bioRxiv preprint doi: https://doi.org/10.1101/349472. this version posted January 17, 2019. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. It is made available under a CC-BY-NC-ND 4.0 International license.

1 Empowering conservation practice with efficient and economical genotyping from poor quality
2 samples using MPCRseq

3 Authors: MeghanaNatesh[1,2 #], Ryan W. Taylor[3,4,#], Nathan Truelove[5],Elizabeth A. Hadly[3],
4 Stephen Palumbi[3,5], Dmitri Petrov[3#*] and Uma Ramakrishnan[1#*]
5 Author Affiliations:
6 [1]National Centre for Biological Sciences, TIFR, Bellary Road, Bangalore - 560065, India
7 [2]Sastra University, Tirumalaisamudram, Thanjavur – 613401, India
8 [3]Department of Biology, Stanford University, Stanford, CA - 94305, USA
9 [4]End2End Genomics LLC, Davis, CA
10 [5]Hopkins Marine Station, Stanford University, Pacific Grove, CA – 93950, USA
11 [#]: Equal contribution
12 *Correspondence: Dmitri Petrov, Department of Biology, 371 Serra St., Stanford University
13 Stanford, CA 94305-5020, USA, dpetrov@stanford.edu
14 and
15 Uma Ramakrishnan, Senior Fellow, Wellcome Trust/DBT India Alliance, NCBS TIFR,
16 Bangalore, 560065, India, uramakri@ncbs.res.in
17
18
19 Running title: Genetic data to empower conservation

bioRxiv preprint doi: https://doi.org/10.1101/349472. this version posted January 17, 2019. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. It is made available under a CC-BY-NC-ND 4.0 International license.

20 Abstract:

21 1. Moderate to high density genotyping (100+ SNPs) is widely used to determine and
22 measure individual identity, relatedness, fitness, population structure and migration in
23 wild populations.
24 2. However, these important tools are difficult to apply when high-quality genetic material
25 is unavailable. Most genomic tools are developed for high quality DNA sources from lab
26 or medical settings. As a result, most genetic data from market or field settings is limited
27 to easily amplified mitochondrial DNA or a few microsatellites.
28 3. To enable genotyping in conservation contexts, we sequenced multiplex PCR products
29 (we call this MPCRseq) from very low-quality DNA extracted from feces, hair, and
30 cooked samples. We demonstrated utility and wide-ranging potential application in
31 endangered wild tigers and tracking commercial trade in Caribbean queen conch.
32 4. We genotyped 100 SNPs from degraded tiger samples to identify individuals, discern
33 close relatives, and detect population differentiation. Co-occurring carnivores do not
34 amplify (e.g. Indian wild dog or Dhole) or are monomorphic (e.g. leopard). 62 SNPs
35 from conch fritters and field-collected samples were used to test relatedness and detect
36 population structure.
37 5. We provide proof-of-concept for a rapid, simple, cost-effective, and scalable method (for
38 both samples and number of loci), a framework that can be applied to other conservation
39 scenarios previously limited by low quality DNA samples. These approaches provide a
40 critical advance for wildlife monitoring and forensics, open the door to field-ready testing,
41 and will strengthen the use of science in policy decisions and wildlife trade.

42

bioRxiv preprint doi: https://doi.org/10.1101/349472. this version posted January 17, 2019. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. It is made available under a CC-BY-NC-ND 4.0 International license.

43 Keywords: conch, conservation genetics, endangered species monitoring, genotyping, multiplex
44 PCR, non-invasive samples, SNPs, tigers

bioRxiv preprint doi: https://doi.org/10.1101/349472. this version posted January 17, 2019. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. It is made available under a CC-BY-NC-ND 4.0 International license.

45 **Introduction**

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

52 In principle, all of these goals can be achieved via genotyping a modest number of loci
53 such as microsatellites or single nucleotide polymorphisms (SNPs). To study endangered species
54 that are rare and elusive, approaches must be able to accommodate non-invasive sources of DNA
55 such as feces, shell, feathers, hair, and saliva, which yield impure, mixed, and/or extremely small
56 amounts of degraded DNA. Moreover, market samples generated by wildlife trade may be
57 processed, cooked, dried, or mixed with other species, again providing low quality and often
58 mixed DNA. Current approaches tend to require relatively large amounts of DNA (Carroll et al.
59 2018, nanograms of DNA) from the target species, or demand expensive and generally
60 inefficient enrichment strategies (Chiou & Bergey, 2018). Approaches designed for lower
61 concentration DNA samples (Kraus et al. 2015) require expensive and specialized equipment.

62 Here we demonstrate that a multiplex PCR approach followed by next-generation
63 sequencing satisfies all the requirements necessary for inexpensive, fast, and easy genotyping of
64 low-quality samples. We illustrate the power of this method for two endangered species in very
65 divergent conservation contexts and real-life settings: genotypes from feces, shed hair, and saliva
66 found on killed prey from wild Indian tigers and from CITES-regulated Caribbean queen conch
67 imported to the US and sold in fried fritters (method schematic Fig. 1a). Methods include DNA

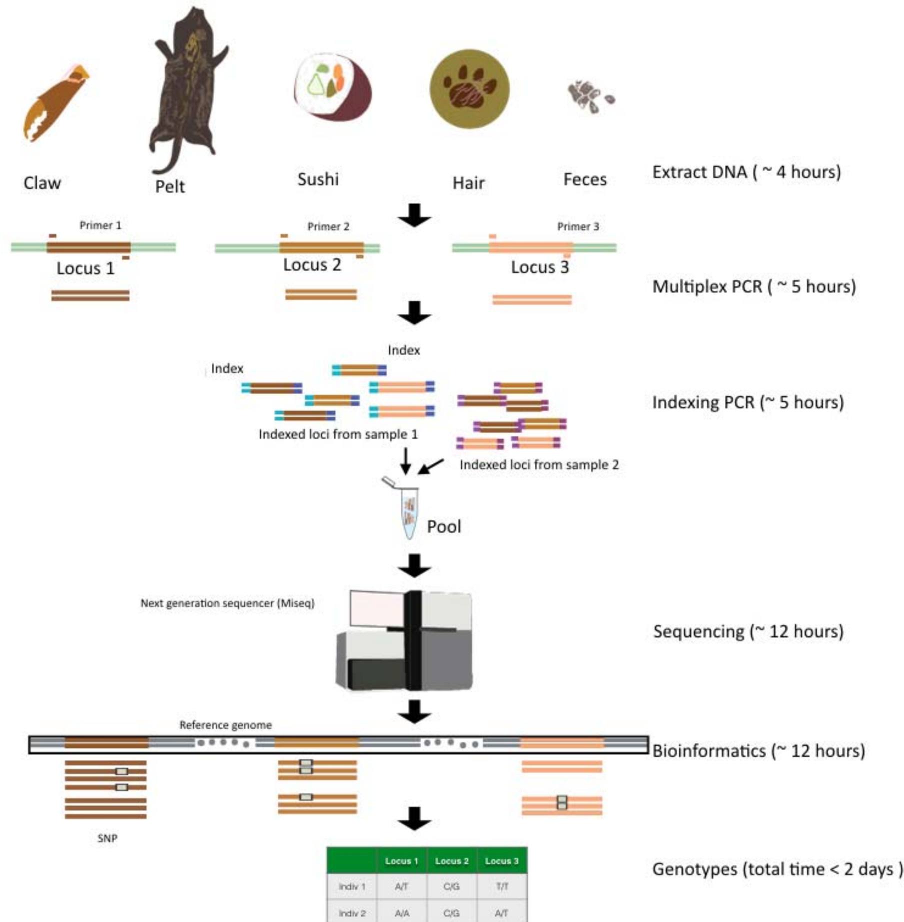
bioRxiv preprint doi: https://doi.org/10.1101/349472. this version posted January 17, 2019. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. It is made available under a CC-BY-NC-ND 4.0 International license.

68 extraction, a multiplex PCR, a second barcoding PCR, Illumina miseq sequencing and
69 bioinformatics for SNP genotyping.

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

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

bioRxiv preprint doi: https://doi.org/10.1101/349472. this version posted January 17, 2019. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. It is made available under a CC-BY-NC-ND 4.0 International license.



86
87
88 **Figure 1a** A schematic showing the protocol and approximate time taken for each step. Samples
89 available for genotyping could include a variety of sources. All steps are described in detail in
90 the main and supplementary text.
91

92 **Methods**

93 *Sample Collection*

94 For tigers, multiple samples from 13 captive tigers (USA zoos) representing different
95 scenarios were used for standardization (details in Table S1). Blood and corresponding fecal
96 swabs from captive tigers, (including one parent-offspring and one sibling pair) were collected in
97 India. Wild tigers were sampled noninvasively from multiple protected areas across India (Table
98 S2).

bioRxiv preprint doi: https://doi.org/10.1101/349472. this version posted January 17, 2019. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. It is made available under a CC-BY-NC-ND 4.0 International license.

99 Scat and saliva (from predator bites on the prey) were sampled by swabbing the surface of the
100 sample with moistened synthetic swabs (Ramón-Laca et al. 2015); tips were stored in lysis buffer
101 in 2ml microcentrifuge. Hair was sampled using forceps and stored in ziplock bags. DNA from
102 legacy fecal samples (collected in alcohol in 2014) were also tested. DNA extraction (first step
103 in Figure 1a) and quantification are described in SM 1. Two leopards and one Dhole samples
104 were included. In most cases, samples were genotyped in triplicate.

105 Tissue samples from live caught Queen Conch were taken from the mantle with sterilized
106 biopsy forceps and preserved in RNALater. Samples from conch fritters purchased from Miami
107 restaurants were frozen until dissected to isolate animal tissue fragments (SM 3a).

108

109

110

111
112
113 **Figure 1b** On the left: a tiger defecating (photo: Himanshu Chhattani), a scrape mark (and
114 associated hair samples, photo: Kaushal Patel), and a tiger hunting (photo: Shantanu Prasad,

bioRxiv preprint doi: https://doi.org/10.1101/349472. this version posted January 17, 2019. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. It is made available under a CC-BY-NC-ND 4.0 International license.

115 associated lick marks). On the right, a conch emerging from its shell, a fisherman holding his
116 catch and a fritter.
117
118 *SNP Identification and filtering*
119 Tigers
120 We identified SNPs from whole genome sequencing of 75 tigers of wild and captive origin from
121 *P. t. tigris, P. t. jacksoni, P. t. altaica, and P. t. sumatrae* subspecies (SM 1a). Alignment,
122 filtering and pruning were used to identify SNPs (SM 1a). We calculated minor allele
123 frequencies (MAF) for each SNP within each subspecies, and retained SNPs with MAF 10, 15,
124 20, 25, and 30 percent. We identified fixed SNPs that differentiated populations. We prioritized
125 SNPs within contigs greater than 10, 5, and 1MB respectively. We selected 10 differentiating
126 SNPs from each subpopulation, and 9992 polymorphic SNPs from each of the aforementioned
127 MAF cutoffs, for a total of 50,000 SNPs.
128 Conch
129 We extracted RNA from 96 *L. gigas* individuals (SM 3a, table S3). Four queen conch individuals
130 (one each from Aruba, Belize, Florida, and St. Eustatius) were imported into TRINITY v2.2.0
131 (Grabherr et al. 2013) to assemble a *de novo* transcriptome (SM 3a).
132 480,962 SNPs were discovered by aligning 96 conch sequences from 6 populations in the
133 Caribbean to the assembled transcriptome (SFG pipeline -
134 https://github.com/bethsheets/Population-Genomics-via-RNAseq, SM 3a).
135
136 *Primer Design*
137 Publically available Primer3 (amplicon size 50-90 bp, primer size 17-25 bp, and Tm of 60-61ºC)
138 was used to design primers for tiger SNPs. Conch primers were designed using the G4C

bioRxiv preprint doi: https://doi.org/10.1101/349472. this version posted January 17, 2019. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. It is made available under a CC-BY-NC-ND 4.0 International license.

139 (Genotyping for Conservation) script library and the same criteria. No attempt was made to
140 identify incompatibilities between primer-pairs and 192 primer pairs were shortlisted for both
141 species (SM 1b, SM 3b). GT-seq indexes and adapters were used (Campbell, Harmon, & Narum,
142 2014).
143
144 *PCR amplification and sequencing*
145 Library preparation consisted of an initial multiplex PCR reaction, a second PCR reaction
146 to add sequencing adapters and indexes, and sample pooling. Sample input DNA volume was
147 adjusted to a maximum of 1ng per reaction. The multiplex PCR simultaneously amplified all
148 target regions for each sample separately (in a 96 well plate). The second PCR reaction added a
149 combination of forward (i5) and reverse (i7) Illumina indexes to uniquely identify each sample.
150 The sequencing library contained equal volumes of each sample's barcoded product and was
151 cleaned with Ampure beads. Sequencing of single 50bp reads was performed on Illumina MiSeq.
152 A detailed protocol is in Supplement 1f. Alignment and genotype calling followed GT-Seq for
153 the conch study (SM3b; Campbell, Harmon, & Narum, 2014) and used standard open source
154 tools for the tiger study (SM1h). Figure 1a illustrates the steps described above.
155 The SNP panels were assessed in multiple ways. For tigers, genotyping success, genotype
156 concordance across replicates, relatedness between pairs of individuals of known and unknown
157 relationship, probability of identity of the SNP panel and population structure were assessed
158 (details in SM 1h). For conch, genotyping success and genotype concordance were tested by
159 comparing SNPs across replicate conches. Genetic distance and ability to assign the conch
160 samples to the correct population was estimated by comparison of the 96 transcriptome samples
161 (details in SM 4).

bioRxiv preprint doi: https://doi.org/10.1101/349472. this version posted January 17, 2019. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. It is made available under a CC-BY-NC-ND 4.0 International license.

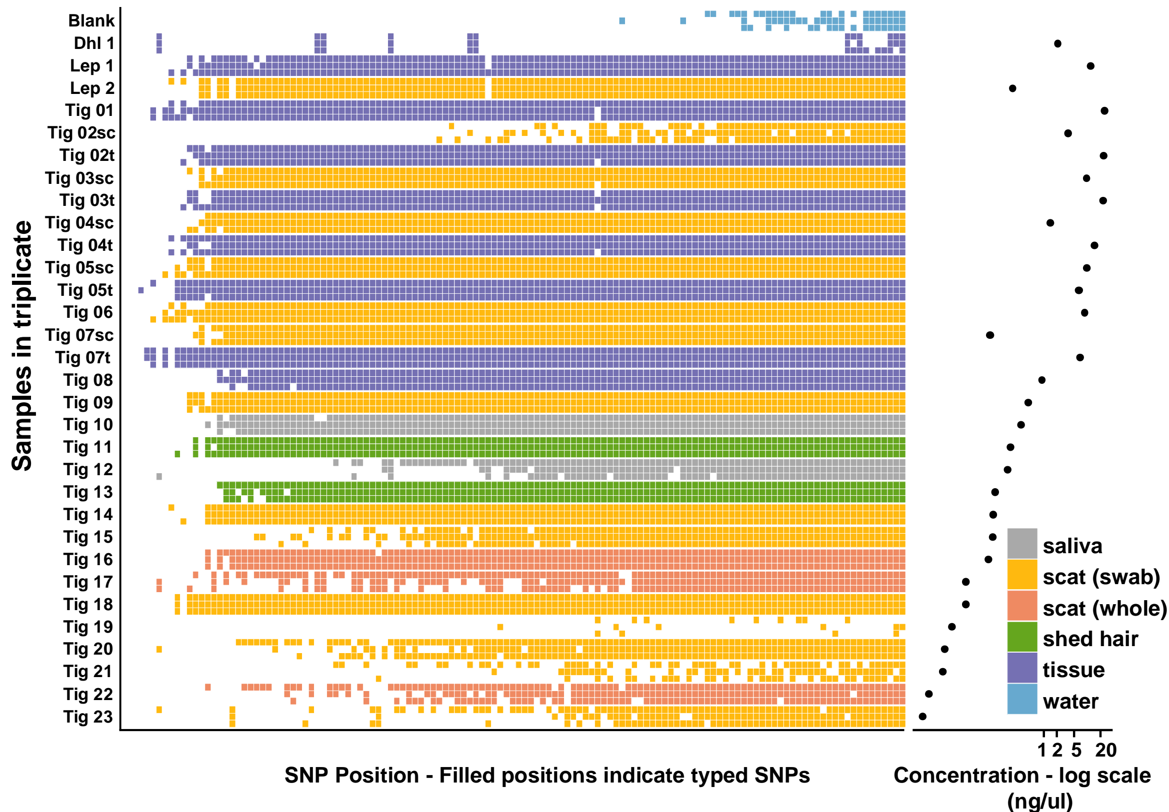
162 **Results**

163 *Tiger*

164 126 targets (66% of the 192 attempted) produced the most consistent results (SM2b,

165 Figure S1, Table S4), and were then tested on noninvasive samples from wild tigers across India

166 and zoo individuals (Fig. 1b, Table S2).



167

168

169 **Figure 2** SNP typing success (in triplicate) for various tiger samples and controls. Filled cells

170 indicate successfully typed SNPs; color indicates sample type. DNA concentrations are on the

171 right.

172

173 The 126 SNP panel for wild tigers had a high overall genotyping success rate (Fig. 2, SM

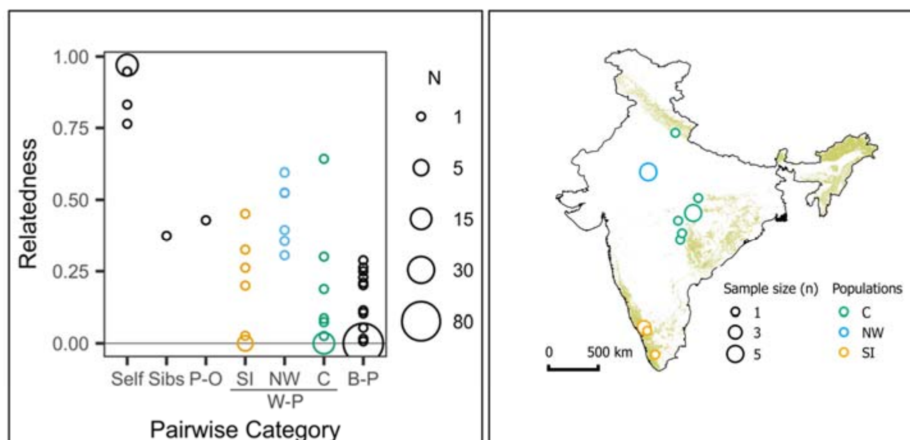
174 2c). An average of 95 SNPs (75%, range: 4 - 114) were successfully typed across all samples.

bioRxiv preprint doi: https://doi.org/10.1101/349472. this version posted January 17, 2019. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. It is made available under a CC-BY-NC-ND 4.0 International license.

175 When tiger DNA was > 0.01ng/ul (all sample types) an average of 105 SNPs (83%, range: 48 -
176 114) were typed.

177 Sample replicates were highly concordant with a high proportion of genotype matches
178 (n=28 triplicates, genotype concordance, mean: 0.957, range: 0.757 - 1.0). Different sample
179 types from the same individual also had highly concordant genotypes (n=5, concordance, mean
180 0.97; range 0.91 - 0.99). Our error rates were comparable to low microsatellite genotyping error
181 rates in some studies (Thaden et al. 2017) or lower than those in other studies of non-invasive
182 samples (Mondol et al. 2014). Our probability of misidentification was vanishingly low (pID_{sibs}
183 = 1.6E-22).

184 The co-occurring carnivores, leopard and dhole, could be distinguished from tiger
185 genotypes. The dhole tissue sample had very poor amplification success (mean across replicates
186 <10 SNPs) as expected. Leopards had high amplification success (mean: 110 SNPs), but SNPs
187 were monomorphic and nearly identical across two individuals (mean: 0.03% mismatches).



188
189 Figure 3: Pairwise relatedness (PI_HAT): Self: three replicates of each sample, and same
190 individual but different sample, Sibs: siblings, P-O: parent offspring pair, W-P: within

bioRxiv preprint doi: https://doi.org/10.1101/349472. this version posted January 17, 2019. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. It is made available under a CC-BY-NC-ND 4.0 International license.

191 population, B-P: between populations. True relatedness unknown for W-P and B-P. Sampling
192 locations and corresponding colours represented in the India map on right.
193
194 The known parent-offspring and sibling pairs (captive individuals, India) had relatedness
195 values close to expected values (0.5, Figure 3). Observed pair-wise relatedness was higher within
196 than between known genetic clusters or populations (see Natesh et al. 2017). As expected,
197 relatedness among individuals from a small, isolated population (NW, Figure 3) was high. Wild
198 individuals in our pilot dataset fell into three genetic clusters/populations as expected (Figure S2).
199
200 *Queen Conch*
201 We tested a 192 primer-pair panel on 279 conch sampled from 14 populations, including
202 48 fried conch fritters from Miami, Florida restaurants. Our SNP success was lower for conch
203 than tigers (SM 4). Approximately half the 192 conch primer-pairs failed to provide data, but 62
204 targets reached an 86% success rate similar to the 126 good tiger targets (Fig. S3). Replicate
205 samples shared 99%-100% of their alleles, and different processed conch samples were
206 genetically identical, suggesting recapture. There were no obvious close relatives among the
207 samples (Fig. S4 and S5). Outlying islands (Aruba, St. Eustatius) were genetically differentiated
208 from the central Caribbean and Florida (F_{ST} = 0.037, 0.048 respectively, Table S5). Samples
209 from the same island group (e.g. Florida, Bahamas) or from the same coast were not
210 differentiated. These patterns parallel a recent survey of queen conch with microsatellites
211 (Truelove et al. 2017).
212 Conch DNA isolated from deep fried fritters showed lower success rates than that
213 sampled by fishermen or from biopsies. However, success was high enough to allow individual

bioRxiv preprint doi: https://doi.org/10.1101/349472. this version posted January 17, 2019. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. It is made available under a CC-BY-NC-ND 4.0 International license.

214 identification and initial population comparison. Twenty-three of the fritters were probably not
215 Queen conch (fewer than 18 SNPs amplified), 8 fritters revealed poor SNP amplification
216 (average 41% success), and 17 fritters were comparable to fresh samples (77% vs 86% success,
217 Fig. S3). These samples revealed lower (average) genetic identity to Florida populations (average
218 78.6 – 80.5 alleles shared, Fig. S6) compared to Puerto Rico, or Andros Island (81.2-82.3, Fig.
219 S6). While positive population identification may require greater geographic sampling and more
220 SNPs (which may require several draft genomes), our pilot data suggest that the fritters we
221 genotyped are less likely to be from the Florida Keys and Nassau. Importantly, high resolution
222 nuclear data can be readily obtained from deeply processed commercial samples to address key
223 conservation needs.
224

225 **Discussion**

226 Our results on these pilot datasets provide proof of concept for multiplex SNP genotyping
227 (MPCRseq) of non-invasive and processed market samples from two species with vastly
228 different physiologies, biologies, ecologies, and conservation challenges. MPCRseq is successful
229 for degraded, cooked, mixed, or small and low-concentration samples (down to 10^{-3} ng DNA/ul),
230 making it an ideal tool for monitoring individuals in populations under rigors of field conditions
231 or commercial markets.

232 Conservation practitioners assume that genetics is expensive. However, MPCRseq is
233 cheap, while providing rich information important for conservation efforts. MPCRseq for a new
234 species of interest would include costs for method development and implementation.
235 Development costs include polymorphic SNP ascertainment and primer design and synthesis.
236 However, note that SNPs have already been identified for many endangered and fisheries species

bioRxiv preprint doi: https://doi.org/10.1101/349472. this version posted January 17, 2019. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. It is made available under a CC-BY-NC-ND 4.0 International license.

237 (e.g. Steiner et al. 2013). If no SNPs have been identified, practitioners could ascertain SNPs
238 using whole genome sequencing (e.g. assemble a genome of reasonable quality ~$2,000,
239 Armstrong et al., 2017) and pooled sequencing of 10 individuals at approximately 50X total
240 coverage, ~ $1000). The upfront cost of primer design and synthesis is between $1,000 to
241 $10,000 (for 100 to 1,000 primer pairs, $10 per primer pair). Once synthesized, primers can be
242 used for 38,000 reactions (~ 400 plates). The continual advance in sequencing and oligo
243 synthesis will drive down these initial development costs. Most important and attractive to
244 conservationists, we estimate implementation costs (for 1000 SNPs) can be as low as $5 per
245 sample (when processing several hundred samples).

246 Increasing the number of SNPs beyond a few hundred can provide additional information.
247 As this was a pilot to test our success rates with degraded DNA, we constrained the number of
248 SNPs that we targeted. Based on the number of SNPs we have identified, it should be possible to
249 target many more. Primers chosen to amplify clusters of closely located SNPs should allow
250 detection of very recent inbreeding using long contiguous runs of homozygosity (Kirin et al.
251 2010). Linked SNPs could generate microhaplotypes, particularly useful in pedigree
252 reconstruction (Baetscher et al. 2018). SNP panels could allow simultaneous species, individual,
253 and diet identification for clusters of species, for instance, all large carnivores and all common
254 prey species in India or sub-Saharan Africa.

255 Effective monitoring of individuals, populations and species is critical to designing rapid
256 conservation action and management of endangered species like tigers. Small, isolated
257 populations will require inbreeding management, genotype tracking for population assessment,
258 and data to assess when individuals should be introduced. Likewise, identification of commercial
259 products from illegal fishing, bush meat hunting or highly processed market samples and food

bioRxiv preprint doi: https://doi.org/10.1101/349472. this version posted January 17, 2019. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. It is made available under a CC-BY-NC-ND 4.0 International license.

260 stalls is an important source of management information. Ability to assay such samples could
261 provide a powerful incentive to follow local conservation laws. Rapid genetic monitoring of
262 endangered species from commonly occurring non-invasive samples will provide a data-pathway
263 to species recovery. We believe that multiplex PCR presents an example of such rapid,
264 accessible, cheap and efficient technology that will make this possible.
265

266 **References**

267 Armstrong, E. E., Taylor, R. W., Prost, S., Blinston, P., Der, E. Van, Madzikanda, H., …Petrov,
268 D. (2017). Entering the era of conservation genomics: Cost-effective assembly of the
269 African wild dog genome using linked long reads. *BioRxiv*.
270 doi:http://dx.doi.org/10.1101/195180.
271 Baetscher, D. S., Clemento, A. J., Ng, T. C., Anderson, C., & Garza, J. C. (2018).
272 Microhaplotypes provide increased power from short-read DNA sequences for relationship
273 inference. *Molecular Ecology Resources*, *18*(November 2017), 296–305. doi:10.1111/1755-
274 0998.12737
275 Campbell, N. R., Harmon, S., & Narum, S. R. (2014). Genotyping-in-Thousands by sequencing
276 (GT-seq): A cost effective SNP genotyping method based on custom amplicon sequencing.
277 *Molecular Ecology Resources*, n/a-n/a. doi:10.1111/1755-0998.12357
278 Carroll, E. L., Bruford, M. W., Dewoody, J. A., Leroy, G., Strand, A., Waits, L., & Wang, J.
279 (2018). Genetic and genomic monitoring with minimally invasive sampling methods.
280 *Evolutionary Applications*, 1–26. doi:10.1111/eva.12600
281 Chiou, K. L., & Bergey, C. M. (2018). Methylation-based enrichment facilitates low-cost ,
282 noninvasive genomic scale sequencing of populations from feces. *Scientific Reports*,

bioRxiv preprint doi: https://doi.org/10.1101/349472. this version posted January 17, 2019. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. It is made available under a CC-BY-NC-ND 4.0 International license.

283 (January), 1–10. doi:10.1038/s41598-018-20427-9

284 Fitak, R. R., Naidu, A., Thompson, R. W., & Culver, M. (2016). A New Panel of SNP Markers
285 for the Individual Identification of North American Pumas. *Journal of Fish and Wildlife*
286 *Management*, *7*(1), 13–27. doi:10.3996/112014-JFWM-080

287 Goodrich, J. M., Lyam, A., Miquelle, D. G., Wibisono, H. T., Kawanishi, K., Pattanavibool, A.,
288 … Karanth, U. K. (2015). Panthera tigris. The IUCN Red List of Threatened Species 2015.
289 *Iucn*, *8235*. doi:e.T15955A50659951. http://dx.doi.org/10.2305/IUCN.UK.2015-
290 2.RLTS.T15955A50659951.en.

291 Grabherr, M. G., Haas, B. J., Yassour, M., Levin, J. Z., Thompson, D. A., Amit, I., … Regev, A.
292 (2013). Trinity: reconstructing a full-length transcriptome without a genome from RNA-Seq
293 data. *Nature Biotechnology*, *29*(7), 644–652. doi:10.1038/nbt.1883.Trinity

294 Kirin, M., Mcquillan, R., Franklin, C. S., Campbell, H., Mckeigue, P. M., & James, F. (2010).
295 Genomic Runs of Homozygosity Record Population History and Consanguinity. *PLoS ONE*,
296 *5*(11), 1–7. doi:10.1371/journal.pone.0013996

297 Kraus, R. H. S., vonHoldt, B., Cocchiararo, B., Harms, V., Bayerl, H., Kühn, R., … Nowak, C.
298 (2015). A single-nucleotide polymorphism-based approach for rapid and cost-effective
299 genetic wolf monitoring in Europe based on noninvasively collected samples. *Molecular*
300 *Ecology Resources*, *15*(2), 295–305. doi:10.1111/1755-0998.12307

301 Mondol, S., Kumar, N. S., Gopalaswamy, A., Sunagar, K., Karanth, K. U., & Ramakrishnan, U.
302 (2014). Identifying species, sex and individual tigers and leopards in the Malenad-Mysore
303 Tiger Landscape, Western Ghats, India. *Conservation Genetics Resources*, 353–361.
304 doi:10.1007/s12686-014-0371-9

305 Natesh, M., Alta, G., Nigam, P., Jhala, Y. V, Zachariah, A., Borthakur, U., … Ramakrishnan, U.

bioRxiv preprint doi: https://doi.org/10.1101/349472. this version posted January 17, 2019. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. It is made available under a CC-BY-NC-ND 4.0 International license.

306 (2017). Conservation priorities for endangered Indian tigers through a genomic lens.
307 *Scientific Reports*, *7*(1), 1–11. doi:10.1038/s41598-017-09748-3
308 Ramón-Laca, A., Soriano, L., Gleeson, D., & Godoy, J. A. (2015). A simple and effective
309 method for obtaining mammal DNA from faeces. *Wildlife Biology*, *21*(4), 195–203.
310 doi:10.2981/wlb.00096
311 Snyder-Mackler, N., Majoros, W. H., Yuan, M. L., Shaver, A. O., Gordon, J. B., Kopp, G. H., …
312 Tung, J. (2016). Efficient Genome-Wide Sequencing and Low Coverage Pedigree Analysis
313 from Non-invasively Collected Samples. *Genetics*, *203*(June), 699–714.
314 doi:10.1534/genetics.116.187492
315 Steiner, C. C., Putnam, A. S., Hoeck, P. E. A., & Ryder, O. A. (2013). Conservation genomics of
316 threatened animal species. *Annual Review of Animal Biosciences*, *1*, 261–281.
317 doi:10.1146/annurev-animal-031412-103636
318 Thaden, A. Von, Cocchiararo, B., Jarausch, A., & Jüngling, H. (2017). Assessing SNP
319 genotyping of noninvasively collected wildlife samples using microfluidic arrays, (May), 1–
320 13. doi:10.1038/s41598-017-10647-w
321 Truelove, N. K., Box, S. J., Aiken, K. A., Boman, E. M., Booker, C. J., Byfield, T. T., …Stoner,
322 A., W. (2017). Isolation by oceanic distance and spatial genetic structure in an
323 overharvested international fishery, 1292–1300. doi:10.1111/ddi.12626
324
325
326 **Acknowledgements:** San Diego Zoo Global, CSC at Oakland Zoo, SF Zoo, Bronx Zoo, WCS
327 Russia, El Paso Zoo, Performing Animal Welfare Society, WCS India, CWS India, WCT,
328 Bannerghatta Zoo, Himanshu, Anubhab, Prachi, Aditya, Arun, Erica Calcagno, Jackie Gai for

bioRxiv preprint doi: https://doi.org/10.1101/349472. this version posted January 17, 2019. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. It is made available under a CC-BY-NC-ND 4.0 International license.

329 tiger samples/data, and forest departments of Rajasthan, Karnataka, Madhya Pradesh and
330 American Zoo Association, Tiger SSP for permissions. Awadhesh Pandit and NCBS NGS
331 facility. Steve Box, and Steve Canty. **Funding:** R43HG009482, Wildlife Conservation Trust
332 grant (UR), Fulbright Nehru grant (2092/F-NAPE/2015,UR) and Wellcome Trust/DBT India
333 Alliance (UR, IA/S/16/2/502714), Summit Foundation and Global Genome Initiative at the
334 Smithsonian Institution (NT, SRP, Steve Box) supported conch work. Author Contributions: MN,
335 RWT, DP, UR, EAH, SP and NT designed the study. MN, RWT and NT conducted labwork and
336 analyses. MN, RWT, DP, UR, EAH, SP wrote the paper. **Competing interests:** RWT and
337 End2End Genomics LLC received NIH small business funding (R43HG009482) to develop tools
338 to study non-model species. **Data Availability:** Dryad repository (XXXXX).