General protocol: Chromogenic Immunohistochemistry (IHC) DAY ONE

- 1. Label all slides w/ pencil.
 - For organoids: do not use slide warmer as it disrupts organoid integrity.
 - For tissues: slide warmer can be used but may cause sections to fall from slides more easily.
- Dewax slides by incubate slides in series of 2 buckets of xylenes for 10 minutes each, 2 buckets of 100% ethanol for 5 minutes each, and 2 buckets of 95% ethanol for 5 minutes each.
- 3. Incubate slides in bucket of distilled water for 10 minutes
 - While slides incubate in water, make 3% peroxide solution by adding 10mL of 30% stock peroxide to a small bucket and filling bucket up to 100mL line with distilled water.
- 4. Incubate slides in bucket of **peroxide** for 10 minutes
 - Blocks endogenous peroxidase activity to allow specificity of DAB reaction.
 - While slides incubate in peroxide, fill vegetable steamer up to maximum line with distilled water and turn steamer on.
 - Preheat steamer ~10 minutes.
- Move slides to a bucket of appropriate antigen retrieval solution*, place bucket in steamer, and steam for one hour.
- 6. After antigen retrieval steam, allow slides to cool in freezer, then transfer to a bucket of room temperature distilled water.

- While slides cool, dilute antibodies in diluent of casein buffer supplemented with 1% Tween.
 - Casein blocks nonspecific antibody binding.
 - Tween increases antibody access to epitopes.
- Place moistened paper towels inside of humidity chamber and flatten them within grooves to accommodate slides.
- 7. Working one slide at a time:
 - 1. Blot water off of slides at a site far from the sample.
 - Draw a wide circle (at least 1cm gap between circle + organoids) around sample with hydrophobic pen.
 - a. Do not press too hard with pen.
 - b. Ensure each circle is complete.
 - 3. Allow pen to dry very briefly then transfer slide into humidity chamber.
 - 4. Add **200µL** of diluted antibody or control solution (diluent only) to each slide.
 - a. If necessary, gently disperse antibody solution with pipette tip just

contacting the solution itself, not touching the sample/slide!

 Place lid on humidity chamber and carefully move the chamber to the +4°C fridge for overnight incubation of primary antibodies.