

CCTGCAGGCTAGGAGGCAATTGTTAATACGACTCACTATAGAGAGTTTGC AAAATATACAGGGGATTATATATAATGGAAAAACAAGAAA
GGAAAAATAGGAGGTTTATCATATGAACGGACCGATTATAATGACTAGAGAAGAGCGTATGAAAAATAGTACATGAAATAAAAGAGAGAAT
ATTAGACAAATATGGTGACGACGTGAAAGCTATTGGAGTGTATGGCTCTCTATGTAGCAATATTACGACCTGGAGGGAAAAGTTATCAG
GCATGCACCTGGTAGCTAGTCTTTAAACCAATAGATTGCATCGGTTTAAAAGGCAAGACCGTCAAATTGCGGGAAAAGGGGTCAACAGCC
GTTTCAGTACCAAGTCTCAGGGGAAACTTTGAGATGGCCTTGCAAAGGGTATGGTAATAAGCTGACGGACATGGTCCTAACCACGCAGCC
AAGTCCTAAGTCAACAGATCTTCTGTTGATATGGATGCAGTTCACAGACTAAATGTCGGTTCGGGGAAGATGTATTCTTCTCATAAGATA
TAGTCGGACCTCTCCTTAATGGGAGCTAGCGGATGAAGTGATGCAACACTGGAGCCGCTGGGAACTAATTTGTATGCGAAAAGTATATTG
ATTAGTTTTGGAGTACTCGTGGTCGTCAGACAGATGGACCGTACTCAGATATCGAGATGATGTGCGTGATGAGTACAGAGGAAGCAGAG
TTCTCTCACGAGTGGACGACGGGTGAATGGAAAGTCGAGGTAAATTTTTGATAGTGAGGAGATATTGTTGGATTACGCTAGTCAAGTGGA
ATCAGATTGGCCTTTAACTCACGGACAATTCTTTTCAATCTTACCGATCTATGATTCAGGTGGATATTTAGAAAAAGTTTACCAAACAG
CAAAAAGTGTGGAGGCGCAAACGTTCCACGACGCGATATGTGCTTTGATTGTTGAGGAATTATTTGAATACGCTGGTAAGTGGCGAAAT
ATTAGAGTTCAGGGTCCTACGACTTTCTTGCCTTCTTTAACTGTGCAAGTAGCGATGGCGGGAGCGATGTTGATTGGCTTGCACCATCG
AATCTGTTACACGACTTCAGCGTCTGTATTAACGGAGGCTGTCAAGCAAAGTGACTTACCATCTGGATACGATCATTATGTCAATTTCG
TAATGTCAGGTCAATTGAGTGATTCAGAAAAGTTGTTGGAGAGTTTGGAAAACCTTTTGGAACGGTATTTCAGGAATGGACGGAACGACAC
GGCTATATAGTTGACGTGTCTAAGCGTATTCTTTTTTGACTCCTCTCTCACTCGAGATAACAGATACTTCGGTATCTGTTATCTGTTTT
TTTTCAACAGATAGCCGCGTTTCGCGGGCTATCTGTTTTTTTTTATAACAATGCTTACTCCCGG

Figure S1. Marker gene cassette of pKTM_IAC. T7 promoter is displayed in red type, *Pcap* promoter and ribosomal binding site is shown by blue type. *KTM* gene coding sequence is shown in yellow highlighting. Intron sequence is underlined. Bold font indicates T7hyb10 transcriptional terminator. Restriction sites flanking the expression cassette are shown by blue highlighting.

KTM_ORIGINAL	1	ATGAATGGACCAATAATAATGACTAGAGAAGAAAGAATGAAGATTGTTCA	50
KTM_MODIFIED	1	ATGAACGGACCGATTATAATGACTAGAGAAGAGCGTATGAAAATAGTACA	50
KTM_ORIGINAL	51	TGAAATTAAGGAACGAATATTGGATAAATATGGGGATGATGTTAAGGCTA	100
KTM_MODIFIED	51	TGAAATAAAAGAGA GAATATTAGACAAATATGGTGACGACGTGAAAGCTA	100
KTM_ORIGINAL	101	TTGGTGTATTATGGCTCTCTTGGTCGTGACTGATGGGCCCTATTCCGGAT	150
KTM_MODIFIED	101	TTGGAGTGTATGGCTCTCTTGGTCGTGACAGATGGACCGTACTCAGAT	150
KTM_ORIGINAL	151	ATTGAGATGATGTGTGTCATGTCAACAGAGGAAGCAGAGTTCAGCCATGA	200
KTM_MODIFIED	151	ATCGAGATGATGTGCGTGATGAGTACAGAGGAAGCAGAGTTCCTCACGA	200
KTM_ORIGINAL	201	ATGGACAACCGGTGAGTGGAAAGTGGAAAGTGAATTTTGATAGCGAAGAGA	250
KTM_MODIFIED	201	GTGGACGACGGTGAATGGAAAGTGGAGGTAAATTTTGATAGTGAGGAGA	250
KTM_ORIGINAL	251	TTCTACTAGATTATGCATCTCAAGGTGGAATCAGATTGGCCGCTTACACAT	300
KTM_MODIFIED	251	TATTGTTGGATTACGCTAGTCAAGTGAATCAGATTGGCCTTAACTCAC	300
KTM_ORIGINAL	301	GGTCAATTTTCTCTATTTTGCCGATTTATGATTCAGGTGGATACTTAGA	350
KTM_MODIFIED	301	GGACAATCTTTCAATCTTACCGATCTATGATTCAGGTGGATATTAGA	350
KTM_ORIGINAL	351	GAAAGTGTATCAAACCTGCTAAATCGGTAGAACCCAAACGTTCCACGATG	400
KTM_MODIFIED	351	AAAAGTTACCAAACAGCAAAAAGTGTGGAGCGCAAACGTTCCACGACG	400
KTM_ORIGINAL	401	CGATTTGTGCCCTTATCGTAGAAGAGCTGTTTGAATATGCAGGCAAATGG	450
KTM_MODIFIED	401	CGATATGTGCTTTGATTGTTGAGGAATATTTGAATACGCTGGTAAGTGG	450
KTM_ORIGINAL	451	CGTAATATTCGTGTGCAAGGACCGACAACATTTCTACCATCCTTGACTGT	500
KTM_MODIFIED	451	CGAAATATTAGAGTTCAGGGTCTACGACTTCTTGCCCTTCTTAACTGT	500
KTM_ORIGINAL	501	ACAGGTAGCAATGGCAGGTGCCATGTTGATTGGTCTGCATCATCGCATCT	550
KTM_MODIFIED	501	GCAAGTAGCGATGGCGGGAGCGATGTTGATTGGCTTGCCATCGAATCT	550
KTM_ORIGINAL	551	GTTATACGACGAGCGCTTCGGTCTTAACTGAAGCAGTTAAGCAATCAGAT	600
KTM_MODIFIED	551	GTTACACGACTTCAGCGTCTGTATTACGGAGGCTGTCAAGCAAAGTGAC	600
KTM_ORIGINAL	601	CTTCCTCAGGTTATGACCATCTGTGCCAGTTCGTAATGTCTGGTCAACT	650
KTM_MODIFIED	601	TTACCATCTGGATACGATCATTTATGTCAATTCGTAATGTGAGTCAATT	650
KTM_ORIGINAL	651	TTCCGACTCTGAGAACTTCTGGAATCGCTAGAGAATTTCTGGAATGGGA	700
KTM_MODIFIED	651	GAGTGATTACAGAAAAGTTGTTGGAGAGTTTGAAAACCTTTTGGAACGGTA	700
KTM_ORIGINAL	701	TTCAGGAGTGGACAGAACGACACGGATATATAGTGGATGTGTCAAAACGC	750
KTM_MODIFIED	701	TTCAGGAATGGACGGAACGACACGGCTATATAGTTGACGTGTCTAAGCGT	750
KTM_ORIGINAL	751	ATACCATTTTGA	762
KTM_MODIFIED	751	ATTCTTTTGA	762

Figure S2. Alignment of native *KTM* gene coding sequence and synthetic *KTM* marker gene coding sequence. Pipe character indicates bases that match between the two sequences, while a dot character indicates a mismatch. Key mismatches targeted by LNA modifications in RT-LAMP primers are shown in bold. F3 and B3 primer binding sites are shown by yellow highlighting. F2 and B2 domains of the FIP and BIP primers, respectively, are indicated with grey highlighting. The splice junction occurs between nucleotides shown in red. Alignment was produced using EMBOSS NEEDLE, with maximum gap opening and gap extension applied.

CCTGCAGGCTAGGAGGCAATTGTT**TAATACGACTCACTATAG**AGAGTTTGC~~AAAA~~TATACAGGGGATTATATATAATGGAAAAACAAGA
AAGGAAAAATAGGAGGTTTTATCATATGGCATCTCAAGCTAAACAGACAGTCCATACTGGTAATACCGTAATGCTTATGATTAAGGT
AAACCTGTAGGTAGAGCTCAATCAGCGTCTGGTACACGTTCTTACGGTACAGAAGGGGTATATGAGATTGGTTCTATCATGCCAC
AAGAACACGTTTACCTTAAGTATGAAGGTGAATTAACAGTAGAACGTCTACGAATGAAAAAAGAGAAGTTTGC~~AAAA~~ATTAGGATA
TGCTTCTCTTGGTGAAGAAATCCTTAAGAAAAGATATTATTGACATTGTTGTTATTGACAACCTTGACTAAAGAAGTGCTTGTGTCA
TATCACGGTTGTAGTGCTAATAACTATGA**ACTTCGTAGCTTCACATAGTAATATGTGTCGAAAAACTTTCTAAACAGGGAAGCC**
TAAGTCTATTAGATATGGTAATCCTGTGCTAAATCAGATTAATAATCTGTAAATGCCAAACGACTAACTGAAACCTACTTGATAA
TTCAAGGGGAGTAAGTGTAGAGCCAAGCGGTAGAAGTTTTAAGTAATTATTATGTAAATCTTCTTTAAATCGAAAAGAAAAGAGT
CCTTTTTTAGGATTGTGATATAGTCTGAACATATATGGAAACATATAGCAGTTCCTAGAGAACGGGCAGTGAGTAGCGACCACTG
TTGAACATAATGGATGAAGCATGGAACACTAATGAAATAACTACTGAAAGC**AT**TC~~AAA~~AGGCGAAGAGTTGTT**CACGGGTGTTG**
TGCCGATCTTAGTAGAATTGGATGGTGACGTTAACGGCCATAAATTTTTCTGTGCGAGGTGAAGGAGAGGGCGACGCGACTAACGG
AAAATTGACATTAAAGTTCATTTGTACGACAGGTAAGTTGCCTGTTCCATGGCCTACTTTAGTGACGACGTTAACATACGGAGTC
CAATGCTTCAGTCGTTATCCAGACCACATGAAGAGACACGACTTTTTCAAAGTGCTATGCCGGAAGGTTATGTACAAGAACGTA
CAATTTCAATCAAAGATGACGGTACTTACAAAACAAGAGCTGAAGTGAATTCGAGGGCGACACTTTGGTGAATAGAATTGAGTT
AAAAGGTATTGATTTCAAAGAGGATGGCAATATCTTAGGACATAAGTTAGAATACAATTTCAACTCACATAATGTGTACATCACT
GCTGACAAACAGAAGAATGGTATCAAAGCAAATTTTAAGATAAGACATAACGTAGAAGATGGAAGTGTT**CAGTTAGCAGACCACT**
ACCAGCAGAATACGCCAATCGGTGACGGACCTGTGTTGTTGCCGGATAATCACTATTTATCTACACAGTCAGTATTATCTAAGGA
CCCGAACGAGAAACGTGACCACATGGTTTTATTGGAGTTTGTAAACAGCAGCTGGCATCACGCACGGCATGGACGAGTTGTACAAA
TAACTCCTCTCTCACTCGAGATAACAGATACTTCGGTATCTGTTATCTGTTTTTTTTTCAACAGATAGCCGCGTTCGCGCGGCTAT
CTGTTTTTTTTTTATAACAATGCTTACT**CCCGG**

Figure S3. Marker gene cassette of pOrf142+gfp_mod. T7 promoter is displayed in red type, *Pcap* promoter and ribosomal binding site is shown by blue type. *Orf142* gene coding sequence (first exon and first 22 bp of second exon) is shown in yellow highlighting. Intron sequence is underlined. Grey sequence indicates spacer to improve primer design characteristics against the transgene. GFP gene is shown with green highlighting, with ablated start codon indicated in lower case. Bold font indicates T7hyb10 transcriptional terminator. Restriction sites flanking the expression cassette are shown by blue highlighting.

Table S1. Oligonucleotide sequences

Oligonucleotide function	Primer name	Primer sequence
pKTM_IAC_v4 transgene RT-LAMP	KTM_IAC_v4F3	GAATATTAGACAAATATGGTGACG
	KTM_IAC_v4FIP_LNA	TCGATATCTGAGTACGGTCCATCTACGTGAAAAGCTATTGGAGT+G*
	KTM_IAC_v4BIP_LNA	CGTGATGAGTACAGAGGAAGCAGCCTCACTATCAAAATTTACCTC+G
	KTM_IAC_v4B3_LNA	TGACTAGCGTAATCCAACA+A
	KTM_IAC_v4LB	ACCACACCTACCACCCTAATAACTAAGAGTGGACGACGGGTGAATG †
Vector backbone DNA and RNA RT-LAMP	ampF3_P1L1	CCTTTTTTGCGGCATTTTGC
	ampB3_P1L1	CGACCGAGTTGCTCTTGC
	ampFIP_P1L1	TGCACCCAACCTGATCTTCAGCATCCTGTTTTTGCTCACCCAG
	ampBIP_P1L1	ATCCTTGAGAGTTTTCGCCCCGAATACCGCGCCACATAGC
	ampLB_P1L1	ACCAACACCTCACATCACACATAATAAGAACGTTTTCCAATGATGAGCACT
pOrf142+gfp_mod transgene RT-LAMP	orf142_GFP_F3	TGTTGTTATTGACAACCTTGACT
	orf142_GFP_B3	GTTAACGTCAACCATCCAATT
	orf142_GFP_FIP	CCTCGACGTTCCATGCTTCATGTGCTTGTGTCATATCACG
	orf142_GFP_BIP	AGTGAGGAGATATTGTTGGATTACGTAAGATCGGCACAACACC
	orf142_GFP_LB	ACCACACCTACCACCCTAATAACTAAGTTCAAAGGCGAAGAGTTGTTCA
<i>P. aeruginosa</i> -specific RT-LAMP	PA_F3	TACAGCGTCGCTCCCG
	PA_B3	TCGACACGCTTCCTCTACC
	PA_FIP	GCCACTGATCGTGGCGAAGACTGAGGGCGCGGAACA
	PA_BIP	CTGACCTGGCGCAGGAAGAACTTGTGGACGGCGATGGTG
	PA_LF	ACAACCCGGTGGTCTACAACA
	PA_LB	ACCAACACCTCACATCACACATAATAGGTTGATCCGCTTGAACAGGC
Transgene control fluorescent probe	IAC UDP Probe	/5' TEX 615/CCTGACCACTTCCGAACCCAACCACTACGACAGACCACACCTACCACCCTAATAACTAA‡
	IAC UDP Quencher	CTGTCGTAGGTGGTTGGGTTCCGGAAGTGGTCAGG/3' Black Hole Quencher®-2/‡
Diagnostic target fluorescent probe	CoV UDP Probe	/5' 6-FAM/CCATCAGCACCAAGACTACCCACCTCGCCACCAAACCAACACCTCACATCACACATAATA‡
	CoV UDP Quencher	TTGGTGGCGAGGTGGGTAGTCTTGGTGCTGATGG/3' Iowa Black® FQ/‡

* Plus sign precedes LNA-modified bases

† Bold font indicates homology tails between loop primers and probes

‡ Sequence from Panpradist, N. *et al. Sci Adv* 7, eabj1281 (2021)