

Nucleus Developer Notes

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MphR Sensing Module

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Abstract

The MphR sensing module consists of the TetR-family allosteric transcription factor (aTF) MphR in the ROSALIND system. The ROSALIND system is based on the regulation of an *in vitro* transcription of a fluorescence activating RNA known as the 3WJdB (three-way junction dimeric Broccoli) aptamer. In the presence of a suitable binding dye (DFHBI-1T), transcription of the 3WJdB aptamer can be measured in real-time. Transcription can be regulated by including the MphR protein in the *in vitro* transcription reaction with a suitably encoded DNA transcription template that includes the MphR binding site (known as the operator sequence, *mphO*). Repression can be relieved through induction of MphR and resulting transcription can be monitored fluorescently (see [References and Resources](#) below for a more thorough description).

Keywords MphR, Macrolide biosensor, ROSALIND, Cell-free transcription, Allosteric transcription factor

1. OVERVIEW

1.0.1. Description of Module

The MphR sensing module consists of the TetR-family allosteric transcription factor (aTF) MphR in the ROSALIND system. The ROSALIND system is based on the regulation of an *in vitro* transcription of a fluorescence activating RNA known as the 3WJdB (three-way junction dimeric Broccoli) aptamer. In the presence of a suitable binding dye (DFHBI-1T), transcription of the 3WJdB aptamer can be measured in real-time. Transcription can be regulated by including the MphR protein in the *in vitro* transcription reaction with a suitably encoded DNA transcription template that includes the MphR binding site (known as the operator sequence, *mphO*). Repression can be relieved through induction of MphR and resulting transcription can be monitored fluorescently (see [References and Resources](#) below for a more thorough description).

MphR induces transcription in the presence of macrolides - a class of antibiotics (e.g., azithromycin, erythromycin) that are widely used in human and animal health and are included on WHO's list of essential medicines. The availability of biological sensors for macrolides will help the discovery and development of new macrolides and macrolide analogues, and enable the ability to monitor the presence of antibiotics in the environment, which give rise to antibiotic resistance. These compounds represent candidates for antifungal, antimicrobial, and antibacterial medicines that are important for human, animal, and planetary health. Such biological sensors can also enable scalable screening and process optimization in biomanufacturing.

1.0.2. Component specifications

Protein: MphR-6xHis

Sequence ([plain text](#)):

MPRPKLKSDDEVLEAATVVLKRCGPiEFTLSGVAKEVGLSRAALIQRFNTRDTLLVRMME
RGVEQVRHYLNAIPiGAGPQGLWEFLQVLVRSMNTRNDFSVNYLISWYELQVPELRTLAI
QRNRAVVEGIRKRLPPGAPAAAELLHLSVIAGATMQWAVDPDGELADHVLAQIAAILCLM
FPEHDDFQLLQPHAKLAAALEHHHHHH

Plasmid: pJBL715 (Backbone: pET28c, Addgene #140385 - UBMTA)

DNA-reporter: T7-mphO-3WjdB-T

Sequence (plain text):

gcggataacaatttcacacaggaaacagctatgaccatgattacgccaagcttgcatgcc
tgcaggctcgactctagataatacgactcactataggagggaatataaccgacgtgactgt
tacatttaggtggccacatactctgatgatccgagacggctcgggtccagatattcgtat
ctgtcgagtagagtgtgggctcggatcattcatggcaagagacggctcgggtccagatatt
cgatatctgtcgagtagagtgtgggctcttgccatgtgtatgtggtagcataacccttg
gggcctctaaacgggtcttgaggggttttttg

Plasmid: pUC19-T7-mphO-3WjdB-T (Backbone: pUC19, Addgene #140386- UBMTA)

2. MATERIALS AND EQUIPMENT

Name	Product	Manufacturer	Part #	Price	Link	Storage Conditions
Spermidine	Spermidine, ≥99% (GC)	Sigma-Aldrich	S2626-5G	\$176	[link]	–25C to –15C
Tris-HCL	Trizma® hydrochloride anhydrous, free-flowing, Redi-Dri™, ≥99.0%	Sigma-Aldrich	RDD009-1KG	\$268.60	[link]	4C to 30C
MgCl2	Magnesium chloride hexahydrate, BioXtra ≥99.0%	Sigma-Aldrich	M2670-100G	\$50.50	[link]	4C to 30C
DTT						
NaCl	Sodium chloride, anhydrous, Redi-Dri™, free-flowing, ACS reagent, ≥99%	Sigma-Aldrich	746398-500G	\$79.70	[link]	4C to 30C
rATP	Adenosine 5'-triphosphate disodium salt hydrate, BioXtra, ≥99% (HPLC), from microbial	Sigma-Aldrich	A7699-1G	\$125.00	[link]	–25C to –15C

Name	Product	Manufacturer	Part #	Price	Link	Storage Conditions
rGTP	Guanosine 5'-triphosphate sodium salt hydrate, ≥ 95% (HPLC), powder	Sigma-Aldrich	G8877-100MG	\$155	[link]	–25C to –15C
rCTP	Cytidine 5'-triphosphate disodium salt, ≥95%	Sigma-Aldrich	C1506-100MG	\$102.00	[link]	–25C to –15C
rUTP	Uridine 5'-triphosphate trisodium salt hydrate, Type IV, ≥93.0% (HPLC)	Sigma-Aldrich	U6750-100MG	\$58.20	[link]	–25C to –15C
0.3 U TIPP	Thermostable Inorganic Pyrophosphatase	New England Biolabs	M0296S	\$82.00	[link]	–25C to –15C
DFHBI-1T (dye)	DFHBI 1T	Tocris Bioscience	5610	\$346.00	[link]	–25C to –15C
MphR (plasmid)	pJBL715	AddGene	140385	\$85.00	[link]	–25C to –15C
T7-mphO-3WJdb-T (plasmid)	pJBL716	AddGene	140386	\$85.00	[link]	–25C to –15C
Forward primer	GCGGATAACAATTCACACAGGAAACAGC					–25C to –15C
Reverse primer	CAAAAAACCCCTCAAGACCCG					–25C to –15C
PCR Kit	Phusion® High-Fidelity PCR Kit	New England Biolabs	E0553S	\$84.00	[link]	–25C to –15C
T7 RNAP*	T7 RNA Polymerase	New England Biolabs	M0251S	\$74.00	[link]	–25C to –15C

* Alternatively, the T7 RNAP is available within the [DNA Distribution](#) as the component pT7-lacO-UTR1-T7RNAP-tT7hyb6

2.1. Usage

2.1.1. Preparation of purified MphR

Purified MphR from the plasmid pJBL715 is described in Jung *et al* (DOI: 10.1038/s41587-020-0571-7) and is likely compatible with the following guides:

- [Grow and Induce Expression Strains](#)
- [Purify Proteins by Ni²⁺ Gravity Column](#)

2.1.2. Preparation of linear DNA templates

The DNA templates used in the ROSALIND system are purified linear PCR amplicons from the plasmid pJBL716 using the forward and reverse primers as described in Jung *et al* (DOI: 10.1038/s41587-020-0571-7).

It is recommended to use the Phusion PCR kit with the following thermocycle parameters: 98 C for 3 min—(98 C for 30 s, 71 C for 30 s, 72 C for 15 s) 25 cycles—72 C for 10 min—hold at 12 C. Purify the product via spin column into MQ water.

Characterize the size and purity of the product by running ~50–100 ng of the purified DNA template on a 2% TAE agarose gel to confirm the size and purity of the product.

2.1.3. Stocks solutions and buffers

Stock solutions

1. Input Template, Dye, aTF and ligand stock concentrations. Add desired amounts per reaction for template, Dye and aTF. (Ligand is added below in step 6).

	Linear DNA Template	Dye	aTF	Ligand
Name	pT7-mphO-3Wjdb-T	DFHBI-1T	MphR-dimer	aTc
Stock concentration	0.5 uM	40.00 mM	210 uM	50 uM
Amount per reaction	0.5 pmol	45.00 nmol	25 pmol	
Volume per reaction	1.0 uL	1.13 uL	0.12 uL	

10x ROSALIND buffer

Reagent	10x concentration [mM]
Spermidine	20
Tris-HCl (pH 8)	400
MgCl ₂	80
DTT	100
NaCl	200

TRIS buffered nucleotides

100 mM rNTPs are buffered in a 11.4 mM solution of TRIS-HCL (pH 7.5) and store at –25C to –15C.

2.1.4. Transcription Mastermix

2. Prepare a mastermix by mixing the following components at room temperature.

Reagent	Vol (uL) for 1 rxn	Vol (uL) for 5 rxn
10X ROSALIND Buffer	2.00	10.00
100 mM ATP	0.57	2.86
100 mM CTP	0.57	2.86
100 mM GTP	0.57	2.86
100 mM UTP	0.57	2.86
0.3 U TIPP	0.15	0.75
DFHBI-1T	1.13	5.63

Reagent	Vol (uL) for 1 rxn	Vol (uL) for 5 rxn
DNA	1.00	5.00
MphR	0.12	0.60
nuc. free H2O	8.32	41.60
Total	15	75.00

- For each desired reaction, aliquot 15 μL of incubated mastermix in each reaction tube.
- Incubate the aliquoted mastermix at 37° C for 15 min to allow MphR:DNA equilibration.
- Meanwhile prepare 1:10 dilution of T7 RNAP stock (stock concentration: 1 $\mu\text{g}/\mu\text{L}$).
- For each desired reaction, add ligand, diluted T7 RNAP, and remaining water according to the table below. (Modify the table according to your experiment design.) Add these components immediately before transferring to a plate reader for measurement.

Ligand Concentrations [pmol]	Volume of Ligand [uL]	Volume of diluted T7 RNAP [uL]	Volume of H2O [uL]
0	0.00	2.00	3.00
25	0.50	2.00	2.50
50	1.00	2.00	2.00
100	2.00	2.00	1.00

3. PERFORMANCE DATA

The performance of the MphR macrolides sensor is characterized using erythromycin - a common antibiotic used to treat a variety of bacterial infections. Here, 12.5 μM of erythromycin (Sigma Aldrich, cat no. [E5389](#)) dissolved in ethanol is added to a ROSALIND reaction containing 208 μM MphR. The fluorescence response is expressed in units of micro-molar equivalent fluorescein (MEF) using a NIST traceable fluorescein standard (Invitrogen, cat no. [F36915](#))

MphR represses transcription until 2.5 μM erythromycin is introduced with a response saturation occurring at approximately 10 μM erythromycin. The kinetic time series data displayed for 12.5 μM erythromycin shows a response of 0.5 MEF within 1 hour.

The complete study wherein the behavior of MphR is characterized using the ROSALIND system can be found Jung *et al* (DOI: 10.1038/s41587-020-0571-7). There, additional macrolides - azithromycin, clarithromycin, and roxithromycin - were also characterized with slightly higher dose response thresholds to obtain saturation. Despite these different saturation thresholds, visible amounts of fluorescence could be observed within similar time frames. The author's of that study note that the "broad range of MphR specificity is expected based on its known promiscuity, and is probably a common feature of xenobiotic-sensing aTFs."

4. RESOURCES AND REFERENCES

- Other Protocols
 - Jung, J. K., Alam, K. K., & Lucks, J. B. (2022). ROSALIND: Rapid Detection of Chemical Contaminants with In Vitro Transcription Factor-Based Biosensors. In Cell-Free Gene Expression (pp. 325–342) [[link](#)]
- Papers

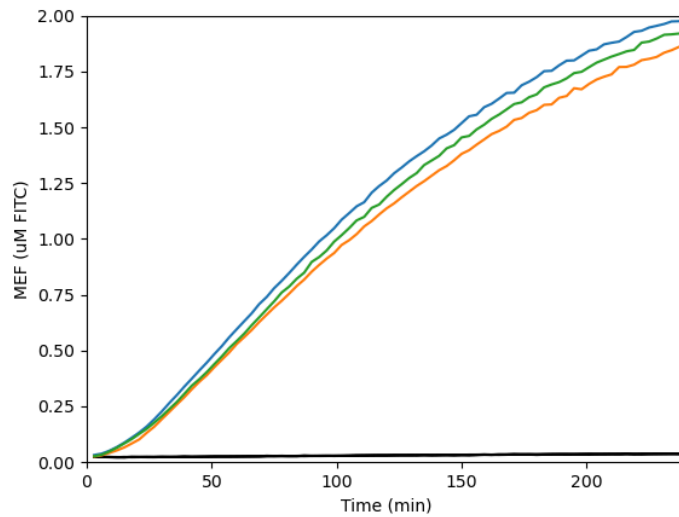


Figure 1. *MphR* can detect erythromycin. The three colored curves represent independent replicates in the presence of 12.5 μM erythromycin. The black curves shows the response with no erythromycin present. Original data from DOI: 10.1038/s41587-020-0571-7

- Alam, K. K., Tawiah, K. D., Lichte, M. F., Porciani, D. & Burke, D. H. A Fluorescent Split Aptamer for Visualizing RNA–RNA Assembly In Vivo. *ACS Synthetic Biology* vol. 6 1710–1721 (2017) [\[link\]](#)
 - Jung, J. K. et al. Cell-free biosensors for rapid detection of water contaminants. *Nature Biotechnology* vol. 38 1451–1459 (2020) [\[link\]](#)
 - Kasey, C. M., Zerrad, M., Li, Y., Cropp, T. A. & Williams, G. J. Development of Transcription Factor-Based Designer Macrolide Biosensors for Metabolic Engineering and Synthetic Biology. *ACS Synthetic Biology* vol. 7 227–239 (2017) [\[link\]](#)
- Resources
 - N/A

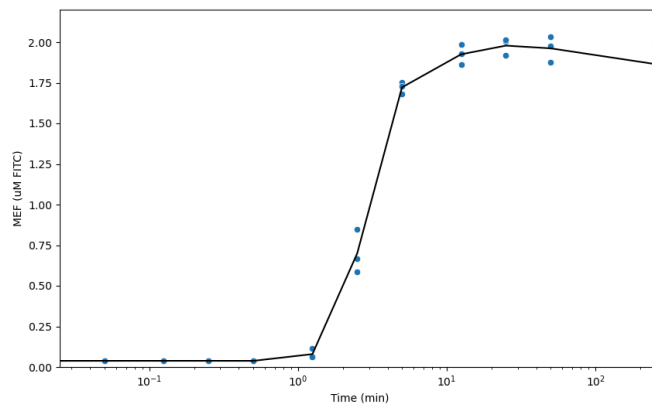


Figure 2. Dose response curve of *MphR* with erythromycin. Each data point is an independent biological replicate. Original data from DOI: 10.1038/s41587-020-0571-7

5. CREDITS

- Developers
 - [Stemloop](#)
- Testers
 - Untested