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7.13 Experimental Microbial Genetics

Fall 2008

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Project List

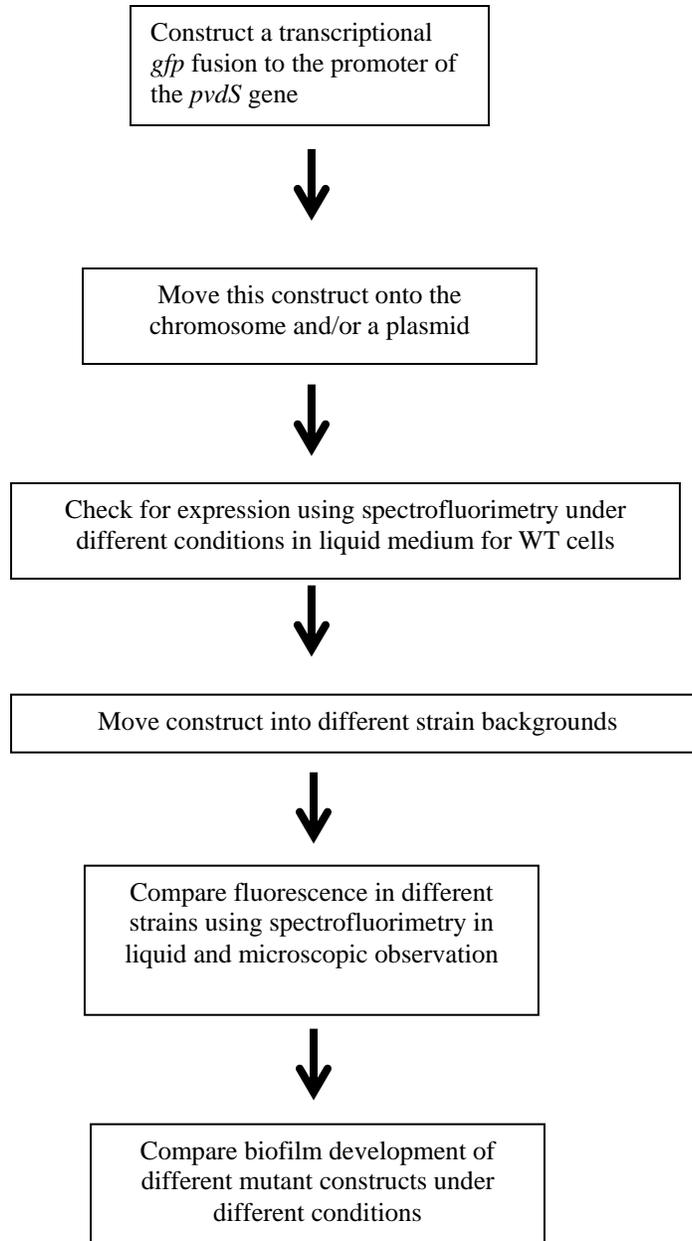
- (1) Determining whether phenazines can support intracellular iron acquisition**
- (2) Selecting and screening for mutations in Fur**
- (3) Determining the mechanism of wrinkling in response to phenazines**
- (4) Screening different CF-lung isolates for phenazine production and identifying their SoxR regulon**
- (5) Identifying genes required for swimming motility**
- (6) Identifying genes required for twitching motility and pili biogenesis**
- (7) Identifying genes required for swarming motility**
- (8) Identifying genes that contribute to antibiotic resistance and phenotypic variation**

Project 1 : Determining whether phenazines can support intracellular iron acquisition

One of the physiological functions that may be important for phenazines is their role in iron acquisition. Through redox reactions with ferric iron, phenazines reduce ferric iron to ferrous iron, which is more bioavailable. In strain PAO1, it has been shown that the ability of *P. aeruginosa* to acquire iron is critical for biofilm formation. In PAO1 (which is compromised in phenazine production), iron acquisition is thought to be achieved mainly by siderophores (high-affinity iron-binding compounds), however, in strain PA14, both siderophores and phenazines appear to be important. One way to test this is to determine whether phenazines affect the intracellular iron pool in PA14. *pvdS* (a gene involved in the biosynthesis of the siderophore, pyoverdine) is activated by a transcription factor that senses intracellular Fe. Students will make a transcriptional *gfp* fusion to *pvdS*, and express it both on a plasmid or in single-copy on the chromosome in different PA14 strains (e.g. wild-type, phenazine mutant, siderophore mutant, and *phz/sid* mutant). Fluorescence spectroscopy and microscopy will be used to monitor gene expression under different conditions.

Key reference: E. Banin, M.L. Vasil, E.P. Greenberg (2006) Iron and *Pseudomonas aeruginosa* biofilm formation, *PNAS*, 102:11076-11081.

Project 1 – Flow Chart

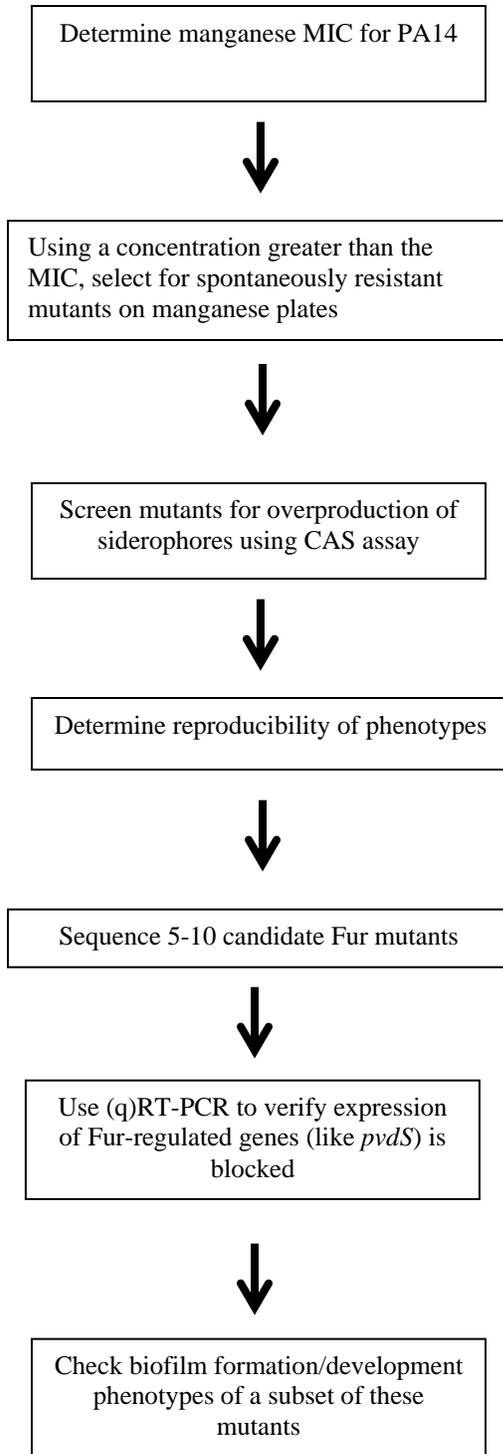


Project 2: Selecting and screening for mutations in Fur

Fur is an iron-dependent transcription factor responsible for turning on the expression of genes involved in iron acquisition (including *pvdS*, see project 1). Its activity appears to be influenced by phenazine production (i.e. in the presence of phenazines, Fur is silenced). In *Pseudomonas aeruginosa*, *fur* appears to be an essential gene (i.e. mutants that cannot make Fur die). However, it is possible to isolate Fur point mutants that are defective in binding DNA yet still competent to bind iron. In this project, students will select for spontaneous Fur missense mutants using a selective medium containing high manganese concentrations. Any mutants that grow under these conditions will be screened for overproduction of siderophores (indicating a defect in Fe-sensitive regulation). Mutants with this phenotype are likely to have a Fur point mutation, and will be sequenced. If time permits, additional characterization of these mutants will be performed, including assaying their biofilm phenotypes under different conditions.

Key reference: Barton *et al.* (1996) Ferric uptake regulator mutants of *Pseudomonas aeruginosa* with distinct alterations in the iron-dependent repression of exotoxin A and siderophores in aerobic and microaerobic environments, *Mol. Microbiol.*, 21:1001-1017.

Project 2 – Flow Chart

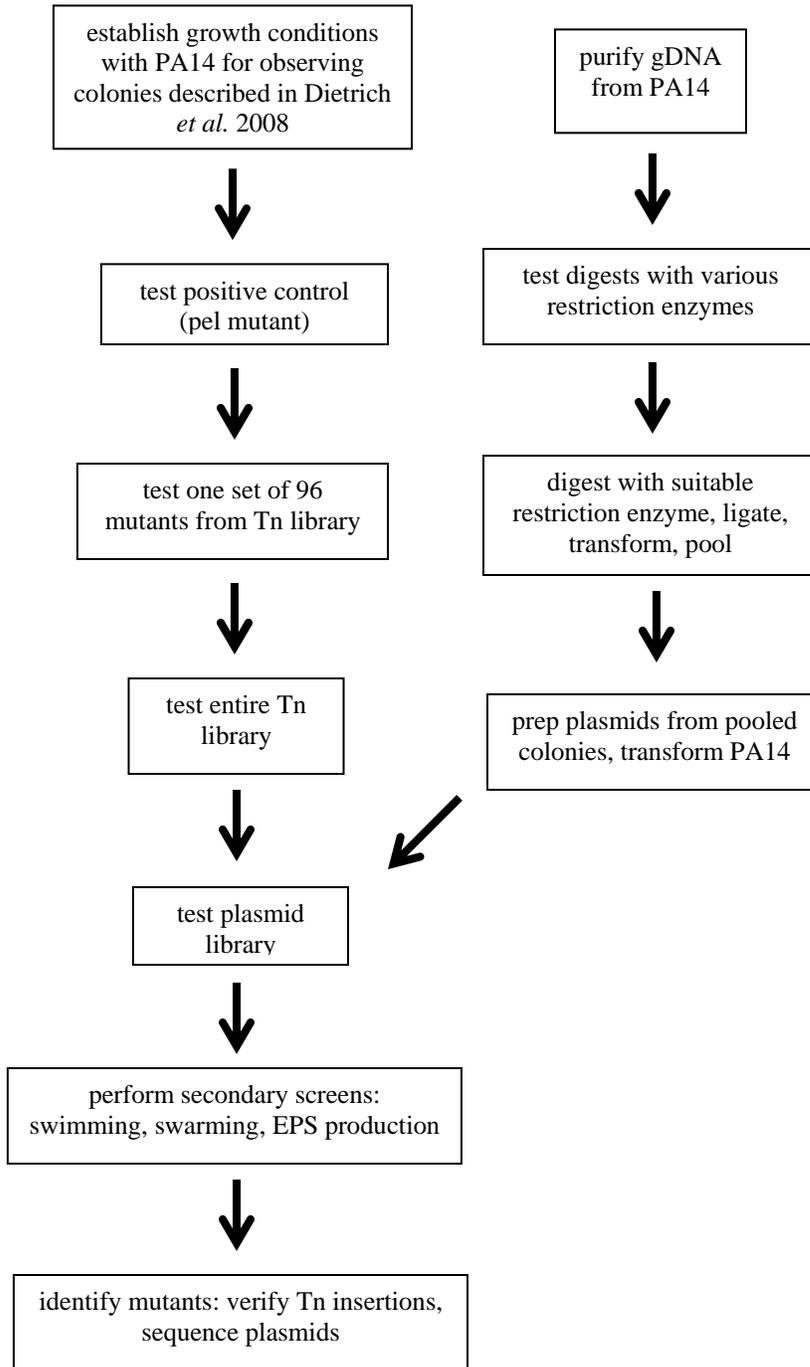


Project 3: Determining the mechanism of wrinkling in response to phenazines

Phenazine production affects *P. aeruginosa* in various ways. One major effect is on community behavior: the architecture of biofilms is dramatically different in mutants that cannot make phenazines compared to the wildtype, or mutants that over-produce phenazines. Whereas phenazine (over)producers make tight smooth colony biofilms, mutants that cannot make phenazines “wrinkle-up” and expand their surface area. Although it is clear that phenazines control this phenotype, we do not understand how they do so. In this project, students will attempt to unravel the mechanism by performing a genetic screen. Starting with the phenazine null mutant (which makes wrinkled colony biofilms), students will generate a transposon library. This library will then be screened for “smooth” colony biofilms. Candidate mutants will be sequenced and analyzed further using bioinformatics as well as other phenotypic screens.

Key reference: L.E.P. Dietrich, T.K. Teal, A. Price-Whelan and D.K. Newman (2008), Redox-active antibiotics control gene expression and community behavior in divergent bacteria, *Science*, 321 (5893): 1203 – 1206.

Project 3 – Flow Chart

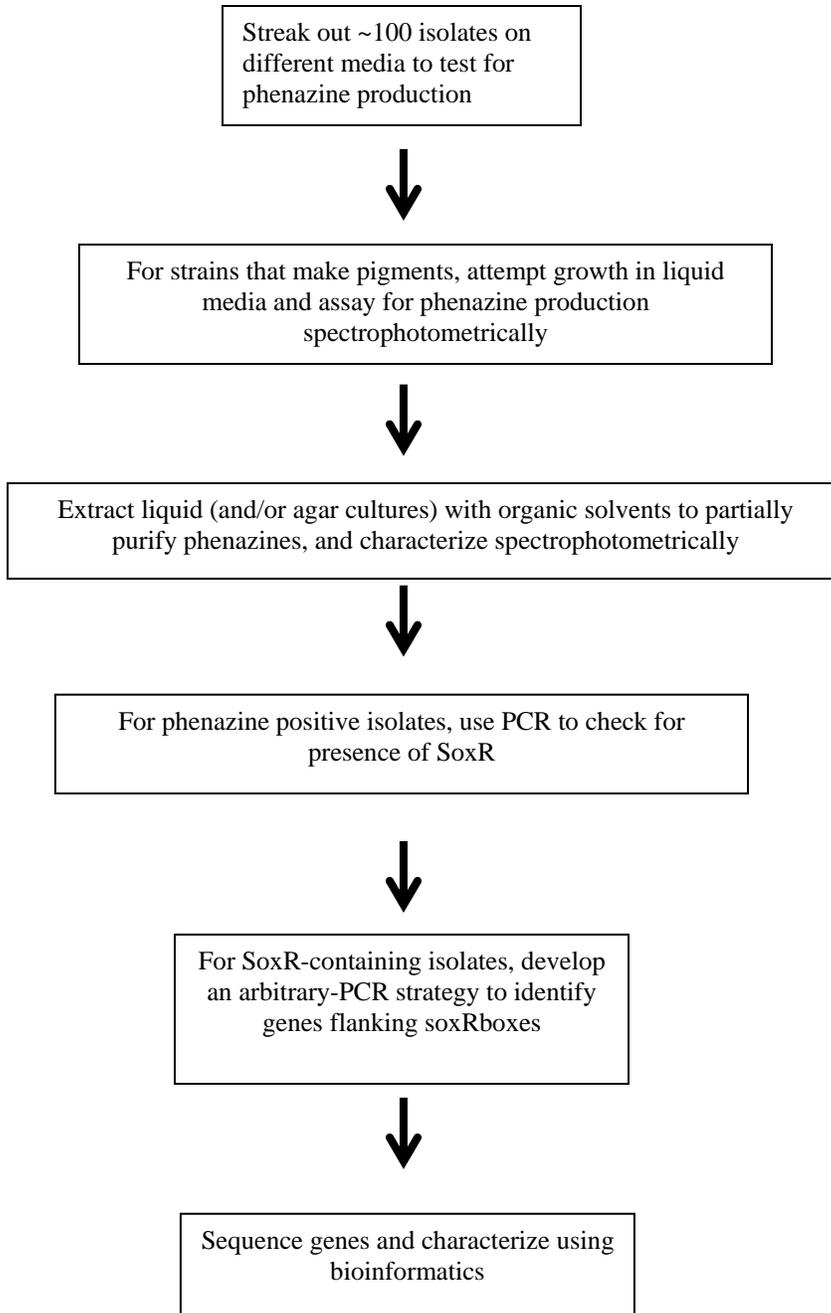


Project 4 : Screening different CF-lung isolates for phenazine production and identifying their SoxR regulon

Phenazines are small, colorful, redox active molecules produced by *Pseudomonas aeruginosa*. While traditionally these molecules have been studied for their antibiotic properties, more recently, it has become clear that they have other, important physiological functions. Phenazine production is known to occur in the lungs of CF patients, but whether this helps *P. aeruginosa* survive in the lung is unknown. To determine whether phenazine production is a trait that is selected for in the lung, students will screen different lung isolates (isolated from different individuals over the course of several years) for phenazine production. In strain PA14, the transcription factor SoxR regulates a small set of genes involved in phenazine trafficking. Thus, for strains found to produce phenazines, students will determine whether they contain the *soxR* gene, and if so, identify genes regulated by SoxR.

Key reference: L.E.P. Dietrich, A. Price-Whelan, A. Petersen, M. Whiteley and D.K. Newman (2006) The phenazine pyocyanin is a terminal signaling factor in the quorum sensing network of *Pseudomonas aeruginosa*, *Mol. Microbiol.*, 61(5): 1308-1321.

Project 4 – Flow Chart



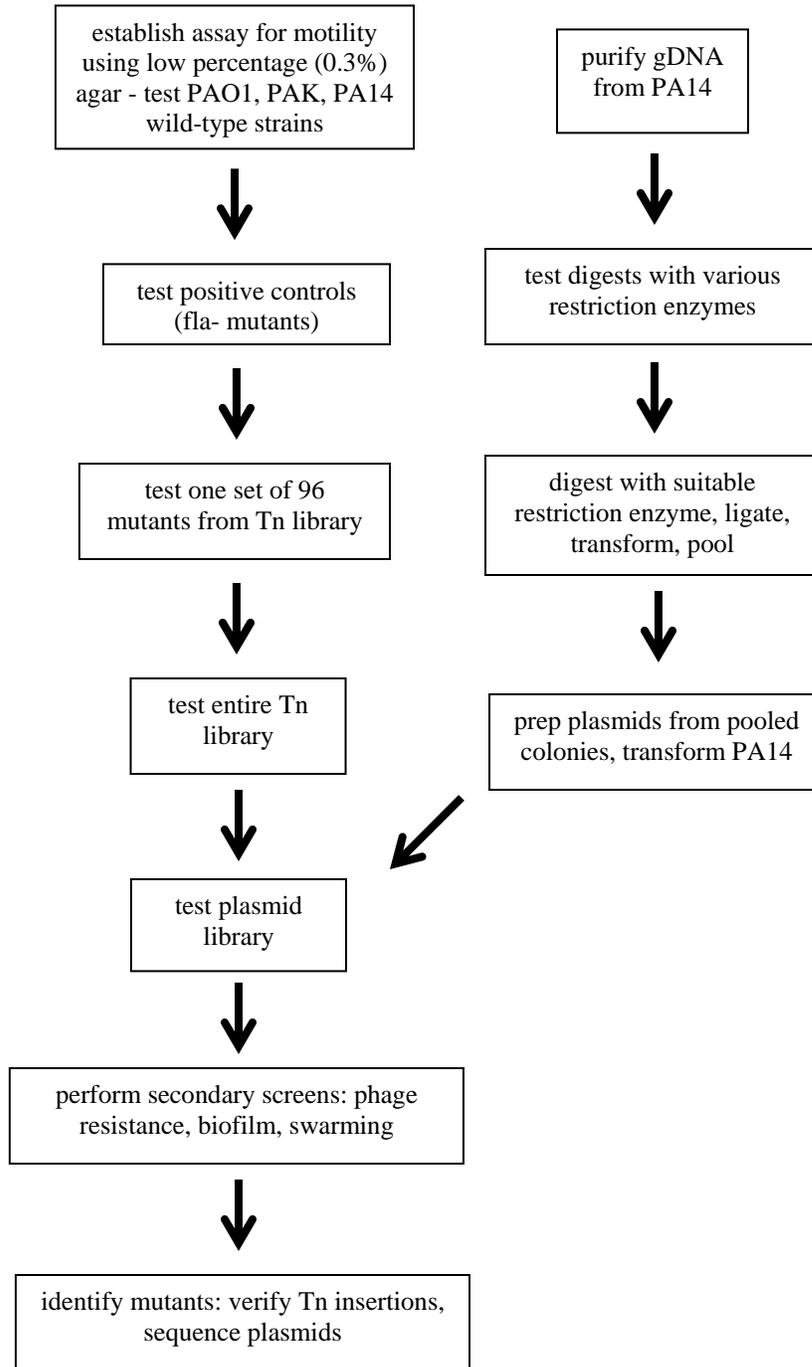
Project 5 : Identifying genes required for swimming motility

One of the primary modes of locomotion for *Pseudomonas aeruginosa* involves flagellar motility, often termed swimming motility. Flagellar-based swimming is a critical aspect of the *Pseudomonas aeruginosa* life cycle, enabling chemotaxis toward food as well as being important for biofilm formation and pathogenesis in cystic fibrosis patients. Many of the genes encoding structural elements of the flagellum are known based on prior genetic screens and analysis of the genome sequence. However, all of the genes that regulate the timing and positioning of flagellar assembly and activity are likely not known. Notably, the *Pseudomonas* flagellum is specifically assembled at a single cell pole and the factors that ensure proper spatial positioning of the flagellum and polar asymmetry are not known. Moreover, recent systematic analysis of deletion strains in *E. coli* led to the identification of dozens of new genes that affect flagellar motility, suggesting our understanding of swimming motility is incomplete.

In this project, students will test the nearly comprehensive library of *P. aeruginosa* transposon mutants for swimming motility defects. A plasmid library of genomic fragments will also be generated and examined to identify genes that affect motility when overexpressed. Mutants identified will be subjected to secondary screens, including those developed in other projects, such as swarming behavior and biofilm formation. Mutants affecting multiple processes are of particular interest as these may help identify key regulatory genes. Genes will be identified by verifying transposon insertion sites or sequencing plasmids.

Key reference: Masduki A, Nakamura J, Ohga T, Umezaki R, Kato J, Ohtake H. Isolation and characterization of chemotaxis mutants and genes of *Pseudomonas aeruginosa*. J Bacteriol. 1995 Feb;177(4):948-52.

Project 5 – Flow Chart

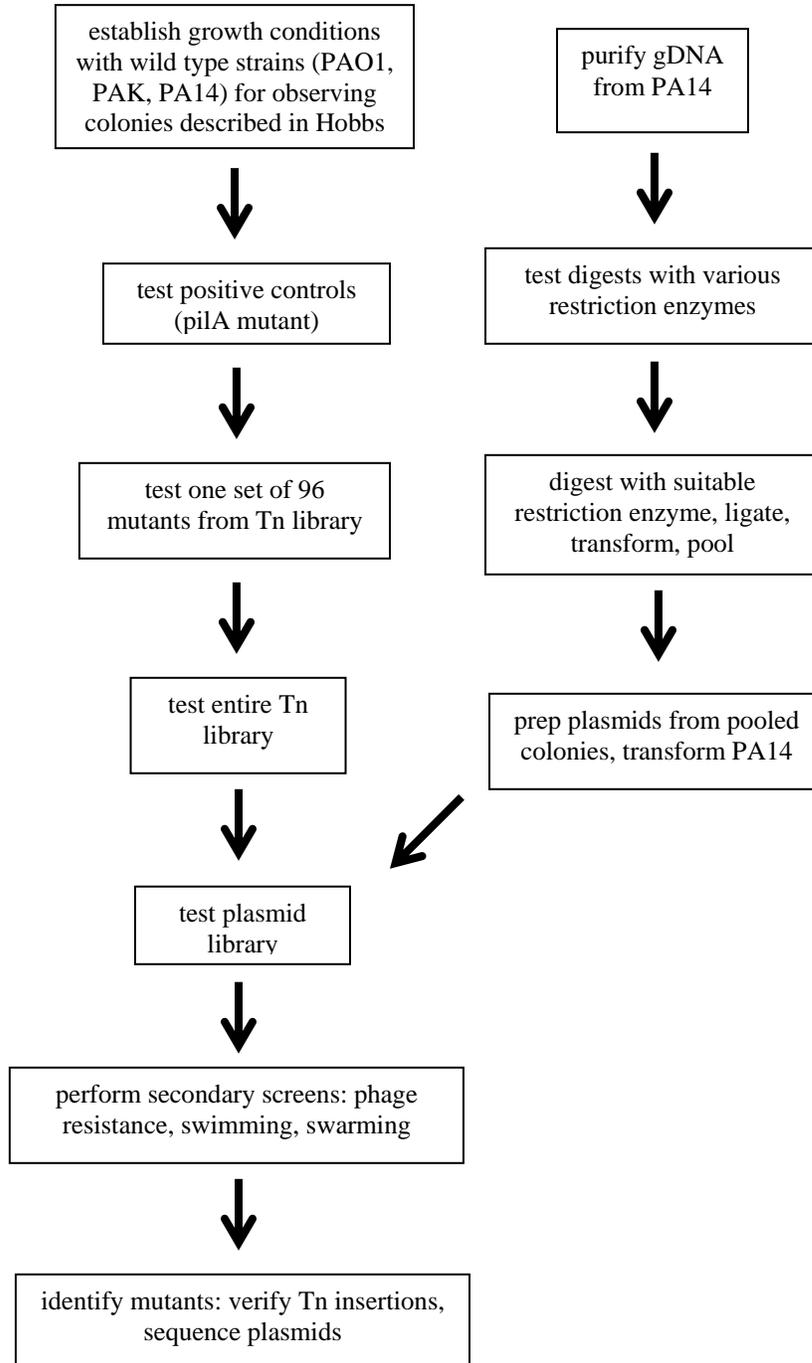


Project 6 : Identifying genes required for twitching motility and pili biogenesis

Type 4 fimbriae (also referred to as pili) are important cell surface structures produced by *Pseudomonas aeruginosa*. The pili mediate attachment to abiotic and biotic surfaces, are often the receptors for bacteriophage, mediate a form of locomotion called twitching motility, are required for biofilm development, and contribute to the virulence of *Pseudomonas aeruginosa*. Many of the genes encoding pilus subunits have been identified, but the full suite of assembly factors and genes responsible for regulating pilus production, extension, and retraction is unknown. This project will take advantage of the nearly complete, ordered PA14 transposon library to screen for genes required for pilus biogenesis, function, and regulation. A plasmid library will also be built and screened for genes that, when overexpressed, affect pilus functions. The screening procedures will take advantage of the previously reported observation (Hobbs et al 1993) that pili and twitching motility are necessary for *Pseudomonas aeruginosa* to form large, rough and flat colonies with a 'ground glass' border when grown on fresh, wet agar plates incubated in humid conditions. Mutants identified will be subject to secondary screening, including phage PO4 resistance and assays developed in other projects such as swimming motility, swarming behavior, and biofilm formation. Mutants affecting multiple processes are of particular interest as possible global regulators of diverse behaviors. Genes will be identified by verifying transposon insertion sites or sequencing plasmids.

key reference: Hobbs M, Collie ES, Free PD, Livingston SP, Mattick JS. PilS and PilR, a two-component transcriptional regulatory system controlling expression of type 4 fimbriae in *Pseudomonas aeruginosa*. Mol Microbiol. 1993 Mar;7(5):669-82.

Project 6 – Flow Chart

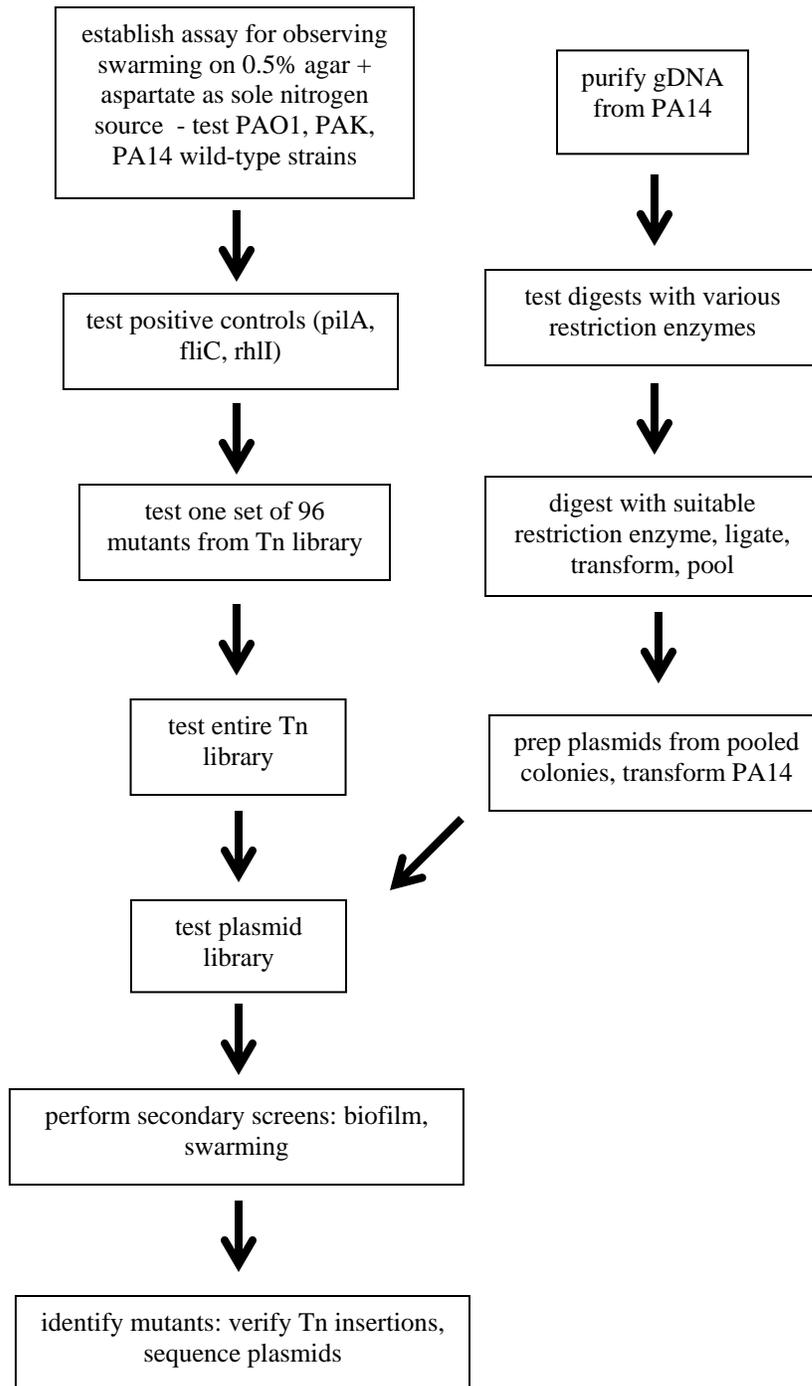


Project 7 : Identifying genes required for swarming motility

In addition to flagellar-based swimming motility and pili-based twitching motility, *Pseudomonas aeruginosa* has yet a third mode of motility, called swarming. On semi-solid surfaces (0.5-0.7% agar) *P. aeruginosa* cells swim in a community-coordinated manner. Swarming cells are typically elongated and contain multiple flagella instead of the usual single flagellum. Swarming also requires growth on media where certain amino acids (glutamate, aspartate, histidine, or proline) are the only nitrogen source. Although distinct from swimming and twitching motility, swarming also requires flagella and pili. Rhamnolipids, a biosurfactant, and cell-to-cell signaling pathways are also thought to be required for this community behavior. In this project, students will screen the comprehensive transposon library for strain PA14 for additional mutants affected in swarming. Additionally, a plasmid library will be built and screened for genes that, when overexpressed, affect swarming. Mutants will be further characterized through other assays, including those developed in other projects, such as biofilm formation, twitching motility, swimming motility, and mucoid colony growth.

Key reference: Köhler T, Curty LK, Barja F, van Delden C, Pechère JC. Swarming of *Pseudomonas aeruginosa* is dependent on cell-to-cell signaling and requires flagella and pili. *J Bacteriol.* 2000 Nov;182(21):5990-6.

Project 7 – Flow Chart



Project 8 : Identifying genes that contribute to antibiotic resistance and phenotypic variation

Antibiotic resistance is a major public health concern. Like many pathogens, *Pseudomonas aeruginosa* can develop resistance to antibiotics making clinical treatment difficult. Some bacteria also contain inherent resistance to a wide range of antibiotics. In many cases, this is thought to be the result of genes that reduce permeability or stimulate drug efflux. However, a comprehensive search for genes that contribute to the intrinsic antibiotic resistance of *Pseudomonas aeruginosa* has not been conducted. Students involved in this project will screen the entire PA14 transposon library for mutants that confer either reduced or increased antibiotic resistance. By plating on media containing antibiotics that inhibit growth of wild-type PA14, mutants that increase resistance can be easily identified. Conversely, by plating on media with antibiotics that permit growth of the wild-type strain, mutants that decrease resistance can be identified. The antibiotics to try include tobramycin and tetracycline. For mutants identified as hyper- or hyposusceptible, the gene responsible will be verified by sequencing of the transposon insertion. Mutant strains will also be subjected to additional analyses, including measurement of minimal inhibitory concentrations (MICs), examination of colony morphology, cross-protection against other antibiotics, and biofilm formation.

Key reference: Fajardo A, Martínez-Martín N, Mercadillo M, Galán JC, Ghysels B, Matthijs S, Cornelis P, Wiehlmann L, Tümmler B, Baquero F, Martínez JL. The neglected intrinsic resistome of bacterial pathogens. PLoS ONE. 2008 Feb 20;3(2):e1619.

Project 8 – Flow Chart

