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SNP genotyping to monitor wild tigers for conservation

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Abstract

Tigers have experienced dramatic range contraction in the recent past and currently occupy only 7% of their historical range [1]. Genetic tools can be used effectively to monitor wild species of conservation concern such as tigers. Such approaches allow us to identify individuals, reconstruct relatedness between them, and monitor connectivity between populations. Microsatellite markers are currently used across many laboratories to study tiger population and conservation genetics. However, non-invasive samples such as feces continue to present challenges with high error rates and low amplification success for such microsatellite loci [2]. In this study, we developed a panel of Single Nucleotide Polymorphism (SNP) markers and experimental pipeline for use with fecal samples from wild tigers. Multiplex PCR followed by Illumina sequencing of pooled, barcoded samples allowed fast implementation of these protocols. A total of 339 SNPs were targeted and amplified in short fragments of 40 base pairs. All samples were run in triplicate to investigate error among replicates. In the first run the protocol was tested using captive tiger fecal samples of different ancestry and a varying target DNA concentration. Following this, non-invasive samples (fecal, saliva and shed hair) collected from multiple field sites across India were tested. Results revealed that samples with very low initial concentration of target DNA (<1ng) had high genotyping success. The observed probability of identity (the probability of obtaining the same genotype for two different individuals) was found to be very low (PID = 2.4E-85, PID sibs = 4.4E-44). Replicate genotypes of the same sample were highly similar with rare occurrence of mismatches. No differences were observed in success rate for the different ancestries. Application of the protocol to fecal samples collected from wild tigers showed that success was independent of sample type. Initial concentration of template DNA seems to govern success, and generally samples with 0.02 ng/µl or higher showed high genotyping success. Replicate genotypes were still highly consistent. In summary, we demonstrate the utility of a highly multiplexed SNP genotyping protocol from non-invasively collected tiger samples. We suggest that such protocols will help in generating data faster, cheaper, while also being compatible across labs.

References

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