A cytosine base editing library screen to explore functionally relevant genes in responses to acidic pH and high temperatures in *Leishmania mexicana*

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CRISPR/Cas tools have allowed parasitology research to make enormous strides in the discovery of gene function. Barcoded libraries of mutants can be screened simultaneously under different conditions to identify genes where mutations incur fitness costs or confer fitness gains. Cytosine base editor (CBE) libraries involve the expression of a CBE fused to a Cas nickase that directs the editing to the intended genes, allowing for the introduction of STOP codons in coding sequences. Here, we used a CBE library of *Leishmania mexicana* promastigotes to discover genes and pathways required for the tolerance of pH and temperature changes. We cultured the CBE promastigote library under five different conditions (pH 5.5, pH 7.5, pH 8.5, in combination with 28°C and 34°C) for at least 25 generations and sampled the DNA at different timepoints to assess fitness. These culture conditions resemble the changes in the parasite's environment during its natural differentiation and infection cycle. By sequencing the sgRNA barcodes we identified distinct sgRNA sets that were enriched or depleted in different conditions, while the non-targeting control sgRNAs remained constant over time. Even though changes in pH had only a small effect on the overall population growth rate at 28°C, out of 7936 targeted genes, we identified 47 sgRNAs sets in pH 5.5 and 82 sgRNAs sets in pH 8.5 that significantly changed their proportions over time compared to the control condition, including some targeting uncharacterized *Leishmania*-specific proteins. By contrast, at 34°C promastigote growth stalled for ~8 days before resuming at a slower growth rate, with longer replication times in pH 5.5 compared to pH 7.5. Particularly at 34 °C and pH 7.5 we found a larger number of enriched sgRNAs sets, targeting genes known to be involved in parasite differentiation, encoding Alba proteins (RNA binding and regulation of expression), several MAP kinases, amastin surface proteins, along with hypothetical proteins of unknown functions. Current work is underway to validate individual hits and study the function of the identified genes in the response to pH and temperature changes.