## Simultaneous Detection of Protein and Transcripts

**•See Lai et al. (2000)**

Modified protocol based on those of O'Neill and Bier (1994) and Sturtevant et al. (1993).

Embryos were fixed and brought into methanol as described by O'Neill and Bier (1994) and then exchanged into BSA-PDT (PBS, 0.3% sodium deoxycholate, 0.2% RNAase-free bovine serum albumin, 0.1% Triton-X). Late third-instar larvae were dissected in PBS and fixed for 30 minutes in PBS, 4% formaldehyde, 50 mM EGTA; after washes in PBS+0.1% Tween, the dissected tissues were rinsed with BSA-PDT. Embryos and discs were then blocked for 2 hours in BSA-PDT. This was followed by incubation in either rabbit anti-Twist (S. Roth, gift from E. Bier, 1:5000 in BSA-PDT) or mouse anti-Cut (mAb 2B10, Developmental Studies Hybridoma Bank, 1:100) primary antibody, washes in BSA-PDT, incubation in either biotinylated goat anti-rabbit (Vector, 1:200 in BSA-PDT) or biotinylated horse anti-mouse (Vector, 1:200) secondary antibody, further washing, and overnight fixation at 4°C. The tissue was then transferred through methanol to ethanol, and subjected to in situ hybridization (as above, except that Proteinase K treatment was reduced to 4 minutes duration). After excess probe was washed off, the tissue was incubated with sheep anti-digoxigenin (Roche #1333089, 1:2000), followed by exposure to a mixture of ALEXA Fluor 594 donkey anti-sheep antibody (Molecular Probes A-11015, 1:200) and ALEXA Fluor 488 neutravidin conjugate [Molecular Probes (no longer available), 1:100]. After extensive washes, discs were mounted in Gel/mount (Biomeda) and embryos were mounted in FluoroGuard Antifade Reagent (Bio-Rad #170-3140). In situ hybridization and protein immunofluorescence signals were captured separately on a Nikon Microphot-FXA microscope, and images were overlaid in Adobe Photoshop.

**Lai, E. C., Bodner, R. and Posakony, J. W.** (2000). The *Enhancer of split* Complex of *Drosophila* includes four Notch-regulated members of the Bearded gene family. *Development* **127**, 3441-3455.
**O'Neill, J. W. and Bier, E.** (1994). Double-label *in situ* hybridization using biotin and digoxigenin-tagged RNA probes. *Biotechniques* **17**, 870, 874-875.
**Sturtevant, M. A., Roark, M. and Bier, E.** (1993). The *Drosophila rhomboid* gene mediates the localized formation of wing veins and interacts genetically with components of the EGF-R signaling pathway. *Genes Dev.* **7**, 961-973.