Received: 1 August 2018 | Accepted: 21 February 2019

DOI: 10.1111/2041-210X.13173

PRACTICAL TOOLS

Methods in Ecology and Evolution BRITISH ECOLOGICAL SOCIETY

Empowering conservation practice with efficient and economical genotyping from poor quality samples

Meghana Natesh[1,2]* | **Ryan W. Taylor**[3,4]* | **Nathan K. Truelove**[5] | **Elizabeth A. Hadly**[3] | **Stephen R. Palumbi**[3,5] | **Dmitri A. Petrov**[3]* | **Uma Ramakrishnan**[1]*

[1]National Centre for Biological Sciences, TIFR, Bangalore, India

[2]Sastra University, Tirumalaisamudram, Thanjavur, India

[3]Department of Biology, Stanford University, Stanford, California

[4]End2End Genomics LLC, Davis, California

[5]Hopkins Marine Station, Stanford University, Pacific Grove, California

Correspondence
Dmitri Petrov
Email: dpetrov@stanford.edu
and
Uma Ramakrishnan
Email: uramakri@ncbs.res.in

Funding information
R43HG009482, Wildlife Conservation Trust grant (UR), Fulbright Nehru grant (2092/F-NAPE/2015, UR) and Wellcome Trust/DBT India Alliance (UR, IA/S/16/2/502714), Summit Foundation and Global Genome Initiative at the Smithsonian Institution (NT, SRP, Steve Box) supported conch work.

Handling Editor: Douglas Yu

Abstract

1. Moderate- to high-density genotyping (100 + SNPs) is widely used to determine and measure individual identity, relatedness, fitness, population structure and migration in wild populations.
2. However, these important tools are difficult to apply when high-quality genetic material is unavailable. Most genomic tools are developed for high-quality DNA sources from laboratory or medical settings. As a result, most genetic data from market or field settings is limited to easily amplified mitochondrial DNA or a few microsatellites.
3. To enable genotyping in conservation contexts, we used next-generation sequencing of multiplex PCR products from very low-quality DNA extracted from faeces, hair and cooked samples. We demonstrated utility and wide-ranging potential application in endangered wild tigers and tracking commercial trade in Caribbean queen conch.
4. We genotyped 100 SNPs from degraded tiger samples to identify individuals, discern close relatives and detect population differentiation. Co-occurring carnivores do not amplify (e.g. Indian wild dog/dhole) or are monomorphic (e.g. leopard). Sixty-two SNPs from conch fritters and field-collected samples were used to test relatedness and detect population structure.
5. We provide proof of concept for a rapid, simple, cost-effective and scalable method (for both samples and number of loci), a framework that can be applied to other conservation scenarios previously limited by low-quality DNA samples. These approaches provide a critical advance for wildlife monitoring and forensics, open the door to field-ready testing, and will strengthen the use of science in policy decisions and wildlife trade.

KEYWORDS
conch, conservation genetics, endangered species monitoring genotyping, multiplex PCR, noninvasive samples, SNPs, tigers

*Equal contribution.

© 2019 The Authors. Methods in Ecology and Evolution © 2019 British Ecological Society

1 | INTRODUCTION

Stemming the tide of global species decline requires continuous monitoring and nimble, adaptive management to promote species recovery. Effective monitoring relies on identifying species presence and the ability to track specific individuals and their familial relationships. While species recovery is critically dependent on tracking individuals and their dynamics locally, integrating data across the species range allows monitoring of global large-scale threats including population range reduction and illegal wildlife trade.

In principle, all of these goals can be achieved via genotyping a modest number of loci such as microsatellites or single nucleotide polymorphisms (SNPs). To study endangered species that are rare and elusive, approaches must be able to accommodate noninvasive sources of DNA such as faeces, shell, feathers, hair and saliva, which yield impure, mixed and/or extremely small amounts of degraded DNA. Moreover, market samples generated by wildlife trade may be processed, cooked, dried or mixed with other species, again providing low-quality and often mixed DNA. Current approaches tend to require relatively large amounts of DNA (Carroll et al., 2018, nanograms of DNA) from the target species, or demand expensive and generally inefficient enrichment strategies (Snyder-Mackler et al., 2016; Chiou & Bergey, 2018). Approaches designed for lower concentration DNA samples (Kraus et al., 2015) require expensive and specialized equipment.

Here we demonstrate that a multiplex PCR approach followed by next-generation sequencing satisfies all the requirements necessary for inexpensive, fast, and easy genotyping of low-quality samples. Our approach is similar to GT-seq (Campbell, Harmon, & Narum, 2014) but can use publicly available software for designing primers and calling SNPs and targets only short fragments in order to succeed with degraded DNA. We illustrate the power of this method for two endangered species in divergent conservation contexts and real-life settings: genotypes from faeces, shed hair and saliva found on killed prey from wild Indian tigers and from CITES-regulated Caribbean queen conch imported to the US and sold in fried fritters (method schematic Figure 1a,b). Methods include DNA extraction, multiplex PCR, a second barcoding PCR, Illumina MiSeq sequencing and bioinformatics for SNP genotyping.

The tiger *Panthera tigris*, a charismatic carnivore, is classified as endangered by the IUCN red list. Distribution across 14 countries (Goodrich et al., 2015) makes it critical that locally collected data be comparable across tiger range. Genotyping of scat or hair, along with rapid forensic testing of confiscated skins or other traded parts can verify species, individual identity and source populations. We developed a multiplex primer set for 192 SNP loci and tested them on faecal, tissue, saliva and hair samples from captive and wild tigers. We also genotyped two sympatric carnivores, the dhole (*Cuon alpinus*), and the leopard (*Panthera pardus*), that may be confused with tigers when targeting noninvasive samples.

Our second example illustrates the use of this approach even when reference genomes are unavailable, and again highlights use in difficult samples: in this case fried conch fritters. Although formerly abundant, the queen conch (*Strombus gigas*) was listed in CITES Appendix II in 1990, which allows for control of trade to reduce over-exploitation. Identifying geographic ancestry of illegally traded queen conch products in Florida markets will aid conservation action that will allow recovery of this formerly lucrative fishery. Because the most direct access to imported conch is from the hundreds of restaurants in Florida, we sought techniques that would allow genotyping from the most abundant menu item, fried conch fritters.

2 | MATERIALS AND METHODS

2.1 | Sample collection

For tigers, multiple samples from 13 captive tigers (USA zoos) representing different scenarios were used for standardization (Supporting Information, Table S1). Blood and corresponding faecal swabs from captive tigers, (including one parent–offspring and one sibling pair) were collected in India. Wild tigers were sampled noninvasively from protected areas across India (Supporting Information, Table S2).

Scat and saliva (from predator bites on the prey) were sampled by swabbing the sample surface with moistened synthetic swabs (Ramón-Laca, Soriano, Gleeson, & Godoy, 2015); tips were stored in lysis buffer in 2 ml microcentrifuge tubes. Hair was sampled using forceps and stored in ziplock bags. DNA from legacy faecal samples (collected in alcohol in 2014) was also tested. DNA extraction (first step in Figure 1a) and quantification are described in SM1. Two leopards and one dhole were included. Most samples were genotyped in triplicate.

Tissue samples from live caught Queen Conch mantle were collected using a sterilized biopsy forceps were preserved in RNALater. Samples from conch fritters purchased from Miami restaurants were frozen until dissected to isolate animal tissue fragments (SM 3a).

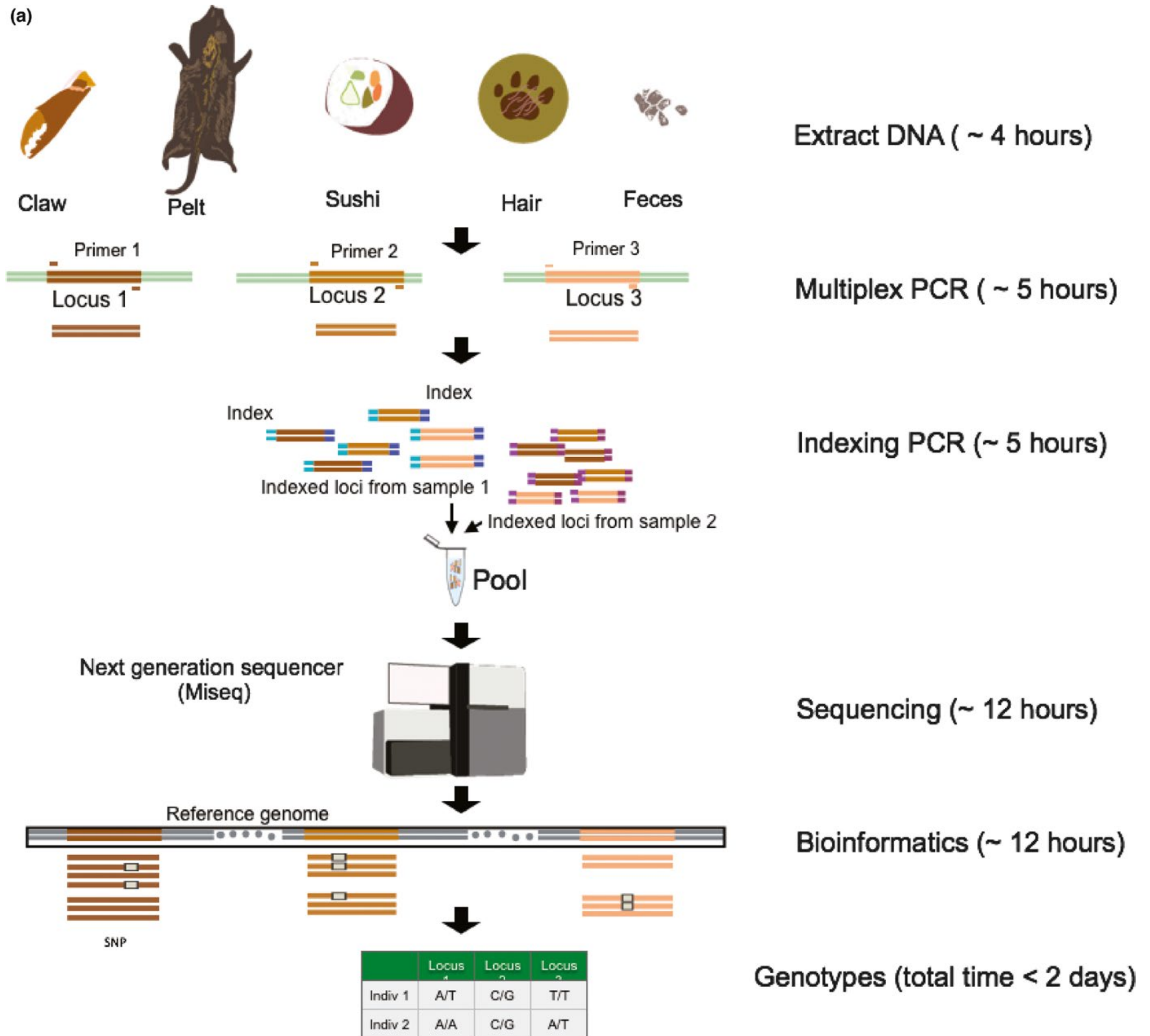
2.2 | SNP Identification and filtering

2.2.1 | Tigers

We identified SNPs from whole genome sequencing of 75 tigers of wild and captive origin from *P. t. tigris*, *P. t. jacksoni*, *P. t. altaica and P. t. sumatrae* subspecies (SM 1a). Alignment, filtering and pruning were used to identify SNPs (SM 1a). We calculated minor allele frequencies (MAF) for each SNP within each subspecies, and retained SNPs with MAF 10, 15, 20, 25 and 30%. We identified fixed SNPs that differentiated populations. We prioritized SNPs within contigs >10, 5 and 1 MB respectively. We selected 10 differentiating SNPs from each subpopulation, and 9992 polymorphic SNPs from each of the aforementioned MAF cut-offs, for a total of 50,000 SNPs.

2.2.2 | Conch

We extracted RNA from 96 *L. gigas* individuals (SM 3a, Supporting Information, Table S3). Four queen conch individuals (one each from Aruba, Belize, Florida and St. Eustatius) were imported into TRINITY v2.2.0 (Grabherr et al., 2013) to assemble a de novo transcriptome (SM 3a).



	Locus 1	Locus 2	Locus 3
Indiv 1	A/T	C/G	T/T
Indiv 2	A/A	C/G	A/T

FIGURE 1 (a) A schematic for protocol and approximate time taken. Details in the main and supplementary text. (b) On the left, a tiger defecating (photo: Himanushu Chhattani), a scrape mark (and associated hair samples, photo: Kaushal Patel) and a tiger hunting (photo: Shantanu Prasad, associated lick marks). On the right, a conch emerging from its shell, a fisherman holding his catch and a fritter

480,962 SNPs were discovered by aligning 96 conch sequences from six populations in the Caribbean to the assembled transcriptome (SFG pipeline -https://github.com/bethsheets/Population-Genomics-via-RNAseq/blob/docs/guide-to-assembly-scripts.md, SM 3a).

2.3 | Primer design

Publically available Primer3 (Untergasser et al., 2012; amplicon size 50–90 bp, primer size 17–25 bp and T_m of 60–61°C) was used to design primers for tiger SNPs. Conch primers were similarly designed with the same criteria as above. No attempt was made to identify incompatibilities between primer pairs; 192 primer pairs were shortlisted for both species (SM 1b, 3b). GT-seq indexes and adapters were used.

2.4 | PCR amplification and sequencing

Library preparation consisted of an initial multiplex PCR reaction, a second PCR reaction to add sequencing adapters and indexes, and sample pooling. Sample input DNA volume was adjusted to a maximum of 1 ng per reaction. The multiplex PCR simultaneously amplified all target regions for each sample separately (in a 96-well plate). The second PCR reaction added a combination of forward (i5) and reverse (i7) Illumina indexes to uniquely identify each sample. The sequencing library contained equal volumes of each sample's barcoded product and was cleaned with Ampure beads. Sequencing of single 150 bp reads was performed on Illumina MiSeq (SM 1f). Alignment and genotype calling followed

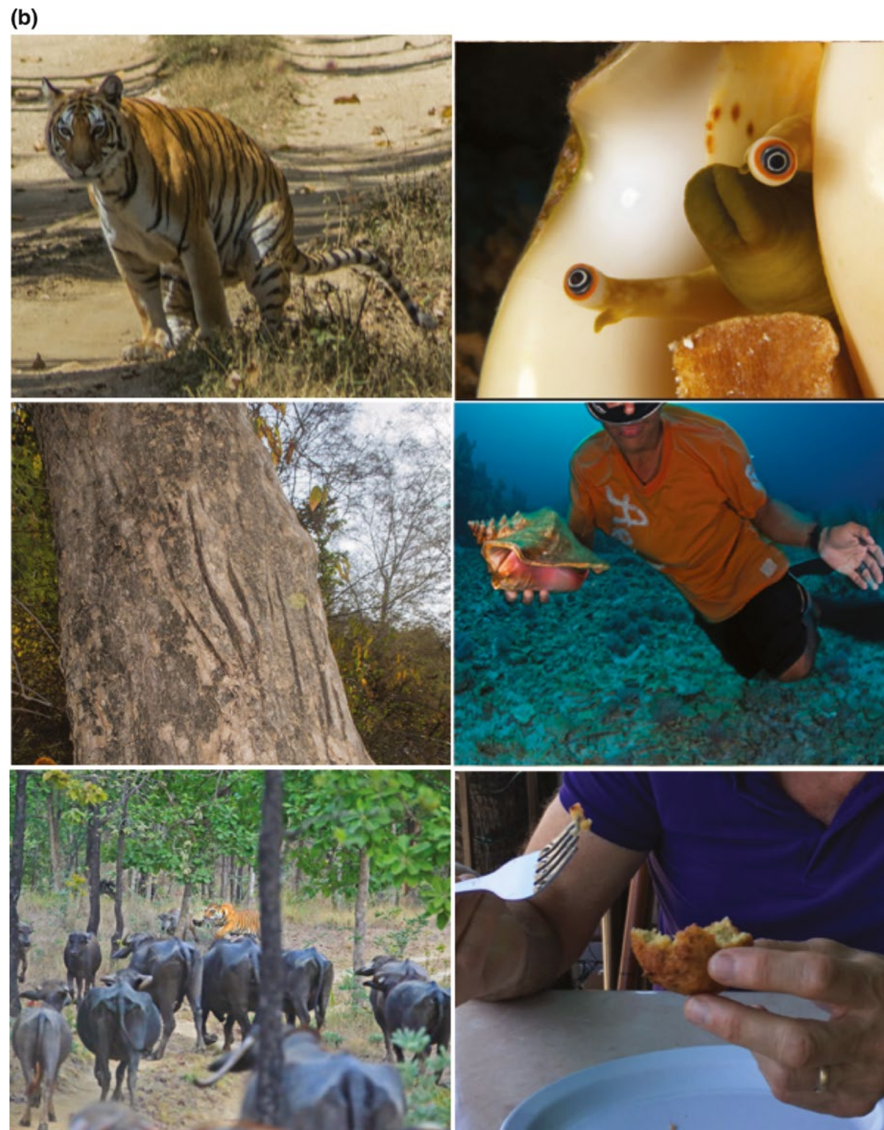


FIGURE 1 (Continued)

GT-Seq for conch (SM 3b) and used standard open source tools for tigers (SM 1h). Figure 1a illustrates the steps described above.

For tigers, genotyping success, genotype concordance across replicates, relatedness between pairs of individuals of known and unknown relationship, probability of identity of the SNP panel and population structure were assessed (SM 1h). For conch, genotyping success and genotype concordance were tested by comparing SNPs across replicate conches. Genetic distance and ability to assign the conch samples to the correct population was estimated by comparison of the 96 transcriptome samples (SM 4).

3 | RESULTS

3.1 | Tiger

One hundred and twenty-six targets (66% of 192 attempted) produced the most consistent results (SM 2b, Supporting Information, Figure S1, Table S4), and were then tested on noninvasive samples from wild tigers across India and zoo individuals (Figure 1b, Supporting Information, Table S2).

The 126 SNP panel for wild tigers had a high overall genotyping success rate (Figure 2, SM 2c). An average of 95 SNPs (75%, range: 4–114) were successfully typed across all samples. When tiger DNA was >0.01 ng/µl (all sample types), an average of 105 SNPs (83%, range: 48–114) were typed.

Sample replicates were highly concordant with a high proportion of genotype matches (n = 28 triplicates, genotype concordance, M: 0.957, range: 0.757–1.0). Different sample types from the same individual also had highly concordant genotypes (n = 5, concordance, M: 0.97; range: 0.91–0.99). Our error rates were comparable to low microsatellite genotyping error rates in some studies (Thaden, Cocchiararo, Jarausch, & Jüngling, 2017) or lower than other noninvasive studies (Valière et al., 2007; Mondol et al., 2014). Our probability of misidentification was vanishingly low (pID_{sibs} = 1.6E-22).

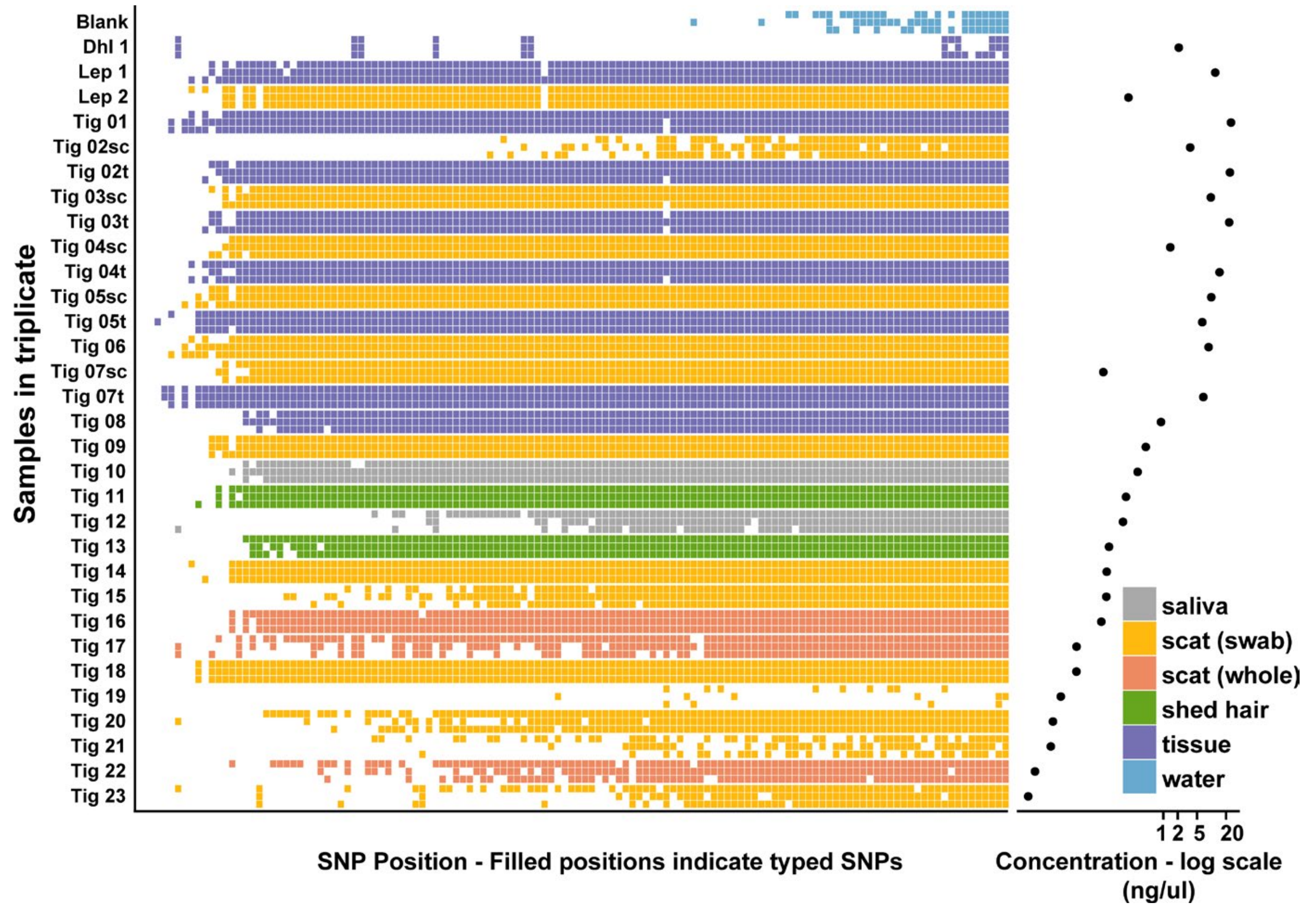


FIGURE 2 SNP typing success (in triplicate) for various tiger samples and controls. Filled cells indicate successfully typed SNPs; colour indicates sample type. DNA concentrations are on the right

The co-occurring carnivores, leopard and dhole, could be distinguished from tiger genotypes. The dhole tissue sample had poor amplification success (mean across replicates <10 SNPs). Leopards had high amplification success (*M*: 110 SNPs), but SNPs were monomorphic and nearly identical across two individuals (*M*: 0.03% mismatches).

The known parent–offspring and sibling pairs (captive individuals, India) had relatedness values close to expected (0.5, Figure 3). Observed pairwise relatedness was higher within than between known genetic clusters (see Natesh et al., 2017). As expected, relatedness among individuals from a small, isolated population (NW, Figure 3) was high. Wild individuals fell into three genetic clusters as expected (Supporting Information, Figure S2).

3.2 | Queen conch

We tested a 192 primer pair panel on 279 conch samples from 14 populations, including 48 fried conch fritters from Miami, Florida restaurants. Our SNP success was lower for conch than tigers (SM 4). Approximately half the 192 conch primer pairs failed to provide data, but 62 targets reached an 86% success rate similar to the 126 good tiger targets (Supporting Information, Figure S3). Replicate samples shared 99%–100% of their alleles, and different processed conch samples were genetically identical, suggesting recapture. There were no obvious close relatives among the samples (Supporting Information, Figures S4 and S5). Outlying islands (Aruba, St. Eustatius) were genetically differentiated from the central Caribbean and Florida (F_{ST} = 0.037, 0.048 respectively, Supporting Information, Table S5). Samples from the same island group (e.g. Florida, Bahamas) or the same coast were not differentiated. These patterns parallel a recent survey of queen conch with microsatellites (Truelove et al., 2017).

Conch DNA from deep-fried fritters had lower success rates than from biopsies. However, success was high enough for individual identification and initial population comparison. Twenty-three fritters were probably not Queen conch (fewer than 18 SNPs amplified), eight fritters revealed poor SNP amplification (average 41% success) and 17 fritters were comparable to fresh samples (77% vs 86% success, Supporting Information, Figure S3). These samples revealed lower (average) genetic identity to Florida populations (average 78.6–80.5 alleles shared, Supporting Information, Figure S6) compared to Puerto Rico, or Andros Island (81.2–82.3, Supporting Information, Figure S6). While positive population identification may require greater geographic sampling and more SNPs (from several whole genomes), our pilot data suggest that the fritters we genotyped are less likely to be from the Florida Keys and Nassau.

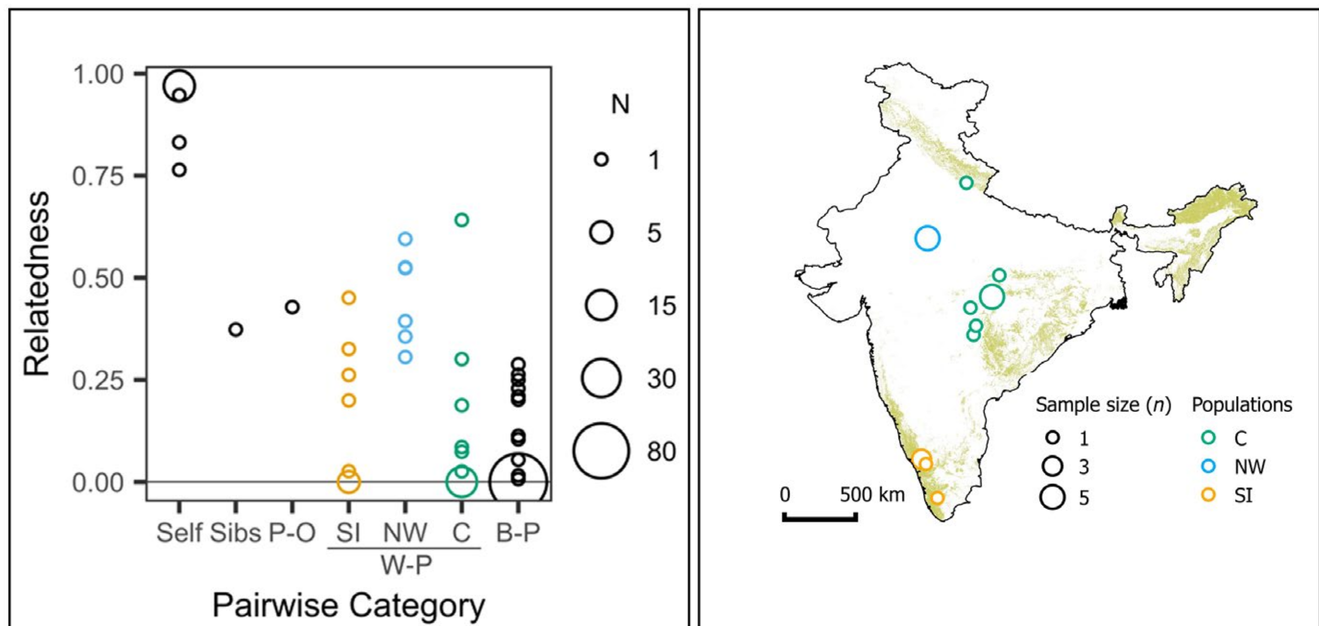


FIGURE 3 Pairwise relatedness (PI_HAT): self: three replicates of each sample; same individual but different sample, Sibs: siblings, P-O: parent–offspring pair, W-P: within population, B-P: between populations. True relatedness unknown for W-P and B-P. Sampling locations and corresponding colours represented in the India map

Importantly, high-resolution nuclear data were readily obtained from processed commercial samples to address key conservation challenges.

4 | DISCUSSION

Our pilot datasets provide proof of concept for multiplex SNP genotyping of noninvasive and processed market samples from two species with vastly different physiologies, ecologies and conservation challenges. The approach is successful for degraded, cooked, mixed, or small and low-concentration samples (down to 10^{-3} ng DNA/µl), making it an ideal tool for monitoring individuals under field conditions or from commercial markets.

Conservation practitioners assume that genetics is expensive. However, our method is cheap, while providing rich information important for conservation. Designing a similar protocol for a new species of interest would include costs for method development and implementation. Development costs include polymorphic SNP ascertainment, primer design and synthesis. However, note that SNPs have already been identified for many endangered and fisheries species (e.g. Steiner, Putnam, Hoeck, & Ryder, 2013). If no SNPs have been identified, practitioners could ascertain SNPs using whole genome sequencing (e.g. genome assembly of reasonable quality ~$2,000, Armstrong et al., 2018) and pooled sequencing of 10 individuals at approximately 50× total coverage, ~$1,000). The upfront cost of primer design and synthesis is between $1,000 and $10,000 (for 100 to 1,000 primer pairs, $10 per primer pair). Once synthesized, primers can be used for 38,000 reactions (~400 plates). The continual advance in sequencing and oligo synthesis will drive down these initial development costs. Most important and attractive to conservationists, we estimate implementation costs (for 1,000 SNPs) can be as low as $5 per sample (when processing several hundred samples).

Increasing the number of SNPs beyond a few hundred can provide additional information. For this pilot, we constrained the number of targeted SNPs, but it should be possible to target many more. Primers chosen to amplify clusters of closely located SNPs should allow detection of very recent inbreeding using long runs of homozygosity (Kirin et al., 2010). Linked SNPs could generate microhaplotypes, particularly useful in pedigree reconstruction (Baetscher, Clemento, Ng, Anderson, & Garza, 2018). SNP panels could allow simultaneous species, individual and diet identification for sets of species, e.g. large carnivores and common prey species in India or sub-Saharan Africa.

Effective monitoring of individuals, populations and species is critical to designing rapid conservation action and management of endangered species like tigers. Small, isolated populations will require inbreeding management, genetics-based population assessment and genetically informed introduction strategies. Likewise, identification of commercial products from illegal fishing, bushmeat hunting or highly processed market samples provides important management information. Ability to assay such samples could provide a powerful incentive to enforce local conservation laws. Rapid genetic monitoring of endangered species from commonly occurring noninvasive samples will provide a data-pathway to species recovery. We believe that multiplex PCR presents an example of such rapid, accessible, cheap and efficient technology that will make this possible.

ACKNOWLEDGEMENTS

San Diego Zoo Global, CSC at Oakland Zoo, SF Zoo, Bronx Zoo, WCS Russia, El Paso Zoo, Performing Animal Welfare Society, WCS India, CWS India, WCT, Bannerghatta Zoo, Himanshu, Anubhab, Prachi, Aditya, Arun, Erica Calcagno, Jackie Gai for tiger samples/ data, and forest departments of Rajasthan, Karnataka, Madhya Pradesh and American Zoo Association, Tiger SSP for permissions, Awadhesh Pandit, NCBS-NGS facility, Steve Box, Steve Canty and Nate Campbell for help with conch scripts.

CONFLICT OF INTEREST

R.W.T. and End2End Genomics L.L.C. received NIH small business funding (R43HG009482) to develop tools to study non-model species.

AUTHORS' CONTRIBUTIONS

M.N., R.W.T., D.P., U.R., E.A.H., S.P. and N.T. designed the study. M.N., R.W.T. and N.T. conducted laboratory work and analyses. M.N., R.W.T., D.P., U.R., E.A.H., S.P. wrote the paper.

DATA ACCESSIBILITY

Raw sequences: available from NCBI (https://www.ncbi.nlm.nih.gov/bioproject/PRJNA516037); primer sequences: supplementary file; scripts for primer design, SNP calling and filtering available on Dryad (tiger, https://doi.org/10.5061/dryad.q15pc00, Natesh 2019) and GitHub (conch, https://doi.org/10.5281/zenodo.2580291).

REFERENCES

Armstrong, E. E., Taylor, R. W., Prost, S., Blinston, P., Meer, E. Van Der, Madzikanda, H., … Petrov, D. (2018). Cost-effective assembly of the African wild dog (Lycaon pictus) genome using linked reads. *GigaScience*, *8*, 1–10. https://doi.org/10.1093/gigascience/giy124

Baetscher, D. S., Clemento, A. J., Ng, T. C., Anderson, C., & Garza, J. C. (2018). Microhaplotypes provide increased power from short-read DNA sequences for relationship inference. *Molecular Ecology Resources*, *18*(2017), 296–305. https://doi.org/10.1111/1755-0998.12737

Campbell, N. R., Harmon, S., & Narum, S. R. (2014). Genotyping-in-Thousands by sequencing (GT-seq): A cost effective SNP genotyping method based on custom amplicon sequencing. *Molecular Ecology Resources*, *15*, 855–867. https://doi.org/10.1111/1755-0998.12357

Carroll, E. L., Bruford, M. W., Dewoody, J. A., Leroy, G., Strand, A., Waits, L., & Wang, J. (2018). Genetic and genomic monitoring with minimally invasive sampling methods. *Evolutionary Applications*, *11*, 1–26. https://doi.org/10.1111/eva.12600

Chiou, K. L., & Bergey, C. M. (2018). Methylation-based enrichment facilitates low-cost, noninvasive genomic scale sequencing of populations from feces. *Scientific Reports*, *January*, 1–10. https://doi.org/10.1038/s41598-018-20427-9

Goodrich, J. M., Lyam, A., Miquelle, D. G., Wibisono, H. T., Kawanishi, K., Pattanavibool, A., … Karanth, U. K. (2015). *Panthera tigris*. The IUCN red list of threatened species 2015. *IUCN*, e.T15955A50659951, 8235. https://doi.org/10.2305/IUCN.UK.2015-2.RLTS.T15955A50659951.en

Grabherr, M. G., Haas, B. J., Yassour, M., Levin, J. Z., Thompson, D. A., Amit, I., … Chen, Z. (2013). Trinity: Reconstructing a full-length transcriptome without a genome from RNA-Seq data. *Nature Biotechnology*, *29*(7), 644–652. https://doi.org/10.1038/nbt.1883.Trinity

Kirin, M., Mcquillan, R., Franklin, C. S., Campbell, H., Mckeigue, P. M., & James, F. (2010). Genomic runs of homozygosity record population history and consanguinity. *PLoS ONE*, *5*(11), 1–7. https://doi.org/10.1371/journal.pone.0013996

Kraus, R. H. S., vonHoldt, B., Cocchiararo, B., Harms, V., Bayerl, H., Kühn, R., … Nowak, C. (2015). A single-nucleotide polymorphism-based approach for rapid and cost-effective genetic wolf monitoring in Europe based on noninvasively collected samples. *Molecular Ecology Resources*, *15*(2), 295–305. https://doi.org/10.1111/1755-0998.12307

Mondol, S., Kumar, N. S., Gopalaswamy, A., Sunagar, K., Karanth, K. U., & Ramakrishnan, U. (2014). Identifying species, sex and individual tigers and leopards in the Malenad-Mysore Tiger Landscape, Western Ghats, India. *Conservation Genetics Resources*, *7*, 353–361. https://doi.org/10.1007/s12686-014-0371-9

Natesh, M. (2019). Data from: Empowering conservation practice with efficient and economical genotyping from poor quality samples. *Dryad Digital Repository*. https://doi.org/10.5061/dryad.q15pc00

Natesh, M., Alta, G., Nigam, P., Jhala, Y. V., Zachariah, A., Borthakur, U., & Ramakrishnan, U. (2017). Conservation priorities for endangered Indian tigers through a genomic lens. *Scientific Reports*, *7*(1), 1–11. https://doi.org/10.1038/s41598-017-09748-3

Ramón-Laca, A., Soriano, L., Gleeson, D., & Godoy, J. A. (2015). A simple and effective method for obtaining mammal DNA from faeces. *Wildlife Biology*, *21*(4), 195–203. https://doi.org/10.2981/wlb.00096

Snyder-Mackler, N., Majoros, W. H., Yuan, M. L., Shaver, A. O., Gordon, J. B., Kopp, G. H., … Tung, J. (2016). Efficient genome-wide sequencing and low coverage pedigree analysis from non-invasively collected samples. *Genetics*, *203*(June), 699–714. https://doi.org/10.1534/genetics.116.187492

Steiner, C. C., Putnam, A. S., Hoeck, P. E. A., & Ryder, O. A. (2013). Conservation genomics of threatened animal species. *Annual Review of Animal Biosciences*, *1*, 261–281. https://doi.org/10.1146/annurev-animal-031412-103636

Thaden, A. V., Cocchiararo, B., Jarausch, A., & Jüngling, H. (2017). Assessing SNP genotyping of noninvasively collected wildlife samples using microfluidic arrays, (May), 1–13. *Scientific Reports*. *7* https://doi.org/10.1038/s41598-017-10647-w

Truelove, N. K., Box, S. J., Aiken, K. A., Boman, E. M., Booker, C. J., Byfield, T. T., … Allan, K. W. (2017). Isolation by oceanic distance and spatial genetic structure in an overharvested international fishery. *Diversity and Distributions*, *23*, 1292–1300. https://doi.org/10.1111/ddi.12626

Untergasser, A., Cutcutache, I., Koressaar, T., Ye, J., Faircloth, B. C., Remm, M., & Rozen, S. G. (2012). Primer3 — new capabilities and interfaces. *Nucleic Acids Research*, *40*(15), 1–12. https://doi.org/10.1093/nar/gks596

Valière, N., Bonenfant, C., Toïgo, C., Luikart, G., Gaillard, J. M., & Klein, F. (2007). Importance of a pilot study for non-invasive genetic sampling: Genotyping errors and population size estimation in red deer. *Conservation Genetics*, *8*(1), 69–78. https://doi.org/10.1007/s10592-006-9149-2

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

How to cite this article: Natesh M, Taylor RW, Truelove NK, et al. Empowering conservation practice with efficient and economical genotyping from poor quality samples. *Methods Ecol Evol*. 2019;00:1–7. https://doi.org/10.1111/2041-210X.13173