

## pRPa<sup>SLi</sup>

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

- Derived from pLEW100 (Wirtz et al, 1999) and p2T7<sup>TAb<sub>lue</sub></sup> (Alibu et al., 2005).
- 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 the tagged *RRNA* spacer in 2T1/TAG<sup>PAC</sup> *T. brucei* after *Ascl* digestion (Alsford et al 2005), giving a transformation efficiency of  $\sim 2.5 \times 10^{-6}$  (Alsford & Horn, 2007).

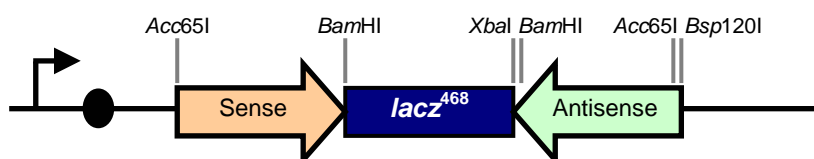
### Primer Design & Cloning

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 pRPa<sup>SLi</sup>MCS<sup>1/2</sup>):

Primer A: 5'-GATC GGGCCC GGTACC -- target specific 5' sequence (20 bases) --  
                   *Bsp120I*      *Acc65I*

Primer B: 5'-GATC TCTAGA GGATCC -- target specific 3' sequence (20 bases) --  
                   *XbaI*      *BamHI*



To confirm the organisation of the stem loop cassette, use the sense fragment cloning restriction enzymes. In the above example, *Acc65I* will excise both fragments and *lacZ*<sup>468</sup>, while *BamHI* will only release *lacZ*<sup>468</sup>.

We regularly use two sequencing primers to confirm correct insertion:

*lacZ*<sup>468</sup> pos 50 towards sense RNAi                      Seq1 5'-AATAGTGGACTCTTGTTC  
*lacZ*<sup>468</sup> pos 420 towards antisense RNAi                Seq2 5'-AAAGGGGATGTGCTGCAAG

### 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 *T. brucei* cell lines expressing TetR and containing a tagged *RRNA* spacer, e.g. 2T1/TAG<sup>PAC</sup> (Alsford et al, 2005).

Upon integration into *T. brucei*, the construct replaces TAG<sup>PAC</sup> and generates a functional *HYG*<sup>R</sup> 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  $\mu$ g ml<sup>-1</sup>) is added to the medium.

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