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Claudia Nose
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A versatile CRISPR/Cas9 editing approach in *Trypanosoma cruzi.*

<u>Vilchez Larrea SC</u>[1,2], Prego A[1], Schoijet AC[1,2], Llanos MA[3], Alberca LN[3], Bellera CL[3], Gavernet L[3], Talevi A[3], Alonso GD[1,2]

[1]INGEBI-CONICET, Ciudad Autónoma de Buenos Aires, Argentina. [2]Facultad de Ciencias Exactas y Naturales, UBA, Ciudad Autónoma de Buenos Aires, Argentina. [3]LiDeB, Facultad de Ciencias Exactas, UNLP, La Plata, Argentina

Resumen

Development of CRISPR/Cas9 as a tool for genomic edition brought a new perspective to the study of *Trypanosoma cruzi*, an organism usually reluctant to other gene editing technologies. Most often, epimastigotes are co-transfected with a single plasmid bearing both the gene for Cas9-GFP expression and a sequence to be translated into a single guide RNA (sgRNA), jointly with a lineal donor DNA encompassing a selection marker flanked by sequences homologous to the target gene. Here, we tested an alternative approach for the generation of Phosphodiesterase (PDE) *knockout* parasites. We obtained epimastigotes from Tul II strain stably expressing Cas9-GFP in the nucleus in all parasite stages, with no detrimental effects on epimastigote growth or differentiation nor on trypomastigote infection capability. These Cas9-GFP epimastigotes were co-transfected with the sgRNA + DNA donor pair, according to the intended gene target. sgRNA were obtained by *in vitro* transcription using a template DNA bearing the specific + scaffold sequence under a T7 promoter. To obtain the donor DNA we designed a “pre-donor” formed by a sequence including several restriction enzyme recognition sites flanked by 30-bp arms homologous to the sequence adjacent sgRNA annealing target. This “pre-donor” allowed to easily generate a variety of donor DNAs by cloning alternative selection markers. DNA extracts (boiling-preps) from 4-day post-transfection cultures were evaluated by PCR using “mixed” primer pairs: while one of the primers annealed to the target gene, the second primer annealed to a sequence in the donor DNA, allowing assessment of its correct insertion in the gene of interest. Advantages of this take on CRISPR/Cas9 edition include its versatility for choosing and switching between alternative selection markers and a quick and affordable generation of the components of the system and analysis of the transfected cultures, while possibly facilitating complementation assays on the KO lines.

Tipo de Presentación

Póster.