# Expression and Localization of a Hypothetical Protein of *Trypanosoma brucei*

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## Introduction

Among the parasitic diseases afflicting sub-Saharan Africa, Trypanosomiasis is of a unique status; for this disease, all possible approaches for control of a typical parasitic disease remain unsatisfactory. To date there is no effective vaccine, just a handful of drugs, while diagnostics are limited to the tedious parasitological methods that are of low sensitivity.

The identification and cellular localization of novel proteins in *Trypanosoma brucei* can provide valuable information for the identification of new drugs, vaccines or diagnostic targets to effectively control this disease. There is now a feasible approach following the publication of the trypanosome genome

## Objectives

To express and localize a hypothetical protein, designated *Tmp10*, which was predicted to be a surface protein using bioinformatics tools.

## Methods

*Tmp10* was cloned from *T. brucei brucei* GVR35, expressed in *E. coli* BL21(DE3) as a partial-length, His-tagged recombinant protein (rTmp10) and purified.

Polyclonal rabbit antibodies against Tmp10 were raised and used to probe Tmp10 by Western blot on *T. brucei brucei* whole cell lysate.

Indirect immunofluorescence microscopy was performed on Trypanosome cells

**This investigation received financial support from Human Frontier Science Program (HFSP) and Grand Challenges Canada**

## Results

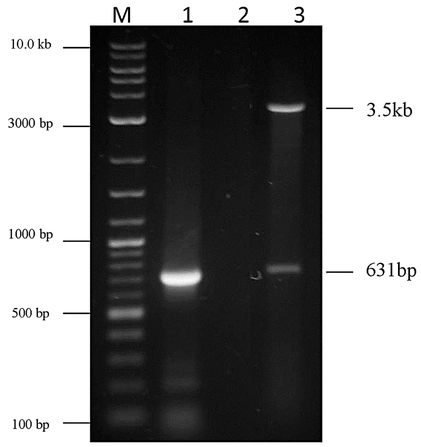


**Figure 1.** Agarose gel detection of the PCR and digestion products of *Tmp10*. PCR yielded a 631 bp product (Lane 1) with no product in the negative control (Lane 2); A BamHI and HindIII double-digest was carried out on the amplicon and the ~3.5kb vector; pQE30. The PCR product was ligated into the pQE30 vector before transforming *E. coli* JM105 cells. Ligation was confirmed by HindIII and BamHI double-digest to release the insert from the vector (Lane 3), and visualized by ethidium bromide (50µg/ml) staining on a 1.0% TAE agarose gel with a NEBTM 2-Log DNA Ladder (Lane M).

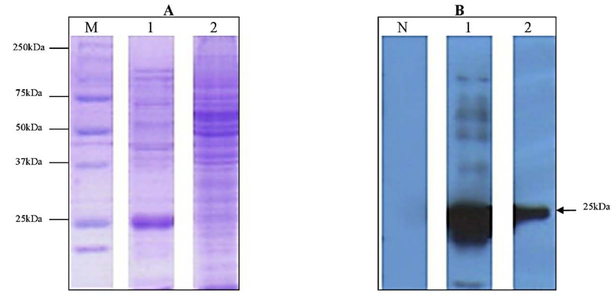


**Figure 2**. SDS-PAGE and Western blot analysis for immunodetection of Tmp10 in *T. brucei brucei* (GVR35) whole cell lysate. The purified recombinant protein (lane 1) and the trypanosome whole cell lysate (lane 2) were subjected to electrophoresis on a polyacrylamide gel. Half the loaded samples were stained with Coomassie blue R250 (Panel A) and samples in the other half transferred to a nitrocellulose membrane for detection by ECL (Panel B). After blocking, membranes were washed and probed with rabbit anti-rTmp10 (1:3000), a preimmune serum assay was included as a negative control. The membranes were then washed, and incubated with HRP-conjugated goat anti-rabbit IgG mAb (1:10,000), and developed with ECL Western blotting detection reagents. The image shows the protein in the whole cell lysate of similar size as the purified recombinant protein (~25kDA). Lane M; Marker, Lane N; Negative control (Preimmune serum)

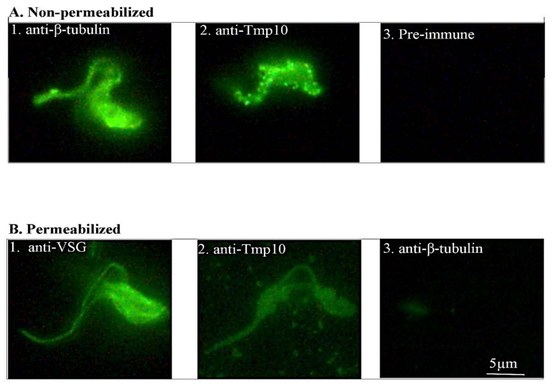


**Figure 3.** Immunofluorescence for localization of the Tmp10 antigen. To determine whether the rabbit antiserum was recognizing a surface or internal antigen, indirect immunofluorescence was performed in two ways. For surface antigens (Panel A), a suspension of intact *T. b. brucei* GVR35 parasites was probed with test serum (1:25 dilution). Pre-immune rabbit serum was added as a negative control (not shown), rabbit anti-VSG was used as a positive control for surface staining of non-permeabilized cells (1), test serum was used to localize Tmp10 (2), mouse anti-β-tubulin mAb (3) was added as a negative control for surface staining to verify membrane integrity. Detection was by FITC conjugated anti-Rabbit IgG (dilutions 1:40) and FITC conjugated anti-mouse IgG for the anti-β-tubulin assay. Panel B shows results of immunofluorescence performed as above except that the cells were permeabilized.

## Conclusion

These results suggest that Tmp10 is ~25kDA surface protein expressed endogenously by *T. brucei brucei*. The study also illustrates the merits of mining genome databases using bioinformatics tools as a viable approach to the identification of novel surface proteins which may have vaccine or diagnostic potential.

## References

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**Fankhauser, N., & Mäser, P.** (2005). Identification of GPI anchor attachment signals by a Kohonen self-organizing map. *Bioinformatics*, *21*(9), 1846–52. doi:10.1093/bioinformatics/bti299 **with disease outbreaks in Uganda in 2007. *Afr J Biotechnol* 2011. 10:3488-3497**

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