SUPPLEMENTARY INFORMATION

A streamlined CRISPR/Cas9 approach for fast and efficient genome editing in *Toxoplasma gondii* and *Besnoitia besnoiti*

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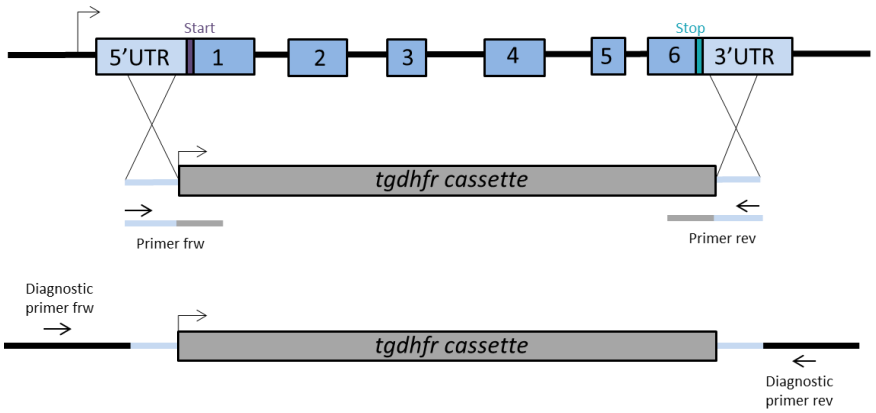

Figure S1: Knockout strategy. The crRNA is designed within the GOI. Homologous regions for the integration of the selection cassette lay in the 5'UTR, respectively in the 3'UTR. This design leads to the excision of the GOI and replacement by the selection cassette.

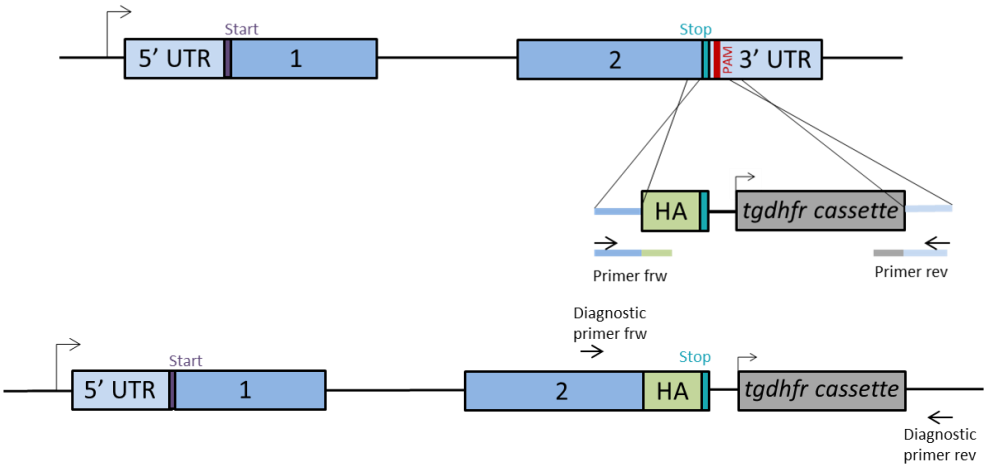

Figure S2: C-terminal tagging strategy. The crRNA is designed downstream of the Stop codon. Homologous regions for the in-frame integration of the PCR product lay right before the Stop codon and the 3' UTR. The integrated PCR product contains the HA-tag followed by a Stop codon and the selection cassette.

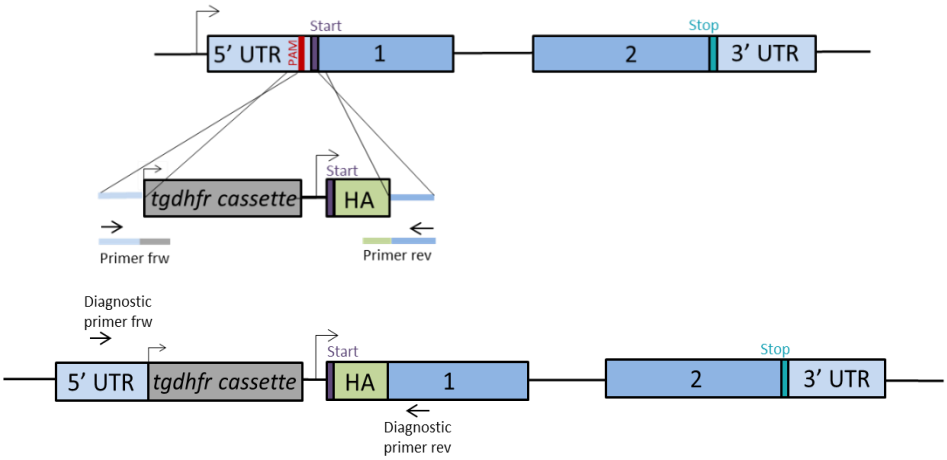

Figure S3: N-terminal tagging strategy. The crRNA is designed upstream of the Start codon. Homologous regions for the in-frame integration of the PCR product lay in the 5' UTR and right downstream of the Start codon. The integrated PCR product contains the selection cassette, followed by the Start codon and the HA-tag.

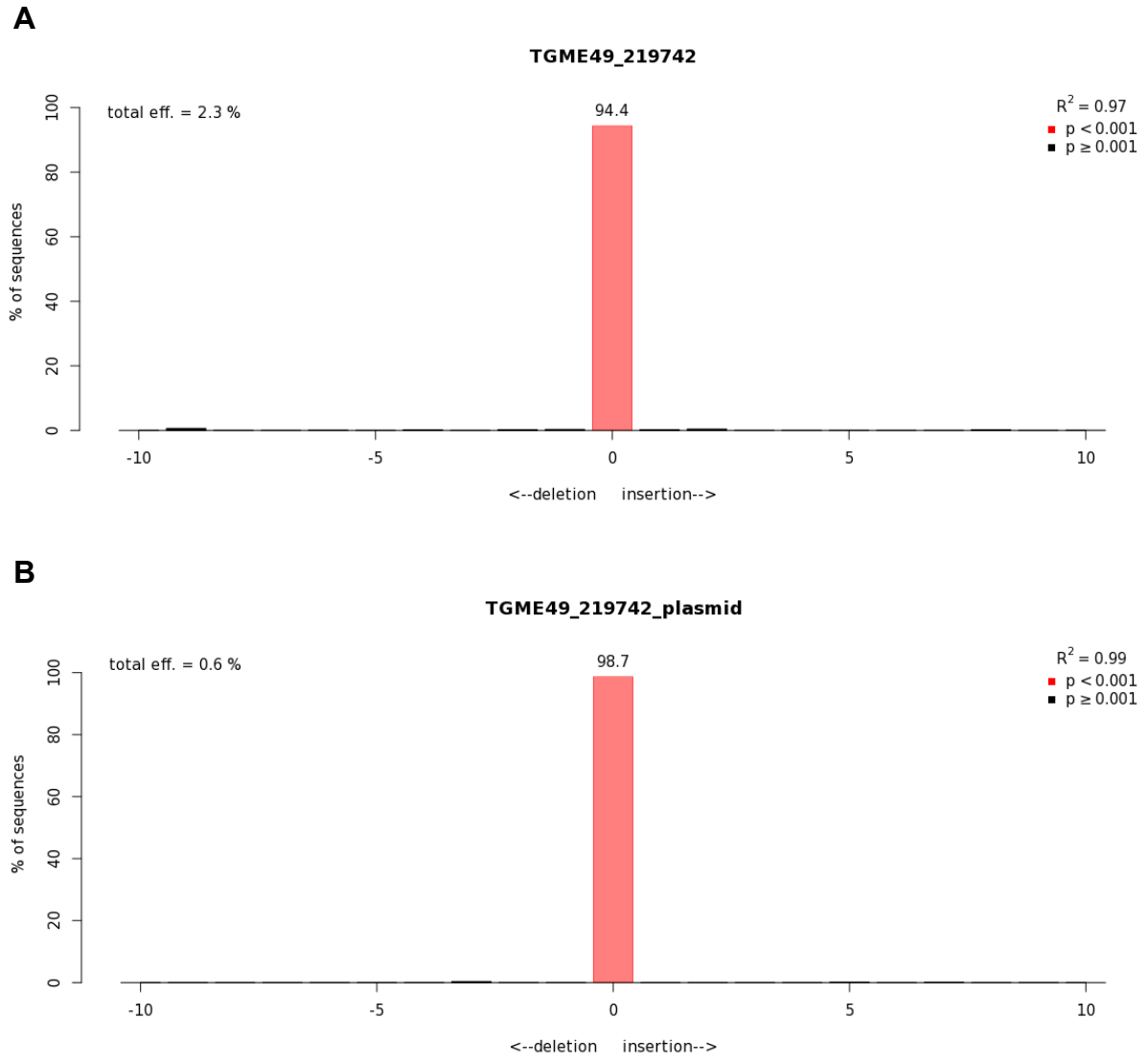

Figure S4: TIDE Indel Spectrum of TGME49_219742. A comparison of the TIDE analysis of TGME49_219742 with the new approach (**A.**) and the conventional 2sgRNA plasmid-directed approach (**B.**). The new approach shows a clear increase of the efficiency.

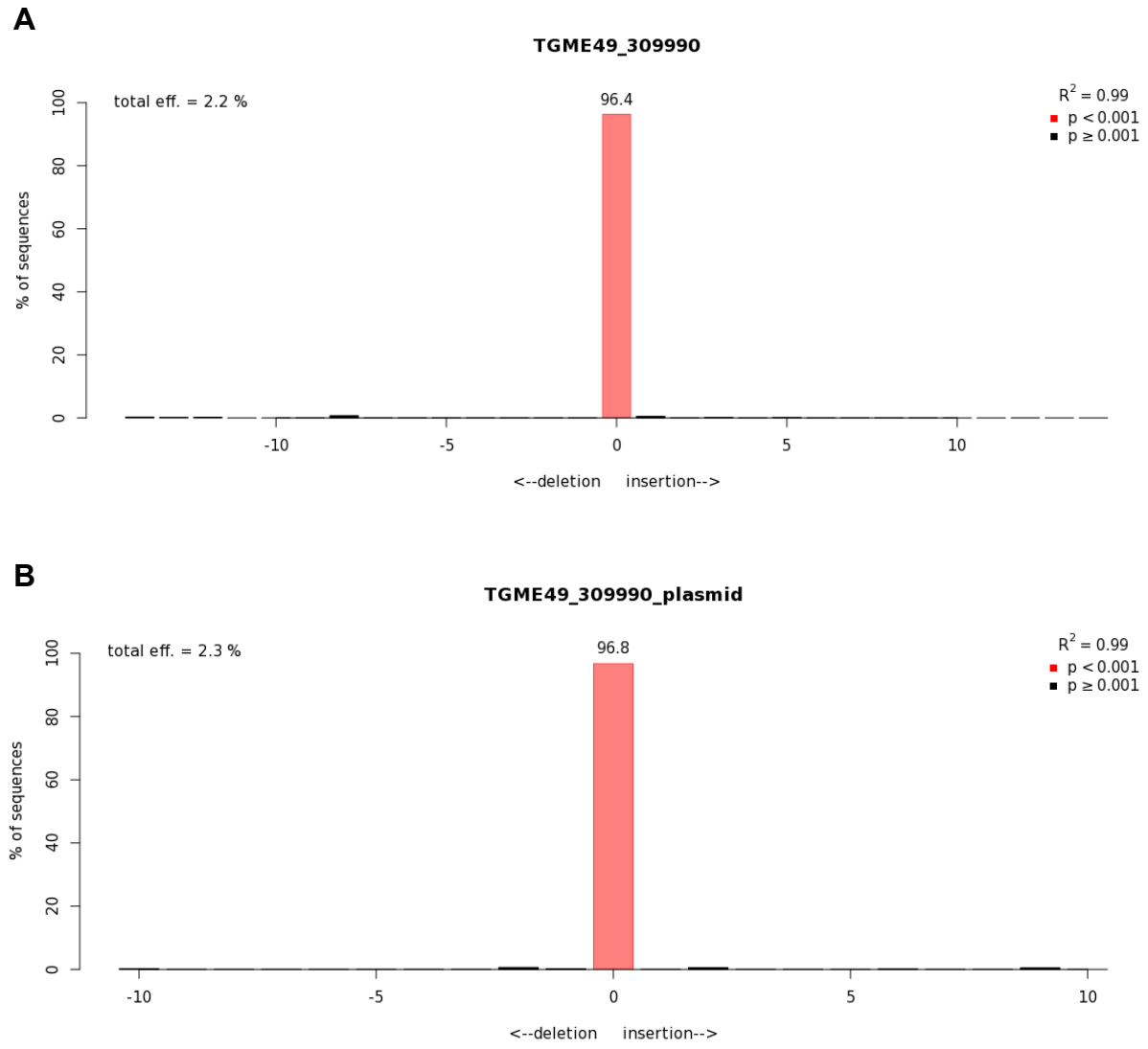

Figure S5: TIDE Indel Spectrum of TGME49_309990. A comparison of the TIDE analysis of TGME49_309990 with the new approach (**A.**) and the conventional 2sgRNA plasmid-directed approach (**B.**). Both approaches show comparable efficiencies.

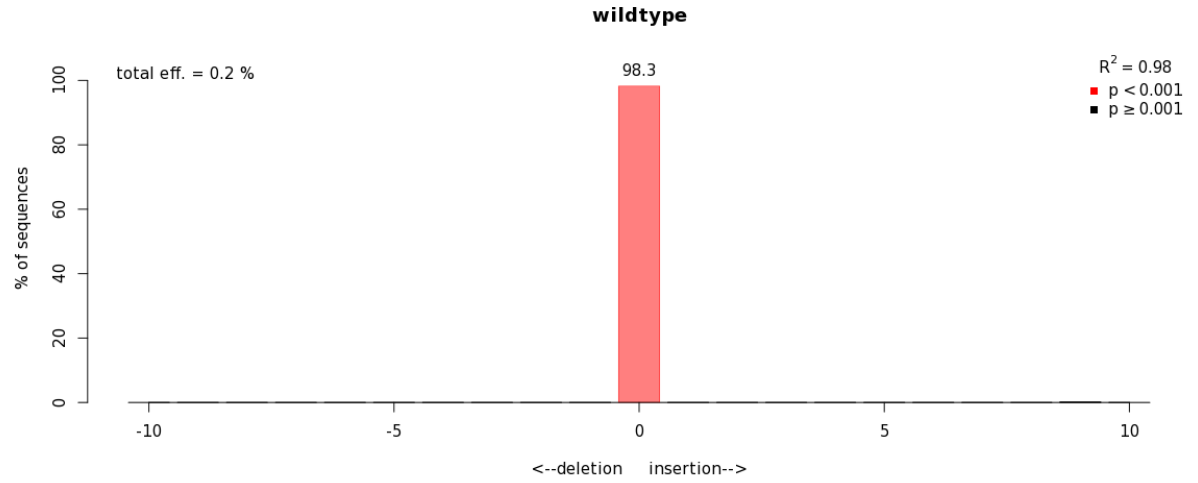

Figure S6: TIDE Indel Spectrum of the wildtype control. The efficiency error was estimated to 0.2 % by TIDE (MOCK transfection).