Identification of Genetically Modified Zebrafish (Danio rerio) by PCR method.

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Introduction

The import, sale and possession of fluorescent transgenic zebrafish offered under the name “GloFish®” in U.S. aquarium shops are not permitted in the European Union. Transgenic zebrafish are harbouring the gfp and/or dsRed genes coding for the green fluorescent protein GFP (originally isolated from the jellyfish Aequorea victoria), red fluorescent protein RFP (from marine sponge Discosoma sp) and also pigments which is a genetic mutant of green fluorescent protein.

In cooperation with Slovak inspectorate we have tested several samples of zebrafish (Danio rerio) which came from Slovak aquarium shops, where the screening controls have been performed.

Various methods of biological sampling were applied: invasive (small tail-clips) and non invasive (wipes from fish mouth on filter-paper or buccal swab) and non invasive (wipes from fish mouth on filter-paper) and the fish genomic DNA was isolated.

Several types of PCR have been performed to determine zebrafish DNA wild type and transgenic zebrafish:
1. to detect amplifiable genomic zebrafish DNA using primers specific for the zebrafish parvalbumin gene (IF232, IF233A and IF233B) and PARforR and PARRev;
2. PCR with primers to specifically amplify the gfp gene – (GFP1F, GFP1R);
3. PCR with primers to specifically amplify the dsRed gene (RFP2206, RFP2207);
4. PCR with primers to specifically amplify the GFP gene (YFP205, YFP206);
5. PCR with primers to specifically amplify transgenic elements NPTII (kanamycin/neomycin antibiotic resistance selectable marker) and the ancestral plasmid – pUC18 (NPTII, pUC18).

Methods

Sampling of biological material

Various methods of biological sampling were applied and tested: invasive (small tail-clips) and non invasive (wipes from fish mouth on filter-paper or buccal swab) and non invasive (wipes from fish mouth on filter-paper). The tail-clips were preserved in ethanol; the samples on filter paper or cotton swab were usable for DNA isolation up to 48 h.

Genomic DNA extraction and PCR

The samples of tail clips were collected by centrifugation, ethanol was removed and samples were dried at 37 °C. Fish genomic DNA from tail clips and from cotton swab or filter paper were isolated by using QIAamp DNA Investigator Kit (Qiagen) and ForensicGEM™ (Zygem, New Zealand). The samples from filter paper or cotton swab were isolated by using QIAamp DNA Investigator Kit and samples were dried at 37 °C.

Various methods of fish genomic DNA isolation from tail-clips and from non invasive wipe sampling techniques were usable and very convenient to use them in terrain by GMO inspectors and DNA can be isolated up to 48h.

In GMO laboratory (Slovakia) we have tested 10 samples of Zebrafish: 5 with tail clips (QI) and 5 – with non invasive wipe sampling techniques (chelex), (zygem) and also yellow fluorescent protein YFP was not detected.

Primers were designed by GMO laboratory (Slovakia) specific for Danio rerio (http://www.gm-inspectorate.gov.uk/documents/Detection_and_traceability_report_011205.pdf)

We have received the same positive results as were detected for transgenic zebrafish in Germany, which contained two events, one containing gfp and the other containing dsRed. No single vector in the literature is known to contain both these genes.

RESULTS

1. The control for amplifiable DNA using primers specific for the zebrafish parvalbumin gene: IFF232, IFF233A and IFF233B. The control for amplifiable isolated DNA was tested by PCR to detect amplifiable genomic DNA using primers specific for the zebrafish parvalbumin gene (IF232, IF233A and IF233B). The DNA was isolated from wt Zebrafish.

2. The control for amplifiable DNA using primers specific for Danio rerio parvalbumin gene PARfor1, PARfor 2 and PARrev (designed by GMO laboratory, Slovakia) and specific for the fish parvalbumin gene: IFF232, IFF233A and IFF233B and TubDAnioF, TubDAnioR.

3. The PCR detection of green (GFP1F,GFP1R) and red (RFP2206, RFP2207) fluorescent protein genes and detection of flanking region (NPTII, pUC18) in samples of Danio rerio designated as DR01 – DR10.

4. Result of the PCR detection in samples of Danio rerio DR01–DR10 for parvalbumin gene (IFF, PAR), green (GFP1F,GFP1R) and red (RFP2206, RFP2207) fluorescent protein genes and detection of flanking region (NPTII, pUC18).

CONCLUSION

In GMO laboratory (Slovakia) we have tested 10 samples of Zebrafish (Danio rerio) designated as DR01 – DR10, which were presumed to be genetically modified.

We verified
• various methods of biological sampling - invasive and non invasive, three methods of fish genomic DNA isolation
• we have tested new PCR primers (TudanioF, TudanioR)

We have received the same positive results as were detected for transgenic zebrafish in Germany, which contained two events, one containing gfp and the other containing dsRed. No single vector in the literature is known to contain both these genes.