

GENOMIC RESOURCES NOTE

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Abstract

This article documents the public availability of (i) transcriptome sequence data and assembly for the rostrum dace (*Leuciscus burdigalensis*) naturally infected by a copepod ectoparasite (*Tracheliastes polycolpus*) and (ii) SNPs identified and validated from RAD sequencing for the Ugandan red colobus (*Procolobus rufomitratus tephrosceles*) using RAD sequencing.

Table 1 contains information on the focal species, data type and resource developed, as well as access details for the data. The authors responsible for each genomic resource are listed in the final

column. Full descriptions of how each resource was developed and tested are uploaded as Supplemental Information with the online version of this manuscript.

Table 1 Information on the focal species, data type and resource developed, as well as access details for the data. The authors responsible for each genomic resource are listed in the final column

Species (no. of individuals)	Data type	Resources	Authors
<i>Leuciscus burdigalensis</i> (8)	Transcriptome sequencing and assembly	Transcriptome sequence data: NCBI BioProject PRJNA264971 Contig assembly: doi:10.5061/dryad.6365v Blast hits (with <i>Danio rerio</i> cDNA database): doi:10.5061/dryad.6365v	Olivier Rey, Géraldine Loot, Olivier Bouchez, Simon Blanchet
<i>Procolobus rufomitratus tephrosceles</i> (24)	SNP discovery and validation from RAD sequencing	RAD sequence data: NCBI Sequence Read Archive SRP050135 SNP information including validation results: http://dx.doi.org/10.5061/dryad.404fh	Maria Jose Ruiz-Lopez, Tony L. Goldberg, Colin A. Chapman, Patrick A. Omeja, James H. Jones, William M. Switzer, Paul D. Etter, Eric A. Johnson, Nelson Ting

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Identification of SNP markers for the endangered Ugandan red colobus (*Procolobus rufomitratu*s *tephrosceles*) using RAD sequencing

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Keywords

Red colobus, SNPs, RAD-Seq, endangered, evolution, conservation genomics

Introduction

Recent progress in DNA sequencing technologies coupled with rapid decreases in per-nucleotide sequencing costs have facilitated the discovery of large numbers of single nucleotide polymorphism (SNPs) both in model and non-model species (Ekblom & Galindo 2011). However, the discovery of SNPs in non-model species is still challenging due to the lack of reference genomes and the computational complexity of whole genome *de novo* assembly. “Reduced representation” approaches that reduce the genome complexity have thus grown in popularity. Among these approaches, Restriction-site-associated DNA sequencing (RAD-Seq; Baird *et al.* 2008; Davey *et al.* 2011) has proven to be a reliable method, enabling direct sequencing of homologous regions evenly distributed throughout the genome and allowing the identification and typing of thousands of SNPs. RAD-Seq has been successfully used in a number of molecular ecology studies, including research in gene flow, population structure, phylogeography, phylogenetics, and adaptation (e.g., Catchen *et al.* 2013a; Hohenlohe *et al.* 2011; Stolting *et al.* 2013; White *et al.* 2013).

Despite the availability of genomic resources across a broad array of primate taxa, none are currently available for any leaf-eating monkey (subfamily Colobinae). Furthermore, although RAD-Seq has been used broadly, it has rarely been used in primates (but see Bergey *et al.* 2013 and Evans *et al.* 2014). Here, we used RAD-Seq to discover genome-wide SNPs for a red colobus monkey (genus *Procolobus*; subgenus *Piliocolobus*) (Grubb *et al.* 2003; Oates & Davies 1994). These are tropical forest-adapted, arboreal, leaf-eating monkeys distributed across Equatorial Africa in 18 different morphotypes. They are among the most threatened of African primates (Oates *et al.* 2008; Struhsaker 2005; Ting 2008a) and are considered a good indicator species because of their sensitivity to habitat loss and hunting by humans (Mittermeier *et al.* 2009; Struhsaker 2005). In addition, they are an emerging model for the study of infectious disease discovery and transmission (e.g., Goldberg *et al.* 2009; Lauck *et al.* 2013; Paige *et al.* 2014). Currently, genetic resources for red colobus (and the colobine subfamily in general) are limited to mitochondrial DNA and microsatellites, which are typically cross-amplified using primers specifically designed for humans (Allen *et al.*

2012; Ting 2008b). Thus, a red colobus genomic resource will assist in conservation efforts by allowing a better understanding of neutral and adaptive genetic diversity, patterns of gene flow, potential adaptation to known pathogens, and how best to construct informed management plans.

We developed a SNP panel for the Ugandan red colobus (*P. rufomitratus tephrosceles*), focusing on the largest viable population of this endangered taxon (Kibale National Park; Chapman *et al.* 2010; Struhsaker 2005). We analyzed 24 individuals using single-end RAD-Seq and obtained 70,773,857 total reads, of which 58,814,906 passed the filtering steps. Using a *de novo* pipeline we identified a total of 173,625 putative RAD loci. To minimize potential drop out we followed the protocol of Davey *et al.* (2013) and selected loci that were present in 90% of individuals. We found 113,376 loci, of which 50,558 were polymorphic. To our knowledge this SNP dataset represents the largest genomic resource for the red colobus available to date.

Data Access

- Red colobus sequence files (fastq) for the 24 individuals analyzed have been made available through the Sequence Read Archive (SRA) repository at NCBI (SRP050135)
- Information on the RAD loci identified is available through Dryad (<http://dx.doi.org/10.5061/dryad.404fh>)

Meta Information

- *Sequencing center:* University of Oregon Genomics Core Facility (Eugene, Oregon, USA).
- *Platform and model:* Illumina HiSeq™ 2000
- *Design description:* Our goal was to obtain a genome-wide panel of SNPs in the Ugandan red colobus monkey. This panel will be the first genomic resource for the species and will facilitate further conservation, ecological, and evolutionary studies. We analyzed blood samples from 24 individuals of a single population at

Kibale National Park (0° 33' N and 30° 21' E) and prepared a single-end RAD-Seq library and analyzed the data using the program STACKs v. 1.1 (available at <http://creskolab.uoregon.edu/stacks/>; Catchen *et al.* 2013b; Catchen *et al.* 2011).

- *Analysis type*: Restriction site associated DNA (RAD) sequencing.
- *Run date*: December 2013

Library

- *Strategy*: Single-end RAD-Sequencing
- *Taxon*: *Procolobus rufomitratu tephrosceles*
- *Sex*: Details in Table 2
- *Tissue*: blood
- *Location*: We collected samples from 2 social groups (Table 2) part of a single population at Kibale National Park, Uganda.
- *Sample handling*: 22 EDTA-treated whole blood samples were collected in 2006 from anesthetized individuals. Plasma was separated in the field by centrifugation and plasma and whole blood samples were stored in liquid nitrogen for transport. Two additional samples were collected in 2010 (Table 1).
- *Additional sample information*: Additional details on sample collection methods can be found in Goldberg *et al.* (2009).
- *Selection*: Reduced representation library using restriction enzyme cutting (*Sbf*I-HF).
- *Layout*: Single end 1x150bp sequencing, V3 chemistry.
- *Library Construction Protocol*: Genomic DNA was extracted from blood samples using QIAamp DNA Mini Kits (Qiagen, Inc., Valencia, California, USA) following the manufacturers protocol. All samples were quantified using the Qubit™ 2.0 High-Sensitivity dsDNA Assay (Life Technologies, Inc., Carlsbad, California, USA) and the mean fragment size of each sample was further assessed in a Fragment Analyzer (Advanced Analytical Technologies, Inc., Ames, Iowa, USA). DNA from the 24 individuals was sequenced as a part of a larger single-end library that included another primate species and a total of 46 monkeys. For

preparing the library 500 ng of genomic DNA from each sample was digested for 60 min at 37°C in a 50 µl reaction volume containing 5 µl 10x CutSmart™ Buffer and 10 units (U) *Sbf*I-HF (New England Biolabs [NEB], Inc., Ipswich, Massachusetts, USA). Each sample was heat inactivated for 20 min at 65°C and allowed to cool at room temperature (RT) overnight. 4 µl of 6 base pair (bp) barcoded *Sbf*I-P1 Adapters (100 nM) were added to each sample followed by 1.0 µl of 10x NEB Buffer 2, 0.6 µl rATP (100 mM, Promega Corporation, Madison, Wisconsin, USA), 0.5 µl (1000 U) T4 DNA ligase (2 million U/ml, NEB), 3.9 µl H₂O and incubated at RT for 30 min. Samples were heat inactivated for 20 min at 65°C and allowed to cool at RT for one hour. 30 µl of the ligation reaction for each sample was combined in sub-libraries according to fragment size. Each pooled sub-library was randomly sheared (Bioruptor® Standard, Diagenode, Denville, New Jersey, USA) to an average size of 500 bp. 30 µl of the sheared product was run on a 1% agarose (Thermo Fisher Scientific, Inc., Waltham, Massachusetts, USA) 1x TBE gel to determine size. The remaining sheared volume for each sub-library was concentrated to 30 µl using Mini-elute columns (QIAGEN) and then purified with 1.0X Agencourt AMPure XP beads (Beckman Coulter, Inc., Brea, California, USA) to remove short fragments and adapter dimers. To polish the ends of the sheared DNA, a Quick Blunting Kit (NEB) was used in a 25 µl reaction volume containing 19 µl of sheared DNA, 2.5 µl 10x Blunting Buffer, 2.5 µl dNTP Mix (1mM) and 1.0 µl Blunt Enzyme Mix. The reactions were incubated at RT for 30 min and the product was purified with 1.0X AMPure XP beads. To add 3' adenine overhangs to the DNA, 19 µl of the previous purified reaction was incubated at 37°C for 30 min in a 25 µl reaction containing 3 µl (10 U) Klenow Fragment (3'-5' exo, NEB), 2.5 µl NEB Buffer 2 and 0.5 µl dATP (10 mM, Fermentas, Thermo Fisher Scientific, Inc., Waltham, Massachusetts, USA). The reaction was cooled at RT for 30 min and purified with 1.0X AMPure XP beads. The modified P2 Illumina© adapter (all rights reserved 2006 Illumina, Inc., San Diego, California, USA; see Etter 2010) was added in 25 µl ligation reaction, including 19 µl of DNA, 1.0 µl of P2 adapter, 2.5 µl of 10x NEB Buffer 2, 0.25 µl rATP (100mM, Promega), 0.5 µl (1000 U) T4 DNA

ligase (2 million U/ml, NEB), and 1.75 μ l H₂O. The reaction was incubated at RT for 30 min and purified with 1.0X AMPure XP beads. Purified sub-libraries were eluted in 51 μ l and quantified using the Qubit™ High-Sensitivity dsDNA Assay. 120 ng (~ 10 ng/individual) of each sub-library was used as template in a 100 μ l PCR amplification with 50 μ l Phusion Hot start Flex 2X Master Mix (NEB) 2X Master Mix and 4 μ l modified Illumina© amplification primer mix (10 μ M, long-P1-forward primer: 5'-AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTC CGATC*T-3', short-P2-reverse primer: 5'-CAAGCAGAAGACGGCATACG*A-3'). PCR conditions included an initial denaturing step at 98° C for 3 min, then 14 cycles of 40 sec at 98° C, 15 sec at 65° C, and 30 sec at 72° C followed by a final extension at 72°C for 5 min. 10 μ l of the sheared product was run on a 1% agarose 1x TBE gel to check the final sub-library size. The remaining amplified product was purified with AMPure XP beads, including a 0.5X size-exclusion step prior to 1.0X purification to equalize the final size of the 4 sub-libraries. Sub-libraries were quantified using Qubit™ 2.0 High-Sensitivity dsDNA Assay, pooled in equimolar concentrations for a final concentration of 10 nM and sequenced in a single lane of the HiSeq™ 2000 following a rapid sequencing Illumina protocol for 150bp single-end reads at the University of Oregon Genomics Core Facility.

Processing

- *Pipeline*- We processed the sequence data, clustered the reads into RAD loci, identified the RAD loci containing SNPs and genotyped all samples using several modules from the STACKS software package version 1.11 (Catchen *et al.* 2013b; Catchen *et al.* 2011). First, we sorted the read pairs by barcode, filtered the read quality and removed any read that did not contain both a correct barcode and the remaining six bases of the *Sbf*I restriction site sequence. The 24 red colobus samples were then analyzed as follows. We identified RAD loci and called SNPs in each individual, created a catalog for all loci across individuals and matched each sample against the previously created catalog using *denovomap.pl*. We used a minimum depth of coverage (-m) of 3 to create stacks, a maximum

nucleotide distance between stacks (-M) of 4 when identifying the loci, and a maximum number of mismatches between sample tags (-n) of 2 when generating the catalog. These parameters were set empirically following Catchen *et al.* (2013b). We enabled the Deleveraging and Removal algorithms to filter out highly repetitive (likely paralogous) loci. These analyses were carried out on the University of Oregon Applied Computational Instrument for Scientific Synthesis (ACISS) server. Then, we used `load_radtags.pl` and `index_radtags.pl` to locally populate and index a MYSQL database of loci. Using the scripts `export_sql.pl` and `populations.pl`, we selected the polymorphic loci that were present in 22 individuals (90%). In addition, using the script `populations.pl` we calculated several genetic diversity parameters (expected and observed heterozygosity, nucleotide diversity and inbreeding coefficient) based on one SNP for each RAD locus for each of the two social groups and the whole population.

- *Runs*: Red colobus sequence files (fastq) for the samples have been made available through the Sequence Read Archive (SRA) repository at NCBI (SRP050135)

Results

- Information on the RAD loci identified was submitted to Dryad (<http://dx.doi.org/10.5061/dryad.404fh>), including information of each locus present in at least 22 individuals and consensus genotypes for each individual.
- Table 1 shows the number of reads obtained in the run and after the quality filtering steps.
- Table 2 shows number of reads obtained after quality filtering per individual, in addition to specific data for each individual, such as sex, social group and date of sampling.
- Table 3 shows several genetic diversity parameters for both social groups and overall. Where H_o is observed heterozygosity, H_e is expected heterozygosity, π is nucleotide diversity, and F_{is} is the inbreeding coefficient of an individual relative to the subpopulation.
- Quality scoring system: phred + 33

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Table 1. Number of reads obtained in the run and after each filtering step.

	Number of Reads
Library	154,469,192
Unidentified barcodes	11,399,135
Other primate species ¹	72,296,200
Red colobus	70,773,857
Retained after quality filtering	58,814,906
Mean per individual	2,948,911

¹ Reads corresponding to the other primate species that were analyzed in the same library than the Red Colobus individuals.

Table 2. Number of reads obtained for each of the red colobus individuals sequenced using RAD-Seq.

ID	Sex	Social Group	Date	Number of reads
R11	Female	Small Camp	2006	2444654
R12	Male	Large Mikana	2006	2623855
R13	Female	Large Mikana	2006	2345512
R21	Male	Large Mikana	2006	1272056
R30	Female	Small Camp	2006	4180405
R31	Female	Small Camp	2006	2219072
R33	Female	Small Camp	2006	3328019
R34	Female	Small Camp	2006	2175606
R40	Male	Small Camp	2006	3696437
R41	Female	Large Mikana	2006	2277670
R49	Male	Large Mikana	2006	2753231
R52	Female	Large Mikana	2006	2184823
R54	Female	Small Camp	2006	2281773
R56	Female	Small Camp	2006	4372857
R6	Male	Large Mikana	2006	2351825
R65	Female	Large Mikana	2006	2120931
R67	Female	Large Mikana	2006	1948210
R70	Female	Large Mikana	2006	1466084
R72	Female	Large Mikana	2006	3080133
R73	Female	Small Camp	2006	1985157
R996	Male	Large Mikana	2006	2623420
R999	Male	Small Camp	2006	1717138
RC54	Female	Small Camp	2010	1899120
RC60	Male	Large Mikana	2010	1466918

Table 3. Mean and standard deviation estimates for heterozygosity, nucleotide diversity, and inbreeding coefficient.

	Ho	He	π	Fis
Large Mikana	0.242	0.248	0.259	0.045
(SD)	0.184	0.165	0.172	0.255
Small Camp	0.252	0.249	0.262	0.027
(SD)	0.192	0.166	0.174	0.253
Both groups	0.247	0.255	0.261	0.045
(SD)	0.167	0.157	0.161	0.216

Ho is observed heterozygosity, He is expected heterozygosity, π is nucleotide diversity, and Fis is the inbreeding coefficient of an individual relative to the subpopulation.