

## B. OVERVIEW OF GENOTYPING ASSAYS AT ZIRC

A number of genotyping strategies have been employed to genotype zebrafish lines. The following types of genotyping assays have been used at ZIRC:

1) An **RFLP** genotyping assay (**R**estriction **F**ragment **L**ength **P**olymorphism) is used to detect a mutation that either creates or abolishes a site recognized by a specific restriction enzyme (Botstein *et al.*, *Am. J. Hum. Genet.* 32: 314-331, 1980). In the RFLP assay, a sequence of interest is first PCR-amplified and then the PCR product is subjected to restriction enzyme digestion. The presence or absence of the mutation is determined by the resulting restriction pattern.

2) A **dCAPS** genotyping assay (**d**erived **C**leaved **A**mplified **P**olymorphic **S**equences) is used to detect a mutation that, together with one or more mismatches introduced into a PCR primer, creates or abolishes a restriction enzyme site in the PCR amplification product (Neff *et al.*, *The Plant Journal* 14(3): 387-392, 1998). The PCR product is subjected to restriction enzyme digestion and the presence or absence of the mutation is determined by the resulting restriction pattern.

3) An **ASA** genotyping assay (**A**llele-**S**pecific **A**mplification) involves designing a PCR primer set in such a way that the 3'-terminal nucleotide of one primer corresponds to the point mutation with respect to its location in the DNA and base pairing. This nucleotide therefore represents a mismatch for the WT template. Because a single mismatch is typically insufficient to achieve a desired level of discrimination, additional mismatches are introduced to further increase this discrimination. If sufficient discrimination is achieved, PCR amplification is detected only for samples carrying the mutation (Newton *et al.*, *Nucleic Acids Research* 17 (7): 2503-2516, 1989; Kwok *et al.*, *Nucleic Acids Research* 18 (4): 999-1005, 1990).

4) **SSLP** markers (z-markers) are used at ZIRC to genotype mutations that have not been cloned yet. Typically, two closely linked SSLP markers that flank the mutation are used for genotyping. These markers display interstrain polymorphisms for a genetic background on which the mutation was induced and a background to which the mutation is outcrossed. An individual fish is considered to carry the mutation if no recombination is detected for both of these markers and the PCR products are specific for the genetic background on which the mutation was originally induced. Whenever possible, the fish identified by SSLP genotyping are verified by phenotyping or other identification procedures to confirm that they are indeed carriers of the mutation.

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For sample preparation and other PCR related protocols, visit <http://zebrafish.org/zirc/documents/protocols.php>

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- 5) An **insertional mutation** is typically genotyped by a primer set designed in such a way that one primer anneals to the insert DNA causing the mutation, and the other primer hybridizes specifically to the genomic region flanking the mutation.
- 6) Transgenic lines for which genomic integration sites are unknown are typically genotyped with **transgene-specific primers**. It is possible that multiple copies of the transgene might have integrated into the genome during transgenesis and that some of these integrations are non-functional. Samples that contain only a non-functional transgene or its fragment will be identified falsely as positive in the genotyping assay. For this reason, it is recommended to use functional assays to verify individuals identified as positive in the genotyping assay.
- 7) Mutations caused by a deletion for which breakpoints have been molecularly characterized are typically genotyped by **deletion-flanking primers**. In this assay, a short PCR amplification product is detected for a DNA template containing the deletion. The PCR product for WT template is larger and will typically not be generated in PCR reactions in which a short elongation time is set for the Taq DNA polymerase.
- 8) Mutations caused by a deletion for which breakpoints have not been characterized are genotyped with genomic markers covered by the deletion. Individual samples obtained from single haploid embryos are typically analyzed in this assay. The absence of marker-specific PCR products demonstrates that the sample contains the deletion. An additional primer set is used as an internal positive control in this assay.
- 9) Mutant samples for a number of alleles are genotyped at ZIRC by **sequencing**. In this assay, a set of primers is designed to PCR-amplify a genomic DNA region containing the mutation. PCR product is then column-purified, combined with either a forward or reverse primer and subjected to sequencing.

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