Novel Divergent Rhabdovirus in Feces of Red Fox, Spain

To the Editor: Rhabdoviruses (family Rhabdoviridae) are enveloped single-stranded negative-sense RNA viruses belonging to the Mononegavirales order. The International Committee on Taxonomy of Viruses recognizes 11 genera (Cytorhabdovirus, Ephemerovirus, Lyssavirus, Nurvirhabdovirus, Nucleorhabdovirus, Perhabdovirus, Sigmavirus, Spirivirus, Tibrovirus, Tupavirus, Vesiculovirus) (1). In addition, many recently described rhabdoviruses remain unassigned.

Rhabdoviruses contain 5 major genes, encoding for nucleoprotein (N), phosphoprotein (P), matrix (M), glycoprotein (G), and RNA-dependent RNA polymerase (L). The Rhabdoviridae family includes pathogens of various animal species, humans, and plants. Viruses of the genus Lyssavirus are the most relevant to public health because they can cause rabies. Bats are the driving force within this genus; foxes and various other species of wild carnivores also can be infected with lyssaviruses and transmit them to humans and dogs (2).

During a viral metagenomic survey, conducted as described previously (3), of fecal samples collected from 4 red foxes (Vulpes vulpes) that were found dead in Álava, Basque Country, Spain, we identified the complete coding sequence and the partial leader and trailer sequence of a novel rhabdovirus, tentatively called red fox fecal rhabdovirus (RFFRV; 15,541 nt, GenBank accession no. KF823814; online Technical Appendix, http://wwwnc.cdc.gov/EID/article/20/12/14-0236-Techapp1.pdf) by mapping 8,287 of the 56,519 sequence reads in the sample of a red fox. A proportion of obtained reads contained sequences that were ≥99% identical to mitochondrial DNA of V. vulpes, which confirmed that the sample was collected from a red fox.

The obtained sequence of RFFRV was partially confirmed by specific primers and Sanger sequencing of PCR amplicons. Five major and 3 minor open reading frames (ORFs) were identified that had a genome organization similar to that of other rhabdoviruses (Figure, panel A). No significant hits were obtained by BLAST analysis (http://blast.ncbi.nlm.gov/Blast.cgi) of N, P, M, and G nucleotide and amino acid sequences, which was reported previously for novel divergent rhabdoviruses (4).

Predicted N, P, and M genes of RFFRV consist of 1,629, 2,490, and 813 nt, respectively, encoding for 543, 830, and 271 aa (online Technical Appendix Table 1). In addition to the absence of significant hits observed by BLAST analysis, no significant sequence homology was observed with known rhabdovirus proteins in pairwise alignments. Furthermore, no conserved motifs were detected in N, P, and M genes of RFFRV that are commonly observed in rhabdoviruses.

However, intergenic regions between all major ORFs contained relatively conserved motifs that could be transcription termination/polyadenylation sequences (A/U) CUCU, similar to other rhabdoviruses (5). Adjacent to this termination signal was a stretch of conserved nucleotides that might function as a transcription initiation signal (online Technical Appendix Table 1).

The amino acid sequence of the G protein consisted of 669 aa and contained an N terminal signal peptide (1-MYHLIVLLVMLGQRA-VA-17), a noncytoplasmic domain (aa 18–646), a transmembrane domain (647-ITAILPPLLALVVG-IMCC-667), and a cytoplasmic tail of 2 aa, similar to other rhabdovirus G proteins as predicted by using Phobius and TMHMM (http://www.cbs.dtu.dk/services/TMHMM) (6,7). We predicted 3 potential glycosylation sites in the ectodomain at positions 38–40.
Between the G and L genes, a complex intergenic region was present that contained 3 ORFs of 246 nt (7,413–7,658 aa), 231 nt (7,716–7,946 aa), and 459 nt (7,893–8,355 aa), of which 2 were overlapping frames (U1–3). Additional ORFs between G and L genes were detected previously in other rhabdoviruses (8,9). We detected transmembrane domains in the amino acid sequences of all 3 additional ORFs, suggesting they might act as viroporin (8,9).

The L gene of RFFRV contained 6,591 nt (2,197 aa). We detected several conserved domains and motifs, including RNA-dependent RNA polymerase, mRNA-capping region, mRNA capping enzyme, and virus-capping methyltransferase. Alignment of the deduced amino acid sequence of the L gene with the L gene of various other viruses belonging to the Mononegavirales order by using MAFFT version 7 (http://mafft.cbrc.jp/alignment/software/) and subsequent phylogenetic reconstruction by using a maximum-likelihood tree (WAG+F+I+G model with 100 bootstrap replicates in MEGA5 [http://www.megasoftware.net]) suggested that this virus belongs to a novel genus of the Rhabdoviridae family. In addition, pairwise identities of the deduced amino acid sequence of the L gene of RFFRV with that of other rhabdoviruses of the Rhabdoviridae family were only ≤ 35% (online Technical Appendix Table 2).

Because the fox was found dead and no tissue samples were collected, whether RFFRV played a role in the animal’s death is unknown. In addition, multiple attempts to isolate this virus on various cell lines of eukaryotes (Vero E6, MDCK, CRFK, N2a, and BHK cells, primary fox kidney cells) failed because of the absence of cytopathic effects and viral replication by quantitative reverse transcription PCR, despite a high number of reads in the original sample. The fox might have acquired the virus through spillover from a small prey, such as a bat, and additional studies are required to elucidate the prevalence, original host, and pathogenic potential of this novel virus.

Acknowledgments

We thank all researchers and institutions for their invaluable help during sampling and for providing the specimens used in this study, especially Patricia Lizarraga, Laura Elorza, Ricardo Gutierrez, and Luis Javier Chueca.

This work was funded by the European Community’s Seventh Framework Program (FP7/2007–2013) under the project “European Management Platform for Emerging and Re-emerging Infectious Disease Entities” European Community grant agreement no. 223498; the Virgo Consortium; and the Niedersachsen-Research Network on Neuroinfectiology of the Ministry of Science and Culture of Lower Saxony, Germany. In addition, this research was funded partially by the Basque Government through the research group on “Systematics, Biogeography and Population Dynamics” (ref. IT317-10; GIC10/76).

A.R.-G. holds a postdoctoral fellowship awarded by the Department of Education, Universities and Research of the Basque Government (ref. DKR-2012-64) and was awarded a short-visit research grant from the ConGenOmics Research networking program of the European Science Foundation to visit the Department of Viroscience, Erasmus Medical Centre and develop the current research project.
Rogier Bodewes,1 Aritz Ruiz-Gonzalez,1 Anita C. Schürch, Albert D.M.E. Osterhaus, and Saskia L. Smits

Author affiliations: Erasmus Medical Centre, Rotterdam, the Netherlands (R. Bodewes, A.C. Schürch, A.D.M.E. Osterhaus, S.L. Smits); University of the Basque Country, Vitoria-Gasteiz, Spain (A. Ruiz-Gonzalez); National Institute for Environmental Protection and Research, Ozzano dell’Emilia, Italy (A. Ruiz-Gonzalez); and Viroclinics Biosciences, Rotterdam (A.D.M.E. Osterhaus, S.L. Smits)

DOI: http://dx.doi.org/10.3201/eid2012.140236

References


Address for correspondence: Rogier Bodewes, Erasmus Medical Centre, Department of Viroscience Dr. Molwaterplein 50, 3015GE Rotterdam, the Netherlands; email: r.bodewes@erasmusmc.nl

Ngari Virus in Goats during Rift Valley Fever Outbreak, Mauritania, 2010

To the Editor: Ngari virus (NRIV) is a single-stranded RNA virus belonging to the family Bunyaviridae, genus Orthobunyavirus. The genome comprises 3 segments, the small (S), medium (M), and large (L) segments, which encode the nucleocapsid (N) protein, the 2 glycoproteins Gn and Gc, and the RNA-dependent RNA-polymerase, respectively. Sequence analysis showed that NRIV is a reassortant between Bunyamwera virus (BUNV) and Batai virus (BATV), both from the genus Orthobunyavirus. S and L segments derived from BUNV, and the M segment derived from BATV (1,2). NRIV is more virulent than BUNV and BATV and is associated with hemorrhagic fever. NRIV was first isolated from Aedes simpsoni mosquitoes in 1979 and from humans in 1993, both in Senegal (3). During 1997 and 1998, humans were affected with hemorrhagic fever diseases in Kenya and Somalia that were caused by Rift Valley fever virus (RVFV) and by NRIV (2,4).

In 2010, during an ongoing RVFV outbreak in Mauritania, we collected 163 serum samples (62 from camels, 8 from cattle, and 93 from small ruminants) (5). RVFV RNA was isolated from serum samples as described previously (5). Further molecular testing of the samples was conducted by a SYBRGreen-based real-time reverse transcription PCR (RT-PCR) adapted from a conventional RT-PCR and based on generic primers (bun_group_forw 5′-CTGCTAAACCCAGCAGTACCTTTGGAC-3′ and bun_group_rev 5′-TGGAGGGTGAGAACCATCGTCAGGAAACTG-3′) that target a 250-nucleotide sequence of the S segment of Bunyamwera serogroup members (6). Real-time RT-PCR was performed in a CFX 96 real-time PCR system (Bio-Rad, Hercules, CA, USA) by using 5 μL RNA with a Quantitect SYBR Green RT-PCR Kit (QIAGEN, Hilden, Germany) in a final volume of 25 μL. Cycling conditions included RT at 50°C for 30 min and 95°C for 15 min, followed by amplification with 44 cycles of 95°C for 15 s, 55°C for 25 s, 72°C for 30 s, and 77°C for 5 s. A melting curve analysis was then performed starting with 95°C for 60 s, and a temperature gradient was conducted from 68°C to 94°C in increments of 0.2°C.

Of the 163 serum samples tested, 2 samples from goats resulted in a positive signal with cycle thresholds of 23 (sample 51) and 28 (sample 65), respectively. Both samples showed similar melting peaks at ~78.2°C and shared the identical partial nucleotide sequence of the S segment. The sequence belonged to the Bunyamwera serogroup, but the short partial sequence was not sufficient for accurate virus determination and identification. For this reason, both serum samples were used to inoculate cell monolayers of Vero E6 cells that were assayed for virus replication. Only sample 51 displayed a cytopathic effect after 72 h and was further analyzed. We isolated the viral RNA from cell culture with TRIzol reagent
Novel Divergent Rhabdovirus in Feces of Red Fox, Spain

Technical Appendix

Technical Appendix Table 1. Characteristics of RFFRV genes and intergenic sequences*

<table>
<thead>
<tr>
<th>Gene</th>
<th>Length, nt</th>
<th>Length, aa</th>
<th>Conserved intergenic sequence between 2 ORFs†</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>1,629</td>
<td>542</td>
<td>UAG-60nt-ACAAAAAACUUACCACCACUUG</td>
</tr>
<tr>
<td>P</td>
<td>2,490</td>
<td>829</td>
<td>UGA-75nt-UCAAAAAACUUACCACCACUUG</td>
</tr>
<tr>
<td>M</td>
<td>813</td>
<td>270</td>
<td>UGA-23nt-ACAAAAAACUUACCACUUG</td>
</tr>
<tr>
<td>G</td>
<td>2,010</td>
<td>669</td>
<td>UGA-70nt-ACAAAAAACCUACCAUG</td>
</tr>
<tr>
<td>U1</td>
<td>246</td>
<td>81</td>
<td>UGA-25nt-ACAAAAAACCUACCUGUGUGUUAG</td>
</tr>
<tr>
<td>U2</td>
<td>231</td>
<td>76</td>
<td>NA</td>
</tr>
<tr>
<td>U3</td>
<td>459</td>
<td>152</td>
<td>UGA-116nt-ACAAAAAACCUACUUACUGUGUUAAUGA</td>
</tr>
<tr>
<td>L</td>
<td>6,591</td>
<td>2,196</td>
<td>NA</td>
</tr>
</tbody>
</table>

*NA, not applicable; ORF, open reading frame; RFFRV, red fox fecal rhabdovirus.
†Start and stopcodon of the flanking genes are underlined.

Technical Appendix Table 2. Pairwise amino acid identities between the L protein of RFFRV and other rhabdoviruses

<table>
<thead>
<tr>
<th>RFFRV</th>
<th>LNYV</th>
<th>BEFV</th>
<th>RABV</th>
<th>IHNV</th>
<th>RYSV</th>
<th>PRV</th>
<th>SIGMAV</th>
<th>VI208</th>
<th>TBIV</th>
<th>BASV</th>
<th>FARV</th>
<th>LBV</th>
<th>SHIBV</th>
<th>OZEV</th>
</tr>
</thead>
<tbody>
<tr>
<td>RFFRV</td>
<td>27</td>
<td>31</td>
<td>34</td>
<td>23</td>
<td>27</td>
<td>33</td>
<td>30</td>
<td>32</td>
<td>31</td>
<td>30</td>
<td>29</td>
<td>35</td>
<td>35</td>
<td>35</td>
</tr>
<tr>
<td>LNYV</td>
<td>NC_007642</td>
<td>27</td>
<td>25</td>
<td>30</td>
<td>23</td>
<td>35</td>
<td>25</td>
<td>25</td>
<td>25</td>
<td>25</td>
<td>25</td>
<td>33</td>
<td>30</td>
<td>30</td>
</tr>
<tr>
<td>BEFV</td>
<td>NC_002526</td>
<td>31</td>
<td>25</td>
<td>45</td>
<td>24</td>
<td>24</td>
<td>60</td>
<td>60</td>
<td>57</td>
<td>57</td>
<td>52</td>
<td>25</td>
<td>46</td>
<td>46</td>
</tr>
<tr>
<td>RABV</td>
<td>SRV9 AAT48626</td>
<td>34</td>
<td>30</td>
<td>45</td>
<td>24</td>
<td>24</td>
<td>46</td>
<td>46</td>
<td>44</td>
<td>46</td>
<td>44</td>
<td>40</td>
<td>29</td>
<td>86</td>
</tr>
<tr>
<td>IHNV</td>
<td>L40883</td>
<td>23</td>
<td>23</td>
<td>24</td>
<td>25</td>
<td>22</td>
<td>24</td>
<td>24</td>
<td>23</td>
<td>23</td>
<td>23</td>
<td>27</td>
<td>27</td>
<td>25</td>
</tr>
<tr>
<td>RYSV</td>
<td>NC_003746</td>
<td>27</td>
<td>25</td>
<td>24</td>
<td>28</td>
<td>22</td>
<td>25</td>
<td>25</td>
<td>23</td>
<td>23</td>
<td>23</td>
<td>30</td>
<td>29</td>
<td>29</td>
</tr>
<tr>
<td>PRV</td>
<td>HM56195</td>
<td>33</td>
<td>25</td>
<td>60</td>
<td>46</td>
<td>24</td>
<td>60</td>
<td>74</td>
<td>54</td>
<td>50</td>
<td>50</td>
<td>26</td>
<td>47</td>
<td>47</td>
</tr>
<tr>
<td>SIGMAV</td>
<td>Q410979</td>
<td>30</td>
<td>25</td>
<td>57</td>
<td>44</td>
<td>24</td>
<td>24</td>
<td>60</td>
<td>59</td>
<td>52</td>
<td>48</td>
<td>23</td>
<td>46</td>
<td>45</td>
</tr>
<tr>
<td>VI208</td>
<td>AAA48441</td>
<td>32</td>
<td>25</td>
<td>60</td>
<td>46</td>
<td>23</td>
<td>74</td>
<td>59</td>
<td>53</td>
<td>51</td>
<td>27</td>
<td>47</td>
<td>46</td>
<td>46</td>
</tr>
<tr>
<td>TBIV</td>
<td>Q024472</td>
<td>31</td>
<td>25</td>
<td>57</td>
<td>44</td>
<td>23</td>
<td>23</td>
<td>54</td>
<td>52</td>
<td>53</td>
<td>52</td>
<td>24</td>
<td>43</td>
<td>43</td>
</tr>
<tr>
<td>BASV</td>
<td>X297815</td>
<td>30</td>
<td>25</td>
<td>52</td>
<td>40</td>
<td>20</td>
<td>50</td>
<td>48</td>
<td>51</td>
<td>52</td>
<td>51</td>
<td>23</td>
<td>41</td>
<td>41</td>
</tr>
<tr>
<td>FARV</td>
<td>HM627182</td>
<td>29</td>
<td>33</td>
<td>25</td>
<td>29</td>
<td>27</td>
<td>30</td>
<td>26</td>
<td>23</td>
<td>27</td>
<td>24</td>
<td>23</td>
<td>28</td>
<td>28</td>
</tr>
<tr>
<td>LBV</td>
<td>JX901139</td>
<td>35</td>
<td>30</td>
<td>46</td>
<td>86</td>
<td>25</td>
<td>28</td>
<td>47</td>
<td>46</td>
<td>47</td>
<td>43</td>
<td>41</td>
<td>28</td>
<td>92</td>
</tr>
<tr>
<td>SHIBV</td>
<td>ADD84511</td>
<td>35</td>
<td>30</td>
<td>46</td>
<td>87</td>
<td>26</td>
<td>29</td>
<td>47</td>
<td>45</td>
<td>46</td>
<td>43</td>
<td>41</td>
<td>28</td>
<td>86</td>
</tr>
<tr>
<td>OZEV</td>
<td>FJ905105</td>
<td>35</td>
<td>30</td>
<td>45</td>
<td>88</td>
<td>25</td>
<td>28</td>
<td>47</td>
<td>45</td>
<td>46</td>
<td>42</td>
<td>41</td>
<td>28</td>
<td>86</td>
</tr>
</tbody>
</table>

Red Fox Fecal Rhabdovirus, Partial Genome (GenBank Accession No. KF823814)*

GGATATCAAGTCCACCAATACCTATATATGTGATGTCATGACATGCACATCATTCTACG
CATACGACTCCAGGAGTACACTAACAAAAAGACGTAACACAGACTTTGGAATTACGT
CAAGTCTAGATTTAAATTGCTGATTTGTTCAACTGATACGATAACGAAAGCAAT
TCGCTACTCTTAAATAGGCGGTAAATGTGCGATATGGGACACCACCATTAAAA
CATCAAGGTTGAGCCCATCACTATCTATTGGGAAATTCCGCTGCAACAGGATAGCAGC
GAAAGGGGGGGCAGAAAGACGTGGGAACGAAAGACATGATTCCGTGGAGTGAAGATG
TTCTGCCCCAGACTGATGCTAAATCTGCTCAATCTCATAGCCGGGAAACCAGAGCC
CCTGAGCTTGAGAAGATCACCATCCAAGATCCGGAAAAAGAGGGCATTTGAAGAA
ATTGGAAAGCAAGTGGGGAGTTTCGCAATTGGGCAAACCTGCTGGTGCACTTGCAAA

*Article DOI: http://dx.doi.org/10.3201/eid2012.140236
ACTGACGACACCAAGAGAAGCTAGAAGAAGGCTGAGCAATCTTGTCGGATCTAGG
GAAGAAGTGCGAGCTATCTGGATTGGCTGTATGCGGATGATAGCAACCTAGTAG
ATATGATGTCGCGGCTTGGTTAACGCGCTTCTGGGCGATTGGGATCGGAGATCTATGG
GTGTGTGGAAGCGCTTTGGAGTGAATGAGAAACACCAGTCTCCTTTTGATGAAAGTGA
AGCGCATTGGAGGAATCTCTGCAGAAAAAACCAAGCCACCTGAAAGAGATGAGTC
AGGTTATTCCGGAAAGGAAAGGGAAATTGGCGAGCAGAAGGTATAAAGAGTGA
GCACCGTTCTCTACTCAAGTGATCTCCTTTCTGGCCAAAGGGGAGCGAGGTGGC
CGTGGAACTCCCATCTCTTGATGAGTGATCGGTCTTCTTCGAAAAGGGGAGAGGC
TGCAGAACTCTTGACACGACACCAAGGAGTTGGAAAGCGCATCGACTGAACAGACG
TAGCCGGATCGCCAGCCGACGCTGAGTCGATCAGTCAGTCGCTGATTATCAGGGAGATC
AGAAATTACCTCTCAGTGGCTCTAGGCTGAAGGCTAGTCCCCACATGGCAGACAAGTAGAT
GCCACTCTAGCCAAATCCGGGCATCAAACCTTAAGCAGTAGGAGCGCAAGCGCCTCTCC
CCCAGATGGAAGGAAACCGGAGTGGAGCCCAAAGGGGATGATCTGAAAGGAGTAG
AGTGGCGCAACCGAGATAGTTGGAGAAATAGAAGGGTTAGCAGGAAGAGGCTGCTGC
TTGAAATGAGAACCACAGAGAAAGGGAGCAGCCTTTCAATCTGATCACAACCTCTGTGAA
AAATTACGTAGCTGGCAGGTCTGGAGCTTGGAAAATCAGAAGTCTCTCCCAA
GCACCGGAGGCTGCTGGATCGGCAATAGGCCACTCCCATAGGCCCAAGCAAGCAGGA
CAGTTATAGGATTCTGTAGGAGTTGGCTTCTCTTCTACCATCAAACCTCTAAAGTCCGGA
TAACGGGGCTATGCAAGACAGATGAGCCGACATGCGATCTGGAAGCAGGATCGCAG
GGATTACCAGCCTGTACCCTCCCCTCCCAGTTGTACCCGGAGTATTACTGCCGAGT
TCTTCAAAAGCTCTCCTTCCCCGAGAAGGCAGGAAGGAAATTCACCGAGAGTTCCG
ACTGATGTTCAGCGGAGTACGAAGAGGCTAGAAGAAGAGGCTCATTGAGGGAG
CCAAATGTTTGACACGACATCTGGAAAGCGATTTGGGCAGCCCGATACTA
TTGGGGTCAGTGATGGGATTTGGGCTGTACATTACAAATCTCCTACCTAAGAG
GGAGAAAAAACCGTCACCAAAAGGAGGATACGCGCCTCCACCTCCATCGGCAC
CTCGAAAAGAAGAGCGGTAATAAATTCAGTGCAAGCTAGGGCAGGCCATAGCCGAC
ACCAGAAGAGTGCTCTGCACCCGCCACCTCTCTAGAGCAGAAGAAACCGGACCCCAAGAG
CTGGATGACTGGGAACACATCAAGGGTTATATCTGCGAGATCGGATAGTTATGG
AATTTCGCAACAAATGGAGGGGAGCTTGCAACACTATTCCATTACATCATGTGTGTGCTGGAG
ACAAAGTTCTCGAGCCATATAATCTCTAAGCGCTAGGGGATTTGCTCACAAGAAGCAGCTAA
AGAATCTTCTTAGGGTTGTGTCAACAACATGGGACAAAGGCTTCCTCCAGCTTTTGATG
AACATTTGACATTAAGAACTACAGAGCAGATCTCAGCTTTTGACAGCTAATTGGGCCAA
TGAAGATAAAAAGCAGAGATGCCAAGGTCCAATGGGAATATACTTCCACAAGGCTAGGA
GAAGCATATGGGATGCTTCTGGGAGGCGCTGCAAGTTGCTACTGCTGAGGCAAGAG
AGGGATTGGACAAAGGATCAGGCTGCTACTAGTCTGGAAGGCTAACACTAAGGCAAG
GGAAAAGTTCCTCCCCCTGGCTGGTTTGTGCAACAACAGAGCAAAAGAGAGCGACAAC
CCCAGCGGCTGGCAAGAGAGCAAGTGAAGGCTGGTTTTAAATCCTAAATGAAATATATTAG
TCTAACCCTCTGAGTGTGATCTTCTGCTCTATATTAGCTTACAAGTAgATGTCTCCCTCTC
TCATCTTTCAAAAAACTAACAACAAGGCTGGAATATGAGCAATTGTACAACTCTTTAGA
CTCTGATGAAATTTGCGACCAAAAGATGTCTTGTGACATCTAATAATCTAAGTATAGGTA
tTGGAATTGTTGACGAGAATGGAAGTGGTAATGCTCAGAGGGCGAGGAAGACACTGCTG
GACACAAATGAGGAGGCTTTGCTTTATCTCCTACATATCCTGAGGCAAAAGAACATATAGT
GAAACACTGCAGCGGAGGCTAAAGAAAGTGTGTTCTGCTATATATCCTAATAACTTTGGA
GTTCTGAGCCTCTTGTATTTCGGAAGATGCGCACATCGCATCAGACATTCTTGAC
GGAATGGAAATGTCTGCAATGAGAAAGCTGGTCTTGCAAATGATGATCTTGTCAACT
AGACCAATGTTGGAGTTTCTCTGATGTTGTATCCGAACTAATCCTCAGTAAATTGT
AGCACCTCTATGCTTTCCGCTTCTAGTGGCAGAGTTACGTACATGATTACGGGGAAT
TTCTGTTGACACAGCTTTGATGACAGAAGCATACATTGGGTGATACAGACATGACATT
GAAGACCGGATAGGCTCAATCATCAATACCCGCTCCCCTGGTTTCTCTAATTGCTTCT
ACACCAACGGCGCTCAAGCCAGACTTCCACCCCCGGACTAGCAGTCGAGAGCTAAA
GGGAAGTTCTTTGCTATCATTGGAGACAAATACATTACGCTTCAAAACGTATAGCGAC
AACACAGTGCTATTTGCTGATGCGGGCCGTGGCTCTCAAGGCAATTAAGCAGAAGTATTA
CCGCTCTGACTTGGATTTTACAAAAAATCAACTAATAACTGTGGTATATAATGTATCA
TCTAATTGTTCTCCTTTGATTGCTTGAAGACAGGACAGTAGCAGAGCCAGCTGACAT
TGAGAGCCGAGCCAAGATCACAGATGAGAGAAGAGGCGTGAGAAAATAAGACG
GGTCTGAGGATTTGAGCTGTGCTTGAGATCCGGCTCAAAGCGCAGCATCCCCACC
TGATGTCGCTCGAGCTACAAAGCGCGCTGCTACATACAGCTGCTCGG
AACATCGCCAGATAAGGTTGGGTTGCTGAGTCTGAATACCTGCTGAGTTGT
GGAGCTCTTTGCTCACCCTGTCTGTGAGAGTGCCCAAGGACACTGAGGGATGTAG
TGCAATCGAGATGCTCCAGGAAAAGGGGATGGCAAAAGCTGACATAGAGTGGCAAGGAACA
CCCAATGGGAGATGATCAGTCTCCGGTTTATAACAAAAATTCGGAATCCGGCTCTCCCTGCA
GCCTCAGCCAAAGCAGACCTTGATCAAATGTACCGAATGGGTGACATCATCCTCGCCAC
AGAAGGAAACGAAGGGGTACAATGCAATCAAGACAATAGAAAGCATCATGGTCGGTT
CTGCACATAAGCACTAGAAGACGAGTCAATTTCCCTACAGGTGACAAATTCTACG
AGTTTATCAGGAAAAGAAGGGCTTTTCAGCTTGCCATCTAGAATCATCGTCTAGTGTCA
TGAGAAAAATCATCCATATAGGGGAGATAGTAACCCCTGAAGTTGCCATAGAGATG
GCAGGATGCTACAGACACTGGGGCCACCAATCATCAATCCTGGTGAGGTCTCCTACT
GCAGTCCGAGAGAAGCGAACTGCAGCCTCTAGCAATGAAAGGCATGAGGCTACAGA
ACTAGCTGCTGATCTCAGATTCTATTTTTGTGGTGGAGGATTTTTGGAAGAGCATGCCAG
GTGGCCCCCGGGAGTGAGATATGAGATGAGCCGGCGACAGATGACAAAGACCTGGTCA
ACAAGTGGGTATCAACTAATCAATTCTCGCCCTGCTACCTCAGTGTCAAGTCAGGA
ACAGCTGGTTTTTCTGTAACATATGACAGCTTATTACGACAAAAATCAAGAAATCCAA
TCCTCAGCCTAAATTCCAGAAGACTCTAGGCTGATTCCCTGCTACTCCGTAAATCAACTC
TCAGTATGCAGAGATCTAGTGAACAAATTTTTGGACTCTATAGACAGCAGCAGGA
AATGGTTAAAAGCAAGCAGACACTTAGTCATCTATTGCGAGAAAAagGCGAGAGCTC
AAGGTAAAAAGGCCTTTTTCCCTATGAGCAGTGACTAATTGACGGGACTTATTTACAA
GCAACCAATACTCAAATTGCAAAGCACATTCTGCGCTACTCCGTAAATTCACATTG
ATGCAGGCGCAAATTGACCTGTGGAAGACTTTCAAAAAGGTGGTCGGTACGCCTGTGAG
TCAAGAGAAATGCTACACATCACATGATCATTGGATTTTGCAAATAAGGAAACACTT
CCAGAGAGAAGAGTCTCAGCCTGGGTTTCCAGATTATGGACAGAGCCTCTCGGGTG
GTCAAAATGTCATATCAGGGACTCATAACTTCTCTTCTCAGGTCGGGCTATGCT
GGAGAAATATAGATATGTTCCTCATTCCGTCTGACAGCAACAATGGCCCTGTGTTGAGC
AGCACAAAAGAGGCTTTTGAAGGATCAGACAGAAGGGATGGAGTGCTGAGGGG
CTTTGCTAATACGCGACTGAGTCTGAGCTCAGATCTGGTCTGATGGGAAAGTGCATAATT
AAGGAGATAACCAGATTACATACCTTGAGTTACCCCTTGGCCAGCTACCATGAAATCTT
CATCGCTTTGCTCGAGAGCGCATAGGCATTGCGAAAATGCAAAACTCAGCTCTACTG
TGTTTCTGAATTGGTCAGAGCAGCTCGGATTAAGAATTAAGCCGAGAGAGCCATGG
ATATCCTCGAGGATGGTTACTACGGGAAGTTCCTGTGATCGACGGATCGGCAAAGA
GGAATGGTGCTTAAAGAACCTCTCGAGGATCCTTTTGGCAATATCAAATGACCTACTCCT
AGCATCTCAAATAGCATCTTTGTGACAGCTATGAGGCGGCTGTGATCCAAT
CATCACAAGCTGCTCCCAATTACATGTTTTCGCTATTTCCTGCCTGCTCCAC
GACTTCTTCTGAATACACAAAAACCTTTGTCCTCATGGGAGCGCTATCTTTCCATACATTCCAGACG
GCTTTATTCGGAACAAACTGCTGTCTGTGGAGACATTACCCAGGCTACAGGGCCCCACAACC
AAGGAATCTCTATCATCATTTTCTGTAGCAAGAGACTGGGCCACCAGCAAGCAATAAGCA
GCAATCGGGCTCTCGGACTAGTACGACCTCCTCAGTCAGGCTCTCTCTTGGAGGTCTTGG
AGGAGCATCATCATTAAAAGTATATGATCAGGCGAGTTTTCTGTACCTCTGCACAGAGGG
CCTACATTGCAAAAGTGGGTTAAGCAAGGGGCAAAACACCTGAGCTAAAGACCA
TTTTCTCCTTTATGGAAGGCGCTTCCCCATACAGTCAAGGCTGTAGTTAGAAACTGCTAG
AGCAACCATGTCGATCAATCTGCAATCTCCTACTCGTCCTGTAAATGATGCTGCAGCG
GTGGTTGAAAGAAAGACATACACACAAGCATGACACGCTGCTGTTAACATGACTGCTG
TGATACGGCGCTTGTCAAGAAAGCAAGATATGCAGGACCCCTCTCTTGAAGATGTGTG
CATCCCTACAGCAGCATCTTCTTTTTGCTCTCTCTCTGTACAGTGCCACCCCCAT
AGGAGTGCGAGAAACAAATGTTGGAAAAATTCTCTCAGGACTAAGACTTTGTTGCCAGA
TTGCTTCTCAGAGAGGGTGCGAGGCGATCGGGAAGAAACTAGTGAAGAGCGAGGT
GAGTTGAAACAACGTATCTAAACAGTCTGCTCGTCTGAGCATGAGGGTT
TGTTTCAGACAAATTGGGTCTGCAAGCTATAAGTCTGGGAAAGTGGGGAGGCGAAATTTG
GGGAGTACAGTGCCTTCACCTGCAAGGCTATTATTGACTAGTGAGTCGCGCAATGACAA
ACGCGGCTCTCAAAGGAAGCCCTGTCTTGGATGATGATGTGCTGCACAGAGG
ACACAAATTGACTCGGATTAGTGATTCGCCGACGATGCGTTTTGTTGAAATCGACAGTCC
GGGAGTTGGGACATACGTTTCTCAGATCTAGAATGCTCTCTCTGGGAAGCGAAATGCGCC
ACCAAATACATAACACTGCACACTCATATTGCGTCTAGTCACAAACGACTATTAGTGAGGGTT
AAGCGCGGAGGGAACAATATCATATTGGTTTGTCCAAGGGCTATTTCGTTATCTACA
AGCATTTAGGGGAGCTAGTGAAGAGGGAGAAATGGGCTCGCTCCAGCCTGCCACCT
TCACCCCTAATTCGGAAGAATGCAGTACCAGTGCCAGTGTTTCAATGGAGCCTTC
AAAACCCGGTATGCCCCGTTTATCAGTGGTTTTATCACCCTGCCCTCCAAGATGTTT
TTAGCTTGGCAGGAATCTCTATGTGAGCTCAGTTCAAGAATCGCTCAGCAATGACTGCTCAG
CCTAAGAGACTATTGTCACACATCGTCTGCTGACAGATGTCAGTCTCTGTTGCCAGACC
CTTCTCGGTTGAGGAGATATCCAGATGCTATTATCCAATGTTTCGCAAGCAAATCATAGCAGTA
ACAAAAACAAATCCTCAAGGAAGATGGACTCTGGGGATACGAACTGCTCATCCCGGT
GTATGCGAGGTTCAACAGTGCTCGTGGAAGGTACTTAAGAGCAAGTTTGATTCTGGAC
TTTGCTATGCTTGTCATTCGGACACTACATTGAAAGCAACGACAGCAGTCACGTCATGCT
GGCTTGTCACGACACTTGAAGAGCACTGACAGCCACATCGGCAAGCCACAGGACT
CAACAAATGCTGGGAGGGGATGCTGAGCAGACGACAGGAGGACTGCTAAGCTGCTAAA
GAGCAGATTCGCTCAAGCGAGATTTCCGATATCGCTATGGAACTTCTCAAGCGCA
GCAGGACAGTCATTATTCGAGCAGTGTGTGTCTCCCATGGAAAGAAATTCATCTACAA
GAATTCCGAGGGAGATGATAGCTCTCCTCCGGAGAGACATGAGAAGGGAATGAACTCC
GGCATATTATAATCGGCTGACATGATGCTGTATGCGCCGCTTCTTTCAAGGAGACACAAC
GGGACCACTAAAAGCACTTGCAGGGGCTGACACTGAGATGAAAAACTCTGTATAG
CAAGCCAGACATTGGAAGAACGAGGAGATCTGAGCAGGCTGCTTGTAGATATGCTT
CAGAAAACACATCAAACCTCTTGTCGTGCGTGTGACTGACTCTCAAAAACACTAACA
AAAAGGCTGCAAGACGTGATCATATATTCTCCTCCGGGAAAGCATACCTGCTATTTTCACTTT
GATACCCGTCAGAGGGCTACTGTATATGGAAACACGCACTCAACAAACAAAGGGCC
TGAATCTCTTTACTTGGAGAAGAGGGAGGAGCTGAGGGAGTGGAGACAAAACCCCTATCA
TGCAATCTTTCCGCCATCTTAAATGCTGCTAAGCGTTCAATACAGATTGCTGTCTGT
CTTGAATACATCCAGCTTCAACCAAAATGTGTCAGTGTGCGGAGATGTAATG
GGGATTTTTGGAGGGCAGATTATTGGGCTCTACCTACGCTGAGTTTTTTCAATTCT
CCTATCTGAAATGCTGACTACGCAGCAGAGGGCTGACCAAGGAATTACCCGCTTG
TGTCGTAAGACTCTCAAAGGTATGCGGAGGATGTCAGATTCCGGAAGATGCTTTAAC
ACTGCTTTCCAGACTTTGTCCTCAGAGAGAGCCTCGAGGTAACGAAATACCTGATG
GAAGAAATGCTTTGAGACGCCAGACCTGCTGCTGCTGAGGAGGACATGAGATGATTCTAT
CAAGAGGTAGGAAATCTTGGACACCTCCTGTTAAGTGGTCCGACAGCGCTCTCAG
TCATAATCAAGCCACTTTGCTGACAGTGACAGGAGGCTCCAGACGTTGGCTGCAATGCGC
ACTTGCAATTCAACATGTTGATTGACTTGGACAGGGACGTCACCAGACGTTGCTGCAATGCGC
TTGAGGTGTACTTGTGAGTCACTCGAATTGAGAGAAAGACAGATTGAGGTGATGACAC
CAGGGATACATATCATCATGTGGAGCTAGACAGCTGAAATCCACGTCACCCTACAGCTC
AATACACAGCTTCCACAGCTGGGTGGTTTCCCTACCTCTGAAAGAGGAGATAGACTCC
GAGCTGTCAGGCCGAGTCAAGCCTACGAGCCACATTAGCAGGATAGAAGATA
AGACCAGAGAGCTGTCAGCGAGTAGAGAGGGAAAGCAAAAAGAGCAGGAGCAGATGG
GGTTGCTGAAATTGCTGCTTGCACTACTTAAGGATCACCACCTATCATGCTATGAACA
ATTCTGTTATCAGGGAACCTCACATGCTGGGAGATTGAGAGCCAGCTTAAAGCGATCGGAGCTGATCGGGCTAATAGGGACTGCTATCGGTGCCTCATGCTTGGTGTCAGCCA
TTTCTCATCCGCATGATTACTGTTTGGTTCTTTGGAACCTTTACAAGAATGGGGTGGATGTGTTTGAGGCAATGATAAAGGGAATATCAAGCCAATTGTGGTCAGAGCCTGGTCAG
GTGAGTCCACTCCTGTAAACTATAAACCACAAAGGAACCTCAAAAAGCCTGTTCATTCTA
TGTTGAGATACTTGGGGGCTTATTGTTGAGCTCTGTGTATTTGATAGAATCATCACTCAAAGGGTACTGGGCTAAGAGAGGATGAACATGGCAGTAGCCACTTGGGAACCGATAC
ATAAGATGGGAGATGAACAGCTGCGTCGAAAGAAATCGATTTCCGGGGGATAAAT
TGATGAATAGCACTTTGATTCTAAAAATAGTATCGAGAAGAAAAACATATATACATATAAAATTAGGAGAATAACTGAAGATCAAAGAGCATTAAAAGAGATCTAAGAGAAAAAGAGAGAAGGACCTGGCAAAAAGCTAAAAGA
TAGAAATAAAGGAAAACAAAGAATAATTTCCTGAGAGGCCAGCACATATAAGGATGATAGG
GATCACATGGAAGCCTCGGAACCTTGAAGCTTTGGTAAGACTAGGAGATGTGGGT

*Coding sequences of identified open reading frames are underlined; deduced amino acid sequences below, overlapping sequence of open reading frames U2 and U3 gene are in italics.

Deduced Amino Acid Sequences RFFRV Genes

N Gene RFFRV

MDHDNEKPSYTSIAEVPDNVAIGSTIYIQGEPIIYFGKSAATGTRKGGAQKDWTKDMIR
GVRVFLPQTDANLLNLIAGETAEPELEKYTIQDPEKKGILKFKFESKWEFANWANLLVDL
QSNTGNIPKGRFPYYSALFSIAIKGAPVLAPAMKDLDGVYVKAPDDLHPLPTGIEWH
GDKISVDEAYIYGAWLIMPRTIKAESKEKDEIAASSKAFTDLRRLLPEITKPVQLSVVV
TQLRLAYHGTCLVPSAYLAAEVAMMRAMNIEYDLKADRECKAGEHFPGCQLRVLQDI
PQYDSEGFWFGFFQVSVLMAEMAGYSALNMHLAGLDIYGTKTIADLRMLINWRCDYNIADEIK
EGPLADDPWRAASYLAPNRITPLSMGKHSIVAYLGLSIQSSAAANISTGAPSPPEGVKM
NELIRKTVDIHAVAVSEWDNDRLQPSTVTTVMIGGQVIPFKGVDPKRVNDLSRMFTQR

Page 10 of 13
QTPLYEVPPHNQRERSPSVSSVHTSSRRDDEGSWEGGNEELLRKLHERRGQYEDTNLGGFYSAT.

**P Gene RFFRV**

MANRVRKIHSVVNDPAEAWQVALQYWAKRILSTDKELLCRITKSMVAHMSVYYGNVKLDCDAVWRVWALLALAWMKSPVDGIALMLALLEFGAKHVQRLRADLSKIGDPRIDDFERLTNTSIARVPKKFKKENSITDTDDTKKLEKAAAILSDLGKKSCTGFCCYADDSENSVGDMMSRVLTASGALGSEDPIGVWKPLELDEKAPYLDEVKRIGGISAEEKSPSPEKKCMRLF

GKKGGKFAAPETSYSAHFPSFSTQVSSKEPPKLKVRGVELPSLDEVIGLLLPKGAAEAELLDDE

TKELEGASAEPELADEHPQLTQSRSDYQGDQNFSTSSVCLKASPPHGSHIDCDSSQIGASNLARASALSPEWKEPDGATKGDDPEEVECANEIVVGGNRRVSEEGCCLNEDTREETGSQLS

DQHPVKNYSSWAERASLESEVSPKHAEEAGSAIGHSHSPQAEEVMDSVGVVASSTIKPL

KSVGTRPMQTDPEHAILEAGSQQITSYLYPPLPVPGVLLLPSKSSLPPAKGKFTEEEGLMLRGRRLGEERLWNVEAKWFDTHLEGIAFKFPGDPDTIGFQLGSYFAQLITNPYLLKREET

KPSKEDHAPPSSAPPRKESVNVKFSQARPSHSQVPTKEDPAHPHPRAEEDPRSWMTGKPSRVYLPGSDDMVFRTNEAELDNFITSCVLEDKFTEPYILKPKELSKEQLKNLLEVVSQH

QKASQLLLCEHLTLRNYKISALTANWAMKIAEMPRSMGIFTRGLGEAYGNGAFWEALQVATAGREGIDKWIRAALVVKANTARGKVPPGPWFVTRAKSDNPSVRARASALFKFNE.

**M Gene RFFRV**

MSNFRYTLMKFASKMSLTSKSKYKVGLGDEGLQSNVNIHEEGEDHTSIYSESPSSKK

KTSRTMRPWVYPTYPEQKNIVRGTLRGARPKKVVLISNLTGVSEPLDFPEVATVSDILD

GMEMSAMRKLVLQMMLSTRPMSGFSDGCGRYSFSCSTCFPSCLGEGFMHDGDFCSTTFDDKTYFGYRLTFEDAIGSNHQYPLLWFHLSYTNALKPDFTPGLAVCRAKGKFLYLDKKYFSFKTYSDVINLLLMGRPKAICKQK.

**G Gene RFFRV**

MYHLIVLLVMLGQRAVAEPAIDARAAKIQYAEERLRNKTGLRLELSRDPQAQSAASPPDVPAKQPATKPAATTAAGTSAPIKVGLPESEYLRQVPFWFTVCESKVPTLTDVVRHL

RKGDKGAHKMLRGTPMGDDHSVYITFSGISAPSRLVVLSESLQEVQRPQLYRVSRLR

SSSTPVRVVLCEVMSAFSPLNVEEMCTGVLGKLSDTSSVTNLNVDPGYVLTMIRVCSC

SAMVRKRWWTWTSFFNVKERLEDWRIPPPDPECESHCEPIFAEVTSAVRGSPDYCYA
U1 Gene RFFRV
MSCLTQDLRKEIREVRVSNSISIQPSVTTLIIIEVILWVGLAYISIALGCHRYLQARIKNSVE
RRQRFRGDGVVLLPDTGPA.

U2 Gene RFFRV
MEEKCSDSFYKELTDAAIEGVTWASPLYTILTAVIIFLILLIFVVAWRAAVIAKIRHRIDE
STARLRPDPFGRGRP.

U3 Gene RFFRV
MSPQHGAALISGVDHPDHFSLIESYPILVQNVAATILVTWLVSFLATDIDFSSVNNLT
DIRGVLIYHLGNEVSEFASHALACVSAQSTSARLTRELEQFRLSGQVTEVTPSSGATGE
LSIPQRYRMLLEKDMLENEMFIAEHSV.

L Gene RFFRV
MEKSFLFDRIPSVLHSLPLREELCFCRDPDKETDYPALIPQPDEFTISRRPELIELRSLTG
AISGVCWCIKLYMRLNWIEAKDMMRAJAEYYQSSTHPEIASDFNVEGLFHSRLDQ
WGENQHPALSQDIWVFAQYSQDAIDNWTYRILAALLWSNHKERGQNQSEFQNSSII
AKAGWGVRAINGVEVEHHKLTKRLGKLTVGAYWCLCELANQPRELRSLLLCEK
DLYLQRWISLFLNCQNAFEGGIPQPKHMLIKYRMGDKIATEGNEGNAIKTIESIMVG
SAHKLQLEDIEPTGDKFYEFITXGLSACHLESSSSAMRKIIHGEIVTPEVAIEMAGCYR
HWGHPINPVGGLTAVRENATAQPLTNERMISLAADLYLLLRSYFEHEHRGWPPGVRY
EAAPEIAKDLFKWSTNPQPGPTSAQVRNWSWFFVYDLSDFKQNEIPILSLISDHSV
GRSALSTMCLKKNLLSSPSRRLQSTLSYAEIDVNNFKLDSIDSTENGLSNDDLVLLEKE
RELKVGRFSLMTYKLYTFTATELYIAKHILPVLPEITMMQQGQIDLWKTFKGAVRTV
SQEKSTHMIHDFKEKWWNFQREESTOPFQIMDRAFWSNVISRTTHNFFSRCFVGYAG
RIDMFPIGLTNWPWCWTGHKGGFEGLRQKGSVGVALLIRHVMRLTGLHGKVIQG