University of Utah
UNDERGRADUATE RESEARCH JOURNAL

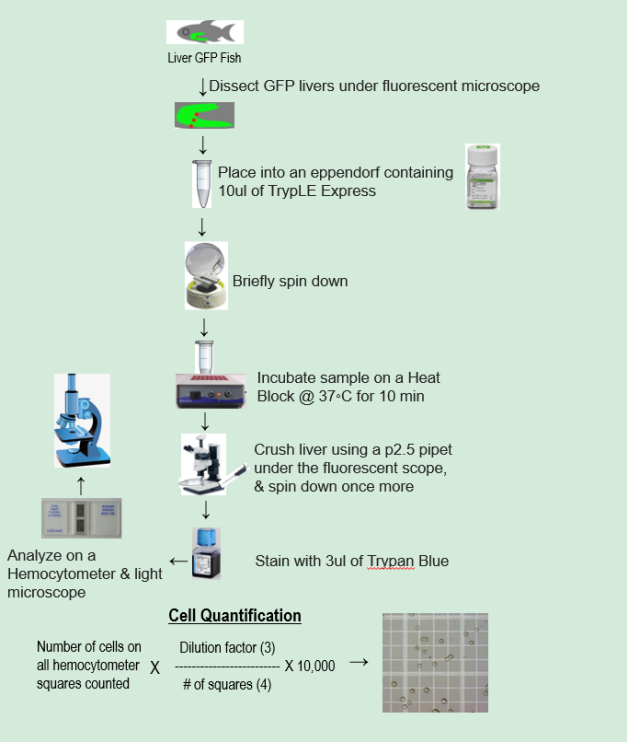
A NEW METHOD FOR QUANTIFYING LIVER SIZE IN ZEBRAFISH BY MECHANICAL ISOLATION AND HEMOCYTOMETER ANALYSIS

Gabriel DeNiro[1], Liam O'Brien[1], Richard Smith[1], Kimberley Evason[1,2]
Huntsman Cancer Institute[1], Department of Pathology[2], University of Utah, Salt Lake City

Background

Over the last several decades, the zebrafish has emerged as a major genetic model in basic science research. Quantification of larval liver size in zebrafish is a pivotal application in studies that target hepatocellular carcinoma, fibrosis, oxidative stress, development, and organ regeneration. Current methods include quantifying liver area or volume based on images generated with confocal or light microscopy. These methods are technically challenging and labor-intensive, and they do not have the ability to distinguish between changes in cell size (hypertrophy) and changes in cell number (hyperplasia). Here we report the development of a new, simple and cost-effective method for quantifying zebrafish liver size via mechanical isolation of cells from florescent livers and cell counting. A major advantage of our new method is the ability to detect changes in cell number, which is particularly relevant for studies of cancer, development, and regeneration.

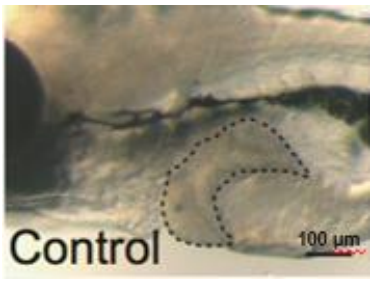
Methods



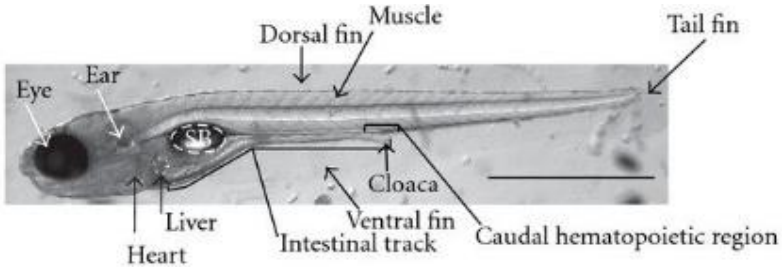
The proposed method is to use zebrafish larvae with fluorescently labelled livers and dissect out the liver under a florescent microscope. Livers are then placed into 1ml Eppendorf tubes with 10 µl of TrypLE Express. Liver samples are then given a quick spin down, then incubated on a heat block at 37°C for 10 minutes. The liver is then brought back under the florescent microscope and using a p2.5 µl pipette, it is gently crushed up into a cellular mass. The sample(s) is then stained with 3 µl of Trypan Blue and analyzed on a hemocytometer under a light microscope. Cells are then quantified using standard hemocytometer cell count analysis.

Current standard method

Shown below is an image of an untreated wild-type (WT) zebrafish larvae at 6 days post-fertilization (dpf), with the liver area being visually outlined and measured. This is the currently the favored method amongst various researchers whom conduct liver size experiments with fish livers.



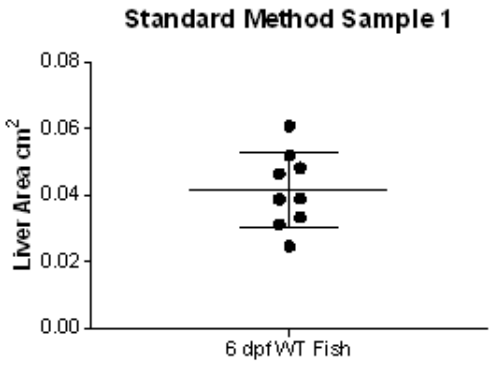
Evason, K. J., *et al.* (2015). Identification of chemical inhibitors of beta]-catenin-driven liver tumorigenesis in zebrafish. *PLoS Genetics*

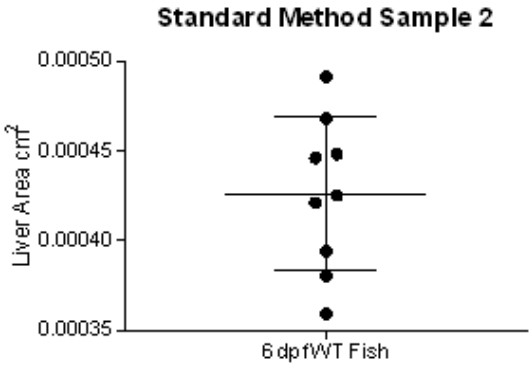


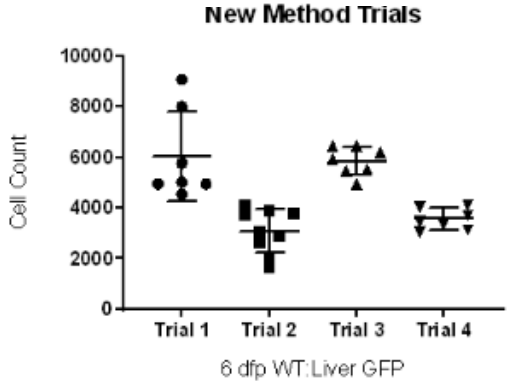
Goldsmith, Jason Jobin, Christian "*Think Small: Zebrafish as a Model System of Human Pathology*" 2012 10.1155/2012/817341 *Journal of Biomedicine & Biotechnology*

Determining Methodological Efficacy

Before any method in research can be put into the scientific community for frequent use, a standard of efficacy must be established to determine if a specific technique is prone to a high error rate. Hence data was collected to determine the error rate generated by the coefficient of variance (CV), from 6 dpf WT fish expressing green fluorescent protein (GFP). Four cell count trials were conducted using this method. Data showed percent CVs of 27.26, 27.9, 9.58 and 11.77. These were compared alongside two sets of randomized sample tests using 6 dpf WT liver data from fellow zebrafish researchers using the standard method. These samples generated CVs of 9.4% and 25.7% respectively.

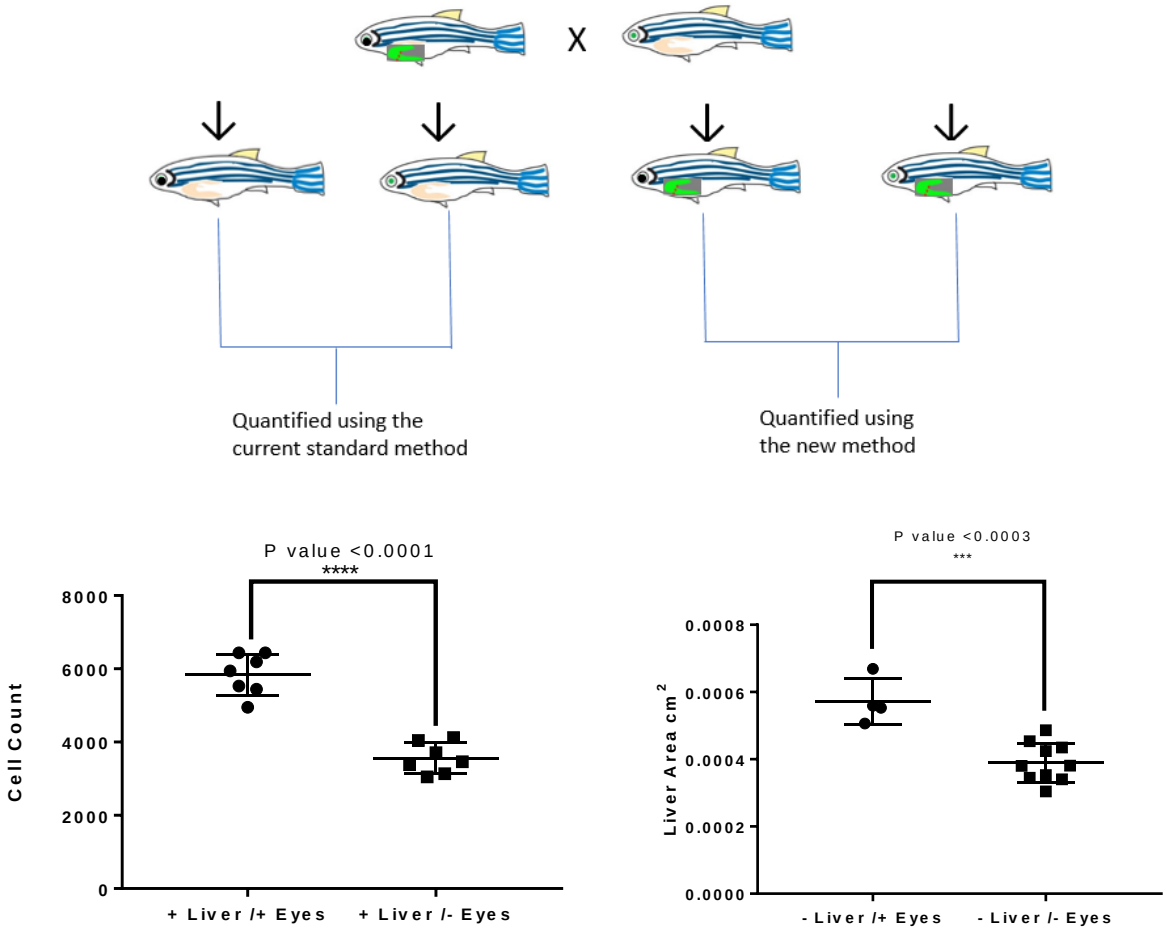






Results

Zebrafish crosses were set up using an even number of WT (AB) fish with GFP fluorescent livers and fish that express hepatocyte-specific activated beta-catenin (fish will express larger livers) with GFP fluorescent eyes. Hence progeny from all clutches will produce different four phenotypes with respect to green liver and eye color; +/+, +/-, -/+, -/-. Fish with fluorescent livers, regardless of eye color, had their livers quantified using the new cell count method. Fish with non-fluorescent livers were quantified using the standard method, measuring liver area and volume from images.



Discussion

Livers quantified using the standard method resulted in a P value of <0.0003 whereas the new cell count method had a P value of <0.0001. Hence there is a clear indication of tight and defined results which can be used to quantify liver size in zebrafish. Furthermore, we are able to use this as a supplementary method alongside the current standard method by measuring liver area, as we are now also able to quantify the number of cells within a specified liver.

References

1. Evason KJ, Francisco MT, Juric V, et al. Identification of Chemical Inhibitors of β-Catenin-Driven Liver Tumorigenesis in Zebrafish. *PLoS Genet*. 2015;11(7):e1005305.
2. Goldsmith, Jason Jobin, Christian 817341 *Think Small: Zebrafish as a Model System of Human Pathology* 2012 (Book)