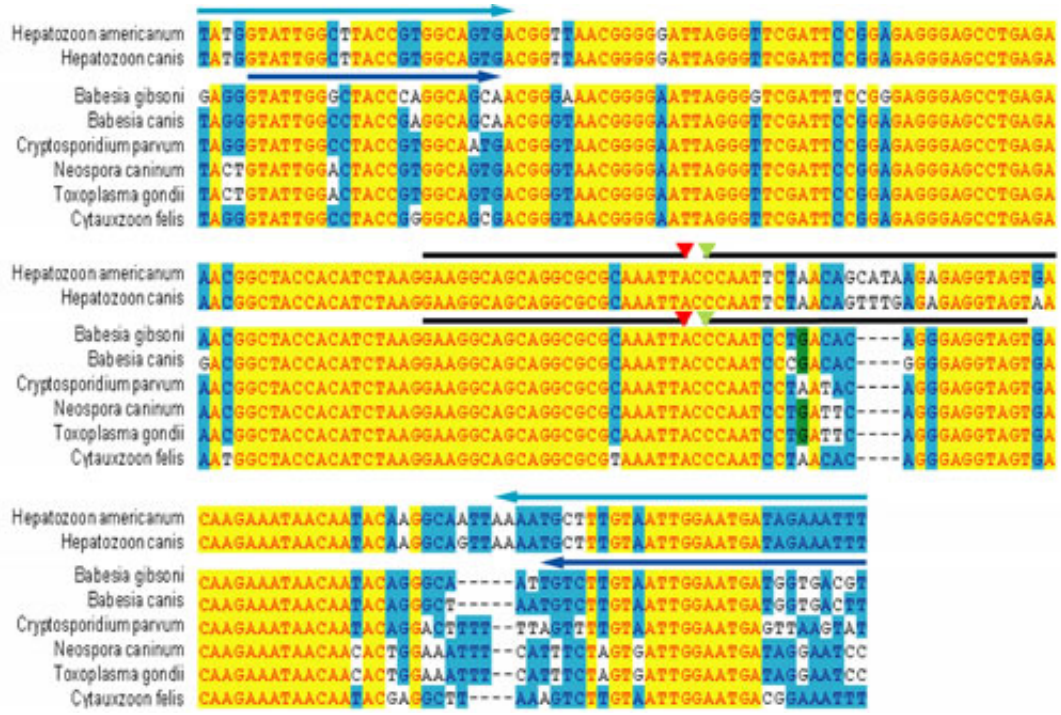
AUBURN UNIVERSITY
COLLEGE OF
VETERINARY MEDICINE

Specificity

All real-time PCR methods in the Auburn University Molecular Diagnostics Laboratory detect targets by measuring light emitted by fluorescent probes.

- The first level of high specificity is provided by highly specific primers.
- The second level of high specificity is provided by fluorescence resonance energy transfer (FRET) probe technology.
- Only simultaneous attachment of two probes to the amplified target molecule will generate red fluorescence.
- Simultaneous probe attachment allows the transfer of energy from the green-fluorescent dye (attached to probe 1) to the red fluorescent dye of probe 2.
- Red light emission is measured to detect and quantify the amplification product.
- Stringent requirement for attachment of both probes combined with highly specific primers generates virtually 100% specificity of each assay.
- The specificity of each assay is validated by DNA sequencing of the standard target.



Real-time PCR design for amplification of the DNA coding for the 18S rRNA of protozoal parasites. The alignment of homologous parasite sequences shows conserved and divergent sequence regions. Primers are shown as lines with arrows (*Hepatozoon americanum* or *H. canis*: light blue; *Babesia gibsoni* or *B. canis*: dark blue). Probes are shown as black lines, with the green triangle indicating the green fluorescent energy donor dye, and the red triangle indicating the energy acceptor dye that emits red fluorescence after stimulation of the donor dye by blue light. Specificity of the primers and probes is ensured by targeting sequence regions that differ between the parasites.