

A depiction of the single crossover integration event of the BSD reporter construct into the tubulin array.

	MES promoter												B							
	22°C				37°C					22°C					37°C					
0	1	2	3	4	0	1	2	3	4	0	1	2	3	4	0	1	2	3	4	hours
4.6	3.9	3.6	3.4	3.6	4.3	4.2	4.0	4.6	5.0	1.5	1.5	1.3	1.2	1	1.7	2.9	4.4	5.7	6.8	BSD mRNA
1.7	1.1	1	1.4	1.2	2.6	5.7	11.7	20.4	25.3	3.5	1.7	1.5	1.5	1.4	3.4	7.6	18.2	29.9	35.5	ESAG7/6 mRNAs

Quantitation of the Northern blotting results shown in Fig.1 relative to α -tubulin mRNA. Numbers in the boxes indicate the levels of BSD and ESAG7/6 mRNAs normalized relative to the amounts of α -tubulin transcript in the samples. The lowest steady-state level is set to 1.



A diagram of the promoterless reporter construct. The backbone of the constructs is pBluescript II KS(+). AGs indicate the *trans*-splice sites upstream and downstream of the BSD coding sequence. The EcoR I restriction site introduced in the middle of the 500 bp ESAG7 CDS fragment was used to linearize the plasmid prior to transfection. The construct drives single crossover integration into the coding sequence of ESAG7 in endogenous BESs, although due to high-degree of sequence identity, analogous integration event into an ESAG6 CDS is also possible.



T. b. rhodesiense strain YTat 1.1, carrying the promoterless reporter construct integrated into endogenous BES was grown at 28°C as described in Fig.1. One flask of the culture was left at 28°C, another placed at 22°C and a third at 37°C and incubated for 4 hours. Northern blotting was performed as in Fig.1. Numbers in the lanes indicate the levels of the detected mRNAs normalized relative to the HSBP1 mRNA amounts in the samples. Asterisk in the ESAG7/6 blot indicates the position of truncated mRNAs resulting from the integration of the construct into ESAG7/6 CDSs.



T. b. brucei TREU927, *T. brucei* Lister 427 and *T. b. rhodesiense* YTat 1.1 procyclic cells were grown at 28°C as described in Fig.1. For each strain, one flask of the cultures was left at 28°C, another placed at 22°C and a third at 37°C and incubated for 4 hours. Northern blotting to detect ESAG7/6 mRNAs was performed as in Fig.1.