Electronic Supplementary Information

Leak-free Million-fold DNA Amplification with Locked Nucleic Acid and Targeted Hybridization in One Pot

K. Komiya,*[a] M. Komori,*[b] C. Noda,[a] S. Kobayashi,[c] T. Yoshimura[b] and M. Yamamura[a]

[a] School of Computing, Tokyo Institute of Technology, Japan

[b] Research and Development, Abbott Japan Co., Ltd., Japan

[c] Department of Communication Engineering and Informatics, the University of Electro-Communications, Japan

S-1. Supplementary Experiment

Investigation of the effects of LNA introduction

We experimentally investigated the effects of LNA introduction on DNA amplification. The DNA sequences without LNA used in the supplementary experiment are shown in Table S1. First, a DNA amplification reaction with the LNA-free converter (Converter 0) and the LNA-free amplifier (Amplifier 0) was implemented. In contrast to the FI plot shown in Fig. 2 for the DNA amplification reaction using the converter and amplifier strands containing multiple LNAs in their sequences, the resulting FI plot showed leak amplification in the absence of input, occurring approximately 20 min earlier than the amplification with 100 fM input (Fig. S1 (A)). Second, a DNA amplification reaction using Converter shown in Table 1 and Amplifier 0 was implemented. The resulting FI plot also showed early leak amplification in the absence of input but slightly later than did the plot of the reaction with 100 fM input (Fig. S1 (B)). Introduction of LNA only into the converter seemed to have a partial suppressive effect, but it was not sufficient to fully suppress leak amplification for more than 2 h.

Next, we investigated the effect of LNA introduction position in the amplifier. One series of DNA amplification reactions were implemented using Converter and one of the amplifiers shown in Table S1, each containing a single LNA in its primer-binding region at the first to tenth position from the 3′ end, termed Amplifier 1 to Amplifier 10. A few of the resulting FI plots showed early leak amplification in the absence of input (Fig. S3 (B), (I), (J)), similarly to that in Fig. S1 (B). LNA introduction at the second, ninth, or tenth position from the 3′ end

appeared to not affect the amplification reaction. In the other FI plots, delayed amplification was observed for all reactions, both in the absence and presence of the input. Notably, in reactions containing Amplifier 6, amplification did not occur for more than 2 h in the absence or presence of 100 fM input (Fig. S3 (F)). LNA introduction at the sixth position significantly delayed the amplification reaction. We confirmed that the positions of LNA in the amplifier affected both correct and leak amplification and that the introduction of only a single LNA did not allow specific suppression of leak amplification.

We then investigated the effect of the combinatorial LNA introduction to the amplifier. Another series of DNA amplification reactions were implemented using Converter and one of the amplifiers shown in Table S2, each containing two LNAs at the positions of four LNAs in Amplifier shown in Table 1. Two resulting FI plots showed early leak amplification in the absence of input (Fig. S4 (A), (E)), similarly to that in Fig. S1 (B). In addition to the combinatorial LNA introduction at the second and tenth positions, that at the first and second positions appeared to not affect the amplification reaction. In contrast, the combinatorial LNA introduction at the first and tenth positions resulted in moderately delayed amplification (Fig. S4 (C)). LNA introduction at the second position might diminish the delayed effect due to LNA introduction at other positions. In the FI plot for LNA introduction at the sixth and tenth positions, the amplification reaction was significantly delayed (Fig. S4 (F)), similarly to that in Fig. S3 (F). Moderately delayed amplification, observed for LNA introduction at the second and sixth positions (Fig. S4 (D)), might be attributed to the effect of LNA at the second position assumed above. However, the effect of the combinatorial LNA introduction did not seem to be the simple synthesis of the effect of single LNA introduction. Specific suppression of leak amplification was not achieved with the introduction of two LNAs.

The other series of DNA amplification reactions were further implemented using Converter and one of the amplifiers shown in Table S2, each containing three LNAs at the positions of four LNAs in Amplifier. One resulting FI plot showed early leak amplification in the absence of input (Fig. S5 (B)), similarly to that in Fig. S1 (B). The combinatorial LNA introduction at the first, second, and tenth positions appeared to not affect the amplification reaction. In the FI plot for LNA introduction at the first, sixth, and tenth positions, the amplification reaction was significantly delayed (Fig. S5 (C)), similarly to that in Fig. S3 (F). In the other FI plots for LNA introduction at the first, second, and sixth positions, and the second, sixth, and tenth positions, slightly and largely delayed amplification was observed, respectively (Fig. S5 (A), (D)). However, specific suppression of leak amplification was not achieved with the introduction of three LNAs.

S-2. Supplementary Figures and Tables

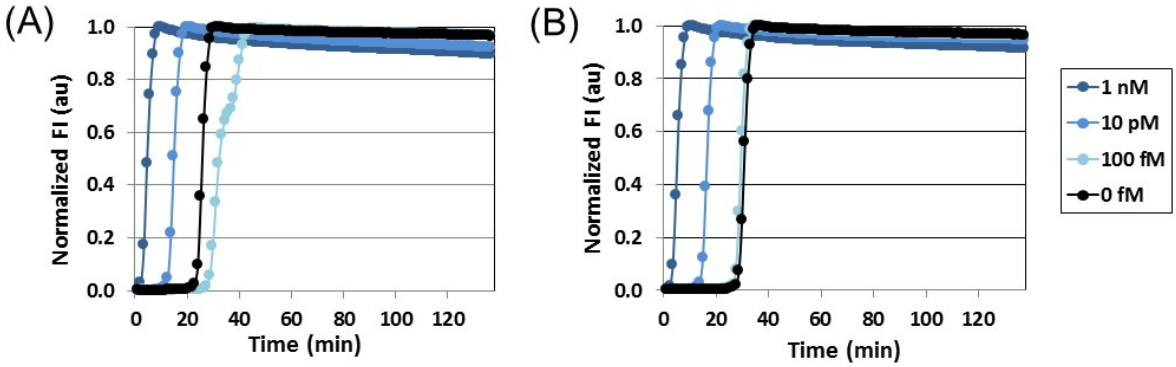


Fig. S1. FI plots of L-TEAM reactions implemented in the absence and presence of input at concentrations from 100 fM to 1 nM with the LNA-free converter and the LNA-free amplifier (A) and with the converter containing two LNAs and the LNA-free amplifier (B).

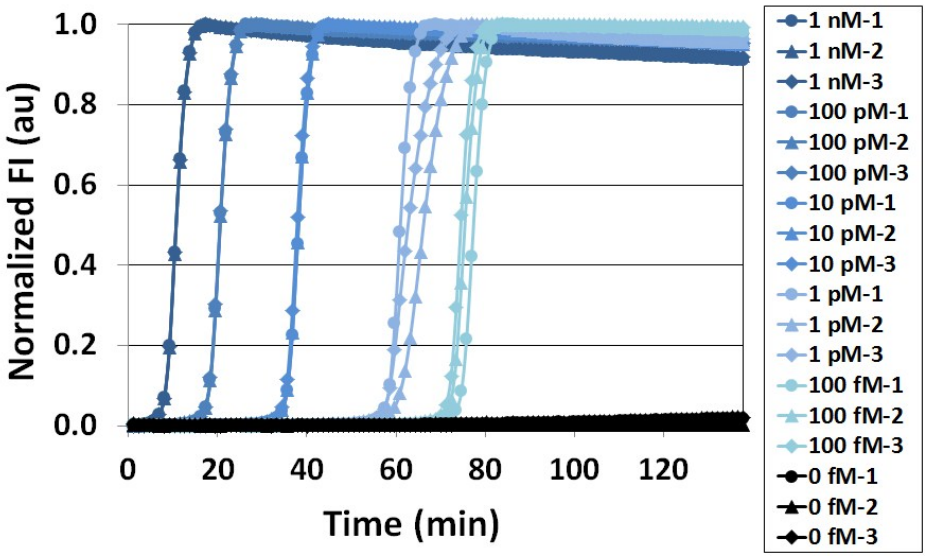


Fig. S2. Resulting normalized FI plot of L-TEAM reactions using Converter and Amplifier shown in Table 1 in the absence and presence of input at concentrations from 100 fM to 1 nM.

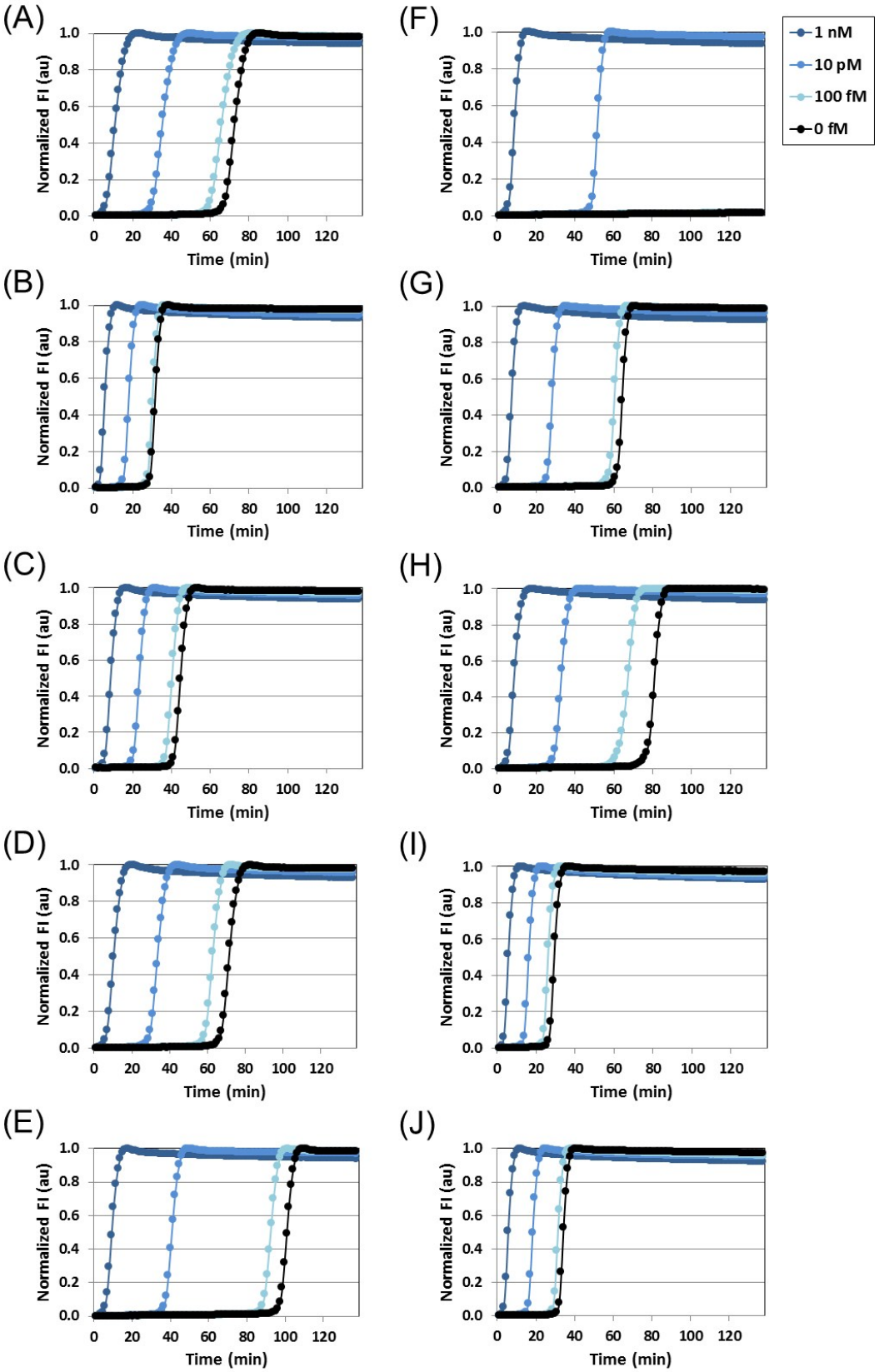


Fig. S3. FI plots of L-TEAM reactions implemented in the absence and presence of input at concentrations from 100 fM to 1 nM with the converter containing two LNAs and Amplifier 1 (A), Amplifier 2 (B), Amplifier 3 (C), Amplifier 4 (D), Amplifier 5 (E), Amplifier 6 (F), Amplifier 7 (G), Amplifier 8 (H), Amplifier 9 (I), and Amplifier 10 (J).

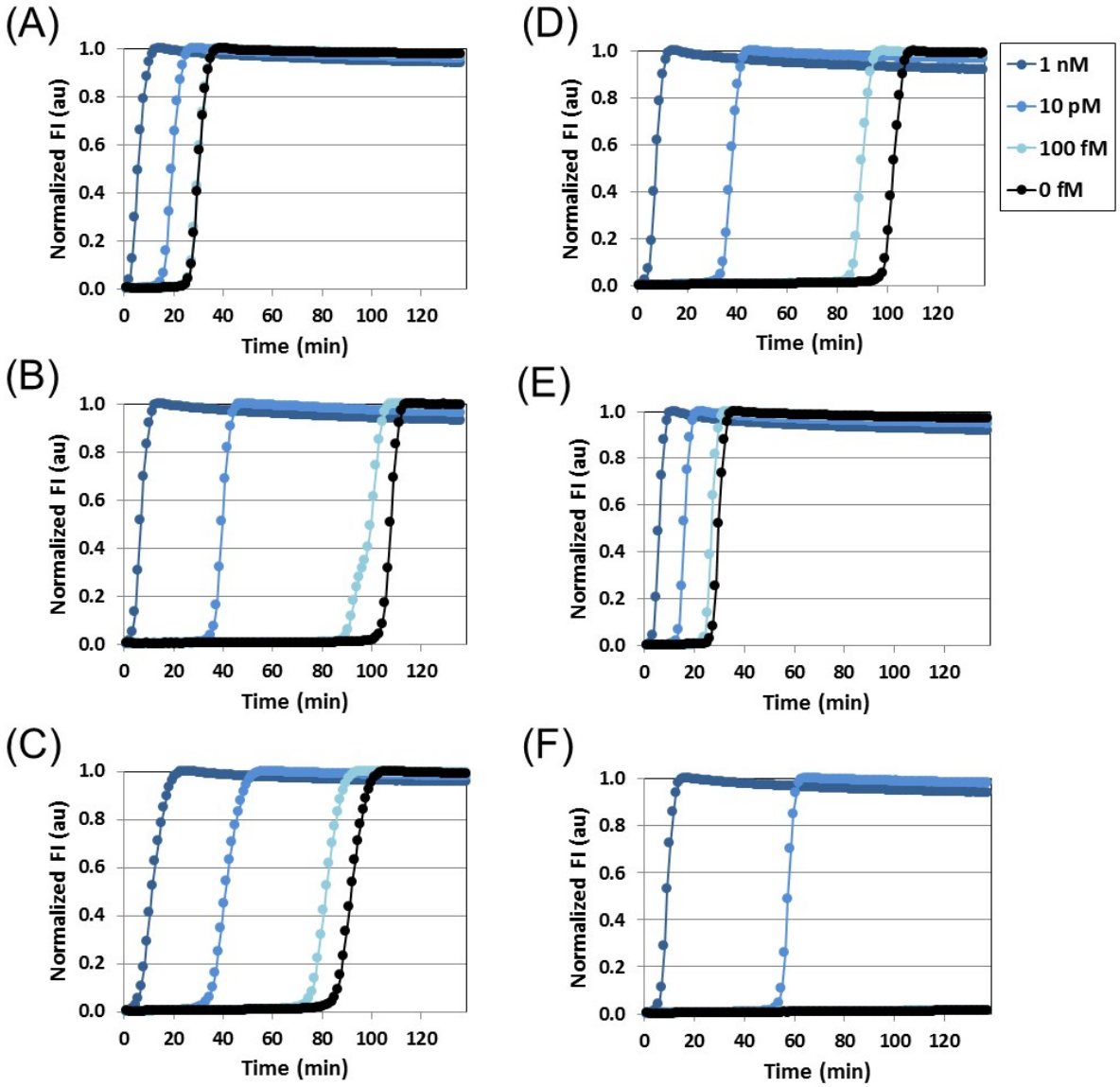
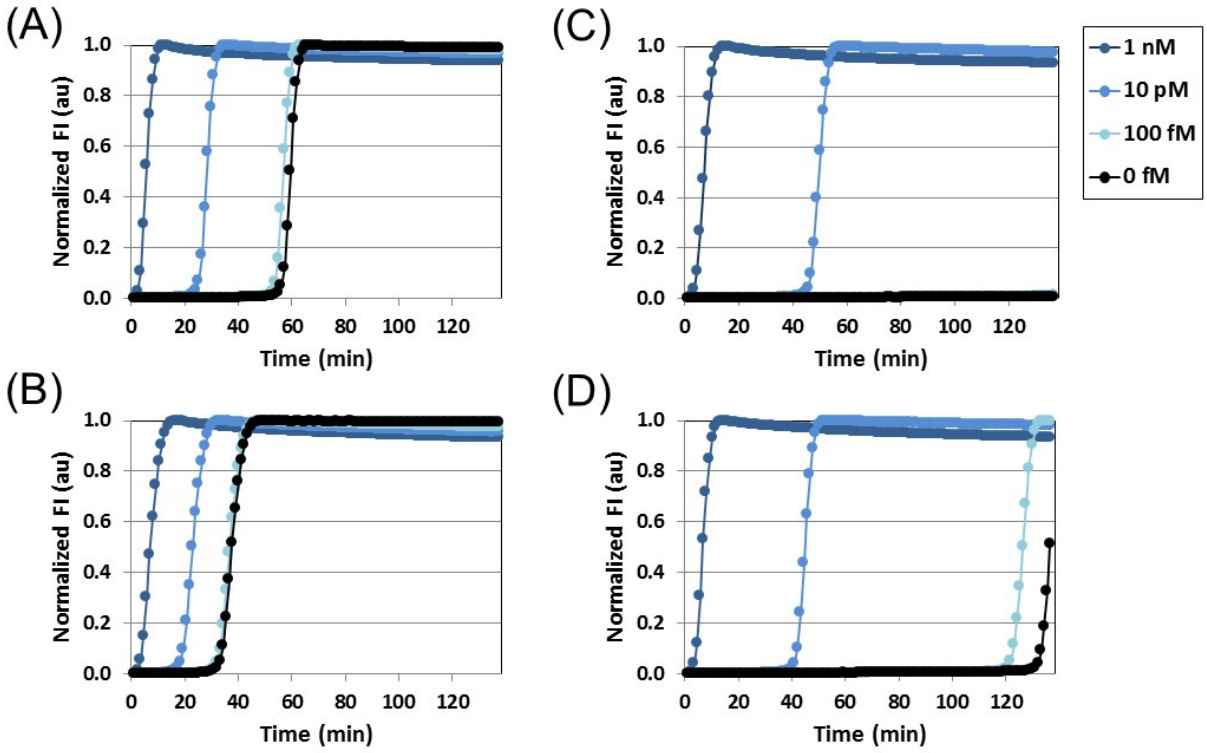


Fig. S4. FI plots of L-TEAM reactions implemented in the absence and presence of input at concentrations from 100 fM to 1 nM with the converter containing two LNAs and Amplifier 1-2 (A), Amplifier 1-6 (B), Amplifier 1-10 (C), Amplifier 2-6 (D), Amplifier 2-10 (E), and Amplifier 6-10 (F).



92

93 Fig. S5. FI plots of L-TEAM reactions implemented in the absence and presence of input at
94 concentrations from 100 fM to 1 nM with the converter containing two LNAs and Amplifier
95 1-2-6 (A), Amplifier 1-2-10 (B), Amplifier 1-6-10 (C), and Amplifier 2-6-10 (D).

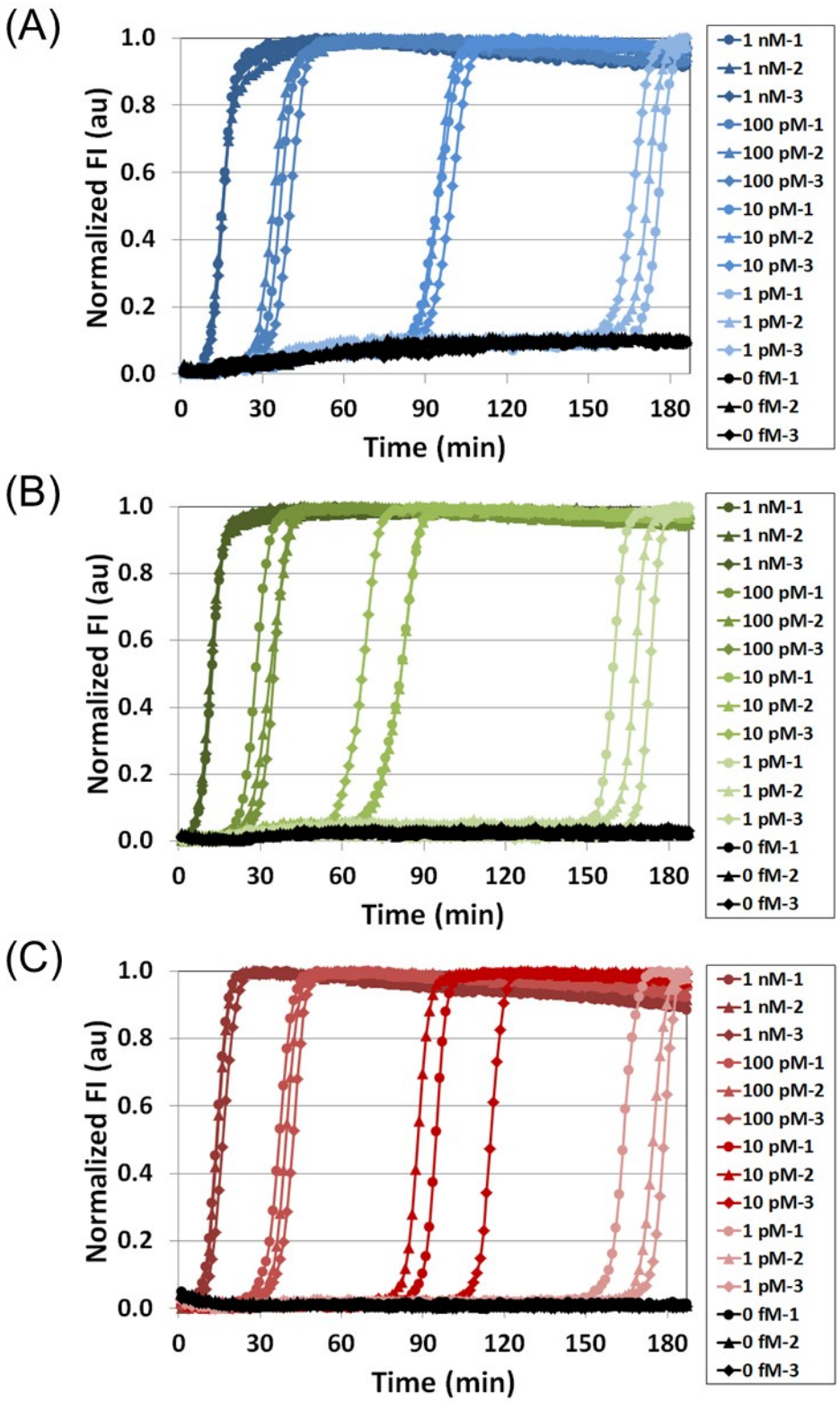
96

Converter 0	5′ AGCCCTGTACAATGCCCTCAGC CTGTTCCTGCTGAACTGAGCCA-(I)-(I) 3′
Amplifier 0	5′ AGCCCTGTACAATGCCCTCAGC AGCCCTGTACAAT-(I)-(I) 3′
Amplifier 1	5′ AGCCCTGTACAATGCCCTCAGC AGCCCTGTACAAT^{L} -(I)-(I) 3′
Amplifier 2	5′ AGCCCTGTACAATGCCCTCAGC AGCCCTGTACAA^{L} T-(I)-(I) 3′
Amplifier 3	5′ AGCCCTGTACAATGCCCTCAGC AGCCCTGTACA^{L} AT-(I)-(I) 3′
Amplifier 4	5′ AGCCCTGTACAATGCCCTCAGC AGCCCTGTAC^{L} AAT-(I)-(I) 3′
Amplifier 5	5′ AGCCCTGTACAATGCCCTCAGC AGCCCTGTA^{L} CAAT-(I)-(I) 3′
Amplifier 6	5′ AGCCCTGTACAATGCCCTCAGC AGCCCTGT^{L} ACAAT-(I)-(I) 3′
Amplifier 7	5′ AGCCCTGTACAATGCCCTCAGC AGCCCTG^{L} TACAAT-(I)-(I) 3′
Amplifier 8	5′ AGCCCTGTACAATGCCCTCAGC AGCCCT^{L} GTACAAT-(I)-(I) 3′
Amplifier 9	5′ AGCCCTGTACAATGCCCTCAGC AGCCC^{L} TGTACAAT-(I)-(I) 3′
Amplifier 10	5′ AGCCCTGTACAATGCCCTCAGC AGCC^{L} CTGTACAAT-(I)-(I) 3′

Table S1. DNA sequences containing no or one LNA, used in the supplementary experiment. Blue and red letters represent the sequences complementary to the signal sequence and the recognition site of Nb.BbvCI, respectively. The positions of LNAs are indicated by the superscripted letter L. (I) represents modification with inverted deoxythymidine at the 3′ ends.

Amplifier 1-2	5′ AGCCCTGTACAATGCCCTCAGC AGCCCTGTACAA^{L} T^{L} -(I)-(I) 3′
Amplifier 1-6	5′ AGCCCTGTACAATGCCCTCAGC AGCCCTGT^{L} ACAAT^{L} -(I)-(I) 3′
Amplifier 1-10	5′ AGCCCTGTACAATGCCCTCAGC AGCC^{L} CTGTACAAT^{L} -(I)-(I) 3′
Amplifier 2-6	5′ AGCCCTGTACAATGCCCTCAGC AGCCCTGT^{L} ACAA^{L} T-(I)-(I) 3′
Amplifier 2-10	5′ AGCCCTGTACAATGCCCTCAGC AGCC^{L} CTGTACAA^{L} T-(I)-(I) 3′
Amplifier 6-10	5′ AGCCCTGTACAATGCCCTCAGC AGCC^{L} CTGT^{L} ACAAT-(I)-(I) 3′
Amplifier 1-2-6	5′ AGCCCTGTACAATGCCCTCAGC AGCCCTGT^{L} ACAA^{L} T^{L} -(I)-(I) 3′
Amplifier 1-2-10	5′ AGCCCTGTACAATGCCCTCAGC AGCC^{L} CTGTACAA^{L} T^{L} -(I)-(I) 3′
Amplifier 1-6-10	5′ AGCCCTGTACAATGCCCTCAGC AGCC^{L} CTGT^{L} ACAAT^{L} -(I)-(I) 3′
Amplifier 2-6-10	5′ AGCCCTGTACAATGCCCTCAGC AGCC^{L} CTGT^{L} ACAA^{L} T-(I)-(I) 3′

Table S2. DNA sequences containing two or three LNAs used in the supplementary experiment. Blue and red letters represent the sequences complementary to the signal sequence and the recognition site of Nb.BbvCI, respectively. The positions of LNAs are indicated by the superscripted letter L. (I) represents modification with inverted deoxythymidine at the 3′ ends.



146

147 Fig. S6. Resulting normalized FI plot of L-TEAM reactions for three distinct input sequences
148 using the corresponding converters and the common Amplifier 3-6 in the absence and
149 presence of the respective inputs at concentrations from 1 pM to 1 nM. Sequences are
150 shown in Table 1. (A) The L-TEAM reactions using Input and Converter. (B) The L-TEAM
151 reactions using Input (2) and Converter (2). (C) The L-TEAM reactions using Input (3) and
152 Converter (3).