http://homepages.lshtm.ac.uk/~ipmbdhor/dhhome.htm
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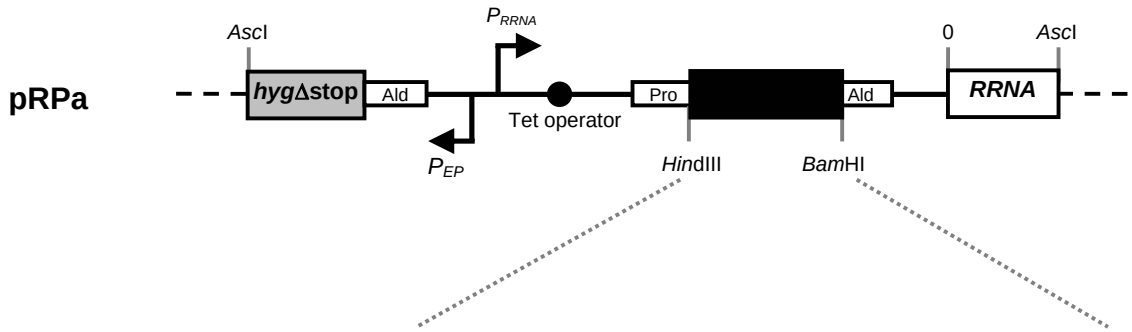
Horn Laboratory, LSHTM

Tagging Plasmid: pRPa^{TAG}



Expression cassettes

GFP$_X$ / Myc$_X$

AAGCTT **ATG-*GFP* or *Myc*** TCTAGA **insert STOP** GGATCC
*Hin*dIII *Xba*I *Bam*HI

x^{GFP}

TTAATTAAGCTT **ATG-insert** TCTAGA ***GFP*-STOP** GGATCC
*Pac*I *Hin*dIII *Xba*I *Bam*HI

x^{6Myc}x

AAGCTTAATTA **ATG** TCTAGA **6*Myc*** CCTAGG **STOP** GGATCC
*Hin*dIII *Pac*I *Xba*I *Avr*II *Bam*HI

Promoter region

RRNA promoter +1 Tet operator *Kpn*I Polypyrimidine tract
...AAGTAGCGCTTACGGCGTACACCCTATCAATGATAGAGTGGTACCCTGCACGCGCCTTCGAGTTTTTTTTCCTTTTCCCCATTTTTTTCAACTTGA
AGACTTCAATTACACCAAAAAGTAAAATTCACAAGCTT -- Expression cassette
SA *Hin*dIII

pRPa^{TAG}

For inducible expression of N/C-terminal human cMYC- (EQKLISEEDL) or N/C-terminal enhanced GFP-tagged proteins in *T. brucei* from a tetracycline-responsive RRNA promoter.

- Derived from pLEW100 (Wirtz et al, 1999) and p2T7^{TAblue} (Alibu *et al*., 2005).
- High fidelity polymerase recommended.
- Integrates at the tagged *RRNA* spacer in 2T1/TAG^{PAC} *T. brucei* following digestion with *Asc*I (Alsford et al 2005), giving a transformation efficiency of ~2.5x10^{-6} (Alsford & Horn, 2007).

Cloning

$^{GFP/MYC}$x — 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]:GGATCC.

x^{GFP} — To ensure that your gene is in frame with the tag, place the last but one codon upstream of the *Xba*I site, i.e. AAGCTT:[start]:[codon]$_{n-1}$:TCTAGA.

x^{6MYC}x — **N-terminal tagging**: clone your gene without a start codon via *Avr*II/*Bam*HI digestion (or without start/stop codons via *Avr*II)
i.e. CCTAGG[codon 2]:[codon]$_n$:[stop]:GGATCC.
C-terminal tagging: clone your gene without a stop codon via *Hin*dIII(or *Pac*I)/*Xba*I digestion (without start/stop codons via *Xba*I)
i.e. AAGCTT:[start]:[codon]$_n$:TCTAGA.

Use *Hind*III / *Bam*HI if you don't want the tag.

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

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

Key features

- Complete sequences 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 *T. brucei* cell lines expressing TetR and containing a tagged *RRNA* spacer, e.g. 2T1/TAG^{PAC} (Alsford et al, 2005).

Upon integration into *T. brucei*, the construct replaces TAG^{PAC} and generates a functional *HYG*^{R} at the previously tagged *RRNA* spacer. The operator binds Tet-repressor in the absence of tetracycline so the inducible RRNA promoter is activated and tagged protein is expressed when tetracycline (1 μg ml^{-1}) is added to the medium.

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).