Title: RNA-seq

Abstract: Among different methods to evaluate gene expression, the high-throughput sequencing of RNA, or RNA-seq. is particularly attractive, as it can be performed and analyzed without relying on prior available genomic information. During RNA-seq, RNA isolated from samples of interest is used to generate a DNA library, which is then amplified and sequenced. Ultimately, RNA-seq can determine which genes are expressed, the levels of their expression, and the presence of any previously unknown transcripts.

Here, JoVE presents the basic principles behind RNA-seq. We then discuss the experimental and analytical steps of a general RNA-seq protocol. Finally, we examine how researchers are currently using RNA-seq, for example, to compare gene expression between different biological samples, or to characterize protein-RNA interactions.

Application Videos:

1. An Experimental and Bioinformatics Protocol for RNA-seq Analyses of Photoperiodic Diapause in the Asian Tiger Mosquito, *Aedes albopictus* (**51961 Thumbnail@7:39-mosquitoes getting homogenized in glass tube**)

Description: The development of many organisms can be influenced by environmental factors, such as light exposure. This article details how RNA-seq can be used to compare gene expression in developmentally delayed and normal insect embryos, respectively produced by mosquitoes that are exposed to light cycles mimicking “short” and “long” days. The results shed light on the differential expression of genes important for the development of this disease-carrying insect.

1. Massively Parallel Reporter Assays in Cultured Mammalian Cells (**51719 Thumbnail @0:52-schematic of MPRA results analysis**)

Description: Regulatory sites play a pivotal role in gene expression, serving as regions to which transcription factors can bind and modulate transcription activity. Here, scientists attached mutated sequences of the same regulatory site to reporter genes “tagged” with unique sequences, and transfected cells with these constructs. By applying RNA-seq to specifically quantify tag sequences—a technique known as “Tag-seq”—researchers were able to understand how changes in regulatory sequences affect gene expression.

1. iCLIP – Transcriptome-wide Mapping of Protein-RNA Interactions with Individual Nucleotide Resolution (**2638 Thumbnail@4:02-talent washing beads in tubes in rack**)

Description: In this video, researchers demonstrate how RNA-seq can be adapted to identify transcripts that are recognized by a protein of interest. Protein-RNA complexes were isolated through a process called “crosslinking and immunoprecipitation” or CLIP, and the protein-associated RNA was extracted, reverse transcribed and sequenced. Careful assessment of the lengths and locations of the resulting sequences allowed researchers to determine the specific nucleotides recognized by the RNA-binding protein.

1. RNA-seq Analysis of Transcriptomes in Thrombin-Treated and Control Human Pulmonary Microvascular Endothelial Cells (**4393 Thumbnail@7:39-40-talent’s hand/flow cell and sequencer**)

Description: Gene expression can be affected by changes in a cell’s environment, such as exposure to a signaling molecule. Here, researchers used RNA-seq to compare gene expression in untreated human blood vessel cells and those exposed to thrombin—a protein involved in blood clotting.

1. PAR-CliP – A Method to Identify Transcriptome-wide the Binding Sites of RNA Binding Proteins (**2034 Thumbnail@8:25-talent with scalpel, removing piece from gel**)

Description: One variation of CLIP, which relies on UV-induced formation of covalent bonds between proteins and their interacting RNAs, makes use of “photoreactive ribonucleoside analogs” that allow more efficient crosslinking between these molecules. A demonstration of this protocol, called PAR-CLIP, is provided here.

Related SciEd Videos:

5038 – Introduction to the Spectrophotometer

5069 – DNA Ligation Reactions

5338 – Invertebrate Lifespan Quantification

5546 – Introduction to Gene Expression