miRNA-Seq

The miRNA molecules are influencing gene expression by post-transcriptional mechanisms and coming to the forefront of interest in the science and medicine field. Explore your miRNAs with our knowledge.

Introduction

miRNA molecules have been a neglected part of the RNA family for a long time. However, recently it has become increasingly clear how important role they play and how they influence gene translation. Micro-RNA, a regulatory factor of all important signaling pathways, influence physiological processes in cells and is often pathological in disease.

IAB Expertise

To perform miRNA analysis requires good quality of miRNA samples. During the library preparation are usually present obstacles associated with the selection of the miRNA fraction from the total RNA sample. Due to its size (18-34 nt), removing long fragments (> 200 bp) and adaptor-dimer sequences from the miRNA library might be limited or impossible. The presence of unwanted dimer molecules leads to 1) a partial or complete loss of library during additional clean-ups and 2) lower or even ruin the descriptive potential of sequencing data. Also, 3) the limited input is more prone to bias the abundance of different miRNA species as more amplification steps in the library preparation protocol are needed. The secondary analysis includes reads trimming, miRNA counting, annotating sequences using miRbase and expression analysis. Please contact us for any special analysis requirements or more information.

Protocol

Sample

Total RNA or miRNA: input 100 ng

Quality Control (RNA)

- RNA quantity: C > 20 ng/µl in total volume > 5 µl (100 ng RNA)
- RNA integrity: Higher RIN score in total RNA isolates refers to the higher quality of NGS libraries. For miRNA isolates, RIN is not evaluated.

Library preparation

- QAIseq miRNA library kit (Fig 1)

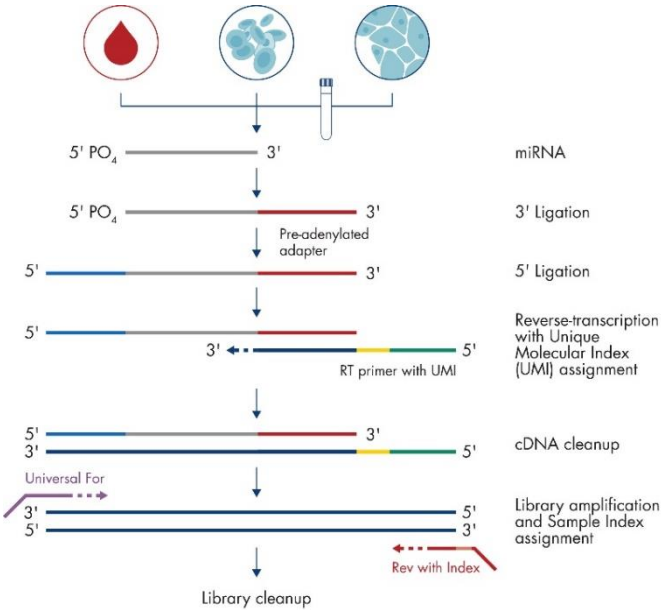

Figure 1

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Library QC

- Quantification (C > 4 nM)
- Quality check by capillary electrophoresis (peak 187 bp with no signal in 157 bp marking the adaptor dimers)
- Normalization of libraries based on molarity

Sequencing

- NovaSeq X Plus (Illumina, USA)
- Pair-end sequencing 2×150 bp in shared experiment (minimal requirement is 1x50 bp)
- %Q30 > 75% (full NovaSeq X Plus specifications)

Results

IAB offers a precise method for the miRNA library preparation, whether from totalRNA or miRNA isolates. Thanks to our expertise, we perform successful sequencing experiments and identify the specific miRNA sequences present in the sample through reads aligned to a reference miRNA database.

Conclusions

Micro-RNA analysis is used especially in the area of determination of the expression level of specific miRNAs in different samples or conditions, identifying novel miRNAs that are not yet known or annotated in databases, exploring the role of miRNAs in various biological processes and diseases or developing of diagnostic or prognostic markers for diseases. Overall, miRNA sequencing is a powerful tool for studying the functions of miRNAs and their potential as therapeutic targets or diagnostic markers.

About IAB

The experienced team follows IAB Exome workflow with strict quality procedures to perform the essential QC steps on your samples and generate reliable and repeatable results.

Illumina

Illumina is a global leader in sequencing and array technologies that are fueling groundbreaking advancements in life science research, translational and consumer genomics, and molecular diagnostics.

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