Monolith Protocol MO-P-026

mRNA – $sRNA_{41}$

Methanosarcina mazei is a model organism for methanoarchaea growing under strictly anaerobic conditions. Next to regulatory proteins, also small noncoding RNAs (sRNAs) are involved in nitrogen (N)-regulation, such as $sRNA_{41}$, which is highly expressed under N-sufficiency. One of its targets is '5'UTR MM2089 mRNA, a polycistronic mRNA encoding for acetyl-CoA-decarbonylase/synthase complexes (ACDS).

RNA – RNA interaction | Nitrogen (N)-regulation

A1. Target/Fluorescent Molecule

'5'UTR MM2089 (mRNA)

A2. Molecule Class/Organism

Polycistronic mRNA
Methanosarcina mazei Gö1

A3. Sequence/Formula

5' Cy3 AGU AGC UGA AGC UUU CAA GAA UCG UCG GUA AAG UGA CUU UAA GUA UAU UUA GGA GGU AAA GCU AAA AUG AGC AAA UUA AC 3'

A4. Purification Strategy/Source

Eurofins Genomics GmbH

A5. Stock Concentration/Stock Buffer

1 µM
2x Structure Buffer (Ambion, Thermo Fisher Scientific)

A6. Molecular Weight/Extinction Coefficient

26.1 kDa

A7. Labeling Strategy

5' Cy3 labeled

A8. Assay Buffer

1x Structure Buffer (Ambion, Thermo Fisher Scientific), 0.02% TWEEN® 20

A9. Labeling Procedure

N/A

A10. Labeling Efficiency

HPLC-purified, 100% labeled RNA

B1. Ligand/Non-Fluorescent Binding Partner

$sRNA_{41}$

B2. Molecule Class/Organism

Noncoding RNA
Methanosarcina mazei Gö1

B3. Sequence/Formula

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5’ GTC TAG CGA ACA GAC GTA AAA ATG GGA AAC GCC TCC TAA ATA TAT TGT ACA AAC TTG CAA TGC
   TGG AAT TCC CCA CCT GTG GTG CAG GCA GGT GTG GAG TTT CAG GTG CAA GGG AGT CAC 3’
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B4. Purification Strategy/Source

In vitro transcription using TranscriptAid T7 High Yield Transcription kit
(Thermo Fisher Scientific, Waltham, MA, USA)

B5. Stock Concentration/Stock Buffer

300 µM
DEPC-H_2O

B6. Molecular Weight/Extinction Coefficient

39.3 kDa

B7. Serial Dilution Preparation

1. Prepare a PCR-rack with 16 PCR tubes. Transfer 10 µL of 2x Structure Buffer and 5 µL of DEPC-H_2O into tube **1**. Then add 5 µL of 300 µM $sRNA_{41}$ and mix by pipetting to obtain 20 µL of a 75 µM $sRNA_{41}$ solution in 1x Structure Buffer.
2. Transfer 10 µL of 1x Structure Buffer into tubes **2** to **16**.
3. Prepare a 1:1 serial dilution by transferring 10 µL from tube to tube. Mix carefully by pipetting up and down. Remember to discard 10 µL from tube **16** to get an equal volume of 10 µL for all samples.
4. Mix 6 µL of 1 µM mRNA with 6 µL of DEPC-H_2O to obtain 12 µL of 500 nM mRNA in 1x Structure Buffer.
5. Add 188 µL 1x Structure Buffer to obtain 200 µL of 30 nM mRNA.
6. Incubate all tubes for 1 min at 95°C followed by 5 min at 4°C.
7. Add 10 µL of 30 nM mRNA to each tube from **16** to **1** and mix by pipetting.
8. Incubate for 15 minutes at 37°C in the dark before loading capillaries.

D1. MST System/Capillaries

Monolith NT.115 Green (NanoTemper Technologies GmbH)
Capillaries Monolith NT.115 (MO-K022, NanoTemper Technologies GmbH)

D2. MST Software

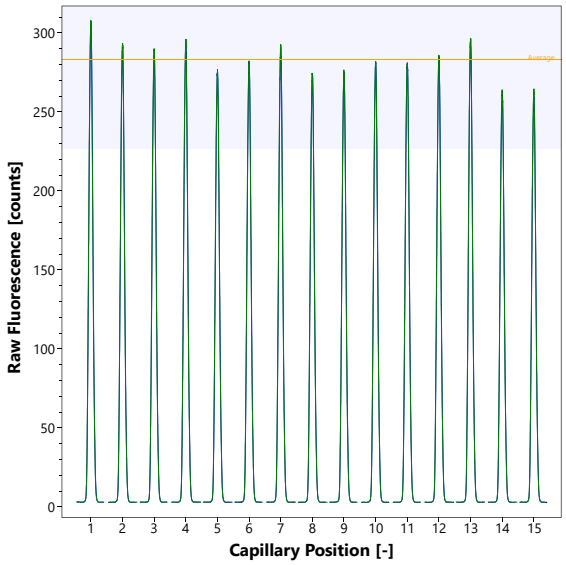
MO.Control v1.6 (NanoTemper Technologies GmbH)
nanotempertech.com/monolith-mo-control-software

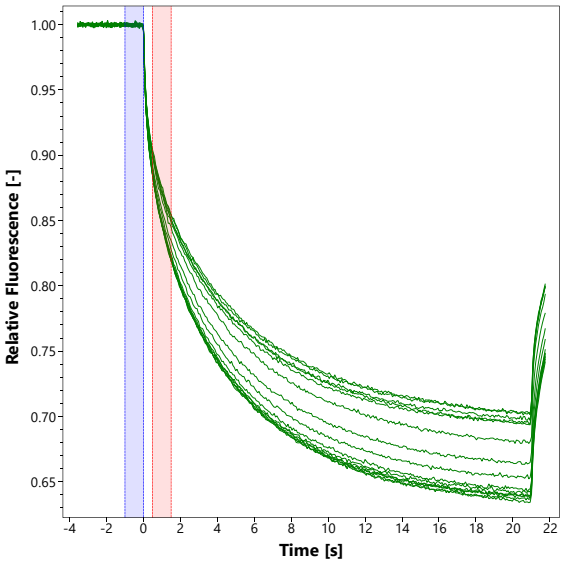
D3. MST Experiment (Assay Buffer/Concentrations/Temperature/MST Power/Excitation Power)

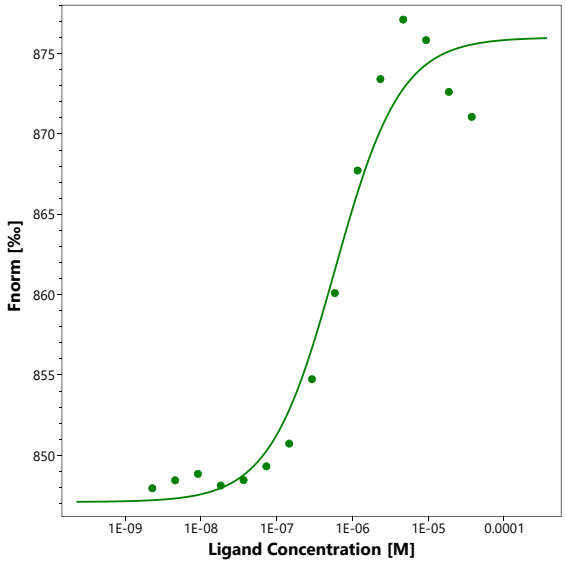
1x Structure buffer, 0.02% TWEEN® 20
15 nM mRNA | 37.5 µM – 1.14 nM $sRNA_{41}$ | 25°C | high MST power | 100% excitation power

D4. MST Results (Capillary Scan/Time Traces/Dose Response)

K_d = 581 nM







D5. Reference Results/Supporting Results

K_d = 631 ± 133 nM Microscale Thermophoresis

Buddeweg et al., Molecular Microbiology 107(5), 595–609 (2018)

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