Whole mount EdU detection of *S. mansoni*

By Jim Collins

***Unless otherwise noted all incubations, washes, and fixation steps should be performed on a rocker with moderate agitation.**

1) Label parasite with EdU (Sigma-Aldrich) either *in vitro* (10 µM for as little as 30 minutes to 24 hours) or in in vivo (100-200 mg/kg by IP injection into mice).
2) Chase EdU for desired period of time. In our experience, for in vitro EdU pulse periods > 4 hours can kill neoblasts, so shorted pulse periods are desired for pulse-chase experiments.
3) Collect mixed sexed *S. mansoni* in media (e.g., Basch Media or DMEM) with 5-10% serum. Initial steps are performed in 15ml conical tubes with 5-10ml of each solution for >50 worms or in 1.7 ml tube for <50 worms.
4) Separate male and female parasites by incubation in a 0.25% solution of the anesthetic ethyl 3-aminobenzoate methanesulfonate (Sigma-Aldrich, St. Louis, MO) dissolved in DMEM+FBS. Alternatively, add 1/10 volume of a 2.5% solution. Rock samples by hand gently for 1-2 minutes or until parasites are relaxed and separated.
5) Kill the parasites in 1ml of 0.6 M $MgCl_2$ for ~1 min
6) Replace $MgCl_2$ with 4% Formaldehyde in PBSTx, incubate 4 hours at RT
7) Rinse 1X with PBSTx.
8) Dehydrate in Methanol and store at -20°C. Samples can be stored for weeks, if not months or years, at -20°C.
9) Rehydrate samples in 50% Methanol solution in PBSTx, 5-10 minutes, RT
10) Incubate in PBSTx, 5-10 minutes, RT
11) Add bleaching solution (9ml H_2O, 500µL Formamide, 250µL 20x SSC, 400µL 30% H_2O_2), incubate 1hr at RT under bright light.
12) Rinse 2x in PBSTx and then incubate in 5ug/ml Proteinase K (Invitrogen) in 1x PBSTx for 45 minutes at RT. **Note: ProK potency appears to vary greatly depending on the source and age of the enzyme. Thus, we suggest empirically determining appropriate enzyme concentration.**
13) Post-fix in 10 ml 4% Formaldehyde in PBSTx, 10 min at RT.
14) Rinse 2x with PBSTx.
15) Add 1ml EdU Detection solution:

789 µL	1x PBS
10 µL	100mM $CuSO_4$
1 µL	10 mM Fluor-conjugated Azide (e.g. Azide-fluor 545, Sigma Aldrich)
200 µL	500mM Asorbic Acid (make fresh)

16) Incubate 30m at RT in the Dark
17) Wash 2-3X with PBSTx
18) Incubate in DAPI (1ug/ml In PBSTx) ~1hr.
19) Clear samples in 80% Glycerol, mount in Vectashield.

Note: If desired, following proteinase K incubation and post-fixation, in situ hybridization can be performed and EdU detection can performed following in situ signal detection.