

# Determination of White-Tailed Deer Agent *groESL* Operon Sequences for Phylogenetic and Diagnostic Applications

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The white-tailed deer agent (WTD agent) is an organism that commonly infects white-tailed deer (*Odocoileus virginianus*) and has been detected by PCR amplification of 16S rRNA gene sequences from blood samples collected from white-tailed deer in southeastern and south central regions of the United States and in California.<sup>1-5</sup> The 16S rDNA sequences reported for the WTD agent are most similar to sequences from *Anaplasma platys* and *A. phagocytophila*, and several of the PCR protocols commonly used for detection of *A. phagocytophila* also amplify WTD agent sequences.<sup>4</sup> The WTD agent has not been propagated in cell culture, and additional gene sequences have not been determined.

To aid in the phylogenetic placement of the WTD agent and to provide an alternate PCR target, we used wide-range *groESL* PCR primers derived from multiple sequence alignments to amplify WTD agent *groESL* heat-shock operon sequences from a platelet-enriched preparation made from the blood of a fawn inoculated with blood samples from three Georgia white-tailed deer. The amplified sequence (1,603 bp) spans the end of *groES*, an intergenic spacer 48 bp in length, and 1,534 bp of *groEL*. The nucleotide sequence was most similar to *groESL* sequences from *A. platys* (83%) and *A. phagocytophila* (80–83%). The deduced amino acid sequence for *groEL* (511 residues) was most similar to sequences inferred from the *groEL* sequences of *A. platys* (97.8% identity) and *A. phagocytophila* (95.8% identity). By comparison, complete *groEL* sequences have been determined for *A. platys* (AAK2609) and *A. phagocytophila* (AAL88677). Both contain 526 amino acid residues and are 96% identical. The similarities correlate with those observed for the respective 16S rDNA sequences, but with the *groESL* nucleotide sequences showing

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greater divergence. A 16S rDNA sequence amplified from the same sample was most similar (98–99%) to sequences previously reported for WTD agent sequences amplified from deer in Georgia and Oklahoma.<sup>2</sup> However, exact homologies could not be determined because the sequences recorded in GenBank contain several ambiguity codes.

To confirm that the putative WTD agent *groESL* sequence could be consistently associated with the WTD agent 16S rDNA sequence, blood samples from four Missouri deer shown to be positive for the WTD agent by nucleotide sequencing of partial 16S rDNA amplicons (496 bp) were tested by nested PCR using *groESL* primers derived from the sequence obtained from the Georgia deer. The partial *groESL* sequences obtained from the Missouri deer (609 bp) were identical to the overlapping sequence obtained from the Georgia deer. The nested *groESL* PCR did not amplify sequences from samples containing *A. phagocytophila* DNA.

An additional PCR target that is less conserved than the 16S rRNA gene should be helpful for studies of natural reservoir hosts and tick vectors of the WTD agent. These animals are often infected with several closely related species that have very similar 16S rDNA sequences.<sup>3,5</sup> It is also likely that novel (undescribed) *Anaplasma* and *Ehrlichia* species may be present.

Analyses of 16S rDNA sequences have indicated that the WTD agent is most closely related to *A. platys* and *A. phagocytophila*.<sup>2</sup> These relationships are further supported by similarities among the *groESL* sequences.

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