

Supplementary materials for one-pot, isothermal LAMP-CRISPR-based assay for *Klebsiella pneumoniae* detection

Table S1. Alternative LAMP primers designed in this study.

Primer	Type		Sequence (5'-3') ^a	Length ^b
Kp2-F3	Forward primer	outer	CTATACCCGGTTGGGATTG	19 nt
Kp2-B3	Backward primer	outer	AATGCCATTACTTTCATCATCA	22 nt
Kp2-FIP	Forward primer	inner	AGCACTGTTGACCTCAACGA- TTC - ACGGGATATCTGACCAGTC	42 nt
Kp2-BIP	Backward primer	inner	GCGGATCTGCAGAAACACTG- CACGAAACAGTCTTCATTCA	40 nt
Kp2-LF	Loop primer	forward	TCCTGTTTTTTAATTCCCC	19 nt
Kp2-LB	Loop primer	backward	CGTCGTGTTGCCCGGCGGT	19 nt
Kp3-F3	Forward primer	outer	ATTGTCACTGAGTAAAACAGAA	22 nt
Kp3-B3	Backward primer	outer	AGGTGATGTTTTTCGGTCAG	19 nt
Kp3-FIP	Forward primer	inner	GCTTTGATGTTTCATTTGCGTTGAG- TTC - TGCTGCAAATGTGGATGG	45 nt
Kp3-BIP	Backward primer	inner	AAGACGGTATCGTCGCATAAA- GAACGATATGATAAATCACCTGC	44 nt
Kp3-LF	Loop primer	forward	TGCGAAGTACCATGCCCGG	19 nt

^a The inserted PAM site (TTC) was in bold. ^b nt, nucleotide.

Table S2. Bacterial strains used in this study.

Bacteria	Strain type (Strain ID)	No. of strains
<i>Klebsiella pneumoniae</i>	Reference strain (ATCC 700603)	1
	Isolated strain	64
<i>Klebsiella oxytoca</i>	Isolated strain	3
<i>Klebsiella aerogenes</i>	Isolated strain	1
<i>Staphylococcus aureus</i>	Reference strain (ATCC 29213)	1
	Isolated strain	2
<i>Staphylococcus haemolyticus</i>	Isolated strain	1
<i>Staphylococcus succinus</i>	Isolated strain	1
<i>Staphylococcus epidermidis</i>	Isolated strain	1
<i>Acinetobacter baumannii</i>	Isolated strain	1
<i>Acinetobacter pizzeri</i>	Isolated strain	1
<i>Acinetobacter junii</i>	Isolated strain	1
<i>Pseudomonas aeruginosa</i>	Reference strain (ATCC 27853)	1
<i>Haemophilus influenzae</i>	Reference strain (ATCC 49247)	1
<i>Moraxella catarrhalis</i>	Isolated strain	1
<i>Escherichia coli</i>	Reference strain (ATCC 25922)	1
<i>Mycobacterium tuberculosis</i>	Reference strain (H37Rv)	1
<i>Streptococcus pneumoniae</i>	Reference strain (ATCC 49619)	1
<i>Streptococcus oralis</i>	Isolated strain	1
<i>Streptococcus salivarius</i>	Reference strain (K12)	1
<i>Streptococcus agalactiae</i>	Isolated strain	1
<i>Streptococcus pyogenes</i>	Reference strain (CGMC 48848)	1
<i>Streptococcus mitis</i>	Isolated strain	1
<i>Streptococcus suis</i>	Reference strain (GZ1)	1
<i>Stenotrophomonas maltophilia</i>	Isolated strain	1
<i>Nocardia farcinica</i>	Reference strain (IFM 10152)	1
<i>Nocardia cyriacigeorgica</i>	Reference strain (DSM 40350)	1
<i>Rhodococcus sp.</i>	Isolated strain	1
<i>Streptomyces sp.</i>	Isolated strain	1
<i>Corynebacterium striatum</i>	Reference strain (ATCC 43751)	1
<i>Corynebacterium simulans</i>	Isolated strain	1
<i>Corynebacterium propinquum</i>	Isolated strain	1
<i>Corynebacterium aurimucosum</i>	Isolated strain	1
<i>Clostridium difficile</i>	Reference strain (ATCC BAA-1803)	1
<i>Enterococcus faecalis</i>	Reference strain (ATCC 29212)	1
<i>Aeromonas caviae</i>	Isolated strain	1
<i>Elizabethkingia anophelis</i>	Isolated strain	1
<i>Ralstonia mannitolilytica</i>	Isolated strain	1
<i>Rothia kristinae</i>	Isolated strain	1
<i>Serratia marcescens</i>	Isolated strain	1

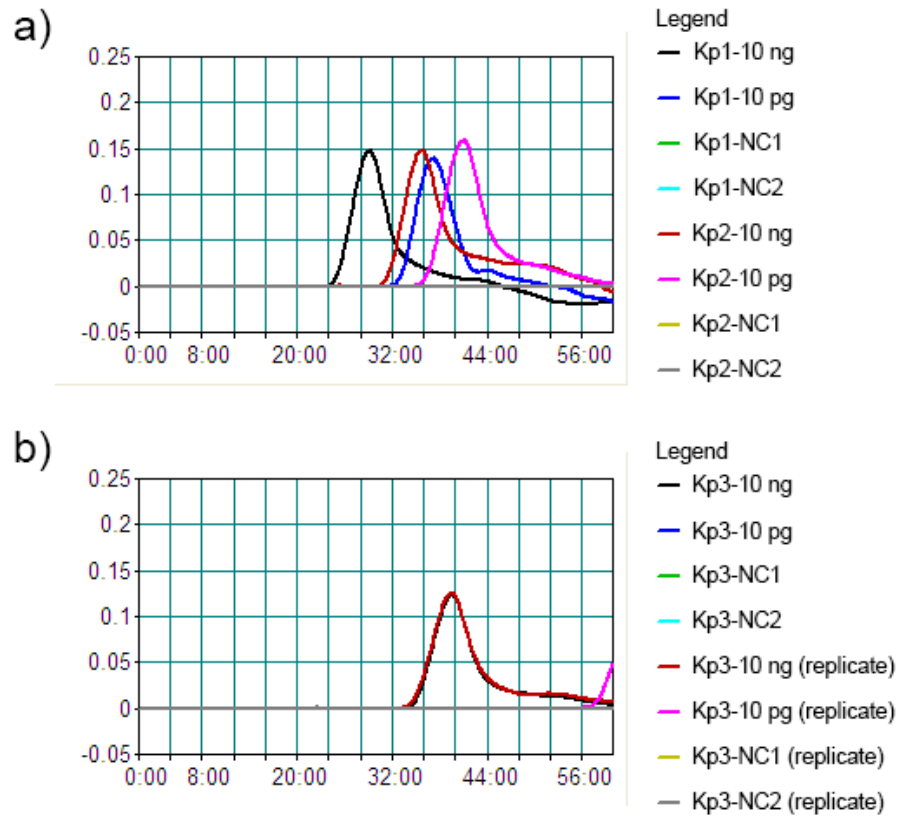


Figure S1. Primer sets screening and confirmation. LAMP detection was conducted at 59 °C for 1 h. **a)** Primer set Kp1 and Kp2. **b)** Primer set Kp3.



Figure S2. The primer set Kp2 and Kp3 design regions. A part of the *rcsA* sequence was shown. Right-pointing arrows and left-pointing arrows indicate sense and complementary strands respectively. **a)** Primer set Kp2. **b)** Primer set Kp3.

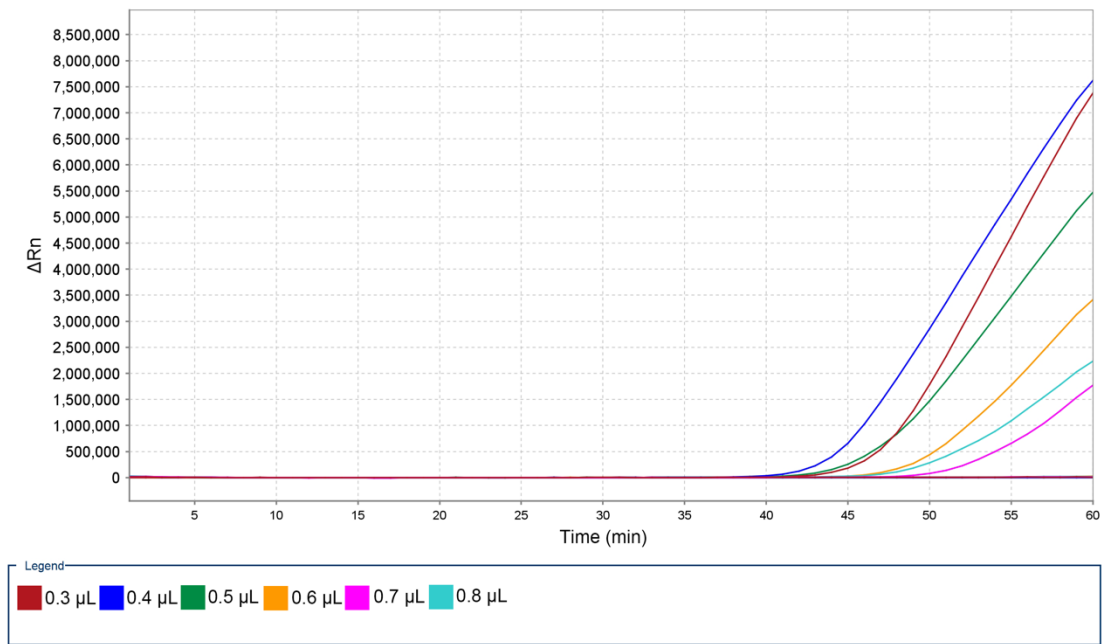


Figure S3. Optimal primer pre-mixture volume of the *K. pneumoniae* CRISPR-top assay. *K. pneumoniae* ATCC 700603 genomic DNA and DW were used as the templates of the positive control and the negative control respectively. PC, positive control; NC, negative control.

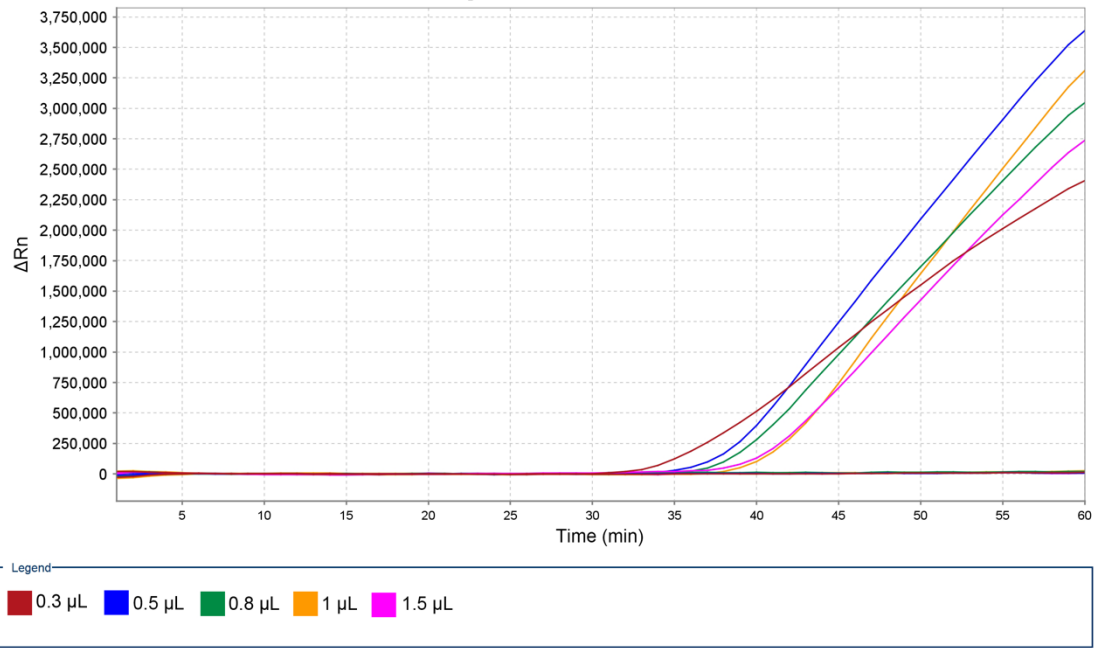


Figure S4. Optimal ssDNA probe concentration of the *K. pneumoniae* CRISPR-top assay. *K. pneumoniae* ATCC 700603 genomic DNA and DW were used as the templates of the positive control and the negative control respectively. PC, positive control; NC, negative control.