pRP^{iTAG}

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.

- High fidelity polymerase recommended.
- Should integrate into any *T. brucei* genome (*RRNA*) following digestion with *Not*I.

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 codon]: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, *Avr*II *Bam*HI	**Insert**	*Avr*II, *Nhe*I, *Spe*I, *Xba*I *Bgl*II

Key features

- Complete sequences available.
- Hygromycin (^{GFP}x, ^{MYC}x, x^{6MYC}x) or Blasticidin (^{MYC}x, x^{6MYC}x, x^{GFP}) 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 wild type cells (for constitutive expression) and any *T. brucei* cell line expressing TetR.

Upon integration into *T. brucei*, the construct lies between *RRNA* spacer sequences. The operator binds Tet-repressor in the absence of tetracycline so the inducible RRNA promoter is activated and tagged protein is expressed in the presence of tetracycline (1 μg ml^{-1}).

New technology development

- 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 can generate variable results. Integrating at a tagged locus can alleviate these effects and improve transformation efficiency (see pRPa and Alsford et al, 2005).

Detection:

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

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

pRP$^{HYG\text{-}iSL}$

For inducible expression of stem loop RNA in *T. brucei* from a tetracycline-responsive RRNA promoter.

- Primers: We use a software tool (RNAit – Redmond et al, 2003) for the selection of RNAi targets that provides primer information and minimises off-target effects (see Durand-Dubief et al, 2003).
- Integrates at a random *RRNA* spacer in *T. brucei* after *Not*I digestion.

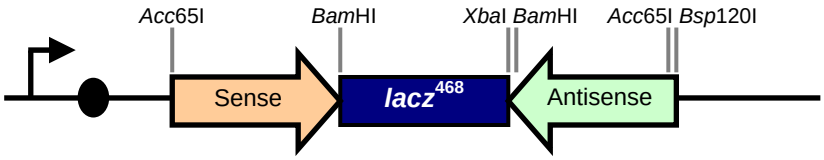
Primer Design & Cloning

A single primer pair is required to generate the two inserts. Each primer contains two restriction sites; the internal sites allow cloning in a sense orientation into MCS1 or 3, while the external sites allow cloning in the antisense orientation into MCS2 or 4.

For example (cloning into pRP$^{HYG\text{-}iSL}$ MCS$^{1/2}$):

Primer A: 5'-GATC GGGCCC GGTACC -- target specific 5'sequence (20 bases) --
*Bsp*120I *Acc*65I

Primer B: 5'-GATC TCTAGA GGATCC -- target specific 3'sequence (20 bases) --
*Xba*I *Bam*HI



To confirm the organisation of the stem loop cassette, use the sense fragment cloning restriction enzymes. In the above example, *Acc*65I will excise both fragments and *lacz*^{468}, while *Bam*HI will only release *lacz*^{468}.

We regularly use two sequencing primers to confirm correct insertion:

lacz^{468} pos 50 towards sense RNAi	Seq1 5'-AATAGTGGACTCTTGTTCCA
l*acz*^{468} pos 420 towards antisense RNAi	Seq2 5'-AAAGGGGGATGTGCTGCAAG

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 components to be exchanged.
- Compatible with any *T. brucei* cell line expressing TetR.
- Upon integration into *T. brucei*, the construct lies between *RRNA* spacer sequences. The operator binds Tet-repressor in the absence of tetracycline so the inducible RRNA promoter is activated and tagged protein is expressed in the presence of tetracycline (1 μg ml^{-1}).

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