Eukaryotic ncRNA Sequencing

Eukaryotic ncRNA-seq uses a next-generation sequencing approach to reveal the profile of non-coding RNAs at certain timepoints or under specific conditions. Sequencing data allows for the identification of present transcript isoforms and their abundance. Novogene's Eukaryotic ncRNA-seq service delivers high quality data, publication-ready analysis results, and personalized analysis pipeline for long non-coding RNA, circular RNA, and small RNA. With our whole transcriptome sequencing service, the potential transcriptional and regulatory networks among different types of RNAs are studied.

Why Novogene?

Extensive experience with over 3000 projects

Industry-leading data quality guarantee

In house pipeline to meet different analysis requirement

Find out more: novogene.com

Sample requirements

Service Type	Sample Type	Library type	Amount	Volume	Concentration	RNA Integrity Number (Agilent 2100)	Purity
lncRNA sequencing	Total RNA	rRNA removal & directional RNA library	≥ 500 ng	≥ 10 μL	≥ 50 ng/μL	≥ 6.5 (Animal); ≥ 6 (Plant & Fungus)	OD260/280 ≥ 2.0 OD260/230 ≥ 2.0 No degradation No contamination
circRNA sequencing		rRNA, linear RNA removal & directional RNA library	≥ 2 μg	≥ 20 μL	≥ 50 ng/μL	≥ 7 (Animal); ≥ 6.5 (Plant & Fungus)	
small RNA sequencing		18~40 bp insert sRNA library	≥ 2 μg	≥ 20 μL	≥ 50 ng/μL		
Whole transcriptome sequencing		rRNA removal & directional RNA library. 18~40 bp insert sRNA library.	≥ 5 μg	≥ 20 μL	≥ 20 ng/μL	≥ 7.5 (Animal); ≥ 7 (Plant & Fungus)	

Sequencing parameters

Service type	Platform	Read length	Recommended data amount	Data quality	Turnaround time
lncRNA sequencing	Illumina NovaSeq 6000	Pair-end 150	9-15Gb per sample	Q30 ≥ 85%	16 working days
circRNA sequencing			9-15Gb per sample		23 working days
sRNA sequencing			10~20M reads per sample		25 working days

Content of analysis

Service type	Analysis	
lncRNA	• lncRNA identification • lncRNA target prediction • Protein-protein interaction • Alternative splicing • SNP/InDel calling	• Raw data quality control • Mapping to reference genome • Quantification • Differential expression analysis • Enrichment analysis (GO & KEGG)
circRNA	• circRNA identification • miRNA binding site prediction	
circRNA	• miRNA identification • miRNA characterization	
WTS	**Standard analysis:** • lncRNA (lncRNA & mRNA) • circRNA • miRNA	• Free joint analysis: • miRNA-mRNA • miRNA-mRNA-lncRNA • miRNA-mRNA-circRNA

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Case study

Integration of lncRNA-miRNA-mRNA reveals novel insights into oviposition regulation in honeybees.

Chen et al., 2017. PeerJ. DOI: 10.7717/peerj.3881.

Research objective:
To explore the expression changes of coding and non-coding RNA in the different oviposition phases of ovary activation in honeybees, as oviposition of the queen is crucial for the reproductive success and development of the colony.

Species: Honeybee *(Apis mellifera)*

Sample collection:
Honeybee ovary RNA from 4 phases (virgin queen, egg-laying queen, egg-laying inhibited queen, and egg-laying recovery queen).

Sequencing strategy:
PE150 & SE50 on Illumina NGS sequencer.

Data amount:
~16.7 Gb raw data per sample for lncRNA & mRNA; 150 M reads for sRNA.

Results (partial results shown)
A number of RNAs were found to be differentially expressed (DE) in the ovaries of honeybees from the 4 phases tested (table 1). Enrichment and functional annotation of the DE RNAs mapped to pathways related to oviposition, including hippo, MAPK, Wnt, notch and mTOR pathways.

Table 1. The number of DE coding and non-coding RNAs identified from each comparison.

Number of differentially expressed RNAs	Ovary activation (Egg-laying queens compared with virgin queens)		Oviposition inhibition (Egg-laying inhibited queens compared with egg-laying queens)		Oviposition recovery (Egg-laying recovery queens compared with egg-laying inhibited queens)	
	Up-regulated	Down-regulated	Up-regulated	Down-regulated	Up-regulated	Down-regulated
mRNAs	3218	2263	266	72	256	241
lncRNAs	224	516	57	31	40	60
miRNAs	39	42	9	4	2	2

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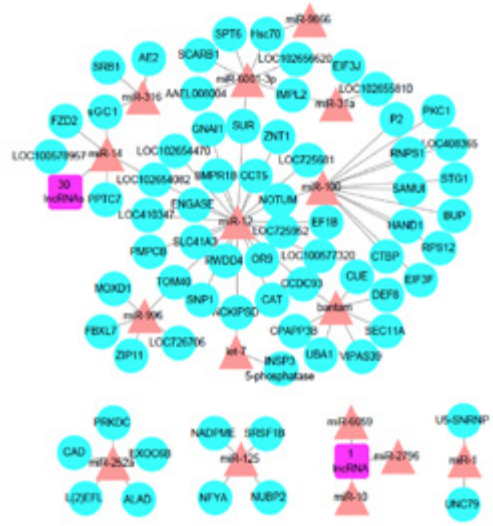

Figure 1
The reproductive associated lncRNA-miRNA-mRNA network.

For example, genes involved in tissue development, hormone biosynthesis and oocyte microtubule cytoskeleton polarisation were upregulated during the ovary activation phase and then downregulated during oviposition inhibition. Many of these DE genes were localised to a quantitative trait locus (QTL) on chromosome 11 previously identified for ovary size.
An integrated analysis of the miRNA-lncRNA-mRNA network revealed a core set of genes that acted as bridges between miRNAs active the identified pathways (fig. 1).

Conclusions

This study revealed the importance of non-coding RNAs in the activation and regulation of oviposition in honey bees. It also revealed the interaction of mRNA and non-coding RNAs with each other, connecting the pathways in which they are involved. Candidate genes for oviposition were also identified.

Publications using Novogene's expertise

Nature, 2020.
Small RNA sequencing revealed various microRNAs involved in ethylene-triggered flowering process in *Aechmea fasciata*

Genes, 2020.
CircRNA Expression Profile during Yak Adipocyte Differentiation and Screen Potential circRNAs for Adipocyte Differentiation

Veterinary Microbiology, 2020.
Identification of functional lncRNAs in pseudorabies virus type II infected cells

BioRxiv, 2019.
An impact of HP1γ on the fidelity of pre-mRNA splicing arises from its ability to bind RNA via intronic repeated sequences

Novogene (UK) Company Limited
25 Cambridge Science Park
Cambridge, CB4 0FW
United Kingdom
www.novogene.com
info@novogene-europe.com

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