



**Figure S11. Experimental validation of multiple primary *trans*-splice sites for *T. brucei* genes.**

Overlay of the number of reads ( $\log_2$ ) from 5'-end- (blue) and 3'-end-enriched (red) libraries aligning to the shown regions of chromosomes IV (A-C) and V (D) covering the ORFs for Tb927.4.1020 (A), Tb927.4.1180 (B), Tb927.4.1600 (C) and Tb927.5.990 (D). SL-containing end-reads are shown as blue or red horizontal lines depending on their orientation (minus or plus strand, respectively). Dashed lines indicate the positions of a nested gene-specific primer and the expected positions of the 3' *trans*-splice sites. Green bars indicate the potential products from an RT-PCR assay with SL and gene-specific primers with the predicted size of the fragments indicated on the left. (E) RT-PCR assay. Poly(A)<sup>+</sup> RNA was reverse transcribed with random primers and the resulting cDNA was used as a template for nested PCR with an identical SL forward primer for both amplification steps. Tb927.5.990 is an example of a gene with highly homogeneous site for SL addition.