Update to: Agarose-Embedded Tissue Arrays for Histologic and Genetic Analysis

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We have made some recent embellishments to the embedding technique and applied it to our work. The main improvement is that we have found several ways to prevent warping and deformation of occasional agarose blocks after processing. First, we avoid the use of "short" programs designed for small tissue samples, which is associated with warping of the blocks. Another way to minimize warping is to minimize the thickness of the agarose (we have recently been pouring between 8 and 9 mL, rather than our original 10 mL of agarose on our tetrad blocks), and to use a minimum amount of agarose to seal the embryos in the block. Finally, warping is also minimized by loading the tissue cassettes in the processor in a horizontal, rather than vertical position; this maximizes the exchange of processing solutions during the liquid exchange steps.

To generate transverse sections, single embryos can be placed in each well in a single direction. After sealing in the agarose block, single side-by-side rows of embryos can be excised and processed. The processed columns are then embedded in paraffin vertically before sectioning. We have recently shown that processing in agarose does not interfere with immunocytochemical analysis of tissue sections (data not shown). This will make possible screens based upon immunocytochemistry.

We have used embedded zebrafish arrays in a parthenogenetic half-tetrad screen for mutations that affect cell differentiation (unpublished observations). Some of the mutants had defects that are not detectable by dissection microscopy of living larvae. We believe that this application represents the first screen for mutations based upon histology.