pT7^{TAG}

For inducible expression of N-terminal human cMYC- (EQKLISEEDL) or enhanced GFP-tagged proteins in *T. brucei* expressing T7 polymerase.

- Derived from pLEW100 (Wirtz et al, 1999) and p2T7^{TAblue} (Alibu *et al*., 2005).
- High fidelity polymerase recommended.
- Should integrate into any *T. brucei* genome (*RRNA* spacer) following digestion with *Not*I.

Cloning
To ensure that your gene is in frame with the tag, place the second codon downstream of the *Xba*I site, i.e. TCTAGA:[codon 2]:[codon]n:[stop codon]:GGATCC.

Use *Hind*III/*Bam*HI (rather than *Xba*I / *Bam*HI) if you don't want the tag.

There are alternatives if the gene contains *Xba*I, *Bam*HI or both:

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

Key features
- Complete sequence available.
- Hygromycin for stable selection.
- All vectors allow inducible expression using tetracycline (or analogues).
- Inducible cassette is independent of selectable marker.
- Modular nature allows tag or other components to be exchanged.
- Compatible with bloodstream (Single Marker Bloodstream form recommended: Wirtz *et al*., 1999 – see Fig. 7) or insect-stage (1313-1333 recommended: Alibu *et al*., 2005) *T. brucei*

Upon integration into *T. brucei*, the construct lies between *RRNA* spacer sequences. The operator binds Tet-repressor in the absence of tetracycline so the T7 promoter is activated and tagged protein is expressed when tetracycline (1 μg ml^{-1}) is added to the medium. T7 terminators prevent transcription from extending into *rDNA*.

New technology development
- Some leaky expression is seen from these vectors. Inducible T7 RNAP expression appears to solve this problem in insect stage cells (Alibu *et al*., 2005).
- These vectors transform bloodstream-form cells at a low efficiency (~10^{-7}) and can integrate in the genome at any one of several ribosomal spacer loci. Subsequent position effects may generate variable results. Integrating at a tagged locus can alleviate these effects and improve transformation efficiency (see pRPa and Alsford et al, 2005).
- Currently the 'stuffer' is indicated by a stretch of 'N's'. Versions we can provide at present should be digested with *Xba*I/*Bam*HI for standard cloning but the vector could be adapted for 'directional cloning' (see Horn, 2005).

Detection:

cMYC	Mouse anti-cMYC, 9E-10 (Santa Cruz; IFA / western blotting) Mouse anti-cMYC, 4A6 (Upstate Biotechnology; western blotting only, as binds the mitotic spindle in *T. brucei*)
eGFP	Rabbit anti-GFP, IgG fraction (Molecular Probes; IFA and western blotting)

Other questions/comments, contact David Horn (david.horn@lshtm.ac.uk).