

Development and comparison of novel multiple cross displacement amplification (MCDA) assays with other nucleic acid amplification methods for SARS-CoV-2 detection

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Supplementary Table 1: List of MCDA, LAMP and rt-PCR primers used in this study. Bolded MCDA primer names are primers shared between 2 or more primer sets. * indicate primers used in rt-PCR for confirmation of complete DNA removal from transcribed RNA. Red text indicates the final primer sets chosen for the MCDA SARS-CoV-2 assay.

MCDA Primer name	Sequence 5' --> 3'	Reference
Region 1 ORF1ab primer set 1		
R1_1_F1*	CTCATGTGGGCGAAATACC	
R1_1_2_F2	ATCGACATAGCGAGTGTATG	
R1_1_2_C1	TTTAGATCGGCGCCGTAACATAG	
R1_1_2_C2	AAGAAAACCTGGAACACTAAACATAGC	
R1_1_2_D1	CACCAGCTCCTTATTACCG	This study
R1_1_D2	AGTGGTGTACCCTGAACT	
R1_1_4_R1*	TCGTCGCCTAAGTCAAATGAC	
R1_1_2_R2	TTGGCACTGATCCTTATGAAG	
R1_1_2_CP1	TTTAGATCGGCGCCGTAACATAGGGCAAGTTCTTCTTCGTAAG*	
R1_1_CP2	AGAAAACCTGGAACACTAAACATAGCCTCCGTTAAGCTCACGCAT	
Region 1 ORF1ab primer set 2		
R1_2_F1	ATGTGGGCGAAATACCAGTG	
R1_1_2_F2	ATCGACATAGCGAGTGTATG	
R1_1_2_C1	TTTAGATCGGCGCCGTAACATAG	
R1_1_2_C2	AAGAAAACCTGGAACACTAAACATAGC	
R1_1_2_D1	CACCAGCTCCTTATTACCG	This study
R1_2_D2	GTGGTGTACCCTGAACT	
R1_2_R1	CGTCGCCTAAGTCAAATGAC	
R1_1_2_R2	TTGGCACTGATCCTTATGAAG	
R1_1_2_CP1	TTTAGATCGGCGCCGTAACATAGGGCAAGTTCTTCTTCGTAAG*	
R1_2_CP2	AGAAAACCTGGAACACTAAACATAGCCTCCGTTAAGCTCACGCAT	
Region 1 ORF1ab primer set 3		
R1_3_F1	TTATGGTTGAGCTGGTAGC	
R1_3_4_F2	TGTTATCGACATAGCGAGTGTATG	
R1_3_C1	GCTCCTTATTACCGTTCTTACGAAG	
R1_3_C2	TTGGCACTGATCCTTATGAAGATTTTC	
R1_3_D1	ACTGGTATTTCGCCACATGA	This study
R1_3_D2	CTGGAACACTAAACATAGCAGTG	
R1_3_R1	ATCGGCGCCGTAACATAGGCGCA	
R1_3_R2	TCATTTGACTTAGGCGACGA	
R1_3_CP1	CTCCTTTATTACCGTTCTTACGAAGAGAACTCGAAGGCATTTCAGT	
R1_3_CP2	TTGGCACTGATCCTTATGAAGATTTTCCTCCGTTAAGCTCACGCAT	
Region 1 ORF1ab primer set 4		
R1_4_F1	AGAACTCGAAGGCATTTCAGTA	
R1_3_4_F2	TGTTATCGACATAGCGAGTGTATG	
R1_4_C1	TTAGATCGGCGCCGTAACATAG	
R1_4_C2	CAAGAAAACCTGGAACACTAAACATAG	
R1_4_D1	CTTACGAAGAAGAACCCTTGC	This study
R1_4_D2	CAGTGGTGTACCCTGAACT	
R1_1_4_R1	TCGTCGCCTAAGTCAAATGAC	
R1_4_R2	TTGGCACTGATCCTTATGAAGAT	
R1_4_CP1	TTAGATCGGCGCCGTAACATAGGCTCATGTGGGCGAAATACCAG*	
R1_4_CP2	AGAAAACCTGGAACACTAAACATAGCCTCCGTTAAGCTCACGCAT	
Region 2 ORF1ab primer set 1		
R2_1_2_F1*	CCACAGTACGTCTACAAGC	
R2_1_2_F2	TATGTGGCAACGGCAGTAC	
R2_1_C1	GTAAGCTTTAGCAGCATCTACAGC	
R2_1_3_4_C2	TGGTCAGGCAATAACAGTTACACC	
R2_1_2_D1	GCACAGAAAGATAATACAGTTG	This study
R2_1_2_D2	AAGCCAATATGGATCAAGAATC	
R2_1_R1*	AGTGATTGGTTGTCCCCAC	
R2_1_R2	AGATGTTGTGTACACACTG	
R2_1_CP1	TTAAGCTTTAGCAGCATCTACAGCTAATGCAACAGAAGTGCCTG	
R2_1_CP2	TGGTCAGGCAATAACAGTTACACCAGACAACACGATGCACCACC	

Region 2 ORF1ab primer set 2

R2_1_2_F1	CCACAGTACGTCTACAAGC	
R2_1_2_F2	TATGTGGCAACGGCAGTAC	
R2_2_C1	CTTTGTAAGCTTTAGCAGCATCTAC	
R2_2_C2	GGTCAGGCAATAACAGTTACACCG	
R2_1_2_D1	GCACAGAAAGATAATACAGTTG	This study
R2_1_2_D2	AAGCCAATATGGATCAAGAATC	
R2_2_R1	CAATTAGTGATTGGTTGTCC	
R2_2_R2	GATGTTGTGTACACACACTGG	
R2_2_CP1	TTTGTAAGCTTTAGCAGCATCTACGTAATGCAACAGAAGTGCCT	
R2_2_CP2	GGTCAGGCAATAACAGTTACACCGAGACAACACGATGCACCACC	

Region 2 ORF1ab primer set 3

R2_3_F1	AGTTTAGCTGCCACAGTACGTC	
R2_3_4_F2	CTATGTGGCAACGGCAGTACA	
R2_3_C1	GTAAGCTTTAGCAGCATCTACAGCAA	
R2_1_3_4_C2	TGGTCAGGCAATAACAGTTACACC	
R2_3_D1	GCACAGAAAGATAATACAGTTGAAT	This study
R2_3_4_D2	GGAAGCCAATATGGATCAAGAA	
R2_3_4_R1	ACACAATTAGTGATTGGTTGTCC	
R2_3_R2	AGATGTTGTGTACACACACTGGT	
R2_3_CP1	AGCTTTAGCAGCATCTACAGCAAGGTAATGCAACAGAAGTGCC	
R2_3_4_CP2	GGTCAGGCAATAACAGTTACACCGACAACACGATGCACCACCA	

Region 2 ORF1ab primer set 4

R2_4_F1	CCTAAATAGAGGTATGGTACTT	
R2_3_4_F2	CTATGTGGCAACGGCAGTACA	
R2_4_C1	TGTAAGCTTTAGCAGCATCTACAGCA	
R2_1_3_4_C2	TGGTCAGGCAATAACAGTTACACC	
R2_4_D1	GCAGGCACCTCTGTTGCATTACC	This study
R2_3_4_D2	GGAAGCCAATATGGATCAAGAA	
R2_3_4_R1	ACACAATTAGTGATTGGTTGTCC	
R2_4_R2	TAAGATGTTGTGTACACACACTG	
R2_4_CP1	TAAGCTTTAGCAGCATCTACAGCAAGTTTAGCTGCCACAGTACG	
R2_3_4_CP2	GGTCAGGCAATAACAGTTACACCGACAACACGATGCACCACCA	

Region 3 N gene primer set 1

R3_1_F1*	AGAATGGAGAACGCAGTGG	
R3_1_F2	AAATACCATCTGGACTGAG	
R3_1_C1	TGCCATGTTGAGTGAGAGCGGTG	
R3_1_C2	TAGCAGTCCAGATGACCAAATTGG	
R3_1_D1	AACCAAGACGCAGTATTATTG	This study
R3_1_D2	CTACCGAAGAGCTACCAGAC	
R3_1_R1*	CCTCGAGGGAATTTAAGGTC	
R3_1_R2	AAGGCGTTCCAATTAACACC	
R3_1_CP1	TGCCATGTTGAGTGAGAGCGGTGAAACAACGTCGGCCCAAGG	
R3_1_CP2	TAGCAGTCCAGATGACCAAATTGGTACCGTCACCACCACGAATTC	

Region 3 N gene primer set 2

R3_2_F1	AAACAACGTCGGCCCAAG	
R3_2_F2	TGGCCAGTTCCTAGGTAG	
R3_2_C1	TTGTCTCGAGGGAATTTAAGGTC	
R3_2_3_C2	ACCGAAGAGCTACCAGACGAATTC	
R3_2_D1	TTGCCATGTTGAGTGAGAGC	This study
R3_2_D2	GTGGTGGTGACCGTAAAATG	
R3_2_R1	CTATTGGTGTAAATTGGAACG	
R3_2_R2	CAGATGACCAAATTGGCTAC	
R3_2_CP1	TTGTCTCGAGGGAATTTAAGGTCAATAACTGCGTCTTGGTTCACC	
R3_2_CP2	CCGAAGAGCTACCAGACGAATTCAGAAATACCATCTTGGACTGA	

Region 3 N gene primer set 3

R3_3_F1	CGATCAAAACAACGTCGGCC	
R3_3_F2	TCTGGCCAGTTCCTAGGTA	
R3_3_C1	TTGTCTCGAGGGAATTTAAGGTCTT	
R3_2_3_C2	ACCGAAGAGCTACCAGACGAATTC	
R3_3_D1	CTTGCCATGTTGAGTGAGAGC	This study
R3_3_D2	GTGGTGGTGACCGTAAAATGAA	
R3_3_R1	CTATTGGTGTAAATTGGAACGCC	
R3_3_R2	TCCAGATGACCAAATTGGCTAC	
R3_3_CP1	TTCTCGAGGGAATTTAAGGTCTTCAATAAATACTGCGTCTTGGTTT	
R3_3_CP2	CGAAGAGCTACCAGACGAATTCAGAAATACCATCTTGGACTGA	

Region 3 N gene primer set 4		
R3_4_F1	GCAGTAACCAGAATGGAGAAC	
R3_4_F2	TTCTGGCCCAGTTCCTAGGTA	
R3_4_C1	CTTGCCATGTTGAGTGAGAGCGGT	
R3_4_C2	CAAATTGGCTACTACCGAAGAGCTAC	
R3_4_D1	GAACCAAGACGCAGTATTATTG	This study
R3_4_D2	AATTCGTGGTGGTGACGGTAA	
R3_4_R1	TCCTCGAGGGAATTTAAGGTCT	
R3_4_R2	AAGGCGTTCCAATTAACACCAATA	
R3_4_CP1	CTTGCCATGTTGAGTGAGAGCGGTCAAAACAACGTGGCCCCAA	
R3_4_CP2	ATTGGCTACTACCGAAGAGCTACTAGAAATACCATCTGGACTG	

LAMP primer name	Sequence 5' --> 3'	
Region 1 ORF1ab primer set		
ORF1a-A-F3	CTGCACCTCATGGTCATGTT	
ORF1a-A-B3	AGCTCGTCGCCTAAGTCAA	
ORF1a-A-FIP	GAGGGACAAGGACACCAAGTGTATGGTTGAGCTGGTAGCAGA	Zhang et al. 2020 Rapid Molecular Detection of SARS-CoV-2 (COVID-19) Virus RNA Using Colorimetric LAMP
ORF1a-A-BIP	CCAGTGGCTTACCGCAAGGTTTTAGATCGGCGCCGTAAC	
ORF1a-A-LF	CCGTAAGAACGGTAATAAAGGAGC	
ORF1a-A-LB	TTCGTAAGAACGGTAATAAAGGAGC	

Region 3 N gene primer set		
GeneN-A-F3	TGGCTACTACCGAAGAGCT	
GeneN-A-B3	TGCAGCATTGTTAGCAGGAT	
GeneN-A-FIP	TCTGGCCCAGTTCCTAGGTAGTCCAGACGAATTCGTGGTGG	Zhang et al. 2020 Rapid Molecular Detection of SARS-CoV-2 (COVID-19) Virus RNA Using Colorimetric LAMP
GeneN-A-BIP	AGACGGCATCATATGGGTTGCACGGGTGCCAATGTGATCT	
GeneN-A-LF	GGACTGAGATCTTTCATTTACCGT	
GeneN-A-LB	ACTGAGGGAGCCTTGAATACA	

rt-PCR primer name	Sequence 5' --> 3'	
Region 3 N gene primer set		
WH-NIC N-F	CGTTTGGTGGACCCTCAGAT	WHO Molecular assays to diagnose COVID-19: Summary table of available protocols- National Institute of Health, Thailand
WH-NIC N-R	CCCCACTGCGTTCTCCATT	
WH-NIC N-P	FAM-CAACTGGCAGTAACCA- BHQ1	

Supplementary Table 2: List of synthesised gene fragments used as DNA/RNA template for MCDA, LAMP and rt-PCR. Blue indicates universal M13 adapters while red depicts the sequence for T7 promoter.

Gene fragment name	Position in NC 045512.2	Gene target	Sequence 5' -> 3'
Region 1	416-931	ORF1ab	TGTA ^{BLUE} AAACGACGGCCAGT ^{RED} TAATACGACTCACTATAGTGTGGCTTAGTAGAAGTTGAAAAGGCGTTTTG CCTCAACTTGAACAGCCCTATGTGTTTCATCAAAACGTTCCGGATGCTCGAAGTGCACCTCATGGTCAATGTTA TGGTTGAGCTGGTAGCAGAAGCTCGAAGGCATTCAGTACGGTCTAGTGGTGAGACACTTGGTGTCTTTG TCCTCATGTGGGCGAAATACCAAGTGGTTACCGCAAGGTTCTTCTCGTAAGAACGGTAATAAAGGAG CTGGTGGCCATAGTTACGGCGCCGATCTAAAGTCAATTTGACTTAGGCGACGAGCTTGGCACTGATCCTT ATGAAGATTTCAAGAAAACCTGGAACACTAAACATAGCAGTGGTGTACCCGTGAACTCATGCGTGAGC TTAACGGAGGGGCATACACTCGTATGTCGATAACAACCTTCTGTGGCCCTGATGGCTACCCTCTTGAGT GCATTAAGACCTTCTAGCACGTGCTGGTAAAGCTTCAATGCACTTTGTCCGAACAACCTGGACTTTATTG ^{BLUE} GTCATAGCTGTTTCCTG
Region 2	12869-13388	ORF1ab	TGTA ^{BLUE} AAACGACGGCCAGT ^{RED} TAATACGACTCACTATAGACTGGTACTATCTATACAGAAGTGAACACCT TGTAGGTTTTGTACAGACACACCTAAAGGTCCTAAAGTGAAGTATTTATACTTTATTAAGGATTAAC AACTAAATAGAGGTATGGTACTTGGTAGTTTAGCTGCCACAGTACGTCTACAAGCTGGTAATGCAACA GAAGTGCCTGCCAATCAACTGTATTATCTTCTGTGCTTTTGTGTAGATGCTGCTAAAGCTTACAAG ATTATCTAGCTAGTGGGGGACAACCAATCACTAATTGTGTAAAGATGTTGTGTACACACACTGGTACTG GTCAGGCAATAACAGTTACACCGGAAGCCAATATGGATCAAGAATCCTTTGGTGGTGCATCGTGTGTC TGTACTGCCGTTGCCACATAGATCATCCAAATCCTAAAGGATTTGTGACTTAAAGGTAAGTATGTAC AAATACCTACAACCTTGTGCTAATGACCCTGTGGGTTTACACTTAAAAACACAGTCTGTACCCTGTGG ^{BLUE} GTAGGTCATAGCTGTTTCCTG
Region 3	28246-28747	N	TGTA ^{BLUE} AAACGACGGCCAGT ^{RED} TAATACGACTCACTATAGTAGATTTCATCTAAACGAACAACTAAAAATGC TGATAATGGACCCAAAATCAGCGAAATGCACCCCGCATTACGTTTGGTGGACCCCTCAGATTCAACTGG CAGTAACCCAGAATGGAGAACGCAAGTGGGGCGCATCAAAACAACGTCGGCCCAAGGTTTACCCAATA ATACTGCTCTTGTGTTACCGCTCTCACTCAACATGGCAAGGAAGACCTTAAATTCCTCGAGGACAAG CGGTTCCAATTAACCAATAGCAGTCCAGATGACCAAAATGGCTACTACCGAAGAGCTACCAGACGA ATTCGTGGTGGTACGGTAAAATGAAAGATTCAGTCCAAGATGGTATTTCTACTACCTAGGAACTGGG CCAGAAGCTGGACTTCCCTATGGTGCTAACAAGACGGCATCATATGGGTTGCAACTGAGGGAGCCTT GAATACACAAAAGATCACATTGGCACCCGCAATCCTGTAACAATGCTGCAATCGTGGT ^{BLUE} CATAGCTG ^{BLUE} TTTCCTG

BLUE = universal M13

RED = T7 promoter