Rapid genotyping of live zebrafish embryos using the Zebrafish Embryo Genotyper device (ZEG)

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Zebrafish Facility Management Workshop, Lisbon, 2018

Ghent Zebrafish Facility

- 6 “semi-closed” recirculating systems (ZebTEC and WTU systems, Tecniplast)
- Housing of 17,000 zebrafish
- ‘Danio Data’ (Fulcrum) zebrafish husbandry management software
- Rotifer-polyculture (Best *et al*., 2010), Micro-artemia (Ocean Nutrition) and dry food (Skretting)

Adult Zebrafish Genotyping

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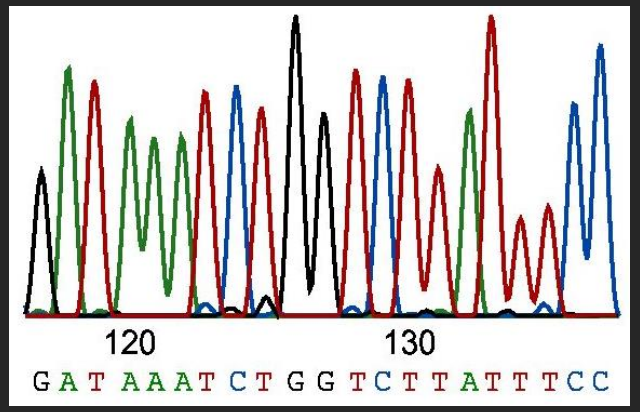
Finclipping of adult zebrafish

Raise fish to adult age

Manual fin clip

DNA extraction/Genotyping



- Requires **space, effort and expenses** of raising more adult fish than needed to ensure obtaining sufficient numbers of animals of the desired genotype
- Finclipping procedure is **time- and labor intensive**
- **Early identification of genotypes needed** in experiments dealing with larval phenotypes and early death or in experiments that require sample pooling

Larval Zebrafish Genotyping

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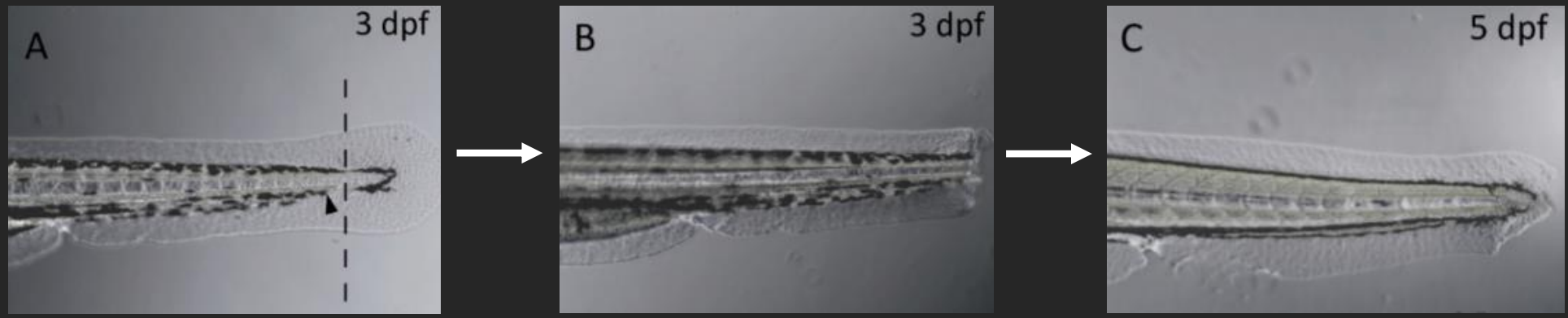
Kosuta *et al.,* 2018
Wilkinson *et al*., 2013

Larval fin clipping

Intact larval fin at 3dfp

Tail fin clipping at 3dp

Fin regrowth at 5dpf



- Sufficient amounts of DNA for **accurate genotyping**, **good survival rates**
- Several **critical steps within the protocol** must be correctly followed to obtain qualitative results (high-precision fin clip; tissue transfer to test tube must be assured)
- Finclipping procedure is **time- and labor intensive on a larger scale**

Larval Zebrafish Genotyping

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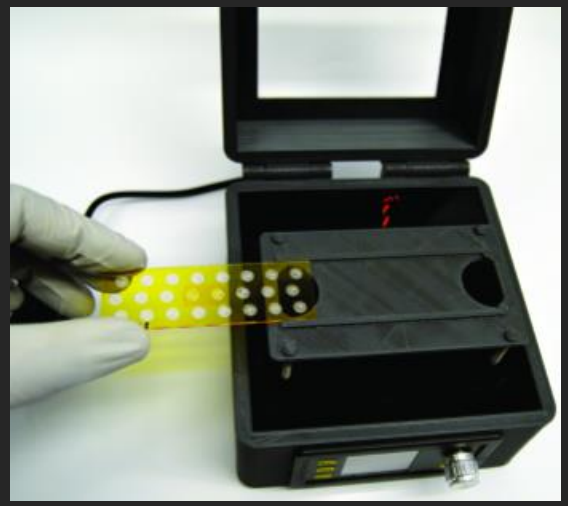
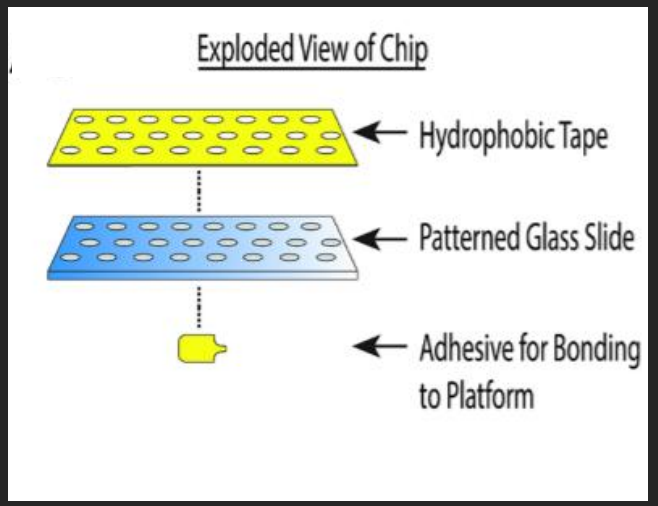
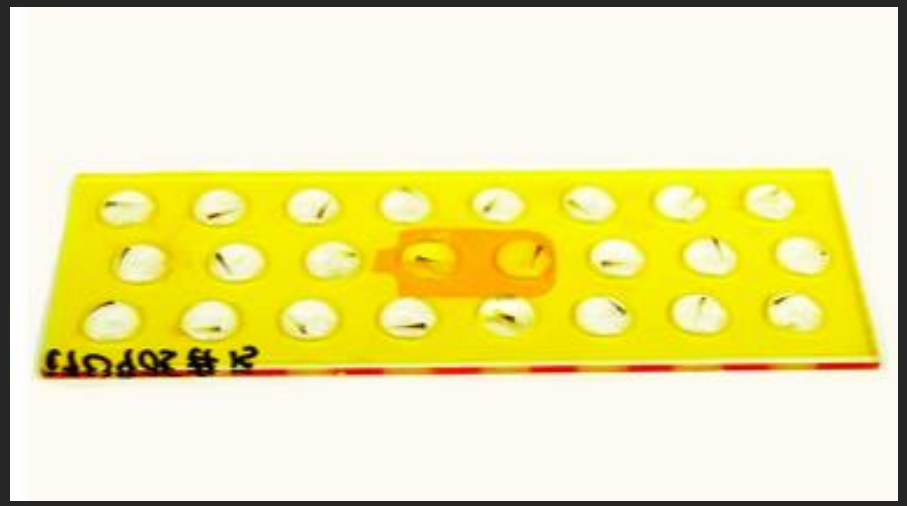
Zebrafish Embryo Genotyper (ZEG)







Lambert et al., 2018 (PlosOne)

Larval Zebrafish Genotyping

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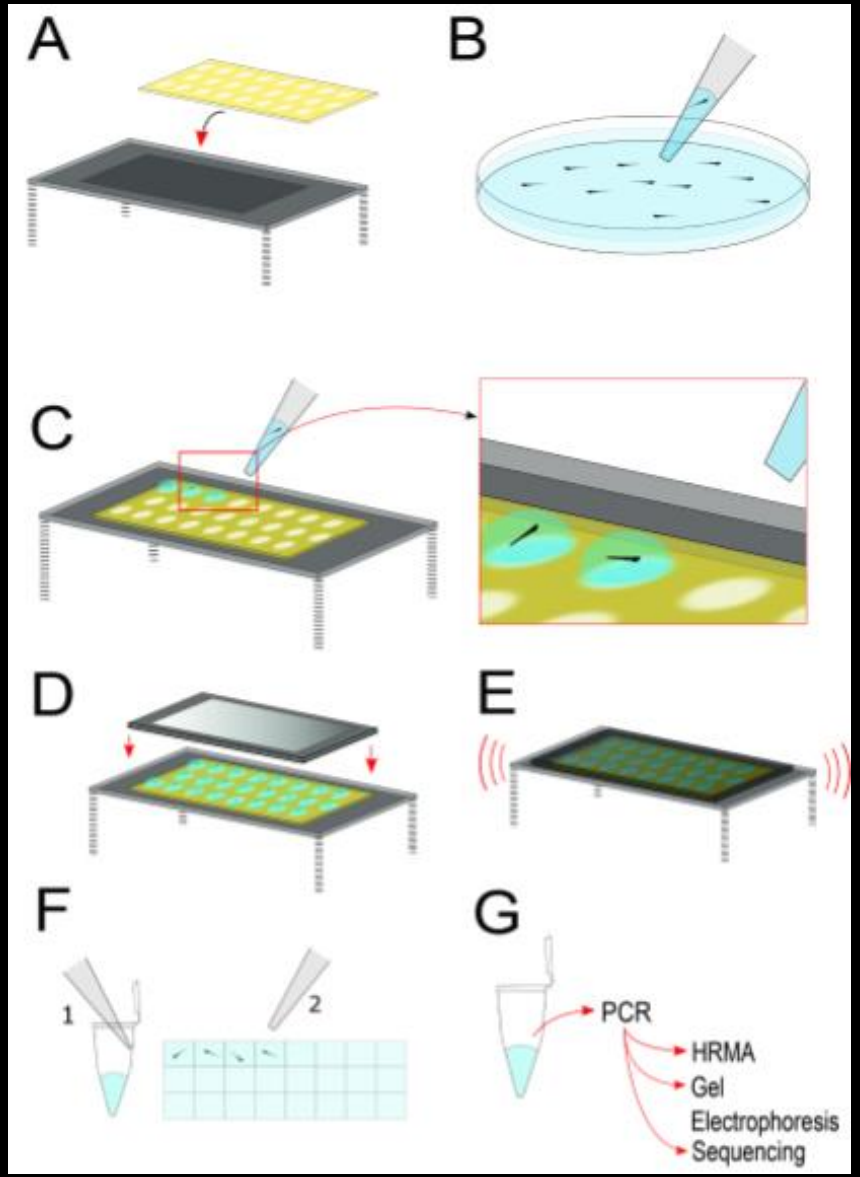
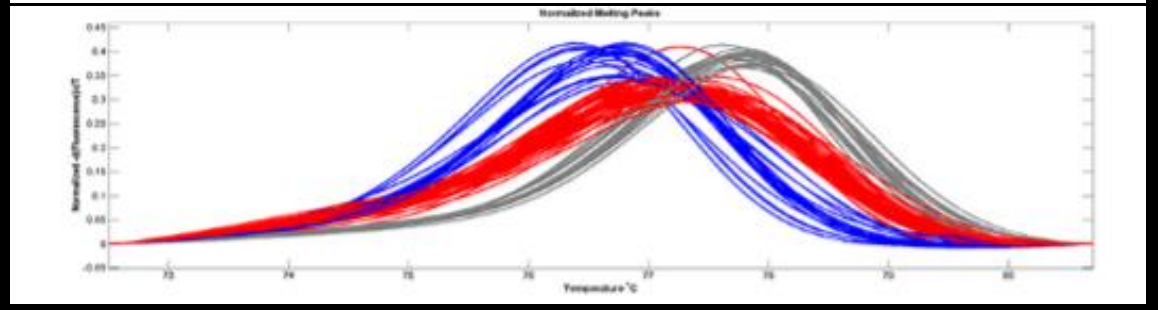
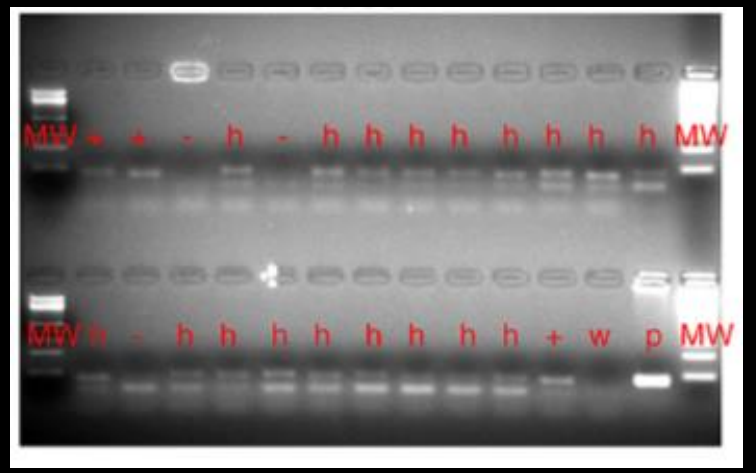
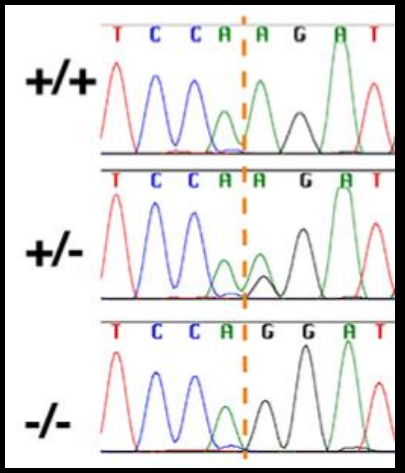
Genetic material extraction using the ZEG

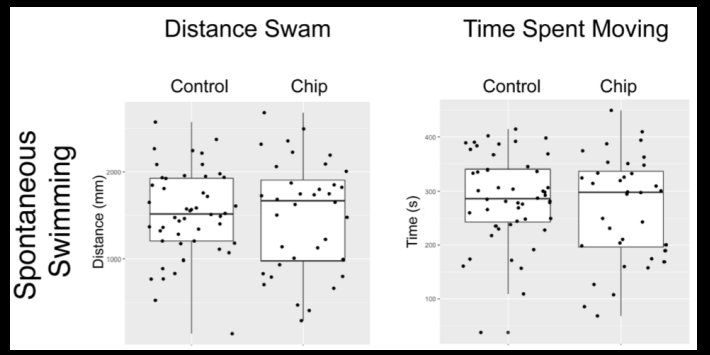


Genetic testing of DNA obtained from the ZEG









- No apparent effects on body morphology and motor behavior at 7dpf or long-term growth and survival (>90%)

Lambert et al., 2018 (PlosOne)

ZEG testing

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Success rate of PCR/genotyping using ZEG solution is high; some general conclusions/trends can be drawn:

- During the ZEG procedure, the embryos are unharmed, with no apparent adverse effects on survival (survival is usually 100%)

- The mean PCR/genotyping success rate is 96%. Only when no PCR amplicon can be detected on gel/fragment analyzer then sequencing fails

- ZEG works equally well for 2dpf and 3dpf embryos, with similar PCR/genotyping success rates. We now mostly use 2dpf embryos and obtain genotypes when fish are 4dpf

ZEG testing

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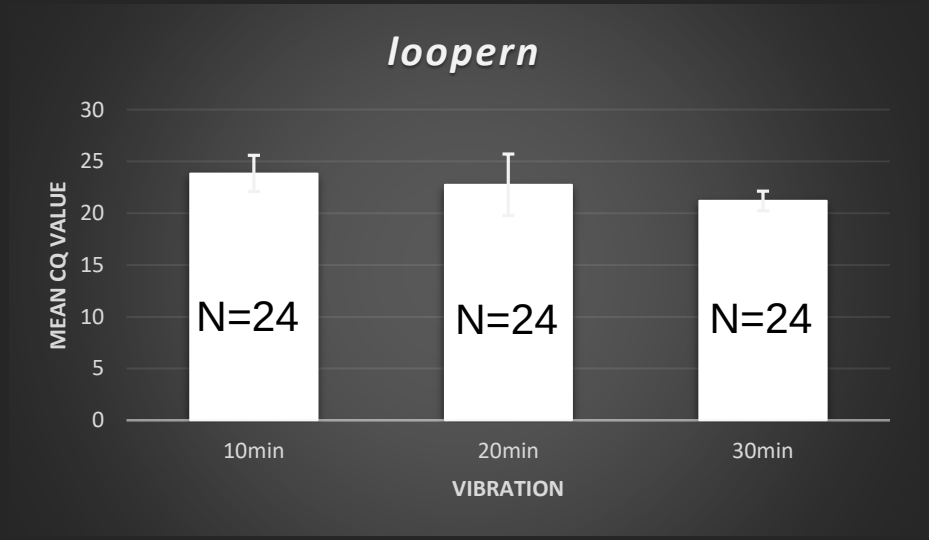
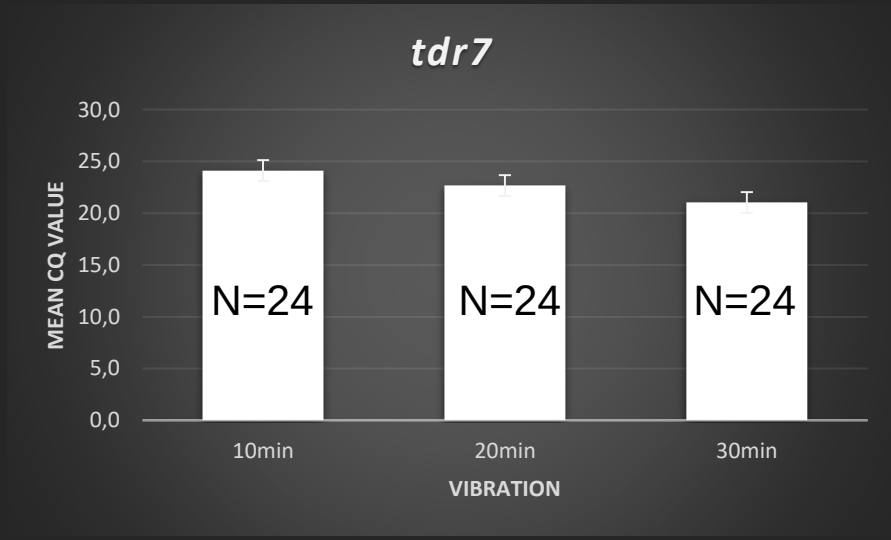
- 5µl of ZEG solution in a 20µL volume PCR works best (highest PCR/genotyping success rates) although 4 or 3 µL also works fine in most cases. Regular touch down PCR programs work well
- With increasing vibration times, more DNA is extracted from embryos





- High correlation between ZEG genotypes and whole embryo genotypes
- ZEG solution works equally well for genotyping with traditional Sanger sequencing and NGS-Miseq sequencing (eg to charactize mosaic embryos from CRISPR experiments)

Conclusions

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- The ZEG device is an automated system that rapidly obtains DNA from zebrafish embryos for genotyping, while keeping the animals alive and intact
- Allows researchers to identify and raise mutants of interest at an early stage of development and therefore saves time, effort and money
- High PCR/sequencing success rate from 2dpf and 3dpf embryos with no or little harm to the embryos up to 30 minutes of vibration
- High correlation between ZEG genotypes and whole embryo genotypes

Acknowledgements

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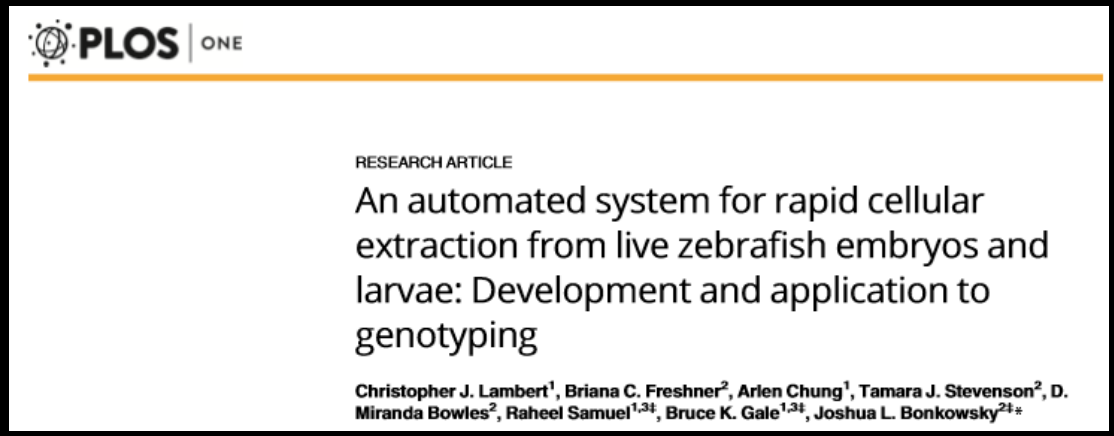
Startup Company ‘Fluidx’ (https://www.wfluidx.com/)


PLOS ONE

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‡*]