# A look back: FISH still fresh after 25 years

It's sometimes easy to forget that molecular biological research was somehow being done before nucleic acid hybridization was commonplace, even before the dawn of the Southern blot. This isn't to say that things were easy, however, and the relatively rudimentary techniques available through much of the 1950s and 1960s made the detailed analysis of genetic and molecular processes quite difficult.

The oocytes of the African clawed frog, *Xenopus laevis*, contain large numbers of nucleoli; the foundation for this phenomenon is the presence of numerous extrachromosomal copies of the genes encoding ribosomal RNA (rRNA). One of the leading investigators studying this 'gene amplification' in the 1960s was Yale researcher Joe Gall, and he and graduate student Mary Lou Pardue sought a technique that would enable them to monitor the process by which these ribosomal genes increase their number.

Ideally, they hoped to mark these genes directly for visual inspection. "Hybridization was the new thing on the block," Pardue explains, "...and one of the dreams that Joe Gall and other people had was to see if it could happen actually in the cell, so that you would be staining some part of the cell with [labeled] RNA." Although RNA preparation was a difficult process, Gall and Pardue were able to use cultured *X. laevis* cells to prepare tritiated rRNA, which they isolated on a sucrose gradient. Upon treatment of the fixed oocytes with purified, hot rRNA, they were pleased to observe clear labeling of the rRNA genes that increased at stages during which these sequences were known to amplify[1]; follow-up experiments confirmed that their '*in situ* hybridization' (ISH) technique offered a practical solution for identifying the chromosomal location of specific sequences.

Early use of ISH was limited by the ability to synthesize and purify appropriate probes. Subsequent work by Pardue in the lab of Max Birnstiel (who had independently helped to develop a similar ISH technique[2]) used sea urchin RNA to probe for histone sequences in *Drosophila melanogaster* chromosomes[3]. "Most people were sort of skeptical," she says. "[But] *Drosophila* had polytene chromosomes that were big enough that you had a target and...given that histones were very conserved, I figured the chance was worth it." The advent of cloning considerably improved the ease with which sequences could be prepared as probe templates. Between this and improved radiolabeling and hybridization techniques, ISH was soon detecting even single-copy sequences.

Radioactive ISH is potent and sensitive, but is potentially vulnerable to high background, and can require days or even weeks of film exposure. One alternative technique bypassed these difficulties by using fluorescently labeled antibodies to recognize specific RNA-DNA hybrids[4], but it wasn't long before this was supplanted by a more direct approach. "It was very difficult to raise the antibody against the RNA-DNA hybrids," explains Joop Wiegant, an investigator at the Leiden University Medical Center. "We spent a lot of effort on that, because at that time you had to immunize rabbits and hope to get a nice antibody, but we failed many times." Wiegant and his colleagues developed a system whereby a fluorophore was chemically coupled to an RNA probe, enabling quick and direct visualization of hybridization. In 1980, they published their first article describing fluorescent *in situ* hybridization (FISH)[5]. This version of FISH, known as the 'direct' approach, remains popular and continues to be expanded through the use of multiple labels—recent work by Wiegant's own group demonstrated the combinatorial use of five different fluorophores to differentially 'paint' each human chromosome arm[6].

'Indirect' FISH, based on the enzymatic or immunological detection of tags incorporated into the probe, also continues to see widespread use. In 1981, David Ward demonstrated the synthesis of modified nucleotide derivatives containing a biotin label, which could be recognized by polymerases for incorporation into probes[7]; these can in turn be detected with anti-biotin antibodies. This strategy is largely identical to the technique used in many modern FISH kits (and the method presented in this issue), although current protocols often use the steroid digoxigenin as an alternative recognition element, or streptavidin-fluorophore conjugates rather than antibodies to detect biotin tags.

With its specificity, clarity and relative rapidity of detection, FISH remains the technique of choice for direct visualization of DNA and RNA at the single cell, tissue and whole-embryo level. Unlike many classical techniques, which plateau or decline in popularity as new technologies supplant them, FISH has only grown more powerful after a quarter of a century, and continues to make important contributions in genomic structure and gene expression studies.

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1. Gall, J.G. & Pardue, M.L. *Proc. Natl. Acad. Sci. USA* **63**, 378–383 (1969).
2. John, H.A. *et al. Nature* **223**, 582–587 (1969).
3. Pardue, M.L. *et al. Chromosoma* **63**, 135–151 (1977).
4. Rudkin, G.T. & Stollar, B.D. *Nature* **265**, 472–473 (1977).
5. Bauman, J.G.J. *et al. Exp. Cell Res.* **128**, 485–490 (1980).
6. Wiegant, J. *et al. Genome Res.* **10**, 861–865 (2000).
7. Langer, P.R. *et al. Proc. Natl. Acad. Sci. USA* **78**, 6633–6637 (1981).