Tcf4 (TCF7L2) Antibody Staining
Adult muscle cryosections
Kardon Lab April 2012 (Malea Murphy)

Protocol:
Flash freeze freshly isolated muscle placed on cork in isopentane (cooled in liquid Nitrogen)
Cryosection (8-12 um thick)

Day 1:
5 min. 4% PFA
2 min. PBS X2
10 min. Citrate Buffer
30 min. Antigen Retrieve (2100 Retriever) in Citrate Buffer
2 min. PBS X2
5 min. in 3% H2O2 in PBS
2 min. PBS X2
1hr TNB buffer @ RT
Overnight in Tcf4 Ab (1:100) in TNB @ 4°C.

Day 2:
5 min. PBS X5
2 hrs. 1st Secondary Ab (Biotin conjugated) in TNB.
2 min. PBS X2
3 hrs. Vectastain ABC Reagent (14 µl of A and 10 µl of B per ml of PBS)
2 min. PBS X2
10 min. TSA Fluorecein or Cy3 in Dilluent Buffer (1:50) (both in TSA box)
2 min. PBS X2
5 min. 4% PFA
2 min. PBS X2
Rinse in water and let slide air dry
coverslip with Fluoromount with added Hoechst (20ul of 10 mg/ml in 25 ml Fluormount)

Reagents:
Tcf-4 (TF7L2) Antibodies:
    Mouse IgG2a (1:100=10ug/ml final concentration): Clone 6H5-3 Millipore 05-511
    Rabbit mAb (1:100=0.7ug/ml final concentration): Clone C48H11 Cell Signaling Technology 2569
    Rabbit antibody is preferred
Biotin conjugated goat α rabbit
    Jackson ImmunoResearch 111-065-144, stock diluted 1:1 in glycerol
    1:500 (1.2 µg/ml) in 5% goat serum in PBS
Citrate Buffer: 1.8 mM Citric Acid and 8.2 mM Sodium Citrate in water
TNB buffer: 0.5% PerkinElmer Blocking Reagent Cat #: FP1020 in 1X PBS (store at -20°C)
Vectastain ABC kit Cat #: PK-6100
PerkinElmer Tyramide Signal Amplification (TSA) Plus
    Fluorescein Cat #: NEL741
    Cyanine 3 Cat #: NEL744
Hoechst (Invitrogen, H1399)
Fluoromount-G (Southern Biotech #0100-01)
2100 Retriever at Electron Microscopy Sciences

All PBS washes and Hoechst staining in 250 ml buckets
1st and 2nd antibodies and block in small volume (200-500ul) on slide, laying flat in humidified darkened plastic tray