pNAT N-terminal in situ tagging constructs

For constitutive expression of N-terminal human 6cMYC- (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 upstream of the tagged locus.

Cloning

^{GFP}x — Clone your targeting fragment without a start codon via *Xba*I/*Bam*HI digestion with the first codon of the targeting fragment immediately downstream of the *Xba*I site.
i.e. [ATG-*GFP*]:TCTAGA:[codon 2]:[codon]n:GGATCC.

^{6MYC}x — Clone your targeting fragment without a start codon via *Avr*II/*Bam*HI digestion.
i.e. [ATG-*6MYC*]:CCTAGG:[codon 2]:[codon]$_n$:GGATCC.

There are alternatives if the gene contains *Xba*I/*Avr*II or *Bam*HI:

Plasmid	*Xba*I/*Avr*II	**Insert**	*Avr*II, *Nhe*I, *Spe*I, *Xba*I
	*Bam*HI		*Bgl*II

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).