

# GENOMICS IN THE JUNGLE

## Course Syllabus

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## Course Introduction

Biological and primatological research is turning to genetic research methods for a deeper look into the biological factors that encode behavior and physiology. We use genetic techniques to determine species delimitations, define populations, understand mating systems, explain behavioral differences in foraging efficiency, screen for disease, conduct paternity studies, evaluate immune status and functioning, and explore microbiome diversity... and these are just a few examples of the full breadth of the field as applied to wildlife biology. The field of genetics is revolutionizing biological research, and in the past few years we have even witnessed the successful deployment of instruments that enable molecular work to be conducted ‘on-the-fly’ and in the field. These new tools are minimizing the hassles and barriers associated with transporting samples around the world to distant labs that possess the equipment and resources to extract, amplify, and sequence DNA. In many ways, this new technology is democratizing wildlife research by empowering field scientists all

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### Location:

**Inkaterra Guides Field Station**

### Course Dates:

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around the world with genetic tools to directly advance their research and conservation initiatives.

This course will take students to the Peruvian Amazon, where they will learn how field research is conducted, assist in sample collection, and then actually extract, amplify, sequence, and interpret genetic data to answer several practical research questions about wildlife ecology and natural history. It will take place at the Inkaterra Field Guides Station, which is the site of the Green Lab, the world's first tropical rainforest molecular genetics laboratory. Participants will go from sample collection to sequence analysis directly in the rainforest, which is the science of the future. This course will provide an introduction to next generation primatologists and biologists, who will gain not only the skills requisite for field research but the technical know-how to employ genetic research tools in the field.

## Case Studies

In this course, we will focus on three specific cases (class size and time permitting) in which cutting-edge genomics can help us solve mysteries common to wildlife research in the field. The ultimate goal of all of these projects will be to use a MinION, a USB-sized powerful sequencer that is revolutionizing how we do genomics in some of the craziest places on the planet.

The MinION has been used to:

- do whole-genome sequencing to identify species with PCRs in under 40 minutes and whole genome amplification in under 100 minutes
- conduct real-time DNA sequencing in a remote rainforest by a course instructor
- sequence DNA offline in the Antarctic and even on the International Space Station in outerspace!

### *Case 1: DNA Fingerprinting*

In 1986, the first criminal was captured on the basis of DNA fingerprinting, a process developed at the University of Leicestershire in the UK. Semen stain samples from two murders

were collected and matched to the same perpetrator. An innocent man accused of the crimes was exonerated, and the real culprit caught. Since then, DNA fingerprinting has revolutionized the way crime scenes are processed and biological samples screened. But DNA fingerprinting is not restricted to forensic science applications alone. You can fingerprint anything, and one way in which we do so in wildlife biology is to use a barcode database.

For this case study, we will collect fecal samples from wild primates and use these known samples to identify a series of unknown samples down to the species. To do this, we will barcode the DNA of each sample, and compare it to a reference database. A second dataset will use a similar process to identify unknown plants, keeping in mind that plants are polyploids and so a bit more complex to barcode.

Skillsets: Sample collection from wild primates and Amazonian plants, sample storage and sterile technique, DNA extraction using multiple kits for comparison, amplification of barcoded regions in the genome, gel electrophoresis to confirm amplicon sizes, sequencing of the regions using a MinION, real-time species identification with the WIMP workflow.

### *Case 2: Does captivity alter primate microbiomes?*

A microbiome is a collection of the genes of all the microbes in a community, and a disturbance of the “normal” microbiome of the gut of an animal (including humans) can result in disease. However, what is normal? Which microbes are present and what do they do? Although much of this information is now known for humans, it is completely unknown in wild primates.

For this case study, we will compare fecal samples collected from a local wildlife rehabilitation center (including animals thought to be healthy and sick) with samples from wild primates living in forests nearby. The goal is to conduct 16S sequencing of all of the bacteria present in these samples. We will also visit the Taricaya Rehabilitation Center to discuss wildlife rehabilitation and the wildlife trade in Peru with veterinarians and keepers on site.

Skillsets: Sample collection (wild and captive), storage and sterile technique, DNA extraction, PCR amplification of the genetic material in the 16S ribosomal subunit, multiplexing of samples and cDNA library prep for sequencing on the MinION, subsequent analysis of data using the WIMP workflow.

### *Case 3: Environmental DNA*

Imagine, if you will, the contents of a primate fecal sample. Beyond thinking about the icky side of things, a fecal sample is as valuable as gold to a research scientist. It contains a multitude of organisms, each with their own unique trace of DNA. For example, represented will be the bacterial microbiome of the animal’s gut, the parasites it might contain, the plant and animal material it consumed, and it’s own gut cells with the host’s DNA. So from one single sample, we can not only identify the producer of the sample but also everything that animal ate. For a wildlife biologist studying hard-to-see and often unhabituated animals, this is indeed, an unappreciated treasure of information. Even the best animal trackers cannot pursue every animal so closely as to see everything it eats, including tiny insects or rare species of vines in the canopy of a rainforest. Enter, genomics in the jungle!

In this case study, we will use markers such as that of the 18s ribosomal subunit to identify all prokaryotic and eukaryotic DNA in a range of fecal samples. Our goal is to ignore the obvious, such as host DNA but to focus on environmental traces of DNA.

Skillsets: Sample preparation and collection, DNA extraction, library preparation, sequencing on the MinION and analysis.

*We will try to answer a few additional questions with all case studies:*

- Can we use the What's In My Pot (WIMP) workflow to accurately classify these species to a reference database in real-time for all of the case studies?
- How many different kinds of samples can we multiplex at one go on a MinION?
- Does the choice of DNA extraction kit affect the outcome?
- Does the length of time we let the MinION run for affect the accuracy of our species identifications?

## Course Objectives

The goals of this course are to give participants advanced training in field techniques important to the collection of biological samples from Amazonian wildlife, their prey and their parasites, all the way to sequencing DNA from these sources.

The course has the following broad objectives:

- \* To engage in both independent and team-based data collection
- \* To teach sample collection techniques from Amazonian wildlife, with a focus on primates as model megafauna
- \* To learn sample storage and clean-lab protocols in the field
- \* To extract DNA in a field laboratory
- \* To test DNA quality and quantify it
- \* To run basic PCRs for a range of markers using multiple protocols on field PCR machines (smaller, lighter, lower-scale and more rugged than typical lab-based machines)
- \* To explore metagenomics in the field using three case studies:
  - \* DNA fingerprinting
  - \* Microbiome metagenomics
  - \* Environmental DNA

# Course Topics

Topic of Study	Activity	Description
<b>I. Introduction</b>		
> Threats to the Amazon in the Madre de Dios Department of Peru; conservation efforts of the Amazon Conservation Association (ACA); conservation efforts of FPI	Lecture	A review of the major conservation approaches in the MDD, including the conservation and research efforts of ACA and FPI.
> Field ethics, safety precautions, rules, and useful tips.	Discussion	Keeping your footprint to a minimum while working with wildlife in the tropics, and ensuring your safety and that of the wildlife around you.
> DNA sequencing and genomics	Lecture/Discussion	An introduction to genomics and its practical applications in the field
> Genomics and Ethics	Discussion	What should we be thinking about when applying genomics to wildlife research? Whose rights come into question? Do different countries regulate genomics in different ways?
<b>II. Navigation and Space Use</b>		
> Basic functions of a handheld GPS and compass	Demonstration	Getting familiar with the most important pieces of equipment you will have in the field.
> Waypoint and track data and how to use them	Exercise	Recording key features of the research station with waypoints and tracks
> Visualizing spatial data	Exercise	Manipulation of GPS data; creating a digital field map (laptop with USB port required for each participant)
<b>III. Collecting Biological Specimens</b>		
> Field methodology: indirect observation and biological sampling	Lecture/Practical Exercise	Tracking primates, identifying plants based on botanical features, and collecting non-contaminated samples from each.
> Field methodology: sample storage and preparation	Lecture/Practical Exercise	Sample collection methods in the field, time-scales for DNA deterioration, and field laboratory sterile technique

Topic of Study	Activity	Description
<b>IV. Basic Field Laboratory Techniques</b>		
> Basic laboratory techniques	Practical Exercise	Regardless of participant background, we will spend a moment practicing good pipetting techniques, sample volume calculations, and gel loading techniques. Repetition is key, getting everyone onto the same skill level
> Laboratory Safety	Lecture/Practical Exercise	Even in the field, lab safety is critical. We will go over protocols, cautionary tales, and specific examples of how not to hurt yourself in a field laboratory.
> Lab recipes	Practical Exercise	We will learn the processes behind the various lab protocols or recipes we will be following in this course
<b>V. Genomics in the Jungle</b>		
> DNA barcoding	Lecture	The history of species identification using barcodes, including some of the most exciting applications in wildlife science
> Environmental DNA	Lecture	DNA is everywhere, in everything - so why would we care about picking up trace DNA? What are the useful applications of this technique?
> Microbiome analyses	Lecture	Microbiome analyses can tell us a great deal about human health - can the same be said for animals? What is a normal monkey gut even look like?
<b>VI. Genomics in a Jungle Lab</b>		
> DNA extraction	Practical Exercise	Whole genome amplifications and genomic DNA extraction
> DNA quantification	Practical Exercise	How much DNA do you have?
> PCRs	Practical Exercise	Amplifying markers for all three case studies using PCRs in a field laboratory
> Gel electrophoresis	Practical Exercise	Testing if your PCR worked using gel electrophoresis and PCR product quantification

Topic of Study	Activity	Description
> Library prep	Practical Exercise	Creation of DNA libraries from samples
> Multiplexing and indexing	Practical Exercise	Minimising sequencing costs by running multiple samples in a single run - multiplexing and indexing samples to tell them apart afterwards
> Using the MinION	Practical Exercise	Learn to run sequences on a MinION device - cutting edge genetic sequencing using nanopore flowcells
> Basic bioinformatics	Practical Exercise	How to interpret data from the MinION - both real-time and post-hoc
<b>V. Excursions and Activities (time and weather permitting)</b>		
> Canopy Walkway	Excursion	Participants will be given the opportunity to climb and view the Amazon rainforest from network of rainforest canopy walkways.
> Claylicks	Excursion	We will visit Lake Sandoval to view the famous macaw and mammal claylicks on site
> Taricaya Rehabilitation Center	Excursion	The illegal wildlife trade is booming in Peru, and rehabilitation centers are the final stop for those animals lucky to be recovered - but is being in captivity changing them irrevocably?
> Guest Lectures	Lecture	The IGFS is a popular location for field researchers. Participants will be given the opportunity to be exposed to various research groups and interests during their time here, including projects that directly emphasize wildlife biology.

# Daily Schedule

Date	Activities	Reading Discussion	Assignments Due
1st July	<b>Arrive in Puerto Maldonado</b> <ul style="list-style-type: none"> <li>- go directly to the IGFS by boat</li> <li>- Settle in, get to know your peers and instructors</li> </ul>		
2nd July	<b>Morning:</b> <ul style="list-style-type: none"> <li>- After breakfast, get on the trails and do a morning hike</li> </ul> <b>Afternoon:</b> <ul style="list-style-type: none"> <li>- DNA: A lab skills refresher</li> </ul> <b>Night:</b> <i>Lectures</i> <ul style="list-style-type: none"> <li>- Introduction to the Madre de Dios, ITA &amp; FPI</li> <li>- Wildlife research - methods and technology</li> <li>- A brief history of genetics and present-day technology</li> </ul>	<i>Discussion:</i> Field ethics, safety, rules, and tips	Lab reports
3rd July	<b>Morning:</b> <ul style="list-style-type: none"> <li>- Explore the canopy walkway</li> <li>- Navigating off trail and tracking primates</li> <li>- Camera traps to detect mammals</li> </ul> <b>Afternoon:</b> <ul style="list-style-type: none"> <li>- Lab safety protocols and recipes</li> </ul> <b>Night:</b> <i>Lectures</i> <ul style="list-style-type: none"> <li>- The pre-genomics era and wildlife biology</li> </ul>	<i>Discussion:</i> Functions of a GPS and Compass	<i>Discussion:</i> How to collect and preserve biological specimens
4th July	<b>Morning:</b> <ul style="list-style-type: none"> <li>- Track primates for fecal samples</li> </ul> <b>Afternoon:</b> <ul style="list-style-type: none"> <li>- Lab: DNA extraction and quantification</li> </ul> <b>Night:</b> <i>Lectures</i> <ul style="list-style-type: none"> <li>- The genomics era and wildlife biology</li> </ul>	<i>Discussion:</i> How to choose the best extraction kit	<i>Lab Skills:</i> Sterile technique, tips for increasing DNA output

Date	Activities	Reading Discussion	Assignments Due
5th July	<b>Morning:</b> - Visit Taricaya Rehab Center - Sample primates in captivity	<i>Discussion:</i> The wildlife trade in Peru	Quiz 1: The history of genomics, DNA extraction, navigation
	<b>Afternoon:</b> - Lab: DNA extraction round 2 - Lab: DNA quantification	<i>Lab Skills:</i> DNA quantification	techniques, basic lab calculations
	<b>Night:</b> <i>Lectures</i> - Quiz 1, Paper discussion	Paper: Pomerantz et al. 2017. Biorxiv	
6th July	<b>Morning and Afternoon:</b> - Split into lab teams - PCR setup and amplifications for various markers - Trail hikes while PCRs are running	Sample collection from primates and plants in the field	
	<b>Night:</b> <i>Lectures</i> - DNA barcoding/fingerprinting		
7th July	<b>Morning and Afternoon:</b> - Split into lab teams - PCR setup and amplifications for various markers - Trail hikes while PCRs are running	Sample collection from primates and plants in the field	
	<b>Night:</b> <i>Lectures</i> - Environmental DNA		
	<b>Morning and Afternoon:</b> - Gel electrophoresis - Trail hikes in between gels	Sample collection from primates and plants in the field	
8th July	<b>Night:</b> <i>Lectures</i> - Microbiomes of wildlife		

Date	Activities	Reading Discussion	Assignments Due
9th July	<p><b>Morning and Afternoon:</b></p> <ul style="list-style-type: none"> <li>- Library prep</li> <li>- Trail hikes in between lab</li> </ul> <p><b>Night:</b> <i>Lectures</i></p> <ul style="list-style-type: none"> <li>- Microbiomes of wildlife</li> </ul>	Sample collection from primates and plants in the field	Quiz 2: Case studies histories, lab methods: DNA extraction, PCRs, gels
10th July	<p><b>Morning and Afternoon:</b></p> <ul style="list-style-type: none"> <li>- Library prep</li> <li>- Trail hikes in between lab</li> <li>- End the day by starting the MinION run</li> </ul> <p><b>Night:</b> <i>Practical Exercise</i></p> <ul style="list-style-type: none"> <li>- Real-time results with the MinION</li> </ul>		
11th July	<p><b>Morning and Afternoon:</b></p> <ul style="list-style-type: none"> <li>- Letting the MinION run overnight</li> <li>- Examining real-time results with WIMP</li> <li>- Eventually stopping the run</li> <li>- Exploring the MinION outputs</li> <li>- Demultiplexing and blasting reference databases as needed</li> </ul> <p><b>Night:</b> <i>Practical Exercise</i></p> <ul style="list-style-type: none"> <li>- Data analysis in groups based on case study</li> </ul>		
12th July	<p><b>Morning:</b></p> <ul style="list-style-type: none"> <li>- Group activities planning presentations of case study outcomes</li> </ul> <p><b>Afternoon:</b></p> <ul style="list-style-type: none"> <li>- Time to practice presentations and prep for final</li> </ul> <p><b>Night:</b> <i>Final Exam</i></p>		Final exam: covers everything learned, focus on the last third of the class

Date	Activities	Reading Discussion	Assignments Due
	<b>Morning:</b> - Final excursions and hikes		
13th July	<b>Afternoon:</b> - Time off to pack  <b>Night:</b> <i>Final presentations: 10 mins per group</i>		
14th July	<b>Depart from Puerto Maldonado</b>		

## Course Work

### *Lab notebook (250 pts)*

Paper notebooks in a lab are soon becoming a thing of the past. We will use Benchling, innovative software that is both free and flexible online, to conduct all the exercises in the course. On the Benchling account for the course will be all lab protocols and templates, pre-designed and ready for use. Participants will simply choose the appropriate template and store all data online. At the end of the course, your Benchling lab notebook will form a part of your grade, as maintaining an accurate record of the work you do in a lab is a crucial part of being a good research scientist.

### *Sample List (100 pts):*

All participants will contribute towards the course sample list, documenting a range of metadata for each sample that will help us identify it in the future. The ongoing sample list will be judged as a group at the end of the course and individual effort to locate and collect samples will also be appreciated.

### *Lab Exercises (250 pts):*

Every case study is going to involve a series of lab sessions, each of which will have reports due at the end. These may be recorded on Benchling or turned in by hand, depending on the exercise. Your ability to complete the exercises, remain enthusiastic and a good team player will determine your grade for each lab exercise.

### *GPS Mapping (100 pts)*

Several activities will involve recording GPS data and manipulating it in the Garmin Basecamp program, which is available for free download. At the end of the course, students will turn in the data from these exercises, which will include tracks made during off-trail hikes and primate follows, as well as a student-generated map of the field station.

## **Reading List** (subject to change)

Pomerantz, A., Penafiel, N., Arteaga, A., Bustamante, L., Pichardo, F., Coloma, L.A., Barrio-Amoros, C.L., Salazar-Valenzuela, D. and Prost, S., 2017. Real-time DNA barcoding in a remote rainforest using nanopore sequencing. bioRxiv, p.189159.

Clayton, J.B., Vangay, P., Huang, H., Ward, T., Hillmann, B.M., Al-Ghalith, G.A., Travis, D.A., Long, H.T., Van Tuan, B., Van Minh, V. and Cabana, F., 2016. Captivity humanizes the primate microbiome. Proceedings of the National Academy of Sciences, p.201521835.

Yildirim, S., Yeoman, C.J., Sipos, M., Torralba, M., Wilson, B.A., Goldberg, T.L., Stumpf, R.M., Leigh, S.R., White, B.A. and Nelson, K.E., 2010. Characterization of the fecal microbiome from non-human wild primates reveals species specific microbial communities. PloS one, 5(11), p.e13963.

Bohmann, K., Evans, A., Gilbert, M.T.P., Carvalho, G.R., Creer, S., Knapp, M., Douglas, W.Y. and De Bruyn, M., 2014. Environmental DNA for wildlife biology and biodiversity monitoring. Trends in Ecology & Evolution, 29(6), pp.358-367.

Mezzasalma, V., Bruni, I., Fontana, D., Galimberti, A., Magoni, C. and Labra, M., 2017. A DNA barcoding approach for identifying species in Amazonian traditional medicine: The case of Piri-Piri. Plant Gene, 9, pp. 1-5.

Janjua, S., Fakhar-I-Abbas, William, K., Malik, I.U. and Mehr, J., 2017. DNA Mini-barcoding for wildlife trade control: a case study on identification of highly processed animal materials. Mitochondrial DNA Part A, 28(4), pp.544-546.

## Grading Criteria

Individual and group assignments will be assessed according to the following point schedule:

Assessment Item	Date Due	Points Possible	Total Points Possible
GPS map and track log	Students are to continuously maintain a GPS tracks log	100	
Lab notebook	Benchling report	250	
Lab exercises	Reports from various exercises, benchling or paper	250	
Sample List	Completion and contribution to a sample collection log	100	1000
Quiz 1		100	
Quiz 2		100	
Final Exam		100	