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Expression of eGFP in Trypanosoma brucei brucei

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Introduction

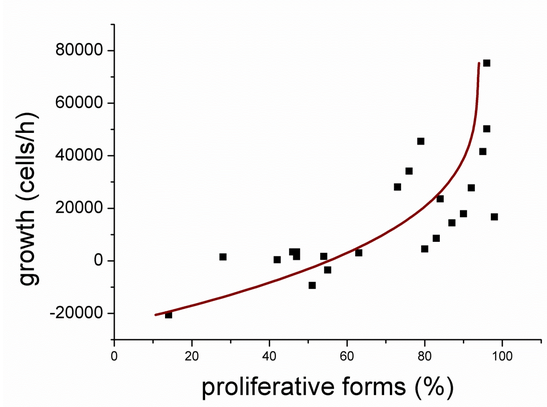
Trypanosoma brucei is the causative agent of Human African Trypanosomiasis (HAT), colloquially known as sleeping sickness. As a flagellate protozoan of the Kinetoplastid order it is closely related to the pathogens of Chagas‘ disease (T. cruzi) and Leishmaniasis (L. spp.)

The infection is transmitted by the Tse-Tse fly. Estimates put the incidence of sleeping sickness at 20.000 cases per year while 70 million people remain at risk [1]. The disease progresses to a meningo-encephalitic stage with an entirely fatal prognosis. Varying morphological stages differing in length can be observed.

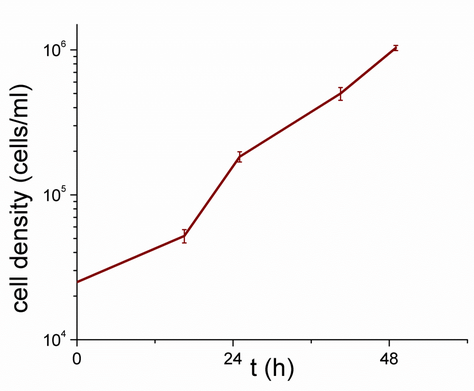
The mechanism of central nervous infection is not yet understood. Fluorescent trypanosomes expressing eGFP may aid in detection of parasites in murine models and in-vitro observations of morphological progression. This necessitates the implementation of both a complex system for in-vitro cultivation and advanced methods of transfection.

Methods

Cultivation of trypanosomes in HMI-9 medium on agarose

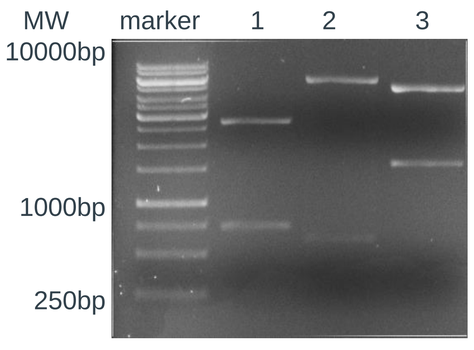


Relation between morphology and growth – trypanosomes in culture show morphological progression akin to the behavior in vivo.

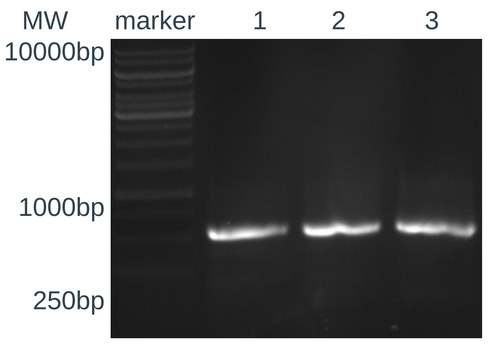


Growth in vitro – the cell density in culture has a generation time of 11,5hrs. Based on previous work by Vassella et al. [2]

Two expression systems for eGFP in trypanosomes



Construction of various vectors.
eGFP was extracted from pLew100 and used in a CloneJET system (lane 2) to introduce BamH1 and HindIII recognition sites. pUB39 and pHD309 were double-digested with BamH1/HindIII to provide ligation sites (lanes 3 and 4)

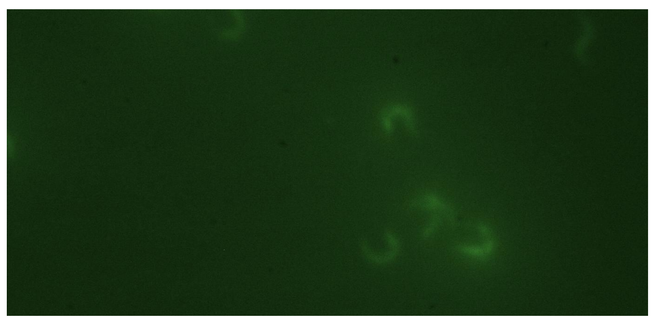


Control of Transfection.

Recombinant strains of Trypanosoma brucei were checked for presence of cDNA (lane 1) and gDNA (lane 2) using eGFP-specific primers. Control on lane 3.

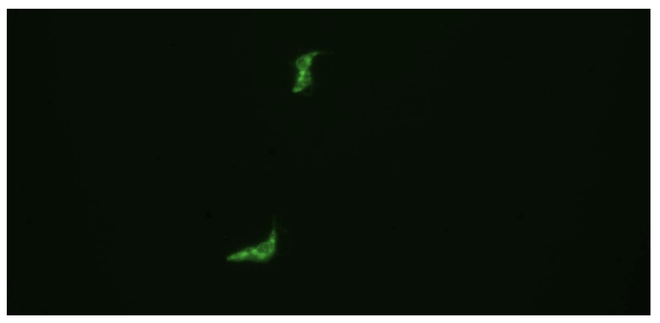
Results

Three fluorescent strains were created, each adapted to a specific use.



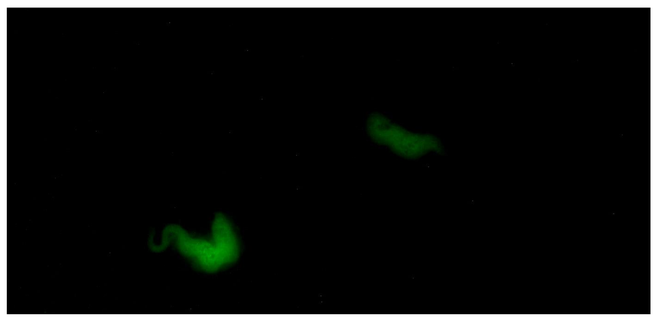
MiTat 221 / pHD309eGFP

A proof-of-concept strain, easy to handle and adapted to laboratory conditions.



MiTat SMB / pUB39eGFP

This strain shows the highest attainable level of expression, subject to tight regulation via a TetOp/Rep system and T7RNAP.



AnTat 1.1 / pHD309eGFP

This wild type strain causes realistic infections in the murine model.

The cells were checked for presence of cDNA and gDNA, as well as level of fluorescence and genomic sequence.

Summary and Outlook

These strains have laid the ground work for further studies. Detection of trypanosomes both through electron and fluorescence microscopy may elucidate the mode of infection, be it through the blood-brain-barrier or the blood-csf-barrier.

Fluorescent parasites also offer ease of observation in-vitro. The various morphologies of Trypanosoma brucei have been shown to be directly linked to the course of infection [3] and may hence be determined more quickly and efficiently.

References

[1] WHO (2013). "Control and surveillance of human African trypanosomiasis." World Health Organization technical report series(984): 1-237.
[2] Vassella, E. and M. Boshart (1996). "High molecular mass agarose matrix supports growth of bloodstream forms of pleomorphic Trypanosoma brucei strains in axenic culture." Molecular and biochemical parasitology **82**(1): 91-105.
[3] Mogk, S., A. Meiwes, et al. (2014). "The lane to the brain: How African trypanosomes invade the CNS." Trends in parasitology. **Epub ahead of print. http://dx.doi.org/10.1016/j.pt.2014.08.002**.

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