Developing a Genomic Protocol for Identification of Tick-borne Pathogens in Laikipia, Kenya

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Abstract
The region of Laikipia, Kenya, is home to a diversity of wild and domestic mammal hosts for ixodid ticks. These tick species act as vectors for numerous infectious diseases, including economically important diseases such as East Coast fever. Due to the effects of tick-borne disease on livestock, cattle in Kenya are treated with acaricides, which reduce tick loads and may also reduce disease for wildlife. Using field-collected adult and nymphal life-stage ticks, I extracted and analyzed pathogen DNA. I developed several functional primer sets for Fluidigm, a microfluidic process consisting of polymerase chain reaction (PCR) analysis followed by MiSeq. PCR products were analyzed for pathogen identity with BLAST and number of sequence reads. The development of this protocol will help to identify and combat tick-borne pathogens around the world.

Methods
Tick collection
Collected ticks with the drag sampling method across a range of land management types in Laikipia, Kenya

Identification to species using published dichotomous keys

DNA extraction
Ticks separated, rinsed in ethanol, and loaded 5 per well
Homogenized using steel beads in 96-well plate using a Qiagen TissueLyzer in protease solution
Extracted and purified tick and pathogen DNA with a E-Z 96 Tissue DNA Kit (Omega)

DNA concentration and alcohol precipitation
DNA concentration using Qubit
Measured concentration of DNA in a Flurometer by measuring fluorescence of dye bound to DNA

Alcohol precipitation
Precipitated DNA from solution using ethanol and salt
Resuspended in EB buffer to increase the concentration

Fluidigm
Primer design
Used NCBI sequences to target conserved regions with areas of variability between species to create primers

Included additional targets for Panama and US

Fluidigm and MiSeq
Created amplions using 2 tagged primer sets
Tagged primers were run perpendicular to samples in 48x48 2D design on a microfluidics chip
Sequenced pooled PCR products using Illumina MiSeq
Identified sequences to species using BLAST

Results
Primer Function
Positive reads yielded 20,000-80,000 sequences
Primers with unknown functionality are still undergoing identification with BLAST

Primers were considered functional if they produced high quality sequences with at least 98% identity for one or more species to which they were targeted

Figure 2. Functionality of tested custom primers.

Successful primers and gene targets
Both more general 16S primers and species-specific primers were effective

18S primers for protozoa were effective
dsb primers for Ehrlichia with positive controls available

SS23S IGS primers for Borrelia were effective
ompB primers for Rickettsia were variably effective

Table 1. Species that were identified using the functional fluidigm primers and the gene targeted by these primers.

Species identification
Maximum of three organisms per read were analyzed
Possible to identify to species level using the species-specific primers as well as the general 16S primers

Species common to Africa, the United States, and Panama were found

Discussion
Fluidigm system was used for the first time for the purpose of tick-borne pathogen identification

Successful identification of 12 tick-borne pathogen species demonstrates potential of this protocol

Use of general primers applicable to the discovery of new pathogens

The current application of this genomic protocol to identify pathogens can be next expanded to identify host-blood meal and tick species

This genomic protocol is likely to become a widely used tool for ecological investigations

Acknowledgments
I am grateful for the guidance and necessary background knowledge provided by Professor Brian Allan and Dr. Page Fredericks. I would also like to thank Dr. Stephen Dolan and Tyler Hedlund for their efforts in identification and tick preparation in this process. Finally, I appreciate the resources and efforts of our NSF-funded collaborative team in Kenya, the Brian Allan Lab, and the Department of Entomology at the University of Illinois.

Introduction
Land use in Kenya
- African savannas are prime areas of livestock-wildlife conflict, including disease, predation, & competition

- Extraordinarily high diversity of tick species present in some areas

- Tick-borne diseases in the area: anaplasmosis, babesiosis, ehrlichiosis, rickettsiosis, and theileriosis

- Traditional method of managing infectious disease transmission: 1) separate cattle and wildlife, and 2) treat cattle with acaricides (tick-specific pesticides)

- Recent research suggests acaricide-treated cattle serve as ‘ecological traps’ for tick control (Keesing et al. 2013)

- New land management strategy: Integrate cattle & wildlife and domestic mammal hosts for Ixodid ticks. These tick species act as vectors for numerous infectious diseases, including economically important diseases such as East Coast fever. Due to the effects of tick-borne disease on livestock, cattle in Kenya are treated with acaricides, which reduce tick loads and may also reduce disease for wildlife. Using field-collected adult and nymphal life-stage ticks, I extracted and analyzed pathogen DNA. I developed several functional primer sets for Fluidigm, a microfluidic process consisting of polymerase chain reaction (PCR) analysis followed by MiSeq. PCR products were analyzed for pathogen identity with BLAST and number of sequence reads. The development of this protocol will help to identify and combat tick-borne pathogens around the world.

- Fluidigm is a microfluidics process that conducts high throughput PCR analysis followed by Illumina MiSeq

- Objective
  To develop a novel application of a high throughput protocol for the simultaneous detection of a diverse suite of tick-vectored pathogens from Laikipia, Kenya.

- Table 1. Species that were identified using the functional fluidigm primers and the gene targeted by these primers.

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  Species common to Africa, the United States, and Panama were found

- Discussion
  Fluidigm system was used for the first time for the purpose of tick-borne pathogen identification
  Successful identification of 12 tick-borne pathogen species demonstrates potential of this protocol
  Use of general primers applicable to the discovery of new pathogens
  The current application of this genomic protocol to identify pathogens can be next expanded to identify host-blood meal and tick species
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- References