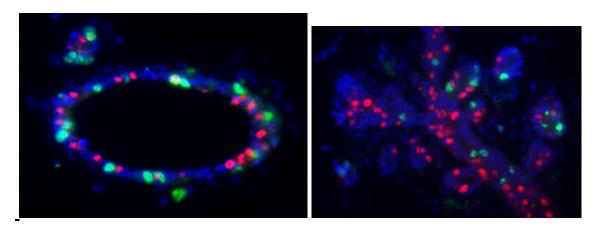
## PR/BrdU double immunofluorescence: Rosen Lab protocol

Reference: TN Seagroves, JP Lydon, RC Hovey, BK Vonderhaar, and JM Rosen. (2000) C/EBPß (CCAAT/Enhancer Binding Protein) Controls Cell Fate Determination during Mammary Gland Development. Molecular Endocrinology **14**: 359-368.

- 1. Two hours prior to tissue collection, all animals are injected with 0.3mg/10 g body weight bromodeoxyuridine (BrdU) cell proliferation labeling reagent (Amersham #RPN 201). Tissue is fixed for 2 hours in 4% paraformaldehyde at 4°C. Embed tissues in paraffin and section onto slides (5-7 µm sections).
- 2. Deparaffinize and rehydrate sections as follows:
  - 3 x 3' Xylene
  - 3 x 2' 100% ethanol
  - 1 x 2' 95%, 80%, 70% ethanol (each)
  - 1 x 5' 1x PBS
- 3. Microwave antigen retrieval:
  - •Place slides in a glass slide holder and fill in the rest of the rack with blank slides (10 total).
  - •Place the rack in 600 ml of 10 mM Sodium Citrate (pH 6; 100 mM stock) in a glass 2L beaker.
  - •Microwave for 20 minutes total, replacing evaporated water after 10 min.
  - •Cool the slides 20 min in the beaker.
  - •Wash 3 x 5' in ddH<sub>2</sub>O, 1 x 5' in 1x PBS
- 4. Shake/wipe off excess PBS and circle all sections with a PAP pen (Research Products International). Do not let the sections dry out.
- 5. Add 50 µl of blocking buffer to each section (5% BSA/0.5% Tween-20 in PBS). Incubate 1-4 hours at room temperature in a humidified chamber.
- 6. Dilute PR antibody at 1:50 and BrdU-FITC antibody at 1:5-1:10 in blocking buffer [rabbit  $\alpha$ -human PR antibody (Dako #A0098), Anti-BrdU FITC (Becton-Dickinson #347583)]. Add 50  $\mu$ l of diluted antibody per section and incubate overnight at room temperature in a humid chamber. KEEP DARK.
- 7. Drain 1° antibody and wash slides 3 x 10′ in 1x PBS.
- 8. Dilute 2° antibody (goat  $\alpha$ -rabbit Texas Red) at 1:1000 in 1x PBS. Add 50  $\mu$ l per section and incubate 1 hour at room temp in a humid chamber.
- 9. Drain 2° antibody and wash slides 3 x 10′ in 1x PBS.
- 10. Mount the sections in 3:1 Vectashield:DAPI (Vector Labs). Coverslip and seal with clear nailpolish.

## Representative images of PR/BrdU immunofluorescence:



The nuclei are blue, the red staining shows PR-positive cells, and the green staining represents BrdU-positive, proliferating cells.

On the left, a 12 week old mouse mammary gland treated for 2 days with E+P. On the right, a mature mouse mammary gland treated for 21 days with an E+P pellet.