B83 A Short Interspersed Nuclear Element (SINE) -Based Multiplex Quantitative Real-Time Polymerase Chain Reaction (qPCR) Assay for Human-Dog-Cat Species Identification and DNA Quantification

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Learning Overview: After attending this presentation, attendees will understand the need for a robust animal species identification and quantification assay, principles of multiplex qPCR assay development, and the performance of SINE-based qPCR assays.

Impact on the Forensic Science Community: This presentation will impact the forensic science community by drawing attention to the use of animal DNA evidence in forensics, SINEs as potential markers for forensic DNA applications, and an assay model that could be implemented in animal-related casework.

Although a majority of households in the United States own a pet, resulting in the likely transfer of animal DNA during crimes, this evidence oftentimes remains unutilized by traditional crime labs, which primarily focus on the extraction, quantification, and Short Tandem Repeat (STR) genotyping of human DNA.[1] Historically, there have been numerous cases in which animal DNA has been used to link suspects to victims and/or crime scenes, sometimes serving as the only evidence producing such linkages and resulting in convictions.[2] One way to incorporate animal DNA analysis in the typical workflow of traditional crime labs is to simultaneously quantify human and animal DNA in an evidence sample rather than in separate reactions, allowing for the determination of the presence and quantity of animal DNA without additional consumption of DNA evidence. This information can then be used to inform the analyst's decision of whether to proceed with STR genotyping and for which species.

The purpose of this research was to develop a multiplex qPCR assay using primers and dual-labeled probes that target species-specific SINEs for the simultaneous quantification of human, dog, and cat DNA in mixed forensic samples. Existing animal qPCR assays typically target species-specific mitochondrial sequences or low-copy nuclear genes, each of which has its own limitations.[1] Mitochondrial assays are more sensitive, but less accurate and predictive of STR genotyping success, whereas low-copy nuclear assays are more accurate and predictive but less sensitive. As repetitive nuclear elements, SINEs possess the advantages of both without their respective limitations. It was thus hypothesized that a SINE-based multiplex qPCR assay will be more sensitive, quantitatively accurate, and reliable than previously published assays for quantifying animal DNA.

First, previously published primers targeting human-, dog-, and cat-specific SINEs were validated via endpoint PCR and agarose gel electrophoresis for species specificity and multiplex compatibility with commercial DNA standards.[3,4] Preliminary results indicate that each primer set does not display cross-amplification in the other two species' DNA templates. Bands of comparable brightness were observed in duplex reactions, indicating that human-, dog-, and cat-specific SINE-targeting primers are multiplex compatible. These validation studies were conducted on the GeneAmp® PCR System 9600 in 10µL reactions containing 1x AmpliTaq Gold™ 360 Master Mix, 0.2µM human primers, 0.3µM canine primers, 0.4µM feline primers, and 10ng of each species' genomic DNA standard. The instrument cycling conditions were an initial hold of 95°C for 12 minutes, 40 cycles at 95°C for 15 seconds, 68°C for 30 seconds, and 72°C for 15 seconds, followed by a final extension at 72°C for 7 minutes.

Then, new primers and dual-labeled probes were designed for multiplexing using National Center for Biotechnology Information's (NCBI's) Primer Basic Local Alignment Search Tool (BLAST) and Primer Express 3.0. Human-, dog-, and cat-specific amplicons are 140, 101, and 98bp respectively. qPCR reactions were conducted using the Invitrogen® Platinum II Taq Hot-Start DNA Polymerase kit. The instrument's cycling conditions were an initial hold of 94°C for two minutes, followed by 40 cycles at 94°C for 15 seconds, and 60°C for one minute. Ten microliter singleplex reactions were conducted on the ABI® 7500 Real Time PCR system and contained 1X Platinum II PCR Buffer, 0.2mM dNTPs, 0.2µM forward and reverse primers, 0.1µM Taqman probe, and 0.8units of Taq. Early results indicate a PCR efficiency of 98.1%, an R^2 value of 0.98, and a detection limit of 10pg.

PCR conditions are being optimized in singleplex reactions for each primer/probe set to minimize the Ct value and maximize the amplification efficiency and linear dynamic range. A multiplex detection assay is in development and being optimized for balanced amplification of multiple targets with amplification curves comparable to their respective singleplex reactions. Forensic conditions are being simulated by performing each assay on DNA extracted from mixtures of human, dog, and cat blood at various concentrations.

Reference(s):

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2. Menotti-Raymond, M.A., David, V.A., and O'Brien, S.J. Pet cat hair implicates murder suspect. *Nature*. 386(1997): 774.
3. Walker, J.A., Kilroy, G.E., Xing, J., Shewale, J., Sinha, S.K., Batzer, M.A. Human DNA quantitation using Alu element-based polymerase chain reaction. *Analytical Biochemistry*. 315(2003): 122-128.
4. Walker, J.A., Hughes, D.A., Hedges, D.J., et al. Quantitative PCR for DNA identification based on genome-specific interspersed repetitive elements. *Genomics*. 83(2004): 518-527.

Animal, DNA, Quantification

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