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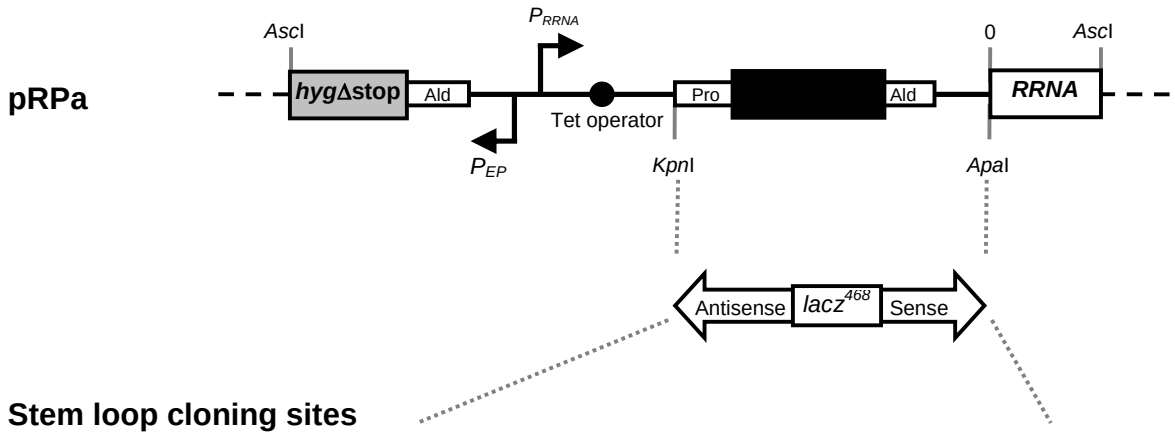
Horn Laboratory, LSHTM

RNAi Plasmid: pRPa^{SL}



Stem loop cloning sites

MCS1/2

GGTA*CCCGGG*CTTAA*GGATCC* lacz^{468} *TCTAGA*AGCTT*CTCGAG*GGGCCC

*Kpn*I *Sma*I *Afl*II *Bam*HI *Xba*I *Hin*dIII *Xho*I *Apa*I

MCS3/4

GGTACCGGTATCGATCAATTGCCCGGG lacz^{468} AGATCTTAAGGCCGGCCTCGAGTCTAGAGGGCCC

*Kpn*I *Age*I *Cla*I * *Mfe*I *Sma*I *Bgl*II *Afl*II *Fse*I *Xho*I *Xba*I *Apa*I

* *Cla*I restriction site blocked by dam methylation

Promoter region & multiple cloning sites

RRNA promoter +1 Tet operator *Kpn*I

...AAGTAGCGCTTACGGCGTACACCCTATCAATGATAGAGTGGTACC -- multiple cloning site

Recommended Sequencing Primers

Seq1 (5' end of *lacz*^{468} position 50 towards MCS1/3) 5′-AATAGTGGACTCTTGTTCCA

Seq2 (3' end of *lacz*^{468} position 420 towards MCS2/4) 5′-AAAGGGGGATGTGCTGCAAG

pRPa^{SLi}

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^{TAblue} (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^{PAC} *T. brucei* after *Asc*I digestion (Alsford et al 2005), giving a transformation efficiency of ~2.5x10^{-6} (Alsford & Horn, 2007).

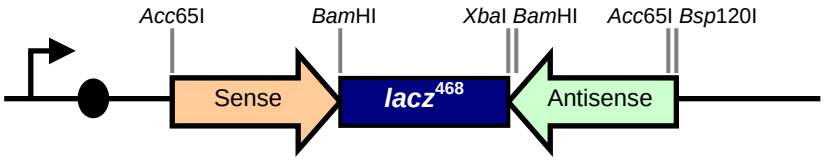
Primer Design & Cloning

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^{SLi}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 *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.

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