EMSA for *T. brucei* TRF

Gel preparation

Pour an 0.8%/0.1x TBE agarose gel: 100 ml in wide mini cell tray with 20-well comb at top.

Binding reaction

Premix for each sample:

- 1 µl 80% Glycerol
- 0.5 µl 1 µg/µl sheared E. coli DNA* (see below)
- 2 µl 150 mM Tris-HCl pH 7.5
- 0.5 µl 100 ng/µl β-casein
- 0.5 µl labeled pTH12 probe (or 1 µl if needed)

Count on 2 extra reactions for your premix. Use one for the 'probe alone' lane (+OG)
* use 1-3 µg E. coli DNA as competitor for crude extracts. Use 0.5 µg for purified fractions or titrate to find desired reduction of background binding for your particular protein.

- Add ddH_2O to each reaction tube to give a FINAL volume of 20 µl (4.5 µl premix
- in example above, protein, competitors, probe, etc.).
- Add premix to each tube (4.5 µl).
- Add protein last. GST-tbTRF recombinant protein: 50-400 ng.
- Mix gently and incubate at RT for 30 min.
- Load onto gel: use OG plus "probe alone" in lane #1, but do NOT put OG in the others.
- Run the gel at 200 V for 30–40 min until the OG is at the very bottom of the gel.
- Dry gel onto DE81/Whatmann, 80°C on gel dryer: expose to film or Phosphorimager O/N at RT.

tbTRF gel shift components

pTH5 competitor plasmid: 100-300 ng uncut

Preparation of sheared *E. coli* DNA

- Dissolve 25 mg E. coli DNA (Sigma D-2001, type VIII) in 10 mM Tris-HCl pH 7.5 at a final concentration of 2.5 mg/ml.
- Sonicate 1 min max output in 5 sec pulses (or syringe-shear 11 times with tuberculin needle) to reach ~400 bp average fragment size. (The efficiency of shearing varies per batch, but best to do less so you do not over-fragment: you can check ~1 µl on a gel after each 1 min cycle of sonication and repeat until desired size range is achieved.
- Add EDTA to 10 mM and SDS to 0.1 %.
- Phenol/chloroform/isoamylacohol extract (shake hard) and spin 10 min at 3,000 rpm in the table top centrifuge. Repeat until the interphase is clean.
- Add 0.1 volume 2 M NaOAc pH 5.5, mix, and 2 volumes of EtOH; precipitate at –20°C; spin 10 min 3,000 rpm. Wash pellet well with 70% EtOH
- Resuspend with TE to reach ~3 mg/ml (estimated).
- Take OD 260 to quantitate.
- Store at –70°C.
- Make 1mg/ml working stock and store at –20°C.