Method for isolation of PCR-ready genomic DNA from zebrafish tissues

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Here we describe a method for the isolation of PCR-ready genomic DNA from various zebrafish tissues that is based on a previously published murine protocol. The DNA solutions are of sufficient quality to allow PCR detection of transgenes from all commonly used zebrafish tissues. In sperm, transgene amplification was successful even when diluted 1000-fold, allowing easy identification of transgenic founders. Given its speed and low cost, we anticipate that the adoption of this method will streamline DNA isolation for zebrafish research.

The zebrafish is a powerful model system in part because of the ease and speed with which transgenic and mutant animals can be generated and characterized. Identification of transgenics or mapping of mutants requires high-throughput DNA isolation for PCR-based genotyping. The zebrafish community at large uses DNA isolation protocols involving proteinase K digestion (zfin.org/zf_info/zfbook/chapt9/9.3.html). The incubation periods vary from 1 hour (1) to overnight with or without DNAzol (Invitrogen Carlsbad, CA, USA; zon.tchlab.org/protocoldna.htm) or resins, at a cost of at least 12 cents per sample (1). Previously, Truett et al. developed a simple method for the isolation of PCR-ready DNA from mouse tissues using hot sodium hydroxide and Tris (HotSHOT) (2). We report here the successful modification of this technique for use in zebrafish. This method achieves PCR-ready DNA in 10–20 min from multiple zebrafish sources including paraformaldehyde-fixed embryos, without the use of proteinase K, DNAzol, or resins, and has a reagent cost of <2.5 cents per sample. Furthermore, we demonstrate that this method generates DNA of sufficient quality to allow for the identification of transgenic males, even when the transgene is expressed in only a small subset of sperm.

The protocol is as follows. Tissues are placed into microcentrifuge tubes or thermal cycler plates containing 50 mM NaOH. The liquid volume should be sufficient to allow for complete submersion of the tissue. For sperm, we use 50 µL, for tails 100 µL, for single embryos/larvae 20–100 µL, and for eggs or multiple embryos/larvae 100–500 µL. The samples are then heated to 95°C until the tissue is noticeably friable. We have found 10 min to be sufficient for embryos and 20 min for paraformaldehyde-fixed embryos or adult tissues. The tubes are cooled to 4°C, and then 1/10th volume of 1 M Tris-HCl, pH 8.0, is added to neutralize the basic solution. The sample is centrifuged to pellet the debris, and the supernatant is immediately ready for use in PCR. One to five microliters solution are used per 25 µL PCR. The sample may be stored for at least 3 months at 4°C (see Figure 1, lane D) or longer at -20°C (data not shown) without compromised efficiency.

To demonstrate the efficiency of this protocol for the isolation of DNA from zebrafish tissues commonly used for genotyping, we isolated sperm, eggs, embryos, and tail from WIK zebrafish transgenic for enhanced green fluorescent protein (EGFP) driven the by p56^{lck} promoter (*lck*::EGFP) (3). DNA was prepared from the tissues as described above. One microliter solution was then used in a 25-µL PCR containing the following reagents at these concentrations: 200 nM each EGFP-specific primers (forward 5′-ATGGTGAGCAAGGGCGAGGA-3′ and reverse 5′-CGTCCTTGAAGA AGATGGTGCG-3′), 1×PCR buffer [10 mM Tris-HCl, pH 8.3, 50 mM KCl, 1.5 mM $MgCl_2$, 0.01% w/v gelatin, 0.01% v/v Tween 20, and 0.01% v/v Nonidet-P40 (NP-40)], 0.2 mM each

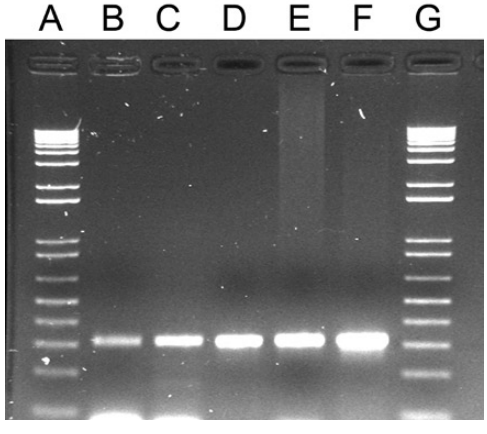

Figure 1. PCR amplification of enhanced green fluorescent protein (EGFP) transgene from zebrafish genomic DNA. DNA was isolated using the modified HotSHOT method. Lanes A and G, 1-kb plus DNA ladder (Invitrogen). DNA source for lanes B–F as follows: B, unfertilized eggs from single clutch; C, 3 h postfertilization embryos from single clutch; D, single 2 day postfertilization larva; E, sperm collected from a single male; F, tail clipping from single animal. PCR fragment size, 311 bp. The DNA sample used for lane D had been stored for 3 months at 4°C prior to PCR. The other samples were freshly isolated.

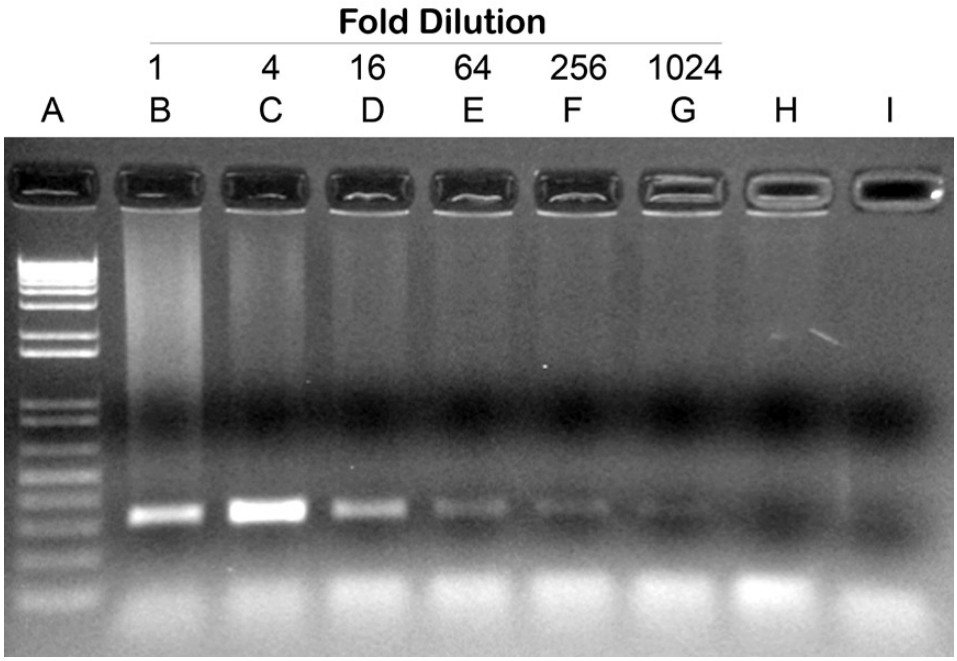

Figure 2. Transgene detection in serial dilutions. Sperm containing one copy each of transgene was serially diluted into wild-type sperm solution. Modified HotSHOT was used to isolate DNA from each dilution, and then PCR was performed for detection of transgene. Lane A, 1-kb plus DNA ladder. DNA source for lanes B–I as follows: B, undiluted transgene-containing sperm; C–G, successive 4-fold sperm dilutions (1×, 4×, 16×, 64×, 256×, and 1024×); H, wild-type DNA; I, water. PCR fragment size, 311 bp.

dATP, dCTP, dGTP, and dTTP, and 0.1 U *Taq* DNA polymerase. Reactions were amplified in a Model PTC-225 Peltier Thermal Cycler (MJ Research, San Francisco, CA, USA) using the following conditions: 95°C for 3 min; 35 cycles of 95°C for 30 s, 60°C for 30 s, and 72°C for 1 min; followed by 72°C for 10 min. Fifteen microliters each reaction were loaded onto a 1% SeaKem LE agarose gel (Cambrex, East Rutherford, NJ, USA) containing ethidium bromide and electrophoresed at 6 V/cm for 45 min in 1× Tris-borate-EDTA (TBE) buffer Tris 90 mM, Boric Acid 90 mM, EDTA 2 mM, pH 8.2 (Sigma-Aldritch, St. Louis, MO). The gel was imaged using a Bio-Rad Gel Doc 2000 apparatus and the Quantity One software (both from Bio-Rad Laboratories, Hercules, CA, USA) (Figure 1). A band matching the target size of 311 bp was present in each lane, indicating that the DNA isolated from each zebrafish tissue was of adequate quality for reliable PCR.

Transgenic zebrafish are generated by injecting DNA constructs into one-cell stage embryos that are then raised to adulthood. Random integration results in transgenic founders that are mosaic for the transgene, most notably in germ cells. The most efficient method for identifying founders is by PCR of genomic DNA isolated from sperm. Thus, DNA isolation methods must provide DNA of sufficient quality to allow for detection of the transgene when diluted in wild-type DNA. To demonstrate that the modified HotSHOT technique meets this requirement, we serially diluted sperm from males homozygous for the *lck*::EGFP transgene into wild-type sperm and then isolated DNA from the dilutions. Five microliters sperm from transgenic males and 10 µL sperm from wild-type Tübingen fish were added to 50 µL and 100 µL 50 mM NaOH, respectively. The solutions were briefly vortex mixed. The wild-type sperm solution was separated into 15-µL aliquots. Five microliters transgenic sperm solution were added to the first aliquot. Serial dilutions were then accomplished using 5 µL each dilution into the next aliquot, resulting in a 1:1024 final dilution. DNA was isolated in each tube as described above. Five microliters each solution were used in 25-µL PCRs as described above. The transgene was detectable even when diluted greater than 1000-fold (Figure 2), indicating that the modified HotSHOT method can be used to detect transgenic males carrying the transgene in as few as 0.1% sperm.

In conclusion, this method is rapid, reliable, and cost-efficient for the isolation of PCR-quality DNA from zebrafish tissues. The DNA solutions are stable at 4°C for at least 3 months and can be kept frozen at -20°C for longer storage. We anticipate that adoption of this protocol among the zebrafish community will speed genotyping, increasing the efficiency of the identification of transgenic animals, the mapping of mutants, and other PCR-based applications.

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COMPETING INTERESTS STATEMENT

The authors declare no competing interests.

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