

Efficient mutation identification in zebrafish by microarray capturing and next generation sequencing

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Abstract

Fish models like medaka, stickleback or zebrafish provide a valuable resource to study vertebrate genes. However, finding genetic variants e.g. mutations in the genome is still arduous. Here we used a combination of microarray capturing and next generation sequencing to identify the affected gene in the *mozartkugel*^{*p11cv*} (*mz*^{*p11cv*}) mutant zebrafish. We discovered a 31-bp deletion in *macf1* demonstrating the potential of this technique to efficiently isolate mutations in a vertebrate genome.

Research highlights

► The maternal-effect mutation *mozartkugel*^{*p11cv*} shows an embryonic polarity defect. ► The mutation localizes to a 770 kb interval on zebrafish chromosome 19. ► DNA capturing and next-generation sequencing identifies a genomic deletion. ► The mutation causes a premature STOP-codon deleting 2483 amino acids of Macf1.

Keywords: Zebrafish; Maternal effect mutation; Embryonic polarity; Embryogenesis; Genomics; Microarray capturing; Next generation sequencing; Microtubule actin crosslinking factor 1 (*macf1*)

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Fig. 1.

The *mozartkugel* (*mzl*) phenotype. Live embryos, 30 min after fertilization, lateral view. (A) The wild-type embryo (wt) forms a blastodisc representing a cap of clear cytoplasm at the animal pole (to the top). (B) The blastodisc is not formed in *mz^{p11cv}* mutants thus lacking animal-vegetal polarity.

Fig. 2.

The *mozartkugel* (*mzl*) locus. (A) Meiotic mapping located the *mz^{p11cv}* mutation on chromosome 19 between the markers z53477 (1.9 cM, centiMorgan; 9 recombinants/475 females) and S11b (0.2 cM; 1 recombinant/475 females). These two markers define a physical interval (red bar) of 0.77 Mb (33'676'248–34'443'857) in the Ensembl zebrafish genome database (http://www.ensembl.org/Danio_rerio; assembly Zv8). (B) Example of sequencing coverage from one Solexa run is indicated per base along the chromosome (Assembly Zv8). Note the low coverage in repeats not represented by probes on the microarray.

Fig. 3.

A genomic deletion is linked to the *mz^{p11cv}* mutation. (A) Zero sequence coverage of mutant DNA (red) indicates a deletion from position 34'405'729 to 34'405'760. Massive-parallel sequencing a +/– genome (blue) shows almost 50% reduction of coverage for the same interval. (B) PCR amplification of a 636 bp genomic fragment covering the deletion from two wild-type (+/+; TÜ), heterozygous (+/–) and *mz^{p11cv}* females (–/–). –Co indicates water control.

Fig. 4.

The *mz^{p11cv}* mutation affects the *macf1* gene. (A) Sequence comparison of wt (+/+) and mutant *macf1* gene (*mz^{p11cv}*). (B) The sequence difference is caused by a 31-bp (green) deletion in the mutant. The deletion generates a mutated amino acid sequence in *mz^{p11cv}* (red) causing a premature STOP in codon 5322 (asterisk). (C) Scheme of the Macf1 protein with a deletion of 2483 amino acids in the *mz^{p11cv}* mutant.

Supplementary data 1.

Supplementary data 2.

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