
# Complementation of Mutant *Pseudomonas aeruginosa* Lacking Periplasmic Proteins PA2807 and PtrA

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By

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# Abstract

The World Health Organization has reported *Pseudomonas aeruginosa* as the second most critical pathogen because of its antibiotic resistance. Upon infection, the host immune response sends macrophages to phagocytize the foreign bodies. Once digested, macrophages commence defense strategies to kill the bacteria. This is achieved by increasing copper levels as toxic anti-microbial effectors in the phagosomes. Normally high levels of copper and other metals will kill bacteria, but *P. aeruginosa* has a highly effective copper homeostasis system. A complex network of copper sensors, regulators and chaperones work in tandem to control the levels of copper in the different cell compartments. Certain periplasmic proteins are upregulated in the presence of high copper levels and assist in maintaining copper homeostasis. Studies have shown that in response to copper stress, mutant cells lacking periplasmic proteins have a different phenotype when compared to the wild strains. Complementing the mutant strains with a vector construct containing the missing gene is necessary to validate the results from previous studies. This study isolated the periplasmic genes PA2807 and PtrA and inserted them into the pHERD 30T vector to transform competent cells that could then be used to complement *P. aeruginosa* mutant strains.

# Acknowledgements

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# Introduction

## Copper: An Essential and Toxic Metal

Essential metals are required for proper function of around one third of proteins and almost 50% of enzymes (1). These proteins and enzymes cannot synthesize essential metals themselves, and so cells have developed various mechanisms for the acquisition of essential metals from their extracellular environment (1). Copper is an example of one of these essential metals. For example, copper is key in the cytochrome c oxidase enzyme in the electron transport chain. Here, copper cycles between its reduced and oxidized states, $Cu^{+}$ and $Cu^{2+}$, transferring electrons to $O_2$ (1,2). Thus, copper is essential for ATP production. Different proteins, called cuproproteins, are exclusively dependent on copper ions as their co-factors. Cuproproteins are involved in the electron transport chain, a host's immune response, and simultaneously part of a pathogenic bacteria's survival mechanism (3).

Even though copper is an essential metal, host cells intentionally exploit the toxicity of high levels of copper as a weapon against bacterial infections (4). Immune responses activate macrophages and phagocytes that harnesses copper ions as antimicrobial agents at the site of infection (3,5). This is done by elevating copper levels (6). When cells create excess levels of copper in the environment of an infection, it leads to oxidative stress (2). High copper levels result in Fenton reactions which convert hydrogen peroxide into toxic free radicals. These products damage the cell membrane through lipid peroxidation, causing the membrane to be more permeable, leading to cell death (7). Excess copper levels also lead to competition between copper ions and other metals. When copper binds in the active sites of metal-dependent proteins, the correct metal co-factor is unable to bind, and therefore the protein can't initiate the necessary

function (8). The toxic copper levels should prevent further infection and kill the invading pathogen, but sometimes that is not the case.

Through evolution, bacteria and other organisms have been able to survive the copper attack by host cells. Bacteria and other infectious microbials already contain proteins that can move and sequester copper as it is a required nutrient. Bacteria use transporters, copper exporting ATPases, copper-binding proteins, chaperones, multi-copper oxidases and other cuproproteins already present to decrease the excess copper levels (4,6). The ability of pathogens to survive copper stress poses a problem (4). Understanding how pathogenic bacteria can reduce copper stress will play a critical role in being able to treat and eliminate bacterial infections.

## *Pseudomonas aeruginosa*

Bacteria, such as *P. aeruginosa*, have several mechanisms that they utilize to cope with metal stress which allows them to strive in environments that would normally be toxic (7). *P. aeruginosa* is a gram-negative and highly opportunistic bacterial pathogen as it is the leading cause of hospital infections (9). This bacterium infects patients with pneumonia, urinary tract infections, cystic fibrosis, as well as immunocompromised patients (10-12).

*P. aeruginosa* uses both intrinsic and acquired resistance mechanisms to avoid the negative effects of copper stress. Intrinsic resistance refers to a mechanism based solely on the inherent structural or functional characteristics encoded for in a cell's genome. the intrinsic resistance of *P. aeruginosa* has been traced back to its outer membrane's low permeability (13). A low membrane permeability decreases the rate that antibiotic molecules can penetrate the cell, thereby diminishing any ability of these antibiotics to be an effective treatment (13). This low permeability provides *P. aeruginosa* with a comparatively high antibiotic tolerance baseline. Other bacteria can be killed with a low dose of antibiotics, but high doses are required to kill *P. aeruginosa*.

Acquired resistance occurs when certain strains or subpopulations of a bacteria are subject to and survive conditions with antimicrobial agents that would normally result in death. There are two types of acquired resistance that are present in the bacterial strains: horizontal genetic transfer and mutational resistance (13). Horizontal genetic transfer can result from conjugation, transformation, or transduction of plasmids that carry antibiotic resistance genes. Mutational resistance occurs spontaneously. An increase in the number of mutations in unrelated genes increases the chances of a bacteria to acquire resistance of certain antibiotics (13).

Researching the genetic makeup of *P. aeruginosa*, whether intrinsic or acquired, and the proteins that are coded for, has become an important area of research. A key part of this research is identifying proteins and their functions that allow *P. aeruginosa* to evade and inhibit a host cell's immune response (13). One example of a well-researched resistance mechanism in *P. aeruginosa* is biofilm formation. When *P. aeruginosa* grows aggregates of cells, called microcolonies, that are encased in a polysaccharide coating on the surface, the cells are referred to as a biofilm (14). Infections caused by biofilms are notoriously difficult to eradicate not only because of their resistance to antimicrobial agents, but because of their resistance to various components of host's immune system (15). Compared to bacteria that cannot form biofilms, bacteria with biofilms are significantly resistant to antibacterial compounds (15,16). This multifactorial resistance mechanism explains why, even with long term treatment, infections caused by *P. aeruginosa* are chronic and present a challenge to treat (16). It has been proposed that the heterogeneous composition of fast-growing and slow-growing cells that make up biofilms could be one of the factors that leads to this multifactorial resistance; some cells are resistant because they are able to express inactivating enzymes and efflux pumps, while others lack the expression of this system (14,16). Other causes of biofilm resistance have been linked to the activation of the general stress

response of *P. aeruginosa* (16). In bacteria, activating their general stress response system increases their resistance to multiple environmental stresses including, but not limited to, oxidative stress, DNA damage, and starvation (16).

## Copper Homeostasis in *P. aeruginosa*

*P. aeruginosa* maintains copper homeostasis through a complex network of proteins. The major proteins involved in copper influx and efflux include copper sensors, regulators, and chaperones. These proteins work in tandem to control the copper levels moving them to and from various cell compartments of *P. aeruginosa* (17). When copper reaches the cytoplasm, a copper defense mechanism is initiated. This defense can be broken down into three stages: scavenging, first responders, and second responders (18). The amount of free copper in the cell is reduced by scavengers binding free copper. The scavengers transport the copper ions to the first responder proteins whose transcription has rapidly increased. Unlike first responders, second responders are not activated during the initial copper shock. The transcription rate of genes that code for second responders are regulated by copper-induced transcription over an extended period copper stress (18).

In the first stage of defense copper chaperones CopZ1 and CopZ2 scavenge free copper and transport it to the first responders (19). Both chaperones bind copper with a 1:1 stoichiometry and have a high affinity for $Cu^{+}$ (17). CopZ1 transports $Cu^{+}$ to CueR, while it is not known where CopZ2 transports $Cu^{+}$ to (20). There is research that supports the development of a metabolic shift occurring; the cell slows down its metabolism so it can focus on responding to the copper stress (18).

The second stage of defense against copper toxicity in *P. aeruginosa* involves first responder proteins. When CopZ1 binds copper, copper efflux regulator CueR is activated even by

the smallest copper concentration (17). Once CueR is activated, it increases the transcription of the CopZ proteins, CopA1 and other genes that are considered first responders due to their fast increase in transcription due to copper stress (17,18). Various genes have been found to be upregulated that have been determined to contribute to copper homeostasis (17). These include PA2807 as a putative $Cu^{+}$ binding protein, PcoA and PtrA, and are all regulated by the CopR regulon (17). Consequently, CopA1 effluxes copper and is assisted by the CopZ proteins. Whether or not CopZ2 delivers copper to CopA2 is unknown (19,20).

In the third stage of response to copper stress the two-component periplasmic copper sensor CopS/CopR is the second responder. The CopS/CopR sensor is activated when elevated copper levels within the periplasm persist (18,21). This two-component system activates other proteins that enable copper export, copper oxidation, and contribute to antibiotic resistance(18). The CopR and CopS proteins work together in the periplasm of *P. aeruginosa* to regulate the levels of free copper in the periplasm. Studies show that CopR and CopS help keep the copper concentration in the periplasm at a constant level. The ability to maintain copper concentrations at this level gives *P. aeruginosa* its survival ability in an otherwise toxic environment (18,20).

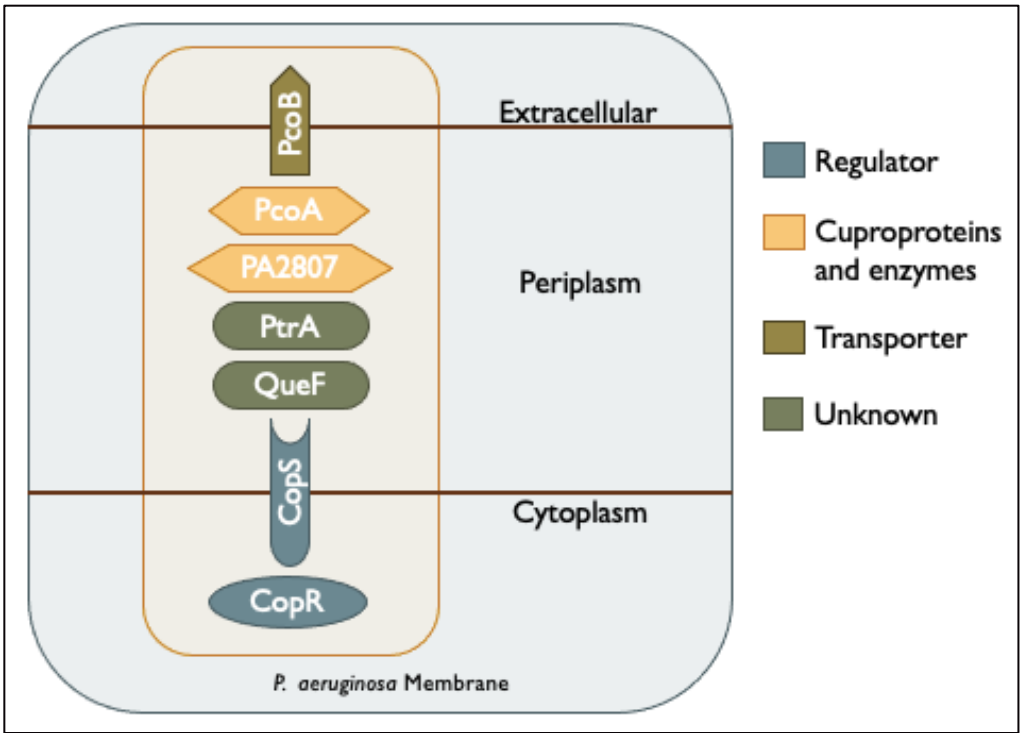


*Figure 1: Model of CopR/CopS Regulon in P. aeruginosa*

The current model and functional classification of proteins involved in copper homeostasis that are controlled by the CopR/CopS regulon are shown in *Figure 1*. Within *P. aeruginosa* various genes are up-regulated or down-regulated in response to copper stress. The CopR/CopS regulon is upregulated in response to copper stress, yet the functions of all the proteins in regulates are not known. These proteins include PA2807, PtrA, PcoA, and QueF.

The protein PcoB is an outer membrane transporter, and proteins CopR and CopS have been classified as regulators (20,21). PA2807 and PcoA have tentatively been put into the enzymatic cuproprotein category, but further research is required to precisely clarify their exact functions. The roles and classifications of the periplasmic proteins PtrA and QueF in the CopR regulon are not well known (20). The function of PtrA has been widely disputed. Some believe it to be a copper-binding protein that contributes to copper scavenging and copper tolerance, but

there has been contradicting results that categorize it transcriptional regulator (18). Research has already shown that the mutant strains response differently than the wild type strains when subjected to different levels of copper stress. The mutants which lacked a specific periplasmic protein died faster than the wild type when coper concentrations were increased. It is necessary to validate these results by complementing the mutants with the gene they are missing in order to recover the wild type phenotype. Verifying these results will aid in producing a clear model of the proteins and mechanisms *P. aeruginosa* activates in response to copper stress will help researchers understand how *P. aeruginosa* is able to survive and thrive in environments that would normally be toxic.

# Materials & Methods

**Bacterial strains, plasmids, and growth conditions:** Bacterial strains, plasmids, and primers sequences can be found in Table 1. The wild type (WT) strain used was *P. aeruginosa* PAO1. The mutant strains used in this study were *ΔPA2807* and *ΔptrA*. All *P. aeruginosa* strains were grown at 37 ºC in Luria-Bertani (LB) medium for 16 hours at 180 rpm. The WT strain was supplemented with 25 µg/mL irgasan while mutant and complemented strains were supplemented with 30 µg/mL gentamicin. *E. coli* strains were grown at 37 ºC and strains were supplemented with the antibiotic corresponding to the resistance of the plasmid. Stock solutions of each strain were made and stored at -80 ºC.

**Primer Design:** APE Software was used to design the primers of the genes of interest (*Table 1*). All genes of interest were inserted downstream of the pBAD promotor in the pHERD 30T Vector. Two primers that would amplify in the forward and reverse direction were made for each gene and vector. Regions of homology between the vector and gene were created for the gene specific primers.

**Plasmid Miniprep:** The Zyppy Plasmid Miniprep Kit manual was followed to separate the plasmid DNA. The concentration and presence of DNA in the collected supernatant was measured using the ThermoNanoDrop. A peak around 250 nm– 260 nm signified DNA was present, which was verified via gel electrophoresis.

**Agarose Gel:** For all gel electrophoresis, 1% agarose gels were made with a 1x TAE buffer, 10 µL EtBr per 100 mL, and 20x TAE was used as the running buffer.

**Polymerase Chain Reaction (PCR):** PCR was used to amplify the pHERD30T vector, the genes of interest and the transformed colonies. The negative control was made with DI water instead of the DNA. PCR products were verified using gel electrophoresis.

**Gel Extraction/Purification of PCR Amplicon Products:** The Machery-Nagel Gel Purification and PCR Clean Up Kit manual was followed to extract the amplified PCR products from the agarose gel. Only strong bands were extracted to optimize nucleic acid concentration. The ThermoNanoDrop instrument was used to determine the concentration of the purified genes and vector.

**Gibson Assembly of Gene of Interest into pHERD Vector:** The general GA protocol was followed to create a Gibson Assembly (GA) based on the concentrations of each gene and vector (*See Table 3)*. The positive control was the empty pHERD30T vector. Water was used for the negative control. The test sample plate had the purified gene and purified vector products.

**Transformation of TOP10 Chemically Competent *E. coli*:** Top10 Competent *E. coli* previously prepared in the Argüello Lab were used for transformations of the GA reaction product. DI water and the empty pHERD30T vector were used for the negative and positive controls, respectively. Based on the optimization of time length for the heat shock, (*Table 4*) the transformation reaction mix was put in the 42 $^{o}$C water bath for 60 seconds.

**Screening for Insertion via PCR and Restriction Enzyme Digest:** Five colonies were inoculated into LB media and grown overnight. The culture was streaked on a plate and used for the Template DNA in PCR. Purified pHERD30T vector was used for the positive control and water for the negative. Gel electrophoresis was used to check the transformation results as well as the results of a restriction enzyme digest.

# Results

## Primer design

Specific vector and gene primers are needed when assembling a vector construct by Gibson Assembly. These primers need to be designed in a way so that they have regions of homology. The regions of homology in the primers helps to produce linear fragments with overlapping base pairs that can be joined together. In this study, the PA2807 and PtrA gene sequences were inserted into the pHERD vector, creating two different vector constructs. All genes of interest were inserted downstream of the pBAD promotor after the first start codon in that reading frame. The vector primers for all the constructs were the same since all the genes were being inserted at the same location. A His tag comprised of six repeating residues was added to the forward vector primer (*Table 1*). The sequence for the vector reverse primer can be found in *Table 1*. The primers for the PA2807 gene were created by inserting the gene sequence immediately after the start codon previously mentioned. For the forward primer, the region of homology was 22 bases in length and located upstream of the start (*Table 1*). The reverse primer contained 21 bases that matched the reverse vector primer as its region of homology (*Table 1*). *Figure 2: PA2807 in pHERD for Gibson Assembly* shows the construct. An enlarged linear section is included to highlight the regions of homology between the vector, gene, and primers.

NEB Builder was used to check that the primers did not cause any breaks in the plasmid construct. The ExPASy Translate tool was used to verify that the protein the sequence coded for contained a His tag. Clustal Omega was used to determine the His-tagged protein was PA2807. Using these online tools verified that the primers would amplify the correct sequences and that the sequences coded for the protein of interest.

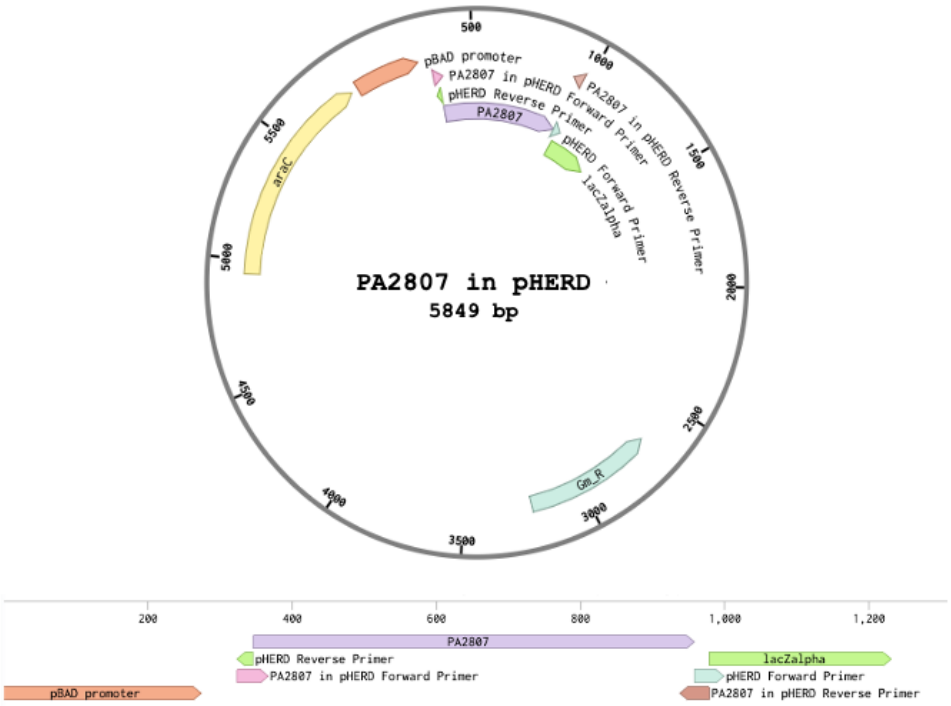


*Figure 2: PA2807 in pHERD for Gibson Assembly*

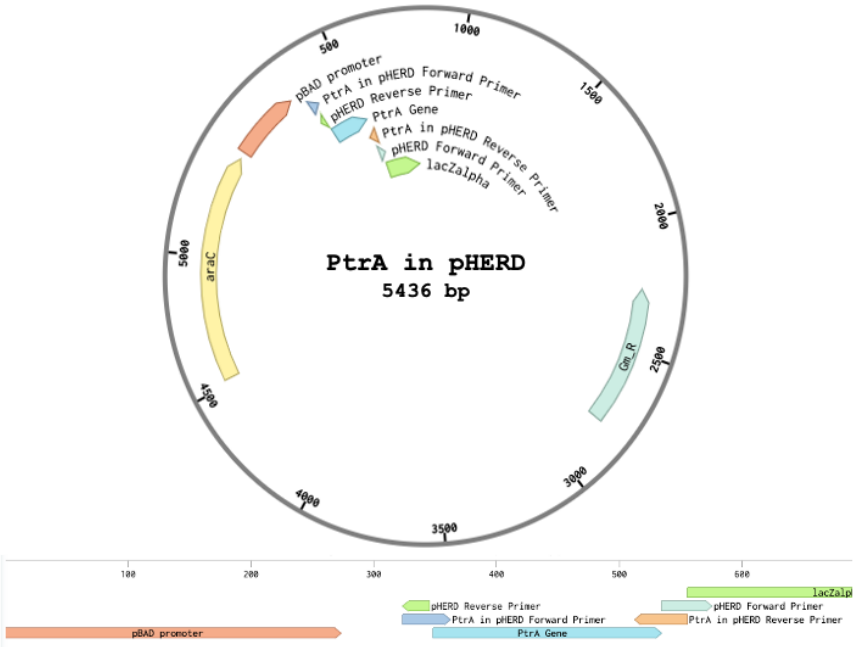


*Figure 3: PtrA in pHERD for Gibson Assembly*

The method described above was used to create primers for PtrA (*See Table 1* and *Figure 3)*, QueF, and PcoA. Even though primers were created for four periplasmic proteins, only PA2807 and PtrA were used for further experimentation in this study.

## Plasmid purification and PCR amplification

The primers that were designed were used to purify and amplify the genes of interest and pHERD in preparation for the Gibson Assembly reaction. The periplasmic genes PA2807 and PtrA in the Argüello lab had previously been inserted into the pUC18 vector. Gel electrophoresis was used to check for the presence of the vectors. *Figure 4A* and 4D shows those results.

DNA polymerase Q5 was used to amplify the pHERD vector as well as the PA2807 and PtrA genes. The vector primers were used to amplify pHERD, while the gene specific primers were used to amplify their respective genes (See Methods and Materials for PCR settings). *Figure 4B, 4C,* and *4E* show the results of the PCR reactions. A 1 kb DNA ladder was used as a standard. The pHERD vector is 5216 bp in length. PA2807 and PtrA are 618 bp and 192 bp, respectively.

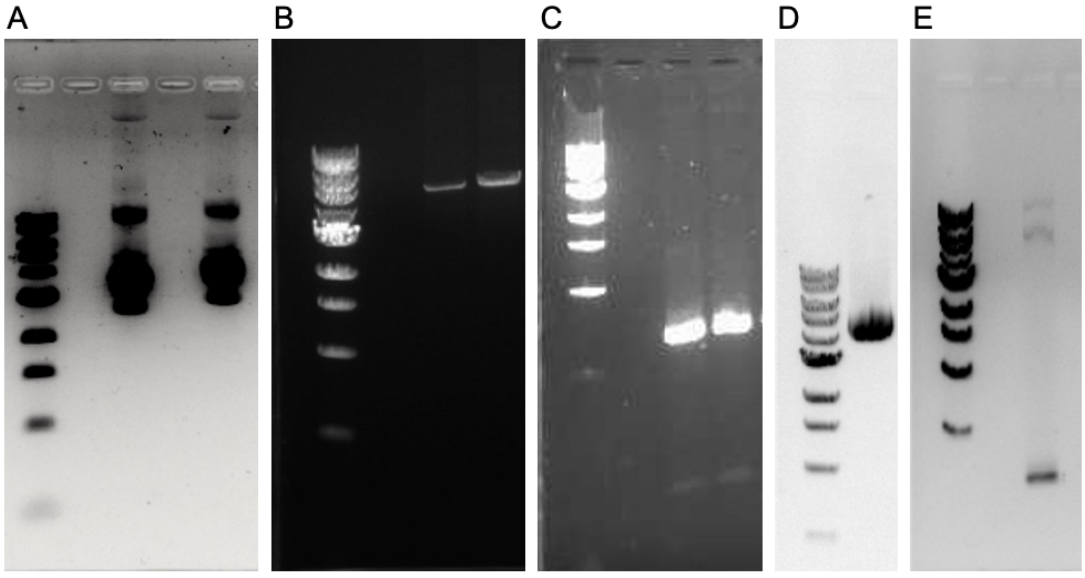


*Figure 4: Plasmid Purification and PCR Amplification Results*

*(A) 1 kb DNA Ladder, and plasmid purification results of pHERD and PA2807 in pUC18. (B) 1 kb DNA Ladder and PCR purification of pHERD vector results. (C) 1 kb DNA Ladder and PCR purification of PA2807 results. (D) 1 kb DNA Ladder and plasmid purification results of PtrA in pUC18. (E) 1 kb DNA Ladder and PCR purification results of PtrA.*

## Gel extraction and purification of PCR amplicons

After verifying that the PCR amplified the genes and vector, the band containing the DNA was extracted and purified. This was completed using the Machery-Nagel Gel Purification Kit. Bands that were not strong were not extracted so that the highest possible concentration of the samples were obtained. Gibson Assembly requires purified genes and vectors with known concentrations so the ThermoNanoDrop was used to determine the concentration of all the extracted bands (*See Table 2*).

## Gibson Assembly, transformation, and colony PCR screening

Based on the concentrations of the genes and vectors, four Gibson Assembly reactions (*See Table 3*) were made per construct. The Gibson Assembly products were transformed into Top10 Competent *E. coli*. To optimize the transformation process, different heat shock times were tested to determine the best time length (*See Table 4*). It was determined that 60 seconds would be the best length of time for the heat shock. All plates were made with LB media and gentamicin. Positive controls consisted of the empty pHERD vector, and negative controls had DI water. Five colonies from each sample plate were picked for colony PCR screening to determine if the transformation grew colonies with the vector construct or not. Taq polymerase was used along with the forward and reverse primers for the gene. *Figure 6* shows the results of the colony screening and *Table 5* reports the number of colonies on each plate.

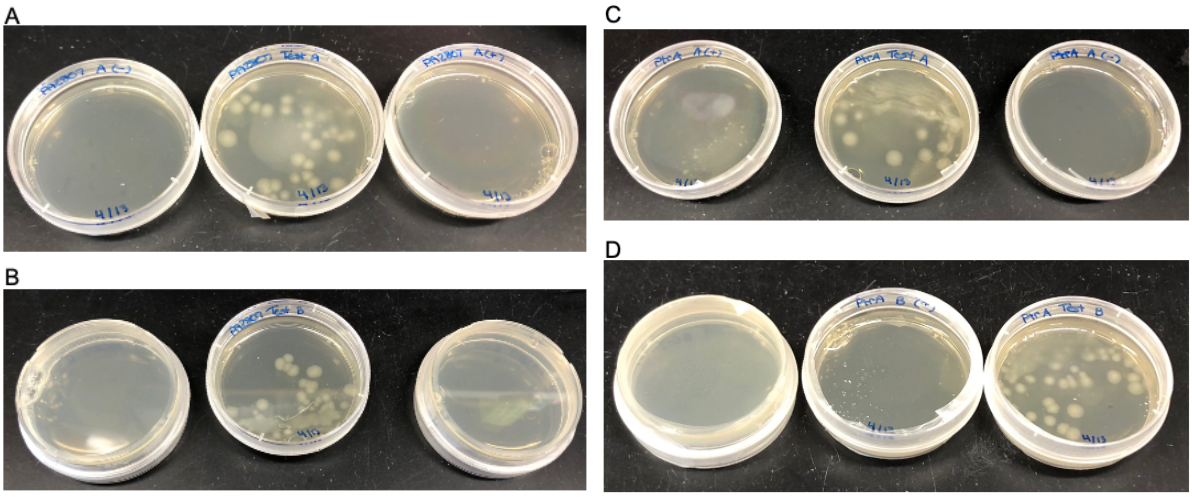


*Figure 5: Transformation Results*

*(A) PA2807 Test Group A: negative control, test sample, positive control. (B) PA2807 Test Group B: negative control, test sample, positive control. (C) PtrA Test Group A: positive control, test sample, negative control. (D) PtrA Test Group B: negative control, positive control, test sample.*

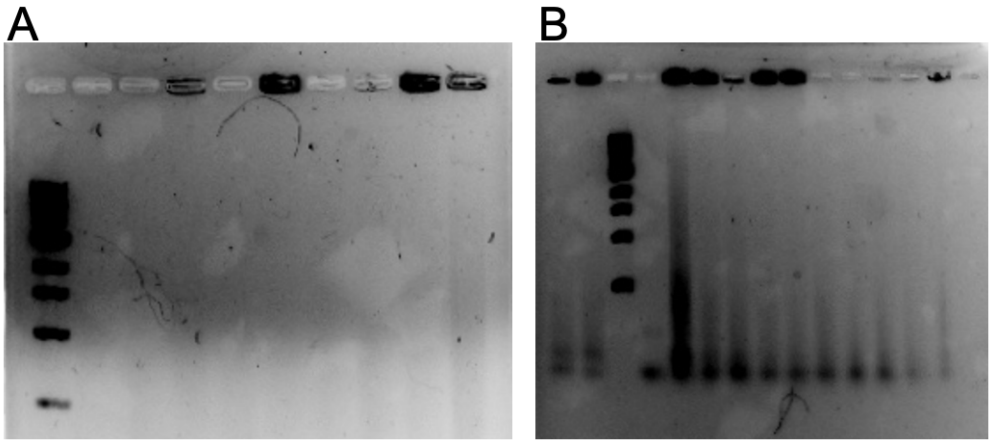


*Figure 6: Colony PCR gel electrophoresis results*

*(A) 1 kb DNA ladder, PA2807 control, PA2807 Group A Colonies 1-5, PA2807 Group B Colonies 1-3. (B) PA2807 Group B Colonies 4-5, 1 kb DNA ladder, PtrA control, PtrA Group A Colonies 1-5, PtrA Group B Colonies 1-5.*

# Discussion

While previous studies have reported a significant change in copper tolerance between mutant and wild type strains of *P. aeruginosa*, these results must be validated by complementing the mutants. A complemented mutant has its missing gene restored and should have the same phenotype as the wild type. To complement the ΔPA2807 and ΔPtrA *P. aeruginosa* strains, each gene of interest was isolated, along with the vector they were to be inserted into. A vector construct was created and transformed into competent *E. coli* cells. Colonies grown from the transformation were screened to determine if they contained the vector construct.

The PCR colony screening did not produce bands at the expected distances. It was expected that the PA2807 colonies would have a band above the 500 bp and 1000 bp mark on the ladder, but the bands were not visible on the first gel (*Figure 6A*). However, bands were visible on the second gel (*Figure 6B*), but they traveled a similar distance to the bands of PtrA. Since PtrA and PA2807 have different molecular weights, bands should not appear at the same distance from the wells. One possibility is that these bands were the primers used in the PCR mix. Considering that both genes and the vector were purified prior to the Gibson Assembly, it is possible that the problem occurred either during the Gibson Assembly, or in the procedures following.

The timeframe of this study did not enable a full investigation into the possible causes for the results presented. Future research involving the periplasmic proteins of *P. aeruginosa* should prioritize analyzing the Gibson Assembly and transformation steps to identify where the problem that led to the reported outcomes occurred, and how to fix it. If the results from future Gibson Assembly reactions yield similar results as this study, a different method should be employed to create the constructs. To verify previous results, complementing the mutants is a necessity, but this cannot be done without being able to consistently make the vector construct with the gene of

interest in it. It is paramount that future research of *P. aeruginosa* periplasmic proteins PA2807 and PtrA can repeatedly produce a viable vector construct to complement the mutant strains with. Complementing the mutants will provide key insight into the pathogenic threat posed by *P. aeruginosa* and its resistance to copper as an antimicrobial agent.

# List of Tables

**Table 1: Bacteria strains, plasmids, and primers used in this study**

| Strains | Relevant Features | |
|---|---|---|
| *Pseudomonas aeruginosa* | | |
| PAO1 | Wild type | |
| ΔPA2807 | $Gm^R$ | |
| ΔPtrA | $Gm^R$ | |
| ΔPcoA | $Gm^R$ | |
| ΔQueF | $Gm^R$ | |
| *Escherichia coli* | | |
| Top10 Competent | | |
| **Plasmids** | **Relevant Features** | |
| pUC18 | $Gm^R$ | |
| pHERD 30T | $Gm^R$ | |
| **Primers** | | |
| **Primer Name** | **Sequence (5'-3')** | **Amplifies** |
| pHERD_His_GA_F | catcatcatcatcattgaggatctgataagaattcgag | pHERD Forward |
| pHERD_ATG_GA_R | catgggtatgtatatctccttc | pHERD Reverse |
| PA2807_pHERD_GA_F | gaaggagatatacatacccatgctcccgacagccagccggcag | PA2807 Forward |
| PA2807_pHERD_His_GA_R | tcaatgatgatgatgatggggctgcacggtcagttgac | PA2807 Reverse |
| PtrA_pHERD_GA_F | gaaggagatatacatacccatgatgcgtaccttcactgcctc | PtrA Forward |
| PtrA_pHERD_His_GA_R | tcaatgatgatgatgatggcagttttccttgtccttcttc | PtrA Reverse |
| PcoA_pHERD_GA_F | gaaggagatatacatacccatgcacagaacttcgcgacgcac | PcoA Forward |
| PcoA_pHERD_His_GA_R | tcaatgatgatgatgatggcagttttccttgtccttcttc | PcoA Reverse |
| QueF_pHERD_GA_F | gaaggagatatacatacccatgcagcatcccgccgaacattc | QueF Forward |
| QueF_pHERD_His_GA_R | tcaatgatgatgatgatgctggcgcaccaggcgtcggttg | QueF Reverse |

**Table 2: Concentrations of isolated genes and vectors**

| Protein/Vector | Amplicon | Concentration |
|---|---|---|
| PA2807 | A 1.1 | 16.2 ng/uL |
| | A 1.2 | 5.8 ng/uL |
| | A 1.3 | 11.3 ng/uL |
| | A 1.4 | 13.4 ng/uL |
| | A 1.5 | 12.3 ng/uL |
| pHERD | A 1.1 | NA |
| | A 1.2 | 18.4 ng/uL |
| | A 1.3 | - 0.2 ng/uL |
| | A 1.4 | NA |
| | A 1.5 | 7.0 ng/uL |
| | A 2.1 | 0.7 ng/uL |
| | A 2.2 | 2.0 ng/uL |
| | A 2.3 | 3.2 ng/uL |
| | A 2.4 | 3.4 ng/uL |
| | A 2.5 | 4.7 ng/uL |
| PtrA | A 2.1 | 3.1 ng/uL |
| | A 2.2 | 10.6 ng/uL |
| | A 2.3 | 9.4 ng/uL |
| | A 2.4 | 9.7 ng/uL |
| | A 2.5 | NA |

**Table 3: Gibson Assembly**

| Gibson Assembly: PA2807 into pHERD | | | | | |
|---|---|---|---|---|---|
| Name | # bp | Amplicon | Concentration | Moles/uL | Mass Required |
| pHERD | 5216 | A 2.4 | 3.4 ng/uL | 0.0976 pmol | 5.1000 ng |
| PA2807 | 618 | A 1.1 | 16.2 ng/uL | 0.0400 pmol | 0.3755 ng |
| Four 5 uL Gibson Assembly Reactions | | | | | |
| pHERD A 2.4 | | | 6.000 uL | | 0.1464 pmol |
| PA2807 A1.1 | | | 0.304 uL | | 0.0030 pmol |
| DI Water | | | 3.6963 uL | | |
| Gibson Assembly Master Mix | | | 10.000 uL | | |
| Totals | | | 20.00 uL | | 0.1494 pmol |
| Gibson Assembly: PtrA into pHERD | | | | | |
| Name | # bp | Amplicon | Concentration | Moles/uL | Mass used |
| pHERD | 5216 | A 2.4 | 3.4 ng/uL | 0.0976 pmol | 5.1000 ng |
| PtrA | 192 | A 2.2 | 10.6 ng/uL | 0.0840 pmol | 1.2330 ng |
| Four 5 uL Gibson Assembly Reactions | | | | | |
| pHERD A 2.4 | | | 6.000 uL | | 0.1464 pmol |
| PtrA A 2.2 | | | 0.142 uL | | 0.0028 pmol |
| DI Water | | | 3.858 uL | | |
| Gibson Assembly Master Mix | | | 10.000 uL | | |
| Totals | | | 20.00 uL | | 0.1492 pmol |

**Table 4: Optimization of Transformation Heat Shock Time**

| pHERD Plasmid Optimization | | | | | | |
|---|---|---|---|---|---|---|
| Plate Designation | 1-A | 1-B | 2-A | 2-B | 3-A | 3-B |
| Vector | pHERD | pHERD | pHERD | pHERD | pHERD | pHERD |
| Heat Shock Time | 30 sec | 30 sec | 45 sec | 45 sec | 75 sec | 75 sec |
| Colony Count | 1 | 0 | 1 | 0 | 7 | 6 |

**Table 5: Transformation Colony Count**

| Transformation of PA2807 and PtrA into Top10 Competent *E. coli* | | | |
|---|---|---|---|
| Plate Identification | (+) Test Plate | (-) Test Plate | Sample Test Plate |
| PA2807 A | 0 | 0 | 35 |
| PA2807 B | 0 | 0 | 24 |
| PtrA A | > 25 | 0 | 13 |
| PtrA B | 6 | 0 | 32 |

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