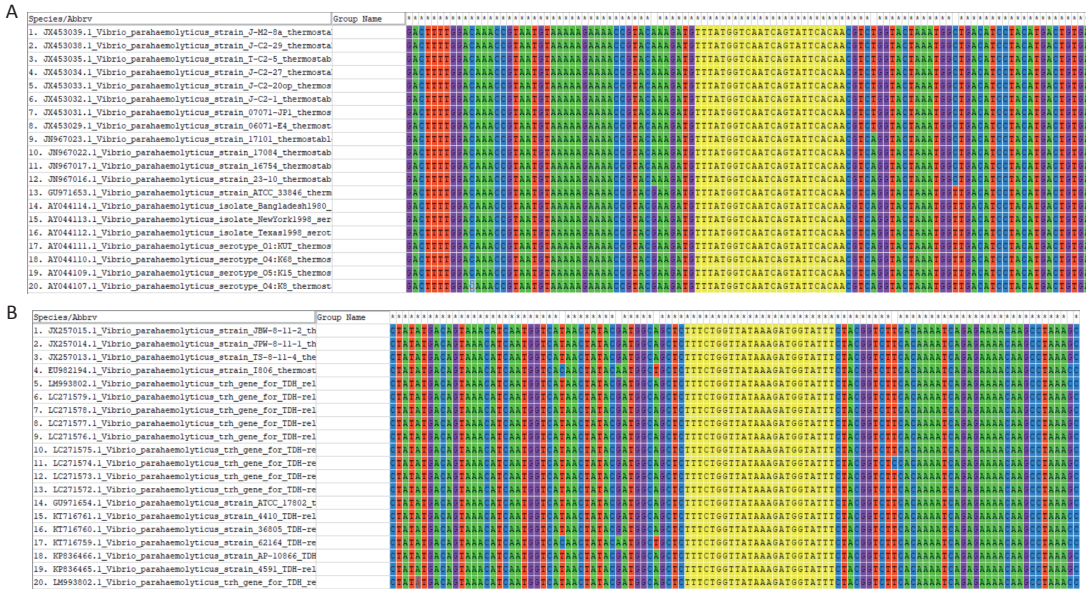
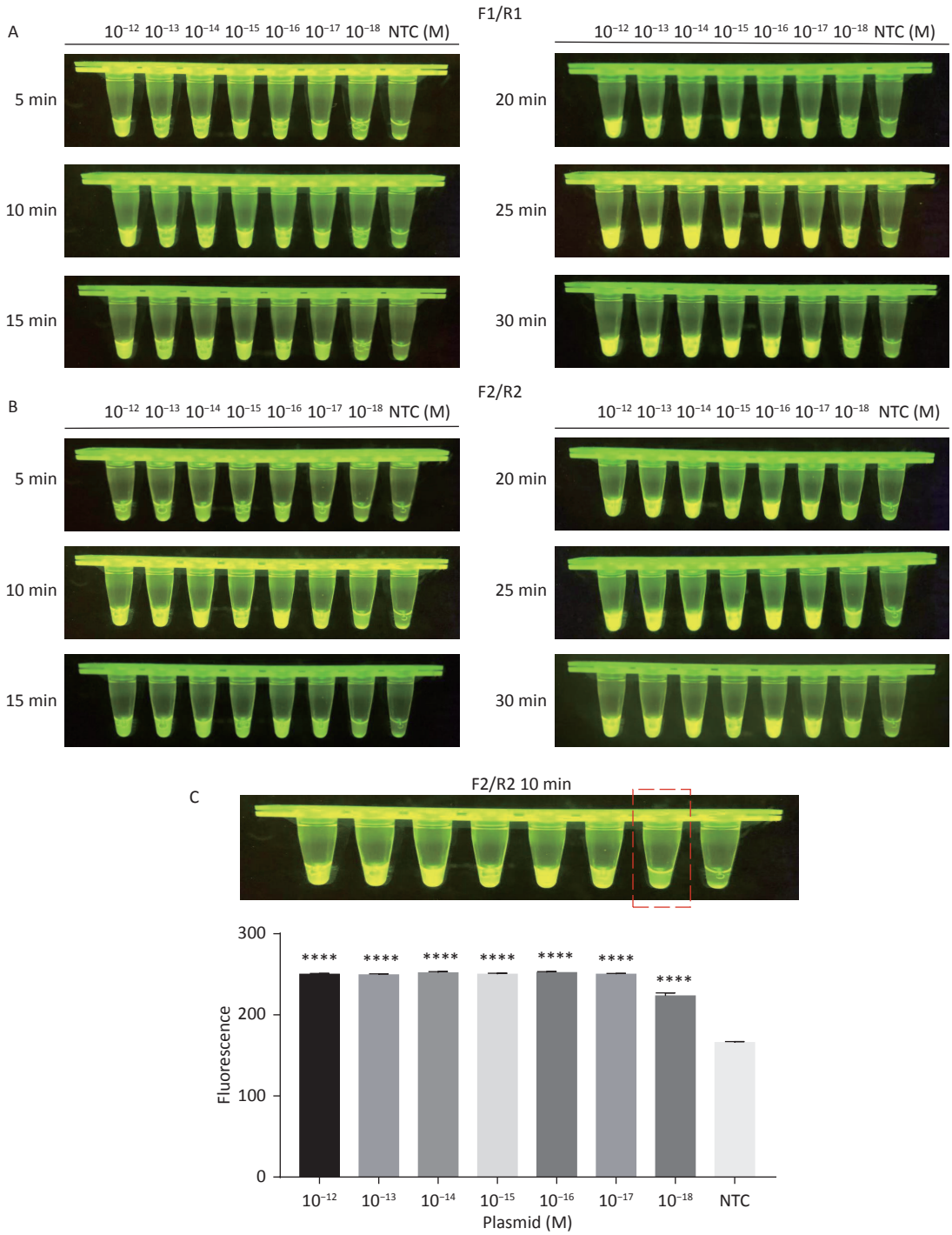
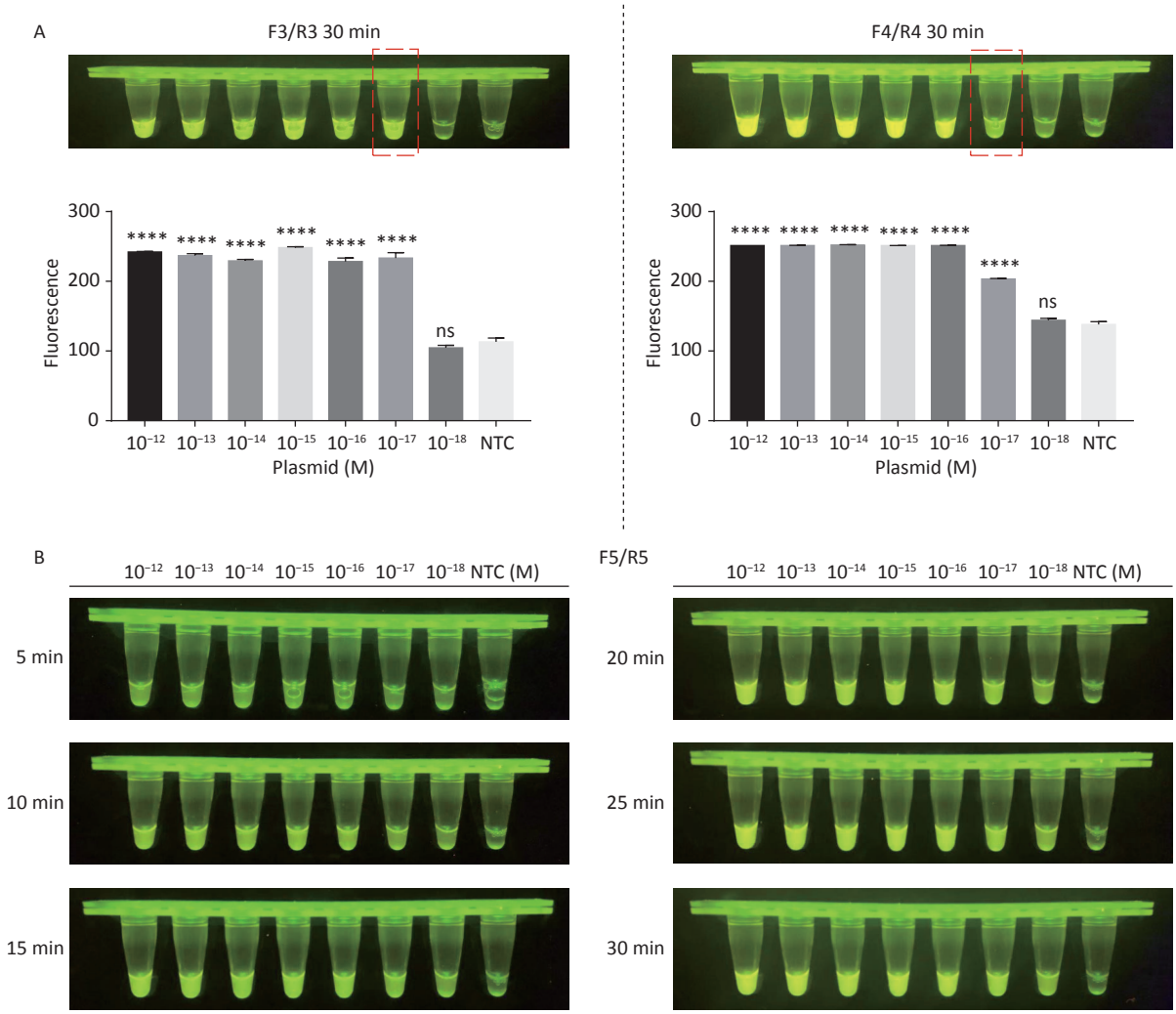


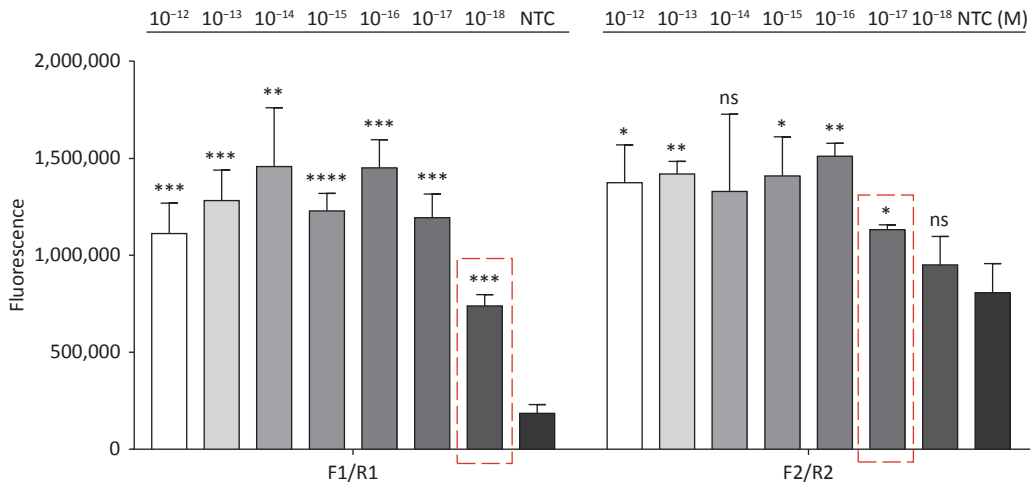

**Supplementary Figure S1.** Sequence alignment of *tdh* and *trh* genes from 20 *V. parahaemolyticus* genotypes: (A) *tdh* genes (B) *trh* genes. The conserved sequences targeted by crRNA were highlighted with yellow color.



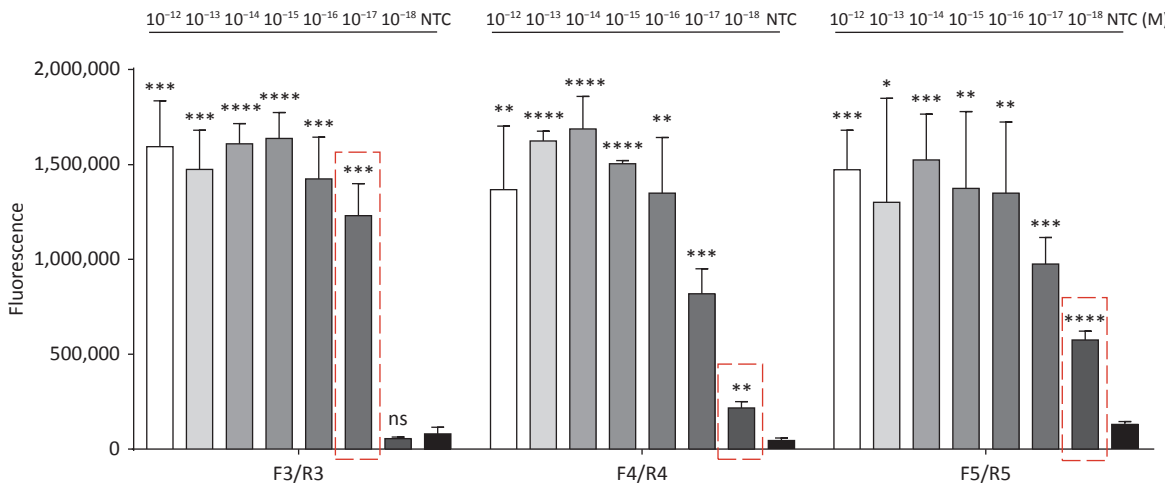

**Supplementary Figure S2.** Sensitivity of the CRISPR/Cas12a-VD method for detecting the *tdh* gene from $10^{-12}$ M to $10^{-18}$ M. (A) Amplified products obtained using F1/R1 were added to the Cas12a-mediated cleavage system and incubated for 5−30 min. (B) Amplified products obtained using F2/R2 were added to the Cas12a-mediated cleavage system and incubated for 5−30 min. (C) Amplified products obtained using F2/R2 were added to the Cas12a-mediated cleavage system and incubated for 10 min. The green fluorescent signal was quantified by ImageJ. $n$ = 3, two-tailed Student's $t$ test; $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, $^{****}P < 0.0001$ and ns not significant; NTC nontarget control; bars represent mean ± SD.



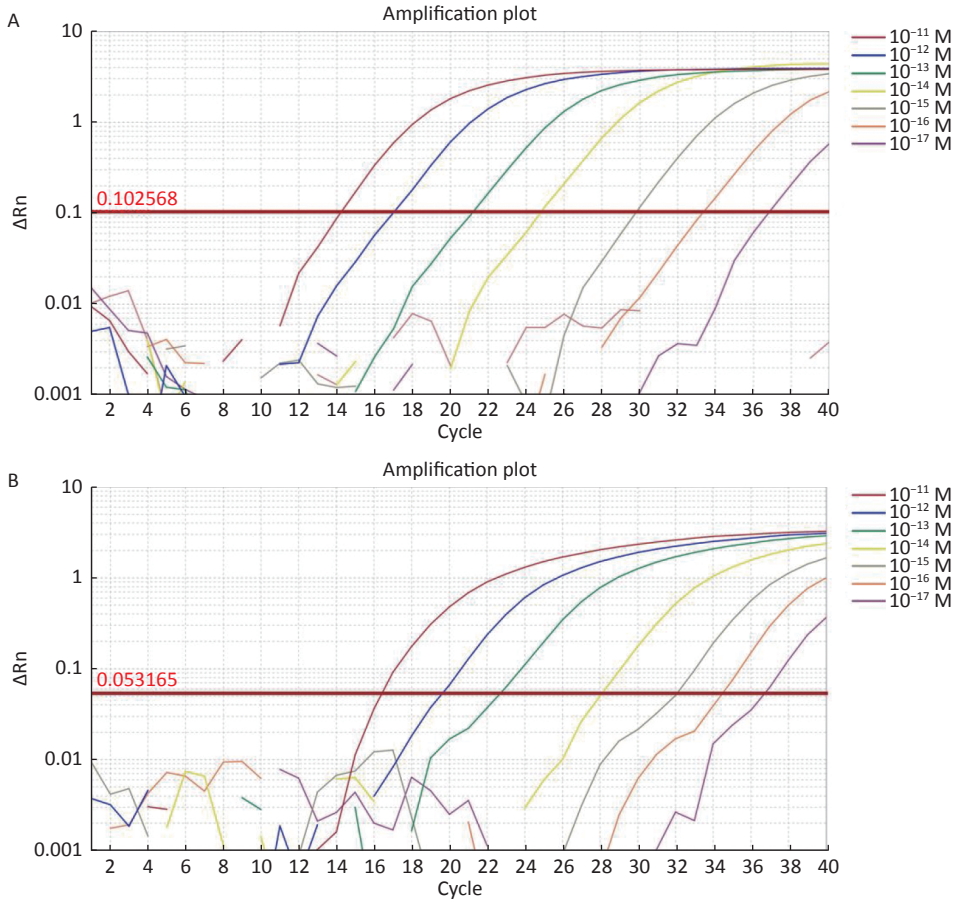

**Supplementary Figure S3.** Sensitivity of the CRISPR/Cas12a-VD method for detecting the *trh* gene from $10^{-12}$ M to $10^{-18}$ M. (A) Amplified products obtained using F3/R3 and F4/R4 were added to the Cas12a-mediated cleavage system and incubated for 30 min. The green fluorescent signal was quantified with ImageJ. (B) Amplified products obtained using F5/R5 were added to the Cas12a-mediated cleavage system and incubated for 5−30 min. $n$ = 3, two-tailed Student's $t$ test; $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, $^{****}P < 0.0001$ and ns not significant; NTC nontarget control; bars represent mean ± SD.



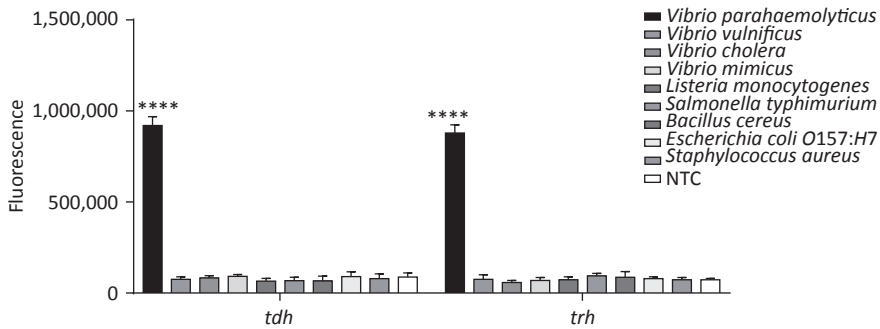

**Supplementary Figure S4.** Sensitivity of the CRISPR/Cas12a coupled with fluorescence readout for detecting the *tdh* gene from $10^{-12}$ M to $10^{-18}$ M using primer pairs F1/R1 and F2/R2. $n$ = 3, two-tailed Student's $t$ test; $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, $^{****}P < 0.0001$ and ns not significant; NTC nontarget control; bars represent mean ± SD.




**Supplementary Figure S5.** Sensitivity of the CRISPR/Cas12a coupled with fluorescence readout for detecting the *trh* gene from $10^{-12}$ M to $10^{-18}$ M using primer pairs F3/R3, F4/R4, and F5/R5. $n$ = 3, two-tailed Student's $t$ test; $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, $^{****}P < 0.0001$ and ns not significant; NTC nontarget control; bars represent mean ± SD.




**Supplementary Figure S6.** (A) The sensitivity of Real-time PCR to detect *tdh* gene from $10^{-11}$ M to $10^{-17}$ M. (B) The sensitivity of Real-time PCR to detect *trh* gene from $10^{-11}$ M to $10^{-17}$ M.




**Supplementary Figure S7.** Specificity of the CRISPR/Cas12a coupled with fluorescence readout for detecting *tdh* and *trh* genes. $n$ = 3, two-tailed Student's $t$ test; $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, $^{****}P < 0.0001$ and ns not significant; NTC, nontarget control; bars represent mean ± SD.

**Supplementary Table S1.** Oligonucleotides used to construct plasmid templates

| Name | Sequence (5' to 3') |
|---|---|
| *tdh* gene fragment | TATTTTGCAAAAAAATCATTTTTATTTATATCCATGTTGGCTG<br>CATTCAAAACATCTGCTTTTGAGCTTCCATCTGTCCCTTTTCC<br>TGCCCCCGGTTCTGATGAGATATTGTTTGTTGTTCGAGATAC<br>AACTTTTAATACCCAAGCTCCGGTCAATGTAAAGGTCTCTGA<br>CTTTTGGACAAACCGTAATGTAAAAAGAAAACCGTACGAAG<br>ATGTTTATGGTCAATCAGTATTCACAACGTCAGGTACTAAAT<br>GGTTGACATCCTACATGACTGTGAACATTAATGATAAAGACT<br>ATACAATGGCAGCGGTGTCTGGCTATAAGAGCGGTCATTCTG<br>CTGTGTTCGTAAAATCAGATCAAGTACAACTTCAACATTCCT<br>ATAATTCTGTAGCTAACTTTGTTGGTGAAGATGAAGGTTCTA<br>TTCCAAGTAAAATGTATTTGGATGAAACTCCAGAATATTTTG<br>TTAATGTAGAAGCATATGAGAGTGGTAGTGGTAATATATTG<br>GTAATGTGTATATCCAACAAAGAATCGTTTTTTGAATGT |
| *trh* gene fragment | ATGAAACTAAAACTCTACTTTGCTTTCAGTTTGCTATTGGTTT<br>CAATGTTTTCAGTATCTAAATCATTCGCGATTGATCTGCCATC<br>AATACCTTTTCCTTCTCCAGGTTCGGCTGAACTGTTATTTGTT<br>GTTAGAAATACAACAATCAAAACTGAATCCCCGGTTAAGGCA<br>ATTGTGGAGGACTATTGGACAAACCGAAACATAAAAAGAAAA<br>CCATACAAAGATGTATACGGTCAATCGGTTTTCACAACAGCAG<br>GTTCAAAGTGGTTAAGCGCCTATATGACAGTAAACATCAATGG<br>TCATAACTATACGATGGCAGCTCTTTCTGGTTATAAAGATGGT<br>ATTTCTACGGTCTTCACAAAATCAGAGAAAACAAGCCTAAAGC<br>AAGACTATTCCTCGGTAAAGTCTTTTGTTGATGACAGCGAAGA<br>ATCAATACCAAGTATAACTTATTTAGATGAAACATCAGAATAC<br>TTTGTTACTGTCGAGGCATATGAGAGCGGCAATGGACATATGT<br>TTGTTATGTGCATTTCCAACAAATTATCATTTGGTGAATGTAAA<br>TCACAAATTTAA |

**Supplementary Table S2.** Primers, CrRNA, and SsDNA reporter used in this study

| Name | Sequence (5' to 3') |
|---|---|
| *tdh*-RPA-F1 | CAACTTTTAATACCCAAGCTCCGGTCAATG |
| *tdh*-RPA-R1 | CAGCAGAATGACCGCTCTTATAGCCAG |
| *tdh*-RPA-F2 | CCGGTTCTGATGAGATATTGTTTGTTGTTC |
| *tdh*-RPA-R2 | GCAGAATGACCGCTCTTATAGCCAGACACC |
| *trh*-RPA-F3 | CAATTGTGGAGGACTATTGGACAAACCGAAAC |
| *trh*-RPA-R3 | CTTTAGGCTTGTTTTCTCTGATTTTGTGAAGACC |
| *trh*-RPA-F4 | GTGGAGGACTATTGGACAAACCGAAACATA |
| *trh*-RPA-R4 | TACCGAGGAATAGTCTTGCTTTAGGCTTGT |
| *trh*-RPA-F5 | GTGGAGGACTATTGGACAAACCGAAACATA |
| *trh*-RPA-R5 | GACTTTACCGAGGAATAGTCTTGCTTTAGG |
| *tdh*-q-F | AAACATCTGCTTTTGAGCTTCCA |
| *tdh*-q-R | CTCGAACAACAAACAATATCTCATCAG |
| *tdh*-q-P | FAM-TGTCCCTTTTCCTGCCCCCGG-TAMRA |
| *trh*-q-F | ATTCGCGATTGATCTGCCAT |
| *trh*-q-R | ATAGTCCTCCACAATTGCCT |
| *trh*-q-P | FAM-CCTTCTCCAGGTTCGGCTGAACTGT-TAMRA |
| *tdh*-crRNA | UAAUUUCUACUAAGUGUAGAUUGGUCAAUCAGUAUUCACAA |
| *trh*-crRNA | UAAUUUCUACUAAGUGUAGAUUGGUUAUAAAGAUGGUAUUU |
| ssDNA reporter | FAM-TTATT-BHQ1 |