Native Elongating Transcript Cap Analysis of Gene Expression

DNAFORM
Precision Gene Technologies

NET-CAGE

5'-RNA sequence service/kit

Quantify all mRNA / ncRNA during transcription
New methods for gene expression

Analysis to enable identification of active enhancers

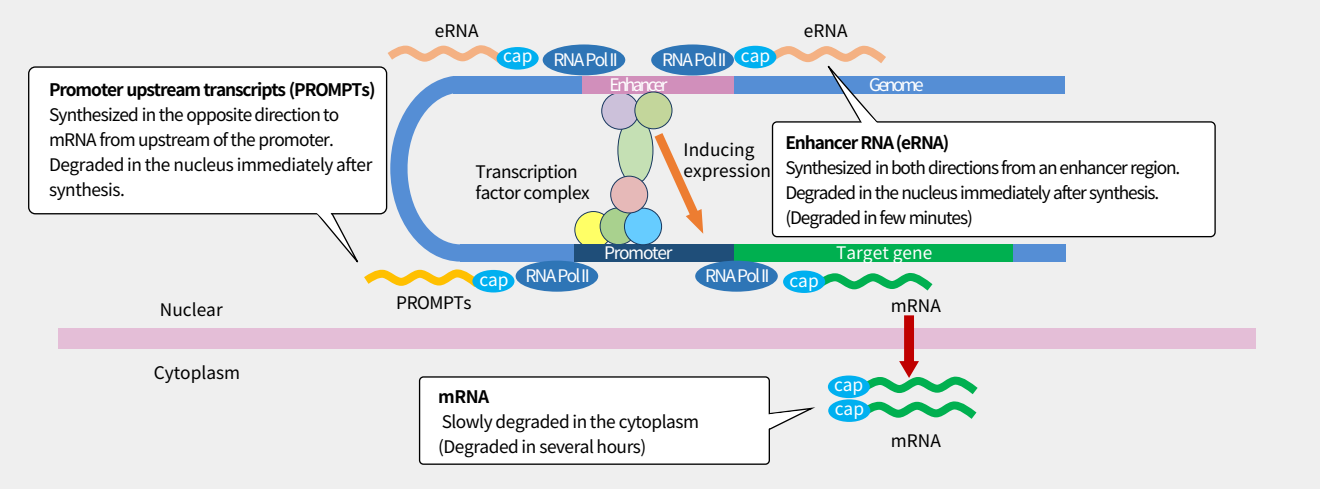
- ☑ Novel gene expression analysis based on nascent RNA (NET-RNA)
- ☑ Captures short-lived eRNA and identifies enhancer regions
- ☑ Gene expression analysis based on instantaneous transcriptional activity
- ☑ Just send the cells or tissues to us, delivered with Bioinformatics analysis

https://cage-seq.com

For Research Use Only

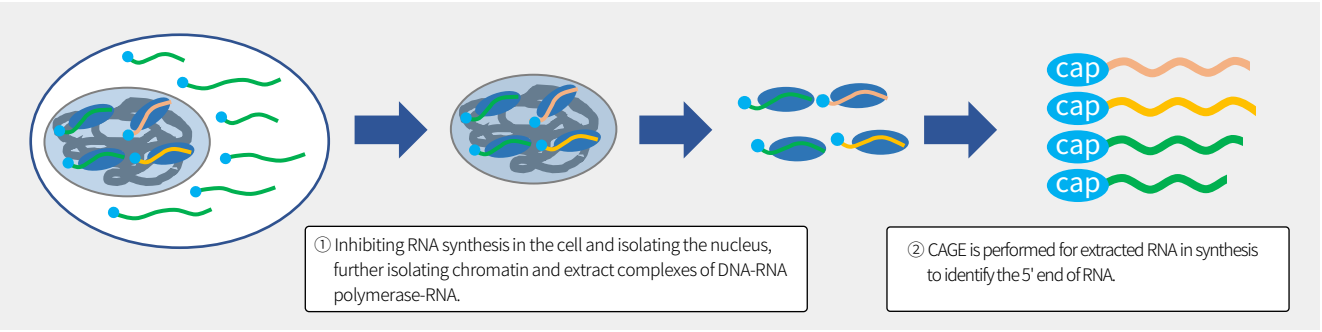
Discovery of short-lived non-coding RNAs

In addition to mRNA, RNA polymerase II is known to transcribe other RNAs (long-non-coding RNAs) such as enhancer RNAs (eRNAs), which are expressed in both directions from the enhancer region, and promoter upstream transcripts (PROMPTs), which are transcribed in the opposite direction to mRNA from upstream of the promoter. eRNAs and PROMPTs have a 5'-end cap structure, are extremely short-lived and are degraded in the nucleus immediately after synthesis.



NET-CAGE : New transcriptome analysis to capture nascent strand RNA

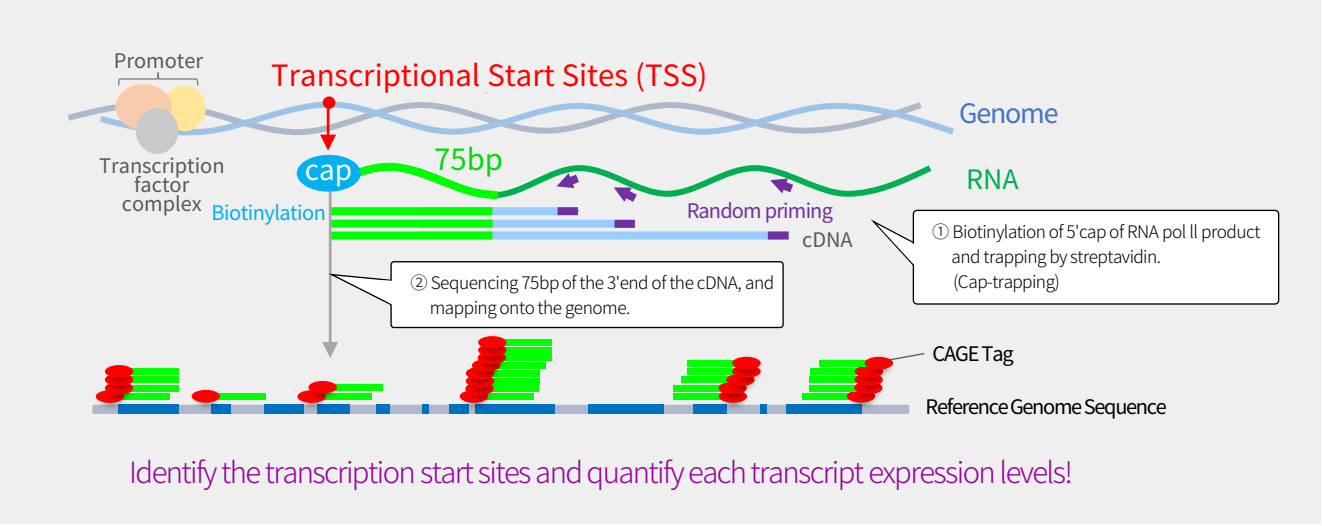
NET-CAGE (Native Elongating Transcript Cap Analysis of Gene Expression)[1] is a technology that concentrates nascent RNA in the nucleus and maps the position of transcription start sites of the nascent RNA on the genome with accuracy of single nucleotide resolution. Not only mRNA, but also short-lived RNAs such as eRNAs and PROMPTs can be captured and quantified.



1: Hirabayashi *et al. Nature Genetics* 2019 volume 51, 1369–1379

What is CAGE?

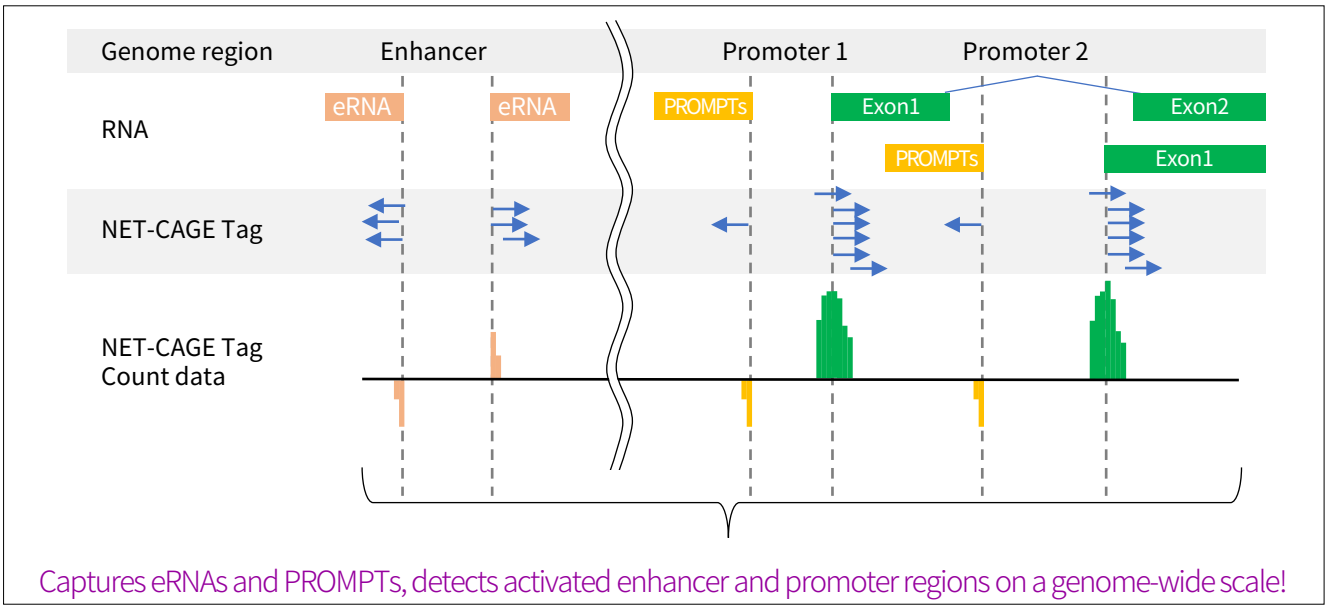
CAGE (Cap Analysis of Gene Expression) is a technology for determine sequence of the 5' end of RNA and mapping it onto the genome based on the "Cap-trapping" method, which captures the Cap structure of RNA polymerase II transcripts.



Identify the transcription start sites and quantify each transcript expression levels!

Detection of active enhancer and promoter regions by NET-CAGE

Transcription start sites of eRNAs that are expressed in both directions from enhancer regions, enables the identification of active enhancers in high resolution. PROMPTs can also be used to identify the extent of activated promoter regions. Furthermore, NET-CAGE capture mRNAs in transcription at the same time, enables to quantify based on the true transcript expression levels of mRNAs.



Captures eRNAs and PROMPTs, detects activated enhancer and promoter regions on a genome-wide scale!

Fig 1. Detecting active enhancer and promoter regions

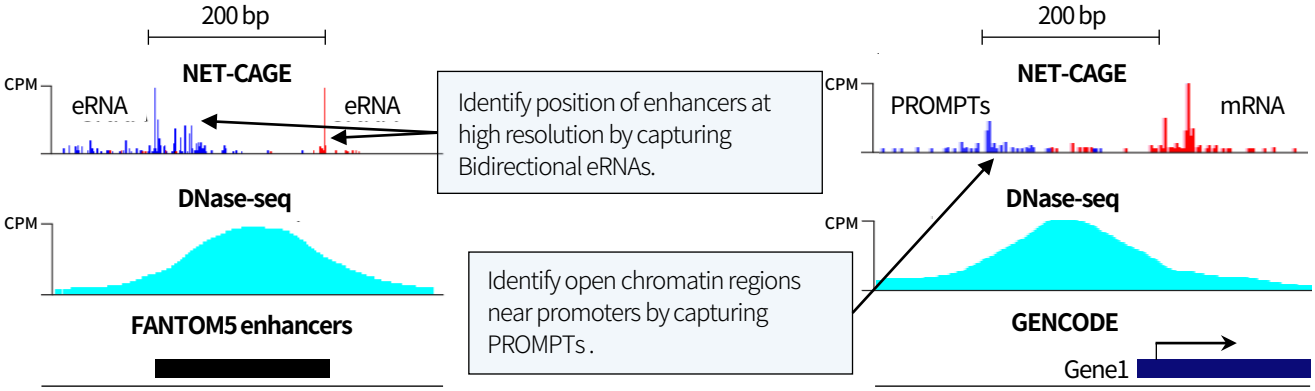
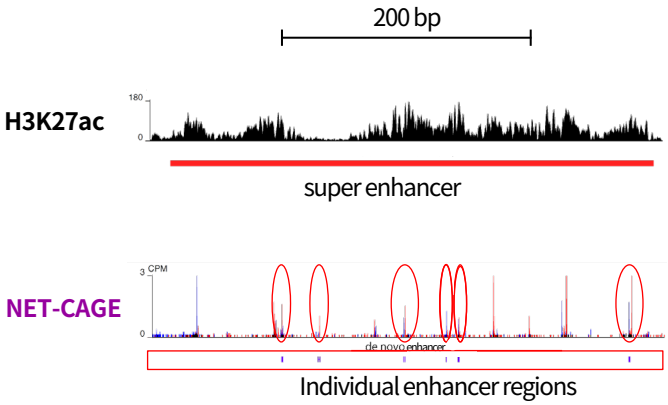
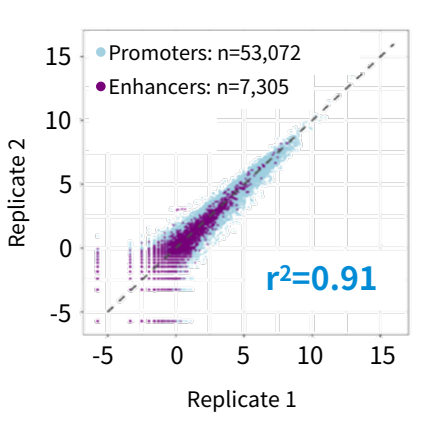


Fig 2. Detecting individual enhancers in a super-enhancer



High-resolution identification of individual enhancer regions in known super-enhancer by NET-CAGE!

High reproducibility



Two replicates of NET-CAGE were performed on the same sample and the expression level were plotted(Log_2CPM).High correlation coefficients are shown.

NET-CAGE Features

1. Activated enhancer and promoter regions can be identified at high resolution.
2. Unlike GRO-seq and PRO-seq, analysis is possible from frozen samples and tissue samples. * 1
3. Gene expression comparison data based on real-time transcriptional activity is also obtain at the same time.
4. Just send the cells or tissue to us. Delivered with Bioinformatics analysis.

* 1 Some tissue types, such as bone and cartilage, may be difficult to analyze with NET-CAGE. Please consult in advance.

Table 1. Features of NET-CAGE and other transcriptome analyses ("N/A" means not applicable)

Purpose of the study	NET-CAGE	CAGE	RNA-seq	ChIP-seq
de novo Gene / ncRNA finding	good	good	good	average
Gene expression quantification	superior	superior	good	N/A
Determing a promoter sites	superior	superior	average	good
Motif finding for the transcription factor binding site	superior	superior	average	superior
Determing the eRNA / PROMPTs	superior	average	N/A	N/A
Determing transcription start / 1st exon site	superior	superior	average	N/A
Determing gene structure (intron/exon, fusion gene, alternative splicing variants)	N/A	N/A	average	N/A
Difficulty of sample adjustment	average	average	good	N/A
Gene expression comparison based on instantaneous transcriptional activity	superior	N/A	N/A	N/A

Project workflow

1. Sample submision

Sample	Cells or tissues*1
Number of cells (for cultured cells)	About 2×10^7 cell*2
Sample condition	Frozen at -80°C

*1 Some tissues may not be analyzed.
Please consult us in advance together with the sample volume.
*2 If possible, please send two tubes of 2×10^7 cells.

2. Library preparation/ sequences

NGS	NextSeq2500/NextSeq500
Amount of data	10-15million reads/sample
Sequencing	50bp/75bp Single-end

Total 5-8 weeks

3. Bioinfomatics analysis

- Raw sequencing data (FASTQ format)
- Genome mapping data (BAM format)
- TSS cluster with anotation
- Differential expression analysis of TSS Clusters ScatterPlot, Heat map, Clustering)
- Comparison of expression with enhancers detected in the RIKEN FANTOM project. Motif search analysis

Ordering information

CAGE library preparation & analysis services

Services	Price [1]
NET-RNA extraction	150 USD/sample
Library preparation [2]	500 USD/sample
Sequencing	250 USD/sample
Bioinformatics analysis	250 USD/sample

1. Shipping : 200 USD/shipment
2. Library is prepared for Illumina-platform

YouTube **Check out our YouTube channel!**
Technical webinar about NET-CAGE
https://www.youtube.com/@user-ky4xw1tz2i/videos

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