PCR-cloning of a *Trypanosoma cruzi* scaffold protein-biotin ligase gene-fusion

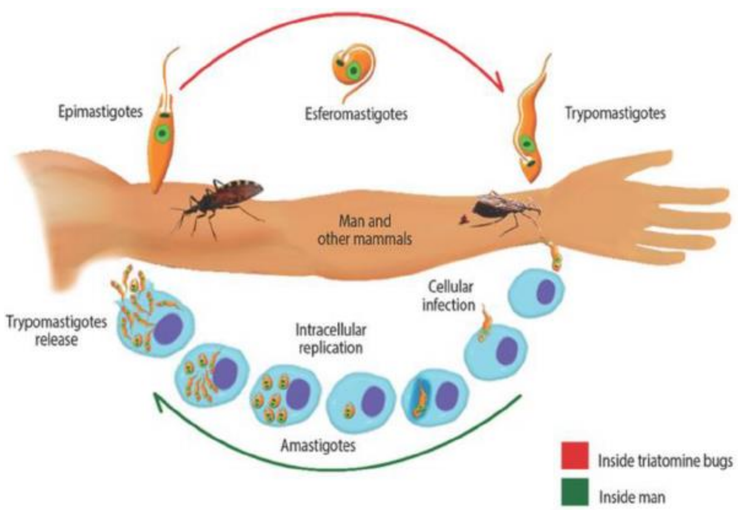
Caegan Jackson, Isabel Stephany-Brassesco, Ben Kelly.

Louisiana State University Health Sciences Center, Department of Microbiology, Immunology, and Parasitology

Introduction

- ***Trypanosoma cruzi* (*T. cruzi*) is an arthropod-transmitted protozoan parasite (Fig.1) that infects, approximately 6 million people and is the etiologic agent of Chagas' disease.**
- **Chagas' patients present with severe cardiac, gastrointestinal, or neurological pathologies that are life-threatening. Those infected reside primarily in Latin America, however it has also been reported in the United States.**
- **There is no vaccine, and current chemotherapies against this disease are inadequate due to their ineffectiveness and toxicity.**
- **To develop effective treatments against this disease, a better understanding of parasite cellular and molecular pathways is expected to identify novel parasite targets for new chemotherapies.**
- **We are studying the molecular function of the *T. cruzi* ribosome-associated scaffold protein TcRACK1. Previous studies indicate these proteins associate with ribosomes in an optimized manner to enhance parasite mitochondrial cytochrome c oxidase (COX) subunit expression for parasite mitochondrial function, hence virulence in the mammalian host.**
- **The goal of this project is to identify ribosomal proteins that interact with TcRACK1. Such interactions are expected to be important in allowing TcRACK1 to regulate *T. cruzi* COX subunit expression for parasite mitochondrial function. To identify these interacting proteins, we will use a transgenic biotin ligase gene-fusion approach.**
- **We will use overlap extension PCR to generate a TcRACK1-TurboID biotin ligase fusion. When this gene fusion is expressed in *T. cruzi*, in the presence of biotin, its biotin ligase activity will ligate the biotin onto all cellular proteins in proximity of TcRACK1-TurboID. These biotinylated proteins, representing TcRACK1-interacting proteins and close neighboring proteins, will be purified by streptavidin-affinity columns and identified by mass spectrometric fingerprinting.**

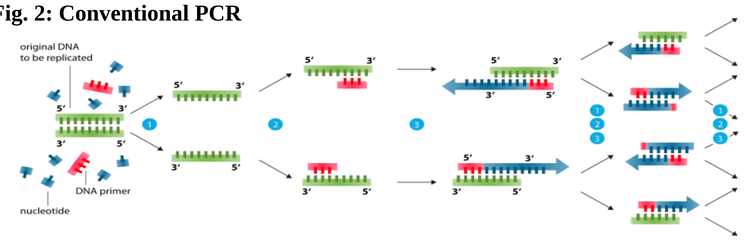
Fig.1: Infectious Life-cycle of *Trypanosoma cruzi*



Methods 1: Conventional PCR

We used conventional PCR (Fig. 2) to amplify 1) the TcRACK1 gene from *T. cruzi* genomic DNA and 2) the TurboID gene (a gift from Dr Chris deGraffenried, Brown University)

Fig. 2: Conventional PCR

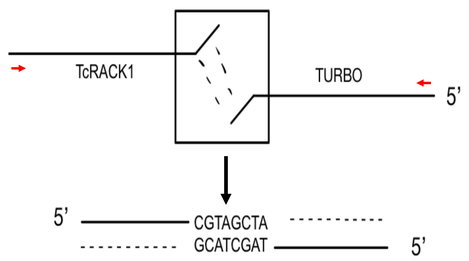


Methods 2: Overlap extension PCR

After generating PCR-amplified TcRACK1 and TurboID products (Fig. 2), we combined them together as templates for overlap extension PCR to generate a TcRACK1-TurboID gene fusion, as shown in Fig. 3.

This gene fusion method relies upon the addition of a short (e.g. 8 bases) sequence onto the 3' end of the downstream TcRACK1 primer and its complementary sequence added to the 5' end of the upstream TurboID primer. Generation of PCR products in the conventional PCR step (Fig. 2), using these primers allows for annealing between the short complementary sequences, generating the fusion product (Fig. 3).

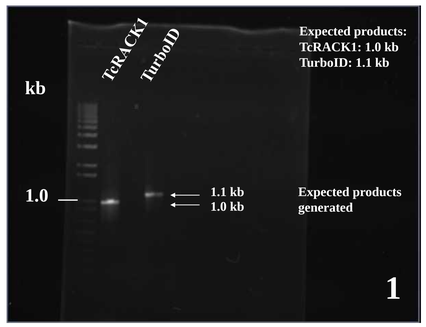
Fig. 3: Overlap Extension PCR

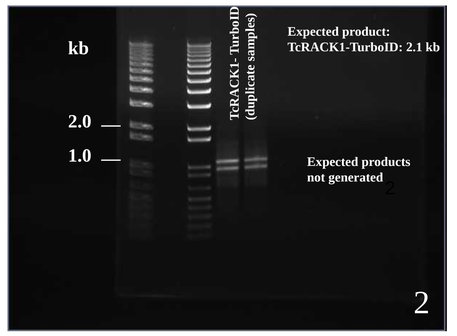


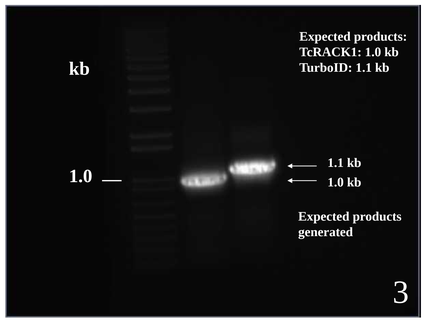
Annealing of TcRACK1 and TurboID products from PCR step 1, the products are then extended and amplified by the DNA polymerase

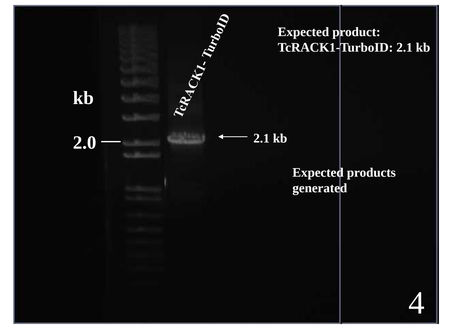
- **Optimization:**
  Primers were redesigned, and polymerase enzyme choice as well as cycling conditions were adjusted to obtain the desired end-products.

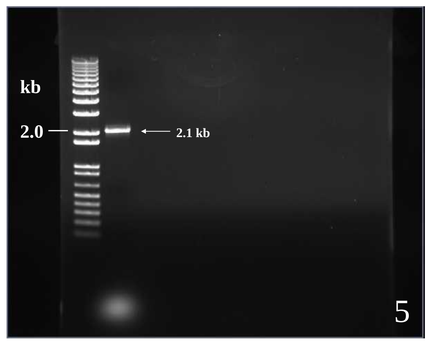
Results: Initial PCRs and Optimization











1. **Initial Experiment, Step 1: PCR amplifications of TcRACK1 and TurboID: Primer set 1; Taq/Vent DNA polymerase.**
2. **Initial Experiment, Step 2: Overlap extension PCR (to generate TcRACK1-TurboID fusion): Primer set 1; Taq/Vent DNA polymerase.**
3. **Optimized Experiment, Step 1: PCR amplifications of TcRACK1 and TurboID: Primer set 2*; Expand DNA polymerase.**
4. **Optimized Experiment, Step 2: Overlap extension PCR (to generate TcRACK1-TurboID fusion): : Primer set 2*; Expand DNA polymerase.**
5. **Purification of the TcRACK1-TurboID fusion for insertion into a *T. cruzi* gene expression plasmid.**

***Primer set 2 were annealing temperature-optimized**

Additional optimizations used

- **Hot start PCR improved the yield of specific PCR products.**
- **Successful amplification of the gene fusion required optimization of primer concentrations and annealing temperature.**
- **Use of the Expand High Fidelity PCR System enzyme resulted in improved levels of specific products.**

Summary & Conclusions

- **Successful generation of the TcRACK1-TurboID fusion was accomplished after redesign of primers and optimization of PCR conditions.**
- **Production of the TcRACK1-TurboID fusion will allow for its transgenic expression in *T. cruzi*, for the identification of TcRACK1 binding proteins.**

This research project was supported by Award Number: DBI-2051440 through the National Science Foundation (NSF), Research Experiences for Undergraduates (REU) Program