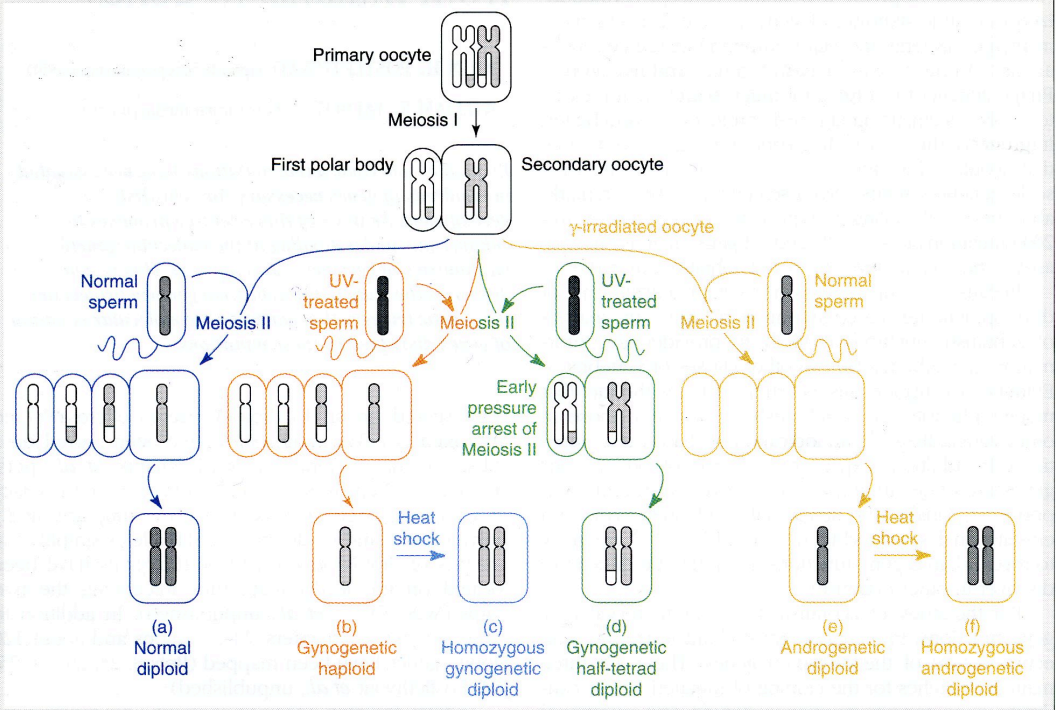
Box 1. Manipulating ploidy of the zebrafish genome



In vitro fertilization techniques can vary the ploidy and parental origin of genome sets in zebrafish, leading to six types of animals useful for genetic analysis. In a primary oocyte, crossing-over occurs in Meiosis I, leading to tetrads with recombinant chromatids. (a) Fertilization of secondary oocytes by normal sperm stimulates the completion of Meiosis II and formation of a normal diploid zygote. (b) Fertilizing secondary oocytes with sperm whose genome has been destroyed with ultraviolet (UV) light activates the egg, stimulates the completion of Meiosis II, and results in the development of a gynogenetic haploid zygote, which can develop into a haploid embryo. (c) Gynogenetic haploids are useful for first-generation mutant screens[2,11] and for the genetic mapping of recessive loci[29]. Haploid embryos develop remarkably normally, but have a specific developmental syndrome including a short body axis and a kinked neural tube; haploids die shortly after hatching, but long after many mutant phenotypes are apparent. Subjecting haploid zygotes to a two minute heat shock, 13 minutes after egg activation, suppresses the embryo's first mitotic cleavage division, leading to a gynogenetic diploid embryo. These animals are homozygous for maternal alleles at all loci. (d) Such embryos can give rise to clonal lines of fish. Whereas heat shock makes gynogenetic diploids that are homozygous for all loci, early pressure (EP) treatment produces gynogenetic half-tetrad diploid animals that are homozygous for only some loci. Freshly ovulated oocytes complete crossing-over and Meiosis I and then commence anaphase of Meiosis II about 90 seconds after fertilization. The application of high pressure at this time dissolves the spindle and suppresses Meiosis II, giving rise to animals that are homozygous for loci proximal to the first cross-over on a chromosome arm. A large number of half-tetrad animals develop normally, survive to sexual maturity and reproduce. Geneticists exploit these animals in first-generation mutant screens[10], in the mapping of loci to centromeres[32,34,46], in the analysis of chromosomal interference in recombination[46,47] and in the production of inbred lines[32]. In addition to these animals that have only maternal chromosomes, geneticists can produce animals that have only paternal chromosomes (e and f). γ-Ray irradiation of oviposited secondary oocytes destroys the maternal genome[60]. Fertilization of these γ-irradiated oocytes with normal sperm yields androgenetic haploid embryos (e). These embryos are useful for comparing rates of recombination in male and female meiosis. The application of heat shock to androgenetic haploids at the time of the first mitotic cleavage division results in homozygous androgenetic diploid embryos (f). Some of these animals survive to adulthood, and can be used to study the role of genetic imprinting in zebrafish. *The Zebrafish Book* details these and other methods[61]. (See Box 2 on page 189 for access to this information and other zebrafish resources over the World Wide Web.)

allow the identification of each individual chromosome and the generation of a standard karyotype (A. Amores, pers. commun.). These preparations should enable the cytogenetic mapping of cloned sequences by fluorescent *in situ* hybridization (FISH). An integrated cytogenetic and linkage map will be helpful for the cloning of zebrafish mutations.

Exploiting genetic tricks to map zebrafish mutations

A standard method to map a mutation in mouse is the genome scan, that is an analysis of 40 or more SSLPs spanning the mouse genome in 60–100 animals from an F_1 backcross or F_2 intercross[44]. This effort can require thousands of PCRs. Even more PCRs are required to utilize the genome scan strategy for mutant mapping in