

## Developing effective markers for censusing of Sumatran and Javan rhinos by local researchers

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The two most endangered species of rhinoceros are the Javan rhinoceros (*Rhinoceros sondaicus*) and the Sumatran rhinoceros (*Dicerorhinus sumatrensis*), both restricted in the wild to small populations in Indonesia. To permit censusing of these two species non-invasively, we developed microsatellite markers for use with fecal DNA. Since the available samples are often limited for endangered species such as rhinos, we developed a bioinformatics routine that would rely on just one or two individuals. Large numbers of next generation sequencing (NGS) reads were generated and screened to find loci with short tandem repeats (STRs or microsatellites), excluding those in repetitive elements. The bioinformatics routine determined whether the locus was polymorphic in the individual(s) sequenced, precluding the need for large-scale laboratory screening of markers to determine which were polymorphic. We modified PCR conditions and routines to maximize success when using DNA from fecal samples, relying on the more widely available samples from black rhinoceros (*Diceros bicornis*) for initial testing of modifications. The Javan rhinoceros historically ranged from the northern Indian subcontinent to the Indonesian islands of Sumatra and Java. The only remaining population of ca. 60 rhinos occurs in Ujung Kulon National Park in Java. For Javan rhinoceros, neither modern nor fecal DNA samples were available. We instead obtained bone samples from museum specimens, isolating their DNA in an ancient DNA facility. Specimens were sequenced for mitochondrial control region to verify their species and their origin in the island of Java. Four DNA samples were subject to NGS, of which one had a large proportion (ca. 40%) of target species DNA. From this museum specimen, 915 potentially polymorphic STR loci were identified using bioinformatics. Primers designed for these loci are initially being tested for their ability to amplify DNA in the related Indian rhinoceros (*R. unicornis*), and will then be tested for polymorphisms in Javan rhinoceros samples collected from the wild. The Sumatran rhinoceros, previously widespread across Southeast Asia, now consists of ca. 100 individuals in isolated populations in Borneo and Sumatra. We used DNA from 2 blood samples from North American zoos for NGS, conducting a similar bioinformatics routine to identify polymorphic STR loci. From 861 potentially polymorphic STR primers identified from NGS, a set of 29 that were variable will be further tested and used to genotype non-invasively collected fecal samples from wild rhinos. Based on PID and PID(sib) values, the Sumatran rhino markers should be sufficient for identification of individuals. For both species, development of genetic markers can aid in conservation and management through assessment of genetic diversity and relatedness, identification of conservation units, and census estimates. Funding from the International Rhino Foundation, US Fish and Wildlife Service, World Wildlife Fund, National Science and Engineering Research Council (Canada), UIUC ACES Office of International Programs Seed Grant and UIUC ACES Graduate Student International Research Grant.