PGR- progesterone receptor (Santa Cruz) Paraffin/ formalin sections

- 1. Deparaffinize:
 - a. Xylene 2 x 5 min
 - b. 100% EtOH 2 x 3 min
 - c. 95% EtOH 2 x 3 min
 - d. 70% EtOH 3 min
 - e. Automation buffer 2 x 5 min
- 2. Heat-Induced Epitope Retrieval Using the Decloaker.
 - a. Fill canister with 1x citrate buffer (10mM Sodium Citrate, 0.05% Tween 20, pH 6.0)
 - i. Tri-sodium citrate (dehydrate) 2.94 g + MQ water 1000 ml, Mix to dissolve. Adjust pH to 6.0 with 1N HCl and then add 0.5 ml of Tween 20 and mix well. Store this solution at room temperature for 3 months or at 4°C for longer storage.
 - b. Place in decloaker with 500 mL of water. Fill the other 2 canisters with 250 mL water
 - c. Use **program 5**: 110°C 15 min cycle wait until the cycle is done
 - d. Let slides cool for an extra 10 min in the decloaker □ and outside the decloaker for another 10 min
 - e. Rinse slide in running dH2O for 3 min
- 3. Block endogenous peroxidase with $3\% H_2O_2$ for 15 minutes.
 - a. Rinse **2 x 5** min in 1x automation buffer (**AB**) –Make fresh--- 50 mM Tris, 20 mM NaCl, 0.05% Tween-20 (50 mL 1 M Tris + 4 mL 5M NaCl + 0.5 mL Tween+ 945.5 mL MQ H_2O)
 - b. Draw a circle around the tissue with Pap pen ~ 5 mm away from the tissue
- Block with 10% Normal Horse Serum in AB (10% NHS) for 20 min RT (50-100 μL/section)
- 5. Block with **Avidin D/Biotin** solutions (Avidin-Biotin blocking Kit, Vector Laboratories, SP2001)
 - a. Avidin D solution for 15 min RT, quick rinse with AB
 - b. Biotin solution for 15 min RT, DO NOT RINSE slides, only wipe excess block
- 6. anti-PR (1:50, SC-398898 mouse monoclonal PR) in 10% NHS at 1h 40 min RT or 4°C O/N
 - a. Rinse 2 x 5 min in AB
- 7. Drain slides and apply Biotinylated horse anti-mouse (1:1000) in 10% NHS, incubate 35 min at RT.
 - a. Rinse 2 x 5 min in AB
- 8. Apply Vectastain R.T.U. Elite (Vector Laboratories, PK7100) 30 min RT.
 - a. Rinse 2 x 5 min in AB Then quick rinse with MQ H₂O
- 9. Apply the **DAB** Chromogen (Dako). Incubate RT 5 min
 - a. Wash with dH₂O & Rinse in running dH₂O for 3 min.
- 10. Counterstain 30 seconds in hematoxylin; rinse slides in tap water until water is clear
 - a. Gently agitate slides in AB until the tissues turn blue (I did 30 sec).
- 11. Dehydrate
 - a. 95% EtOH 1 x 3 min
 - b. 100% EtOH 3 x 3 min
 - c. Xylene 2 x 5 min & Coverslip with Permount®