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DISCOVERY IN EVOLUTION IN DISCOVERY™

GeneX Expression in Human Eye
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GeneX Expression Summary

Here are slides exposed to emulsion for one, two and four weeks from the in situ hybridization (ISH) experiment using GeneX to probe sections of human eye. Due to size limitations of the glass slides used, the eye was divided into anterior and posterior halves. The anterior half contained the lens, cornea and ciliary bodies. The posterior half contained the retina and a portion of the optic nerve. Both halves contained sclera.

In summary, GeneX expression was detected in the cornea near the limbic epithelium. GeneX expression was not detected in sclera, retina, optic nerve, lens or ciliary bodies. GAPDH was used as a positive control. GAPDH expression was detected in corneal epithelium and endothelium.

It would be interesting to investigate the expression of GeneX during development in the mouse cornea to see how its expression pattern changes with maturation of the cornea.

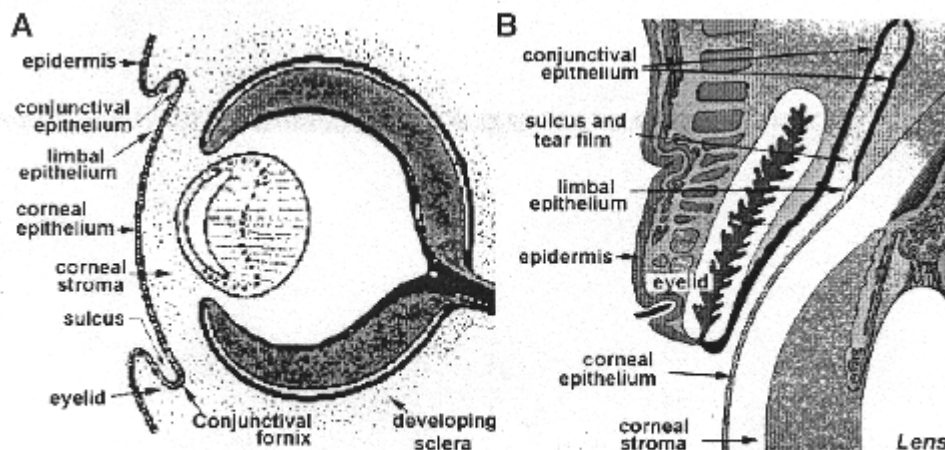


Fig. 1. Schematic description of the ocular surface epithelial zone. (A) The ocular surface at fetal week seven. (B) The adult ocular surface.

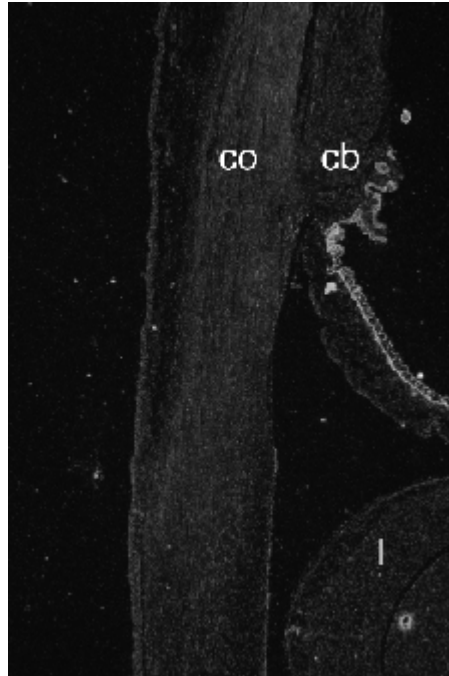
Figure 1 from Wolosin, J.M. et al. 2004 *Int. J. Dev. Biol.* 48:981-991 diagrams the orientation of the results presented in this report. The corneal epithelium is to the left of the micrograph, the ciliary body adjacent to the limbic epithelium is at the top of the micrograph, and the lens is at the lower right.

Odd numbered glass slides were hybridized to the antisense (AS) probe that detects the mRNA. Even numbered glass slides were hybridized to the sense probe that does not hybridize but shows the background level of silver grains for the assay. The image labels indicate the glass slide number (#) the probe used and the magnification.

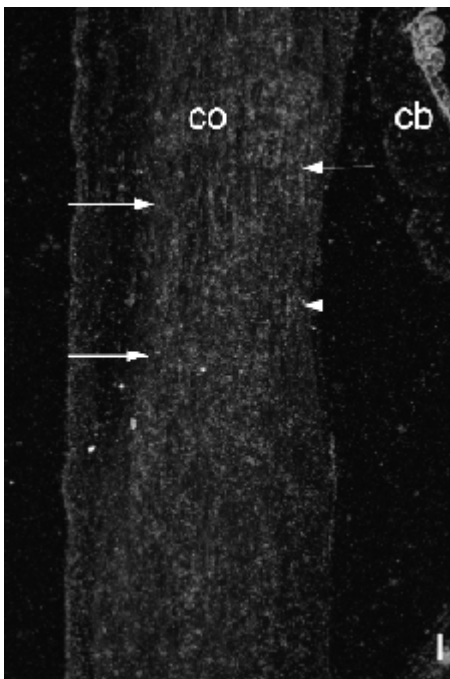
GeneX Expression Results



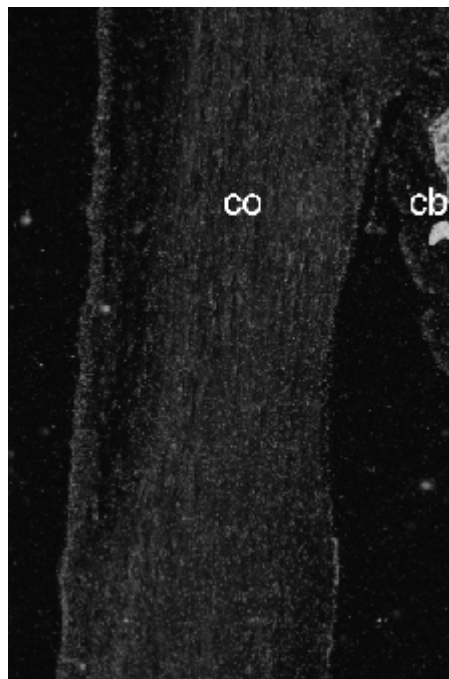
Slide 01 # 07 AS 2.5x



Slide 03 # 08 S 2.5x



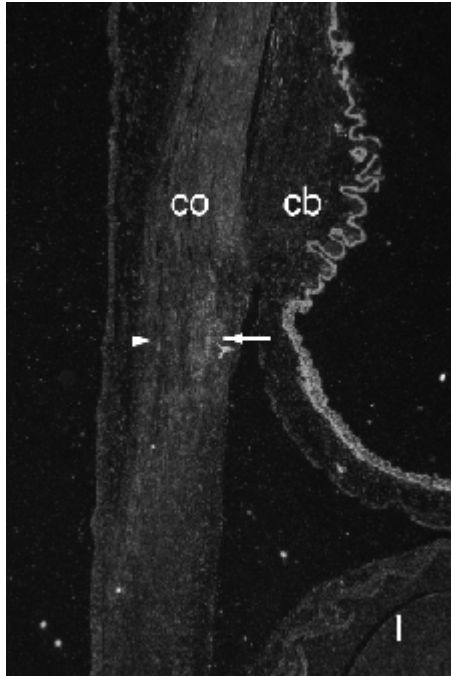
Slide 02 # 07 AS 5x



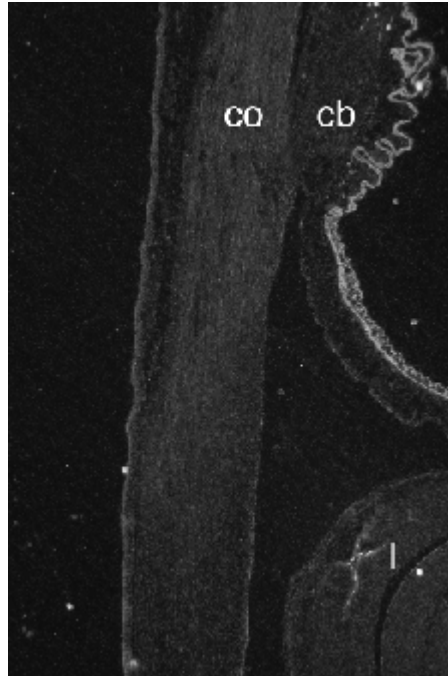
Slide 04 # 08 S 5x

Slide 01 # 07 AS 2.5x and Slide 02 # 07 AS 5x show darkfield images of the same section. Arrows point to the region of positive ISH signal (where; co=cornea, cb=ciliary body, l=lens). Slide 03 # 08 S 2.5x and Slide 04 # 08 S 5x show the corresponding sense control results. The background level of silver grains for this assay was low.

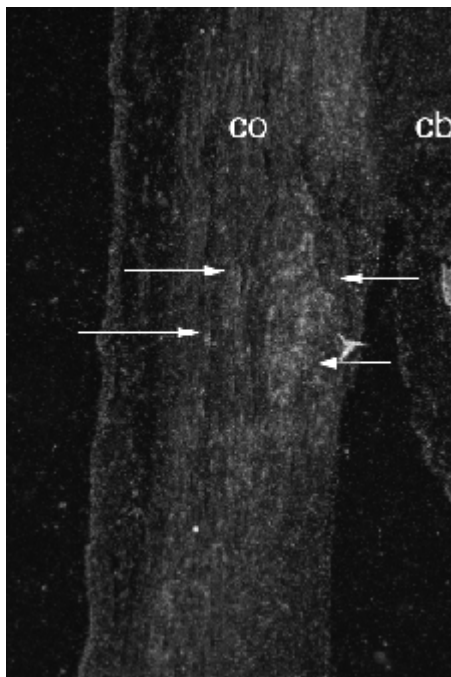
GeneX Expression Results (continued)



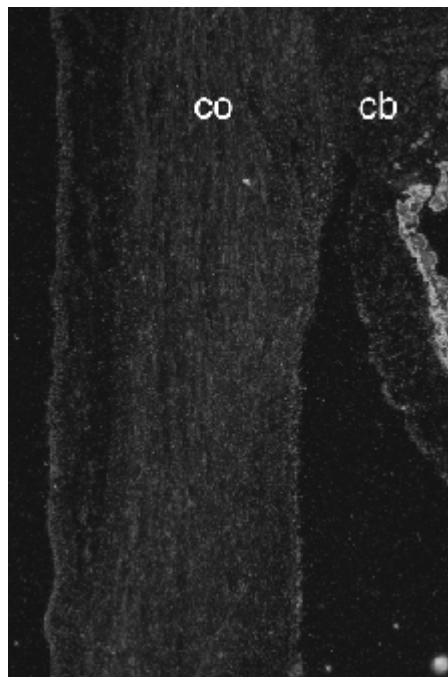
Slide 05 # 09 AS 2.5x



Slide 07 # 10 S 2.5x



Slide 06 # 09 AS 5x



Slide 08 # 10 S 5x

Slide 05 # 09 AS 2.5x and Slide 06 # 09 AS 5x show darkfield images of the next section in the series. Arrows point to the region of positive ISH signal. Slide 07 # 10 S 2.5x and Slide 08 # 10 S 5x show the corresponding sense control results.

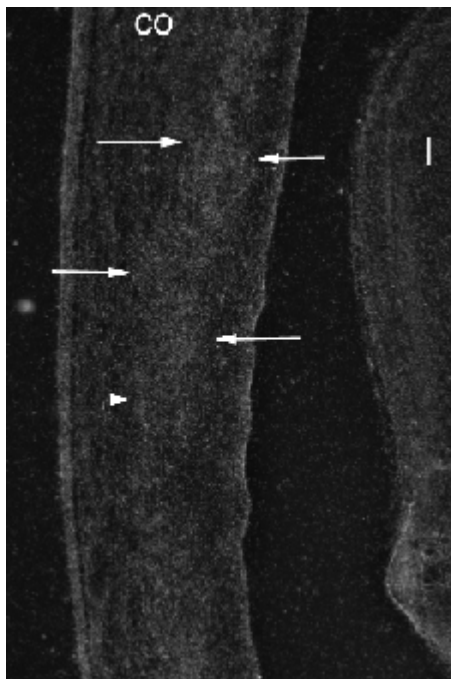
GeneX Expression Results (continued)



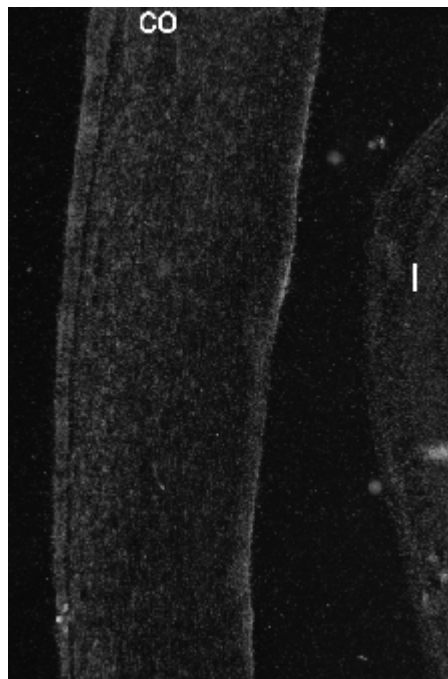
Slide 09 # 11 AS 2.5x



Slide 11 # 12 S 2.5x



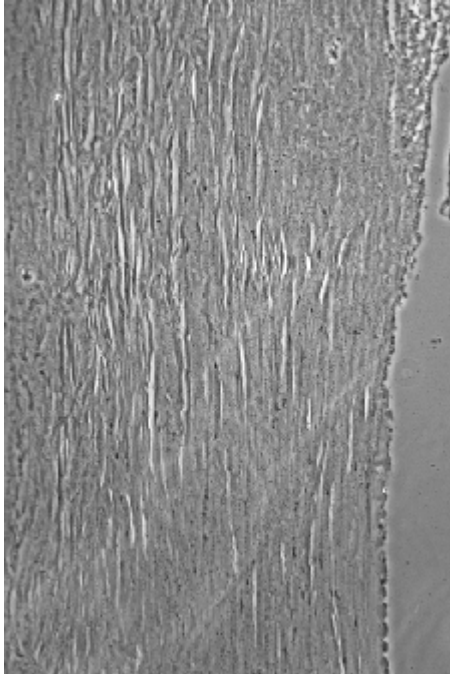
Slide 10 # 11 AS 5x



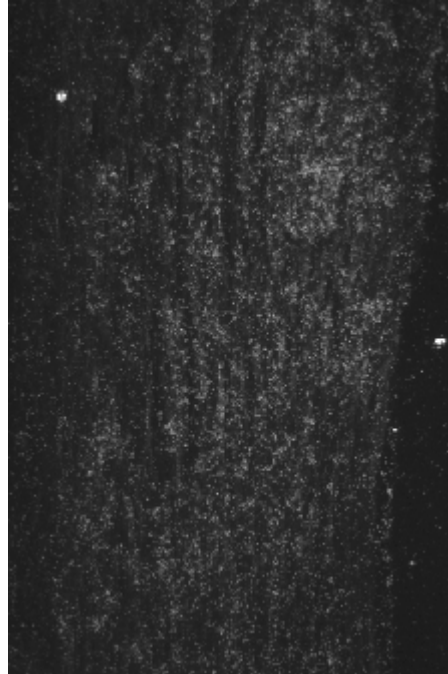
Slide 12 # 12 S 5x

Slide 09 # 11 AS 2.5x and Slide 10 # 11 AS 5x show darkfield images of the next section in the series. Arrows point to the region of positive ISH signal. Slide 11 # 12 S 2.5x and Slide 12 # 12 S 5x show the corresponding sense control results.

GeneX Expression Results (continued)



Slide 13 # 07 AS 10x

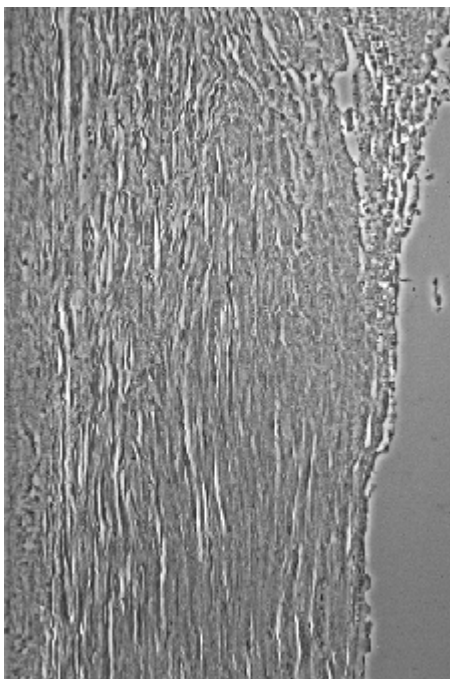


Slide 14 # 07 AS DF 10x

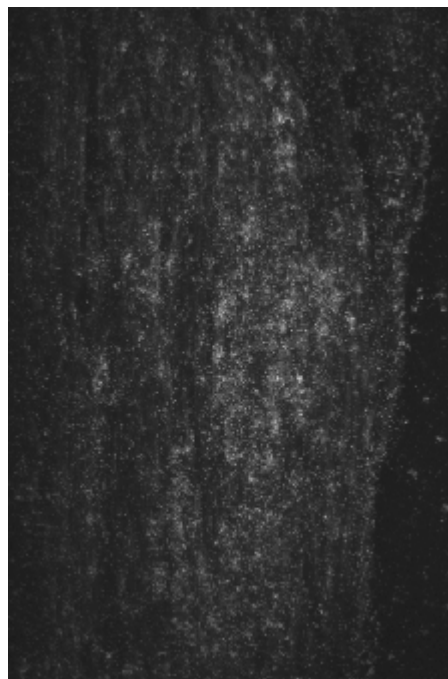
The regions of the corneal stroma that were positive for GeneX expression were photographed at 10x as phase contrast and darkfield images.

Slide 13 # 07 AS 10x and Slide 14 # 07 AS DF 10x show the ISH signal in the corneal stroma near the ciliary body.

Slide 15 # 09 AS 10x and Slide 16 # 09 AS DF 10x show the next slide in the series.

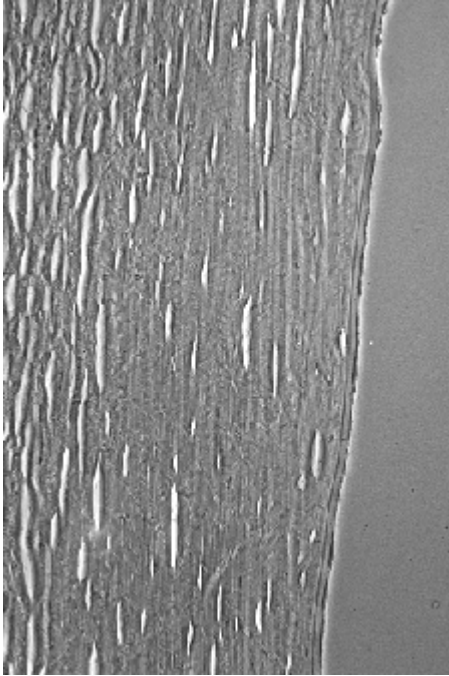


Slide 15 # 09 AS 10x.gif

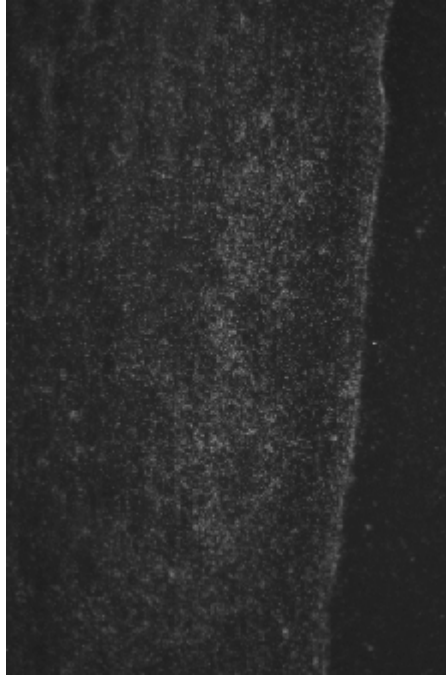


Slide 16 # 09 AS DF 10x

GeneX Expression Results (continued)



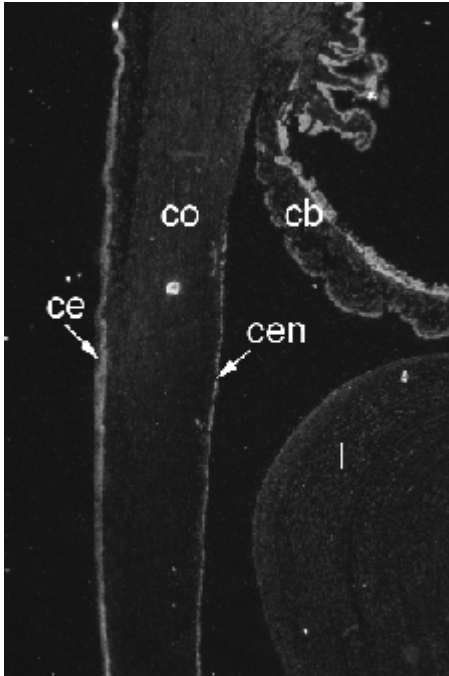
Slide 17 # 11 AS 10x



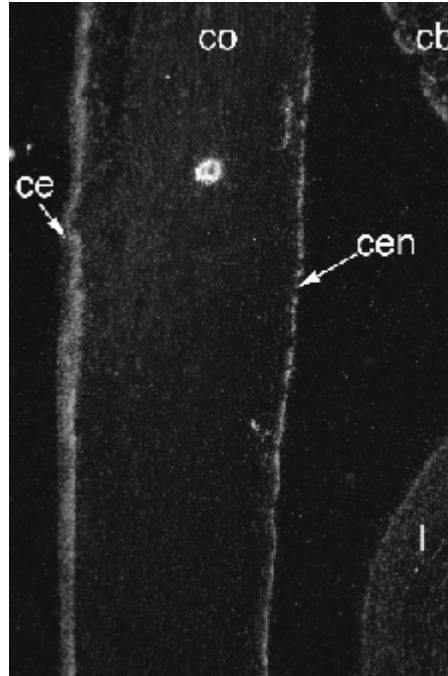
Slide 18 # 11 AS DF 10x

Slide 17 # 11 AS 10x and Slide 18 # 11 AS DF 10x show the third slide in the series.

GAPDH Expression Results



Slide 19 # 15 GAPDH AS 2.5x



Slide 20 # 15 GAPDH AS 5x

Slide 19 #15 GAPDH AS 2.5x and Slide 20 # 15 GAPDH AS 5x show darkfield images of the same section that was hybridized with the positive control probe, GAPDH. The positive corneal epithelium (ce) and corneal endothelium (cen) are indicated by arrows.

Methods, Storage & Viewing

Methodology

Tissue Fixation, Embedding and Pretreatment

Tissues were fixed in 4% paraformaldehyde in phosphate buffered saline (PBS) overnight, dehydrated and infiltrated with paraffin. 5 to 7 Micron serial sections were mounted on gelatinized slides. 1 to 3 Sections were mounted/slide, deparaffinized in xylene, rehydrated and post-fixed. The sections were digested with proteinase K, post-fixed, treated with triethanolamine/acetic anhydride, washed and dehydrated.

cRNA probe preparation

The cRNA transcripts were synthesized according to manufacturer's conditions (Ambion) and labelled with ³⁵S-UTP (>1000 Ci/mmol; Amersham). cRNA transcripts larger than 200 nucleotides were subjected to alkali hydrolysis to give a mean size of 70 bases for efficient hybridization.

Hybridization and Washing Procedures

Sections were hybridized overnight at 52°C in 50% deionized formamide, 0.3 M NaCl, 20 mM Tris-HCl pH 7.4, 5 mM EDTA, 10 mM NaH₂PO₄, 10% dextran sulfate, 1X Denhardt's, 50 µg/ml total yeast RNA, and 50-75,000 cpm/µl ³⁵S-labelled cRNA probe. The tissue was subjected to stringent washing at 65°C in 50% formamide, 2X SSC, 10 mM DTT and washed in PBS before treatment with 20 µg/ml RNase A at 37°C for 30 minutes. Following washes in 2X SSC and 0.1X SSC for 10 minutes at 37°C, the slides were dehydrated and dipped in Kodak NTB-2 nuclear track emulsion and exposed for one week in light-tight boxes with dessicant at 4°C.

Imaging

Photographic development was carried out in Kodak D-19. Slides were counterstained lightly with toluidine blue and analyzed using both light- and darkfield optics of a Zeiss Axiophot microscope. Sense control cRNA probes (identical to the mRNAs) always gave background levels of hybridization signal.

Storage & Rehydration

For any section that is "crystallized", it may be repaired by allowing the coverslip to fall off after soaking in xylene for 24-48 hours. Rehydrate the slide to 70% EtOH. Redehydrate the slide in 80%, 95% and 2x 100% EtOH for 2 minutes each. After three changes of xylene, mount the coverslip with Cytoseal (VWR Scientific) or other comparable mounting medium. Using the same method, coverslips can be removed for histological staining to take brightfield micrographs. Histological stains that require acidic conditions may dissolve silver grains. Overstaining may obscure the silver grains. Any excess mounting medium or residual emulsion on the back of the slide can be removed with a single-edged razor. Dry the recoverslipped slides flat for 24 hours. These slides can be stored indefinitely at room temp.

Viewing Original Slides

The results are best viewed with darkfield illumination, but with a 20x or 40x phase-contrast objective, the silver grains can be localized over particular cell groups. The antisense probe (AS) detects the mRNA and the sense control probe (S) shows the background level of silver grains for the experiments. Enclosed are TIFF image files labeled with the tissue and whether the result was with the antisense or sense probe; a, b, c, etc. indicate a different region of the section on the same glass slide. Scans of a one millimeter micrometer, taken with the same objective as the figures, are provided if scale bars are required. The large lines on the micrometer represent 100 microns. All micrographs are 10x.