

## In-Situ Hybridisation



Head **Richard Poulson**

### Staff

Toby Hunt

Pooja Seedhar

Rosemary E Jeffery

This Service exists principally to help researchers localise the sites of expression of specific mRNAs in histological sections of normal or diseased tissue of any species.

The Service has significant expertise, having hybridised over 82,000 sections of tissues that have been fixed and embedded routinely, with a very high rate of success for detection of mRNAs using riboprobes labelled with <sup>35</sup>S (or <sup>3</sup>H for higher spatial resolution). We find isotopic probes are usually informative at the first attempt, provided that probe and tissue quality are adequate, although we have continued to explore alternative techniques for the demonstration of miRNA.

Increasingly we are called on to use genome- or chromosome-specific probes to establish the origin of cells in tumour xenografts or after cell transplantation, or in chimaeras generated by blastocyst fusion. We are able to combine immunohistochemistry or cytogenetic ISH with detection of an abundant mRNA using non-isotopic *in situ* hybridisation and multichannel imaging (Figure 1).

Our results allow researchers to identify the cell type or types in which a gene is expressed, and in what circumstances. *In situ* analysis can make an important contribution to validation of other data, e.g. [Armstrong et al., *Arterioscler Thromb Vasc Biol.* 2008 Sep;28(9):1640-6].

Semi-quantitative scoring can reveal whether the level of expression is related to tumour development or treatment in pre-clinical models, e.g. we have used *in situ* hybridisation for VEGFR2 mRNA and detected altered expression in endothelial cells following treatment of adenomas with a tyrosine kinase inhibitor [Alferez et al., *Mol Cancer Ther.* 2008 Mar;7(3):590-8].

One of the greatest advantages of *in situ* hybridisation is that patterns of expression of several genes can be compared and contrasted with each-other using just a few sections of tissues that can be in very short supply, whether relatively rare clinical specimens (e.g. [Jones et al., *J Pathology* 2008;216: 408-417]), or very small early lesions in mouse cancer models (e.g. [Segditsas et al., *Hum Mol Genet.* 2008 17(24):3864-75]).

A User Committee considers applications for projects or training made by researchers from the London Research Institute and other CRUK laboratories so that we undertake around 25 projects each year.

Our intranet pages show examples of results and offer advice on how to design probes so that they are specific.

The ISH Service team is always pleased to help researchers with their project.

Publications listed on page 135



Figure 1. Combined detection of PODXL mRNA and both X and Y chromosomes to determine the origin of podocytes in a female mouse following male bone marrow transplantation; a) monochrome transmitted light image showing formazan-detected mRNA in the cytoplasm of podocytes (P) on the periphery of a renal glomerulus; b) plus image planes showing FISH signals for X (red) and Y (green) chromosomes and DNA (DAPI = blue); c) false colouring of panel a in orange to enhance visibility of mRNA signal reveals the central podocyte appears to be of bone marrow origin.