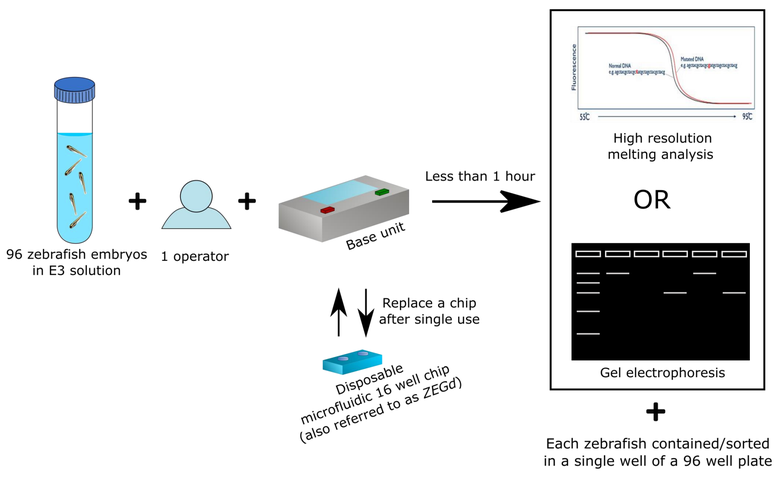
A Microfluidic Device for DNA Extraction from Live Zebrafish Embryos for Rapid Genotyping

Contact: info@wfluidx.com Website: www.wfluidx.com

Background

Zebrafish are extensively used by biomedical researchers as a model organism for determination of genetic and biochemical pathways, identification of basic biological mechanisms, and preclinical drug discovery. Genotyping is necessary for a variety of drug screens, for use with phenotyping, and developments in functional and knock-in/knock-out genomics. However, in most situations genotyping is constrained by manual procedures, which limits throughput and requires either sacrifice of the animal or waiting until they are several months of age. Our goal was to develop a device that could be automated to rapidly, accurately, and efficiently collect genetic material sufficient for genotyping from live zebrafish embryos.

Methods

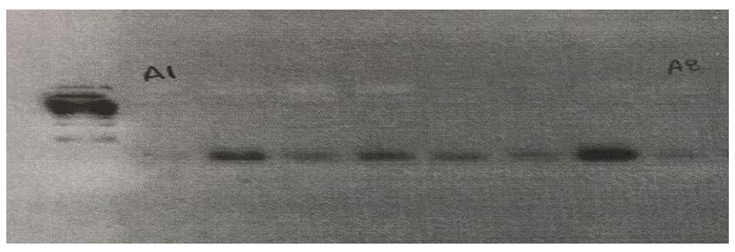


Current Protocol

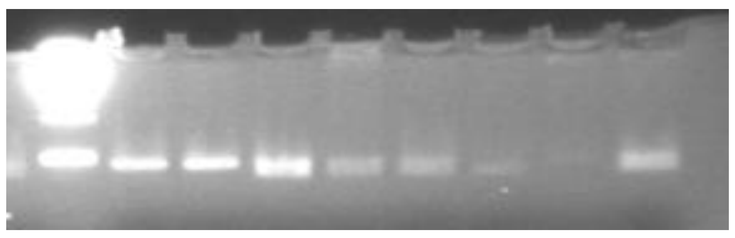
1. Load chip into base unit
2. Load embryos (72hpf) to chip chamber
   Use standard pipette + wide bore tip
3. Run cell extraction protocol
   10 minutes (automated)
4. Collect cell samples
   Use standard pipette
5. Relocate fish to well plate
   Use disposable transfer pipette

The extracted samples are now ready for PCR and do not require DNA isolation. Following PCR, the product can be analyzed using gel electrophoresis or high resolution melt analysis (HRMA). HRMA is a new technology used to identify variations in DNA sequences as low as a single nucleotide. This is done by detecting minute differences in DNA denaturation at specific temperatures.

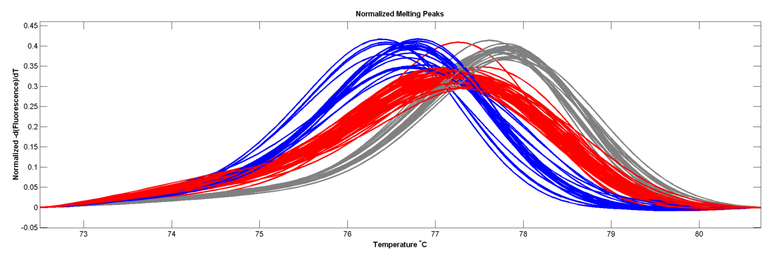
Results: Genotyping



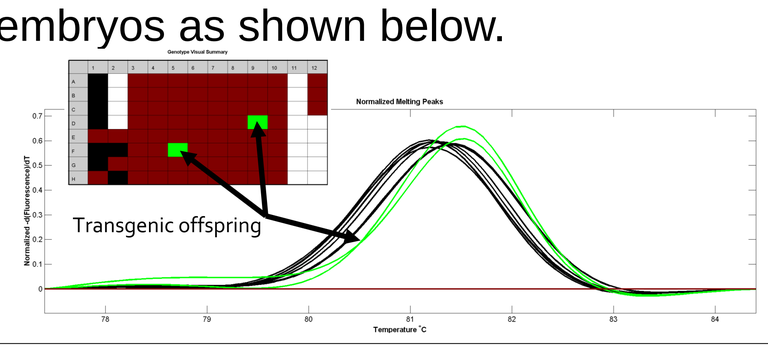
Genotyping can be completed using gel electrophoresis or HRMA. The gel shown above was completed using DNA samples generated by the ZEG device on abcd1 splice variant (SNP) Zebrafish Embryos. The gel below was completed using 72hpf embryos (abcd1-zc90).



Alternatively, HRMA can be used in conjunction with the ZEG device to genotype live zebrafish embryos. The HRMA plot below demonstrates the capability to genotype samples taken from live 72hpf zebrafish embryos.



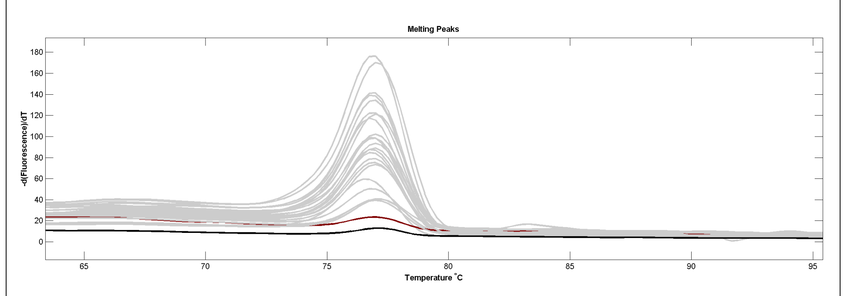
This enabling technology has shown particular value in screening for founder transgenic fish. Rare transgenic offspring can be determined at the embryonic stage without sacrificing embryos as shown below.



Results: High Throughput

DNA was extracted from 504 live embryos in 3 hours and 28 minutes by a team of 6 people. The total time including genotyping using HRMA was 4 hours and 39 minutes. The genotyping sensitivity was 72% and embryo survival was 87%. The technology has since evolved and should a similar experiment be repeated, we expect DNA extraction from 500 live embryos could be done by a single user in less than 8 hours.

Results: Single User



Current prototype system is capable of extracting DNA from 24 live embryos in less than 20 minutes with >95% sensitivity and 100% embryo survival. The HRMA plot above shows melting peaks for embryos (abcd1-sa509) processed simultaneously on a single chip by a single user.

Conclusions

This device shows great promise as an alternative to current methods of DNA collection from zebrafish embryos. DNA samples collected from live zebrafish embryos using this technology can be analyzed using PCR with standard gel electrophoresis or HRMA protocols resulting in both high sensitivity (>90%) and embryo survival (>90%). The current technology is capable of collecting DNA from 24 live embryos in less than 20 minutes with a single user. Ongoing research shows higher throughput is possible.

Future Directions

Ongoing research and future work aims to further refine the instrument's capacity for higher throughput, application in other species, automation, and integration with PCR technology. We envision an instrument capable receiving live zebrafish embryos which then automatically extracts DNA, performs PCR, and outputs embryos with correlated samples for each live embryo at a rate of 96 fish per hour. Such an instrument would be an enabling technology for expediting zebrafish research involving genotyping.

Acknowledgements

We are very grateful for and extend our appreciation to Bonkowsky Lab and Gale Lab at the University of Utah. This research was supported by funding from National Institute of Health, USA (Grant no. 1R43OD023027-01).