pNAT C-terminal in situ tagging constructs

For constitutive expression of C-terminal human 12cMYC- (EQKLISEEDL) or enhanced GFP-tagged proteins in *T. brucei* from the native locus.

- High fidelity polymerase recommended.
- Integration occurs by a single crossover at the native locus following linearisation at a unique restriction site in the cloned sequence, resulting in duplication of the targeting fragment downstream of the tagged locus.

Cloning

$x^{GFP/12MYC}$ Clone the targeting fragment without a stop codon via *Hin*dIII(or *Asc*I)/*Xba*I digestion with the last codon of the targeting fragment immediately upstream of the *Xba*I site.
i.e. AAGCTT:[targeting fragment]:TCTAGA:[*GFP/12MYC* STOP]:GGATCC.

There are alternatives if the gene contains *Xba*I or *Asc*I:

Plasmid		**Insert**	
	*Xba*I		*Avr*II, *Nhe*I, *Spe*I
	*Asc*I		*Afl*III
	*Hin*dIII		

Key features

- Complete sequences available.
- Hygromycin or Blasticidin versions available.
- Modular nature allows tag or other components to be exchanged.
- Compatible with wild type cells or any other *T. brucei* cell line.

Detection:

cMYC	Mouse anti-cMYC, 9E-10 (Source Bioscience; IFA / western blotting)
	Mouse anti-cMYC, 4A6 (Upstate Biotech; WB only; in IFA binds *T. brucei* spindle)
eGFP	Rabbit anti-GFP, (Molecular Probes; IFA and western blotting)

Other questions/comments, contact Sam Alsford (sam.alsford@lshtm.ac.uk).