B141 The RoarPlex – A Novel Tetranucleotide Microsatellite and Sex Identification Panel

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After attending this presentation, attendees will recognize the natural research barriers wildlife genetic studies pose and how to successfully overcome them to learn more about the species of focus. A better understanding of the benefits tetranucleotide microsatellite markers provide in comparison to their dinucleotide counterparts will also be accomplished through the analysis of the designed RoarPlex.

Due to the constantly evolving and largely emerging nature of wildlife forensics, the RoarPlex could have a tremendous influence on future studies involving large felid species. This presentation will impact the forensic science community by providing exposure to the wildlife forensics field and illustrating what can be accomplished using a forensic skill set to aid in wildlife conservation efforts.

The snow leopard (*Panthera uncia*) is an elusive species native to the mountainous regions of Central and South Asia. Targeted for its fur and bones or to protect the livestock upon which they prey, the snow leopard is an endangered species that requires immediate conservation action. Due to the snow leopard's cryptic nature, research based on direct observations required for effective conservation is problematic and can be supplemented by non-invasive surveys and genetic analysis.

A multiplex containing eight tetranucleotide microsatellite markers and a sex-determining region Y marker was developed to aid in the investigation of illegal killings of snow leopards and to assist with population abundance and distribution studies on a larger geographic scale. Previously, studies relied on dinucleotide repeats that were complicated to score and difficult to combine across data sets. Dinucleotide microsatellite markers are vulnerable to errors associated with high stutter percentages and misinterpretation of adenylylation stutter. The previous panel also included only four microsatellite markers in one reaction. Tetranucleotide repeats drastically reduce the errors caused by slippage and decrease the visual impact of adenylylation stutter, making for unambiguous allele interpretation. The addition of more microsatellite markers in one reaction exponentially increases the individual identification information available, a component comparable to the Combined DNA Index System (CODIS) increasing the number of core loci accepted. An allelic ladder was developed to ensure accurate allele designation across laboratories, with validation according to the Scientific Working Group on DNA Analysis Methods (SWGDAM) and the International Society of Forensic Genetics (ISFG) recommendations. To accomplish the multiplex design, 16 tetranucleotide microsatellite markers originating in the domestic cat (*Felis catus*) were screened. Results indicated that 15 markers could be successfully amplified using the available primer sets due to common ancestry and similar genome structure. Using M13 labeling, the 15 amplified microsatellites were fluorescently tagged and genotyped. Resulting peaks of the successful microsatellites were more distinct and allele calling was simplified. An informative multiplex containing eight of the most optimal microsatellite markers was constructed through selective data analysis, primer redesign, and compatibility determination. A sex-determining region Y marker was inserted into the reaction mix to simultaneously accomplish sex identification. The tetranucleotide panel provided more information, including sex, in one reaction, reducing the cost, error, and time required to perform the assay.

The enhanced panel and allelic ladder simultaneously improved research methods, assisted with transboundary initiatives, and enabled data sharing, thereby increasing the impact of population studies. Despite multiplex design being focused on the snow leopard, initial studies indicate that the RoarPlex could be successful in obtaining the individual identification of other large felids, including the bobcat (*Lynx rufus*), lion (*Panthera leo*), and cheetah (*Acinonyx jubatus*), expanding its utility. Due to the constantly evolving and largely emerging nature of wildlife forensics, the RoarPlex could have a tremendous influence on future studies involving large felid species.

STR, Tetranucleotide, Multiplex