Inducible cell lines:

There are a number of options: Also see **George Cross** and **Christine Clayton** web-sites.

We have experience with the Single Marker Bloodstream line from George Cross and this line was also used to target >200 genes as part of the **TrypanoFAN** project.

We have also developed vectors to make inducible cell lines as outlined below:
Using bloodstream-form cells, we had two main objectives:

[1] To optimise the system for increased throughput.

A major objective following on from genome sequencing is to characterise large numbers of *T. brucei* genes. Vector and cell optimisation will facilitate this process.

[2] To make a T7RNAP-independent inducible expression system.

T7RNAP has been used widely in *T. brucei* and facilitates the use of head-to-head T7 promoters for the generation of RNAi constructs in one step (the single sub-unit polymerase is able to efficiently traverse a template in both directions to generate dsRNA). Because we are specifically interested in establishing assays that report loss-of-silencing. To do this we are using a T7-independent system so we can distinguish between native or heterologous polymerase-based loss-of-silencing. T7RNAP also displays toxicity when expressed at high level (Alsford et al., 2005).

Components available:

- Tetracycline repressor (TetR): We use pHD1313 from Christine Clayton (Alibu et al, 2005).
- Inducible *RRNA* promoter **[2]**: We have constructs for expression of cMYC- or GFP-tagged proteins – tet inducible only if TetR is expressed (Alsford et al., 2005).
- Vectors to generate a cell line with a single tagged ribosomal spacer: This requires two electroporation steps but increases subsequent transfection efficiency **[1]** and eliminates position effects **[1+2]** (Alsford et al, 2005; Alsford & Horn, 2007). Vectors for RNAi and GFP-tagging are available for use in these '*RRNA* spacer-tagged' cells.

Future developments:

Several developments are needed, planned or underway to improve the 'tool-kit'. We may also test our vectors in the genome reference strain, TREU 927 (we have used Lister 427 previously). New systems that may benefit the research community will be made available ASAP.

Contact david.horn@lshtm.ac.uk with comments or for further details.

References

Alibu VP, Storm L, Haile S, Clayton C. and Horn D. (2005) A doubly inducible system for RNA interference and rapid RNAi plasmid construction in *Trypanosoma brucei*. *Mol. Biochem. Parasitol.* **139**: 75-82.

Alsford S, Kawahara T, Glover L and Horn D. (2005) Tagging a *T. brucei RRNA* locus improves stable transfection efficiency and circumvents inducible expression position effects. *Mol. Biochem. Parasitol.* **144**: 142-148.

Alsford S and Horn D. (2007) RNA polymerase I transcription stimulates homologous recombination in *Trypanosoma brucei*. *Mol. Biochem. Parasitol.* **153**: 77-79.