MOLECULAR COMPARISONS OF *BABESIA ODOCOILEI* USING THE INTERNAL TRANSCRIBED SPACERS OF RIBOSOMAL RNA

A Thesis

by

LORIEN SCHOELKOPF

Submitted to the Office of Graduate Studies of
Texas A&M University
in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

August 2004

Major Subject: Veterinary Parasitology

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Approved as to style and content by:

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ABSTRACT

Molecular Comparisons of *Babesia odocoilei* Using the Internal Transcribed Spacers of Ribosomal RNA. (August 2004)

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Chair of Advisory Committee: Dr. Patricia J. Holman

Babesia odocoilei is an intraerythrocytic apicomplexan parasite which infects cervidae, sometimes causing babesiosis. It is vectored by the tick *Ixodes scapularis* and is distributed throughout the southeastern United States. The geographic and host range continue to extend as new incidence of infection is detected.

A genomic DNA region spanning the internal transcribed spacer 1 (ITS1), 5.8S rRNA gene, and ITS2 of ribosomal RNA (rRNA) from 18 *B. odocoilei* isolates (speciation confirmed by small subunit rRNA analysis) was amplified using the polymerase chain reaction, cloned and sequenced. The isolates originated from 6 different cervidae or bovidae hosts in various U.S. geographic areas. Included in the analysis was a previously described reindeer *B. odocoilei*-like isolate, RD61, which showed only 99.0% identity in SSU rRNA analysis to *B. odocoilei*. Percent identity pairwise comparisons among the samples were calculated for both the full ITS1-5.8S-ITS2 and individual genomic regions. Identity values for all comparisons ranged from 90% to 100%, with the exception of RD61, which showed no higher than 88% identity for all gene regions.

An analysis of fixed differences identified in the ITS1 and ITS2 gene regions of all clones revealed 21 fixed differences in ITS1, and only 11 in ITS2. Most isolates were found to have 2 overall patterns of fixed differences, although some had 1 or 3.

Phylogenetic analysis of all sequences for the entire ITS1-5.8S-ITS2 gene region placed most isolates into 2 distinct groups corresponding to those observed in the analysis of fixed differences. This suggested the presence of at least 2 rRNA transcription units in *B. odocoilei*.

ITS analysis failed to demonstrate host or geographic differences that might serve to pinpoint the source of outbreaks of *B. odocoilei* in farmed and managed host animals. This failure might result from genetic recombination of ITS genomic regions during the tick vector stage. Lack of conspecificity between the RD61 isolate and *B. odocoilei* was supported by this study; however, more data are needed to clarify the taxonomic status of this *B. odocoilei*-like isolate.

DEDICATION

I would never have made it to Texas A&M University without the constant support and inspiration from my family, most especially my father and mother. Mom and Dad, you have always been there for me, from my first day of school to my thesis seminar and defense. I am so happy to make you proud with the work I have done here and will continue to do, and there is no one else in the world to whom I could dedicate this thesis. We have so much to look forward to!

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To my fellow labmates Kylie Bendele, Angela Spencer, Allison James and Paulette Waters, I will always say that I was extremely fortunate for the privilege of working alongside all of you, and for your companionship and support along the way. You helped my difficult transition into a new town and life more than you may ever know. I also must thank Angie personally for her friendship and advice, which I will always treasure.

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CHAPTER I

INTRODUCTION

The genus *Babesia* is one of the most important constituents of the class Piroplasmasida, order Piroplasmorida, and family Babesiidae. These erythrocytic apicomplexan parasites have influenced both the veterinary and medical communities. In 1893, Smith and Kilbourne discovered that *Babesia bigemina*, a causative agent of bovine babesiosis, was transmitted by the tick *Boophilus annulatus*. This revelation was a breakthrough in the history of parasitology, as it was the first proof that an arthropod was the vector of any disease agent. Ticks from several genera are now known to be vectors and reservoirs of numerous *Babesia* spp. transmissible to an array of mammals (Levine, 1985).

Members of the Piroplasmasida represent a moderately consistent group of vertebrate blood cell parasites, which can be either piriform, or pear-shaped, round, amoeboid, or rod-shaped in morphology. All are found in the erythrocytes, though some genera may also have a leukocytic stage. *Babesia* spp. have polar rings, subpellicular microtubules, and perhaps micronemes as well, all characteristics of apicomplexans. No flagella or cilia are present, and no spores or oocysts are formed. Locomotion is either by gliding or body flexion. In the vertebrate host blood stage, reproduction is asexual by division, and parasites are heteroxenous and vectored by Ixodid ticks (Levine, 1973).

This thesis follows the style of the Journal of Wildlife Diseases.

Babesia are transmitted to the vertebrate host by ticks which became infected by ingesting *Babesia* within erythrocytes. After a multiplication cycle and presumed gamogony and syngamy in the tick gut, the parasites penetrate the gut wall and travel via the hemolymph to the ovary, where they invade the developing eggs (Levine, 1985). The parasites become infective in the hatched ticks and, after further development in the salivary glands, are transmitted by larval, nymphal, or adult ticks (Levine, 1985). The lumen of the salivary glands become full of thousands of individual sporozoites and are inoculated into the vertebrate host as the tick feeds. This 'transovarial transmission' results from infection of the next generation of ticks through the ovary, to the ova, to the larvae, and leads to actual transmission of the parasite by the bite of the offspring of the initially infected adult tick (Kingston, 1981).

Babesia can also be transmitted transstadially, a stage-to-stage transmission in which the ticks infected in one stage transmit the parasites by later stages of the same tick. Depending on the species, *Babesia* can be picked up by a larval tick and transmitted by the nymph, or it can be picked up by a nymph and transmitted by the adult. Parasites proliferate in the phagocytes of the body cavity of the tick, and form pseudocysts, or club-shaped stages, which exit the host cell and travel to the tick muscle cells. Tick muscles remain unchanged during metamorphosis, and thus *Babesia* parasites remain there and continue to divide by binary fission. The parasites can also be retained and proliferate in the epithelial cells of the tick gut, which also remains unchanged during molting. As the adult tick begins to feed on its host, parasites migrate to the salivary glands, and further develop for two to three more days. The parasites then undergo a series of binary fissions, and enter the cells of the salivary gland acini. They continue to multiply, filling the host

cell with thousands of diminutive parasites. They then become vermiform, leave the host cell, and migrate to the lumen of the gland, where the now infective *Babesia* sporozoites are injected into a new host when the tick takes a blood meal. Upon injection into the host, the *Babesia* invade erythrocytes, where they undergo division (Levine, 1985). After division, the organisms leave the host cells and penetrate another erythrocyte and repeat the cycle (Kingston, 1981). Some vertebrate hosts remain infected for life.

Babesia parasites are the cause of babesiosis, a potentially fatal disease with generally higher death rates in livestock adults than in young animals. The clinical signs are usually similar in different hosts. There is typically fever, malaise, listlessness, anorexia, and severe anemia caused by the destruction of the erythrocytes that is accompanied by hemoglobinuria. Icterus develops, and the spleen and liver become enlarged. Diarrhea or constipation and yellow feces are also common. Without treatment, affected animals become emaciated and often die (Levine, 1973). If no illness occurs as a result of infection the host may maintain babesiasis as a chronic infection.

Piroplasmosis in white-tailed deer (*Odocoileus virginianus chiriquensis* Allen) was originally detected in Panama by observation of parasites in Giemsa stained brain smears of hunter-killed animals (Clark, 1918). Subsequent experiments by Clark and Zetek (1925) showed clinical piroplasmosis in white-tailed deer that was transmissible to a calf and a brocket deer (*Mazama sartorii repertica* Goldman) by *Boophilus microplus* (syn. *Margaropus annulatus australis*) ticks.

Emerson and Wright (1968; 1970) first isolated an unknown *Babesia* from white-tailed deer (*Odocoileus virginianus*) in Texas, which was later designated *Babesia odocoilei*. The organism was not transmissible to sheep, goats or splenectomized calves,

but the infection caused severe anemia and was fatal in a splenectomized deer (Waldrup, 1991). Infections caused by certain *Babesia* species, including *B. odocoilei*, can exist without causing serious disease problems, and the fact that parasitemias have been found in healthy, immature deer suggests that a situation of enzootic stability may exist (Callow, 1977; Perry et al., 1985a). *Babesia odocoilei* is the only named species reported from white-tailed deer (WTD), although Spindler et al. (1958) described a *Babesia bigemina*-like isolate in WTD in New Mexico.

Babesia odocoilei is widely distributed throughout parts of the southeastern United States. Early studies identified the parasite in the pineywoods of eastern Texas (Robinson et al., 1967). Further studies led to the extension of the *B. odocoilei* range northward and westward into the post oak-savannah of Texas, Oklahoma, and Virginia (Waldrup et al., 1989a and 1989b; Perry et al., 1985b). The geographic range was recently extended to include Minnesota (MN), where a caribou (*Rangifer tarandus*) suffered a fatal case of babesiosis due to *B. odocoilei* (Holman et al., 1994 and 2000; Petrini et al., 1995). The parasite was also isolated from WTD in the same area of MN, and a *B. odocoilei*-like parasite was found in southern California bighorn sheep (*Ovis canadensis nelsoni*) (Holman et al., 2000; Goff et al., 1993). Additionally, *B. odocoilei* has been detected in elk (*Cervus elaphus canadensis*) herds from Indiana, Minnesota, Wisconsin and New Hampshire by culturing the parasite from both subclinically and clinically infected animals (Gallatin et al., 2003; pers. comm., P.J. Holman).

Morphologically, *B. odocoilei* is quite similar to other small *Babesia* spp., most notably the European parasites *Babesia divergens* and *Babesia capreoli*. *Babesia divergens* is a well-known parasite of cattle (Genus *Bos*) that has been shown to infect a

variety of hosts under both experimental and natural conditions. These include reindeer (*Rangifer tarandus tarandus*), wild sheep (*Ovis musoni*), fallow deer (*Dama dama*), red deer (*Cervus elaphus elaphus*), and roe deer (*Capreolus capreolus*) (Enigk and Friedhoff, 1962a, b; 1963; Nilsson et al., 1965; Gray et al., 1990). Gerbils, hamsters, rats, chimpanzees, and humans are among the diverse hosts also known to be susceptible to *B. divergens* (Canning et al., 1976; Entrican et al., 1979; Lewis et al., 1980; Liddell et al., 1980; Gray et al., 1985; Garnham and Bray, 1959; Gorenflot and Piette, 1976). *Babesia capreoli*, while morphologically and serologically similar to *B. divergens*, is distinct in its host specificity, infecting only roe deer, red deer, and sheep (Nikol'skii and Pozov, 1972; Adam et al., 1976; Purnell et al., 1981). Whether *B. capreoli* is infectious to reindeer is unknown.

Intraerythrocytic *B. odocoilei* and *B. capreoli* parasites from experimentally infected sika deer (*Cervus nippon*) closely resemble one another, and both parasites are frequently located along the margin of the erythrocyte (Gray et al., 1991). This location is commonly referred to as the accolé position, and is comparable to that described for *B. divergens* in bovine blood at both the light and ultrastructural microscopy levels (Friedhoff and Scholtyseck, 1977; Gorenflot et al., 1991).

The ixodid tick *Ixodes scapularis* (syn. *Ixodes dammini*; Oliver et al., 1993) was shown to experimentally transmit *B. odocoilei* transstadially (Waldrup et al., 1990). Additionally, *B. odocoilei* DNA was found to be the prevalent piroplasm in the salivary glands of *I. scapularis* ticks obtained from extremely infested sites in Maine, Massachusetts, and Wisconsin (Armstrong et al., 1998). *Ixodes scapularis* was found on WTD in the Dismal Swamps in Virginia, but not on *B. odocoilei*-infected deer

(Sonenshine, 1979; Perry et al., 1985b). Other ticks have been conjectured to vector *B. odocoilei*. Engorged female *Dermacentor albipictus* ticks were present on an elk that succumbed to babesiosis in Texas, and a tentative identification of the isolate as *B. odocoilei* was made, and later confirmed, based on immunofluorescent antibody assays and morphology (Holman et al., 1994 and 2000). However, further studies are required to determine if *D. albipictus* is a competent vector of *B. odocoilei.*

Babesia odocoilei is closely related to *B. divergens* and *B. capreoli* (Gray et al., 1991; Holman et al., 2000). Ixodid ticks vector the three parasites; the European ixodid tick, *Ixodes ricinus*, is the vector of *B. divergens*, and has also been incriminated as a vector of *B. capreoli* (Donnelly and Pierce, 1975; Adam et al., 1976). Given that both *B. capreoli* and *B. divergens* are transmitted both transstadially and transovarially (Joyner et al., 1963), it is likely that both modes are also utilized by *B. odocoilei*. However, to date only transstadial transmission has been proven for *B. odocoilei*, with retention of the parasite between the nymphal and adult tick stages (Waldrup et al., 1990).

Laboratory-reared nymphal *I. scapularis* ticks were placed on a *B. odocoilei*-infected WTD, and the replete nymphs were collected and held in favorable conditions until they molted to adults (Waldrup et al., 1989a). These adult *I. scapularis* were then allowed to feed on a second 6-month-old deer previously determined to be free of babesial infection by stained blood smear examination and by the lack of specific antibody using the indirect fluorescent antibody test. Piroplasms from the second deer were noted in peripheral blood smears 6 days after tick infestation, and specific antibody was present 26 days following the infestation. The experiment was similarly repeated using a third

infection-free deer, thus proving that *B. odocoilei* was transstadially transmitted from deer to deer by *I. scapularis* (Waldrup et al., 1990).

Some of the first methods developed to distinguish different *Babesia* species were serologic analyses, such as the indirect fluorescent antibody (IFA) and immunoprecipitation assays. The IFA test has been used in the detection of antibodies reactive to *B. divergens* in red deer (*Cervus elaphus elaphus*) and *B. capreoli* in roe deer (*Capreolus capreolus*) (Latif and Adam, 1973; Blancou, 1983). It has also been used in the diagnosis of bovine babesiosis (Todorovic and Long, 1976). Immunoprecipitation assays are sensitive tests, especially when using ^{35}S-labelled proteins (Barbet et al., 1983). Using this technique, *Babesia bovis* and *Babesia bigemina* antigens were detected and distinguished from each other, and antigenic diversity was even observed between different stocks of the same species (Passos, 1998).

Waldrup (1991) used the IFA test to determine serologic reactivity to *B. odocoilei* and *B. bovis* of deer sera collected from a range of areas in Texas. This was done to clarify the serologic relationship between these two parasites, to establish the geographic range of each, and to ascertain serologic reactivity and prevalence rates of deer sera acquired throughout Texas to both *Babesia* spp. In vitro cultures of *B. odocoilei* and *B. bovis* were used as the sources of antigen for the tests. It was also ensured that sera from deer infected with *Theileria cervi* or inoculated with *Anaplasma marginale* vaccine did not cross-react with either the *B. odocoilei* or *B. bovis* antigen. As many as 71% of WTD in Texas have *T. cervi* at any given time, and anaplasmosis has been previously reported in WTD, although the infection is more prevalent in mule deer and black-tailed deer (Howe, 1970; Robinson et al., 1967; Waldrup et al., 1989b; Waldrup 1991). Results

of the IFA test for *B. odocoilei* antibody activity showed the highest prevalence in the Gulf Coast (51%), the Gulf Slope (84%), and the Southwestern Prairie (57%) regions. The overall prevalence rate for *B. bovis* in deer was much lower, 1%, with activity only detected in 1 of 27 (3%) WTD in the Robert Kerr Wildlife Management Area (Edwards Plateau), and 2 of 50 (4%) WTD in the Welder Wildlife Refuge (Gulf Coast) (Waldrup, 1991).

Serologic analyses, though at times able to make a distinction between *Babesia* spp. isolates, are not always conclusive, and involve rather tedious work. There can also be background problems, and these may affect the performance of the assays. Additionally, immunological tests will only detect exposure, not current infection status, and when performing an analysis of a herd of animals, they may not be an optimal choice. This was evident in a study of an outbreak of *B. odocoilei* in a herd of North American elk (*Cervus elaphus*) in Indiana (Gallatin et al., 2003). The herd was screened for the parasites through microscopic evaluation of Giemsa-stained blood smears, cultures and immunofluorescent antibody (IFA) testing, which would indicate the presence of serum antibodies against *B. odocoilei*. Any positive test resulted in imidocarb treatment for that animal. Of the complete herd, 58% of the elk were positive, and were reevaluated six weeks following the treatment. None of the elk showed detectable organisms in the blood smears, yet yielded positive results by IFA analysis. No sudden deaths or a reappearance of clinical signs occurred. Hence, if the second assessment of the animals had been based purely on serologic analysis, current babesial infection would have been suspected, and the elk may have been misdiagnosed. Clearly, new techniques are needed to definitively ascertain current infection status in suspected cases of babesiosis.

Holman et al. (2000) compared a previously established isolate of *B. odocoilei* (*B. odocoilei*-E, so named due to its geographic origin, the Gus Engeling Wildlife Management Area in East Texas) with caribou and North American elk (*Cervus elaphus canadensis*) *Babesia* spp. isolates that had caused fatal infections and high circulating parasitemias (Holman et al., 1988). Immunofluorescent antibody tests were performed using the methods of Goff et al. (1993), and immunoprecipitation assays using those of Barbet et al. (1983). Despite the fact that both serologic analyses revealed antigenic variation, the presence of shared antigens among the three *Babesia* spp. was observed.

Genetic markers have been utilized for identification and diagnosis of these apicomplexan parasites and the diseases they cause. The small subunit ribosomal RNA (SSU rRNA) gene is currently the foremost marker for identification of the piroplasms, and is one of the principal methods of classification of these parasites.

Holman et al. (2000) also compared the caribou, elk and *B. odocoilei*-E isolates against each other by experimental infection and SSU rRNA gene nucleotide sequence analysis. Experimental infection in yearling male red deer (*Cervus elaphus elaphus*), a closely related subspecies of the North American elk, showed no clinical discrepancies among the isolates. SSU rRNA genes of the three samples were amplified, and compared amongst each other as well as against a *B. divergens* isolate originating from an infected cow in County Wicklow, Ireland (Purnell et al., 1976). The elk and caribou *Babesia* spp. isolates were found to possess SSU rRNA gene sequences indistinguishable from the *B. odocoilei*-E isolate, proving conclusively that they were both indeed *B. odocoilei*. Thus, antigenic discrepancies shown by two different immunological tests accordingly reveal that these traditional methods of characterizing these parasites are not able to absolutely define

whether the caribou and the elk *Babesia* spp. isolates were indeed *B. odocoilei*, or even whether they were conspecific (Holman et al., 2000).

Extensive molecular studies have been done with *Theileria* species, haemoprotozoans that infect ruminants, which are closely related to *Babesia* spp. Benign *Theileria* species from Asia and North America were sequenced through the SSU rRNA V4 variable region to provide a better understanding of the phylogenetic relationships among these isolates (Chae et al., 1998a). The samples came from bovine hosts in Japan, Korea and the United States, and cervid hosts in the United States and Canada. This study resulted in the classification of seven different nucleotide sequence patterns (Types A through G); the cervine isolates represent a species separate from the bovine isolates. As there were several sequence types noted in most of the bovine *Theileria* isolates, it was concluded that mixed species, subspecies populations and/or multiple genotypes may well be present in cattle (Chae et al., 1998a).

The SSU rRNA gene nucleotide-sequence analysis was used to definitively identify both benign and moderately pathogenic *Theileria* isolates from cattle and deer originating from different geographic regions (Chae et al., 1999a). Six divergent groups in two major divisions, each division with a common ancestor, were determined upon construction of a phylogenetic tree. Presumed geographic diversity was noted in only Korean bovine *Theileria* spp. (Types C and H), and African *Theileria mutans*. United States bovine *Theileria* isolates in the study were proven not to be *T. mutans*, as previously thought, since they possess *Theileria buffeli* (Type A or D) SSU rRNA gene sequences.

An additional study (Chae et al., 1999b) confirmed *T. cervi* infection in North American WTD and elk based on SSU rRNA V4 variable region analysis. Previous

analyses had discovered two sequence types, F and G, in *T. cervi* from WTD and elk; this study confirmed both types in two deer and two elk isolates. Microheterogeneity was present in the Type G gene only, resulting in the designation of Subtypes G1, G2 and G3, while Type F was highly conserved. The Type F variable regions could eventually be utilized to design specific polymerase chain reaction (PCR) primers (Chae et al., 1999b).

Schnittger et al. (2000) used the SSU rRNA gene to resolve phylogenetic relationships between *Theileria* and *Babesia* isolates. A *Theileria lestoquardi*-like isolate fatal to sheep and goats in northwestern China was compared to other *Theileria* and *Babesia* species, in an attempt to resolve its close association with *T. lestoquardi*. The unknown isolate appeared to be most closely related to *T. buffeli*, yet clearly divergent from *T. lestoquardi*. *Theileria lestoquardi* was found to be most closely related to *Theileria annulata* and *T. buffeli*. The confirmed SSU rRNA sequence of the new Chinese parasite was then used to design specific polymerase chain reaction (PCR) primers to amplify genomic DNA (gDNA) of this organism, an important step as this study ultimately concluded that the Chinese isolate was an as yet unrecognized *Theileria* species.

Cossio-Bayugar et al. (2002) confirmed infection of *T. buffeli* (Type A) in cattle in Michigan by SSU rRNA gene sequence analysis. Previously, *T. buffeli* had only been reported in animals in Texas, Missouri and North Carolina.

Another study using SSU rRNA analysis by Holman et al. (2002) cultured an isolate from reindeer, designated RD61, which was morphologically similar to *B. odocoilei*. Serum from four different reindeer from the same herd all reacted equally strongly to *B. odocoilei* and the RD61 parasites when an IFA test was performed. Gene sequence analysis of the SSU rRNA showed 99.0% identity to that of *B. odocoilei*.

Yet another study isolated and sequenced the SSU gene sequences from both human and wildlife *Babesia* species infections from California and Washington, and performed a phylogenetic analysis that included Asian and African isolates (Kjemtrup et al., 2000). Sequence comparisons revealed that isolates from the human cases were exceptionally similar or, in some cases, indistinguishable, from the isolates from the western wildlife species, particularly those found in mule deer (*Odocoileus hemionus*). The results supported the hypothesis that large ungulates could serve as reservoirs for human infection, and a phylogenetic analysis further demonstrated this in showing the western United States piroplasm isolates in their own distinct clade apart from the Asian and African ones (Kjemtrup et al. 2000).

Other gene markers are showing promise in distinguishing the relationships among both human and animal apicomplexans, such as heat shock-related proteins (hsps) (Ruef et al., 2000). They are highly conserved functional proteins, with homology across their entire length, and thus offer a reasonable target for phylogenetic analyses (Lindquist and Craig, 1988). The hsps assist parasites as they are subjected to stress when invading and adapting to a new host environment, and are even known to have a chaperone function, forming complexes with an assorted group of cellular proteins and peptides (Polla, 1991; Heike et al., 1996).

There are several advantages of using this gene target, as opposed to the SSU rRNA gene target. The conserved sets of genes allow expansion of the size of the data set while still retaining homology. This larger amount of data for alignments and analysis, the use of amino acid sequences rather than those of nucleic acids, and the fact that a conserved, functional protein is used as opposed to conserved secondary structure of a

transcribed product, are just a few of the reasons that this alternative method of evaluating apicomplexan relationships may be advantageous (Ruef et al., 2000). A phylogenetic study by Ruef et al. (2000) using *B. bovis* and other apicomplexan hsps showed strong support for the monophyly of the piroplasms in the genus *Theileria*, and paraphyly of the genus *Babesia*.

Beta-tubulin, a crucial cytoskeleton gene, is gaining acceptance as another candidate molecular marker for speciation. This conserved molecular target seems to contain enough genetic variation to propose a dependable species identification method (Cacciò et al., 2000). A beta-tubulin gene fragment was amplified by PCR from nine different haemoparasitic isolates, *Theileria sergenti*, *T. annulata*, *Babesia bigemina*, *Babesia bovis*, *Babesia major*, *Babesia equi*, *Babesia caballi*, *B. divergens*, and *Babesia microti* by Cacciò et al. (2000). Within this amplified gene fragment is an intron that varies extensively in both length and sequence. Two separate assays were developed: one to differentiate the species directly on the basis of the size of the PCR products and one that further utilized a simple PCR-restriction fragment length polymorphism (RFLP) protocol to differentiate species not able to be defined based on the first assay.

Size variation in the products of the first assay suggested the presence of introns having different lengths in the different species. Electrophoretic separation of the amplification products resulted in the immediate identification of species associated with either horses (*B. caballi* and *B. equi*) or humans (*Bx. divergens* and *B. microti*). An additional nested PCR assay using newly designed primers presented the same results as this primary PCR assay. However, the bovine parasites were not distinguishable in either the regular or nested assays, leading to the development of the PCR-RFLP protocol.

Digestion of the PCR products with the endonuclease *Rsa*I generated specific patterns for each species that allowed for easy differentiation among the equine, human and bovine species. Therefore, the variable introns that interrupt the conserved beta-tubulin genes show enough variation to allow speciation of apicomplexans (Cacciò et al., 2000).

Recent work has shown that the internal transcribed spacers (ITS) of ribosomal DNA (rDNA) are not only species specific, but may provide discrimination among parasites at the subspecies level (reviewed by Prichard and Tait, 2001). A key advantage of this potential genomic target is that it includes highly conserved segments in the coding regions as well as hypervariable spacer sequences (Zahler et al., 1998). Zietara et al. (2001) isolated the complete sequences of the ITS rDNA regions of four subgenera of *Gyrodactylus*. Much molecular variation was expressed in the ITS1 and ITS2 regions, as opposed to morphological variation, expressed in the size and shape of the attachment apparatus. Thus the ITS data allowed new insight to the molecular phylogeny of *Gyrodactylus*, indicating either that the ITS region evolves fast in *Gyrodactylus*, or that the genus consists of groups of a higher taxonomic level than previously recognized (Zietara et al., 2001).

Sequencing of the SSU and LSU (large subunit) rRNA genes, as well as the ITS genes, of several *Theileria parva lawrencei* and *T. parva parva* isolates showed that the 5.8S gene sequences of all eleven *T. parva* isolates were identical, but the ITS regions of both *T. p. parva* and *T. p. lawrencei* contained different combinations of identifiable sequence segments (Collins and Allsopp, 1999). As this resulted in an assortment of segments in any one isolate, it was inferred that the two populations undergo genetic recombination, deriving from gene pools that are not entirely discrete.

Adam et al. (2000) investigated differing degrees of ITS1 variability in several isolates of *Cyclospora cayetanensis*, an apicomplexan protozoan that is an important source of epidemic and endemic human diarrhea. Isolates obtained from Guatemala, where an outbreak occurred in 1996, were compared with Guatemalan and Peruvian isolates from endemic regions. All the isolates from the outbreak contained identical ITS1 sequences, in accordance with their single source of origin, while one of the two Guatemalan isolates and two Peruvian ones contained multiple ITS1 sequences. It was conjectured that the sequence inconsistencies exist due to either variability of the ITS1 region within the genome of a single clone, or representation of multiple clones originating from a single clinical source (Adam et al., 2000).

Zahler et al. (1998) isolated and sequenced the first and second internal transcribed spacers, ITS1 and ITS2, along with the intervening 5.8S coding region of the rRNA gene (Fig. 2.1), in eight *Babesia canis* isolates. The isolates were of disparate geographic origins, vector specificity, and pathogenicity to dogs (*Canis familiaris*). Their study was conducted to determine whether the genetic differences among the isolates concurred with the currently proposed subspecies levels, *B. canis canis*, *B. canis vogeli*, and *B. canis rossi*. The samples used were two *B. canis canis* isolates each from Germany and Hungary, three *B. canis vogeli* isolates (one from Egypt and two from Spain), and one *B. canis rossi* isolate from South Africa. There was little or no genetic variation observed within the subspecies, and the genetic variation between the subspecies was indeed congruent with the existing taxonomical classifications. The sequences separated into three distinguishable genotypic groups that showed identities amongst each other of no more than 82% (*B. canis canis* and *B. canis vogeli*). Comparisons to an equine *Babesia* isolate,

B. caballi, resulted in identities no higher than 69%. Therefore, the tripartite division of *B. canis* was proven and retained, and equally important, the advantages of the ITS1-5.8S-ITS2 genes as a practical genomic target were put into practice and shown to be effective in taxonomically distinguishing organisms at and below the species level. It is currently unknown whether genetic recombination between *B. canis* subspecies occurs at the tick level when they feed on dually infected dogs.

Thus, analysis of the ITS1-5.8S-ITS2 region appears to be a potentially advantageous and significant gene marker that could be useful for identifying subgroups of *B. odocoilei* based on mammalian host or geographic origin. The optimal molecular assay for diagnosis of infections caused by both *Babesia* and *Theileria* species should combine the high sensitivity of the PCR reaction, which is needed for the detection of asymptomatic carriers, with the concurrent identification of the species, which requires an appropriate and informative marker. In addition, the ITS1-5.8S-ITS2 DNA region may provide a diagnostic tool for determining the source of infection when outbreaks of babesiosis occur in managed herds.

To date, a study of the ITS1-5.8S-ITS2 genes of *B. odocoilei* has yet to be done, and whether these gene markers are appropriate for future diagnostic work, and perhaps phylogenetic studies, remains unknown. For these reasons and based on the results and questions from previous studies, the current project was undertaken to sequence the ITS1-5.8S-ITS2 genes from *B. odocoilei* and a *B. odocoilei*-like isolate based on SSU rRNA gene analysis.

CHAPTER II

MATERIALS AND METHODS

Babesia spp. isolates

The *Babesia* spp. isolates used in this study covered a wide range of both infected host species and geographic regions (Table 2.1). *Babesia* spp. previously described included *B. odocoilei* isolates (*Bodo* E, *Bodo* B) from white-tailed deer (*Odocoileus virginianus*) in Texas, a caribou (*Rangifer tarandus caribou*) in Minnesota (MN Carib), an elk (*Cervus elaphus*) in Texas (TX Elk 1), an elk and a reindeer (*Rangifer tarandus tarandus*) in Wisconsin (WIS Elk 1 and WIS Rein, respectively), a desert bighorn sheep (*Ovis canadensis nelsoni*) in California (BH 1), and an elk in Indiana (IN Elk); and a *Babesia* sp. (RD61) from a reindeer in California (Holman et al., 2000; Holman et al., 2003; Goff et al., 1993; Gallatin et al., 2003). Isolates *Bodo* E, *Bodo* B, MN Carib, TX Elk 1, WIS Elk 1, WIS Rein, and IN Elk were previously identified as *B. odocoilei*; all share identical SSU rRNA gene sequences (GenBank Accession No. U16369; Holman et al., 2000; Holman et al., 2002; Holman et al., 2003; Gallatin et al., 2003). RD61 is closely related to *B. odocoilei*, the SSU rRNA gene sequence (GenBank Accession No. AF411337) varying in only 18 base positions from that of *B. odocoilei* (GenBank Accession No. U16369) (Holman et al., 2002).

In addition, *Babesia* isolates from elk in New Hampshire and Wisconsin; from reindeer in New York and Pennsylvania; from white-tailed deer in Massachusetts, Oklahoma, and Minnesota; and 2 isolates from musk ox in Minnesota were included. SSU rRNA gene sequence analysis was used to identify each isolate as to *Babesia* species as described below.

TABLE 2.1. *Babesia* isolates used in this study. Host, geographic origin, clinical signs, how they were acquired (descrip/ref) and GenBank database accession numbers are provided.

Isolate Name	Host	Geographic area	Clinical Signs	Description/References	ITS Genbank Numbers	SSU GenBank Numbers
Bodo B-a	White-tailed deer	East Texas	Normal	Cultured from a naturally infected adult deer on the Brushy Creek Experimental Ranch in the Gulf Slope area of Texas (Holman et al., 2000); a definitively established isolate of *B.odocoilei*		U16369 (Holman et al., 2000)
Bodo B-b				Duplicate DNA extraction		
Bodo E	White-tailed deer	East Texas	Normal	Cultured from an infected blood sample drawn from a 1.5-yr-old male white-tailed deer killed by a hunter at the Gus Engeling Wildlife Management Area (Holman et al., 1988); a definitively established isolate of *B. odocoilei*	Cl. 1 – AY339753 Cl. 2 – AY339754 Cl. 3 – AY339755 (Holman et al., 2003)	U16369 (Holman et al., 2000)
TX Elk 1-a	Elk	Del Rio, TX	Acute babesiosis; died	Cultured from elk in a farmed herd in S. TX; first report of naturally acquired acute fatal babesiosis in elk under management near Del Rio TX; confirmed as *B. odocoilei* using SSU rRNA gene sequence (Holman et al., 2000)	Cl. 1 – AY339751 Cl. 2 – AY339752 Cl. 3 – AY339759 (Holman et al., 2003)	AY339760 (Holman et al., 2003)
TX Elk 1-b				Duplicate DNA extraction		
MN Carib	Caribou	AppleValley, Minnesota	Fatal babesiosis	Cultured from caribou in the MN Zoological Garden; first report of naturally acquired acute fatal babesiosis in caribou under management (Holman et al., 2000)	Cl. 1 – AY339756 Cl. 2 – AY339757 Cl. 3 – AY339758 (Holman et al., 2003)	AY339761 (Holman et al., 2003)
WIS Elk 1	Elk	West Wisconsin	Sick with suspected hemoparasite infection	Cultured from 2 6-yr. old male elk in a farmed herd in W. WIS (Holman et al., 2003)	Cl. 1 – AY339747 Cl. 2 – AY345121 Cl. 3 – AY339748 (Holman et al., 2003)	AY294206 (Holman et al., 2003)
WIS Elk 2	Elk	Wisconsin	Babesiosis	Cultured from elk in a farmed herd in WIS		
WIS Rein	Reindeer	North Wisconsin	Acute babesiosis; died	Obtained from blood of 7-mo. old female reindeer in a farmed herd in N. WIS (Holman et al., 2003)	Cl. 1 – AY339749 Cl. 2 – AY339750 Cl. 3 – AY345122 (Holman et al., 2003)	AY237638 (Holman et al., 2003)
BH 1-a	Desert Bighorn Sheep	San Bernardino Mountains, CA	Normal	Cultured from bighorn sheep in a resident herd in S. CA (Goff et al., 1993); first isolation of *B. odocoilei* in state of CA		AY661502
BH 1-b	Desert Bighorn Sheep	San Bernardino Mountains, CA	Normal	Blood stabilate of bighorn sheep in a resident herd in S. CA (Goff et al., 1993)		AY661502
NH Elk	Elk	New Hampshire	Babesiosis	Cultured from bull elk in a farmed herd; first report of *B. odocoilei* in state of NH		AY661503
IN Elk	Elk	Central Indiana	Fatal babesiosis	Cultured from bull elk with fatal babesiosis in a farmed herd in IN (Gallatin et al., 2003)		
OK WTD	White-tailed deer	Oklahoma	Normal; dual infection with Theileria cervi	Cultured from captive 2-yr. old white-tailed deer		

TABLE 2.1. Continued.

Isolate Name	Host	Geographic area	Clinical Signs	Description/References	ITS Genbank Numbers	SSU GenBank Numbers
WTD MA	White-tailed deer	Massachusetts	Normal	Obtained from blood of free-ranging white-tailed deer		
NY Rein 1	Reindeer	New York	Fatal, acute babesiosis	Obtained from reindeer blood; first report of *B. odocoilei* in state of NY		AY661504
NY Rein 2	Reindeer	New York	Fatal, acute babesiosis	Obtained from reindeer blood		AY661505
MN MO1	Musk Ox	Apple Valley, Minnesota	Fatal, acute babesiosis	Obtained from musk ox blood; first report of *B. odocoilei* in adult male musk ox		AY661507
MN MO2	Musk Ox	Apple Valley, Minnesota	Fatal, acute babesiosis	Obtained from musk ox blood; yearling male musk ox.		AY661508
MN WTD	White-tailed deer	Apple Valley, Minnesota	Normal	Obtained from blood of free-ranging white-tailed deer in a managed area		
PA Rein	Reindeer	Pennsylvania	Fatal, acute babesiosis	Obtained from reindeer blood; first report of *B. odocoilei* in Pennsylvania; 2-yr. old female reindeer		AY661506
RD61	Reindeer	Applegate, Placer County, CA	Concurrent infection with Listeria; died of unknown causes not *Babesia*-related	Cultured from naturally infected CA reindeer (No. 61) ; morphologically similar to *B. odocoilei*; SSU rRNA gene sequencing (Holman et al., 2002) showed 99.0% identity to *B. odocoilei*	Cl. 1 – AY339744 Cl. 2 – AY339745 Cl. 3 – AY339746 (Holman et al., 2003)	AF411337 (Holman et al., 2002)

DNA Extraction

Purified DNA samples previously obtained from isolates *Bodo* B, *Bodo* E, MN Carib, TX Elk 1, WIS Elk 1, WIS Rein, and CA RD61 were used in this study (Holman et al., 2000; Holman et al., 2002; Holman et al., 2003). In addition, duplicate extractions were made from frozen blood for isolates *Bodo* B and TX Elk 1; the original samples were signified by "a" and the duplicates, "b." DNA was purified from cultured BH 1 (BH 1-a) and from original blood stabilate (BH 1-b), the latter kindly provided by W. Goff, USDA/ARS, Pullman, WA. Genomic DNA was also purified from the newly acquired isolates from cultures or infected blood. The culture method used was described previously (Holman et al., 2003).

Genomic DNA was purified using a standard phenol-chloroform extraction method facilitated by the use of Phase Lock Gel tubes (Phase Lock Gel System, Eppendorf AG, Hamburg, Germany) as follows. Heavy and Light Phase Lock Gel (PLG) tubes were prepared by centrifuging at 9000 X g for 10 min. Infected RBC pellets were washed 3X in PBS by centrifugation at 600 X g and either immediately used for DNA extraction or frozen at –80 C until use. Frozen samples were quickly thawed at 37 C prior to use. Following the transfer of 0.2 ml RBC or thawed RBC lysate to the pre-spun Light PLG tube, an equal volume of lysis buffer (10 mM Tris, pH 7.5; 1 mM EDTA, pH 8.0; 10% SDS) was added and the mixture incubated at room temperature until complete lysis of the erythrocytes occurred. RNAse A was then added to a final concentration of 50 µg/ml, and then the mixture was incubated at 37 C for 1 hr. Following the incubation, Proteinase K was added to a final concentration of 100 µg/ml (Proteinase K stock solution 20 mg/ml in water; 5 µl/ml lysate added), and the mixture incubated either for 3 hr at 50 C with

occasional swirling or overnight at 25 C. The mixture was allowed to cool to 25 C, and an equal volume of Tris-equilibrated phenol was added to the tube, which was then mixed on a tube rotator (Dynal Rotamix, Dynal, Inc., New Hyde Park, NY) at 25 rpm for 5 min. The tube was centrifuged for 2 min at 9000 X g. The aqueous phase was reextracted with an equal volume of Tris-equilibrated phenol, then mixed and centrifuged as above. The aqueous phase was then extracted using an equal volume of 50:50 chloroform/iso-amyl alcohol:phenol (24 parts chloroform to 1 part iso-amyl alcohol), then mixed and centrifuged as above. The aqueous phase was transferred to a Heavy PLG tube and extracted with an equal volume of chloroform/iso-amyl alcohol, then mixed and centrifuged as above. The top aqueous layer in the tube was measured and transferred to a sterile microtube and 3 M NaOAC was added to a final concentration of 0.3 M. After mixing, 2.5 to 3 volumes of cold absolute ethanol were added and mixed, and the DNA was allowed to precipitate overnight at –80 C. The following day, the microtube was centrifuged at 7 C for 30 minutes at 9300 X g. The supernatant was removed and the pellet was rinsed with 500 µl of cold 70% ethanol by centrifugation as above. The ethanol was removed from the remaining pellet, which was dried overnight at room temperature, and then resuspended the next day in 20-50 µl TE buffer (0.1 M tris(hydroxymethyl)aminomethane and 2 mM EDTA, pH 8.0), the amount depending on the size of the pellet.

SSU rRNA Gene Sequence

SSU rRNA gene sequences were obtained for all new isolates by amplifying and sequencing the gene as described below, except for WIS Elk 2. The WIS Elk 2 SSU rRNA

gene was sequenced from previously prepared cloned plasmid DNA (unpublished data, P.J. Holman). For the remaining samples, the SSU rRNA genes were amplified from approximately 50-100 ng template genomic DNA using 1 pmol each primers A and B (Fig. 2.1) in a 25 µl reaction volume (Sogin, 1990). The polymerase chain reaction (PCR) mixture also contained PCR Buffer (40 mM Tricine-KOH, 15 mM KOAc, 3.5 mM $Mg(OAC)_2$, 3.75 µg/ml BSA, 0.005% Tween 20, 0.005% Nonidet-P40), dNTP Mix (0.2 mM each of dATP, dCTP, dGTP, and dTTP), *Taq*DNA Polymerase Mix (BD TITANIUM *Taq*DNAPolymerase, proofreading polymerase, and BD TaqStart Antibody at 1.1 µg/µl) and PCR-Grade water according to manufacturer's instructions (BD Advantage 2 PCR Kit, BD Biosciences Clontech, Palo Alto, CA). The amplification profile for the primary PCR was: initial denaturation at 96 C for 3 min, followed by 30 cycles of denaturation at 94 C for 10 sec, annealing at 60 C for 10 sec and extension at 72 C for 2 min, with a final extension at 72 C for 10 min and then hold at 4 C (PCR Express or Sprint thermocycler; Hybaid, Ashford, UK). A second, nested reaction was used for the MN WTD isolate with the reaction volume, reagents, and amplification profile the same as above, except that the template DNA consisted of 1 µl of the primary PCR product, and primers AN50 (5'-GCTTGTCTTAAAGATTAAGCCATGC-3') and BN1700 (5'-CGACTTCTCCTTCCTTTAAGTGATAAG-3') were used (Fig. 2.1). Primary and nested products were separated by electrophoresis through a 1% agarose gel, alongside a 100 BP marker (Invitrogen Corp., Carlsbad, CA). The agarose gel was subsequently stained with ethidium bromide to visualize the bands by UV transillumination.

BH 1-a, BH 1-b, NH Elk, and MN MO 1 SSU rRNA genes were directly sequenced from the primary SSU rRNA gene products. For each, 2 to 5 (depending on the

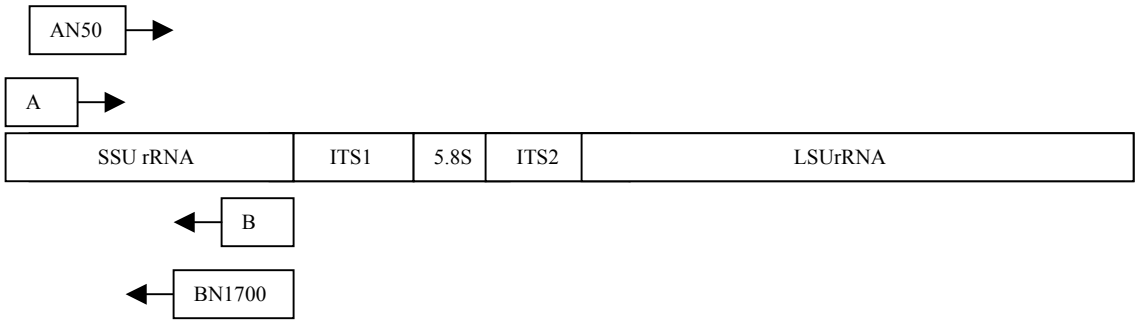

FIGURE 2.1. Schematic drawing showing positions and directions of primers used for amplification of the SSU rRNA gene region. Primary PCR primers include A and B, and nested PCR primers include AN50 and BN1700.

amount of amplicon obtained) primary SSU rDNA PCR products were pooled, column purified (QIAquick PCR Purification Kit, Qiagen Inc., Valencia, CA) and quantitated by agarose gel electrophoresis alongside a mass marker (High Mass DNA Ladder, Invitrogen Corp., Carlsbad, CA), which ranged from 5 to 100 ng/µl. Approximately 200 ng purified amplicon was used in each sequencing reaction described below.

Isolates OK WTD, WTD MA, MN MO 2, MN WTD, NY Rein 1, NY Rein 2, and PA Rein were cloned prior to sequencing. Each amplicon was ligated into a plasmid vector, pCR 2.1-TOPO, and *Escherichia coli* chemically competent cells (TOP10F, One Shot) were transformed according to manufacturer's instructions (TOPO TA Cloning, Invitrogen Corp., Carlsbad, CA). If the PCR product was over 24 hr old at the time of ligation, the product was incubated with 2X Qiagen *Taq* PCR Master Mix (*Taq*DNAPolymerase, Qiagen PCR Buffer (3 mM $MgCl_2$), 400 µM of each dNTP, Qiagen Inc., Valencia, CA) for 15 min at 72 C, to add single deoxyadenosine (A) overhangs to the 3' ends of the SSU-DNA and thus ensure that it would be ligated efficiently with the

plasmid vector; amplicons less than 24 hr old were directly ligated into the vector. The ligation mixture was incubated at room temperature for 30 min. The *E. coli* was thawed on ice for 15 min and 2 µl of the ligation mixture was added to the cells, then the tube was held on ice for another 30 min. The cells were then heat shocked for 30 sec at 42 C, and 250 µl SOC medium was added. The tube was incubated for 1 hr at 37 C in an incubator-shaker at 200 rpm (Queue Orbital Shaker, Queue Systems, Inc., Columbia, SC). Finally, the *E. coli* suspension was spread onto two LB (Luria Broth Agar, Sigma-Aldrich Co., St. Louis, MO) plates containing Kanamycin (50 mg/ml in 0.9% sodium chloride, Sigma-Aldrich Co., St. Louis, MO) and X-Gal (5-Bromo-4-Chloro-3-Indolyl-β-D-Galactopyranoside, 40 mg/ml, Fisher Scientific, Fair Lawn, NJ). The plates were incubated overnight at 37 C.

Colony PCR was performed on 14 colonies the following day to screen for the insert SSU-DNA. A portion of each colony was added to 9 µl sterile water in a 0.2 ml PCR tube. One tube containing 10 µl water served as a negative control. The tubes were incubated for 10 min at 96 C, then placed on ice and 11 µl PCR master mix was added to each. The master mix consisted of 2X Qiagen *Taq* PCR Master Mix (Qiagen Inc., Valencia, CA) with 1 pmol each M13 Forward (-20) (5-GTAAAACGACGGCCAG-3') and M13 Reverse (5'-CAGGAAACAGCTATGAC-3') primers. The cycling program used was initial denaturation at 94 C for 10 min, followed by 30 cycles of denaturation at 94 C for 1 min, annealing at 50 C for 1 min and extension at 72 C for 1 min, with a final extension at 72 C for 10 min and then hold at 4 C. The products were checked on an agarose gel as described above.

Five clones containing the desired insert were expanded in an overnight broth culture, and then plasmid DNA (pDNA) purified according to manufacturer's instructions (Qiagen Miniprep Kit, Qiagen Inc., Valencia, CA). The purified pDNA was quantitated by electrophoresis on an agarose gel alongside a plasmid DNA sample of known concentration (pTZ Marker, 230 ng/µl, Sigma-Aldrich Co., St. Louis, MO). To confirm the presence of the correct size insert, the pDNA was digested using the restriction enzyme *Eco*R I (Invitrogen Corp., Carlsbad, CA). In each of 5 tubes, 1 µl pDNA, 0.5 µl *Eco*R I enzyme, 0.5 µl 10X Buffer (50 mM Tris-HCl (pH 8.0), 10 mM $MgCl_2$, 100 mM NaCl, Invitrogen Corp., Carlsbad, CA), and 3 µl sterile water were added, and then all tubes were incubated at 37 C for 1 hr, with stirring every 15 min. To each tube was then added 2 µl of 5X gel loading solution (0.05% bromphenol blue, 40% sucrose, 0.1 M EDTA pH 8, 0.5% sodium lauryl sulfate, Sigma-Aldrich Co., St. Louis, MO). The 7 µl samples were each electrophoresed through an agarose gel as described above, alongside a 100 BP marker (Invitrogen Kit, Invitrogen Corp., Carlsbad, CA).

The full nucleotide sequences were obtained from clones by sequencing with primers 528F (5'-CGGTAATTCCAGCTCC-3'), M13 Forward (-20) and M13 Reverse. Primers 528F, AN50 and BN1700 were used to directly obtain the sequences from PCR amplicons. All sequencing reactions (dGTP Big Dye terminator ready reaction; PE Applied Biosystem, Norwalk, CT) were performed by automated methods (Applied Biosystems 3100 genetic analyzer with DNA analysis software version 3.7) through services at Texas A&M University at either the Gene Technologies Lab in the Department of Biology, or the DNA Technologies Lab in the Department of Veterinary Pathobiology.

BLAST searches (NCBI) were performed for all of the SSU rRNA gene sequences obtained to determine the identity of the respective parasites.

ITS rRNA Gene Sequence

To acquire the ITS sequences, a DNA fragment spanning the ITS1-5.8S-ITS2 region (Fig. 2.2) was amplified from the template DNA using PCR. Generic eukaryotic forward strand primers 1055F (5'-GGTGGTGCATGGCCG-3') or ITSF (5'-GAGAAGTCGTAACAAGGTTTCCG-3'), derived from the SSU rRNA gene, and reverse strand primers ITSR (5'-GGTCCGTGTTTCAAGACGG-3') or LSUR50 (5'-GCTTCACTCGCCGTTACTAGG-3'), derived from the large subunit (LSU) rRNA gene, were used in primary PCRs (Fig 2.2). If a single band was not obtained, a secondary PCR was done using nested generic forward ITS primer 1200F (5'-CAGGTCTGTGATGCT-3'), ITSF (5'-GAGAAGTCGTAACAAGGTTTCCG-3'), or Bo1550F (5'-CCCGAAAGGGCTGG-3'), all derived from the SSU rRNA gene, and reverse nested primer LSUR300 (5'-TWGCGCTTCAATCCC-3'), LSUR50 (5'-GCTTCACTCGCCGTTACTAGG-3'), or BoLSUR10 (5'-CAGCGGGATAGCCTC-3'), all derived from the LSU rRNA gene (Fig. 2.2). The primer sets used for each isolate are shown in Table 2.2.

The ITS PCR mixes were composed as described above. The amplification profile for the primary ITS PCR was: initial denaturation at 96 C for 3 min, followed by 35 cycles of denaturation at 94 C for 30 sec, annealing at 55 (primers ITSF and LSUR50, 1055F and ITSR), 57 (primers 1055F and ITSR) or 60 C (primers ITSF and LSUR50) for 30 sec and extension at 72 C for 2 min, with a final extension at 72 C for 10 min and then hold at 4 C.

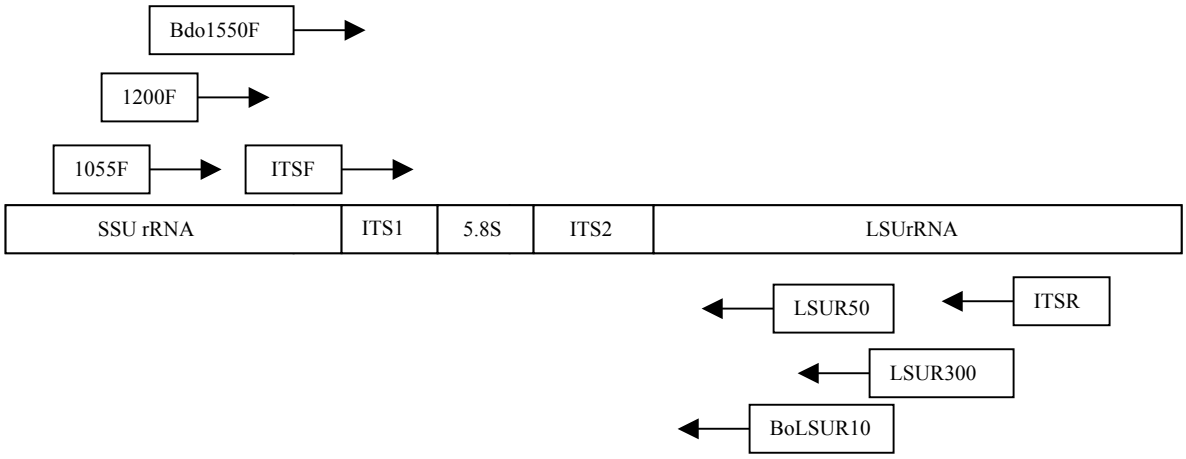

FIGURE 2.2**.** Schematic drawing showing positions and directions of primers used for amplification of the ITS1-5.8S-ITS2 gene region. These PCR primers include 1055F, ITSR, 1200F, LSUR50, LSUR300, ITSR, Bdo1550F, and BoLSUR10.

The amplification profile for the nested ITS PCR was: initial denaturation at 96 C for 3 min, followed by 30 cycles of denaturation at 94 C for 10 sec, annealing at 52 C (primers Bo1550F and BoLSUR10), 55 C (primers1200F and LSUR300, ITSF and LSUR300, ITSFand LSUR50), 57 C (primers 1200F and LSUR300) or 60 C (primers ITSF and LSUR50) for 10 sec and extension at 72 C for 2 min, with a final extension at 72 C for 10 min and then hold at 4 C. The details for each isolate are listed in Table 2, except for isolates *Bodo* E, WIS Elk 2, RD61, and TX Elk 1-a, which were provided to this study as nested PCR products (unpublished results, P.J. Holman). All primary and nested ITS-PCR products were electrophoresed through an agarose gel as described above, alongside a 100 BP marker (Invitrogen Kit, Invitrogen Corp., Carlsbad, CA).

All PCR products were cloned as described above, except that the ligation mixture was incubated at room temperature for 5 min instead of 30 min, and the NH Elk nested

PCR products were column purified prior to ligation (QIAquick PCR Purification Kit, Qiagen Inc.).

Sequencing reactions and sequencing were performed as described above, but using primers ITSFN (5'-GTGAACCTGCGGAAGG-3'), ITSF, LSUR50, M13 Forward (-20) and M13 Reverse. The ITS sequences were aligned and compared among each other using Sequencher 3.11 software. Percent identities of both the entire ITS1-5.8S-ITS2 DNA segments, and each gene separately, were determined using the GeneStream program (http://www2.igh.cnrs.fr/).

TABLE 2.2. ITS-PCR details for *Babesia* isolates. Particulars for acquiring the ITS1-5.8S-ITS2 gene region for each isolate are provided.

Isolate	DNA Source	Primary PCR Primers	Annealing Temperature, Primary PCR	Sequence From Primary PCR?	Secondary Nested PCR Primers	Annealing temperature, Secondary PCR	Sequence from Secondary PCR?
Bodo B-a	Culture	ITSF, LSUR50	55 C	Yes	NA [a]	NA	No
Bodo B-b	Culture	1055F, ITSR	55 C	No	ITSF, LSUR50	60 C	Yes
TX Elk 1-b	Culture	1055F, ITSR	55 C	No	1200F, LSUR300	55 C	Yes
MN Carib	Culture	1055F, ITSR	55 C	No	ITSF, LSUR300	55 C	Yes
WIS Elk 1	Culture	1055F, ITSR	55 C	No	1200F, LSUR300	57 C	Yes
WIS Rein	Blood	1055F, ITSR	55 C	No	ITSF, LSUR300	55 C	Yes
BH 1-a	Culture	1055F, ITSR	55 C	No	1200F, LSUR300	55 C	Yes
BH 1-b	Stabilate	1055F, ITSR	55 C	No	1200F, LSUR300	55 C	Yes
NH Elk	Culture	1055F, ITSR	55 C	No	1200F, LSUR300	55 C	Yes [b]
IN Elk	Culture	1055F, ITSR	55 C	No	1200F, LSUR300	55 C	Yes
OK WTD	Culture	1055F, ITSR	55 C	No	1200F, LSUR300	55 C	Yes

TABLE 2.2. Continued.

Isolate	DNA Source	Primary PCR Primers	Annealing Temperature, Primary PCR	Sequence From Primary PCR?	Secondary Nested PCR Primers	Annealing temperature, Secondary PCR	Sequence from Secondary PCR?
WTD MA	Blood	1055F, ITSR	57 C	No	1200F, LSUR300	57 C	Yes
NY Rein 1	Blood	1055F, ITSR	55 C	No	Bo1550F, BoLSUR10	52 C	Yes
NY Rein 2	Blood	1055F, ITSR	55 C	No	1200F, LSUR300	55 C	Yes
MN MO 1	Blood	ITSF, LSUR50	60 C	Yes	NA	NA	No
MN MO 2	Blood	1055F, ITSR	55 C	No	ITSF, LSUR50	55 C	Yes
MN WTD	Blood	1055F, ITSR	55 C	No	ITSF, LSUR300	55 C	Yes
PA Rein	Blood	1055F, ITSR	55 C	No	ITSF, LSUR50	55 C	Yes

[a] NA = not applicable.
[b] The nested ITS-PCR products were pooled and column purified prior to ligation.

CHAPTER III

RESULTS

All *Babesia* sp. isolates used in this study possessed SSU rRNA gene sequences identical to that of *Babesia odocoilei* (GenBank accession no. U16369), except for RD61, the reindeer isolate from California (AF411337).

DNA was purified from each isolate and the ITS1-5.8S-ITS2 gene region was successfully amplified by PCR and cloned. Duplicate sets of clones were obtained from *Bodo* B (*Bodo* B-a and *Bodo* B-b), TX Elk (TX Elk 1-a and TX Elk 1-b) and BH 1 (BH 1-a and BH 1-b) isolates using different batches of DNA. At least three clones from each amplicon were sequenced. Four clones were sequenced from MN Carib, TX Elk 1-a, NY Rein 2 and *Bodo* E, and five clones from MN WTD.

The *B. odocoilei* ITS1-5.8S-ITS2 gene region ranged from 818 to 827 base pairs (bp) in length, with the ITS1 from 414 to 420 bp and the ITS2 from 250 to 253 bp. The California reindeer RD61 *Babesia* sp. possessed an ITS1-5.8S-ITS2 gene region of 835 bp, with the ITS1 423 bp and ITS2 253 bp in length. The 5.8S gene region, consisting of 159 bp, was identical for all isolates, including RD61, except for minor microhetero-geneity (14 occurrences as single base polymorphisms among all 63 clones). No fixed differences were found in the 5.8S gene sequence.

Percent identity pairwise comparisons among the samples were calculated for the full ITS1-5.8S-ITS2 gene region, and for the individual ITS1 and ITS2 regions (Appendix A). From this data comparisons were done based on: 1) Isolates, 2) Clones, 3) Host, 4) Geographic origin, 5) Culture versus blood derived parasite DNA clones, and 6) Fatal versus nonfatal host infections.

Genetic variation between the California RD61 *Babesia* sp. and the various *B. odocoilei* isolates was compared. RD61, which is distinct from *B. odocoilei* based on SSU rRNA gene sequence (99.0% identity between RD61 and *B. odocoilei*), was also consistently distinct in the ITS region from all *B. odocoilei* isolates in this study. While identities between the *B. odocoilei* isolates ranged from 93.3% to 99.9% in the entire ITS1-5.8S-ITS2 gene region, 90.2% to 99.8% in ITS1 and 92.0% to 100.0% in ITS2, the highest percent identity between RD61 and any isolate was only 88.2% in ITS1-5.8S-ITS2 (with OK WTD and *Bodo* E), 85.8% in ITS1 (with OK WTD), and 87.6% in ITS2 (with *Bodo* E).

Percent identity among clones from the same *Babesia* sp. isolate for the ITS1-5.8S-ITS2 region ranged from 93.6 for *Bodo* B-b clones 1 and 21 to 100.0 for both MN MO1 clones 5 and 8 and MN WTD clones 1 and 14. ITS1 identities ranged from 91.4% between *Bodo* B-b clones 14 and 21 to 100.0% between MN MO1 clones 5 and 8, NY Rein 1 clones 4 and 12, RD61 clones 5 and 8 and *Bodo* E clones 1 and 3. Ranges in ITS2 were slightly narrower, from 93.2% between *Bodo* B-b clones 1, 14 and 21, to 100.0% between MN Carib clones 3, 8 and 10, MN MO1 clones 5 and 8, MN MO2 clones 6 and 14, *Bodo* B-b clones 1 and 14, TX Elk 1-a clones 7 and 18, TX Elk 1-b clones 1 and 2, RD61 clones 2 and 5, WIS Elk 2 clones 10 and 14, *Bodo* E clones 1 and 3, IN Elk clones 11 and 14 and MN WTD clones 6 and 14.

Parallel comparisons of *B. odocoilei* and RD61 were also done based on the percent identities within and between the animal host of the isolate. The isolates from reindeer, elk, white-tailed deer or musk ox were compared among each other. The CA bighorn sheep (BH 1-a, BH 1-b) and MN Carib were not included since only one isolate from each

host was available for this study. The highest and lowest ranges in each gene region are listed in Table 3.1, and do not include intraclonal comparisons. Reindeer isolates included PA Rein, WIS Rein, NY Rein 1 and NY Rein 2; elk isolates, WIS Elk 1, TX Elk 1-a, TX Elk 1-b, NH Elk, WIS Elk 2 and IN Elk; white-tailed deer isolates, *Bodo* B-a, *Bodo* B-b, OK WTD, WTD MA, *Bodo* E and MN WTD; and musk ox isolates, MN MO1 and MN MO2. Again, the percent identities for *B. odocoilei* within a particular host in both ITS1, ITS2 and the entire ITS1-5.8S-ITS2 range from approximately 90% to 100% for all animal hosts of origin. Table 3.1 also lists comparisons within the reindeer host including the RD61 isolate, and these percent identities in all gene regions range from approximately 83% to 98%. The highest and lowest percent identity ranges between animal hosts are listed in Table 3.2, and do not include intraclonal comparisons. For all gene regions, this data ranges from approximately 91% to 100% for all interhost comparisons.

In order to carry out the geographic comparisons, all isolates were placed into one of five geographic areas – California, Minnesota, Northeastern United States, Texas-Oklahoma, and Wisconsin-Indiana. California isolates included RD61, BH 1-a and BH 1-b; Minnesota isolates, MN Carib, MN MO1, MN MO2 and MN WTD; Northeastern United States isolates, PA Rein, WTD MA, NH Elk, NY Rein 1 and NY Rein 2; Texas-Oklahoma isolates, *Bodo* B-a, *Bodo* B-b, TX Elk 1-a, TX Elk 1-b, OK WTD and *Bodo* E; and Wisconsin-Indiana isolates, WIS Elk 1, WIS Rein, WIS Elk 2 and IN Elk. The highest and lowest ranges in each gene region are listed in Table 3.3, and do not include intraclonal comparisons. Although the samples from California were limited and included disparate data leading to results unlike the other geographic regions, they are still included

TABLE 3.1. Parasite ITS identity comparisons within a host species. The lowest percent identity and the highest percent identity found between isolates from the same species of vertebrate host are given for the full ITS1-5.8S-ITS2 region and ITS1 and ITS2 only.

	Low Value ITS1-5.8S-ITS2	High Value ITS1-5.8S-ITS2	Low Value ITS1	High Value ITS1	Low Value ITS2	High Value ITS2
Reindeer	95.0% WIS Rein vs NY Rein 2	98.2% PA Rein vs NY Rein 1	92.1% WIS Rein vs NY Rein 1 and 2	98.3% PA Rein vs WIS Rein	93.6% PA Rein vs NY Rein 1	98.4% PA Rein vs NY Rein 1;NY Rein 1 vs NY Rein 2
RD61 vs Reindeer	86.5% WIS Rein vs RD61	87.8% NY Rein 1 vs RD61	82.5% WIS Rein vs RD61	84.7% NY Rein 1 vs RD61	84.6% WIS Rein vs RD61	86.5% NY Rein 1 vs RD61
Elk	94.1% WIS Elk 1 vs TX Elk 1-a	98.2% WIS Elk 1 vs NH Elk	90.2% WIS Elk 1 vs TX Elk 1-a	98.8% WIS Elk 1 vs NH Elk	92.4% TX Elk 1-a vs IN Elk	99.2% WIS Elk 1 vs NH Elk
White-tailed deer	93.3% *Bodo* B-b vs *Bodo* E	98.3% *Bodo* B-b vs WTD MA	90.5% WTD MA vs *Bodo* E	98.1% *Bodo* B-a and B-b vs WTD MA;WTD MA vs MN WTD	92.8% *Bodo* B-a and B-b vs *Bodo* E;OK WTD vs WTD MA	98.8% *Bodo* B-b vs WTD MA;WTD MA vs *Bodo* E and MN WTD
Musk Ox	97.7% MN MO1 vs MN MO2	99.9% MN MO1 vs MN MO2	96.4% MN MO1 vs MN MO2	99.8% MN MO1 vs MN MO2	98.4% MN MO1 vs MN MO2	100.0% MN MO1 vs MN MO2

in the analysis. Percent identities in both ITS1, ITS2 and the entire ITS1-5.8S-ITS2 range from approximately 91% to 100% for all geographic areas. The highest and lowest percent identity ranges among geographic areas are listed in Table 3.4, and do not include intraclonal comparisons or RD61. For all gene regions, this data ranges from approximately 90% to 100% for all interarea comparisons.

The effect of culture versus blood source of the parasite on clonal variation was also evaluated. ITS1-5.8S-ITS2 comparisons of cultured isolates ranged from 93.6%

TABLE 3.2. Parasite ITS identity comparisons between host species. The lowest percent identity and the highest percent identity found between isolates from paired host species are given for the full ITS1-5.8S-ITS2 region and ITS1 and ITS2 only.

	Low Value ITS1-5.8S-ITS2	High Value ITS1-5.8S-ITS2	Low Value ITS1	High Value ITS1	Low Value ITS2	High Value ITS2
Reindeer vs Elk	93.6% NY Rein 1 vs NH Elk	98.4% NY Rein 1 vs WIS Elk 2	91.2% WIS Rein vs WIS Elk 1 and 2	98.6% PA Rein vs WIS Elk 2; NY Rein 2 vs NH Elk	93.6% PA Rein vs TX Elk 1-a	99.6% WIS Rein vs WIS Elk 2
Reindeer vs White-tailed deer	93.9% PA Rein and NY Rein 1 vs *Bodo* E	98.8% WIS Rein vs MN WTD; NY Rein 1 vs WTD MA	90.7% WIS Rein vs *Bodo* E	98.6% NY Rein 1 vs *Bodo* B-a	92.8% PA Rein and NY Rein 2 vs *Bodo* B-a and B-b	99.6% WIS Rein vs MN WTD; NY Rein 1 vs WTD MA
Reindeer vs Musk Ox	95.2% WIS Rein vs MN MO2	98.9% NY Rein 1 vs MN MO2	91.9% WIS Rein 1 vs MN MO1 and 2	98.3% NY Rein 1 vs MN MO2	94.4% NY Rein 1 vs MN MO2	100.0% NY Rein 1 vs MN MO1
Reindeer vs Bighorn Sheep	93.7% vs WIS Rein	97.6% vs WIS Rein	91.4% vs WIS Rein	97.3% vs WIS Rein	93.6% vs WIS Rein and NY Rein 1	97.6% vs WIS Rein and NY Rein 1
Elk vs White-tailed deer	93.6% TX Elk 1-a vs *Bodo* B-b	98.3% WIS Elk 2 vs WTD MA and MN WTD	90.5% WIS Elk 2 vs *Bodo* E	98.8% IN Elk vs *Bodo* B-a	92.1% NH Elk vs WTD MA	100.0% TX Elk 1-a and 1-b vs *Bodo* B-b
Elk vs Musk Ox	94.1% TX Elk 1-a vs MN MO2	98.6% IN Elk vs MN MO2	91.4% WIS Elk 1 vs MN MO2	98.8% IN Elk vs MN MO2	95.2% TX Elk 1-a vs MN MO1 and 2	99.2% WIS Elk 1 vs MN MO1 and 2; NH Elk vs MN MO1 and 2
Elk vs Bighorn Sheep	93.5% vs TX Elk 1-a	97.0% vs NH Elk	91.2% vs TX Elk 1-a	98.3% vs WIS Elk 1	92.8% vs TX Elk 1-a	97.6% vs IN Elk
White-tailed deer vs Musk Ox	93.7% *Bodo* E vs MN MO2	99.2% WTD MA vs MN MO2	90.5% *Bodo* E vs MN MO1 and 2	98.6% WTD MA vs MN MO2	93.2% *Bodo* B-a and B-b vs MN MO1 and 2	99.6% WTD MA vs MN MO1 and 2

TABLE 3.2. Continued.

	Low Value ITS1-5.8S-ITS2	High Value ITS1-5.8S-ITS2	Low Value ITS1	High Value ITS1	Low Value ITS2	High Value ITS2
White-tailed deer vs Bighorn Sheep	94.5% vs *Bodo* B-a	97.7% vs MN WTD	91.7% vs OK WTD	96.9% vs MN WTD	93.2% vs WTD MA	98.4% vs MN WTD
Musk Ox vs Bighorn Sheep	94.1% vs MN MO2	95.5% vs MN MO2	90.7% vs MN MO1 and 2	93.6% vs MN MO2	96.0% vs MN MO1 and 2	97.6% vs MN MO1

(*Bodo* B-b clones 1 and 21, *Bodo* B-b clones 14 and 21) to 99.9% (TX Elk 1-a clones 7 and 18, TX Elk 1-b clones 1 and 2, *Bodo* E clones 1, 3 and 6, IN Elk clones 11 and 14), and isolates obtained from blood ranged from 95.2% (MN WTD clones 4 and 9) to 100.0% (MN MO1 clones 5 and 8, MN WTD clones 1 and 14). ITS1 was somewhat more variable, with cultured isolates ranging from 91.4% (*Bodo* B-b clones 14 and 21) to 100.0% (*Bodo* E clones 1 and 3, RD61 clones 5 and 8), and blood isolates from 92.2% (WTD MA clones 5 and 9) to 100.0% (MN MO1 clones 5 and 8, NY Rein 1 clones 4 and 12, MN WTD clones 1 and 14). ITS2 comparison values were similar to those obtained for the full gene region, with cultured isolates ranging from 93.2% (*Bodo* B-b clones 1 and 21, *Bodo* B-b clones 14 and 21) to 100.0% (MN Carib clones 3, 8 and 10, *Bodo* B-b clones 1 and 14, TX Elk 1-a clones 7 and 18, TX Elk 1-b clones 1 and 2, RD61 clones 2 and 5, WIS Elk 2 clones 10 and 14, *Bodo* E clones 1, 3 and 6, IN Elk clones 11 and 14), and blood isolates ranging from 95.2% (NY Rein 1 clones 7 and 12, WTD MA clones 3 and 9) to 100.0% (MN MO1 clones 5 and 8, MN MO2 clones 6 and 14, MN WTD clones 1 and 4).

TABLE 3.3. Comparisons among parasite isolates within geographic regions.

	Low Range ITS1-5.8S-ITS2	High Range ITS1-5.8S-ITS2	Low Range ITS1	High Range ITS1	Low Range ITS2	High Range ITS2
CA	87.1% BH 1-a vs RD61	87.3% BH 1-a and 1-b vs RD61	83.9% BH 1-a vs RD61	84.6% BH 1-b vs RD61	83.9% BH 1-b vs RD61	85.1% BH 1-a and 1-b vs RD61
MN	94.2% MN Carib vs MN WTD	99.9% MN MO1 vs 2	91.7% MN Carib vs MN WTD	99.8% MN MO1 vs 2	94.8% MN Carib vs MN WTD, MN MO1 and 2	100.0% MN MO1 vs 2
NE US	93.6% NH Elk vs NY Rein 1	98.8% NY Rein 1 vs WTD MA	91.9% NH Elk vs NY Rein 1 and WTD MA; NY Rein 1 vs WTD MA	98.6% NH Elk vs NY Rein 2	92.1% NH Elk vs WTD MA	99.6% NH Elk and NY Rein 1 vs WTD MA
TX-OK	93.3% *Bodo* B-b vs *Bodo* E	97.8% TX Elk 1-a and 1-b vs OK WTD	91.0% TX Elk 1-a vs *Bodo* E	98.3% TX Elk 1-a and 1-b vs OK WTD	92.8% *Bodo* B-a and B-b vs *Bodo* E	100.0% *Bodo* B-b vs TX Elk 1-a and 1-b
WIS-IN	94.3% WIS Elk 1 vs WIS Rein	98.2% WIS Rein vs WIS Elk 2	91.2% WIS Elk 1 and 2 vs WIS Rein	97.9% WIS Elk 2 vs IN Elk	94.8% WIS Elk 2 vs IN Elk	99.6% WIS Rein vs WIS Elk 2

Finally, the effect of fatal versus nonfatal host infections on isolate variation was evaluated. The isolates were separated into fatal or nonfatal infection status, and comparisons were done among them. Again, the RD61 isolate was not included due to the disparate data. The highest and lowest ranges in each gene region are listed in Table 3.5, and do not include intraclonal comparisons. Parasite isolates obtained from fatal host infections were TX Elk 1-a, TX Elk 1-b, MN Carib, WIS Rein, NY Rein 1, NY Rein 2, MN MO1, MN MO2, PA Rein and IN Elk; from nonfatal host infections, *Bodo* B-a,

TABLE 3.4. Comparisons among *B. odocoilei* isolates between geographic regions.

	Low Range ITS1-5.8S-ITS2	High Range ITS1-5.8S-ITS2	Low Range ITS1	High Range ITS1	Low Range ITS2	High Range ITS2
CA vs MN	93.3% vs MN Carib	97.7% vs MN WTD	90.7% vs MN MO1	96.9% vs MN WTD	92.4% vs MN Carib	98.4% vs MN WTD
CA vs NE US	93.8% vs NY Rein 1	97.0% vs NH Elk	91.6% vs PA Rein	97.6% vs NH Elk	93.2% vs WTD MA	97.6% vs NY Rein 1
CA vs TX-OK	93.5% vs TX Elk 1-a	97.1% vs OK WTD	91.2% vs TX Elk 1-a	97.1% vs TX Elk 1-a	92.8% vs TX Elk 1-a	97.6% vs OK WTD
CA vs WIS-IN	93.7% vs WIS Rein	97.6% vs WIS Rein	91.4% vs WIS Rein	98.3% vs WIS Elk 1	93.6% vs WIS Rein	97.6% vs WIS Rein
MN vs NE US	94.2% MN Carib vs NH Elk	99.0% MN MO1 vs WTD MA	91.5% MN Carib vs NH Elk	98.3% MN MO1 vs WTD MA	94.0% MN Carib vs PA Rein; MN MO2 and MN WTD vs WTD MA	100.0% MN MO1 vs NY Rein 1
MN vs TX-OK	93.4% MN Carib vs *Bodo* E	98.3% MN Carib vs TX Elk 1-b	90.5% MN Carib, MN MO1 and 2 vs *Bodo* E	98.1% MN MO2 vs *Bodo* B-a	93.2% MN MO1 and 2 vs *Bodo* B-a and B-b	99.2% MN Carib vs TX Elk 1-a and 1-b; MN MO1 and 2 vs *Bodo* B-b
MN vs WIS-IN	93.9% MN Carib vs WIS Elk 1	98.8% MN WTD vs WIS Rein	90.3% MN Carib vs WIS Elk 1	98.8% MN MO2 vs IN Elk	92.0% MN Carib vs IN Elk	99.6% MN WTD vs WIS Rein
NE US vs TX-OK	93.9% PA Rein and NY Rein 1 vs *Bodo* E	98.3% WTD MA vs *Bodo* B-b	90.5% WTD MA vs *Bodo* E	98.6% NY Rein 1 vs *Bodo* B-a	92.8% PA Rein and NY Rein 2 vs *Bodo* B-a and B-b; WTD MA vs OK WTD	99.2% PA Rein vs OK WTD
NE US vs WIS-IN	94.2% NY Rein 1 vs WIS Elk 1	98.4% NY Rein 1 vs WIS Elk 2	91.4% NH Elk vs WIS Rein	98.8% NH Elk vs WIS Elk 1	93.7% NH Elk vs IN Elk	99.2% NH Elk vs WIS Elk 1
TX-OK vs WIS-IN	93.9% *Bodo* E vs WIS Elk 2	98.1% *Bodo* B-b vs WIS Rein	90.2% TX Elk 1-a vs WIS Elk 1	98.8% *Bodo* B-a vs IN Elk	92.4% TX Elk 1-a vs IN Elk	98.8% OK WTD vs IN Elk

TABLE 3.5. Comparisons among *B. odocoilei* isolates within the type of host animal infections, fatal versus nonfatal.

	Low Range ITS1-5.8S-ITS2	High Range ITS1-5.8S-ITS2	Low Range ITS1	High Range ITS1	Low Range ITS2	High Range ITS2
Fatal infections	94.0% MN Carib vs WIS Rein	99.9% MN MO1 vs 2	91.6% TX Elk 1-a and 1-b vs MN MO1 and 2	99.8% MN MO1 vs 2	92.0% MN Carib vs IN Elk	100.0% NY Rein 1 vs MN MO2; MN MO1 vs 2
Nonfatal infections	93.3% *Bodo* B vs *Bodo* E	98.3% *Bodo* B vs WTD MA; WTD MA and MN WTD vs WIS Elk 2	90.5% *Bodo* E vs WTD MA and WIS Elk 2	98.8% NH Elk vs WIS Elk 1	92.1% NH Elk vs WTD MA and MN WTD	99.6% NH Elk vs WTD MA and MN WTD

Bodo B-b, *Bodo* E, BH 1-a, BH 1-b, NH Elk, OK WTD, WTD MA, MN WTD, WIS Elk 1 and WIS Elk 2. The percent identities in both ITS1, ITS2 and the entire ITS1-5.8S-ITS2 range from approximately 91% to 100% for all host infections. The highest and lowest percent identity ranges between geographic areas are listed in Table 3.6, and do not include intraclonal comparisons. For all gene regions, this data ranges from approximately 90% to 100% for all interinfection comparisons.

A direct analysis of sequence variation was performed to analyze fixed differences identified in the ITS1 and ITS2 gene regions (the few nucleotide differences found in the 5.8S gene region were attributed to random polymorphisms due to either PCR or sequencing error**,** and, accordingly, the 5.8S region was not included in the direct analysis). DNA regions showing fixed differences ranged from 1 to 27 base pairs in length, and were identified in a full alignment of sequences from all the isolates and clones (Appendix B). The location of each run of fixed differences was numbered, and each particular sequence

TABLE 3.6. Comparisons among *B. odocoilei* isolates between the type of host animal infections, fatal versus nonfatal.

	Low Range ITS1-5.8S-ITS2	High Range ITS1-5.8S-ITS2	Low Range ITS1	High Range ITS1	Low Range ITS2	High Range ITS2
Fatal infections v. Nonfatal infections	93.3% MN Carib vs BH 1-a	99.2% MN MO2 vs WTD MA	90.2% TX Elk 1-a vs WIS Elk 1	98.8% IN Elk vs *Bodo* B-a	92.4% MN Carib vs BH 1-a and 1-b	100.0% TX Elk 1-a and 1-b vs *Bodo* B-b

type in a run was assigned a lowercase letter (Tables 3.7 and 3.8). An example comparison of RD61 clone 2 and *Bodo* E clone 1 shows fixed differences and their designations (Fig. 3.1). A tabulation of the results for all of the isolates (and clones) is shown in Tables 3.9-3.15.

In ITS1, 21 fixed differences were identified. The fixed differences analysis showed only 1 overall pattern each for isolates RD61, NY Rein 2, BH 1-a and BH 1-b (Table 3.9); 2 patterns were found in isolates MN Carib, PA Rein, WIS Rein, WIS Elk 1, NH Elk, *Bodo* E, IN Elk, MN WTD, TX Elk 1-a, TX Elk 1-b, NY Rein 1, MN MO1 and MN MO2 (Table 3.10); three patterns were found in isolates OK WTD, WTD MA and WIS Elk 2 (Table 3.11). In some cases, occasionally a single base polymorphism found in one fixed difference of an otherwise identical sequence pattern group would give it a different designation at that location. In the summation of ITS1 sequence patterns found in each isolate, single base differences were discounted and the sequence denoted as the predominant type. Those single base differences observed in a sequence pattern predominant in a particular isolate are identified by asterisks in Tables 3.9, 3.10 and 3.11.

TABLE 3.7. Fixed differences in ITS1.

ITS1	At base pair position:	a	b	c	d	e	f	g	h	j	k	l
1	37-42	CTGTTG	CTGTTA	CCGTTG	CCGTTA	TTGTTA	CTGTCA					
2	51-56	AGCTCT	AGCTGT	GGCTGT	AG::GT	AGGTC:	AGCTTT					
3	61-62	CT	TT	CC								
4	82-84	GCG	GCA	CCG	GTG							
5	94-96	TGT	AGT	ACC								
6	104-106	TAG	CAA									
7	166	T	C									
8	180-181	CT	:T	CA								
9	207-214	TCCGGCG G	CACGGCG G	TTCGGCG G	TCCGGCG A	TCCGGTGG	TTCGGTG G	CACGGTG G				
10	219	A	G									
11	234-235	TC	CC	TT								
12	244-270	GTTG:GTG T A::CTGGTG CGTGAGC AC	GGTGTGT GT AATCTGTT A CCTTG:TA G	GGTGTGT GT AATCTGTT A CTTTG:TA G	GTTG:ATG T A::CTGGTG CGTGAGC AC	TTGGTGT GG::TCTGTT G CTCCGGTAA	GGTGCGT GT AATCTGTT A CCTT:GTA G	GGTGTGT GT AATCTGTT A CTTT:ATA G	GGTGCGT GT AATCTGTT A CTCT:GTA G	GTTA:GTGT A::CTGGTG CGCGAGCA C	GGTGCGT GT AATCTGTT A CTTT:GTA G	
13	271-283	CGGTACT GCACCA	CGGTATT GCACCA	CTGTGA::C ATTA	CTGTGA::C ATCA	CTCGCC:GC ATCG						
14	284-287	CTGG	CTAG	CCAA	ATGG	CTAA	CCAG	GCGG	GTGG	CTGA		
15	311-316	TCATGA	TGATGA	TCACGG	TCATGG	CTATAG						
16	328	T	A									
17	339	C	T									
18	354-362	TTTTGACT G	GT::GACT A	TT::GACTG	TTTTGGCT G	TTTTGACCG	GT::GACT G	TTCTGACT G				
19	389-394	A:TTTT	T:ATTT	TGTTAT	T:TTTT	T:GTTT	G:TTT:	G:TTTT	TGTTTT	TATTAT	TGTTAC	AATTA T
20	396-399	GCTA	GTGG	GCTG	GCAA							
21	416-417	TG	TT	CT								

TABLE 3.8. Fixed differences in ITS2.

ITS2	At base pair position:	a	b	c	d	e
1	595	G	A			
2	608-609	AT	GC	AC	GT	
3	613-618	TTACTA	TTACCA	CTACCG	TTACCG	TCGCCA
4	622-627	TATCGG	TATCGA	CGCTGG	CGCCGG	CACTTG
5	643	A	C			
6	658-660	GAA	GGA	ATG	GTG	
7	758	A	G			
8	770-772	GTC	GTA	ATC	GCC	
9	780-790	TGCGATATGGC	CGTGGTGCGGC	CGCGATATGGC	CGTGATGCGGC	TGCGATGCGGC
10	798-805	TAATGCGT	TGGTACAT	TAATGCAT	TAGTGCAT	TGATGCGT
11	826	C	T			

		f	g	h	j
1	595				
2	608-609				
3	613-618	CTACCA	CACTGG		
4	622-627	CACCGG			
5	643				
6	658-660				
7	758				
8	770-772				
9	780-790	TGCGC:GCAGT	TGCGGTATGGC		
10	798-805	TGATGCAT	AAATGCGT	TAATGCGC	TAATGTGT
11	826				

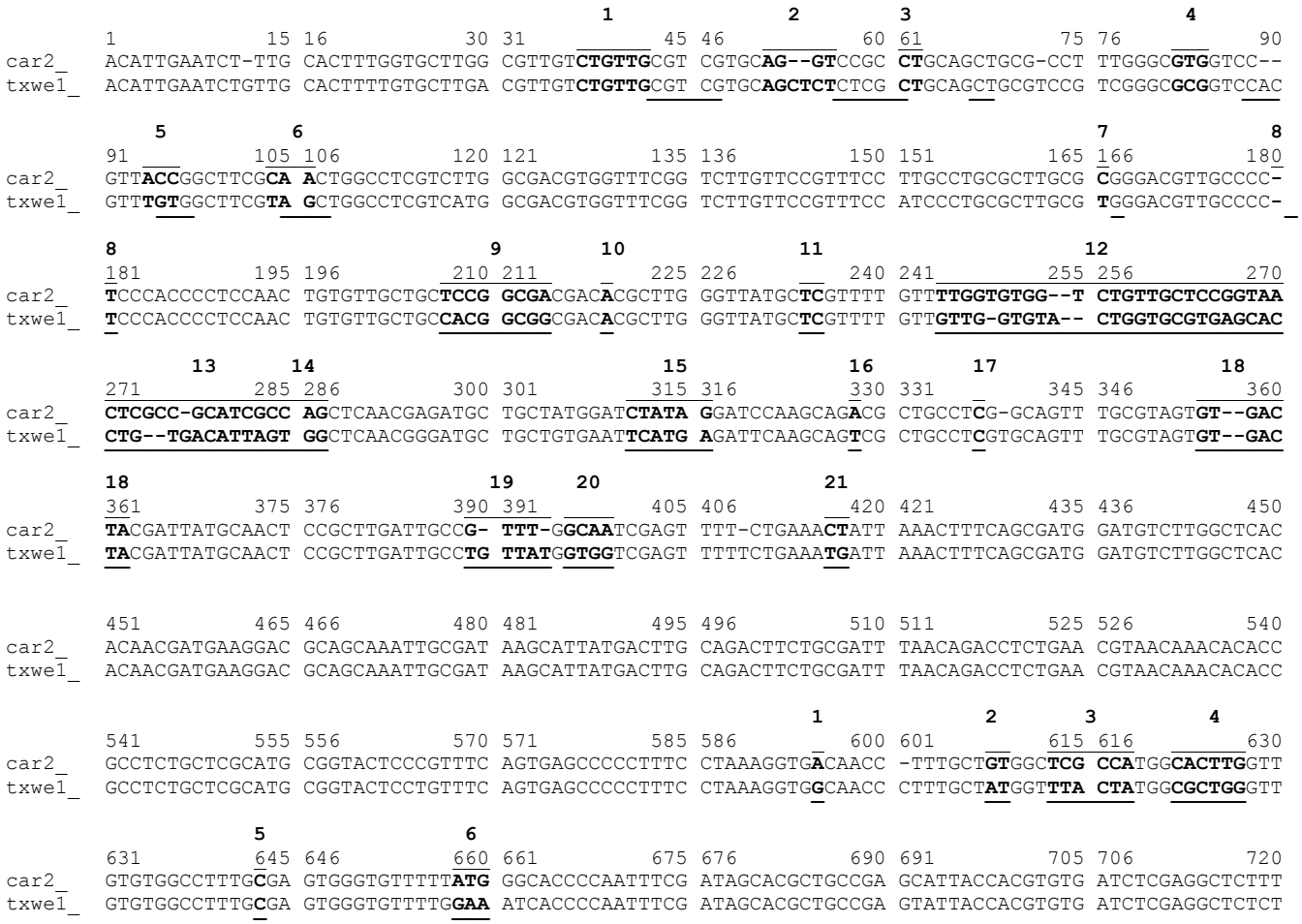

FIGURE 3.1. An example of the alignment used in the direct analysis for sequence variation. Sequences for RD61 clone 2 (car2) and *Bodo* E clone 1 (txwe1) are shown, including fixed differences and their designations.

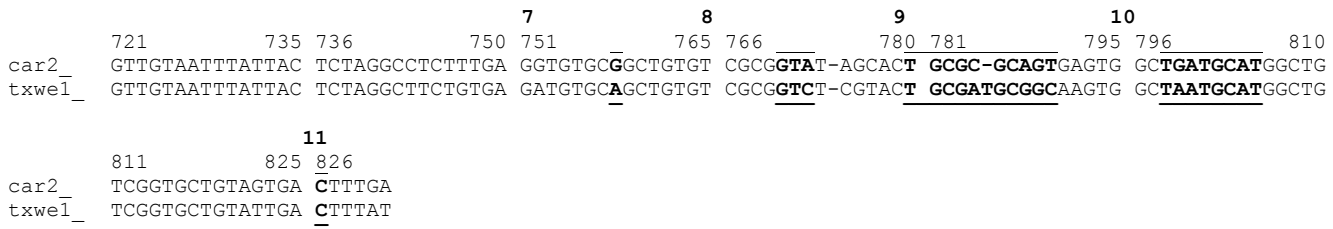

FIGURE 3.1. Continued.

TABLE 3.9. Isolates with one overall ITS1 sequence pattern. The ITS1 sequence patterns obtained by ITS1 direct analysis of sequence variation are shown for each isolate.

Isolate	Fixed Differences																				
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
RD61 Cl.2	a	e	a	d	c	b	b	b	d	a	a	e	e	f[a]	f	b	a	b	f	d	c
RD61 Cl.5,8	a	e	a	d	c	b	b	b	d	a	a	e	e	c	f	b	a	b	f	d	c
NY Rein 2 Cl.1,3,5,8	b	a	a	a	a	a	a	b	c	a	a	c	b	b	a	a	a	c	b	a	a
BH 1-b Cl.2,BH 1-a Cl.10,14	a	a	a	a[b]	a	a	a	b	a	a	a	a	c	g	a	a	b	d	b	a	a
BH 1-a Cl.11,BH 1-b Cl.5,8	a	a	a	c	a	a	a	b	a	a	a	a	c	g	a	a	b	d	b	a	a

[a] Single base difference between "c" and "f" pattern. Only difference in sequence pattern among 3 clones.
[b] Single base difference converts "c" to "a" pattern. Only difference in sequence pattern among 3 clones.

The actual base pair differences found in each of these fixed difference sites are shown in Table 3.12.

The *Bodo* B-a and *Bodo* B-b isolates contained up to 3 identifiable ITS1 patterns that appeared to result from recombination, as evident in Table 3.13. For the first 5 fixed difference sites, *Bodo* B-a clones 3 and 5 and *Bodo* B-b clone 21 had one pattern (b a a a a), and *Bodo* B-b clones 1 and 14 and *Bodo* B-a clone 6 had another pattern (a b a b b). The last positions, 9-21, also showed the same pattern (a a a b a b a a a a e a a) for *Bodo* B-a clones 3 and 5 and *Bodo* B-b clone 21, and another pattern (d a a d c d a b a a c b a) for *Bodo* B-b clones 1 and 14. *Bodo* B-a clone 6, however, did not match *Bodo* B-b

TABLE 3.10. Isolates with two overall ITS1 sequence patterns. The ITS1 sequence patterns obtained by ITS1 direct analysis of sequence variation are shown for each isolate.

	Fixed Differences																				
Isolate	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
MN Carib Cl.10	a	a	a	a	a	a	a	b	a	a	a	c	a	e	d	a	a	f	h	b	a
MN Carib Cl.3, 8,12	e	b	a	a	a	a	a	a	a	a	a	c	a	e	d	a	a	a	a	a	a
PA Rein Cl.3	a	c	a	a	a	a	a	a	c	a	a	c	a	f	a	a	a	a	a	b	a
PA Rein Cl.8,9	a	c	a	a	a	a	a	a	f	a	a	b	a	a	a	a	a	a	a	b	a
WIS Rein Cl.24	a	c	a	a	a	a	a	b	c	a	a	c	a	a	a	a	a	a	a	b	b
WIS Rein Cl.25,27	a	a	a	a	a	a	a	b	d	a	a	a	c	d	a	a	a	a	a	b	a
WIS Elk 1 Cl.8	d	b	a	a	b	a	a	a	b	b	a	a	c	d	a	a	a	a	b	a	a
WIS Elk 1 Cl.9,10	c	b	a	a	a	a	a	b	e	a	a	a	c	d	a	a	a	a	b	a	a
NH Elk Cl.80	c	b	a	a	a	a	a	c	a	a	a	a	c	d	a	a	a	a	a	a	a
NH Elk Cl.74,79	b	a	a	a	b	b	a	b	e	a	a	b	b	b	a	a	a	c	b	a	a
*Bodo*E Cl.2	b	f	a	a	b	a	a	a	a	a	c	a	c	h	a	a	a	b	c	b	a
*Bodo*E Cl.1,3,6	a	a	a	a	a	a	a	b	b	a	a	a	c	h	a	a	a	b	c	b	a
IN Elk Cl.6	b	a	a	a	a	a	a	a	a	a	a	b	a	a	a	a	a	a	b	a	a
IN Elk Cl.11	b	a	a	a	a	a	a	a	a	a	a	h[a]	a	b	a	a	a	a	a	b	b
IN Elk Cl.14	b	a	a	a	a	a	a	a	a	a	a	k[a]	a	b	a	a	a	a	a	b	b
MN WTD Cl.14	b	a	a	a	b[b]	a	a	a	b	a	b	f	a	a	a	a	a	a	d	a	b
MN WTD Cl.1	b	a	a	a	a	a	a	a	b	a	b	f	a	a	a	a	a	a	d	a	b
MN WTD Cl.4	f[c]	a	a	a	a	a	a	a	b	a	b	f	a	a	a	a	a	a	d	a	b
MN WTD Cl.9	b	a	a	b[b]	b	a	a	b	a	a	a	j	c	d	a	a	a	a	g	b	a
MN WTD Cl. 11	b	a	a	a	b	a	a	b	a	a	a	j	c	d	a	a	a	a	g	b	a
TX Elk 1-a Cl.7, 14, TX Elk 1-b Cl.8	b	a	a	a	a	a	a	b	c	a	a	a	d	c	c	a	a	a	a	a	a
TX Elk 1-a Cl.18	b	a	a	d[d]	a	a	a	b	c	a	a	a	d	c	c	a	a	a	a	a	a
TX Elk 1-a Cl.24, TX Elk 1-b Cl.1	a	d	a	a	a	a	a	b	a	a	a	c	a	a	c	a	a	a	c	b	a

TABLE 3.10. Continued.

Isolate	Fixed Differences																				
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
TX Elk 1-b Cl.2	a	d	a	a	a	a	a	b	a	a	a	c	a	j[e]	c	a	a	a	c	b	a
NY Rein 1 Cl.4,12	a	b	a	a	b	a	a	b	b	a	a	b	a	c	a	a	a	a	g	b	a
NY Rein 1 Cl.7	c	b	a	a	b	a	a	a	c	a	a	b	a	b	a	a	a	e	b	c	a
MN MO1 Cl.5,8	c	b	a	a	b[b]	a	a	a	g	b[b]	a	c	a	f	b	a	a	a	a	b	b
MN MO2 Cl.5	c	b	b[b]	a	b[b]	a	a	a	b[f]	b[b]	a	c	a	f	b	a	a	a	a	b	b
MN MO2 Cl.6	c	a	b[b]	a	b[b]	a	a	a	f	a	a	b	a	a	a	a	a	a	d	a	a
MN MO1 Cl.4	c	a	a	a	a	a	a	a	f	b[b]	a	b	a	a	a	a	a	a	d	a	a
MN MO2 Cl.14	c	a	a	a	a	a	a	a	f	a	a	b	a	a	a	a	a	a	d	a	a

[a] Single base difference converts "h" and "k" to same pattern. Only difference in sequence pattern among clones.
[b] Single base difference converts "b" to "a" pattern.
[c] Single base difference converts "f" to "b" pattern.
[d] Single base difference converts "d" to "a" pattern.
[e] Single base difference converts "j" to "a" pattern.
[f] Single base difference converts "b" to "g" pattern.

clones 1 and 14 in these locations, but instead matched *Bodo* B-a clones 3 and 5 and *Bodo* B-b clone 21 (Table 3.13).

In ITS2, 11 fixed differences were identified, and up to 3 distinct fixed difference patterns per isolate were also revealed in ITS2, although there was much more conservation in this shorter gene region. Isolates NY Rein 2, *Bodo* E, BH 1-a, BH 1-b and MN Carib were found to have only 1 pattern (Table 3.14); NY Rein 1, PA Rein, WIS Rein, WIS Elk 1, *Bodo* B-a, *Bodo* B-b, RD61, TX Elk 1-a, TX Elk 1-b, NH Elk, WIS Elk 2, IN Elk, MN MO1, MN MO 2 and MN WTD had 2 patterns (Table 3.15); isolates OK WTD and WTD MA had 3 patterns (Table 3.16). Single base differences

TABLE 3.11. Isolates with three overall ITS1 sequence patterns. The ITS1 sequence patterns obtained by ITS1 direct analysis of sequence variation are shown for each isolate.

	Fixed Differences																				
Isolate	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
OK WTD Cl.3	a	a	a	a	a	a	a	b	d	a	b	g	a	e	a	a	a	a	j	b	a
OK WTD Cl.8	a	a	a	b	b	a	a	b	c	a	a	a	d	c	d	a	a	a	a	b	a
OK WTD Cl.11	b	b	a	b	b	a	a	b	b	a	a	a	d	c	c	a	a	a	b	b	a
WTD MA Cl.3	a	b	c	a	a	a	a	a	a	a	a	b	a	a	a	a	a	a	b	b	b
WTD MA Cl.5	d	a	a	a	b	a	a	a	a	a	a	d	c	d	a	a	a	a	l	b	a
WTD MA Cl.9	c	b	a	a	a	a	a	a	e	a	a	b	a	e	b	a	a	a	a	a	a
WIS Elk 2 Cl.14	d	b	a	a	a	a	a	a	b	a	a	b	b	e	a	a	a	a	b	a	a
WIS Elk 2 Cl.10	d	b	a	b	a	a	a	a	b	a	a	b	b	e	a	a	a	a	b	c	a
WIS Elk 2 Cl.13	a	a	a	a	a	a	a	a	c	a	a	b	a	a	a	a	a	a	d	b	a

are identified by asterisks in Tables 3.14, 3.15 and 3.16, and are shown in the sequences in Table 3.18. There were no isolates showing distinct recombination, as there were in ITS1.

The recombination events evident in ITS1 for *Bodo* B-a and *Bodo* B-b continued through the ITS2 region, as shown in Table 3.17. In ITS1, positions 9-21 showed a match between *Bodo* B-a clone 6 and *Bodo* B-a clones 3 and 5 and *Bodo* B-b clone 21. ITS2 demonstrates a conservation of this trend; *Bodo* B-a clones 3, 5 and 6 and *Bodo* B-b clone 21 show one pattern (a b c a a a a a b b a), while *Bodo* B-b clones 1 and 14 show another (a a d c a a a a a e a).

TABLE 3.12. Nucleotide difference changes in ITS1 sequences containing 1, 2 and 3 types per isolate, and for *Bodo* B isolates.

Location (1 type/isolate)	Sequence (1 type/isolate)	Location (2 types/isolate)	Sequence (2 types/isolate)	Location (3 types/isolate)	Sequence (3 types/isolate)
4a	GCG	1b	CTGTTA	4a	GCG
4c	CCG	1f	CTGTCA	4d	GTG
14c	CCAA	4a	GCG	14a	CTGG
14f	CCAG	4b	GCA	14j	CTGA
		5a	TGT		
		5b	AGT		
		12b	TGGTGTGTGTAATC TGTTACCTTGTAG		
		12h	TGGTGCGTGTAATC TGTTACTCTGTAG		
		12k	TGGTGCGTGTAATC TGTTACTTTGTAG		

Location (Bodo B isolates)	Sequence (Bodo B isolates)	Location (MN MO isolates)	Sequence (MN MO isolates)
4a	GCG	3a	CT
4b	GCA	3b	TT
8a	CT	9b	CACGGCGG
8b	:T	9g	CACGGTGG
18a	TTTTGACTG		
18g	TTCTGACTG		
19c	TGTTAT		
19k	TGTTAC		

TABLE 3.13. Apparent recombination in *Bodo* B ITS1. Results of direct analysis of sequence variation in ITS1.

	Fixed Differences																				
Isolate	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
Bodo B-a Cl.3	b	a	a	a	a	a	a	b	a	a	a	b	a	b	a	a	a	g[a]	e	a	a
Bodo B-a Cl.5	b	a	a	a	a	a	a	b	a	a	a	b	a	b	a	a	a	a	e	a	a
Bodo B-b Cl.21	b	a	a	a	a	a	b	b	a	a	a	b	a	b	a	a	a	a	e	a	a
Bodo B-b Cl.1	a	b	a	b	b	a	a	b	d	a	a	d	c	d	a	b	a	a	c	b	a
Bodo B-b Cl.14	a	b	a	b	b	a	a	a[b]	d	a	a	d	c	d	a	b	a	a	k[c]	b	a
Bodo B-a Cl.6	a	b	a	a[b]	b	a	a	b	a	a	a	b	a	b	a	a	a	a	e	a	a

[a] Single base difference converts "g" to "a" pattern.
[b] Single base difference converts "a" to "b" pattern.
[c] Single base difference converts "k" to "c" pattern.

All clone sequences for the entire ITS1-5.8S-ITS2 gene region were aligned using a ClustalW 1.8 Program (http://searchlauncher.bcm.tmc.edu/multi-align/multi-align.html). The alignment (Appendix B) was then used to create a phylogenetic Neighbor-Joining tree, shown both in Fig. 3.2 and Appendix C, using bootstrap resampling (Paup 4.0b10 software program).

The RD61 isolates, used as an outgroup, formed one clade, clearly separated from ITS1, most likely due to the fast that the ITS2 gene region is shorter and more conserved than the ITS1 gene region. Some *B. odocoilei* isolates are grouped together as clonal groups, and some separate into different groups, correlating to the same distributions seen in the direct analysis of sequence variation based on fixed differences. Clones of NY Rein 2 (nyr2), PA Rein (par), BH 1-a and BH 1-b (casa and casb), and *Bodo* E (txwe) formed single groups for each isolate. Clones of isolates TX Elk 1-a and

TABLE 3.14. Isolates with one overall ITS2 sequence pattern. The ITS2 sequence patterns obtained by ITS2 direct analysis of sequence variation are shown for each isolate.

Isolate	Fixed Differences										
	1	2	3	4	5	6	7	8	9	10	11
NY Rein 2 Cl.1,3,5,8	a	a	b	g	a	a	a	a	g	a	a
Bodo E Cl.1,2,3,6	a	a	a	c	b	a	a	a	e	c	a
BH 1-a Cl.10,14, BH 1-b Cl.2,5,8	a	b	d	f	a	b	a	c	a	d	a
BH 1-a Cl.11	a	b	d	g[a]	a	b	a	c	a	d	a
MN Carib Cl.3,8,10	b	a	b	a	a	a	a	a	b	a	a
MN Carib Cl.12	b	a	b	b[b]	a	a	a	a	b	a	a

[a] Single base difference converts "g" to "f" pattern.
[b] Single base difference converts "b" to "a" pattern.

TX Elk 1-b (txea and txeb) and those of MN Carib (mnc), MN WTD (mnw), NH Elk (nhe), IN Elk (ine), NY Rein 1 (nyr1), WIS Rein (wir), WTD MA (maw) and WIS Elk 1 (wie1) each separated into two distinct groups, as seen in the direct sequence analysis. WIS Elk 2 (wie2) and *Bodo* B (a and b) (txwba and txwbb) each distribute into 2 groups as in the direct sequence analysis. MN MO1 (mnmo1) clones 5 and 8 and MN MO2 (mnmo2)

clone 8 group together, versus clones 5 and 8 placed closer together, which is consistent with the ITS2 direct sequence analysis. The direct sequence analysis shows differences among MN MO1 clone 4 and MN MO2 clones 6 and 14, which in the tree results in the MN MO2 clones branching together separately from the MN MO1 clone. OK WTD

(okw)clones 3, 8 and 11 all show different types in both ITS1 and ITS2 direct sequence analyses, and in the tree clones 8 and 11 group together, while clone 3 is separate.

In summary, the phylogenetic tree based on ITS region nucleotide sequences shows no clear separation of isolates due to vertebrate host, geographic region, or other factor. The tree does show that the majority of the isolates have two distinct ITS sequence types, and that within these types, clones of an isolate are more like each other than other isolates that share that type.

TABLE 3.15. Isolates with two overall ITS2 sequence patterns. The ITS2 sequence patterns obtained by ITS2 direct analysis of sequence variation are shown for each isolate.

	Fixed Differences												Fixed Differences										
Isolate	1	2	3	4	5	6	7	8	9	10	11	Isolate	1	2	3	4	5	6	7	8	9	10	11
NY Rein 1 Cl.7	a	a	d	f	a	a	a	a	a	a	a	TX Elk 1-a Cl.7,18, TX Elk 1-b Cl.8	a	a	a	a	a	a	a	a	d	a	a
NY Rein 1 Cl.4	b	b	c	c	a	a	a	a	b	a	a	TX Elk 1-a Cl.14,24, TX Elk 1-b Cl.1,2	a	a	a	a	a	a	a	a	b	a	a
NY Rein 1 Cl.12	b	b	c	c	a	a	a	a	b	h[a]	a												
												MN MO1 Cl.4, MN MO2 Cl.6,14	a	a	d	c	a	a	a	a	a	a	a
PA Rein Cl.3	b	a	d	f	a	a	a	a	a	b	a	MN MO1 Cl.5,8, MN MO2 Cl.5	a	a	d	f	a	a	a	a	a	a	a
PA Rein Cl.8,9	b	a	d	c	a	a	a	a	a	c	a												
												NH Elk Cl.80	a	a	b	a	a	a	a	b	a	g	a
WIS Rein Cl.24	a	a	d	c	a	a	a	a	b	e	a	NH Elk Cl.74,79	a	a	d	d	a	a	a	a	c	a	a
WIS Rein Cl.25,27	a	b	d	c	a	a	a	a	a	a	a												
												WIS Elk 2 Cl.13	a	a	b	g	a	a	a	a	b	c	a
WIS Elk 1 Cl.8	a	a	d	a	a	a	a	a	a	a	a	WIS Elk 2 Cl.10,14	a	b	d	c	a	a	a	a	c	a	a
WIS Elk 1 Cl.9	a	a	b	c	a	a	a	a	c	h[a]	a												
WIS Elk 1 Cl.10	a	a	b	c	a	a	a	a	c	a	a	IN Elk Cl.6	a	a	c	c	a	a	a	a	a	b	a
												IN Elk Cl.11,14	b	b	c	f	a	a	a	a	a	a	a
Bodo B-b Cl.1,14	a	a	d	c	a	a	a	a	a	e	a												
Bodo B-b Cl.21	a	b	c	a	a	a	b[b]	a	b	b	a												
Bodo B-a Cl.3,6	a	b	c	a	a	a	a	a	b	b	a												
Bodo B-a Cl.5	a	b	c	a	a	a	a	d[c]	b	b	a												

TABLE 3.15. Continued.

Isolate	Fixed Differences 1	2	3	4	5	6	7	8	9	10	11	Isolate	Fixed Differences 1	2	3	4	5	6	7	8	9	10	11
RD61 Cl.2,5	b	d	e	e	b	c	b	b	f	f	a												
RD61 Cl.8	b	d	c	e	b	d	b	b	f	f	a												
MN WTD Cl.11	a	b	d	f	a	a	a	a	a	a	a												
MN WTD Cl.9	a	c[d]	d	f	a	a	a	a	a	j[e]	a												
MN WTD Cl.1,4,14	a	a	d	c	a	a	a	a	b	a	a												

[a] Single base difference converts "h" to "a" pattern.
[b] Single base difference converts "b" to "a" pattern.
[c] Single base difference converts "d" to "a" pattern.
[d] Single base difference converts "c" to "b" pattern.
[e] Single base difference converts "j" to "a" pattern.

TABLE 3.16. Isolates with three overall ITS2 sequence patterns. The ITS2 sequence patterns obtained by ITS2 direct analysis of sequence variation are shown for each isolate.

Isolate	Fixed Differences 1	2	3	4	5	6	7	8	9	10	11
OK WTD Cl.3	a	a	b	a	a	a	a	a	c	a	b
OK WTD Cl.8	a	b	f	f	a	a	a	a	a	a	a
OK WTD Cl.11	a	a	d	f	a	a	a	a	a	b	a
WTD MA Cl.3	b	a	d	c	a	a	a	a	b	b	b
WTD MA Cl.5	a	a	b	c	a	a	a	a	e	a	a
WTD MA Cl.9	a	a	d	d	a	a	a	a	a	a	a

TABLE 3.17. Recombination in *Bodo* B. Clone *Bodo* B-a Cl.6 most closely shares the same sequence pattern vs *Bodo* B-b Cl.1 and *Bodo* B-b Cl.14 in ITS1, positions 1-8. For ITS1 positions 9-21, and the complete ITS2 region, this clone matches *Bodo* B-a Cl. 3, *Bodo* B-a Cl.5, and *Bodo* B-a Cl.21.

	Fixed Differences																														
	ITS1																					ITS2									
Isolate	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	1	2	3	4	5	6	7	8	9	10	11
Bodo B-a Cl.3	b	a	a	a	a	a	a	b	a	a	a	b	a	b	a	a	a	g[a]	e	a	a	a	b	c	a	a	a	a	a	b	b	a
Bodo B-a Cl.5	b	a	a	a	a	a	a	b	a	a	a	b	a	b	a	a	a	a	e	a	a	a	b	c	a	a	a	a	d[e]	b	b	a
Bodo B-b Cl.21	b	a	a	a	a	a	b	b	a	a	a	b	a	b	a	a	a	a	e	a	a	a	b	c	a	a	a	b[d]	a	b	b	a
Bodo B-b Cl.1	a	b	a	b	b	a	a	b	d	a	a	d	c	d	a	b	a	a	c	b	a	a	a	d	c	a	a	a	a	a	e	a
Bodo B-b Cl.14	a	b	a	b	b	a	a	a[b]	d	a	a	d	c	d	a	b	a	a	k[c]	b	a	a	a	d	c	a	a	a	a	a	e	a
Bodo B-a Cl.6	a	b	a	a[b]	b	a	a	b	a	a	a	b	a	b	a	a	a	a	e	a	a	a	b	c	a	a	a	a	a	b	b	a

[a] Single base difference converts "g" to "a" pattern.
[b] Single base difference converts "a" to "b" pattern.
[c] Single base difference converts "k" to "c" pattern.
[d] Single base difference converts "b" to "a" pattern.
[e] Single base difference converts "d" to "a" pattern.

TABLE 3.18. Nucleotide difference changes in ITS2 sequences containing 1 and 2 types per isolate.

Location (1 type/isolate)	Sequence (1 type/isolate)	Location (2 types/isolate)	Sequence (2 types/isolate)
4f	CACCGG	7a	A
4g	CACTGG	7b	G
4a	TATCGG	8a	GT:
4b	TATCGA	8d	GC:
		10a	TAATGCGT
		10h	TAATGCGC
		10j	TAATGTGT

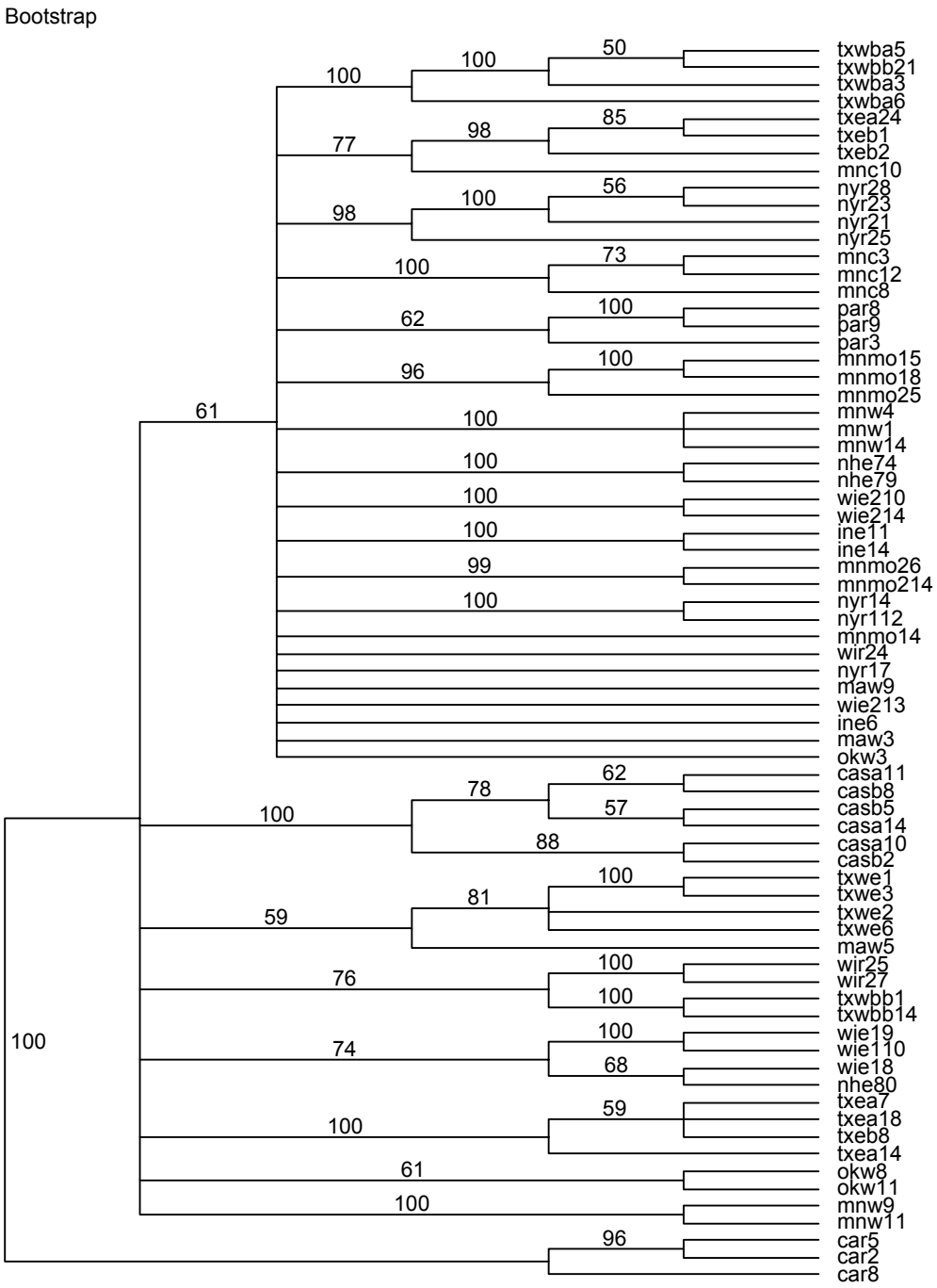

FIGURE 3.2. Neighbor-joining phylogenetic tree with bootstrapping. The California Reindeer RD61 *Babesia* sp. served as the outgroup. The different isolates are designated by number to more clearly show the separate placement of different clones from the same isolate.

CHAPTER IV

DISCUSSION AND SUMMARY

The ITS1-5.8S-ITS2 ribosomal RNA gene region contains both highly conserved and variable regions now considered to be criteria for discrimination among parasites at a subspecies level (reviewed by Prichard and Tait, 2001).

Zahler et al. (1998) analyzed the ITS1-5.8S-ITS2 rRNA gene region in eight *Babesia canis* isolates of disparate geographic origins, vector specificity and pathogenicity to dogs (*Canis familiaris*) in order to determine whether genetic differences concurred with currently proposed subspecies designations - *B. canis canis*, *B. canis vogeli* and *B. canis rossi*. Definite genetic variation between the subspecies congruent with the existing taxonomic classifications was observed, with the sequences separating into three distinguishable genotypic groups. The percent identity among these three groups ranged from 70-82% for the entire ITS1-5.8S-ITS2 gene region. Little or no variation was observed within each subspecies. Polymorphism between this gene region in the *B. canis* isolates and an equine *Babesia caballi* isolate was on the same order of magnitude as that found among the *B. canis* subspecies. Thus, the partitions of *B. canis* were not only proven, but separate species status was even suggested in place of the current subspecies designations. This study established the possibility of utilizing the ITS1-5.8S-ITS2 gene region as a standard for grouping together and assigning subspecies or even species status to interrelated *Babesia* organisms.

The results from the Zahler study confirming taxonomic divisions for one *Babesia* sp. using molecular analysis consequently led to the idea that a similar study could be undertaken for *Babesia odocoilei* isolates, also of differing geographic origin and pathogenicity to hosts. It was anticipated that genotypic groups could be discerned for *B. odocoilei* as well using ITS as a genetic marker.

Babesia odocoilei has been described in a number of different deer hosts including the original host, white-tailed deer, and caribou and elk (Spindler et al., 1958; Emerson and Wright 1968; 1970; Holman et al., 1994; Holman et al., 2000). During the course of this study, this parasite was also found to infect reindeer and two members of the bovidae, bighorn sheep and musk ox (Holman et al., 2003; unpublished data). As these different hosts were recognized, the known geographic range of the parasite was also extended. Originally described in Texas, the parasite is now known to be cosmopolitan across the southern U.S., as well as in the Great Lakes region, New York, Pennsylvania and New Hampshire. It has also been identified in California.

Frequently, babesiosis outbreaks with one or more fatalities occur in farmed or managed deer with no previous history of the disease (Holman et al., 1994; 2000; 2003). This study was undertaken to determine if the ITS1-5.8S-ITS2 gene region could be used as a marker to trace the source of *Babesia* sp. infection when these outbreaks occur. The isolates used in this study covered a wide range of both geographic regions and hosts infected.

To assess the baseline variation that might be expected in the ITS gene region within a *Babesia* sp. isolate, intraclonal comparisons based on sequence percent

identities were first determined for each isolate. For the full ITS1-5.8S-ITS2 gene region, these percent identities ranged from 93.6-100.0%. Ranges in the ITS1 gene region were from 91.4-100.0%, and ranges in ITS2, the smaller and more conserved gene region, were from 93.2-100.0%. Isolates for which duplicate clone sets were made showed higher percent identities in all gene regions among themselves compared to other sequences. This was most notable in ITS2. For all isolates, higher conservation was consistently found in the ITS2 versus the ITS1 gene region. The disparity in sequences among clones of an isolate deemed it necessary to include at least three, and in some cases four (Minnesota Caribou, Texas Elk 1-a, New York Reindeer 2, Texas *B. odocoilei*-E WTD) or five (Minnesota WTD), clones per isolate when determining percent identities and in sequence analysis.

The California reindeer *Babesia* sp., RD61, which is distinct from *B. odocoilei* based on SSU rRNA gene sequence (99.0% identity between RD61 and *B. odocoilei*), was also distinct in the ITS region from all *B. odocoilei* isolates in this study. Identities between the *B. odocoilei* isolates ranged from 93.3-99.9% for the entire ITS1-5.8S-ITS2 gene region, whereas the highest percent identity between RD61 and *B. odocoilei* was only 88.2% (Appendix A).

Pairwise comparisons of the full ITS1-5.8S-ITS2 gene region and the ITS1 or ITS2 regions only were performed to determine if any sequence segregation exists based on vertebrate host, geographic location of the infection, fatal versus nonfatal disease and culture- versus blood-derived parasites. None of the above factored into the results obtained. The values for these comparisons were no different from the intraclonal

variation observed. These conclusions were supported by the phylogenetic tree constructed using the full ITS1-5.8S-ITS2 gene region (Fig. 3.2), where the isolates did not segregate into groups based on vertebrate host, geographic area, clinical manifestation, or source of parasite.

An alignment of the sequences obtained in this study revealed that there were a limited number of variable sites reflecting fixed differences throughout the ITS1 and ITS2 gene regions. The 5.8S gene region was identical in all isolates, except for random single base heterogeneity observed in some clones. There were no fixed differences in 5.8S gene sequences among the isolates. Random single base heterogeneity was also found occasionally throughout the ITS1 and ITS2 regions. As these variations were both inconsistent and random, it is likely that they are a result of PCR and/or sequencing errors.

Analysis of the fixed differences in ITS1 and ITS2 revealed that most of the isolates had two distinct sequence patterns, although two isolates had only one pattern and three appeared to have three patterns. Some of the fixed differences were point mutations such that in some cases, a sequence pattern differed from the predominant in a particular isolate due to a single base variation. When only one base difference occurred, the sequences were considered as a single pattern.

The phylogenetic tree (Fig. 3.2) was inferred using the Neighbor-Joining/UPGMA method, bootstrap resampling and the RD61 isolates as the outgroup. This program produces a reliable phylogeny if the rates of evolution are reasonably constant among the different lineages (Sokal and Sneath, 1963), as would be expected

with this group of closely related parasites. RD61 was confirmed as an outgroup, branching separately from the *B. odocoilei* isolates. The *B. odocoilei* isolates either grouped together as clonal groups or separated into different groups, usually correlating to the same distributions seen in the direct analysis of sequence variation based on fixed differences. As mentioned above, the phylogenetic tree did not characterize *Babesia* species based on animal hosts and geographic areas. Indeed, similarity in ITS sequences was shown among isolates from diverse hosts and geographic separation. Thus, the tree topology concurred with the other analyses that no segregation of isolates can be inferred from the ITS sequence data.

The clones of each *B. odocoilei* isolate generally separate into two categories occupying different positions in the tree, which may be adjacent or divergent. Within these categories, clones of an isolate are more similar to each other than to other isolates that also occupy that position, with the exception of New Hampshire Elk isolate clone 80. Despite the variation seen in some isolates in the fixed differences pattern analysis, which at times resulted in three or more patterns, when analyzed in direct comparison to all the other sequences each isolate separated into two categories of clones in the phylogenetic tree, except for the Massachusetts WTD isolate. This division into two categories suggests that there are at least two rRNA transcription units present in *B. odocoilei*.

In the analysis based on fixed differences, Texas *B. odocoilei*-B-a WTD and Texas *B. odocoilei*-B-b WTD isolates contained up to 3 identifiable patterns that appear to result from recombination in the ITS1 and ITS2 gene regions (Table 3.16).

Recombination in the ITS regions of *Theileria parva* was previously reported by Collins and Allsopp (1999). Isolation and sequencing of *Theileria parva* subspecies, *Theileria parva lawrencei* and *Theileria parva parva*, showed that the 5.8S gene sequences of eleven *T. parva* isolates were identical, but the ITS regions of both *T. p. parva* and *T. p. lawrencei* contained different combinations of identifiable sequence segments. This data led to a conclusion of the other extreme from that garnered in the *B. canis* study (Zahler et al., 1998); namely, that the resulting assortment of segments in any one isolate made it impossible to definitively distinguish isolates based solely on ITS1 and ITS2 sequences (Collins and Allsopp 1999). Collins and Allsopp suggested that genetic recombination of populations, derived from mingled gene pools, could account for such diverse data.

It is possible that the recombination evident in the Texas *B. odocoilei*-B isolate in this study occurs in an area where the incidence of *Babesia* infection and tick infestation in the white-tailed deer population is quite high. Texas *B. odocoilei*-B was isolated from a naturally infected white-tailed deer exhibiting no clinical signs of illness, suggesting that a situation of enzootic stability is present on the Brushy Creek Experimental Ranch, TX. Furthermore, the high seropositive prevalence rate to *B. odocoilei* (80%) in resident deer (Waldrup et al., 1989a) supports the likelihood of endemicity in that region. In such a situation, there would be opportunity for recombination to occur.

A highly endemic region will lead to a substantial proportion of *Babesia*-infected juveniles in the white-tailed deer population while they are still protected by maternal antibodies and/or age related immunity factors (possibly fetal hemoglobin), which then culminate in an adult population that is predominantly immune to disease by *B.*

odocoilei (Perry et al., 1985b). A population of white-tailed deer carrying the parasites in such a prevalent area could lead to considerable recombination events in the population and therefore, an assortment of sequence clones in a single isolate. Indeed, the Texas *B. odocoilei*-B WTD isolates showed evidence of recombination throughout the ITS1-5.8S-ITS2 gene region (Table 3.16). Knowing that the endemic region does in fact exist in Texas, the direct sequence analysis data provides more evidence to support the conclusion that recombination of ITS1 and ITS2 segments of *B. odocoilei* does occur in the tick gut.

Initially, it was thought that perhaps the disparity found in the first set of clones from the Texas *B. odocoilei*-B isolate might be due to mutations that may have occurred during lengthy storage at 4 C, thus a second set of clones was derived from infected blood cryopreserved in liquid nitrogen. A total of seven clones were sequenced; a high level of variation was found among the second set of clones also. Therefore, the variation observed is inherent in this particular isolate.

Another Texas white-tailed deer isolate, *B. odocoilei*-E, originated from the Gus Engeling Wildlife Management Area, TX, which showed an intermediate prevalence rate to *B. odocoilei* (50%) (Waldrup et al., 1989a). Although two sequence types were observed for Texas *B. odocoilei*-E WTD in the direct analysis, the phylogenetic tree places them on adjacent branches, rather than in discrete groups as with Texas *B. odocoilei*-B, indicating that they are more conserved. This may imply either that the prevalence of *Babesia* and tick infection in the white-tailed deer population at the Gus Engeling Wildlife Management Area is not as high as in the Brushy Creek area, or that

there could be a more closed population of white-tailed deer in the Gus Engeling area, with less introduction of new individuals or ticks so there is less variation than in the Brushy Creek area.

Seropositive prevalence rates to *B. odocoilei* were also determined for parts of Oklahoma. The Oklahoma WTD isolate was obtained from a captive 2-year old white-tailed deer in Payne County, where the rates were as high as 75% (Waldrup et al., 1989a). The high occurrence of *B. odocoilei* in Oklahoma may also be reflected in the direct analysis of fixed differences, in which 3 different patterns were discernable in both ITS1 and ITS2. Again, it appears that increased genetic recombination results in an area with a high rate of *Babesia* infection and tick infestation in the white-tailed deer population.

Three of the isolates in the present study, Minnesota Caribou, Minnesota Musk Ox 1 and Minnesota Musk Ox 2, originated from a zoo in Apple Valley, Minnesota. The white-tailed deer isolate from Minnesota was a naturally infected, free-ranging animal exhibiting no clinical signs of illness, and was collected in the vicinity of the zoo. All three isolates exhibited 2 consistent patterns in both ITS1 and ITS2. A serosurvey of animals in the zoo was carried out in 1993 (pers. comm., P.J. Holman). No positive animals were found, and no subsequent cultures from a variety of zoo animals were positive for *B. odocoilei*. Therefore, although there is obvious concurrent *Babesia* and tick infection occurring in the Apple Valley area, the lack of recombination and the negative serosurvey results implies that the zoo area may not be endemic as is the case in Texas.

Genetic exchange is clearly more likely to occur in regions of endemicity where prevalence of infection in ticks is high since gamogony occurs in the vector. However, epidemiological data encompassing areas of both high and low incidence are needed to prove or disprove the hypothesis that more variation is found within isolates in such regions.

The most remote isolates in the study, RD61 and Bighorn Sheep, were from Northern and Southern California, respectively. RD61 originated from Placer County, near Sacramento, and Bighorn Sheep originated from the San Bernardino Mountains, near Los Angeles. In the direct analysis of fixed differences, the Bighorn Sheep clones contained only one pattern in both ITS1 and ITS2. The RD61 clones exhibited one pattern in ITS1 and two patterns in ITS2, but the two patterns evident in the RD61 clones were due to variation in only 2 fixed difference sites out of 11. Hence, it appears as though the parasite genetic recombination prevalent in Texas, and perhaps Oklahoma as well, is not occurring in California. The white-tailed deer population is much lower in this state than in Texas and Oklahoma. Their range extends throughout the continental United States, but is much lower in arid portions of the West and Southwest, where they coexist with mule deer, especially common in the higher elevations (Smith, 1991; Downing, 1987).

However, these are not the only isolates to exhibit just one pattern in the fixed difference analysis; this is true for New York Reindeer 2 in both ITS1 and ITS2, and Texas *B. odocoilei*-E WTD and Minnesota Caribou in ITS2. ITS2 is a more conserved region compared with ITS1, so it may not be unusual to see conservation of patterns in

the clones in this gene region. Additionally, there may be recombination events occurring in the New York Reindeer 2 isolate that were not picked up in this study. More data is needed to make definite conclusions regarding the factors contributing to recombination of ITS segments in *B. odocoilei*.

Based on the ITS data, it appears that *B. odocoilei* possesses at least two rDNA units. The numbers of rDNA transcriptional units determined for other *Babesia* species range from two to four, depending on the species. There are two units in *Babesia microti* and *Babesia rodhaini*, three units in *Babesia bigemina* and *Babesia bovis*, and possibly four units in *B. canis* (Dalrymple 1990; Reddy et al., 1991; Dalrymple et al., 1992). *Babesia bovis* has three highly conserved rDNA units that are probably single copy, each separated from the others by at least 16 kb DNA (Dalrymple 1990; Reddy et al., 1991), similar to the gene organization in *Plasmodium* spp., which have a small number of units dispersed through the genome (McCutchan, 1986). Each rDNA unit is composed of the SSU rRNA gene, ITS1, 5.8S gene, ITS2, and the large subunit ribosomal gene. Unlike many other organisms, these units are not tandemly repeated.

The data acquired in this study imply that there exist at least two transcriptional units in *B. odocoilei*, the same number found in both *B. rodhaini* and *B. microti.* These species, however, fall into the category of "uncertain taxonomic standing," ie. the *Babesia sensu lata,* and are phylogenetically distinct from the "true *Babesia,*" ie. the *Babesia sensu stricto* (Ellis et al., 1992; Holman et al., 2000). Morphologically and on the basis of SSU rRNA gene sequence analysis, of the named *Babesia* spp., *B. odocoilei*

most closely resembles the small parasite, *Babesia divergens* (Holman et al., 2000). To date the number of transcriptional rDNA units in *B. divergens* has not been determined.

Although multiple rDNA units do exist in *Babesia* species, thus probably in *B. odocoilei* as well, to date the evidence suggests that these units do not comprise clearly discrete sets of RNA genes that are expressed differentially depending on the stage of the parasite, as is the case for *Plasmodium* species. In *Plasmodium* spp., the ITS sequences are identical at 80-91% of the positions among the genes expressed during the asexual stage and 75% between the genes expressed during sporogony, with just 42-57% identity between the two types (Rogers et al., 1995). The SSU rRNA genes from the same set of genes, however, showed no sequence variation from a single genotype. Up to 10% variation was found among geographically distinct strains.

Currently, it is unknown whether the rRNA genes of *B. odocoilei* are in tandem or located on different chromosomes. Most likely, the array will be similar to the organization found in *B. bovis*. However, rDNA units in the hemoparasite *T. parva* have been cloned and mapped out to 2 separate chromosomes, and 2 different ITS sequences were obtained upon cloning a single isolate (Kibe et al., 1994). Multiple *T. parva* ITS sequences were confirmed by Collins and Allsopp (1999), who noted the futility of attempting classification of *T. parva* subspecies based solely upon ITS sequences, a view shared for *B. odocoilei* by the results of the current study.

The data from this study support lack of conspecificity between the California reindeer RD61 isolate and *B. odocoilei.* Although RD61 and *B. odocoilei* are morphologically similar and indirect fluorescent antibody (IFA) testing to *B. odocoilei*

showed equally strong reactions with both anti-*B. odocoilei* and serum from the RD61-infected reindeer, small subunit ribosomal RNA (SSU rRNA) gene-sequence analysis showed only 99.0% gene identity to *B. odocoilei* (Holman et al., 2002). Furthermore, RD61 was consistently distinct from all other isolates in this ITS-based study. Indeed, lower percent identities were observed in this study between RD61 and all the *B. odocoilei* isolates than among the *B. odocoilei* isolates. While the known *B. odocoilei* isolates ranged in identity from 93.3-99.9% in the entire ITS1-5.8S-ITS2 gene region, 90.2-99.8% in ITS1 and 92.0-100.0% in ITS2, the highest percent identity between RD61 and any isolate was only 88.4% in the ITS1-5.8S-ITS2 gene region, 85.8% in ITS1 and 87.6% in ITS2. Although regions of sequence conservation between some *B. odocoilei* clones and the RD61 clones were evident in the fixed differences analysis, the overall lower identity values indicate that RD61 is not conspecific with *B. odocoilei*.

Thus, these rDNA data support the separation of RD61 from *B. odocoilei.* Among the *B. canis* subspecies, ITS comparisons were no higher than 82.0%, which suggests that the anomalous ITS data for the RD61 isolate in this study may be indicative of a *B. odocoilei* subspecies similar to those of *B. canis*. The SSU sequences for the three *B. canis* subspecies are approximately 95-98% identical (Allsopp et al., 1994; Cacciò et al., 2002), compared to 99% for RD61 versus *B. odocoilei*. This study, combined with previous sequencing data, show that eighteen *B. odocoilei* isolates from different vertebrate hosts and different geographic regions share identical SSU rRNA gene sequences. Although this alone is not conclusive as to whether a 1% difference in SSU gene identities is evidence of two distinct species, or subspecies, the additional

genetic heterogeneity shown in the ITS region supports the case for two distinct *Babesia* isolates.

The ITS1-5.8S-ITS2 gene region has been studied in other protozoan parasites, including *Entamoeba* species (Som et al., 2000). *Entamoeba* rRNA genes are arranged on circular extrachromosomal DNA molecules and, unlike the *Babesia* rRNA, the ITS2 sequences were more variable than the ITS1 sequences. ITS nucleotide sequence differences were found among individual *Entamoeba* species. SSU rRNA sequences for *Entamoeba histolytica* and *Entamoeba dispar* (GenBank Accession numbers X56991 and Z49256, respectively) share 98-99% identity (Genestream analysis), which is comparable to the difference seen between RD61 and *B. odocoilei*. ITS1, 5.8S and ITS2 analysis between the two *Entamoeba* species showed sequence differences equivalent to a percent identity of 89%. Thus, two closely related *Entamoeba* species show differences in the SSU rRNA and the ITS gene regions comparable to those of the RD61 isolate and *B. odocoilei* species. However, it is still unknown whether this difference, along with that in the ITS, reflect differences among distinct species versus subspecies.

It is interesting to note that as more *B. odocoilei* infections and fatalities are reported, these are more prevalently occurring in zoos, herded farms or managed wildlife areas. The parasite continues to emerge in new animal hosts and geographic areas; since commencing the present study, *B. odocoilei* has been newly reported in musk ox from Minnesota, elk from New Hampshire and reindeer from New York and Pennsylvania. As the rate of cases, both fatal and nonsymptomatic, seems to be increasing, the shipment of animals nationwide is also increasing. Parasite infections leading to clinical

disease in male elk are more prevalent during the rut season, which happens to fall at the same time of year as the high tick activity season for the vector, *I. scapularis*. If this also coincides with the shipment of an elk, the stress of the move, the introduction of a new animal into an enzootic area or the arrival of an animal carrying the parasite can lead to devastating outbreaks in these animals. The recent report of a 7-month-old female reindeer dying of acute babesiosis is also troubling; whether this is an isolated incident or typical for reindeer is unknown (Holman et al., 2003). If age-related immunity to babesiosis is not a characteristic of reindeer, tick control on reindeer farms must be strictly followed.

Equally remarkable is the emergence of musk ox (*Ovibos moschatus*) as a new host for *B. odocoilei* infection during the course of this study. The Bighorn Sheep (*Ovis canadensis nelsoni*) *Babesia* sp. isolate was previously described, but at the time was not confirmed to be *B. odocoilei* (Goff et al., 1993). Thus the emergence of musk ox as the second bovidae host to be susceptible to *B. odocoilei* infection is significant. These two isolates from the bovidae hosts did not group together in the phylogenetic tree, but this is not surprising due to the extreme sequence variation observed in this study. Additionally, the geographic areas of both isolates are quite removed, especially that of Bighorn Sheep, which originated from the San Bernardino Mountains in southern California and was one of the most isolated samples in the study.

Certain initial questions from the beginning of this study remain unanswered. It was hoped that the ITS gene regions could be used as a gene target for determining the source of outbreaks of *B. odocoilei* in farmed and managed cervids. Clearly, these gene

regions did not show any trends based on animal host, geographic area, or type of infection, and more variation was observed than was ever expected. The inability to separate isolates using the ITS gene regions has been shown for *T. parva* as well. The RD61 isolate, already known to be different from *B. odocoilei*, indeed showed itself to be a distinct organism from the *B. odocoilei* isolates in all analyses and comparisons. Particularly important is the possible evidence in both direct sequence and phylogenetic analyses for at least two rRNA transcription units, which have already been shown to exist in several other *Babesia* species. Equally noteworthy is the amount of data analyzed in this study. Nineteen distinct isolates and a minimum of three clones per isolate were evaluated. Although some clones were identical to others, this was typically not the case, and the level of variation observed was staggering. For proper scientific accuracy, it is critical to include many isolates when carrying out similar phylogenetic studies that attempt to define one or more species.

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APPENDIX A

ALL	Carib3	Carib8	Carib10	Carib12	PA3	PA8	PA9	MO4	MO5	MO8	BH10	BH11	BH14	BH2-2	BH2-5	BH2-8	WR24	WR25	WR27	WP8	WP9	WP10	BB3	BB5
Carib3	100.0	99.6	97.9	99.3	96.9	96.9	96.9	97.0	96.7	96.7	93.7	93.6	93.6	93.9	93.9	93.9	97.1	94.2	94.2	94.9	94.9	94.9	97.2	97.3
Carib8		100.0	98.1	99.4	97.0	97.0	97.0	97.1	96.9	96.9	93.9	93.7	93.7	94.0	94.0	94.0	97.2	94.3	94.3	95.1	95.1	95.1	97.3	97.5
Carib10			100.0	97.7	96.5	96.5	96.5	96.4	96.4	96.4	93.4	93.3	93.3	93.5	93.5	93.5	97.0	94.3	94.3	93.9	94.3	94.3	96.9	96.9
Carib12				100.0	96.6	96.6	96.6	96.7	96.5	96.5	93.5	93.4	93.4	93.6	93.6	93.6	96.9	94.0	94.0	94.7	94.7	94.7	97.0	97.1
PA3					100.0	98.6	98.6	97.5	98.1	98.1	94.8	94.7	94.7	94.9	94.9	94.9	97.6	95.3	95.3	94.7	94.8	94.8	96.5	96.6
PA8						100.0	99.8	98.7	98.1	98.1	94.7	94.8	94.6	94.8	94.8	94.8	98.1	96.1	96.1	95.2	95.9	95.9	96.0	96.1
PA9							100.0	98.7	98.1	98.1	94.7	94.8	94.6	94.8	94.8	94.8	98.1	96.1	96.1	95.2	95.9	95.9	96.0	96.1
MO4								100.0	98.2	98.2	95.3	95.4	95.2	95.4	95.4	95.4	97.5	96.0	96.0	96.3	96.7	96.7	96.6	96.7
MO5									100.0	100.0	94.5	94.3	94.3	94.6	94.6	94.6	97.3	95.4	95.4	95.9	95.4	95.4	95.7	95.8
MO8										100.0	94.5	94.3	94.3	94.6	94.6	94.6	97.3	95.4	95.4	95.9	95.4	95.4	95.7	95.8
BH10											100.0	99.1	99.4	99.4	99.6	99.4	93.8	97.4	97.4	97.0	97.4	97.4	94.6	94.7
BH11												100.0	99.0	99.5	99.5	99.8	94.0	97.6	97.6	96.8	97.6	97.6	94.5	94.6
BH14													100.0	99.3	99.5	99.3	93.7	97.3	97.3	96.8	97.3	97.3	94.5	94.6
BH2-2														100.0	99.5	99.8	94.0	97.6	97.6	97.1	97.6	97.6	94.7	94.8
BH2-5															100.0	99.8	94.0	97.6	97.6	97.1	97.6	97.6	94.7	94.8
BH2-8																100.0	94.0	97.6	97.6	97.1	97.6	97.6	94.7	94.8
WR24																	100.0	95.5	95.5	94.3	95.3	95.3	96.5	96.6
WR25																		100.0	99.8	97.2	98.1	98.1	94.2	94.4
WR27																			100.0	97.2	98.1	98.1	94.3	94.4
WP8																				100.0	98.2	98.2	94.5	94.6
WP9																					100.0	99.8	94.4	94.6
WP10																						100.0	94.4	94.6
BB3																							100.0	99.6
BB5																								100.0

ALL	BB6	BB2-	BB2-	BB2-.	TXE7	TXE1	TXE1	TXE2	TXE2	TXE2	TXE2	NH74	NH73	NH86	OK3	OK8	OK11	Bz4	Bz7	Bz12	Dc1	Dc3	Dc5	Dc8
Carib	97.6	93.8	93.8	97.2	96.6	96.3	96.5	97.2	97.6	97.7	96.5	96.7	96.6	95.3	97.1	94.9	94.7	97.1	97.2	97.0	97.2	97.0	97.2	97.1
Carib	97.7	93.9	93.9	97.3	96.7	96.4	96.6	97.3	97.7	97.8	96.6	96.9	96.7	95.4	97.2	95.1	94.8	97.2	97.3	97.1	97.3	97.1	97.3	97.2
Carib	97.1	93.9	93.9	96.7	95.8	95.4	95.7	97.8	98.2	98.3	95.7	96.6	96.5	94.2	97.5	95.1	94.6	97.2	96.6	97.1	97.1	96.9	97.1	97.0
Carib	97.3	93.5	93.5	97.0	96.4	96.0	96.3	97.0	97.3	97.5	96.3	96.5	96.4	95.1	96.9	94.7	94.5	96.9	97.0	96.7	97.0	96.7	97.0	96.9
PA3	97.1	95.1	95.1	96.5	94.9	94.3	94.8	96.0	96.4	96.3	94.8	96.5	96.4	94.9	96.9	95.7	96.3	97.0	97.9	96.9	97.0	96.7	97.0	96.9
PA8	96.6	95.7	95.7	96.0	94.9	94.3	94.8	96.3	96.6	96.5	94.8	97.2	97.1	95.3	96.9	95.7	95.5	97.5	98.2	97.3	97.2	97.0	97.2	97.1
PA9	96.6	95.7	95.7	96.0	94.9	94.3	94.8	96.3	96.6	96.5	94.8	97.2	97.1	95.3	96.9	95.7	95.5	97.5	98.2	97.3	97.2	97.0	97.2	97.1
MO4	97.0	95.5	95.5	96.6	95.5	94.9	95.4	96.4	96.7	96.6	95.4	98.1	97.9	95.9	96.9	95.5	95.2	97.0	98.8	96.9	98.1	97.8	98.1	97.9
MO5	96.5	95.2	95.2	95.7	94.9	94.3	94.8	96.1	96.5	96.4	94.8	97.1	97.0	95.3	97.0	95.9	96.0	97.5	98.4	97.3	97.0	96.7	97.0	96.9
MO8	96.5	95.2	95.2	95.7	94.9	94.3	94.8	96.1	96.5	96.4	94.8	97.1	97.0	95.3	97.0	95.9	96.0	97.5	98.4	97.3	97.0	96.7	97.0	96.9
BH10	94.9	96.4	96.4	94.6	96.5	95.9	96.4	93.6	94.0	93.8	96.4	94.9	94.8	96.8	94.2	97.0	97.0	94.1	95.2	94.0	95.0	94.8	95.0	94.9
BH11	94.8	96.5	96.5	94.5	96.4	95.7	96.2	93.5	93.8	93.7	96.2	94.8	94.7	96.7	94.1	96.8	96.8	94.2	95.1	94.1	95.2	94.9	95.2	95.0
BH14	94.8	96.2	96.2	94.5	96.4	95.7	96.2	93.5	93.8	93.7	96.2	94.8	94.7	96.7	94.1	96.8	96.8	94.0	95.1	93.8	94.9	94.7	94.9	94.8
BH2-.	95.1	96.5	96.5	94.7	96.6	96.0	96.5	93.7	94.1	94.0	96.5	95.0	94.9	97.0	94.3	97.1	97.1	94.2	95.3	94.1	95.2	94.9	95.2	95.0
BH2-	95.1	96.5	96.5	94.7	96.6	96.0	96.5	93.7	94.1	94.0	96.5	95.0	94.9	97.0	94.3	97.1	97.1	94.2	95.3	94.1	95.2	94.9	95.2	95.0
BH2-	95.1	96.5	96.5	94.7	96.6	96.0	96.5	93.7	94.1	94.0	96.5	95.0	94.9	97.0	94.3	97.1	97.1	94.2	95.3	94.1	95.2	94.9	95.2	95.0
VR2	97.1	95.1	95.1	96.5	95.3	94.9	95.2	97.0	97.3	97.2	95.2	96.5	96.4	94.5	96.6	95.1	94.9	97.7	97.2	97.6	97.0	96.7	97.0	96.9
VR2	94.7	98.1	98.1	94.3	96.7	96.1	96.6	94.6	94.9	94.8	96.6	95.2	95.0	97.5	95.3	97.9	97.0	95.3	95.5	95.2	95.3	95.0	95.3	95.2
VR2	94.7	98.1	98.1	94.3	96.7	96.1	96.6	94.5	94.8	94.7	96.6	95.2	95.0	97.5	95.3	97.9	97.0	95.3	95.5	95.2	95.3	95.0	95.3	95.2
VP8	95.1	97.3	97.3	94.5	97.1	96.5	97.0	94.1	94.5	94.3	97.0	95.4	95.3	98.2	94.7	96.6	97.5	94.3	96.4	94.2	95.5	95.3	95.5	95.4
VP5	95.0	97.7	97.7	94.4	97.2	96.6	97.1	94.6	94.9	94.8	97.1	96.1	96.0	98.2	95.2	97.0	97.0	94.7	96.3	94.8	96.0	95.8	96.0	95.9
VP16	95.0	97.7	97.7	94.4	97.2	96.6	97.1	94.6	94.9	94.8	97.1	96.1	96.0	98.2	95.2	97.0	97.0	94.7	96.3	94.6	96.0	95.8	96.0	95.9
BB3	99.2	93.6	93.6	99.5	95.4	95.0	95.3	96.6	97.0	96.9	95.3	96.9	96.7	94.2	96.3	94.4	94.8	96.9	96.7	96.7	96.9	96.6	96.9	96.7
BB5	99.3	93.7	93.7	99.6	95.5	95.2	95.4	96.7	97.1	97.0	95.4	94.7	96.7	94.3	96.4	94.6	94.9	97.0	96.9	96.9	96.9	96.6	96.9	96.7
BB6	**100.0**	94.5	94.5	99.2	95.5	95.2	95.4	97.0	97.3	97.2	95.4	97.1	97.0	94.8	96.6	95.0	95.4	97.7	97.6	97.6	96.9	96.6	96.9	96.7
BB2-1		**100.0**	99.5	93.6	95.8	95.2	95.6	94.2	94.6	94.5	95.6	94.8	94.7	97.0	94.8	96.7	97.1	94.6	95.7	94.5	94.7	94.5	94.7	94.6
BB2-14			**100.0**	93.6	95.8	95.2	95.6	94.2	94.6	94.5	95.6	94.8	94.7	97.0	94.8	96.7	97.1	94.6	95.7	94.5	94.7	94.5	94.7	94.6
BB2-21				**100.0**	95.4	93.6	95.3	96.6	97.0	96.9	95.3	96.7	96.6	94.2	96.3	94.4	94.8	96.9	96.7	96.7	96.7	96.5	96.7	96.6
TXE7					**100.0**	99.4	99.9	95.9	96.3	96.4	99.9	95.3	95.2	97.3	95.5	97.8	97.4	95.2	95.4	95.0	95.7	95.4	95.7	95.5
TXE14						**100.0**	99.3	95.5	95.9	96.0	99.3	94.7	94.6	96.7	94.9	97.2	96.8	94.8	94.8	94.7	95.3	95.0	95.3	95.2
TXE18							**100.0**	95.8	96.1	96.3	99.8	95.2	95.0	97.2	95.4	97.7	97.3	95.0	95.3	94.9	95.5	95.3	95.5	95.4
TXE24								**100.0**	99.6	99.5	95.8	96.0	95.9	94.3	97.3	94.7	94.6	96.5	96.5	96.4	96.5	96.3	96.5	96.4
TXE2-1									**100.0**	99.9	96.1	96.4	96.3	94.7	97.7	95.1	94.9	96.9	96.9	96.7	96.9	96.6	96.9	96.7
TXE2-2										**100.0**	96.3	96.3	96.1	94.6	97.8	95.2	95.1	97.0	96.7	96.9	96.7	96.5	96.7	96.6
TXE2-8											**100.0**	95.2	95.0	97.2	95.4	97.7	97.3	95.0	95.3	94.9	95.5	95.3	95.5	95.4
NH74												**100.0**	99.6	94.8	96.9	95.0	95.3	96.6	98.1	96.5	98.3	98.1	98.3	98.2
NH73													**100.0**	94.7	96.7	94.9	95.2	96.5	97.9	96.4	98.2	97.9	98.2	98.2
NH86														**100.0**	95.0	96.8	96.4	93.8	95.9	93.6	95.2	94.9	95.2	95.1
OK3															**100.0**	95.5	95.2	96.4	97.3	96.3	97.1	96.9	97.1	97.0
OK8																**100.0**	97.9	95.9	95.9	95.8	95.4	95.2	95.4	95.3
OK11																	**100.0**	95.4	96.0	95.3	95.3	95.0	95.3	95.2
Bz4																		**100.0**	97.2	99.9	96.5	96.3	96.5	96.4
Bz7																			**100.0**	97.1	98.1	97.8	98.1	97.9
Bz12																				**100.0**	96.4	96.1	96.4	96.3
Dc1																					**100.0**	99.5	99.8	99.6
Dc3																						**100.0**	99.5	99.4
Dc5																							**100.0**	99.6
Dc8																								**100.0**

ALL	R61-2	R61-5	R61-8	MA3	MA5	MA9	WH10	WH13	WH14	BoE1	BoE2	BoE3	BoE6	IN6	IN11	IN14	MO2-5	MO2-6	MO2-14	MW1	MW4	MW9	MW11	MW14
Carib3	87.0	87.2	86.7	96.9	94.8	97.7	97.0	97.5	97.0	93.7	93.6	93.7	93.9	96.6	97.2	97.3	96.5	97.0	97.1	97.6	97.5	94.5	94.5	97.6
Carib8	87.2	87.3	87.1	97.0	95.0	97.8	97.1	97.6	97.1	93.9	93.7	93.9	94.0	96.7	97.3	97.5	96.6	97.1	97.2	97.7	97.6	94.6	94.6	97.7
Carib10	87.4	87.5	87.3	96.7	94.6	96.6	96.1	97.6	95.9	95.0	94.3	95.0	95.2	95.8	96.9	97.0	96.1	96.4	96.5	96.7	96.6	94.6	94.6	96.7
Carib12	86.8	86.9	86.7	96.6	94.6	97.5	96.7	97.2	96.7	93.5	93.4	93.5	93.6	96.4	97.0	97.1	96.3	96.7	96.9	97.3	97.2	94.2	94.2	97.3
PA3	87.0	86.8	86.9	97.6	94.9	97.7	96.9	97.5	96.6	94.3	93.9	94.3	94.5	97.6	97.9	98.1	97.8	97.5	97.6	96.5	96.4	95.3	95.3	96.5
PA8	87.1	87.0	87.0	97.8	95.8	98.4	97.6	98.2	97.3	95.2	94.7	95.2	95.3	97.8	97.9	98.1	97.6	98.7	98.8	97.5	97.3	95.8	95.8	97.5
PA9	87.1	87.0	87.0	97.8	95.8	98.4	97.6	98.2	97.3	95.2	94.7	95.2	95.3	97.8	97.9	98.1	97.6	98.7	98.8	97.5	97.3	95.8	95.8	97.5
MO4	87.3	87.1	87.2	97.2	95.9	99.0	98.2	98.1	98.2	95.1	94.5	95.1	95.2	98.4	97.6	97.7	97.7	99.8	99.9	98.3	98.2	95.8	95.8	98.3
MO5	87.0	86.8	86.9	96.7	95.5	98.6	97.6	97.2	97.3	94.6	94.0	94.6	94.7	96.7	98.1	98.2	99.5	97.9	98.1	97.2	97.1	95.7	95.7	97.2
MO8	87.0	86.8	86.9	96.7	95.5	98.6	97.6	97.2	97.3	94.6	94.0	94.6	94.7	96.7	98.1	98.2	99.5	97.9	98.1	97.2	97.1	95.7	95.7	97.2
BH10	87.2	87.1	87.1	94.2	96.6	94.9	94.9	94.6	94.9	96.4	95.6	96.4	96.5	95.4	95.2	95.1	94.2	95.3	95.4	94.6	94.5	97.3	97.6	94.6
BH11	87.3	87.2	87.2	94.3	96.7	94.8	95.1	94.7	95.1	96.5	95.8	96.5	96.6	95.5	95.1	94.9	94.1	95.4	95.5	94.7	94.6	97.2	97.4	94.7
BH14	87.2	87.1	87.1	94.1	96.5	94.8	94.8	94.5	94.8	96.2	95.5	96.2	96.4	95.3	95.1	94.9	94.1	95.2	95.3	94.5	94.3	97.2	97.4	94.5
BH2-2	87.3	87.2	87.2	94.3	96.7	95.1	95.1	94.7	95.1	96.5	95.8	96.5	96.6	95.5	95.3	95.2	94.3	95.4	95.5	94.7	94.6	97.4	97.7	94.7
BH2-5	87.3	87.2	87.2	94.3	96.7	95.1	95.1	94.7	95.1	96.5	95.8	96.5	96.6	95.5	95.3	95.2	94.3	95.4	95.5	94.7	94.6	97.4	97.7	94.7
BH2-8	87.3	87.2	87.2	94.3	96.7	95.1	95.1	94.7	95.1	96.5	95.8	96.5	96.6	95.5	95.3	95.2	94.3	95.4	95.5	94.7	94.6	97.4	97.7	94.7
WR24	86.9	86.8	86.5	98.1	95.4	97.2	96.9	98.2	96.6	94.8	94.1	94.8	94.9	96.9	97.2	97.3	97.1	97.5	97.6	97.9	97.8	95.0	95.0	97.9
WR25	87.3	87.2	87.2	94.9	98.2	95.7	95.7	95.5	95.4	97.3	96.6	97.3	97.5	95.4	96.4	96.4	95.2	96.0	96.1	95.3	95.2	98.5	98.8	95.3
WR27	87.5	87.3	87.3	94.9	98.2	95.7	95.7	95.5	95.4	97.3	96.6	97.3	97.5	95.4	96.4	96.4	95.2	96.0	96.1	95.3	95.2	98.5	98.8	95.3
WP8	86.6	86.5	86.5	94.3	97.8	95.7	96.0	94.7	96.0	96.6	96.2	96.6	96.7	95.5	95.2	95.2	95.7	96.7	96.1	95.7	95.5	97.7	97.7	95.7
WP9	87.4	87.3	87.1	95.2	98.2	96.4	96.1	95.5	96.1	97.2	96.6	97.2	97.3	95.9	95.2	95.2	94.9	96.7	96.9	95.8	95.7	97.7	97.7	95.8
WP10	87.4	87.3	87.1	95.2	98.2	96.4	96.1	95.5	96.1	97.2	96.6	97.2	97.3	95.9	95.2	95.2	94.9	96.7	96.9	95.8	95.7	97.7	97.7	95.8
BB3	87.3	87.2	87.5	97.1	94.1	96.6	96.7	97.1	96.7	93.8	93.4	93.8	94.0	97.6	96.9	97.0	95.4	96.6	96.7	97.2	97.1	94.6	94.8	97.2
BB5	87.3	87.2	87.2	97.2	94.2	96.7	96.9	97.2	96.9	93.8	93.4	93.8	94.0	97.7	97.0	97.1	95.5	96.7	96.9	97.3	97.2	94.7	94.9	97.3
BB6	87.8	87.7	87.7	97.7	94.5	97.2	97.1	97.5	97.1	94.1	93.7	94.1	94.2	97.7	97.0	97.1	96.3	97.0	97.1	97.3	97.2	94.9	95.2	97.3
BB2-1	87.4	87.2	87.3	95.1	98.3	95.2	95.1	95.1	94.8	97.1	97.0	97.1	97.2	95.3	95.2	95.2	94.9	95.5	95.7	94.8	94.7	97.8	97.8	94.8
BB2-14	87.2	87.1	87.1	95.1	98.3	95.2	95.1	95.1	94.8	97.1	97.0	97.1	97.2	95.3	95.2	95.2	94.9	95.5	95.7	94.8	94.7	97.8	97.8	94.8
BB2-21	87.7	87.6	87.8	97.1	94.1	96.6	96.7	97.1	96.7	93.7	93.3	93.7	93.8	97.6	96.9	97.0	95.4	96.6	96.7	97.2	97.1	94.6	94.8	97.2
TXE7	87.8	87.9	87.7	94.5	97.1	95.7	95.2	95.8	95.2	96.5	95.9	96.5	96.6	94.9	95.3	95.3	94.7	95.5	95.7	95.9	95.8	97.0	97.0	95.9
TXE14	87.3	87.4	87.2	94.1	96.5	95.1	94.6	95.4	94.6	95.9	95.3	95.9	96.0	94.3	94.7	94.7	94.1	94.9	95.1	95.5	95.4	96.4	96.4	95.5
TXE18	87.9	88.0	87.8	94.3	97.0	95.5	95.1	95.7	95.1	96.4	95.8	96.4	96.5	94.8	95.2	95.2	94.6	95.4	95.5	95.8	95.7	96.8	96.8	95.8
TXE24	86.4	86.3	86.1	96.6	94.9	96.1	95.8	97.6	95.5	94.8	94.1	94.8	94.9	95.9	96.4	96.5	95.9	96.4	96.5	96.7	96.6	94.7	94.7	96.7
TXE2-1	86.8	86.7	86.4	97.0	95.3	96.5	96.1	97.9	95.9	95.2	94.5	95.2	95.3	96.3	96.7	96.9	96.3	96.7	96.9	97.1	97.0	95.1	95.1	97.1
TXE2-2	86.7	86.8	86.6	96.9	95.2	96.6	96.3	97.8	96.0	95.1	94.3	95.1	95.2	96.1	96.6	96.7	96.1	96.6	96.7	97.0	96.9	94.9	94.9	97.0
TXE2-8	87.7	87.8	87.5	94.3	97.0	95.5	95.1	95.7	95.1	96.4	95.8	96.4	96.5	94.8	95.2	95.2	94.6	95.4	95.5	95.8	95.7	96.8	96.8	95.8
NH74	87.9	87.8	87.8	96.5	95.3	98.1	97.8	96.9	97.8	94.7	94.4	94.7	94.8	97.7	97.1	97.2	96.6	98.1	98.2	97.5	97.3	95.7	95.7	97.5
NH79	87.9	87.8	87.8	96.5	95.3	98.1	97.8	96.9	97.8	94.7	94.4	94.7	94.7	97.6	97.1	97.2	96.6	98.1	98.2	97.3	97.2	95.5	95.5	97.3
NH80	86.9	86.8	86.5	94.1	97.6	96.0	95.1	94.9	95.1	96.2	95.9	96.2	96.4	95.1	95.3	95.3	95.1	95.9	96.0	94.9	94.8	97.5	97.5	94.9
OK3	86.5	86.6	86.3	96.5	95.1	97.2	96.9	97.1	96.6	94.6	93.9	94.6	94.7	96.5	97.2	97.3	96.7	96.9	97.0	96.5	96.4	95.1	95.1	96.5
OK8	88.1	88.2	88.2	94.2	97.3	95.7	95.4	95.5	95.2	96.5	95.9	96.5	96.6	94.9	96.5	96.5	95.7	95.5	95.7	94.7	94.6	97.9	98.2	94.7
OK11	87.6	87.7	87.7	95.4	97.1	95.4	95.8	95.2	95.5	96.7	96.1	96.7	96.8	95.9	95.5	95.5	95.8	95.2	95.3	95.1	94.9	97.6	97.6	95.1
Bz4	87.7	87.8	87.8	97.5	95.0	97.2	97.8	97.7	97.6	94.8	94.0	94.8	94.9	96.5	97.5	97.6	97.2	97.0	97.1	97.7	97.6	95.3	95.5	97.7
Bz7	87.4	87.3	87.3	97.2	95.9	98.8	98.4	97.8	98.2	94.7	94.5	94.7	94.8	97.9	97.8	97.9	98.2	98.8	98.9	97.6	97.5	96.0	96.0	97.6
Bz12	87.6	87.7	87.7	97.3	94.8	97.1	97.7	97.6	97.5	94.7	93.9	94.7	94.8	96.4	97.3	97.5	97.1	96.9	97.0	97.6	97.5	95.2	95.4	97.6
Dc1	87.7	87.6	87.4	96.5	95.4	97.6	97.7	97.6	97.7	95.2	94.6	95.2	95.3	97.7	97.3	97.5	96.7	98.1	98.2	97.6	97.5	95.4	95.4	97.6
Dc3	87.5	87.4	87.3	96.3	95.2	97.3	97.5	97.3	97.5	94.9	94.3	94.9	95.0	97.5	97.1	97.2	96.5	97.8	97.9	97.3	97.2	95.2	95.2	97.3
Dc5	87.7	87.6	87.4	96.5	95.4	97.6	97.7	97.6	97.7	95.2	94.6	95.2	95.3	97.7	97.3	97.5	96.7	98.1	98.2	97.6	97.5	95.4	95.4	97.6
Dc8	87.6	87.5	87.4	96.4	95.3	97.5	97.6	97.5	97.6	95.0	94.4	95.0	95.2	97.6	97.2	97.3	96.6	97.9	98.1	97.5	97.3	95.3	95.3	97.5
R61-2	100.0	99.9	98.0	87.6	87.4	87.3	86.8	87.7	86.9	88.2	87.7	88.2	88.4	87.3	87.4	87.3	86.7	87.1	87.4	87.5	87.3	87.5	87.7	87.5
R61-5		100.0	98.2	87.5	87.3	87.4	86.9	87.6	87.0	88.1	87.5	88.1	88.2	87.1	87.3	87.2	86.6	87.0	87.3	87.3	87.2	87.3	87.6	87.3
R61-8			100.0	87.2	87.1	87.4	86.9	87.3	87.0	87.9	87.3	87.9	88.0	87.4	87.6	87.5	86.6	87.0	87.3	87.1	87.0	87.3	87.6	87.1
MA3				100.0	95.3	97.0	97.0	98.2	96.7	94.7	94.3	94.7	94.8	97.8	97.0	97.1	96.5	97.2	97.3	97.8	97.7	94.7	94.7	97.8
MA5					100.0	95.6	95.5	95.9	95.3	97.8	97.8	97.8	97.9	95.5	95.8	95.8	95.3	95.9	96.0	95.7	95.5	98.2	98.2	95.7
MA9						100.0	98.3	97.3	98.3	94.5	94.1	94.5	94.6	97.9	97.8	97.9	98.1	99.0	99.2	97.7	97.6	95.7	95.7	97.7
WH10							100.0	97.2	99.5	94.7	94.1	94.7	94.8	97.7	97.6	97.7	97.3	98.2	98.3	97.9	97.8	95.4	95.7	97.9
WH13								100.0	97.0	95.5	94.9	95.5	95.7	97.5	97.3	97.5	97.0	98.1	98.2	98.3	98.2	95.5	95.5	98.3
WH14									100.0	94.5	93.9	94.5	94.6	97.7	97.3	97.5	97.1	98.2	98.3	97.9	97.8	95.2	95.4	97.9
BoE1										100.0	98.5	99.8	99.9	94.7	94.6	94.6	94.3	95.1	95.2	95.2	95.1	97.1	97.1	95.2

MV14	BcE2	BcE3	BcE6	IN6	IN11	IN14	MO2-5	MO2-6	MO2-14	MV1	MV4	MV5	MV11	MV14
BcE2	**100.0**	98.5	98.7	94.5	94.3	94.3	93.7	94.5	94.6	94.5	94.3	96.8	96.8	94.5
BcE3		**100.0**	99.9	94.7	94.6	94.6	94.3	95.1	95.2	95.2	95.1	97.1	97.1	95.2
BcE6			**100.0**	94.8	94.7	94.7	94.5	95.2	95.3	95.3	95.2	97.2	97.2	95.3
IN6				**100.0**	97.5	97.6	96.5	98.4	98.6	97.8	97.7	95.4	95.4	97.8
IN11					**100.0**	99.9	97.8	97.6	97.7	97.5	97.3	96.5	96.7	97.5
IN14						**100.0**	97.9	97.7	97.8	97.6	97.5	96.4	96.6	97.6
MO2-5							**100.0**	97.7	97.6	97.0	96.9	95.4	95.4	97.0
MO2-6								**100.0**	99.9	98.3	98.2	95.8	95.8	98.3
MO2-14									**100.0**	98.4	98.3	95.9	95.9	98.4
MV1										**100.0**	99.9	95.3	95.3	100.0
MV4											**100.0**	95.2	95.2	99.9
MV5												**100.0**	99.8	95.3
MV11													**100.0**	95.3
MV14														**100.0**

ITS1	*Carib3*	*Carib8*	*Carib10*	*Carib12*	*PA3*	*PA8*	*PA9*	*MO4*	*MO5*	*MO8*	*BH10*	*BH11*	*BH14*	*BH2-2*	*BH2-5*	*BH2-8*	*WR24*	*WR25*	*WR27*	*WP8*	*WP9*	*WP10*	*BB3*	*BB5*
Carib3	100.0	99.5	96.7	99.3	97.1	96.7	96.9	96.9	95.9	95.9	91.9	91.7	91.7	92.2	91.7	91.9	96.2	91.9	92.2	92.2	92.6	92.4	97.1	97.4
Carib8		100.0	96.7	99.3	97.1	96.7	96.9	96.9	95.9	95.9	91.9	91.7	91.7	92.2	91.7	91.9	96.2	91.9	92.2	92.2	92.6	92.4	97.1	97.4
Carib10			100.0	96.4	96.7	96.2	96.4	95.9	95.5	95.5	91.4	91.2	91.2	91.7	91.2	91.4	96.2	92.4	92.6	90.3	91.7	91.4	96.7	96.7
Carib12				100.0	96.9	96.4	96.7	96.7	95.7	95.7	91.7	91.4	91.4	91.9	91.4	91.7	95.9	91.7	91.9	91.9	92.4	92.2	96.9	97.1
PA3					100.0	98.6	98.8	96.9	97.6	97.6	92.1	91.9	91.9	92.4	91.9	92.1	98.1	93.1	93.3	91.6	92.4	92.1	96.2	96.4
PA8						100.0	99.8	97.8	97.1	97.1	91.9	91.6	91.6	92.1	91.6	91.9	98.1	93.3	93.6	91.9	93.1	92.8	96.2	96.4
PA9							100.0	98.1	97.4	97.4	92.1	91.9	91.9	92.4	91.9	92.1	98.3	93.6	93.8	92.1	93.3	93.1	96.4	96.7
MO4								100.0	96.9	96.9	93.1	92.8	92.8	93.3	92.8	93.1	96.4	92.6	92.8	93.6	94.3	94.0	97.4	97.6
MO5									100.0	100.0	90.9	90.7	90.7	91.2	90.7	90.9	96.7	91.9	92.1	92.6	92.1	91.9	95.0	95.2
MO8										100.0	90.9	90.7	90.7	91.2	90.7	90.9	96.7	91.9	92.1	92.6	92.1	91.9	95.0	95.2
BH10											100.0	99.3	99.8	99.8	99.8	99.5	91.6	96.9	97.1	96.6	98.1	97.8	92.9	93.1
BH11												100.0	99.0	99.5	99.5	99.8	91.4	96.6	96.9	96.4	97.8	97.6	92.6	92.9
BH14													100.0	99.5	99.5	99.3	91.4	96.6	96.9	96.4	97.8	97.6	92.6	92.9
BH2-2														100.0	99.5	99.8	91.9	97.1	97.3	96.9	98.3	98.1	93.1	93.3
BH2-5															100.0	99.3	91.4	96.6	96.9	96.4	97.8	97.6	92.6	92.9
BH2-8																100.0	91.6	96.9	97.1	96.6	98.1	97.8	92.9	93.1
WR24																	100.0	93.1	93.3	91.2	92.3	92.1	95.7	95.9
WR25																		100.0	99.8	95.9	97.3	97.1	92.3	92.6
WR27																			100.0	96.1	97.6	97.3	92.6	92.8
WP8																				100.0	98.1	97.8	92.4	92.6
WP9																					100.0	99.8	93.3	93.5
WP10																						100.0	93.1	93.3
BB3																							100.0	99.8
BB5																								100.0

ITS1	BB6	BB2-1	BB2-14	BB2-21	TXE7	TXE14	TXE18	TXE24	TXE2-1	TXE2-2	TXE2-8	NH74	NH79	NH80	OK3	OK8	OK11	Bz4	Bz7	Bz12	Dc1	Dc3	Dc5	Dc8
Carib3	97.6	91.2	91.0	97.1	94.3	94.0	94.0	95.2	95.9	96.2	94.3	96.2	95.9	93.3	96.2	93.1	92.8	96.4	96.9	96.4	97.1	96.9	96.9	96.9
Carib8	97.6	91.2	91.0	97.1	94.3	94.0	94.0	95.2	95.9	96.2	94.3	96.2	95.9	93.3	96.2	93.1	92.8	96.4	96.9	96.4	97.1	96.9	96.9	96.9
Carib10	96.9	91.7	91.5	96.4	92.8	92.6	92.6	96.7	97.4	97.6	92.8	96.2	95.9	91.5	97.1	93.6	92.8	96.9	95.9	96.9	97.1	96.9	96.9	96.9
Carib12	97.4	91.0	90.8	96.9	94.0	93.8	93.8	95.0	95.7	95.9	94.0	95.9	95.7	93.1	95.9	92.8	92.6	96.2	96.7	96.2	96.9	96.7	96.7	96.7
PA3	97.1	92.4	92.1	96.2	93.3	93.1	93.1	95.7	96.4	96.2	93.3	95.2	95.0	93.1	96.7	94.0	93.1	97.6	97.4	97.6	96.7	96.4	96.4	96.4
PA8	97.1	92.6	92.4	96.2	92.9	92.6	92.6	95.7	96.4	96.2	92.9	95.7	95.5	93.3	96.2	93.6	92.6	97.1	97.4	97.1	96.2	95.9	95.9	95.9
PA9	97.4	92.9	92.6	96.4	93.1	92.9	92.9	95.9	96.0	96.4	93.1	95.9	95.7	93.6	96.4	93.8	92.9	97.4	97.6	97.4	96.4	96.2	96.2	96.2
MO4	97.8	91.9	91.7	97.4	93.6	93.3	93.3	95.5	96.7	95.9	93.6	96.9	96.7	94.0	95.7	92.9	91.9	96.2	98.1	96.2	97.4	97.1	97.1	97.1
MO5	96.4	91.7	91.4	95.0	91.9	91.6	91.6	94.5	95.2	95.0	91.9	95.0	94.7	92.4	95.5	93.1	93.1	97.6	96.9	97.6	95.2	95.0	95.0	95.0
MO8	96.4	91.7	91.4	95.0	91.9	91.6	91.6	94.5	95.2	95.0	91.9	95.0	94.7	92.4	95.5	93.1	93.1	97.6	96.9	97.6	95.2	95.0	95.0	95.0
BH10	93.3	95.9	95.7	92.9	96.9	96.6	96.6	91.4	92.1	91.9	96.9	92.6	92.3	97.3	91.9	96.1	95.9	91.9	92.4	91.9	93.3	93.1	93.1	93.1
BH11	93.1	95.7	95.4	92.6	96.6	96.4	96.4	91.2	91.9	91.6	96.6	92.3	92.1	97.1	91.7	95.9	95.6	91.6	92.2	91.6	93.1	92.8	92.8	92.8
BH14	93.1	95.7	95.4	92.6	96.6	96.4	96.4	91.2	91.9	91.6	96.6	92.3	92.1	97.1	91.7	95.9	95.6	91.6	92.2	91.6	93.1	92.8	92.8	92.8
BH2-2	93.6	96.1	95.9	93.1	97.1	96.9	96.9	91.6	92.4	92.1	97.1	92.8	92.6	97.6	92.2	96.4	96.1	92.1	92.6	92.1	93.5	93.3	93.3	93.3
BH2-5	93.1	95.7	95.4	92.6	96.6	96.4	96.4	91.2	91.9	91.6	96.6	92.3	92.1	97.1	91.7	95.9	95.6	91.6	92.2	91.6	93.1	92.8	92.8	92.8
BH2-8	93.3	95.9	95.7	92.9	96.9	96.6	96.6	91.4	92.1	91.9	96.9	92.6	92.3	97.3	91.9	96.1	95.9	91.9	92.4	91.9	93.3	93.1	93.1	93.1
WR24	96.6	91.9	91.7	95.7	92.6	92.4	92.4	95.7	96.4	96.2	92.6	94.7	94.5	92.6	96.2	93.3	92.4	96.6	96.4	96.6	96.2	95.9	95.9	95.9
WR25	92.8	97.3	97.1	92.3	96.4	96.1	96.1	92.4	93.1	92.8	96.4	91.6	91.4	97.6	93.1	97.1	95.9	92.4	92.1	92.4	92.3	92.1	92.1	92.1
WR27	93.1	97.6	97.3	92.6	96.6	96.4	96.4	92.4	93.1	92.8	96.6	91.9	91.6	97.8	93.3	97.3	96.1	92.6	92.4	92.6	92.6	92.3	92.3	92.3
WP8	93.3	96.4	96.1	92.4	95.9	95.7	95.7	90.2	91.0	90.7	95.9	92.4	92.1	97.8	90.8	95.2	96.6	91.9	93.6	91.9	92.8	92.6	92.6	92.6
WP9	94.3	96.9	96.6	93.3	96.6	96.4	96.4	91.6	92.4	92.1	96.6	93.3	93.1	98.8	92.2	95.9	96.1	91.9	93.8	91.9	93.5	93.3	93.3	93.3
WP10	94.0	96.6	96.4	93.1	96.4	96.1	96.1	91.4	92.1	91.9	96.4	93.1	92.8	98.6	91.9	95.6	95.9	91.7	93.6	91.7	93.3	93.1	93.1	93.1
BB3	98.6	91.7	91.4	99.5	93.5	93.3	93.3	95.7	96.4	96.2	93.5	97.6	97.4	92.8	96.2	92.3	92.3	96.2	97.1	96.2	98.1	97.8	97.8	97.8
BB5	98.8	91.9	91.7	99.8	93.8	93.5	93.5	95.9	96.7	96.4	93.8	97.6	97.4	93.1	96.4	92.6	92.6	96.4	97.4	96.4	98.1	97.8	97.8	97.8
BB6	100.0	93.1	92.9	98.6	93.5	93.3	93.3	96.2	96.9	96.7	93.5	97.8	97.6	93.8	96.7	93.3	93.3	97.6	98.6	97.6	97.8	97.6	97.6	97.6
BB2-1		100.0	99.3	91.7	94.7	94.5	94.5	91.9	92.6	92.4	94.7	91.2	91.0	96.9	92.4	95.9	95.9	92.2	92.6	92.2	91.4	91.2	91.2	91.2
BB2-14			100.0	91.4	94.5	94.2	94.2	91.7	92.4	92.1	94.5	91.0	90.7	96.6	92.2	95.7	95.7	91.9	92.4	91.9	91.2	91.0	91.0	91.0
BB2-21				100.0	93.5	93.3	93.3	95.7	96.4	96.2	93.5	97.6	97.4	92.8	96.2	92.3	92.3	96.2	97.1	96.2	98.1	97.8	97.8	97.8
TXE7					100.0	99.8	99.8	92.1	92.9	93.1	100.0	92.8	92.6	96.9	92.6	98.3	97.8	93.1	92.8	93.1	94.0	93.8	93.8	93.8
TXE14						100.0	99.5	91.9	92.6	92.9	99.8	92.6	92.3	96.6	92.4	98.1	97.6	92.8	92.6	92.8	93.8	93.5	93.5	93.5
TXE18							100.0	91.9	92.6	92.9	99.8	92.6	92.3	96.6	92.4	98.1	97.6	92.8	92.6	92.8	93.8	93.5	93.5	93.5
TXE24								100.0	99.3	99.0	92.1	94.5	94.3	91.2	96.4	92.4	92.4	95.5	95.2	95.5	95.5	95.2	95.2	95.2
TXE2-1									100.0	99.8	92.9	95.2	95.0	91.9	97.1	93.1	93.1	96.2	95.9	96.2	96.2	95.9	95.9	95.9
TXE2-2										100.0	93.1	95.0	94.7	91.7	97.4	93.3	93.3	96.4	95.7	96.4	95.9	95.7	95.7	95.7
TXE2-8											100.0	92.8	92.6	96.9	92.6	98.3	97.8	93.1	92.8	93.1	94.0	93.8	93.8	93.8
NH74												100.0	99.8	92.1	95.5	92.1	92.3	95.7	96.9	95.7	98.6	98.3	98.3	98.3
NH79													100.0	91.9	95.2	91.9	92.1	95.4	96.7	95.4	98.3	98.1	98.1	98.1
NH80														100.0	91.7	96.1	95.4	91.9	93.6	91.9	92.8	92.6	92.6	92.6
OK3															100.0	93.3	92.8	96.4	96.2	96.4	96.4	96.2	96.2	96.2
OK8																100.0	98.1	94.3	93.1	94.3	92.8	92.6	92.6	92.6
OK11																	100.0	94.5	93.1	94.5	92.8	92.6	92.6	92.6
Bz4																		100.0	97.1	100.0	95.9	95.7	95.7	95.7
Bz7																			100.0	97.1	97.4	97.1	97.1	97.1
Bz12																				100.0	95.9	95.7	95.7	95.7
Dc1																					100.0	99.8	99.8	99.8
Dc3																						100.0	99.5	99.5
Dc5																							100.0	99.5
Dc8																								100.0

ITS1	R61-2	R61-5	R61-8	MA3	MA5	MA9	WH10	WH13	WH14	BoE1	BoE2	BoE3	BoE6	IN6	IN11	IN14	MO2-5	MO2-6	MO2-14	MW1	MW4	MW9	MW11	MW14
Carib3	83.5	83.7	83.7	96.4	91.9	98.1	97.1	96.4	97.1	90.7	90.7	90.7	90.7	97.8	97.4	97.6	95.9	96.9	97.1	96.9	96.7	91.9	91.9	96.9
Carib8	83.5	83.7	83.7	96.4	91.9	98.1	97.1	96.4	97.1	90.7	90.7	90.7	90.7	97.8	97.4	97.6	95.9	96.9	97.1	96.9	96.7	91.9	91.9	96.9
Carib10	84.3	84.6	84.6	96.4	91.7	96.2	95.7	96.9	95.2	93.5	92.3	93.5	93.5	96.4	96.9	97.1	95.5	95.9	96.2	95.5	95.2	92.4	92.4	95.5
Carib12	83.2	83.5	83.5	96.2	91.7	97.8	96.9	96.2	96.9	90.5	90.5	90.5	90.5	97.6	97.1	97.4	95.7	96.7	96.9	96.7	96.4	91.7	91.7	96.7
PA3	83.2	83.0	83.0	97.4	92.6	97.1	96.4	97.8	95.9	91.9	91.2	91.9	91.9	96.9	97.8	98.1	97.6	96.9	97.1	96.2	95.9	92.6	92.6	96.2
PA8	83.1	82.8	82.8	97.8	92.9	97.6	96.4	98.3	95.9	92.1	91.4	92.1	92.1	97.4	97.4	97.6	96.7	97.8	98.1	96.7	96.4	93.1	93.1	96.7
PA9	83.3	83.1	83.1	98.1	93.1	97.8	96.7	98.6	96.2	92.4	91.7	92.4	92.4	97.6	97.6	97.8	96.9	98.1	98.3	96.9	96.7	93.3	93.3	96.9
MO4	83.8	83.5	83.5	97.1	92.6	98.3	97.1	98.1	97.1	91.9	91.0	91.9	91.9	98.6	96.7	96.9	96.4	99.5	99.8	97.8	97.6	92.6	92.6	97.8
MO5	83.2	83.0	83.0	96.7	92.4	97.4	96.4	96.4	95.9	91.4	90.5	91.4	91.4	95.7	97.1	97.4	99.5	96.4	96.7	96.2	95.9	91.9	91.9	96.2
MO8	83.2	83.0	83.0	96.7	92.4	97.4	96.4	96.4	95.9	91.4	90.5	91.4	91.4	95.7	97.1	97.4	99.5	96.4	96.7	96.2	95.9	91.9	91.9	96.2
BH10	84.4	84.1	84.1	92.6	96.4	92.2	92.1	92.6	92.1	96.4	95.2	96.4	96.4	94.0	92.4	92.2	90.9	93.1	93.3	92.8	92.6	96.6	96.6	92.8
BH11	84.1	83.9	83.9	92.4	96.1	91.9	91.9	92.4	91.9	96.1	94.9	96.1	96.1	93.8	92.2	91.9	90.7	92.8	93.1	92.6	92.4	96.4	96.4	92.6
BH14	84.1	83.9	83.9	92.4	96.1	91.9	91.9	92.4	91.9	96.1	94.9	96.1	96.1	93.8	92.2	91.9	90.7	92.8	93.1	92.6	92.4	96.4	96.4	92.6
BH2-2	84.6	84.4	84.4	92.8	96.6	92.4	92.4	92.8	92.4	96.6	95.4	96.6	96.6	94.3	92.6	92.4	91.2	93.3	93.6	93.1	92.8	96.9	96.9	93.1
BH2-5	84.1	83.9	83.9	92.4	96.1	91.9	91.9	92.4	91.9	96.1	94.9	96.1	96.1	93.8	92.2	91.9	90.7	92.8	93.1	92.6	92.4	96.4	96.4	92.6
BH2-8	84.4	84.1	84.1	92.6	96.4	92.2	92.1	92.6	92.1	96.4	95.2	96.4	96.4	94.0	92.4	92.2	90.9	93.1	93.3	92.8	92.6	96.6	96.6	92.8
WR24	82.7	82.5	82.5	97.4	92.1	96.2	95.5	97.4	95.0	91.9	90.7	91.9	91.9	96.4	97.4	97.6	96.7	96.4	96.7	96.2	95.9	92.6	92.6	96.2
WR25	83.9	83.7	83.7	93.1	97.6	92.2	91.6	93.6	91.2	96.9	95.7	96.9	96.9	93.1	93.8	93.8	91.9	92.6	92.8	92.4	92.1	98.1	98.1	92.4
WR27	84.4	84.1	84.1	93.3	97.8	92.4	91.9	93.8	91.4	97.1	95.9	97.1	97.1	93.7	94.0	94.0	92.1	92.8	93.1	92.6	92.4	98.3	98.3	92.6
WP8	83.2	83.0	83.0	92.4	97.3	92.4	93.8	92.1	93.8	95.9	95.4	95.9	95.9	93.8	92.1	92.1	92.6	93.1	93.3	93.6	93.3	96.6	96.6	93.6
WP9	84.1	83.9	83.9	93.3	97.3	93.8	93.3	92.8	93.3	96.4	95.4	96.4	96.4	94.3	92.6	92.6	91.6	94.3	94.5	93.1	92.8	97.1	97.1	93.1
WP10	83.9	83.7	83.7	93.1	97.1	93.6	93.1	92.6	93.1	96.1	95.2	96.1	96.1	94.0	92.4	92.4	91.4	94.0	94.3	92.8	92.6	96.9	96.9	92.8
BB3	84.7	84.4	84.4	96.7	92.1	97.1	96.9	96.9	96.9	92.1	91.4	92.1	92.1	98.6	96.9	97.1	95.0	97.4	97.6	97.4	97.1	92.8	92.8	97.4
BB5	84.7	84.4	84.4	96.9	92.4	97.4	97.1	97.1	97.1	92.1	91.4	92.1	92.1	98.8	97.1	97.4	95.2	97.6	97.8	97.6	97.4	93.1	93.1	97.6
BB6	85.4	85.2	85.2	97.6	92.6	98.1	97.4	97.4	97.4	92.4	91.9	92.4	92.4	98.6	96.9	97.1	96.4	97.8	98.1	97.4	97.1	93.3	93.3	97.4
BB2-1	84.2	84.0	84.0	93.1	98.1	91.5	91.7	92.9	91.2	96.6	96.6	96.6	96.6	92.6	92.6	92.6	91.7	91.9	92.1	91.7	91.4	97.3	97.3	91.7
BB2-14	83.7	83.5	83.5	92.9	97.8	91.2	91.4	92.6	91.0	96.4	96.4	96.4	96.4	92.4	92.4	92.4	91.4	91.7	91.9	91.4	91.2	97.1	97.1	91.4
BB2-21	84.7	84.4	84.4	96.7	92.1	97.1	96.9	96.9	96.9	92.1	91.4	92.1	92.1	98.6	96.9	97.1	95.0	97.4	97.6	97.4	97.1	92.8	92.8	97.4
TXE7	85.1	85.3	85.3	92.1	95.9	93.6	93.1	93.1	93.1	95.2	94.2	95.2	95.2	94.0	93.6	93.6	91.9	93.6	93.8	93.6	93.3	96.4	96.4	93.6
TXE14	84.9	85.1	85.1	91.9	95.7	93.3	92.8	92.9	92.8	94.9	94.0	94.9	94.9	93.8	93.3	93.3	91.6	93.3	93.6	93.3	93.1	96.1	96.1	93.3
TXE18	84.9	85.1	85.1	91.9	95.7	93.3	92.8	92.9	92.8	94.9	94.0	94.9	94.9	93.8	93.3	93.3	91.6	93.3	93.6	93.3	93.1	96.1	96.1	93.3
TXE24	82.5	82.2	82.2	96.2	91.9	94.7	94.5	96.4	94.0	92.1	91.0	92.1	92.1	96.2	95.9	96.2	94.5	95.5	95.7	95.0	94.7	92.1	92.1	95.0
TXE2-1	83.2	82.9	82.9	96.9	92.6	95.5	95.2	97.1	94.7	92.8	91.7	92.8	92.8	96.9	96.7	96.9	95.2	96.2	96.4	95.7	95.5	92.8	92.8	95.7
TXE2-2	82.9	83.2	83.2	96.7	92.4	95.7	95.5	96.9	95.0	92.6	91.4	92.6	92.6	96.7	96.4	96.7	95.0	95.9	96.2	95.5	95.2	92.6	92.6	95.5
TXE2-8	85.1	85.3	85.3	92.1	95.9	93.6	93.1	93.1	93.1	95.2	94.2	95.2	95.2	94.0	93.6	93.6	91.9	93.6	93.8	93.6	93.3	96.4	96.4	93.6
NH74	85.6	85.3	85.3	95.9	92.1	96.7	96.7	95.9	96.7	91.8	91.6	91.8	91.8	97.8	95.9	96.2	94.5	96.9	97.1	96.4	96.2	92.6	92.6	96.4
NH79	85.3	85.1	85.1	95.7	91.9	96.4	96.4	95.7	96.4	91.6	91.4	91.6	91.6	97.6	95.7	95.9	94.3	96.7	96.9	96.2	95.9	92.3	92.3	96.2
NH80	83.9	83.7	83.7	93.1	97.6	94.1	93.1	93.1	93.1	95.9	95.4	95.9	95.9	94.0	93.3	93.3	92.4	94.0	94.3	93.3	93.1	97.1	97.1	93.3
OK3	82.9	83.2	83.2	96.7	92.4	96.2	95.9	96.7	95.5	92.4	91.2	92.4	92.4	96.7	96.9	97.1	95.5	95.7	95.9	95.7	95.5	92.2	92.2	95.7
OK8	85.6	85.8	85.8	92.9	96.6	92.8	92.4	93.8	91.9	95.9	94.9	95.9	95.9	92.9	93.8	93.8	93.1	92.9	93.1	92.4	92.1	97.1	97.1	92.4
OK11	84.4	84.6	84.6	93.1	96.4	92.1	93.8	92.9	93.3	96.1	95.2	96.1	96.1	93.1	93.1	93.1	93.1	91.9	92.1	92.9	92.6	96.6	96.6	92.9
Bz4	84.4	84.7	84.7	96.9	91.9	96.9	97.1	97.1	96.7	92.6	91.2	92.6	92.6	96.4	96.7	96.9	97.6	96.2	96.4	96.4	96.2	93.3	93.3	96.4
Bz7	84.0	83.8	83.8	97.6	93.1	97.8	98.1	97.6	97.6	91.7	91.4	91.7	91.7	98.1	96.7	96.9	96.9	98.1	98.3	96.9	96.7	92.6	92.6	96.9
Bz12	84.4	84.7	84.7	96.9	91.9	96.9	97.1	97.1	96.7	92.6	91.2	92.6	92.6	96.4	96.7	96.9	97.6	96.2	96.4	96.4	96.2	93.3	93.3	96.4
Dc1	84.3	84.0	84.0	96.4	92.4	96.7	97.4	96.9	97.4	92.8	91.9	92.8	92.8	98.3	96.9	97.1	95.2	97.4	97.6	97.1	96.9	92.6	92.6	97.1
Dc3	84.0	83.8	83.8	96.2	92.1	96.4	97.1	96.7	97.1	92.6	91.6	92.6	92.6	98.1	96.7	96.9	95.0	97.1	97.4	96.9	96.7	92.3	92.3	96.9
Dc5	84.0	83.8	83.8	96.2	92.1	96.4	97.1	96.7	97.1	92.6	91.6	92.6	92.6	98.1	96.7	96.9	95.0	97.1	97.4	96.9	96.7	92.3	92.3	96.9
Dc8	84.0	83.8	83.8	96.2	92.1	96.4	97.1	96.7	97.1	92.6	91.6	92.6	92.6	98.1	96.7	96.9	95.0	97.1	97.4	96.9	96.7	92.3	92.3	96.9
R61-2	100.0	99.8	99.8	84.3	83.3	84.0	82.8	83.8	83.1	84.8	83.9	84.8	84.8	84.5	84.2	83.9	83.2	83.5	84.0	84.0	83.8	84.6	84.6	84.0
R61-5		100.0	100.0	84.0	83.0	84.2	83.1	83.5	83.3	84.6	83.7	84.6	84.6	84.2	83.9	83.7	83.0	83.3	83.8	83.8	83.5	84.4	84.4	83.8
R61-8			100.0	84.0	83.0	84.2	83.1	83.5	83.3	84.6	83.7	84.6	84.6	84.2	83.9	83.7	83.0	83.3	83.8	83.8	83.5	84.4	84.4	83.8
MA3				100.0	93.1	96.9	96.9	98.1	96.4	92.4	91.9	92.4	92.4	98.1	97.6	97.8	96.7	97.1	97.4	97.1	96.9	93.1	93.1	97.1
MA5					100.0	92.2	92.6	93.1	92.1	96.6	96.9	96.6	96.6	93.6	93.8	93.8	92.4	92.6	92.9	92.4	92.1	98.1	98.1	92.4
MA9						100.0	97.6	96.9	97.6	91.0	90.5	91.0	91.0	97.8	96.9	97.1	96.9	98.3	98.6	96.9	96.7	92.1	92.1	96.9
WH10							100.0	96.7	99.0	91.9	91.0	91.9	91.9	97.8	96.4	96.7	96.4	97.1	97.4	97.4	97.1	92.1	92.1	97.4
WH13								100.0	96.2	92.9	91.9	92.9	92.9	98.1	97.6	97.8	96.4	98.1	98.3	97.4	97.1	93.6	93.6	97.4
WH14									100.0	91.4	90.5	91.4	91.4	97.8	95.9	96.2	95.9	97.1	97.4	97.4	97.1	91.6	91.6	97.4
BoE1										100.0	97.8	100.0	100.0	92.4	92.4	92.4	91.4	91.9	92.1	92.4	92.1	96.9	96.9	92.4

ITS1	*BoE2*	*BoE3*	*BoE6*	*IN6*	*IN11*	*IN14*	*MO2-5*	*MO2-6*	*MO2-14*	*MW1*	*MW4*	*MW9*	*MW11*	*MW14*
BoE2	**100.0**	97.8	97.8	92.1	92.1	92.1	90.5	91.0	91.2	91.2	91.0	96.6	96.6	91.2
BoE3		**100.0**	100.0	92.4	92.4	92.4	91.4	91.9	92.1	92.4	92.1	96.9	96.9	92.4
BoE6			**100.0**	92.4	92.4	92.4	91.4	91.9	92.1	92.4	92.1	96.9	96.9	92.4
IN6				**100.0**	97.6	97.8	95.7	98.6	98.8	98.6	98.3	93.6	93.6	98.6
IN11					**100.0**	99.8	97.1	96.7	96.9	97.6	97.4	94.0	94.0	97.6
IN14						**100.0**	96.2	96.9	97.1	97.8	97.6	93.8	93.8	97.8
MO2-5							**100.0**	96.4	96.2	96.2	95.9	91.9	91.9	96.2
MO2-6								**100.0**	99.8	97.8	97.6	92.6	92.6	97.8
MO2-14									**100.0**	98.1	97.8	92.8	92.8	98.1
MW1										**100.0**	99.8	92.8	92.8	100.0
MW4											**100.0**	92.6	92.6	99.8
MW9												**100.0**	100.0	92.8
MW11													**100.0**	92.8
MW14														**100.0**

ITS2	Carib3	Carib8	Carib10	Carib12	PA3	PA8	PA9	MO4	MO5	MO8	BH10	BH11	BH14	BH2-2	BH2-5	BH2-8	WR24	WR25	WR27	WP8	WP9	WP10	BB3	BB5
Carib3	100.0	100.0	100.0	99.2	94.8	95.6	95.2	95.6	96.4	96.4	94.0	93.2	93.6	93.2	94.0	93.6	97.2	94.8	94.4	96.8	96.0	96.4	96.0	96.0
Carib8		100.0	100.0	99.2	94.8	95.6	95.2	95.6	96.4	96.4	94.0	93.2	93.6	93.2	94.0	93.6	97.2	94.8	94.4	96.8	96.0	96.4	96.0	96.0
Carib10			100.0	99.2	94.8	95.6	95.2	95.6	96.4	96.4	94.0	93.2	93.6	93.2	94.0	93.6	97.2	94.8	94.4	96.8	96.0	96.4	96.0	96.0
Carib12				100.0	94.0	94.8	94.4	94.8	95.6	95.6	93.2	92.4	92.8	92.4	93.2	92.8	96.4	94.0	93.6	96.0	95.2	95.6	95.2	95.2
PA3					100.0	97.6	97.2	96.8	97.6	97.6	96.8	96.0	96.4	96.0	96.8	96.4	95.2	96.0	95.6	96.4	95.6	96.0	94.8	94.8
PA8						100.0	99.6	99.2	98.4	98.4	96.8	96.8	96.4	96.0	96.8	96.4	96.8	98.4	98.0	97.6	98.0	98.4	93.2	93.2
PA9							100.0	98.8	98.0	98.0	96.4	96.4	96.0	95.6	96.4	96.0	96.4	98.0	97.6	97.2	97.6	98.0	92.8	92.8
MO4								100.0	99.2	99.2	96.8	96.8	96.4	96.0	96.8	96.4	97.6	99.2	98.8	98.4	98.8	99.2	93.2	93.2
MO5									100.0	100.0	97.6	96.8	97.2	96.8	97.6	97.2	96.8	98.4	98.0	98.8	98.0	98.4	94.0	94.0
MO8										100.0	97.6	96.8	97.2	96.8	97.6	97.2	96.8	98.4	98.0	98.8	98.0	98.4	94.0	94.0
BH10											100.0	99.2	99.6	99.2	100.0	99.6	94.4	97.6	97.2	96.4	95.6	96.0	94.8	94.8
BH11												100.0	98.8	99.2	99.2	99.6	94.4	97.6	97.2	95.6	95.6	96.0	94.0	94.0
BH14													100.0	98.8	99.6	99.2	94.0	97.2	96.8	96.0	95.2	95.6	94.4	94.4
BH2-2														100.0	99.2	99.6	93.6	96.8	96.4	95.6	94.8	95.2	94.0	94.0
BH2-5															100.0	99.6	94.4	97.6	97.2	96.4	95.6	96.0	94.8	94.8
BH2-8																100.0	94.0	97.2	96.8	96.0	95.2	95.6	94.4	94.4
WR24																	100.0	96.8	96.4	96.0	97.2	97.6	95.6	95.6
WR25																		100.0	99.6	97.6	98.0	98.4	94.0	94.0
WR27																			100.0	97.2	97.6	98.0	93.6	93.6
WP8																				100.0	97.2	97.6	94.4	94.4
WP9																					100.0	99.6	92.8	92.8
WP10																						100.0	93.2	93.2
BB3																							100.0	99.2
BB5																								100.0

ITS2	BB6	BB2-1	BB2-14	BB2-21	TXE7	TXE14	TXE18	TXE24	TXE2-1	TXE2-2	TXE2-8	NH74	NH79	NH80	OK3	OK8	OK11	Bz4	Bz7	Bz12	Dc1	Dc3	Dc5	Dc8
Carib3	96.4	94.8	94.8	96.0	98.8	98.8	98.8	99.2	99.2	99.2	98.8	96.0	96.4	96.0	97.2	95.2	94.8	96.8	96.4	96.4	96.0	96.0	96.4	96.0
Carib8	96.4	94.8	94.8	96.0	98.8	98.8	98.8	99.2	99.2	99.2	98.8	96.0	96.4	96.0	97.2	95.2	94.8	96.8	96.4	96.4	96.0	96.0	96.4	96.0
Carib10	96.4	94.8	94.8	96.0	98.8	98.8	98.8	99.2	99.2	99.2	98.8	96.0	96.4	96.0	97.2	95.2	94.8	96.8	96.4	96.4	96.0	96.0	96.4	96.0
Carib12	95.6	94.0	94.0	95.2	98.0	98.0	98.0	98.4	98.4	98.4	98.0	95.2	95.6	95.2	96.4	94.4	94.0	96.0	95.6	95.6	95.2	95.2	95.6	95.2
PA3	95.2	96.8	96.8	94.8	94.4	93.6	94.4	94.0	94.0	94.0	94.4	96.4	96.8	94.8	95.2	95.6	99.2	94.0	97.6	93.6	95.6	95.6	96.0	95.6
PA8	93.6	98.4	98.4	93.2	95.2	94.4	95.2	94.8	94.8	94.8	95.2	98.0	98.4	95.6	96.0	96.4	97.6	96.4	98.4	96.0	97.2	97.2	97.6	97.2
PA9	93.2	98.0	98.0	92.8	94.8	94.0	94.8	94.4	94.4	94.4	94.8	97.6	98.0	95.2	95.6	96.0	97.2	96.0	98.0	95.6	96.8	96.8	97.2	96.8
MO4	93.6	99.2	99.2	93.2	96.0	95.2	96.0	95.6	95.6	95.6	96.0	98.8	99.2	96.4	96.8	97.2	97.6	96.4	99.2	96.0	98.0	98.0	98.4	98.0
MO5	94.4	98.4	98.4	94.0	96.8	96.0	96.8	96.4	96.4	96.4	96.8	98.8	99.2	99.2	97.6	98.0	98.4	95.6	100.0	95.2	98.0	98.0	98.4	98.0
MO8	94.4	98.4	98.4	94.0	96.8	96.0	96.8	96.4	96.4	96.4	96.8	98.8	99.2	99.2	97.6	98.0	98.4	95.6	100.0	95.2	98.0	98.0	98.4	98.0
BH10	95.2	96.0	96.0	94.8	94.4	93.6	94.4	94.0	94.0	94.0	94.4	96.4	96.8	94.8	95.2	97.2	97.6	94.8	97.6	94.4	95.6	95.6	96.0	95.6
BH11	94.4	96.0	96.0	94.0	93.6	92.8	93.6	93.2	93.2	93.2	93.6	95.6	96.0	94.0	94.4	96.4	96.8	94.8	96.8	94.4	95.6	95.6	96.0	95.6
BH14	94.8	95.6	95.6	94.4	94.0	93.2	94.0	93.6	93.6	93.6	94.0	96.0	96.4	94.4	94.8	96.8	97.2	94.4	97.2	94.0	95.2	95.2	95.6	95.2
BH2-2	94.4	95.2	95.2	94.0	93.6	92.8	93.6	93.2	93.2	93.2	93.6	95.6	96.0	94.0	94.4	96.4	96.8	94.0	96.8	93.6	94.8	94.8	95.2	94.8
BH2-5	95.2	96.0	96.0	94.8	94.4	93.6	94.4	94.0	94.0	94.0	94.4	96.4	96.8	94.8	95.2	97.2	97.6	94.8	97.6	94.4	95.6	95.6	96.0	95.6
BH2-8	94.8	95.6	95.6	94.4	94.0	93.2	94.0	93.6	93.6	93.6	94.0	96.0	96.4	94.4	94.8	96.8	97.2	94.4	97.2	94.0	95.2	95.2	95.6	95.2
WR24	96.0	97.6	97.6	95.6	96.8	96.8	96.8	97.2	97.2	97.2	96.8	97.2	97.6	94.0	95.2	94.8	96.0	98.0	96.8	97.6	96.4	96.4	96.8	96.4
WR25	94.4	98.4	98.4	94.0	95.2	94.4	95.2	94.8	94.8	94.8	95.2	98.0	98.4	95.6	96.0	98.0	96.8	97.2	98.4	96.8	97.2	97.2	97.6	97.2
WR27	94.0	98.0	98.0	93.6	94.8	94.0	94.8	94.4	94.4	94.4	94.8	97.6	98.0	95.2	95.6	97.6	96.4	96.8	98.0	96.4	96.8	96.8	97.2	96.8
WP8	94.8	97.6	97.6	94.4	97.2	96.4	97.2	96.8	96.8	96.8	97.2	97.6	98.0	97.6	98.0	96.8	97.2	94.8	98.8	94.4	97.2	97.2	97.6	97.2
WP9	93.2	98.0	98.0	92.8	96.4	95.6	96.4	96.0	96.0	96.0	96.4	98.4	98.8	96.0	97.2	96.8	96.4	96.0	98.0	96.4	97.6	97.6	98.0	97.6
WP10	93.6	98.4	98.4	93.2	96.8	96.0	96.8	96.4	96.4	96.4	96.8	98.8	99.2	96.4	97.6	97.2	96.8	96.4	98.4	96.0	98.0	98.0	98.4	98.0
BB3	99.6	93.2	93.2	99.2	95.6	95.6	95.6	96.0	96.0	96.0	95.6	93.6	94.0	92.9	94.0	94.4	95.6	96.0	94.0	95.6	92.8	92.8	93.2	92.8
BB5	99.6	93.2	93.2	99.2	95.6	95.6	95.6	96.0	96.0	96.0	95.6	93.6	94.0	92.9	94.0	94.4	95.6	96.0	94.0	95.6	92.8	92.8	93.2	92.8
BB6	100.0	93.6	93.6	99.6	96.0	96.0	96.0	96.4	96.4	96.4	96.0	94.0	94.4	93.3	94.4	94.8	96.0	96.4	94.4	96.0	93.2	93.2	93.6	93.2
BB2-1		100.0	100.0	93.2	95.2	94.4	95.2	94.8	94.8	94.8	95.2	98.0	98.4	95.6	96.0	96.4	97.6	95.6	98.4	95.2	97.2	97.2	97.6	97.2
BB2-14			100.0	93.2	95.2	94.4	95.2	94.8	94.8	94.8	95.2	98.0	98.4	95.6	96.0	96.4	97.6	95.6	98.4	95.2	97.2	97.2	97.6	97.2
BB2-21				100.0	95.6	95.6	95.6	100.0	96.0	100.0	95.6	93.6	94.0	92.9	94.0	94.4	95.6	96.0	94.0	95.6	92.8	92.8	93.2	92.8
TXE7					100.0	99.2	100.0	99.6	99.6	99.6	100.0	96.4	96.8	96.4	97.6	95.6	95.2	95.6	96.8	95.2	95.6	95.6	96.0	95.6
TXE14						100.0	99.2	99.6	99.6	99.6	99.2	95.6	96.0	95.6	96.8	94.8	94.4	95.6	96.0	95.2	95.6	95.6	96.0	95.6
TXE18							100.0	99.6	99.6	99.6	100.0	96.4	96.8	96.4	97.6	95.6	95.2	95.6	96.8	95.2	95.6	95.6	96.0	95.6
TXE24								100.0	100.0	100.0	99.6	96.0	96.4	96.0	97.2	95.2	94.8	96.0	96.4	95.6	96.0	96.0	96.4	96.0
TXE2-1									100.0	100.0	99.6	96.0	96.4	96.0	97.2	95.2	94.8	96.0	96.4	95.6	96.0	96.0	96.4	96.0
TXE2-2										100.0	99.6	96.0	96.4	96.0	97.2	95.2	94.8	96.0	96.4	95.6	96.0	96.0	96.4	96.0
TXE2-8											100.0	96.4	96.8	96.4	97.6	95.6	95.2	95.6	96.8	95.2	95.6	95.6	96.0	95.6
NH74												100.0	99.6	96.0	97.2	96.8	97.2	96.0	98.8	95.6	96.8	96.8	97.2	96.8
NH79													100.0	96.4	97.6	97.2	97.6	96.4	99.2	96.0	97.2	97.2	97.6	97.2
NH80														100.0	97.2	96.0	95.6	92.9	99.2	92.5	96.0	96.0	96.4	96.0
OK3															100.0	96.4	96.0	94.0	97.6	93.6	96.4	96.4	96.8	96.4
OK8																100.0	96.4	96.0	98.0	95.6	96.8	96.8	97.2	96.8
OK11																	100.0	94.0	98.4	93.6	96.4	96.4	96.8	96.4
Bz4																		100.0	95.6	99.6	95.2	95.2	95.6	95.2
Bz7																			100.0	95.2	98.0	98.0	98.4	98.0
Bz12																				100.0	94.8	94.8	95.2	94.8
Dc1																					100.0	99.6	99.6	99.2
Dc3																						100.0	99.6	99.6
Dc5																							100.0	99.6
Dc8																								100.0

ITS2	R61-2	R61-5	R61-8	MA3	MA5	MA9	WH10	WH13	WH14	BoE1	BoE2	BoE3	BoE6	IN6	IN11	IN14	MO2-5	MO2-6	MO2-14	MW1	MW4	MW9	MW11	MW14
Carib3	85.3	85.3	84.9	96.0	96.8	96.0	95.2	98.0	95.2	95.6	95.2	95.6	95.6	92.8	95.6	95.6	95.6	95.6	95.6	97.6	97.6	95.6	95.6	97.6
Carib8	85.3	85.3	84.9	96.0	96.8	96.0	95.2	98.0	95.2	95.6	95.2	95.6	95.6	92.8	95.6	95.6	95.6	95.6	95.6	97.6	97.6	95.6	95.6	97.6
Carib10	85.3	85.3	84.9	96.0	96.8	96.0	95.2	98.0	95.2	95.6	95.2	95.6	95.6	92.8	95.6	95.6	95.6	95.6	95.6	97.6	97.6	95.6	95.6	97.6
Carib12	84.5	84.5	84.1	95.2	96.0	95.2	94.4	97.2	94.4	94.8	94.4	94.8	94.8	92.0	94.8	94.8	94.8	94.8	94.8	96.8	96.8	94.8	94.8	96.8
PA3	85.0	85.0	85.5	96.4	95.6	97.2	95.6	95.2	95.6	95.2	94.8	95.2	95.2	97.2	96.8	96.8	96.8	96.8	96.8	94.8	94.8	96.8	96.8	94.8
PA8	85.8	85.8	86.3	96.4	98.0	98.8	98.0	96.8	98.0	97.6	97.2	97.6	97.6	97.2	97.6	97.6	97.6	99.2	99.2	97.2	97.2	97.6	97.6	97.2
PA9	85.4	85.4	85.9	96.0	97.6	98.4	97.6	96.4	97.6	97.2	96.8	97.2	97.2	96.8	97.2	97.2	97.2	98.8	98.8	96.8	96.8	97.2	97.2	96.8
MO4	85.0	85.0	85.5	95.6	98.8	99.6	98.8	96.8	98.8	97.6	97.2	97.6	97.6	97.2	97.6	97.6	98.4	100.0	100.0	98.0	98.0	98.4	98.4	98.0
MO5	85.0	85.0	85.5	94.8	98.0	99.6	98.0	96.8	98.0	96.8	96.4	96.8	96.8	96.4	98.4	98.4	99.2	99.2	99.2	97.2	97.2	99.2	99.2	97.2
MO8	85.0	85.0	85.5	94.8	98.0	99.6	98.0	96.8	98.0	96.8	96.4	96.8	96.8	96.4	98.4	98.4	99.2	99.2	99.2	97.2	97.2	99.2	99.2	97.2
BH10	84.6	84.6	85.1	94.0	95.6	97.2	97.2	95.2	97.2	95.2	94.8	95.2	95.2	95.6	97.6	97.6	96.8	96.8	96.8	94.8	94.8	97.6	98.4	94.8
BH11	84.6	84.6	85.1	94.0	95.6	96.4	97.2	95.2	97.2	95.2	94.8	95.2	95.2	95.6	96.8	96.8	96.0	96.8	96.8	94.8	94.8	96.8	97.6	94.8
BH14	84.6	84.6	85.1	93.6	95.2	96.8	96.8	94.8	96.8	94.8	94.4	94.8	94.8	95.2	97.2	97.2	96.4	96.4	96.4	94.4	94.4	97.2	98.0	94.4
BH2-2	83.9	83.9	84.3	93.2	94.8	96.4	96.4	94.4	96.4	94.4	94.0	94.4	94.4	94.8	96.8	96.8	96.0	96.0	96.0	94.0	94.0	96.8	97.6	94.0
BH2-5	84.6	84.6	85.1	94.0	95.6	97.2	97.2	95.2	97.2	95.2	94.8	95.2	95.2	95.6	97.6	97.6	96.8	96.8	96.8	94.8	94.8	97.6	98.4	94.8
BH2-8	84.3	84.3	84.7	93.6	95.2	96.8	96.8	94.8	96.8	94.8	94.4	94.8	94.8	95.2	97.2	97.2	96.4	96.4	96.4	94.4	94.4	97.2	98.0	94.4
WR24	85.7	85.7	86.1	98.0	98.0	97.2	97.2	98.4	97.2	96.8	96.4	96.8	96.8	95.6	95.2	95.2	96.0	97.6	97.6	99.6	99.6	96.0	96.0	99.6
WR25	85.0	85.0	85.5	94.8	98.0	98.8	99.6	96.0	99.6	96.8	96.4	96.8	96.8	96.4	98.4	98.4	97.6	99.2	99.2	97.2	97.2	98.4	99.2	97.2
WR27	84.6	84.6	85.1	94.4	97.6	98.4	99.2	95.6	99.2	96.4	96.0	96.4	96.4	96.0	98.0	98.0	97.2	98.8	98.8	96.8	96.8	98.0	98.8	96.8
WP8	83.9	83.9	84.3	94.0	97.2	98.4	97.2	95.6	97.2	96.0	95.6	96.0	96.0	95.6	97.2	97.2	98.0	98.4	98.4	96.4	96.4	98.0	98.0	96.4
WP9	85.0	85.0	84.6	95.2	98.4	98.4	98.4	97.2	98.4	97.2	96.8	97.2	97.2	96.0	96.4	96.4	97.2	98.8	98.8	97.6	97.6	97.2	97.2	97.6
WP10	85.4	85.4	85.0	95.6	98.8	98.8	98.8	97.6	98.8	97.6	97.2	97.6	97.6	96.4	96.8	96.8	97.6	99.2	99.2	98.0	98.0	97.6	97.6	98.0
BB3	83.7	83.7	84.9	96.0	93.6	93.6	94.4	95.6	94.4	93.2	92.8	93.2	93.2	94.4	94.8	94.8	93.2	93.2	93.2	95.2	95.2	94.0	94.8	95.2
BB5	83.7	83.7	84.9	96.0	93.6	93.6	94.4	95.6	94.4	93.2	92.8	93.2	93.2	94.4	94.8	94.8	93.2	93.2	93.2	95.2	95.2	94.0	94.8	95.2
BB6	84.1	84.1	85.3	96.4	94.0	94.0	94.8	96.0	94.8	93.6	93.2	93.6	93.6	94.8	95.2	95.2	93.6	93.6	93.6	95.6	95.6	94.4	95.2	95.6
BB2-1	85.0	85.0	85.5	95.6	98.0	98.8	98.0	96.0	98.0	96.8	96.4	96.8	96.8	97.2	96.8	96.8	97.6	99.2	99.2	97.2	97.2	97.6	97.6	97.2
BB2-14	85.0	85.0	85.5	95.6	98.0	98.8	98.0	96.0	98.0	96.8	96.4	96.8	96.8	97.2	96.8	96.8	97.6	99.2	99.2	97.2	97.2	97.6	97.6	97.2
BB2-21	84.5	84.5	85.7	96.0	93.6	93.6	94.4	95.6	94.4	93.2	92.8	93.2	93.2	94.4	94.8	94.8	93.2	93.2	93.2	95.2	95.2	94.0	94.8	95.2
TXE7	84.5	84.5	84.1	94.8	97.2	96.4	95.6	97.6	95.6	96.8	96.4	96.8	96.8	93.2	95.2	95.2	96.0	96.0	96.0	97.2	97.2	96.0	96.0	97.2
TXE14	84.1	84.1	83.7	94.8	96.4	95.6	94.8	97.6	94.8	96.0	95.6	96.0	96.0	92.4	94.4	94.4	95.2	95.2	95.2	97.2	97.2	95.2	95.2	97.2
TXE18	84.5	84.5	84.1	94.8	97.2	96.4	95.6	97.6	95.6	96.8	96.4	96.8	96.8	93.2	95.2	95.2	96.0	96.0	96.0	97.2	97.2	96.0	96.0	97.2
TXE24	84.5	84.5	84.1	95.2	96.8	96.0	95.2	98.0	95.2	96.4	96.0	96.4	96.4	92.8	94.8	94.8	95.6	95.6	95.6	97.6	97.6	95.6	95.6	97.6
TXE2-1	84.5	84.5	84.1	95.2	96.8	96.0	95.2	98.0	95.2	96.4	96.0	96.4	96.4	92.8	94.8	94.8	95.6	95.6	95.6	97.6	97.6	95.6	95.6	97.6
TXE2-2	84.5	84.5	84.1	95.2	96.8	96.0	95.2	98.0	95.2	96.4	96.0	96.4	96.4	92.8	94.8	94.8	95.6	95.6	95.6	97.6	97.6	95.6	95.6	97.6
TXE2-8	84.5	84.5	84.1	94.8	97.2	96.4	95.6	97.6	95.6	96.8	96.4	96.8	96.8	93.2	95.2	95.2	96.0	96.0	96.0	97.2	97.2	96.0	96.0	97.2
NH74	84.2	84.2	84.6	95.2	97.6	99.2	98.4	96.4	98.4	96.4	96.0	96.4	96.4	96.0	97.2	97.2	98.0	98.8	98.8	97.6	97.6	98.0	98.0	97.6
NH79	84.6	84.6	85.0	95.6	98.0	99.6	98.8	96.8	98.8	96.8	96.4	96.8	96.8	96.4	97.6	97.6	98.4	99.2	99.2	98.0	98.0	98.4	98.4	98.0
NH80	83.5	83.5	83.1	92.1	96.0	96.8	95.2	94.8	95.2	94.8	94.4	94.8	94.8	93.7	95.6	95.6	96.4	96.4	96.4	94.4	94.4	96.4	96.4	94.4
OK3	83.8	83.8	83.5	94.0	96.4	97.2	96.4	96.0	96.4	95.2	94.8	95.2	95.2	94.0	96.0	96.0	96.8	96.8	96.8	95.6	95.6	96.8	96.8	95.6
OK8	84.6	84.6	85.1	92.8	96.8	96.8	97.6	95.6	97.6	95.6	95.2	95.6	95.6	95.2	98.8	98.8	97.2	97.2	97.2	95.2	95.2	98.0	98.8	95.2
OK11	85.0	85.0	85.5	96.4	96.4	96.4	96.4	96.0	96.4	96.0	95.6	96.0	96.0	98.0	96.8	96.8	97.6	97.6	97.6	95.6	95.6	97.6	97.6	95.6
Bz4	85.3	85.3	86.5	96.8	96.8	96.8	97.6	97.2	97.6	95.6	95.2	95.6	95.6	94.4	97.2	97.2	94.8	96.4	96.4	98.4	98.4	95.6	96.4	98.4
Bz7	85.0	85.0	85.5	94.8	98.0	99.6	98.0	96.8	98.0	96.8	96.4	96.8	96.8	96.4	98.4	98.4	99.2	99.2	99.2	97.2	97.2	99.2	99.2	97.2
Bz12	84.9	84.9	86.1	96.4	96.4	96.4	97.2	96.8	97.2	95.2	94.8	95.2	95.2	94.0	96.8	96.8	94.4	96.0	96.0	98.0	98.0	95.2	96.0	98.0
Dc1	85.9	85.9	85.6	94.4	97.6	97.6	96.8	97.2	96.8	96.4	96.0	96.4	96.4	95.2	96.4	96.4	97.2	98.0	98.0	96.8	96.8	97.2	97.2	96.8
Dc3	86.3	86.3	85.9	94.8	98.0	98.0	97.2	97.6	97.2	96.8	96.4	96.8	96.8	95.6	96.8	96.8	97.6	98.0	98.0	96.8	96.8	97.2	97.2	96.8
Dc5	86.1	86.1	85.8	94.8	98.0	98.0	97.2	97.6	97.2	96.8	96.4	96.8	96.8	95.6	96.8	96.8	97.6	98.4	98.4	97.2	97.2	97.6	97.6	97.2
Dc8	85.9	85.9	85.6	94.4	97.6	97.6	96.8	97.2	96.8	96.4	96.0	96.4	96.4	95.2	96.4	96.4	97.6	98.0	98.0	96.8	96.8	97.2	97.2	96.8
R61-2	**100.0**	100.0	94.4	85.9	87.1	85.5	85.5	87.1	85.5	87.6	87.1	87.6	87.6	84.7	85.9	85.9	85.1	85.0	85.0	85.3	85.3	84.3	85.0	85.3
R61-5		**100.0**	94.4	85.9	87.1	85.5	85.5	87.1	85.5	87.6	87.1	87.6	87.6	84.7	85.9	85.9	85.1	85.0	85.0	85.3	85.3	84.3	85.0	85.3
R61-8			**100.0**	85.5	86.8	86.0	86.0	85.9	86.0	87.2	86.8	87.2	87.2	86.0	87.2	87.2	85.6	85.5	85.5	85.7	85.7	84.7	85.5	85.7
MA3				**100.0**	96.0	95.2	95.2	97.2	95.2	95.6	95.2	95.6	95.6	96.0	94.0	94.0	94.0	95.6	95.6	97.6	97.6	94.0	94.0	97.6
MA5					**100.0**	98.4	97.6	98.0	97.6	98.8	98.4	98.8	98.8	96.0	96.4	96.4	97.2	98.8	98.8	98.4	98.4	97.2	97.2	98.4
MA9						**100.0**	98.4	96.4	98.4	97.2	96.8	97.2	97.2	96.8	98.0	98.0	98.8	99.6	99.6	97.6	97.6	98.8	98.8	97.6
WH10							**100.0**	96.4	100.0	96.4	96.0	96.4	96.4	96.0	98.0	98.0	97.2	98.8	98.8	97.6	97.6	98.0	98.8	97.6
WH13								**100.0**	96.4	97.6	97.2	97.6	97.6	94.8	95.2	95.2	96.0	96.8	96.8	98.8	98.8	96.0	96.0	98.8
WH14									**100.0**	96.4	96.0	96.4	96.4	96.0	98.0	98.0	97.2	98.8	98.8	97.6	97.6	98.0	98.8	97.6
BoE1										**100.0**	99.6	100.0	100.0	95.6	95.2	95.2	96.0	97.6	97.6	97.2	97.2	96.0	96.0	97.2

ITS2	*BoE2*	*BoE3*	*BoE6*	*IN6*	*IN11*	*IN14*	*MO2-5*	*MO2-6*	*MO2-14*	*MW1*	*MW4*	*MW9*	*MW11*	*MW14*
BoE2	**100.0**	99.6	99.6	95.2	94.8	94.8	95.6	97.2	97.2	96.8	96.8	95.6	95.6	96.8
BoE3		**100.0**	100.0	95.6	95.2	95.2	96.0	97.6	97.6	97.2	97.2	96.0	96.0	97.2
BoE6			**100.0**	95.6	95.2	95.2	96.0	97.6	97.6	97.2	97.2	96.0	96.0	97.2
IN6				**100.0**	95.6	95.6	95.6	97.2	97.2	95.2	95.2	95.6	95.6	95.2
IN11					**100.0**	100.0	97.6	97.6	97.6	95.6	95.6	98.4	99.2	95.6
IN14						**100.0**	97.6	97.6	97.6	95.6	95.6	98.4	99.2	95.6
MO2-5							**100.0**	98.4	98.4	96.4	96.4	98.4	98.4	96.4
MO2-6								**100.0**	100.0	98.0	98.0	98.4	98.4	98.0
MO2-14									**100.0**	98.0	98.0	98.4	98.4	98.0
MW1										**100.0**	100.0	96.4	96.4	100.0
MW4											**100.0**	96.4	96.4	100.0
MW9												**100.0**	99.2	96.4
MW11													**100.0**	96.4
MW14														**100.0**

APPENDIX B

				1	2	3	4
		1 15	16 30	31 45	46 60	61 75	76 90
1	car2_	ACATTGAATCT-TTG	CACTTTGGTGCTTGG	CGTTGT**CTGTTG**CGT	CGTGC**AG--GT**CCGC	**CT**GCAGCTGCG-CCT	TTGGGC**GTG**GTCC--
2	car5_	ACATTGAATCT-TTG	CACTTTGGTGCTTGG	CGTTGT**CTGTTG**CGT	CGTGC**AG--GT**CCGC	**CT**GCAGCTGCG-CCT	TTGGGC**GTG**GTCC--
3	car8_	ACATTGAATCT-TTG	CACTTTGGTGCTTGG	CGTTGT**CTGTTG**CGT	CGTGC**AG--GT**CCGC	**CT**GCAGCTGCG-CCT	TTGGGC**GTG**GTCC--
4	mnc3_	ACATTGAATCTGTTG	CACTTTTGTGCTCGA	CGTTGT**TTGTTA**CGT	CGTGC**AGCTGT**CTCG	**CT**GCAGCTGCGTCCG	TCGGGC**GCG**GTCCAC
5	mnc8_	ACATTGAATCTGTTG	CACTTTTGTGCTTGA	CGTTGT**TTGTTA**CGT	CGTGC**AGCTGT**CTCG	**CT**GCAGCTGCGTCCG	TCGGGC**GCG**GTCCAC
6	mnc12_	ACATTGAATCTGTTG	CACTTTTGTGCTTGA	CGTTGT**TTGTTA**CGT	CGTGC**AGCTGT**CTCG	**CT**GCAGCTGCGTCCG	TCGGGC**GCG**GTCCAC
7	par3_	ACATTGAATCTGTTG	CACTTTTGTGCTTGA	CGTTGT**CTGTTG**CGT	CGTGC**GGCTGT**CTCG	**CT**GCAGCTGCGTCCG	TCGGGC**GCG**GTCCAC
8	par8_	ACATTGAATCTGTTG	CACTTTTGTGCTTGA	CGTTGT**CTGTTG**CGT	CGTGC**GGCTGT**CTCG	**CT**GCAGCTGCGTCCG	TCGGGC**GCG**GTCCAC
9	par9_	ACATTGAATCTGTTG	CACTTTTGTGCTTGA	CGTTGT**CTGTTG**CGT	CGTGC**GGCTGT**CTCG	**CT**GCAGCTGCGTCCG	TCGGGC**GCG**GTCCAC
10	mnmo14_	ACATTGAATCTGTTG	CACTTTTGTGCTTGA	CGTTGT**CCGTTG**CGT	CGTGC**AGCTCT**CTCG	**CT**GCAGCTGCGTCCG	TCGGGC**GCG**GTCCAC
11	mnmo15_	ACATTGAATCTGTTG	CACTTTTGTGCTTGA	CGTTGT**CCGTTG**CGT	CGTGC**AGCTGT**CTCG	**CT**GCAGCTGCGTCCG	TCGGGC**GCG**GTCCAC
12	mnmo18_	ACATTGAATCTGTTG	CACTTTTGTGCTTGA	CGTTGT**CCGTTG**CGT	CGTGC**AGCTGT**CTCG	**CT**GCAGCTGCGTCCG	TCGGGC**GCG**GTCCAC
13	wir24_	ACATTGAATCTGTTG	CACTTTTGTGCTTGA	CGTTGT**CTGTTG**CGT	CGTGC**GGCTGT**CTCG	**CT**GCAGCTGCGTCCG	TCGGGC**GCG**GTCCAC
14	txwba3_	ACATTGAATCTGTTG	CACTTTTGTGCTTGA	CGTTGC**CTGTTA**CGT	CGTGC**AGCTCT**CTCG	**CT**GCAGCTGCGTCCG	TCGGGC**GCG**GTCCAC
15	txwba5_	ACATTGAATCTGTTG	CACTTTTGTGCTTGA	CGTTGC**CTGTTA**CGT	CGTGC**AGCTCT**CTCG	**CT**GCAGCTGCGTCCG	TCGGGC**GCG**GTCCAC
16	txwba6_	ACATTGAATCTGTTG	CACTTTTGTGCTTGA	CGTTGT**CTGTTG**CGT	CGTGC**AGCTGT**CTCG	**CT**GCAGCTGCGTCCG	TCGGGC**GCG**GTCCAC
17	txwbb21_	ACATTGAATCTGTTG	CACTTTTGTGCTTGA	CGTTGC**CTGTTA**CGT	CGTGC**AGCTCT**CTCG	**CT**GCAGCTGCGTCCG	TCGGGC**GCG**GTCCAC
18	mnc10_	ACATTGAATCTGTTG	CACTTTTGTGCTTGA	CGTTGT**CTGTTG**CGT	CGTGC**AGCTCT**CTCG	**CT**GCAGCTGCGTCCG	TCGGGC**GCG**GTCCAC
19	wir25_	ACATTGAATCTGTTG	CACTTTTGTGCTTGA	CGTTGT**CTGTTG**CGT	CGTGC**AGCCCT**CTCG	**CT**GCAGCTGCGTCCG	TCGGGC**GCG**GTCCAC
20	wir27_	ACATTGAATCTGTTG	CACTTTTGTGCTTGA	CGTTGT**CTGTTG**CGT	CGTGC**AGCTCT**CTCG	**CT**GCAGCTGCGTCCG	TCGGGC**GCG**GTCCAC
21	wie19_	ACATTGAATCTGTTG	CACTTTTGTGCTTGA	CGTTGT**CCGTTG**CGT	CGTGC**AGCTGT**CTCG	**CT**GCAGCTGCGTCCG	TCGGGC**GCG**GTCCAC
22	wie110_	ACATTGAATCTGTTG	CACTTTTGTGCTTGA	CGTTGT**CCGTTG**CGT	CGTGC**AGCTGT**CTCG	**CT**GCAGCTGCGTCCG	TCGGGC**GCG**GTCCAC
23	casa10_	ACATTGAATCTGTTG	CACTTTTGTGCTTGA	CGTTGT**CTGTTG**CGT	CGTGC**AGCTCT**CTCG	**CT**GCAGCTGCGTCCG	TCGGGC**GCG**GTCCAC
24	casa11_	ACATTGAATCTGTTG	CACTTTTGTGCTTGA	CGTTGT**CTGTTG**CGT	CGTGC**AGCTCT**CTCG	**CT**GCAGCTGCGTCCG	TCGGGC**CCG**GTCCAC
25	casb5_	ACATTGAATCTGTTG	CACTTTTGTGCTTGA	CGTTGT**CTGTTG**CGT	CGTGC**AGCTCT**CTCG	**CT**GCAGCTGCGTCCG	TCGGGC**CCG**GTCCAC
26	casb8_	ACATTGAATCTGTTG	CACTTTTGTGCTTGA	CGTTGT**CTGTTG**CGT	CGTGC**AGCTCT**CTCG	**CT**GCAGCTGCGTCCG	TCGGGC**CCG**GTCCAC
27	casb2_	ACATTGAATCTGTTG	CACTTTTGTGCTTGA	CGTTGT**CTGTTG**CGT	CGTGC**AGCTCT**CTCG	**CT**GCAGCTGCGTCCG	TCGGGC**GCG**GTCCAC
28	casa14_	ACATTGAATCTGTTG	CACTTTTGTGCTTGA	CGTTGT**CTGTTG**CGT	CGTGC**AGCTCT**CTCG	**CT**GCAGCTGCGTCCG	TCGGGC**GCG**GTCCAC
29	txea7_	ACATTGAATCTGTTG	CACTTTTGTGCTTGA	CGTTGT**CTGTTA**CGT	CGTGC**AGCTCT**CTCG	**CT**GCAGCTGCGTCCG	TCGGGC**GCG**GTCCAC
30	txea18_	ACATTGAATCTGTTG	CACTTTTGTGCTTGA	CGTTGT**CTGTTA**CGT	CGTGC**AGCTCT**CTCG	**CT**GCAGCTGCGTCCG	TCGGGC**GTG**GTCCAC
31	txea14_	ACATTGAATCTGTTG	CACTTTTGTGCTTGA	CGTTGT**CTGTTA**CGT	CGTGC**AGCTCT**CTCG	**CT**GCAGCTGCGTCCG	TCGGGC**GCG**GTCCAC
32	wie18_	ACATTGAATCTGTTG	CACTTTTGTGCTTGA	CGTTGT**CCGTTA**CGT	CGTGC**AGCTGT**CTCG	**CT**GCAGCTGCGTCCG	TCGGGC**GCG**GTCCAC
33	txwbb1_	ACATTGAATCTGTTG	CACTTTTGTGCTTGA	CGTTGT**CTGTTG**CGT	CGCGC**AGCTGT**CTCG	**CT**GCAGCTGCGTCCG	TCGGGC**GCG**GTCCAC
34	txwbb14_	ACATTGAATCTGTTG	CACTTTTGTGCTTGA	CGTTGT**CTGTTG**CGT	CGTGC**AGCTGT**CTCG	**CT**GCAGCTGCGTCCG	TCGGGC**GCG**GTCCAC
35	txea24_	ACATTGAATCTGTTG	CACTTTTGTGCTTGA	CGTTGT**CTGTTG**CGT	CGTGC**AGC-CT**CTCG	**CT**GCAGCTGCGTCCG	TCGGGC**GCG**GTCCAC
36	txeb8_	ACATTGAATCTGTTG	CACTTTTGTGCTTGA	CGTTGT**CTGTTA**CGT	CGTGC**AGCTCT**CTCG	**CT**GCAGCTGCGTCCG	TCGGGC**GCG**GTCCAC

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                                                   1                  2          3                            4
               1              15 16            30 31         45 46          60 61            75 76          90
37 okw11_    ACATTGAATCTGTTG CACTTTTGTGCTTGA CGTTGTCTGTTACGT CGTGCAGCTGTCTCG CTGCAGCTGCGTCCG TCGGGCGCGGTCCAC
38 okw8_     ACATTGAATCTGTTG CACTTTTGTGCTTGA CGTTGTCTGTTGCGT CGTGCAGCTCTCTCG CTGCAGCTGCGTCCG TCGGGCGCGGTCCAC
39 mnw9_     ACATTGAATCTGTTG CACTTTTGTGCTTGA CGTTGTCTGTTACGT CGTGCAGCTCTCTCG CTGCAGCTGCGTCCG TCGGGCGCGGTCCAC
40 mnw11_    ACATTGAATCTGTTG CACTTTTGTGCTTGA CGTTGTCTGTTACGT CGTGCAGCTCTCTCG CTGCAGCTGCGTCCG TCGGGCGCGGTCCAC

41 maw5_     ACATTGAATCTGTTG CACTTTTGTGCTTGA CGTTGTCCGTTACGT CGTGCAGCTCTCTCG CTGCAGCTGCGTCCG TCGGGCGCGGTCCAC
42 nhe80_    ACATTGAATCTGTTG CACTTTTGTGCTTGA CGTTGTCCGTTGCGT CGTGCAGCTGTCTCG CTGCAGCTGCGTCCG TCGGGCGCGGTCCAC
43 nhe74_    ACATTGAATCTGTTG CACTTTTGTGCTTGA CGTTGTCTGTTACGT CGTGCAGCTCTCTCG CTGCAGCTGCGTCCG TCGGGCGCGGTCCAC
44 nhe79_    ACATTGAATCTGTTG CACTTTTGTGCTTGA CGTTGTCTGTTACGT CGTGCAGCTCTCTCG CTGCAGCTGCGTCCG TCGGGCGCGGTCCAC

45 nyr21_    ACATTGAATCTGTTG CACTTTTGTGCTTGA CGTTGTCTGTTACGT CGTGCAGCTCTCTCG CTGCAGCTGCGTCCG TCGGGCGCGGTCCAC
46 nyr25_    ACATTGAATCTGTTG CACTTTTGTGCTTGA CGTTGTCTGTTACGT CGTGCAGCTCTCTCG CTGCAGCTGCGTCCG TCGGGCGCGGTCCAC
47 nyr28_    ACATTGAATCTGTTG CACTTTTGTGCTTGA CGTTGTCTGTTACGT CGTGCAGCTCTCTCG CTGCAGCTGCGTCCG TCGGGCGCGGTCCAC
48 nyr23_    ACATTGAATCTGTTG CACTTTTGTGCTTGA CGTTGTCTGTTACGT CGTGCAGCTCTCTCG CTGTAGCTGCGTCCG TCGGGCGCGGTCCAC

49 wie210_   ACATTGAATCTGTTG CACTTTTGTGCTTGA CGTTGTCCGTTACGT CGTGCAGCTGTCTCG CTGCAGCTGCGTCCG TCGGGCGCAGTCCAC
50 wie214_   ACATTGAATCTGTTG CACTTTTGTGCTTGT CGTTGTCCGTTACGT CGTGCAGCTGTCTCG CTGCAGCTGCGTCCG TCGGGCGCGGTCCAC
51 nyr17_    ACATTGAATCTGTTG CACTTTTGTGCTTGA CGTTGTCCGTTGCGT CGTGCAGCTGTCTCG CTGCAGCTGCGTCCG TCGGGCGCGGTCCAC
52 maw9_     ACATTGAATCTGTTG CACTTTTGTGCTTGA CGTTGTCCGTTGCGT CGTGCAGCTGTCTCG CTGCAGCTGCGTCCG TCGGGCGCGGTCCAC

53 wie213_   ACATTGAATCTGTTG CACTTTTGTGCTTGA CGTTGTCTGTTGCGT CGTGCAGCTCTCTCG CTGCAGCTGCGTCCG TCGGGCGCGGTCCAC
54 ine6_     ACATTGAATCTGTTG CACTTTTGTGCTTGA CGTTGTCTGTTACGT CGTGCAGCTCTCTCG CTGCAGCTGCGTCCG TCGGGCGCGGTCCAC
55 maw3_     GCATTGAATCTGTTG CACTTTTGTGCTTGA CGTTGTCTGTTGCGT CGTGCAGCTGTCTCG CCGCAGCTGCGTCCG TCGGGCGCGGTCCAC
56 ine11_    ACATTGAATCTGTTG CACTTTTGTGCTTGA CGTTGTCTGTTACGT CGTGCAGCTCTCTCG CTGCAGCTGCGTCCG TCGGGCGCGGTCCAC

57 ine14_    ACATTGAATCTGTTG CACTTTTGTGCTTGA CGTTGTCTGTTACGT CGTGCAGCTCTCTCG CTGCAGCTGCGTCCG TCGGGCGCGGTCCAC
58 mnmo25_   ACATTGAATCTGTTG CACTTTTGTGCTTGA CGTTGTCCGTTGCGT CGTGCAGCTGTCTCG TTGCAGCTGCGTCCG TCGGGCGCGGTCCAC
59 mnmo26_   ACATTGAATCTGTTG CACTTTTGTGCTTGA CGTTGTCCGTTGCGT CGTGCAGCTCTCTCG TTGCAGCTGCGTCCG TCGGGCGCGGTCCAC
60 mnmo214_  ACATTGAATCTGTTG CACTTTTGTGCTTGA CGTTGTCCGTTGCGT CGTGCAGCTCTCTCG CTGCAGCTGCGTCCG TCGGGCGCGGTCCAC

61 mnw1_     ACATTGAATCTGTTG CACTTTTGTGCTTGA CGTTGTCTGTTACGT CGTGCAGCTCTCTCG CTGCAGCTGCGTCCG TCGGGCGCGGTCCAC
62 mnw4_     ACATTGAATCTGTTG CACTTTTGTGCTTGA CGTTGTCTGTCACGT CGTGCAGCTCTCTCG CTGCAGCTGCGTCCG TCGGGCGCGGTCCAC
63 mnw14_    ACATTGAATCTGTTG CACTTTTGTGCTTGA CGTTGTCTGTTACGT CGTGCAGCTCTCTCG CTGCAGCTGCGTCCG TCGGGCGCGGTCCAC
64 nyr14_    ACATTGAATCTGTTG CACTTTTGTGCTTGA CGTTGTCTGTTGCGT CGTGCAGCTGTCTCG CTGCAGCTGCGTCCG TCGGGCGCGGTCCAC

65 nyr112_   ACATTGAATCTGTTG CACTTTTGTGCTTGA CGTTGTCTGTTGCGT CGTGCAGCTGTCTCG CTGCAGCTGCGTCCG TCGGGCGCGGTCCAC
66 txeb1_    ACATTGAATCTGTTG CACTTTTGTGCTTGA CGTTGTCTGTTGCGT CGTGCAGC-CTCTCG CTGCAGCTGCGTCCG TCGGGCGCGGTCCAC
67 txeb2_    ACATTGAATCTGTTG CACTTTTGTGCTTGA CGTTGTCTGTTGCGT CGTGCAGC-CTCTCG CTGCAGCTGCGTCCG TCGGGCGCGGTCCAC
68 okw3_     ACATTGAATCTGTTG CACTTTTGTGCTTGA CGTTGTCTGTTGCGT CGTGCAGCTCTCTCG CTGCAGCTGCGTCCG TCGGGCGCGGTCCAC

69 txwe1_    ACATTGAATCTGTTG CACTTTTGTGCTTGA CGTTGTCTGTTGCGT CGTGCAGCTCTCTCG CTGCAGCTGCGTCCG TCGGGCGCGGTCCAC
70 txwe3_    ACATTGAATCTGTTG CACTTTTGTGCTTGA CGTTGTCTGTTGCGT CGTGCAGCTCTCTCG CTGCAGCTGCGTCCG TCGGGCGCGGTCCAC
71 txwe6_    ACATTGAATCTGTTG CACTTTTGTGCTTGA CGTTGTCTGTTGCGT CGTGCAGCTCTCTCG CTGCAGCTGCGTCCG TCGGGCGCGGTCCAC
72 txwe2_    ACATTGAATCTGTTG CACTTTTGTGCTTGA CGTTGTCTGTTACGT CGTGCAGCTTTCTCG CTGCAGCTGTGTCCG TCGGGCGCGGTCCAC
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              5         6                                                                                7             8
          91 ___       105 106          120 121          135 136          150 151          165 166          180
1  car2_     GTTACCGGCTTCGCA ACTGGCCTCGTCTTG GCGACGTGGTTTCGG TCTTGTTCCGTTTCC TTGCCTGCGCTTGCG CGGGACGTTGCCCC-
2  car5_     GTTACCGGCTTCGCA ACTGGCCTCGTCTTG GCGACGTGGTTTCGG TCTTGTTCCGTTTCC TTGCCTGCGCTTGCG CGGGACGTTGCCCC-
3  car8_     GTTACCGGCTTCGCA ACTGGCCTCGTCTTG GCGACGTGGTTTCGG TCTTGTTCCGTTTCC TTGCCTGCGCTTGCG CGGGACGTTGCCCC-
4  mnc3_     GTTTGTGGCTTCGTA GCTGGCCTCGTCATG GCGACGTGGTTTCGG TCTTGTTCCGTTTCC ATCCCTGCGCTTGCG TGGGACGTTGCCCCC

5  mnc8_     GTTTGTGGCTTCGTA GCTGGCCCCGTCATG GCGACGTGGTTTCGG TCTTGTTCCGTTTCC ATCCCTGCGCTTGCG TGGGACGTTGCCCCC
6  mnc12_    GTTTGTGGCTTCGTA GCTGGCCTCGTCATG GCGACGTGGTTTCGG TCTTGTTCCGTTTCC ATCCCTGCGTTTGCG TGGGACGTTGCCCCC
7  par3_     GTTTGTGGCTTCGTA GCTGGCCTCGTCATG GCGACGTGGTTTCGG TCTTGTTCCGTTTCC ATCCCTGCGCTTGCG TGGGACGTTGCCCCC
8  par8_     GTTTGTGGCTTCGTA GCTGGCCTCGTCATG GCGACGTGGTTTCGG TCTTGTTCCGTTTCC ATCCCTGCGCTTGCG TGGGACGTTGCCCCC

9  par9_     GTTTGTGGCTTCGTA GCTGGCCTCGTCATG GCGACGTGGTTTCGG TCTTGTTCCGTTTCC ATCCCTGCGCTTGCG TGGGACGTTGCCCCC
10 mnmo14_   GTTTGTGGCTTCGTA GCTGGCCTCGTCATG GCGACGTGGTTTCGG TCTTGTTCCGTTTCC ATCCCTGCGCTTGCG TGGGACGTTGCCCCC
11 mnmo15_   GTTAGTGGCTTCGTA GCTGGCCTCGTCATG GCGACGTGGTTTCGG TCTTGTTCCGTTTCC ATCCCTGCGCTTGCG TGGGACGTTGCCCCC
12 mnmo18_   GTTAGTGGCTTCGTA GCTGGCCTCGTCATG GCGACGTGGTTTCGG TCTTGTTCCGTTTCC ATCCCTGCGCTTGCG TGGGACGTTGCCCCC

13 wir24_    GTTTGTGGCTTCGTA GCTGGCCTCGTCATG GCGACGTGGTTCCGG TCTTGTTCCGTTTCC ATCCCTGCGCTCGCG TGGGACGTTGCCCC-
14 txwba3_   GTTTGTGGTTTCGTA GCTGGCCTCGTCATG GCGACGTGGTTTCGG TCTTGTTCCGTTTCC ATCCCTGCGCTTGCG TGGGACGTTGCCCC-
15 txwba5_   GTTTGTGGTTTCGTA GCTGGCCTCGTCATG GCGACGTGGTTTCGG TCTTGTTCCGTTTCC ATCCCTGCGCTTGCG TGGGACGTTGCCCC-
16 txwba6_   GTTAGTGGCTTCGTA GCTGGCCTCGTCATG GCGACGTGGTTTCGG TCTTGTTCCGTTTCC ATCCCTGCGCTTGCG TGGGACGTTGCCCC-

17 txwbb21_  GTTTGTGGTTTCGTA GCTGGCCTCGTCATG GCGACGTGGTTTCGG TCTTGTTCCGTTTCC ATCCCTGCGCTTGCG CGGGACGTTGCCCC-
18 mnc10_    GTATGTGGCTTCGTA GCTGGCCTCGTCATG GCGACGTGGTTTCGG TCTTGTTCCGTTTCC ATCCCTGCGCTTGCG TGGGACGTTGCCCC-
19 wir25_    GTTTGTGGCTTCGTA GCTGGCCTCGTCATG GCGACGTGGTTTCGG TCTTGTTCCGTTTCC ATCCCTGCGCTTGCG TGGGACGTTGCCCC-
20 wir27_    GTTTGTGGCTTCGTA GCTGGCCTCGTCATG GCGACGTGGTTTCGG TCTTGTTCCGTTTCC ATCCCTGCGCTTGCG TGGGACGTTGCCCC-

21 wie19_    GTTTGTGGCTTCGTA GCTGGCCTCGTCATG GCGACGTGGTTTCGG TCTTGTTCCGTTTCC ATCCCTGCGCTTGCG TGGGACGTTGCCCC-
22 wie110_   GTTTGTGACTTCGTA GCTGGCCTCGTCATG GCGACGTGGTTTCGG TCTTGTTCCGTTTCC ATCCCTGCGCTTGCG TGGGACGTTGCCCC-
23 casa10_   GTTTGTGGCTTCGTA GCTGGTCTCGTCATG GCGACGTGGTTTCGG TCTTGTTCCGTTTCC ATCCCTGCGCTTGCG TGGGACGTTGCCCC-
24 casa11_   GTTTGTGGCTTCGTA GCTGGCCTCGTCATG GCGACGTGGTTTCGG TCTTGTTCCGTTTCC ATCCCTGCGCTTGCG TGGGACGTTGCCCC-

25 casb5_    GTTTGTGGCTTCGTA GCTGGTCTCGTCATG GCGACGTGGTTTCGG TCTTGTTCCGTTTCC ATCCCTGCGCTTGCG TGGGACGTTGCCCC-
26 casb8_    GTTTGTGGCTTCGTA GCTGGCCTCGTCATG GCGACGTGGTTTCGG TCTTGTTCCGTTTCC ATCCCTGCGCTTGCG TGGGACGTTGCCCC-
27 casb2_    GTTTGTGGCTTCGTA GCTGGCCTCGTCATG GCGACGTGGTTTCGG TCTTGTTCCGTTTCC ATCCCTGCGCTTGCG TGGGACGTTGCCCC-
28 casa14_   GTTTGTGGCTTCGTA GCTGGTCTCGTCATG GCGACGTGGTTTCGG TCTTGTTCCGTTTCC ATCCCTGCGCTTGCG TGGGACGTTGCCCC-

29 txea7_    GTTTGTGGCTTCGTA GCTGGCCTCGTCATG GCGACGTGGTTTCGG TCTTGTTCCGTTTCC ATCCCTGCGCTTGCG TGGGACGTTGCCCC-
30 txea18_   GTTTGTGGCTTCGTA GCTGGCCTCGTCATG GCGACGTGGTTTCGG TCTTGTTCCGTTTCC ATCCCTGCGCTTGCG TGGGACGTTGCCCC-
31 txea14_   GTTTGTAGCTTCGTA GCTGGCCTCGTCATG GCGACGTGGTTTCGG TCTTGTTCCGTTTCC ATCCCTGCGCTTGCG TGGGACGTTGCCCC-
32 wie18_    GTTAGTGGCTTCGTA GCTGGCCTCGTCATG GCGACGTGGTTTCGG TCTTGTTCCGTTTCC ATCCCTGCGCTTGCG TGGGACGTTGCCCCC

33 txwbb1_   GTTAGTGGCTTCGTA GCTGGCCTCGTCATG GCGACGTGGTTTCGG TCTTGTTCCGTTTCC ATCCCTGCGCTTGCG TGGGACGTTGCCCCC
34 txwbb14_  GTTAGTGGCTTCGTA GCTGGCCTCGTCATG GCGACGTGGTTTCGG TCTTGTTCCGTTTCC ATCCCTGCGCTTGCG TGGGACGTTGCCCCC
35 txea24_   GTTTGTGGCTTCGTA GCTGGCCTCGTCATG GCGACGTGGTTTCGG TCTCGTTCCGTTTCC ATCCCTGCGCTTGCG TGGGACGTTGCCCC-
36 txeb8_    GTTTGTGGCTTCGTA GCTGGCCTCGTCATG GCGACGTGGTTTCGG TCTTGTTCCGTTTCC ATCCCTGCGCTTGCG TGGGACGTTGCCCC-
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                5         6                                                                    7            8
             91 ___     105 106         120 121         135 136         150 151         165 166          180
37 okw11_   GTTAGTGGCTTCGTA GCTGGCCTCGTCATG GCGACGTGGTTTCGG TCTTGTTCCGTTTCC ATCCCTGCGCTTGCG TGGGACGTTGCCCC-
38 okw8_    GTTAGTGGCTTCGTA GCTGGCCTCGTCATG GCGACGTGGTTTCGG TCTTGTTCCGTTTCC ATCCCTGCGCTTGCG TGGGACGTTGCCCC-
39 mnw9_    GTTAGTGGCTTCGTA GCTGGCCTCGTCATG GCGACGTGGTTTCGG TCTTGTTCCGTTTCC ATCCCTGCGCTTGCG TGGGACGTTGCCCC-
40 mnw11_   GTTAGTGGCTTCGTA GCTGGCCTCGTCATG GCGACGTGGTTTCGG TCTTGTTCCGTTTCC ATCCCTGCGCTTGCG TGGGACGTTGCCCC-

41 maw5_    GTTAGTGGCTTCGTA GCTGGCCTCGTCATG GCGACGTGGTTTCGG TCTTGTTCCGTTTCC ATCCCTGCGCTTGCG TGGGACGTTGCCCCC
42 nhe80_   GTTTGTGGCTTCGTA GCTGGCCTCGTCATG GCGACGTGGTTTCGG TCTTGTTCCGTTTCC ATCCCTGCGCTTGCG TGGGACGTTGCCCCC
43 nhe74_   GTTAGTGGCTTCGCA ACTGGCCTCGTCATG GCGACGTGGTTTCGG TCTTGTTCCGTTTCC ATCCCTGCGCTTGCG TGGGACGTTGCCCC-
44 nhe79_   GTTAGTGGCTTCGCA ACTGGCCTCGTCATG GCGACGTGGTTTCGG TCTTGTTCCGTTTCC ATCCCTGCGCTTGCG TGGGACGTTGCCCC-

45 nyr21_   GTTTGTGGCTTCGTA GCTGGCCTCGTCATG GCGACGTGGTTTCGG TCTTGTTCCGTTTCC ATCCCTGCGCTTGCG TGGGACGTTGCCCC-
46 nyr25_   GTTTGTGGCTTCGTA GCTGGCCTCGTCATG GCGACGTGGTTTCGG TCTTGTTCCGTTTCC ATCCCTGCGCTTGCG TGGGACGTTGCCCC-
47 nyr28_   GTTTGTGGCTTCGTA GCTGGCCTCGTCATG GCGACGTGGTTTCGG TCTTGTTCCGTTTCC ATCCCTGCGCTTGCG TGGGACGTTGCCCC-
48 nyr23_   GTTTGTGGCTTCGTA GCTGGCCTCGTCATG GCGACGTGGTTTCGG TCTTGTTCCGTTTCC ATCCCTGCGCTTGCG TGGGACGTTGCCCC-

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50 wie214_  GTTTGTGGCTTCGTA GCTGGCCTCGTCATG GCGACGTGGTTTCGG TCTTGTTCCGTTTCC ATCCCTGCGCTTGCG TGGGACGTTGCCCCC
51 nyr17_   GTTAGTGGCTTCGTA GCTGGCCTCGTCATG GCGACGTGGTTTCGG TCTTGTTCCGTTTCC ATCCCTGCGCTTGCG TGGGACGTTGCCCCC
52 maw9_    GTTTGTGGCTTCGTA GCTGGCCTCGTCATG GCGACGTGGTTTCGG TCTTGTTCCGTTTCC ATCCCTGCGCTTGCG TGGGACGTTGCCCCC

53 wie213_  GTTTGTGGCTTCGTA GCTGGCCTCGTCATG GCGACGTGGTTTCGG TCTTGTTCCGTTTCC ATCCCTGCGCTTGCG TGGGACGTTGCCCCC
54 ine6_    GTTTGTGGCTTCGTA GCTGGCCTCGTCATG GCGACGTGGTTTCGG TCTTGTTCCGTTTCC ATCCCTGCGCTTGCG TGGGACGTTGCCCCC
55 maw3_    GTTTGTGGCTTCGTA GCTGGCCTCGTCATG GCGACGTGGTTTCGG TCTTGTTCCGTTTCC ATCCCTGCGCTTGCG TGGGACGTTGCCCCC
56 ine11_   GTTTGTGGCTTCGTA GCTGGCCTCGTCATG GCGACGTGGTTTCGG TCTTGTTCCGTTTCC ATCCCTGCGCTTGCG TGGGACGTTGCCCCC

57 ine14_   GTTTGTGGCTTCGTA GCTGGCCTCGTCATG GCGACGTGGTTTCGG TCTTGTTCCGTTTCC ATCCCTGCGCTTGCG TGGGACGTTGCCCCC
58 mnmo25_  GTTAGTGGCTTCGTA GCTGGCCTCGTCATG GCGACGTGGTTTCGG TCTTGTTCCGTTTCC ATCCCTGCGCTTGCG TGGGACGTTGCCCCC
59 mnmo26_  GTTTGTGGCTTCGTA GCTGGCCTCGTCATG GCGACGTGGTTTCGG TCTTGTTCCGTTTCC ATCCCTGCGCTTGCG TGGGACGTTGCCCCC
60 mnmo214_GTTTGTGGCTTCGTA GCTGGCCTCGTCATG GCGACGTGGTTTCGG TCTTGTTCCGTTTCC ATCCCTGCGCTTGCG TGGGACGTTGCCCCC

61 mnw1_    GTTTGTGGCTTCGTA GCTGGCCTCGTCATG GCGACGTGGTTTCGG TCTTGTTCCGTTTCC ATCCCTGCGCTTGCG TGGGACGTTGCCCCC
62 mnw4_    GTTTGTGGCTTCGTA GCTGGCCTCGTCATG GCGACGTGGTTTCGG TCTTGTTCCGTTTCC ATCCCTGCGCTTGCG TGGGACGTTGCCCCC
63 mnw14_   GTTTGTGGCTTCGTA GCTGGCCTCGTCATG GCGACGTGGTTTCGG TCTTGTTCCGTTTCC ATCCCTGCGCTTGCG TGGGACGTTGCCCCC
64 nyr14_   GTTAGTGGCTTCGTA GCTGGCCTCGTCATG GCGACGTGGTTTCGG TCTTGTTCCGTTTCC AT-CCTGCGCTTGCG TGGGACGTTGCCCC-

65 nyr112_  GTTAGTGGCTTCGTA GCTGGCCTCGTCATG GCGACGTGGTTTCGG TCTTGTTCCGTTTCC AT-CCTGCGCTTGCG TGGGACGTTGCCCC-
66 txeb1_   GTTTGTGGCTTCGTA GCTGGCCTCGTCATG GCGACGTGGTTTCGG TCTTGTTCCGTTTCC ATCCCTGCGCTTGCG TGGGACGTTGCCCC-
67 txeb2_   GTTTGTGGCTTCGTA GCTGGCCTCGTCATG GCGACGTGGTTTCGG TCTTGTTCCGTTTCC ATCCCTGCGCTTGCG TGGGACGTTGCCCC-
68 okw3_    GTTTGTGGCTTCGTA GCTGGCCTCGTCATG GCGACGTGGTTTCGG TCTTGTTCCGTTTCC ATCCCTGCGCTTGCG TGGGACGTTGCCCC-

69 txwe1_   GTTTGTGGCTTCGTA GCTGGCCTCGTCATG GCGACGTGGTTTCGG TCTTGTTCCGTTTCC ATCCCTGCGCTTGCG TGGGACGTTGCCCC-
70 txwe3_   GTTTGTGGCTTCGTA GCTGGCCTCGTCATG GCGACGTGGTTTCGG TCTTGTTCCGTTTCC ATCCCTGCGCTTGCG TGGGACGTTGCCCC-
71 txwe6_   GTTTGTGGCTTCGTA GCTGGCCTCGTCATG GCGACGTGGTTTCGG TCTTGTTCCGTTTCC ATCCCTGCGCTTGCG TGGGACGTTGCCCC-
72 txwe2_   GTTAGTGGCTTCGTA GCTGGCCTCGTCATG GCGACGTGGTTTCGG TCTTGTTCCGTTTCC ATCCCTGCGCTTGCG TGGGACGTTGCCCCC
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              8                                  9      10                11                     12
              181           195 196           210 211   _     225 226       __    240 241           255 256          270
1  car2_      TCCCACCCCTCCAAC TGTGTTGCTGCTCCG GCGACGACACGCTTG GGTTATGCTCGTTTT GTTTTGGTGTGG--T CTGTTGCTCCGGTAA
2  car5_      TCCCACCCCTCCAAC TGTGTTGCTGCTCCG GCGACGACACGCTTG GGTTATGCTCGTTTT GTTTTGGTGTGG--T CTGTTGCTCCGGTAA
3  car8_      TCCCACCCCTCCAAC TGTGTTGCTGCTCCG GCGACGACACGCTTG GGTTATGCTCGTTTT GTTTTGGTGTGG--T CTGTTGCTCCGGTAA
4  mnc3_      TCCCACCCCTCCAAC TGTGTTGCTGCTCCG GCGGCGACACGCTTG GGTTATGCTCGTTTT GTTGGTGTGTGTAAT CTGTTACTTTG-TAG

5  mnc8_      TCCCACCCCTCCAAC TGTGTTGCTGCTCCG GCGGCGACACGCTTG GGTTATGCTCGTTTT GTTGGTGTGTGTAAT CTGTTACTTTG-TAG
6  mnc12_     TCCCACCCCTCCAAC TGTGTTGCTGCTCCG GCGGCGACACGCTTG GGCTATGCTCGTTTT GTTGGTGTGTGTAAT CTGTTACTTTG-TAG
7  par3_      TCCCACCCCTCCAAC TGTGTTGCTGCTTCG GCGGCGACACGCTTG GGTTATGCTCGTTTT GTTGGTGTGTGTAAT CTGTTACTTTG-TAG
8  par8_      TCCCACCCCTCCAAC TGTGTTGCTGCTTCG GTGGCGACACGCTTG GGTTATGCTCGTTTT GTTGGTGTGTGTAAT CTGTTACCTTG-TAG

9  par9_      TCCCACCCCTCCAAC TGTGTTGCTGCTTCG GTGGCGACACGCTTG GGTTATGCTCGTTTT GTTGGTGTGTGTAAT CTGTTACCTTG-TAG
10 mnmo14_    TCCCACCCCTCCAAC TGTGTTGCTGCTTCG GTGGCGACGCGCTTG GGTTATGCTCGTTTT GTTGGTGTGTGTAAT CTGTTACCTTG-TAG
11 mnmo15_    TCCCACCCCTCCAAC TGTGTTGCTGCCACG GTGGCGACGCGCTTG GGTTATGCTCGTTTT GTTGGTGTGTGTAAT CTGTTACTTTG-TAG
12 mnmo18_    TCCCACCCCTCCAAC TGTGTTGCTGCCACG GTGGCGACGCGCTTG GGTTATGCTCGTTTT GTTGGTGTGTGTAAT CTGTTACTTTG-TAG

13 wir24_     TCCCACCCCTCCAAC TGTGTTGCTGCTTCG GCGGCGACACGCTTG GGTTATGCTCGTTTC GTTGGTGTGTGTAAT CTGTTACTTTG-TAG
14 txwba3_    TCCCACCCCTCCAAC TGTGTTGCTGCTCCG GCGGCGACACGCTTG GGTTATGCTCGTTTT GTTGGTGTGTGTAAT CTGTTACCTTG-TAG
15 txwba5_    TCCCACCCCTCCAAC TGTGTTGCTGCTCCG GCGGCGACACGCTTG GGTTATGCTCGTTTT GTTGGTGTGTGTAAT CTGTTACCTTG-TAG
16 txwba6_    TCCCACCCCTCCAAC TGTGTTGCTGCTCCG GCGGCGACACGCTTG GGTTATGCTCGTTTT GTTGGTGTGTGTAAT CTGTTACCTTG-TAG

17 txwbb21_   TCCCACCCCTCCAAC TGTGTTGCTGCTCCG GCGGCGACACGCTTG GGTTATGCTCGTTTT GTTGGTGTGTGTAAT CTGTTACCTTG-TAG
18 mnc10_     TCCCACCCCTCCAAC TGTGTTGCTGCTCCG GCGGCGACACGCTTG GGTTATGCTCGTTTT GTTGGTGTGTGTAAT CTGTTACTTTG-TAG
19 wir25_     TCCCACCCCTCCAAT TGTGTTGCTGCTCCG GCGACGACACGCTTG GGTTATGCTCGTTTT GTTGTTG-GTGTA-- CTGGTGCGTGAGCAC
20 wir27_     TCCCACCCCTCCAAT TGTGTTGCTGCTCCG GCGACGACACGCTTG GGTTATGCTCGTTTT GTTGTTG-GTGTA-- CTGGTGCGTGAGCAC

21 wie19_     TCCCACCCCTCCAAC TGTGTTGCTGCTCCG GTGGCGACACGCTTG GGTTATGCTCGTTTT GTTGTTG-GTGTA-- CTGGTGCGTGAGCAC
22 wie110_    TCCCACCCCTCCAAC TGTGTTGCTGCTCCG GTGGCGACACGCTTG GGTTATGCTCGTTTT GTTGTTG-GTGTA-- CTGGTGCGTGAGCAC
23 casa10_    TCCCACCCCTCCAAC TGTGTTGCTGCTCCG GCGGCGACACGCTTG GGTTATGCTCGTTTT GTTGTTG-GTGTA-- CTGGTGCGTGAGCAC
24 casa11_    TCCCACCCCTCCAAC TGTGTCGCTGCTCCG GCGGCGACACGCTTG GGTTATGCTCGTTTT GTTGTTG-GTGTA-- CTGGTGCGTGAGCAC

25 casb5_     TCCCACCCCTCCAAC TGTGTTGCTGCTCCG GCGGCGACACGCTTG GGTTATGCTCGTTTT GTTGTTG-GTGTA-- CTGGTGCGTGAGCAC
26 casb8_     TCCCACCCCTCCAAC TGTGTTGCTGCTCCG GCGGCGACACGCTTG GGTTATGCTCGTTTT GTTGTTG-GTGTA-- CTGGTGCGTGAGCAC
27 casb2_     TCCCACCCCTCCAAC TGTGTTGCTGCTCCG GCGGCGACACGCTTG GGTTATGCTCGTTTT GTTGTTG-GTGTA-- CTGGTGCGTGAGCAC
28 casa14_    TCCCACCCCTCCAAC TGTGTTGCTGCTCCG GCGGCGACACGCTTG GGTGATGCTCGTTTT GTTGTTG-GTGTA-- CTGGTGCGTGAGCAC

29 txea7_     TCCCACCCCTCCAAC TGTGTTGCTGCTTCG GCGGCGACACGCTTG GGTTATGCTCGTTTT GTTGTTG-GTGTA-- CTGGTGCGTGAGCAC
30 txea18_    TCCCACCCCTCCAAC TGTGTTGCTGCTTCG GCGGCGACACGCTTG GGTTATGCTCGTTTT GTTGTTG-GTGTA-- CTGGTGCGTGAGCAC
31 txea14_    TCCCACCCCTCCAAC TGTGTTGCTGCTTCG GCGGCGACACGCTTG GGTTATGCTCGTTTT GTTGTTG-GTGTA-- CTGGTGCGTGAGCAC
32 wie18_     TCCCACCCCTCCAAC TGTGTTGCTGCCACG GCGGCGACGCGCTTG GGTTATGCTCGTTTT GTTGTTG-GTGTA-- CTGGTGCGTGAGCAC

33 txwbb1_    TCCCACCCCTCCAAC TGTGTTGCTGCTCCG GCGACGACACGCTTG GGTTATGCTCGTTTT GTTGTTG-ATGTA-- CTGGTGCGTGAGCAC
34 txwbb14_   TCCCACCCCTCCAAC TGTGTTGCTGCTCCG GCGACGACACGCTTG GGTTATGCTCGTTTT GTTGTTG-ATGTA-- CTGGTGCGTGAGCAC
35 txea24_    TCCACCCCCTCCGAC TGTGTTGCTGCTCCG GCGGCGACACGCTTG GGTTATGCTCGTTTT GTTGGTGTGTGTAAT CTGTTACTTTG-TAG
36 txeb8_     TCCCACCCCTCCAAC TGTGTTGCTGCTTCG GCGGCGACACGCTTG GGTTATGCTCGTTTT GTTGTTG-GTGTA-- CTGGTGCGTGAGCAC
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         8                                      9          10                 11                   12
         181           195 196           210 211     _     225 226        __   240 241            255 256           270
37 okw11_   TCCCACCCCTCCAAC TGTGTTGCTGCCACG GCGGCGACACGCTTG GGTTATGCTCGTTTT GTTGTTG-GTGTA-- CTGGTGCGTGAGCAC
38 okw8_    TCCCACCCCTCCAAC TGTGTTGCTGCTTCG GCGGCGACACGCTTG GGTTATGCTCGTTTT GTTGTTG-GTGTG-- CTGGTGCGTGAGCAC
39 mnw9_    TCCCACCCCTCCAAC TGTGTTGCTGCTCCG GCGGCGACACGCTTG GGTTATGCTCGTTTT GTTGTTA-GTGTA-- CTGGTGCGCGAGCAC
40 mnw11_   TCCCACCCCTCCAAC TGTGTTGCTGCTCCG GCGGCGACACGCTTG GGTTATGCTCGTTTT GTTGTTA-GTGTA-- CTGGTGCGCGAGCAC

41 maw5_    TCCCACCCCTCCAAC TGTGTTGCTGCTCCG GCGGCGACACGCTTG GGTTATGCTCGTTTT GTTGTTG-ATGTA-- CTGGTGCGTGAGCAC
42 nhe80_   ACCCACCCCTCCAAC TGTGTTGCTGCTCCG GCGGCGACACGCTTG GGTTATGCTCGTTTT GTTGTTG-GTGTA-- CTGGTGCGTGAGCAC
43 nhe74_   TCCCACCCCTCCAAC TGTGTTGCTGCTCCG GTGGCGACACGCTTG GGTTATGCTCGTTTT GTTGGTGTGTGTAAT CTGTTACCTT-GTAG
44 nhe79_   TCCCACCCCTCCAAC TGTGTTGTTGCTCCG GTGGCGACACGCTTG GGTTATGCTCGTTTT GTTGGTGTGTGTAAT CTGTTACCTT-GTAG

45 nyr21_   TCCCACCCCTCCAAC TGTGTTGCTGCTTCG GCGGCGACACGCTTG GGTTATGCTCGTTTT GTTGGTGTGTGTAAT CTGTTACTTT-GTAG
46 nyr25_   TCCCACCCCTCCAAC TGTGTTGCTGCTTCG GCGGCGACACGCTTG GGTTATGCTCGTTTT GGTGGTGTGTGTAAT CTGTTACTTT-GTAG
47 nyr28_   TCCCACCCCTCCAAC TGTGTTGCTGCTTCG GCGGCGACACGCTTG GGTTATGCTCGTTTT GTTGGTGTGTGTAAT CTGTTACTTT-GTAG
48 nyr23_   TCCCACCCCTCCAAC TGTGTTGCTGCTTCG GCGGCGACACGCTTG GGTTATGCTCGTTTT GTTGGTGTGTGTAAT CTGTTACTTT-GTAG

49 wie210_  TCCCACCCCTCCAAC TGTGTTGCTGCCACG GCGGCGACACGCTTG GGTTATGCTCGTTTT GTTGGTGTGTGTAAT CTGTTACCTT-GTAG
50 wie214_  TCCCACCCCTCCAAC TGTGTTGCTGCCACG GCGGCGACACGCTTG GGTTATGCTCGTTTT GTTGGTGTGTGTAAT CTGTTACCTT-GTAG
51 nyr17_   TCCCACCCCTCCAAC TGTGTTGCTGCTTCG GCGGCGACACGCTTG GGTTATGCTCGTTTT GTTGGTGTGTGTAAT CTGTTACCTT-GTAG
52 maw9_    TCCCACCCCTCCAAC TGTGTTGCTGCTCCG GTGGCGACACGCTTG GGTTATGCTCGTTTT GTTGGTGTGTGTAAT CTGTTACCTT-GTAG

53 wie213_  TCCCACCCCTCCAAC TGTGTTGCTGCTTCG GCGGCGACACGCTTG GGTTATGCTCGTTTT GTTGGTGTGTGTAAT CTGTTACCTT-GTAG
54 ine6_    TCCCACCCCTCCAAC TGTGTTGCTGCTCCG GCGGCGACACGCTTG GGTTATGCTCGTTTT GTTGGTGTGTGTAAT CTGTTACCTT-GTAG
55 maw3_    TCCCACCCCTCCAAC TGTGTTGCTGCTCCG GCGGCGACACGCTTG GGTTATGCTCGTTTT GTTGGTGTGTGTAAT CTGTTACCTT-GTAG
56 ine11_   TCCCACCCCTCCAAC TGTGTTGCTGCTCCG GCGGCGACACGCTTG GGTTATGCTCGTTTT GTTGGTGCGTGTAAT CTGTTACTCT-GTAG

57 ine14_   TCCCACCCCTCCAAC TGTGTTGCTGCTCCG GCGGCGACACGCTTG GGTTATGCTCGTTTT GTTGGTGCGTGTAAT CTGTTACTTT-GTAG
58 mnmo25_  TCCCACCCCTCCAAC TGTGTTGCTGCCACG GCGGCGACGCGCTTG GGTTATGCTCGTTTT GTTGGTGTGTGTAAT CTGTTACTTT-GTAG
59 mnmo26_  TCCCACCCCTCCAAC TGTGTTGCTGCTTCG GTGGCGACACGCTTG GGTTATGCTCGTTTT GTTGGTGTGTGTAAT CTGTTACCTT-GTAG
60 mnmo214_ TCCCACCCCTCCAAC TGTGTTGCTGCTTCG GTGGCGACACGCTTG GGTTATGCTCGTTTT GTTGGTGTGTGTAAT CTGTTACCTT-GTAG

61 mnw1_    TCCCACCCCTCCAAC TGTGTTGCTGCCACG GCGGCGACACGCTTG GGTTATGCCCGTTTT GTTGGTGCGTGTAAT CTGTTACCTT-GTAG
62 mnw4_    TCCCACCCCTCCAAC TGTGTTGCTGCCACG GCGGCGACACGCTTG GGTTATGCCCGTTTT GTTGGTGCGTGTAAT CTGTTACCTT-GTAG
63 mnw14_   TCCCACCCCTCCAAC TGTGTTGCTGCCACG GCGGCGACACGCTTG GGTTATGCCCGTTTT GTTGGTGCGTGTAAT CTGTTACCTT-GTAG
64 nyr14_   TCCCACCCCTCCAAC TGTGTTGCTGCCACG GCGGCGACACGCTTG GGTTATGCTCGTTTT GTTGGTGTGTGTAAT CTGTTACCTT-GTAG

65 nyr112_  TCCCACCCCTCCAAC TGTGTTGCTGCCACG GCGGCGACACGCTTG GGTTATGCTCGTTTT GTTGGTGTGTGTAAT CTGTTACCTT-GTAG
66 txeb1_   TCCCACCCCTCCGAC TGTGTTGCTGCTCCG GCGGCGACACGCTTG GGTTATGCTCGTTTT GTTGGTGTGTGTAAT CTGTTACTTT-GTAG
67 txeb2_   TCCCACCCCTCCGAC TGTGTTGCTGCTCCG GCGGCGACACGCTTG GGTTATGCTCGTTTT GTTGGTGTGTGTAAT CTGTTACTTT-GTAG
68 okw3_    TCCCACCCCTCCAAC TGTGTTGCTGCTCCG GCGACTACACGCTTG GGTTATGCCCGTTTT GTTGGTGTGTGTAAT CTGTTACTTT-ATAG

69 txwe1_   TCCCACCCCTCCAAC TGTGTTGCTGCCACG GCGGCGACACGCTTG GGTTATGCTCGTTTT GTTGTTG-GTGTA-- CTGGTGCGTGAGCAC
70 txwe3_   TCCCACCCCTCCAAC TGTGTTGCTGCCACG GCGGCGACACGCTTG GGTTATGCTCGTTTT GTTGTTG-GTGTA-- CTGGTGCGTGAGCAC
71 txwe6_   TCCCACCCCTCCAAC TGTGTTGCTGCCACG GCGGCGACACGCTTG GGTTATGCTCGTTTT GTTGTTG-GTGTA-- CTGGTGCGTGAGCAC
72 txwe2_   TCCCACCCCTCCAAC TGTGTTGCTGCTCCG GCGGCGACACGCTAG GGTTATGCTTGTTTT GTTGTTG-GTGTA-- CTGGTGCGTGAGCAC
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                  13        14                              15             16       17                 18
              271        285 286          300 301        315 316          330 331        345 346        360
1  car2_      CTCGCC-GCATCGCC AGCTCAACGAGATGC TGCTATGGATCTATA GGATCCAAGCAGACG CTGCCTCG-GCAGTT TGCGTAGTGT--GAC
2  car5_      CTCGCC-GCATCGCC AACTCAACGAGATGC TGCTATGGATCTATA GGATCCAAGCAGACG CTGCCTCG-GCAGTT TGCGTAGTGT--GAC
3  car8_      CTCGCC-GCATCGCC AACTCAACGAGATGC TGCTATGGATCTATA GGATCCAAGCAGACG CTGCCTCG-GCAGTT TGCGTAGTGT--GAC
4  mnc3_      CGGTACTGCACCACT AACTCAACGGGATGC TGCTGTGAATTCATG GGATTCAAGCAGTCG CTGCCTCGTGCAGTT TGCGTAGTTTTTGAC

5  mnc8_      CGGTACTGCACCACT AACTCAACGGGATGC TGCTGTGAATTCATG GGATTCAAGCAGTCG CTGCCTCGTGCAGTT TGCGTAGTTTTTGAC
6  mnc12_     CGGTACTGCACCACT AACTCAACGGGATGC TGCTGTGAATTCATG GGATTCAAGCAGTCG CTGCCTCGTGCAGTT TGCGTAGTTTTTGAC
7  par3_      CGGTACTGCACCACC AGCTCAACGGGATGC TGCTGTGAATTCATG AGATTCAAGCAGTCG CTGCCTCGTACAGTT TGCGTAGTTTTTGAC
8  par8_      CGGTACTGCACCACT GGCTCAACGGGATGC TGCTGTGAATTCATG AGATTCAAGCAGTCG CTGCCTCGTGCAGTT TGCGTAGTTTTTGAC

9  par9_      CGGTACTGCACCACT GGCTCAACGGGATGC TGCTGTGAATTCATG AGATTCAAGCAGTCG CTGCCTCGTGCAGTT TGCGTAGTTTTTGAC
10 mnmo14_    CGGTACTGCACCACT GGCTCAACGGGATGC TGCTGTGAATTCATG AGATTCAAGCAGTCG CTGCCTCGTGCAGTT TGCGTAGTTTTTGAC
11 mnmo15_    CGGTACTGCACCACC AGCTCAACGGGATGC TGCTGTGAATTGATG AGATTCAAGCAGTCG CTGCCTCGTGCAGTT TGCGTAGTTTTTGAC
12 mnmo18_    CGGTACTGCACCACC AGCTCAACGGGATGC TGCTGTGAATTGATG AGATTCAAGCAGTCG CTGCCTCGTGCAGTT TGCGTAGTTTTTGAC

13 wir24_     CGGTACTGCACCACT GGCTCAACGGGATGC TGCTGTGAATTCATG AGATTCAAGCAGTCG CTGCCTCGTGCAGTT TGCGTAGTTTTTGAC
14 txwba3_    CGGTACTGCACCACT AGCTCAACGGGATGC TGCTGTGAATTCATG AGATTCAAGCAGTCG CTGCCTCGTGCAGTT TGCGTAGTTTCTGAC
15 txwba5_    CGGTACTGCACCACT AGCTCAACGGGATGC TGCTGTGAATTCATG AGATTCAAGCAGTCG CTGCCTCGTGCAGTT TGCGTAGTTTTTGAC
16 txwba6_    CGGTACTGCACCACT AGCTCAACGGGATGC TGCTGTGAATTCATG AGATTCAAGCAGTCG CTGCCTCGTGCAGTT TGCGTAGTTTTTGAC

17 txwbb21_   CGGTACTGCACCACT AGCTCAACGGGATGC TGCTGTGAATTCATG AGATTCAAGCAGTCG CTGCCTCGTGCAGTT TGCGTAGTTTTTGAC
18 mnc10_     CGGTACTGCACCACT AACTCAACGGGATGC TGCTGTGAATTCATG GGATTCAAGCAGTCG CTGCCTCGTGCAGTT TGCGTAGTGT--GAC
19 wir25_     CTGTGA--CATTAAT GGCTCAACGGGATGC TGCTGTGAATTCATG AGATTCAAGCAGTCG CTGCCTCGTGCAGTT TGCGTAGTTTTTGAC
20 wir27_     CTGTGA--CATTAAT GGCTCAACGGGATGC TGCTGTGAATTCATG AGATTCAAGCAGTCG CTGCCTCGTGCAGTT TGCGTAGTTTTTGAC

21 wie19_     CTGTGA--CATTAAT GGCTCAACGGGATGC TGCTGTGAATTCATG AGATTCAAGCAGTCG CTGCCTCGTGCAGTT TGCGTAGTTTTTGAC
22 wie110_    CTGTGA--CATTAAT GGCTCAACGGGATGC TGCTGTGAATTCATG AGATTCAAGCAGTCG CTGCCTCGTGCAGTT TGCGTAGTTTTTGAC
23 casa10_    CTGTGA--CATTAGC GGCTCAACGGGATGC TGCTGTGAATTCATG AGATTCAAGCAGTCG CTGCCTTGTGCAGTT TGCGTAGTTTTTGGC
24 casa11_    CTGTGA--CATTAGC GGCTCAACGGGATGC TGCTGTGAATTCATG AGATTCAAGCAGTCG CTGCCTTGTGCAGTT TGCGTAGTTTTTGGC

25 casb5_     CTGTGA--CATTAGC GGCTCAACGGGATGC TGCTGTGAATTCATG AGATTCAAGCAGTCG CTGCCTTGTGCAGTT TGCGTAGTTTTTGGC
26 casb8_     CTGTGA--CATTAGC GGCTCAACGGGATGC TGCTGTGAATTCATG AGATTCAAGCAGTCG CTGCCTTGTGCAGTT TGCGTAGTTTTTGGC
27 casb2_     CTGTGA--CATTAGC GGCTCAACGGGATGC TGCTGTGAATTCATG AGATTCAAGCAGTCG CTGCCTTGTGCAGTT TGCGTAGTTTTTGGC
28 casa14_    CTGTGA--CATTAGC GGCTCAACGGGATGC TGCTGTGAATTCATG AGATTCAAGCAGTCG CTGCCTTGTGCAGTT TGCGTAGTTTTTGGC

29 txea7_     CTGTGA--CATCACC AACTCAACGGGATGC TGCTGTGAATTCACG GGATTCAAGCAGTCG CTGCCTCGTGCAGTT TGCGTAGTTTTTGAC
30 txea18_    CTGTGA--CATCACC AACTCAACGGGATGC TGCTGTGAATTCACG GGATTCAAGCAGTCG CTGCCTCGTGCAGTT TGCGTAGTTTTTGAC
31 txea14_    CTGTGA--CATCACC AACTCAACGGGATGC TGCTGTGAATTCACG GGATTCAAGCAGTCG CTGCCTCGTGCAGTT TGCGTAGTTTTTGAC
32 wie18_     CTGTGA--CATTAAT GGCTCAACGGGATGC TGCTGTGAATTCATG AGATTCAAGCAGTCG CTGCCTCGTGCGGTT TGCGTAGTTTTTGAC

33 txwbb1_    CTGTGA--CATTAAT GGCTCAACGGGATGC TGCTGTGAATTCATG AGATTCAAGCAGACG CTGCCTCGTGCAGTT TGCGTAGTTTTTGAC
34 txwbb14_   CTGTGA--CATTAAT GGCTCAACGGGATGC TGCTGTGAATTCATG AGATTCAAGCAGACG CTGCCTCGTGCAGTT TGCGTAGTTTTTGAC
35 txea24_    CGGTACTGCACCACT GGCTCAACGGGATGC TGCTGTGAATTCACG GGATTCAAGCAGTCG CTGCCTCGTGCAGTT TGCGTAGTTTTTGAC
36 txeb8_     CTGTGA--CATCACC AACTCAACGGGATGC TGCTGTGAATTCACG GGATTCAAGCAGTCG CTGCCTCGTGCAGTT TGCGTAGTTTTTGAC
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            13    14                      15             16     17              18
            271           285 286           300 301           315 316           330 331           345 346           360
37 okw11_   CTGTGA--CATCACC AACTCAACGGGATGC TGCTGTGAATTCACG GGATTCAAGCAGTCG CTGCCTCGTGCAGTT TGCGTAGTTTTTGAC
38 okw8_    CTGTGA--CATCACC AACTCAACGGGATGC TGCTGTGAATTCATG GGATTCAAGCAGTCG CTGCCTCGTGCAGTT TGCGTAGTTTTTGAC
39 mnw9_    CTGTGA--CATTAAT GGCTCAACGGGATGC TGCTGTGAATTCATG AGATTCAAGCAGTCG CTGCCTCGTGCAGTT TGCGTAGTTTTTGAC
40 mnw11_   CTGTGA--CATTAAT GGCTCAACGGGATGC TGCTGTGAATTCATG AGATTCAAGCAGTCG CTGCCTCGTGCAGTT TGCGTAGTTTTTGAC

41 maw5_    CTGTGA--CATTAAT GGCTCAACGGGATGC TGCTGTGAATTCATG AGATTCAAGCAGTCG CTGCCTCGTGCAGTT TGCGTAGTTTTTGAC
42 nhe80_   CTGTGA--CATTAAT GGCTCAACGGGATGC TGCTGTGAATTCATG AGATTCAAGCAGTCG CTGCCTCGTGCAGTT TGCGTAGTTTTTGAC
43 nhe74_   CGGTATTGCACCACT AGCTCAACGGGATGC TGCTGTGAATTCATG AGATTCAAGCAGTCG CTGCCTCGTGCAGTT TGCGTAGTTT--GAC
44 nhe79_   CGGTATTGCACCACT AGCTCAACGGGATGC TGCTGTGAATTCATG AGATTCAAGCAGTCG CTGCCTCGTGCAGTT TGCGTAGTTT--GAC

45 nyr21_   CGGTATTGCACCACT AGCTCAACGGGATGC TGCTGTGAATTCATG AGATTCAAGCAGTCG CTGCCTCGTGCAGTT TGCGTAGTTT--GAC
46 nyr25_   CGGTATTGCACCACT AGCTCAACGGGATGC TGCTGTGAATTCATG AGATTCAAGCAGTCG CTGCCTCGTGCAGTT TGCGTAGTTT--GAC
47 nyr28_   CGGTATTGCACCACT AGCTCAACGGGATGC TGCTGTGAATTCATG AGATTCAAGCAGTCG CTGCCTCGTGCAGTT TGCGTAGTTT--GAC
48 nyr23_   CGGTATTGCACCACT AGCTCAACGGGATGC TGCTGTGAATTCATG AGATTCAAGCAGTCG CTGCCTCGTGCAGTT TGCGTAGTTT--GAC

49 wie210_  CGGTATTGCACCACT AACTCAACGGGATGC TGCTGTGAATTCATG AGATTCAAGCAGTCG CTGCCTCGTGCAGTT TGCGTAGTTTTTGAC
50 wie214_  CGGTATTGCACCACT AACTCAACGGGATGC TGCTGTGAATTCATG AGATTCAAGCAGTCG CTGCCTCGTGCAGTT TGCGTAGTTTTTGAC
51 nyr17_   CGGTACTGCACCACT AGCTCAACGGGATGC TGCTGTGAATTCATG AGATTCAAGCAGTCG CTGCCTCGTGCAGTT TGCGTAGTTTTTGAC
52 maw9_    CGGTACTGCACCACT AACTCAACGGGATGC TGCTGTGAATTGATG AGATTCAAGCAGTCG CTGCCTCGTGCAGTT TGCGTAGTTTTTGAC

53 wie213_  CGGTACTGCACCACT GGCTCAACGGGATGC TGCTGTGAATTCATG AGATTCAAGCAGTCG CTGCCTCGTGCAGTT TGCGTAGTTTTTGAC
54 ine6_    CGGTACTGCACCACT GGCTCAACGGGATGC TGCTGTGAATTCATG AGATTCAAGCAGTCG CTGCCTCGTGCAGTT TGCGTAGTTTTTGAC
55 maw3_    CGGTACTGCACCACT GGCTCAACGGGATGC TGCTGTGAATTCATG AGATTCAAGCAGTCG CTGCCTCGTGCAGTT TGCGTAGTTTTTGAC
56 ine11_   CGGTACTGCACCACT AGCTCAACGGGATGC TGCTGTGAATTCATG AGATTCAAGCAGTCG CTGCCTCGTGCAGTT TGCGTAGTTTTTGAC

57 ine14_   CGGTACTGCACCACT AGCTCAACGGGATGC TGCTGTGAATTCATG AGATTCAAGCAGTCG CTGCCTCGTGCAGTT TGCGTAGTTTTTGAC
58 mnmo25_  CGGTACTGCACCACC AGCTCAACGGGATGC TGCTGTGAATTGATG AGATTCAAGCAGTCG CTGCCTCGTGCAGTT TGCGTAGTTTTTGAC
59 mnmo26_  CGGTACTGCACCACT GGCTCAACGGGATGC TGCTGTGAATTCATG AGATTCAAGCAGTCG CTGCCTCGTGCAGTT TGCGTAGTTTTTGAC
60 mnmo214_ CGGTACTGCACCACT GGCTCAACGGGATGC TGCTGTGAATTCATG AGATTCAAGCAGTCG CTGCCTCGTGCAGTT TGCGTAGTTTTTGAC

61 mnw1_    CGGTACTGCACCACT GGCTCAACGGGATGC TGCTGTGAATTCATG AGATTCAAGCAGTCG CTGCCTCGTGCAGTT TGCGTAGTTTTTGAC
62 mnw4_    CGGTACTGCACCACT GGCTCAACGGGATGC TGCTGTGAATTCATG AGATTCAAGCAGTCG CTGCCTCGTGCAGTT TGCGTAGTTTTTGAC
63 mnw14_   CGGTACTGCACCACT GGCTCAACGGGATGC TGCTGTGAATTCATG AGATTCAAGCAGTCG CTGCCTCGTGCAGTT TGCGTAGTTTTTGAC
64 nyr14_   CGGTACTGCACCACC AACTCAACGGGATGC TGCTGTGAATTCATG AGATTCAAGCAGTCG CTGCCTCGTGCAGTT TGCGTAGTTTTTGAC

65 nyr112_  CGGTACTGCACCACC AACTCAACGGGATGC TGCTGTGAATTCATG AGATTCAAGCAGTCG CTGCCTCGTGCAGTT TGCGTAGTTTTTGAC
66 txeb1_   CGGTACTGCACCACT GGCTCAACGGGATGC TGCTGTGAATTCACG GGATTCAAGCAGTCG CTGCCTCGTGCAGTT TGCGTAGTTTTTGAC
67 txeb2_   CGGTACTGCACCACT GACTCAACGGGATGC TGCTGTGAATTCACG GGATTCAAGCAGTCG CTGCCTCGTGCAGTT TGCGTAGTTTTTGAC
68 okw3_    CGGTACTGCACCACT AACTCAACGGGATGC TGCTGTGAATTCATG AGATTCAAGCAGTCG CTGCCTCGTGCAGTT TGCGTAGTTTTTGAC

69 txwe1_   CTGTGA--CATTAGT GGCTCAACGGGATGC TGCTGTGAATTCATG AGATTCAAGCAGTCG CTGCCTCGTGCAGTT TGCGTAGTGT--GAC
70 txwe3_   CTGTGA--CATTAGT GGCTCAACGGGATGC TGCTGTGAATTCATG AGATTCAAGCAGTCG CTGCCTCGTGCAGTT TGCGTAGTGT--GAC
71 txwe6_   CTGTGA--CATTAGT GGCTCAACGGGATGC TGCTGTGAATTCATG AGATTCAAGCAGTCG CTGCCTCGTGCAGTT TGCGTAGTGT--GAC
72 txwe2_   CTGTGA--CATTAGT GGCTCAACGGGATGC TGCTGTGAATTCATG AGATTCAAGCAGTCG CTGCCTCGTGCAGTT TGCGTAGTGT--GAC
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              18                                   19      20                       21
              361           375 376           390 391               405 406           420
1  car2_      TACGATTATGCAACT CCGCTTGATTGCCG- TTT-GGCAATCGAGT TTT-CTGAAACTATT
2  car5_      TACGATTATGCAACT CCGCTTGATTGCCG- TTT-GGCAATCGAGT TTT-CTGAAACTATT
3  car8_      TACGATTATGCAACT CCGCTTGATTGCCG- TTT-GGCAATCGAGT TTT-CTGAAACTATT
4  mnc3_      TGCGATTATGCAACT CCGCTTGATTGCCA- TTTTGGCTATCGAGT TTTTCTGAAATGATT

5  mnc8_      TGCGATTATGCAACT CCGCTTGATTGCCA- TTTTGGCTATCGAGT TTTTCTGAAATGATT
6  mnc12_     TGCGATTATGCAACT CCGCTTGATTGCCA- TTTTGGCTATCGAGT TTTTCTGAAATGATT
7  par3_      TGCGATTATGCAACT CCGCTTGATTGCCA- TTTTGGTGGTCGAGT TTTTCTGAAATGATT
8  par8_      TGCGATTATGCAACT CCGCTTGGTTGCCA- TTTTGGTGGTCGAGT TTTTCTGAAATGATT

9  par9_      TGCGATTATGCAACT CCGCTTGATTGCCA- TTTTGGTGGTCGAGT TTTTCTGAAATGATT
10 mnmo14_    TGCGATTATGCAACT CCGCTTGATTGCCT- TTTTGGCTATCGAGT TTTTCTGAAATGATT
11 mnmo15_    TGCGATTATGCAACT CCGCTTGATTGCCA- TTTTGGTGGTCGAGT TTTTCTGAAATTATT
12 mnmo18_    TGCGATTATGCAACT CCGCTTGATTGCCA- TTTTGGTGGTCGAGT TTTTCTGAAATTATT

13 wir24_     TGCGATTATGCAACT CCGCTTGATTGCCA- TTTTGGTGGTCGAGT TTTTCTGAAATTATT
14 txwba3_    TGCGATTATGCAACT CCGCTTGATTGCCT- GTTTGGCTATCGAGT TTTTCTGAAATGATT
15 txwba5_    TGCGATTATGCAACT CCGCTTGATTGCCT- GTTTGGCTATCGAGT TTTTCTGAAATGATT
16 txwba6_    TGCGATTATGCAACT CCGCTTGATTGCCT- GTTTGGCTATCGAGT TTTTCTGAAATGATT

17 txwbb21_   TGCGATTATGCAACT CCGCTTGATTGCCT- GTTTGGCTATCGAGT TTTTCTGAAATGATT
18 mnc10_     TGCGATTATGCAACT CCGCTTGATTGCCTG TTTTGGTGGTCGAGT TTTTCTGAAATGATT
19 wir25_     TGCGATTATGCAACT CCGCTTGATTGCCA- TTTTGGTGGTCGAGT TTTTCTGAAATGATT
20 wir27_     TGCGATTATGCAACT CCGCTTGATTGCCA- TTTTGGTGGTCGAGT TTTTCTGAAATGATT

21 wie19_     TGCGATTATGCAACT CCGCTTGATTGCCT- ATTTGGCTATCGAGT TTTTCTGAAATGATT
22 wie110_    TGCGATTATGCAACT CCGCTTGATTGCCT- ATTTGGCTATCGAGT TTTTCTGAAATGATT
23 casa10_    TGCGATTATGCAACT CCGCTTGATTGCCT- ATTTGGCTATCGAGT TTTTCTGAAATGATT
24 casa11_    TGCGATTATGCAACT CCGCTTGATTGCCT- ATTTGGCTATCGAGT TTTTCTGAAATGATT

25 casb5_     TGCGATTATGCAACT CCGCTTGATTGCCT- ATTTGGCTATCGAGT TTTTCTGAAATGATT
26 casb8_     TGCGATTATGCAACT CCGCTTGATTGCCT- ATTTGGCTATCGAGT TTTTCTGAAATGATT
27 casb2_     TGCGATTATGCAACT CCGCTTGATTGCCT- ATTTGGCTATCGAGT TTTTCTGAAATGATT
28 casa14_    TGCGATTATGCAACT CCGCTTGATTGCCT- ATTTGGCTATCGAGT TTTTCTGAAATGATT

29 txea7_     TGCGATTATGCAACT CCGCTTGATTGCCA- TTTTGGCTATCGAGT TTTTCTGAAATGATT
30 txea18_    TGCGATTATGCAACT CCGCTTGATTGCCA- TTTTGGCTATCGAGT TTTTCTGAAATGATT
31 txea14_    TGCGATTATGCAACT CCGCTTGATTGCCA- TTTTGGCTATCGAGT TTTTCTGAAATGATT
32 wie18_     TGCGATTATGCAACT CCGCTTGATTGCCT- ATTTGGCTATCGAGT TTTTCTGAAATGATT

33 txwbb1_    TGCGATTATGCAACT CCGCTTGATTGCCTG TTATGGTGGTCGAGT TTTTCTGAAATGATT
34 txwbb14_   TGCGATTATGCAACT CCGCTTGATTGCTTG TTACGGTGGTCGAGT TTTTCTGAAATGATT
35 txea24_    TGCGATTATGCAACT CCGCTTGATTGCCTG TTATGGTGGTTGAGT TTTTCTGAAATGATT
36 txeb8_     TGCGATTATGCAACT CCGCTTGATTGCCA- TTTTGGCTATCGAGT TTTTCTGAAATGATT
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18 **19** **20** **21**

361 375 376 390 391 405 406 420

37 okw11_ **TG**CGATTATGCAACT CCGCTTGATTGCC**T- ATTT**G**GTGG**TCGAGT TTTTCTGAAA**TG**ATT
38 okw8_ **TG**CGATTATGCAACT CCGCTTGATTGCC**A- TTTT**G**GTGG**TCGAGT TTTTCTGAAA**TG**ATT
39 mnw9_ **TG**CGATTATGCAACT CCGCTTGATTGCC**G- TTTT**G**GTGG**TCGAGT TTTTCTGAAA**TG**ATT
40 mnw11_ **TG**CGATTATGCAACT CCGCTTGATTGCC**G- TTTT**G**GTGG**TCGAGT TTTTCTGAAA**TG**ATT

41 maw5_ **TG**CGATTATGCAACT CCGCTTGATTGCC**AA TTAT**G**GTGG**TCGAGT TTTTCTGAAA**TG**ATT
42 nhe80_ **TG**CGATTATGCAACT CCGCTTGATTGCC**A- TTTT**G**GCTA**TCGAGT TTTTCTGAAA**TG**ATT
43 nhe74_ **TG**CGATTATGCAACT CCGCTTGATTGCC**T- ATTT**G**GCTA**TCGAGT TTTTCTGAAA**TG**ATT
44 nhe79_ **TG**CGATTATGCAACT CCGCTTGATTGCC**T- ATTT**G**GCTA**TCGAGT TTTTCTGAAA**TG**ATT

45 nyr21_ **TG**CGATTATGCAACT CCGCTTGATTGCC**T- ATTT**G**GCTA**TCGAGT TTTTCTGAAA**TG**ATT
46 nyr25_ **TG**CGATTATGCAACT CCGCTTGATTGCC**T- ATTT**G**GCTA**TCGAGT TTTTCTGAAA**TG**ATT
47 nyr28_ **TG**CGATTATGCAACT CTGCTTGATTGCC**T- ATTT**G**GCTA**TCGAGT TTTTCTGAAA**TG**ATT
48 nyr23_ **TG**CGATTATGCAACT CCGCTTGATTGCC**T- ATTT**G**GCTA**TCGAGT TTTTCTGAAA**TG**ATT

49 wie210_ **TG**CGATTATGCAACT CCGCTTGATTGCC**T- ATTT**G**GCTG**TCGAGT TTTTCTGAAA**TG**ATT
50 wie214_ **TG**CGATTATGCAACT CCCCTTGATTGCC**T- ATTT**G**GCTA**TCGAGT TTTTCTGAAA**TG**ATT
51 nyr17_ **CG**CGATTATGCAACT CCGCTTGATTGCC**T- ATTT**G**GCTG**TCGAGT TTTTCTGAAA**TG**ATT
52 maw9_ **TG**CGATTATGCAACT CCGCTTGATTGCC**A- TTTT**G**GCTA**TCGAGT TTTTCTGAAA**TG**ATT

53 wie213_ **TG**CGATTATGCAACT CCGCTTGATTGCC**T- TTTT**G**GTGG**TCGGGT TCTTCTGAAA**TG**ATT
54 ine6_ **TG**CGATTATGCAACT CCGCTTGATTGCC**T- ATTT**G**GCTA**TCGAGT TTTTCTGAAA**TG**ATT
55 maw3_ **TG**CGATTATGCAACT CCGCTTGATTGCC**T- ATTT**G**GTGG**TCGAGT TTTTCTGAAA**TT**ATT
56 ine11_ **TG**CGATTATGCAACT CCGCTTGATTGCC**A- TTTT**G**GTGG**TCGAGT TTTTCTGAAA**TT**ATT

57 ine14_ **TG**CGATTATGCAACT CCGCTTGATTGCC**A- TTTT**G**GTGG**TCGAGT TTTTCTGAAA**TT**ATT
58 mnmo25_ **TG**CGATTATGCAACT CCGCTTGATTGCC**A- TTTT**G**GTGG**TCGAGT TTTTCTGAAA**TT**ATT
59 mnmo26_ **TG**CGATTATGCAACT CCGCTTGATTGCC**T- TTTT**G**GCTA**TCGAGT TTTTCTGAAA**TG**ATT
60 mnmo214_**TG**CGATTATGCAACT CCGCTTGATTGCC**T- TTTT**G**GCTA**TCGAGT TTTTCTGAAA**TG**ATT

61 mnw1_ **TG**CGATTATGCAACT CCGCTTGATTGCC**T- TTTT**G**GCTA**TCGAGT TTTTCTGAAA**TT**ATT
62 mnw4_ **TG**CGATTATGCAACT CCGCTTGATTGCC**T- TTTT**G**GCTA**TCGAGT TTTTCTGAAA**TT**ATT
63 mnw14_ **TG**CGATTATGCAACT CCGCTTGATTGCC**T- TTTT**G**GCTA**TCGAGT TTTTCTGAAA**TT**ATT
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65 nyr112_ **TG**CGATTATGCAACT CCGCTTGATTGCC**G- TTTT**G**GTGG**TCGAGT TTTTCTGAAA**TG**ATT
66 txeb1_ **TG**CGATTATGCAACT CCGCTTGATTGCC**TG TTAT**G**GTGG**TTGAGT TTTTCTGAAA**TG**ATT
67 txeb2_ **TG**CGATTATGCAACT CCGCTTGATTGCC**TG TTAT**G**GTGG**TTGAGT TTTTCTGAAA**TG**ATT
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71 txwe6_ **TA**CGATTATGCAACT CCGCTTGATTGCC**TG TTAT**G**GTGG**TCGAGT TTTTCTGAAA**TG**ATT
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2  car5_        AAACTTTCAGCGATG GATGTCTTGGCTCAC ACAACGATGAAGGAC GCAGCAAATTGCGAT AAGCATTATGACTTG CAGACTTCTGCGATT
3  car8_        AAACTTTCAGCGATG GATGTCTTGGCTCAC ACAACGATGAAGGAC GCAGCAAATTGCGAT AAGCATTATGACTTG CAGACTTCTGCGATT
4  mnc3_        AAACTTTCAGCGATG GATGTCTTGGCTCAC ACAACGATGAAGGAC GCAGCAAATCGCGAT AAGCATTATGACTTG CAGACTTCTGCGATT

5  mnc8_        AAACTTTCAGCGATG GATGTCTTGGCTCAC ACAACGATGAAGGAC GCAGCAAATTGCGAT AAGCATTATGACTTG CAGACTTCTGCGATT
6  mnc12_       AAACTTTCAGCGATG GATGTCTTGGCTCAC ACAACGATGAAGGAC GCAGCAAATTGCGAT AAGCATTATGACTTG CAGACTTCTGCGATT
7  par3_        AAACTTTCAGCGATG GATGTCTTGGCTCAC ACAACGATGAAGGAC GCAGCAAATTGCGAT AAGCATTATGACTTG CAGACTTCTGCGATT
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9  par9_        AAACTTTCAGCGATG GATGTCTTGGCTCAC ACAACGATGAAGGAC GCAGCAAATTGCGAT AAGCATTATGACTTG CAGACTTCTGCGATT
10 mnmo14_      AAACTTTCAGCGATG GATGTCTTGGCTCAC ACAACGATGAAGGAC GCAGCAAATTGCGAT AAGCATTATGACTTG CAGACTTCTGCGATT
11 mnmo15_      AAACTTTCAGCGATG GATGTCTTGGCTCAC ACAACGATGAAGGAC GCAGCAAATTGCGAT AAGCATTATGACTTG CAGACTTCTGCGATT
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13 wir24_       AAACTTTCAGCGATG GATGTCTTGGCTCAC ACAACGATGAAGGAC GCAGCAAATTGCGAT AAGCATTATGACTTG CAGACTTCTGCGATT
14 txwba3_      AAACTTTCAGCGATG GATGTCTTGGCTCAC ACAACGATGAAGGAC GCAGCAAATTGCGAT AAGCATTATGACTTG CAGACTTCTGCGATT
15 txwba5_      AAACTTTCAGCGATG GATGTCTTGGCTCAC ACAACGATGAAGGAC GCAGCAAATTGCGAT AAGCATTATGACTTG CAGACTTCTGCGATT
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17 txwbb21_     AAACTTTCAGCGATG GATGTCTTGGCTCAC ACAACGATGAAGGAC GCAGCAAATTGCGAT AAGCATTATGACTTG CAGACTTCTGCGATT
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22 wie110_      AAACTTTCAGCGATG GATGTCTTGGCTCAC ACAACGATGAAGGAC GCAGCAAATTGCGAT AAGCATTATGACTTG CAGACTTCTGCGATT
23 casa10_      AAACTTTCAGCAATG GGTGTCTTGGCTCAC ACAACGATGAAGGAC GCAGCAAATTGCGAT AAGCATTATGACTTG CAGACTTCTGCGATT
24 casa11_      AAACTTTCAGCGATG GATGTCTTGGCTCAC ACAACGATGAAGGAC GCAGCAAATTGCGAT AAGCATTATGACTTG CAGACTTCTGCGATT

25 casb5_       AAACTTTCAGCGATG GATGTCTTGGCTCAC ACAACGATGAAGGAC GCAGCAAATTGCGAT AAGCATTATGACTTG CAGACTTCTGCGATT
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27 casb2_       AAACTTTCAGCGATG GATGTCTTGGCTCAC ACAACGATGAAGGAC GCAGCAAATTGCGAT AAGCATTATGACTTG CAGACTTCTGCGATT
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29 txea7_       AAACTTTCAGCGATG GATGTCTTGGCTCAC ACAACGATGAAGGAC GCAGCAAATTGCGAT AAGCATTATGACTTG CAGACTTCTGCGATT
30 txea18_      AAACTTTCAGCGATG GATGTCTTGGCTCAC ACAACGATGAAGGAC GCAGCAAATTGCGAT AAGCATTATGACTTG CAGACTTCTGCGATT
31 txea14_      AAACTTTCAGCGATG GATGTCTTGGCTCAC ACAACGATGAAGGAC GCAGCAAATTGCGAT AAGCATTATGACTTG CAGACTTCTGCGATT
32 wie18_       AAACTTTCAGCGATG GATGTCTTGGCTCAC ACAACGATGAAGGAC GCAGCAAATTGCGAT AAGCATTATGACTTG CAGACTTCTGCGATT

33 txwbb1_      AAACTTTCAGCGATG GATGTCTTGGCTCAC ACAACGGTGAAGGAC GCAGCAAATTGCGAT AAGCATTATGACTTG CAGACTTCTGCGATT
34 txwbb14_     AAACTTTCAGCGATG GATGTCTTGGCTCAC ACAACGATGAAGGAC GCAGCAAATTGCGAT AAGCATTATGACTTG CAGACTTCTGCGATT
35 txea24_      AAACTTTCAGCGATG GATGTCTTGGCTCAC ACAACGATGAAGGAC GCAGCAAATTGCGAT AAGCATTATGACTTG CAGACTTCTGCGATT
36 txeb8_       AAACTTTCAGCGATG GATGTCTTGGCTCAC ACAACGATGAAGGAC GCAGCGAATTGCGAT AAGCATTATGACTTG CAGACTTCTGCGATT
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37 okw11_    AAACTTTCAGCGATG GATGTCTTGGCTCAC ACAACGATGAAGGAC GCAGCAAATTGCGAT AAGCATTATGACTTG CAGACTTCTGCGATT
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43 nhe74_    AAACTTTCAGCGATG GATGTCTTGGCTCAC ACAACGATGAAGGAC GCAGCAAATTGCGAT AAGCATTATGACTTG CAGACTTCTGCGATT
44 nhe79_    AAACTTTCAGCGATG GATGTCTTGGCTCAC ACAACGATGAAGGAC GCAGCAAATTGCGAT AAGCATTATGACTTG CAGACTTCTGCGATT

45 nyr21_    AAACTTTCAGCGATG GATGTCTTGGCTCAC ACAACGATGAAGGAC GCAGCAAATTGCGAT AAGCATTATGACTTG CAGACTTCTGCGATT
46 nyr25_    AAACTTTCAGCGATG GATGTCTTGGCTCAC ACAACGATGAAGGAC GCAGCAAATTGCGAT AAGCATTATGACTTG CAGACTTCTGCGATT
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48 nyr23_    AAACTTTCAGCGATG GATGTCTTGGCTCAC ACAACGATGAAGGAC GCAGCAAATTGTGAT AAGCATTATGACTTG CAGACTTCTGCGATT

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51 nyr17_    AAACTTTCAGCGATG GATGTCTTGGCTCAC ACAACGATGAAGGAC GCAGCAAATTGCGAT AAGCATTATGACTTG CAGACTTCTGCGATT
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53 wie213_   AAACTTTCAGCGATG GATGTCTTGGCTCAC ACAACGATGAAGGAC GCAGCAAATTGCGAT AAGCATTATGACTTG CAGACTTCTGCGATT
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55 maw3_     AAACTTTCAGCGATG GATGTCTTGGCTCAC ACAACGATGAAGGAC GCAGCAAATTGCGAT AAGCATTATGACTTG CAGACTTCTGCGATT
56 ine11_    AAACTTTCAGCGATG GATGTCTTGGCTCAC ACAACGATGAAGGAC GCAGCAAATTGCGAT AAGCATTATGACTTG CAGACTTCTGCGATT

57 ine14_    AAACTTTCAGCGATG GATGTCTTGGCTCAC ACAACGATGAAGGAC GCAGCAAATTGCGAT AAGCATTATGACTTG CAGACTTCTGCGATT
58 mnmo25_   AAACTTTCAGCGATG GATGTCTTGGCTCAC ACAACGATGAAGGAC GCAGCAAATTGCGAT AAGCATTATGACTTG CAGACTTCTGCGATT
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61 mnw1_     AAACTTTCAGCGATG GATGTCTTGGCTCAC ACAACGATGAAGGAC GCAGCAAATTGCGAT AAGCATTATGACTTG CAGACTTCTGCGATT
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66 txeb1_    AAACTTTCAGCGATG GATGTCTTGGCTCAC ACAACGATGAAGGAC GCAGCAAATTGCGAT AAGCATTATGACTTG CAGACTTCTGCGATT
67 txeb2_    AAACTTTCAGCGATG GATGTCTTGGCTCAC ACAACGATGAAGGAC GCAGCAAATTGCGAT AAGCATTATGACTTG CAGACTTCTGCGATT
68 okw3_     AAACTTTCAGCGATG GATGTCTTGGCTCAC ACAACGATGAAGGAC GCAGCAAATTGCGAT AAGCATTATGACTTG CAGACTTCTGCGATT

69 txwe1_    AAACTTTCAGCGATG GATGTCTTGGCTCAC ACAACGATGAAGGAC GCAGCAAATTGCGAT AAGCATTATGACTTG CAGACTTCTGCGATT
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2  car5_      TAACAGACCTCTGAA CGTAACAAACACACC GCCTCTGCTCGCATG CGGTACTCCCGTTTC AGTGAGCCC
3  car8_      CAACAGACCTCTGAA CGTAACAAACACACC GCCTCTGCTCGCATG CGGTACTCCCGTTTC AGTGAGCCC
4  mnc3_      TAACAGACCTCTGAA CGTAACAAACACACC GCCTCTGCTCGCATG CGGTACTCCCGTTTC AGTGAGCCC

5  mnc8_      TAACAGACCTCTGAA CGTAACAAACACACC GCCTCTGCTCGCATG CGGTACTCCCGTTTC AGTGAGCCC
6  mnc12_     TAACAGACCTCTGAA CGTAACAAACACACC GCCTCTGCTCGCATG CGGTACTCCCGTTTC AGTGAGCCC
7  par3_      TAACAGACCTCTGAA CGTAACAAACACACC GCCTCTGCTCGCATG CGGTACTCCCGTTTC AGTGAGCCC
8  par8_      TAACAGACCTCTGAA CGTAACAAACACACC GCCTCTGCTCGCATG CGGTACTCCCGTTTC AGTGAGCCC

9  par9_      TAACAGACCTCTGAA CGTAACAAACACACC GCCTCTGCTCGCATG CGGTACTCCCGTTTC AGTGAGCCC
10 mnmo14_    TAACAGACCTCTGAA CGTAACAAACACACC GCCTCTGCTCGCATG CGGTACTCCCGTTTC AGTGAGCCC
11 mnmo15_    TAACAGACCTCTGAA CGTAACAAACACACC GCCTCTGCTCGCATG CGGTACTCCCGTTTC AGTGAGCCC
12 mnmo18_    TAACAGACCTCTGAA CGTAACAAACACACC GCCTCTGCTCGCATG CGGTACTCCCGTTTC AGTGAGCCC

13 wir24_     TAACAGACCTCTGAA CGTAACAAACACACC GCCTCTGCTCGCATG CGGTACTCCCGTTTC AGTGAGCCC
14 txwba3_    TAACAGACCTCTGAA CGTAACAAACACACC GCCTCTGCTCGCATG CGGTACTCCCGTTTC AGTGAGCCC
15 txwba5_    TAACAGACCTCTGAA CGTAACAAACACACC GCCTCTGCTCGCATG CGGTACTCCCGTTTC AGTGAGCCC
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17 txwbb21_   TAACAGACCTCTGAA CGTAACAAACACACC GCCTCTGCTCGCATG CGGTACTCCCGTTTC AGTGAGCCC
18 mnc10_     TAACAGACCTCTGAA CGTAACAAACACACC GCCTCTGCTCGCATG CGGTACTCCCGTTTC AGTGAGCCC
19 wir25_     TAACAGACCTCTGAA CGTAACAAACACACC GCCTCTGCTCGCATG CGGTACTCCCGTTTC AGTGAGCCC
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21 wie19_     TAACAGACCTCTGAA CGTAACAAACACACC GCCTCTGCTCGCATG CGGTACTCCCGTTTC AGTGAGCCC
22 wie110_    TAACAGACCTCTGAA CGTAACAAACACACC GCCTCTGCTCGCATG CGGTACTCCCGTTTC AGTGAGCCC
23 casa10_    TAACAGACCTCTGAA CGTAACAAACACACC GCCTCTGCTCGCATG CGGTACTCCCGTTTC AGTGAGCCC
24 casa11_    TAACAGACCTCTGAA CGTAACAAACACACC GCCTCTGCTCGCATG CGGTACTCCCGTTTC AGTGAGCCC

25 casb5_     TAACAGACCTCTGAA CGTAACAAACACACC GCCTCTGCTCGCATG CGGTACTCCCGTTTC AGTGAGCCC
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28 casa14_    TAACAGACCTCTGAA CGTAACAAACGCACC GCCTCTGCTCGCATG CGGTACTCCCGTTTC AGTGAGCCC

29 txea7_     TAACAGACCTCTGAA CGTAACAAACACACC GCCTCTGCTCGCATG CGGTACTCCCGTTTC AGTGAGCCC
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35 txea24_    TAACAGACCTCTGAA CGTAACAAACACACC GCCTCTGCTCGCATG CGGTACTCCCGTTTC AGTGAGCCC
36 txeb8_     TAACAGACCTCTGAA CGTAACAAACACACC GCCTCTGCTCGCATG CGGTACTCCCGTTTC AGTGAGCCC
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38 okw8_     TAACAGACCTCTGAA CGTAACAAACACACC GCCTCTGCTCGCATG CGGTACTCCCGTTTC AGTGAGCCC
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41 maw5_     TAACAGACCTCTGAA CGTAACAAACACACC GCCTCTGCTCGCATG CGGTACTCCCGTTTC AGTGAGCCC
42 nhe80_    TAACAGACCTCTGAA CGTAACAAACACACC GCCTCTGCTCGCATG CGGTACTCCCGTTTC AGTGAGCCC
43 nhe74_    TAACAGACCTCTGAA CGTAACAAACACACC GCCTCTGCTCGCATG CGGTACTCCCGTTTC AGTGAGCCC
44 nhe79_    TAACAGACCTCTGAA CGTAACAAACACACC GCCTCTGCTCACATG CGGTACTCCCGTTTC AGTGAGCCC

45 nyr21_    TAACAGACCTCTGAA CGTAACAAACACACC GCCTCTGCTCGCATG CGGTACTCCCGTTTC AGTGAGCCC
46 nyr25_    TAACAGACCTCTGAA CGTAACAAACACACC GCCTCTGCTCGCATG CGGTACTCCCGTTTC AGTGAGCCC
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52 maw9_     TAACAGACCTCTGAA CGTAACAAACACACC GCCTCTGCTCGCATG CGGTACTCCCGTTTC AGTGAGCCC

53 wie213_   TAACAGACCTCTGAA CGTAACAAACACACC GCCTCTGCTCGCATG CGGTACTCCCGTTTC AGTGAGCCC
54 ine6_     TAACAGACCTCTGAA CGTAACAAACACACC GCCTCTGCTCGCATG CGGTACTCCCGTTTC AGTGAGCCC
55 maw3_     TAACAGACCTCTGAA CGTAACAAACACACC GCCTCTGCTCGCATG CGGTACTCCCGTTTC AGTGAGCCC
56 ine11_    TAACAGACCTCTGAA CGTAACAAACACACC GCCTCTGCTCGCATG CGGTACTCCCGTTTC AGTGAGCCC

57 ine14_    TAACAGACCTCTGAA CGTAACAAACACACC GCCTCTGCTCGCATG CGGTACTCCCGTTTC AGTGAGCCC
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63 mnw14_    TAACAGACCTCTGAA CGTAACAAACACACC GCCTCTGCTCGCATG CGGTACTCCCGTTTC AGTGAGCCC
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67 txeb2_    TAACAGACCTCTGAA CGTAACAAACACACC GCCTCTGCTCGCATG CGGTACTCCCGTTTC AGTGAGCCC
68 okw3_     TAACAGACCTCTGAA CGTAACAAACACACC GCCTCTGCTCGCATG CGGTACTCCCGTTTC AGTGAGCCC

69 txwe1_    TAACAGACCTCTGAA CGTAACAAACACACC GCCTCTGCTCGCATG CGGTACTCCTGTTTC AGTGAGCCC
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71 txwe6_    TAACAGACCTCTGAA CGTAACAAACACACC GCCTCTGCTCGCATG CGGTACTCCCGTTTC AGTGAGCCC
72 txwe2_    TAACAGACCTCTGAA CGTAACATACACACC GCCTCTGCTCGCATG CGGTACTCCCGTTTC AGTGAGCCC
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                            1              2       3          4                   5                6
                585 586     _ 600 601     __  615 616  ______630 631         _645 646         ___660 661        675
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2  car5_     CCTTTC CTAAAGGTGACAACC -TTTGCTGTGGCTCG CCATGGCACTTGGTT GTGTGGCCTTTGCGA GTGGGTGTTTTTATG GGCACCCCAATTTCG
3  car8_     CCTTTC CTAAAGGTGACAACC -TTTGCTGTGGTCTA CCGTGGCACTTGGTT GTGTGGCCTTTGCGA GTGGGTGTTTTCGTG GGCACCCCAATTTCG
4  mnc3_     CCTTTC CTAAAGGTGACAACC CTTTGCTATGGTTTA CCATGGTATCGGGTT GTGTGGCCTTTGAGA GTGGGTGTTTTGGAA ATCACCCCAATTTCG

5  mnc8_     CCTTTC CTAAAGGTGACAACC CTTTGCTATGGTTTA CCATGGTATCGGGTT GTGTGGCCTTTGAGA GTGGGTGTTTTGGAA ATCACCCCAATTTCG
6  mnc12_    CCTTTC CTAAAGGTGACAACC CTTTGCTATGGTTTA CCATGGTATCGAGTT GTGTGGCCTTTGAGA GTGGGTGTTTTGGAA ATCACCCCAATTTCG
7  par3_     CCTTTC CTAAAGGTGACAACC CTTTGCTATGGTTTA CCGTGGCACCGGGTT GTGTGGCCTTTGAGA GTGGGTGTTTTGGAA ATCACCCCAATTTCG
8  par8_     CCTTTC CTAAAGGTGACAACC CTTTGCTATGGTTTA CCGTGGCGCTGGGTT GTGTGGCCTTTGAGA GTGGGTGTTTTGGAA ATCACCCCAATTTCG

9  par9_     CCTTTC CTAAAGGTGACAACC CTTTGCTATGGTTTA CCGTGGCGCTGGGTT GTGTGGCCTTTGAGA GTGGGTGTTTTGGAA ATCACCCCAATTTCG
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12 mnmo18_   CCTTTC CTAAAGGTGGCAACC CTTTGCTATGGTTTA CCGTGGCACCGGGTT GTGTGGCCTTTGAGA GTGGGTGTTTTGGAA ATCACCCCAATTTCG

13 wir24_    CCTTTC CTAAAGGTGGCAACC CTTTGCTATGGTTTA CCGTGGCGCTGGGTT GTGTGGCCTTTGAGA GTGGGTGTTTTGGAA ATCACCCCAATTTCG
14 txwba3_   CCTTTC CTAAAGGTGGCAACC CTTTGCTGCGGTCTA CCGTGGTATCGGGTT GTGTGGCCTTTGAGA GTGGGTGTTTTGGAA ATCACCCCAATTTCG
15 txwba5_   CCTTTC CTAAAGGTGGCAACC CTTTGCTGCGGTCTA CCGTGGTATCGGGTT GTGTGGCCTTTGAGA GTGGGTGTTTTGGAA ATCACCCCAATTTCG
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17 txwbb21_  CCTTTC CTAAAGGTGGCAACC CTTTGCTGCGGTCTA CCGTGGTATCGGGTT GTGTGGCCTTTGAGA GTGGGTGTTTTGGAA ATCACCCCAATTTCG
18 mnc10_    CCTTTC CTAAAGGTGACAACC CTTTGCTATGGTTTA CCATGGTATCGGGTT GTGTGGCCTTTGAGA GTGGGTGTTTTGGAA ATCACCCCAATTTCG
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23 casa10_   CCTTTC CTAAAGGTGGCAACC CTTTGCTGCGGTTTA CCGTGGCACCGGGTT GTGTGGCCTTTGAGA GTGGGTGTTTTGGGA ATCACCCCAATTTCG
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28 casa14_   CCTTTC CTAAAGGTGGCAACC CTTTGCTGCGGTTTA CCGTGGCACCGGGTT GTGTGGCCTTTGAGA GTGGGTGTTTTGGGA ATCACCCCAATTTCG

29 txea7_    CCTTTC CTAAAGGTGGCAACC CTTTGCTATGGTTTA CTATGGTATCGGGTT GTGTGGCCTTTGAGA GTGGGTGTTTTGGAA ATCACCCCAATTTCG
30 txea18_   CCTTTC CTAAAGGTGGCAACC CTTTGCTATGGTTTA CTATGGTATCGGGTT GTGTGGCCTTTGAGA GTGGGTGTTTTGGAA ATCACCCCAATTTCG
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35 txea24_   CCTTTC CTAAAGGTGGCAACC CTTTGCTATGGTTTA CTATGGTATCGGGTT GTGTGGCCTTTGAGA GTGGGTGTTTTGGAA ATCACCCCAATTTCG
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1 **2** **3** **4** **5** **6**

585 586 _ 600 601 __ 615 616 ______630 631 645 646 660 661 675

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41 maw5_ CCTTTC CTAAAGGTG**G**CAACC CTTTGCT**AT**GGT**TTA CCA**TGG**CGCTGG**GTT GTGTGGCCTTTG**A**GA GTGGGTGTTTTG**GAA** ATCACCCCAATTTCG
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45 nyr21_ CCTTTC CTAAAGGTG**G**CAACC CTTTGCT**AT**GGT**TTA CCA**TGA**CACTGG**GTT GTGTGGCCTTTG**A**GA GTGGGTGTTTTG**GAA** ATCACCCCAATTTCG
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53 wie213_ CCTTTC CTAAAGGTG**G**CAACC CTTTGCT**AT**GGT**TTA CCA**TGG**CACTGG**GTT GTGTGGCCTTTG**A**GA GTGGGTGTTTTG**GAA** ATCACCCCAATTTCG
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57 ine14_ CCTTTC CTAAAGGTG**A**CAACC CTTTGCT**GC**GGT**CTA CCG**TGG**CACCGG**GTT GTGTGGCCTTTG**A**GA GTGGGTGTTTTG**GAA** ATCACCCCAATTTCG
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69 txwe1_ CCTTTC CTAAAGGTG**G**CAACC CTTTGCT**AT**GGT**TTA CTA**TGG**CGCTGG**GTT GTGTGGCCTTTG**C**GA GTGGGTGTTTTG**GAA** ATCACCCCAATTTCG
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3  car8_    ATAGCACGCTGCCGA GCATTACCACGTGTG ATCTCGAGGCTCTTT GTTGTAATTTATTAC TCTAGGCCTCTTTGA GGTGTGCGGCTGCGT
4  mnc3_    ATAGCACGCTGCCGA GTATTACCACGTGTG ATCTCGAGGCTCTCT GTTGTAATTTATTAC TCTAGGCTTCTGTGA GATGTGCAGCTGTGT

5  mnc8_    ATAGCACGCTGCCGA GTATTACCACGTGTG ATCTCGAGGCTCTCT GTTGTAATTTATTAC TCTAGGCTTCTGTGA GATGTGCAGCTGTGT
6  mnc12_   ATAGCACGCTGCCGA GTATTACCACGTGTG ATCTCGAGGCTCTCT GTTGTAATTTATTAC CCTAGGCTTCTGTGA GATGTGCAGCTGTGT
7  par3_    ATAGCACGCTGCCGA GTATTACCACGTGTG ATCTCGAGGCTCTCT GTTGTAATTTATTAC TCTAGGCTTCTGTGA GATGTGTAGCTGTGT
8  par8_    ATAGCACGCTGCCGA GTATTACCACGTGTG ATCTCGAGGCTCTCT GTTGTAATTTATTAC TCTAGGCTTCTGTGA GATGTGCAGCTGTGT

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13 wir24_   ATAGCACGCTGCCGA GTATTACCACGTGTG ATCTCGAGGCTCTCT GTTGTAATTTATTAC TCTAGGCTTCTGTGA GATGTGCAGCTGTGT
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18 mnc10_   ATAGCACGCTGCCGA GTATTACCACGTGTG ATCTCGAGGCTCTCT GTTGTAATTTATTAC TCTAGGCTTCTGTGA GATGTGCAGCTGTGT
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23 casa10_  ATAGCACGCTGCCGA GTATTACCACGTGTG ATCTCGAGGCTCTCT GTTGTAATTTATTAC TCTAGGCTTCTGTGA GATGTGCAGCTGTGT
24 casa11_  ATAGCACGCTGCCGA GTATTACCACGTGTG GTCTCGAGGCTCTCT GTTGTAATTTATTAC TCTAGGCTTCTGTGA GATGTGCAGCTGTGT

25 casb5_   ATAGCACGCTGCCGA GTATTACCACGTGTG ATCTCGAGGCTCTCT GTTGTAATTTATTAC TCTAGGCTTCTGTGA GATGTGCAGCTGTGT
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36 txeb8_   ATAGCACGCTGCCGA GTATTACCACGTGTG ATCTCGAGGCTCTCT GTTGTAATTTATTAC TCTAGGCTTCTGTGA GATGTGCAGCTGTGT
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           676          690 691          705 706          720 721          735 736          750 751     _   765
37 okw11_  ATAGCACGCTGCCGA GTATTACCACGTGTG ATCTCGAGGCTCTCT GTTGTAATTTATTAC TCTAGGCTTCTGTGA GATGTGCAGCTGTGT
38 okw8_   ATAGCACGCTGCCGA GTATTACCACGTGTG ATCTCGAGGCTCTCT GTTGTAATTTATTAC TCTAGGCTTCTGTGA GATGTGCAGCTGTGT
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		766 ... 780	781 ... 795	796 ... 810	811 ... 825	826
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6	mnc12_	CGCG**GTC**T-CGTAC**C**	**GTGGTGCGGC**AAGTG	GC**TAATGCGT**GGCTG	TCGGTGCTGTATTGA	**C**TTTAT
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9	par9_	CGCG**GTC**T-CGTAC**T**	**GCGATATGGC**AAGTG	GC**TAATGCAT**GGCTG	TCGGTGCTGTATTGA	**C**TTTAT
10	mnmo14_	CGCG**GTC**T-CGTAC**T**	**GCGATATGGC**AAGTG	GC**TAATGCGT**GGCTG	TCGGTGCTGTATTGA	**C**TTTAT
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12	mnmo18_	CGCG**GTC**T-CGTAC**T**	**GCGATATGGC**AAGTG	GC**TAATGCGT**GGCTG	TCGGTGCTGTATTGA	**C**TTTAT
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39 mnw9_    CGCGGTCT-CGTACT GCGATATGGCAAGTG GCTAATGTGTGGCTG TCGGTGCTGTATTGA CTTTAT
40 mnw11_   CGCGGTCT-CGTACT GCGATATGGCAAGTG GCTAATGCGTGGCTG TCGGTGCTGTATTGA CTTTAT

41 maw5_    CGCGGTCT-CGTACT GCGATGCGGCAAGTG GCTAATGCGTGGCTG TCGGTGCTGTATTGA CTTTAT
42 nhe80_   CACGGTACTCGTACT GCGATATGGCAAGTG GCAAATGCGTGGCTG TCGGTGCTGTATTGA CTTTAT
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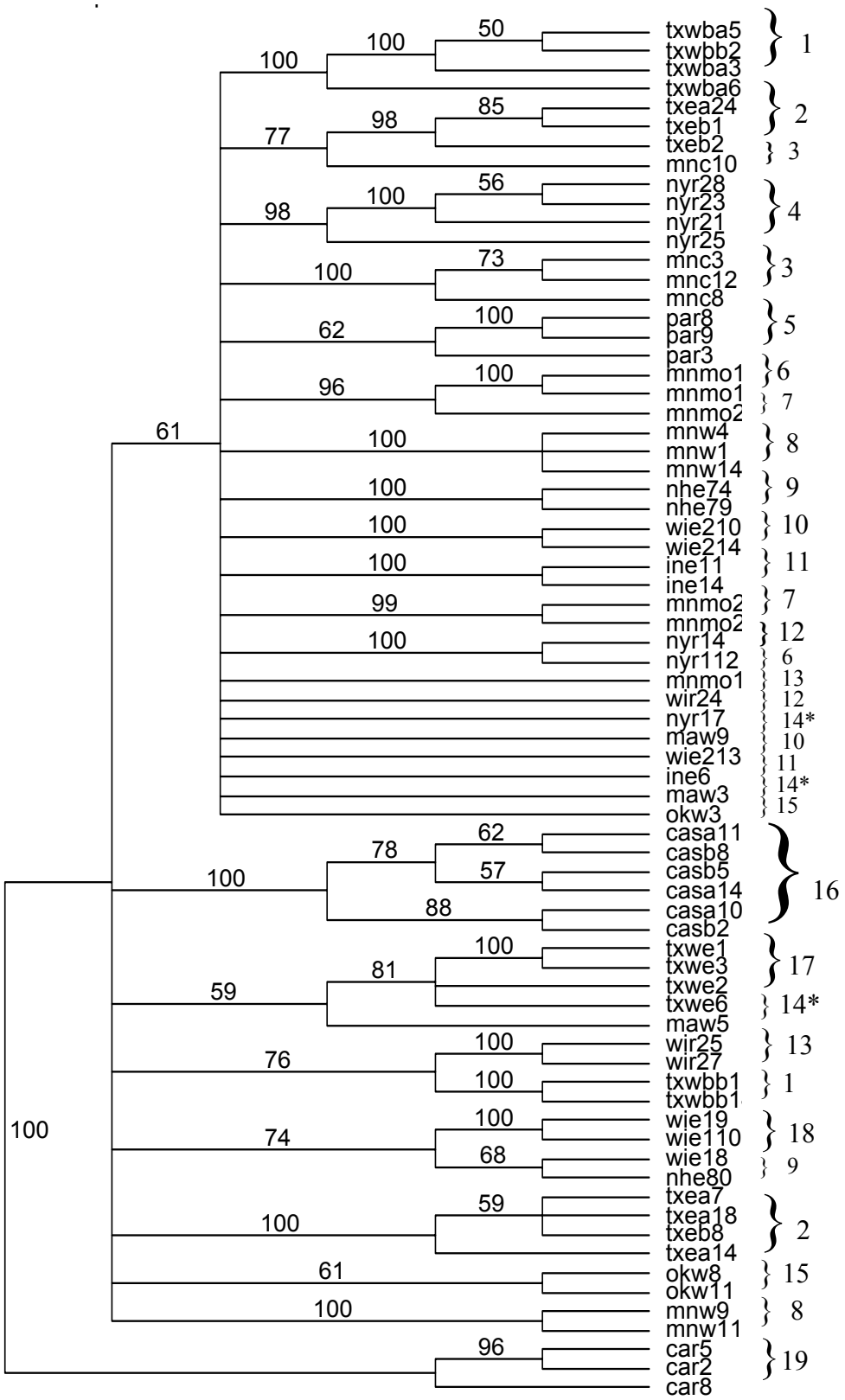
APPENDIX C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2002	B.S. Biology, University of Texas at Dallas, Richardson, Texas, USA.

PUBLICATIONS

HOLMAN, P. J., K. G. BENDELE, L. SCHOELKOPF, R. L. JONES-WITTHUHN, AND S. O. JONES. 2003. Ribosomal RNA analysis of *Babesia odocoilei* isolates from farmed reindeer (*Rangifer tarandus tarandus*) and elk (*Cervus elaphus canadensis*) in Wisconsin. Parasitology Research 91: 378-383.

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