A High-Throughput Assay To Measure Whole Body Metabolic Rate Using Zebrafish Larvae.

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1 OVERVIEW

Regulation of whole body metabolism and energy homeostasis has been shown to require signaling between multiple organs, such as the brain liver, and gut. To identify genetic programs that determine metabolic rate, as well as compounds that can modify it, we have developed a high-throughput, whole animal assay. Since acid production is directly correlated to metabolic rate, we exploited this fact to develop a novel non-invasive colorimetric assay for acid secretion by individual zebrafish larvae in a 96-well plate format.

Using this assay we detected a 3-fold increase in metabolic rate that accompanied development between 24 and 96 hours post fertilization. In addition, we detected dynamic changes in metabolic rate in response to different conditions such as temperature and drug treatments. Finally, this assay was used to measure metabolic rate in the progeny of fish known to carry a recessive mutation in a gene required for ribosome biogenesis (*npo*$^{fw07\text{-}g}$), which would be expected to reduce energy consumption. We found a strong correlation ($p<10^{-6}$) between reduced metabolic rate and genotype even before the developmental defect was visually evident.

2 INTRODUCTION

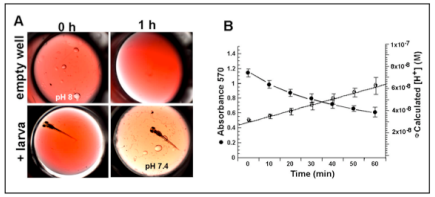
Energy imbalance underlies many of the most common diseases afflicting Western society such as obesity, cardiovascular disease and diabetes. Understanding the molecular network mediating energy imbalance is therefore of critical importance in preventing such diseases. Energy intake and energy expenditure are two critical parameters that determine energy imbalance. Our study focuses on energy expenditure since it has received less attention and due to the inherent difficulty of measuring whole animal metabolic rate. To enable large-scale measurements of energy expenditure (metabolism) in a vertebrate, we chose zebrafish as the platform of choice and developed an assay to directly measure metabolic rate. The objective of this work was to develop a high-throughput whole-animal assay system that measure metabolic rates in zebrafish.

3 METHODS

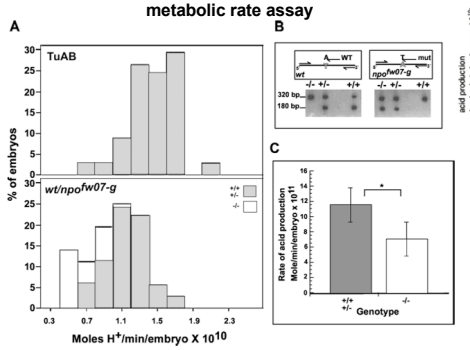
The metabolic rate assay is explained in detail in the published reference (Makky et al).

RESULTS

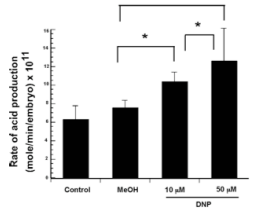
4 Fig. 1 Embryo metabolism acidifies the media



5 Fig. 2 Genotype-phenotype correlation using metabolic rate assay

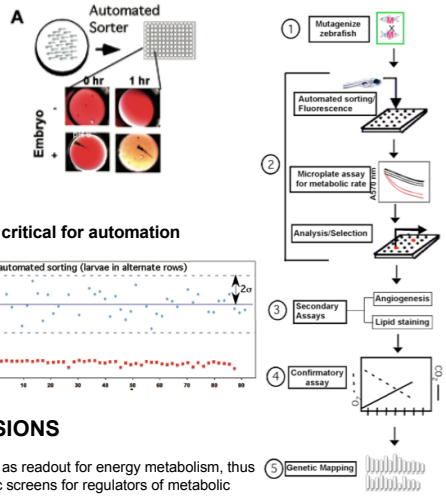


6 Fig. 3 DNP treatment of fish alters metabolic rate

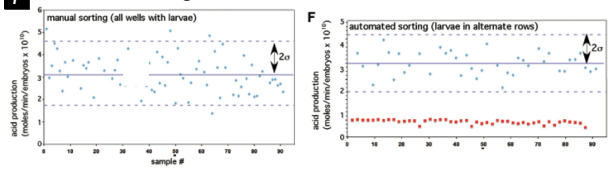


RESULTS

8 Fig. 4 Automation plan



7 Fig. 5 COPAS sorter is critical for automation



9 CONCLUSIONS

Whole animal acid secretion can be used as readout for energy metabolism, thus enabling high-throughput chemical and genetic screens for regulators of metabolic rate in a vertebrate.

10 ACKNOWLEDGEMENTS AND REFERENCE

This research was supported by start-up seed funds from the Children's Research Institute (CRI) at the Medical College of Wisconsin. Data presented here is taken from our publication Makky et al, The Journal of Biomolecular Screening, 2008 Dec; 13(10): 960-7.