Supplementary information

Simple and rapid colorimetric detection of serum lncRNA biomarkers for diagnosis of pancreatic cancer

Supplementary tables and figures:

Primer 2	Sequences (5'-3')	Concentrations
F3	CTTTCTGGCCGTTTCACCA	0.2μM
B3	TGCTGCCTGTGTCTCTACG	0.2μM
FIP	GGAACAGGGGCCTAGAACCCTACTCCCTCCAAGTGGCATTG	1.6μM
BIP	ACATACTGGTCAGACACGGCTGCTCGCATGGCAGTTCTCAT	1.6μM
LF	CTGTCTCTTTGTCACTGTGAGTTTT	0.8μM
LB	GAGGCCAAGGTCAAGTTGAAAGT	0.8μM

Table S1. Sequences and concentrations of HOTTIP primers for loop-mediated isothermal amplification.

Temperature			55˚C	60˚C	65˚C
Primers	F3/B3	Positive control	-	-	-
		Negative control	-	-	-
	F3/B3 + LF/LB	Positive control	-	-	-
		Negative control	-	-	-
	F3/B3 + FIP/BIP	Positive control	-	+	+
		Negative control	-	-	-
	F3/B3 + LF/LB + FIP/BIP	Positive control	+	+	+
		Negative control	+	+	+

Figure S1. Effect of temperature and primer combinations on LAMP reaction. LAMP products were analysed under agarose gel electrophoresis. F3/B3 primers with or without LF/LB primers could not generate ladder-like multiple bands. Primer combination including all three primer pairs generated false positive results due to non-specificity of loop primers. Only F3/B3 with FIP/BIP produced true amplifications above 55°C, therefore was selected for subsequent LAMP reactions. +: multiple bands obtained from LAMP; -: no band or single band obtained from LAMP.

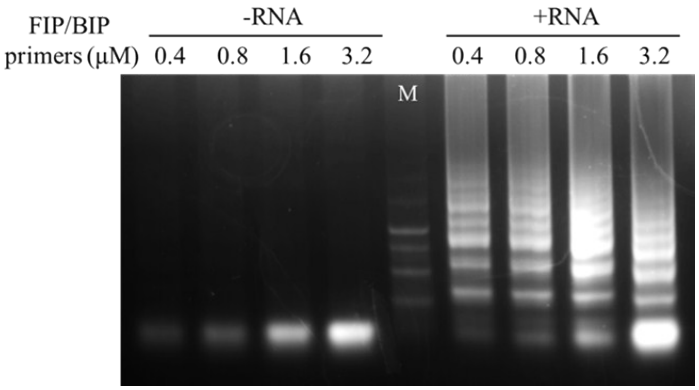

Figure S2. Optimization on FIP and BIP primer concentrations. Optimized inner primer concentration was 1.6μM.

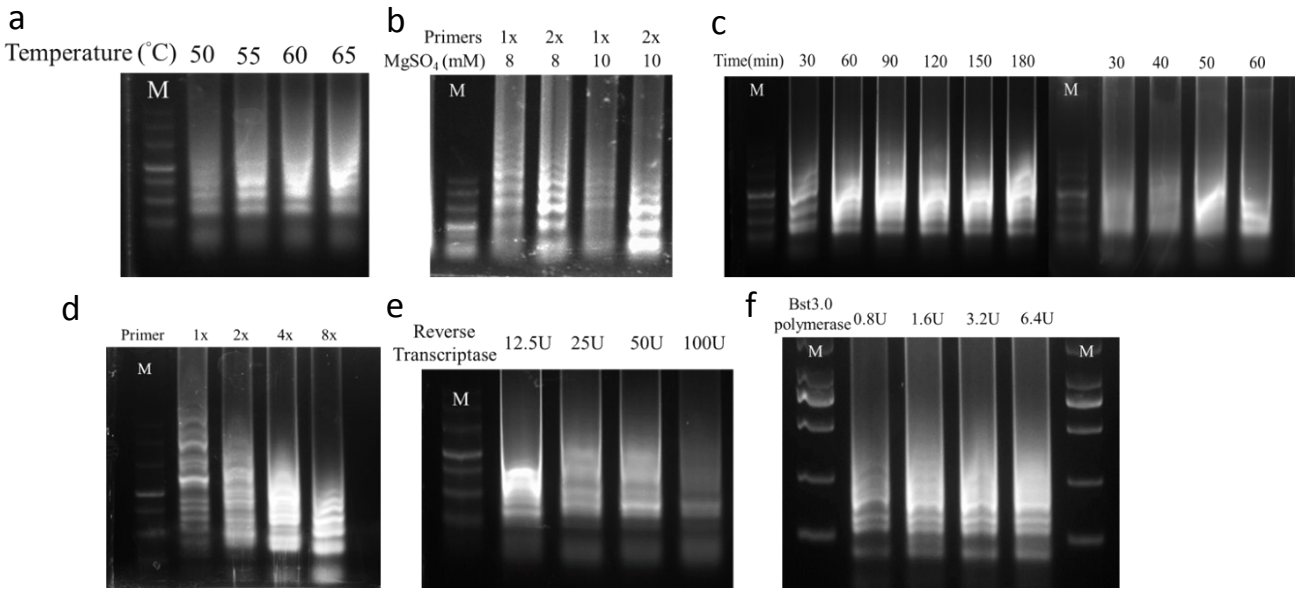

Figure S3. Optimization of LAMP conditions with different (a) temperature; (b) MgSO4; (c) incubation time; (d) primer concentration; (e) reverse transcriptase; (f) Bst3.0 polymerase. Optimized LAMP reaction was performed with 8mM MgSO4, 1x Primers, 12.5U Reverse transcriptase, 1.6U Bst3.0 polymerase at 60°C for 60min.

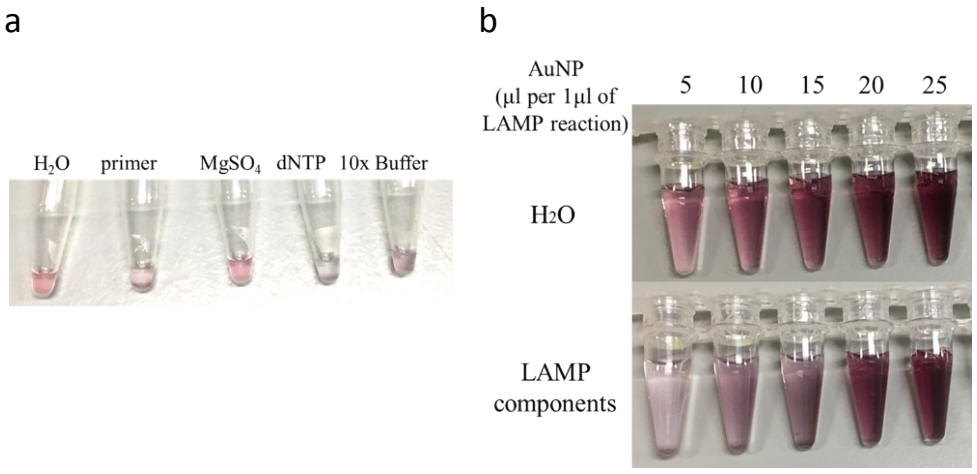

Figure S4. Optimization of (+)AuNP volume. (a) Effect of LAMP reaction components including primers, $MgSO_4$, dNTP and 10x buffer on 5 µL AuNP. Nanoparticles may be precipitated by primers and dNTP. (b) Overcoming interference of LAMP components using different ratios of LAMP product to (+)AuNPs. Optimized volume was 20 µL for each µL of LAMP reaction product.