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METHODS AND RESOURCES ARTICLE

A simple, economical protocol for DNA extraction and amplification where there is no lab

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Abstract Genetic analyses are well suited to address many research questions in the study of wild populations, yet species of interest often have distributions that are geographically distant from molecular laboratories, necessitating potentially lengthy transport of biological specimens. Performing basic genetic analyses on site would avoid the project delays and risks of sample quality decline associated with transport, as well as allow original specimens to remain in the country of origin. Further, diagnostic genetic assays performed in the field could provide real-time information allowing for more nimble adjustments to research plans and use of resources. To this end, we developed protocols for reliably performing front-end genetics bench work in the field, without the requirements of electricity or permanent shelter. We validated these protocols on buccal swabs collected during routine capturing of sifaka lemurs (*Propithecus verreauxi*) at Bezà Mahafaly Special Reserve in Southwest Madagascar and faecal samples collected from captive sifakas (*P. coquereli*) at the Duke Lemur Center. Our basic protocol pipeline involves a chelating resin based DNA extraction followed by whole genome amplification or polymerase chain reaction using reagents stored at ambient temperature and portable, compact equipment powered by a lightweight solar panel. We achieved a high success rate (>80%) in downstream procedures, demonstrating the promise of such protocols for performing basic genetic analyses in a broad range of field situations.

Keywords Population genetics · Genotyping · Sex-typing · Field methods · Capacity building · Madagascar

Introduction

Genetic analyses are a valuable tool in the study of wild populations and, as a result, have been widely adopted to address questions related to conservation (Taberlet et al. 1997; Goossens et al. 2005; Kohn et al. 2006; Bergl and Vigilant 2008; Allendorf and Luikart 2009; Quéméré et al. 2010; Gray et al. 2014; Caragiulo et al. 2015), demographic and population history (Thalmann et al. 2011; Ruegg et al. 2013), social and reproductive organization (Griffin et al. 2003; Castro et al. 2004; Bradley et al. 2005; Alberts et al. 2006), pathogen load (Pallen 2014; Wedrowicz et al. 2016), and phenotype status of ecologically relevant traits like color vision phenotypes (Jacobs et al. 2017) or immune system profile (Schwensow et al. 2008). However, most genetic assays are performed in specialized molecular laboratories that are often distant from the species' natural geographic distributions. Thus, the biological samples from which genetic material is derived frequently need to be transported internationally. There are several disadvantages to this situation. First, without reliable refrigeration

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or freezer systems, it can be challenging to adequately store specimens to avoid degradation at field sites or during transport. Further, for the many endangered species that are protected by international laws and treaties, transport requires permits that can often be difficult to obtain, even when research is expressly aimed at conservation, resulting in project delays that can further compromise sample quality. The removal of samples from the country of origin also generally precludes the involvement of local collaborators and students in the genetic components of research projects. Finally, prolonged periods between sample collection and dissemination of results can hinder conservation efforts, such as surveys of elusive species. In these and similar cases, real-time and in situ information provided by diagnostic genetic assays (e.g., species identity, sex-typing) could be highly beneficial, obviating travel between the lab and the field and potentially allowing for the active direction of expeditions while "on the ground."

To address these issues, we developed and tested protocols for basic genetics laboratory work under field conditions, without access to temperature control, an electrical grid, or permanent shelter.

Specifically, we used portable equipment that was powered by a lightweight, fold-up solar panel, along with reagents kept at ambient temperatures, to test a DNA extraction protocol. Extractions were then followed by either whole genome amplification or diagnostic sex-typing polymerase chain reaction (PCR) experiments. We tested these protocols at a remote field site [Bezà Mahafaly Special Reserve (BMSR)] in Madagascar and at a naturalistic captive site [Duke Lemur Center (DLC)] in North Carolina using buccal swab and faecal samples collected from sifaka lemurs (genus *Propithecus*). We obtained a high level of success, suggesting these protocols can yield rapid results and facilitate international sharing of samples without the need for establishing costly on-site laboratories or freezer storage.

Materials and methods

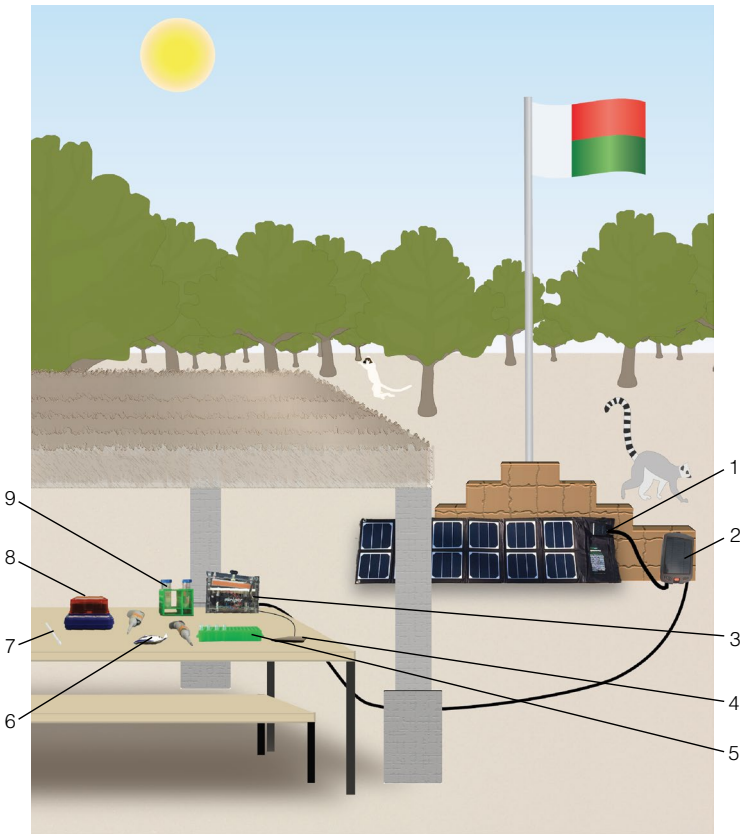
Field sites and sample collection

Remote field site: BMSR encompasses 4600 hectares of dry forest in Southwest Madagascar and is home to four species of lemur. The field site is 5 h or more from Toliara, the nearest major city, by car, depending on the conditions of the roads, which vary seasonally. The BMSR sifaka (*Propithecus verreauxi*) population has been the subject of continuous research since 1984 (Richard et al. 2002; Sussman et al. 2012), but the reserve is off the electrical grid and there is no freezer storage. This long-term project includes the annual capturing and marking of unmarked animals. Animals are demobilized using a tranquilizer dart launched from a Dan-Inject blowgun. Typically, unmarked yearlings and immigrants are captured, and a range of qualitative and quantitative measurements are taken from each anaesthetized animal. The animals are also given collars bearing an ID number prior to their release (Richard et al. 2002). We collected buccal cells using foam-tipped swabs from individuals while anaesthetized during the August capture season. August corresponds to the austral winter and the dry season in this region of Madagascar. Sunshine was plentiful, with around 9 h of direct sunlight reaching the solar panel per day. One DNA extraction per buccal swab was performed within an hour of collection.

Naturalistic captive site: We also tested our protocol on faecal samples, which we collected from captive animals at the DLC in North Carolina, a research site that partially mimics naturalistic habitat and conditions (Zehr et al. 2014). Faecal samples were collected from a closely related species of sifaka lemur (*Propithecus coquereli*) housed at the DLC. Samples were collected in the morning shortly after the sifakas awake and become active. Fresh faeces were collected immediately upon deposit from the floors of enclosures, which included both interior concrete floors (cleaned daily), and from the forest floor of natural habitat enclosures. Faecal pellets were gently swabbed with a foam-tipped swab dipped in sterile DNase-free water. Swabs were then returned to the sterile wrapper and placed in a small plastic bag stored at ambient indoor temperature (~25 °C) until extraction.

Equipment and set-up

See Fig. 1 for a schematic of the equipment set-up. All heat block and temperature cycling steps were performed using a miniPCR™ machine, which is a portable thermocycler weighing 0.45 kg. The miniPCR was programmed via an application on an android tablet (Google Nexus, weight: 0.44 kg). The miniPCR and tablet were powered by a solar battery (Apollo Pro 23000mAh battery, weight: 0.64 kg, made by PowerAdd). We implemented gel electrophoresis for the sex-typing assay on-site using a portable gel box with built-in power source and blue light illuminator (blueGel™, weight: 0.34 kg, made by miniPCR). The gel box was powered by a 27,000 mAh battery with an AC outlet (weight: 1.08 kg, made by ChargeTech). Batteries were charged from two fold-up solar panels (a 40 W panel, weight: 1.08 kg, made by PowerAdd, and a 60 W panel, weight: 1.63 kg, made by AllPowers). Standard laboratory pipettes and sample racks were used. The total weight of this equipment was around 10 kg and the total cost, excluding pipettes, was just over US$2000.



1 Fold-up solar panel *Poweradd 40W solar charger*

2 Solar battery *PowerAdd Apollo Pro 23000mAh solar battery*

3 Portable PCR machine *miniPCR*

4 Android tablet running miniPCR app

5 Shelf stable whole genome amplification kit *illustra Ready-to-go GenomiPhi whole genome amplification* or PCR shelf stable PCR reagents *(MasterMix, primers, BSA, H_2O)*

6 Break-n-Shake Instant Cold Pack *Dynarex*

7 Swabs *Foam tip*

8 Portable gel rig *miniPCR*

9 10% Chelex Solution *Biorad Chelex 100 resin diluted in ddH_2O*

Fig. 1 Depiction of equipment and field setting

DNA extraction

Swabs were swirled for a few seconds in 1.5 ml tubes containing approximately 300 µl of Chelex® 100 chelating resin (BioRad) in solution and then discarded. For buccal swabs, a 10% Chelex solution was prepared, and for faecal swabs, a 20% Chelex solution was used to counteract potential inhibition from compounds in the faeces. Chelex solution was then split between at least two 0.2 ml PCR tubes that were subsequently incubated at 95 °C for 10 min in the miniPCR unit. Samples were set to cool for 10 min, after which time, the supernatant was used as template in downstream amplification steps (Fig. 2; see Supplemental File 1 for protocol sheet). Quantification of DNA extractions was possible using a NanoDrop 2000 (Thermo-Fisher Scientific) spectrophotometer at the captive site.

Amplification

Option A: whole genome amplification and downstream PCR

Four whole genome amplifications (WGAs) per individual were performed immediately following DNA extraction

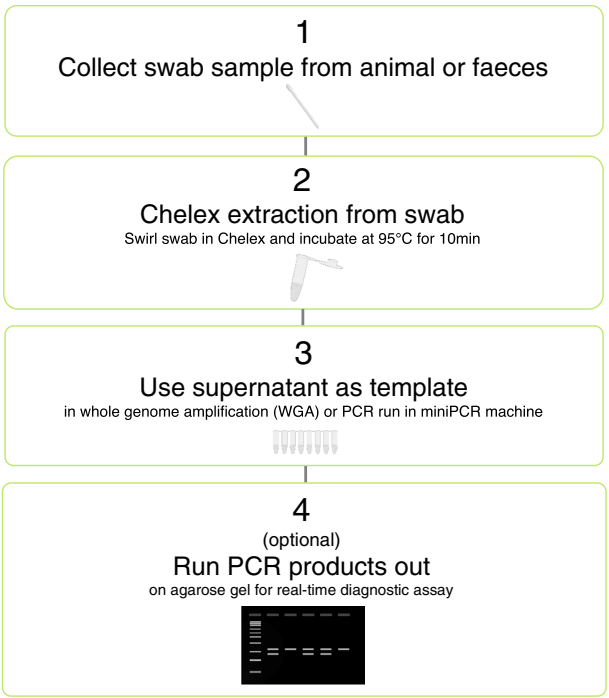


Fig. 2 Flow chart of the basic protocol

from buccal swabs using the illustra Ready-to-go GenomiPhi™ v3 whole genome amplification kit (GE Life Sciences). All reagents in this kit are shelf stable and the protocol consists of three basic incubation steps: a denaturation step, an amplification step, and a deactivation step. Denaturation step: In a strip of 0.2 ml PCR tubes, 10 ul denaturation buffer was added, followed by 8 µl sterile DNase-free water, and 2 µl of template DNA. Samples were incubated for 3 min at 95 °C after which they were cooled on instant ice packs (Dynarex). In the meantime, a cooling program was begun on the miniPCR that was set up for incubation at the lowest temperature setting (20 °C), which results in continuous blowing of the miniPCR fans. Kit-provided strip tubes containing reagent cakes were placed in the miniPCR unit with the lid left open, and the 20 µl samples were then transferred to these tubes. Amplification step: all samples were incubated at 30 °C for 90 min in the miniPCR. Deactivation step: samples were incubated at 65 °C for 10 min in the miniPCR and then removed for storage at ambient temperature. All steps in this protocol and the preceding extraction from the buccal swabs were performed at a basic picnic table.

WGAs were kept at ambient temperature during storage in the field (~30 °C during the day) and transported back to the United States within 5–15 days, after which they were stored at −80 °C. Success of WGAs was assessed via visualization on a 2% agarose gel pre-stained with GelRed (Biotium). WGAs were then used as template in downstream PCRs targeting seven microsatellite loci that had already been characterized in this sifaka population (Lawler et al. 2001). Microsatellite PCRs using fluorescently labelled primers were performed in duplicate for each sample. Amplicons were run out on an agarose gel to confirm amplification of products of the target length and fragment analysis was performed using capillary electrophoresis with an ABI 3730xl 96-Capillary Genetic Analyzer at the DNA Analysis Facility at Yale University. GeneMarker v2.6.3 (SoftGenetics) was used to visualize the electropherograms and call genotypes. Homozygote genotypes were confirmed in a minimum of four independent PCRs. Allele frequency analysis was performed using CERVUS 3.0 (Kalinowski et al. 2007).

Option B: direct PCR

PCR reactions targeting the UTX/UTY sex-typing loci (Villesen and Fredsted 2006) were performed in the miniPCR using template from the faecal swabs. Most reactions were performed directly after extraction. This assay uses three primers to target an X-Y homologous portion of the ubiquitously transcribed tetratricopeptide repeat protein gene (Villesen and Fredsted 2006). The two target regions differ in length by 41 bp (X = 127 bp and Y = 86 bp) so that they may be visually distinguished via gel electrophoresis. Males will present as heterozygotes (two distinct bands) while females present as homozygotes (one band) (Fig. 3).

Reactions included 4 µl template DNA, 12.5 µl of EZ PCR Master Mix (miniPCR), 2 µl Ambion Ultrapure non-acetylated Bovine Serum Albumin (40 µg), 1 µl UTY/UTX primer (forward primer for both UTX and UTY regions), 4 µl UTY reverse primer, and 0.25 µl UTX reserve primer (all at a concentration of 10 µM), and 1.25 µl sterile DNase-free water. The Master Mix, primers, and BSA were stored frozen prior for use in these PCRs, but all of these reagents are stable at ambient temperature for a month or longer (based on manufacturer specifications and direct experience). Cycling conditions were as follows: 94 °C for 30 s, 59 °C for 40 s, 72 °C for 60 s for 35 cycles preceded by an activation step at 95 °C for 15 m and followed by a final extension step at 72 °C for 7 min. PCR products were visualized on a 2% agarose gel pre-stained with GelGreen (Biotium) in a portable gel box with built-in power source and blue light illuminator (blueGel, weight: 0.75 lbs, made by miniPCR). Gels were pre-made and stored wrapped in tinfoil inside a sealed plastic bag at room temperature for up to 2 weeks.

Results and discussion

During the August 2015 capture season at BMSR, we collected buccal swabs from 26 anaesthetized sifakas (21 yearlings and five adults) from which we extracted DNA and performed WGAs. The WGAs performed in field conditions showed a high rate of successful amplification (72% overall), as determined by gel electrophoresis visualization. Downstream microsatellite PCRs performed using successful WGAs as template also showed a high rate of successful amplification. We were able to call genotypes successfully from fragment analyses of the amplicons 89% of the time. (individual locus success rates were 80–97%; Table 1). These outcomes are similar to those we typically observe following our traditional microsatellite genotyping workflow based on silica column extractions from exported

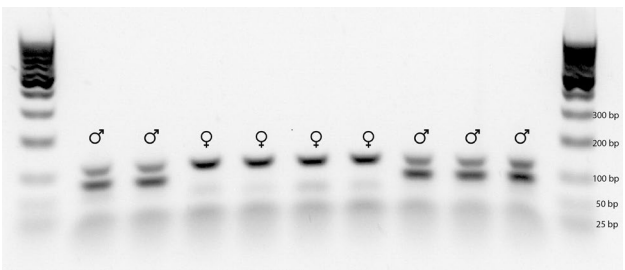


Fig. 3 Gel showing successful amplification of sex-typing PCR

Table 1 Protocol success rates

Procedure	Test N	Success rate	
		Test	Typical
Trial 1: Buccal WGA			
Microsatellite genotyping success rate			
WGAs	104	72%	87%
Locus			
PV1	40	85%	86%
PV4	40	78%	77%
PV6	40	97%	87%
PV8	40	95%	91%
PV14	40	87%	91%
PV15	40	93%	87%
PV16	40	87%	89%
Total	280	89%	87%
Trial 2: Faecal PCR			
Sex-typing PCRs	42	94%	91%
Median DNA concentration (ng/µl)		53[a]	15
Median DNA molecular weight (ng)		3975[a]	2100

Trial 1: WGA success rate was determined as visible DNA following gel electrophoresis and microsatellite genotyping success rate determined as callable genotypes from electropherograms resulting from fragment analysis. Trial 2: Sex-typing PCR success rate was determined by visualization via gel electrophoresis. Typical success rates determined by these same procedures from "typical" sifaka samples in our hands (from gDNAs derived from tissue samples exported to the US and processed using traditional methods in the laboratory) are also shown

[a]Total DNA, likely includes high levels of bacterial DNA

tissue samples (Table 1). Genotypes for these individuals were not obtained using our traditional workflow because original samples remain in Madagascar, precluding a direct comparison. However, two adult males in our sample had been captured during previous field seasons and genotype data generated from our traditional protocol was available. In these cases, the genotypes derived for these individuals from both protocols matched, except in the case of one allele at one locus in which the estimated length was shifted by 2 bp, likely reflecting an error in allele calling due to stutter. Our sample size was too low to estimate deviation from Hardy–Weinberg, but the rates of observed versus expected heterozygosity did not markedly differ from what we typically observe.

We tried a variation on the incubation step of the WGA protocol to potentially conserve electricity in which we left samples at ambient temperature, which was around 30 °C, for 90 min rather than incubating them inside the miniPCR. We found that the WGAs incubated in the miniPCR amplified much more successfully (92% success rate) than those incubated at ambient temperature (43%), as determined by gel electrophoresis. In contrast, we found no relationship between the number of days that WGAs were stored at ambient temperature and amplification success.

Fresh faeces were collected from 11 sifakas at the DLC that were used in the faecal swab and downstream sex-typing PCR protocol. The median concentration of faecal swab extractions was 53 ng/µl, as measured on the NanoDrop spectrophotometer, which measures total DNA concentration and, in the case of faecal-derived DNAs like these, could include relatively high concentrations of bacterial DNA. The median volume of supernatant was 75 µl. 94% of the UTX/UTY PCRs performed using supernatant directly after extraction from freshly collected samples showed amplification, as determined by gel electrophoresis visualization. PCRs using template DNA from extractions or samples stored at ambient temperature overnight or longer did not amplify as reliably; however, successful amplification was achieved from faecal swabs that were up to 13 days old. Sex genotypes based on multiple amplifications matched the known sex of the animals. One of the challenges we faced was optimizing extractions from non-invasively collected samples. While at BMSR, we opportunistically collected ten faecal samples that were used in our extraction-WGA protocol. In place of the swab, we dunked and swirled the faecal pellet in the tube containing Chelex solution with tweezers. However, few of the WGAs performed from these extractions showed amplification, as determined by gel electrophoresis, after returning to the lab. In our subsequent attempts at the DLC, we found that swabbing the exterior of the pellet to be critical as this likely disproportionately targeted host cells and avoided inhibiting compounds. We also increased the concentration of the Chelex solution used in faecal swab extractions from 10 to 20% to more effectively remove compounds in the faecal samples that could potentially inhibit downstream PCR.

The equipment proved functional, resilient to our field conditions, and easily transportable. Solar energy was sufficient to perform the planned number of analyses, and rates of DNA extraction and amplification were high. The PowerAdd and ChargeTech batteries functioned fully when left in direct sun, however additional types of lithium ion batteries proved unable to withstand similar temperatures during charging.

Our success was likely in part due to favourable field conditions; specifically, these included plentiful sunshine and an arid climate without extreme heat, which benefits sample preservation. In contrast, overcast climate or denser forest conditions could present challenges for obtaining sufficient power. Nevertheless, our protocol could likely be adjusted to suit such conditions with alternative equipment. For example, we also successfully ran the miniPCR from a car battery.

These experiments were also performed during a relatively short field season, with 15 days being the longest time period that reagents and WGAs were kept at ambient temperature. It is uncertain how well WGA integrity would hold up over longer periods without freezing. We tried storing WGAs on FTA cards, which could potentially extend the longevity of nucleic acid at ambient temperatures; however, attempts to extract WGA template from the cards failed. Similarly, pre-cast agarose gels are unlikely to hold up well during longer field excursions and in those cases it would be preferable to heat agarose solutions to cast fresh gels in the field. Alternatively, a number of suppliers offer gel cassettes that might be used.

Our protocols resulted in a success rate comparable or superior to the rate we typically observe in equivalent analyses performed in a dedicated molecular lab using samples transported internationally from BMSR. Recent technological developments in lab equipment, solar energy, and reagent chemistry will increasingly enable the incorporation of genetic analyses into field projects. A number of portable and semi-portable technologies have been used in molecular analyses, particularly for disease diagnostics (Marx 2015), and some of these technologies have allowed for genetic analyses to be performed in field laboratories (Bunting et al. 2014). Further, emerging portable genomics devices like the Oxford Nanopore MinION could greatly advance field researchers' capacity to obtain vast amounts of genetic data from wild organisms while in the field. These technologies currently depend on reagents that require freezing, but could potentially be used at field sites with solar and/or portable freezer options.

Our protocol offers a method that is highly portable, can be performed immediately upon sample collection in the field, and has the potential to be powered by green energy. These protocols also have an advantage in avoiding research delays related to permit applications. For example, U.S. Fish and Wildlife Services and the Center for Disease Control do not currently require permits for the import of synthetic nucleic acids. Further, the relative low cost and ease of use of the components of this "portable lab" could provide a means for researchers to incorporate genetic components into projects that previously did not include these analyses, without having to invest in a molecular lab. With some training, these analyses could also potentially be performed by local students or assistants, providing an opportunity for capacity building and community involvement.

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Compliance with ethical standards

Conflict of interest The authors have no conflicts of interest to declare.

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