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Tammy Stevenson, Bonkowsky Lab
Raheel Samuel and Chris Lambert, WFluidx

wFluidx
know your fish

The ZEG: A New Device For Rapid Live Zebrafish Embryo Genotyping

https://www.wfluidx.com/

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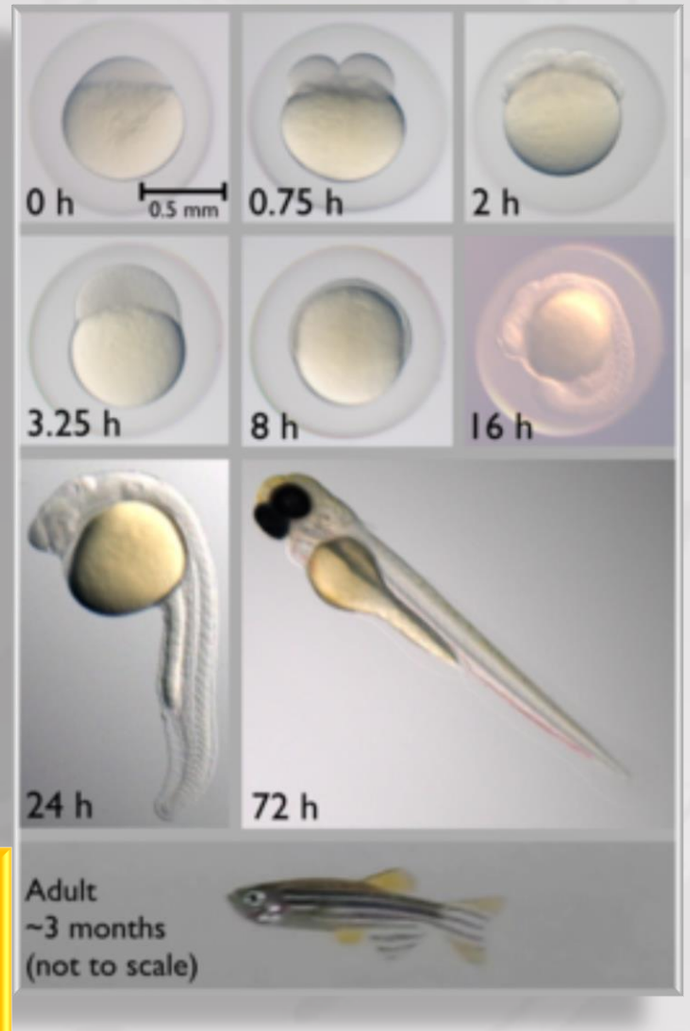
Zebrafish: of course!



Zebrafish may be the next ally in the fight against opioid addiction, U. study show

Septem

Artificial neural networks could be used to provide insight into biological

July 2019, Har

Zebrafish show true colors as models for autism sleep studies

July 2019, Nature "Neural signatures of sleep in zebrafish."

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So many lines, so little time

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Zebrafish International Resource Center (ZIRC)

Browse **38702** Zebrafish Lines at ZIRC

CRISPR/Cas9 technology –
increased number of lines without fluorescent markers for screening

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Genotyping: Fin Clip

3 Months

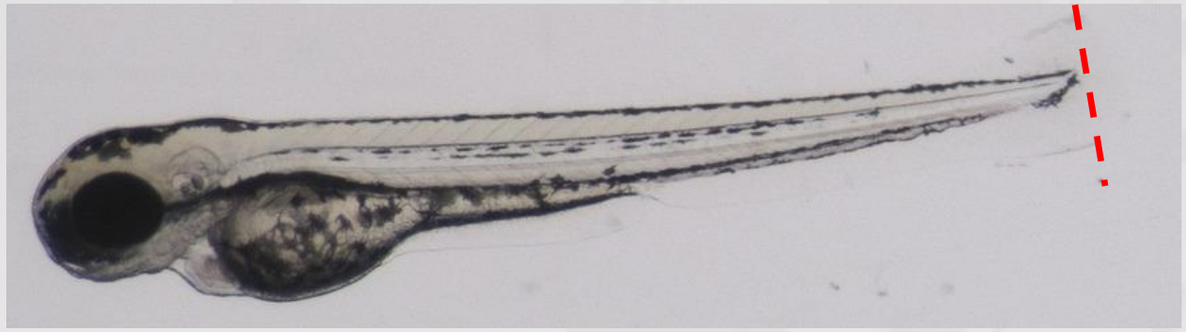
64 mm

Disadvantages of Current Methods:

Adult fin clip

- Must raise fish to at least 6 weeks
- Nursery space and costs
- Time-consuming
- Risk to fish health – invasive procedure

72 Hours



1.9mm

Larval fin clip

- More technically challenging
- Time consuming
- Risk to fish health – invasive procedure
- Potential to alter swimming behavior, immune response, and more
- Can do on fixed larvae; also time-consuming

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A Microfluidic Approach to Genetic Material Extraction

Bruce Gale lab
(Engineering)

Josh Bonkowsky lab
(Pediatric Neurology)

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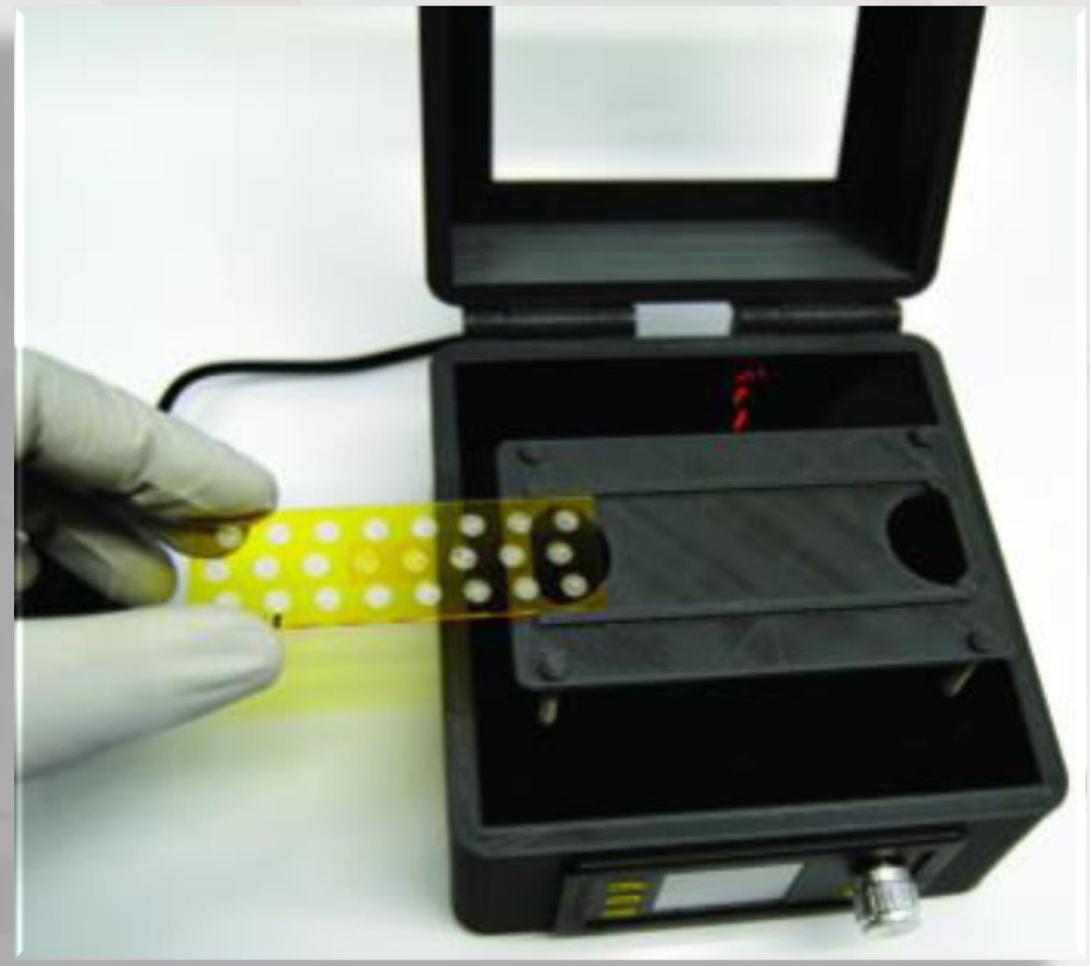
ZEG Microfluidic System

- Fast: 96 Embryos per hour
- 24 hpf – 7 dpf
- Minimal Training
- Simple to Use
- Non-invasive
- >90% Sensitivity
- >95% Survival



Chris Lambert, Raheel Samuel, Arlen Chung – Gale lab

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Benefits of live embryo genotyping with the ZEG

1. Non-destructive isolation of genetic material
2. Faster and easier than adult or embryo fin clipping, saving personnel time and costs
3. Raise only the fish you really want, decreasing nursery costs and saving tank space in CZAR nursery
   - Screen for homozygous mutant fish
   - Screen crispants and other embryos without fluorescent markers
   - Screen gal4 lines
4. Genotype fish prior to doing experiments, and get useful data from every fish (e.g. behavior, Westerns, antibody staining, *in situ*, and more)

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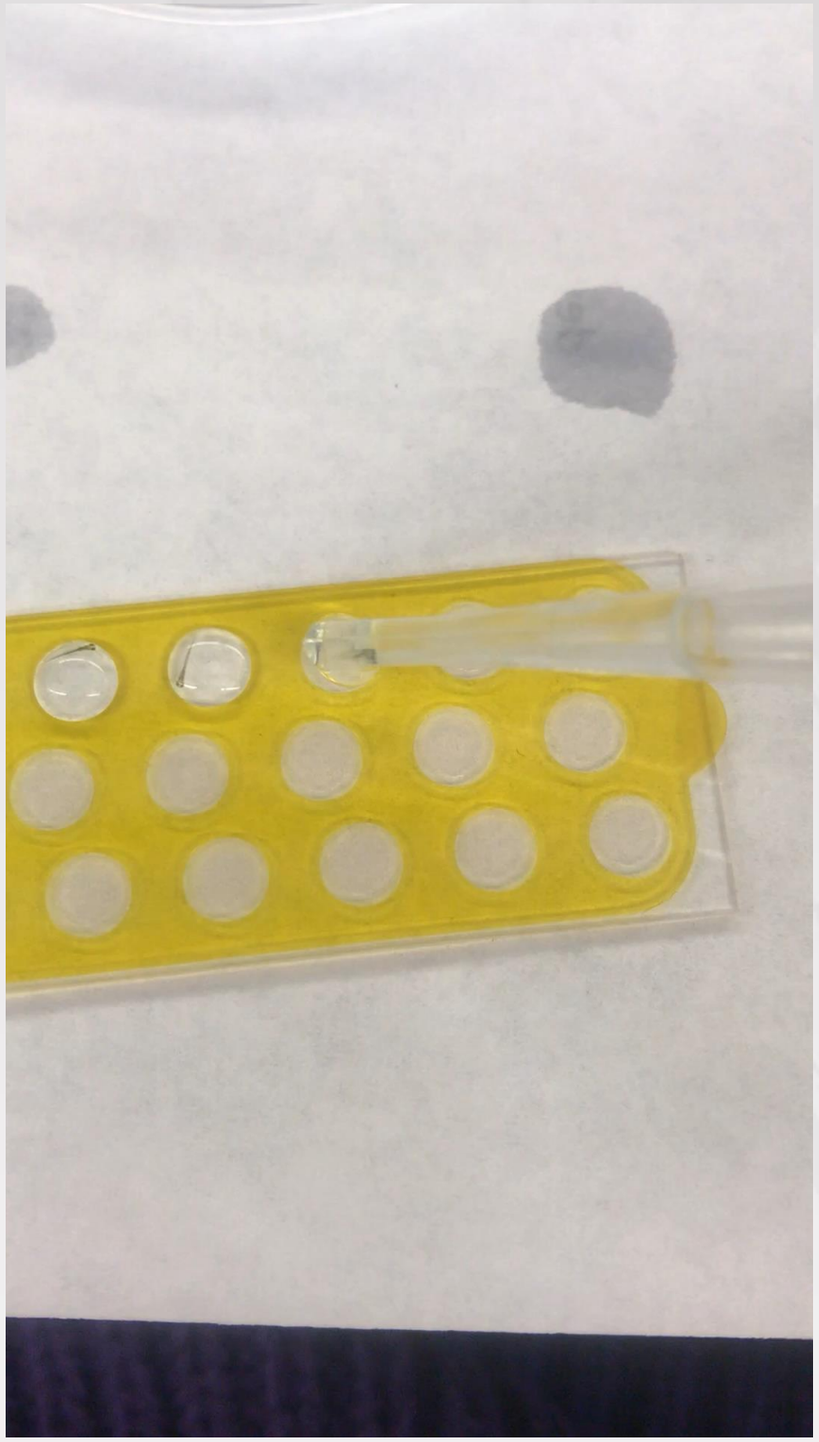
ZEG: Protocol



1. Load 24 embryos into chip chambers
    - P20 with wide bore tip; in12 µL E3
    - ~3 minutes
2. Run genetic material (GM) extraction protocol
    - 7 minutes
    - Load next chip while it runs
3. Collect GM samples for PCR
    - ~3 minutes; yield 10-12 µL E3
    - 8 channel pipette
4. Transfer fish to 96-well plate
    - ~3 minutes

Lambert CJ, Freshner BC, Chung A, Stevenson TJ, Bowles DM, Samuel R, et al. (2018) An automated system for rapid cellular extraction from live zebrafish embryos and larvae: Development and application to genotyping. PLoS ONE 13(3): e0193180. https://doi.org/10.1371/journal.pone.0193180

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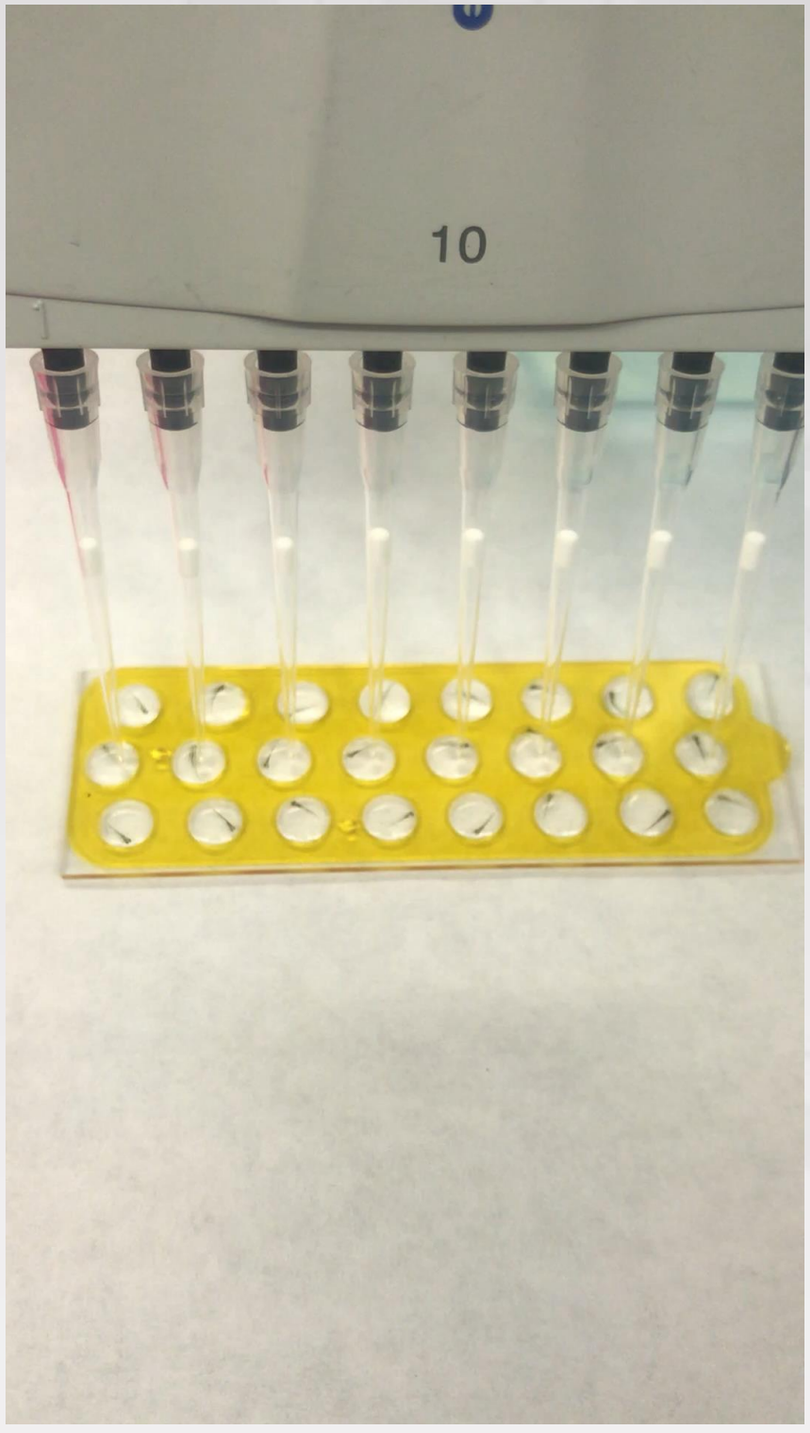

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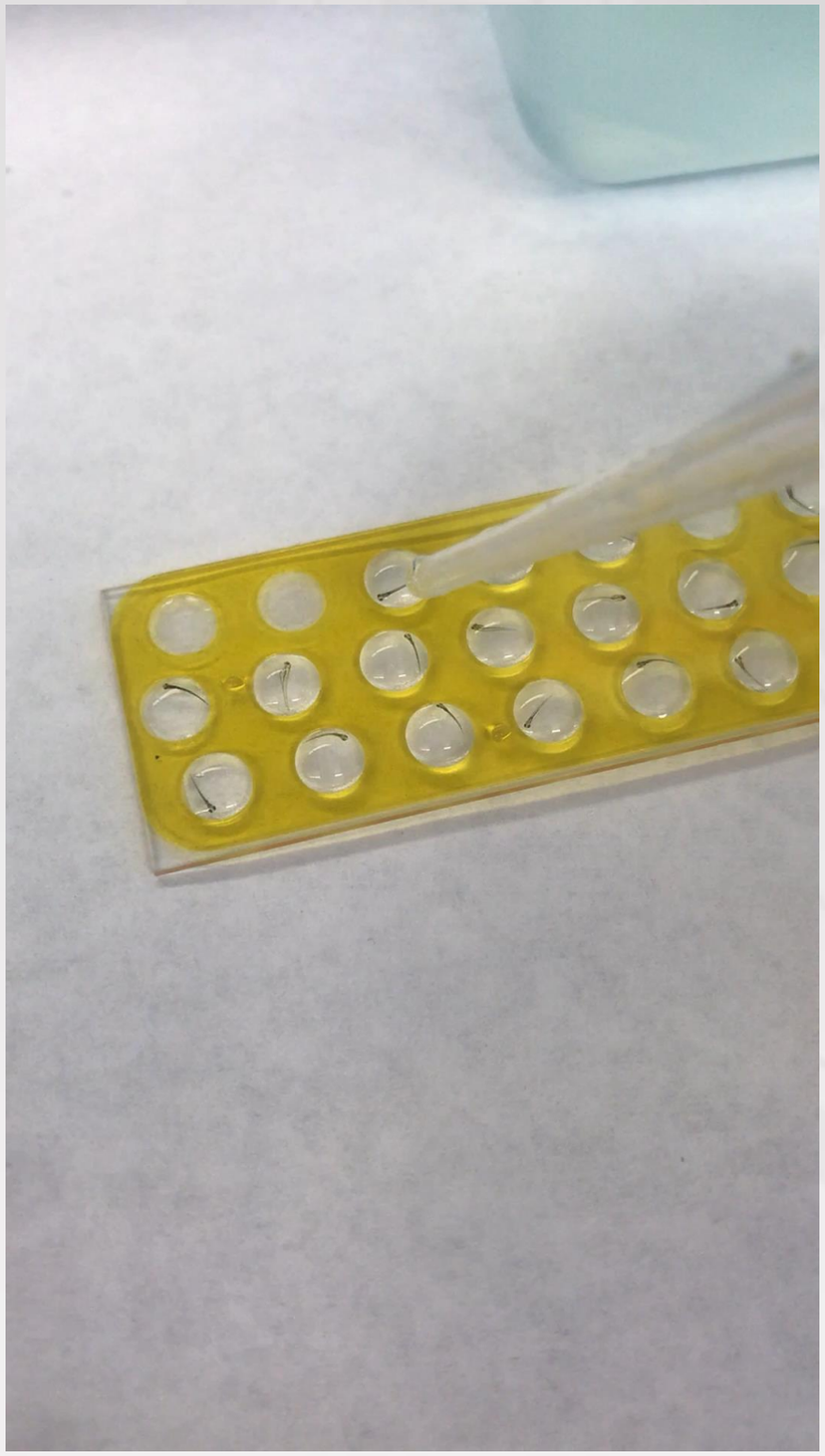

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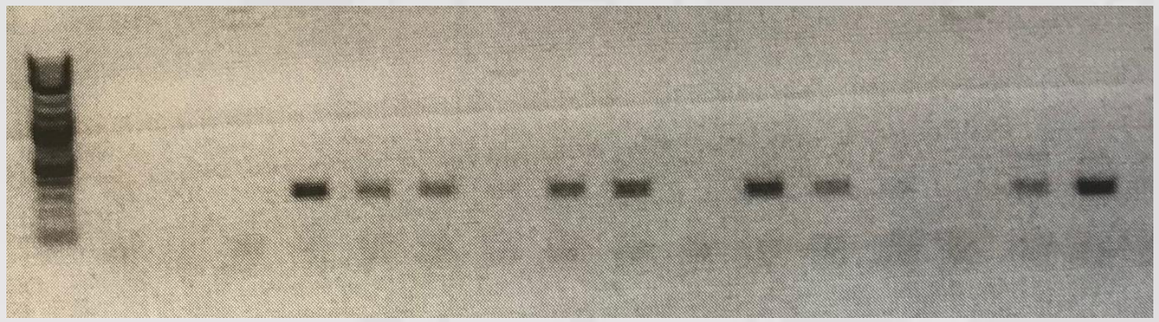
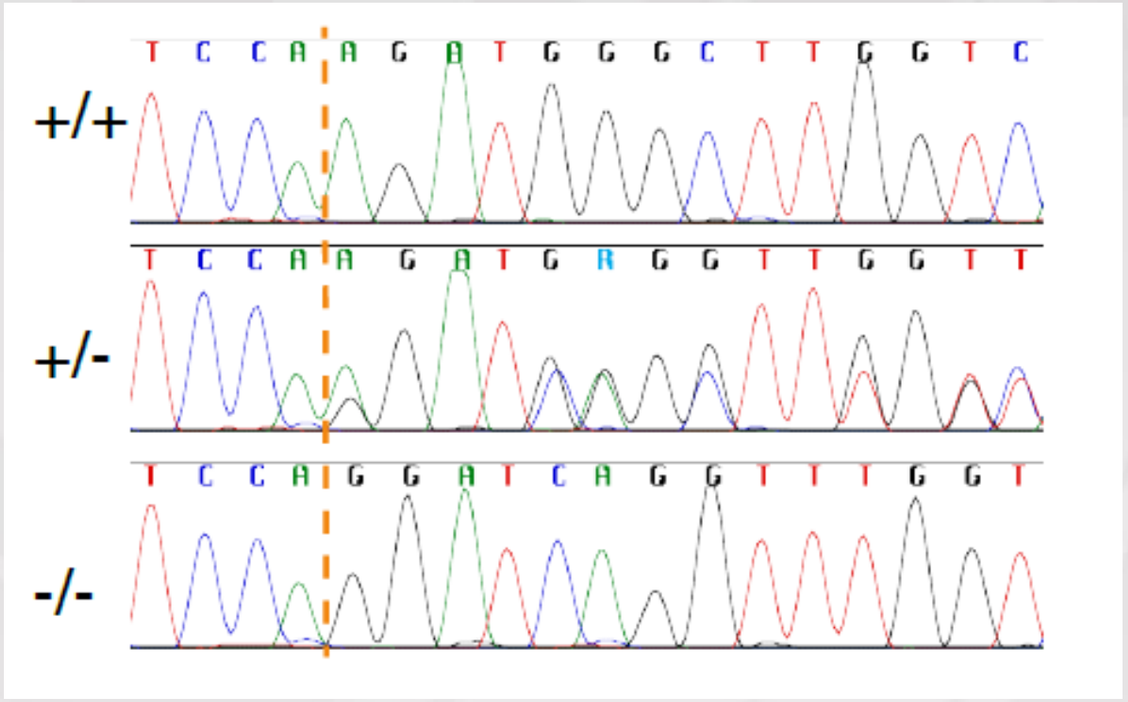
Downstream Applications

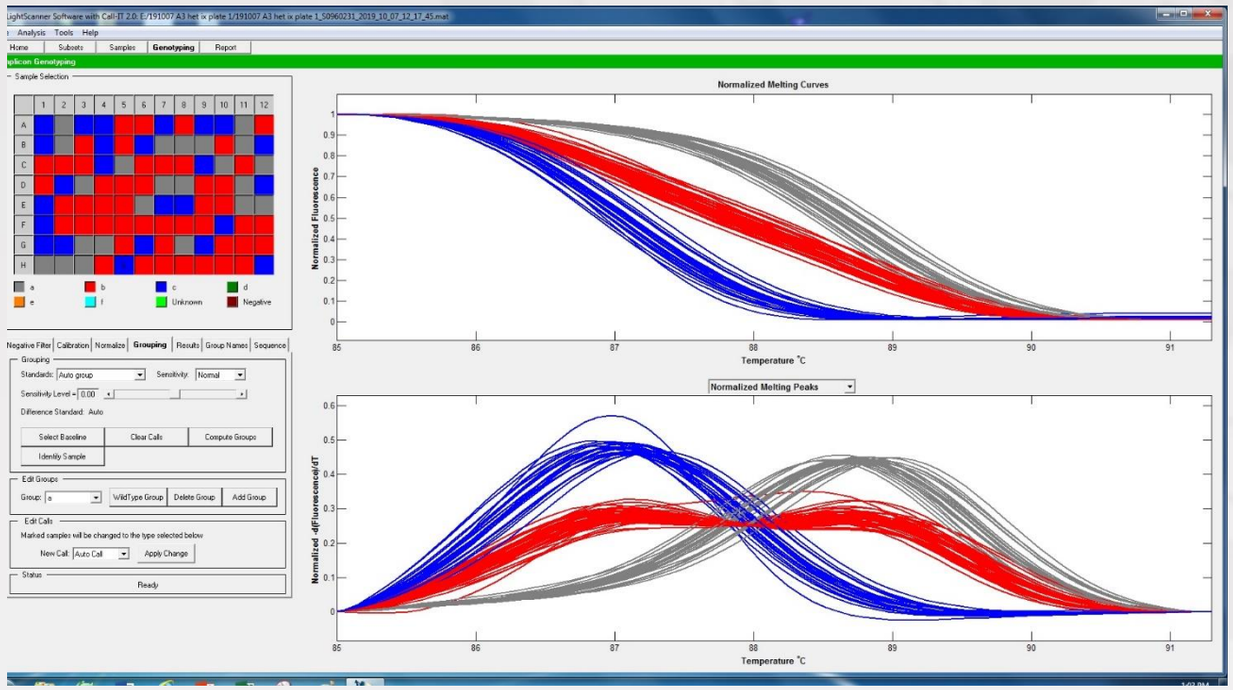
PCR followed by:



Gel electrophoresis



Sanger sequencing



HRMA

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Results reported in our paper

RESEARCH ARTICLE

An automated system for rapid cellular extraction from live zebrafish embryos and larvae: Development and application to genotyping

Christopher J. Lambert[1], Briana C. Freshner[2], Arlen Chung[1], Tamara J. Stevenson[2], D. Miranda Bowles[2], Raheel Samuel[1,3‡], Bruce K. Gale[1,3‡], Joshua L. Bonkowsky[2‡*]

1 Department of Mechanical Engineering, University of Utah, Salt Lake City, Utah, United States of America, **2** Department of Pediatrics, University of Utah School of Medicine, Salt Lake City, Utah, United States of America, **3** Nanonc Inc., Salt Lake City, Utah, United States of America

Table 1. Results comparing chip and device designs. Testing results comparing two different micro-abrasion chip and device designs.

Test Description	Sensitivity	Survival	n (embryos)
Chip—20uL PDMS Chamber on Roughened Glass; Processed on Shaker Plate for 10 min at 100rpm;	51%	81%	96
Chip—Hydrophobic layer on Roughened Glass; Processed on Coin Vibration Motor System for 10 min	94%	94%	>200

https://doi.org/10.1371/journal.pone.0193180.t001

- 94% survival
- 94% sensitivity of PCR
- n > 200 embryos

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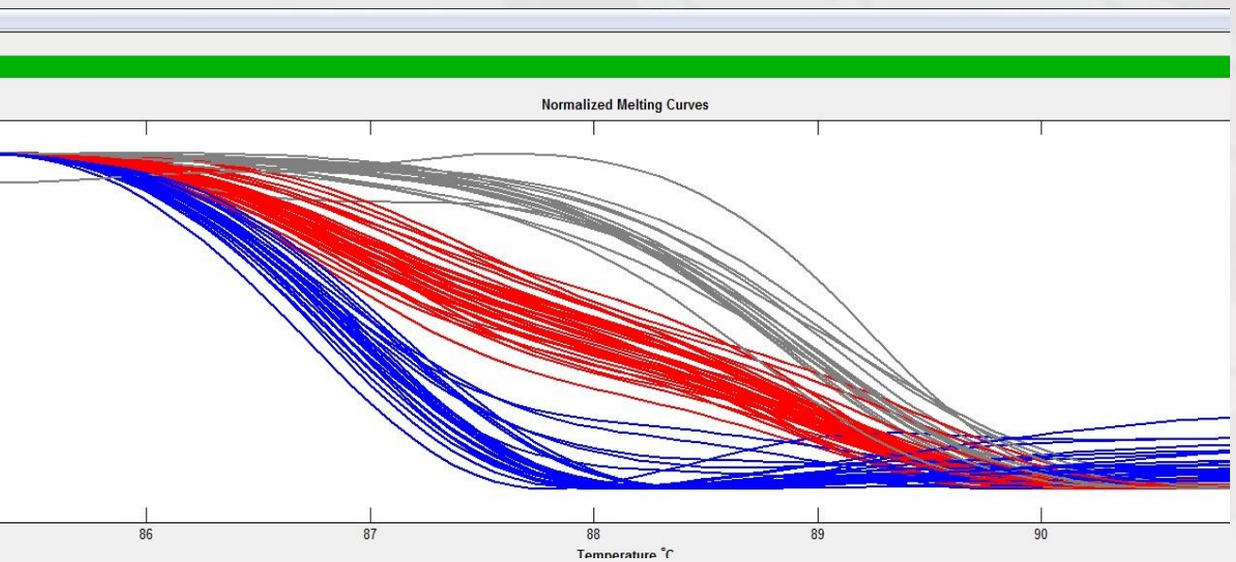
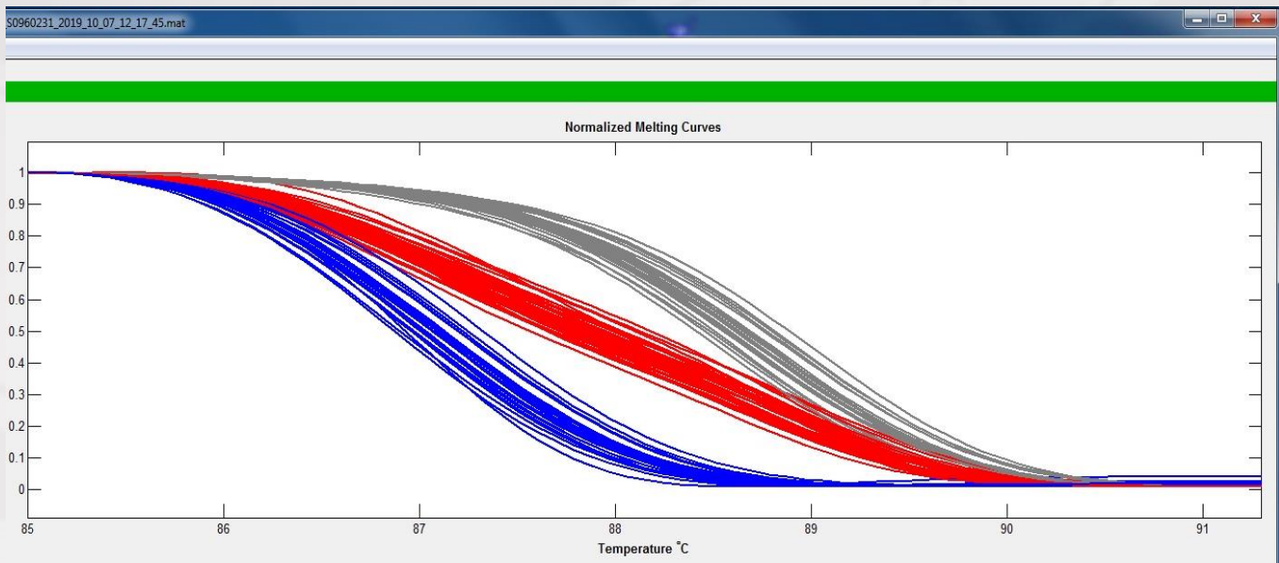
Typical Results

(in my hands, last 6 months)

- >95% survival for 48hpf-7dpf
- 95.8% PCR success
- n=742
- 40 cycles of PCR is best

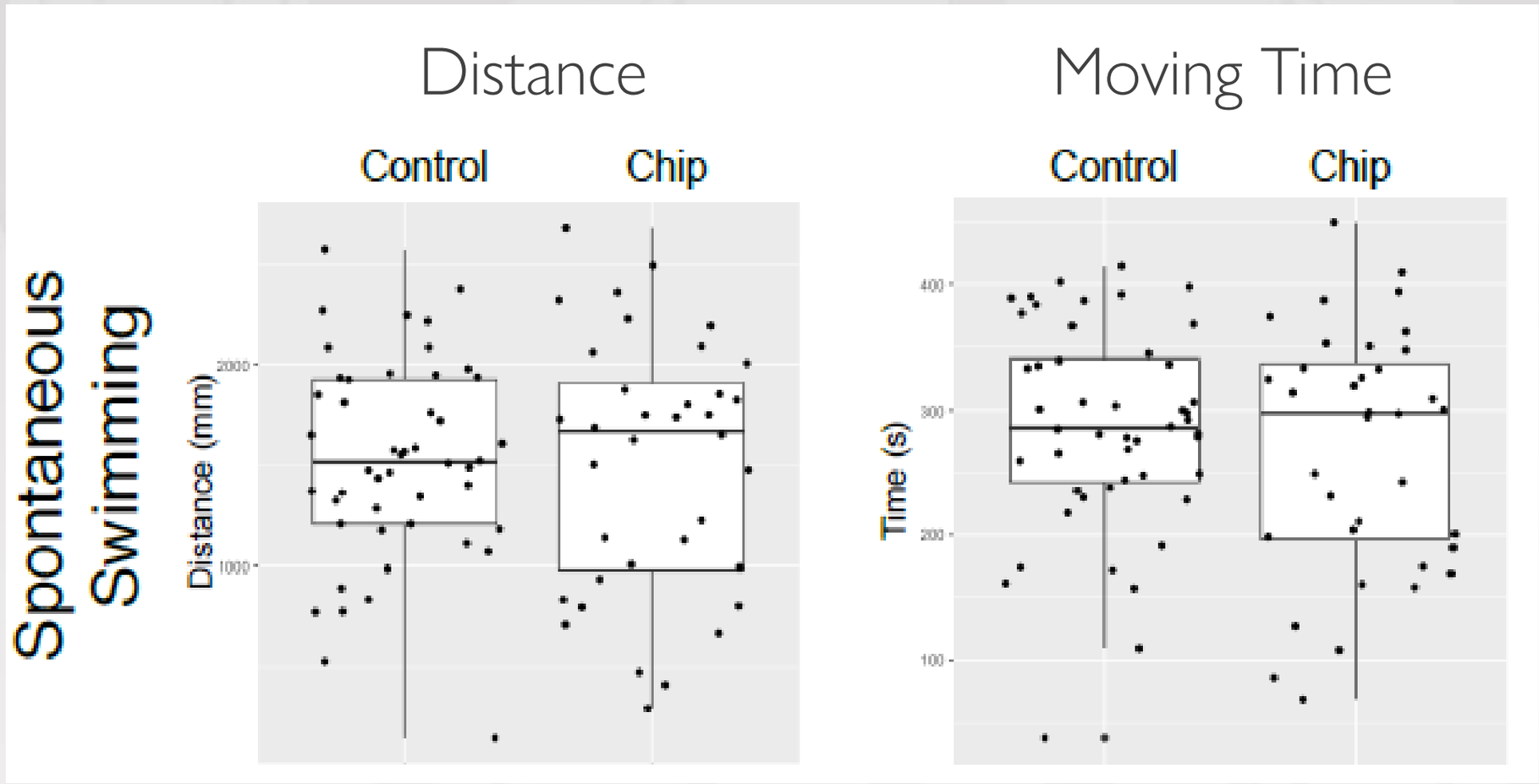


30 cycles



40 cycles

Behavior Studies



No apparent effects on body morphology or motor behavior at 7 dpf or on long-term growth, survival, and fertility at 90 dpf

Conclusions

- The ZEG device is a device for rapidly obtaining DNA from individual live zebrafish embryos for genotyping.
- Enables early stage identification of mutants expanding research potential and reducing husbandry costs.
- High PCR success rates; many downstream applications
- 100% correlation between ZEG genotypes and whole embryo genotypes.
- Visit www.wFluidx.com for more information or to purchase the device and/or chips

Future research

- Improved yield of genetic material
- Automation – robotic loading and unloading with Opentrons
- 96-well format instead of 24-well

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Acknowledgements

Gale lab

- Bruce Gale
- Chris Lambert
- Raheel Samuel
- Arlen Chung

Bonkowsky lab

- Josh Bonkowsky
- Briana Freshner
- Miranda Bowles
- Regan Stephenson

Thank you