

## 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 *Sac*I 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* lowall CryptoDB-28 genome to avoid off-target effects.

**Publication:** [A Stem-Cell-Derived Platform Enables Complete \*Cryptosporidium\* Development In Vitro and Genetic Tractability](#)