

## **Using de novo transcriptome for identification genes involved in cryotolerance in the turtle leech *Ozobranchus jantseanus*.**

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The turtle leech *Ozobranchus jantseanus* is capable to withstand instant freezing and long-term storage under super-low temperature [1]. In contrast to known cryotolerant invertebrates the turtle leeches does not require prolonged pre-conditioning and resist immediate freezing, the post-freezing restoration of activity take less than 20 min. We compared transcriptome data from control samples (before freezing) and samples after storage in super low temperatures (−80°C). De-novo transcriptome assembly of was carried out using paired-end reads from Illumina HiSeq 2000. The RNA-Seq dataset was represented by control samples (before freezing), 30min.of thawing samples, 3.5h. of thawing samples, 24 h. of thawing samples. In order to understand molecular mechanisms involved in thawing processes we performed Gene Ontology (GO) analysis of transcriptome data. Comparative GO analysis was done in R statistical computing environment [2] (<http://www.R-project.org/>) using Bioconductor [3] (<http://www.bioconductor.org/>) software packages.

We observed that among the most up-regulated annotated transcripts during process of thawing 29 transcripts are belongs to products with peptidase inhibitor activity (GO:0030414) or peptidase regulator activity (GO:0061134) functions. The majority of up-regulated during thawing process annotated transcripts coded products predicted to be located in extracellular regions or on cell surfaces. We suppose that among them, there are some proteins taking part in processes of reparation and

regeneration after freezing and further in vitro studies will be done for deeper understanding of this process.

We did not observe any transcripts resembling known anti-freeze proteins. As the leech, in contrast to known animals with inducible cryo- and anhydro-resistance, is capable to withstanding instant freezing, we assume that some of the abundant biomolecules in the leech body would contribute to this unusual tolerance. Among the Top-100 highly expressed transcripts in control, 23% are represented by products of unknown orphan Ozobranchus-specific genes. Furthermore, 6% of them have ORF. Another possibility to be explored is potential contributed the host (turtle) blood, or some of its components to the cryo-resistance, as even short term starving is known to drastically decrease the ability of the leech to tolerate freezing.

The work is performed according to the Russian Government Program of Competitive Growth of KFU and supported by JSPS a N<sup>o</sup>14-04-92116.

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