HYBRIDOMA MAKING. There are several ways to make hybridomas, some using proprietary myeloma cell mutants. The most popular free technology uses a mouse plasma cell myeloma that has lost both rearranged H and L loci so it no longer makes antibody, and also lacks hypoxanthine-guanine phosphoribosyl transferase (HGPRT). There are two ways for a cell to make purines for nucleic acid synthesis, *de novo* and the salvage pathway. HGPRT is required for salvage, forcing the mutant myeloma cells to survive on *de novo* synthesis.

Spleen cells from an immunized mouse are fused to the myeloma cells with polyethylene glycol, and then placed in hypoxanthine-aminopterin-thymidine (HAT) containing growth medium. Aminopterin poisons *de novo* purine synthesis, so unfused myeloma cells die; but myeloma cells that have fused with a B cell get its HGPRT, and hybrids survive. Unfused B cells die after a few days, leaving only hybridomas. Once the desired clone is identified and isolated it is imperative to grow up a large batch and freeze aliquots, as the hybrid genome can be unstable, and anyway you'd hate to lose your hybridoma after all that work.