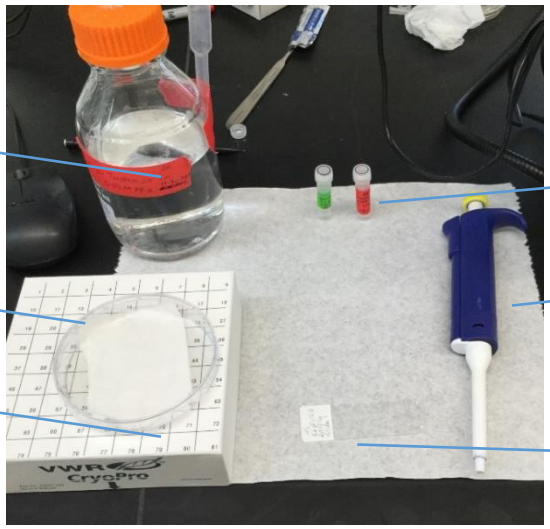
Visual Protocol for immunohistochemistry (IHC)

(February 2016)

IHC staining is a 2-day process. Please note that many factors (e.g., the concentration of the solution, washing/blocking time and etc) may be different depending on things you want to stain, types of antibodies, types of sample tissues, ways of tissue preparation and etc. You need to talk to Siddharth before starting. This is an example protocol for IHC staining for glucocorticoid receptors with sectioned samples of 3 dpf zebrafish embryos on a Fisher Superfrost Plus slide.

Main materials you need



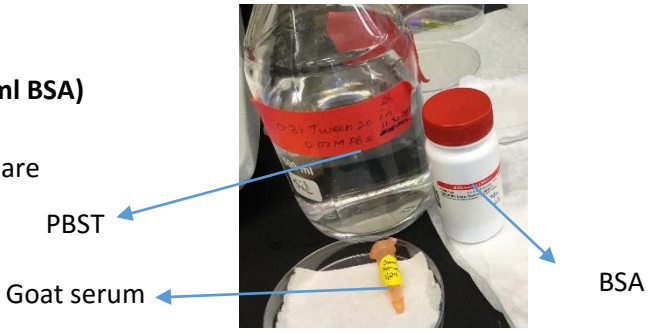
Other things you need: BSA (Albumin from bovine serum, kept in the side pocket in 4°C fridge), goat serum (kept in -20 °C), primary antibody (anti-GR rabbit, kept in 4°C fridge), secondary antibody (goat anti-rabbit, kept in -20°C fridge), transfer pipet, paper towels

Solutions you need to make: Blocking solution (2% goat serum in PBST + 2mg/ml BSA), Primary ab solution (1:100 concentration), Secondary ab solution (1:300 concentration)

How to make solutions

1. **Block solution (2% goat serum in PBST + 2mg/ml BSA)**
   You need this in DAY1 and DAY 2
   To make 2ml (2000ul) of blocking solution, prepare the following and mix well.

PBST	1960 ul
Goat Serum (100%)	40 ul
BSA	4mg



2. **Primary antibody solution (anti-GR rabbit, 1:100 in blocking PBST)** and **Secondary antibody solution (goat anti-rabbit/Alexa 568, 1:300 in blocking PBST).**
   Making 600 ul of the 1° ab and 1500 ul of the 2° ab solutions, prepare the following and mix well.

Primary (1°) ab solution (600 ul) 1:100	
Block solution	594 ul
1° ab (Anti-GR rabbit)	6 ul

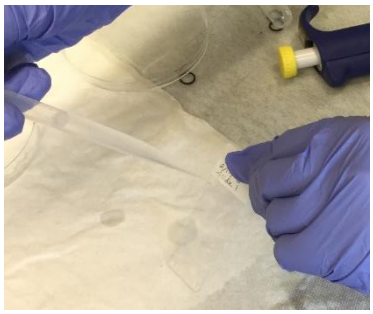
You need this in DAY 1.

Secondary (2°) ab solution (1500 ul) 1:300	
Block solution	1495 ul
2° ab (Goat anti-rabbit)	5 ul

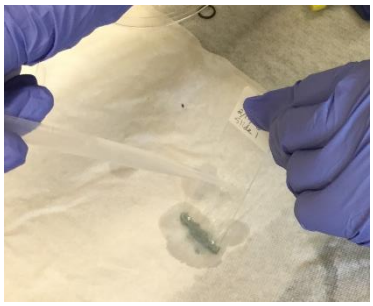
You need this in DAY 2.

DAY 1

1) Obtain your sample (sectioned on the slide) from 4°C fridge (or after drying if you section and stain on the same day), and place it on the warmer for 30 min (35-47°C).
2) Manually wash slides 2x10 minutes (2 times for 10 min) with **PBST (0.3% Tween in PBS).**
   - To wash, add PBST to slide at an angle for a quick wash, then cover slide with PBST and let sit for 10 minutes. Repeat this 2 times. You use about 1ml of PBST each time.
   - Alternatively, if you have a lot of slides, place the slides in rinse container, add PBST, and then place on shaker for 10 min



Holding the slide with angle



Wash each section with PBST with angles

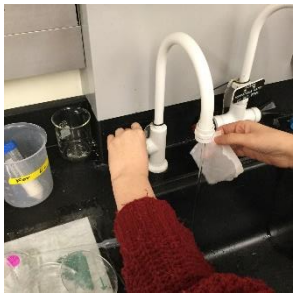
put the slide in flat and add more PBST. Leave it for 10 min.

3) Remove PBST by holding up the slide with angles. Clean the back of slide with KimWipe.
4) Incubate the slide in **block solution (2% goat serum in PBST + 2mg/ml BSA)** for 2h at room temperature
   - Add ~125-300 ul block solution to each slide (enough to cover your slides). Add a bit more solution to the top your slide as the solution tends to move towards the bottom over time. Cover and let sit for 2h.

Apply blocking solution to each samples

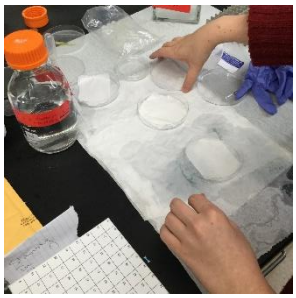
Cover the slide and let sit for 2 h

5) Wash 3 x 10 min in PBST by using the same technique described in (2).
6) Incubate in **primary antibody solution (anti-GR rabbit, 1:100 in blocking PBST)** overnight at 4° C, in wet box.
   - To create wet box for slide(s) use a plastic container such as a petri dish (1 slide/petri dish) with a damp paper towel covering the bottom of the dish. Place your slide(s) face-up on top of the paper towel.



Put dH2O on paper towel

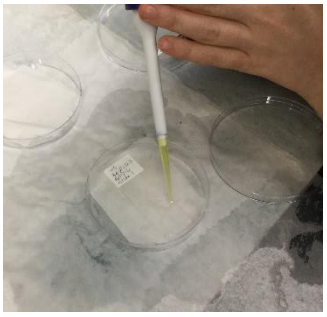
Remove excessed dH2O



Place it on a petri-dish

Put the slide on it

   - Add enough primary antibody solution to cover each slide (~125-300 ml). Add more solution to your upper sections, as the solution tends to move towards the bottom of the slides.
   - Cover and store in fridge.



Apply the 1° ab solution

Cover the dish and keep it in 4°C fridge for overnight

DAY 2

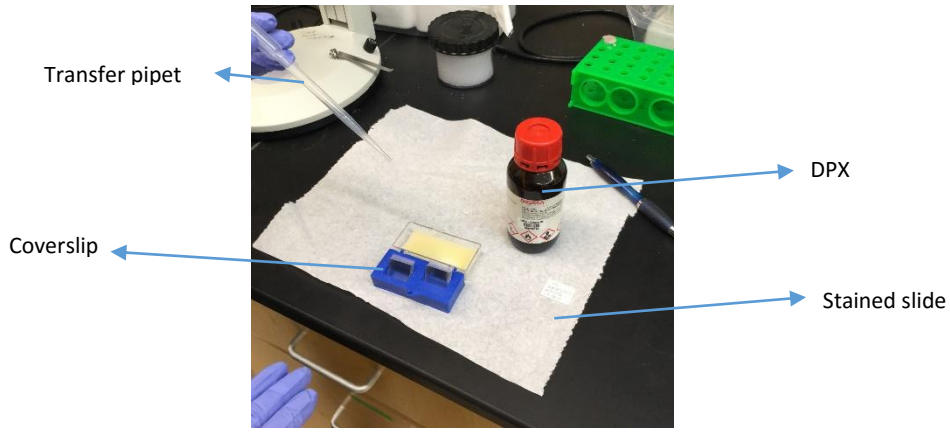
7) Wash 3 x 10 min in PBST by using the same technique described in (2).
8) Incubate in **secondary antibody solution (goat anti-rabbit/Alexa 568, 1:300 in blocking PBST)** at room temperature for 2h in dark. Add a similar amount of solution to cover each slide.

Apply blocking solution to each samples

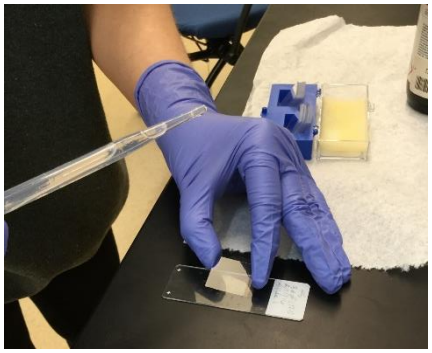
Cover the slide and let sit for 2 h

9) Wash 2 x 5 min in PBST, plus quick wash as needed until all dirt/extraneous particles are removed.

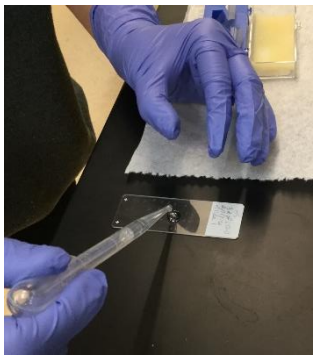
10) Mount slides using DPX Mountant.



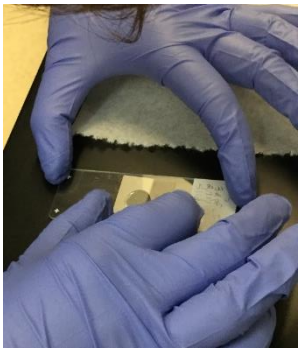
- Using gloves, carefully remove coverslip and plan where coverslips will fit over sections (up to 2 coverslips per slide).
- Slowly draw DPX into transfer pipette. If there are air bubbles visible in the pipette, slowly release DPX back into its container until all air bubbles have been cleared from the pipette.
- Place one drop of DPX into the center of where your coverslip will go on the slide. Carefully place coverslip on top and quickly arrange as needed. Dry them in room temperature and store mounted slides at 4° C.



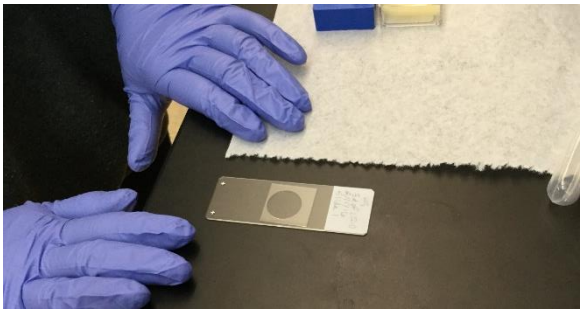
Plan where coverslips will fit



Apply one drop of DPX



Place the coverslip on the top of DPX



Wait until DPX spreads over sample sections under the coverslip.