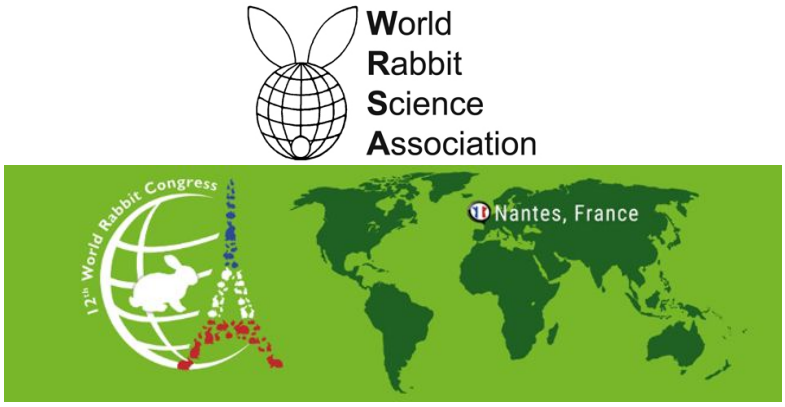

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REX RABBIT ILLUMINA SEQUENCING AND BIOINFORMATICS ANALYSIS ON miRNA IN TESTES

Full text of the communication

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Poster

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REX RABBIT ILLUMINA SEQUENCING AND BIOINFORMATICS ANALYSIS ON miRNA IN TESTES

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ABSTRACT

miRNA as a kind of micromolecule non-coding RNA with the length of about 22 bp plays an important regulatory role in reproductive biology and spermatogenesis. This research uses Illumina sequencing technology to conduct miRNA sequencing on three seven-month adult male rabbits , aiming at evaluating the quality of high throughput sequencing in testes rabbit miRNA data mining, and exploring the biological characteristics of miRNA in testes tissues. Results show that, the quality , the purity of total RNA and integrity of the total RNA were good, which can meet the requirements of construction of small RNA library and the applicability of obtained sequence in subsequent miRNA identification analysis.Uniq reads accounted for 24.17 % of the total sequences. The length of RNA was mostly between 22 - 31 nt, which takes up more than 89.32 % of pure sequences. Nonetheless, 82.86 % of cleaned sequence were not mapping to previously known long and short RNA. miRNA sequence length imbalance may be related to the action mechanism and specific functions of miRNA in testes tissues.
Key words: Rex rabbit ; testes ; high throughput sequencing ; miRNA

INTRODUCTION

miRNA plays an important regulatory role in reproductive biology and spermatogenesis(
M. Ran et al) This research uses Illumina sequencing technology to conduct miRNA sequencing on adult male rabbits to explore the biological characteristics of miRNA in testes tissues.

MATERIALS AND METHODS

Sample collection and preparation

Rex rabbit testes sampling and total RNA extraction three healthy seven-month male Rex rabbit were selected for sample collection from the Rex rabbit breeding farm of Sichuan in Dayi county, Chengdu city, China. The testes tissues were quickly collected and immediately placed in liquid nitrogen for the preservation of total RNA .

Extraction of total RNA

Total RNA from the sample was extracted using Trizol reagent ®(Invitrogen,USA) kit, and the operation steps were carried out according to the operation instructions. The degradation and integrity of total RNA was monitored on 1% agarose gels. Three testes total RNA qualified for integrity tests were put into three RNA pools according to the same concentration, and the total RNA were sent to Beijing Novogene company for sequencing.

Library generation and sequencing

A total amount of 3 μg total RNA per sample was used as input material for the small RNA library. Raw data (raw reads) of fastq format were firstly processed through custom perl and python scripts. The small RNA tags were mapped to reference sequence by Bowtie without mismatch to analyze their expression and distribution on the reference. Mapped small RNA tags were used to looking for known miRNA. miRBase20.0 was used as reference, modified software mirdeep2) and srna -tools-cli were used to obtain the potential miRNA and draw the secondary structures. Custom scripts were used to

obtain the miRNA counts as well as base bias on the first position of identified miRNA with certain length and on each position of all identified miRNA respectively.

RESULTS AND DISCUSSION

Sequencing data quality

Table 1 Summary of data cleaning of tags produced by small RNA sequencing

Type of reads	total_reads	N% > 10%	low quality	5_adapter_contamine	3_adapter_null or insert_null	with ployA/T/G/C	clean reads	Unique reads
Counts (3 samples	11,480,993 ±1,095,276	91 ±8	12886 ±788	1067 ±203	124004 ±19961	41247 ±6674	11301699 ±1095019	2766967 ±228029
Percent	100.00 ±0.00%	0.00 ±0.00%	0.11 ±0.01%	0.01 ±0.00%	1.09 ±0.21%	0.36 ±0.05%	98.43 ±0.25%	24.17 ±1.88%

Unique reads accounted for 24.17 % of the total sequences (Table 1) . The lengths of clean sequences were mostly between 22 - 31 nt (Figure 1), which was a typical Dicer enzyme cutting products (Y. Lee et al). The 29 nt sequence accounted for 21.08 % of the total sequence, followed by 30 nt (18.45 %) and 28 nt (10.61%).

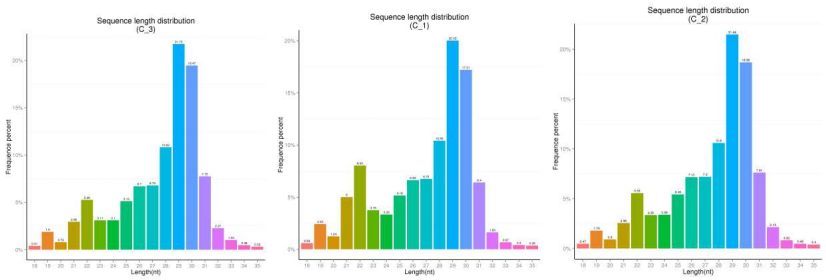

Figure1 Length distribution of clean sequences in the three testes samples

Classification annotation

In the sequencing library, in order to further analyze the sequence types of microRNA obtained, after genome positioning, we classified and annotated the clean reads with more than 18 nt in the sequencing library (Table 2). The non-coding RNA such as rRNA, tRNA, snRNA, snoRNA and repeat RNA, which accounted for 11.29 % of the total clean reads, only 0.08 % of the sequences in the sequencing library were annotated as known miRNAs, indicating that the proportion of miRNAs in the sequencing sequence was relatively low, while the unmatched sequence accounted for 82.86 %, which might be candidate sequences of new miRNAs.

Table 2 Classification annotation of small RNAs

Types	total	known_ miRNA	rRNA	tRNA	snRNA	snoRNA	repeat	novel_ miRNA	exon:+	exon:-	intron:+	intron:-	other
Count (3 samples	2191836 ±155058	1861 ±47	2460 ±376	466 ±29	2163 ±367	4526 ±617	236677 ±9784	766 ±23	4105 ±404	436 ±24	60609 ±3904	60319 ±3043	1817447 ±156680
Percent (%)	100.0 ±0.0	0.08 ±0.01	0.11 ±0.02	0.02± 0.00	0.10 ±0.10	0.21 ±0.02	10.85 ±1.19	0.03 ±0.01	0.19 ±0.01	0.02 ±0.00	2.77 ±0.05	2.76 ±0.13	82.86 ±1.35

DISSCUSSION

RNA-seq technology can accurately obtain the expression of each specific miRNA by counting the determined sequence, which has the advantages of wide coverage and low cost. Meanwhile, it effectively solves the technical problems of low abundance and tissue-specific miRNA identification,

as well as the interference of cDNA clone by high expression RNA (Wold B, et al.). At present, RNA-seq technology has been widely used in the analysis of miRNA expression profiles in different tissues, development stages and physiological states of pigs, cattle, sheep and poultry, and a large number of miRNAs with different expressions have been successfully screened. However, the accuracy of miRNA expression profile depends on the quality of sequencing data. The ratio of the total amount of sequences obtained by sequencing to the sequence types obtained in this study total / unique can better explain the depth of sequencing. If the depth of sequencing is not enough, it can not better reflect the expression of small RNA in the samples. In this study, the total / unique ratio of Library sequencing was 24.17 %. In addition, the proportion of tRNA in sequencing results can directly reflect the quality of samples. Generally, the proportion of rRNA in animal samples with better quality should be less than 40 %, and that in plant samples should be less than 60 %. In this study, only 0.02 % of tRNA was sequenced, while the unmatched sequence accounted for 82.86 %, which might be the candidate sequence of new miRNAs in testes tissues, because many references (Ballester et al., 2010, Baranda-Avila et al., 2010, Estellé et al., 2006, Saenz-de-Juano et al., 2013, Xue, 2014) reported gene expression only in female rabbit reproductive organs.

CONCLUSIONS

Results show that, the quality, the purity of total RNA and integrity of the total RNA were good, which can meet the requirements of construction of small RNA library and the applicability of obtained sequence in subsequent miRNA identification analysis. Unique reads accounted for 24.17 % of the total sequences The length of total number is mostly between 22 - 31 nt, which takes up more than 89.32 % of pure sequences. 82.86 % of cleaned sequence were not mapping to any previously known RNA in the reference genome annotation. miRNA sequence length imbalance may be related to the action mechanism and specific functions of miRNA in testes tissues.

ACKNOWLEDGMENTS:

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Rex Rabbit Illumina Sequencing and Bioinformatics Analysis on miRNA in testes

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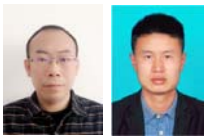

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Context and objectives: miRNA plays an important regulatory role in reproductive biology and spermatogenesis. This research uses Illumina sequencing technology to conduct miRNA sequencing on adult male rabbits to explore the biological characteristics of miRNA in testes tissues.

Methods : Rex rabbit testes sampling and total RNA extraction three healthy seven-month male Rex rabbit were selected for sample collection and preparation. Total RNA from the samples were extracted, qualified, Library generation and sequencing. A total amount of 3 μg total RNA per sample was used as input material for the small RNA library.Raw data (raw reads) of fastq format were firstly processed through custom perl and python scripts. The small RNA tags were mapped to reference sequence by Bowtie without mismatch to analyze their expression and distribution on the reference. Mapped small RNA tags were used to looking for known miRNA. miRBase20.0 was used as reference, modified software mirdeep2 and srna -tools-cli were used to obtain the potential miRNA and draw the secondary structures. Custom scripts were used to obtain the miRNA counts as well as base bias on the first position of identified miRNA with certain length and on each position of all identified miRNA respectively.

Results : The quality, the purity of total RNA and integrity of the total RNA were good, which can meet the requirements of construction of small RNA library and the applicability of obtained sequence in subsequent miRNA identification analysis. Unique reads accounted for 24.17 % of the total sequences. The length of total number is mostly between 22 - 31 nt, which takes up more than 89.32 % of pure sequences. 82.86 % of cleaned sequence were not mapping to any previously known RNA in the reference genome annotation.

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Take home message : In this study, only 0.02 % of tRNA was sequenced, while the unmatched sequence accounted for 82.86 %, which might be the candidate sequence of new miRNAs in testes tissues, because many references reported gene expression only in female rabbit reproductive organs.