Introducing Chagas’ Disease Reservoirs with PCR and Next-Generation DNA Sequencing

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Abstract

American trypanosomiasis (Chagas’ Disease) is a zoonotic vector-borne disease caused by the protozoan Trypanosoma cruzi, and is an important cause of mortality and morbidity in Latin America. T. cruzi circulates between reservoir hosts (birds, rodents, and human) and hematophagous insect vectors. Parasites are susceptible to the disease once infected with the parasite by contact with the infected insect vector, ingestion of food or drink contaminated with the parasite, or by transovarian transmission, or by transplacental infection with infected blood or tissue transplants. Research has shown that blood meal analysis, via standard PCR and sequencing, allows for identifying host reservoirs known to the species level, but these techniques are limited because: 1) they often cannot identify multiple blood meals within a vector, 2) they cannot be used to simultaneously detect vector infection with trypanosomes or co-infection with other pathogens, and 3) they may require a relatively large amount of vertebrate DNA, which may be degraded in the insect vector. The objective of this study is to standardize next generation sequencing methodologies for simultaneous blood meal species identification and trypanosome infection within kissing bugs. Rhodnius pallescens, a triatomine vector of Chagas’ disease.

To pipeline samples for next-generation sequencing, we need to quantify the amount of DNA extracted from R. pallescens, normalize the DNA concentrations, and optimize the PCR conditions for each portion of the next generation “Tagging” technique. The samples were put in three different groups consisting of high, medium, and low concentration DNA. At the first round of PCR, there were a series of tests with a known insect and reservoir DNA, which may be degraded in the insect vector. The objective of this study is to standardize next generation sequence methodologies for simultaneous blood meal species identification and trypanosome infection within kissing bugs, Rhodnius pallescens, a triatomine vector of Chagas’ disease.

Methods

DNA was extracted from the kissing bug vectors, Rhodnius pallescens in various habitats (deforested and forested areas) in rural Panama.

Blood meal components

R. pallescens potentially infected with trypanosomal species (e.g. T. cruzi and T. rangeli)

1st round of PCR

The first round of PCR allows for an inner tag to be placed on the DNA sample. The primers, “Trypanosoma Gen’ (12-S. or Cyb-I). The 12-S and Cyb-I are designed for vertebrate DNA amplification. The trypanosome primers amplify the different species of trypanosomes. This outer tag allows for well identification (within a 6 well plate) to occur in the Taggimatrix. Next-Generation Sequencing (NGS) technique

2nd round of PCR

The second round of PCR allows for an outer tag to be placed on the DNA sample. “Trypanosoma Gen’” (12-S. or Cyb-I). The 12-S and Cyb-I are designed for vertebrate DNA amplification. The trypanosome primers amplify the different species of trypanosomes. This outer tag allows for well identification (within a 6 well plate) to occur in the Taggimatrix. Next-Generation Sequencing (NGS) technique

Sequencing

All samples have unique molecular tags and are pooled together and run as one sequencing run. Because these samples only account for a small portion of the run, the total cost of sequencing hundreds of these samples is low.

Analysis

Once sequences have been obtained, sequences are submitted for database searches and a BLAST search then identifies the vertebrate species and trypanosomes in the blood meal. By comparing the new sequences to known sequences, sequences come back and trypanosomes/host species are identified.

End Result

Happy REU students with expertise in NGS techniques

Trypanosomes and blood meal identification in insect vectors

Objectives

• Optimize PCR procedures for vertebrate blood meal amplification in insect vectors while excluding insect DNA.

• Run PCR for identification of Trypanosoma app. Presence

• Run PCR for the 12-S and Cyb-I amplification of mammalian DNA in sampled blood meals

• Perform Next-Generation Sequencing for Cytochrome-c and 12-S to identify blood meal contents for mammalian species.

• Analyze sequences of mammalian hosts and perform a BLAST search for species identification

• Correlate results with the environmental habitat of the insect sample

Discussion

• Most of the DNA that was worked with had very low concentrations.

• With both samples of pure mammalian DNA, amplification was easily achieved for both cytochrome-c and 12S, without amplifying contaminating insect DNA. However, the amount of mammalian DNA in the insect blood meal amplification increased cytochrome-c numbers. Cytochrome-c was not successfully amplified from insects with blood meals and 12-S amplification was possible mainly in fresh insect samples.

• Amplified trypanosome DNA was used to amplify the 12-S samples, a note-worthy observation. We were using the 12-S to amplify the samples but did not amplify the same samples. Primer design is extremely important.

• The percentage of trypanosome infection was much lower than previous studies have shown, the trypanosome primers may not be the ideal primers that would work best on all the samples. This is also a significant reduction in samples size from previous studies.

Future Directions

• Continue to perfect the PCR settings for the 12-S primer and start to experiment with the different filter primers. The filter primers went well with the 12-S amplification of the insects.

• Since all results are based on the new group of samples, tests with the color group can begin. PCR conditions and primer design can be optimized to deal with these new DNA samples.

• Preliminary tests can be done with the trypanosome primers with the old group of samples. Several control positives for these samples did produce PCR products when the amplifications were repeated.

• Bioinformatic analysis of the sequences can occur once the samples are amplified. This can be analyzed and compared to known sequences through a BLAST search.

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