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MICROBIAL GENETICS

C. parvum gets the CRISPR upgrade

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New research has described the first system for genetic modification of *Cryptosporidium*, single-celled parasites that cause diarrhoeal disease.

Young children and immunocompromised individuals are particularly vulnerable to cryptosporidiosis, a condition for which there is no vaccine and a paucity of treatment options. The dearth of robust molecular tools to study *Cryptosporidium* has hampered research efforts aimed at tackling this important pathogen.

Factors that have hindered the development of methods to genetically manipulate *Cryptosporidium* species include the lack of a long-term *in vitro* culture system, the likely absence of non-homologous recombination in *Cryptosporidium* species and an insufficiency of well-characterized drugs and associated resistance genes for positive selection of transfected cells. In particular, *Cryptosporidium* is resistant to antifolates, a class of drug commonly used in other Apicomplexa — such as *Toxoplasma* and *Plasmodium* species — to select for genetically modified parasites.

Vinayak and colleagues addressed each of these insufficiencies in a series of experiments that culminated in the generation of stable transgenic *Cryptosporidium parvum* parasites. First, they optimized a method for transient transfection of a nanoluciferase reporter construct using electroporation of sporozoites, and established that incorporation of the neomycin resistance marker reduced parasite susceptibility to paromomycin. To achieve long-term maintenance of transfected parasites, a method for direct delivery of cultured sporozoites to the intestines of mice was developed. In parallel, to increase the efficiency and stability of genetic modification, they built a CRISPR–Cas9 system using the *C. parvum* U6 RNA promoter to drive guide RNA expression and the *Streptococcus pyogenes cas9* gene flanked by *C. parvum* regulatory sequences. Combined, these advances enabled the transfection, propagation and paromomycin-based selection of nanoluciferase-positive parasites.

This system was used to establish a drug screening platform using nanoluciferase reporter parasites — an approach that was more sensitive than previous PCR-based techniques to quantitate parasite survival — and to demonstrate the basis of *C. parvum* resistance to antifolates. Here, the authors achieved targeted deletion of the gene for thymidine kinase, which is absent in other apicomplexan genera, to show that this enzyme provides an alternative route for thymidine monophosphate synthesis and thereby enables *C. parvum* to tolerate high doses of antifolate drugs. Together, these applications establish foundations for both drug screening and drug target validation through genetic modification, highlighting just two of many new possibilities now open to researchers of this neglected tropical disease.

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