Supporting Information

Reduction of Background-Triggered Amplification in Lesion-Induced DNA Amplification (LIDA)

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Table S1. Oligonucleotide Sequences

DNA-I	5' – TTG TTA AAT ATT GAT AAG – 3'
DNA-IIa$_p$	5' – p(Ab) ATT TAA CAA – 3'
DNA-IIb	5' – CTT ATC AA – 3'
DNA-Ia	5' – T_FTG TTA AAT – 3'
DNA-Ib$_p$	5' – pATT GAT AAG – 3'

Ab = model abasic group; P = phosphate; T_F = fluorescein modified thymine.

Quantifying the ligation yields. The percent yield for every ligation reaction was calculated from fluorescent polyacrylamide gel images obtained after the electrophoretic separation of ligation reaction according to our previous work. The equation used to calculate the *% conversion* of the product at each data point based on the conversion of the fluorescent reactant band (DNA-Ia) to the ligation product was:

$$\%\ Conversion = \frac{Product\ Band}{Product\ Band + Reactant\ Band} \times 100\%$$

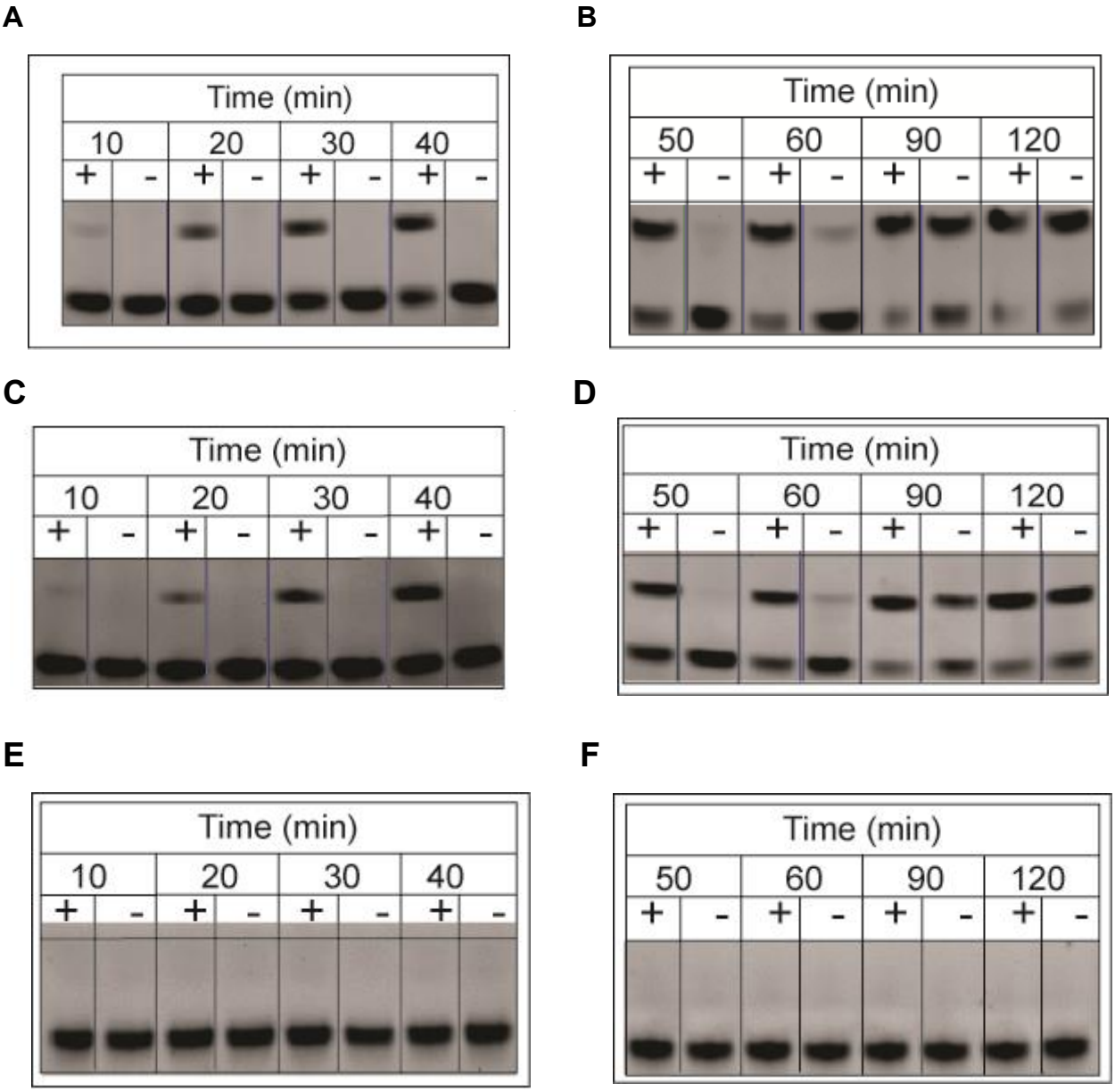
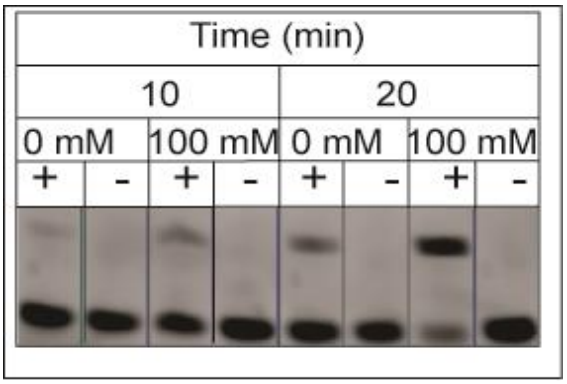


Figure S1. Representative fluorescent images of denaturing polyacrylamide gels after electrophoretic separation of aliquots of LIDA amplification initiated with 14 nM (+) and 0 nM (-) **DNA-I** correspond to experimental data show in Figure 3 A and B. Gels A and B correspond to reactions with 5 mM ATP, gels C and D to reactions with 7.5 mM ATP, and gels E and F to reactions with 10 mM ATP. *Experimental Conditions:* 14 nM DNA-I, 1.4 μM Ia, 2.8 μM IIa_P , 2.8 μM Ib_P, 2.8 μM IIb, 50 mM TRIS-HCl (pH 7.5), 10 mM $MgCl_2$, **Varying ATP concentration**, 2000 CEU T4 DNA ligase, 30 °C.

A


Time (min)
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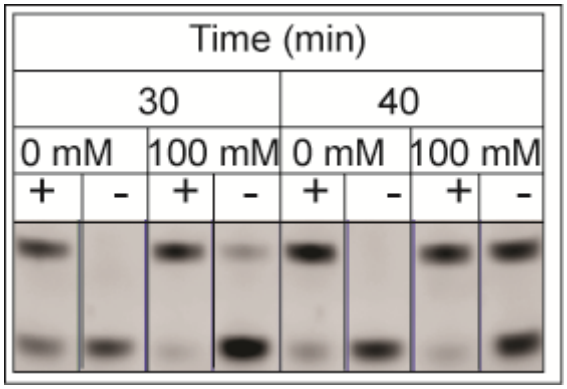
20
0 mM
100 mM
0 mM
100 mM
+
-
+
-
+
-
+
-

B


Time (min)
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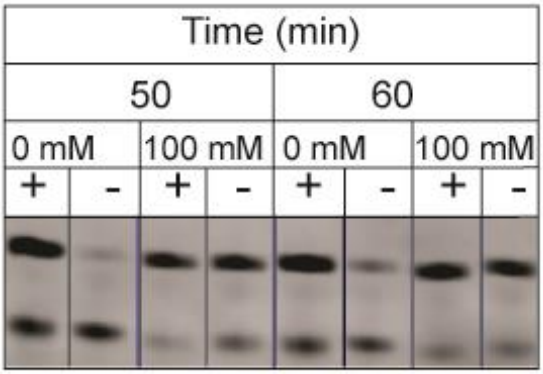
0 mM
100 mM
+
-
+
-
+
-
+
-

C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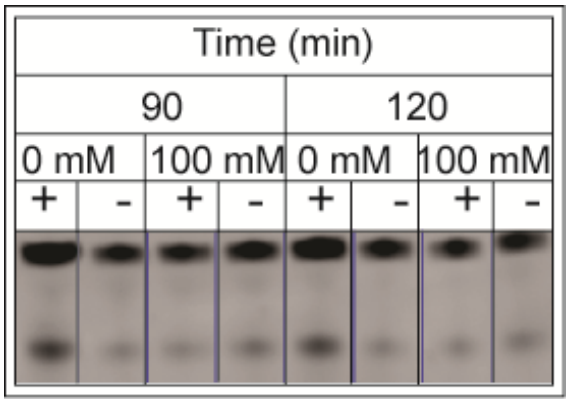
0 mM
100 mM
+
-
+
-
+
-
+
-

D


Time (min)
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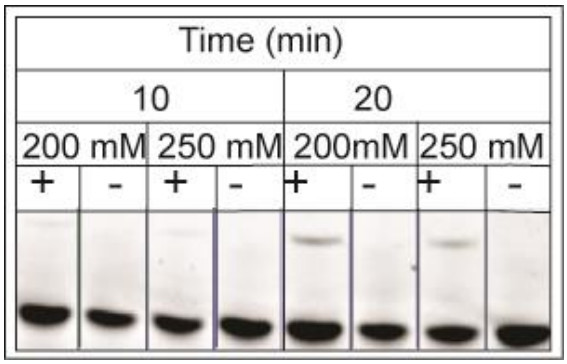
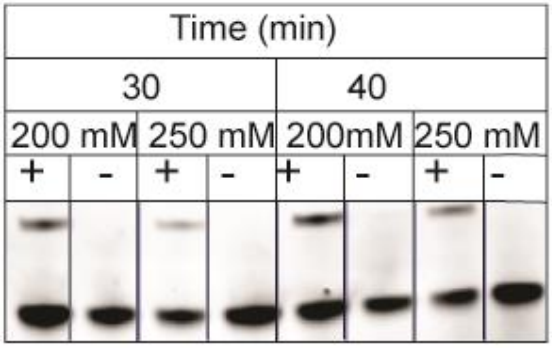
0 mM
100 mM
0 mM
100 mM
+
-
+
-
+
-
+
-

E


Time (min)
10
20
200 mM
250 mM
200mM
250 mM
+
-
+
-
+
-
+
-

F


Time (min)
30
40
200 mM
250 mM
200mM
250 mM
+
-
+
-
+
-
+
-

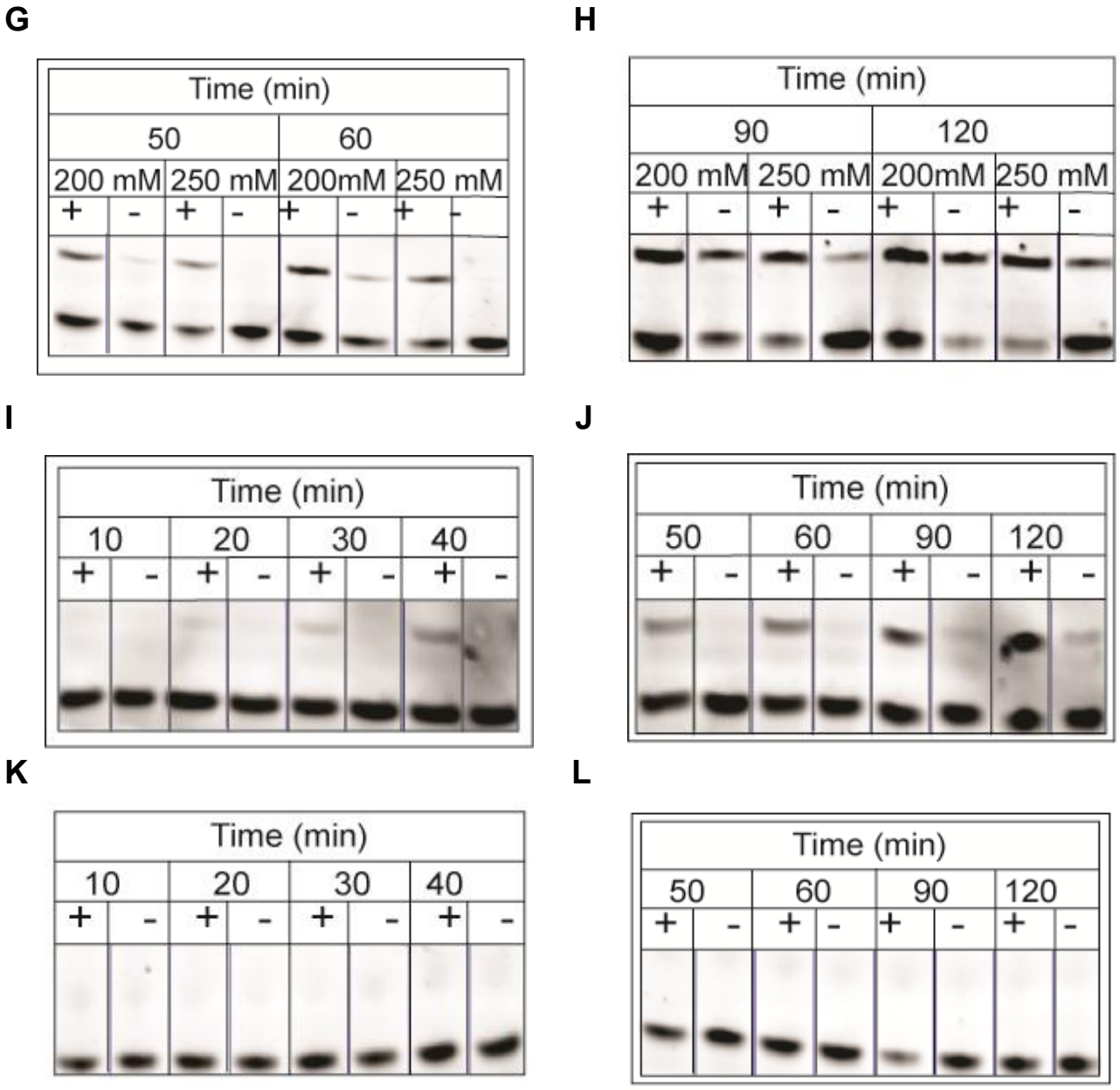


Figure S2. Representative fluorescent images of denaturing polyacrylamide gels after electrophoretic separation of aliquots of LIDA amplification initiated with 14 nM (+) and 0 nM (-) **DNA-I** correspond to experimental data show in Figure 4 A and B. Gels (A, B, C, D) correspond to reactions with 0 mM NaCl and 100 mM NaCl, (E, F, G, H) to reactions with 200 mM and 250 mM (traces not shown in the Figure 4A and B), (I, J) to reactions with 300 mM NaCl, and (K, L) to reactions with 400 mM NaCl. *Experimental Conditions:* 14 nM DNA-I, 1.4 μM Ia, 2.8 μM IIa_P , 2.8 μM Ib_P, 2.8 μM IIb, 50 mM TRIS-HCl (pH 7.5), 10 mM $MgCl_2$, 1 mM ATP, **Varying NaCl concentration**, 2000 CEU T4 DNA ligase, 30 °C.

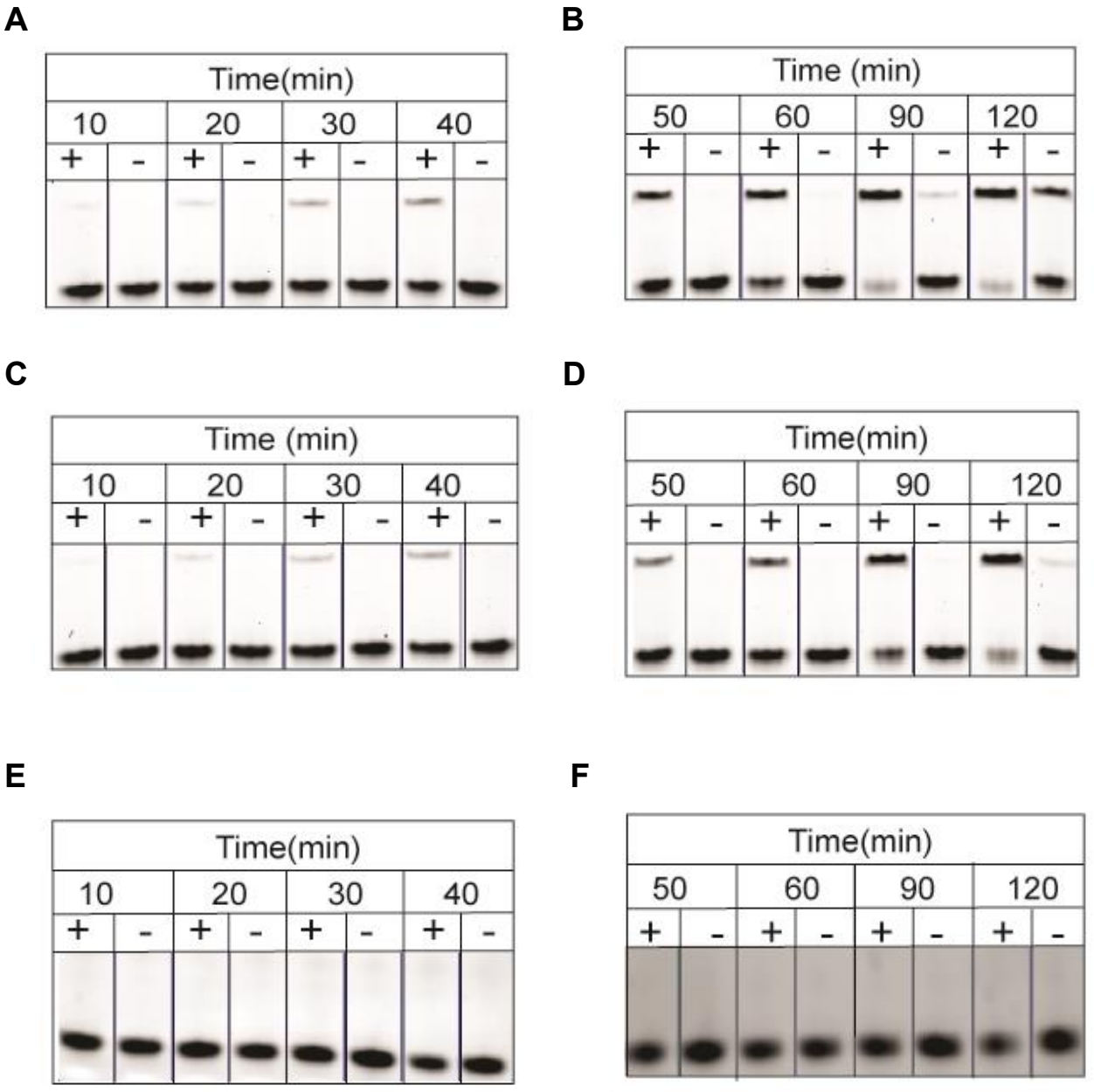


Figure S3. Representative fluorescent images of denaturing polyacrylamide gels after electrophoretic separation of aliquots of LIDA amplification initiated with 14 nM (+) and 0 nM (-) **DNA-I** correspond to experimental data show in Figure 5 A and B. Gels A and B correspond to reactions with 5 mM $MgCl_2$, gels C and D to reactions with 2.5 mM $MgCl_2$, and gels E and F to reactions with 1 mM $MgCl_2$. *Experimental Conditions:* 14 nM DNA-I, 1.4 μM Ia, 2.8 μM IIa_P , 2.8 μM Ib_P, 2.8 μM IIb, 50 mM TRIS-HCl (pH 7.5), **Varying $MgCl_2$ concentration**, 1 mM ATP, 2000 CEU T4 DNA ligase, 30 °C.

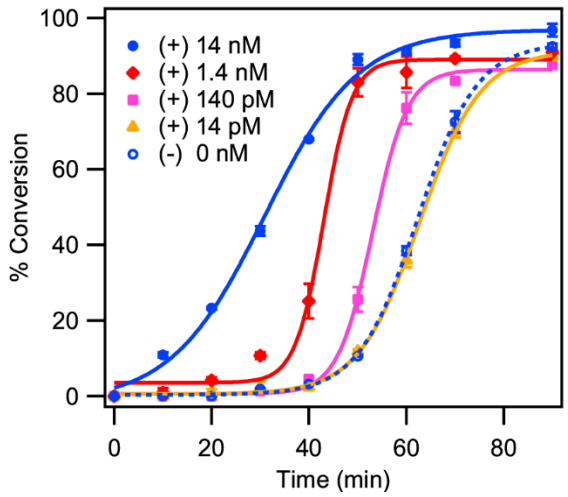


Figure S4. Representative kinetic traces of cross-catalytic formation of DNA-I using our standard conditions[1] (based on typical commercial ligase buffer conditions) initiated by different concentrations of DNA-I target. *Experimental Conditions*: 1.4 μM **DNA-Ia;** 2.8 μM **DNA-IIa$_p$**; 2.8 μM **DNA-IIb**; 2.8 μM **DNA-Ib$_p$**; 50 mM TRIS-HCl (pH 7.5), 2000 CEU T4 DNA ligase, 1 mM ATP, 10 mM $MgCl_2$, 30 °C.

The above experiment aimed to assess the target concentration that could be reliably distinguished from background-triggered amplification, under our standard buffer conditions that we have used in our previous work.[1]

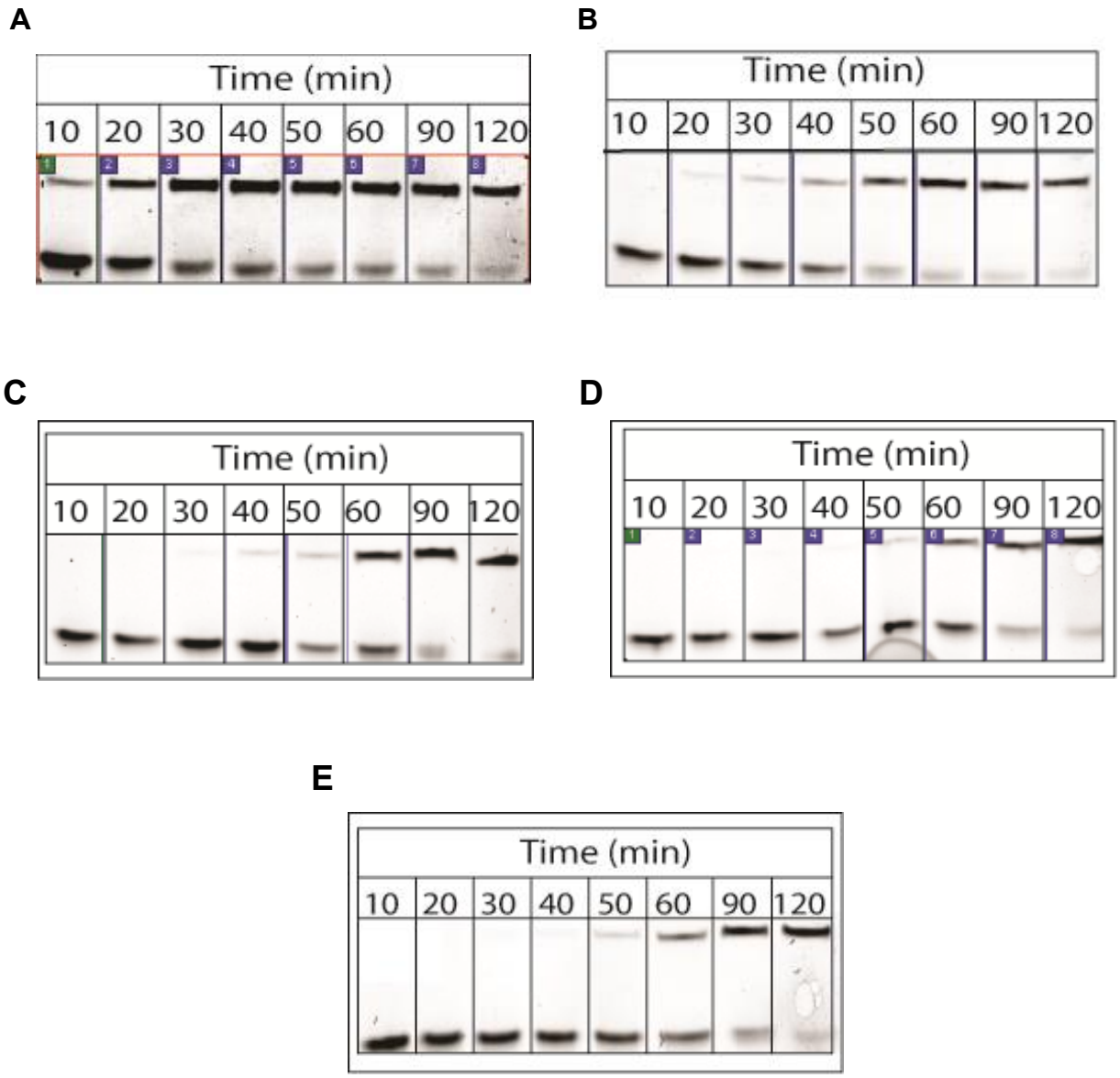


Figure S5. Representative gel images correspond to the graph above in Figure S4 (A) 14 nM, (B) 1.4 nM, (C) 140 pM, (D) 14 pM , (E) 0 nM **DNA-I .** *Top band*: F-DNA-I; *Bottom Band:* DNA-Ia. The number above the lane refers to the number of minutes the LIDA reaction had proceeded. *Experimental Conditions*: 1.4 µM **DNA-Ia;** 2.8 µM **DNA-IIa$_P$**; 2.8 µM **DNA-IIb**; 2.8 µM **DNA-Ib$_P$**; 50 mM TRIS-HCl (pH 7.5), 2000 CEU T4 DNA ligase, 1 mM ATP, 10 mM $MgCl_2$, 30 °C.

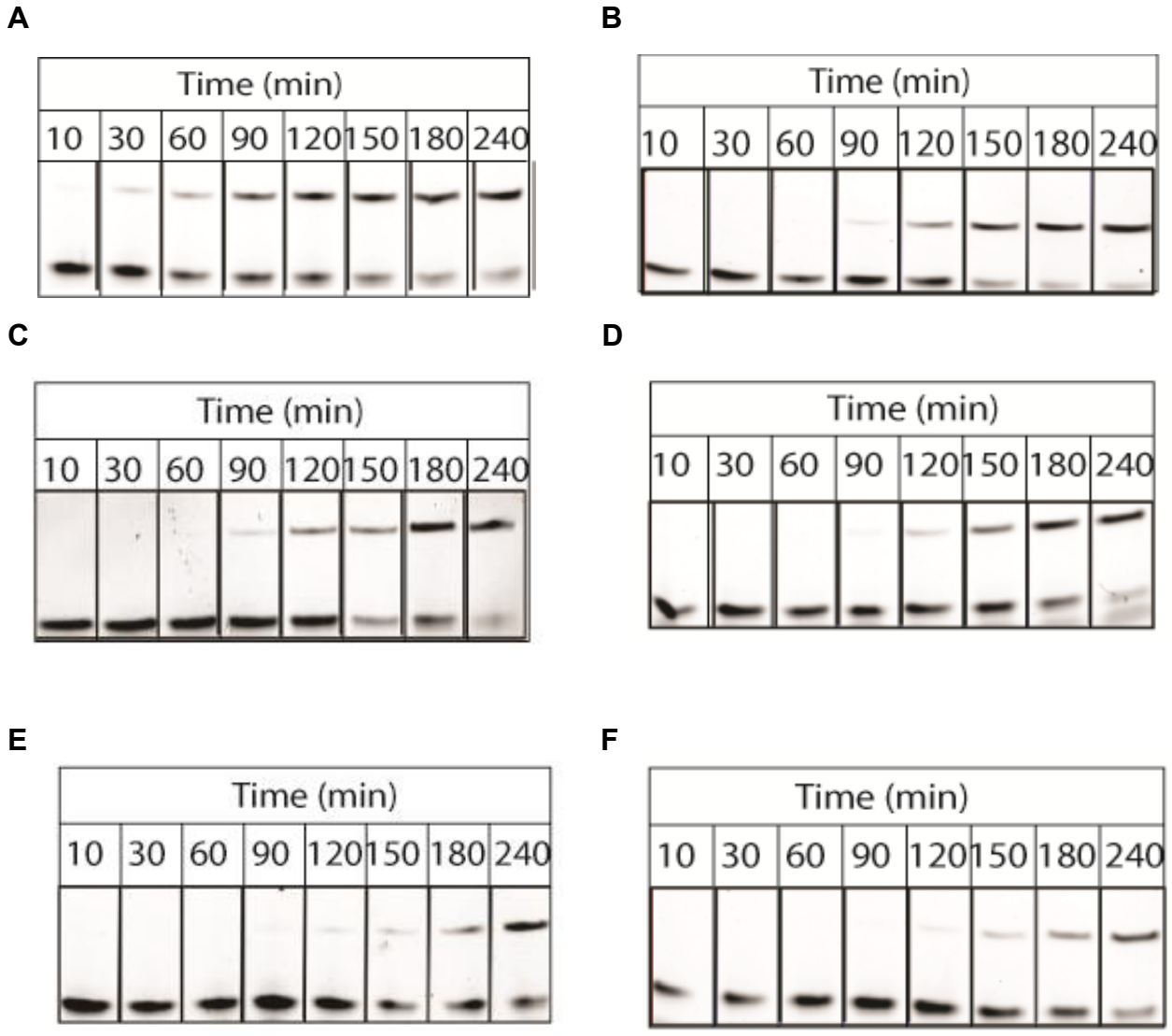


Figure S6. Representative fluorescent images of denaturing polyacrylamide gels after electrophoretic separation of aliquots of LIDA amplification under optimized conditions initiated with different amounts of **DNA-I** correspond to graph show in Figure 6A. (A) 14 nM, (B) 140 pM, (C) 14 pM, (D) 14 fM , (E) 140 aM, (F) 0 nM **DNA-I**. *Top band*: F-DNA-I; *Bottom Band:* DNA-Ia. The number above the lane refers to the number of minutes the LIDA reaction had proceeded. *Experimental Conditions*: 1.4 µM **DNA-Ia**; 2.8 µM **DNA-IIa$_p$**; 2.8 µM **DNA-IIb**; 2.8 µM **DNA-Ib$_p$**; 50 mM TRIS-HCl (pH 7.5), 2000 CEU T4 DNA ligase, 1 mM ATP, 2.5 mM $MgCl_2$, 30 °C.

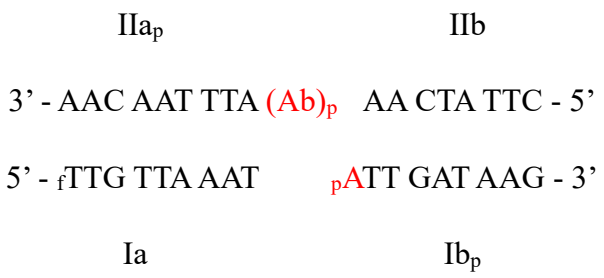


Figure S7. Primers used in our LIDA process. The pseudo-blunt end ligation reaction involves the ligation of a 5'- abasic (Ab) to the 3'OH of **IIb**.[2] This (Ab) forms a 5'- deoxyadenosine (A) single base overhang show in red, which we refer to as a pseudo-blunt end given the lack of interaction between the abasic and adenine.

References

(1) Kausar, A.; Mitran, C. J.; Li, Y.; Gibbs-Davis, J. M. *Angewandte Chemie International Edition* **2013**, *52* (40) , 10577–10581.

(2) Park, H.; Parshotam, S.; Hales, S. C.; Mittermaier, A. K.; Gibbs, J. M. *Chemistry – A European Journal* **2023**, *29* (33) , e202300080.