Supporting Information for

# An Interlocked DNA Cascade System for Universal Probe-based Melting Curve Analysis

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**Theoretical calculation model**

During the initial stage, three main reactions happened in the system:

1. probe + bridge →probe/bridge;
2. bridge + target →bridge/target;
3. probe + bridge + target →probe/bridge/ target.

Since the initial concentration of probe, bridge and target were 100 nM, thus

$$[target] + [bridge/target] + [probe/bridge/target] = 100\ nM \quad (1.1)$$

$$[probe] + [probe/bridge] + [probe/bridge/target] = 100\ nM \quad (1.2)$$

$$[bridge] + [probe/bridge] + [probe/bridge/target] = 100\ nM \quad (1.3)\backslash*$$

MERGEFORMAT

Here for presentation simplicity, we set the [probe/bridge], [probe/target], [probe/bridge/ target] to be x, y, z. Thus:

$$[probe] = 100\,nM - x - z \quad (1.4)$$

$$[WT] = 100\,nM - y - z \quad (1.5)$$

$$[bridge] = 100\,nM - x - y - z \quad (1.6)$$

Since

$$\begin{cases} x = k_1 \cdot [probe] \cdot [bridge] = e^{\frac{-\Delta G_1}{R \cdot T}} \cdot (100\,nM - x - z) \cdot (100\,nM - x - y - z) \\ y = k_2 \cdot [target] \cdot [bridge] = e^{\frac{-\Delta G_2}{R \cdot T}} \cdot (100\,nM - y - z) \cdot (100\,nM - x - y - z) \\ z = k_3 \cdot [target] \cdot [bridge] \cdot [probe] = e^{\frac{-\Delta G_3}{R \cdot T}} \cdot (100\,nM - x - z) \cdot (100\,nM - y - z) \cdot (100\,nM - x - y - z) \end{cases}$$

(1.7)

Where $k_1$, $k_2$, $k_3$ were the equilibrium constants for reaction 1, 2, 3 and $\Delta G_1$, $\Delta G_2$, $\Delta G_3$ were the free

energy change of reaction 1, 2, 3.

The analytical solution of equation (1.7) is complex. For convenience of the subsequent description, we simply set the solution of equation (1.7) to be ($x_1$, $y_1$, $z_1$).

When no substrate was added to the system, only one main reaction happened:

1. probe + bridge →probe/bridge;

Since

$$[probe] + [probe/bridge] = 100\ nM \tag{2.1}$$

$$[bridge] + [probe/bridge] = 100\ nM \tag{2.2}$$

we set the [probe/bridge] to be x, thus

$$[probe] = [bridge] = 100\ nM - x \tag{2.3}$$

$$x = k_1 \cdot [probe] \cdot [bridge] = e^{\frac{-\Delta G_1}{R \cdot T}} \cdot (100\ nM - x)^2 \tag{2.4}$$

Similarly, we set the solution of equation (2.4) to be $x_2$.

Thus

$$\frac{[probe/bridge/target]}{[probe/bridge]} = \frac{z_1}{x_2} \tag{2.5}$$

Next, we used a computer program to solve equations (1.7 and 2.4) numerically. The programs were written in MATLAB:

```
function L=calculate(z1,z2,z3)%z1, z3, z3 refers to ΔG1, ΔG2, ΔG3;
r=8.314;
t=310.15;
y1=exp(-z1/(r*t));
y2=exp(-z2/(r*t));
y3=exp(-z3/(r*t));
```

```
syms x1 x2 x3 p;
[solp0,solp1,solp2] = solve (y1*(10^-7-x1-x3)*(10^-7-x1-x2-x3)-x1==0,y2*(10^-7-x2-x3)*(10^-7-x1-x2-x3)-x2==0,y3*(10^-7-x1-x3)*(10^-7-x2-x3)*(10^-7-x1-x2-x3)-x3==0,x1,x2,x3);
a1=double(solp0);
a2=double(solp1);
a3=double(solp2);
A=[a1,a2,a3];
n=size(A);
for i=1:n(1)
    if isreal(A(i,1))==1
        if A(i,1)>0&&A(i,1)<10^-7
          if isreal(A(i,2))==1
            if A(i,2)>0&&A(i,2)<10^-7
                if isreal(A(i,3))==1
                    if A(i,3)>0&&A(i,3)<10^-7
                        y=A(i,:);
                        break
                    end
                end
            end
          end
        end
```

```
        end
    end
    q=y1*(10^-7-p)^2;
    r=double(solve(q==p));
    if r(1)>0&&r(1)<10^-7
        R=r(1);
        if r(2)>0&&r(2)<10^-7
            R=r(2);
        end
    end
    L=y(3)/R;
```

With this program, we could directly predict the ratio by inputting the values of $\Delta G_1$, $\Delta G_2$, $\Delta G_3$. The results were shown as follows:

Table S1. The calculation results of different reaction systems used in this work

| Probe | Bridge | Target | $\Delta G_1$ (j/mol) | $\Delta G_2$ (j/mol) | $\Delta G_3$ (j/mol) | Triplex/ duplex |
|---|---|---|---|---|---|---|
| Probe | bridge-1 | wt-1 | -57027.92 | -53931.76 | -132298.08 | 1.0483 |
| Probe | bridge-2 | wt-1 | -48618.08 | -78450.00 | -131754.16 | 1.163 |
| Probe | bridge-3 | wt-1 | -54015.44 | -56776.88 | -135477.92 | 1.0918 |
| Probe | bridge-4 | wt-1 | -45270.88 | -27070.48 | -99118.96 | 1.3721 |
| Probe | bridge-5 | wt-1 | -53806.24 | -50333.52 | -129034.56 | 1.0939 |
| Probe | bridge-6 | wt-2 | -49162.00 | -58743.36 | -130331.60 | 1.2518 |

| | | | | | | |
|---|---|---|---|---|---|---|
| Probe | bridge-7 | wt-2 | -47195.52 | -31631.04 | -110081.04 | 1.347 |

| Probe | Bridge | Target | $\Delta G_1$ (j/mol) | $\Delta G_2$ (j/mol) | $\Delta G_3$ (j/mol) | Triplex/ duplex |
|---|---|---|---|---|---|---|
| Probe | bridge-8 | wt-3 | -52467.36 | -48157.84 | -130540.80 | 1.1254 |
| Probe | bridge-9 | wt-3 | -51212.16 | -44685.12 | -127068.08 | 1.1619 |
| Probe | bridge-10 | wt-4 | -61462.96 | -59747.52 | -140289.52 | 1.0201 |
| Probe | bridge-11 | wt-4 | -56986.08 | -42634.96 | -123135.12 | 1.1667 |
| Probe | bridge-12 | wt-5 | -55103.28 | -64182.56 | -135896.32 | 1.0713 |
| Probe | bridge-13 | wt-5 | -52425.52 | -57822.88 | -129578.48 | 1.2225 |
| Probe | bridge-14 | wt-6 | -53178.64 | -40961.36 | -123344.32 | 1.1030 |
| Probe | bridge-15 | wt-6 | -41045.04 | -26275.52 | -99336.48 | 1.5126 |

In order to compare with experimental results in a better way, we modified the mathematical model to calculate the sum of the concentration of triplex and duplex after adding target over the concentration of duplex without adding target.

Thus

$$\frac{[probe/bridge/target]+[probe/bridge]}{[probe/bridge]}=\frac{x_1+z_1}{x_2} \tag{3.1}$$

Next, we changed the last line of the previous program to "L=(y(1)+y(3))/R" to calculate the ratio. The results were shown as follows:

Table S2. The calculation results of different reaction systems used in this work

| Probe | Bridge | Target | $\Delta G_1$ (j/mol) | $\Delta G_2$ (j/mol) | $\Delta G_3$ (j/mol) | Triplex/ duplex |
|---|---|---|---|---|---|---|
| Probe | bridge-1 | wt-1 | -57027.92 | -53931.76 | -132298.08 | 1.0487 |
| Probe | bridge-2 | wt-1 | -48618.08 | -78450.00 | -131754.16 | 1.1635 |
| Probe | bridge-3 | wt-1 | -54015.44 | -56776.88 | -135477.92 | 1.0924 |
| Probe | bridge-4 | wt-1 | -45270.88 | -27070.48 | -99118.96 | 1.3727 |
| Probe | bridge-5 | wt-1 | -53806.24 | -50333.52 | -129034.56 | 1.0946 |
| Probe | bridge-6 | wt-2 | -49162.00 | -58743.36 | -130331.60 | 1.2529 |
| Probe | bridge-7 | wt-2 | -47195.52 | -31631.04 | -110081.04 | 1.3481 |

| Probe | Bridge | Target | $\Delta G_1$ (j/mol) | $\Delta G_2$ (j/mol) | $\Delta G_3$ (j/mol) | Triplex/ duplex |
|---|---|---|---|---|---|---|
| Probe | bridge-8 | wt-3 | -52467.36 | -48157.84 | -130540.80 | 1.1258 |
| Probe | bridge-9 | wt-3 | -51212.16 | -44685.12 | -127068.08 | 1.1624 |
| Probe | bridge-10 | wt-4 | -61462.96 | -59747.52 | -140289.52 | 1.0209 |
| Probe | bridge-11 | wt-4 | -56986.08 | -42634.96 | -123135.12 | 1.1675 |
| Probe | bridge-12 | wt-5 | -55103.28 | -64182.56 | -135896.32 | 1.0720 |
| Probe | bridge-13 | wt-5 | -52425.52 | -57822.88 | -129578.48 | 1.2231 |
| Probe | bridge-14 | wt-6 | -53178.64 | -40961.36 | -123344.32 | 1.1040 |
| Probe | bridge-15 | wt-6 | -41045.04 | -26275.52 | -99336.48 | 1.5132 |

Table S3. DNA sequences used in this work.

| Name | Sequences (5'→3') |
|---|---|
| Probe | FAM-GGCGCGGGCCCAGCCCCTACCGCGCC-BHQ1 |
| WT-1 | GTAAAAGACATGACAGCGATACTTTCCCAGAGC |
| MT-1 | GTAAAAGACATGACAGTGATACTTTCCCAGAGC |
| WT-2 | TTTGCTGAGAATAACAGCTTCTAGCTCCATC |
| MT-2 | TTTGCTGAGAATAACAACTTCTAGCTCCATC |
| WT-3 | GCAATACCATTCAGGACATAGGCATGGGCAA |
| MT-3 | GCAATACCATTCAGGATATAGGCATGGGCAA |
| WT-4 | GGACATTACCACAGATCCTACAGGCATTAAA |
| MT-4 | GGACATTACCACAGACCCTACAGGCATTAAA |
| WT-5 | ATGCACCTGTAACAATAGCTGAGTCTTGG |
| MT-5 | ATGCACCTGTAACAACAGCTGAGTCTTGG |
| WT-6 | TGGTGTTCATCAAATGAGTGATC |
| MT-6 | TGGTGTTCATCAAGTGAGTGATC |
| WT-1-L | GCAACTGGAGCCAAGAAGAGTAACAAGCCAAATGAACAGACAAGTAA AAGACATGACAGCGATACTTTCCCAGAGCTGAAGTTAACAAATGCACC TGGTTC |
| MT-1-L | CCTGCAACTGGAGCCAAGAAGAGTAACAAGCCAAATGAACAGACAAG TAAAAGACATGACAGTGATACTTTCCCAGAGCTGAAGTTAACAAATGC ACCTGGTTC |

| Name | Sequences (5'→3') |
|---|---|
| WT-4-L | CACACAAATTACTAGCATCAGGAATGAAGGAGAGGACATTACCACAGA TCCTACAGGCATTAAAAAGAGAACAGGCAAATTATCATGAACTACTTA TGCTAATAAAC |
| MT-4-L | CACACAAATTACTAGCATCAGGAATGAAGGAGAGGACATTACCACAGA CCCTACAGGCATTAAAAAGAGAACAGGCAAATTATCATGAACTACTTA TGCTAATAAAC |
| Bridge-1 | GCGCGACGGGAAAGTATCACTGTCATGTCGCGCGGTAGGGGCTGGGC |
| Bridge-2 | CCGCGCTGAGGGAAAGTATCACTGTCATGTCAGCCGGTAGGGGCTGGG C |
| Bridge-3 | CCGCGCATGGGGAAAGTATCACTGTCATGTCATGCGCGGTAGGGGCTG GGC |
| Bridge-4 | CCGCGCGACAAAGTATCACTGTCATGTCGCGCGGTAGGGGCTGGGC |
| Bridge-5 | CCGCGCATGGAAAGTATCACTGTCATGTCATGCGCGGTAGGGGCTGGC |
| Bridge-6 | CCGCGCTGAGGAGCTAGAAGTTGTTATTCTCAGCGCGGTAGGGGCTGC |
| Bridge-7 | CCGCGCGAGGCTAGAAGTTGTTATTCTCGCGCGGTAGGGGCTGGGC |
| Bridge-8 | CCGCGCCCACCATGCCTATATCCTGAATGGGCGCGGTAGGGGCTGGGC |
| Bridge-9 | CCGCGCCCAATGCCTATATCCTGAATGGGCGCGGTAGGGGCTGGGC |
| Bridge-10 | CCGCGCTACGCCTGTAGGGTCTGTGGTAGCGCGGTAGGGGCTGGGC |
| Bridge-11 | CCGCGCTACCCTGTAGGGTCTGTGGTAGCGCGGTAGGGGCTGGGC |
| Bridge-12 | CCGCGCACCGACTCAGCTGTTGTTACAGGTGCGCGGTAGGGGCTGGGC |

| Name | Sequences (5'→3') |
|---|---|
| Bridge-13 | CCGCGCACCACTCAGCTGTTGTTACAGGTGCGCGGTAGGGGCTGGGC |
| Bridge-14 | CCGCGCGGTTCACTCACTTGATGAACACCGCGCGGTAGGGGCTGGGC |
| Bridge-15 | CCGCGCGGTACTCACTTGATGAACACCGCGCGGTAGGGGCTGGGC |
| FP-1 | GCAACTGGAGCCAAGAAGA |
| RP-1 | GAACCAGGTGCATTTGTTAAC |
| FP-2 | CACACAAATTACTAGCATCAG |
| RP-2 | GTTTATTAGCATAAGTAGTTCAT |
| ssDNA-1 | TTTTACTTGTCTGTTCATTTGGCTTGTTACTCTTCTTGGCTCCAGTTGCA GG |
| ssDNA-2 | CTGGTATTTGAACACTTAGTAAAAGAACCAGGTGCATTTGTTAACTTCA GCTCTGGG |
| ssDNA-3 | GTCCTCTCCTTCATTCCTGATGCTAGTAATTTGTGTGTTTTCTCT TTG TTC TTA ATC AAT ATA GC |
| ssDNA-4 | GATATAGTTGTGTTTATTAGCATAAGTAGTTCATGATAATTTGCCTGTT CTCTT TTTAA |

The point mutations in the sequences are indicated in red.

## 1. Optimization of the bridge design of five other mutation points

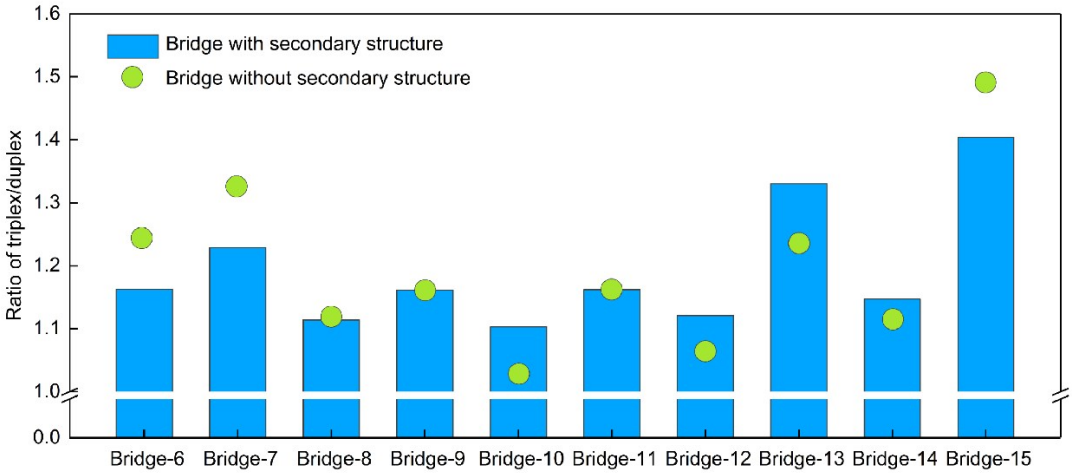


Figure S1. Choose the best Bridge strands of *BRCA1*/c.5152+66G>A, *RCA1*/c.2612C>T, *BRCA2*/c.7397T>C, *BRCA2*/c.8755-66T>C, *BRCA2*/c.2971A>G mutation points by comparing the calculated values with the experimental values.

## 2. Schematic illustration of the ssDNA strand strategy

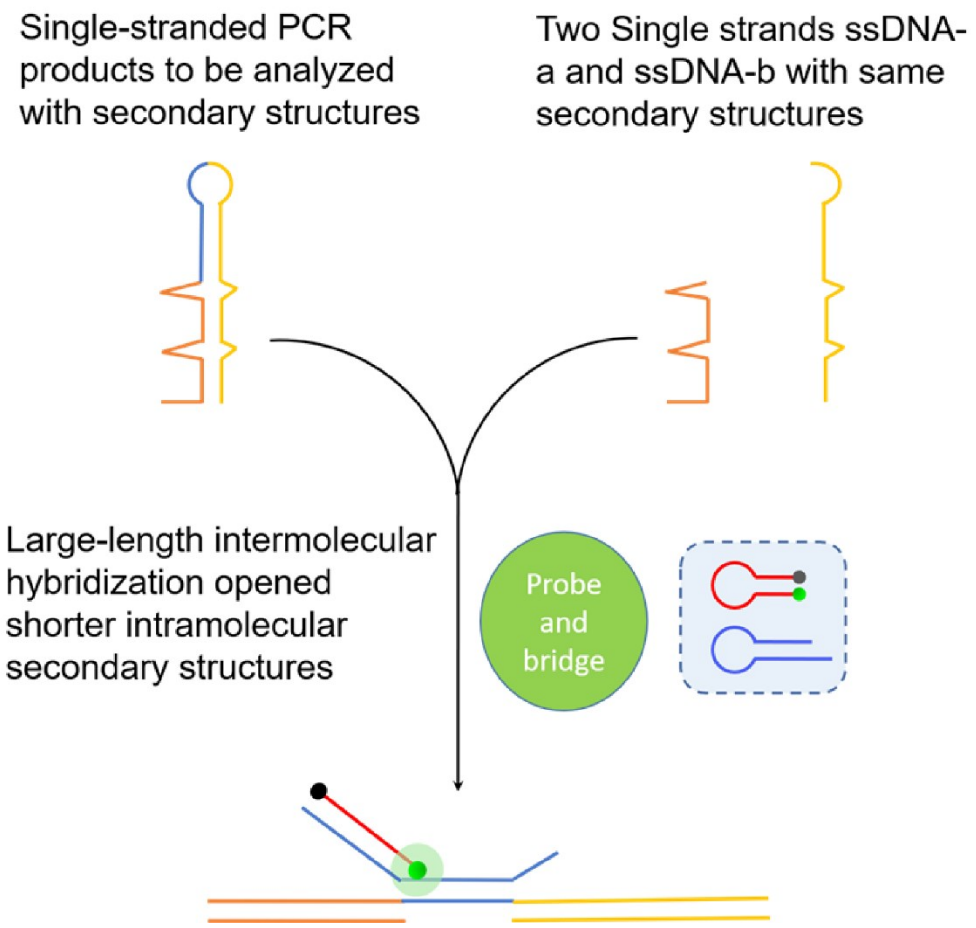


Figure S2. two single-stranded strands ssDNA-a and ssDNA-b were designed to eliminate the influence of the secondary structure on the detection performance. After asymmetric PCR of genomic DNA, ssDNA-a and ssDNA-b were added at 1, 2, 4, and 8 times the bridge concentration. The results showed 4 times the concentration of ssDNA-a and ssDNA-b can get a better discrimination effect.

**3. Sanger sequencing of mutations from genomic DNA of ovarian cancer patient and normal genomic DNA from normal after asymmetric PCR.**

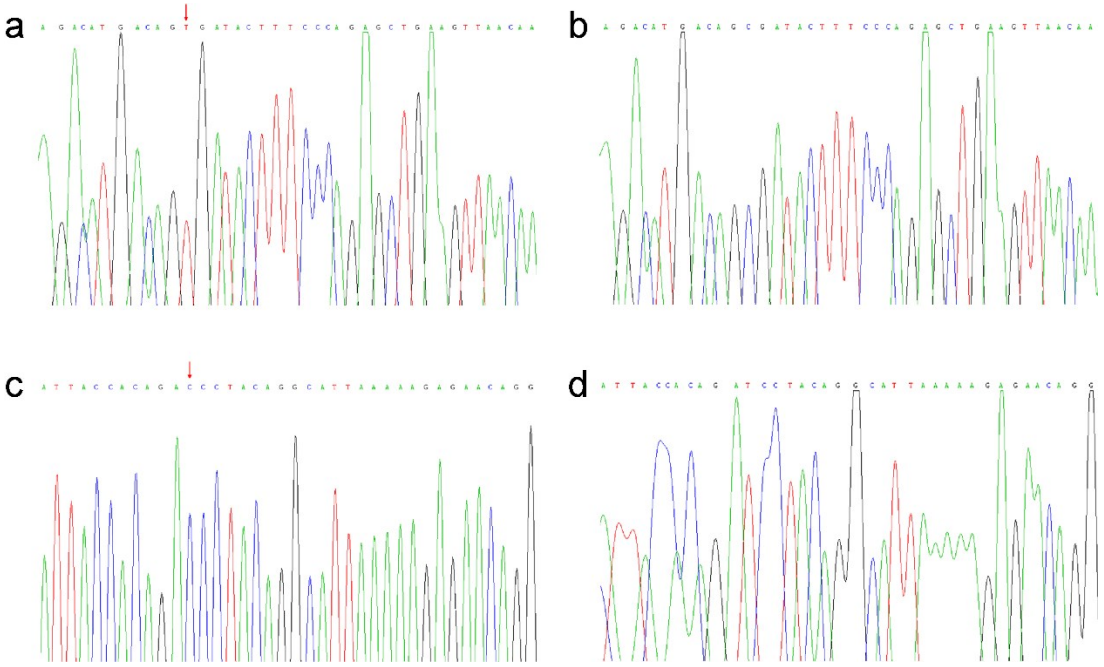


Figure S3. (a) *BRCA1*/c.2082C>T mutation from genomic DNA of ovarian cancer patient 1. (b) *BRCA1*/c.2082C>T of normal genomic DNA from normal people. (c) *BRCA2*/c.7397T>C mutation from genomic DNA of ovarian cancer patient 2. (d) *BRCA2*/c.7397T>C of normal genomic DNA from normal people. (Mutated bases are marked with red arrows).