

# Epigenomics in *Trypanosoma cruzi*: Exploring immunodetection techniques to study DNA methylation

## **Introduction**

The Chagas disease is still one of the most deadly diseases in South America today. The parasite responsible for this disease is the *Trypanosoma Cruzi* which is carried by the triatomine bug. The disease affects millions of people in central and South America [1]. One intriguing characteristic of the parasite is its ability to thrive and replicate within muscle cells for 30 years before killing its host. In 30% of the cases, individuals develop severe damage to the digestive tract, cardiac and smooth muscles. The disease agent exhibits three phenotypic characteristics during its life cycle. These include the amastigote form, which is found inside of the host cell and is replicative, the epimastigotes which are extra cellular and also replicative and then the infective form of the protozoan, the trypomastigote [2]. A lot of research efforts are being made to understand the complex biological functions of this parasite. However, information on the epigenomic events like DNA methylation is limited.

While some data show that methylation in trypanosomes have some kind of effect on DNA protein interaction and DNA conformation and subsequent effect of gene expression, the extent of the effect is still not known; specific genes that are up regulated or down regulated in the presence of a demethylation agent like (5azaC) and the extent to which it affect the parasitic ability of the protozoan remains unknown.

It has been found that there are methylated bases in the *T.Cruzi* genome specifically, 5-methylcytosine ( $m^5\text{Cyt}$ ) and possibly N6- methyladenine ( $m^6\text{Ade}$ ) [2]. The occurrence of methylated bases are in sites that are different from CpG sites as they are characterized in other organisms. In *T. Cruzi*, methylated bases are found in regions possibly XCGX. [2]. Building on some of this evidence, our quest this past summer was to identify genes that are under the control of a Methylation. In this task, we sought to block methylation with the use of a potent demethylation agent, namely 5-azaCytidine (5-azaC). The drug works by blocking the action a DNA methyltransferase that works tranfering a methyl group to the DNA. The DNA methyltransferase use S-adenosyl methionine as a methyl donor.

## Methods

Several experiments that have been done with Trypanosomes have used different methods to detect the stutus of methylation in this this parasite. Interestingly, different methods have yeilded different results.

In our previous experiments we used microarray technology to detect upregulation and downregulation of certain genes, the downside of this technique is that specific genes and the location of the genes that were under the control of methylation was not known. In this experiment we would use an immunodetection technique that would use a specific antibody prepared against methylated cytosine in the DNA of the parasite. This technique has been used to detect methylated cytosines in the genomic DNA of *Drosophila melanogaster*[x1]. In order to confirm the methylated status of the identified genes they would be treated with sodium bisulfite.

### Possible results and implications

Since this would be the first use of an immunodetection technique used in this project we are quite sure we would identify methylated genes in the genome of this parasite.

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