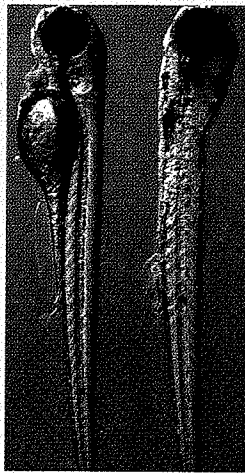


Have a Heart



Obtaining pure organ preparation by microdissection from small organisms, such as the zebrafish, *Danio rerio*, is itself a sufficiently arduous task. Isolating such organs in the same manner from the fish in its embryonic stage is near impossible. With this technical hurdle to mount, **Burns et al.** developed a simple and straightforward isolation technique, described on p. 274 of this issue, which yielded good quantities of almost homogeneous zebrafish heart. The basic setup included a fine, large-gauge needle and syringe, clamped securely over a microfuge tube containing the zebrafish embryos (those between

and 2 and 4 days postfertilization were used). Making use of the shear forces created by repeatedly drawing up and expelling the solution containing suspended embryos, the yolk sac could be broken, and the embryos fragmented. Filtering through two grades of nylon mesh generated a highly pure suspension of zebrafish hearts. The procedure was sufficiently vigorous to produce a good yield, but also gentle enough that intact organs were obtained that, when warmed to room temperature in media, demonstrated spontaneous rhythmic contractions. Further proof of the purity of the extract and success of the technique was provided through tissue-specific quantitative RT-PCR analysis.

Thinking Outside the BOXTO

The development of new, brighter, and more stable fluorescent molecules and DNA dyes covering a broader emission range has dramatically increased the flexibility for experimental design available to scientists. Multicolor fluorescent in situ hybridization, more complex fluorescence resonance energy transfer (FRET) setups, and enhanced multiplex real-time PCR are just some examples. **Lind et al.**, on p. 315 of this issue, describe one such case of increased flexibility, in which they make use of a recently developed dsDNA binding dye for melting curve analysis in conjunction with established real-time PCR methodologies with the commonly used FAM fluor. The dye, BOXTO, a member of the BEBO family of groove-binding cyanine dyes, was developed for use in real-time PCR applications as a potential alternative to SYBR® Green. Now, the authors further extend the application of BOXTO by showing that it can be used in combination with sequence-specific FAM-labeled probes. While the latter are adept at detecting only targeted PCR product, post-PCR melting curve analysis using BOXTO can identify the presence of aberrant products such as nonspecific products and primer-dimers. Since the emission wavelength of BOXTO is sufficiently different from that of FAM (552 and 521 nm, respectively), their signals will not overlap, enabling facile detection of both in the same reaction tube. BOXTO did not significantly alter the efficiency of the PCR, although a small affect on the C_T value was seen.

Totally Tubular

Single-walled carbon nanotubes are increasingly employed in biomedical research; these applications frequently require functionalization of the nanostructures. In a report on p. 295, **Didenko and Baskin** describe an enzymatic approach for labeling nanotubes with quantum dots. The strategy is based upon the tyramide-horseradish peroxidase reaction, in which the enzyme reacts with tyramide conjugates to generate radicals that can mediate attachment to a substrate of choice, in this case the nanotube. Unfortunately, using a tyramide-fluorophore conjugate does not result in labeling, as the nanotube quenches the proximate fluorophore. Instead, the authors performed the reaction with a tyramide-biotin conjugate and mixed the resulting biotinylated nanotubes with streptavidin-linked quantum dots. They found that the extent of labeling could be determined by agarose electrophoresis, as nanotubes, unlike free quantum dots, cannot enter the gel. Electron microscopy revealed quantum dots decorating the nanotubes in a "beads-on-a-string" configuration. In fluorescence microscopy, ropes and individual nanotubes could be observed. This functionalization method should allow straightforward production of labeled nanotubes that can be applied to the study of single molecules; in addition, the technique provides a general strategy for bioconjugation of single-walled carbon nanotubes.

