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Reconstruction of a genome-scale metabolic model for chicken whole embryo considering transcriptomics data

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Motivation and Aim: One of the rapidly developing modern biological fields, in which advanced technologies are most likely and in-demand developed, is synthetic biology. The main goal of the research field is the creation of new pro- and eukaryotic organs with desired properties based on *de novo* genome synthesis or their deep reorganization using genetic engineering (GE) approaches, bioengineering, systems biology and bioinformatics. Genetic modifications of model lab animals are mainly focused on basic research, while the application of GE technologies in investigations of livestock including chicken plays a key role in the capability to significantly extend existed or create a new niche market. As a perspective, the development of livestock with modified economically useful traits, genetically resistant to hereditary and infectious diseases too is considered. However, a lack of both efficient approaches in the identification of target genes, their regulatory elements determining desired traits and powerful mathematical models enabling quantitative predictions of GE modifications hinders the implementation of this approach in livestock breeding.

Methods and Algorithms: *Hypometabolic experiments:* As an experimental model, we used three groups: 1) normally developing chicken embryos (38.5C, stages HH1, HH4, HH16 and HH24); 2) embryos in cooling-induced (38.5C—>21C) hypometabolic torpor and 3) embryos with re-started normal metabolism (1 hour after 21C—>38.5C). Total RNA was extracted from 36 embryos in total (4 embryonic stages x 3-time point x 3 replicates).

CAGE-seq experimental data and its processing: CAGE-seq libraries from the total RNA of the embryos were prepared according to the manufacturer's protocol (DNAform, https://www.dnaform.jp/en/products/library/cage_kit/tech/). Single-read sequences were analyzed for quality and overrepresented adapter sequences with the theFastQC tool. Quality filtering and adapter trimming were performed with the Trimmomatic tool v0.39. Read mapping on the chicken genome GRCg6a was performed with the STAR local alignment tool. CAGE tag start sites (CTSS) for each sample were imputed using the PromoterPipeline aggregation script from the C1 CAGE protocol. Peak clustering was performed with the Decomposition-based Peak Identification (DPI1) tool. Bidirectional enhancers were identified using the pipeline by Andersson et

al, 2014. Statistical significance of differential expression of TSS and CTSS peaks was calculated using the DeSeq2 tool.

Genome-scale metabolic model reconstruction: We utilized the recently published generic genome-scale metabolic model for chicken [1]. Then we fine-tuned the model for chicken whole embryo knowledge and data based on the original transcriptomics data and BioUML platform [2]. The model simulations and analysis was conducted with COBRApy [3] integrated into the platform, whereas Escher web-tool was used to build a metabolic network with fluxes distribution across the map [4].

Results: As a result we specified the model for chicken whole embryo metabolic data. We also simulated the metabolic model when glycogen is used as a single carbon substrate for growth, with a growth rate of 0.34 h^{-1} on RPMI medium. Flux distributions in gluconeogenesis and glycolysis pathways are demonstrated in Fig. 1.

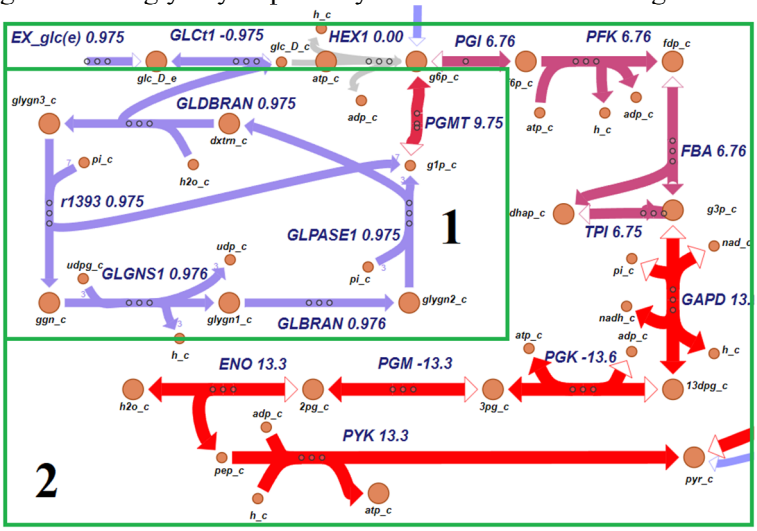

Fig. 1. A genome-scale metabolic network with fluxes distribution across the map predicted by the developed model. The color of arcs in the figure reflects the flux value. The map is constructed in the Escher web-tool [4]. With numbers are indicated two pathways: 1 – glycogenesis pathway, 2 – glycolysis pathway

Conclusion: The use of systems biology methods and approaches provides an opportunity to predict the role of various genome modifications on phenotypic traits and their effect on cells. In this regard, the development of genome-scale metabolic models is the most promising approach in the application of GE technologies.

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