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Data in Brief

MicroRNA of the fifth-instar posterior silk gland of silkworm identified by Solexa sequencing

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ABSTRACT

No special studies have been focused on the microRNA (miRNA) in the fifth-instar posterior silk gland of *Bombyx mori*. Here, using next-generation sequencing, we acquired 93.2 million processed reads from 10 small RNA libraries. In this paper, we tried to thoroughly describe how our dataset generated from deep sequencing which was recently published in BMC genomics. Results showed that our findings are largely enriched silkworm miRNA depository and may benefit us to reveal the miRNA functions in the process of silk production.

Specifications: Where applicable, please follow the Ontology for Biomedical Investigations: http://obi-ontology.org/page/Main_Page

Organism/cell line/tissue	Posterior silk gland of *Bombyx mori*
Sex	Half males and half females
Sequencer or array type	Illumina Genome Analyzer IIx
Data format	Raw data: FA, TXT, PNG
Experimental factors	Instar, strain, rearing condition
Experimental features	Sequencing 10 total RNA samples from the posterior silk gland of different strains and developmental stage using Illumina Solexa technology. Four strains of silkworm (Q, B, QB and BQ) with different two development stages (stage 1: fourth instar molting to day 2 of fifth instar; stage 2: fifth instar day 3 to day 8 before spinning, according to our previous gene expression cluster analysis), and two strains (R1 and J1) from entire period (stage 1 + stage 2).
Consent	N/A
Sample source location	Yuhangtang Road 866, Hangzhou, Zhejiang province, China

Direct link to deposited data

Please click on the following link: http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE56380

Experimental design, materials and methods

Experimental design

In order to obtain more miRNA profiling and eliminate strain-specific effects [1], we selected six domesticated silkworm strains (Q, Qiufeng; B, Baiyu; QB, Qiufeng × Baiyu; and BQ, Baiyu × Qiufeng, R1, and J1) and employed next generation sequencing platform to determine the novel and conserved miRNAs hidden in the posterior silk gland of silkworm. In addition, according to gene expression cluster analysis [2], the third day of fifth-instar larva (V3) is a key time point for silk synthesis and rapid cell growth. Thus, animals were collected from three stages: (1) stage 1: fourth instar molting to day 2 of fifth instar from Q, B, QB, and BQ; (2) stage 2: fifth instar day 3 to day 8 before spinning also from Q, B, QB, and BQ; and (3) entire stage: stages 1 + 2 from R1 and J1 (Fig. 1).

Sample preparation

The healthy animals were reared at 25 °C on a natural diet of clean mulberry leaves to the scheduled time. Posterior silk glands were

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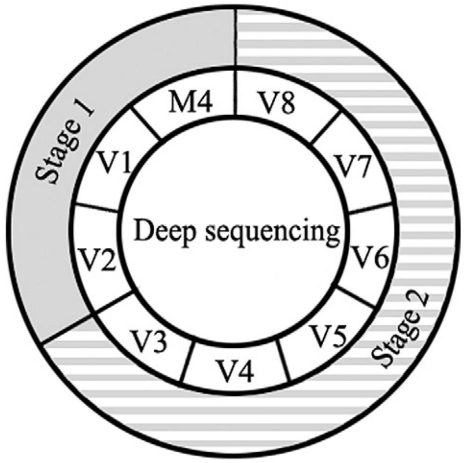

Fig. 1. The silk gland from the fourth molting to the fifth instar day 8. M4 to V8 represent nine consecutive days of the silk glands' developmental stages from the fourth molting larva to the fifth-instar larvae (V1 to V8).

dissected daily in 0.7% NaCl at low temperature, rinsed with DEPC-treated water, and promptly stored in liquid nitrogen.

Deep-sequencing

Following the manufacturer's instructions, posterior silk glands were collected, and total RNA was extracted with Trizol reagent (Invitrogen, Carlsbad, CA, USA). After quantified and examined with the Agilent 2100 bioanalyzer (Agilent Technologies, Palo Alto, CA) (Fig. 2), total RNA of the desired size range (18–30 nt) was size-fractionated on a 15% PAGE gel and ligated with 5′ RNA adapter (5′-GUU-CAGAGUUCUACAGUCCGACGAUC-3′) using T4 RNA ligase. Ligated RNA was size-fractionated on a 15% agarose gel to obtain 40–60 nt fraction. Subsequently, the 3′RNA adapter (5′-pUC-GUAUGCCGUCUUCUGC UUGidT-3′; p, phosphate; idT, inverted deoxythymidine) was also ligated with T4 RNA ligase. After size-fractionated on a 10% agarose gel, the 70–90 nt fraction excised and was subjected to RT-PCR. After amplified for 15 cycles, the PCR products were separated on agarose gels.

The RT-PCR products were sequenced on the Illumina platform (Beijing Genomics Institute or BGI, Shenzhen) [3,4] and 35 nt small RNA reads were generated. After removed low quality reads, contaminants, poly A, and adapter sequences with perl script (Table 1), clean reads with the size range of 18–30 nt were retrieved and submitted to NCBI's Gene Expression Omnibus (GEO) with the accession number GSE 56380.

Conclusion

Combining results from deep sequencing, microaary assay and bioinformatics analysis, we identified 728 novel miRNAs (including 55 miRNA/miRNA* duplex and 709 Bm-specific miRNAs expressed in the posterior silk gland in the period of the fourth-instar molting to the fifth-instar (day 8 before) spinning. These findings pave a way for further functional elucidation of these miRNAs and their targets in silk production [5].

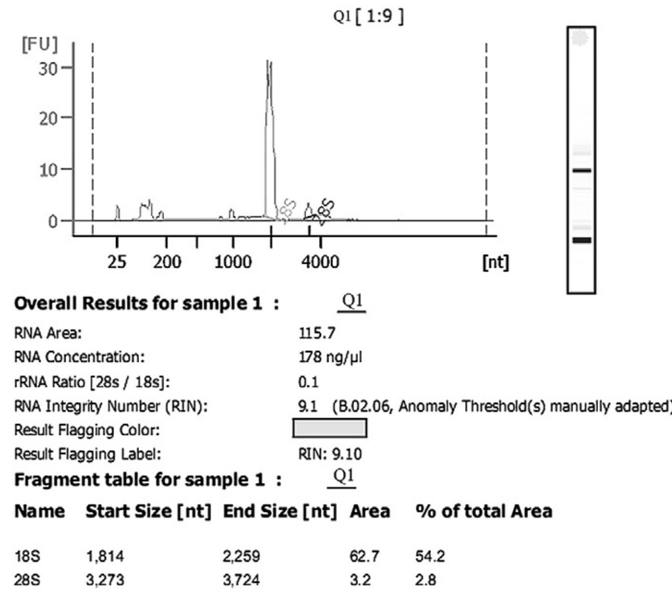

Fig. 2. RNA detecting result by Agilent 2100 bioanalyzer.

Acknowledgment

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