Gamma Ray Mutagenesis

(Source: C. Walker)
Gamma rays are an effective mutagen for zebrafish embryos. -rays are known to produce many kinds of chromosomal damage such as small deletions, large deletions, chromosome inversions and rearrangements. We have used -rays to produce mutations in three developmental stages of zebrafish: sperm, eggs, and 3 hpf blastulae. In all cases, the mutagenized embryos are raised to adulthood and the germ line is screened for interesting new mutations. The specific site mutation frequency is very similar for all three stages

Egg: 4 x 10^{-5}/chromosome set/rad
Sperm: 4 x 10^{-5}/chromosome set/rad
Blastula: 1.2 x 10^{-5}/chromosome set/rad

Presently, we use -rays to mutagenize at the sperm stage and at the blastula stage.

-irradiated sperm
Sperm are pooled from a number of wild-type males into Hank's solution at the same concentration used for EMBRYO PRODUCTION BY IN VITRO FERTILIZATION (see page 2.14). Keeping the sperm on ice, the sperm is put into the chamber of a Gammatory-M irradiator with a cesium-137 source and given a dose of approximately 250-300 rads. The irradiated sperm is used to fertilize wild-type eggs. Approximately 25% of the embryos survive to adulthood.

The advantages of -irradiating sperm are:
1. The sperm does not have as much non-chromosomal material to be damaged by the -rays as eggs.
2. The mutation is present in 25%-50% of the germ line of the mutagenized female and, therefore, can be recognized and recovered easily.
3. -ray sperm do not tend to produce male adults as frequently as -ray embryos do.

-irradiated 3 hpf blastulae
Wild-type eggs are squeezed and fertilized by wild-type sperm. The time of fertilization is noted. At 120 minutes, the embryos are irradiated in a small glass vial at a dose of 250-300 rads. Batches of eggs fertilized 4 minutes earlier and later can be pooled before irradiation. Survival ranges between 25%-50%.

A high percentage of males develop from -irradiating blastulae. The percentage is linear with the dose (250-300 rads produces about 75-80% males).

Blastula stage embryos contain several germ line progenitor cells, from 1 to 10, providing more chromosome sets as targets for mutagenesis. The mutant clone size ranges from 5%-50% of the haploid germ cells.

We have found that embryos older than 3 hpf are considerably more resistant to -rays as a mutagen suggesting that there may be a repair mechanism at work after cleavage stages.

It is presently unknown whether -rays damage the sperm DNA in the same manner that they damage blastula DNA.