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PLASMIDS FOR CRYPTOSPORIDIUM PARVUM

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These plasmids are for performing CRISPR/Cas9 gene editing in Cryptosporidium parvum

To generate a CRISPR/Cas9 plasmid for use in *C. parvum*, researchers used restriction cloning with SacI to insert the *C. parvum* U6 gene into a pUC19 vector to create pUC19-CpU6. We then inserted the *C. parvum* actin promoter (984 bp upstream of cgd5_3160) upstream of Cas9-NLS-GFP, followed by the *C. parvum* actin 3' UTR region (562 bp downstream of cgd5_3160) into puc19-CpU6 by Gibson assembly to create pACT1:Cas9-GFP. This plasmid (pACT1:Cas9-GFP) was further modified by inserting the thymidine kinase (TK, cgd5_4440) guide RNA (sgRNA) and the tracrRNA amplified from the Aldolase_Cas9 plasmid downstream of the CpU6 promoter using Gibson assembly to create pACT1:Cas9-GFP, U6:sgTK (Addgene 122852). The plasmid pACT1:Cas9-GFP, U6:sgUPRT (Addgene 122853) was generated by replacing the TK sgRNA with a sgRNA targeting the *C. parvum* uracil phosphoribosyltransferase gene (*uprt*, cgd1_1900) using Q5 site-directed mutagenesis. The UPRT sgRNA was designed using the Eukaryotic Pathogen CRISPR guide RNA/DNA Design tool searching against the *C. parvum* IowaII CryptoDB-28 genome to avoid off-target effects.

Publication: A Stem-Cell-Derived Platform Enables Complete Cryptosporidium Development In Vitro and Genetic Tractability