World Journal of Advanced Research and Reviews
eISSN: 2581-9615 CODEN (USA): WJARAI
Cross Ref DOI: 10.30574/wjarr
Journal homepage: https://wjarr.com/

(RESEARCH ARTICLE)

Check for updates

An Insight into the Active sites of the RNA Polymerase and Proofreading Exonuclease of the Human Respiratory Syncytial Virus

Peramachi Palanivelu *

Department of Molecular Microbiology, School of Biotechnology, Madurai Kamaraj University, Madurai – 625 021, India.

World Journal of Advanced Research and Reviews, 2023, 18(03), 842–858

Publication history: Received on 06 May 2023; revised on 13 June 2023; accepted on 15 June 2023

Article DOI: https://doi.org/10.30574/wjarr.2023.18.3.1159

Abstract

Human respiratory syncytial virus (hRSV) is one of the triple epidemic viruses that causes infections of the respiratory tract and lungs. For multiplication of the virus in human cells, its RNA polymerase is the crucial enzyme and it forms a part of a large protein (LP). The LP is a multicomponent and multifunctional protein harboring at least 3 different enzymes. The RNA polymerase belongs to RNA-dependent RNA polymerase (RdRp) (EC: 2.7.7.48) type and performs the synthesis of both mRNAs (transcription) and genomic RNA (gRNA) (replication). In addition to the RNA polymerase, the LP also harbours two more enzymes, viz. enzymes for cap addition and cap methylation of mRNAs. The polymerase domain of the LP is analyzed for its active site amino acids and its proofreading (PR) domain. Two polymerase active site regions and a DEDD-superfamily of 3'→5' PR exonuclease active site domain are identified in the polymerase region. The signature metal-binding motifs, viz. -GDNQ- and –SDD- which are commonly found in the RdRps of all the (-) strand RNA viral pathogens are also found in the hRSV RNA polymerase. The two highly conserved polymerase catalytic core regions identified by sequence similarity are in close agreement with other DNA/RNA polymerases already reported and hence, proposed to function in the nucleotidyl transfer reactions. Presence of the two catalytic regions also suggest that the polymerase may function in a dual mode, one for transcription and the other one for replication, using the same invariant catalytic Mg^{2+}-binding –GDNQ- and –SDD- motifs.

Keywords: Human respiratory syncytial virus; RNA polymerase; Metal-binding motifs; Polymerase active sites; Proofreading exonuclease; Proofreading exonuclease active site.

1. Introduction

Respiratory syncytial virus (RSV), also known as human respiratory syncytial virus or human orthopneumovirus, is a highly contagious virus that causes infections of the respiratory tract. It is one of the triple epidemic viruses along with Severe Acute Respiratory Syndrome-Coronaviruses (SARS-CoVs) and human influenza viruses (HInVs). Millions of people every year are hospitalized due to RSV infections and tens of thousands die. It infects almost all children before 2 years of age, resulting in the hospitalization of large number of children worldwide [1]. In babies, aged between around one month to one year, RSV is globally the second-highest cause of death, behind malaria [2]. The hRSV infection affects not only young children, but also older adults and immunocompromised patients causing significant morbidity and mortality. Thus, it is a major burden on pediatric and older adult health-care services around the world. Following initial infection via the eyes or nose, the virus infects the epithelial cells (which are a thin continuous protective layer that line the outer surfaces of organs and blood vessels throughout the body and also the inner surfaces of cavities of many internal organs) of the upper and lower respiratory tracts, causing inflammation, cell damage, and airway obstruction [3]. Of all the children, 0.5% to 2.0% of them are hospitalized with lower respiratory tract infections, 50% to 90% develop bronchiolitis and 5% to 40% develop pneumonia. The incubation period varies from 2 to 8 days, depending upon various factors, like the age of the patient, other underlying lung disease such as bronchopulmonary dysplasia, congenital heart disease, asthma, cardiopulmonary disease, etc. A large systematic review estimated that hRSV caused

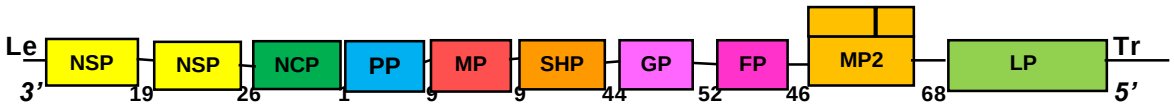
* Corresponding author: Peramachi Palanivelu.

33.1 million episodes of RSV acute lower respiratory tract infections, 3.2 million hospital admissions, and 59,600 in-hospital deaths in 2015, globally. Importantly, 99% of deaths due to hRSV, occur in low- and middle-income countries [4].

It is a major pathogen for which there is no vaccine or clinically effective treatment [5]. Aberrant immune responses to natural infection after immunization with a formalin-inactivated whole-virus RSV vaccine were shown to cause vaccine-enhanced disease in infants [6]. Therefore, multiple RSV vaccines are in development using a variety of traditional as well as modern technologies, including mRNA-based vaccines. Recently, the Food and Drug Administration, USA, has approved GSK's Arexvy, the first RSV vaccine, a recombinant subunit vaccine, for use in the USA for use in people aged 60 years and older [7]. It is designed to the prefusion state of F protein of the RSV. The vaccine was found to be 94% effective at preventing severe disease in senior persons.

As all RNA viruses employ the crucial enzyme, the RdRp, for their multiplication in human cells, and therefore most of the antiviral drug designs are targeted at this enzyme to control the spread of hRSVs. Ribavirin is the only antiviral medication currently licensed for the treatment of RSV in children [8]. (It is a guanosine analog that acts by inhibiting viral RNA synthesis and capping). However, the use of ribavirin remains controversial due to unclear evidence regarding its efficacy and concerns about its toxicity to the exposed staff members. Therefore, there is an urgent need for more effective therapeutic agents for hRSV, including small-molecular antivirals. However, these efforts are hampered by limited structural information on the RdRp catalytic core(s) and its catalytic mechanism. In the present analysis, attempts are made to understand the catalytic core(s) of this enzyme, its PR exonuclease active site and its possible catalytic mechanism, which will pave the way to designing an effective and safe antiviral(s) for hRSVs.

The hRSV is a single-stranded, (-) strand, enveloped RNA virus. Unlike HinVs, it possesses a non-segmented genome and hence, it belongs to the same family of non-segmented (-) strand RNA viral pathogens like Ebola and rabies. It belongs to the Genus: Pneumovirus, Subfamily: Pneumovirinae, Family: Paramyxoviridae of the Order: Mononegavirales. A representative hRSV genome is 15,222 nucleotides (nt) with 44 nts leader (Le) at the 3'- untranslated region (UTR) and 155 nts trailer (Tr) at the 5'-UTR. The RSV genome contains 10 genes encoding 11 proteins (Fig. 1). The M2 gene has two overlapping ORFs, generating M2.1 (a transcription processivity factor) and M2.2 (a protein that governs the switch from transcription to replication). The first two transcribed genes are the nonstructural proteins NSP1 and NSP2, which together inhibit apoptosis and interferon responses [9]. The nuclear capsid protein, NCP is a basic protein that wraps up the RNA genome and protects the genome from degradation and also shields it from recognition by host cell receptors that initiate immune responses. The PP is a tetrameric phosphoprotein that regulates the RNA synthesis through interacting with LP and thus, serves as an essential polymerase cofactor. The hRSV virion's envelope harbours three proteins, viz. a fusion glycoprotein (FP), an attachment glycoprotein (GP) and a small hydrophobic (SHP) protein. The FP and GP are in greater abundance than the SHP. The SHP forms a pentameric ion channel and is suggested to be involved in delaying apoptosis in infected cells. The viral envelope is supported by a layer of matrix protein (MP) and MP2.1 (a zinc-binding transcription anti-terminator), and MP2.2 (a regulatory factor involved in the balancing of transcription and replication). The FP in the outer envelope of the virion is highly conserved among different hRSV strains, making it a potential vaccine target and the present successful vaccine is designed against it. Transcriptional mapping studies have shown that gene transcription of hRSV occurs in a sequential manner in the following order: NSP1, NSP2, NCP, PP, MP, SHP, GP, FP, MP2 (MP2.1, MP2.2) and LP [10] (Fig. 1). The RNA polymerase of LP and the transcription processivity factor MP2.1 and the protein that governs the switch from transcription to replication, MP2.2, are found at the ends of the genomic RNA.



Adapted from [10]

Figure 1 A schematic diagramme showing the arrangement of genes in the hRSV genome

The RSV binds to the chemokine receptor, CX3CR1, present on the cell surface of ciliated epithelial cells, through the attachment GP. Subsequently, the FP mediates the fusion of the viral membrane with the host cell membrane and releases the nucleocapsid into the host cell cytoplasm. In the cytoplasm, the viral genome is transcribed, translated, and replicated, unlike HInVs, where it is replicated in the nucleus. Each of the RSV genes is transcribed, capped and polyadenylated by the RdRp and enzymes of the LP. All the mRNAs are translated in the cytoplasm. The GP and FP are glycosylated in the Golgi of the host cell and added to the incomplete viral particles to make complete, mature viral

particles during the exit from the host cell membrane. The mature viral particles are released from the host cells through the process called budding. PCR techniques detect the hRSV in nasopharyngeal swabs with high sensitivity and specificity [11]. Only the RdRp of the LP is analyzed in detail and reported in this communication.

Table 1 Numbers of nucleotides and amino acids of the proteins of hRSV

Protein/RNA	Nucleotides (nt)	Amino acids
Leader (Le) (3′-UTR)	**44**	-
NSP1	532	139
NSP2	503	124
NCP	1203	391
PP	914	241
MP	958	256
SHP	410	64
GP	923	298
FP	1903	574
M2 (M2.1/M2.2)	961	284 (194/90)
LP	6578	2165
Trailer (Tr) (5′-UTR)	**155**	-

Total length of the virion RNA is 15,222 nt [10]

1.1. Salient Features of the RdRp of the hRSVs

The RdRp of the hRSV is a crucial enzyme and performs both viral genome replication and mRNA transcriptions. It is a ~ 250 kDa protein and is part of the LP. The LP harbours three enzymes, viz. i) for nucleotide polymerization (the RdRp), ii) for cap addition, the capping enzyme polyribonucleotidyl transferase, (the PRNTase), and iii) for cap methylation, the methyl transferase, (the MTase) (Fig. 2). Interestingly, all three related activities for mRNA synthesis are integrated into a single large polypeptide and function as a multifunctional enzyme as found in most DNA polymerase I (e.g., DNA pol I of *E. coli* [12]. Thus, the LP not only synthesizes mRNAs but also possesses enzymes which co-transcriptionally add a cap to each transcript and methylate it. In hRSV, the mRNA caps are synthesized using unconventional reactions: firstly, the cap is formed by the PRNTase, but not by a guanylyltransferase, which is distinct from eukaryotes and all other virus families. Secondly, the cap is methylated at the 2′-O position first, followed by the N-7 position, which is in the opposite order of cap methylations of mammalian mRNAs. The tetrameric phosphoprotein (PP) serves as an essential polymerase cofactor [13] and helps in the catalysis of the three distinct enzymatic activities.

The viral RNA polymerase binds to the genomic RNA at two different sites in the 3'- Le promoter region and initiates either genome replication starting at $\mathbf{U_1}$ or mRNA transcription starting at $\mathbf{C_3}$ of the 3'-end of the viral RNA sequence ($\mathbf{U_1}G_2\mathbf{C_3}$). The replicative RNA initiated at position 1 of the Le and Tr promoters contains 5' pppApC.

In the transcription mode, the RdRP initiates *de novo* RNA synthesis by recognizing a single promoter within the Le region at the 3'-end of the negative-sense genome and sequentially synthesizes mRNAs of the linear array of genes. The RNA polymerase performs sequential transcription of all the mRNAs using a 'termination-reinitiation' mechanism responding to 'gene-start' and 'gene-end' signals. The PRNTase adds the cap structure when the nascent RNA chain length has reached a few nucleotides and the 2'-O methylation of ribose on the mRNA cap precedes and facilitates subsequent Guanine-N-7 methylation and is accomplished by the MTase. The polyadenylation of subgenomic mRNAs is performed by a stuttering mechanism at a slippery, short poly-U-rich region at the gene-ends of each viral gene. The LP needs the PP as the cofactor for processivity and the MP2-1 as the anti-termination protein.

When enough NCPs are made, the RdRp changes to replication mode. The MP2-2 acts as a negative regulator of transcription. During replication, the + complementary strand of the RNA (antigenome) is synthesized first from the Le promoter (found within the first 11 nt of the Le sequence) at its 3'-end. Subsequently, the – strand (viral genome) is synthesized from the antigenome. For viral genome synthesis, the Tr promoter at the 3'-end of the antigenome is used which is identical in sequence to the Le for 11 of the first 13 nt and signals. The replicative RNA initiated at position 1 ($\mathbf{U_1}G_2\mathbf{C_3}$) of the Le and Tr promoters contains 5' pppApC. It should be noted that the RdRp replicates the whole viral genome without recognizing the gene ends and transcriptional signals.

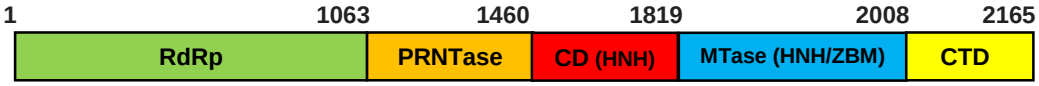


Figure 2 A schematic diagramme showing various domains of the LP of hRSVs

PRNtase, Polyribonucleotidyltransferase; CD, Connecting domain; HNH, HNH-endonuclease domain; MTase, Mononegavirus-type SAM-dependent 2'-O-MTase/7'-N-MTase. ZBM, Zinc-binding motif; CTD, Carboxyl terminal domain.

The core hRSV RNA polymerase consists of LP and PP of molecular masses 250 and 27 kDa, respectively. LP and PP together are sufficient for the polymerase to synthesize RNA and respond appropriately to gene-start and gene-end signals with the RNA template. However, the LP-PP complex requires M2-1 (194 kDA) as a transcription elongation factor to be fully processive [14]. When enough NCPs are made, the RDRp switches to replication mode. However, M2-1 is not required for RNA replication. Thus, the viral components required for RSV's transcription and replication modes are the RNA template, the core polymerase LP-PP-M2-1 and LP-PP-NCP, respectively [15] (Fig. 3).

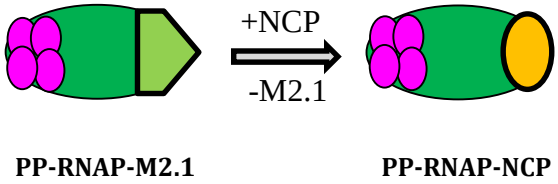


Figure 3 A schematic diagramme showing the organization of heterotrimeric transcription/replication complexes of the hRSV's RNA polymerase (RNAP).

The cryo-EM showed that the RdRp domain adopted a conventional "fingers-palm-thumb" right-hand fold as reported for most of the other RNA and DNA polymerases. The palm subdomain is positively charged and found at the edge of the putative nucleoside triphosphate (NTP) entrance channel. The interactions of the PP with the LP are extensively analyzed by Cryo-EM by Cao et al [16]. They found that the PP is negatively charged and the interactions between LP and PP were dictated partly by electrostatic complementarity.

2. Materials and methods

Protein sequence data of the LP from various hRSV strains were obtained from PUBMED and SWISS-PROT databases. The advanced version of Clustal Omega was used for protein sequence analysis. The highly conserved motifs identified by the bioinformatics analysis, and along with the data already available from biochemical, SDM, and cryo-EM analyses on the LP, the possible amino acids that make-up the RNA polymerase and PR exonuclease active sites are proposed.

3. Results and discussion

3.1. MSA Analysis of the Polymerase of the RSVs

hRSVs are broadly classified into two distinct antigenic groups, viz. A and B, and each contains several distinct subgroups based on the antigenic and genomic sequence differences, especially of the G and F glycoproteins which also show epidemiological differences. Although the subtypes cocirculate, one subtype generally predominates the other depending on the region and climate [17]. Fig. 4 shows the MSA of the LP from different antigenic groups of the hRSVs, (only the regions required for discussions are shown here). In the present study, LP sequences from both the groups are mixed and analyzed by MSA. hRSVs A and B sequences are highlighted in yellow and light blue, respectively. Interestingly, the LPs of all viral strains from both the groups are highly conserved from N- to C-terminals, but only with minor variations. Important motifs identified by MSA analysis are: Motif I, -^{134}**K**E**K**D**KI**K-, which is similar to the finger priming loop (FPL), –**K**L**K**R**R**A- of BP1 subunit of the of human influenza viral RNA polymerase [18]; Motif II, a highly conserved K rich motif, -^{403}**K**xx**K**xx**K**xx- (x represents any amino acid); which is immediately followed by the Motif III, a –^{412}**GDN**N- motif; Motif IV, a–^{810}**GDN**Q- motif (shown to involve in the catalytic Mg^{2+}-binding) and Motif V, a –^{1032}**SDD**-, the commonly found metal-binding motif which is reported from the RdRps of other (-) strand RNA viruses [18]. The RdRp from the RSVs harbours two additional glycine residues that form a –^{775}**G**Gxx**G**- motif (Motif VI), which is also found in the RdRp domains of LPs from hMPV, Ebola, VSV, and other non-segmented negative-sense RNA viruses [19].

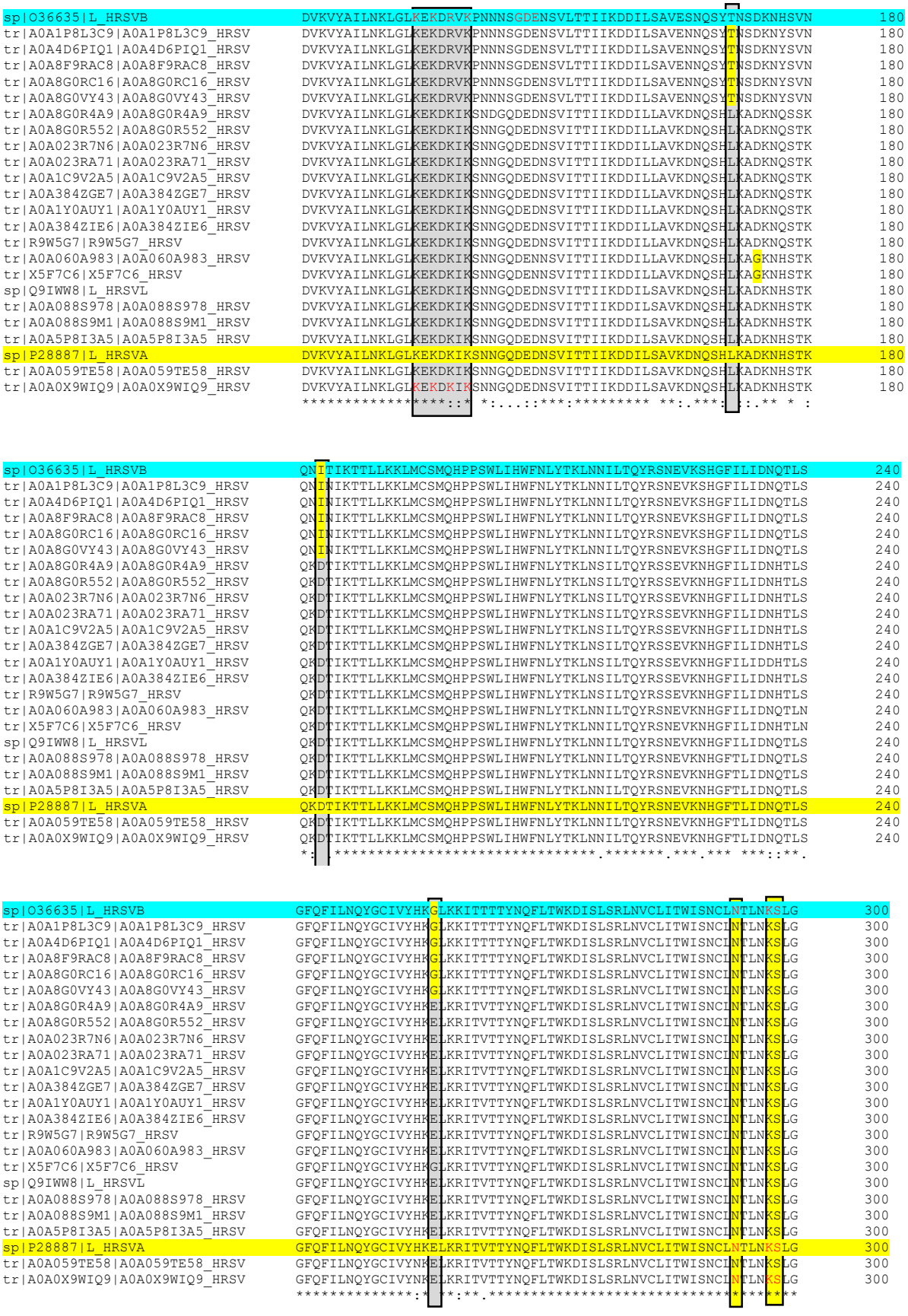
Therefore, it is suggested that the palm subdomain is placed between two glycine residues on either side which likely confer the necessary flexibility of this region to accommodate the template RNA and incoming NTPs in the catalytic site [20]. In addition to the above conserved motifs, two highly conserved polymerase catalytic core regions (highlighted in yellow) and a complete and an incomplete PR exonuclease active site domains are also found (highlighted in light blue).

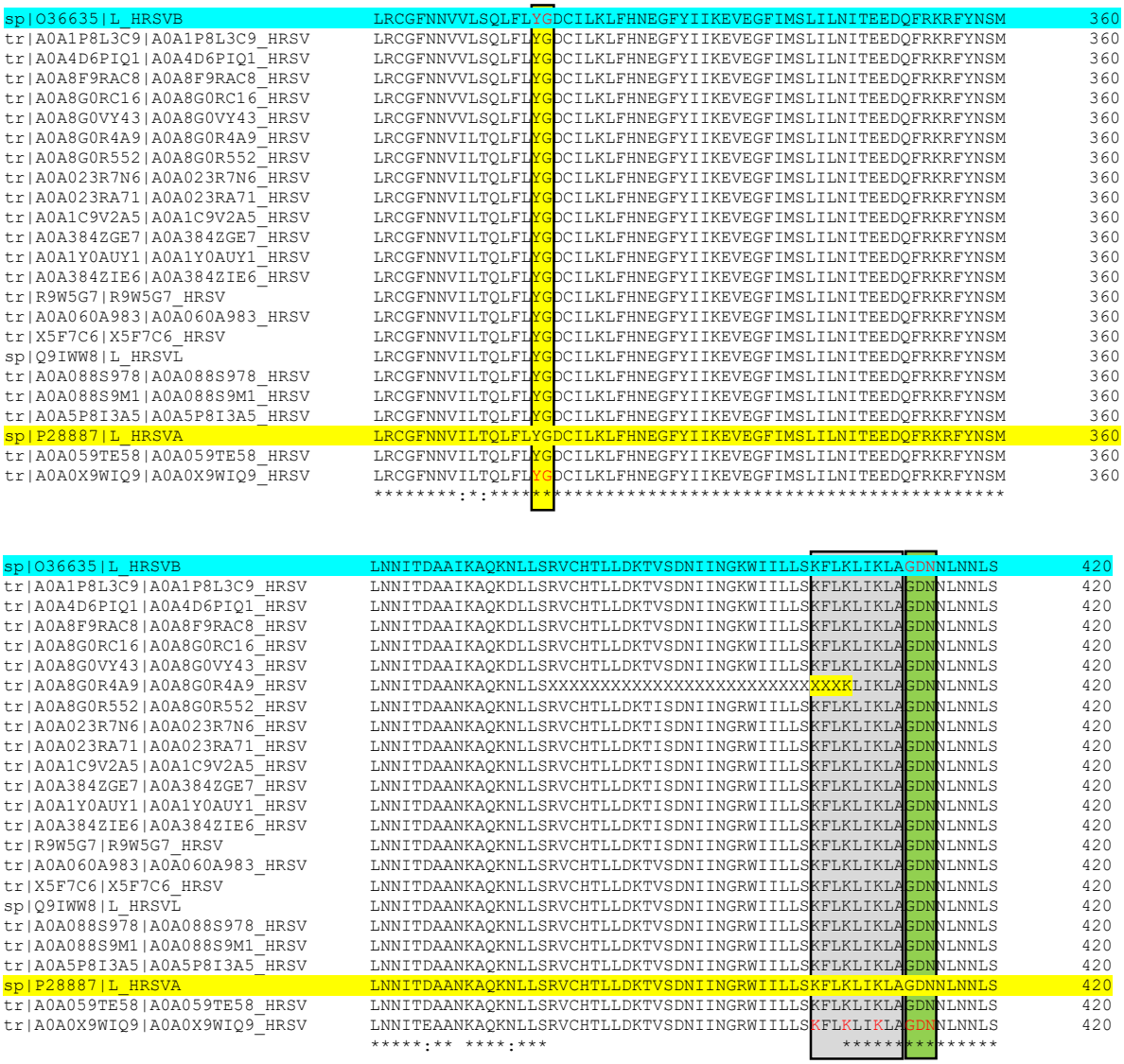
It is interesting to note that all the RSV B group viral strains show distinct variations from A group strains by replacing T to N at 65; T o L at 169; I to D at 183; G to E at 257; N to R at 511; S to P at 610; H to L at 741; S to N at 970; G to N at 1021; L to T at 1088 and T to L at 1159 (highlighted in light grey) in the RdRp domain (Fig. 3).

CLUSTAL O (1.2.4) MSA of RNA polymerases of the hRSVs

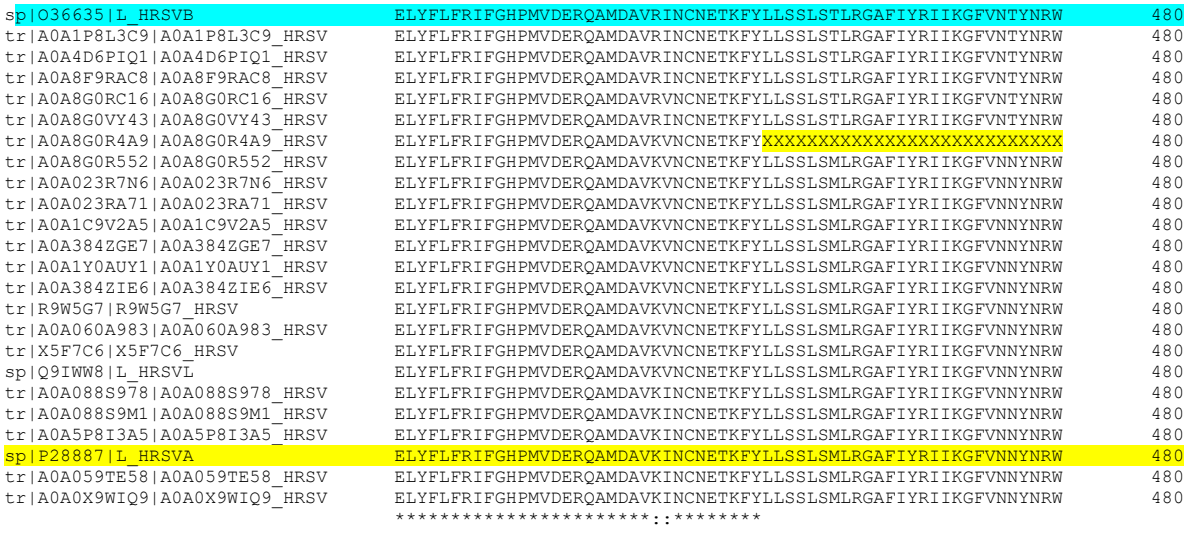
```
sp|O36635|L_HRSVB                MDPIINGNSANVYLTDSYLKGVISFSECNALGSYLFNGPYLKNDYTNLISRQSPLLEHMN   60
tr|A0A1P8L3C9|A0A1P8L3C9_HRSV    MDPIINGSSANVYLTDSYLKGVISFSECNALGSYLFNGPYLKNDYTNLISRQSPLLEHMN   60
tr|A0A4D6PIQ1|A0A4D6PIQ1_HRSV    MDPIINGSSANVYLTDSYLKGVISFSECNALGSYLFNGPYLKNDYTNLISRQSPLLEHMN   60
tr|A0A8F9RAC8|A0A8F9RAC8_HRSV    MDPIINGSSANVYLTDSYLKGVISFSECNALGSYLFNGPYLKNDYTNLISRQSPLIEHMN   60
tr|A0A8G0RC16|A0A8G0RC16_HRSV    MDPIINGSSANVYLTDSYLKGVISFSECNALGSYLFNGPYLKNDYTNLISRQSPLIEHMN   60
tr|A0A8G0VY43|A0A8G0VY43_HRSV    MDPIINGSSANVYLTDSYLKGVISFSECNALGSYLFNGPYLKNDYTNLISRQSPLIEHMN   60
tr|A0A8G0R4A9|A0A8G0R4A9_HRSV    MDPIISGNSANVYLTDSYLKGVISFSECNALGSYIFNGPYLKNDYTNLISRQNPLIEHIN   60
tr|A0A8G0R552|A0A8G0R552_HRSV    MDPIISGNSANVYLTDSYLKGVISFSECNALGSYIFNGPYLKNDYTNLISRQNPLIEHIN   60
tr|A0A023R7N6|A0A023R7N6_HRSV    MDPIISGNSANVYLTDSYLKGVISFSECNALGSYIFNGPYLKNDYTNLISRQNPLIEHIN   60
tr|A0A023RA71|A0A023RA71_HRSV    MDPIISGNSANVYLTDSYLKGVISFSECNALGSYIFNGPYLKNDYTNLISRQNPLIEHIN   60
tr|A0A1C9V2A5|A0A1C9V2A5_HRSV    MDPVISGNSANVYLTDSYLKGVISFSECNALGSYIFNGPYLKNDYTNLISRQNPLIEHIN   60
tr|A0A384ZGE7|A0A384ZGE7_HRSV    MDPIISGNTANVYLTDSYLKGVISFSECNALGSYIFNGPYLKNDYTNLISRQNPLIEHIN   60
tr|A0A1Y0AUY1|A0A1Y0AUY1_HRSV    MDPIISGNSANVYLTDSYLKGVISFSECNALGSYIFNGPYLKNDYTNLISRQNPLIEHIN   60
tr|A0A384ZIE6|A0A384ZIE6_HRSV    MDPIISGNSANVYLTDSYLKGVISFSECNALGSYIFNGPYLKNDYTNLISRQNPLIEHIN   60
tr|R9W5G7|R9W5G7_HRSV            MDPIISGNSANVYLTDSYLKGVISFSECNALGSYIFNGPYLKNDYTNLISRQNPLIEHIN   60
tr|A0A060A983|A0A060A983_HRSV    MDPIINGNSANVYLTDSYLKGVISFSECNALGSYIFNGPYLKNDYTNLISRQNPLIEHIN   60
tr|X5F7C6|X5F7C6_HRSV            MDPIINGNSANVYLTDSYLKGVISFSECNALGSYIFNSPYLKNDYTNLISRQNPLIEHIN   60
sp|Q9IWW8|L_HRSVL                MDPIINGNSANVYLTDSYLKGVISFSECNALGSYIFNGPYLKNDYTNLISRQNPLIEHMN   60
tr|A0A088S978|A0A088S978_HRSV    MDPIINGNSANVYLTDSYLKGVISFSECNALGSYIFNGPYLKNDYTNLISRQNPLIEHMN   60
tr|A0A088S9M1|A0A088S9M1_HRSV    MDPIINGNSANVYLTDSYLKGVISFSECNALGSYIFNGPYLKNDYTNLISRQNPLIEHMN   60
tr|A0A5P8I3A5|A0A5P8I3A5_HRSV    MDPIINGNSANVYLTDSYLKGVISFSECNALGSYIFNGPYLKNDYTNLISRQNPLIEHMN   60
sp|P28887|L_HRSVA                MDPIINGNSANVYLTDSYLKGVISFSECNALGSYIFNGPYLKNDYTNLISRQNPLIEHMN   60
tr|A0A059TE58|A0A059TE58_HRSV    MDPIINGNSANVYLTDSYLKGVISFSECNALGSYIFNGPYLKNDYTNLISRQNPLIEHIN   60
tr|A0A0X9WIQ9|A0A0X9WIQ9_HRSV    MDPIINGNSANVYLTDSYLKGVISFSECNALGSYIFNGPYLKNDYTNLISRQNPLIEHIN   60
                                 ***:*.*.:*************************:**.**************.**:**:*

sp|O36635|L_HRSVB                LKKLTTTQSLISRYHKGELKLEEPTYFQSLLMTYKSMSSSEQIATTNLLKKIIRRAIEIS   120
tr|A0A1P8L3C9|A0A1P8L3C9_HRSV    LKKLTTTQSLISRYHKGELKLEEPTYFQSLLMTYKSMSSSEQIATTNLLKKIIRRAIEIS   120
tr|A0A4D6PIQ1|A0A4D6PIQ1_HRSV    LKKLTTTQSLISRYHKGELKLEEPTYFQSLLMTYKSMSSSETIATTNLLKKIIRRAIEIS   120
tr|A0A8F9RAC8|A0A8F9RAC8_HRSV    LKKLTTTQSLISRYHKGELKLEEPTYFQSLLMTYKSMSSSEQIATTNLLKKIIRRAIEIS   120
tr|A0A8G0RC16|A0A8G0RC16_HRSV    LKKLTTTQSLISRYHKGELKLEEPTYFQSLLMTYKSMSSSEQIATTNLLKKIIRRAIEIS   120
tr|A0A8G0VY43|A0A8G0VY43_HRSV    LKKLTTTQSLISRYHKGELKLEEPTYFQSLLMTYKSMSSSEQIATTNLLKKIIRRAIEIS   120
tr|A0A8G0R4A9|A0A8G0R4A9_HRSV    LKKLNITQSLISKCHKGEIKIEEPTYFQSLLMTYKSMTSSEQTTTTNLLKKIIRRAIEIS   120
tr|A0A8G0R552|A0A8G0R552_HRSV    LKKLNITQSLISKYHKGEIKIEEPTYFQSLLMTYKSMTSSEQTTTTNLLKKIIRRAIEIS   120
tr|A0A023R7N6|A0A023R7N6_HRSV    LKKLNITQSLISKYHKGEIKIEEPTYFQSLLMTYKSMTSSEQTTTTNLLKKIIRRAIEIS   120
tr|A0A023RA71|A0A023RA71_HRSV    LKKLNITQSLISKYHKGEIKIEEPTYFQSLLMTYKSMTSSEQTTTTNLLKKIIRRAIEIS   120
tr|A0A1C9V2A5|A0A1C9V2A5_HRSV    LKKLNITQSLISKYHKGEIKIEEPTYFQSLLMTYKSMTSSEQTTTTNLLKKIIRRAIEIS   120
tr|A0A384ZGE7|A0A384ZGE7_HRSV    LKKLNITQSLISKYHKGEIKIEEPTYFQSLLMTYKSMTSSEQTTTTNLLKKIIRRAIEIS   120
tr|A0A1Y0AUY1|A0A1Y0AUY1_HRSV    LKKLNITQSLISKYHKGEIKIEEPTYFQSLLMTYKSMTSSEQTTTTNLLKKIIRRAIEIS   120
tr|A0A384ZIE6|A0A384ZIE6_HRSV    LKKLNITQSLISKYHKGEIKIEEPTYFQSLLMTYKSMTSSEQTTTTNLLKKIIRRAIEIS   120
tr|R9W5G7|R9W5G7_HRSV            LKKLNITQSLISKYHKGEIKIEEPTYFQSLLMTYKSMTSSEQTTTTNLLKKIIRRAIEIS   120
tr|A0A060A983|A0A060A983_HRSV    LKKLNITQSLISKYHKGEIKIEEPTYFQSLLMTYKSMTSSEQITTTNLLKKIIRRAIEIS   120
tr|X5F7C6|X5F7C6_HRSV            LKKLNITQSLISKYHKGEIKIEEPTYFQSLLMTYKSMTSSEQITTTNLLKKIIRRAIEIS   120
sp|Q9IWW8|L_HRSVL                LKKLNITQSLISKYHKGEIKLEEPTYFQSLLMTYKSMTSLEQIATTNLLKKIIRRAIEIS   120
tr|A0A088S978|A0A088S978_HRSV    LKKLNITQSLISKYHKGEIKLEEPTYFQSLLMTYKSMTSSEQIATTNLLKKIIRRAIEIS   120
tr|A0A088S9M1|A0A088S9M1_HRSV    LKKLNITQSLISKYHKGEIKLEEPTYFQSLLMTYKSMTSSEQIATTNLLKKIIRRAIEIS   120
tr|A0A5P8I3A5|A0A5P8I3A5_HRSV    LKKLNITQSLISKYHKGEIKLEEPTYFQSLLMTYKSMTSSEQIATTNLLKKIIRRAIEIS   120
sp|P28887|L_HRSVA                LKKLNITQSLISKYHKGEIKLEEPTYFQSLLMTYKSMTSSEQIATTNLLKKIIRRAIEIS   120
tr|A0A059TE58|A0A059TE58_HRSV    LKKLNVTQSLISKYHKGEIKLEEPTYFQSLLMTYKSMTSSEQIATTNLLKKIIRRAIEIS   120
tr|A0A0X9WIQ9|A0A0X9WIQ9_HRSV    LKKLNITQSLISKYHKGEIKLEEPTYFQSLLMTYKSMTSSELIATTNLLKKIIRRAIEIS   120
                                 *****.******: ****:*:*****************:* *  :****************
```


sp|O36635|L_HRSVB DVKVYAILNKLGLKEKDRVKPNNNSGDENSVLTTIIKDDILSAVESNQSYTNSDKNHSVN 180
tr|A0A1P8L3C9|A0A1P8L3C9_HRSV DVKVYAILNKLGLKEKDRVKPNNNSGDENSVLTTIIKDDILSAVENNQSYTNSDKNYSVN 180
tr|A0A4D6PIQ1|A0A4D6PIQ1_HRSV DVKVYAILNKLGLKEKDRVKPNNNSGDENSVLTTIIKDDILSAVENNQSYTNSDKNYSVN 180
tr|A0A8F9RAC8|A0A8F9RAC8_HRSV DVKVYAILNKLGLKEKDRVKPNNNSGDENSVLTTIIKDDILSAVENNQSYTNSDKNYSVN 180
tr|A0A8G0RC16|A0A8G0RC16_HRSV DVKVYAILNKLGLKEKDRVKPNNNSGDENSVLTTIIKDDILSAVENNQSYTNSDKNYSVN 180
tr|A0A8G0VY43|A0A8G0VY43_HRSV DVKVYAILNKLGLKEKDRVKPNNNSGDENSVLTTIIKDDILSAVENNQSYTNSDKNYSVN 180
tr|A0A8G0R4A9|A0A8G0R4A9_HRSV DVKVYAILNKLGLKEKDKIKSNDGQDEDNSVITTIIKDDILLAVKDNQSHLKADKNQSSK 180
tr|A0A8G0R552|A0A8G0R552_HRSV DVKVYAILNKLGLKEKDKIKSNNGQDEDNSVITTIIKDDILLAVKDNQSHLKADKNQSTK 180
tr|A0A023R7N6|A0A023R7N6_HRSV DVKVYAILNKLGLKEKDKIKSNNGQDEDNSVITTIIKDDILLAVKDNQSHLKADKNQSTK 180
tr|A0A023RA71|A0A023RA71_HRSV DVKVYAILNKLGLKEKDKIKSNNGQDEDNSVITTIIKDDILLAVKDNQSHLKADKNQSTK 180
tr|A0A1C9V2A5|A0A1C9V2A5_HRSV DVKVYAILNKLGLKEKDKIKSNNGQDEDNSVITTIIKDDILLAVKDNQSHLKADKNQSTK 180
tr|A0A384ZGE7|A0A384ZGE7_HRSV DVKVYAILNKLGLKEKDKIKSNNGQDEDNSVITTIIKDDILLAVKDNQSHLKADKNQSTK 180
tr|A0A1Y0AUY1|A0A1Y0AUY1_HRSV DVKVYAILNKLGLKEKDKIKSNNGQDEDNSVITTIIKDDILLAVKDNQSHLKADKNQSTK 180
tr|A0A384ZIE6|A0A384ZIE6_HRSV DVKVYAILNKLGLKEKDKIKSNNGQDEDNSVITTIIKDDILLAVKDNQSHLKADKNQSTK 180
tr|R9W5G7|R9W5G7_HRSV DVKVYAILNKLGLKEKDKIKSNNGQDEDNSVITTIIKDDILLAVKDNQSHLKADKNQSTK 180
tr|A0A060A983|A0A060A983_HRSV DVKVYAILNKLGLKEKDKIKSNNGQDENNSVITTIIKDDILLAVKDNQSHLKAGKNHSTK 180
tr|X5F7C6|X5F7C6_HRSV DVKVYAILNKLGLKEKDKIKSNNGQDENNSVITTIIKDDILLAVKDNQSHLKAGKNHSTK 180
sp|Q9IWW8|L_HRSVL DVKVYAILNKLGLKEKDKIKSNNGQDEDNSVITTIIKDDILSAVKDNQSHLKADKNHSTK 180
tr|A0A088S978|A0A088S978_HRSV DVKVYAILNKLGLKEKDKIKSNNGQDEDNSVITTIIKDDILSAVKDNQSHLKADKNHSTK 180
tr|A0A088S9M1|A0A088S9M1_HRSV DVKVYAILNKLGLKEKDKIKSNNGQDEDNSVITTIIKDDILSAVKDNQSHLKADKNHSTK 180
tr|A0A5P8I3A5|A0A5P8I3A5_HRSV DVKVYAILNKLGLKEKDKIKSNNGQDEDNSVITTIIKDDILSAVKDNQSHLKADKNHSTK 180
sp|P28887|L_HRSVA DVKVYAILNKLGLKEKDKIKSNNGQDEDNSVITTIIKDDILSAVKDNQSHLKADKNHSTK 180
tr|A0A059TE58|A0A059TE58_HRSV DVKVYAILNKLGLKEKDKIKSNNGQDEDNSVITTIIKDDILSAVKDNQSHLKADKNHSTK 180
tr|A0A0X9WIQ9|A0A0X9WIQ9_HRSV DVKVYAILNKLGLKEKDKIKSNNGQDEDNSVITTIIKDDILSAVKDNQSHLKADKNHSTK 180
sp|O36635|L_HRSVB QNITIKTTLLKKLMCSMQHPPSWLIHWFNLYTKLNNILTQYRSNEVKSHGFILIDNQTLS 240
tr|A0A1P8L3C9|A0A1P8L3C9_HRSV QNINIKTTLLKKLMCSMQHPPSWLIHWFNLYTKLNNILTQYRSNEVKSHGFILIDNQTLS 240
tr|A0A4D6PIQ1|A0A4D6PIQ1_HRSV QNINIKTTLLKKLMCSMQHPPSWLIHWFNLYTKLNNILTQYRSNEVKSHGFILIDNQTLS 240
tr|A0A8F9RAC8|A0A8F9RAC8_HRSV QNINIKTTLLKKLMCSMQHPPSWLIHWFNLYTKLNNILTQYRSNEVKSHGFILIDNQTLS 240
tr|A0A8G0RC16|A0A8G0RC16_HRSV QNINIKTTLLKKLMCSMQHPPSWLIHWFNLYTKLNNILTQYRSNEVKSHGFILIDNQTLS 240
tr|A0A8G0VY43|A0A8G0VY43_HRSV QNINIKTTLLKKLMCSMQHPPSWLIHWFNLYTKLNNILTQYRSNEVKSHGFILIDNQTLS 240
tr|A0A8G0R4A9|A0A8G0R4A9_HRSV QKDTIKTTLLKKLMCSMQHPPSWLIHWFNLYTKLNSILTQYRSSEVKNHGFILIDNHTLS 240
tr|A0A8G0R552|A0A8G0R552_HRSV QKDTIKTTLLKKLMCSMQHPPSWLIHWFNLYTKLNSILTQYRSSEVKNHGFILIDNHTLS 240
tr|A0A023R7N6|A0A023R7N6_HRSV QKDTIKTTLLKKLMCSMQHPPSWLIHWFNLYTKLNSILTQYRSSEVKNHGFILIDNHTLS 240
tr|A0A023RA71|A0A023RA71_HRSV QKDTIKTTLLKKLMCSMQHPPSWLIHWFNLYTKLNSILTQYRSSEVKNHGFILIDNHTLS 240
tr|A0A1C9V2A5|A0A1C9V2A5_HRSV QKDTIKTTLLKKLMCSMQHPPSWLIHWFNLYTKLNSILTQYRSSEVKNHGFILIDNHTLS 240
tr|A0A384ZGE7|A0A384ZGE7_HRSV QKDTIKTTLLKKLMCSMQHPPSWLIHWFNLYTKLNSILTQYRSSEVKNHGFILIDNHTLS 240
tr|A0A1Y0AUY1|A0A1Y0AUY1_HRSV QKDTIKTTLLKKLMCSMQHPPSWLIHWFNLYTKLNSILTQYRSSEVKNHGFILIDDHTLS 240
tr|A0A384ZIE6|A0A384ZIE6_HRSV QKDTIKTTLLKKLMCSMQHPPSWLIHWFNLYTKLNSILTQYRSSEVKNHGFILIDNHTLS 240
tr|R9W5G7|R9W5G7_HRSV QKDTIKTTLLKKLMCSMQHPPSWLIHWFNLYTKLNSILTQYRSSEVKNHGFILIDNHTLS 240
tr|A0A060A983|A0A060A983_HRSV QKDTIKTTLLKKLMCSMQHPPSWLIHWFNLYTKLNNILTQYRSNEVKNHGFILIDNQTLN 240
tr|X5F7C6|X5F7C6_HRSV QKDTIKTTLLKKLMCSMQHPPSWLIHWFNLYTKLNNILTQYRSNEVKNHGFILIDNHTLN 240
sp|Q9IWW8|L_HRSVL QKDTIKTTLLKKLMCSMQHPPSWLIHWFNLYTKLNNILTQYRSNEVKNHGFILIDNQTLS 240
tr|A0A088S978|A0A088S978_HRSV QKDTIKTTLLKKLMCSMQHPPSWLIHWFNLYTKLNNILTQYRSNEVKNHGFTLIDNQTLS 240
tr|A0A088S9M1|A0A088S9M1_HRSV QKDTIKTTLLKKLMCSMQHPPSWLIHWFNLYTKLNNILTQYRSNEVKNHGFTLIDNQTLS 240
tr|A0A5P8I3A5|A0A5P8I3A5_HRSV QKDTIKTTLLKKLMCSMQHPPSWLIHWFNLYTKLNNILTQYRSNEVKNHGFTLIDNQTLS 240
sp|P28887|L_HRSVA QKDTIKTTLLKKLMCSMQHPPSWLIHWFNLYTKLNNILTQYRSNEVKNHGFTLIDNQTLS 240
tr|A0A059TE58|A0A059TE58_HRSV QKDTIKTTLLKKLMCSMQHPPSWLIHWFNLYTKLNNILTQYRSNEVKNHGFTLIDNQTLS 240
tr|A0A0X9WIQ9|A0A0X9WIQ9_HRSV QKDTIKTTLLKKLMCSMQHPPSWLIHWFNLYTKLNNILTQYRSNEVKNHGFTLIDNQTLS 240
sp|O36635|L_HRSVB GFQFILNQYGCIVYHKGLKKITTTTYNQFLTWKDISLSRLNVCLITWISNCLNTLNKSLG 300
tr|A0A1P8L3C9|A0A1P8L3C9_HRSV GFQFILNQYGCIVYHKGLKKITTTTYNQFLTWKDISLSRLNVCLITWISNCLNTLNKSLG 300
tr|A0A4D6PIQ1|A0A4D6PIQ1_HRSV GFQFILNQYGCIVYHKGLKKITTTTYNQFLTWKDISLSRLNVCLITWISNCLNTLNKSLG 300
tr|A0A8F9RAC8|A0A8F9RAC8_HRSV GFQFILNQYGCIVYHKGLKKITTTTYNQFLTWKDISLSRLNVCLITWISNCLNTLNKSLG 300
tr|A0A8G0RC16|A0A8G0RC16_HRSV GFQFILNQYGCIVYHKGLKKITTTTYNQFLTWKDISLSRLNVCLITWISNCLNTLNKSLG 300
tr|A0A8G0VY43|A0A8G0VY43_HRSV GFQFILNQYGCIVYHKGLKKITTTTYNQFLTWKDISLSRLNVCLITWISNCLNTLNKSLG 300
tr|A0A8G0R4A9|A0A8G0R4A9_HRSV GFQFILNQYGCIVYHKELKRITVTTYNQFLTWKDISLSRLNVCLITWISNCLNTLNKSLG 300
tr|A0A8G0R552|A0A8G0R552_HRSV GFQFILNQYGCIVYHKELKRITVTTYNQFLTWKDISLSRLNVCLITWISNCLNTLNKSLG 300
tr|A0A023R7N6|A0A023R7N6_HRSV GFQFILNQYGCIVYHKELKRITVTTYNQFLTWKDISLSRLNVCLITWISNCLNTLNKSLG 300
tr|A0A023RA71|A0A023RA71_HRSV GFQFILNQYGCIVYHKELKRITVTTYNQFLTWKDISLSRLNVCLITWISNCLNTLNKSLG 300
tr|A0A1C9V2A5|A0A1C9V2A5_HRSV GFQFILNQYGCIVYHKELKRITVTTYNQFLTWKDISLSRLNVCLITWISNCLNTLNKSLG 300
tr|A0A384ZGE7|A0A384ZGE7_HRSV GFQFILNQYGCIVYHKELKRITVTTYNQFLTWKDISLSRLNVCLITWISNCLNTLNKSLG 300
tr|A0A1Y0AUY1|A0A1Y0AUY1_HRSV GFQFILNQYGCIVYHKELKRITVTTYNQFLTWKDISLSRLNVCLITWISNCLNTLNKSLG 300
tr|A0A384ZIE6|A0A384ZIE6_HRSV GFQFILNQYGCIVYHKELKRITVTTYNQFLTWKDISLSRLNVCLITWISNCLNTLNKSLG 300
tr|R9W5G7|R9W5G7_HRSV GFQFILNQYGCIVYHKELKRITVTTYNQFLTWKDISLSRLNVCLITWISNCLNTLNKSLG 300
tr|A0A060A983|A0A060A983_HRSV GFQFILNQYGCIVYHKELKRITVTTYNQFLTWKDISLSRLNVCLITWISNCLNTLNKSLG 300
tr|X5F7C6|X5F7C6_HRSV GFQFILNQYGCIVYHKGLKRITVTTYNQFLTWKDISLSRLNVCLITWISNCLNTLNKSLG 300
sp|Q9IWW8|L_HRSVL GFQFILNQYGCIVYHKELKRITVTTYNQFLTWKDISLSRLNVCLITWISNCLNTLNKSLG 300
tr|A0A088S978|A0A088S978_HRSV GFQFILNQYGCIVYHKELKRITVTTYNQFLTWKDISLSRLNVCLITWISNCLNTLNKSLG 300
tr|A0A088S9M1|A0A088S9M1_HRSV GFQFILNQYGCIVYHKELKRITVTTYNQFLTWKDISLSRLNVCLITWISNCLNTLNKSLG 300
tr|A0A5P8I3A5|A0A5P8I3A5_HRSV GFQFILNQYGCIVYHKELKRITVTTYNQFLTWKDISLSRLNVCLITWISNCLNTLNKSLG 300
sp|P28887|L_HRSVA GFQFILNQYGCIVYHKELKRITVTTYNQFLTWKDISLSRLNVCLITWISNCLNTLNKSLG 300
tr|A0A059TE58|A0A059TE58_HRSV GFQFILNQYGCIVYNKELKRITVTTYNQFLTWKDISLSRLNVCLITWISNCLNTLNKSLG 300
tr|A0A0X9WIQ9|A0A0X9WIQ9_HRSV GFQFILNQYGCIVYNKELKRITVTTYNQFLTWKDISLSRLNVCLITWISNCLNTLNKSLG 300


sp|O36635|L_HRSVB LRCGFNNVVLSQLFLYGDCILKLFHNEGFYIIKEVEGFIMSLILNITEEDQFRKRFYNSM 360
tr|A0A1P8L3C9|A0A1P8L3C9_HRSV LRCGFNNVVLSQLFLYGDCILKLFHNEGFYIIKEVEGFIMSLILNITEEDQFRKRFYNSM 360
tr|A0A4D6PIQ1|A0A4D6PIQ1_HRSV LRCGFNNVVLSQLFLYGDCILKLFHNEGFYIIKEVEGFIMSLILNITEEDQFRKRFYNSM 360
tr|A0A8F9RAC8|A0A8F9RAC8_HRSV LRCGFNNVVLSQLFLYGDCILKLFHNEGFYIIKEVEGFIMSLILNITEEDQFRKRFYNSM 360
tr|A0A8G0RC16|A0A8G0RC16_HRSV LRCGFNNVVLSQLFLYGDCILKLFHNEGFYIIKEVEGFIMSLILNITEEDQFRKRFYNSM 360
tr|A0A8G0VY43|A0A8G0VY43_HRSV LRCGFNNVVLSQLFLYGDCILKLFHNEGFYIIKEVEGFIMSLILNITEEDQFRKRFYNSM 360
tr|A0A8G0R4A9|A0A8G0R4A9_HRSV LRCGFNNVILTQLFLYGDCILKLFHNEGFYIIKEVEGFIMSLILNITEEDQFRKRFYNSM 360
tr|A0A8G0R552|A0A8G0R552_HRSV LRCGFNNVILTQLFLYGDCILKLFHNEGFYIIKEVEGFIMSLILNITEEDQFRKRFYNSM 360
tr|A0A023R7N6|A0A023R7N6_HRSV LRCGFNNVILTQLFLYGDCILKLFHNEGFYIIKEVEGFIMSLILNITEEDQFRKRFYNSM 360
tr|A0A023RA71|A0A023RA71_HRSV LRCGFNNVILTQLFLYGDCILKLFHNEGFYIIKEVEGFIMSLILNITEEDQFRKRFYNSM 360
tr|A0A1C9V2A5|A0A1C9V2A5_HRSV LRCGFNNVILTQLFLYGDCILKLFHNEGFYIIKEVEGFIMSLILNITEEDQFRKRFYNSM 360
tr|A0A384ZGE7|A0A384ZGE7_HRSV LRCGFNNVILTQLFLYGDCILKLFHNEGFYIIKEVEGFIMSLILNITEEDQFRKRFYNSM 360
tr|A0A1Y0AUY1|A0A1Y0AUY1_HRSV LRCGFNNVILTQLFLYGDCILKLFHNEGFYIIKEVEGFIMSLILNITEEDQFRKRFYNSM 360
tr|A0A384ZIE6|A0A384ZIE6_HRSV LRCGFNNVILTQLFLYGDCILKLFHNEGFYIIKEVEGFIMSLILNITEEDQFRKRFYNSM 360
tr|R9W5G7|R9W5G7_HRSV LRCGFNNVILTQLFLYGDCILKLFHNEGFYIIKEVEGFIMSLILNITEEDQFRKRFYNSM 360
tr|A0A060A983|A0A060A983_HRSV LRCGFNNVILTQLFLYGDCILKLFHNEGFYIIKEVEGFIMSLILNITEEDQFRKRFYNSM 360
tr|X5F7C6|X5F7C6_HRSV LRCGFNNVILTQLFLYGDCILKLFHNEGFYIIKEVEGFIMSLILNITEEDQFRKRFYNSM 360
sp|Q9IWW8|L_HRSVL LRCGFNNVILTQLFLYGDCILKLFHNEGFYIIKEVEGFIMSLILNITEEDQFRKRFYNSM 360
tr|A0A088S978|A0A088S978_HRSV LRCGFNNVILTQLFLYGDCILKLFHNEGFYIIKEVEGFIMSLILNITEEDQFRKRFYNSM 360
tr|A0A088S9M1|A0A088S9M1_HRSV LRCGFNNVILTQLFLYGDCILKLFHNEGFYIIKEVEGFIMSLILNITEEDQFRKRFYNSM 360
tr|A0A5P8I3A5|A0A5P8I3A5_HRSV LRCGFNNVILTQLFLYGDCILKLFHNEGFYIIKEVEGFIMSLILNITEEDQFRKRFYNSM 360
sp|P28887|L_HRSVA LRCGFNNVILTQLFLYGDCILKLFHNEGFYIIKEVEGFIMSLILNITEEDQFRKRFYNSM 360
tr|A0A059TE58|A0A059TE58_HRSV LRCGFNNVILTQLFLYGDCILKLFHNEGFYIIKEVEGFIMSLILNITEEDQFRKRFYNSM 360
tr|A0A0X9WIQ9|A0A0X9WIQ9_HRSV LRCGFNNVILTQLFLYGDCILKLFHNEGFYIIKEVEGFIMSLILNITEEDQFRKRFYNSM 360

sp|O36635|L_HRSVB LNNITDAAIKAQKNLLSRVCHTLLDKTVSDNIINGKWIILLSKFLKLIKLAGDNNLNNLS 420
tr|A0A1P8L3C9|A0A1P8L3C9_HRSV LNNITDAAIKAQKDLLSRVCHTLLDKTVSDNIINGKWIILLSKFLKLIKLAGDNNLNNLS 420
tr|A0A4D6PIQ1|A0A4D6PIQ1_HRSV LNNITDAAIKAQKDLLSRVCHTLLDKTVSDNIINGKWIILLSKFLKLIKLAGDNNLNNLS 420
tr|A0A8F9RAC8|A0A8F9RAC8_HRSV LNNITDAAIKAQKDLLSRVCHTLLDKTVSDNIINGKWIILLSKFLKLIKLAGDNNLNNLS 420
tr|A0A8G0RC16|A0A8G0RC16_HRSV LNNITDAAIKAQKDLLSRVCHTLLDKTVSDNIINGKWIILLSKFLKLIKLAGDNNLNNLS 420
tr|A0A8G0VY43|A0A8G0VY43_HRSV LNNITDAAIKAQKDLLSRVCHTLLDKTVSDNIINGKWIILLSKFLKLIKLAGDNNLNNLS 420
tr|A0A8G0R4A9|A0A8G0R4A9_HRSV LNNITDAANKAQKNLLSXXXXXXXXXXXXXXXXXXXXXXXXXXXXKLIKLAGDNNLNNLS 420
tr|A0A8G0R552|A0A8G0R552_HRSV LNNITDAANKAQKNLLSRVCHTLLDKTISDNIINGRWIILLSKFLKLIKLAGDNNLNNLS 420
tr|A0A023R7N6|A0A023R7N6_HRSV LNNITDAANKAQKNLLSRVCHTLLDKTISDNIINGRWIILLSKFLKLIKLAGDNNLNNLS 420
tr|A0A023RA71|A0A023RA71_HRSV LNNITDAANKAQKNLLSRVCHTLLDKTISDNIINGRWIILLSKFLKLIKLAGDNNLNNLS 420
tr|A0A1C9V2A5|A0A1C9V2A5_HRSV LNNITDAANKAQKNLLSRVCHTLLDKTISDNIINGRWIILLSKFLKLIKLAGDNNLNNLS 420
tr|A0A384ZGE7|A0A384ZGE7_HRSV LNNITDAANKAQKNLLSRVCHTLLDKTISDNIINGRWIILLSKFLKLIKLAGDNNLNNLS 420
tr|A0A1Y0AUY1|A0A1Y0AUY1_HRSV LNNITDAANKAQKNLLSRVCHTLLDKTISDNIINGRWIILLSKFLKLIKLAGDNNLNNLS 420
tr|A0A384ZIE6|A0A384ZIE6_HRSV LNNITDAANKAQKNLLSRVCHTLLDKTISDNIINGRWIILLSKFLKLIKLAGDNNLNNLS 420
tr|R9W5G7|R9W5G7_HRSV LNNITDAANKAQKNLLSRVCHTLLDKTISDNIINGRWIILLSKFLKLIKLAGDNNLNNLS 420
tr|A0A060A983|A0A060A983_HRSV LNNITDAANKAQKNLLSRVCHTLLDKTVSDNIINGRWIILLSKFLKLIKLAGDNNLNNLS 420
tr|X5F7C6|X5F7C6_HRSV LNNITDAANKAQKNLLSRVCHTLLDKTVSDNIINGRWIILLSKFLKLIKLAGDNNLNNLS 420
sp|Q9IWW8|L_HRSVL LNNITDAANKAQKNLLSRVCHTLLDKTVSDNIINGRWIILLSKFLKLIKLAGDNNLNNLS 420
tr|A0A088S978|A0A088S978_HRSV LNNITDAANKAQKNLLSRVCHTLLDKTVSDNIINGRWIILLSKFLKLIKLAGDNNLNNLS 420
tr|A0A088S9M1|A0A088S9M1_HRSV LNNITDAANKAQKNLLSRVCHTLLDKTVSDNIINGRWIILLSKFLKLIKLAGDNNLNNLS 420
tr|A0A5P8I3A5|A0A5P8I3A5_HRSV LNNITDAANKAQKNLLSRVCHTLLDKTVSDNIINGRWIILLSKFLKLIKLAGDNNLNNLS 420
sp|P28887|L_HRSVA LNNITDAANKAQKNLLSRVCHTLLDKTVSDNIINGRWIILLSKFLKLIKLAGDNNLNNLS 420
tr|A0A059TE58|A0A059TE58_HRSV LNNITDAANKAQKNLLSRVCHTLLDKTVSDNIINGRWIILLSKFLKLIKLAGDNNLNNLS 420
tr|A0A0X9WIQ9|A0A0X9WIQ9_HRSV LNNITEAANKAQKNLLSRVCHTLLDKTVSDNIINGRWIILLSKFLKLIKLAGDNNLNNLS 420


sp|O36635|L_HRSVB ELYFLFRIFGHPMVDERQAMDAVRINCNETKFYLLSSLSTLRGAFIYRIIKGFVNTYNRW 480
tr|A0A1P8L3C9|A0A1P8L3C9_HRSV ELYFLFRIFGHPMVDERQAMDAVRINCNETKFYLLSSLSTLRGAFIYRIIKGFVNTYNRW 480
tr|A0A4D6PIQ1|A0A4D6PIQ1_HRSV ELYFLFRIFGHPMVDERQAMDAVRINCNETKFYLLSSLSTLRGAFIYRIIKGFVNTYNRW 480
tr|A0A8F9RAC8|A0A8F9RAC8_HRSV ELYFLFRIFGHPMVDERQAMDAVRINCNETKFYLLSSLSTLRGAFIYRIIKGFVNTYNRW 480
tr|A0A8G0RC16|A0A8G0RC16_HRSV ELYFLFRIFGHPMVDERQAMDAVRVNCNETKFYLLSSLSTLRGAFIYRIIKGFVNTYNRW 480
tr|A0A8G0VY43|A0A8G0VY43_HRSV ELYFLFRIFGHPMVDERQAMDAVRINCNETKFYLLSSLSTLRGAFIYRIIKGFVNTYNRW 480
tr|A0A8G0R4A9|A0A8G0R4A9_HRSV ELYFLFRIFGHPMVDERQAMDAVKVNCNETKFYXXXXXXXXXXXXXXXXXXXXXXXXXXX 480
tr|A0A8G0R552|A0A8G0R552_HRSV ELYFLFRIFGHPMVDERQAMDAVKVNCNETKFYLLSSLSMLRGAFIYRIIKGFVNNYNRW 480
tr|A0A023R7N6|A0A023R7N6_HRSV ELYFLFRIFGHPMVDERQAMDAVKVNCNETKFYLLSSLSMLRGAFIYRIIKGFVNNYNRW 480
tr|A0A023RA71|A0A023RA71_HRSV ELYFLFRIFGHPMVDERQAMDAVKVNCNETKFYLLSSLSMLRGAFIYRIIKGFVNNYNRW 480
tr|A0A1C9V2A5|A0A1C9V2A5_HRSV ELYFLFRIFGHPMVDERQAMDAVKVNCNETKFYLLSSLSMLRGAFIYRIIKGFVNNYNRW 480
tr|A0A384ZGE7|A0A384ZGE7_HRSV ELYFLFRIFGHPMVDERQAMDAVKVNCNETKFYLLSSLSMLRGAFIYRIIKGFVNNYNRW 480
tr|A0A1Y0AUY1|A0A1Y0AUY1_HRSV ELYFLFRIFGHPMVDERQAMDAVKVNCNETKFYLLSSLSMLRGAFIYRIIKGFVNNYNRW 480
tr|A0A384ZIE6|A0A384ZIE6_HRSV ELYFLFRIFGHPMVDERQAMDAVKVNCNETKFYLLSSLSMLRGAFIYRIIKGFVNNYNRW 480
tr|R9W5G7|R9W5G7_HRSV ELYFLFRIFGHPMVDERQAMDAVKVNCNETKFYLLSSLSMLRGAFIYRIIKGFVNNYNRW 480
tr|A0A060A983|A0A060A983_HRSV ELYFLFRIFGHPMVDERQAMDAVKVNCNETKFYLLSSLSMLRGAFIYRIIKGFVNNYNRW 480
tr|X5F7C6|X5F7C6_HRSV ELYFLFRIFGHPMVDERQAMDAVKVNCNETKFYLLSSLSMLRGAFIYRIIKGFVNNYNRW 480
sp|Q9IWW8|L_HRSVL ELYFLFRIFGHPMVDERQAMDAVKVNCNETKFYLLSSLSMLRGAFIYRIIKGFVNNYNRW 480
tr|A0A088S978|A0A088S978_HRSV ELYFLFRIFGHPMVDERQAMDAVKINCNETKFYLLSSLSMLRGAFIYRIIKGFVNNYNRW 480
tr|A0A088S9M1|A0A088S9M1_HRSV ELYFLFRIFGHPMVDERQAMDAVKINCNETKFYLLSSLSMLRGAFIYRIIKGFVNNYNRW 480
tr|A0A5P8I3A5|A0A5P8I3A5_HRSV ELYFLFRIFGHPMVDERQAMDAVKINCNETKFYLLSSLSMLRGAFIYRIIKGFVNNYNRW 480
sp|P28887|L_HRSVA ELYFLFRIFGHPMVDERQAMDAVKINCNETKFYLLSSLSMLRGAFIYRIIKGFVNNYNRW 480
tr|A0A059TE58|A0A059TE58_HRSV ELYFLFRIFGHPMVDERQAMDAVKINCNETKFYLLSSLSMLRGAFIYRIIKGFVNNYNRW 480
tr|A0A0X9WIQ9|A0A0X9WIQ9_HRSV ELYFLFRIFGHPMVDERQAMDAVKINCNETKFYLLSSLSMLRGAFIYRIIKGFVNNYNRW 480


sp|O36635|L_HRSVB PTLRNAIVLPLRWLNYYKLNTYPSLLEITENDLIILSGLRFYREFHLPKKVDLEMIINDK 540
tr|A0A1P8L3C9|A0A1P8L3C9_HRSV PTLRNAIVLPLRWLNYYKLNTYPSLLEITENDLIILSGLRFYREFHLPKKVDLEMIINDK 540
tr|A0A4D6PIQ1|A0A4D6PIQ1_HRSV PTLRNAIVLPLRWLNYYKLNTYPSLLEITENDLIILSGLRFYREFHLPKKVDLEMIINDK 540
tr|A0A8F9RAC8|A0A8F9RAC8_HRSV PTLRNAIVLPLRWLNYYKLNTYPSLLEITENDLIILSGLRFYREFHLPKKVDLEMIINDK 540
tr|A0A8G0RC16|A0A8G0RC16_HRSV PTLRNAIVLPLRWLNYYKLNTYPSLLEITENDLIILSGLRFYREFHLPKKVDLEMIINDK 540
tr|A0A8G0VY43|A0A8G0VY43_HRSV PTLRNAIVLPLRWLNYYKLNTYPSLLEITENDLIILSGLRFYREFHLPKKVDLEMIINDK 540
tr|A0A8G0R4A9|A0A8G0R4A9_HRSV XXXXXXXXXXXXXXXXXXXXXXXXXXXELTERDLIVLSGLRFYREFRLPKKVDLEMIINDK 540
tr|A0A8G0R552|A0A8G0R552_HRSV PTLRNAIVLPLRWLTYYKLNTYPSLLELTERDLIVLSGLRFYREFRLPKKVDLEMIINDK 540
tr|A0A023R7N6|A0A023R7N6_HRSV PTLRNAIVLPLRWLTYYKLNTYPSLLELTERDLIVLSGLRFYREFRLPKKVDLEMIINDK 540
tr|A0A023RA71|A0A023RA71_HRSV PTLRNAIVLPLRWLTYYKLNTYPSLLELTERDLIVLSGLRFYREFRLPKKVDLEMIINDK 540
tr|A0A1C9V2A5|A0A1C9V2A5_HRSV PTLRNAIVLPLRWLTYYKLNTYPSLLELTERDLIVLSGLRFYREFRLPKKVDLEMIINDK 540
tr|A0A384ZGE7|A0A384ZGE7_HRSV PTLRNAIVLPLRWLTYYKLNTYPSLLELTERDLIVLSGLRFYREFRLPKKVDLEMIINDK 540
tr|A0A1Y0AUY1|A0A1Y0AUY1_HRSV PTLRNAIVLPLRWLTYYKLNTYPSLLELTERDLIVLSGLRFYREFRLPKKVDLEMIINDK 540
tr|A0A384ZIE6|A0A384ZIE6_HRSV PTLRNAIVLPLRWLTYYKLNTYPSLLELTERDLIVLSGLRFYREFRLPKKVDLEMIINDK 540
tr|R9W5G7|R9W5G7_HRSV PTLRNAIVLPLRWLTYYKLNTYPSLLELTERDLIVLSGLRFYREFRLPKKVDLEMIINDK 540
tr|A0A060A983|A0A060A983_HRSV PTLRNAIVLPLRWLTYYKLNTYPSLLELTERDLIVLSGLRFYREFRLPKKVDLEMIINDK 540
tr|X5F7C6|X5F7C6_HRSV PTLRNAVVLPLRWLTYYKLNTYPSLLELTERDLIVLSGLRFYREFRLPKKVDLEMIINDK 540
sp|Q9IWW8|L_HRSVL PTLRNAIVLPLRWLTYYKLNTYPSLLELTERDLIVLSGLRFYREFRLPKKVDLEMIINDK 540
tr|A0A088S978|A0A088S978_HRSV PTLRNAIVLPLRWLTYYKLNTYPSLLELTERDLIVLSGLRFYREFRLPKKVDLEMIINDK 540
tr|A0A088S9M1|A0A088S9M1_HRSV PTLRNAIVLPLRWLTYYKLNTYPSLLELTERDLIVLSGLRFYREFRLPKKVDLEMIINDK 540
tr|A0A5P8I3A5|A0A5P8I3A5_HRSV PTLRNAIVLPLRWLTYYKLNTYPSLLELTERDLIVLSGLRFYREFRLPKKVDLEMIINDK 540
sp|P28887|L_HRSVA PTLRNAIVLPLRWLTYYKLNTYPSLLELTERDLIVLSGLRFYREFRLPKKVDLEMIINDK 540
tr|A0A059TE58|A0A059TE58_HRSV PTLRNAIVLPLRWLTYYKLNTYPSLLELTERDLIVLSGLRFYREFRLPKKVDLEMIINDK 540
tr|A0A0X9WIQ9|A0A0X9WIQ9_HRSV PTLRNAIVLPLRWLTYYKLNTYPSLLELTERDLIVLSGLRFYREFRLPKKVDLEMIINDK 540
sp|O36635|L_HRSVB AISPPKDLIWTSFPRNYMPSHIQNYIEHEKLKFSESDRSRRVLEYYLRDNKFNECDLYNC 600
tr|A0A1P8L3C9|A0A1P8L3C9_HRSV AISPPKDLIWTSFPRNYMPSHIQNYIEHEKLKFSESDRSRRVLEYYLRDNKFNECDLYNC 600
tr|A0A4D6PIQ1|A0A4D6PIQ1_HRSV AISPPKDLIWTSFPRNYMPSHIQNYIEHEKLKFSESDRSRRVLEYYLRDNKFNECDLYNC 600
tr|A0A8F9RAC8|A0A8F9RAC8_HRSV AISPPKDLIWTSFPRNYMPSHIQNYIEHEKLKFSESDRSRRVLEYYLRDNKFNECDLYNC 600
tr|A0A8G0RC16|A0A8G0RC16_HRSV AISPPKDLIWTSFPRNYMPSHIQNYIEHEKLKFSESDRSRRVLEYYLRDNKFNECDLYNC 600
tr|A0A8G0VY43|A0A8G0VY43_HRSV AISPPKDLIWTSFPRNYMPSHIQNYIEHEKLKFSESDRSRRVLEYYLRDNKFNECDLYNC 600
tr|A0A8G0R4A9|A0A8G0R4A9_HRSV AISPPKNLIWTSFPRNYMPSHIQNYIEHEKLKFSDSDKSRRVLEYYLRDNKFNECDLHNC 600
tr|A0A8G0R552|A0A8G0R552_HRSV AISPPKNLIWTSFPRNYMPSHIQNYIEHEKLKFSDSDKSRRVLEYYLRDNKFNECDLHNC 600
tr|A0A023R7N6|A0A023R7N6_HRSV AISPPKNLIWTSFPRNYMPSHIQNYIEHEKLKFSDSDKSRRVLEYYLRDNKFNECDLYNC 600
tr|A0A023RA71|A0A023RA71_HRSV AISPPKNLIWTSFPRNYMPSHIQNYIEHEKLKFSDSDKSRRVLEYYLRDNKFNECDLYNC 600
tr|A0A1C9V2A5|A0A1C9V2A5_HRSV AISPPKNLIWTSFPRNYMPSHIQNYIEHEKLKFSDSDKSRRVLEYYLRDNKFNECDLYNC 600
tr|A0A384ZGE7|A0A384ZGE7_HRSV AISPPKNLIWTSFPRNYMPSHIQNYIEHEKLKFSDSDKSRRVLEYYLRDNKFNECDLHNC 600
tr|A0A1Y0AUY1|A0A1Y0AUY1_HRSV AISPPKNLIWTSFPRNYMPSHIQNYIEHEKLKFSDSDKSRRVLEYYLRDNKFNECDLYNC 600
tr|A0A384ZIE6|A0A384ZIE6_HRSV AISPPKNLIWTSFPRNYMPSHIQNYIEHEKLKFSDSDKSRRVLEYYLRDNKFNECDLHNC 600
tr|R9W5G7|R9W5G7_HRSV AISPPKNLIWTSFPRNYMPSHIQNYIEHEKLKFSESDKSRRVLEYYLRDNKFNECDLHNC 600
tr|A0A060A983|A0A060A983_HRSV AISPPKNLIWTSFPRNYMPSHIQNYIEHEKLKFSESDKSRRVLEYYLRDNKFNECDLYNC 600
tr|X5F7C6|X5F7C6_HRSV AISPPKNLIWTSFPRNYMPSHIQNYIEHEKLKFSESDKSRRVLEYYLRDNKFNECDLYNC 600
sp|Q9IWW8|L_HRSVL AISPPKNLIWTSFPRNYMPSHIQNYIEHEKLKFSESDKSRRVLEYYLRDNKFNECDLYNC 600
tr|A0A088S978|A0A088S978_HRSV AISPPKNLIWTSFPRNYMPSHIQNYIEHEKLKFSESDKSRRVLEYYLRDNKFNECDLYNC 600
tr|A0A088S9M1|A0A088S9M1_HRSV AISPPKNLIWTSFPRNYMPSHIQNYIEHEKLKFSESDKSRRVLEYYLRDNKFNECDLYNC 600
tr|A0A5P8I3A5|A0A5P8I3A5_HRSV AISPPKNLIWTSFPRNYMPSHIQNYIEHEKLKFSESDKSRRVLEYYLRDNKFNECDLYNC 600
sp|P28887|L_HRSVA AISPPKNLIWTSFPRNYMPSHIQNYIEHEKLKFSESDKSRRVLEYYLRDNKFNECDLYNC 600
tr|A0A059TE58|A0A059TE58_HRSV AISPPKNLIWTSFPRNYMPSHIQTYIEHEKLKFSESDKSRRVLEYYLRDNKFNECDLYNC 600
tr|A0A0X9WIQ9|A0A0X9WIQ9_HRSV AISPPKNLIWTSFPRNYMPSHIQNYIEHEKLKFSESDKSRRVLEYYLRDNKFNECDLYNC 600
sp|O36635|L_HRSVB VVNQSYLNNSNHVVSLTGKERELSVGRMFAMQPGMFRQIQILAEKMIAENILQFFPESLT 660
tr|A0A1P8L3C9|A0A1P8L3C9_HRSV VVNQSYLNNSNHVVSLTGKERELSVGRMFAMQPGMFRQIQILAEKMIAENILQFFPESLT 660
tr|A0A4D6PIQ1|A0A4D6PIQ1_HRSV VVNQSYLNNSNHVVSLTGKERELSVGRMFAMQPGMFRQIQILAEKMIAENILQFFPESLT 660
tr|A0A8F9RAC8|A0A8F9RAC8_HRSV VVNQSYLNNSNHVVSLTGKERELSVGRMFAMQPGMFRQIQILAEKMIAENILQFFPESLT 660
tr|A0A8G0RC16|A0A8G0RC16_HRSV VVNQSYLNNSNHVVSLTGKERELSVGRMFAMQPGMFRQIQILAEKMIAENILQFFPESLT 660
tr|A0A8G0VY43|A0A8G0VY43_HRSV VVNQSYLNNSNHVVSLTGKERELSVGRMFAMQPGMFRQIQILAEKMIAENILQFFPESLT 660
tr|A0A8G0R4A9|A0A8G0R4A9_HRSV VVNQSYLNNPNHVVSLTGKERELSVGRMFAMQPGMFRQVQILAEKMIAENILQFFPESLT 660
tr|A0A8G0R552|A0A8G0R552_HRSV VVNQSYLNNPNHVVSLTGKERELSVGRMFAMQPGMFRQVQILAEKMIAENILQFFPESLT 660
tr|A0A023R7N6|A0A023R7N6_HRSV VVNQSYLNNPNHVVSLTGKERELSVGRMFAMQPGMFRQVQILAEKMIAENILQFFPESLT 660
tr|A0A023RA71|A0A023RA71_HRSV VVNQSYLNNPNHVVSLTGKERELSVGRMFAMQPGMFRQVQILAEKMIAENILQFFPESLT 660
tr|A0A1C9V2A5|A0A1C9V2A5_HRSV VVNQSYLNNPNHVVSLTGKERELSVGRMFAMQPGMFRQVQILAEKMIAENILQFFPESLT 660
tr|A0A384ZGE7|A0A384ZGE7_HRSV VVNQSYLNNPNHVVSLTGKERELSVGRMFAMQPGMFRQVQILAEKMIAENILQFFPESLT 660
tr|A0A1Y0AUY1|A0A1Y0AUY1_HRSV IVNQSYLNNPNHVVSLTGKERELSVGRMFAMQPGMFRQVQILAEKMIAENILQFFPESLT 660
tr|A0A384ZIE6|A0A384ZIE6_HRSV VVNQSYLNNPNHVVSLTGKERELSVGRMFAMQPGMFRQVQILAEKMIAENILQFFPESLT 660
tr|R9W5G7|R9W5G7_HRSV VVNQSYLNNPNHVVSLTGKERELSVGRMFAMQPGMFRQVQILAEKMIAENILQFFPESLT 660
tr|A0A060A983|A0A060A983_HRSV VVNQSYLNNPNHVVSLTGKERELSVGRMFAMQPGMFRQVQILAEKMIAENILQFFPESLT 660
tr|X5F7C6|X5F7C6_HRSV VVNQSYLNNPNHVVSLTGKERELSVGRMFAMQPGMFRQVQILAEKMIAENILQFFPESLT 660
sp|Q9IWW8|L_HRSVL VVNQSYLNNPNHVVSLTGKERELSVGRMFAMQPGMFRQVQILAEKMIAENILQFFPESLT 660
tr|A0A088S978|A0A088S978_HRSV VVNQSYLNNPNHVVSLTGKERELSVGRMFAMQPGMFRQVQILAEKMIAENILQFFPESLT 660
tr|A0A088S9M1|A0A088S9M1_HRSV VVNQSYLNNPNHVVSLTGKERELSVGRMFAMQPGMFRQVQILAEKMIAENILQFFPESLT 660
tr|A0A5P8I3A5|A0A5P8I3A5_HRSV VVNQSYLNNPNHVVSLTGKERELSVGRMFAMQPGMFRQVQILAEKMIAENILQFFPESLT 660
sp|P28887|L_HRSVA VVNQSYLNNPNHVVSLTGKERELSVGRMFAMQPGMFRQVQILAEKMIAENILQFFPESLT 660
tr|A0A059TE58|A0A059TE58_HRSV VVDQSYLNNPNHVVSLTGKERELSVGRMFAMQPGMFRQVQILAEKMIAENILQFFPESLT 660
tr|A0A0X9WIQ9|A0A0X9WIQ9_HRSV VVDQSYLNNPNHVVSLTGKERELSVGRMFAMQPGMFRQVQILAEKMIAENILQFFPESLT 660

```
sp|O36635|L_HRSVB                LKLLYKEYAGIGHKLKGTETYISRDMQFMSKTIQHNGVYYPASIKKVLRVGPWINTILDD 900
tr|A0A1P8L3C9|A0A1P8L3C9_HRSV    LKLLYKEYAGIGHKLKGTETYISRDMQFMSKTIQHNGVYYPASIKKVLRVGPWINTILDD 900
tr|A0A4D6PIQ1|A0A4D6PIQ1_HRSV    LKLLYKEYAGIGHKLKGTETYISRDMQFMSKTIQHNGVYYPASIKKVLRVGPWINTILDD 900
tr|A0A8F9RAC8|A0A8F9RAC8_HRSV    LKLLYKEYAGIGHKLKGTETYISRDMQFMSKTIQHNGVYYPASIKKVLRVGPWINTILDD 900
tr|A0A8G0RC16|A0A8G0RC16_HRSV    LKLLYKEYAGIGHKLKGTETYISRDMQFMSKTIQHNGVYYPASIKKVLRVGPWINTILDD 900
tr|A0A8G0VY43|A0A8G0VY43_HRSV    LKLLYKEYAGIGHKLKGTETYISRDMQFMSKTIQHNGVYYPASIKKVLRVGPWINTILDD 900
tr|A0A8G0R4A9|A0A8G0R4A9_HRSV    LKLLYKEYAGIGHKLKGTETYISRDMQFMSKTIQHNGVYYPASIKKVLRVGPWINTILDD 900
tr|A0A8G0R552|A0A8G0R552_HRSV    LKLLYKEYAGIGHKLKGTETYISRDMQFMSKTIQHNGVYYPASIKKVLRVGPWINTILDD 900
tr|A0A023R7N6|A0A023R7N6_HRSV    LKLLYKEYAGIGHKLKGTETYISRDMQFMSKTIQHNGVYYPASIKKVLRVGPWINTILDD 900
tr|A0A023RA71|A0A023RA71_HRSV    LKLLYKEYAGIGHKLKGTETYISRDMQFMSKTIQHNGVYYPASIKKVLRVGPWINTILDD 900
tr|A0A1C9V2A5|A0A1C9V2A5_HRSV    LKLLYKEYAGIGHKLKGTETYISRDMQFMSKTIQHNGVYYPASIKKVLRVGPWINTILDD 900
tr|A0A384ZGE7|A0A384ZGE7_HRSV    LKLLYKEYAGIGHKLKGTETYISRDMQFMSKTIQHNGVYYPASIKKVLRVGPWINTILDD 900
tr|A0A1Y0AUY1|A0A1Y0AUY1_HRSV    LKLLYKEYAGIGHKLKGTETYISRDMQFMSKTIQHNGVYYPASIKKVLRVGPWINTILDD 900
tr|A0A384ZIE6|A0A384ZIE6_HRSV    LKLLYKEYAGIGHKLKGTETYISRDMQFMSKTIQHNGVYYPASIKKVLRVGPWINTILDD 900
tr|R9W5G7|R9W5G7_HRSV            LKLLYKEYAGIGHKLKGTETYISRDMQFMSKTIQHNGVYYPASIKKVLRVGPWINTILDD 900
tr|A0A060A983|A0A060A983_HRSV    LKLLYKEYAGIGHKLKGTETYISRDMQFMSKTIQHNGVYYPASIKKVLRVGPWINTILDD 900
tr|X5F7C6|X5F7C6_HRSV            LKLLYKEYAGIGHKLKGTETYISRDMQFMSKTIQHNGVYYPASIKKVLRVGPWINTILDD 900
sp|Q9IWW8|L_HRSVL                LKLLYKEYAGIGHKLKGTETYISRDMQFMSKTIQHNGVYYPASIKKVLRVGPWINTILDD 900
tr|A0A088S978|A0A088S978_HRSV    LKLLYKEYAGIGHKLKGTETYISRDMQFMSKTIQHNGVYYPASIKKVLRVGPWINTILDD 900
tr|A0A088S9M1|A0A088S9M1_HRSV    LKLLYKEYAGIGHKLKGTETYISRDMQFMSKTIQHNGVYYPASIKKVLRVGPWINTILDD 900
tr|A0A5P8I3A5|A0A5P8I3A5_HRSV    LKLLYKEYAGIGHKLKGTETYISRDMQFMSKTIQHNGVYYPASIKKVLRVGPWINTILDD 900
sp|P28887|L_HRSVA                LKLLYKEYAGIGHKLKGTETYISRDMQFMSKTIQHNGVYYPASIKKVLRVGPWINTILDD 900
tr|A0A059TE58|A0A059TE58_HRSV    LKLLYKEYAGIGHKLKGTETYISRDMQFMSKTIQHNGVYYPASIKKVLRVGPWINTILDD 900
tr|A0A0X9WIQ9|A0A0X9WIQ9_HRSV    LKLLYKEYAGIGHKLKGTETYISRDMQFMSKTIQHNGVYYPASIKKVLRVGPWINTILDD 900
                                 ************************************************************

sp|O36635|L_HRSVB                FKVSLESIGSLTQELEYRGES LLCSLIFRNIWLYNQIALQLRNHALCNNKLYLDILKVLK 960
tr|A0A1P8L3C9|A0A1P8L3C9_HRSV    FKVSLESIGSLTQELEYRGES LLCSLIFRNIWLYNQIALQLRNHALCNNKLYLDILKVLK 960
tr|A0A4D6PIQ1|A0A4D6PIQ1_HRSV    FKVSLESIGSLTQELEYRGES LLCSLIFRNIWLYNQIALQLRSHALCNNKLYLDILKVLK 960
tr|A0A8F9RAC8|A0A8F9RAC8_HRSV    FKVSLESIGSLTQELEYRGES LLCSLIFRNIWLYNQIALQLRNHALCNNKLYLDILKVLK 960
tr|A0A8G0RC16|A0A8G0RC16_HRSV    FKVSLESIGSLTQELEYRGES LLCSLIFRNIWLYNQIALQLRNHALCNNKLYLDILKVLK 960
tr|A0A8G0VY43|A0A8G0VY43_HRSV    FKVSLESIGSLTQELEYRGES LLCSLIFRNIWLYNQIALQLRNHALCNNKLYLDILKVLK 960
tr|A0A8G0R4A9|A0A8G0R4A9_HRSV    FKVSLESIGSLTQELEYRGES LLCSLIFRNXXXXXQIALQLKNHALCNNKLYLDILKVLK 960
tr|A0A8G0R552|A0A8G0R552_HRSV    FKVSLESIGSLTQELEYRGES LLCSLIFRNVWLYNQIALQLKNHALCNNKLYLDILKVLK 960
tr|A0A023R7N6|A0A023R7N6_HRSV    FKVSLESIGSLTQELEYRGES LLCSLIFRNVWLYNQIALQLKNHALCNNKLYLDILKVLK 960
tr|A0A023RA71|A0A023RA71_HRSV    FKVSLESIGSLTQELEYRGES LLCSLIFRNVWLYNQIALQLKNHALCNNKLYLDILKVLK 960
tr|A0A1C9V2A5|A0A1C9V2A5_HRSV    FKVSLESIGSLTQELEYRGES LLCSLIFRNVWLYNQIALQLKNHALCNNKLYLDILKVLK 960
tr|A0A384ZGE7|A0A384ZGE7_HRSV    FKVSLESIGSLTQELEYRGES LLCSLIFRNVWLYNQIALQLKNHALCNNKLYLDILKVLK 960
tr|A0A1Y0AUY1|A0A1Y0AUY1_HRSV    FKVSLESIGSLTQELEYRGES LLCSLIFRNVWLYNQIALQLKNHALCNNKLYLDILKVLK 960
tr|A0A384ZIE6|A0A384ZIE6_HRSV    FKVSLESIGSLTQELEYRGES LLCSLIFRNVWLYNQIALQLKNHALCNNKLYLDILKVLK 960
tr|R9W5G7|R9W5G7_HRSV            FKVSLESIGSLTQELEYRGES LLCSLIFRNVWLYNQIALQLKNHALCNNKLYLDILKVLK 960
tr|A0A060A983|A0A060A983_HRSV    FKVSLESIGSLTQELEYRGES LLCSLIFRNVWLYNQIALQLKNHALCNNKLYLDILKVLK 960
tr|X5F7C6|X5F7C6_HRSV            FKVSLESIGSLTQELEYRGES LLCSLIFRNVWLYNQIALQLKNHALCNNKLYLDILKVLK 960
sp|Q9IWW8|L_HRSVL                FKVSLESIGSLTQELEYRGES LLCSLIFRNVWLYNQIALQLKNHALCNNKLYLDILKVLK 960
tr|A0A088S978|A0A088S978_HRSV    FKVSLESIGSLTQELEYRGES LLCSLIFRNVWLYNQIALQLKNHALCNNKLYLDILKVLK 960
tr|A0A088S9M1|A0A088S9M1_HRSV    FKVSLESIGSLTQELEYRGES LLCSLIFRNVWLYNQIALQLKNHALCNNKLYLDILKVLK 960
tr|A0A5P8I3A5|A0A5P8I3A5_HRSV    FKVSLESIGSLTQELEYRGES LLCSLIFRNVWLYNQIALQLKNHALCNNKLYLDILKVLK 960
sp|P28887|L_HRSVA                FKVSLESIGSLTQELEYRGES LLCSLIFRNVWLYNQIALQLKNHALCNNKLYLDILKVLK 960
tr|A0A059TE58|A0A059TE58_HRSV    FKVSLESIGSLTQELEYRGES LLCSLIFRNVWLYNQIALQLKNHALCNNKLYLDILKVLK 960
tr|A0A0X9WIQ9|A0A0X9WIQ9_HRSV    FKVSLESIGSLTQELEYRGES LLCSLIFRNVWLYNQIALQLKNHALCNNKLYLDILKVLK 960
                                 ******************** ********     ******:.***************** 

sp|O36635|L_HRSVB                HLKTFFNL DSIDMALSLYMNLPMLFGGGDPNLLYRSFYRRTPDFLTEAIVHSVFVLSYYT 1020
tr|A0A1P8L3C9|A0A1P8L3C9_HRSV    HLKTFFNL DSIDTALSLYMNLPMLFGGGDPNLLYRSFYRRTPDFLTEAIVHSVFVLSYYT 1020
tr|A0A4D6PIQ1|A0A4D6PIQ1_HRSV    HLKTFFNL DSIDTALSLYMNLPMLFGGGDPNLLYRSFYRRTPDFLTEAIVHSVFVLSYYT 1020
tr|A0A8F9RAC8|A0A8F9RAC8_HRSV    HLKTFFNL DSIDTALSLYMNLPMLFGGGDPNLLYRSFYRRTPDFLTEAIVHSVFVLSYYT 1020
tr|A0A8G0RC16|A0A8G0RC16_HRSV    HLKTFFNL DSIDTALSLYMNLPMLFGGGDPNLLYRSFYRRTPDFLTEAIVHSVFVLSYYT 1020
tr|A0A8G0VY43|A0A8G0VY43_HRSV    HLKTFFNL DSIDTALSLYMNLPMLFGGGDPNLLYRSFYRRTPDFLTEAIVHSVFVLSYYT 1020
tr|A0A8G0R4A9|A0A8G0R4A9_HRSV    HLKTFFNL DNIDTALTLYMNLPMLFGGGDPNLLYRSFYRRTPDFLTEAIVHSVFILSYYT 1020
tr|A0A8G0R552|A0A8G0R552_HRSV    HLKTFFNL DNIDTALTLYMNLPMLFGGGDPNLLYRSFYRRTPDFLTEAIVHSVFILSYYT 1020
tr|A0A023R7N6|A0A023R7N6_HRSV    HLKTFFNL DNIDTALTLYMNLPMLFGGGDPNLLYRSFYRRTPDFLTEAIVHSVFILSYYT 1020
tr|A0A023RA71|A0A023RA71_HRSV    HLKTFFNL DNIDTALTLYMNLPMLFGGGDPNLLYRSFYRRTPDFLTEAIVHSVFILSYYT 1020
tr|A0A1C9V2A5|A0A1C9V2A5_HRSV    HLKNFFNL DNIDTALTLYMNLPMLFGGGDPNLLYRSFYRRTPDFLTEAIVHSVFILSYYT 1020
tr|A0A384ZGE7|A0A384ZGE7_HRSV    HLKTFFNL DNIDTALTLYMNLPMLFGGGDPNLLYRSFYRRTPDFLTEAIVHSVFILSYYT 1020
tr|A0A1Y0AUY1|A0A1Y0AUY1_HRSV    HLKTFFNL DNIDTALTLYMNLPMLFGGGDPNLLYRSFYRRTPDFLTEAIVHSVFILSYYT 1020
tr|A0A384ZIE6|A0A384ZIE6_HRSV    HLKTFFNL DNIDTALTLYMNLPMLFGGGDPNLLYRSFYRRTPDFLTEAIVHSVFILSYYT 1020
tr|R9W5G7|R9W5G7_HRSV            HLKTFFNL DNIDTALTLYMNLPMLFGGGDPNLLYRSFYRRTPDFLTEAIVHSVFILSYYT 1020
tr|A0A060A983|A0A060A983_HRSV    HLKTFFNL DNIDTALTLYMNLPMLFGGGDPNLLYRSFYRRTPDFLTEAIVHSVFILSYYT 1020
tr|X5F7C6|X5F7C6_HRSV            HLKTFFNL DNIDTALTLYMNLPMLFGGGDPNLLYRSFYRRTPDFLTEAIVHSVFILSYYT 1020
sp|Q9IWW8|L_HRSVL                HLKTFFNL DNIDTALTLYMNLPMLFGGGDPNLLYRSFYRRTPDFLTEAIVHSVFILSYYT 1020
tr|A0A088S978|A0A088S978_HRSV    HLKTFFNL DNIDTALTLYMNLPMLFGGGDPNLLYRSFYRRTPDFLTEAIVHSVFILSYYT 1020
tr|A0A088S9M1|A0A088S9M1_HRSV    HLKTFFNL DNIDTALTLYMNLPMLFGGGDPNLLYRSFYRRTPDFLTEAIVHSVFILSYYT 1020
tr|A0A5P8I3A5|A0A5P8I3A5_HRSV    HLKTFFNL DNIDTALTLYMNLPMLFGGGDPNLLYRSFYRRTPDFLTEAIVHSVFILSYYT 1020
sp|P28887|L_HRSVA                HLKTFFNL DNIDTALTLYMNLPMLFGGGDPNLLYRSFYRRTPDFLTEAIVHSVFILSYYT 1020
tr|A0A059TE58|A0A059TE58_HRSV    HLKTFFNL DNIDTALTLYMNLPMLFGGGDPNLLYRSFYRRTPDFLTEAIVHSVFILSYYT 1020
tr|A0A0X9WIQ9|A0A0X9WIQ9_HRSV    HLKTFFNL DNIDTALTLYMNLPMLFGGGDPNLLYRSFYRRTPDFLTEAIVHSVFILSYYT 1020
                                 ***.***** .** **:*************************************:***** 
```


RdRp
PRNTase

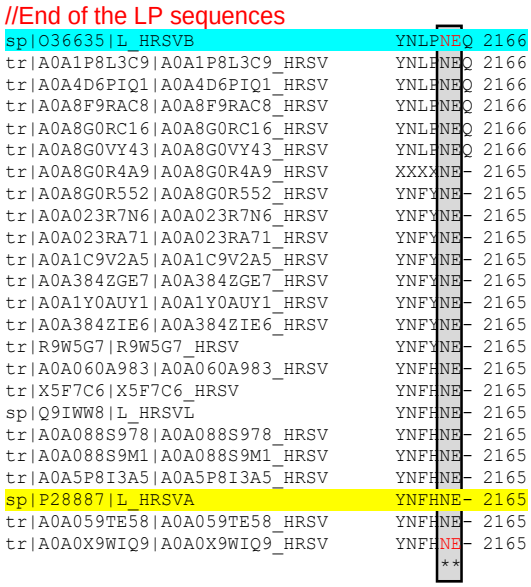


Figure 4 MSA of the RNA polymerases of the LP from hRSVs

O36635|L_HRSVB, type B (Strain B1)
A0A4D6PIQ1_HRSV, type B
A0A8G0RC16_HRSV, type B
A0A8G0R4A9_HRSV, type A
A0A023R7N6_HRSV
A0A1C9V2A5_HRSV
A0A1Y0AUY1_HRSV, type A
R9W5G7_HRSV
X5F7C6_HRSV, type A
A0A088S978_HRSV, type MinA
A0A5P8I3A5_HRSV, type MaxL
A0A059TE58_HRSV
A0A1P8L3C9_HRSV
A0A8F9RAC8_HRSV, type B
A0A8G0VY43_HRSV, type B
A0A8G0R552_HRSV, type A
A0A023RA71_HRSV
A0A384ZGE7_HRSV, type A
A0A384ZIE6_HRSV, type A
A0A060A983_HRSV, type A
Q9IWW8|L_HRSVL, type A (Strain Long)
A0A088S9M1_HRSV, type MinB
P28887|L_HRSVA, type A (Strain A2)
A0A0X9WIQ9_HRSV

4. Active Site Analyses of the RNA Polymerase of hRSVs

4.1. Metal-binding sites

The catalytic metal-binding sites were extensively studied by SDM, cryo-EM and other techniques. Two amino acids, viz. the **–DN-** in the metal-binding -^{810}G**DN**Q- motif of hRSV have been confirmed by the above techniques. Cao et al [16] have shown by Cryo-EM analyses that the RNA synthesis was observed with the wild-type RNA polymerase, but not in the RNA polymerase mutant, ^{811}**D**→A and it was found to be catalytically inactive. The next amino acid, -^{812}**N**- in that motif was also found to be essential for RNA polymerase synthesis [21]. They found by SDM analysis that the RNA polymerase with ^{812}**N**→A mutation did not catalyze *de novo* RNA synthesis from the +1 and +3 sites. Gilman et al [19] by cryo-EM studies have shown that the -^{700}**D**- from motif A (highlighted in dark blue) was also located at the catalytic centre. Therefore, they suggested that **D**^{811}, **N**^{812}, and **D**^{700} are responsible for coordinating the two magnesium ions required for catalysis of phosphodiester bond formation [19-21].

Similar results were obtained with the –GDNQ- motif in the RdRps of Ebola, rabies, measles and mumps, where all belong to (-) strand, non-segmented RNA viruses like hRSVs. Schmidt and Hoenen [22] have shown by MSA that -GDN- is the invariant motif found in all the (-) strand RNA viral RdRps like Ebola, rabies, measles mumps and human influenza. (Table 1). By SDM experiments, Schmidt and Hoenen [22] have further confirmed that the triple mutation, –^{741}G**DNQ**- → -^{741}G**AAA**- in Ebola viral RdRp abolished both the replication as well as transcription activities. Furthermore, Tchesnokov et al [23] found that the **D**^{742}→A mutant in the –G**D**^{742}NQ- motif of Ebola viral RdRp lacked the ability to extend the primer in the presence of Mg^{2+} and resulted in the absence of polymerase activity. Similar observations were made by Schnell and Conzelmann [24] on another human pathogenic (-) strand RNA virus, viz. the rabies virus. They found by SDM experiments that any modification of the –^{728}GDNQ- motif by conservative and non-conservative amino

acids resulted in complete loss of the RNA polymerase activity. A similar additional -^{412}GDNN- motif (highlighted in light green) is also identified in the polymerase region of hRSVs by MSA (Fig. 3) and its role is not clear now.

Interestingly, the second metal-binding motif identified is the –SDD- motif. The -^{1032}P/**SDD**- motif that is found here, is also found in all (-) strand RNA viruses along with the –GDNQ- motif (the P substitution is found only B group hRSVs). The –SDD- motif was also found to be essential for the polymerase activity by SDM experiments. In human influenza viruses (a (-) strand RNA virus but segmented type), it was found that replacement of the invariant amino acids, S^{444}→P, D^{445}→G and D^{446}→N of the -^{444}**SDD**- motif abolished the RNA polymerase activity completely [25]. Furthermore, by minireplicon assays, they confirmed that any mutation altering the –^{444}SDD- sequence rendered the polymerase nonfunctional [18]. Therefore, its possible involvement in metal-binding is based on sequence similarity and SDM analysis with other (-) strand RNA viruses.

Table 2 Catalytic region(s) in the viral, prokaryotic and eukaryotic RNA/DNA polymerases

Polymerase Type	Catalytic Region(s)
SSU RNA and DNA Polymerases	
Viral T7 SSU RNA pol	-$_{620}$WLAY^{-8}GVTR^{-4}SVTK^{631}R^{1}SVMTLAY^{8}GS- [24]
Viral SP6 SSU RNA pol	-$_{612}$WDSI^{-8}GITR^{-4}SLTKK^{1}PVMTLPY^{8}GS-
Mitochondrial SSU RNA pol (Yeast)	-TR^{-4}KVVKQ^{1}TVMTNVY^{8}GV--
Chloroplast SSU pol (ARATH)	-DR^{-4}KLVKQ^{1}TVMTSVY^{8}GV-
E. coli DNA pol I (SSU)	-^{753}QR^{-4}RSAK^{758}A^{1}INFGLIY^{8}GM- [25]
Initiation Subunits of Prokaryotic and Eukaryotic MSU DdRps (mRNAs)	
E. coli MSU RNAP β subunit	-$_{539}$TR^{-8}ERAGFEVRD^{1}VHPTHY^{7}GRV$_{558}$-
S. cerevisiae MSU RNAP II Rpb2 subunit	-$_{851}$FR^{-5}SLFFRS^{1}YMDQEKKY^{9}GMSI$_{870}$-
Human MSU RNAP II Rpb2 subunit	-$_{806}$FR^{-5}SVFYRS^{1}YKEQESKK^{9}GFDQ$_{825}$-
Elongation Subunits of Prokaryotic and Eukaryotic MSU DdRps (mRNAs)	
E. coli MSU RNAP β' subunit	-$_{833}$NSV^{-6}DAVKVRS^{1}VVSC^{5}DTDFGVC^{12}AHC^{15}Y^{16}G^{17}RDL$_{861}$-
S. cerevisiae MSU RNAP II Rpb1 subunit	-$_{55}$DPR^{-6}LGSIDRN^{1}LKC^{4}QTC^{7}QEGMNEC^{14}PGHF^{18}G^{19}HI$_{84}$-
Human MSU RNAP II Rpb1 subunit	-$_{59}$DPR^{-6}QGVIERT^{1}GRC^{4}QTC^{7}AGNMTEC^{14}PGHF^{18}G^{19}HI$_{88}$-
Active Site Regions in Prokaryotic and Eukaryotic DNA Polymerases#	
T7 DNA polymerase (Rep)	-^{622}AYGVTR^{-4}SVTK^{631}R^{1}SVMTLAY^{639}G^{9}SKEFG [26]
P. furiosus DNA polymerase	-$_{478}$ILLDYR^{-5}QKAIKL^{1}LANSFY^{7}GYYGYAK-
Yeast DNA polymerase α (Rep)	-$_{933}$RVQCDIR^{-5}QQALKL^{1}TANSMY^{7}GCLGYVN-
Human DNA polymerase α (Rep)	-$_{939}$ILQYDIR^{-5}QKALKL^{1}TANSMY^{7}GCLGFSY-
Human DNA polymerase δ (Rep) (Catalytic subunit)	-$_{683}$RQVLDGR^{-5}QLALKV^{1}SANSVY^{7}GFTGAQV-
Human DNA polymerase ε (Rep) (Catalytic subunit)	-^{801}YDSLQ^{-4}LAHKC^{1}ILNSFY^{7}GVVMR-
Yeast DNA polymerase ε (Rep) (Catalytic subunit)	-^{816}YDSLQ^{-4}LAHKV^{1}ILNSFY^{831}G^{8}YVMR- [27]
Human DNA polymerase γ (Rep)	-$_{917}$TTVGISR^{-4}EHAKI^{1}FNYGRIY^{8}GAGQPFAER-
(+) Strand RNA Viruses- (SARS-Coronaviruses)	
RdRps (NSP12)	
SARS-CoV-1	-STMTNR^{-5}QFHQKL^{1}LKSIAATRGATVVIGTSKFY^{21}GG$_{597}$-
SARS-CoV-2	-STMTNR^{-5}QFHQKL^{1}LKSIAATRGATVVIGTSKFY^{21}GG$_{597}$- [13]
MERS-CoV	-STMTNR^{-5}QYHQKM^{1}LKSMAATRGATCVIGTTKFY^{21}GG$_{598}$-
(-) Strand, Segmented RNA Viruses	
PB1 Catalytic Subunits of the RdRps [WJARR PB1 subunit]	
Human influenza A virus	-^{256}ETLAR^{-5}SICEKL^{1}EQSGLPV^{8}GGN- #gRNA -^{477}MSKKK^{-5}SYINRT^{1}GTFEFTSFFYRY^{13}GFV- #mRNA
Human influenza B virus	-^{256}ENLAK^{-5}NICENL^{1}EQSGLPV^{8}GGN- #gRNA -^{666}RTKRN^{-5}RSILNT^{1}DQRNMILEEQCY^{13}AKCCX$_{5}$CF- #mRNA
Human influenza C virus	-^{258}ETVAQK^{-4}ICEKL^{1}KESGLPV^{8}GGN- #gRNA -^{464}IRRFN^{-4}AVCKL^{1}IGINMSLEKSY^{12}GSL- #mRNA
(-) Strand, Non-segmented RNA Virus	
Respiratory Syncytial Viruses	-^{291}CLN^{-4}TLNKS^{1}LGLRCGFNNVILTQLFLY^{18}GD- (catalytic core 1)
	-^{640}QVQ^{-5}ILAEKM^{1}IAENILQFFPESLTRY^{17}GD- (catalytic core 2)

Adapted from Palanivelu [19 and references therein].
gRNA, genomic RNA; Rep, Replicase. *P. furiosus, Pyrococcus furiosus*
#gRNAs, similar to eukaryotic DNA pols and #mRNAs, similar to multisubunit (MSU) RNA pols.
The active site amino acids confirmed by SDM and by other techniques are highlighted in dark blue.

4.2. Polymerase Core Catalytic Regions

The catalytic core regions generally consist of three components, viz. a template–binding pair, a basic catalytic amino acid (which initiates catalysis by proton abstraction) and usually a basic amino acid at -4 to -5 position from the catalytic amino acid for nucleotide selection (Table 2). Interestingly, two different catalytic core regions are identified in the RNA polymerase of hRSVs. The first catalytic core (-^{293}**N**$^{-4}$----**K**$_{S1}$----------**Y**^{18}**G**-) and the second catalytic core (-^{640}**Q**$^{-5}$-----**K**$_{M1}$----------**Y**^{17}**G**---) are found in the polymerase region suggesting possibly one for gRNA and the other for mRNA syntheses (Table 2). In both cases, an invariant -YG- pair is identified as the template–binding pair and the catalytic amino acid as an invariant K. The nucleotide selection amino acid N is at -4 and Q is at -5 positions, respectively. The first core, very close to the proposed FPL, may involve in replication, whereas the second catalytic core may involve in transcription.

4.3. PR Exonuclease Domains of the hRSV RNA polymerase

There are two types of PR exonucleases to correct misincorporated nucleotides during polymerization. They belong to DEDD- and PHP-superfamilies of exonucleases: They are either found on the same polypeptide along with the DNA/RNA polymerase domains as multifunctional enzymes (e.g.), DNA pol I, or, found as a part of the tightly associated enzyme in a multienzyme complex (e.g.), ε-subunit of the DNA pol III, ExoN of the SARS-CoVs and PA subunit of the RNA polymerase in HinVs [12, 26, 27]. These two superfamilies are invariably found/associated in/with both DNA/RNA replicases to repair any error during the replications [28, 29]. Whereas the DEDD-superfamily is reported from all kingdoms of life, the PHP-superfamily has been reported only from the bacterial kingdom, but recently in the viral kingdom by Palanivelu [30]. The DEDD-superfamily consists of two subfamilies, viz. DEDDy and DEDDh, depending upon whether they employ an invariant Y or a H as the proton acceptor to initiate catalysis [28]. Like other RNA/DNA polymerases, the RNA polymerase from the hRSVs exhibits two DEDD-superfamily of PR exonuclease domains, one is complete and the other one is incomplete (highlighted in light blue (Fig. 4). In the incomplete one, the essential D, preceding the proton acceptor (H), is found missing. In the complete one, all the 5 essential active site amino acids are in place and belong to DEDD(H)-subfamily (Table 3). It is interesting to note that the PR exonucleases of RNase T and ε-subunit of the bacterial replicases (DNA pols III) also belong to the same DEDD(H)-subfamily and the involvement of these active site amino acids was confirmed by SDM experiments and X-ray crystallographic analysis in the above enzymes. [30 and references therein].

Table 3 shows a summary of the identified DEDD-superfamily active site amino acids from human pathogenic viruses, compared with the same superfamily, reported from different bacterial and other viral sources.

Table 3 DEDD-(Y/H) superfamily active site amino acids from various sources

Exo-Family	Consensus AS Pattern	Proton Acceptor	Catalytic Metal ion*	Zn-Binding site(s)
DEDD(Y/H)-family				
DNA pol I	-D^{355}xE^{357}-D^{424}-Y^{497}-D^{501}-	Tyr	Zn^{2+}	1
DNA pol II	-D^{156}xE-D^{229}-Y^{331}-D^{335}-	Tyr	Zn^{2+}	1
RNase D	-D^{28}xE^{30}-D^{85}-Y^{151}-D^{155}-	Tyr	Zn^{2+}	1
DNA pol III, ε-subunit	-D^{12}xE^{14}-D^{103}-H^{162}-D^{167}-	His	Zn^{2+}	1
RNase T	-D^{23}xE^{25}-D^{125}-H^{181}-D^{186}-	His	Zn^{2+}	1
SARS-CoV-1 ExoN/ACE2^	-DxE-D-H-D-	His	Zn^{2+}	2
MERS-CoV ExoN/DPP4	-DxE-D-H-D-	His	Zn^{2+}	2
SARS-CoV-2 ExoN//ACE2	-DxE-D-H-D-	His	Zn^{2+}	2
HCoV-NL63 ExoN/ACE2	-DxE-D-H-D-	His	Zn^{2+}	2
Human Influenza A virus	-DxE-D-H-D-	His	Zn^{2+}	1
Human Influenza B virus	-DxE-D-H-D-	His	Zn^{2+}	1
Human Influenza C virus	-DxE-D-H-D-	His	Zn^{2+}	1
Respiratory syncytial viruses	-DxD-D-H-D-	His	Zn^{2+}	1

Adapted from Palanivelu [30]
AS, Active site; *Water-bound Zn^{2+}
Active site amino acids, proved by SDM is highlighted in dark blue
hRSV PR exonuclease active site amino acid patterns are highlighted in yellow
^Similar amino acids, in the active sites of SARS-CoVs and Human CoVs were also reported [[26] too.
ACE2, Angiotensin-Converting Enzyme 2; DPP4, Dipeptidyl peptidase 4.

Figure 5 shows the proposed amino acids in the active site of the PR exonuclease domain of hRSV.

DEDD(H)-superfamily -^{664}**D**LE^{666}----------L**D**^{734}---------D**H**^{829}A→3 aa←^{833}**D**YL---

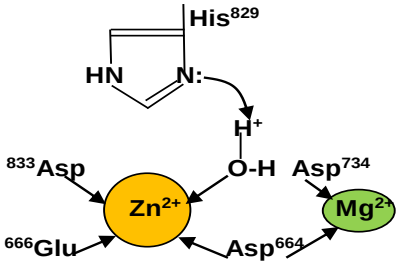


Figure 5 Proposed amino acids at the active site of the PR exonuclease domain of hRSV

Table 4 shows the DEDD-superfamily exonuclease active site amino acids and their distance conservations from human RNA pathogens and prokaryotic and eukaryotic replicases.

Table 4 DEDD(Y/H)-superfamily PR exonuclease active site amino acids and their distance conservations.

Prokaryotic Replicases	
DNA pol III (*Tth*) ε-subunit	-^{77}DLE----------FD^{161}--------CH^{214}R→4 aa$^{\#}$←^{219}DVE-
DNA pol III (*E. coli*) ε-subunit	-^{12}DLE----------FD^{103}--------CH^{162}R→4 aa ←^{167}DVE-
Eukaryotic Replicases	
DNA pol ε cat. subunit (*Sc*)	-^{290}DIE---------- FD^{388}---------EY^{473}S→3 aa←^{477}DAV-
DNA pol δ cat. subunit (*Hs*)	-^{316}DIE---------- FD^{402}--------- VY^{511}C→3 aa←D^{515}AY-
RNA Viral Replicases	
(+) Strand RNA viruses	
SARS-CoV-1 ExoN/ACE2	-^{90}DVE----------ID^{243}---------AH^{268}V→4 aa←^{273}DAI-
MERS-CoV ExoN/DPP4	-^{90}DVE----------ID^{243}---------AH^{268}V→4 aa←^{273}DAI-
SARS-CoV-2 ExoN//ACE2	-^{90}DVE----------ID^{243}---------AH^{268}V→4 aa←^{273}DAI-
(-) Strand-segmented RNA viruses	
Influenza A virus (H1N1)	-^{347}DIE----------LD^{425}---------SH^{510}L→3 aa←^{514}DTD-
Influenza B virus	-^{194}DIE----------LD^{420}---------SH^{506}L→3 aa←^{510}DTD-
Influenza C virus	-^{84}DLE----------ID^{288}---------SH^{494}L→3 aa←^{498}DDG-
(-) Strand-non-segmented RNA viruses	
Respiratory syncytial viruses*	-^{664}DLE----------LD^{425}---------DH^{829}A→3 aa←^{833}DYL-
Prokaryotic Replicases	
DNA pol III (*Tth*) ε-subunit	-^{77}DLE----------FD^{161}--------CH^{214}R→4 aa$^{\#}$←^{219}DVE-
DNA pol III (*E. coli*) ε-subunit	-^{12}DLE----------FD^{103}--------CH^{162}R→4 aa ←^{167}DVE-
Eukaryotic Replicases	
DNA pol ε cat. subunit (*Sc*)	-^{290}DIE---------- FD^{388}---------EY^{473}S→3 aa←^{477}DAV- [31]
DNA pol δ cat. subunit (*Hs*)	-^{316}DIE---------- FD^{402}--------- VY^{511}C→3 aa←D^{515}AY- [32]
RNA Viral Replicases	
(+) Strand RNA viruses	
SARS-CoV-1 ExoN/ACE2	-^{90}DVE----------ID^{243}---------AH^{268}V→4 aa←^{273}DAI-
MERS-CoV ExoN/DPP4	-^{90}DVE----------ID^{243}---------AH^{268}V→4 aa←^{273}DAI-
SARS-CoV-2 ExoN//ACE2	-^{90}DVE----------ID^{243}---------AH^{268}V→4 aa←^{273}DAI-
(-) Strand-segmented RNA viruses	
Influenza A virus (H1N1)	-^{347}DIE----------LD^{425}---------SH^{510}L→3 aa←^{514}DTD-
Influenza B virus	-^{194}DIE----------LD^{420}---------SH^{506}L→3 aa←^{510}DTD-
Influenza C virus	-^{84}DLE----------ID^{288}---------SH^{494}L→3 aa←^{498}DDG-
(-) Strand-non-segmented RNA viruses	
Respiratory syncytial viruses*	-^{664}DLE----------LD^{425}---------DH^{829}A→3 aa←^{833}DYL-

Adapted from Palanivelu [30]
Tth, Thermus thermophilus; Sc, Saccharomyces cerevisiae; Hs, Homo sapiens.
ACE2, Angiotensin-Converting Enzyme 2; DPP4, Dipeptidyl peptidase 4.
#The 3-4 amino acid gap is maintained between the last invariant Y/H and D.

*The hRSV PR exonuclease active site amino acids are highlighted in yellow.
Active site amino acids proved by SDM analysis are highlighted in dark blue and by X-ray are highlighted in light blue.

5. Conclusions

The RNA polymerase of hRSVs is analyzed for their catalytic cores, metal-binding motifs and PR exonuclease active sites. The present study identified two polymerase catalytic cores, at least two possible metal-binding motifs and a PR exonuclease active site. Presence of two possible catalytic cores suggest that the hRSV polymerase may follow a dual mode, i.e., it could use one for viral gRNA synthesis (replication) and the other for mRNA synthesis (transcription) as suggested for human influenza viruses [19]. The two typical invariant catalytic metal-binding motifs, viz. –GDNQ- and –SDD- reported in other (-) strand RNA viruses are also found in the RNA polymerase of hRSVs. It is suggested it could use the same metal-binding motifs for both gRNA and mRNA syntheses. Interestingly, a PR exonuclease active site is also identified and it belongs to the DEDD-superfamily of PR exonucleases. These data will be useful in designing structure-based antiviral drugs, targeting the polymerase active sites for the treatment of hRSVs in the future.

Compliance with ethical standards

Acknowledgments

The author wishes to thank Dr. N. Srinivasan, Former Professor, Department of Endocrinology, Post Graduate Institute of Basic Medical Sciences, University of Madras, Chennai, for his useful suggestions on the manuscript.

Disclosure of conflict of interest

The author has declared that no competing interests exist.

References

[1] Glezen WP, Taber LH, Frank AL, Kasel JA. Risk of primary infection and reinfection with respiratory syncytial virus. Am. J. Dis. Child. 1986; 140:543–546.

[2] Drew L. Will a new wave of RSV vaccines stop the dangerous virus? Pfizer, GSK and Moderna are ahead in the race to produce vaccines against respiratory syncytial virus — what will be their impact? Nature. 2023; 614: 620.

[3] Griffiths C, Drews SJ, Marchant DJ. Respiratory Syncytial Virus: Infection, Detection, and New Options for Prevention and Treatment. Clin Microbiol Rev. 2017; 30: 277–319.

[4] Shi T, McAllister DA, O'Brien KL, Simoes EAF, Madhi SA, Gessner BD, Polack FP, et al. RSV Global Epidemiology Network, Global, regional, and national disease burden estimates of acute lower respiratory infections due to respiratory syncytial virus in young children in 2015: A systematic review and modelling study. Lancet. 2017; 390: 946–958.

[5] Crank MC, Ruckwardt TJ, Chen M, Morabito KM, Phung E, Costner PJ, Holman LSA, Hickman SP, et al., A proof of concept for structure-based vaccine design targeting RSV in humans. Science. 2019; 365: 505–509.

[6] Kapikian AZ, Mitchell RH, Chanock RM, Shvedoff RA, Stewart CE. An epidemiologic study of altered clinical reactivity to respiratory syncytial (RS) virus infection in children previously vaccinated with an inactivated RS virus vaccine. Am. J. Epidemiol. 1969; 89: 405–421.

[7] Valero MV. 'A good day': FDA approves world's first RSV vaccine. Nature. 2023

doi: https://doi.org/10.1038/d41586-023-01529-5

[8] Ventre K, Randolph AG. Ventre K (eds.). Ribavirin for respiratory syncytial virus infection of the lower respiratory tract in infants and young children. The Cochrane Database of Systematic Reviews (1): doi:10.1002/14651858.CD000181.pub3 (2007).

[9] Battles MB, McLellan JS. Respiratory syncytial virus entry and how to block it. Nat Rev, (Microbiol Rev). 2019;17: 233-237.

[10] Collins PL, Fearns R, Graham BS. Respiratory Syncytial Virus: Virology, Reverse Genetics, and Pathogenesis of Disease. Curr Top Microbiol Immunol. 2013; 372: 3–38. doi:10.1007/978-3-642-38919-1_1.

[11] Mammas IN, Drysdale SB, Rath B, Theodoridou M, Papaioannou G, Papatheodoropoulou A, koutsounaki E, et al. Update on current views and advances on RSV infection (Rev). Int J Mol Med, 2020; 46: 509–520.

[12] Palanivelu P. DNA polymerases – An insight into their active sites and mechanism of action, In: Recent Advances in Biological Research, Vol 1, Chapter 2, pp 1-39, SCIENCEDOMAIN International Book Publishers, UK. ISBN: 9788193422441, DOI: 10.9734/bpi/rabr/v1; 2019.

[13] Grosfeld H, Hill MG, Collins PL. RNA replication by respiratory syncytial virus (RSV) is directed by the N, P, and L proteins; transcription also occurs under these conditions but requires RSV superinfection for efficient synthesis of full-length mRNA. J. Virol. 1995; 69: 5677–5686.

[14] Collins PL, Hill MG, Cristina J, Grosfeld H. Transcription elongation factor of respiratory syncytial virus, a non-segmented negative-strand RNA virus. Proc Natl Acad Sci. USA. 1996;93: 81-85.

[15] Fearns R, Deval J. New antiviral approaches for respiratory syncytial virus and other mononegaviruses: Inhibiting the RNA polymerase. Antiviral Res. 2016;134: 63-76.

[16] Cao D, Gao Y, Roesler C, Rice S, D'Cunha P, Zhuang L, Slack J, et al. Cryo-EM structure of the respiratory syncytial virus RNA polymerase. Nat Commun. 2020; 11: 1-9.

[17] Tan L, Lemey P, Houspie L, Viveen MC, Jansen NJG, van Loon AM, Wiertz E, van Bleek, GM, et al. Genetic Variability among Complete Human Respiratory Syncytial Virus Subgroup A Genomes: Bridging Molecular Evolutionary Dynamics and Epidemiology. PLoS ONE 7(12): e51439. ttps://doi.org/10.1371/journal.pone.0051439

[18] Palanivelu P. An Insight into the Active Sites of the Catalytic Basic Protein Subunit PB1 of the RNA Polymerase of Human Influenza Viruses, World J Adv Res Rev. 2022; 17: 625–565.

[19] Gilman MSA, Liu C, Fung A, Behera I, Jordan P, Rigaux P, Ysebaert N, et al. Structure of the Respiratory Syncytial Virus Polymerase Complex. Cell. 2019; 179:193–204.

[20] Garriga D, Ferrer-Orta C, Querol-Audí J, Oliva B, Verdaguer N. Role of motif B loop in allosteric regulation of RNA-dependent RNA polymerization activity. J. Mol. Biol. 2013; 425:2279–2287.

[21] Noton SL, Nagendra K, Dunn EF, Mawhorter ME, Yu Q, Fearns R. Respiratory Syncytial Virus Inhibitor AZ-27 Differentially Inhibits Different Polymerase Activities at the Promoter. J Virol. 2015; 89: 7786–7798.

[22] Schmidt ML, Hoenen T. Characterization of the catalytic center of the Ebola virus L polymerase. PLoS Negl Trop Dis. 2017; 11:1-14.

[23] Tchesnokov EP, Raeisimakiani P, Ngure M, Marchant D, Götte M. Recombinant RNA-Dependent RNA Polymerase Complex of Ebola Virus. Sci Rep. 2018; 8:3970-3979.

[24] Schnell MJ, Conzelmann KK. Polymerase activity of in vitro mutated rabies virus L protein. Virology. 1995; 214:522-530. doi: 10.1006/viro.1995.0063.

[25] Chu C, Fan S, Li C, Macken C, Kim JH, Hatta M, et al. Functional Analysis of Conserved Motifs in Influenza Virus PB1 Protein. PLoS ONE. 2012; 7: e36113, doi:10.1371/journal.pone.0036113

[26] Palanivelu P. An overview of the proofreading functions in bacteria and SARS-Coronaviruses. Int J Biochem Res Rev. 2021; 30: 33-62.

[27] Granoff A, Webster R (Editors) Encyclopedia of Virology (Second Edition) Elsevier, 1999, eBook ISBN: 9780080547978.

[28] Zuo Y, Deutscher MP. Exoribonuclease superfamilies: structural analysis and phylogenetic distribution. Nucleic Acids Res. 2001; 29:1017–1026.

[29] Nagpal S, Nair DT. The PHP domain of PolX from Staphylococcus aureus aids high fidelity DNA synthesis through the removal of misincorporated deoxyribo-, ribo- and oxidized nucleotides. Nat Sci Rep. 2021; 11:4178.

[30] Palanivelu P. Identification of DEDD- and PHP-Superfamilies of Proofreading Exonucleases in the Acidic Protein Subunit PA of RNA Polymerase of Human Influenza Viruses, World J Rec Res Rev. 2022; 16: 804–824.

[31] Pinto MN, ter Beek J, Ekanger LA, Erik Johansson E, Barton JK. The [4Fe4S] Cluster of Yeast DNA Polymerase ε Is Redox Active and Can Undergo DNA-Mediated Signaling. J Am Chem Soc.: https://doi.org/10.1021/jacs.1c07150

[32] Pavlov YI, Maki S, Maki H, Kunkel TA. Evidence for interplay among yeast replicative DNA polymerases alpha, delta and epsilon from studies of exonuclease and polymerase active site mutations. BMC Biol. 2004; 2:1-13.