Function of the MRE11 and EXO1 nucleases and RECQ2 helicase in DNA end resection and double strand breaks repair in *Trypanosoma brucei*

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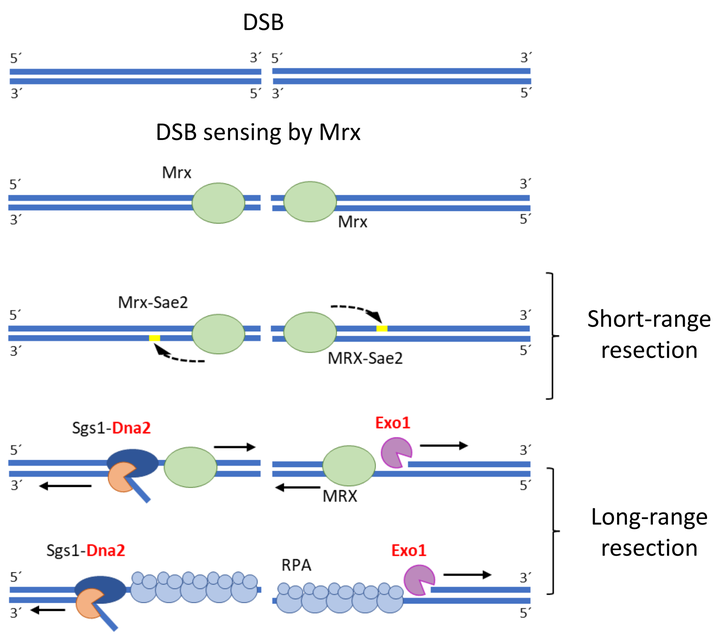
INTRODUCTION

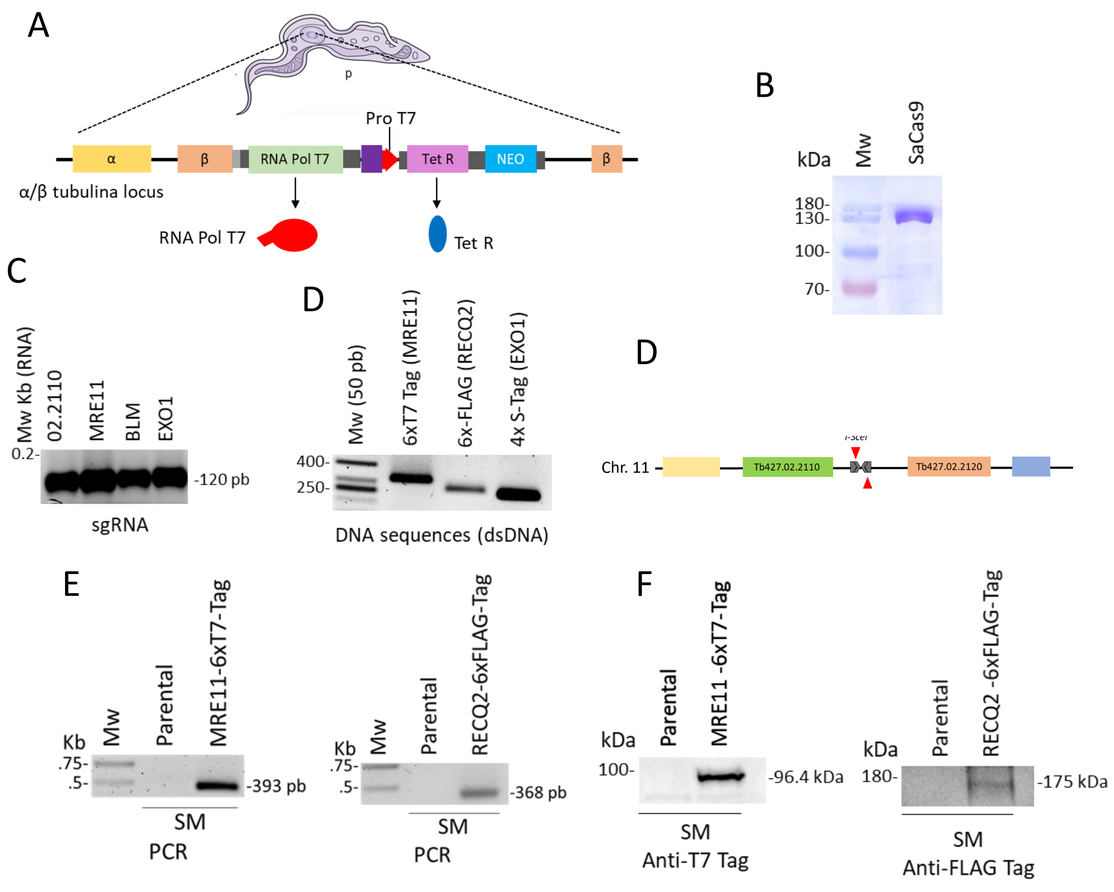
DNA double strand breaks (DSBs) are one of the most toxic forms of DNA damage. These can arise accidentally during normal cell metabolism or after exposure of cells to DNA-damaging agents. In eukaryotes, DSBs are repaired by homologous recombination (HR), non-homologous end-joining (NHEJ) and microhomology-mediated end joining (MMEJ). In HR and MMEJ, the 5´ DNA strands of the DSBs are nucleolytically degraded through a process termed DNA end resection. This process, critical for repair pathway choice by HR or MMEJ and checkpoint signaling, is driven by the MRE11, DNA2 and EXO1 nucleases and generate 3'-ended single-stranded DNA tails with different lengths of homology sequences. In most eukaryotes, repair by HR and MMEJ is conserved, including *Trypanosoma brucei*. In this parasite, DNA end resection is not well understood. So, in this context, the present research project intends to characterize the functions of the major nucleases, MRE11 and EXO1, and RECQ2 helicase in DNA resection and repair of DSBs.



Schematic representation of DNA end resection process in yeast.

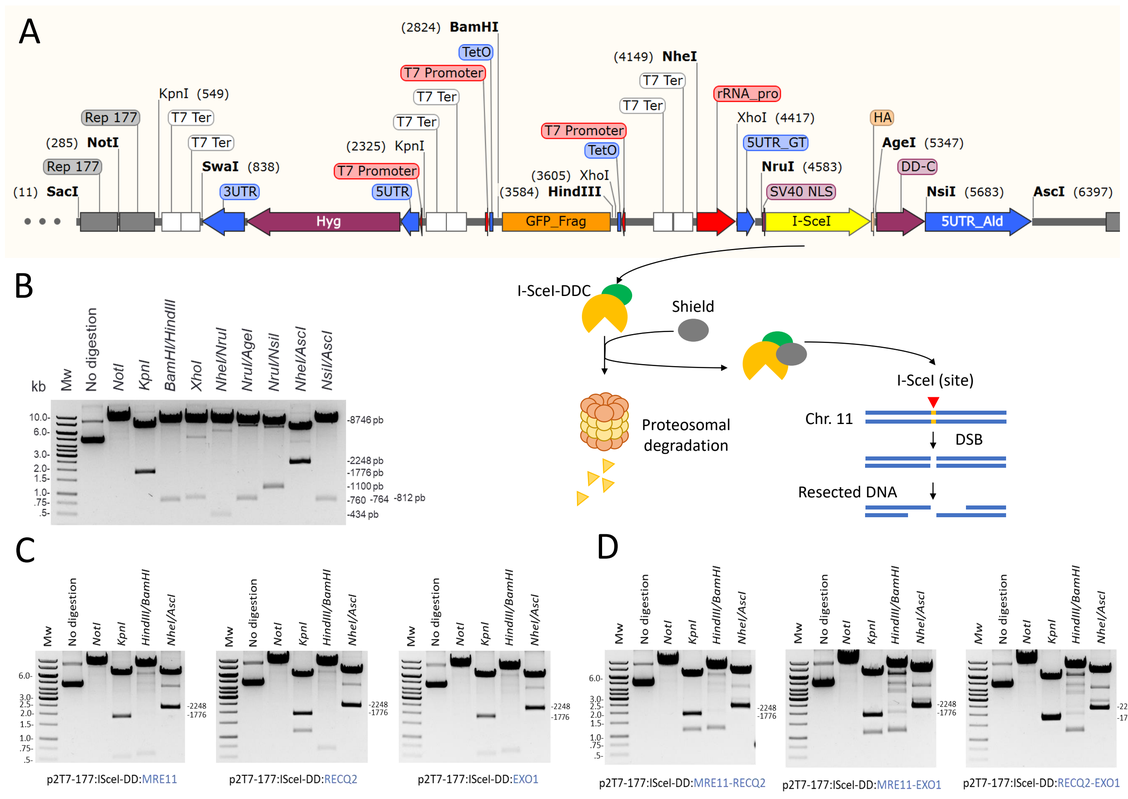
RESULTS

Generation of a *T. brucei* cell line with tagged nucleases and recognition sites for *I-SceI using a* CRISPR/Cas9 alternative method



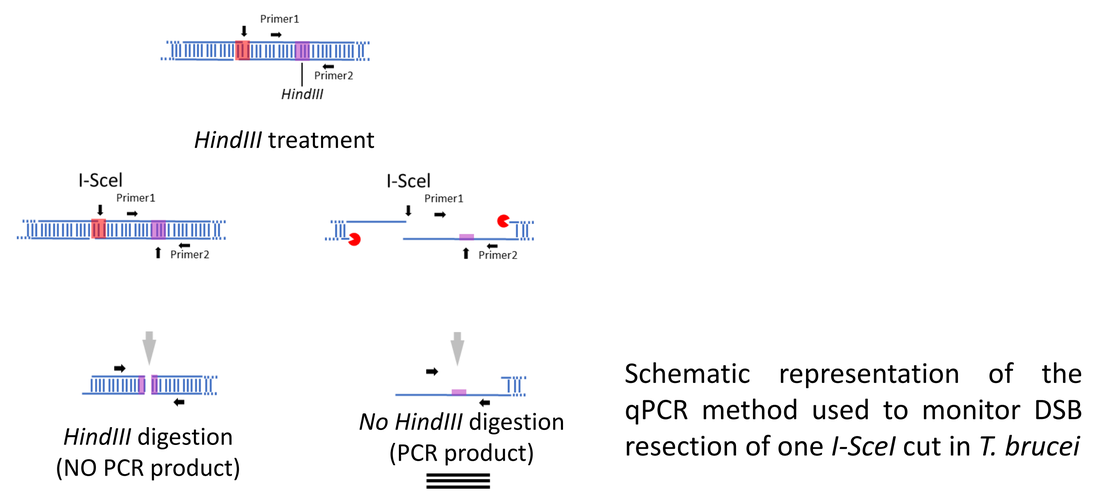
(A) Schematic representation of *T. brucei* 427 single-marker bloodstream forms used in our experiments. The *T. brucei* BSF SM expresses T7 RNA polymerase and the tetracycline repressor that are used to produce double-stranded RNA for RNAi knockdown of target genes or for expression of genes. (B) Purified *Staphylococcus aureus* Cas9 (SaCas9). (C) Detection of sgRNAs synthesized *in vitro*. (D) Donor DNA templates with 30 bp homology arms. (E) Schematic representation of the sites for *I-Sce*I on chromosome 11. (F) Detection of fused genes with tags (MRE11 6xT7 tag and RECQ2 6x FLAG tag) in *T. brucei* SM by PCR. (F) Detection of tagged proteins in *T. brucei* SM.

Generation of a vector for the conditional expression of *I-SceI* and RNA interference for nucleases



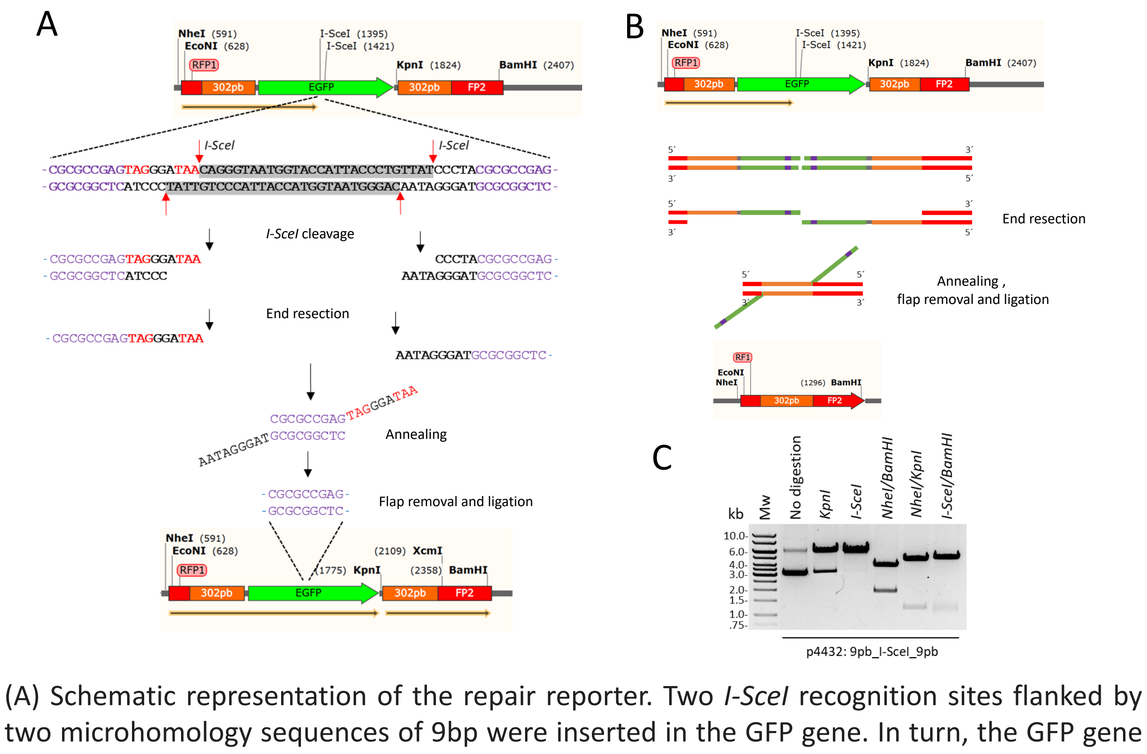
(A) Schematic representation of a vector for the conditional expression of *I-SceI* and RNAi for nucleases involved in DNA end resection. The *I-SceI* gene was fused to a destabilization domain (DD) in the vector for its conditional expression. (B) Restriction enzyme digestion analysis of vector p2T7 177 Hyg I-SceI-DD before the cloning of the nuclease (or helicase) fragments for RNAi or after cloning (C-D). The fragments were cloned into the vector individually (C) or fused with another fragment (D) for knockdown of one or two proteins, respectively.

Quantification of DNA end resection by qPCR from *I-SceI* cut



Schematic representation of the qPCR method used to monitor DSB resection of one *I-SceI* cut in *T. brucei*

Construction of a reporter vector to measure the balance between HR and MMEJ repair



(A) Schematic representation of the repair reporter. Two *I-SceI* recognition sites flanked by two microhomology sequences of 9bp were inserted in the GFP gene. In turn, the GFP gene was flanked by two truncated parts of the RFP gene that shares 302 bp of homologous sequence. The stabilization of *I-SceI* generates a DSB; if the damage is resolved by MMEJ, cells will express the GFP protein (A), while if it is repaired using homologous sequence by HR, cells will express the RFP gene (B). (C) Restriction enzyme digestion analysis of vector p4432 9 bp_ISceI_9 bp can be integrated in alpha/beta tubulin locus in *T. brucei*.

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