Near single-cell transcriptomics analysis of differential expression in *Helix lucorum* statocysts under microgravity

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Helix lucorum snail is a classical model object for studies of the nervous system functions [1,2]. In order to understand the genome mechanisms of the gravity reception in the snail nervous system we performed a near single-cell transcriptomics analysis of space flight induced differential expression in *Helix* statocysts [2].

There were 8 animals in two equal groups of snails – that flied into space (n=4) and remained on Earth (n=4), some 13 cells were used per every sample. We performed a full novel transcriptome assembly based on the total mRNA sequenced by means of Ion Proton System. Two assembly programs with different settings were used to fine tune the results. Near 60% of reads per sample were mapped to the best assembly (Table 1).

All the calculations were performed on the PC based on 4-cores 8-threads Intel Core i7-3820, 64 GB RAM running Debian GNU/Linux 7.0 wheezy (3.2.0-4-amd64), that proved sufficient, despite the common confidence that a special computing cluster is needed for the task.

assembly	ass	sc	sc+wns	Trinity	wns
all contigs	59273	59308	58142	248182	2065601
contigs >= 1000 bp	5162	5165	5694	4813	32349
whole length	32118642	32130804	32323607	93622478	338447864
length >= 1000 bp	6973004	6974353	8077421	6500058	57218292
greatest contig	4575	4575	8758	5230	15939
GC (%)	41.78	41.78	41.83	41.64	41.05
N50	614	614	636	371	222
N75	432	432	438	283	100
L50	17007	17013	16007	82100	306406
L75	32726	32736	31479	154857	913587
N per 100 Kbp	1.24	1.24	1.24	0.00	0.00

avg. mapping %	49,75	49,76	52,2	61,1	41.5

Table 1. The characteristics of the assemblies obtained: ass - assembly by the RNASpades program with the default settings; sc - assembly by the RNASpades program using the command line option "single cell analysis"; sc + wns - assembly by the RNASpades program using the "single cell analysis" command line option with the option "rely on the ready assembly", in this case – the total transcriptome of the whole nervous system; Trinity – assembly by Trinity program with the default settings; wns – assembly of the total transcriptome of the whole nervous system.

The differential expression analysis revealed more than 15 000 “genes” when mapped to the best assembly by RNASpades and more then 45 000 “genes” for Trinity assembly, that could be due to more coarse assembly by RNASpades (see assemblies statistics in Table 1). Most of them except a few dozens are expressed only in one or another group with zero expression in the counterpart. The functional analysis of these “genes” is in progress, although with little prospect of success due to diverged proteome of snails compared to known proteins in the common model organisms in annotated databases.

More than 40 significantly differentially expressed (i.e. down-regulated by space flight) genes were expressed in both groups. Most of them relate to the cell reception and different stages of intracellular signaling pathways, including gene expression regulation.

Interestingly they were no significantly differently expressed genes if transcripts were mapped to the whole nervous system transcriptome assembly provided by our colleagues, and the overall portion of the mapped reads was less by nearly 20%.

The data obtained indicates that genes that are differently expressed are specific to the statocysts themselves and are probably related to the gravity reception.

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