at gd 14 or later (15), while the latter requires some expertise in cytogenetics. A technique for the identification of segmental trisomy progeny in the Ts65DN mouse (an additional mouse model of Down syndrome) using interphase FISH of tail fibroblasts has also been reported (21). Our procedure, though similar, uses a commercially available probe generated from PCR-amplified DNA from FACS-sorted chromosomes (Certificate of Analysis Information for Mouse Chromosome 16-specific Paint; Cambio). Whole chromosome paints for murine chromosomes 1–19, X and Y are also commercially available for use in detection of chromosomal abnormalities in mice (http://www.cambio.co.uk).

The recent sequencing of human chromosome 21 provides great impetus for research on the molecular basis of Down syndrome (10). Due to the partial synteny between mouse chromosome 16 and human chromosome 21, the Ts 16 mouse has been used as a model for the study of aspects of this syndrome (4,6,8,12,13,19). Although the Ts 16 mouse used in the present studies exhibits perinatal mortality, it provides a valuable animal model to examine aspects of the Down syndrome phenotype such as aberrant craniofacial development. Mouse chromosome 16 has partial synteny with human 22q11, a region implicated in DiGeorge syndrome (14). Cardiac defects in the Ts 16 mouse also make it useful in the study of the formation of the atrioventricular junctions and atrial and ventricular septation (22). Analysis of aberrant gene expression in the tissues affected in these syndromes and defects will likely contribute to our understanding of the molecular and cellular basis of the associated malformations.

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## De-Chorionation of Fixed Zebrafish Embryos

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The zebrafish has become an important model for investigating vertebrate development because of a number of attractive characteristics, including high reproductive potential and the optical clarity and accessibility of the embryos. The embryo is surrounded by a protec-

tive envelope, the chorion, and many procedures require the removal of this envelope to improve penetration to the embryo by reagents, antibodies, or nucleic acids. Established methods for doing this include mechanical removal of the chorions, usually with fine tweezers or tungsten needles (2) and enzymatic digestion with proteases to weaken and permeabilize the envelope (2). Although the mechanical method works reasonably well with fixed embryos, we have still found this method to be relatively tedious and time consuming because each embryo must be dealt with individually and the chorion must be torn and then removed. We have also found that this results in some damaged embryos. Protease digestion works fairly well on live embryos at the somite stage of development or older, but it does not work well on fixed embryos. Consequently, our experiments involving fixed embryos, in situ hybridizations in particular, have suffered from low numbers of embryos or damaged embryos, which result in an increased background.

We have devised a simple, quick, and yet surprisingly efficient mechanism for removing the chorions from zebrafish embryos. The principle is simple: the embryo is drawn up into a broken glass micropipet that has a diameter wider than the embryo itself but narrower than the chorion. The jagged glass edges of the needle snag, tear, and remove the chorion as the embryo passes undamaged up the capillary tube. When the embryo is expelled from the micropipet, it is protected from the jagged glass by a fluid cushion. A number of embryos can be aspirated into the pipet at the same time (in a row), making the de-chorionation process quite rapid.

The de-chorionating micropipet is fashioned from a thin-walled capillary tube that has an inside diameter larger than the intact chorion, such as Kwik-Fil™ capillaries model TW150-4 (World Precision Instruments, Sarasota, FL, USA). The tubes are then heated and pulled to produce a long narrow taper similar to microinjection pipets. Automatic pipet pullers, such as the Kopf™ Vertical Pipette Puller model 700D (David Kopf Instruments, Tujunga, CA, USA) work well, but simply heating the capillary tubes over a bunsen burner and stretching by hand produces an equally effective micropipet. The micropipet tip is then broken by squeezing it with fine forceps at a point where the internal diameter at the tip is larger than the embryo but smaller than the chorion. We have found that the more ragged and damaged the end the better (Figure 1A). Not every needle will break well or work efficiently, so this step may require a number of attempts. However, once a batch of effective needles has been prepared, they may be used repeatedly.

Once the needle has been made, it is then inserted into a holder connected to a mouth pipet consisting of a short length of narrow tubing connecting a mouthpiece to the needle holder. As a needle holder, we use a 1-mL pipet tip from an adjustable pipettor cut off at a point that provides a hole just large enough to form a snug fit for the capillary tube. The pipet tip is inserted onto the tubing of the mouth pipet. The assembled apparatus is then used to draw the embryos up and down the capillary tube by mouth pressure, which catches the chorion and tears it off as the embryo moves up the capillary tube (Figure 1, B and C).

Embryos de-chorionated in this way appeared to be undamaged, but we then

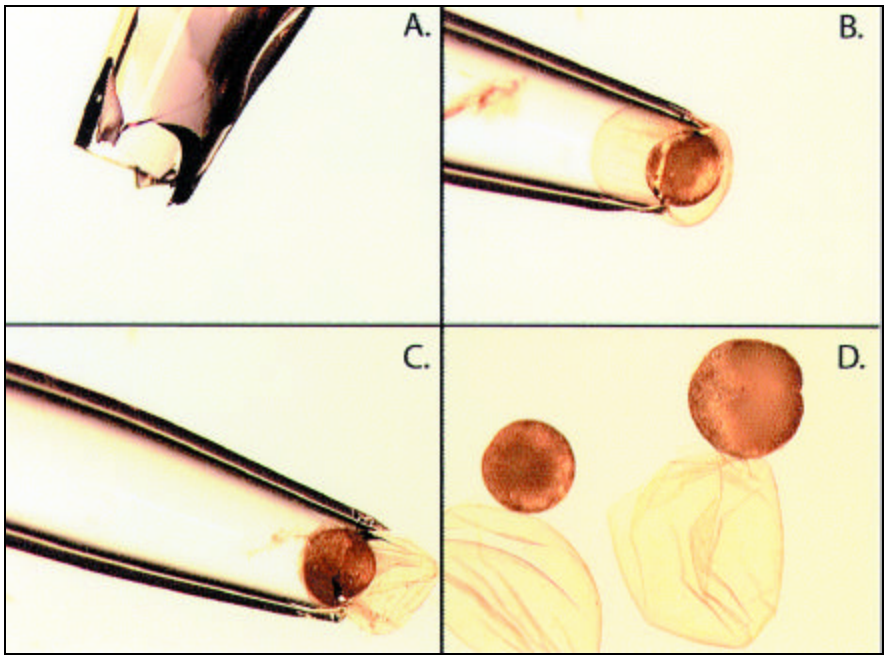


**Figure 1. Removal of zebrafish chorions.** The end of an efficient chorion-removing needle is shown in panel A. Panels B and C show how the chorion is distorted and torn by being drawn up and down the needle, and panel D shows the embryos and the chorions after removal.

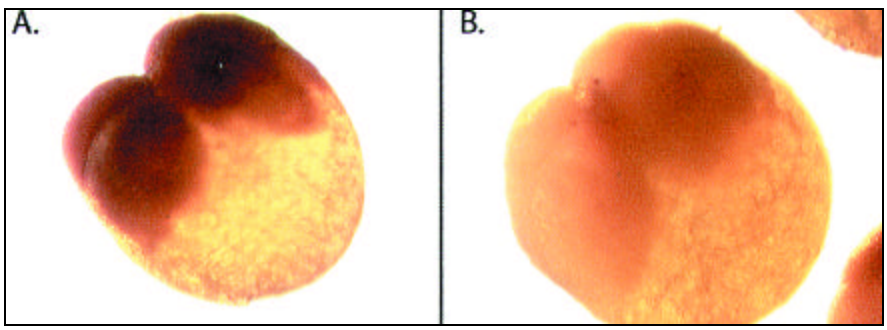


**Figure 2. Four cell zebrafish embryos de-chorionated and hybridized with a zebrafish-specific sequence.** Panel A is hybridized with the antisense strand, and panel B is hybridized with the sense strand.

used them in in situ hybridization experiments to ensure that they had enough integrity to be used for practical applications. The embryos were hybridized with RNA transcribed from a cloned, zebrafish-specific sequence that is expressed in early embyos (unpublished data). The RNA was labeled with digoxigenin, and hybridizations were carried out essentially as described by Westerfield (2) lacking post-hybridization RNase treatments. As shown in Figure 2, the embryos retained their integrity and showed appropriate staining having a positive signal with the antisense sequence but no signal and low background staining in embryos hybridized with the sense strand. Therefore, embryos de-chorionated in this fashion appear to be completely practical for use in in situ hybridizations and, by extension, other protocols as well.

We have described here a method to efficiently remove chorions from early-stage zebrafish embryos and have successfully used this technique to remove the chorions from unfertilized eggs, several cleavage stages, blastula and gastrula stage embryos fixed with a variety of fixatives including 4% paraformaldehyde (2), and Altmann's fixative (1). Very few embryos were damaged by the chorion removal, and they worked well in in situ hybridization experiments. The same procedure should be easily adaptable to a number of different types of embryos and/or stages just by varying the diameter of the tip of the glass needle. We have also tried this technique on live embryos, but it is less efficient, particularly for younger embryos (less than 12 h post-fertilization). Although the chorions are removed, the embryo tends to be damaged more often by the aspiration process.

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