# mRNA Production of Gene 50 in JacoRen57

Kaytlyn Harms and Dr. Elizabeth Heeg
**Biology Department, Northwestern College**

For further details and Materials/Methods

**ABSTRACT:** Mycobacteriophages are viruses that infect mycobacteria; little is known about their cis-regulatory transcriptional control elements. In previous work, we identified and characterized a regulatory region (40,670- 40,951 bp) in JacoRen57, an AB cluster mycobacteriophage. The regulatory region appears to play a role in regulating the expression of Gene 50. Utilizing qRT-PCR, we investigated further to see if Gene 50 mRNA is produced during an active infection of *Mycobacterium smegmatis* by JacoRen57.

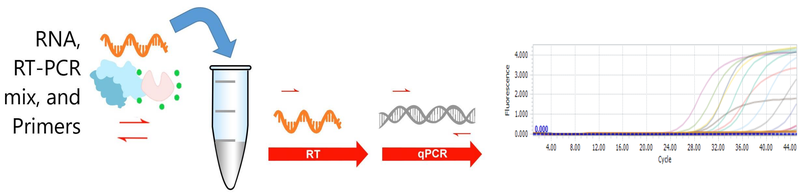



***Figure 1: qRT-PCR Overview.*** qRT-PCR is a biochemical tool that is used to measure gene transcription. The LightCycler® EvoScript RNA SYBR® Green I Master (Roche) is designed for sensitive, high-specificity and precision one-step quantification. RNA is converted to DNA using site specific primers, then as DNA is amplified, SYBR Green dye binds the minor groove of newly synthesized double-stranded DNA causing the dye to increase in fluorescence. This fluorescence is recorded by a fluorimeter and provides the ability to quantify the original template.

## INTRODUCTION

Bacteriophage, JacoRen57, was discovered by Tanner Rensink in February 2018 and the genomic sequencing was completed in August 2018 as part of the SEA-Phages research program at Northwestern College. It was categorized in phage cluster AB and has a lytic lifecycle. It's morphotype is *Siphoviridae*, meaning it has an icosahedral, capsid head and a six-subunit tail which are coded for separately within the genome (King et al., 2012). JacoRen57 is a mycobacteriophage which selectively infects bacteria within the genus *Mycobacterium*. Specifically, the host of this phage is *Mycobacterium smegmatis* mc$^2$155. Sequencing revealed JacoRen57's double stranded DNA genome is 70,300 bp long and consists of 73 genes: 57 forward genes and 16 reverse genes. We observed that one of the gap regions (40,644 - 40,975 bp) between reverse and forward genes possessed minimal coding potential (Almail et al., 2019) (Phagesdb, 2021). From in vitro investigations, we have identified this area as a regulatory region for Gene 50 (Almail et al., 2019). The product of this gene appears to be an ATP-dependent recombinase. It exhibits homology with known proteins including RecA-like recombinase and DnaB (Leipe et al., 2000).

Additional investigation of the RNA and protein expression of Gene 50 is needed. We sought to examine Gene 50 mRNA production during an active infection of *M. smegmatis* utilizing qRT-PCR. To do this we first established a timeline of infection and replication, designed primers to measure Gene 50 expression, and carried out qRT-PCR.

### STEP 1 – Establishing a Timeline of Viral Infection

Before examining if Gene 50 is expressed during JacoRen57 infection, we needed to identify the eclipse phase of the phage – the time between successful cell infection and new virion production. Determining the timeline of viral infection associated with a JacoRen57 infection of *M. smegmatis* enabled us to identify time points to extract samples which would most likely contain our target mRNA. Using plaque assays we noted the eclipse phase occurred from approximately 60 minutes to 120 minutes after introduction of the virus (Figure 2).

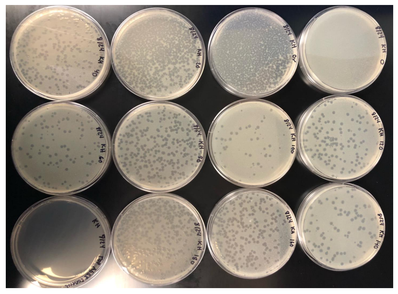


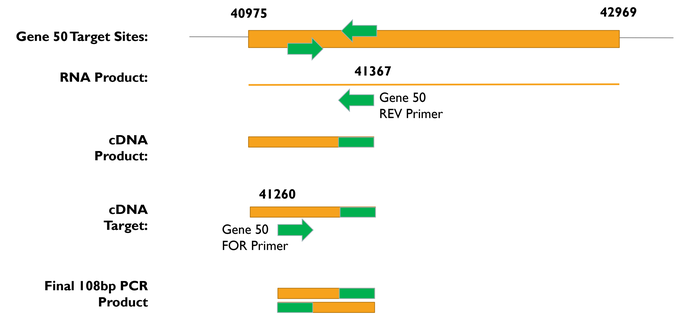
***Figure 2: Establishing Eclipse Phase.*** *M. smegmatis* was infected with JacoRen57 in liquid culture. Every 20 minutes a sample was removed & centrifuged. Viruses that had not entered the host would remain in the supernatant which was then utilized in a plaque assay. The decrease of plaques from one time point to the next indicates virions had entered their hosts in the liquid culture.

### STEP 2 – RNA Isolation of Selected Samples

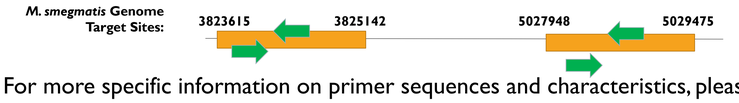
RNA isolation was carried out as described in Prasad et al., 2019. Protoplast buffer was prepared using 1.5 mL of 15 mM Tris-HCl (pH 8), 45 mL of 0.45 M sucrose, 8 mL of 8 mM EDTA, 0.4 g of 4 mg/mL lysozyme, and added dI$H_2O$ for a final volume of 100 mL. 5 mL of protoplast buffer was used to suspend infection pellets. The samples were then incubated at 37°C for 1 hour to promote cell lysis. The suspensions were centrifuged (4000 rpm) for 10 minutes at 4°C to collect the pelleted RNA. RNA was purified from the pellet using the Qiagen RNeasy Mini Kit (Qiagen, 2019). DNA was eliminated from the sample using DNAse. This procedure results in the isolation of both viral and *M. smegmatis* RNA. Isolated RNA was then stored at -80°C until qRT-PCR (see QR Code for more details).

### STEP 3 – Primer Design

Utilizing the JacoRen57 Genome (GenBank ID: MK279840) we created primers to target the region of Gene 50 to give us an amplicon of 108 bp.




We initially utilized primers cited in Prasad et al., 2019, but they had inconsistent qRT-PCR results (Figure 4). We subsequently designed primers to anneal to16S rRNA portions of the *M. smegmatis* genome (target sites seen below). The purpose of these primers are to act as a loading control.




For more specific information on primer sequences and characteristics, please scan the QR code.

### STEP 4 – Testing Gene 50 Primer with Viral DNA

To test the Gene 50 primers, we set-up a qPCR experiment with different dilutions of the high titer lysate, with and without primers and with and without template (dI$H_2O$). Fluorescent quantification was confirmed using gel electrophoresis.. We saw evidence of product in the undiluted high titer lysate leading to the conclusion Gene 50 primers worked when the Gene 50 DNA template is present.

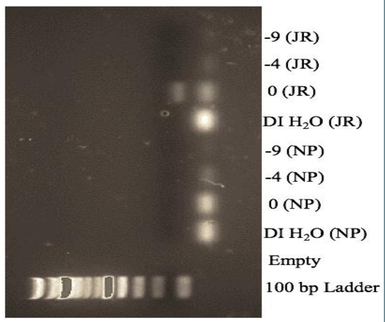



***Figure 3: Confirming Primers.*** The numbers represent the dilution of JacoRen57 high titer lysate. NP, no primers; JR, JacoRen57 primers.

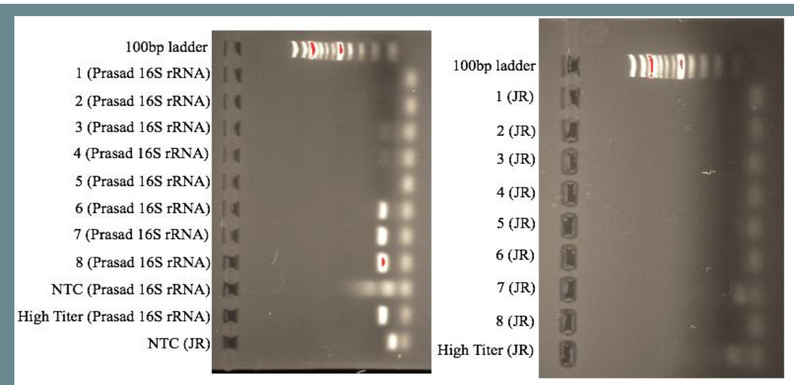



***Figure 4: qRT-PCR.*** The numbers represent the samples from the timeline experiment which were loaded into each reaction. NTC, no template control; JR, Gene 50 primers; Prasad 16S rRNA, Prasad 16S rRNA primers.

### STEP 5 – qRT-PCR Examining Gene 50 Expression

After confirming the Gene 50 primers worked with viral DNA, we wanted to see if we could find Gene 50 in any of our timeline samples. Using the viral high titer as a positive control, we observed a product of the expected amplicon size in sample 7. During the same experiment, we noticed that our loading controls, samples with the Prasad 16S rRNA primers, produced inconsistent results. This led to the redesigning of our loading control primers and running additional qRT-PCRs.

## FUTURE DIRECTIONS

### Protein Expression, Purification, and Antibody Production in Mice

After confirming the presence of Gene 50 mRNA in the active infections, we can begin investigating the protein it codes for. We will express a HIS-tagged Gene 50 in *E. coli* and then utilize $Ni^{2+}$ columns to purify. The purified protein will be used to generate antibodies in mice. Specifically, we will inject this protein into BALB/c mice for them to raise an immune response against the Gene 50 protein product. These antibodies will be collected from serum and used in additional expression studies during *M. smegmatis* infections with JacoRen57.


## SOURCES

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