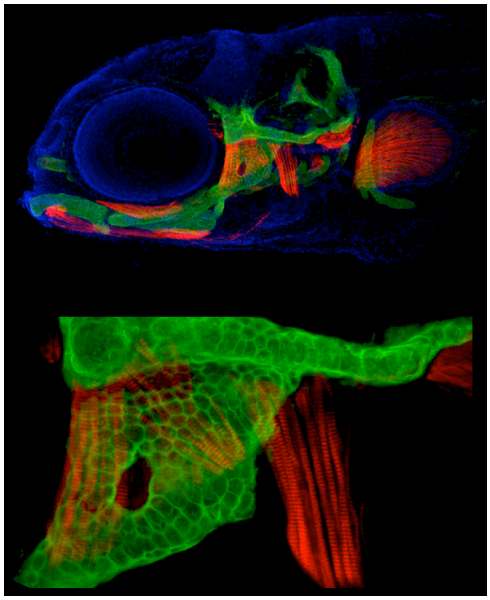
Musculoskeletal staining: col2 60 to 120hrs Henry Roehl

overnight fixation in 4% PFA/PBST rinse - 4X 5min in PBST - Put through MeOH series to store at -20C in 100%.
Day 1
reverse MeOH series, wash in PBST
digest with 10 ug/ml ProK for 1hr at RT rinse 3X in PBST (could try trypsin instead)
1hr block 10% NBCS PBST
overnight in 1:500 anti-col2a(II-II6B3) and 1:500 anti-sarcomere (MF20) in 10% NBCS PBST
Day 2
rinse 4X 30min in PBST
1hr block 10% NBCS PBST
overnight in 1:500 anti-IgG1/HRP (II-II6B3 is this type of monoclonal) and 1:500 anti-sarcomere (MF20) in 10% NBCS PBST
Day 3
rinse 4X 30min in PBST
the following steps should be done avoiding bright light (I use an empty Vectashield box)
incubate in 0.5ml staining solution (fluorescein) for 30min at RT - invert every 10min
rinse 6X10min PBST
incubate 10min in 0.1M HCl
rinse 3X5 min PBST
fix 30min RT - rinse 4X 5min in PBST
1hr block 10% NBCS PBST
overnight in 1:500 anti-IgG2B/HRP (MF20 is this type of monoclonal) in 10% NBCS PBST
Day 4
rinse 4X 30min in PBST
incubate in 0.5ml staining solution (cyanine-3) for 30min at RT - invert every 10min
rinse 6X10min PBST
fix 4% PFA/PBST 30min RT - rinse 2X 5min in PBST
transfer to Vectasheild



Staining solution: use substrate at concentration recommended by manufacturer – dilute in 0.5ml PBST then add 1ul of 3%H2O2 Mix, use immediately (can use less per sample)
Should be able to stain for 15min, this may reduce background
Antibodies: II-II6B3 and MF20 are from Developmental Studies Hybridoma Bank
Goat antiIgG1(#1070-05) and IgG2B(#1090-05) are from Biozol (Southern Biotechnology). HRP substrates are from NEN. Vectashield is from Vector
Substrates: SAT705A001EA/CY5 SAT701001EA/Fluorescein SAT704A001EA/CY3 all from Perkin Elmer. Molecular probes sells Alexa conjugates, you can also make your own: http://www.jhc.org/cgi/content/full/46/6/771
Storage notes: Antibodies at 4C, HRP substrates at 4C or -20C, make sure H2O2 is not too old (store at 4C in dark), store fixed embryos in MeOH at –20C for months, store stained larvae for months in Vectashield at 4C and dark.
Other notes:
1) Cii-C1, anti-col2 also works and is IgG 2a (conditions not optimized.
2) Alternatively you could probably use normal fluorescent secondaries also from Southern(not HRP conjugates). Using this HRP protocol the fluorescence is really strong but also expensive.
3) A4.1025 (anti-myosin) also works for muscle and is an IgG 2a
4) 1ul Toto 3 in 300ul Vectashield will stain nuclei in the CY5 channel (can photograph in this).
5) Use sequential scanning to avoid bleed through