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# Title: Identification of consensus hairpin loop structure among the negative sense sub-genomic RNAs of SARS-CoV-2

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Abstract:

SARS-CoV-2 is the causative agent of worldwide pandemic disease COVID-19. SARS-CoV-2 bears positive sense RNA genome, that have organized and complex pattern of replication/transcription process including the generation of subgenomic RNAs. Transcription regulatory sequences (TRS) have important role in the pausing of replication/transcription and generation of subgenomic RNAs. In the present bioinformatics analysis a consensus secondary structure was identified among negative sense subgenomic RNAs at the adjacent of initiation codon. This study proposed that consensus structured domain could involve in mediating the long pausing of replication/transcription complex and responsible for subgenomic RNA production.

## Introduction:

COVID-19 is the worldwide pandemic disease caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) that carries a positive sense, single strand RNA genome with the size of 29.9 kb (Kim et al., 2020; Zhu et al., 2020a). The RNA genome undergoes complex pattern of replication/transcription process inside the host environment. The SARS-CoV-2 enters the host cell and undergoes replication of positive sense RNA genome, where negative sense RNA genome is generated, that subsequently serve as template for positive sense RNA in order to translate the viral proteins and packaging of RNA genome in to virion particles. SARS-CoV-2 RNA genome bears conserved transcriptional regulatory sequences (TRS) of 6-7 nucleotides(Yang et al., 2020). The TRS is present at the immediate upstream of initiation codon, where replication/transcription complex is paused (Alexandersen et al., 2020; Kim et al., 2020). RdRp has the important property to backtracking that causes long pausing at specific site (Dulin et al., 2015; Malone et al., 2021). Therefore, in addition to complete RNA genome, a nested set of negative sense subgenomic RNAs is also generated including S subgenomic, 3a subgenomic, E subgenomic, M subgenomic, 6 subgenomic, 7a subgenomic, 7b subgenomic, 8 subgenomic, N subgenomic and 10 subgenomic. These

canonical subgenomic RNAs are thought to be generated through the complex mechanism that involves pausing of negative sense RNA synthesis by RNA dependent RNA polymerase (RdRp) (Alexandersen et al., 2020; Kim et al., 2020; Mohammadi-Dehcheshmeh et al., 2021; Yang et al., 2020).

Although, the conserved role of TRS has been identified/proposed in the pausing of transcription, however, the role of RNA secondary structure has not been identified in this perspective. Therefore, in this study investigation of conserved secondary structure pattern was performed nearby the initiation codon/TRS that might be important in pausing of transcription during negative sense RNA synthesis of the SARS-CoV-2 genome.

The analysis of negative sense subgenomic RNAs was objectively performed in this study because of following important reason. The negative sense RNA is firstly generated through the replication/transcription of positive sense RNA genome. The negative sense RNA will serve as template for positive sense RNA genome and subgenomic messenger RNAs (or subgenomic RNAs) (Sawicki et al., 2007; Yang et al., 2020). According to previous research model, it was suggested that strand exchange or 5′ leader (5′ UTR) sequence to body (RNA) fusion occurs at TRS during negative sense RNA synthesis (Kim et al., 2020). Therefore, the consensus secondary structural motifs nearby the TRS might be important in several perspectives. In this study the bioinformatics analysis of negative sense subgenomic RNAs was performed that revealed a consensus hairpin loop secondary structure. Simultaneously, we validate the finding in parallel comparison with SARS-CoV and MERS-CoV. SARS-CoV-2 genome has 79.5% sequence similarity with SARS-CoV (Lu et al., 2020; Zhu et al., 2020b). Within this context it is significant to reveal the specific hairpin domain at the adjacent of initiation codon/TRS that is consistently present in SARS-CoV-2 and SARS-CoV subgenomic RNAs. In this perspective, research report proposed that consensus hairpin

loop secondary structure during the synthesis of negative sense RNA mediates important role in transcriptional pausing and generation of subgenomic RNAs.

**Results:**

**The negative sense sub-genomic RNAs bear conserved hairpin domain at immediate downstream of initiation codon**

Study by Alexandersen et al., 2021, determined the presence and abundance of subgenomic RNAs from the COVID-19 patient samples. Authors mapped the NGS reads to subgenomic RNAs in order to identify the abundance of particular subgenomic RNA from the patient samples(Alexandersen et al., 2020). NGS reads mapped results revealed that Orf7a and N subgenomic were in high abundance whereas Orf8, Orf6, and E subgenomic were relatively low (in an increasing order). These subgenomic RNAs are present several fold higher in comparison to whole genome fragments. Therefore, the present study seek to identify the possible conserved regions nearby the transcription regulatory sequences (TRS) or initiation codon those could mediate significant roles in the generation or expression of sub-genomic RNAs.

Therefore, in order to identify the consensus secondary structure, negative sense subgenomic RNAs were aligned by using LocARNA webserver. The significant length of upstream and downstream region (with respect to initiation codon) was considered for the analysis, with the total length of ~420 nt. Sequence alignment revealed a consensus hairpin domain (~35 to 60 nt; depending on the particular subgenomic RNA) among different negative sense sub-genomic RNAs (Figure 1, Table 1). This domain is present at the immediate downstream of initiation codon. The important features could be noted as the distance between initiation codon and consensus hairpin domain (Table 1). The structured hairpin domain of negative sense subgenomic RNAs might be of intrinsic functioning in either pausing /backtracking of RdRp at the immediate downstream of

99 initiation codon. The conserved hairpin domain could also have role in template
100 switching during transcription, although the role of conserved TRS has only been
101 identified in recombination events (Yang et al., 2021). The conserved TRS is AACGAAC,
102 that is highlighted in green (as GUUCGUU; negative sense) in the analyzed sequences
103 of negative sense subgenomic RNAs (supplementary data file).

104 **The Orf7a subgenomic, N subgenomic, E subgenomic, Orf6 subgenomic and Orf8**
105 **subgenomic bears distinct secondary structure domain near the TRS:**

106 The sequence alignment of negative sense subgenomic RNAs revealed single hairpin
107 domain. However, it was required to perform secondary structure prediction of each
108 subgenomic RNA sequence to examine the possibility of consensus secondary structure
109 formation. The secondary structure prediction was performed by using Vienna RNA
110 webserver, and determined whether particular subgenomic RNA adopts a specific
111 hairpin structure or not. Data obtained from sequence alignment and secondary
112 structure prediction indicates that Orf7a subgenomic, N subgenomic, E subgenomic,
113 Orf6 subgenomic and Orf8 subgenomic bears distinct and long hairpin domain with high
114 probability of formation (Table 1, Figure 2A). These structural features could possibly be
115 correlated with the previous study by Alexandersen et al; which described the high
116 abundance of Orf7a subgenomic and N subgenomic RNAs whereas Orf8 subgenomic,
117 Orf6 subgenomic, and E subgenomic were relatively low (Alexandersen et al., 2020). In
118 addition, the remaining subgenomic RNAs (S subgenomic, Orf7b, Orf3a and M
119 subgenomic) were reported to be very low or near zero level abundance (Alexandersen et
120 al., 2020). The underlying reason of low level of NGS reads could be correlated with
121 their respective hairpin structure (Figure 2B) and other properties mentioned in Table
122 1. These features include: hairpin loop splits in to subdomains, hairpin loop merged with
123 initiation codon, length and probability of hairpin domain formation. Overall, the

124 formation of distinct hairpin structure might be the underlying feature in the generation
125 of negative sense sub-genomic RNAs.

126 Importantly, the highest similarity (and intermediate alignment) was observed between
127 Orf7a and N subgenomic RNAs, as both subgenomic RNAs were placed together in guide
128 tree. It provides significant relevance and correlation from the previously reported high
129 abundant reads of N and Orf7a subgenomic (Alexandersen et al., 2020). In this
130 perspective, we propose that conserved secondary structural elements might be a
131 determining factor in transcriptional pausing and backtracking of RdRp that leads to
132 subgenomic RNA production.

133 **Analysis of consensus secondary structure prediction in SARS-CoV subgenomic RNAs**

134 It is important to validate or examine the possibility of consensus secondary structure in
135 closely related enveloped viruses (beta-coronaviruses). Within this context, we further
136 analyzed the subgenomic RNA sequences of SARS-CoV. We identified a distinct hairpin
137 structure domain from the sequence alignment of subgenomic RNA sequences of SARS-
138 CoV (Figure 4). The SARS-CoV subgenomic RNAs bears a distinct hairpin domain at the
139 immediate downstream of TRS, as observed in the case of subgenomic RNAs of SARS-
140 CoV-2 (Supplementary data file). However, after analysis it was observed that an
141 additional domain could be considered in the case of some of the subgenomic RNAs of
142 SARS-CoV as it falls at the immediate upstream of TRS, whereas for some of the specific
143 subgenomic RNAs the second downstream domain could additionally be considered (as
144 the consensus region falls at the immediate downstream of TRS).

145 **Discussion**

146 The present finding revealed the conserved hairpin structure that is present in negative
147 sense sub-genomic RNAs of SARS-CoV-2. The significance of conserved hairpin
148 structure could be of two fold reasons. Firstly, the particular hairpin loop secondary

149 structure could have intrinsic functioning in the pausing of replication/transcription of
150 negative sense subgenomic RNAs mediated by RdRp. In addition, the conserved
151 secondary structure could facilitate template switching (by unknown mechanism) at
152 TRS during transcription, that involve the joining of 5′UTR to RNA body sequence.
153 Further experimental studies could be performed to identify the much precise role of the
154 conserved secondary structure nearby the initiation codon/TRS in the generation of
155 negative sense subgenomic RNAs and maintenance of viral infection.

156 **Methods:**

157 **Extraction of SARS-CoV-2 RNA genome and subgenomic RNAs**

158 The complete genome sequence of SARS-CoV-2 isolate Wuhan-Hu-1 (NCBI Reference
159 Sequence: NC_045512.2) was used in this study, and specific regions (upstream and
160 downstream) corresponding to particular subgenomic RNA were extracted. The
161 nucleotides sequences were converted in to reverse complement that served as negative
162 sense subgenomic RNA. The negative sense RNAs were used in alignment and
163 secondary structure prediction analysis.

164 To analyze the subgenomic RNAs SARS-CoV, Bat coronavirus (BtCoV/279/2005),
165 complete genome was used.

166 **RNA secondary structural similarity by using LocARNA webserver**

167 LocARNA-Alignment and Folding webserver align the input RNA sequences and
168 simultaneously fold them (Raden et al., 2018; Will et al., 2012). This webserver was used
169 in the present study because it folds the RNA by using very realistic energy models as
170 used by RNAfold of the Vienna RNA package. The sequences of negative sense
171 subgenomic RNAs were aligned by using LocARNA webserver. The alignment type was
172 global and in standard mode. The alignment was used to identify the consensus

173 secondary structures elements between negative sense subgenomic RNA sequences of
174 SARS-CoV-2.

175 **Secondary structure prediction by using Vienna RNA webserver**

176 Secondary structure prediction of subgenomic RNA sequences was performed by using
177 Vienna RNA webserver (Hofacker, 2003). The subset of aligned sequence region (~285
178 nt) respective to each subgenomic RNA was submitted for structure prediction by using
179 Vienna RNA webserver. The prediction results are included in supplementary data file.

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| Sr. No. | Type of subgenomic RNA | Location of hairpin structure (from the downstream of initiation codon) | Length of hairpin loop structure with probability | Hairpin structure is distinct or not (merged with different loop carrying initiation codon) | Sequence alignment matches with predicted structure (probability) | Probability of formation of distinct secondary structure |
|---|---|---|---|---|---|---|
| 1 | S subgenomic | Includes ~28 nt of coding region | ~55 nt | No (merged with initiation codon) | Yes | Less |
| 2 | *3a subgenomic | ~10 nt | ~58 nt | Yes | Yes | Moderate |
| 3 | ***E subgenomic | ~47 nt | ~52 nt | Yes | Yes | High |
| 4 | *M subgenomic | ~8 nt | Splits in three small hairpin loops | No (first loop merged with initiation codon) | No (three distinct hairpin structures) | Moderate |
| 5 | **Orf6 subgenomic | ~41 nt | ~47 nt | Yes | Yes | High |
| 6 | ****Orf7a subgenomic | ~30 nt | ~35 nt | Yes | Yes | High |
| 7 | Orf7b subgenomic | Includes ~6 nt of coding sequence | Splits in two hairpin loop structure | No (first loop merged with initiation codon) | No (two distinct hairpin structures) | Moderate |
| 8 | **Orf8 subgenomic | ~20 nt | ~57 nt | Yes | Yes | Moderate |

| | | | | | | |
|---|---|---|---|---|---|---|
| 9 | ****N subgenomic | ~1st nucleotide of initiation codon | ~60 nt | Yes | Yes | Moderate |

224 **Table 1**: The table presents the description of analyzed negative sense subgenomic
225 RNAs. The description is drawn from the combined output of sequence alignment
226 followed by secondary structure prediction results. The number of asterisks (*) denotes
227 to the abundance level for each subgenomic RNA detected by NGS reads mapped to the
228 sequences and no asterisk is given to particular subgenomic that was detected at very
229 low or near zero level) (Alexandersen et al., 2020).

230 **Figure legend**:

231 **Figure 1**

232 The figure presents the output of alignment result of negative sense subgenomic RNAs
233 of SARS-CoV-2 that was done through the LocARNA webserver. The alignment result
234 revealed the consensus sequence and hairpin structure (with double headed arrow)
235 among negative sense subgenomic RNAs. This consensus structure is present at the
236 immediate downstream of initiation codon or TRS (mentioned with double headed
237 arrow).

238 **Figure 2**

239 Secondary structure of consensus sequence of respective negative sense subgenomic
240 RNAs. The secondary structure of each sequence was predicted by using Vienna RNA
241 webserver. The respective portion of hairpin loop is shown with probability in color
242 coding from 0 to 1. Secondary structure of consensus alignment sequence of each
243 subgenomic RNA is shown in panel A and B. Panel A involves consensus secondary
244 structure from subgenomic RNAs those have been detected or mapped at considerable
245 level through NGS reads mapping (Alexandersen et al., 2020). Panel B involves
246 consensus structure of subgenomic RNAs those have been detected at very low or zero

247 level through NGS reads mapping. The complete secondary structure of each
248 subgenomic RNA is provided in supplementary data file.

249 **Figure 3**

250 The figure presents the output of alignment result of negative sense subgenomic RNAs
251 of SARS-CoV that was done through the LocARNA webserver. The alignment result
252 revealed the consensus sequence and hairpin structure (with double headed arrow)
253 among negative sense subgenomic RNAs. This consensus structure is present at the
254 immediate downstream of initiation codon or TRS (mentioned with double headed
255 arrow). In addition, the TRS sequence (GUUCGUU) is also shown just before the
256 consensus hairpin domain.

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                                   ...................(((((((.................
Orf6a   UCACAAGUAGCGAG---------------UGUU-AUCAGUGCCAAGAAAAGAAUAAUUUU 44
Orf7a   --------AGUUUA---------------GGUGAAACUGAUCUGGCACGUAACUGAUAGA 37
S       GCAAAAUAAAC-AC---------------CAUCAUUAAAUGGUAGGACAGGGUUAUCA-- 42
Orf7b   GGGUCAUCAACUACAUAUGGUUGAUGUUGAGUACAUGACUGUAAACUACAUUCU------ 54
Orf8b   AGGAAACUGUAUAAUUACCGAUAUCGAUGUACUGAAUGGGUGA----------------- 43
E       AU------------------------------CAGGAACUCUAGAAGAA------------ 19
M       AUUCUGUAAACAGCAGCAAGCACAAAACAAGCUAAAGUUA----------CUGGCCAUAA 50
Orf3a   A-------------------------------------CCCUUGGAGAGUG---------- 14
N       GAAC---------------------------------GCCUUGUCCUCGAGGGAAUUUAAGGU 30
        .........10........20........30........40........50........6

        ....................))))))).................(((((((....)))))
Orf6a   CAUGUUCGUUUAAUCAAUCUCCAUUGGUUGCUCUUCAUCUAAUUGAG-AAUAUUUAUU-C 102
Orf7a   CGUGUUUUAC--------GCCGUCAGGACAAGCAAAAGCAAAUUGAG-UGCUAAAGCAAG 88
S       ---AACCUCUUAGUA-----CCAUUGGUCCCAGAGACAUGUAU--AG-CAUGGAACCAAG 91
Orf7b   UGGUGAAAUGCAG-----CUACAGUUGUGAUGAUUCCUAAGAAAACA-AGAAAUUUC-AU 107
Orf8b   ------UUUAGAACCAGCCUCAUCCACGCACAAUUCAAUUAAAGGUGCUGAUUUUCUAGC 97
E       ------------------UUCAGAUUUUUAACACGAGAGUAAACGUAAAAAGAAGGUUUU 61
M       CAGCCAGAGGAAAAU-------UAACUUAAUUAUAUACAAAAACCUAUUCCUGUUGGCAU 103
Orf3a   -CUAGUUGCCAUCUCUUUUUGAGGGUUAU-------------------------------- 42
N       CUUCCUUGCCAUGUUGAG-UGAGAGCGGU-------------------------------- 58
        0........70........80........90........100.......110.......1

        )))...................................(((((((((...............
Orf6a   UCAG-UUAGUGACUUAGAU---AAAUUUUUAAUUAUGAGGUUUAU-------GAUGUAAU 151
Orf7a   UCAG---UGCAAAUUUGUUAUCAGCUAGAGGAUGAAAUGGUGAAUUGCCCUCGUAUGUUC 145
S       UAAC-AUUGGAAA------AGAAAGGUAAGAACAAGUCCUGAGUUG--------------- 130
Orf7b   GUUC-GUUUAGGCGUGACAAGUUUC----------AUUAUGAUCUUG--------------- 143
Orf8b   UCCU-ACUCUAAUAUACCAUUUAGAAUAGAAGUGAAUAGGACACGGG------------U 144
E       ACA--AGACUCACGU------------------UAACAAUAUUUGCAG------------- 88
M       AGGCAAAUUG--------UAGAAGACAAAUCCAUGUAAGGAAUAG---------------- 140
Orf3a   ---GAUUUUGGAAGCGCUCUGAAAAACAGCAAGAAGUGCAACGCCAACAAUAAGCCAUCC 99
N       ---GAACCA----AGACGCAGUAUUAUUGGGUAAACCUUGGGGCCGAC------------ 99
        20.......130.......140.......150.......160.......170.......1

        ..........................))))))))).......................((
Orf6a   CAAGAUUCCA-AAUGGAA-ACUUUAAAAGUCCUCAUAAUAAUUAGUAAUAUCUCUGCUAU 209
Orf7a   CAGAAGAGC--AAGGUUC-UUUUAAAAGUACUGUUGUACCUCUAACA-------CACUCU 195
S       -----------------A-AUGUAAAACUGAGGAUCUGAAAACUUUGUCAGGGUAAUAAA 172
Orf7b   CAGUUC-----AAGUGAG-AACCAAAAGAUAAUAAGCAUAAUUAAAACAAGG--AAUAGC 195
Orf8b   CAUCAACUAC-AUAUGGU-UGAUGUUGAGUA---------------------------CAU 176
E       CAGUACGCAC-ACAAUCGAAGCGCAGUAAGGAUGGCUAGUGUAACUAGCAAGAAUACCAC 147
M       -GAAACCUAU-UACUAGG--UUCCAUUGUUCA-----------AGGAGCUUUUUAAGCUC 185
Orf3a   GAAAGGGAGUGAGGCUUGUAUCGGUAUCGUUGC--------------------------AG 134
N       ----------GUUGUUUUGAUCGC--GCCCCAC--------------------------UG 122
        80.......190.......200.......210.......220.......230.......2

        (((((.............................................)))))))...
Orf6a   AGUAAC--CUGAAAGU-C-AACGAGAU----GA-AACAUCUGUUGUCAC-UUACUGUAC- 258
Orf7a   UGGUA---GUGA-----U-AA-AGCUCACAAGU-AGCGAGUGUUAUCAG-UGCCAAGAA- 242
S       CACCACGUGUGAAAGAAU-UAGUGUAUGCAGG---GGGUAAUUGAGUUC-UGGUUGUAA- 226
Orf7b   AGAAAGGCUAAAAAGCACAAAUAGAAGUCAAUU-AAUGAAAGUUCAAUC-AUUCUGU--- 250
Orf8b   GACUG---------UAAACUACAUUCU-----U-GGUGAAAUGCAGCUA-CAGUUGUGAU 220
E       GAAAGCAAGAAAAAGAA--GUACGCUAUUAACU-AUUAACGUACCUGUCUCUUCCGAAAC 204
M       UUCAAC--GGUAA-----------------------------UAGUACCGUUGGA----- 209
Orf3a   UAGC----GCGAA--CAAAAUCUGA-AGGAGUAGCAUCCUUGAUUUCACCUUGCUUCAAA 187
N       CGUU----CUCCAUUCUGGUUACUGCCAG--UUGAAUCUGAGGGUCCACCAAACGUAA-- 174
        40.......250.......260.......270.......280.......290.......3

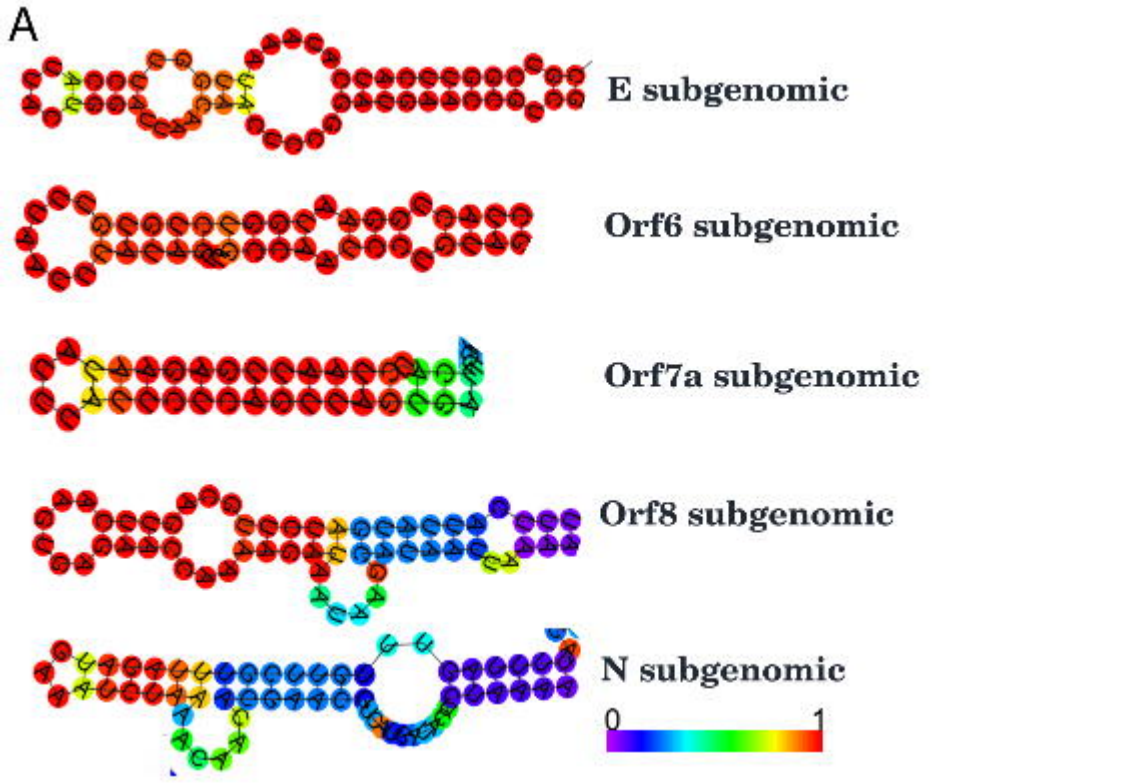
        ...........................................................(
Orf6a   -AAGCA--------------------------AAGCAAU----AUUGUCACUGC------U 282
Orf7a   -AAGAAUAAUUU-------UCAUGUUCGUUUAAUCAAUCUCCAUUGGUUGCUC------U 288
S       -GAUUAACA-------------------------CACUGACUAGAGA---CUA------G 251
Orf7b   ---------------------------------------------------CU------U 253
Orf8b   GAUUCCUAAGAA-------AACAAGAAAUUUCAUGUUCGUUUAGGCGUGACAA------G 267
E       GAAUGAGUACAUAAGUUCGUACUCAUCAGCUUGUGCUUACAAAGGCACGCUAGUAGUCGU 264
M       ------------------------------------------AUCUGCCAUGGCUAAAAU 227
Orf3a   GUUACAGUUCCAAUUGUGAAGAUUCUCAUAAACAAAUCCAUAAGUU------CGUUUAUG 241
N       -UGCGGGGUGCAUUUCGC-------UGAUUUUG---------GGGU------CCAUUAUC 211
        00.......310.......320.......330.......340.......350.......3

        (((.((((..((((......((((((.......)))))).........))))).))).....
Orf6a   AC-UGGAA-UGGU-------CUGUGUUUAAUUUAUAG----UU----GCCAAUCC----- 320
Orf7a   UCAUCUAA-UUGA-------GAAU-----AUUUAUUC----------UCAGUUAG----- 320
S       UGGCAAUA-AAACAAGAAAAACAAACA------UUGU----UC----GUUUAGUUGUUAA 296
Orf7b   UCUUUUGAGUGUGAAGCAAAGUGUU----AUAAACAGUAUUGC----CGCAACAA--UAA 303
Orf8b   UUUCAUUAUGAUCUUGC-AGUUC-----AAGUGAGAACCAAAA----GAUAAUA----AG 313
E       CGUCGGU-UCAUCAUAAAUUGGUUCCAUUACUGGAUUAACAACUCCGGAUGAACCG---- 319
M       UAAAGUUCCAAACAGAAAAACUAA-UAUAAUAUUUAGUUC-------GUUUAGACCAGAA 279
Orf3a   UGUAAUGUAAUUUGACUCCUUUGAGCACUG-GCUCAGAGU-CGU-----CUUCAUCAAAU 294
N       AGACAUUUUAGUUUGUUCGUUUAGAUG-AA-AUCUAAAAC-AACACGAACGUCAUGAUAC 268
        60.......370.......380.......390.......400.......410.......4

        ...........))))...........................................
Orf6a   -----------UGUAGCGACUGUAUGCAGCAAAACCUGAGUCA--CCUG----------- 356
Orf7a   -----------UGA----------------------CUUAGAUA--AAUU----------- 335
S       ---CAAGAACAUCACUAGA--------AAUAACAACUCUGUUG--UUUUCUCUAAUUAUA 343
Orf7b   ---GAAAAAUUGGAGAGUAAAGUUCU---UGAACUUCCUCUUG--UCUGAUGAACAGUUU 355
Orf8b   ---CAUAAUUAAAACAAGGAA------------------UAGCAGAAAGGCUAAAAA---- 349
E       -----------UCGAUUG----------------------------UGUG---------- 330
M       GAUCAGGAACUCUAGAAGAAUUCAGAUUUUUAACACGAGAGUAAACGUAAAAAGAAGGUU 339
Orf3a   UUGCA------GCAGGAUCCACAAGAACAACAGCCCUUGAGACAACUACAGCAACUGGUC 348
N       UCUAA------AAAGUCUUCAUAGAACGAACAACGCACUACAAGACUACCCAAUUUAGGU 322
        20.......430.......440.......450.......460.......470.......4

        ...........................................................
Orf6a   -------CUACACGCUGCGAAG----CUC----------------CCAAUUUGUAAUAAG 389
Orf7a   -------UUUAAU-UAUGAGGU----UUAUGAUGUAAUCAAGAUUCCAAAUGGAAACUUU 383
S       AGUCUACCUUUACUAAGAAGAG----AUAAAAUCAUAUCAU---------------UGAUU 385
Orf7b   AGGUGAAACUGAUCUGGCACGU----AACUGAU------------------------- 384
Orf8b   -----------GCACAAAUAGA----AGUCAAUU------AAUGAAAGUUCAAUCAUUCU 388
E       ----AAUUUGGACAUGUUCUUCAGGCUCAUCAACA-------------AUUUUAUUGUAG 373
M       UUACAAGACUCAC-----GUUAACAAUAUUGCAGCAGUACGCACACAAUCGAAGCGCAGU 394
Orf3a   AUACAGC----AAAGCAUAAUUGUCACCAUUACUAUGGCAAUCAAGCCA----------- 393
N       UCCUGGCAAUUAAUUGUAAAGGUAAACAGGAAACUGUAUAAUUACCGAUAUCGAUGUAC 382
        80.......490.......500.......510.......520.......530.......5

        ...........................................
Orf6a   AAAGCGUUC--GUG--AUGUAGCAACAGUGAUU------U------C 420
Orf7a   AAAAGUCCU--CAU--AAUAAUUAGUAAUAUCUCUGCUAU------A 420
S       UGACCUUCU--UUU----AAAGACAUAACAGCAGUACCCC------U 420
Orf7b   ---AGACGU--GUUUUACGCCGUCAGGACAAGCAAAAGCA------A 420
Orf8b   GUCUUUCUU--UUGAGUGUGAAGCAAAGUGUUA-------------U 420
E       AUGAAGAAGGUAACAUGUUCAACACCAGUGUCUGUACUCAAUUGAGU 420
M       AAGGAUGGCUAGUGUAA-----------------------CUAGCAAGA 420
Orf3a   ---------GCUAUAAAACC--------UAGCCAAAUGUAC---CAU 420
N       UGAAUGGGUGAUUUAGAACC---------AGCCUCAUCCACGCACAA 420
        40.......550.......560.......570.......580.....
```


A
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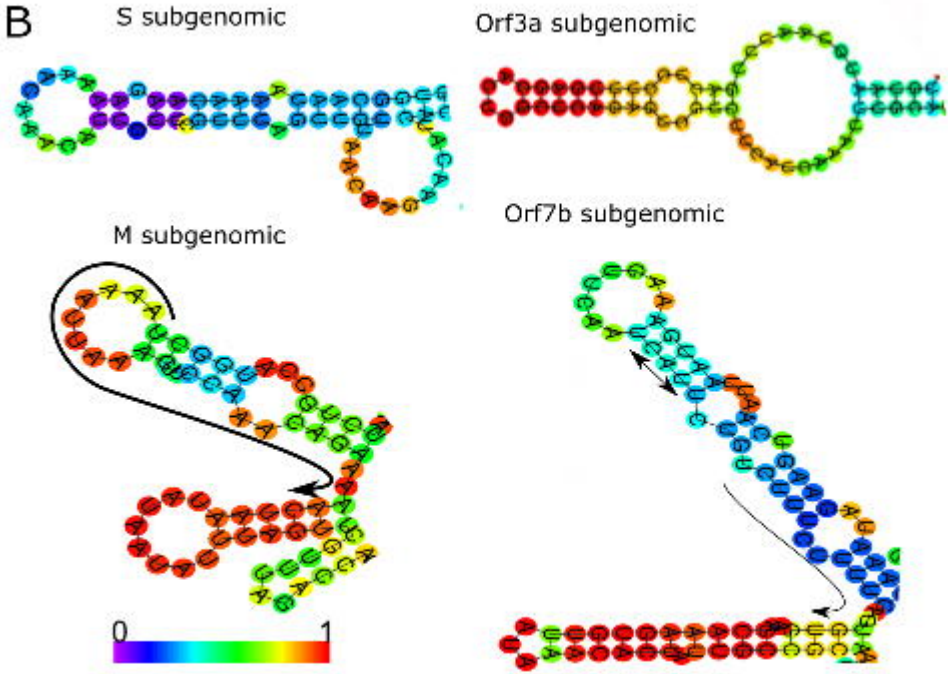
B
S subgenomic
Orf3a subgenomic
M subgenomic
Orf7b subgenomic
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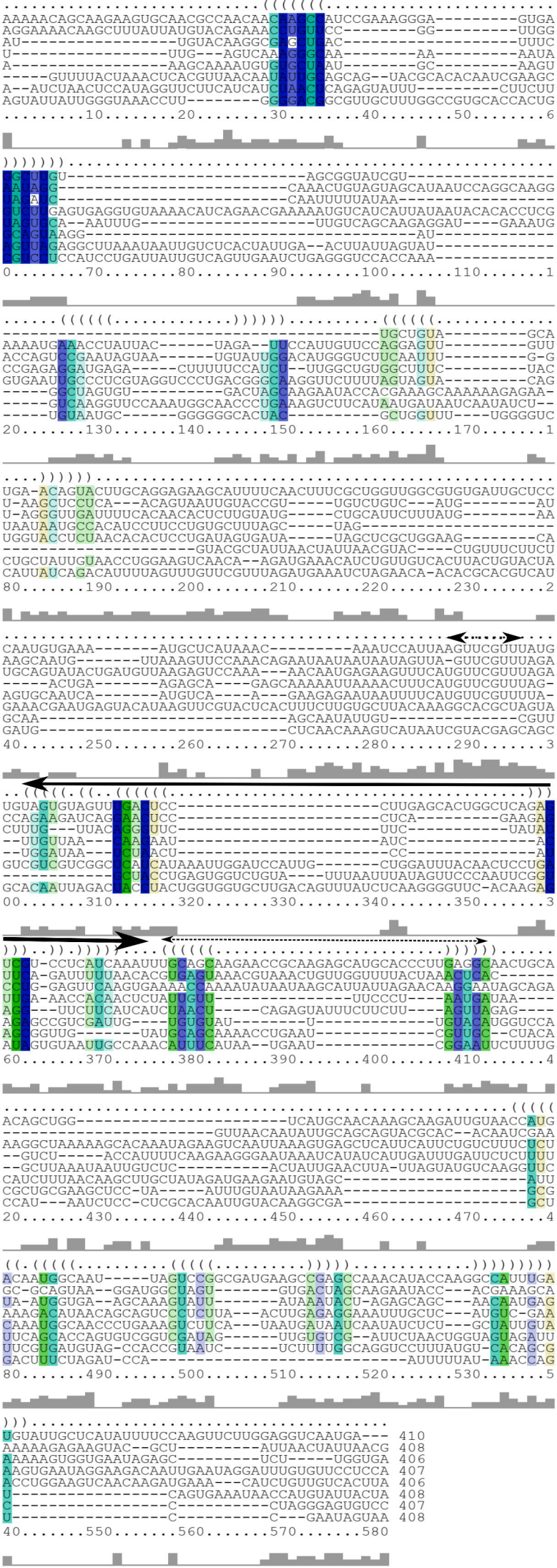
Orf3a
M
Orf8
S
Orf7
E
Orf6
N