Using CRISPR to hunt for the parasite *T. tenax*

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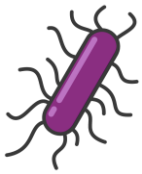
Periodontitis is the major cause of tooth loss in adults (Jin et al., 2016). Its cause is still debated.

Trichomonas tenax is found in association with periodontitis (Bisson et al., 2019). Though, its role is unclear.

Outdated methods of detection hinder research into *T. tenax* as a cause of periodontitis (Marty et al., 2017).

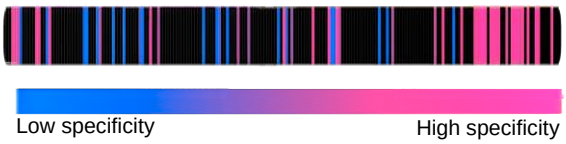
Cas12a protein purified

Escherichia coli were transformed with a plasmid containing the Cas12a gene (Addgene plasmid #90096). The *E. coli* were cultured, with the Cas12a protein extracted and then purified via affinity chromatography.



Cas12a, a CRISPR-associated protein, uses guide RNA to bind target DNA and cleave a fluorescent reporter.
A guide that binds *T. tenax* DNA will allow for detection of the parasite (Swarts, 2019).
Cas12a has been shown to be more sensitive than PCR and more suitable for point-of-care use (Low et al., 2023).

Targets within 6 kb region of *T. tenax* genome

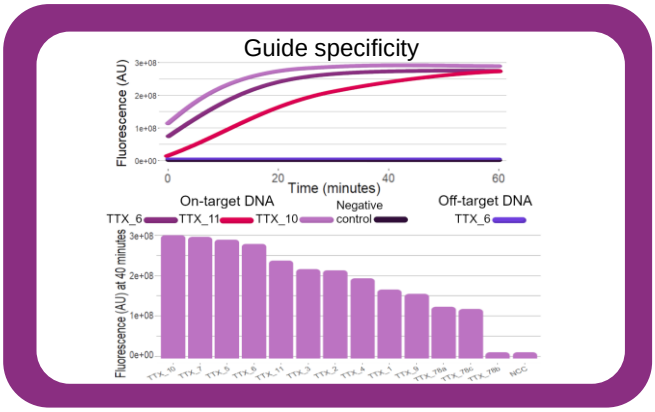


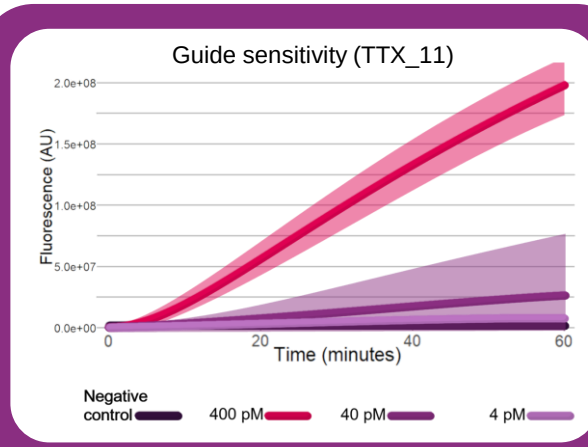
Potential targets identified

225 potential targets, identified within a 6 kb region of the *T. tenax* genome, were compared to the human and *T. vaginalis* genomes. Based on published literature, a specificity score was devised and calculated for each guide. These were then mapped onto the *T. tenax* genome (left). Guide RNAs designed for the 14 targets with the highest specificity scores were synthesised by Integrated DNA Technologies alongside synthetic *T. tenax* DNA.

Guides tested for specificity

Each guide, with Cas12a protein and a fluorescent probe, was tested against on-target and off-target DNA (examples right-top). One guide (TTX_8) showed non-specific activity and was rejected. The remaining 13 guides were ranked by their fluorescent output at 40 minutes (right-bottom).





Guides tested for sensitivity

The six highest performing guides from the specificity testing were tested against serial dilutions of on-target DNA. The results for one guide (TTX_11) are shown (left), with shading indicating the 95% confidence interval. TTX_6 and TTX_10 showed the lowest limit of detection at 4 pM on-target DNA.

Next steps: Lateral flow assay

The assay successfully detected *T. tenax* DNA. An amplification step may improve sensitivity and allow repackaging as a lateral flow test strip for point-of-care use with genomic DNA isolated from patient samples (Low et al., 2023).

References

1. Bisson, C., Dridi, S. M., & Machouart, M. (2019). Assessment of the role of Trichomonas tenax in the etiopathogenesis of human periodontitis: A systematic review. *PLoS ONE, 14*(12), 1–20. https://doi.org/10.1371/journal.pone.0226266
2. Jin, L. J., Lamster, I. B., Greenspan, J. S., Pitts, N. B., Scully, C., & Warnakulasuriya, S. (2016). Global burden of oral diseases: emerging concepts, management and interplay with systemic health. *Oral Diseases, 22*(7), 609-619. https://doi.org/10.1111/odi.12428
3. Low, S. J., O'Neill, M. T., Kerry, W. J., Krysiak, M., Papadakis, G., Whitehead, L. W., Savic, I., Prestedge, J., Williams, L., Cooney, J. P., Tran, T., Lim, C. K., Caly, L., Towns, J. M., Bradshaw, C. S., Fairley, C., Chow, E. P. F., Chen, M. Y., Pellegrini, M., . . . Williamson, D. A. (2023). Rapid detection of monkeypox virus using a CRISPR-Cas12a mediated assay: a laboratory validation and evaluation study. *Lancet Microbe, 4*(10), e800-e810. https://doi.org/10.1016/S2666-5247(23)00148-9
4. Marty, M., Lemaitre, M., Kémoun, P., Morrier, J., & Monsarrat, P. (2017). Trichomonas tenax and periodontal diseases: a concise review. *Parasitology, 144*(11), 1417-1425. https://doi.org/doi:10.1017/S0031182017000701
5. Swarts, D. C. (2019). Making the cut(s): How Cas12a cleaves target and non-target DNA. *Biochemical Society Transactions, 47*(5), 1499–1510. https://doi.org/10.1042/BST20190564

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