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Sequencing and Data Analysis of Prokaryotic 5’-Transcript Ends.
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Abstract

Next generation sequencing technology has dramatically changed the way gene expression profiles are carried out. Studying the 5'-transcript ends of prokaryotes genes has been difficult until recently due to technical difficulties in enriching for mRNAs that lack 3' poly(A) tails and 5' cap. Using E.coli as a model, we are trying to develop a simple process to sequence and analyze prokaryotic 5'-transcription start site (5'TSS) libraries. We have been testing construction parameters such as 1) the volume of RNA SPRI used for enzyme reaction clearance, 2) the concentrations of hexamer-3'adapter for reverse transcription (RT) as well as 3) different ways alone or combined to remove all kinds of rRNA. Our approach to library construction and the subsequent prediction of transcription start sites should contribute to genome annotation and cell biology research.

1. 5’-TSS Library Construction Procedure

Total RNA

Ribonuclease or MicroExpress and 5’ Terminator
Exonuclease to remove 23s, 16s, 5s rRNA and mono-phosphate mRNA.

OH---------
P__________
PPP-------

5’adapter-3’adapter

SuperScript III

Tobacco Acid Phosphatase (TAP)

OH-------
P-------
PPP-----

T4 RNA ligase 1

5’adapter

cDNA

PCR

Library QC

gPCR

5’adapter-3’adapter

SAM-to-BAM

Upload:

Sequencing file
Reference genome file
Barcode file

Process

Barcoded Reads

Map with Bowtie for Illumina

Display with IGV

Galaxy tools is available at https://galaxy.jgi-psf.org

2. Procedure of Data Analysis using Galaxy Programs

3. Comparison of Different Ratios of RNA SPRI Beads for reaction Clearance

Different volume of RNA SPRI beads were used after enzyme reactions: (1)CNUC, 1:1.0; (2)CNUB, 1:1.4; (3) CNUF, 1:0.8; and (4) CNUA, 1:1.8. Results show that 1:1.8 has been difficult until recently due to technical difficulties in enriching for mRNAs that lack 3' poly(A) tails and 5' cap. Using E.coli as a model, we are trying to develop a simple process to sequence and analyze prokaryotic 5'-transcription start site (5'TSS) libraries. We have been testing construction parameters such as 1) the volume of RNA SPRI used for enzyme reaction clearance, 2) the concentrations of hexamer-3'adapter for reverse transcription (RT) as well as 3) different ways alone or combined to remove all kinds of rRNA. Our approach to library construction and the subsequent prediction of transcription start sites should contribute to genome annotation and cell biology research.

4. Comparison of Different Concentrations of Hexmer-3’adapter Used in RT

5. Comparison of Different Methods for Removing rRNA

Different methods of removing rRNA and RNA were compared: (1) COWN, non treatment; (2) COWO, MicroExpress alone; (3) COWP, MicroExpress plus Terminator Exonuclease; (4) COWS, Terminator Exonuclease alone; (5) COWT, Ribo-Zero alone and (6) COWU, Ribo-Zero plus Terminator Exonuclease. Figure (A) shows some species of 16s and 23s RNA can’t be removed by MicroExpress and Terminator Exonuclease. Figure (B) shows the best results were obtained by using Ribo-Zero combined with Terminator Exonuclease.

We would like to thank Edward Kirton for Galaxy technical support