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Research Article

Feline calicivirus. Molecular Detection with Primers Design

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Received: June 01, 2020; **Published:** June 25, 2020

Abstract

One of the infectious agents causing the Feline Viral Respiratory Complex is *Feline calicivirus* (FCV), participating in one of the main diseases that affect domestic felines, causing high morbidity rates and low mortality, mainly in kittens.

FCVV specifically affects the *Felidae* family and mainly the domestic cat, however, infection has also been described in wild cats. This virus is small in size (27 - 40 nm), uncovered, with an icosahedral capsid and a single-stranded RNA genome, positive polarity and approximately 7700 base pairs. This genome has three Open Reading Frames (ORFs) and ORF 2 contains the gene that codes for the VP1 capsid protein, a detection target in this study.

The diagnosis is made mainly based on the clinical signs of the affected cats, however, its interpretation is usually doubtful, as there is a low correlation between the symptoms and the presence of the virus.

Although the presence of FCV in Chile has already been described, its detection based on the primers design in line with the information available in the Genbank® is still a viable alternative when considering the geographical distribution of the different strains of FVC already described.

Thus, the alignment of 10 VP1 sequences according to their geographical distribution yielded a single common nucleotide zone that was used as a candidate sequence for the primers by the OligoPerfect Design software from ThermoFisher Scientific®. The RT-PCR protocol used was effective, since on the one and clear DNA band were obtained without nonspecific amplification in positive controls and no amplification in negative and reagent controls. The utility of the primers was reflected by the BLAST software.

Therefore, it is suggested to subsequently use the protocol in real samples, as a complementary diagnostic alternative in national Veterinary Medicine.

Keywords: *Diagnosis; Primers Design; FCV; RT-PCR*

Background

Feline calicivirus (FCV)

It is one of the most frequently reported pathogens causing oral and upper respiratory disease in cats. The virus has a worldwide distribution, is resistant to disinfection and can persist for up to a month in the environment. Furthermore, it is highly contagious, and the disease associated with FCV causes high morbidity and, in general, low mortality; these are only occasional cases of a more virulent disease [1,2].

FCV is a relatively specific pathogen in its host, predominantly infecting domestic cats. There is more limited evidence supporting the infection of some non-domestic members of the *Felidae* family [3,4].

Although FCVs demonstrate significant antigenic variation, seroneutralization studies using various polyclonal antibodies have shown substantial cross-reactivity to various strains. Due to this antigenic overlap, all FCV isolates were considered to belong to a single serotype [5-7].

Classified in the genus *Vesivirus* of the *Caliciviridae* family [8], the FCV corresponds to a small virus (27 - 40 nm) [9] not enveloped in an icosahedral capsid, with a single RNA genome positive polarity chain, approximately 7700 base pairs with three Open Reading Frames (ORFs) [10]. ORF2 encodes a capsid protein (VP1) and contains both conserved and variable sequences.

Comparative analysis of ORF2 sequences has been used to determine phylogenetic relationships between the various FCV strains [10]. The sequences of the genes encoding the FCV capsid demonstrate some variation. On average, the identity of the ORF2 nucleotide sequence is approximately 80%, and the amino acid identity of VP1 is 88% [8]. Finally, the FCV isolates appear to belong to a single genotype [11,12].

Etiopathogenesis

Cats are infected via the oronasal and conjunctival lines by contact with contaminated nasal or oral secretions, and the primary site of replication is the oropharynx. Transient viremia is established in 3 - 4 days, with a course of approximately 2 to 3 weeks. The virus induces necrosis of epithelial cells; vesicles, located on the margin of the tongue, progress to ulcers and in other affected regions, the dermis is infiltrated with neutrophils [2,13].

FCV usually affects other tissues, causing pneumonia and lameness [14]. On the other hand, the pathogenesis of virulent systemic disease caused by FCV (FCV-SV) differs considerably from the classic picture; these strains cause generalized vasculitis, multi-organ failure, and death in up to two-thirds of infected cats [15,16].

The pathogenesis of FCV-SV infection is unknown and may include viral evolution and/or immunomediated components, as well as environmental and management factors [17]. After recovery, most cats clear the infection within 30 days; a few transmit the virus for much longer, possibly for life. This carrier state persists, and viral spread can occur from the oropharynx for weeks to months, likely due to amino acid changes in the virus capsid protein, allowing FCV variants to escape the host immune response and thus persist [1,11].

Symptomatology

In FCV infection, considerable strain diversity can lead to some variation in clinical signs. The classic sign is oral ulceration, typically on the tongue; Other signs characteristic of upper respiratory tract disease such as sneezing, nasal/ocular discharge and conjunctivitis are less common than those seen in VHF-1 infection but remain frequent findings [18].

With some FCV strains, lameness and pyrexia may be characteristic, with or without respiratory/oral disease; Other strains can induce interstitial pneumonia with infection of alveolar macrophages [19], which occurs particularly in kittens, manifesting itself through dyspnea, cough, fever, and depression, even leading to death. Furthermore, FCV infection is associated with chronic stomatitis, although its exact role in the condition is unclear [1].

Thus, some hypervirulent strains of CFV-SV have emerged in North America and in several European countries [20]. In addition to upper respiratory tract disease, affected cats demonstrate varying degrees of pyrexia, anorexia and jaundice, in addition to cutaneous edema and ulcerative dermatitis, the latter due to deep vasculitis. The mortality rate of FCV-SV is high and usually more than half of infected cats can die because of severe vasculitis, hepatocellular necrosis, disseminated vascular coagulation, or other symptoms of the condition [21,22].

Adult cats are usually more severely affected than kittens, and on the ground, the disease is seen in both vaccinated and unvaccinated individuals. Each outbreak appears to be caused by a different strain; so far none of these FCV-SVs appear to have become widely established in the population [23,24].

Epidemiology

In Chile, Cerda., *et al.* [25] carried out a study in which FCV was isolated from samples of oropharyngeal secretions from cats with respiratory symptoms; A protocol for the development of the PCR was defined, a test that allowed molecular identification of the calicivirus and the acting strain in Chile. The identified strain was coincident with the sequences that possess the nitrogenous bases of the vaccine strain F9 and a European strain; The ordering of the latter was the model used to synthesize the first one used to recognize the native virus and the content of commercial vaccines applied in the country. This allows us to infer that the calicivirus strains that cause the disease in cats are widely spread worldwide. Currently there are no studies on the prevalence of the disease in the country.

Immunization

The most effective prevention measure is done through vaccination; In Chile there are four vaccines authorized by the Livestock Agricultural Service for veterinary use, which contain the live attenuated *Feline calicivirus* virus vaccine strain F9, in addition to the attenuated feline infectious rhinotracheitis and feline panleukopenia viruses [26].

Diagnosis

Due to the asymptomatic carrier phase, and the fact that viruses in live vaccines can occasionally be disseminated post vaccination, special caution should be used when interpreting positive FCV results due to poor correlation. between the presence of the virus and clinical signology [27,28]. FCV-SV, on the other hand, is diagnosed based on clinical signs, high contagion and mortality, and based on the isolation of the same strain from the blood of several sick cats (evaluated by genomic sequencing of hypervariable regions in the capsid gene) [1,29].

The diagnostic methods are as follows.

Viral isolation: It is a useful method to detect FCV infection; indicates the presence of virus replication and has the advantage of being less sensitive to the effect of variation between strains than RT-PCR. The virus can be isolated from nasal, conjunctival or oropharyngeal swabs, but viral isolation may fail due to a low virion load in the sample, inactivation of the virus during its transfer, or due to the presence of antibodies in extracellular fluids that prevent virus replication *in vitro* [13,30].

Serology: Antibodies against FCV can be detected by viral neutralization or by ELISA Seroprevalence is generally high in the cat population due to natural infection and vaccination. Consequently, the presence of specific antibodies is not useful to diagnose the infection [13,31,32].

Nucleic acid detection: PCR assays have been developed to detect FCV RNA in conjunctival and oral swabs, blood, skin scrapings, and lung tissue, depending on the clinical form and outcome of the disease. Diagnostic sensitivity depends on both the starters and the strain, due to the high variability of the viral genome [1,33]. Therefore, molecular analyzes should be validated using a panel of different strains to minimize false negative results. RT-PCR has the advantage of identifying unique virus strains and has been shown to be useful in molecular epidemiology and outbreak investigations [11,29]. However, consistent genetic markers associated with virulence, specifically in FCV-SV strains, are not yet available [23].

In this work, the use of RT-PCR as a molecular diagnostic technique for the detection of FCV was proposed, using *in silico*-designed primers by determining common regions of the capsid protein gene of different virus strains. In this way, the results obtained could establish an alternative protocol to the existing ones.

Materials and Methods

The experimental work was carried out in the laboratories of Animal Virology and Microbiology of the Department of Preventive Animal Medicine of the Faculty of Veterinary and Livestock Sciences of the University of Chile.

Controls

For the implementation of RT-PCR, the attenuated *Feline calicivirus* F9 strain, present in the FELIGEN CRP® vaccine (Virbac®), was used as a positive control. As negative control, 5 RNA samples from canine distemper virus available in the laboratory were included. Nuclease-free water was used as the reagent control.

Extraction of viral RNA

RNA extraction was carried out using the TRIZOL® LS Extraction Kit (Invitrogen®). This procedure consisted of preparing a mixture of 0.25 mL of sample with 0.75 mL of the reagent, which has the function of breaking the viral envelope, leaving the RNA free. The mixture could incubate at 2°C for 5 minutes and thereafter, 0.2 mL of chloroform was added, stirred for 15 seconds and the incubation was repeated at 22°C for 2 to 3 minutes. Then, it was centrifuged at 12,000xg for 15 minutes at 4°C. The product was separated into two phases, where the RNA was located in the upper phase (aqueous phase). The latter was transferred to a new tube, causing the RNA to precipitate when mixing with 0.5 mL of isopropyl alcohol and allowing to incubate at 22°C for 10 minutes for its subsequent centrifugation at 12,000xg for 10 minutes at 4°C. The supernatant was removed, washing the RNA precipitate with 75% ethanol, vortexing and centrifuging at 7,500xg for 5 minutes at 4°C. Finally, the precipitate was vacuum-dried for 10 minutes, resuspended in 100 µL of nuclease-free water and stored at -18° to -20°C.

Primer design

The primers for the reaction were designed *in silico* from conserved regions that code for the capsid protein gene [11], identified by aligning nucleotide sequences of 10 FCV strains, described in different geographical locations, to have greater genomic variability.

The following sequences were selected by consulting the Genbank® database: 255 (U07130.1), UTCVM-NH12 (AY996863.1), UTCVM-NH2 (AY560114.1), USDA (AY560118.1), FCVV- U2 (AY053460.1), TIG-1 (KU373057.1), FCVV2024 (AF479590.1), F65 (AF109465.1), JOK63 (AF109466.1), GD (GU214989.1) (Annex 1). These sequences were aligned using the Clustal Ω program [34] (Annex 2), in order to determine the common regions and based on these, the partitions were designed with the OligoPerfect™ Designer [35] open access program. The selection criteria included a GC close to 50%, a ΔTm of < 3°C and a length of 20 bases. The synthesis of these primers was commissioned to the company IDT®.

Annex 1: *FCV VP1 sequences used, by Genbank® access number*

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> U07130.1
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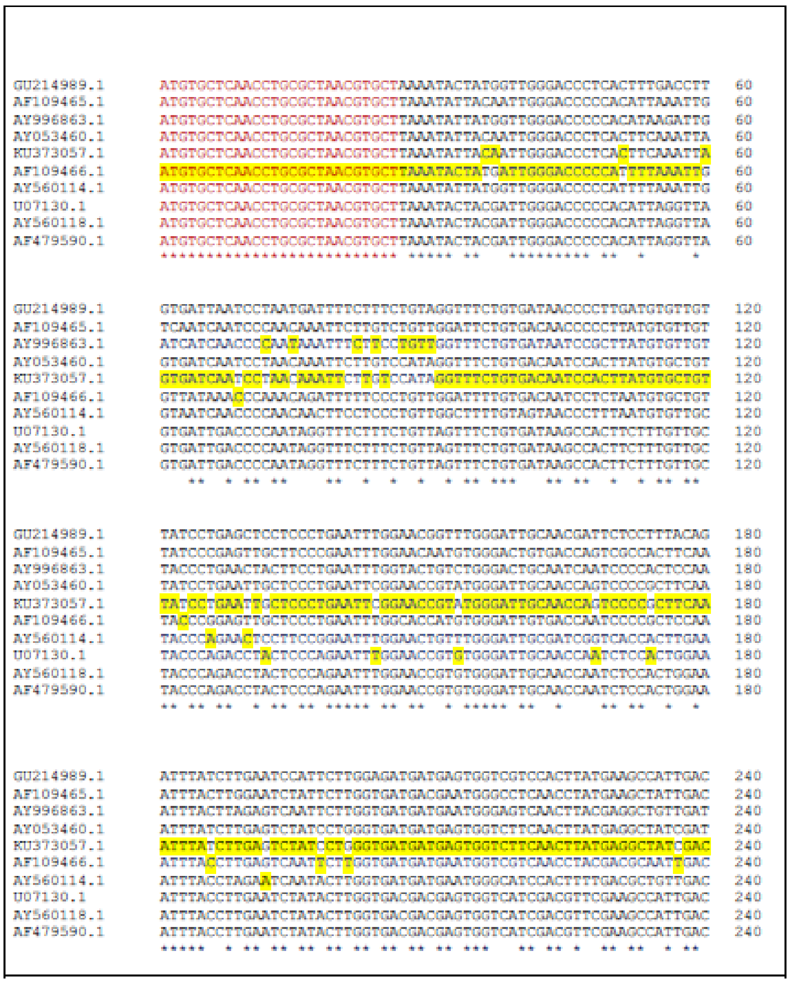
Annex 2: *Alignment according to Clustal Ω and choice of consensus sequence.*



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AY996863.1      ATGTGCTCAACCTGCGCTAACGTGCTTAAATATTATGGTTGGGACCCCCACATAAGATTG   60
AY053460.1      ATGTGCTCAACCTGCGCTAACGTGCTTAAATATTACAATTGGGACCCTCACTTCAAATTA   60
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AF109466.1      ATGTGCTCAACCTGCGCTAACGTGCTTAAATACTATGATTGGGACCCCCATTTTAAATTG   60
AY560114.1      ATGTGCTCAACCTGCGCTAACGTGCTTAAATATTATGGTTGGGACCCCCATTTTAAATTG   60
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KU373057.1      GTGATCAATCCTAACAAATTCTTGTCCATAGGTTTCTGTGACAATCCACTTATGTGCTGT   120
AF109466.1      GTTATAAACCCAAACAGATTTTTCCCTGTTGGATTTTGTGACAATCCTCTAATGTGCTGT   120
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AF109466.1      TACCCGGAGTTGCTCCCTGAATTTGGCACCATGTGGGATTGTGACCAATCCCCGCTCCAA   180
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U07130.1        TACCCAGACCTACTCCCAGAATTTGGAACCGTGTGGGATTGCAACCAATCTCCACTGGAA   180
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AF479590.1      TACCCAGACCTACTCCCAGAATTTGGAACCGTGTGGGATTGCAACCAATCTCCACTGGAA   180
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GU214989.1      ATTTATCTTGAATCCATTCTTGGAGATGATGAGTGGTCGTCCACTTATGAAGCCATTGAC   240
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AY996863.1      ATTTACTTAGAGTCAATTCTTGGTGATGATGAATGGGAGTCAACTTACGAGGCTGTTGAT   240
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KU373057.1      ATTTATCTTGAGTCTATCCTGGGTGATGATGAGTGGTCTTCAACTTATGAGGCTATCGAC   240
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AF479590.1      ATTTACCTTGAATCTATACTTGGTGACGACGAGTGGTCATCGACGTTCGAAGCCATTGAC   240
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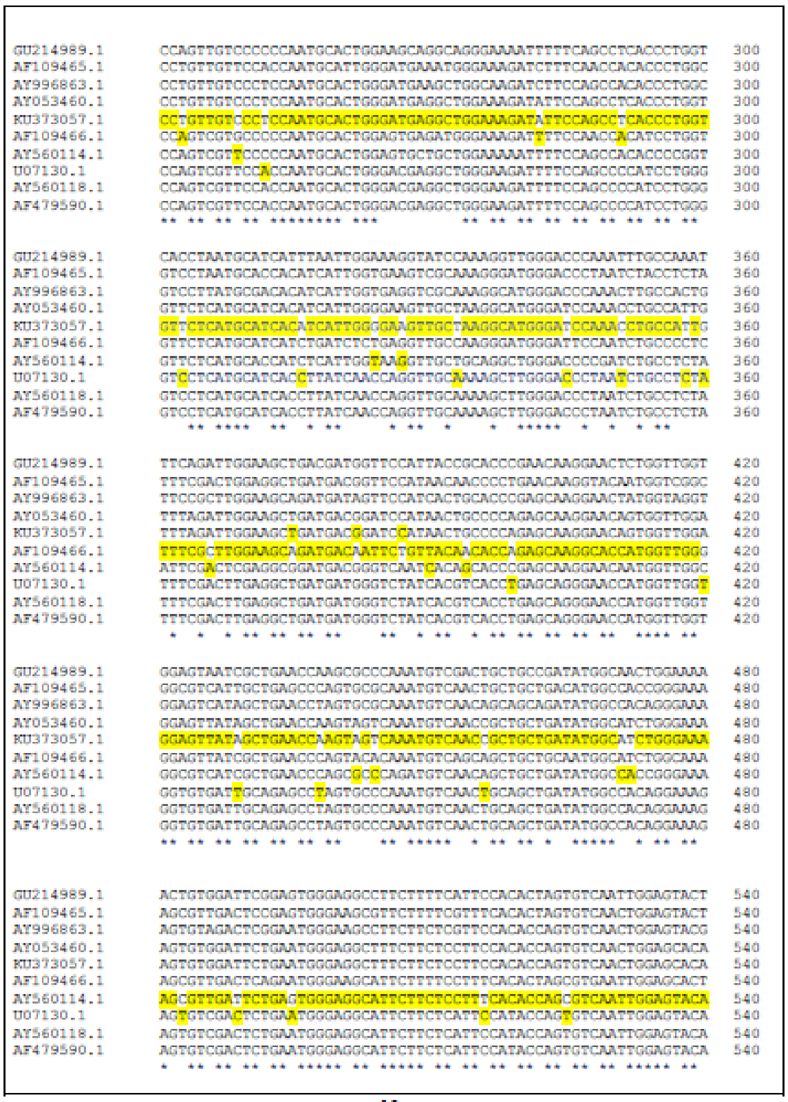

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KU373057.1      CCTGTTGTCCCTCCAATGCACTGGGATGAGGCTGGAAAGATATTCCAGCCTCACCCTGGT   300
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AF479590.1      CCAGTCGTTCCACCAATGCACTGGGACGAGGCTGGGAAGATTTTCCAGCCCCATCCTGGG   300
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AY996863.1      GTCCTTATGCGACACATCATTGGTGAGGTCGCAAAGGCATGGGACCCAAACTTGCCACTG   360
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AF479590.1      GTCCTCATGCATCACCTTATCAACCAGGTTGCAAAAGCTTGGGACCCTAATCTGCCTCTA   360
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AY560118.1      GGTGTGATTGCAGAGCCTAGTGCCCAAATGTCAACTGCAGCTGATATGGCCACAGGAAAG   480
AF479590.1      GGTGTGATTGCAGAGCCTAGTGCCCAAATGTCAACTGCAGCTGATATGGCCACAGGAAAG   480
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GU214989.1      ACTGTGGATTCGGAGTGGGAGGCCTTCTTTTCATTCCACACTAGTGTCAATTGGAGTACT   540
AF109465.1      AGCGTTGACTCCGAGTGGGAAGCGTTCTTTTCGTTTCACACTAGTGTCAACTGGAGTACT   540
AY996863.1      AGTGTAGACTCGGAATGGGAAGCCTTCTTCTCGTTCCACACCAGTGTCAACTGGAGTACG   540
AY053460.1      AGTGTGGATTCTGAATGGGAGGCTTTCTTCTCCTTCCACACCAGTGTCAACTGGAGCACA   540
KU373057.1      AGTGTGGATTCTGAATGGGAGGCTTTCTTCTCCTTCCACACCAGTGTCAACTGGAGCACA   540
AF109466.1      AGCGTTGACTCAGAATGGGAAGCATTCTTTTCCTTTCACACTAGCGTGAATTGGAGCACT   540
AY560114.1      AGCGTTGATTCTGAGTGGGAGGCATTCTTCTCCTTTCACACCAGCGTCAATTGGAGTACA   540
U07130.1        AGTGTCGACTCTGAATGGGAGGCATTCTTCTCATTCCATACCAGTGTCAATTGGAGTACA   540
AY560118.1      AGTGTCGACTCTGAATGGGAGGCATTCTTCTCATTCCATACCAGTGTCAATTGGAGTACA   540
AF479590.1      AGTGTCGACTCTGAATGGGAGGCATTCTTCTCATTCCATACCAGTGTCAATTGGAGTACA   540
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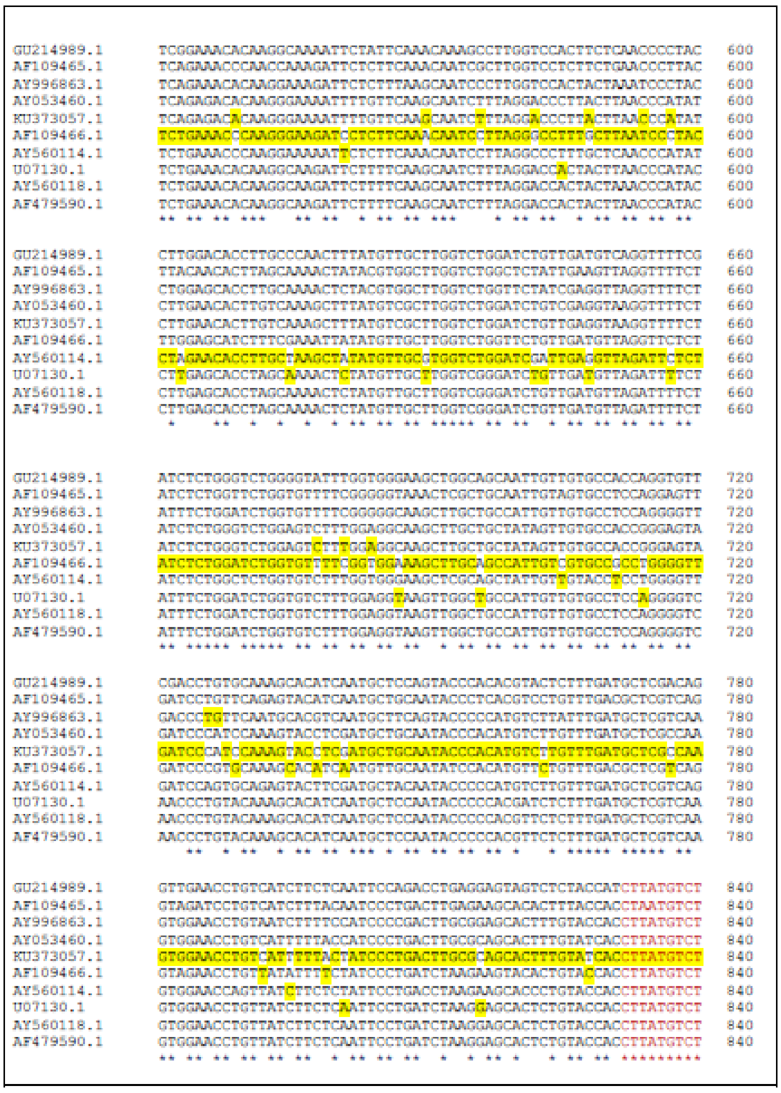

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GU214989.1      TCGGAAACACAAGGCAAAATTCTATTCAAACAAAGCCTTGGTCCACTTCTCAACCCCTAC   600
AF109465.1      TCAGAAACCCAACCAAAGATTCTCTTCAAACAATCGCTTGGTCCTCTTCTGAACCCTTAC   600
AY996863.1      TCAGAAACACAAGGAAAGATTCTCTTTAAGCAATCCCTTGGTCCACTACTAAATCCCTAC   600
AY053460.1      TCAGAGACACAAGGGAAAATTTTGTTCAAGCAATCTTTAGGACCCTTACTTAACCCATAT   600
KU373057.1      TCAGAGACACAAGGGAAAATTTTGTTCAAGCAATCTTTAGGACCCTTACTTAACCCATAT   600
AF109466.1      TCTGAAACCCAAGGGAAGATCCTCTTCAAACAATCCTTAGGGCCTTTGCTTAATCCCTAC   600
AY560114.1      TCTGAAACCCAAGGAAAAATTCTCTTCAAACAATCCTTAGGCCCTTTGCTCAACCCATAT   600
U07130.1        TCTGAAACACAAGGCAAGATTCTTTTCAAGCAATCTTTAGGACCACTACTTAACCCATAC   600
AY560118.1      TCTGAAACACAAGGCAAGATTCTTTTCAAGCAATCTTTAGGACCACTACTAAACCCATAC   600
AF479590.1      TCTGAAACACAAGGCAAGATTCTTTTCAAGCAATCTTTAGGACCACTACTTAACCCATAC   600
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GU214989.1      CTTGGACACCTTGCCCAACTTTATGTTGCTTGGTCTGGATCTGTTGATGTCAGGTTTTCG   660
AF109465.1      TTACAACACTTAGCAAAACTATACGTGGCTTGGTCTGGCTCTATTGAAGTTAGGTTTTCT   660
AY996863.1      CTGGAGCACCTTGCAAAACTCTACGTGGCTTGGTCTGGTTCTATCGAGGTTAGGTTTTCT   660
AY053460.1      CTTGAACACTTGTCAAAGCTTTATGTCGCTTGGTCTGGATCTGTCGAGGTAAGGTTTTCT   660
KU373057.1      CTTGAACACTTGTCAAAGCTTTATGTCGCTTGGTCTGGATCTGTTGAGGTAAGGTTTTCT   660
AF109466.1      TTGGAGCATCTTTCGAAATTATATGTTGCTTGGTCTGGTTCTGTTGATGTTAGGTTCTCT   660
AY560114.1      CTAGAACACCTTGCTAAGCTATATGTTGCGTGGTCTGGATCGATTGAGGTTAGATTCTCT   660
U07130.1        CTTGAGCACCTAGCAAAACTCTATGTTGCTTGGTCGGGATCTGTTGATGTTAGATTTTCT   660
AY560118.1      CTTGAGCACCTAGCAAAACTCTATGTTGCTTGGTCGGGATCTGTTGATGTTAGATTTTCT   660
AF479590.1      CTTGAGCACCTAGCAAAACTCTATGTTGCTTGGTCGGGATCTGTTGATGTTAGATTTTCT   660
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GU214989.1      ATCTCTGGGTCTGGGGTATTTGGTGGGAAGCTGGCAGCAATTGTTGTGCCACCAGGTGTT   720
AF109465.1      ATCTCTGGTTCTGGTGTTTTCGGGGGTAAACTCGCTGCAATTGTAGTGCCTCCAGGAGTT   720
AY996863.1      ATTTCTGGATCTGGTGTTTTCGGGGGCAAGCTTGCTGCCATTGTTGTGCCTCCAGGGGTT   720
AY053460.1      ATCTCTGGGTCTGGAGTCTTTGGAGGCAAGCTTGCTGCTATAGTTGTGCCACCGGGAGTA   720
KU373057.1      ATCTCTGGGTCTGGAGTCTTTGGAGGCAAGCTTGCTGCTATAGTTGTGCCACCGGGAGTA   720
AF109466.1      ATCTCTGGATCTGGTGTTTTCGGTGGAAAGCTTGCAGCCATTGTCGTGCCGCCTGGGGTT   720
AY560114.1      ATCTCTGGCTCTGGTGTCTTTGGTGGGAAGCTCGCAGCTATTGTTGTACCTCCTGGGGTT   720
U07130.1        ATTTCTGGATCTGGTGTCTTTGGAGGTAAGTTGGCTGCCATTGTTGTGCCTCCAGGGGTC   720
AY560118.1      ATTTCTGGATCTGGTGTCTTTGGAGGTAAGTTGGCTGCCATTGTTGTGCCTCCAGGGGTC   720
AF479590.1      ATTTCTGGATCTGGTGTCTTTGGAGGTAAGTTGGCTGCCATTGTTGTGCCTCCAGGGGTC   720
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GU214989.1      CGACCTGTGCAAAGCACATCAATGCTCCAGTACCCACACGTACTCTTTGATGCTCGACAG   780
AF109465.1      GATCCTGTTCAGAGTACATCAATGCTGCAATACCCTCACGTCCTGTTTGACGCTCGTCAG   780
AY996863.1      GACCCTGTTCAATGCACGTCAATGCTTCAGTACCCCCATGTCTTATTTGATGCTCGTCAA   780
AY053460.1      GATCCCATCCAAAGTACCTCGATGCTGCAATACCCACATGTCTTGTTTGATGCTCGCCAA   780
KU373057.1      GATCCCATCCAAAGTACCTCGATGCTGCAATACCCACATGTCTTGTTTGATGCTCGCCAA   780
AF109466.1      GATCCCGTGCAAAGCACATCAATGTTGCAATATCCACATGTTCTGTTTGACGCTCGTCAG   780
AY560114.1      GATCCAGTGCAGAGTACTTCGATGCTACAATACCCCCATGTCTTGTTTGATGCTCGTCAG   780
U07130.1        AACCCTGTACAAAGCACATCAATGCTCCAATACCCCCACGATCTCTTTGATGCTCGTCAA   780
AY560118.1      AACCCTGTACAAAGCACATCAATGCTCCAATACCCCCACGTTCTCTTTGATGCTCGTCAA   780
AF479590.1      AACCCTGTACAAAGCACATCAATGCTCCAATACCCCCACGTTCTCTTTGATGCTCGTCAA   780
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GU214989.1      GTTGAACCTGTCATCTTCTCAATTCCAGACCTGAGGAGTAGTCTCTACCATCTTATGTCT   840
AF109465.1      GTAGATCCTGTCATCTTTACAATCCCTGACTTGAGAAGCACACTTTACCACCTAATGTCT   840
AY996863.1      GTGGAACCTGTAATCTTTTCCATCCCCGACTTGCGGAGCACTTTGTACCACCTTATGTCT   840
AY053460.1      GTGGAACCTGTCATTTTTACCATCCCTGACTTGCGCAGCACTTTGTATCACCTTATGTCT   840
KU373057.1      GTGGAACCTGTCATTTTTACTATCCCTGACTTGCGCAGCACTTTGTATCACCTTATGTCT   840
AF109466.1      GTAGAACCTGTTATATTTTCTATCCCTGATCTAAGAAGTACACTGTACCACCTTATGTCT   840
AY560114.1      GTGGAACCAGTTATCTTCTCTATTCCTGACCTAAGAAGCACCCTGTACCACCTTATGTCT   840
U07130.1        GTGGAACCTGTTATCTTCTCAATTCCTGATCTAAGGAGCACTCTGTACCACCTTATGTCT   840
AY560118.1      GTGGAACCTGTTATCTTCTCAATTCCTGATCTAAGGAGCACTCTGTACCACCTTATGTCT   840
AF479590.1      GTGGAACCTGTTATCTTCTCAATTCCTGATCTAAGGAGCACTCTGTACCACCTTATGTCT   840
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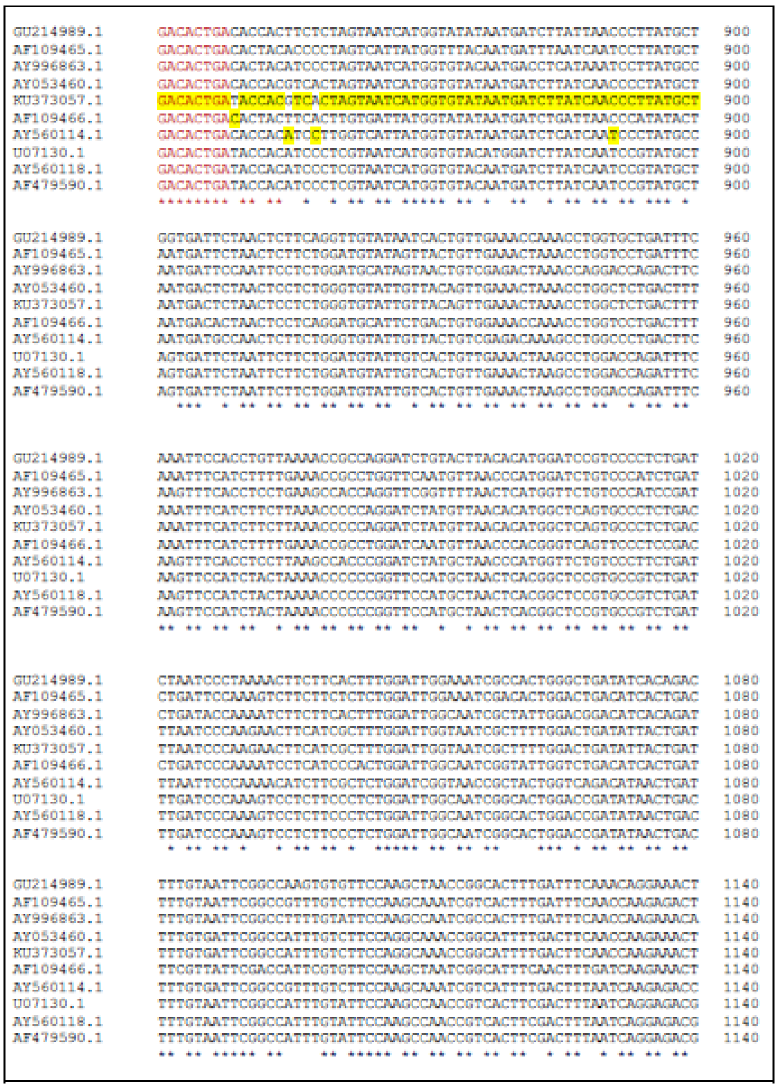

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GU214989.1      GACACTGACACCACTTCTCTAGTAATCATGGTATATAATGATCTTATTAACCCTTATGCT   900
AF109465.1      GACACTGACACTACACCCCTAGTCATTATGGTTTACAATGATTTAATCAATCCTTATGCT   900
AY996863.1      GACACTGACACTACATCCCTAGTAATCATGGTGTACAATGACCTCATAAATCCTTATGCC   900
AY053460.1      GACACTGACACCACGTCACTAGTAATCATGGTGTATAATGATCTTATCAACCCCTATGCT   900
KU373057.1      GACACTGATACCACGTCACTAGTAATCATGGTGTATAATGATCTTATCAACCCTTATGCT   900
AF109466.1      GACACTGACACTACTTCACTTGTGATTATGGTATATAATGATCTGATTAACCCATATACT   900
AY560114.1      GACACTGACACCACATCCTTGGTCATTATGGTGTATAATGATCTCATCAATCCCTATGCC   900
U07130.1        GACACTGATACCACATCCCTCGTAATCATGGTGTACATGGATCTTATCAATCCGTATGCT   900
AY560118.1      GACACTGATACCACATCCCTCGTAATCATGGTGTACAATGATCTTATCAATCCGTATGCT   900
AF479590.1      GACACTGATACCACATCCCTCGTAATCATGGTGTACAATGATCTTATCAATCCGTATGCT   900
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GU214989.1      GGTGATTCTAACTCTTCAGGTTGTATAATCACTGTTGAAACCAAACCTGGTGCTGATTTC   960
AF109465.1      AATGATTCTAACTCTTCTGGATGTATAGTTACTGTTGAAACTAAACCTGGTCCTGATTTC   960
AY996863.1      AATGATTCCAATTCCTCTGGATGCATAGTAACTGTCGAGACTAAACCAGGACCAGACTTC   960
AY053460.1      AATGACTCTAACTCCTCTGGGTGTATTGTTACAGTTGAAACTAAACCTGGCTCTGACTTT   960
KU373057.1      AATGACTCTAACTCCTCTGGGTGTATTGTTACAGTTGAAACTAAACCTGGCTCTGACTTT   960
AF109466.1      AATGACACTAACTCCTCAGGATGCATTCTGACTGTGGAAACCAAACCTGGTCCTGACTTT   960
AY560114.1      AATGATGCCAACTCTTCTGGGTGTATTGTTACTGTCGAGACAAAGCCTGGCCCTGACTTC   960
U07130.1        AGTGATTCTAATTCTTCTGGATGTATTGTCACTGTTGAAACTAAGCCTGGACCAGATTTC   960
AY560118.1      AGTGATTCTAATTCTTCTGGATGTATTGTCACTGTTGAAACTAAGCCTGGACCAGATTTC   960
AF479590.1      AGTGATTCTAATTCTTCTGGATGTATTGTCACTGTTGAAACTAAGCCTGGACCAGATTTC   960
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GU214989.1      AAATTCCACCTGTTAAAACCGCCAGGATCTGTACTTACACATGGATCCGTCCCCTCTGAT   1020
AF109465.1      AAATTTCATCTTTTGAAACCGCCTGGTTCAATGTTAACCCATGGATCTGTCCCATCTGAT   1020
AY996863.1      AAGTTTCACCTCCTGAAGCCACCAGGTTCGGTTTTAACTCATGGTTCTGTCCCATCCGAT   1020
AY053460.1      AAATTTCATCTTCTTAAACCCCCAGGATCTATGTTAACACATGGCTCAGTGCCCTCTGAC   1020
KU373057.1      AAATTTCATCTTCTTAAACCCCCAGGATCTATGTTAACACATGGCTCAGTGCCCTCTGAC   1020
AF109466.1      AAATTTCATCTTTTGAAACCGCCTGGATCAATGTTAACCCACGGGTCAGTTCCCTCCGAC   1020
AY560114.1      AAGTTTCACCTCCTTAAGCCACCCGGATCTATGCTAACCCATGGTTCTGTCCCTTCTGAT   1020
U07130.1        AAGTTCCATCTACTAAAACCCCCCGGTTCCATGCTAACTCACGGCTCCGTGCCGTCTGAT   1020
AY560118.1      AAGTTCCATCTACTAAAACCCCCCGGTTCCATGCTAACTCACGGCTCCGTGCCGTCTGAT   1020
AF479590.1      AAGTTCCATCTACTAAAACCCCCCGGTTCCATGCTAACTCACGGCTCCGTGCCGTCTGAT   1020
                ** ** ** **  * ** ** ** ** **  *  * ** ** ** ** ** ** ** **

GU214989.1      CTAATCCCTAAAACTTCTTCACTTTGGATTGGAAATCGCCACTGGGCTGATATCACAGAC   1080
AF109465.1      CTGATTCCAAAGTCTTCTTCTCTCTGGATTGGAAATCGACACTGGACTGACATCACTGAC   1080
AY996863.1      CTGATACCAAAATCTTCTTCACTTTGGATTGGCAATCGCTATTGGACGGACATCACAGAT   1080
AY053460.1      TTAATCCCAAGAACTTCATCGCTTTGGATTGGTAATCGCTTTTGGACTGATATTACTGAT   1080
KU373057.1      TTAATCCCAAGAACTTCATCGCTTTGGATTGGTAATCGCTTTTGGACTGATATTACTGAT   1080
AF109466.1      CTGATCCCAAAATCCTCATCCCACTGGATTGGCAATCGGTATTGGTCTGACATCACTGAT   1080
AY560114.1      TTAATTCCCAAAACATCTTCGCTCTGGATCGGTAACCGCTACTGGTCAGACATAACTGAT   1080
U07130.1        TTGATCCCAAAGTCCTCTTCCCTCTGGATTGGCAATCGGCACTGGACCGATATAACTGAC   1080
AY560118.1      TTGATCCCAAAGTCCTCTTCCCTCTGGATTGGCAATCGGCACTGGACCGATATAACTGAC   1080
AF479590.1      TTGATCCCAAAGTCCTCTTCCCTCTGGATTGGCAATCGGCACTGGACCGATATAACTGAC   1080
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GU214989.1      TTTGTAATTCGGCCAAGTGTGTTCCAAGCTAACCGGCACTTTGATTTCAAACAGGAAACT   1140
AF109465.1      TTTGTAATTCGGCCGTTTGTCTTCCAAGCAAATCGTCACTTTGATTTCAACCAAGAGACT   1140
AY996863.1      TTTGTAATTCGGCCTTTTGTATTCCAAGCCAATCGCCACTTTGATTTCAACCAAGAAACA   1140
AY053460.1      TTTGTGATTCGGCCATTTGTCTTCCAGGCAAACCGGCATTTTGACTTCAACCAAGAAACT   1140
KU373057.1      TTTGTGATTCGGCCATTTGTCTTCCAGGCAAACCGGCATTTTGACTTCAACCAAGAAACT   1140
AF109466.1      TTCGTTATTCGACCATTCGTGTTCCAAGCTAATCGGCATTTCAACTTTGATCAAGAAACT   1140
AY560114.1      TTTGTGATTCGGCCGTTTGTCTTCCAAGCAAATCGTCATTTTGACTTTAATCAAGAGACC   1140
U07130.1        TTTGTAATTCGGCCATTTGTATTCCAAGCCAACCGTCACTTCGACTTTAATCAGGAGACG   1140
AY560118.1      TTTGTAATTCGGCCATTTGTATTCCAAGCCAACCGTCACTTCGACTTTAATCAGGAGACG   1140
AF479590.1      TTTGTAATTCGGCCATTTGTATTCCAAGCCAACCGTCACTTCGACTTTAATCAGGAGACG   1140
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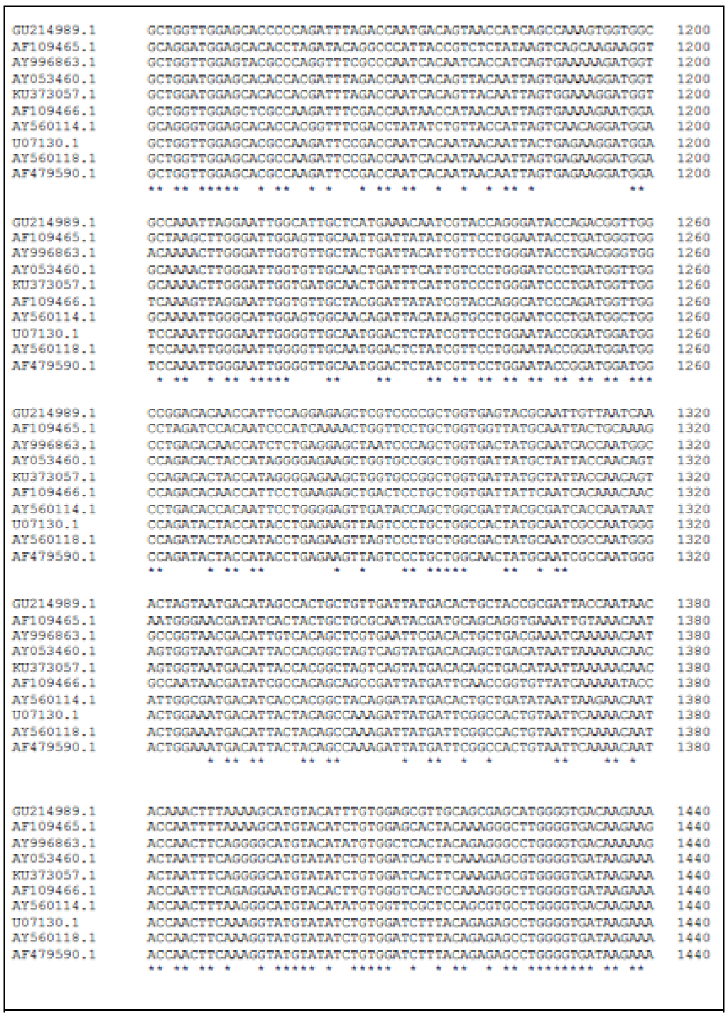

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AF109465.1        GCAGGATGGAGCACACCTAGATACAGGCCCATTACCGTCTCTATAAGTCAGCAAGAAGGT   1200
AY996863.1        GCTGGTTGGAGTACGCCCAGGTTTCGCCCAATCACAATCACCATCAGTGAAAAAGATGGT   1200
AY053460.1        GCTGGATGGAGCACACCACGATTTAGACCAATCACAGTTACAATTAGTGAAAAGGATGGT   1200
KU373057.1        GCTGGATGGAGCACACCACGATTTAGACCAATCACAGTTACAATTAGTGGAAAGGATGGT   1200
AF109466.1        GCTGGTTGGAGCTCGCCAAGATTTCGACCAATAACCATAACAATTAGTGAAAAGAATGGA   1200
AY560114.1        GCAGGGTGGAGCACACCACGGTTTCGACCTATATCTGTTACCATTAGTCAACAGGATGGA   1200
U07130.1          GCTGGTTGGAGCACGCCAAGATTCCGACCAATCACAATAACAATTACTGAGAAGGATGGA   1200
AY560118.1        GCTGGTTGGAGCACGCCAAGATTCCGACCAATCACAATAACAATTAGTGAGAAGGATGGA   1200
AF479590.1        GCTGGTTGGAGCACGCCAAGATTCCGACCAATCACAATAACAATTAGTGAGAAGGATGGA   1200
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GU214989.1        GCCAAATTAGGAATTGGCATTGCTCATGAAACAATCGTACCAGGGATACCAGACGGTTGG   1260
AF109465.1        GCTAAGCTTGGGATTGGAGTTGCAATTGATTATATCGTTCCTGGAATACCTGATGGGTGG   1260
AY996863.1        ACAAAACTTGGGATTGGTGTTGCTACTGATTACATTGTTCCTGGGATACCTGACGGGTGG   1260
AY053460.1        GCAAAACTTGGGATTGGTGTTGCAACTGATTTCATTGTCCCTGGGATCCCTGATGGTTGG   1260
KU373057.1        GCAAAACTTGGGATTGGTGATGCAACTGATTTCATTGTCCCTGGGATCCCTGATGGTTGG   1260
AF109466.1        TCAAAGTTAGGAATTGGTGTTGCTACGGATTATATCGTACCAGGCATCCCAGATGGTTGG   1260
AY560114.1        GCAAAATTGGGCATTGGAGTGGCAACAGATTACATAGTGCCTGGAATCCCTGATGGCTGG   1260
U07130.1          TCCAAATTGGGAATTGGGGTTGCAATGGACTCTATCGTTCCTGGAATACCGGATGGATGG   1260
AY560118.1        TCCAAATTGGGAATTGGGGTTGCAATGGACTCTATCGTTCCTGGAATACCGGATGGATGG   1260
AF479590.1        TCCAAATTGGGAATTGGGGTTGCAATGGACTCTATCGTTCCTGGAATACCGGATGGATGG   1260
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GU214989.1        CCGGACACAACCATTCCAGGAGAGCTCGTCCCCGCTGGTGAGTACGCAATTGTTAATCAA   1320
AF109465.1        CCTAGATCCACAATCCCATCAAAACTGGTTCCTGCTGGTGGTTATGCAATTACTGCAAAG   1320
AY996863.1        CCTGACACAACCATCTCTGAGGAGCTAATCCCAGCTGGTGACTATGCAATCACCAATGGC   1320
AY053460.1        CCAGACACTACCATAGGGGAGAAGCTGGTGCCGGCTGGTGATTATGCTATTACCAACAGT   1320
KU373057.1        CCAGACACTACCATAGGGGAGAAGCTGGTGCCGGCTGGTGATTATGCTATTACCAACAGT   1320
AF109466.1        CCAGACACAACCATTCCTGAAGAGCTGACTCCTGCTGGTGATTATTCAATCACAAACAAC   1320
AY560114.1        CCTGACACCACAATTCCTGGGGAGTTGATACCAGCTGGCGATTACGCGATCACCAATAAT   1320
U07130.1          CCAGATACTACCATACCTGAGAAGTTAGTCCCTGCTGGCCACTATGCAATCGCCAATGGG   1320
AY560118.1        CCAGATACTACCATACCTGAGAAGTTAGTCCCTGCTGGCGACTATGCAATCGCCAATGGG   1320
AF479590.1        CCAGATACTACCATACCTGAGAAGTTAGTCCCTGCTGGCAACTATGCAATCGCCAATGGG   1320
                  **     * ** **        *  *    ** *****    **  * **

GU214989.1        ACTAGTAATGACATAGCCACTGCTGTTGATTATGACACTGCTACCGCGATTACCAATAAC   1380
AF109465.1        AATGGGAACGATATCACTACTGCTGCGCAATACGATGCAGCAGGTGAAATTGTAAACAAT   1380
AY996863.1        GCCGGTAACGACATTGTCACAGCTCGTGAATTCGACACTGCTGACGAAATCAAAAACAAT   1380
AY053460.1        AGTGGTAATGACATTACCACGGCTAGTCAGTATGACACAGCTGACATAATTAAAAACAAC   1380
KU373057.1        AGTGGTAATGACATTACCACGGCTAGTCAGTATGACACAGCTGACATAATTAAAAACAAC   1380
AF109466.1        GCCAATAACGATATCGCCACAGCAGCCGATTATGATTCAACCGGTGTTATCAAAAATACC   1380
AY560114.1        ATTGGCGATGACATCACCACGGCTACAGGATATGACACTGCTGATATAATTAAGAACAAT   1380
U07130.1          ACTGGAAATGACATTACTACAGCCAAAGATTATGATTCGGCCACTGTAATTCAAAACAAT   1380
AY560118.1        ACTGGAAATGACATTACTACAGCCAAAGATTATGATTCGGCCACTGTAATTCAAAACAAT   1380
AF479590.1        ACTGGAAATGACATTACTACAGCCAAAGATTATGATTCGGCCACTGTAATTCAAAACAAT   1380
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GU214989.1        ACAAACTTTAAAAGCATGTACATTTGTGGAGCGTTGCAGCGAGCATGGGGTGACAAGAAA   1440
AF109465.1        ACCAATTTTAAAAGCATGTACATCTGTGGAGCACTACAAAGGGCTTGGGGTGACAAGAAG   1440
AY996863.1        ACCAACTTCAGGGGCATGTACATATGTGGCTCACTACAGAGGGCCTGGGGTGACAAAAAG   1440
AY053460.1        ACTAATTTCAGGGGCATGTATATCTGTGGATCACTTCAAAGAGCGTGGGGTGATAAGAAA   1440
KU373057.1        ACTAATTTCAGGGGCATGTATATCTGTGGATCACTTCAAAGAGCGTGGGGTGATAAGAAA   1440
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AY560114.1        ACCAACTTTAAGGGCATGTACATATGTGGTTCGCTCCAGCGTGCCTGGGGTGACAAGAAA   1440
U07130.1          ACCAACTTCAAAGGTATGTATATCTGTGGATCTTTACAGAGAGCCTGGGGTGATAAGAAA   1440
AY560118.1        ACCAACTTCAAAGGTATGTATATCTGTGGATCTTTACAGAGAGCCTGGGGTGATAAGAAA   1440
AF479590.1        ACCAACTTCAAAGGTATGTATATCTGTGGATCTTTACAGAGAGCCTGGGGTGATAAGAAA   1440
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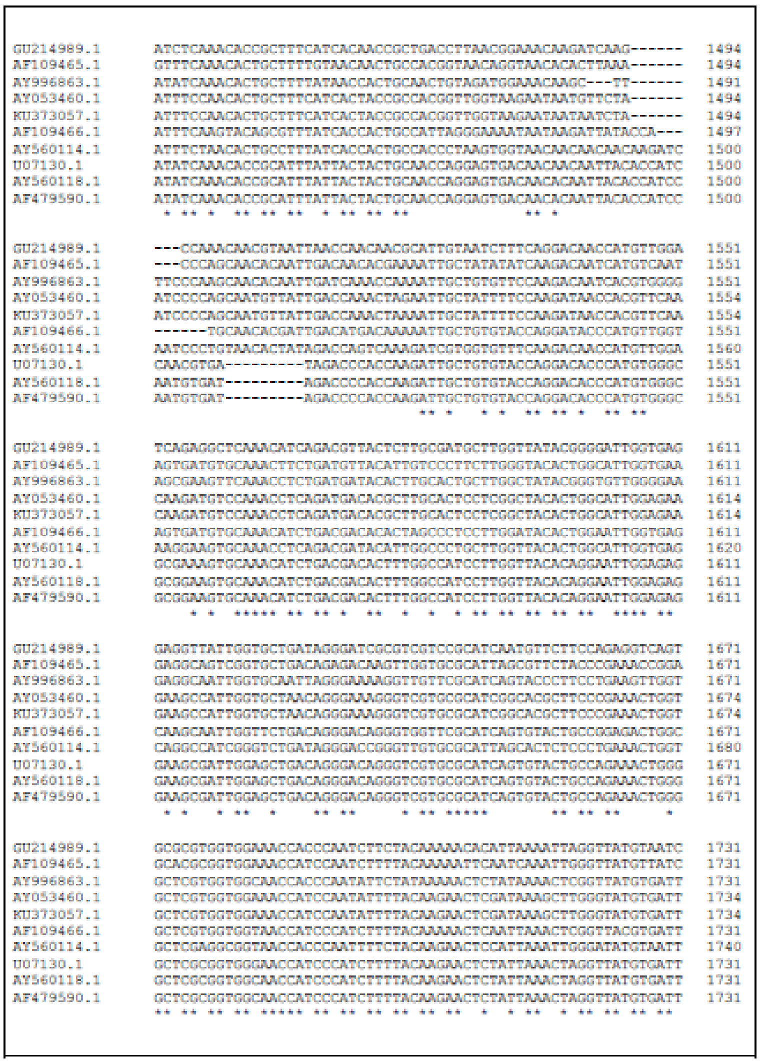

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AF109465.1        GTTTCAAACACTGCTTTTGTAACAACTGCCACGGTAACAGGTAACACACTTAAA------   1494
AY996863.1        ATATCAAACACTGCTTTTATAACCACTGCAACTGTAGATGGAAACAAGC---TT------   1491
AY053460.1        ATTTCCAACACTGCTTTCATCACTACCGCCACGGTTGGTAAGAATAATGTTCTA------   1494
KU373057.1        ATTTCCAACACTGCTTTCATCACTACCGCCACGGTTGGTAAGAATAATAATCTA------   1494
AF109466.1        ATTTCAAGTACAGCGTTTATCACCACTGCCATTAGGGAAAATAATAAGATTATACCA---   1497
AY560114.1        ATTTCTAACACTGCCTTTATCACCACTGCCACCCTAAGTGGTAACAACAACAACAAGATC   1500
U07130.1          ATATCAAACACCGCATTTATTACTACTGCAACCAGGAGTGACAACAACAATTACACCATC   1500
AY560118.1        ATATCAAACACCGCATTTATTACTACTGCAACCAGGAGTGACAACACAATTACACCATCC   1500
AF479590.1        ATATCAAACACCGCATTTATTACTACTGCAACCAGGAGTGACAACACAATTACACCATCC   1500
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GU214989.1        ---CCAAACAACGTAATTAACCAACAACGCATTGTAATCTTTCAGGACAACCATGTTGGA   1551
AF109465.1        ---CCCAGCAACACAATTGACAACACGAAAATTGCTATATATCAAGACAATCATGTCAAT   1551
AY996863.1        TTCCCAAGCAACACAATTGATCAAACCAAAATTGCTGTGTTCCAAGACAATCACGTGGGG   1551
AY053460.1        ATCCCCAGCAATGTTATTGACCAAACTAGAATTGCTATTTTCCAAGATAACCACGTTCAA   1554
KU373057.1        ATCCCCAGCAATGTTATTGACCAAACTAAAATTGCTATTTTCCAAGATAACCACGTTCAA   1554
AF109466.1        ------TGCAACACGATTGACATGACAAAAATTGCTGTGTACCAGGATACCCATGTTGGT   1551
AY560114.1        AATCCCTGTAACACTATAGACCAGTCAAAGATCGTGGTGTTTCAAGACAACCATGTTGGA   1560
U07130.1          CAACGTGA---------TAGACCCACCAAGATTGCTGTGTACCAGGACACCCATGTGGGC   1551
AY560118.1        AATGTGAT---------AGACCCCACCAAGATTGCTGTGTACCAGGACACCCATGTGGGC   1551
AF479590.1        AATGTGAT---------AGACCCCACCAAGATTGCTGTGTACCAGGACACCCATGTGGGC   1551
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GU214989.1        TCAGAGGCTCAAACATCAGACGTTACTCTTGCGATGCTTGGTTATACGGGGATTGGTGAG   1611
AF109465.1        AGTGATGTGCAAACTTCTGATGTTACATTGTCCCTTCTTGGGTACACTGGCATTGGTGAA   1611
AY996863.1        AGCGAAGTTCAAACCTCTGATGATACACTTGCACTGCTTGGCTATACGGGTGTTGGGGAA   1611
AY053460.1        CAAGATGTCCAAACCTCAGATGACACGCTTGCACTCCTCGGCTACACTGGCATTGGAGAA   1614
KU373057.1        CAAGATGTCCAAACCTCAGATGACACGCTTGCACTCCTCGGCTACACTGGCATTGGAGAA   1614
AF109466.1        AGTGATGTGCAAACATCTGACGACACACTAGCCCTCCTTGGATACACTGGAATTGGTGAG   1611
AY560114.1        AAGGAAGTGCAAACCTCAGACGATACATTGGCCCTGCTTGGTTACACTGGCATTGGTGAG   1620
U07130.1          GCGAAAGTGCAAACATCTGACGACACTTTGGCCATCCTTGGTTACACAGGAATTGGAGAG   1611
AY560118.1        GCGGAAGTGCAAACATCTGACGACACTTTGGCCATCCTTGGTTACACAGGAATTGGAGAG   1611
AF479590.1        GCGGAAGTGCAAACATCTGACGACACTTTGGCCATCCTTGGTTACACAGGAATTGGAGAG   1611
                      * *  ***** ** ** *  **  *  *  * ** ** ** ** **  **** **

GU214989.1        GAGGTTATTGGTGCTGATAGGGATCGCGTCGTCCGCATCAATGTTCTTCCAGAGGTCAGT   1671
AF109465.1        GAGGCAGTCGGTGCTGACAGAGACAAGTTGGTGCGCATTAGCGTTCTACCCGAAACCGGA   1671
AY996863.1        GAGGCAATTGGTGCAATTAGGGAAAAGGTTGTTCGCATCAGTACCCTTCCTGAAGTTGGT   1671
AY053460.1        GAAGCCATTGGTGCTAACAGGGAAAGGGTCGTGCGCATCGGCACGCTTCCCGAAACTGGT   1674
KU373057.1        GAAGCCATTGGTGCTAACAGGGAAAGGGTCGTGCGCATCGGCACGCTTCCCGAAACTGGT   1674
AF109466.1        CAAGCAATTGGTTCTGACAGGGACAGGGTGGTTCGCATCAGTGTACTGCCGGAGACTGGC   1671
AY560114.1        CAGGCCATCGGGTCTGATAGGGACCGGGTTGTGCGCATTAGCACTCTCCCTGAAACTGGT   1680
U07130.1          GAAGCGATTGGAGCTGACAGGGACAGGGTCGTGCGCATCAGTGTACTGCCAGAAACTGGG   1671
AY560118.1        GAAGCGATTGGAGCTGACAGGGACAGGGTCGTGCGCATCAGTGTACTGCCAGAAACTGGG   1671
AF479590.1        GAAGCGATTGGAGCTGACAGGGACAGGGTCGTGCGCATCAGTGTACTGCCAGAAACTGGG   1671
                   * *   * **  *    ** **     * ** *****       ** ** **     *

GU214989.1        GCGCGTGGTGGAAACCACCCAATCTTCTACAAAAACACATTAAAATTAGGTTATGTAATC   1731
AF109465.1        GCACGCGGTGGAAACCATCCAATCTTTTACAAAAATTCAATCAAATTGGGTTATGTTATC   1731
AY996863.1        GCTCGTGGTGGCAACCACCCAATATTCTATAAAAACTCTATAAAACTCGGTTATGTGATT   1731
AY053460.1        GCTCGTGGTGGAAACCATCCAATATTTTACAAGAACTCGATAAAGCTTGGGTATGTGATT   1734
KU373057.1        GCTCGTGGTGGAAACCATCCAATATTTTACAAGAACTCGATAAAGCTTGGGTATGTGATT   1734
AF109466.1        GCTCGTGGTGGTAACCATCCCATCTTTTACAAAAACTCAATTAAACTCGGTTACGTGATT   1731
AY560114.1        GCTCGAGGCGGTAACCACCCAATTTTCTACAAGAACTCCATTAAATTGGGATATGTAATT   1740
U07130.1          GCTCGCGGTGGGAACCATCCCATCTTTTACAAGAACTCTATTAAACTAGGTTATGTGATT   1731
AY560118.1        GCTCGCGGTGGCAACCATCCCATCTTTTACAAGAACTCTATTAAACTAGGTTATGTGATT   1731
AF479590.1        GCTCGCGGTGGCAACCATCCCATCTTTTACAAGAACTCTATTAAACTAGGTTATGTGATT   1731
                  ** ** ** ** ***** ** ** ** ** ** **  *  * **  * ** ** ** **
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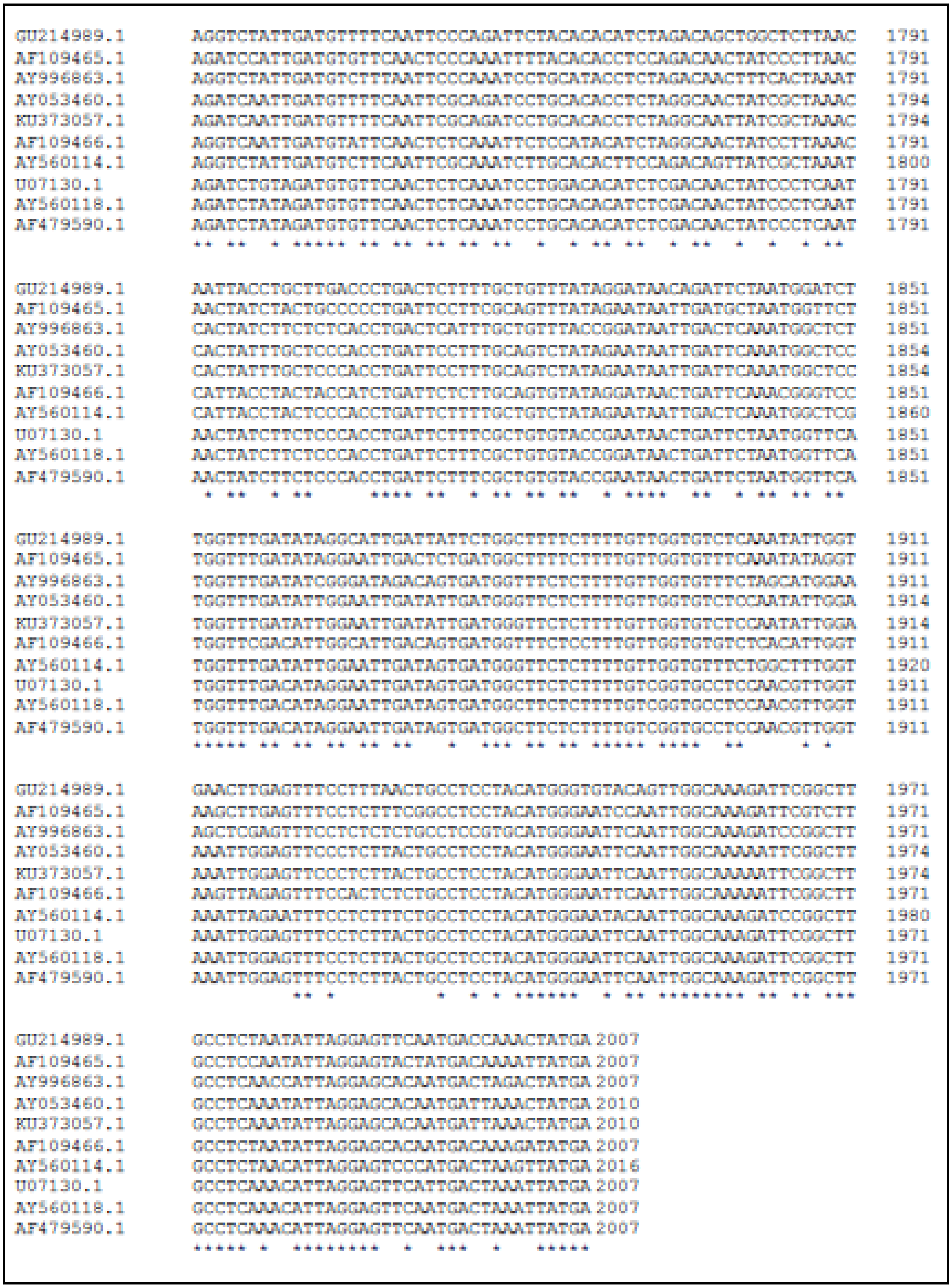

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GU214989.1      AGGTCTATTGATGTTTTCAATTCCCAGATTCTACACACATCTAGACAGCTGGCTCTTAAC   1791
AF109465.1      AGATCCATTGATGTGTTCAACTCCCAAATTTTACACACCTCCAGACAACTATCCCTTAAC   1791
AY996863.1      AGGTCTATTGATGTCTTTAATTCCCAAATCCTGCATACCTCTAGACAACTTTCACTAAAT   1791
AY053460.1      AGATCAATTGATGTTTTCAATTCGCAGATCCTGCACACCTCTAGGCAACTATCGCTAAAC   1794
KU373057.1      AGATCAATTGATGTTTTCAATTCGCAGATCCTGCACACCTCTAGGCAATTATCGCTAAAC   1794
AF109466.1      AGGTCAATTGATGTATTCAACTCTCAAATTCTCCATACATCTAGGCAACTATCCTTAAAC   1791
AY560114.1      AGGTCTATTGATGTCTTCAATTCGCAAATCTTGCACACTTCCAGACAGTTATCGCTAAAT   1800
U07130.1        AGATCTGTAGATGTGTTCAACTCTCAAATCCTGGACACATCTCGACAACTATCCCTCAAT   1791
AY560118.1      AGATCTATAGATGTGTTCAACTCTCAAATCCTGCACACATCTCGACAACTATCCCTCAAT   1791
AF479590.1      AGATCTATAGATGTGTTCAACTCTCAAATCCTGCACACATCTCGACAACTATCCCTCAAT   1791
                ** **  * ***** ** ** ** ** **  *  * ** **  * **  *  *  * **

GU214989.1      AATTACCTGCTTGACCCTGACTCTTTTGCTGTTTATAGGATAACAGATTCTAATGGATCT   1851
AF109465.1      AACTATCTACTGCCCCCTGATTCCTTCGCAGTTTATAGAATAATTGATGCTAATGGTTCT   1851
AY996863.1      CACTATCTTCTCTCACCTGACTCATTTGCTGTTTACCGGATAATTGACTCAAATGGCTCT   1851
AY053460.1      CACTATTTGCTCCCACCTGATTCCTTTGCAGTCTATAGAATAATTGATTCAAATGGCTCC   1854
KU373057.1      CACTATTTGCTCCCACCTGATTCCTTTGCAGTCTATAGAATAATTGATTCAAATGGCTCC   1854
AF109466.1      CATTACCTACTACCATCTGATTCTCTTGCAGTGTATAGGATAACTGATTCAAACGGGTCC   1851
AY560114.1      CATTACCTACTCCCACCTGATTCTTTTGCTGTCTATAGAATAATTGACTCAAATGGCTCG   1860
U07130.1        AACTATCTTCTCCCACCTGATTCTTTCGCTGTGTACCGAATAACTGATTCTAATGGTTCA   1851
AY560118.1      AACTATCTTCTCCCACCTGATTCTTTCGCTGTGTACCGGATAACTGATTCTAATGGTTCA   1851
AF479590.1      AACTATCTTCTCCCACCTGATTCTTTCGCTGTGTACCGAATAACTGATTCTAATGGTTCA   1851
                 * **  * **     **** **  * ** ** **  * ****  **  * ** ** **

GU214989.1      TGGTTTGATATAGGCATTGATTATTCTGGCTTTTCTTTTGTTGGTGTCTCAAATATTGGT   1911
AF109465.1      TGGTTTGATATAGGAATTGACTCTGATGGCTTTTCTTTTGTTGGTGTTTCAAATATAGGT   1911
AY996863.1      TGGTTTGATATCGGGATAGACAGTGATGGTTTCTCTTTTGTTGGTGTTTCTAGCATGGAA   1911
AY053460.1      TGGTTTGATATTGGAATTGATATTGATGGGTTCTCTTTTGTTGGTGTCTCCAATATTGGA   1914
KU373057.1      TGGTTTGATATTGGAATTGATATTGATGGGTTCTCTTTTGTTGGTGTCTCCAATATTGGA   1914
AF109466.1      TGGTTCGACATTGGCATTGACAGTGATGGTTTCTCCTTTGTTGGTGTGTCTCACATTGGT   1911
AY560114.1      TGGTTTGATATTGGAATTGATAGTGATGGGTTCTCTTTTGTTGGTGTTTCTGGCTTTGGT   1920
U07130.1        TGGTTTGACATAGGAATTGATAGTGATGGCTTCTCTTTTGTCGGTGCCTCCAACGTTGGT   1911
AY560118.1      TGGTTTGACATAGGAATTGATAGTGATGGCTTCTCTTTTGTCGGTGCCTCCAACGTTGGT   1911
AF479590.1      TGGTTTGACATAGGAATTGATAGTGATGGCTTCTCTTTTGTCGGTGCCTCCAACGTTGGT   1911
                ***** ** ** ** ** **   *  *** ** ***** ****  **     * *

GU214989.1      GAACTTGAGTTTCCTTTAACTGCCTCCTACATGGGTGTACAGTTGGCAAAGATTCGGCTT   1971
AF109465.1      AAGCTTGAGTTTCCTCTTTCGGCCTCCTACATGGGAATCCAATTGGCAAAGATTCGTCTT   1971
AY996863.1      AGCTCGAGTTTCCTCTCTCTGCCTCCGTGCATGGGAATTCAATTGGCAAAGATCCGGCTT   1971
AY053460.1      AAATTGGAGTTCCCTCTTACTGCCTCCTACATGGGAATTCAATTGGCAAAAATTCGGCTT   1974
KU373057.1      AAATTGGAGTTCCCTCTTACTGCCTCCTACATGGGAATTCAATTGGCAAAAATTCGGCTT   1974
AF109466.1      AAGTTAGAGTTTCCACTCTCTGCCTCCTACATGGGAATTCAATTGGCAAAAATTCGGCTT   1971
AY560114.1      AAATTAGAATTTCCTCTTTCTGCCTCCTACATGGGAATACAATTGGCAAAGATCCGGCTT   1980
U07130.1        AAATTGGAGTTTCCTCTTACTGCCTCCTACATGGGAATTCAATTGGCAAAGATTCGGCTT   1971
AY560118.1      AAATTGGAGTTTCCTCTTACTGCCTCCTACATGGGAATTCAATTGGCAAAGATTCGGCTT   1971
AF479590.1      AAATTGGAGTTTCCTCTTACTGCCTCCTACATGGGAATTCAATTGGCAAAGATTCGGCTT   1971
                         ** *         *  * * ****** *  * ** ******** ** ** ***

GU214989.1      GCCTCTAATATTAGGAGTTCAATGACCAAACTATGA 2007
AF109465.1      GCCTCCAATATTAGGAGTACTATGACAAAATTATGA 2007
AY996863.1      GCCTCAACCATTAGGAGCACAATGACTAGACTATGA 2007
AY053460.1      GCCTCAAATATTAGGAGCACAATGATTAAACTATGA 2010
KU373057.1      GCCTCAAATATTAGGAGCACAATGATTAAACTATGA 2010
AF109466.1      GCCTCTAATATTAGGAGCACAATGACAAAGATATGA 2007
AY560114.1      GCCTCTAACATTAGGAGTCCCATGACTAAGTTATGA 2016
U07130.1        GCCTCAAACATTAGGAGTTCATTGACTAAATTATGA 2007
AY560118.1      GCCTCAAACATTAGGAGTTCAATGACTAAATTATGA 2007
AF479590.1      GCCTCAAACATTAGGAGTTCAATGACTAAATTATGA 2007
                ***** *  ********  *  ***  *   *****
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Reaction mixture

To perform the RT-PCR technique, the Invitrogen® "SuperScript™ III One-Step RT-PCR System with Platinum™ Taq" kit was used following the procedure guidelines indicated by the manufacturer: 25 µL of "2X Reaction Mix" (Buffer containing 0.4 mM of each deoxyribonucleotide triphosphate (dNTP) and 3.2 mM $MgSO_4$), 10 µL of the RNA template, 2 µL of each primer, 2 µL of "SuperScript™ III RT/Platinum ™ Taq Mix" and free water of nucleases achieving a total volume of 50 µL.

DNA amplification

To carry out the reaction, an Apollo thermocycler (CLP, USA) with 96 wells of 0.2 mL each was used. The amplification program was started with reverse transcription performed at 48°C for 30 minutes followed by initial denaturation and activation of the *Taq* Platinum™ at 95°C for 10 minutes. A sequence of 40 cycles was then performed (denaturation at 95°C for 30 seconds, alignment at 55°C * (determined by a temperature gradient thermocycler) for 30 seconds; elongation at 72°C for 30 seconds) and an elongation finish at 72°C for 7 minutes.

Visualization of amplified products

It was performed by electrophoresis on 2% agarose gel (Winkler®) in Tris acetate EDTA (TAE) buffer. 5 µL of the RT-PCR reaction product were mixed with 1 µL of the commercial loading product (6X Mass Ruler Loading Dye Solution, Fermentas®) to verify the progress of migration of the DNA bands. The commercial product Maestrogen®) was used as a marker of molecular size which contains DNA fragments between 100 and 3000 bp. Electrophoresis was performed at 90 volts for 45 minutes. After this, the incubation in gel with ethidium bromide (0.5 µL/mL) (Fermelo®) was carried out for 30 minutes and then it was visualized under ultraviolet light in a transilluminator (Transiluminator UVP®). Finally, the gel was digitally photographed.

Determination of the percentage of nucleotide identity

To determine the percentage of nucleotide identity, one of the samples that were positive to the RT-PCR reaction were sent to the Sequencing Center of the company Genytec Ltda. The resulting nucleotide sequences were aligned using the Clustal Ω program and a sequence was generated consensus. Subsequently, this sequence was entered the BLAST program [36] and the nucleotide sequences with the highest percentages of nucleotide identity were identified.

Biosafety measures

In order to carry out the laboratory work, security measures were followed in accordance with the biosecurity levels established for the Microbiology and Animal Virology laboratories. These included the use of clean and sterile material, the use of a closed white apron and disposable latex gloves. Because the visualization of the amplified products involved the use of Ethidium Bromide and the use of a UV light transilluminator, use was made of an acrylic plate and glasses with a UV filter in order to protect the observer's vision. After finishing the work, the gel dipped in ethidium bromide was removed, since the latter compound has a mutagenic effect.

Results

Implement an RT-PCR assay for molecular diagnosis of the FCV capsid protein gene

Alignment of used sequences

Table 1 shows the common area according to the alignment using Clustal Omega.

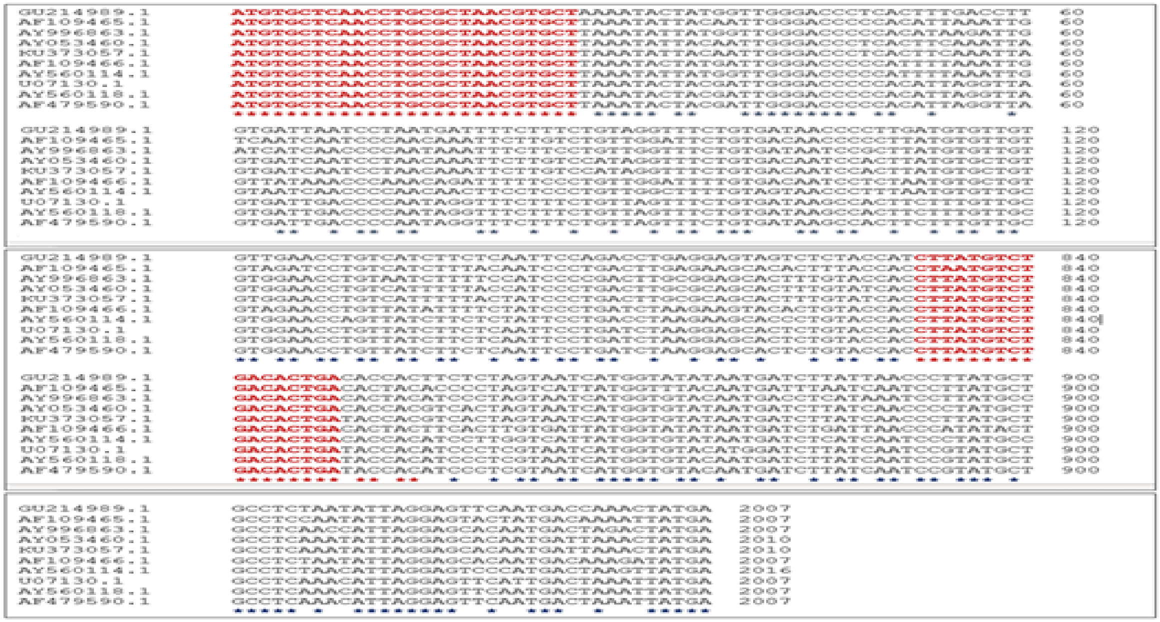
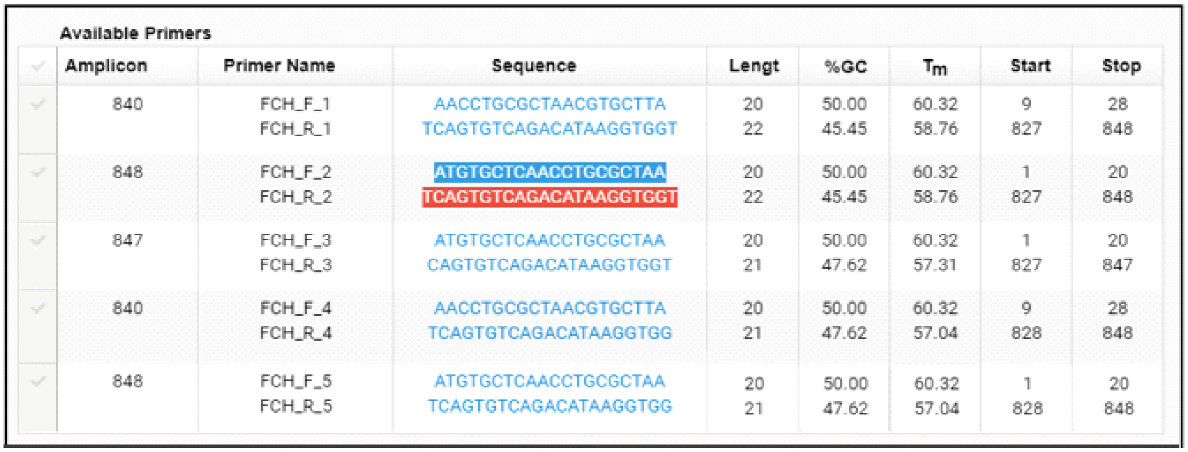


Table 1: *Synthesis of FCV VP1 sequence alignment available from Genbank®. In red, the beginning and end of the candidate sequence for the design of partitions.*

Primer design

Table 2 shows the primers proposed by the OligoPerfect Design software.



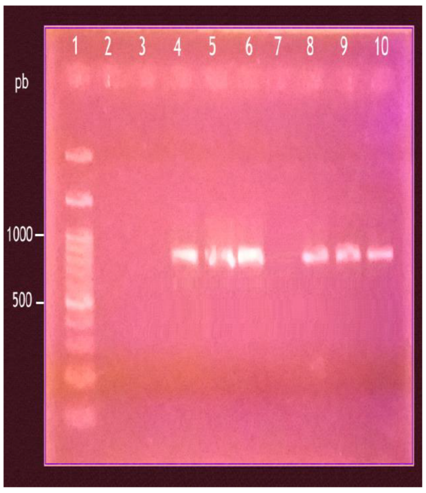
Available Primers

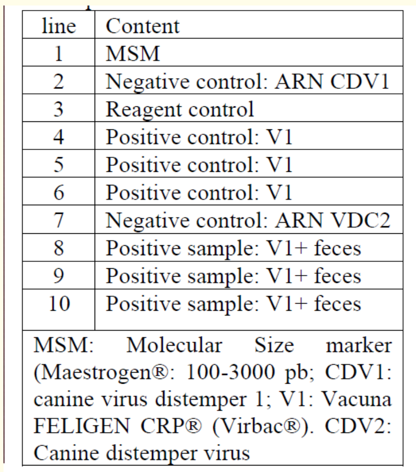
Amplicon	Primer Name	Sequence	Lengt	%GC	T_m	Start	Stop
840	FCH_F_1 FCH_R_1	AACCTGCGCTAACGTGCTTA TCAGTGTCAGACATAAGGTGGT	20 22	50.00 45.45	60.32 58.76	9 827	28 848
848	FCH_F_2 FCH_R_2	ATGTGCTCAACCTGCGCTAA TCAGTGTCAGACATAAGGTGGT	20 22	50.00 45.45	60.32 58.76	1 827	20 848
847	FCH_F_3 FCH_R_3	ATGTGCTCAACCTGCGCTAA CAGTGTCAGACATAAGGTGGT	20 21	50.00 47.62	60.32 57.31	1 827	20 847
840	FCH_F_4 FCH_R_4	AACCTGCGCTAACGTGCTTA TCAGTGTCAGACATAAGGTGG	20 21	50.00 47.62	60.32 57.04	9 828	28 848
848	FCH_F_5 FCH_R_5	ATGTGCTCAACCTGCGCTAA TCAGTGTCAGACATAAGGTGG	20 21	50.00 47.62	60.32 57.04	1 828	20 848

Table 2: *Features of the partitions proposed by the Invigogen® Oligoperfect Design software: sequence, size, % GC and Tm.*

RT-PCR reaction

In figure 1 it is possible to observe the bands originated in the RT-PCR reaction of the controls, where clear bands were observed both in the cases of positive controls and for each one of the stool samples mixed with V1. No bands were observed in the negative controls or in the reagent control.



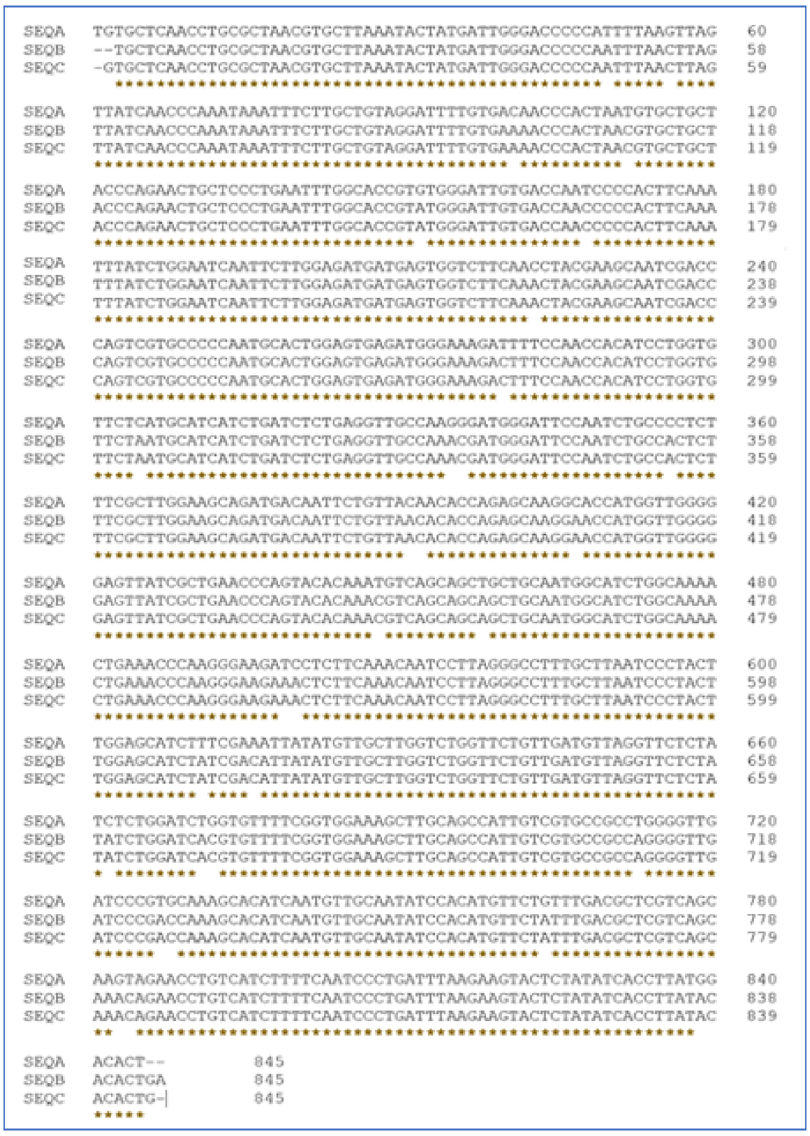


line	Content
1	MSM
2	Negative control: ARN CDV1
3	Reagent control
4	Positive control: V1
5	Positive control: V1
6	Positive control: V1
7	Negative control: ARN VDC2
8	Positive sample: V1+ feces
9	Positive sample: V1+ feces
10	Positive sample: V1+ feces
MSM: Molecular Size marker (Maestrogen®: 100-3000 pb; CDV1: canine virus distemper 1; V1: Vacuna FELIGEN CRP® (Virbac®). CDV2: Canine distemper virus	

Figure 1: *2% Agarose gel electrophoresis.*

Identify the specificity of the RT-PCR assay by the percentage of nucleotide identity of the RT-PCR product with respect to official reference sequences stored in the Genbank®

To determine the sequence of the amplified fragment, the PCR product from lane 10 was sent to the company Genytec Ltda. The alignment of the sequences obtained (Table 3) allowed defining a consensus sequence: FACH (Table 4), which according to BLAST coincides in 92% with the VP1 gene of the *Feline calicivirus* strain JOK63 (Table 5).



```
SEQA    TGTGCTCAACCTGCGCTAACGTGCTTAAATACTATGATTGGGACCCCCATTTTAAGTTAG   60
SEQB    --TGCTCAACCTGCGCTAACGTGCTTAAATACTATGATTGGGACCCCCAATTTAACTTAG   58
SEQC    -GTGCTCAACCTGCGCTAACGTGCTTAAATACTATGATTGGGACCCCCAATTTAACTTAG   59
          *********************************************** ***** ****

SEQA    TTATCAACCCAAATAAATTTCTTGCTGTAGGATTTTGTGACAACCCACTAATGTGCTGCT   120
SEQB    TTATCAACCCAAATAAATTTCTTGCTGTAGGATTTTGTGAAAACCCACTAACGTGCTGCT   118
SEQC    TTATCAACCCAAATAAATTTCTTGCTGTAGGATTTTGTGAAAACCCACTAACGTGCTGCT   119
        **************************************** ********** ********

SEQA    ACCCAGAACTGCTCCCTGAATTTGGCACCGTGTGGGATTGTGACCAATCCCCACTTCAAA   180
SEQB    ACCCAGAACTGCTCCCTGAATTTGGCACCGTATGGGATTGTGACCAACCCCCACTTCAAA   178
SEQC    ACCCAGAACTGCTCCCTGAATTTGGCACCGTATGGGATTGTGACCAACCCCCACTTCAAA   179
        ******************************* *************** ************

SEQA    TTTATCTGGAATCAATTCTTGGAGATGATGAGTGGTCTTCAACCTACGAAGCAATCGACC   240
SEQB    TTTATCTGGAATCAATTCTTGGAGATGATGAGTGGTCTTCAAACTACGAAGCAATCGACC   238
SEQC    TTTATCTGGAATCAATTCTTGGAGATGATGAGTGGTCTTCAAACTACGAAGCAATCGACC   239
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SEQA    CAGTCGTGCCCCCAATGCACTGGAGTGAGATGGGAAAGATTTTCCAACCACATCCTGGTG   300
SEQB    CAGTCGTGCCCCCAATGCACTGGAGTGAGATGGGAAAGACTTTCCAACCACATCCTGGTG   298
SEQC    CAGTCGTGCCCCCAATGCACTGGAGTGAGATGGGAAAGACTTTCCAACCACATCCTGGTG   299
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SEQA    TTCTCATGCATCATCTGATCTCTGAGGTTGCCAAGGGATGGGATTCCAATCTGCCCCTCT   360
SEQB    TTCTAATGCATCATCTGATCTCTGAGGTTGCCAAACGATGGGATTCCAATCTGCCACTCT   358
SEQC    TTCTAATGCATCATCTGATCTCTGAGGTTGCCAAACGATGGGATTCCAATCTGCCACTCT   359
        **** *****************************  ******************* ****

SEQA    TTCGCTTGGAAGCAGATGACAATTCTGTTACAACACCAGAGCAAGGCACCATGGTTGGGG   420
SEQB    TTCGCTTGGAAGCAGATGACAATTCTGTTAACACACCAGAGCAAGGAACCATGGTTGGGG   418
SEQC    TTCGCTTGGAAGCAGATGACAATTCTGTTAACACACCAGAGCAAGGAACCATGGTTGGGG   419
        ******************************  ************** *************

SEQA    GAGTTATCGCTGAACCCAGTACACAAATGTCAGCAGCTGCTGCAATGGCATCTGGCAAAA   480
SEQB    GAGTTATCGCTGAACCCAGTACACAAACGTCAGCAGCAGCTGCAATGGCATCTGGCAAAA   478
SEQC    GAGTTATCGCTGAACCCAGTACACAAACGTCAGCAGCAGCTGCAATGGCATCTGGCAAAA   479
        *************************** ********* **********************

SEQA    CTGAAACCCAAGGGAAGATCCTCTTCAAACAATCCTTAGGGCCTTTGCTTAATCCCTACT   600
SEQB    CTGAAACCCAAGGGAAGAAACTCTTCAAACAATCCTTAGGGCCTTTGCTTAATCCCTACT   598
SEQC    CTGAAACCCAAGGGAAGAAACTCTTCAAACAATCCTTAGGGCCTTTGCTTAATCCCTACT   599
        ******************  ****************************************

SEQA    TGGAGCATCTTTCGAAATTATATGTTGCTTGGTCTGGTTCTGTTGATGTTAGGTTCTCTA   660
SEQB    TGGAGCATCTATCGACATTATATGTTGCTTGGTCTGGTTCTGTTGATGTTAGGTTCTCTA   658
SEQC    TGGAGCATCTATCGACATTATATGTTGCTTGGTCTGGTTCTGTTGATGTTAGGTTCTCTA   659
        ********** **** ********************************************

SEQA    TCTCTGGATCTGGTGTTTTCGGTGGAAAGCTTGCAGCCATTGTCGTGCCGCCTGGGGTTG   720
SEQB    TATCTGGATCACGTGTTTTCGGTGGAAAGCTTGCAGCCATTGTCGTGCCGCCAGGGGTTG   718
SEQC    TATCTGGATCACGTGTTTTCGGTGGAAAGCTTGCAGCCATTGTCGTGCCGCCAGGGGTTG   719
        * ********  **************************************** *******

SEQA    ATCCCGTGCAAAGCACATCAATGTTGCAATATCCACATGTTCTGTTTGACGCTCGTCAGC   780
SEQB    ATCCCGACCAAAGCACATCAATGTTGCAATATCCACATGTTCTATTTGACGCTCGTCAGC   778
SEQC    ATCCCGACCAAAGCACATCAATGTTGCAATATCCACATGTTCTATTTGACGCTCGTCAGC   779
        ******  *********************************** ****************

SEQA    AAGTAGAACCTGTCATCTTTTCAATCCCTGATTTAAGAAGTACTCTATATCACCTTATGG   840
SEQB    AAACAGAACCTGTCATCTTTTCAATCCCTGATTTAAGAAGTACTCTATATCACCTTATAC   838
SEQC    AAACAGAACCTGTCATCTTTTCAATCCCTGATTTAAGAAGTACTCTATATCACCTTATAC   839
        **  ******************************************************

SEQA    ACACT--            845
SEQB    ACACTGA            845
SEQC    ACACTG-|           845
        *****
```
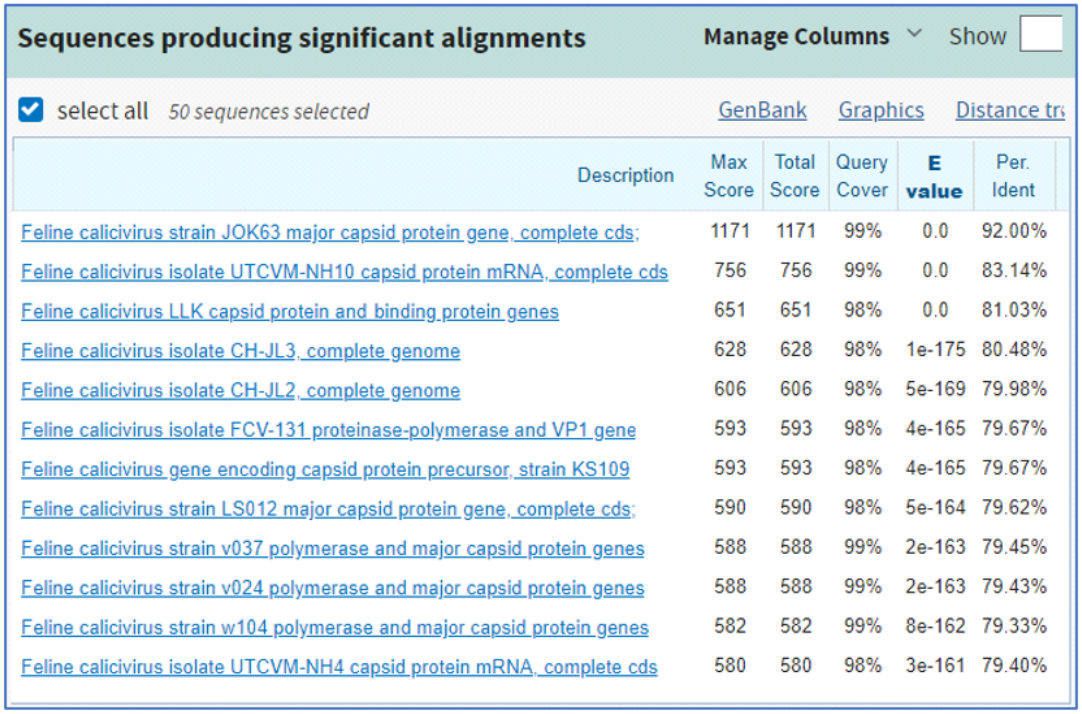

Table 3: *Alignment according to Clustal Omega. Sequences obtained from Genytec Ltda.*

```
>FACH
GTGCTCAACCTGCGCTAACGTGCTTAAATACTATGATTGGGACCCCCAATTTAACTTAGTTATCAACCCAAA
TAAATTTCTTGCTGTAGGATTTTGTGAAAACCCACTAACGTGCTGCTACCCAGAACTGCTCCCTGAATTTGG
CACCGTATGGGATTGTGACCAACCCCCACTTCAAATTTATCTGGAATCAATTCTTGGAGATGATGAGTGGTC
TTCAAACTACGAAGCAATCGACCCAGTCGTGCCCCCAATGCACTGGAGTGAGATGGGAAAGACTTTCCAACC
ACATCCTGGTGTTCTAATGCATCATCTGATCTCTGAGGTTGCCAAACGATGGGATTCCAATCTGCCACTCTT
TCGCTTGGAAGCAGATGACAATTCTGTTAACACACCAGAGCAAGGAACCATGGTTGGGGGAGTTATCGCTGA
ACCCAGTACACAAACGTCAGCAGCAGCTGCAATGGCATCTGGCAAAAGCGTTGACTCAGAATGGGAAGCACT
CTTATCCTTTCACACTAGCGTGAATTGGAGCACTTCTGAAACCCAAGGGAAGAAACTCTTCAAACAATCCTT
AGGGCCTTTGCTTAATCCCTACTTGGAGCATCTATCGACATTATATGTTGCTTGGTCTGGTTCTGTTGATGT
TAGGTTCTCTATATCTGGATCACGTGTTTTCGGTGGAAAGCTTGCAGCCATTGTCGTGCCGCCAGGGGTTGA
TCCCGACCAAAGCACATCAATGTTGCAATATCCACATGTTCTATTTGACGCTCGTCAGCAAACAGAACCTGTC
ATCTTTTCAATCCCTGATTTAAGAAGTACTCTATATCACCTTATACACACTG
```

Table 4: *FACH consensus sequence. Lab result for use in BLAST software.*



Description	Max Score	Total Score	Query Cover	E value	Per. Ident
Feline calicivirus strain JOK63 major capsid protein gene, complete cds;	1171	1171	99%	0.0	92.00%
Feline calicivirus isolate UTCVM-NH10 capsid protein mRNA, complete cds	756	756	99%	0.0	83.14%
Feline calicivirus LLK capsid protein and binding protein genes	651	651	98%	0.0	81.03%
Feline calicivirus isolate CH-JL3, complete genome	628	628	98%	1e-175	80.48%
Feline calicivirus isolate CH-JL2, complete genome	606	606	98%	5e-169	79.98%
Feline calicivirus isolate FCV-131 proteinase-polymerase and VP1 gene	593	593	98%	4e-165	79.67%
Feline calicivirus gene encoding capsid protein precursor, strain KS109	593	593	98%	4e-165	79.67%
Feline calicivirus strain LS012 major capsid protein gene, complete cds;	590	590	98%	5e-164	79.62%
Feline calicivirus strain v037 polymerase and major capsid protein genes	588	588	99%	2e-163	79.45%
Feline calicivirus strain v024 polymerase and major capsid protein genes	588	588	99%	2e-163	79.43%
Feline calicivirus strain w104 polymerase and major capsid protein genes	582	582	99%	8e-162	79.33%
Feline calicivirus isolate UTCVM-NH4 capsid protein mRNA, complete cds	580	580	98%	3e-161	79.40%

Table 5: *Significant alignments with respect to the FACH consensus sequence, according to BLAST.*

Discussion

The development of this work raised the use of the PCR technique prior to reverse transcription as a diagnostic alternative for the detection of FCV, using *in silico* primers design considering at least 10 FCV sequences according to their geographical distribution.

In this sense, although the current diagnosis of FCV by means of RT-PCR may involve starters used from the literature [37-39] the strategy used seems be suitable for the detection of FCV present in a commercial vaccine and additionally in a mixture of the vaccine and feces from a healthy cat, with the idea of verifying the effect that a different matrix or inhibitors could involve that could interfere with the values of sensitivity and specificity attributable to Polymerase Chain Reaction (Erlich, 1991; Harris, 1997).

Thus, the results obtained suggest that the use of at least 10 official FCV VP1 nucleotide sequences stored in the Genbank® was a successful initial strategy, since the consensus sequence obtained - around 2700 nucleotides - turned out to be a possible candidate to use as a template for the design of the partitions, in which all those areas that did not present common spaces or that did not allow finding 20 nucleotide primers or primers were excluded.

Subsequently, it was possible to obtain primer sequences according to the Oligoperfect Design software from ThermoFisher Scientific®, which predicted the obtaining of 840 base pair DNA fragments, which was corroborated by the visualization of amplicons with a size greater than 800 bp.

The implementation of an RT-PCR technique that only generates specific bands in positive controls and no presence of DNA fragments or amplicons when using negative controls or nuclease-free water in the 2% agarose gel, corroborates an effective choice of primers.

Additionally, the sample sent to be sequenced in triplicate indicated a high percentage of nucleotide identity (PIN > 92) with respect to the official Genbank® sequence for VP1 of the JOK63 strain of *Feline calicivirus* and a PIN range between 76 and 83 for the others. 99 FCV sequences compared, which also suggests an excellent choice of primers.

A not less important aspect to consider in the implemented technique is the low interference, almost null, of the matrix effect in the detection of FCV, since the samples mixed with feces give positive results to such an extent that one of them (well 10 of the electrophoresis) was sent to sequence.

Although for virological diagnosis, the sampling should consider the use of lingual, nasal, conjunctival and pharyngeal swabs [40], including a sample consisting of a mixture of vaccine and feces of a healthy cat, represented an opportunity to verify the matrix effect, since FCV has been detected in a dog feces sample [41].

Finally, the results obtained in this study would suggest that the strategy of choosing a common area -present in 10 nucleotide sequences of the VP1 gene of FCV-as a possible candidate for the design of *in silico* starters was successful, corroborated both by sharp detection and specific as per the sequencing of the DNA fragment obtained.

Conclusion

The developed PCR protocol can be suggested as a basis for the complementary and alternative molecular diagnosis of FCV.

Bibliography

1. Radford A., *et al*. "Feline calicivirus infection. ABCD guidelines on prevention and management". *Journal of Feline Medicine and Surgery* (2009).
2. Henzel A., *et al*. "Epidemiological status of felid herpesvirus type-1 and feline calicivirus infections in Brazil". *Ciência Rural* (2015).
3. Kadoi K., *et al*. "A strain of calicivirus isolated from lions with vesicular lesions on tongue and snout". *New Microbiologica* 20 (1997): 141-148.

4. Radford A., *et al*. "Feline Calicivirus". *Veterinary Research* (2007).
5. Povey C and Ingersoll J. "Cross-protection among feline caliciviruses". *Infection and Immunity* 11.5 (1975): 877-885.
6. Poulet H., *et al*. "Immunisation With a Combination of Two Complementary Feline Calicivirus Strains Induces a Broad Cross-Protection Against Heterologous Challenges". *Veterinary Microbiology* (2005).
7. Porter C., *et al*. "Comparison of the Ability of Feline Calicivirus (FCV) Vaccines to Neutralise a Panel of Current UK FCV Isolates". *Journal of Feline Medicine and Surgery* (2008).
8. Pesavento P., *et al*. "Molecular Virology of Feline Calicivirus". *Veterinary Clinics of North America* 38 (2008): 775-786.
9. ICTV. "International Committee On Taxonomy Of Viruses". ICTV Ninth Report; 2009 Taxonomy (2019).
10. Prikhodko V., *et al*. "Genetic characterization of feline calicivirus strains associated with varying disease manifestations during an outbreak season in Missouri (1995–1996)". *Virus Genes* 48 (2014): 96-110.
11. Coyne K., *et al*. "Large scale spatial and temporal genetic diversity of feline calicivirus". *Journal of Virology* 86.20 (2012): 11356-11367.
12. Pereira J., *et al*. "Molecular characterization of feline calicivirus variants from multicat household and public animal shelter in Rio de Janeiro, Brazil". *The Brazilian Journal of Microbiology* (2018).
13. Gaskell R., *et al*. "Feline respiratory disease". *Infectious Diseases of the Dog and Cat* (2006):145-154.
14. Dawson S., *et al*. "Acute arthritis of cats associated with feline calicivirus infection". *Research in Veterinary Science* 56 (1994): 133-143.
15. Hurley K., *et al*. "An outbreak of virulent systemic feline calicivirus disease". *American Veterinary Medical Association* 224.2 (2004): 241-249.
16. Coyne K., *et al*. "Lethal outbreak of a disease associated with feline calicivirus infection in cats". *Veterinary Record* 158 (2006): 544-550.
17. Hurley K. "Virulent Calicivirus infection in cats". In: Proceedings American College of Veterinary Internal Medicine Congress. Louisville, Kentucky (2006).
18. Afonso M., *et al*. "Feline Upper Respiratory Infections". In: Ettinger S, Feldman E, Cote E (Editions.). textbook of veterinary internal medicine: diseases of the dog and the cat, eighth edition. St. Louis, Missouri: Elsevier (2017): 2521-2529.
19. Monne J., *et al*. "Alveolar macrophages are the main target cells in feline calicivirus associated pneumonia". *The Veterinary Journal* 201.2 (2014): 156-165.
20. Reynolds B., *et al*. "A nosocomial outbreak of feline calicivirus associated virulent systemic disease in France". *Journal of Feline Medicine and Surgery* 11 (2009): 633-644.
21. Cohn L. "Feline respiratory disease complex". *Veterinary Clinics of North America: Small Animal Practice* 41.6 (2011): 1273-1289.

22. Deschamps JY., *et al*. "Nosocomial feline calicivirus-associated virulent systemic disease in a veterinary emergency and critical care unit in France". *JFMS Open Reports* (2015).
23. Ossiboff R., *et al*. "Feline caliciviruses (FCVVs) isolated from cats with virulent systemic disease possess in vitro phenotypes distinct from those of other FCVV isolates". *Journal of General Virology* 88.2 (2007): 506-517.
24. UQ VETS. "Virulent Systemic Feline Calicivirus (VS-FCV)". *School of Veterinary Science* (2016).
25. Cerda L., *et al*. "Identificación del calicivirus felino en Chile, Caliciviridae. Boletín Veterinario Oficial N°9". Servicio Agrícola Ganadero, División de Protección Pecuaria, Chile (2009): 1-6.
26. SAG. Servicio Agrícola Y Ganadero. Medicamentos veterinaries (2018).
27. Sykes J., *et al*. "Detection and strain differentiation of feline calicivirus in conjunctival swabs by RTPCR of the hypervariable region of the capsid protein gene". *Archives of Virology* 147 (1998): 1321-1334.
28. Ruch R., *et al*. "Results of molecular diagnostic assays targeting feline herpesvirus-1 and feline calicivirus in adult cats administered modified live vaccines". *Journal of Feline Medicine and Surgery* 13.8 (2011): 541-545.
29. Berger A., *et al*. "Feline calicivirus and other respiratory pathogens in cats with Feline calicivirus related symptoms and in clinically healthy cats in Switzerland". *BMC Veterinary Research* (2015).
30. Henzel A., *et al*. "Isolation and identification of feline calicivirus and feline herpesvirus in Southern Brazil". *The Brazilian Journal of Microbiology* (2012).
31. Lappin M., *et al*. "Use of serologic tests to predict resistance to feline herpesvirus 1, feline calicivirus, and feline parvovirus infection in cats". *American Veterinary Medical Association* 220.1 (2002): 38-42.
32. Bergmann M., *et al*. "Antibody Response to Feline Calicivirus Vaccination in Healthy Adult Cats". *Viruses* 11.8 (2019): 702.
33. Wilhelm S and Truyen U. "Real-time reverse transcription polymerase chain reaction assay to detect a broad range of feline calicivirus isolates". *Journal of Virological Methods* 133 (2006): 105-108.
34. Clustal Ω. Multiple Sequence Alignment (2020).
35. Oligoperfect Design. Thermo Fisher Scientific (2020).
36. BLAST. Basic Local Alignment Search Tool (2020).
37. Sykes J., *et al*. "Detection of feline calicivirus, feline herpesvirus 1 and Chlamydia psittaci mucosal swabs by multiplex RT-PCR/PCR". *Veterinary Microbiology* 81.2 (2001): 95-108.
38. Ohe K., *et al*. "Detection of Feline calicivirus (FCVV) from vaccinated cats and phylogenetic analysis of its capsid genes". *Veterinary Research Communications* 30.3 (2006): 293-305.
39. Hou J., *et al*. "European molecular epidemiology and strain diversity of feline calicivirus". *Veterinary Record* 178.5 (2016): 114-121.

40. González M. "Aislamiento y análisis filogenético de Calicivirus felino desde gatos domésticos (Felis silvestris catus) de la Región Metropolitana, Chile. Memoria para la obtención del titulo de Médico Veterinario". Universidad de Chile, Facultad de Ciencias Veterinarias y Pecuarias (2020): 76.

41. Di Martino B., *et al*. "Characterization of a strain of feline calicivirus isolated from a dog faecal sample". *Veterinary Microbiology* 139 (2009): 52-57.

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