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Microbial RNA Sequencing

Prokaryotic mRNAs are polycistronic (polygenic) and usually carry information for the synthesis of several polypeptides from a single mRNA. Prokaryotic RNA-seq is used to reveal the presence and quantity of RNA at a given moment in a single species. Meta-transcriptome analysis offers a method to sequence transcripts in a natural population or community (e.g., soil, water, or gut), and release gene expression profiling, taxonomic components, functional enrichment and more.

Why Novogene?

Extensive experience with over 3000 projects

Industry-leading data quality guarantee

In house pipeline to meet different analysis requirement

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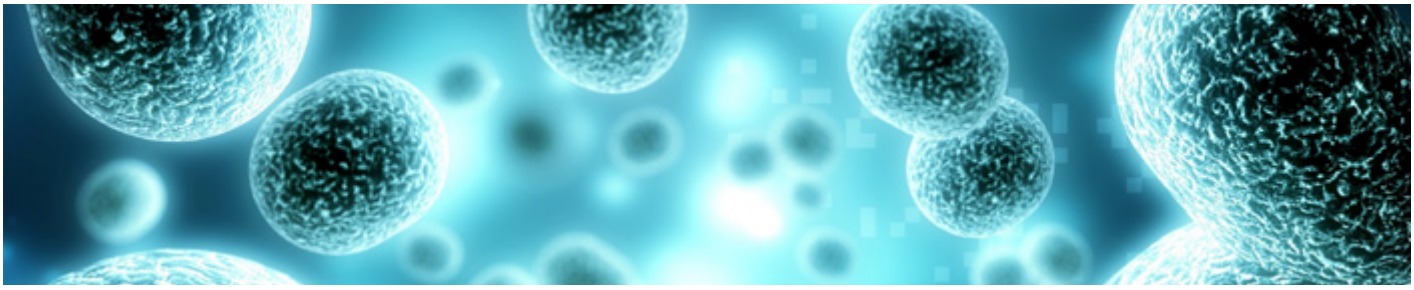
Sample requirements

Service Type	Sample Type	Amount	Volume	Concentration	RNA Integrity number	Purity
Prokaryotic RNA-seq	Total RNA	≥ 2 μg	≥ 20 ng	≥ 50 ng/ μL	≥ 6.0; Flat base line	OD260/280≥ 2.0 OD260/230 ≥ 2.0 No degradation No contamination
Meta-transcriptome					≥ 6.5; Flat base line	

Sequencing parameters

Service type	Library type	Platform	Read length	Recommended data amount	Data quality	Turnaround time
Prokaryotic RNA-seq	rRNA removal & directional RNA library	Illumina NovaSeq 6000	Pair-end 150	≥ 2Gb per sample	Q30 ≥ 85%	16 working days
Meta-transcriptome	rRNA removal & directional/ non-directional RNA library			≥ 12Gb per sample		20 working days



Publications using Novogene's expertise

bioRxiv, 2020.
Identification of Avramr1 from Phytophthora infestans using long read and cDNA pathogen-enrichment sequencing (PenSeq)

Animals, 2019.
Age-Dependent Expression of MyHC Isoforms and Lipid Metabolism-Related Genes in the Longissimus Dorsi Muscle of Wild and Domestic Pigs

Applied and Environmental Microbiology, 2019.
l-Rhamnose Metabolism in Clostridium beijerinckii Strain DSM 6423

Journal of Oral Pathology & Medicine, 2018.
Copy number variation: A prognostic marker for young patients with squamous cell carcinoma of the oral tongue

Scientific Reports, 2018.
Csde1 binds transcripts involved in protein homeostasis and controls their expression in an erythroid cell line

PLOS ONE, 2018.
Strap associates with Csde1 and affects expression of select Csde1-bound transcripts

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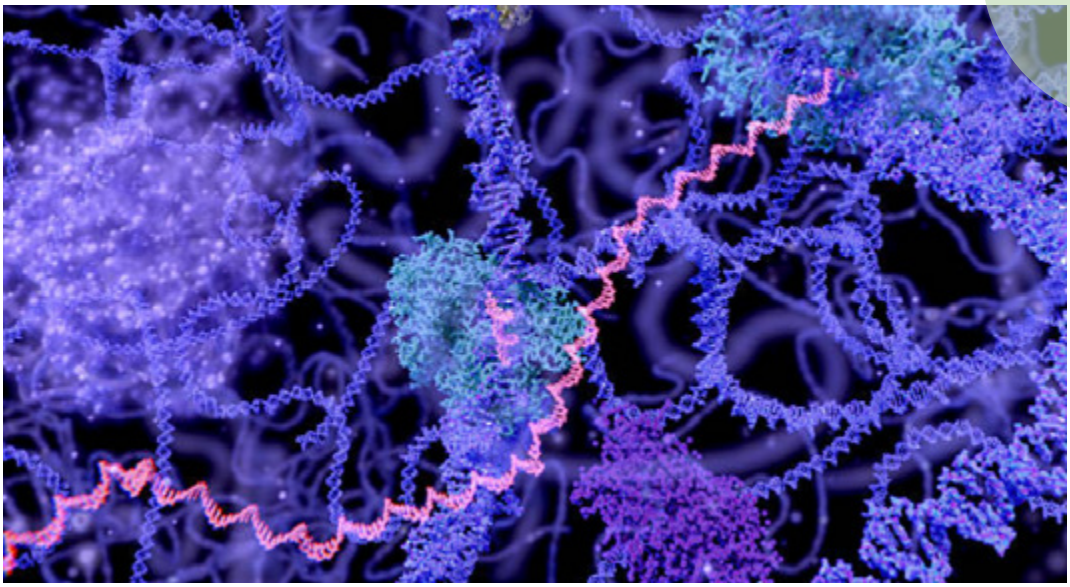
Case study

Global mapping transcriptional start sites revealed both transcriptional and post-transcriptional regulation of cold adaptation in the methanogenic archaeon Methanolobus psychrophilus.

Li et al., 2015. Scientific Reports. DOI: 10.1038/srep09209



Research objective:

To investigate an integrated transcriptional and post-transcriptional regulation for cold adaptation in a psychrophilic methanogen.

Sample collection:

Total RNA extracted from *M. psychrophilus* cultured at 8°C and 18°C.

Sequencing strategy:

Single-end sequencing for dRNA-seq libraries and paired-end sequencing for whole-transcript libraries. Both were sequenced on the Illumina HiSeq 2000 platform which produced reads of 100bp.

Data amount:

104 million reads in total.

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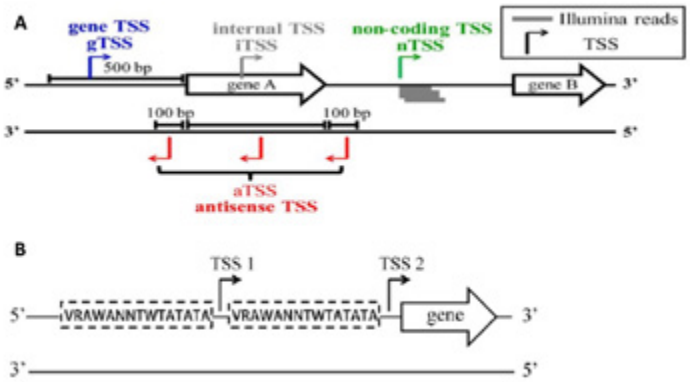

Figure 1
(A) Schematic explanation of the various types of TSSs identified in *M. psychrophilus.* Gene (g); internal (i); antisense (a); and non-coding (n) RNA. Grey lines represent the Illumina reads. (B) Schematic of the promoters and gTSSs used by a given ORF. The broken line frames identify potential promoter features.

Results

Most prokaryotic mRNAs have short (20-40nt) untranslated regions (UTRs), but 51% of the mRNAs of *M. psychrophilus* are above 50nt, implying that post-transcriptional regulation (PTR) of these mRNAs may be significant to this particular psychrophilic methanogen. mRNA secondary structure responds faster to cold than proteins, so the transcription start sites (TSSs) and how they change in response to cold adaptation were examined.

219 genes contained multiple TSSs, and 84 genes exhibited the temperature-regulated gTSS that resulted in alternative 5' UTR expression. Masking of ribosome binding sites (RBSs) by stem-loop formation was detected in response to cold, and many sRNAs were also induced by cold conditions, indicating the role of transcriptional and post-transcriptional regulation of RNA molecules in cold adaptation of the psychrophilic methanogen.

Conclusions

This study showed a very dynamic transcriptome of *M. psychrophilus* in response to cold. The multiple types of TSS identified gave insight into the mechanism used for cold adaptation, which is a transcriptional and post-transcriptional one, rather than translation, as mRNA can respond much more rapidly to environmental signals than protein. Depending on where along the UTR transcription is initiated, RNA molecules can be transcribed with shorter UTRs resulting in regular or over-expression, or with longer UTRs with stem-loops and other RBS shielding structures to reduce expression levels.

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