SUPPLEMENTARY DATA

Elucidation of leak-resistance DNA hybridization chain reaction with universality and extensibility

Shaofei Li$^{1,2,4}$, Pan Li$^1$, Meihong Ge$^{1,4}$, Hongzhi Wang$^{1,3}$, Yizhuang Cheng$^{1,4}$, Gan Li$^5$, Qiang Huang$^5$, Huan He$^1$, Chentai Cao$^{1,4}$, Dongyue Lin$^{1,4}$, Liangbao Yang$^{1,3,*}$

$^1$ Center of Medical Physics and Technology, Hefei Institutes of Physical Science, Chinese Academy of Sciences, Hefei, Anhui 230031, China
$^2$ School of Life Science, Anhui University, Hefei, Anhui 230601, China
$^3$ Cancer Hospital, Chinese Academy of Sciences, Hefei, Anhui 230031, China
$^4$ Department of Chemistry, University of Science and Technology of China, Hefei, Anhui 230026, China
$^5$ State Key Laboratory of Genetic Engineering, School of Life Sciences, Fudan University, Shanghai 200438, China

* To whom correspondence should be addressed. Email: lbyang@iim.ac.
Contents

List of Supplementary Texts:
Text S1. Molecular dynamics simulation

List of Supplementary Figures:
Figure S1. Native polyacrylamide gel electrophoresis and SERS analysis method
Figure S2. Effect of stem length on DNA assembly behavior
Figure S3. Effect of reversed stem length on DNA assembly behavior
Figure S4. Leakage of DNA hairpins based on other sequences
Figure S5. Effect of free energy on DNA assembly behavior
Figure S6. Leakage of hairpin sequences at different temperature
Figure S7. Linear fit of free energy versus length of the sequences at different temperature
Figure S8. Effects of toehold and loop on DNA assembly behavior
Figure S9. Stability analysis of enlarged loops
Figure S10. Stability analysis of other enlarged loops
Figure S11. Effects of DNA concentration and reaction condition on DNA assembly behavior
Figure S12. Effect of initiator on DNA assembly behavior
Figure S13. Isolation and characterization of exosomes
Figure S14. Specific signal amplification of miRNA

List of Supplementary Tables:
Table S1. Evolution of the hairpin based on Dirks and Pierce’s
Table S2. Evolution of the hairpin based on reversed stem
Table S3. Evolution of the other hairpin based on Dirks and Pierce’s
Table S4. Evolution of the other DNA Hairpin sequences
Table S5. Evolution of the hairpin based on changed toehold
Table S6. Modified sequences in stem region by mutation and substitution
Table S7. DNA sequences used in effect of toehold and loop length on DNA assembly behavior
Table S8. DNA sequences used in effect of initiator on HCR
Table S9. DNA Fairpin for detection of miRNA family
Supplementary Text S1. Molecular dynamics simulation

Molecular dynamics was used to simulate the states of DNA in a solvent environment. First, a 3D atomic model of DNA was built with the web server 3D-Nus. Then, in each simulation system, a water cube with a DNA model in its centre was established, and the distance from the cube edges to the model surface was kept to a minimum of 12 Å. To achieve an ionic concentration of 0.15 M, the appropriate proportion of water was replaced with Na\(^+\) and Cl\(^-\) ions, with extra Na\(^+\) ions included for charge neutralization.

All simulations were performed with the program Gromacs-5.0.7, and periodic conditions were applied. The pressure was balanced with standard atmospheric pressure by the Berendsen method, and the temperature was maintained at 300 K by velocity rescaling. The particle mesh Ewald (PME) method was used to calculate the electrostatic interactions, and the cut-off radius for both electrostatic and van der Waals interactions was set to 14 Å. All bonds involved in the system were constrained with the LINCS algorithm, and a time step of 2.0 fs was used.

The force field was selected scrupulously from a series, and eventually Parmbsc1 was selected for use in all the simulations, since it performed perfectly in the DNA atomistic simulations(1). The general form of the molecular force field is:

\[
U = U_b + U_\theta + U_\phi + U_X + U_{vdW} + U_{el}
\]

\[
= \frac{1}{2} \sum_i k_b (r_i - r_{i0})^2 + \frac{1}{2} \sum_i k_\theta (\theta_i - \theta_{i0})^2 + \frac{1}{2} \sum_i V_n (1 + \cos(n\phi - \delta))
\]

\[
+ \frac{1}{2} \sum_i k_\chi \chi^2 + \sum_i \sum_{j=i+1} 4\varepsilon_{ij} \left[ \left( \frac{\sigma_{ij}}{r_{ij}} \right)^{12} - \left( \frac{\sigma_{ij}}{r_{ij}} \right)^6 \right] + \sum_i \sum_{j=i+1} \frac{q_i q_j}{4\pi \varepsilon_0 \varepsilon_r r_{ij}}
\]

where \(r_{i0}, \theta_{i0}\) stands for standard values of bond length and angle; \(k_b, k_\theta\) stands for elastic coefficient of harmonic potential energy; \(n\) stands for the rotation period of the dihedral angle; \(\delta\) stands for the phase; \(V_n, k_\chi\) stands for the height of the barrier; \(\varepsilon_{ij}\) stands for the depth of the potential well of the interaction between atom \(i\) and \(j\); \(\sigma_{ij}\) stands for the distance between two atoms when the potential energy of van der Waals is minimum; \(q_i q_j\) stands for the charge of atom \(i\) and \(j\) respectively; and \(r_{ij}\) stands for the distance between atom \(i\) and \(j\).

The parameterization processes of the force field Parmbsc1 uses a Monte Carlo method to avoid changes in other torsional parameters. It efficiently maintains the force field by fitting the QM-MM difference or residual energy to a Fourier series in the third order(2). It can be represented as follows:

\[
E_{dih} = E_{QM} - E_{ffbsc0(\chi=0)} \quad (I)
\]

\[
E_{dih} = \sum_{torsions} \sum_{n} \frac{V_n}{2} \left[ 1 + \cos(n\phi - \alpha) \right] \quad (II)
\]
Supplementary Figure S1. Native polyacrylamide gel electrophoresis and SERS analysis method. (A) Native polyacrylamide gel electrophoresis with the coated gold nanoparticles. (B) Electrophoretic gel surface in the slot. (C) Nanoparticles characterized by transmission electron microscope. (D) The diagram of SERS detection.
Supplementary Figure S2. Effect of stem length on DNA assembly behavior. (A) and (B) Native polyacrylamide gel electrophoresis analysis as control. (A) The hairpin monomers were analyzed, and no leakage products were produced in the gel loading well. (B) Stem lengths of 13 to 20 bp were tested in response to initiator. Hybridization products were blocked in the gel loading well. x represents the values 13, 15 and 17–20. I represents the initiator sequence.
Supplementary Figure S3. Effect of reversed stem length on DNA assembly behavior. (A) Evolution of the hairpin design. The reversed stem length was changed. bp represents the base pairs. (B) Native polyacrylamide gel electrophoresis analysis for the monomer of DNA hairpin. (C) Native polyacrylamide gel electrophoresis analysis. Normal hybridization products were blocked in the gel loading well. x represents the values 15-20, and l-b represents the initiator sequence. (D) SERS analysis for leakage in the gel loading well. The green band indicates the marker of adenine at approximately 732 cm\(^{-1}\). Spectra a-c correspond to lanes 1-3 in Figure 2D respectively, and d-i correspond to lanes 1-6 in Figure 2E respectively.
Supplementary Figure S4. Leakage of DNA hairpins based other sequences. (A), (B), (C) and (D) Native polyacrylamide gel electrophoresis analysis for leakage. Leakage products were blocked in the gel loading well. (A) The other DNA hairpins based on Dirks and Pierce’s original design were also evolved by changing stem length and toehold base composition. H1C-13 to H1C-18 had the same toehold. HC1-18A, HC1-16A, HC1-14A and HC1-13A had the same toehold. HC1-18B, HC1-16B, HC1-14B and HC1-13A had the same toehold. That leakage occurred in the stem lengths of 13 to 14 bp but not in the longer stem, and three toeholds did not show difference in leakage. (B) The other DNA Hairpin sequences based on miRNA were analyzed. (C) and (D) The DNA Hairpin sequences based on changed toehold were designed.
Supplementary Figure S5. Effect of free energy on DNA assembly behavior. (A) Native polyacrylamide gel electrophoresis assay. Sequences were modified in the stem region by mutation and substitution, and leakage occurred in sequences with higher free energy. Leakage products were blocked in the gel loading well. (B) The free energies of modified sequences. Leakage energies were marked in red. (C) and (D) SERS analysis for leakage in the gel loading well. The green band indicates the marker of adenine at approximately 732 cm$^{-1}$. (C) Spectra a-d correspond to lanes 6-9 in Figure 3B respectively. Spectra e-h correspond to lanes 3-4 and 6-9 in Figure 3C respectively. (D) Spectra a-c correspond to lanes 3-5 in panel A respectively.
Supplementary Figure S6. Leakage of hairpin sequences at different temperature. (A), (C) and (E) Native polyacrylamide gel electrophoresis analysis for leakage products at 25 °C. (B), (D) and (F) Comparison of free energy of sequences at different temperatures. The red value is the free energy of the leakage sequence. Leakage decreased accordingly as the temperature dropped.
Supplementary Figure S7. Linear fit of free energy versus length of the sequences at different temperature. All of them showed a good linear relationship between the free energy of the secondary structure sequence and its length. (A) Sequences from H1-7 to H1-20. (B) Sequences from H2-7 to H2-20. (C) Sequences from H1-10b to H2-20b. (D) Sequences from H2-10b to H2-20b. (E) Sequences derived from HR1. (F) Sequences derived from HR2.
Supplementary Figure S8. Effects of toehold and loop on DNA assembly behavior. (A) Native polyacrylamide gel electrophoresis analysis. Four hairpin species with 8-nt exposed toehold and 8-nt loop were investigated, and no significant leakage occurred. (B) Minimum free energy structures of DNA hairpins with extended loops. Loops of three hairpin species based on H1b-17, H1-18a-17b and H1-181a-17b were enlarged by inserting bases. The enlarged loops did not form additional secondary structures.
Supplementary Figure S9. Stability analysis of enlarged loops. (A) and (B) Native polyacrylamide gel electrophoresis assay for leakage with enlarged hairpin after incubation at 37 °C. No leakage products were blocked in the gel loading well. (A) For 24 h. Hx represents the hairpins H1b-17 and H2b-17. (B) For 48 h. Hy represents the hairpins H1-18a-17b and H2-18a-17b. Hz represents the hairpins H1-181a-17b and H2-181a-17b. (D)-(J) Native polyacrylamide gel electrophoresis assay for hybridization. T-01 or T-02 was incubated with corresponding hairpin. The red triangle indicated the consuming hairpins. The yellow-green triangle indicated the formed dimmers. (C) and (G) In the ratio of 3:2 for 2 h. (D) and (H) In the ratio of 3:2 for 12 h. (E) and (I) In the ratio of 2:3 for 2 h. (F) and (J) In the ratio of 2:3 for 12 h.
**Supplementary Figure S10.** Stability analysis of other enlarged loops. (A)-(F) Native polyacrylamide gel electrophoresis assay for hybridization. T-18a-01 or T-181a-01 was incubated with corresponding hairpin for 48 h. The consumption and products tended to occur in the systems of hairpins with longer loops. The red triangle indicated the consuming hairpins. The yellow-green triangle indicated the formed dimmers. (A) and (D) In the ratio of 3:2. (B) and (E) In the ratio of 2:3. (C) and (F) In the ratio of 3:2. (G) and (H) Molecular dynamics simulations for E18. The molecular conformation of the loop was shown in yellow. (G) Occasionally exposed state of the loop. (H) Restored state of the loop.
Supplementary Figure S11. Effects of DNA concentration and reaction condition on DNA assembly behavior. (A)-(F) Native polyacrylamide gel electrophoresis analysis for leakage. Three hairpin species were investigated without initiators in the reaction buffer at 37 °C, and no significant change in leakage. (A), (C) and (E) For 48 h. (B), (D) and (F) For 72 h. (G) and (H)
Native polyacrylamide gel electrophoresis assay showing the effect of ions on leakage. The symbol + represents the addition of extra salt concentration based on the reaction buffer. (G) Lanes 1–4 and 6-8: assembly efficiency for H1-18a-17b, H2-18a-17b and I-18a; Lanes 5 and 9: leakage evaluation for H1-18a-17b and H2-18a-17b. (H) Lanes 1–4 and 6-8: assembly efficiency for H1-181a-17b, H2-181a-17b and I-181a; Lanes 5 and 9: leakage evaluation for H1-181a-17b and H2-181a-17b.
Supplementary Figure S12. Effect of initiator on DNA assembly behavior. (A) SERS showed the hybridization products. The green band indicated the marker of adenine at approximately 732 cm$^{-1}$. Spectra a–d correspond to lanes 5–8 in Figure 6C respectively. (B)-(E) Native polyacrylamide gel electrophoresis assay for the hybridization products. The shortened initiators with a constant toehold length were incubated in the reaction buffer at 37 °C. The values from -2 to -9 represent the number of truncated nucleotides for initiators. The listed sequences are in Supplementary Table S8. (B) The hairpins are H1C-18 and H2C-18, and the initiators are from T-18-2 to T-18-8 respectively. They correspond to lanes 1–7 respectively. (C) The hairpins are H1-18a-17b and H2-18a-17b, and the initiators are from T-18a-3 to T-18a-8 respectively. They correspond to lanes 1–6 respectively. (D) The hairpins are H1d-20 and H2d-20, and the initiators are from T-20-3 to T-20-8. They correspond to lanes 1–6 respectively. (E) The hairpins are H1E-20 and H2E-20, and the initiators are from T-E20-4 to T-E20-9. They correspond to lanes 1–6 respectively.
Supplementary Figure S13. Isolation and characterization of exosomes. (A) Size distribution of exosomes analysed by dynamic light scattering. The size was distributed around approximately 100 nm. (B) SDS polyacrylamide gel electrophoresis assay for the proteins of urinary exosomes. Lane 1: protein molecular weight standard; Lane 2: proteins of urinary exosomes. The red triangle indicates an obvious band at approximately 60 kDa, which might include the exosome-specific external CD63 protein.
Supplementary Figure S14. Specific signal amplification of miRNA. (A) Hybridization products detected by using SERS. Unmarked is negative controls, including HR1-17C, HR2-17C, RNA and bank. The marker band of adenine around 732 cm$^{-1}$ appears. (B) The detection of hsa-miR-21-5p from nine patients. Lane 10: blank. (C) Native polyacrylamide gel electrophoresis assay for detection of highly similar miRNA. Lane1-4: H1-7b and H2-7b were used as signal amplifiers; lane5-8: H1-7c and H2-7c were used as signal amplifiers; lane9-12: H1-7e and H2-7e were used as signal amplifiers; and lane13-16: H1-7i and H2-7i were used as signal amplifiers. The target could trigger the hybridization reaction only in the corresponding design system. (D) SERS showed the hybridization products. The green band indicated the marker of adenine at approximately 732 cm$^{-1}$. Spectra of H1-7b+H2-7b+7b, H1-7c+H2-7c+7c, H1-7e+H2-7e+7e, H1-7i+H2-7i+7i correspond to lane 1, 6, 11 and 16 in panel C respectively.
**Supplementary Table S1. Evolution of the hairpin based on Dirks and Pierce’s design**

| Name | Strand sequences (5’ to 3’) | Free energy of secondary structure (-kcal mol⁻¹) | Stem GC% |
|------|-----------------------------|-----------------------------------------------|---------|
|      |                             | 37 °C  |          | 25 °C |
| I    | AGTCTAGGATTCGGCGTG**GTTAA**  |        |          |       |
| H1-20| TTAACCACGCGCAATCTTAGACTATCAAAGTATAGTCTAGGATTCGGCGTG | 23.27 | 28.72 | 50.00 |
| H1-19| TTAACCACGCGCAATCTTAGACTATCAAAGTATAGTCTAGGATTCGGCGTG | 22.19 | 27.34 | 47.37 |
| H1-18| TTAACCACGCGCAATCTTAGACTATCAAAGTATAGTCTAGGATTCGGCGTG | 21.81 | 26.76 | 55.56 |
| H1-17| TTAACCACGCGCAATCTTAGACTATCAAAGTATAGTCTAGGATTCGGCGTG | 20.73 | 25.41 | 58.52 |
| H1-15| TTAACCACGCGCAATCTTAGACTATCAAAGTATAGTCTAGGATTCGGCGTG | 17.99 | 22.16 | 60.00 |
| H1-13| TTAACCACGCGCAATCTTAGACTATCAAAGTATAGTCTAGGATTCGGCGTG | 15.93 | 19.58 | 61.54 |
| H1-11| TTAACCACGCGCAATCTTAGACTATCAAAGTATAGTCTAGGATTCGGCGTG | 13.01 | 16.15 | 63.64 |
| H1-10| TTAACCACGCGCAATCTTAGACTATCAAAGTATAGTCTAGGATTCGGCGTG | 11.51 | 14.40 | 60.00 |
| H1-9 | TTAACCACGCGCAATCTTAGACTATCAAAGTATAGTCTAGGATTCGGCGTG | 10.43 | 13.03 | 66.67 |
| H1-7 | TTAACCACGCGCAATCTTAGACTATCAAAGTATAGTCTAGGATTCGGCGTG | 8.53  | 10.66 | 85.71 |
| H2-20| ATAGTCTAGGATTCGGCGTG**GTTAA**ACGCGCAATCTTAGACTATACTTTG | 23.68 | 28.93 | 50.00 |
| H2-19| TAGTCTAGGATTCGGCGTG**GTTAA**ACGCGCAATCTTAGACTATACTTTG | 22.93 | 27.92 | 47.37 |
| H2-18| AGTCTAGGATTCGGCGTG**GTTAA**ACGCGCAATCTTAGACTATACTTTG | 22.22 | 26.97 | 55.56 |
| H2-17| GTCTAGGATTCGGCGTG**GTTAA**ACGCGCAATCTTAGACTATACTTTG | 21.33 | 26.10 | 58.82 |
| H2-15| CTAGGATTCGGCGTG**GTTAA**ACGCGCAATCTTAGACTATACTTTG | 18.69 | 22.78 | 60.00 |
| H2-13| AGGATTCGGCGTG**GTTAA**ACGCGCAATCTTAGACTATACTTTG | 16.34 | 19.80 | 61.54 |
| H2-11| GATTCGGCGTG**GTTAA**ACGCGCAATCTTAGACTATACTTTG | 13.61 | 16.85 | 58.33 |
| H2-10| ATTCGGCGTG**GTTAA**ACGCGCAATCTTAGACTATACTTTG | 11.92 | 14.62 | 60.00 |
| H2-9 | TTCGGCGTG**GTTAA**ACGCGCAATCTTAGACTATACTTTG | 10.68 | 13.14 | 66.67 |
| H2-7 | CGCGTG**GTTAA**ACGCGCAATCTTAGACTATACTTTG | 9.23  | 11.27 | 85.71 |

The underlined bases at the end constituted the exposed toehold, and the blue ones at the middle constituted the sequestered toehold. The red value is the free energy of the leakage sequence. I is the initiator sequence.
The stem regions of DNA hairpins based on Dirks and Pierce's original design were reversed. The underlined bases at the end constituted the exposed toehold, and the blue ones at the middle constituted the sequestered toehold. The red value is the free energy of the leakage sequence. I-b is the initiator sequence.

| Name   | Strand sequences (5’ to 3’)                                           | Free energy of secondary structure (-kcal mol⁻¹) | Stem GC% |
|--------|---------------------------------------------------------------------|-----------------------------------------------|----------|
|        |                                                                     | 37°C          | 25°C         |          |
| I-b    | GGTTAA                                                             | 23.90         | 29.10        | 50.00    |
| H1b-20 | TTAACCTCAGATCTAAGCGGCACATCAAAGTATGTCGGCTTAGGATCTGA                 | 22.82         | 27.72        | 52.63    |
| H1b-19 | TTAACCTCAGATCTAAGCGGCACATCAAAGTATGTCGGCTTAGGATCTGA                 | 21.77         | 26.43        | 55.55    |
| H1b-18 | TTAACCTCAGATCTAAGCGGCACATCAAAGTATGTCGGCTTAGGATCTGA                 | 19.93         | 24.29        | 52.94    |
| H1b-17 | TTAACCTCAGATCTAAGCGGCACATCAAAGTATGTCGGCTTAGGATCTGA                 | 18.88         | 23.00        | 56.25    |
| H1b-16 | TTAACCTCAGATCTAAGCGGCACATCAAAGTATGTCGGCTTAGGATCTGA                 | 16.64         | 20.50        | 53.33    |
| H1b-15 | TTAACCTCAGATCTAAGCGGCACATCAAAGTATGTCGGCTTAGGATCTGA                 | 14.47         | 17.97        | 50.00    |
| H1b-14 | TTAACCTCAGATCTAAGCGGCACATCAAAGTATGTCGGCTTAGGATCTGA                 | 10.39         | 13.39        | 41.67    |
| H1b-10 | TTAACCTCAGATCTAAGCGGCACATCAAAGTATGTCGGCTTAGGATCTGA                 | 7.71          | 10.13        | 40.00    |
| H2b-20 | ATGTGGCGCTTAGGATCTGAAGTTAAATCAGATCTTAAGCGGCACATACTTTG             | 24.23         | 29.44        | 50.00    |
| H2b-19 | TGGCGCGCTTAGGATCTGAAGTTAAATCAGATCTTAAGCGGCACATACTTTG             | 22.99         | 28.03        | 52.63    |
| H2b-18 | TGGCGCGCTTAGGATCTGAAGTTAAATCAGATCTTAAGCGGCACATACTTTG             | 22.29         | 27.25        | 55.56    |
| H2b-17 | TGGCGCGCTTAGGATCTGAAGTTAAATCAGATCTTAAGCGGCACATACTTTG             | 20.10         | 24.59        | 52.94    |
| H2b-16 | GCGGCTTAGGATCTGAAGTTAAATCAGATCTTAAGCGGCACATACTTTG             | 19.40         | 23.82        | 56.25    |
| H2b-15 | GCGGCTTAGGATCTGAAGTTAAATCAGATCTTAAGCGGCACATACTTTG             | 17.26         | 21.23        | 53.33    |
| H2b-14 | GCGGCTTAGGATCTGAAGTTAAATCAGATCTTAAGCGGCACATACTTTG             | 14.99         | 18.79        | 50.00    |
| H2b-12 | GCGGCTTAGGATCTGAAGTTAAATCAGATCTTAAGCGGCACATACTTTG             | 11.01         | 14.13        | 41.67    |
| H2b-10 | GCGGCTTAGGATCTGAAGTTAAATCAGATCTTAAGCGGCACATACTTTG             | 8.37          | 10.83        | 40.00    |
### Supplementary Table S3. Evolution of the other hairpin based on Dirks and Pierce’s

| Name  | Strand sequences (5' to 3') | Free energy of secondary Structure at 37 °C (-kcal mol⁻¹) | Stem GC% |
|-------|-----------------------------|----------------------------------------------------------|----------|
| H1C-18 | TAACAGAAAGCCAAACCAGAAGAGGTGTGAGCTTCTGTGTAATCGAGTAGAAGAAAGCCAAACC | 19.70 | 38.89 |
| H1C-18A | TAACAGAAAGCCAAACCAGAAGAGGTGTGAGCTTCTGTGTAATCGAGTAGAAGAAAGCCAAACC | 19.50 | 38.89 |
| H1C-18B | TAACAGAAAGCCAAACCAGAAGAGGTGTGAGCTTCTGTGTAATCGAGTAGAAGAAAGCCAAACC | 19.30 | 38.89 |
| H1C-16 | ACAAGAAGCCAAACCAGAAGAGGTGTGAGCTTCTGTGTAATCGAGTAGAAGAAAGCCAAACC | 17.60 | 43.75 |
| H1C-16A | ACAAGAAGCCAAACCAGAAGAGGTGTGAGCTTCTGTGTAATCGAGTAGAAGAAAGCCAAACC | 17.70 | 43.75 |
| H1C-16B | ACAAGAAGCCAAACCAGAAGAGGTGTGAGCTTCTGTGTAATCGAGTAGAAGAAAGCCAAACC | 17.30 | 43.75 |
| H1C-15 | ACAAGAAGCCAAACCAGAAGAGGTGTGAGCTTCTGTGTAATCGAGTAGAAGAAAGCCAAACC | 16.25 | 46.67 |
| H1C-14 | ACAAGAAGCCAAACCAGAAGAGGTGTGAGCTTCTGTGTAATCGAGTAGAAGAAAGCCAAACC | 17.60 | 43.75 |
| H1C-14A | ACAAGAAGCCAAACCAGAAGAGGTGTGAGCTTCTGTGTAATCGAGTAGAAGAAAGCCAAACC | 17.50 | 43.75 |
| H1C-14B | ACAAGAAGCCAAACCAGAAGAGGTGTGAGCTTCTGTGTAATCGAGTAGAAGAAAGCCAAACC | 19.30 | 38.89 |
| H1C-13 | ACAAGAAGCCAAACCAGAAGAGGTGTGAGCTTCTGTGTAATCGAGTAGAAGAAAGCCAAACC | 19.41 | 42.86 |
| H1C-13A | ACAAGAAGCCAAACCAGAAGAGGTGTGAGCTTCTGTGTAATCGAGTAGAAGAAAGCCAAACC | 13.71 | 46.15 |
| H1C-13B | ACAAGAAGCCAAACCAGAAGAGGTGTGAGCTTCTGTGTAATCGAGTAGAAGAAAGCCAAACC | 13.61 | 46.15 |
| H2C-18 | TCTCTCTGTGTGAGCTTCTGTGTAATCGAGTAGAAGAAAGCCAAACC | 18.98 | 38.89 |
| H2C-18A | TCTCTCTGTGTGAGCTTCTGTGTAATCGAGTAGAAGAAAGCCAAACC | 19.20 | 38.89 |
| H12-18B | GAGAGAGGTGTGAGCTTCTGTGTAATCGAGTAGAAGAAAGCCAAACC | 19.22 | 38.89 |
| H2C-16 | TCTCTCTGTGTGAGCTTCTGTGTAATCGAGTAGAAGAAAGCCAAACC | 17.67 | 43.75 |
| H2C-16A | TCTCTCTGTGTGAGCTTCTGTGTAATCGAGTAGAAGAAAGCCAAACC | 17.62 | 43.75 |
| H2C-16B | GAGAGAGGTGTGAGCTTCTGTGTAATCGAGTAGAAGAAAGCCAAACC | 17.64 | 43.75 |
| H2C-15 | TCTCTCTGTGTGAGCTTCTGTGTAATCGAGTAGAAGAAAGCCAAACC | 16.53 | 46.67 |
| H2C-14 | TCTCTCTGTGTGAGCTTCTGTGTAATCGAGTAGAAGAAAGCCAAACC | 14.78 | 42.86 |
| H2C-14A | TCTCTCTGTGTGAGCTTCTGTGTAATCGAGTAGAAGAAAGCCAAACC | 14.73 | 42.86 |
| H2C-14B | GAGAGAGGTGTGAGCTTCTGTGTAATCGAGTAGAAGAAAGCCAAACC | 14.75 | 42.86 |
| H2C-13 | TCTCTCTGTGTGAGCTTCTGTGTAATCGAGTAGAAGAAAGCCAAACC | 13.78 | 46.15 |
| H2C-13A | GAGAGAGGTGTGAGCTTCTGTGTAATCGAGTAGAAGAAAGCCAAACC | 13.73 | 46.15 |
| H2C-13B | GAGAGAGGTGTGAGCTTCTGTGTAATCGAGTAGAAGAAAGCCAAACC | 13.75 | 46.15 |

The other hairpin based on Dirks and Pierce’s were designed. The underlined bases at the end constituted the exposed toehold, and the blue ones at the middle constituted the sequestered toehold. The red value is the free energy of the leakage sequence.
### Supplementary Table S4. Evolution of the other DNA Hairpin sequences

| Name                | Strand sequences (5’ to 3’) | Free energy of secondary structure (kcal mol⁻¹) | Stem GC% |
|---------------------|-----------------------------|-----------------------------------------------|----------|
|                     |                             | 37 °C | 25 °C |                   |
| hsa-miR-21-5p       | UAGCUUAUCAGACUGAUUGUAAG     |       |       |                    |
| HR1-17C             | TCAACA TCGTCTGATAAGCTCTCAAAATG TTAGCTCTATCGACTGA | 17.23 | 21.67 | 41.17              |
| HR1-17T             | TCAACA TCGTCTGATAAGCTCTCAAAATG TTAGCTCTATCGACTGA | 16.47 | 20.91 | 35.29              |
| HR1-16              | TCAACA TCGTCTGATAAGCTCTCAAAATG TTAGCTCTATCGACTGA | 15.39 | 19.53 | 37.50              |
| HR1-15              | TCAACA TCGTCTGATAAGCTCTCAAAATG TTAGCTCTATCGACTGA | 15.01 | 18.94 | 40.00              |
| HR1-14              | TCAACA TCGTCTGATAAGCTCTCAAAATG TTAGCTCTATCGACTGA | 13.93 | 17.59 | 42.86              |
| HR1-13              | TCAACA TCGTCTGATAAGCTCTCAAAATG TTAGCTCTATCGACTGA | 11.69 | 15.10 | 38.46              |
| HR1-12              | TCAACA TCGTCTGATAAGCTCTCAAAATG TTAGCTCTATCGACTGA | 10.01 | 13.10 | 33.33              |
| HR1-11              | TCAACA TCGTCTGATAAGCTCTCAAAATG TTAGCTCTATCGACTGA | 9.01  | 11.83 | 36.36              |
| HR2-17T             | TATAGCTTATCGACTGATGTGATCGTCTGATAAGCTTATACCTTTG | 17.73 | 22.26 | 35.29              |
| HR2-17C             | TATAGCTTATCGACTGATGTGATCGTCTGATAAGCTTATACCTTTG | 18.68 | 23.51 | 41.18              |
| HR2-16              | TATAGCTTATCGACTGATGTGATCGTCTGATAAGCTTATACCTTTG | 16.98 | 21.25 | 37.50              |
| HR2-15              | AGCTTATCGACTGATGTGATCGTCTGATAAGCTTATACCTTTG | 16.27 | 20.30 | 40.00              |
| HR2-14              | AGCTTATCGACTGATGTGATCGTCTGATAAGCTTATACCTTTG | 15.38 | 19.43 | 42.86              |
| HR2-13              | AGCTTATCGACTGATGTGATCGTCTGATAAGCTTATACCTTTG | 13.24 | 16.85 | 38.46              |
| HR2-12              | AGCTTATCGACTGATGTGATCGTCTGATAAGCTTATACCTTTG | 11.71 | 14.35 | 33.33              |
| HR2-11              | AGCTTATCGACTGATGTGATCGTCTGATAAGCTTATACCTTTG | 10.70 | 13.55 | 36.36              |
| hsa-let-7a-5p       | UAGGUAGGUAGGUUUGUAGUAG     |       |       |                    |
| H1-7a-16            | AACTAT ACACAACCCTACCTCACA AAAAGTTGAGGTAGTTGTTG | 16.60 | 43.75 |                   |
| H1-7a-15            | AACTAT ACACAACCCTACCTCACA AAAAGTTGAGGTAGTTGTTG | 15.55 | 46.67 |                   |
| H1-7a-14            | AACTAT ACACAACCCTACCTCACA AAAAGTTGAGGTAGTTGTTG | 14.06 | 42.86 |                   |
| H1-7a-13            | AACTAT ACACAACCCTACCTCACA AAAAGTTGAGGTAGTTGTTG | 12.97 | 46.15 |                   |
| H2-7a-16            | TGAGGTAGTTGTTGTTAGTTACACA CCTAATCTACCCAAGTTTTCCT | 17.19 | 43.75 |                   |
| H2-7a-15            | TGAGGTAGTTGTTGTTAGTTACACA CCTAATCTACCCAAGTTTTCCT | 16.49 | 46.67 |                   |
| H2-7a-14            | AGGTAGTTGTTGTTAGTTACACA CCTAATCTACCCAAGTTTTCCT | 14.80 | 42.86 |                   |
| H2-7a-13            | AGGTAGTTGTTGTTAGTTACACA CCTAATCTACCCAAGTTTTCCT | 13.91 | 46.15 |                   |
| hsa-miR-18a-5p      | TATCTG CACCTGACTGACCTCACA AAAAGTTGAGGTGCACTCAGTG | 16.13 | 46.67 |                   |
| H1-18a-15           | TATCTG CACCTGACTGACCTCACA AAAAGTTGAGGTGCACTCAGTG | 15.13 | 50.00 |                   |
| H1-18a-14           | TATCTG CACCTGACTGACCTCACA AAAAGTTGAGGTGCACTCAGTG | 14.05 | 53.85 |                   |
| H1-18a-13           | TATCTG CACCTGACTGACCTCACA AAAAGTTGAGGTGCACTCAGTG | 14.05 | 53.85 |                   |
| H1-18a-12 | **FATCTG** CACTAGATGCACCAAAGTGTGCATCTAGTG | 12.21 | 50.0 |
| H2-18a-15 | AAGGTCATCCTAGTGCAGATACACTAGATGCACCTTTACTTTG | 16.34 | 46.67 |
| H2-18a-14 | AGGGTCATCCTAGTGCAGATACACTAGATGCACCTACTTTG | 15.34 | 50.0 |
| H2-18a-13 | GGTGCATCTAGTGCAGATACACTAGATGCACCACTTTG | 14.45 | 53.85 |
| H2-18a-12 | GTCATCTAGTGCAGATACACTAGATGCACACTTTG | 12.61 | 50.0 |

| hsa-miR-20a-5p | UAAAGUGCUUUAUAGUCAGGUAG |
| H1-20a-17 | **CTACCT** GCACTATAAACACTTTCACAAGTTAAAAGTCATTTATAGTC | 16.63 | 35.29 |
| H1-20a-15 | **CTACCT** GCACTATAAACACTTTCACAAGTTAAAAGTCATTTATAGTC | 15.25 | 40.0 |
| H2-20a-17 | AAAGTGCTTTAGTGGCAAGTTGAAGCATAATAGCCTTTTAC | 17.08 | 35.29 |
| H2-20a-15 | AAGTGCCTTATAGTGGCAAGTTGAAGCATAATAGCCTTTTAC | 15.37 | 40.0 |

| hsa-miR-29a-3p | UAGCACAACUUGAAUAUCGGIUUA |
| H1-29a-16 | **AACGGA** TTTCAGATGTTGCTAAAACAAGTTTTAGCACCACATCTGAAA | 16.24 | 37.5 |
| H1-29a-15 | **AACGGA** TTTCAGATGTTGCTAAAACAAGTTTTAGCACCACATCTGAAA | 15.24 | 40.0 |
| H2-29a-16 | TTAGCACCATCGTAAAATCCTGGTTTTTCAGTAGTGGCTAAACTTTG | 15.96 | 37.5 |
| H2-29a-15 | TAGCACCATCGTAAAATCCTGGTTTTTCAGTAGTGGCTAAACTTTG | 15.45 | 40.0 |

| hsa-miR-30a-5p | UGUAACAAUCCUCGACUGGAAG |
| H1-30a-16 | **CTTCCG** GTCGAGGATTTTACACAAAGTTTGGCACCACACTTCGAC | 16.75 | 43.75 |
| H1-30a-15 | **CTTCCG** GTCGAGGATTTTACACAAAGTTTGGCACCACACTTCGAC | 15.70 | 46.67 |
| H2-30a-16 | TGTAACATCTCGAACGGAAGGTGCGAGGATTTTACAACCTTTG | 18.26 | 43.75 |
| H2-30a-15 | GTAACATCCATCGACTGGAAGGTCGAGGATTTTACAACCTTTG | 17.56 | 46.67 |

| hsa-miR-95-3p | UUCAACGGGUAUUUAUUGAGCA |
| H1-95-16 | **TGCTCA** ATAAATACCCCGTTGAACAAAGTTTTTCAACGGGATTTAT | 15.16 | 31.25 |
| H2-95-16 | TTCAACCGGATTATTTTAGGCAATTTATAACCCCGTTGAAACCTTTG | 16.97 | 31.25 |

| hsa-miR-181a-5p | AACAUUAACACGCUUCGUGGUGUAG |
| H1-181a-15 | **ACTCAG** CGCACAGGTTTGGAATGCAAAAGTTTCTTACACGCTTGT | 17.79 | 53.33 |
| H1-181a-13 | **ACTCAG** CGCACAGGTTTGGAATGCAAAAGTTTCTTACACGCTTGT | 15.06 | 53.85 |
| H2-181a-15 | CATTCAAGCTGTCGTTGAGTGCAGACGCGTGGTAAATGACTTTG | 18.41 | 53.33 |
| H2-181a-13 | TTTCAACGCTGTCGTTGAGTGCAGACGCGTGGTAAATGACTTTG | 15.23 | 53.85 |

| hsa-miR-302a-3p | UAAUGUCUUUCAUGUUUUUGUGA |
| H1-302a-15 | **TCACCA** AAAACATGGAAGCAAAAGTATATGCTTCCACTTTT | 16.01 | 40.0 |
| H1-302a-14 | **TCACCA** AAAACATGGAAGCAAAAGTATATGCTTCCACTTTT | 14.93 | 42.86 |
The other DNA Hairpin sequences based on miRNA were designed. The underlined bases at the end constituted the exposed toehold, and the blue ones at the middle constituted the sequestered toehold. The red value is the free energy of the leakage sequence. miRNA sequences were from the database of miRTarBase: (http://mirtarbase.mbc.nctu.edu.tw/php/index.php)

|      | Sequence                        | Free Energy | Secondary Structure |
|------|---------------------------------|-------------|---------------------|
| H2-302a-15 | AGTCTCCATTTTGGTAAACATGGAACTTACCTGTTC | 17.38       | ACTTTG              |
| H2-302a-14 | GTGCTCCCATTTTGGTAAACATGGAAAGCACACTTTG | 16.49       | ACTTTG              |
Supplementary Table S5. Evolution of the hairpin based on changed toehold

| Name     | Strand sequences (5’ to 3’)                                                                 | Free energy of secondary Structure at 37 °C (-kcal mol⁻¹) | Stem GC% |
|----------|--------------------------------------------------------------------------------------------|----------------------------------------------------------|----------|
| I-18a    | TAAGGTGCATCTAGTGCAGATAG                                                                    |                                                          |          |
| H1-18a-18b | CTATCTGCACATAGTGCACCCTTagCATACGGTAAAGGTGCATCTAGTGC                                        | 20.68                                                   | 50.0     |
| H1-18a-17b | CTATCTGCACATAGTGCACCCTAGTCAGATAGGCAGTCATCTAGTGC                                          | 18.74                                                   | 47.06    |
| H1-18a-15b | CTATCTGCACATAGTGCACCCTAGTCAGATAGGCAGTCATCTAGTGC                                          | 17.16                                                   | 53.33    |
| H1-18a-14b | CTATCTGCACATAGTGCACCCTAGTCAGATAGGCAGTCATCTAGTGC                                          | 16.38                                                   | 57.14    |
| H1-18a-13b | CTATCTGCACATAGTGCACCCTAGTCAGATAGGCAGTCATCTAGTGC                                          | 14.54                                                   | 53.85    |
| H1-18a-12b | CTATCTGCACATAGTGCACCCTAGTCAGATAGGCAGTCATCTAGTGC                                          | 12.6                                                    | 50.0     |
| H1-18a-11b | CTATCTGCACATAGTGCACCCTAGTCAGATAGGCAGTCATCTAGTGC                                          | 11.65                                                   | 54.55    |
| H1-18a-10b | CTATCTGCACATAGTGCACCCTAGTCAGATAGGCAGTCATCTAGTGC                                          | 9.54                                                    | 50.0     |
| H2-18a-18b | GTAAAGGTGCACATCTAGTGCAGATAGGCCACTAGATGCACCCTACGCTGTC                                      | 20.38                                                   | 50.0     |
| H2-18a-17b | GTAAAGGTGCACATCTAGTGCAGATAGGCCACTAGATGCACCCTACGCTGTC                                      | 19.19                                                   | 47.06    |
| H2-18a-15b | AGTGCACATCTAGTGCAGATAGGCCACTAGATGCACCCTACGCTGTC                                          | 17.19                                                   | 53.33    |
| H2-18a-14b | GTGCACATCTAGTGCAGATAGGCCACTAGATGCACCCTACGCTGTC                                          | 16.08                                                   | 57.14    |
| H2-18a-13b | GTGCACATCTAGTGCAGATAGGCCACTAGATGCACCCTACGCTGTC                                          | 14.24                                                   | 53.85    |
| H2-18a-12b | GTGCACATCTAGTGCAGATAGGCCACTAGATGCACCCTACGCTGTC                                          | 12.47                                                   | 50.0     |
| H2-18a-11b | GTGCACATCTAGTGCAGATAGGCCACTAGATGCACCCTACGCTGTC                                          | 11.35                                                   | 54.55    |
| H2-18a-10b | CATCTAGTGCAGATAGGCCACTAGATGCACCCTACGCTGTC                                                | 9.03                                                    | 50.0     |

I-181     | AACATTCAAGCCGTCTCGGTTGAGT                      |                                                          |          |
| H1-181-17b | ACTCACCGACAGCGTTGGAATGCGCATACAACTTAGCTTGC   | 19.73                                                   | 47.06    |
| H1-181-15b | ACTCACCGACAGCGTTGGAATGCGCATACAACTTAGCTTGC | 17.69                                                   | 53.33    |
| H1-181-14b | ACTCACCGACAGCGTACTTGGCACTTGGCACTTGGCACTTGG | 15.84                                                   | 50.0     |
| H1-181-13b | ACTCACCGACAGCGTTGGAATGCGCATACAACTTAGCTTGC | 14.96                                                   | 53.85    |
| H1-181-11b | ACTCACCGACAGCGTTGGAATGCGCATACAACTTAGCTTGC | 13.06                                                   | 63.64    |
| H1-181-10b | ACTCACCGACAGCGTTGGAATGCGCATACAACTTAGCTTGC | 11.21                                                   | 60.0     |
| H1-181-9b  | ACTCACCGACAGCGTTGGAATGCGCATACAACTTAGCTTGC | 10.21                                                   | 66.67    |
| H1-181-8b  | ACTCACCGACAGCGTTGGAATGCGCATACAACTTAGCTTGC | 9.17                                                    | 75.0     |
| H2-181-17b | AACATTCAACCGCTCCTGGTTAATGTTTCCATACAACTTAGCTTGGCACTTGGCACTTGGCACTTGGCACTTGGCACTTGGCACTTGG | 20.38 | 47.06 |
| H2-181-15b | CATTCACCGCTCCTGGTTAATGTTTCCATACAACTTAGCTTGGCACTTGGCACTTGGCACTTGGCACTTGGCACTTGGCACTTGG | 17.93 | 53.33 |
| H2-181-14b | ATTCAACCGCTCCTGGTTAATGTTTCCATACAACTTAGCTTGGCACTTGGCACTTGGCACTTGGCACTTGGCACTTGGCACTTGG | 16.49 | 50.0 |
| H2-181-13b | TTCAACCGCTCCTGGTTAATGTTTCCATACAACTTAGCTTGGCACTTGGCACTTGGCACTTGGCACTTGGCACTTGGCACTTGG | 15.12 | 53.85 |
The DNA Hairpin sequences based on changed toehold were designed. The underlined bases at the end constituted the exposed toehold, and the blue ones at the middle constituted the sequestered toehold. The red value is the free energy of the leakage sequence. I-18a is the corresponding DNA sequence of hsa-miR-18a-5p. I-181a is the corresponding DNA sequence of hsa-miR-181a-5p.

| Sequence       | Underlined | Blue            | Red  | Green |
|----------------|------------|-----------------|------|-------|
| H2-181a-11b    | CAACGCTGTCG| GTGAGTCGACAGCGTTGTATGG | 13.30 | 63.64 |
| H2-181a-10b    | AACGCTGTCG| GTGAGTCGACAGCGTTGTATGG | 11.86 | 60.0  |
| H2-181a-9b     | ACGCTGTCG| GTGAGTCGACAGCGTTGTATGG | 10.86 | 66.67 |
| H2-181a-8b     | CGCTGTCG  | GTGAGTCGACAGCGTTGTATGG | 9.41  | 75.0  |
Supplementary Table S6. Modified sequences in stem region by mutation and substitution

| Name  | Strand sequences (5’ to 3’)                             | Free energy of secondary Structure at 37 °C (-kcal mol⁻¹) |
|-------|----------------------------------------------------------|----------------------------------------------------------|
| H1b-17| TTAACC TCAGATCTAAGCCGCAACAGGTTGCGGCTTTAGGATCTGA          | 19.93                                                   |
| M-1   | TTAACC TAAGATCTAAGCCGCAACAGGTTGCGGCTTTAGGATCTGA          | 17.94                                                   |
| M-2   | TTAACC TCAGATCTCAAGCCGCAACAGGTTGCGGCTTTAGGATCTGA         | 16.95                                                   |
| M-3   | TTAACC TCAGATCTAAGCCGCAACAGGTTGCGGCTTTAGGATCTGA          | 16.44                                                   |
| M-4   | TTAACC TCAGATCTAAGCCGCAACAGGTTGCGGCTTTAGGATCTGA          | 16.44                                                   |
| M-5   | TTAACC TAAGATCTCAAGCCGCAACAGGTTGCGGCTTTAGGATCTGA         | 14.96                                                   |
| M-6   | TTAACC TCAGATCTAAGCCGCAACAGGTTGCGGCTTTAGGATCTGA          | 14.04                                                   |
| M-7   | TTAACC TAAGATCTAAGCCGCAACAGGTTGCGGCTTTAGGATCTGA          | 14.45                                                   |
| M-8   | TTAACC TCAGATCTCAAGCCGCAACAGGTTGCGGCTTTAGGATCTGA         | 13.46                                                   |
| H1-15 | TTAACCCACGCGCAATTCTGGCAACAGGTTGCGGCTTTAGGATCTGA          | 17.99                                                   |
| H1-15-1| TTAACCCACGCGCAATTCTGGCAACAGGTTGCGGCTTTAGGATCTGA         | 16.71                                                   |
| H1-15-2| TTAACCCACGCGCAATTCTGGCAACAGGTTGCGGCTTTAGGATCTGA         | 16.03                                                   |
| H1-15-3| TTAACCCACGCGCAATTCTGGCAACAGGTTGCGGCTTTAGGATCTGA         | 15.32                                                   |
| H1-15-4| TTAACCCACGCGCAATTCTGGCAACAGGTTGCGGCTTTAGGATCTGA         | 15.38                                                   |
| H1-15-5| TTAACCCACGCGCAATTCTGGCAACAGGTTGCGGCTTTAGGATCTGA         | 16.32                                                   |
| H1-15-6| TTAACCCACGCGCAATTCTGGCAACAGGTTGCGGCTTTAGGATCTGA         | 16.37                                                   |
| H1-15-7| TTAACCCACGCGCAATTCTGGCAACAGGTTGCGGCTTTAGGATCTGA         | 15.88                                                   |
| H1-15-8| TTAACCCACGCGCAATTCTGGCAACAGGTTGCGGCTTTAGGATCTGA         | 16.38                                                   |
| H1-15-9| TTAACCCACGCGCAATTCTGGCAACAGGTTGCGGCTTTAGGATCTGA         | 13.93                                                   |
| H1-15-10| TTAACCCACGCGCAATTCTGGCAACAGGTTGCGGCTTTAGGATCTGA        | 13.62                                                   |
| H2-15 | CTAGATCTCAGGCTTTGCGGCTTTAGGATCTGA                       | 18.69                                                   |
| H2-15-1| TTAGGATCTGCGCGTGCGGCTTTAGGATCTGA                       | 16.71                                                   |
| H2-15-2| CTATTATGCGCTTTGCGGCTTTAGGATCTGA                       | 16.73                                                   |
| H2-15-3| CTATTATGCGCTTTGCGGCTTTAGGATCTGA                       | 16.02                                                   |
| H2-15-4| CTATTATGCGCTTTGCGGCTTTAGGATCTGA                       | 16.08                                                   |
| H2-15-5| CTATTATGCGCTTTGCGGCTTTAGGATCTGA                       | 17.02                                                   |
| H2-15-6| CTATTATGCGCTTTGCGGCTTTAGGATCTGA                       | 17.07                                                   |
| H2-15-7| CTATTATGCGCTTTGCGGCTTTAGGATCTGA                       | 16.58                                                   |
| H2-15-8| CTATTATGCGCTTTGCGGCTTTAGGATCTGA                       | 17.08                                                   |
| H2-15-9| CTATTATGCGCTTTGCGGCTTTAGGATCTGA                       | 14.38                                                   |
The changed nucleotides were marked in red. The red value is the free energy of the leakage sequence.
Supplementary Table S7. DNA sequences used in effect of toehold and loop length on DNA assembly behaviour

| Name        | Strand sequences (5' to 3')                                                                 | Free energy of secondary Structure at 37 °C (kcal mol⁻¹) |
|-------------|------------------------------------------------------------------------------------------|--------------------------------------------------------|
| H1b-17-8T   | TTAACCTAGATCCTAAGGCCGCAAAAGTTGCGGTATTAGGATCTGA                                          | 19.93                                                  |
| H2b-17-8T   | ATGTGCAGGCTTAAGGACAGTTGAAGCTAGATCCTAAGGCCGCAATTTTCAAGGCATCTAGTGA                        | 19.63                                                  |
| H1-17-8T    | TTAACCTAGATCCTAAGGCCGCAAAAGTTGCGGTATTAGGATCTGA                                          | 19.93                                                  |
| H2-17-8T    | ATGTCTAGGATTCGGGTGGGTATAAGCCGGAATCTAGCATAACTTTTCAAGGCATCTAGTGA                          | 19.63                                                  |
| H1-18a-17b-8T | TCTATACTGCACCTAGATCCTAAGGCCGCAAAAGTTGCGGTATTAGGATCTGA                               | 19.63                                                  |
| H2-18a-17b-8T | TTAACCTAGATCCTAAGGCCGCAAAAGTTGCGGTATTAGGATCTGA                                      | 19.63                                                  |
| HR1-17C-8T  | CATACAACGTACGTCTGATAAGCTACCAAGGTCTCCGACTAGCTATACGAAGCTACACCAAGGTCTCCGACTAGCTATACGA   | 19.63                                                  |
| HR2-17C-8T  | GTAGCGTTAATGACACTGATGTTAGTAAGCTACTCTAGATCTGA                                         | 19.63                                                  |
| H1b-17      | TTAACCTAGATCCTAAGGCCGCAAAAGTTGCGGTATTAGGATCTGA                                          | 19.93                                                  |
| E1          | TCAGATCTCCTAAGGCCGAATACCGTTCGAAGCTAGATCCTAAGGCCGCAAAAGTTGCGGTATTAGGATCTGA              | 19.63                                                  |
| E3          | TCAGATCTCCTAAGGCCGAATACCGTTCGAAGCTAGATCCTAAGGCCGCAAAAGTTGCGGTATTAGGATCTGA              | 19.63                                                  |
| E5          | TCAGATCTCCTAAGGCCGAATACCGTTCGAAGCTAGATCCTAAGGCCGCAAAAGTTGCGGTATTAGGATCTGA              | 19.63                                                  |
| E7          | TCAGATCTCCTAAGGCCGAATACCGTTCGAAGCTAGATCCTAAGGCCGCAAAAGTTGCGGTATTAGGATCTGA              | 19.63                                                  |
| E9          | TCAGATCTCCTAAGGCCGAATACCGTTCGAAGCTAGATCCTAAGGCCGCAAAAGTTGCGGTATTAGGATCTGA              | 19.63                                                  |
| E13         | TCAGATCTCCTAAGGCCGAATACCGTTCGAAGCTAGATCCTAAGGCCGCAAAAGTTGCGGTATTAGGATCTGA              | 19.63                                                  |
| E15         | TCAGATCTCCTAAGGCCGAATACCGTTCGAAGCTAGATCCTAAGGCCGCAAAAGTTGCGGTATTAGGATCTGA              | 19.63                                                  |
| E18         | TCAGATCTCCTAAGGCCGAATACCGTTCGAAGCTAGATCCTAAGGCCGCAAAAGTTGCGGTATTAGGATCTGA              | 19.63                                                  |
| T-01        | TCAGATCTCCTAAGGCCGAATACCGTTCGAAGCTAGATCCTAAGGCCGCAAAAGTTGCGGTATTAGGATCTGA              | 19.63                                                  |
| T-02        | TCAGATCTCCTAAGGCCGAATACCGTTCGAAGCTAGATCCTAAGGCCGCAAAAGTTGCGGTATTAGGATCTGA              | 19.63                                                  |
| H1-18a-17b  | CTACCTGAACCTAGATGACCTCCTAAGGCCGCAAAAGTTGCGGTATTAGGATCTGA                               | 19.63                                                  |
| 18a-E1      | GCAGATCTCCTAAGGCCGAATACCGTTCGAAGCTAGATCCTAAGGCCGCAAAAGTTGCGGTATTAGGATCTGA              | 19.63                                                  |
| 18a-E3      | GCAGATCTCCTAAGGCCGAATACCGTTCGAAGCTAGATCCTAAGGCCGCAAAAGTTGCGGTATTAGGATCTGA              | 19.63                                                  |
| 18a-E5      | GCAGATCTCCTAAGGCCGAATACCGTTCGAAGCTAGATCCTAAGGCCGCAAAAGTTGCGGTATTAGGATCTGA              | 19.63                                                  |
| 18a-E7      | GCAGATCTCCTAAGGCCGAATACCGTTCGAAGCTAGATCCTAAGGCCGCAAAAGTTGCGGTATTAGGATCTGA              | 19.63                                                  |
| 18a-E9      | GCAGATCTCCTAAGGCCGAATACCGTTCGAAGCTAGATCCTAAGGCCGCAAAAGTTGCGGTATTAGGATCTGA              | 19.63                                                  |
| 18a-E12     | GCAGATCTCCTAAGGCCGAATACCGTTCGAAGCTAGATCCTAAGGCCGCAAAAGTTGCGGTATTAGGATCTGA              | 19.63                                                  |
| 18a-E15     | GCAGATCTCCTAAGGCCGAATACCGTTCGAAGCTAGATCCTAAGGCCGCAAAAGTTGCGGTATTAGGATCTGA              | 19.63                                                  |
| 18a-E18     | GCAGATCTCCTAAGGCCGAATACCGTTCGAAGCTAGATCCTAAGGCCGCAAAAGTTGCGGTATTAGGATCTGA              | 19.63                                                  |

S29
The underlined bases at the end constituted the exposed toehold, and the blue ones at the middle constituted the sequestered toehold. Inserting bases were highlighted in red.
**Supplementary Table S8. DNA sequences used in effect of initiator on HCR**

| Name | Strand sequences (5' to 3') |
|------|-----------------------------|
| H1b-17 | **TTAACC**TCAGATCCTAAGCCGCAAAAGTTGCGGCTTAGGATCTGGA** |
| H2b-17 | **TGCGGCTTAGGATCTGAG**GGTFAAACAGATCTAAGCCGCAACTTTG |
| T-1   | **TGCGGCTTAGGATCTGAGGTA** |
| T-2   | **TGCGGCTTAGGATCTGAGGTT** |
| T-3   | **TGCGGCTTAGGATCTGAGG** |
| T-4   | **TGCGGCTTAGGATCTGAGGG** |
| T-5   | **GTGCCGGCTTAGGATCTGAGGTTAA** |
| T-6   | **GCGGCTTAGGATCTGAGGTTAA** |
| T-7   | **GCGGCTTAGGATCTGAGGTTAA** |
| T-8   | **GCGGCTTAGGATCTGAGGTTAA** |
| T-9   | **GCGGCTTAGGATCTGAGGTTAA** |
| T-10  | **GCGGCTTAGGATCTGAGGTTAA** |
| T-11  | **GCGGCTTAGGATCTGAGGTTAA** |
| T-12  | **GCGGCTTAGGATCTGAGGTTAA** |
| H1C-18 | **TAACAAAGAAGCCAAACGAGATTTGGGCTTTGCTTTGTTTA**CCTGAT |
| H2C-18 | **CATCTGCGTTTGGCTTTGCTTTGTTTAATCAGGTAACAAGAAGCCAAAC** |
| T-18-2 | **ATCAGGTAACAAGAAGCCAAA** |
| T-18-3 | **ATCAGGTAACAAAGAAGCCAAA** |
| T-18-4 | **ATCAGGTAACAAGAAGCCAAA** |
| T-18-5 | **ATCAGGTAACAAGAAGCCAAA** |
| T-18-6 | **ATCAGGTAACAAAGAAGCCAAA** |
| T-18-7 | **ATCAGGTAACAAGAAGCCAAA** |
| T-18-8 | **ATCAGGTAACAAGAAGCCAAA** |
| H1-18a-17b | **CTATCTGCACTAGATGCACCTTAGATGCTAGTGCTTTTGAAGGTA** |
| H2-18a-17b | **TAAGGTCATCTAAGGCGACATAGATGACACTAGCATCCTTAGGTA** |
| T-18a-3 | **GGTGCATCTAGTGCGATAC** |
| T-18a-4 | **GTGCATCTAGTGCGTAG** |
| T-18a-5 | **TGCAATCGAGTGCGATAC** |
| T-18a-6 | **GCTACTATGCAGTAG** |
| T-18a-7 | **CATACTATGCAGTAG** |
| T-18a-8 | **ATCTAGTGCGATAC** |

S31
The underlined bases at the end constituted the exposed toehold, and the blue ones at the middle constituted the sequestered toehold.
Supplementary Table S9. DNA Fairpin for detection of miRNA family

| Name          | Strand sequences (5' to 3')                          | Free energy of secondary structure at 37 °C (-kcal mol⁻¹) |
|---------------|-----------------------------------------------------|----------------------------------------------------------|
| hsa-let-7d-5p | AGAGGUAGGUUUGGUUGCAUAGUU                          |                                                          |
| H1-7d-0       | AACTATGCAACCTACTACCTCTCAAAGTAGAGGTAGATTGTTGC        | 16.00                                                    |
| H2-7d-0       | AGAGGTAGTTAGGTTGCTAAGGTTGCAACCTACTACCTCTAACCTTCA   | 21.69                                                    |
| H1-7d         | AACTAT GCAACCTACTACCTCTCAAAGTAGAGGTAGATTGTTGC       | 19.60                                                    |
| H2-7d         | GTTAGGTTAGGTTGCTAAGGTTGCAACCTACTACCTTCA ACTTTG      | 20.01                                                    |
| hsa-let-7b-5p | AGAGGUAGGUUGGUUGGUUU                                |                                                          |
| H1-7b-0       | ACTTTGAAACACACAACCTACTTAGGTAATAGGTTGTTGGT          | 17.37                                                    |
| H2-7b-0       | ACTTTGAAACACACAACCTACTACCTCAA ACTTTG              | 17.24                                                    |
| H1-7b         | AGTAGGTTAGGTGGTTGCTGCAAACCTACTACCTCTGCAACCTACTACCTCA | 21.69                                          |
| H2-7b         | ACTTTGAACACACAACCTACTACCTCAA ACTTTG      | 21.42                                                    |
| hsa-let-7a-5p | AGAGGUAGGUUGGUUGGUUU                                |                                                          |
| H1-7a-0       | AACTATACAACCTACTACCTCA ACAAAGTTGAGGTAGATTGTTG      | 16.60                                                    |
| H2-7a-0       | TGAAGTATAGTGTTGAGTCAACCCACTACTACCTCA ACTTTG         | 16.01                                                    |
| H1-7a         | AACTATACAACCTACTACCTCA ACTTTG                      | 21.02                                                    |
| H2-7a         | GAGTAGGTTAGGTGGTTGCTAAGGTTGCAACCTACTACCTTCA ACTTTG | 20.78                                                    |
| hsa-let-7c-5p | AGAGGUAGGUUGGUUGGUUU                                |                                                          |
| H1-7c-0       | ATAGTAGGTTGTAATGTTCTCAAAGTTAACATACAACCTACTACCTCA  | 17.30                                                    |
| H2-7c-0       | ACTTTGAAACACACAACCTACTACCTCAA ACTTTG              | 17.24                                                    |
| H1-7c         | ATAGTAGGTTGTAATGTTCTCAAAGTTAACATACAACCTACTACCTCA  | 21.42                                                    |
| H2-7c         | ACTTTG CAGTTAACCCATACACCTACTACCTGTAATGTTGATGTTGTTGC | 22.02                                                    |
| hsa-let-7e-5p | AGAGGUAGGUUGGUUGGUUU                                |                                                          |
| H1-7e-0       | AACTATACAACCTCTACTACCTCA ACAAAGTTGAGGTAGGGTGGT     | 15.56                                                    |
| H2-7e-0       | AACTATACAACCTCTACTACCTCA ACAAAGTTGAGGTAGGGTGGT     | 15.50                                                    |
| H1-7e         | AACTATACAACCTCTACTACCTCA ACCTCA ACCTCA AACTTCA      | 21.69                                                    |
| H2-7e         | GCCAACTATAACAACCTCTTACCTGTAACACCTCTACCTCA GCCACTTTG | 21.42                                                    |
| hsa-let-5p    | AGAGGUAGGUUGGUUGGUUU                                |                                                          |
| H1-7i-0       | ATAGTAGGTTGTTGCTGTCAAAGTTAACATACAACCTACTACCTCA    | 16.70                                                    |
| H2-7i-0       | ACTTTGAACACACAACCTACTACCTGTAATGTTGCTGTTT          | 16.64                                                    |
| H1-7i         | ATAGTAGGTTGTTGCTGTCAAAGTTAACACACAACACCACTACTACCTCA | 20.59                                                    |
| H2-7i         | ACTTTG CAGTTAACACACAACCTCTTCAGGTTGAGGTGGTGGTTG     | 20.32                                                    |

The underlined bases at the middle were the loop as the sequestered toehold and those at the end constituted the exposed toehold. Added bases were highlighted in red.
References
1. Ivani, I., Dans, P. D., Noy, A., et al. (2016) Parmbsc1: a refined force field for DNA simulations. *Nature methods*, **13**, 55-58.
2. Pérez, A., Marchán. I., Svozil, D., et al. (2007) Refinement of the AMBER force-field for nucleic acid simulations: Improving the representation of a/c conformations. *Biophysical J*, **92**, 3817-3829.