.

| Index | ID# | Duplex       | Folding Free Energy Change (kcal/mol) | Reference |
|-------|-----|--------------|---------------------------------------|-----------|
| 149   | D1  | UGCGCAa aACGCGU | -9.60 (12)                            | (12)      |
| 150   | D2  | UGCGCAc cACGCGU | -9.11 (12)                            | (12)      |
| 151   | D3  | UGCGCAg gACGCGU | -9.79 (12)                            | (12)      |
| 152   | D4  | UGCGCAu uACGCGU | -9.27 (12)                            | (12)      |
| 153   | D5  | AUGCAUa aUACGUA | -6.06 (12)                            | (12)      |
| 154   | D6  | AUGCAUc cUACGUA | -4.98 (12)                            | (12)      |
| 155   | D7  | AUGCAUg gUACGUA | -6.06 (12)                            | (12)      |
| 156   | D8  | AUGCAUu uUACGUA | -4.91 (12)                            | (12)      |
| 157   | D9  | CCGGa aGGCC   | -6.85 (3)                             | (3)       |
| 158   | D10 | CCGGa aGGCC   | -6.84 (3)                             | (3)       |
| 159   | D11 | CCGGc cGGCC   | -5.25 (4)                             | (4)       |
| 160   | D12 | CCGGg gGGCC   | -7.05 (4)                             | (4)       |
| 161   | D13 | CCGGu uGGCC   | -5.81 (4)                             | (4)       |
| 162   | D14 | CCGGu uGGCC   | -5.76 (3)                             | (3)       |
| 163   | D15 | GCGCa aCGCG   | -7.92 (11)                            | (11)      |
| 164   | D16 | GGCCa aCCGG   | -9.01 (4)                             | (4)       |
| 165   | D17 | GCGCc cCGCG   | -6.16 (11)                            | (11)      |
| 166   | D18 | GGGCc cCCGG   | -6.96 (4)                             | (4)       |
|   |   |   |   |   |
|---|---|---|---|---|
| 167 | D19 | GCGCg  
gCGCG | -7.70 | (11) |
| 168 | D20 | GGCCg  
gCCGG | -8.88 | (4) |
| 169 | D21 | GGCCu  
uCCGG | -8.16 | (5) |
| 170 | D22 | GCGu  
uCGCG | -6.93 | (11) |
| 171 | D23 | GGCu  
uCGG | -7.98 | (4) |
| 172 | D24 | aUGCGCA  
ACGCUa | -8.72 | (12) |
| 173 | D25 | cUCAUGA  
AGUACUc | -4.09 | Unpublished |
| 174 | D26 | aAUGCAU  
UACGUa | -5.38 | (12) |
| 175 | D27 | cAUGCAU  
UACGUac | -5.23 | (12) |
| 176 | D28 | gAUGCAU  
UACGUag | -5.44 | (12) |
| 177 | D29 | uAUGCAU  
UACGUau | -5.11 | (12) |
| 178 | D30 | aCCGG  
GGCCa | -5.60 | (3) |
| 179 | D31 | cCCGG  
GGCCc | -5.27 | Unpublished |
| 180 | D32 | gCCGG  
GGCCg | -4.90 | (5) |
| 181 | D33 | uCCGG  
GGCCu | -4.84 | (5) |
| 182 | D34 | aGGCC  
CGCGa | -5.81 | (5) |
| 183 | D35 | cGGCC  
CGCGc | -5.79 | (5) |
| 184 | D36 | cGCGC  
CGCGc | -5.27 | (11) |
| 185 | D37 | gGC GC  
CGCGg | -4.55 | (11) |
| 186 | D38 | uGGCC  
CGCGu | -5.17 | (5) |
| 187 | D39 | uGCGC  
CGCGu | -5.08 | (11) |
|   |   |   |   |   |
|---|---|---|---|---|
| 188 | D40 | GCCCc cCCGG | -7.22 | (4) |
| 189 | D41 | GCCGGGUa aUGGCCG | -11.37 | (8) |
| 190 | D42 | GCCGCGa aCGCCGC | -14.63 | (14) |
| 191 | D43 | GCCCGCa aCUGGCG | -12.17 | (14) |
| 192 | D44 | GCCGCGp pGCGCCGA | -8.73 | (27) |
| 193 | D45 | GCCGGGAg gAGGCCG | -8.44 | (27) |
### Supplementary Table 3: Terminal Mismatch Duplex Experiment List

Note: The top strand is shown 5’ to 3’. The bottom strand is shown 3’ to 5’. Unpaired nucleotides are in lower case.

| Index | ID# | Duplex | Folding Free Energy Change (kcal/mol) | Reference |
|-------|-----|--------|--------------------------------------|-----------|
| 194   | T1  | aUGCGCAa<br> aACGCGUa | -9.68 (13) |           |
| 195   | T2  | aUGCGCAc<br> cACGCGUa | -9.42 (13) |           |
| 196   | T3  | aUGCGCAg<br> gACGCGUa | -9.77 (13) |           |
| 197   | T4  | aGCgCa<br> aCGCGa | -7.64 (28) |           |
| 198   | T5  | cGCgCc<br> aCCGGc | -8.30 (28) |           |
| 199   | T6  | gGCCGa<br> aCGCGg | -7.52 (28) |           |
| 200   | T7  | uGGCCc<br> cCCGGu | -6.90 (28) |           |
| 201   | T8  | gGCgGg<br> gGCgGg | -7.71 (28) |           |
| 202   | T9  | cGCgCu<br> uCCGGc | -8.09 (28) |           |
| 203   | T10 | uGCgCu<br> uCGGu | -7.01 (8) |           |
| 204   | T11 | aCCGGa<br> aGGCCa | -6.73 (29) |           |
| 205   | T12 | gCGGGa<br> aGGCCg | -7.03 (8) |           |
| 206   | T13 | aCCGGc<br> cGGCCa | -6.72 (29) |           |
| 207   | T14 | aCGGg<br> gGGCCa | -7.71 (29) |           |
| 208   | T15 | gCGGGg<br> gGGCCg | -7.48 (8) |           |
| 209   | T16 | aUCCGGGa<br> aGGCCUa | -7.94 (30) |           |
| 210   | T17 | aUGCCCgG<br> gCCCgGUA | -9.72 (30) |           |
| 211   | T18 | uUGCCGGu<br> uGCGGUu | -9.70 (31) |           |
|   |   |   |   |
|---|---|---|---|
| 212 | T19 | aAUGCAUa aUACGUAa | -6.61 (13) |
| 213 | T20 | cAUGCAUa aUACGUAc | -6.25 (13) |
| 214 | T21 | gAUGCAUa aUACGUAg | -6.93 (13) |
| 215 | T22 | aAUGCAUc cUACGUAa | -6.17 (13) |
| 216 | T23 | cAUGCAUc cUACGUAc | -5.90 (13) |
| 217 | T24 | uAUGCAUc cUACGUAu | -5.66 (13) |
| 218 | T25 | aAUGCAUg gUACGUAa | -6.96 (13) |
| 219 | T26 | gAUGCAUg gUACGUAg | -7.02 (13) |
| 220 | T27 | cAUGCAUu uUACGUAu | -5.91 (13) |
| 221 | T28 | uAUGCAUu uUACGUAu | -5.75 (13) |
| 222 | T29 | aGCGUa aUGCGa | -3.97 (30) |
| 223 | T30 | aGGCGCUg gUCGCGGa | -9.38 (30) |
| 224 | T31 | gGCCGGUg gUGGCCGg | -10.79 (30) |
| 225 | T32 | uGCCGGUu uUGGCCGu | -9.97 (31) |
| 226 | T33 | aGCGCg gCGCGa | -7.47 (27) |
Supplementary Table 4: Hairpin Loop Experiment List

Note: The sequence is shown 5’ to 3’. Unpaired nucleotides are in lower case.

| Index | ID# | Duplex                     | Folding Free Energy Change (kcal/mol) | Reference |
|-------|-----|----------------------------|--------------------------------------|-----------|
| 227   | H1  | GGAauaUCC                  | 0.64                                 | (32)      |
| 228   | H2  | GGAGaaaUUCC                | -0.93                                | (30)      |
| 229   | H3  | GGCauaGCC                  | -0.96                                | (32)      |
| 230   | H4  | GGGaaaUCC                  | 0.33                                 | (30)      |
| 231   | H5  | GGGauaCCC                  | -0.21                                | (32)      |
| 232   | H6  | GGGAUACaaaGUAUCCA          | -6.30                                | (33)      |
| 233   | H7  | GGGAUACcccGUAUCCA          | -4.64                                | (33)      |
| 234   | H8  | GGGAUACuuuGUAUCCA          | -7.03                                | (33)      |
| 235   | H9  | GGUauaACC                  | 0.58                                 | (32)      |
| 236   | H10 | GGUauaGCC                  | 0.87                                 | (30)      |
| 237   | H11 | GCGauuaUGC                 | -0.32                                | (30)      |
| 238   | H12 | GCGGauuaUCGC               | -1.41                                | (30)      |
| 239   | H13 | GGAauuaUCC                 | -0.06                                | (32)      |
| 240   | H14 | GCauuaGCC                  | -2.52                                | (32)      |
| 241   | H15 | GGaccaUCC                  | -1.81                                | (30)      |
| 242   | H16 | GGauuACC                   | -1.28                                | (32)      |
| 243   | H17 | GGGAUACaaaGUAUCCA          | -7.60                                | (33)      |
| 244   | H18 | GGGAUACcccGUAUCCA          | -4.30                                | (33)      |
| 245   | H19 | GGGAUACuuuGUAUCCA          | -8.16                                | (33)      |
| 246   | H20 | GGUauuaACC                 | -1.79                                | (32)      |
| 247   | H21 | GGUauuaGCC                 | 0.17                                 | (30)      |
| 248   | H22 | GGUgcaaaGCC                | -1.44                                | (30)      |
| 249   | H23 | GGCgagaGCC                 | -3.31                                | (31)      |
| 250   | H24 | GGCgcgaGCC                 | -3.50                                | (31)      |
| 251   | H25 | GCCgggaGCC                 | -3.23                                | (31)      |
|    |   |                |   |   |
|----|---|----------------|---|---|
| 252 | H26 | GGCgugaGCC     | -3.16 | (31) |
| 253 | H27 | GGCgaaGCC      | -3.19 | (31) |
| 254 | H28 | GGCgcaaaGCC    | -3.35 | (31) |
| 255 | H29 | GGCggaGCC      | -3.23 | (31) |
| 256 | H30 | GGCguaaGCC     | -3.53 | (31) |
| 257 | H31 | GCCACuuuuGUCC  | -4.20 | (34) |
| 258 | H32 | GGAGuucgCUCC   | -4.05 | (34) |
| 259 | H33 | GGACguuuGUCC   | -4.43 | (34) |
| 260 | H34 | GCGgaagaUGC    | -0.28 | (30) |
| 261 | H35 | GAaauuuUACC    | 0.42  | (32) |
| 262 | H36 | GCauauaGCC     | -2.38 | (32) |
| 263 | H37 | GCauuuuGCC     | -2.66 | (32) |
| 264 | H38 | GGauuuuGCC     | -1.70 | (32) |
| 265 | H39 | GGauuuuGCC     | -0.70 | (30) |
| 266 | H40 | GGauuuuUCC     | -7.72 | (33) |
| 267 | H41 | GGGAUACaaaaaGUAUCCA | -4.10 | (33) |
| 268 | H42 | GGGAUACccccccGUAUCCA | -8.13 | (33) |
| 269 | H43 | GGGAUACuuuuuGUUCCA | 0.25  | (32) |
| 270 | H44 | GGauuuuACC     | -0.57 | (30) |
| 271 | H45 | GGauuuuGCC     | 1.60  | (28) |
| 272 | H46 | ACCgacacaGGU   | -3.35 | (35) |
| 273 | H47 | AGGauauuaUCC    | -2.38 | (35) |
| 274 | H48 | AGGUauuaaGCU    | -2.21 | (35) |
| 275 | H49 | CGGuuaauuCCG    | -1.95 | (28) |
| 276 | H50 | CUCUacaccaAGAG  | -1.78 | (28) |
| 277 | H51 | GCGgugaaUAGC    | -0.69 | (30) |
| 278 | H52 | GGuuaauuUAGC    | 0.35  | (30) |
| 279 | H53 | GGuuaauuUAGC    | -0.73 | (35) |
| 280 | H54 | GGuuaauuUAGC    | -1.72 | (28) |
|    |   |                  |     |     |
|----|---|------------------|-----|-----|
| 281| H55| GGCauaaauaGCC    | -2.76 | (35) |
| 282| H56| GGCauaaucGCC     | -2.56 | (28) |
| 283| H57| GGCauaaugGCC     | -3.11 | (28) |
| 284| H58| GGCcuauaaGCC     | -2.15 | (28) |
| 285| H59| GGCcuauaucGCC    | -2.38 | (28) |
| 286| H60| GGCcuauauGCC     | -1.82 | (28) |
| 287| H61| GGCguauaaGCC     | -3.47 | (35) |
| 288| H62| GGCguauaugGCC    | -3.85 | (28) |
| 289| H63| GGCuuaauaGCC     | -2.89 | (28) |
| 290| H64| GGCuuaauuGCC     | -6.23 | (28) |
| 291| H65| GGGauaaauUCC     | -1.42 | (35) |
| 292| H66| GGUuauaaACC      | -0.34 | (35) |
| 293| H67| GGUuauaaGCC      | -0.47 | (35) |
| 294| H68| GGUugauauaACC    | -1.54 | (28) |
| 295| H69| GGUugauaaGCC     | -1.85 | (28) |
| 296| H70| GGUugauaugACC    | -1.04 | (28) |
| 297| H71| GGUugauaugGCC    | -1.29 | (30) |
| 298| H72| GUGguauaaCAC     | -1.08 | (28) |
| 299| H73| GUGguauaaUAC     | 0.94  | (28) |
| 300| H74| GGAuuaauuUCC     | -1.29 | (31) |
| 301| H75| GGUuuaauuACC     | -0.83 | (31) |
| 302| H76| GGUuuaauuGCC     | -0.53 | (31) |
| 303| H77| GCGguauaugCGC    | -2.54 | (31) |
| 304| H78| GGAguaaugUCC     | -1.16 | (31) |
| 305| H80| GCGguauaugUGC    | -0.80 | (31) |
| 306| H81| GCGAauaaauaUCGC  | -2.41 | (32) |
| 307| H82| GGCauaaauaGCC    | -2.11 | (32) |
| 308| H83| GGGacggacaUCC    | -1.03 | (30) |
| 309| H84| GGGauaaauaCCC    | -2.12 | (32) |
|   |   |   |   |   |
|---|---|---|---|---|
| 310 | H85 | GGGauaaauaUCC | -0.22 | (30) |
| 311 | H86 | GGGAUACaaaaaaGUAUCCA | -7.19 | (33) |
| 312 | H87 | GGGAUACcccccccGUAUCCA | -2.91 | (33) |
| 313 | H88 | GGGAUACuuuuuuuGUAUCCA | -7.38 | (33) |
| 314 | H89 | GGUauaaaauaACC | -0.41 | (32) |
| 315 | H90 | GGUauaaaauaGCC | 0.56 | (30) |
| 316 | H91 | GGUguaaaaaGCC | -0.66 | (30) |
| 317 | H92 | GCGaauucauaUGC | -0.65 | (30) |
| 318 | H93 | GCGgauggaagaGCG | -1.28 | (30) |
| 319 | H94 | G6AauaaauaUCC | -2.19 | (32) |
| 320 | H95 | GGCauaaaauaGCG | -2.16 | (32) |
| 321 | H96 | GGGauaaaauaCCC | -2.35 | (32) |
| 322 | H97 | GGGauaaaauaUCC | -1.65 | (30) |
| 323 | H98 | GGUauucauaGCG | -0.08 | (30) |
| 324 | H99 | GGUauaaaauaACC | 0.51 | (32) |
| 325 | H100 | GGUauaaaauaGCC | 0.03 | (30) |
| 326 | H101 | GCGAauaaauaUCGC | -2.23 | (32) |
| 327 | H102 | GCGUauaaaauaACGC | -1.08 | (32) |
| 328 | H103 | GGAauaaaauaUCC | 0.25 | (32) |
| 329 | H104 | GGCauaaaauaGCG | -2.14 | (32) |
| 330 | H105 | GGGauaaaauaCCC | -2.09 | (32) |
| 331 | H106 | GGGAUACaaaaaaGUAUCCA | -5.55 | (33) |
| 332 | H107 | GGGAUACcccccccGUAUCCA | -2.10 | (33) |
| 333 | H108 | GGGAUACuuuuuuuuuGUAUCCA | -8.05 | (33) |
| 334 | H126 | G6ACaacGUCC | -1.32 | (36) |
| 335 | H127 | G6AGuuaCUCC | -0.92 | (36) |
| 336 | H132 | GGCuacgGCC | -3.22 | (31) |
| 337 | H133 | GGCucccgGCC | -3.95 | (31) |
| 338 | H134 | GGCuucgGCC | -4.09 | (31) |
|   |   |   |   |
|---|---|---|---|
| 339 | H135 | GAAGAcaguguUCUUC | -5.52 | (37) |
| 340 | H136 | GAAGAcagugcUCUUC | -5.44 | (37) |
| 341 | H137 | GAAGAcaguacUCUUC | -4.44 | (37) |
| 342 | H138 | GGAcagugcUCC | -1.02 | (31) |
| 343 | H139 | GGAcagugaUCC | -1.59 | (31) |
| 344 | H141 | GGACuuugGUCC | -3.79 | (38) |
| 345 | H148 | GGACuucgGUCC | -6.31 | (34) |
| 346 | H149 | GGACuuugGUCC | -4.30 | (34) |
| 347 | H150 | GGACuacgGUCC | -5.68 | (34) |
| 348 | H152 | GGACcaagGUCC | -4.02 | (39) |
| 349 | H153 | GGACccagGUCC | -3.83 | (39) |
| 350 | H154 | GGACcgcagGUCC | -3.67 | (39) |
| 351 | H155 | GGACcuagGUCC | -3.64 | (39) |
| 352 | H156 | GGACcacgGUCC | -3.56 | (39) |
| 353 | H158 | GGACcgacGUCC | -3.67 | (39) |
| 354 | H159 | GGACcuacGUCC | -4.77 | (39) |
| 355 | H160 | GGACuaacGUCC | -3.61 | (39) |
| 356 | H161 | GGACuacgGUCC | -3.61 | (39) |
| 357 | H163 | GGACuaagGUCC | -3.76 | (39) |
| 358 | H164 | GGACuacgGUCC | -4.88 | (39) |
| 359 | H165 | GGACuaccGUCC | -4.45 | (39) |
| 360 | H166 | GGACuacgGUCC | -4.42 | (39) |
| 361 | H167 | GGACuuacGUCC | -4.78 | (39) |
| 362 | H170 | GGACcaacGUCC | -1.77 | (39) |
Supplementary Table 5: Internal Loop Duplex Experiment List

Note: The top strand is shown 5’ to 3’. The bottom strand is shown 3’ to 5’. Unpaired nucleotides are in lower case. The underscore character (_) does not represent a nucleotide. It is used to maintain alignment between the top and bottom strands.

| Index | ID# | Duplex        | Folding Free Energy Change (kcal/mol) | Reference |
|-------|-----|---------------|--------------------------------------|-----------|
| 363   | I1  | GAGcGAG       | -5.63                                | (23)      |
| 364   | I2  | GAGaGAG       | -5.32                                | (23)      |
| 365   | I3  | GAGcGAG       | -5.22                                | (23)      |
| 366   | I4  | GAGuGAG       | -5.24                                | (23)      |
| 367   | I5  | GAGaGAG       | -4.95                                | (23)      |
| 368   | I6  | GAGaGAG       | -4.85                                | (23)      |
| 369   | I7  | GAGcGAG       | -4.75                                | (23)      |
| 370   | I8  | GAGuGAG       | -4.75                                | (23)      |
| 371   | I9  | GCCuCGG       | -8.23                                | (23)      |
| 372   | I10 | GCCuCGG       | -7.90                                | (23)      |
| 373   | I11 | GCCuCGG       | -7.79                                | (23)      |
| 374   | I12 | GCCuCGG       | -8.15                                | (23)      |
| 375   | I13 | GCCuCGG       | -6.31                                | (23)      |
| 376   | I14 | GCCuUCG       | -5.99                                | (23)      |
| 377   | I15 | GUGuUCG       | -3.80                                | (23)      |
| 378   | I16 | GCCuUCG       | -4.98                                | (23)      |
| 379   | I17 | GCAuUCG       | -4.55                                | (23)      |
|    |   |                                                                 |    |    |    |
|----|---|----------------------------------------------------------------|----|----|----|
| 380| I18| GCUuACG GCauUGC                                                   | -4.47 | (23) |
| 381| I19| CGCaGCG GCaCGC                                                   | -6.08 | (17) |
| 382| I20| GCGaCCG CGCaGGC                                                  | -6.67 | (23) |
| 383| I21| GGCaGCC CGaCCG                                                   | -7.83 | (23) |
| 384| I22| GgCUGAG CgGACUC                                                  | -6.87 | (23) |
| 385| I23| CGgCAUG GCgGUAC                                                  | -6.07 | (23) |
| 386| I24| GUGgCAG CACgGUC                                                  | -6.66 | (23) |
| 387| I25| GCGgCGC CCGgGCg                                                  | -9.28 | (23) |
| 388| I26| CGCgGCg CGGgGCG                                                  | -8.14 | (23) |
| 389| I27| UGAcCUCA ACUGaGAG                                                 | -7.94 | (17) |
| 390| I28| GAGaGGAG CUCgUCUC                                                | -5.83 | (22) |
| 391| I29| GAGuGGAG CUCuUCUC                                                | -6.26 | (22) |
| 392| I30| GAGaGGAG CUCaUCUC                                                | -5.27 | (22) |
| 393| I31| GAGgUGAG CUCaGCUC                                                | -4.97 | (22) |
| 394| I32| GAGuUGAG CUCgGCUC                                                | -4.67 | (22) |
| 395| I33| GAGuUGAG CUCuGCUC                                                | -4.69 | (22) |
| 396| I34| GAGaUGAG CUCaGCUC                                                | -4.36 | (22) |
| 397| I35| GAGUaGAG CUCgGCUC                                                | -6.11 | (22) |
| 398| I36| GAGUgGAG CUCGaCU                                                  | -6.09 | (22) |
| 399| I37| GAGUuGAG CUCguCU                                                  | -6.24 | (22) |
| 400| I38| GAGUCGAG CUCGcCU                                                  | -5.56 | (22) |
|   |   | GAGUcGAG CUCGaCUC  | -6.00 | (22) |
|---|---|-------------------|-------|-----|
| 401| I39| GAGUaGAG CUCGaCUC | -5.15 | (22) |
| 402| I40| GAGGaGAG CUCUGcUC | -6.56 | (22) |
| 403| I41| GAGGuGAG CUCUuCUC | -5.21 | (22) |
| 404| I42| GAGGcGAG CUCUaCUC | -5.39 | (22) |
| 405| I43| GAGGaGAG CUCUaCUC | -5.74 | (22) |
| 406| I44| GAGGcGAG CUCUaCUC | -5.83 | (22) |
| 407| I45| GAGGaGAG CUCUaCUC | -5.42 | (22) |
| 408| I46| UGACa_CUC ACUGaaGAGU | -6.43 | (17) |
| 409| I47| UGACa_CUC ACUGaaGAGU | -6.80 | (40) |
| 410| I48| UGAGa_GUCA ACUCaaCAGU | -6.60 | (17) |
| 411| I49| UGACa_CUC ACUGagGAGU | -8.32 | (40) |
| 412| I50| UGACa_CUC ACUGagGAGU | -6.44 | (24) |
| 413| I51| UGACa_CUC ACUGaaGAGU | -7.42 | (40) |
| 414| I52| UGACa_CUC ACUGaaGAGU | -8.01 | (40) |
| 415| I53| UGAGaGUCA ACUCgaCAGU | -6.98 | (40) |
| 416| I54| UGACc_CUC ACUGaaGAGU | -7.40 | (40) |
| 417| I55| UGACc_CUC ACUGaaGAGU | -6.60 | (40) |
| 418| I56| UGACc_CUC ACUGccGAGU | -6.62 | (40) |
| 419| I57| UGACc_CUC ACUGccGAGU | -7.16 | (40) |
| 420| I58| UGACc_CUC ACUGccGAGU | -7.35 | (40) |
| 421| I59| UGACc_CUC ACUGagGAGU | -7.92 | (40) |
|   |   |   |   |   |
|---|---|---|---|---|
| 422 | I60 | UGACg_CUCA ACUggaGAGU | -8.32 | (40) |
| 423 | I61 | UGACu_CUCA ACUGccGAGU | -6.85 | (40) |
| 424 | I62 | UCACu_CUGA AGUGcuGACU | -5.91 | (24) |
| 425 | I63 | UGACu_CUCA ACUGucGAGU | -7.54 | (40) |
| 426 | I64 | UGACu_CUCA ACUGuuGAGU | -7.90 | (40) |
| 427 | I65 | AGGCu_CGGA UCCGuuGCCU | -10.43 | (24) |
| 428 | I66 | GCGagCGC CGGaGCG | -8.46 | (18) |
| 429 | I67 | GCGuuCGC CGCuuGCG | -7.66 | (19) |
| 430 | I68 | CGGcaCCG GCCacGGC | -5.49 | (19) |
| 431 | I69 | CUgcucGAG UACucGUC | -2.32 | (19) |
| 432 | I70 | CGGcuCCG GCCucGCG | -5.57 | (19) |
| 433 | I71 | GCGacCGC CGCaGCG | -6.23 | (19) |
| 434 | I72 | GCGaaCGC CGCaGCG | -5.66 | (19) |
| 435 | I73 | GGCagGCC CCGgaCGG | -9.44 | Unpublished |
| 436 | I74 | CGCagCGC GCGGaCGC | -7.76 | (41) |
| 437 | I75 | CGCuuCGC GCGuuCGC | -7.18 | (42) |
| 438 | I76 | UGCggGCA ACGggCGU | -5.02 | (42) |
| 439 | I77 | CGCaCGC GCGacCGC | -5.69 | (42) |
| 440 | I78 | CGCcucGCG GCGCuGCG | -5.45 | (42) |
| 441 | I79 | CGCucCGG GCGCuCGC | -5.38 | (42) |
| 442 | I80 | CGCccCGG GCGccCGC | -5.13 | (42) |
|   |   | Sequence       | Area (kcal/Mole) | References |
|---|---|----------------|-----------------|------------|
|443| I81| GGCacGCC       | -6.97           | 42         |
|444| I82| CGCaGCG        | -5.44           | 17         |
|445| I83| GCUGaAGC       | -5.88           | 18         |
|446| I84| GCUuAGC        | -5.50           | 19         |
|447| I85| GGUcaACC       | -4.14           | 19         |
|448| I86| GGUcuACC       | -3.86           | 19         |
|449| I87| GGUucACC       | -3.21           | 19         |
|450| I88| GCUaaAGC       | -3.82           | 19         |
|451| I89| GGAgaUCC       | -6.41           | 18         |
|452| I90| GCAgaUGC       | -5.61           | 18         |
|453| I91| GCAuuUGC       | -5.61           | 19         |
|454| I92| GACcaUCC       | -4.01           | 19         |
|455| I93| GGAacUCC       | -3.89           | 19         |
|456| I94| GCAaaUCC       | -3.52           | 19         |
|457| I95| GGCgaGCC       | -9.69           | 41         |
|458| I96| GAGgaGAG       | -6.91           | 22         |
|459| I97| GAGaaGAG       | -5.74           | 22         |
|460| I98| GAGuaGAG       | -4.26           | 22         |
|461| I99| GAGaaGAG       | -4.61           | 22         |
|462| I100| UGAGaaGUC      | -7.04           | 17         |
|463| I101| GAGaaGAG      | -5.39           | 22         |
|   |   |   |   |   |
|---|---|---|---|---|
| 464 | I102 | GAGagGAG CUCgaCUC | -5.92 | (22) |
| 465 | I103 | GAGugGAG CUCuaCUC | -4.37 | (22) |
| 466 | I104 | GAGcgGAG CUCaaCUC | -5.99 | (22) |
| 467 | I105 | GAGagGAG CUCcaCUC | -6.19 | (22) |
| 468 | I106 | GAGagGAG CUCaaCUC | -5.23 | (22) |
| 469 | I107 | GAGguGAG CUCauCUC | -4.77 | (22) |
| 470 | I108 | GAGauGAG CUCguCUC | -3.88 | (22) |
| 471 | I109 | GAGuuGAG CUCuuCUC | -5.88 | (22) |
| 472 | I110 | GAGauGAG CUCcuCUC | -4.96 | (22) |
| 473 | I111 | GAGauGAG CUCauCUC | -3.85 | (22) |
| 474 | I112 | GAGaaGAG CUCgcCUC | -4.49 | (22) |
| 475 | I113 | GAGuaGAG CUCucCUC | -5.41 | (22) |
| 476 | I114 | GAGuuGAG CUCucCUC | -5.10 | (22) |
| 477 | I115 | GAGacGAG CUCguCUC | -4.69 | (22) |
| 478 | I116 | GAGgcGAG CUCacCUC | -4.47 | (22) |
| 479 | I117 | GAGacGAG CUCgcCUC | -4.53 | (22) |
| 480 | I118 | GAGucGAG CUCucCUC | -5.31 | (22) |
| 481 | I119 | GAGacGAG CUCccCUC | -3.34 | (22) |
| 482 | I120 | GAGacGAG CUCacCUC | -3.03 | (22) |
| 483 | I121 | GAGgcGAG CUCaaCUC | -5.14 | (22) |
| 484 | I122 | GAGacGAG CUCgaCUC | -4.81 | (22) |
|   |   |   |   |   |
|---|---|---|---|---|
| 485 | I123 | GAGucGAG | CUCuaCUC | -5.24 | (22) |
| 486 | I124 | GAGacGAG | CUCcaCUC | -4.14 | (22) |
| 487 | I125 | GAGacGAG | CUCaaCUC | -4.58 | (22) |
| 488 | I126 | GAGgaGAG | CUCaaCUC | -5.55 | (22) |
| 489 | I127 | GAGaaGAG | CUCgaCUC | -4.90 | (22) |
| 490 | I128 | GAGuaGAG | CUCuaCUC | -3.77 | (22) |
| 491 | I129 | GAGaaGAG | CUCaaCUC | -4.71 | (22) |
| 492 | I130 | GGAagUCC | CCUgaAGG | -4.64 | (43) |
| 493 | I131 | GGUagACC | CCagaUGG | -5.13 | (43) |
| 494 | I132 | GCAggUGC | CGUggACG | -4.43 | (25) |
| 495 | I133 | GCUggAGC | CGAggUCG | -4.26 | (25) |
| 496 | I134 | GUGgcGUG | CACguCAC | -5.30 | (25) |
| 497 | I135 | GAGcgGAG | CUCugCUC | -5.69 | (25) |
| 498 | I136 | GAGcgGAG | CUCagCUC | -5.70 | (25) |
| 499 | I137 | GAGgcGAG | CUCguCUC | -5.66 | (25) |
| 500 | I138 | CUGgaGUC | GACggCAG | -5.00 | (25) |
| 501 | I139 | GAGgcGAG | CUCgaCUC | -5.57 | (25) |
| 502 | I140 | CUGagGUC | GACggCAG | -5.11 | (25) |
| 503 | I141 | GUGgaGUG | CACgaCAC | -4.63 | (25) |
| 504 | I142 | GUGagGUG | CACagCAC | -4.49 | (25) |
| 505 | I143 | GAGguGAG | CUCgcCUC | -5.02 | (25) |
|   |   |   |   |   |   |
|---|---|---|---|---|---|
| 506 | I144 | GAGugGAG CUCcgCUC | -4.98 | (25) |
| 507 | I145 | GAGguGAG CUCguCUC | -4.56 | (25) |
| 508 | I146 | GAGugGAG CUCugCUC | -3.86 | (25) |
| 509 | I147 | GAGuuuGAG CUCccCUC | -3.54 | (22) |
| 510 | I148 | GAGcgGAG CUCcaCUC | -5.92 | (22) |
| 511 | I149 | GAGcaGAG CUCagCUC | -5.60 | (22) |
| 512 | I150 | CGCaaGCG GCGaaCGC | -5.44 | (17) |
| 513 | I151 | CCACg__CUCC GGUgaaaGAGG | -9.65 | (24) |
| 514 | I152 | CGACg__GCAG GCUgaaCGUC | -8.34 | (24) |
| 515 | I153 | UCAGc__GUGA AGUCcauCACU | -5.73 | (24) |
| 516 | I154 | CGACa__GCAG GCUgaaCGUC | -8.01 | (24) |
| 517 | I155 | CCACa__CUCC GGUgaaaGAGG | -9.18 | (24) |
| 518 | I156 | UCCGa__CGCA AGGCaaGCGU | -9.02 | (24) |
| 519 | I157 | GGCu__CGG CGGuuuGCC | -6.03 | (24) |
| 520 | I158 | UGACu__CUCA ACUGcuuGAGU | -6.77 | (24) |
| 521 | I159 | UCACu__CUGA AGUCcuuGACU | -5.30 | (24) |
| 522 | I160 | UGACa__CUCA ACUGaacGAGU | -6.59 | (24) |
| 523 | I161 | UGAGa__GUCA ACUCcgaCAGU | -6.55 | (24) |
| 524 | I162 | UGACa__CUCA ACUGaaGAGU | -6.55 | (17) |
| 525 | I163 | UGACc__CUCA ACUGcuuGAGU | -6.34 | (24) |
| 526 | I164 | AGGCu__CGGA UCCguuuGCCU | -9.25 | (24) |
| 527 | I165 | UGAGa__GUCA  
     |      | ACUCaaaCAGU | -6.16 | (17) |
| 528 | I166 | UCCGa__CGCA  
     |      | AGGCggaACGU | -8.13 | (24) |
| 529 | I167 | AAGGCu__CGGAA  
     |      | UUCCGuuuGCCUU | -11.60 | (24) |
| 530 | I168 | UCCUg__UGCA  
     |      | AGGAgagACGU | -6.99 | (24) |
| 531 | I169 | UCCUa__UGCA  
     |      | AGGAgagACGU | -6.63 | (24) |
| 532 | I170 | UCCUa__UGCA  
     |      | AGGGaagaACGU | -6.56 | (24) |
| 533 | I171 | AAGGUc__UGGA  
     |      | UUCCAuuuACCUU | -7.87 | (24) |
| 534 | I172 | UCCUg__UGCA  
     |      | AGGGaagaACGU | -6.17 | (24) |
| 535 | I173 | UCCUa__UGCA  
     |      | AGGGaagaACGU | -6.18 | (24) |
| 536 | I174 | UCCUa__UGCA  
     |      | AGGAgaaACGU | -6.09 | (24) |
| 537 | I175 | UCCUa__UGCA  
     |      | AGGAgaaACGU | -6.09 | (24) |
| 538 | I176 | ACCUc__UGGC  
     |      | UGGAacaAACG | -5.02 | (24) |
| 539 | I177 | CCUCUc__GUUGA  
     |      | GGAGAaaaCCGCU | -9.86 | (24) |
| 540 | I178 | GAGUg__UGAC  
     |      | CUCGaagGCUG | -5.07 | (43) |
| 541 | I179 | GAGCga__CGAC  
     |      | CUCGaagGCUG | -10.51 | (24) |
| 542 | I180 | CCACgg__CUUG  
     |      | GGUGagaGAGG | -9.84 | (24) |
| 543 | I181 | GAGCaa__CGAC  
     |      | CUCGaagGCUG | -9.23 | (24) |
| 544 | I182 | CGACga__CGAG  
     |      | CUCGgaaCGUC | -8.44 | (24) |
| 545 | I183 | CCUCUgc__GUUGA  
     |      | GGAGAaaaCCGCU | -11.57 | (24) |
| 546 | I184 | UGACuu__CUCA  
     |      | ACUGuuuGAGU | -7.36 | (24) |
| 547 | I185 | GAGCag__CGAC  
     |      | CUCGgaaGCUG | -8.80 | (24) |
|    | Codon | Sequence                  | ΔG (kcal/mol) | Ref |
|----|-------|---------------------------|---------------|-----|
| 548| I186  | GAGG_ga.CGAC             | -8.77         | 24  |
| 549| I187  | CCAG_gg.CUCC             | -9.22         | 24  |
| 550| I188  | GAGCaa.CGAC             | -8.61         | 24  |
| 551| I189  | UGACuu.CUCA             | -6.99         | 24  |
| 552| I190  | GAGCag.CGAC             | -8.50         | 24  |
| 553| I191  | UCACuu.CUGA             | -5.34         | 24  |
| 554| I192  | ACCUgc.UUGC             | -7.33         | 24  |
| 555| I193  | UCAGcc.GUGA             | -5.11         | 24  |
| 556| I194  | UGAGaa.GUCA             | -6.59         | 17  |
| 557| I195  | UGAGaa.GUCA             | -6.58         | 24  |
| 558| I196  | GAGCag.CGAC             | -8.14         | 24  |
| 559| I197  | CUGUgg.AC_              | -5.45         | 24  |
| 560| I198  | GAGCaa.CGAC             | -7.99         | 24  |
| 561| I199  | GAGUaa.CGAC             | -7.96         | 43  |
| 562| I200  | GAGUga.UGAC             | -6.99         | 43  |
| 563| I201  | GAGCaa.UGAC             | -7.48         | 43  |
| 564| I202  | CUGUau.GACG             | -6.58         | 43  |
| 565| I203  | GAGUaa.CGAC             | -6.62         | 43  |
| 566| I204  | GAGUaa.UGAC             | -5.68         | 43  |
| 567| I205  | GAGUga.UGAC             | -5.52         | 43  |
| 568| I206  | GAGCaa.UGAC             | -6.23         | 43  |
|   |   |   |   |   |   |
|---|---|---|---|---|---|
| 569 | I207 | GAGUag\_UGAC CUCGaaaGCUG | -4.79 | (43) |
| 570 | I208 | GAGUaa\_UGAC CUCGaaaGCUG | -4.70 | (43) |
| 571 | I209 | UGACuuCUCA ACU GCCucuGAGU | -6.31 | (24) |
| 572 | I210 | CGACga\_GCA GCUG aagCGUC | -9.77 | (44) |
| 573 | I211 | UGACA_a\_CUCA ACUGaaaGAGU | -6.12 | (17) |
| 574 | I212 | UGAGa__GUCA ACUCaaaGAGU | -5.56 | (17) |
| 575 | I213 | UGAGaaaGUCA ACUCaaaGAGU | -6.67 | (17) |
| 576 | I214 | UGACaa__CUCA ACUGaaaGAGU | -6.07 | (17) |
| 577 | I215 | UGAGaa__GUCA ACUCaaaGAGU | -6.00 | (17) |
| 578 | I216 | UGACa_____CUCA ACUGaaaGAGU | -5.71 | (17) |
| 579 | I217 | UGAGa____GUCA ACUCaaaGAGU | -5.30 | (17) |
| 580 | I218 | UGACcaaaCUCA ACUGaaaGAGU | -7.14 | (17) |
| 581 | I219 | UGACaaaCUCA ACUGaacGAGU | -7.17 | (17) |
| 582 | I220 | CGCaaaGCCGGa a CGC | -4.88 | (17) |
| 583 | I221 | CGGaaaCCCG GCCaaaaGCC | -4.64 | (17) |
| 584 | I222 | GCGaaaCGCG GCCaaaaGCC | -4.27 | (26) |
| 585 | I223 | CGCaaaGCCCGa a GCC | -5.85 | (26) |
| 586 | I224 | CGGaaaCGCG GCCaaaaGCC | -5.00 | (26) |
| 587 | I225 | CGCauaGGCG GCCa uGCGa uGCG | -6.05 | (26) |
| 588 | I226 | CGCauaGGCG GCCaaaGCC | -6.14 | (26) |
| 589 | I227 | GAGUgaaUGAC CUCAagaGCUG | -7.01 | (26) |
|   |   | Sequence                  | Intron | Stability | (nt) |
|---|---|---------------------------|--------|-----------|------|
| 590 | I228 | GAGCagaCGAC             | CUCGagaGCUG | -8.36 | (26) |
| 591 | I229 | CGCagaGGC               | GCGaugCCG | -6.33 | (26) |
| 592 | I230 | GAGCguaCGAC            | CUCGauaGCUG | -8.48 | (26) |
| 593 | I231 | CGCaaaGGC               | GCGauaCCG | -6.56 | (26) |
| 594 | I232 | GAGCagaCGAC            | CUCGauaGCUG | -8.78 | (26) |
| 595 | I233 | GAGCagaCGAC            | CUCGauaGCUG | -8.73 | (26) |
| 596 | I234 | CGGcacCCG              | GCCcacGGC | -4.70 | (26) |
| 597 | I235 | GAGCggaCGAC            | CUCGauaGCUG | -8.89 | (26) |
| 598 | I236 | CGCaaaGGC              | GCGaaaCCG | -6.78 | (26) |
| 599 | I237 | GCNgaaUGC              | GUuagACG | -4.58 | (26) |
| 600 | I238 | CGCucuGGC              | GCGucuCCG | -6.90 | (26) |
| 601 | I239 | GAGCaaacCGAC          | CUCGauaGCUG | -9.12 | (26) |
| 602 | I240 | CGCaaacGGC            | GCGaacCCG | -6.98 | (26) |
| 603 | I241 | GAGCcgacAGAC          | CUCGagaGCUG | -9.13 | (26) |
| 604 | I242 | CGACgcaCGAC           | GCUuaaaCGUC | -8.43 | (26) |
| 605 | I243 | CGGaacaCCG           | GCGacaGGC | -5.07 | (26) |
| 606 | I244 | GAGCugCCGAC           | CUCGuauGCUG | -9.26 | (26) |
| 607 | I245 | GAGCgaCGAC            | CUCGauaGCUG | -9.23 | (26) |
| 608 | I246 | GAGCgaaCGAC           | CUCGaaaGCUG | -9.32 | (26) |
| 609 | I247 | GCUuaaaAGC           | CAAagUCG | -5.13 | (26) |
| 610 | I248 | GAGCgaaCGAC           | CUCGauaGCUG | -9.34 | (26) |
|    |    | DNA Sequence                  | RNA Sequence                  | ΔG (kcal/mole) | Ref. |
|----|----|------------------------------|------------------------------|---------------|------|
| 611 | I249 | GAGCgagCGAC CUCGauaGCUG | -9.45 (26) | |
| 612 | I250 | GCCgaaGCC CGGaagCGG | -7.74 (26) | |
| 613 | I251 | CGGaagCGC GCCguaaGCG | -6.33 (26) | |
| 614 | I252 | GAGCgagCGAC CUCGaaGCU | -9.60 (26) | |
| 615 | I253 | GAGCgagCGAC CUCGaagGCUG | -9.64 (26) | |
| 616 | I254 | CGCgaaGCC GCGaccCCG | -7.48 (26) | |
| 617 | I255 | GAGCuuguCGAC CUCGuauGCUG | -9.72 (26) | |
| 618 | I256 | CGCgaaGCC GCGaaaCCG | -7.67 (26) | |
| 619 | I257 | CGGaagCGC GCCgaaGCG | -6.60 (26) | |
| 620 | I258 | GCGuuuGC CCGuucuGGC | -6.46 (26) | |
| 621 | I259 | CGCucuGGC GCGuuuCGC | -7.83 (26) | |
| 622 | I260 | CGACgcaGCAG CUGaagCGUC | -9.22 (26) | |
| 623 | I261 | CGCuuuGGC GCGucuCCG | -7.85 (26) | |
| 624 | I262 | CGCaagGCC GCGgaaGC | -6.02 (26) | |
| 625 | I263 | GCGgaaCGG CGCaugGCC | -6.65 (26) | |
| 626 | I264 | CGCuuuGGC GCGuuuCGC | -7.84 (26) | |
| 627 | I265 | CGCgaaGCC GCGaugCCG | -7.97 (26) | |
| 628 | I266 | GAGCgagCGAC CUCGagaGCUG | -10.12 (26) | |
| 629 | I267 | CGCauaGCC GCGaagCCG | -7.98 (26) | |
| 630 | I268 | GCGgaaCGC CGCaagGCC | -6.75 (26) | |
| 631 | I269 | GCGguaCGG CGCaugGCC | -7.73 (26) | |
| #   | Gene   | Sequence                  | Score  | Ref   |
|-----|--------|---------------------------|--------|-------|
| 632 | I270   | CGCaaaGGC                | -8.26  | (26)  |
| 633 | I271   | GAGCgaaCGAC              | -10.55 | (26)  |
| 634 | I272   | CGCgaaGGC                | -8.41  | (26)  |
| 635 | I273   | GAGCgaaCGAC              | -10.75 | (26)  |
| 636 | I274   | CGCgaaGGC                | -8.65  | (26)  |
| 637 | I275   | GAGCagaCGAC              | -10.98 | (26)  |
| 638 | I276   | GAGCcgaCGAC              | -11.02 | (26)  |
| 639 | I277   | CGCagaGGC                | -9.01  | (26)  |
| 640 | I278   | CGACcgaGCAG              | -10.55 | (26)  |
| 641 | I279   | GAGCggaCGAC              | -11.32 | (26)  |
| 642 | I280   | CGCgaaGGC                | -10.28 | (26)  |
| 643 | I281   | GAGCggaCGAC              | -12.48 | (26)  |
| 644 | I282   | GCgagCGG                 | -6.68  | (41)  |
| 645 | I283   | GCaaaCGG                 | -4.20  | (41)  |
| 646 | I284   | GCagGCGG                 | -6.33  | (41)  |
| 647 | I285   | GCgagCGG                 | -4.01  | (41)  |
| 648 | I286   | GCgagGCA                 | -6.78  | (41)  |
| 649 | I287   | GCgagGCU                 | -5.84  | (41)  |
| 650 | I288   | CAGgaCUG                 | -6.13  | (18)  |
| 651 | I289   | GAGgaCUC                 | -7.00  | (18)  |
| 652 | I290   | GCgacCGG                 | -9.66  | (18)  |
| ID  | Amino Acid  | Nucleotide Sequence       | Stability | Ref. |
|-----|-------------|---------------------------|-----------|------|
| 653 | I291        | GCUGaGGC CGGagUGC         | -6.70     | (18) |
| 654 | I292        | GCGgaUGC CGUagGCG         | -4.63     | (18) |
| 655 | I293        | GCUaaGGC CGGaaUCG         | -4.71     | (19) |
| 656 | I295        | UGACu_CUC ACUGcuGAGU      | -9.50     | (40) |
| 657 | I296        | UGAGa_GUCA ACUCagCAGU     | -8.74     | (40) |
| 658 | I297        | UGAGa_GUCA ACUCggCAGU     | -8.64     | (40) |
| 659 | I301        | CUGUgaUGAC GACGagGCUG     | -8.45     | (43) |
| 660 | I302        | GAGUaaCGAC CUCGaaGCUG     | -7.87     | (43) |
| 661 | I303        | GAGUgaUGAC CUCGaaGCUG     | -6.72     | (43) |
| 662 | I304        | GCGaugUGC CGUgaGCg        | -3.84     | (43) |
| 663 | I305        | GAGCaUGAC CUCGaaGCUG      | -7.12     | (43) |
| 664 | I306        | CUGUagGCAG GACGgaUGUC     | -5.50     | (43) |
| 665 | I307        | GAGUagUGAC CUCGaaGCUG     | -5.18     | (43) |
| 666 | I308        | GAGUaaUGAC CUCGaaGCUG     | -5.22     | (43) |
Supplementary Table 6: Multibranch Loop Duplex Experiment List

Note: The top strand is shown 5’ to 3’. The bottom strand is shown 5’ to 3’. Unpaired nucleotides are in lower case. The underscore character (_) is used to delimit parts of the sequence that form different helices without intervening unpaired nucleotides.

| Index | ID# | Duplex | Folding Free Energy Change (kcal/mol) | Reference |
|-------|-----|--------|--------------------------------------|-----------|
| 667   | M1  | GGAG\_CGGCuucgGCC\_GACG CGUCaaCUCC | -5.42 (45) |           |
| 668   | M2  | GGAGaCGGCuucgGCC\_GACG CGUCauaCUCC | -4.05 (45) |           |
| 669   | M3  | GGAGaCGGCuucgGCC\_GCAG CUGCaauaCUCC | -5.85 (45) |           |
| 670   | M4  | GGAGgCGCuucgGCCuGACG CGUCcauaCUCC | -6.01 (45) |           |
| 671   | M5  | GGAGaCGCuucgGCCGcGACG CGUCauaCUCC | -4.78 (45) |           |
| 672   | M6  | GGAGgCGCuucgGCCGuGACG CGUCauaCUCC | -6.17 (45) |           |
| 673   | M7  | GGAG\_CGGCuucgGCC\_GACG CGUC\_CUCC | -5.71 (45) |           |
| 674   | M8  | GGAGaCGCuucgGCCGcGACG CGUCccuaCUCC | -4.59 (45) |           |
| 675   | M9  | GGAG\_CGGCuucgGCC\_GACG CGUCauaCUCC | -5.43 (45) |           |
| 676   | M10 | GGAG\_CGGCuucgGCC\_GACG CGUccauaCUCC | -5.92 (45) |           |
| 677   | M11 | GGAGaCGCuucgGCCGcGACG CGUccauaCUCC | -5.25 (45) |           |
| 678   | M12 | GGAGaCGCuucgGCCGaGACG CGUccauaCUCC | -5.91 (45) |           |
| 679   | M13 | GGCAG\_CGGCuucgGCCG\_GGAG GCUCC\_CUGCC | -8.38 (46) |           |
| 680   | M14 | GGCAG\_CGGCuucgGCCG\_GGAG GCUCCaaCUCC | -10.41 (46) |         |
| 681   | M15 | GGCAG\_CGGCuucgGCCG\_GGAG GCUCCaaCUCC | -11.51 (46) |         |
| 682   | M16 | GGCAG\_CGGCuucgGCCG\_GGAG CCUCaaCUCC | -11.48 (46) |         |
| 683   | M17 | GGCAG\_CGGCuucgGCCG\_GGAG CCUCauaCUCC | -12.88 (46) |         |
| 684   | M18 | GGCAG\_CGGCuucgGCCG\_GGAG CCUCaaCUCC | -12.47 (46) |         |
| 685   | M19 | GGCAG\_CGGCuucgGCCG\_GGAG CCUC\_CUGCC | -8.80 (46) |           |
| 686   | M20 | GGCAG\_CGGCuucgGCCG\_GGAG CCUC\_CUGCC | -10.54 (46) |         |
| 687   | M21 | GGCAG\_CGGCuucgGCCG\_GGAG CCUCaaCUCC | -11.13 (46) |         |
|    |   |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
|----|----|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 688| M22| GGCAGaGCGCuucgGCGC\_GGAGG CCUCaaaCUGGCC | -10.69 | (46) |
| 689| M23| GGCAGaGCGCuucgGCGC\_GGAGG CCUCauaCUGGCC | -11.35 | (46) |
| 690| M24| GGCAGaGCGCuucgGCGC\_GGAGG CCUCaaaCUUGCC | -10.98 | (46) |
| 691| M25| GGCAGaGCGCuucgGCGC\_GGAGG CCUC\_CUUGCC | -9.72 | (46) |
| 692| M26| GGCAGaGCGCuucgGCGC\_GGAGG CCUCCaCUGGCC | -11.59 | (46) |
| 693| M27| GGCAGaGCGCuucgGCGC\_GGAGG CCUCaaCUUGCC | -11.86 | (46) |
| 694| M28| GGCAGaGCGCuucgGCGC\_GGAGG CCUCaCUUGCC | -11.26 | (46) |
| 695| M29| GGCAGaGCGCuucgGCGC\_GGAGG CCUCauaCUGGCC | -11.48 | (46) |
| 696| M30| GGCAGaGCGCuucgGCGC\_GGAGG CCUCaaCUUGCC | -10.97 | (46) |
| 697| M31| GGCAGaGCGCuucgGCGCaGGAGG CCUC\_CUUGCC | -11.66 | (46) |
| 698| M32| GGCAGaGCGCuucgGCGCaGGAGG CCUCCaCUUGCC | -12.89 | (46) |
| 699| M33| GGCAGaGCGCuucgGCGCaGGAGG CCUCaaCUUGCC | -13.05 | (46) |
| 700| M34| GGCAGaGCGCuucgGCGCaGGAGG CCUCaaCUUGCC | -13.15 | (46) |
| 701| M35| GGCAGaGCGCuucgGCGCaGGAGG CCUCauaCUGGCC | -12.93 | (46) |
| 702| M36| GGCAGaGCGCuucgGCGCaGGAGG CCUCaaCUUGCC | -12.37 | (46) |
| 703| M37| GGCAGaGCGCuucgGCGCaGGAGG CCUC\_CUUGCC | -12.50 | (46) |
| 704| M38| GGCAGaGCGCuucgGCGCaGGAGG CCUCCaCUUGCC | -13.15 | (46) |
| 705| M39| GGCAGaGCGCuucgGCGCaGGAGG CCUCaaCUUGCC | -12.88 | (46) |
| 706| M40| GGCAGaGCGCuucgGCGCaGGAGG CCUCaaCUUGCC | -12.64 | (46) |
| 707| M41| GGCAGaGCGCuucgGCGCaGGAGG CCUCauaCUGGCC | -13.23 | (46) |
| 708| M42| GGCAGaGCGCuucgGCGCaGGAGG CCUCaaCUUGCC | -12.95 | (46) |
| 709| M43| GGCAG\_UCGcuucgGCGA\_GGAGG CCUC\_CUUGCC | -9.15 | (46) |
| 710| M49| GGCAGaUCGcuucgGCGA\_GGAGG CCUC\_CUUGCC | -9.84 | (46) |
| 711| M50| GGCAGaUCGcuucgGCGA\_GGAGG CCUCCaCUUGCC | -11.79 | (46) |
| 712| M51| GGCAGaUCGcuucgGCGA\_GGAGG CCUCaaCUUGCC | -12.38 | (46) |
| 713| M52| GGCAGaUCGcuucgGCGA\_GGAGG | -12.00 | (46) |
|    |   |    |    |
|----|----|----|----|
| 714 | M53 | GCCGaugCCGcuucgGCCG_A_GAGG | -12.06 (46) |
| 715 | M54 | GCCGaugCCGcuucgGCCG_A_GAGG | -11.91 (46) |
| 716 | M55 | GCCG_CGGCcuucgGGCG_CGCGgcaaaGCGC_GGAGG | -16.48 (46) |
| 717 | M56 | GCCG_CGGCcuucgGGCG_CGCGgcaaaGCGC_GGAGG | -13.90 (46) |
| 718 | M57 | GCCG_CGGCcuucgGGCG_CGCGgcaaaGCGC_GGAGG | -12.40 (46) |
| 719 | M58 | GCCG_CGGCcuucgGGCG_CGCGgcaaaGCGC_GGAGG | -11.19 (46) |
| 720 | M59 | GCCG_CGGCcuucgGGCG_CGCGgcaaaGCGC_GGAGG | -10.46 (46) |
| 721 | M60 | GCCG_CGGCcuucgGGCG_CGCGgcaaaGCGC_GGAGG | -13.12 (46) |
| 722 | M61 | GCCG_CGGCcuucgGGCG_CGCGgcaaaGCGC_GGAGG | -15.40 (46) |
| 723 | M62 | GCCG_CGGCcuucgGGCG_CGCGgcaaaGCGC_GGAGG | -13.52 (46) |
| 724 | M63 | GCCG_CGGCcuucgGGCG_CGCGgcaaaGCGC_GGAGG | -11.86 (46) |
| 725 | M64 | GCCG_CGGCcuucgGGCG_CGCGgcaaaGCGC_GGAGG | -12.31 (46) |
| 726 | M65 | GCCG_CGGCcuucgGGCG_CGCGgcaaaGCGC_GGAGG | -11.18 (46) |
| 727 | M66 | GCCG_CGGCcuucgGGCG_CGCGgcaaaGCGC_GGAGG | -13.58 (46) |
| 728 | M67 | GCCG_CGGCcuucgGGCG_CGCGgcaaaGCGC_GGAGG | -13.36 (46) |
| 729 | M68 | GCCG_CGGCcuucgGGCG_CGCGgcaaaGCGC_GGAGG | -14.76 (46) |
| 730 | M69 | GCCG_CGGCcuucgGGCG_CGCGgcaaaGCGC_GGAGG | -13.01 (46) |
| 731 | M70 | GCCG_CGGCcuucgGGCG_CGCGgcaaaGCGC_GGAGG | -12.59 (46) |
| 732 | M71 | GCCG_CGGCcuucgGGCG_CGCGgcaaaGCGC_GGAGG | -12.66 (46) |
| 733 | M72 | GCCG_CGGCcuucgGGCG_CGCGgcaaaGCGC_GGAGG | -11.77 (46) |
| 734 | M73 | GCCG_CGGCcuucgGGCG_CGCGgcaaaGCGC_GGAGG | -12.65 (46) |
| 735 | M74 | GCCG_CGGCcuucgGGCG_CGCGgcaaaGCGC_GGAGG | -12.77 (46) |
Supplementary Table 7: Bulge Loop Duplex Experiment List

Note: The top strand is shown 5’ to 3’. The bottom strand is shown 3’ to 5’. Lower case letters in the top strand indicate unpaired nucleotides. Lower case letters in the bottom strand indicate nucleotides that can base pair with multiple possible nucleotides in the top strand. The underscore character (_) is used to maintain alignment between the top and bottom strands.

| Index | ID# | Duplex | Folding Free Energy Change (kcal/mol) | Reference |
|-------|-----|--------|---------------------------------------|-----------|
| 736   | B1  | GCGaGCG CGC_CGC | -6.76                                  | (14)      |
| 737   | B2  | GCGuGCG CGC_CGC | -6.51                                  | (14)      |
| 738   | B3  | CGCaCGC GCG_GCG | -6.85                                  | (14)      |
| 739   | B4  | GCGaaGCG CGC___CGC | -5.17                                | (14)      |
| 740   | B5  | GCGuuGCG CGC___CGC | -5.01                                | (14)      |
| 741   | B6  | CGCaGGCG GCG__GCG | -5.33                                  | (14)      |
| 742   | B7  | GCGaaaGCG CGC___CGC | -4.76                                | (14)      |
| 743   | B8  | GCGuuuGCG CGC___CGC | -4.85                                | (14)      |
| 744   | B9  | CGCaGGCG GCG__GCG | -6.63                                  | (14)      |
| 745   | B12 | GCGaaGGCa aCGC__CGC | -6.64                                | (14)      |
| 746   | B13 | CGCaGGCa aCGC__GCG | -7.16                                  | (14)      |
| 747   | B14 | GCGaaaGCGa aCGC___CGC | -5.39                               | (14)      |
| 748   | B15 | CGCaGGCa aCGC___GCG | -7.38                                  | (14)      |
| 749   | B18 | GCGaaGUCa aCGC___CAG | -6.60                                 | (14)      |
| 750   | B19 | GCGaaGUCa aCGC___CAG | -5.07                                 | (14)      |
| 751   | B20 | CCACaCUACC GGUAA_GAUUGG | -9.76                             | (47)      |
| 752   | B21 | GCAaGAGG CGUG_CUCC | -9.07                                  | (47)      |
|   |   |   |   |   |   |
|---|---|---|---|---|---|
| 753| B22| GACCaUGUC | CUGG_ACAG | -8.16 | (47)|
| 754| B23| UGAGaGUCA | ACUC_CAGU | -7.82 | (47)|
| 755| B24| UGACaCUCA | ACUG_GAGU | -7.17 | (47)|
| 756| B25| GACUaUGUC | CUGA_ACAG | -6.06 | (47)|
| 757| B26| CAUGUgACUAC | GUACA__UGAUG | -8.89 | (47)|
| 758| B27| UGACgCUCA | ACUG_GAGU | -7.57 | (47)|
| 759| B28| GACUgUGUC | CUGA_ACAG | -7.40 | (47)|
| 760| B29| GACAgAGUC | CUGU_UCAG | -6.63 | (47)|
| 761| B30| GCACuGAGG | CGUG_CUCC | -9.63 | (47)|
| 762| B31| ACUGuGAGU | UGAC_CUCA | -8.06 | (47)|
| 763| B32| UGACuCUCA | ACUG_GAGU | -7.79 | (47)|
| 764| B33| GACAuAGUC | CUGU_UCAG | -6.48 | (47)|
| 765| B34| CAUGAcGCUAC | GUACU_CGAUG | -10.58 | (47)|
| 766| B35| CAUGUcACUAC | GUACA__UGAUG | -8.61 | (47)|
| 767| B36| UGAgcGUCA | ACUC_CAGU | -8.41 | (47)|
| 768| B37| GACUcUGUC | CUGA_ACAG | -7.29 | (47)|
| 769| B38| UCCUcGAAC | AGGA_CUUG | -6.69 | (47)|
| 770| B39| GACAcAGUC | CUGU_UCAG | -5.93 | (47)|
| 771| B40| GACCaaGUC | CUGG_uCAG | -9.40 | (47)|
| 772| B41| GACGaGUC | CUGC_uCAG | -8.24 | (47)|
| 773| B42| GACaaaGUC | CUG_uuCAG | -6.87 | (47)|
Supplementary Table 8: Coaxial Stacking Experiment List

Note: The top strand is shown 5’ to 3’. The bottom strand is shown 3’ to 5’. Unpaired nucleotides are in lower case. The top strand forms a hairpin loop whose stem can coaxially stack with the stem formed by base pairing with the bottom strand.

| Index | ID# | Duplex | Folding Free Energy Change (kcal/mol) | Reference |
|-------|-----|--------|----------------------------------------|-----------|
| 785   | X1  | GGACGCAGUGGCaaaaaGCCACUGCCUGA      | -7.79      | (48)       |
| 786   | X2  | GGACGCAGUGGCaaaaaGCCACUGCCUG       | -6.28      | (48)       |
| 787   | X3  | GGACGCAGUGGCgcaaGCCACUCCUGA        | -7.41      | (48)       |
| 788   | X4  | GGACGCAGUGGCgcaaGCCACUCCUG         | -5.87      | (48)       |
| 789   | X5  | GGACGCAGUGGCgcaaGCCACGA            | -5.69      | (48)       |
| 790   | X6  | GGACGCAGUGGCgcaaGCCACGA            | -6.67      | (48)       |
| 791   | X7  | GGACACAGUGGCaaaaaGCCACUGCCUGG      | -7.79      | (48)       |
| 792   | X8  | GGACCCAGUGGCaaaaaGCCACUGCCUC       | -5.64      | (48)       |
| 793   | X9  | GGACCAGUGGCaaaaaGCCACUGCCUGC       | -6.41      | (48)       |
| 794   | X10 | GGACGCAGUGGCaaaaaGCCACUGpCCUGAa    | -6.34      | (48)       |
| 795   | X11 | GGACGCAGUGGCaaaaasGCCACUGaCCUGA   | -6.18      | (48)       |
| 796   | X12 | GGACGCAGUGGCaaaaaGCCACUGpCCUGA    | -6.50      | (48)       |
| 797   | X13 | GGACGCAGUGGCaaaaaGCCACUGaCCUGp    | -5.64      | (48)       |
| 798   | X14 | GGACGCAGUGGCaaaaaGCCACUGAgCCUG    | -5.43      | (48)       |
| 799   | X15 | GGACGCAGUGGCgcaaGCCACUaCCUGA      | -6.08      | (48)       |
| 800   | X16 | GGACGCAGUGGCgcaaGCCACUGpCCUGAa    | -6.22      | (48)       |
| 801   | X17 | GGAGCCAGUGGCaaaaaGCCACUGaCCUCa    | -5.12      | (48)       |
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