Supplemental material

Effects of secondary structures of DNA templates on the quantification of qPCR

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**CONTENT**

Supplemental Tables S1-4

Supplemental Figures S1-6



Figure S1. Preparation scheme of double stranded DNA templates used in this study. The DNA templates were the ligation products from ‘TemA’, ‘TemB’, and ‘TemC’ in Tables S1-3, and the certain hairpin structures exist in ‘TemA’ (except TemAN). The splint A-B and splint B-C were the auxiliary DNA to ligate ‘TemA’ with ‘TemB’, and ‘TemB’ with ‘TemC’, respectively.

Table S1 Sequence of DNA used in different stem length assays for internal and external hairpins

|  |  |
| --- | --- |
| **Name** | **Sequence（5′-3′）** |
| TemAS6 | GCTCATCACGCAG**GCTGTG*tcgtacgttagtcctccgat*CA** |
| TemAS9 | GCTCATCACGCAG**GCTGTGACC*tcgtacgttagtcctccgat*GGTCA** |
| TemAS12 | GCTCATCACGCAG**GCTGTGGTGACC*tcgtacgttagtcctccgat*GGTCAC** |
| TemAS15 | GCTCATCACGCAG**GCTGTGGTGGTGACC*tcgtacgttagtcctccgat*GGTCACCAC** |
| TemAS20 | AGTCTCATGCTCATCACGCAG**GCTGTGGGACGACGGTGACC*tcgtacgttagtcctccgat*GGTCACCGTC** |
| **In**PriS (F/R) | GCTCATCACGCAG**GCTGTG/**GACTCATTGCTCGGCTTACCT |
| **Ex**PriS6 (F/R) | ***cgttagtcctccgat*CACAGC/**GACTCATTGCTCGGCTTACCT |
| **Ex**PriS9-20 (F/R) | ***cgttagtcctccgat*GGTCAC/**GACTCATTGCTCGGCTTACCT |
| TemAN | CATCACGCAGGCTGTGACCTCGTATGTTAGTCGAATTTAATAATATCTTCAGATAGTGA |
| PriN (F/R) | CATCACGCAGGCTGTGACC/GACTCATTGCTCGGCTTACCT |
| TemB1 | **CAGC**GGTGAAGTTGGGTTTCTTTCAAGAACATGTTCTACACAGACATATC |
| TemB2 | **CACAGC**GGTGAAGTTGGGTTTCTTTCAAGAACATGTTCTACACAGACATATC |
| TemB3 | **GTCCCACAGC**GGTGAAGTTGGGTTTCTTTCAAGAACATGTTCTACACAGACATATC |
| TemC | GGAAAGTGTTATCAAGCTACGAGGTAAGCCGAGCAATGAGTC |
| SplintA-B | ACC**GCTGTGACC*at*** |
| SplintA-B′ | ACC**GCTGTG*atcgg*** |
| SplintA-BN | ACCGCTGTCACTAT |
| SplintB-C | ACTTTCCGATATGT |

Note:

(1) TemAS6/AS9, TemB1, and TemCwere used to prepare templates with hairpins with 6 or 9-bp stem, the TemAS12/AS15, TemB2, and TemCwere used to prepare templates with hairpins with 12 or 15-bp stem, and the TemAS20, TemB3, and TemCwere used to prepare templates with hairpins with 20-bp stem. **In**PriS was used as primers for amplifying templates with inernal hairpins, and **Ex**PriS6and **Ex**PriS9-20 were used as primers for amplifying templates with external hairpins with 6-bp and 9~20-bp stems, respectively.

(2) The template control with no hairpins was prepared by TemAN, TemB1 and TemC.

(3) All TemAs and TemBs were ligated by SplintA-B, except that TemAN and TemB1 were ligated by SplintA-BN. Tem B1/B2/B3 and Tem C were ligated by SplintB-C.

Table S2 Sequence of DNA used in different loop size assays for internal and external hairpins

|  |  |
| --- | --- |
| **Name** | **Sequence（5′-3′）** |
| **In**TemAL5 | CATCACGCAG**GCTGTGACC*tcgat*GGTCA** |
| **In**TemAL15 | CATCACGCAG**GCTGTGACC*tcgtactcttccgat*GGTCA** |
| **In**TemAL25 | CATCACGCAG**GCTGTGACC*tcgtacgttagtcgtgtcctccgat*GGTCA** |
| **In**TemAL35 | CATCACGCAG**GCTGTGACC*tcgtacgttagtcgaatttaataatatcttccgat*GGTCA** |
| **In**PriL (F/R) | CATCACGCAG**GCTGTGACC**/GACTCATTGCTCGGCTTACCT |
| **Ex**TemAL10 | CATCACGCAG**GCTGTGACC*tcgagagcat*GGTCA** |
| **Ex**TemAL20 | CATCACGCAG**GCTGTGACC*tcgacagttagtcctccgat*GGTCA** |
| **Ex**TemAL30 | CATCACGCAG**GCTGTGACC*tcgtacgttagtcgaatttagtcttccgat*GGTCA** |
| **Ex**TemAL40 | CATCACGCAG**GCTGTGACC*tcgtacgttagtcgaatttaataatacggtctcttccgat*GGTCA** |
| **Ex**PriL10 (F/R) | ***tcgagagcat*GGTCACAGC/**GACTCATTGCTCGGCTTACCT |
| **Ex**PriL20 (F/R) | ***gtcctccgat*GGTCACAGC/**GACTCATTGCTCGGCTTACCT |
| **Ex**PriL30 (F/R) | ***agtcttccgat*GGTCACAGC/**GACTCATTGCTCGGCTTACCT |
| **Ex**PriL40 (F/R) | ***tctcttccgat*GGTCACAGC/**GACTCATTGCTCGGCTTACCT |
| PriN (F/R) | CATCACGCAGGCTGTGACC/GACTCATTGCTCGGCTTACCT |
| TemAN | CATCACGCAGGCTGTGACCTCGTATGTTAGTCGAATTTAATAATATCTTCAGATAGTGA |
| TemB1 | **CAGC**GGTGAAGTTGGGTTTCTTTCAAGAACATGTTCTACACAGACATATC |
| TemC | GGAAAGTGTTATCAAGCTACGAGGTAAGCCGAGCAATGAGTC |
| SplintA-B | ACC**GCTGTGACC*at*** |
| SplintA-B′ | ACC**GCTGTG*atcgg*** |
| SplintA-BN | ACCGCTGTCACTAT |
| SplintB-C | ACTTTCCGATATGT |

Note:

(1) **In**TemAL5/AL15/AL25/AL35, TemB1 and TemCwere used to prepare templates with internal hairpins with 5, 15, 25, and 35-nt loop, respectively, and **In**PriL was used as primers for their qPCR

(2) **Ex**TemAL10/AL20/AL30/AL40, TemB1 and TemCwere used to prepare templates with external hairpins with 10, 20, 30, and 40-nt loop, respectively, and **Ex**PriL10/PriL20/PriL30/PriL40, were used as primers for their qPCR, respectively.

(3) The template control with no hairpins was prepared by TemAN, TemB1 and TemC.

(4) All TemAs and TemBs were ligated by SplintA-B, except that **Ex**TemAL10 and TemB1 were ligated by SplintA-B′, and TemAN and TemB1 were ligated by SplintA-BN. Tem B1 and Tem C were ligated by SplintB-C.

Table S3 Sequence of DNA used in the different primer-binding site assay

|  |  |
| --- | --- |
| **Name** | **Sequence（5′-3′）** |
| **Loc**TemA | GTCTCATGCTCATCACGCAG**GCTGTGACC*tcgtactcttccgat*GGTCA** |
| TemB1 | **CAGC**GGTGAAGTTGGGTTTCTTTCAAGAACATGTTCTACACAGACATATC |
| TemC | GGAAAGTGTTATCAAGCTACGAGGTAAGCCGAGCAATGAGTC |
| SplintA-B | ACC**GCTGTGACC*at*** |
| SplintB-C | ACTTTCCGATATGT |
| **Loc**Pri1 (F/R) | TGCTCATCACGCAG**GCTGT/**GACTCATTGCTCGGCTTACCT |
| **Loc**Pri2 (F/R) | CATCACGCAG**GCTGTGACC/**GACTCATTGCTCGGCTTACCT |
| **Loc**Pri3 (F/R) | CGCAG**GCTGTGACC*tcgta*/**GACTCATTGCTCGGCTTACCT |
| **Loc**Pri4 (F/R) | ***tcgtactcttccgat*GGTCA/**GACTCATTGCTCGGCTTACCT |
| **Loc**Pri5 (F/R) | ***actcttccgat*GGTCACAGC/**GACTCATTGCTCGGCTTACCT |
| **Loc**Pri6 (F/R) | ***cagt*GGTCACAGC**GGTGAA**/**GACTCATTGCTCGGCTTACCT |
| **Loc**PriN (F/R) | GTCTCATGCTCATCACGCAG**/**GACTCATTGCTCGGCTTACCT |

Note:

(1) **Loc**TemA, TemB1, and TemC were used to prepare templates for various primer-binding stie assays, and **Loc**Pri1~6were the primers annealing to different sites towards hairpins.

(2) SplintA-B was used to ligate **Loc**TemA and TemB1. SplintB-C was used to ligate Tem B1 and Tem C.

(3) **Loc**Pri1, **Loc**Pri2, **Loc**Pri3, **Loc**Pri4, **Loc**Pri5, **Loc**Pri6, and **Loc**PriN were named as #1, #2, #3, #4, #5, #6, and #N in the main text.

Table S4 Sequence of DNA used in the quantification assay of toxR gene

|  |  |
| --- | --- |
| **Name** | **Sequence（5′-3′）** |
| **Vp**PriC (F/R) | ATTGACGCCTCTGCTAATGAG**/**TACGCAAATCGGTAGTAATAGTG |
| **VpIn**Pri (F/R) | CGTTACCAGTGGAAGTAATTGC**/**TCATACGAGTGGTTGCTGTCAT |
| **VpEx**Pri (F/R) | AGTTCCGTCAGATTGGTGAGTA**/**TTACTTCCACTGGTAACGAGTC |

Note: **Vp**PriC, **VpIn**Pri, and **VpEx**Pri, were the primers of standard curve assays of qPCR fortoxR gene in *Vibrio paraheamoliticus*, which was used to amplify the amplicons with no, internal, and external hairpins (named as toxR-c, toxR-1 and toxR-2 in the main text, respectively).



Figure S2. The sequence information of templates with internal hairpins with 6-, 9-, 12-, 15-, or 20-bp stems in Figure 2. The loop of each hairpin was 20 nt. The black letters are the sequences of amplicons (the letters in bold are the sequences of stems). The red letters show the forward primers, and the parts double-underlined in black are for the binding region of reverse primer.



Figure S3. The sequence information of templates with internal hairpins with 5-, 15-, 25-, or 35-nt loops in Figure 3. The stem length of each hairpin was 9 bp. The black letters are the sequences of amplicons (the lowercase letters are the loop sequence). The red letters show the forward primers, and the parts double-underlined in black are for the binding sites of reverse primers.



Figure S4. The sequence information of templates with external hairpins with 6-, 9-, 12-, 15-, or 20-bp stems in Figure 4. The loop of each hairpin was 20 nt. The black letters are the sequences of amplicons (the letters in bold are the sequences of stems). The red letters show the forward primers, and the parts double-underlined in black are for the binding sites of reverse primers.



Figure S5. The sequence information of templates with external hairpins with 10-, 20-, 30-, or 40-nt loops in Figure 5. The stem length of each hairpin was 9 bp. The black letters are the sequences of amplicons (the lowercase letters are the loop sequence). The red letters show the forward primers, and the parts double-underlined in black are for the binding sites of reverse primers.



Figure S6. The sequence information of 3 amplicons of *Vibrio Parahaemoliticus*, used in Figure 8. In (A), (B) and (C), amplicons toxR-c (with no hairpins), toxR-1 (with internal hairpins), and toxR-2 (with external hairpins), are shown. The black letters are the sequences of 3 amplicons. The red letters show the forward primers, and the parts double-underlined in black are for the binding sites of reverse primers.