

HOMOLOGY ANALYSIS OF NEW DELHI METALLO- β -LACTAMASE (NDM-1) GENE SEQUENCES FROM *ENTEROBACTER*

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Review

SUMMARY

New Delhi metallo- β -lactamase (NDM-1), as a broad-spectrum β -lactamase, is able to inactivate almost all β -lactams. NDM-producers have been detected in extensively pathogen worldwide. The objective of this study was to analyze the homology of NDM-

1-encoding genes based on the bla_{NDM-1} gene sequences downloaded from GenBank. The phylogenetic tree analysis showed that the bla_{NDM-1} gene sequences divided into two different branches with similarity score only 54. A common sequence of 242bps at length was extracted from branch-1, and 469bps common sequence was extracted

from branch-2, respectively. The subsequent detection of NDM-1 harboring clinical isolates with PCR and real-time PCR molecular techniques should be based on the two common sequences of branch-1 and branch-2.

Key words: New Delhi metallo- β -lactamase (NDM-1), Emerging pathogen, Resistance, Homology analysis, sequences contrasting

1. INTRODUCTION

Carbapenem-hydrolysing β -lactamases such as carbapenemases in bacterial clinical isolates are of increasing concern, that's because they often also confer resistance to most other β -lactam antimicrobial agents (1). Metallo- β -lactamases (MBLs), as one class of carbapenemases, which belongs to ambler class B metallo-enzyme groups, plays an important role in the multidrug resistance of *Enterobacteriaceae*. In the past, the prevalence of MBLs producing clinical isolates was increasing worldwide, and most of the MBL-encoding genes such as bla_{IMP} and bla_{VIM} etc. reside on class 1 integrons and plasmids that usually confer high mobility to these genetic elements (2, 3, 4, 5). Therefore, early detection of MBL-producing organisms is of crucial importance for prevention of their inter- and intrahospital dissemination, not only in institutions with high prevalences of such isolates but also in those in which phenotypes of resistance have never been detected (6).

Recently, a new class B enzyme, New Delhi metallo- β -lactamase (NDM-1), as a broad-spectrum β -lactamase, which is able to inactivate all β -lactams except aztreonam (7). Since the first report of NDM-producing *Klebsiella pneumoniae* from Sweden in December 2009 (7), NDM-producers have been detected in extensive pathogen all over the world (8, 9, 10, 11). Especially, the NDM-1 not only harbored in *Enterobacteriaceae* isolates, but also have been detected in *Enterococcus* (12). NDM-1 producers bring several additional factors such as high mobility, spreading producers, and the host-pathogen pathogenicity, which are deeply disconcerting for public health worldwide (13). Therefore, there is an urgent international need to rapid detect NDM-1 producers in any health care facility to prevent their further spread.

Various criteria for screening MBL-producers have been suggested (6, 14), such as Etest MBL strip (15), double-disk synergy test (DDST) and combined disk (CD) assay (6), of

which the susceptibility testing results were recorded according to the Clinical and Laboratory Standards Institute (CLSI) guidelines as modified in June 2010 (16). However, such methods are time-consuming and don't discriminate between NDM-1 and other MBLs. So the detection of the bla_{NDM-1} gene based on PCR and real-time PCR molecular techniques are rapid methods to screening and identifying the carriers of NDM-1 producers (13, 17). At present, the PCR and real-time PCR techniques are always established based on a certain sequence from a certain bacteria strain, some other strains may be escaped from the screening and detecting of NDM-1 producers.

The aim of this study was to evaluate the homology of bla_{NDM-1} gene from *Enterobacteriaceae* and *Enterococcus*, and find out the common sequence of bla_{NDM-1} gene from different host-pathogen for the subsequent PCR and real-time PCR detection.

2. MATERIALS AND METHODS

The source of NDM-1 gene se-

GenBank No	Source	Length (bp)	Country	Reference*
HQ605724	Acinetobacter baumannii strain 10051750	417	China	UP
HQ256747	Enterococcus faecium strain ZW031-2	813	China	UP
HQ917683	Escherichia coli strain MH01	710	Canada	[37]
HQ451074	Escherichia coli strain HK-01 plasmid pNDM-HK	813	HK China	[38]
AB571289	Escherichia coli megaplasmid	475	India	POD
HQ162469	Escherichia coli strain 271 insertion sequence ISSen4	813	Australia	[11]
HQ284043	Klebsiella pneumoniae strain DU1301/10	736	Singapore	[39]
HQ284042	Klebsiella pneumoniae strain DU7433/10	752	Singapore	[39]
FN396876	Klebsiella pneumoniae plasmid pKpANDM-1	813	India	[7]
HQ738352	Klebsiella pneumoniae strain VF408/10	813	Malaysia	UP
HQ171206	Klebsiella pneumoniae strain CMC Micro VB1	469	India	DS
AB614355	Stenotrophomonas maltophilia megaplasmid	813	India	UP
FR820591	Pseudomonas aeruginosa strain MMA533	1059	Serbia	[28]
FR820590	Pseudomonas aeruginosa strain MMA83	1059	Serbia	[28]
JN157767	Klebsiella pneumoniae strain MSNDM116	719	India	UP
JF838352	Acinetobacter baumannii strain D442	813	China	DS
HQ652609	Acinetobacter baumannii strain A65	536	China	UP
HQ652608	Klebsiella pneumoniae subsp. ozaenae strain K16	528	China	UP
AB604954	Klebsiella pneumoniae strain 419	915	Japan	UP
AB604953	Escherichia coli strain NDM-1 Dok01	920	Japan	UP
JF922606	Escherichia coli strain DVR22 insertion sequence ISAbA125	813	Spain	[29]
JF836807	Acinetobacter baumannii	403	India	UP
HQ328085	Klebsiella pneumoniae clone KPM_nasey	813	France	[31]

Table 1 Characteristics of 23 bla_{NDM-1} gene sequences obtained from GenBank database in NCBI. * DS: Direct Submission; UP: Unpublished; POD: Published Only in Database

quences. We searched the GenBank database in National Center of Biotechnology Information (NCBI) website (<http://www.ncbi.nlm.nih.gov>) with search term of “NDM-1”, and got the NDM-1 sequences from variety of *Enterobacteriaceae* and *Enterococcus* submitted before August 1, 2011.

Sequences analysis. The NDM-1 sequences were contrasted with bio-software Clustal W2 provided by European Molecular Biology Laboratory (<http://www.ebi.ac.uk/Tools/clustalw2/index.html>). The phylogenetic tree was established to study the characteristics of the NDM-1 sequences. The similarity scores were analyzed and the common sequence was also extracted.

3. RESULTS

Total homology analysis of bla_{NDM-1} genes

We have extracted 23 bla_{NDM-1} gene sequences from NDM-1-harboring clinical isolates, of which 9 for *Klebsiella pneumoniae*, 6 for *Escherichia coli*, 4 for *Acinetobacter baumannii*, 2 for *Pseudomonas aeruginosa*, one for *Stenotrophomonas maltophilia* and *Enterococcus faecium* (showed in Table 1). The total homology of these 23 bla_{NDM-1} gene

sequences showed that there have sourced from two different branches respectively (Figure 1a), we called branch-1 and branch-2. Branch-1 including 18 sequences with the accession number of HQ328085, AB604953, AB604954, HQ284042, JF838352, HQ284043, HQ605724, JN157767, FR820590, FR820591, JF922606, HQ451074, HQ917683, HQ652608, JF836807, AB614355, HQ256747 and AB571289. While branch-2 including 5 sequences: HQ171206, HQ652609, HQ738352, HQ162469 and FN396876. The similarity scores in branch-1 were from 60 to 100, most of which were 99 and 100, while in branch-2, all the similar scores were 100. However, the similarity scores between branch-1 and branch-2 just from 43 to 54 (Figure 1b).

The sequences characteristics of branch-1 and branch-2

The sequences contrasting analysis of branch-1 and branch-2 have

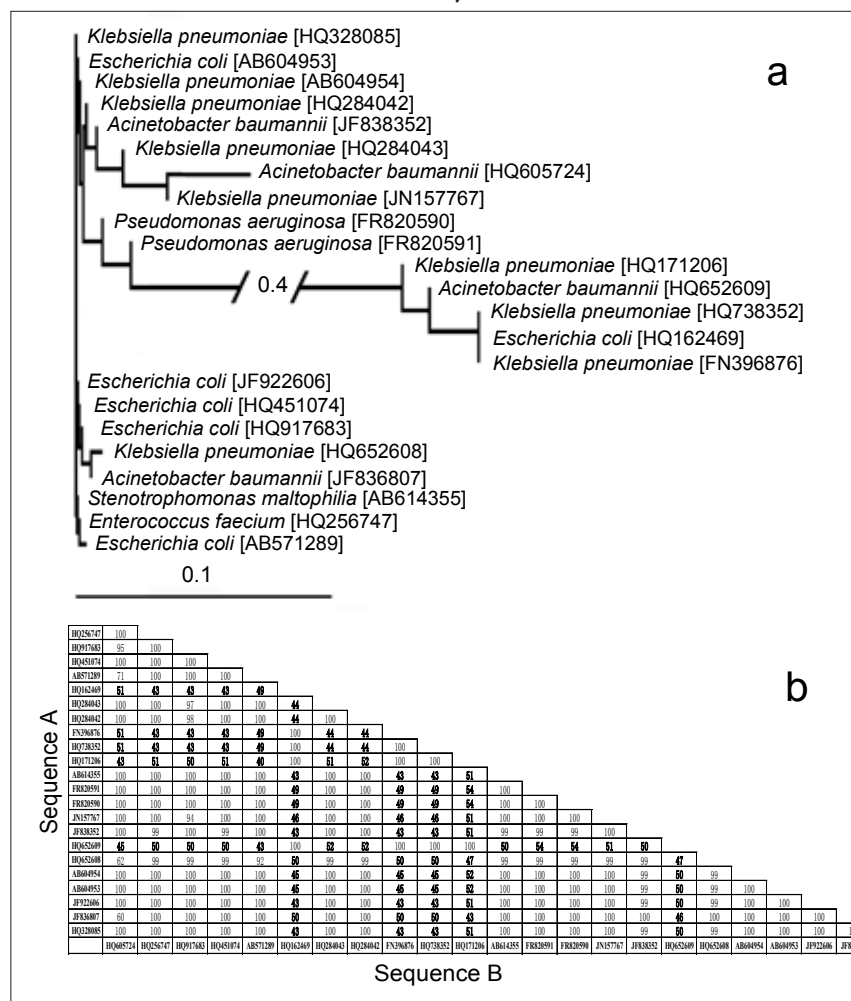


Figure 1 Results of homology analysis of bla_{NDM-1} gene sequences. a. Phylogenetic tree obtained for 23 bla_{NDM-1} gene sequences. b. Similarity scores contrasted with 23 bla_{NDM-1} gene sequences. The scores contrasted between branch-1 and branch-2 are showed in bold.

GenBank No	Positions*
AB571289	57-298 bp
AB614355	248-489 bp
AB604954	293-534 bp
HQ328085	248-489 bp
HQ652608	19-260 bp
JF836807	1-242 bp
HQ917683	156-397 bp
HQ256747	248-489 bp
FR820591	350-591 bp
AB604953	297-538 bp
JF922606	248-489 bp
FR820590	350-591 bp
HQ451074	248-489 bp
HQ284042	210-451 bp
JF838352	248-489 bp
HQ284043	204-445 bp
JN157767	204-445 bp
HQ605724	176-417 bp

Table 2. The positions of common sequence in 18 bla_{NDM-1} gene sequences including in branch-1. * Position numbers correspond to the nucleotides of the coding sequences.

been processed respectively, and the results of contrasting analysis showed in Supplementary Figure S1 and Figure S2. We have extracted a common sequence at length of 242bp from branch-1 (Figure 2), and the common sequence positions showed in Table 2. A 469bp common sequence has been extracted from branch-2 (Figure 3), the positions of common sequence showed in Table 3. The similarity between the common sequences of branch-1 and branch-2 have also contrasted, and the score only 54 (showed in Supplementary Figure S3).

4. DISCUSSION

Carbapenemase-expressing *Enterobacteriaceae* and *Enterococcus* are of great threat to the global health system, that's because this class of antibiotics is of major importance as a last-line therapy of nosocomial infections (17). The emergence of the novel carbapenemase, NDM-1, was widely noticed and even reported by popular media, and the prevalence of NDM-1 producing strains have made the medical staff feeling worrisome. To present, the bla_{NDM-1}-harboring strains have been detected in multiple areas: India (18, 19), China (20), United States (21), Japan (22, 23), Singapore (24), Canada (25, 26), Australia (27), United Kingdom (8, 9), Serbia (28), Spain (29), France

(30, 31), and other European countries (32) etc. and in multiple clinical isolates: *Acinetobacter baumannii* (33), *Acinetobacter lwofii* (34), *Klebsiella pneumoniae* (35), *Escherichia coli* (29, 36), *Pseudomonas aeruginosa* (28), *Stenotrophomonas maltophilia* and *Enterococcus faecium* etc. (show in Table 1). So it is need to analyse the homology for the epidemiological study. Our study showed that there are two branches of bla_{NDM-1} encoding gene in NDM-1 producing pathogen. In some countries with great population such as India and China, both branches were detected, while in the country of small populations such as Japan, Singapore

GenBank No	Positions*
HQ162469	134-602 bp
FN396876	134-602 bp
HQ738352	134-602 bp
HQ652609	34-502 bp
HQ171206	1-469 bp

Table 3. The positions of common sequence in 5 bla_{NDM-1} gene sequences including in branch-2. * Position numbers correspond to the nucleotides of the coding sequences.

and Serbia, just one branch was detected. In addition, in some other countries such as France, Malaysia, Australia, Spain, and Canada, just one clinical isolate reported at present.

PCR and real-time PCR techniques are regarded as rapid methods in molecular diagnostics, and the real-time PCR may represent the current gold standard. The use of synthetic gene as positive control reaction demonstrates that the PCR assay works are functional, however, there might still have a false-negative result because the PCR and real-time PCR techniques are processed based on a certain sequence from a certain strain. That made some other strains which only single or several bases different from the certain sequence escaped from screening and detecting. The results of this study demonstrated that there are two branches with low similarity in bla_{NDM-1}, that's to say we should establish the PCR and real-time PCR techniques based on these two common sequences of branch-1 and branch-2 to increase the detect-

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001-GCGGCCGCTGCTGGTGGTCGATACCGCTGGACCGATGA-040
041-CCAGACCGCCAGATCCTCAACTGGATCAACGAGAGATC-080
081-AACCTGCCGCTCGCGTGGCGGTGGTGACTCAGCGCATC-120
121-AGGACAAGATGGGCGGTATGGACCGCGTGCATGCGCGGG-160
161-GATTGCGACTTATGCCAATCGCTGTGCAACAGCCTGCC-200
201-CCGCAAGAGGGGATGTTGCGCGCAACACAGCCTGACTT-240
241-TC -242
```

Figure 2 The common sequence of branch-1.

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001-TCAGTGTCCGATCACCAGATGTCGGAGCGACTTGGCCT-040
041-TGCTGTCTTGTATCAGGCAGCCACAAAAGCGATGTCGGT-080
081-GCCGTGATCCCAACGGTATATTGCTACTGGTGGCGCC-120
121-GGGCCGGGTAAATACCTTGAGCGGGCCAAAGTTGGCG-160
161-CGGTGTCTGGTTCGACCCAGCCATGGCGGGCAAAGTCAG-200
201-GCTGTGTTGGCGCAACCACTCCCTCTTGGGGGAAGC-240
241-TGGTTGCAACCGATTGGCATAAGTCGAATCCCGCCG-280
281-CATGCAGCGCTCCATACCGCCATCTTGTCTGTATGCGC-320
321-GTAGTACCACCGCCAGCGCCAGCGCAGGTGATCTCC-360
361-TGCTGATCCAGTTGAGGATCTGGGCGGTCTGGTATCGG-400
401-TCCAGGCGGTATCGACACCAGCAGCGCGCCATCCT-440
441-GACGATCAAACCGTTGGAAGCGACTGCC -469
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Figure 3. The common sequence of branch-2.

ing ratio of true-positive in bla_{NDM-1} harboring clinical isolates.

Although the present study analyzed all bla_{NDM-1} gene sequences have been submitted to the GenBank to date, there still have some other bla_{NDM-1} gene sequences have not yet submitted to the GenBank, or some other NDM-1-encoding genes have not detected at present. We can use the PCR and real-time PCR protocols based on the two

Sequence number used in this study	Accession number in GenBank
sequence1	HQ605724
sequence2	HQ256747
sequence3	HQ917683
sequence4	HQ451074
sequence5	AB571289
sequence6	HQ162469
sequence7	HQ284043
sequence8	HQ284042
sequence9	FN396876
sequence10	HQ738352
sequence11	HQ171206
sequence12	AB614355
sequence13	FR820591
sequence14	FR820590
sequence15	JN157767
sequence16	JF838352
sequence17	HQ652609
sequence18	HQ652608
sequence19	AB604954
sequence20	AB604953
sequence21	JF922606
sequence22	JF836807
sequence23	HQ328085
sequence24	Common sequence of branch-1
sequence25	Common sequence of branch-2

Table S1. Sequence accession numbers used in this study

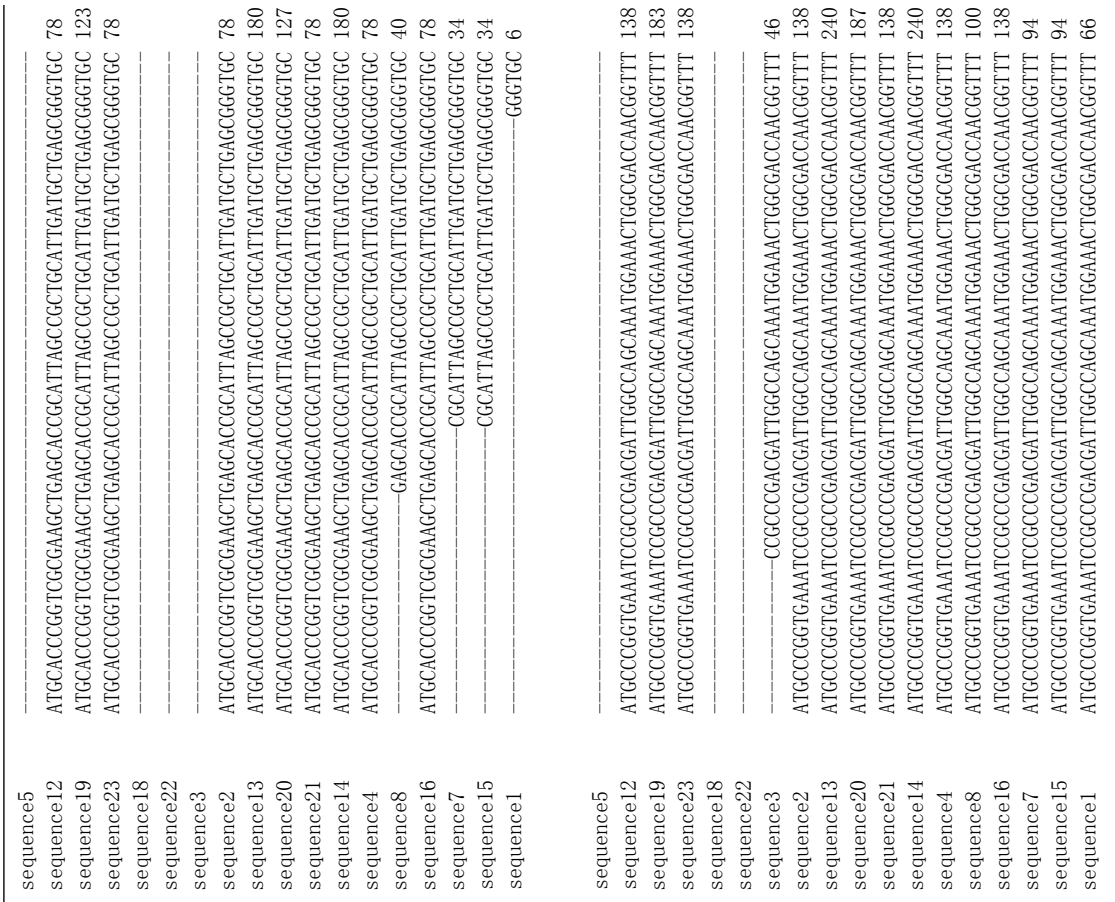


Figure S1 Continued.

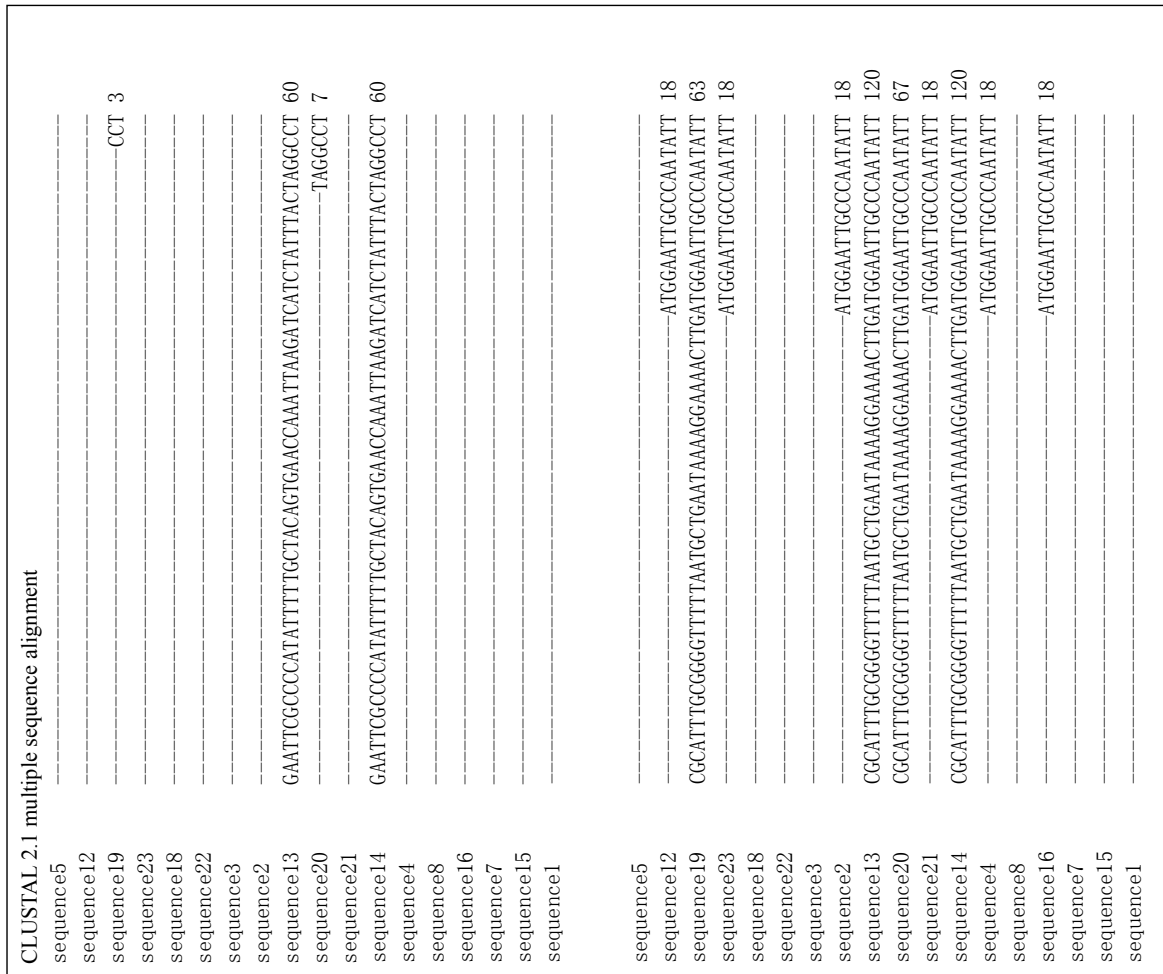


Figure S1 Result of sequence contrasting of 18 *bla*_{NDM-1} gene sequences including in branch-1. The common sequence is emphasized with “**”.

sequence5	GGCGGTATGACGCGCTGCAATGCGGGGGGATTCGCACTTATGCAATGGTGTGCGAAC	247
sequence12	GGCGGTATGACGCGCTGCAATGCGGGGGGATTCGCACTTATGCAATGGTGTGCGAAC	438
sequence19	GGCGGTATGACGCGCTGCAATGCGGGGGGATTCGCACTTATGCAATGGTGTGCGAAC	483
sequence23	GGCGGTATGACGCGCTGCAATGCGGGGGGATTCGCACTTATGCAATGGTGTGCGAAC	438
sequence18	GGCGGTATGACGCGCTGCAATGCGGGGGGATTCGCACTTATGCAATGGTGTGCGAAC	209
sequence22	GGCGGTATGACGCGCTGCAATGCGGGGGGATTCGCACTTATGCAATGGTGTGCGAAC	191
sequence3	GGCGGTATGACGCGCTGCAATGCGGGGGGATTCGCACTTATGCAATGGTGTGCGAAC	346
sequence2	GGCGGTATGACGCGCTGCAATGCGGGGGGATTCGCACTTATGCAATGGTGTGCGAAC	438
sequence13	GGCGGTATGACGCGCTGCAATGCGGGGGGATTCGCACTTATGCAATGGTGTGCGAAC	540
sequence20	GGCGGTATGACGCGCTGCAATGCGGGGGGATTCGCACTTATGCAATGGTGTGCGAAC	487
sequence21	GGCGGTATGACGCGCTGCAATGCGGGGGGATTCGCACTTATGCAATGGTGTGCGAAC	438
sequence14	GGCGGTATGACGCGCTGCAATGCGGGGGGATTCGCACTTATGCAATGGTGTGCGAAC	540
sequence4	GGCGGTATGACGCGCTGCAATGCGGGGGGATTCGCACTTATGCAATGGTGTGCGAAC	438
sequence8	GGCGGTATGACGCGCTGCAATGCGGGGGGATTCGCACTTATGCAATGGTGTGCGAAC	400
sequence16	GGCGGTATGACGCGCTGCAATGCGGGGGGATTCGCACTTATGCAATGGTGTGCGAAC	438
sequence7	GGCGGTATGACGCGCTGCAATGCGGGGGGATTCGCACTTATGCAATGGTGTGCGAAC	394
sequence15	GGCGGTATGACGCGCTGCAATGCGGGGGGATTCGCACTTATGCAATGGTGTGCGAAC	394
sequence1	GGCGGTATGACGCGCTGCAATGCGGGGGGATTCGCACTTATGCAATGGTGTGCGAAC	366

sequence5	CAGTTGCCCGCCGCAAGAGGGGATGGTTGGGGGCAACACAGCCCTGACTTTGGCGGCCAAT	307
sequence12	CAGTTGCCCGCCGCAAGAGGGGATGGTTGGGGGCAACACAGCCCTGACTTTGGCGGCCAAT	498
sequence19	CAGTTGCCCGCCGCAAGAGGGGATGGTTGGGGGCAACACAGCCCTGACTTTGGCGGCCAAT	543
sequence23	CAGTTGCCCGCCGCAAGAGGGGATGGTTGGGGGCAACACAGCCCTGACTTTGGCGGCCAAT	498
sequence18	CAGTTGCCCGCCGCAAGAGGGGATGGTTGGGGGCAACACAGCCCTGACTTTGGCGGCCAAT	269
sequence22	CAGTTGCCCGCCGCAAGAGGGGATGGTTGGGGGCAACACAGCCCTGACTTTGGCGGCCAAT	251
sequence3	CAGTTGCCCGCCGCAAGAGGGGATGGTTGGGGGCAACACAGCCCTGACTTTGGCGGCCAAT	406
sequence2	CAGTTGCCCGCCGCAAGAGGGGATGGTTGGGGGCAACACAGCCCTGACTTTGGCGGCCAAT	498
sequence13	CAGTTGCCCGCCGCAAGAGGGGATGGTTGGGGGCAACACAGCCCTGACTTTGGCGGCCAAT	600
sequence20	CAGTTGCCCGCCGCAAGAGGGGATGGTTGGGGGCAACACAGCCCTGACTTTGGCGGCCAAT	547
sequence21	CAGTTGCCCGCCGCAAGAGGGGATGGTTGGGGGCAACACAGCCCTGACTTTGGCGGCCAAT	498
sequence14	CAGTTGCCCGCCGCAAGAGGGGATGGTTGGGGGCAACACAGCCCTGACTTTGGCGGCCAAT	600
sequence4	CAGTTGCCCGCCGCAAGAGGGGATGGTTGGGGGCAACACAGCCCTGACTTTGGCGGCCAAT	498
sequence8	CAGTTGCCCGCCGCAAGAGGGGATGGTTGGGGGCAACACAGCCCTGACTTTGGCGGCCAAT	460
sequence16	CAGTTGCCCGCCGCAAGAGGGGATGGTTGGGGGCAACACAGCCCTGACTTTGGCGGCCAAT	454
sequence7	CAGTTGCCCGCCGCAAGAGGGGATGGTTGGGGGCAACACAGCCCTGACTTTGGCGGCCAAT	454
sequence15	CAGTTGCCCGCCGCAAGAGGGGATGGTTGGGGGCAACACAGCCCTGACTTTGGCGGCCAAT	454
sequence1	CAGTTGCCCGCCGCAAGAGGGGATGGTTGGGGGCAACACAGCCCTGACTTTGGCGGCCAAT	417

Figure S1 Continued.

sequence5	CTGGTGGTTCGATACCGCTGACCGGATACCAGACCGCCAGATCTCAACTGGATCAAG	127
sequence12	CTGGTGGTTCGATACCGCTGACCGGATACCAGACCGCCAGATCTCAACTGGATCAAG	318
sequence19	CTGGTGGTTCGATACCGCTGACCGGATACCAGACCGCCAGATCTCAACTGGATCAAG	363
sequence23	CTGGTGGTTCGATACCGCTGACCGGATACCAGACCGCCAGATCTCAACTGGATCAAG	318
sequence18	CTGGTGGTTCGATACCGCTGACCGGATACCAGACCGCCAGATCTCAACTGGATCAAG	89
sequence22	CTGGTGGTTCGATACCGCTGACCGGATACCAGACCGCCAGATCTCAACTGGATCAAG	71
sequence3	CTGGTGGTTCGATACCGCTGACCGGATACCAGACCGCCAGATCTCAACTGGATCAAG	226
sequence2	CTGGTGGTTCGATACCGCTGACCGGATACCAGACCGCCAGATCTCAACTGGATCAAG	318
sequence13	CTGGTGGTTCGATACCGCTGACCGGATACCAGACCGCCAGATCTCAACTGGATCAAG	420
sequence20	CTGGTGGTTCGATACCGCTGACCGGATACCAGACCGCCAGATCTCAACTGGATCAAG	367
sequence21	CTGGTGGTTCGATACCGCTGACCGGATACCAGACCGCCAGATCTCAACTGGATCAAG	318
sequence14	CTGGTGGTTCGATACCGCTGACCGGATACCAGACCGCCAGATCTCAACTGGATCAAG	420
sequence4	CTGGTGGTTCGATACCGCTGACCGGATACCAGACCGCCAGATCTCAACTGGATCAAG	318
sequence8	CTGGTGGTTCGATACCGCTGACCGGATACCAGACCGCCAGATCTCAACTGGATCAAG	280
sequence16	CTGGTGGTTCGATACCGCTGACCGGATACCAGACCGCCAGATCTCAACTGGATCAAG	318
sequence7	CTGGTGGTTCGATACCGCTGACCGGATACCAGACCGCCAGATCTCAACTGGATCAAG	274
sequence15	CTGGTGGTTCGATACCGCTGACCGGATACCAGACCGCCAGATCTCAACTGGATCAAG	274
sequence1	CTGGTGGTTCGATACCGCTGACCGGATACCAGACCGCCAGATCTCAACTGGATCAAG	246

sequence5	CAGGAGATCAACTGCCCGTGGCGGTTGGTGAATCAACCGGATCAGGACAAAGATG	187
sequence12	CAGGAGATCAACTGCCCGTGGCGGTTGGTGAATCAACCGGATCAGGACAAAGATG	378
sequence19	CAGGAGATCAACTGCCCGTGGCGGTTGGTGAATCAACCGGATCAGGACAAAGATG	423
sequence23	CAGGAGATCAACTGCCCGTGGCGGTTGGTGAATCAACCGGATCAGGACAAAGATG	378
sequence18	CAGGAGATCAACTGCCCGTGGCGGTTGGTGAATCAACCGGATCAGGACAAAGATG	149
sequence22	CAGGAGATCAACTGCCCGTGGCGGTTGGTGAATCAACCGGATCAGGACAAAGATG	131
sequence3	CAGGAGATCAACTGCCCGTGGCGGTTGGTGAATCAACCGGATCAGGACAAAGATG	286
sequence2	CAGGAGATCAACTGCCCGTGGCGGTTGGTGAATCAACCGGATCAGGACAAAGATG	378
sequence13	CAGGAGATCAACTGCCCGTGGCGGTTGGTGAATCAACCGGATCAGGACAAAGATG	480
sequence20	CAGGAGATCAACTGCCCGTGGCGGTTGGTGAATCAACCGGATCAGGACAAAGATG	427
sequence21	CAGGAGATCAACTGCCCGTGGCGGTTGGTGAATCAACCGGATCAGGACAAAGATG	378
sequence14	CAGGAGATCAACTGCCCGTGGCGGTTGGTGAATCAACCGGATCAGGACAAAGATG	480
sequence4	CAGGAGATCAACTGCCCGTGGCGGTTGGTGAATCAACCGGATCAGGACAAAGATG	378
sequence8	CAGGAGATCAACTGCCCGTGGCGGTTGGTGAATCAACCGGATCAGGACAAAGATG	340
sequence16	CAGGAGATCAACTGCCCGTGGCGGTTGGTGAATCAACCGGATCAGGACAAAGATG	378
sequence7	CAGGAGATCAACTGCCCGTGGCGGTTGGTGAATCAACCGGATCAGGACAAAGATG	334
sequence15	CAGGAGATCAACTGCCCGTGGCGGTTGGTGAATCAACCGGATCAGGACAAAGATG	334
sequence1	CAGGAGATCAACTGCCCGTGGCGGTTGGTGAATCAACCGGATCAGGACAAAGATG	306

Figure S1 Continued.

sequence5	GGCTGCCGTGATCAAGGACAGCAGGCCAAGTGGCTCGCTGGCAATCTCGGT-----475
sequence12	GGCTGCCGTGATCAAGGACAGCAGGCCAAGTGGCTCGCTGGCAATCTCGGTGATGCGGACACT 678
sequence19	GGCTGCCGTGATCAAGGACAGCAGGCCAAGTGGCTCGCTGGCAATCTCGGTGATGCGGACACT 723
sequence23	GGCTGCCGTGATCAAGGACAGCAGGCCAAGTGGCTCGCTGGCAATCTCGGTGATGCGGACACT 678
sequence18	GGCTGCCGTGATCAAGGACAGCAGGCCAAGTGGCTCGCTGGCAATCTCGGTGATGCGGACACT 449
sequence22	GGCTGCCGTGATCAAGGACAGCAGGCCAAGTGGCTCGCTGGCAATCTCGGTGATGCGGACACT 403
sequence3	GGCTGCCGTGATCAAGGACAGCAGGCCAAGTGGCTCGCTGGCAATCTCGGTGATGCGGACACT 586
sequence2	GGCTGCCGTGATCAAGGACAGCAGGCCAAGTGGCTCGCTGGCAATCTCGGTGATGCGGACACT 678
sequence13	GGCTGCCGTGATCAAGGACAGCAGGCCAAGTGGCTCGCTGGCAATCTCGGTGATGCGGACACT 780
sequence20	GGCTGCCGTGATCAAGGACAGCAGGCCAAGTGGCTCGCTGGCAATCTCGGTGATGCGGACACT 727
sequence21	GGCTGCCGTGATCAAGGACAGCAGGCCAAGTGGCTCGCTGGCAATCTCGGTGATGCGGACACT 678
sequence14	GGCTGCCGTGATCAAGGACAGCAGGCCAAGTGGCTCGCTGGCAATCTCGGTGATGCGGACACT 780
sequence4	GGCTGCCGTGATCAAGGACAGCAGGCCAAGTGGCTCGCTGGCAATCTCGGTGATGCGGACACT 678
sequence8	GGCTGCCGTGATCAAGGACAGCAGGCCAAGTGGCTCGCTGGCAATCTCGGTGATGCGGACACT 640
sequence16	GGCTGCCGTGATCAAGGACAGCAGGCCAAGTGGCTCGCTGGCAATCTCGGTGATGCGGACACT 678
sequence7	GGCTGCCGTGATCAAGGACAGCAGGCCAAGTGGCTCGCTGGCAATCTCGGTGATGCGGACACT 634
sequence15	GGCTGCCGTGATCAAGGACAGCAGGCCAAGTGGCTCGCTGGCAATCTCGGTGATGCGGACACT 634
sequence1	-----
sequence5	GAGCACTACGCCCGCTCAGCCGGCGGCTTTGGTGGCGGCTTCCCAAGGCCAAGCATGATC 738
sequence12	GAGCACTACGCCCGCTCAGCCGGCGGCTTTGGTGGCGGCTTCCCAAGGCCAAGCATGATC 738
sequence19	GAGCACTACGCCCGCTCAGCCGGCGGCTTTGGTGGCGGCTTCCCAAGGCCAAGCATGATC 738
sequence23	GAGCACTACGCCCGCTCAGCCGGCGGCTTTGGTGGCGGCTTCCCAAGGCCAAGCATGATC 738
sequence18	GAGCACTACGCCCGCTCAGCCGGCGGCTTTGGTGGCGGCTTCCCAAGGCCAAGCATGATC 509
sequence22	-----
sequence3	GAGCACTACGCCCGCTCAGCCGGCGGCTTTGGTGGCGGCTTCCCAAGGCCAAGCATGATC 646
sequence2	GAGCACTACGCCCGCTCAGCCGGCGGCTTTGGTGGCGGCTTCCCAAGGCCAAGCATGATC 738
sequence13	GAGCACTACGCCCGCTCAGCCGGCGGCTTTGGTGGCGGCTTCCCAAGGCCAAGCATGATC 840
sequence20	GAGCACTACGCCCGCTCAGCCGGCGGCTTTGGTGGCGGCTTCCCAAGGCCAAGCATGATC 787
sequence21	GAGCACTACGCCCGCTCAGCCGGCGGCTTTGGTGGCGGCTTCCCAAGGCCAAGCATGATC 738
sequence14	GAGCACTACGCCCGCTCAGCCGGCGGCTTTGGTGGCGGCTTCCCAAGGCCAAGCATGATC 840
sequence4	GAGCACTACGCCCGCTCAGCCGGCGGCTTTGGTGGCGGCTTCCCAAGGCCAAGCATGATC 738
sequence8	GAGCACTACGCCCGCTCAGCCGGCGGCTTTGGTGGCGGCTTCCCAAGGCCAAGCATGATC 700
sequence16	GAGCACTACGCCCGCTCAGCCGGCGGCTTTGGTGGCGGCTTCCCAAGGCCAAGCATGATC 738
sequence7	GAGCACTACGCCCGCTCAGCCGGCGGCTTTGGTGGCGGCTTCCCAAGGCCAAGCATGATC 694
sequence15	GAGCACTACGCCCGCTCAGCCGGCGGCTTTGGTGGCGGCTTCCCAAGGCCAAGCATGATC 694
sequence1	-----

Figure S1 Continued.

sequence5	GGCTGGGTGAAACAGCAGCAGCCGGCCCAACTTTGGCCCGCTCAAGGTAATTTACCCCGGC 367
sequence12	GGCTGGGTGAAACAGCAGCAGCCGGCCCAACTTTGGCCCGCTCAAGGTAATTTACCCCGGC 558
sequence19	GGCTGGGTGAAACAGCAGCAGCCGGCCCAACTTTGGCCCGCTCAAGGTAATTTACCCCGGC 603
sequence23	GGCTGGGTGAAACAGCAGCAGCCGGCCCAACTTTGGCCCGCTCAAGGTAATTTACCCCGGC 558
sequence18	GGCTGGGTGAAACAGCAGCAGCCGGCCCAACTTTGGCCCGCTCAAGGTAATTTACCCCGGC 329
sequence22	GGCTGGGTGAAACAGCAGCAGCCGGCCCAACTTTGGCCCGCTCAAGGTAATTTACCCCGGC 311
sequence3	GGCTGGGTGAAACAGCAGCAGCCGGCCCAACTTTGGCCCGCTCAAGGTAATTTACCCCGGC 466
sequence2	GGCTGGGTGAAACAGCAGCAGCCGGCCCAACTTTGGCCCGCTCAAGGTAATTTACCCCGGC 558
sequence13	GGCTGGGTGAAACAGCAGCAGCCGGCCCAACTTTGGCCCGCTCAAGGTAATTTACCCCGGC 660
sequence20	GGCTGGGTGAAACAGCAGCAGCCGGCCCAACTTTGGCCCGCTCAAGGTAATTTACCCCGGC 607
sequence21	GGCTGGGTGAAACAGCAGCAGCCGGCCCAACTTTGGCCCGCTCAAGGTAATTTACCCCGGC 558
sequence14	GGCTGGGTGAAACAGCAGCAGCCGGCCCAACTTTGGCCCGCTCAAGGTAATTTACCCCGGC 660
sequence4	GGCTGGGTGAAACAGCAGCAGCCGGCCCAACTTTGGCCCGCTCAAGGTAATTTACCCCGGC 558
sequence8	GGCTGGGTGAAACAGCAGCAGCCGGCCCAACTTTGGCCCGCTCAAGGTAATTTACCCCGGC 520
sequence16	GGCTGGGTGAAACAGCAGCAGCCGGCCCAACTTTGGCCCGCTCAAGGTAATTTACCCCGGC 558
sequence7	GGCTGGGTGAAACAGCAGCAGCCGGCCCAACTTTGGCCCGCTCAAGGTAATTTACCCCGGC 514
sequence15	GGCTGGGTGAAACAGCAGCAGCCGGCCCAACTTTGGCCCGCTCAAGGTAATTTACCCCGGC 514
sequence1	-----
sequence5	CCCGGCCACACAGTGACAATATACCGTTGGGATGACGGCACCGACATCGCTTTTGGT 427
sequence12	CCCGGCCACACAGTGACAATATACCGTTGGGATGACGGCACCGACATCGCTTTTGGT 618
sequence19	CCCGGCCACACAGTGACAATATACCGTTGGGATGACGGCACCGACATCGCTTTTGGT 663
sequence23	CCCGGCCACACAGTGACAATATACCGTTGGGATGACGGCACCGACATCGCTTTTGGT 618
sequence18	CCCGGCCACACAGTGACAATATACCGTTGGGATGACGGCACCGACATCGCTTTTGGT 389
sequence22	CCCGGCCACACAGTGACAATATACCGTTGGGATGACGGCACCGACATCGCTTTTGGT 371
sequence3	CCCGGCCACACAGTGACAATATACCGTTGGGATGACGGCACCGACATCGCTTTTGGT 526
sequence2	CCCGGCCACACAGTGACAATATACCGTTGGGATGACGGCACCGACATCGCTTTTGGT 618
sequence13	CCCGGCCACACAGTGACAATATACCGTTGGGATGACGGCACCGACATCGCTTTTGGT 720
sequence20	CCCGGCCACACAGTGACAATATACCGTTGGGATGACGGCACCGACATCGCTTTTGGT 667
sequence21	CCCGGCCACACAGTGACAATATACCGTTGGGATGACGGCACCGACATCGCTTTTGGT 618
sequence14	CCCGGCCACACAGTGACAATATACCGTTGGGATGACGGCACCGACATCGCTTTTGGT 720
sequence4	CCCGGCCACACAGTGACAATATACCGTTGGGATGACGGCACCGACATCGCTTTTGGT 618
sequence8	CCCGGCCACACAGTGACAATATACCGTTGGGATGACGGCACCGACATCGCTTTTGGT 580
sequence16	CCCGGCCACACAGTGACAATATACCGTTGGGATGACGGCACCGACATCGCTTTTGGT 574
sequence7	CCCGGCCACACAGTGACAATATACCGTTGGGATGACGGCACCGACATCGCTTTTGGT 574
sequence15	CCCGGCCACACAGTGACAATATACCGTTGGGATGACGGCACCGACATCGCTTTTGGT 574
sequence1	-----

Figure S1 Continued.

sequence5	-----	sequence12	-----
sequence19	GTGATGAGCCATTCCGCCCCCGGATAGCGGCGCGCAATGACTCATACGGCCCGCATGGCC	sequence19	GATGTACAGAG----- 915
sequence23	GTGATGAGCCATTCCGCCCCCGGATAGCGGCGCGCAATGACTCATACGGCCCGCATGGCC	sequence23	-----
sequence18	GTGATGAGCCATTCCGCCCCCGGATAGCGGCGCGCAATGACTCATACGGCCCGCATGGCC	sequence18	-----
sequence22	-----	sequence22	-----
sequence3	GTGATGAGCCATTCCGCCCCCGGATAGCGGCGCGCAATGACTCATACGGCCCGCATGGCC	sequence3	-----
sequence2	GTGATGAGCCATTCCGCCCCCGGATAGCGGCGCGCAATGACTCATACGGCCCGCATGGCC	sequence2	-----
sequence13	GTGATGAGCCATTCCGCCCCCGGATAGCGGCGCGCAATGACTCATACGGCCCGCATGGCC	sequence13	GATGTACAGAGCGGTTTTATGCGAAGCTGGGCTTTCGACGAGTTGGAAGGATCGCGGC
sequence20	GTGATGAGCCATTCCGCCCCCGGATAGCGGCGCGCAATGACTCATACGGCCCGCATGGCC	sequence20	GATGTACAGAG----- 920
sequence21	GTGATGAGCCATTCCGCCCCCGGATAGCGGCGCGCAATGACTCATACGGCCCGCATGGCC	sequence21	-----
sequence14	GTGATGAGCCATTCCGCCCCCGGATAGCGGCGCGCAATGACTCATACGGCCCGCATGGCC	sequence14	GATGTACAGAGCGGTTTTATGCGAAGCTGGGCTTTCGACGAGTTGGAAGGATCGCGGC
sequence4	GTGATGAGCCATTCCGCCCCCGGATAGCGGCGCGCAATGACTCATACGGCCCGCATGGCC	sequence4	-----
sequence8	GTGATGAGCCATTCCGCCCCCGGATAGCGGCGCGCAATGACTCATACGGCCCGCATGGCC	sequence8	-----
sequence16	GTGATGAGCCATTCCGCCCCCGGATAGCGGCGCGCAATGACTCATACGGCCCGCATGGCC	sequence16	-----
sequence7	GTGATGAGCCATTCCGCCCCCGGATAGCGGCGCGCAATGACTCATACGGCCCGCATGGCC	sequence7	-----
sequence15	GTGATGAGCCATTCCGCCCCCGGATAGCGGCGCGCAATGACTCATACGGCCCGCATGGCC	sequence15	-----
sequence1	-----	sequence1	-----
sequence5	-----	sequence5	-----
sequence12	GACAAGCTGGCGTGA----- 813	sequence12	-----
sequence19	GACAAGCTGGCGTGA----- 813	sequence19	-----
sequence23	GACAAGCTGGCGTGA----- 813	sequence23	-----
sequence18	-----	sequence18	-----
sequence22	-----	sequence22	-----
sequence3	GAC----- 709	sequence3	-----
sequence2	GACAAGCTGGCGTGA----- 813	sequence2	-----
sequence13	GACAAGCTGGCGTGA----- 813	sequence13	TGGATGATCCTGCAGCGCGCGGTTTTGAGCTCGAATTC 1059
sequence20	GACAAGCTGGCGTGA----- 813	sequence20	-----
sequence21	GACAAGCTGGCGTGA----- 813	sequence21	-----
sequence14	GACAAGCTGGCGTGA----- 813	sequence14	TGGATGATCCTGCAGCGCGCGGTTTTGAGCTCGAATTC 1059
sequence4	GACAAGCTGGCGTGA----- 813	sequence4	-----
sequence8	-----	sequence8	-----
sequence16	GACAAGCTGGCGTAG----- 813	sequence16	-----
sequence7	-----	sequence7	-----
sequence15	-----	sequence15	-----
sequence1	-----	sequence1	-----

Figure S1 Continued.

Figure S1 Continued.

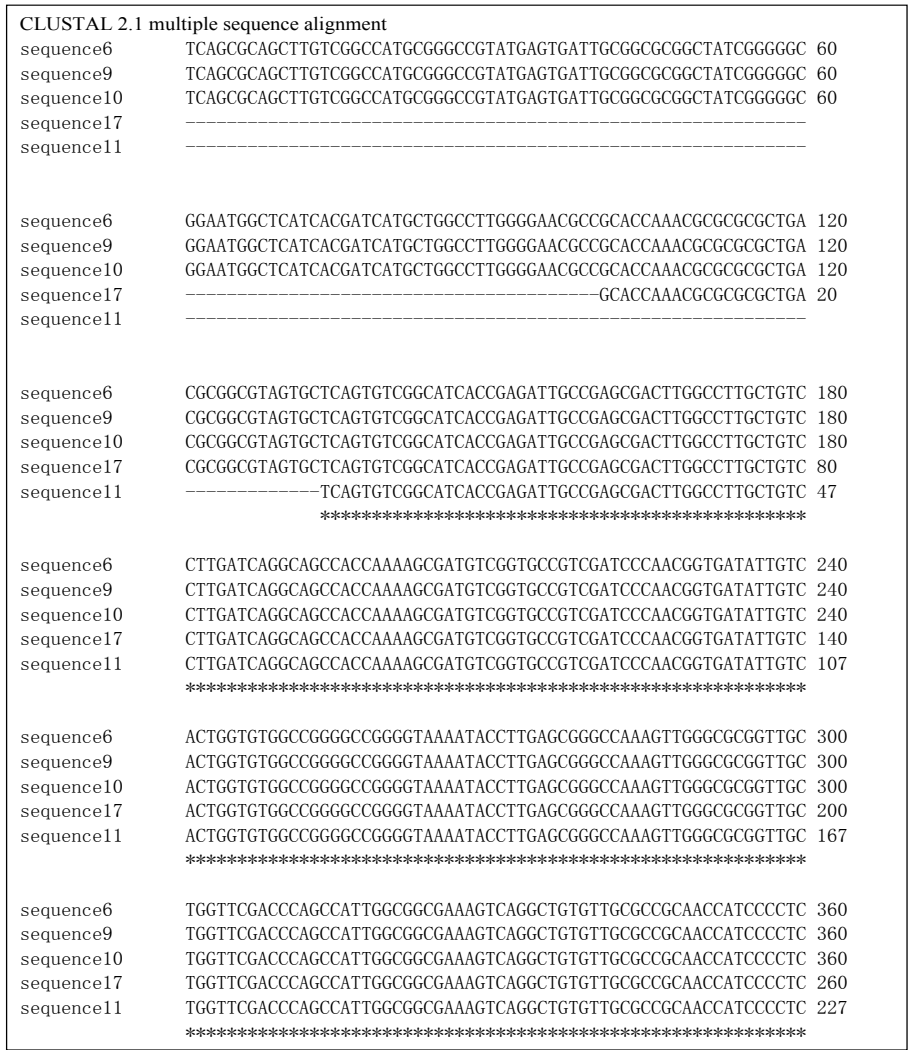
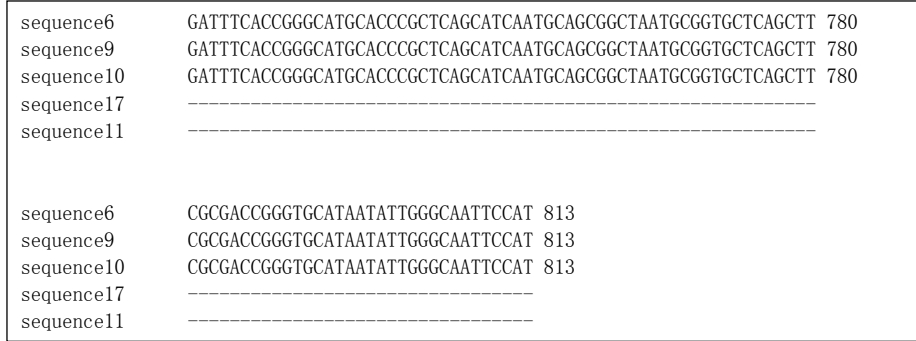


Figure S2 Result of sequence contrasting of 5 *bla*_{NDM-1} gene sequences including in branch-2. The common sequence is emphasized with “*”.



common sequences of this study as a rapid detection and identification methods to control the hospital infection and the spreading of these resistant determinants. After the rapid detection, the DDST, CD assay and Etest MBL strip can be used for the identification of MBLs with negative isolates screened by PCR and real-time PCR to identify the false-negative *bla*_{NDM-1} harboring isolates.

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