

Sequencing the Genome of *Pseudomonas fluorescens* L5.1-96: — Engaging Undergraduate Students in Science



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Background

P. fluorescens L5.1-96 is a plant growth-promoting rhizosphere bacterium that is a superior colonizer of wheat roots and is an effective biocontrol agent of a wide range of soilborne fungal plant pathogens, notably *Gaeumannomyces graminis* var. *tritici*, which causes Take-All Disease of wheat.

Sequencing the genome of this strain will help identify genes important in colonization and persistence of populations of the strain on wheat roots.

ComGen is an innovative project supported by an NSF CCLI grant. As part of this **mini-graduate school experience** Community College students isolate plasmids from a genomic library, assess DNA quality, and perform DNA sequencing reactions and analyses.

Preliminary sequencing results have identified several unique genes in strain L5.1-96 not present in two previously sequenced strains (Pf-5 and Pf01) of fluorescent *Pseudomonas* spp.

Materials & Methods

Bacterial Strain

Pseudomonas fluorescens strain L5.1-96 provided by Root Disease and Biological Control Unit, USDA-ARS, Pullman WA

Plasmid Library

Generated by: Amplicon Express, Pullman WA
Total Number of clones: 18,432
Total Number of 384 well plates: 48
Average Insert Sizes: 2.7 and 2.2 kb
Cloning site: EcoRV (Blunt)
DNA fragmented by: Hydroshear
Cloning Vector: pSmart Lucigen vectorSnpou
Competent Cell: *E. coli* DH10B T1-R

DNA Isolation

Cultures grown at 37°C for 16-24 hours in Luria-Burtani broth with 30 ug /ml of kanamycin
Bio-Rad Quantum Prep® Plasmid Miniprep Kit

Sequencing Reactions

BigDye® Terminator v3.1 Cycle Sequencing Kits (10ul reactions)

PCR

Bio-rad MyCycler™
Program:
96°C 4 minutes
25 cycles:
96°C 10 seconds
50° 5 seconds
60° 4 minutes
hold at 4°C

PCR clean up

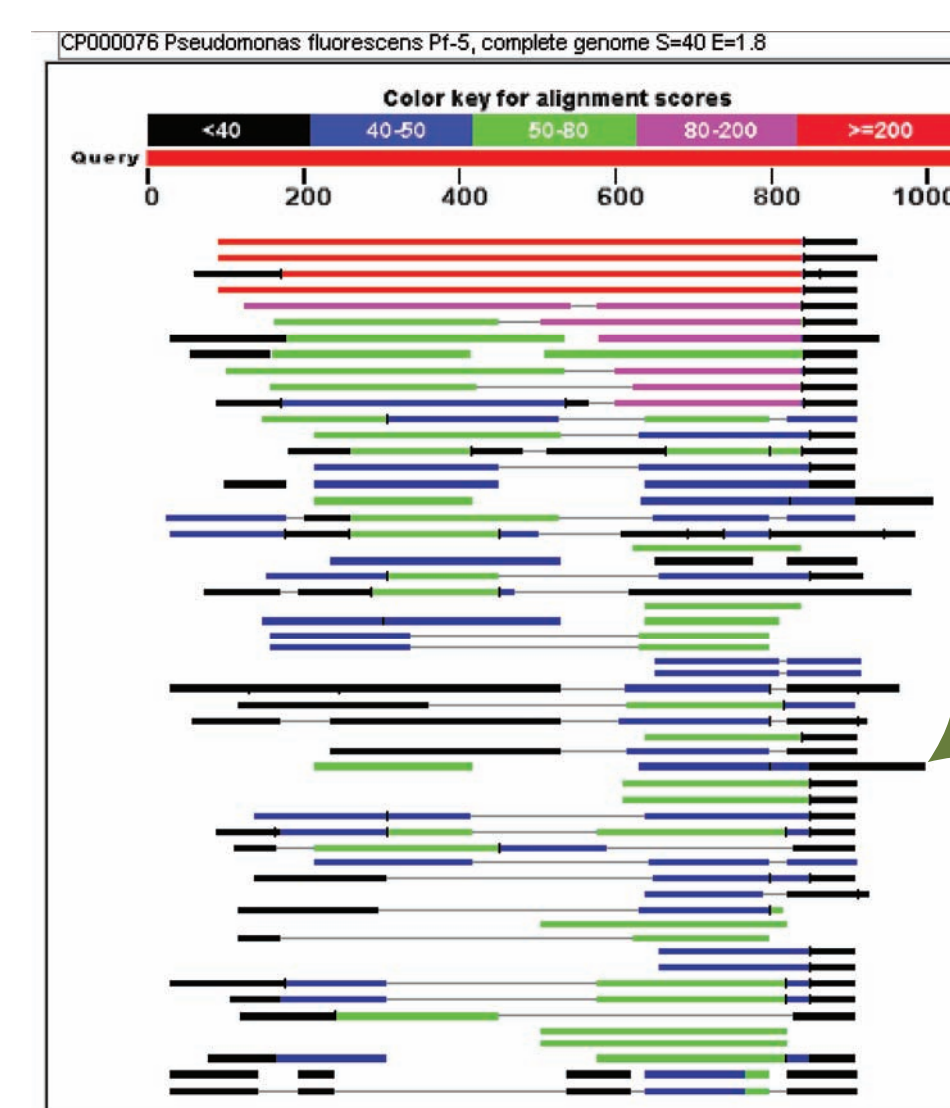
BigDye® XTerminator™ Purification Kit

DNA Sequencer

Applied Biosystems 3130

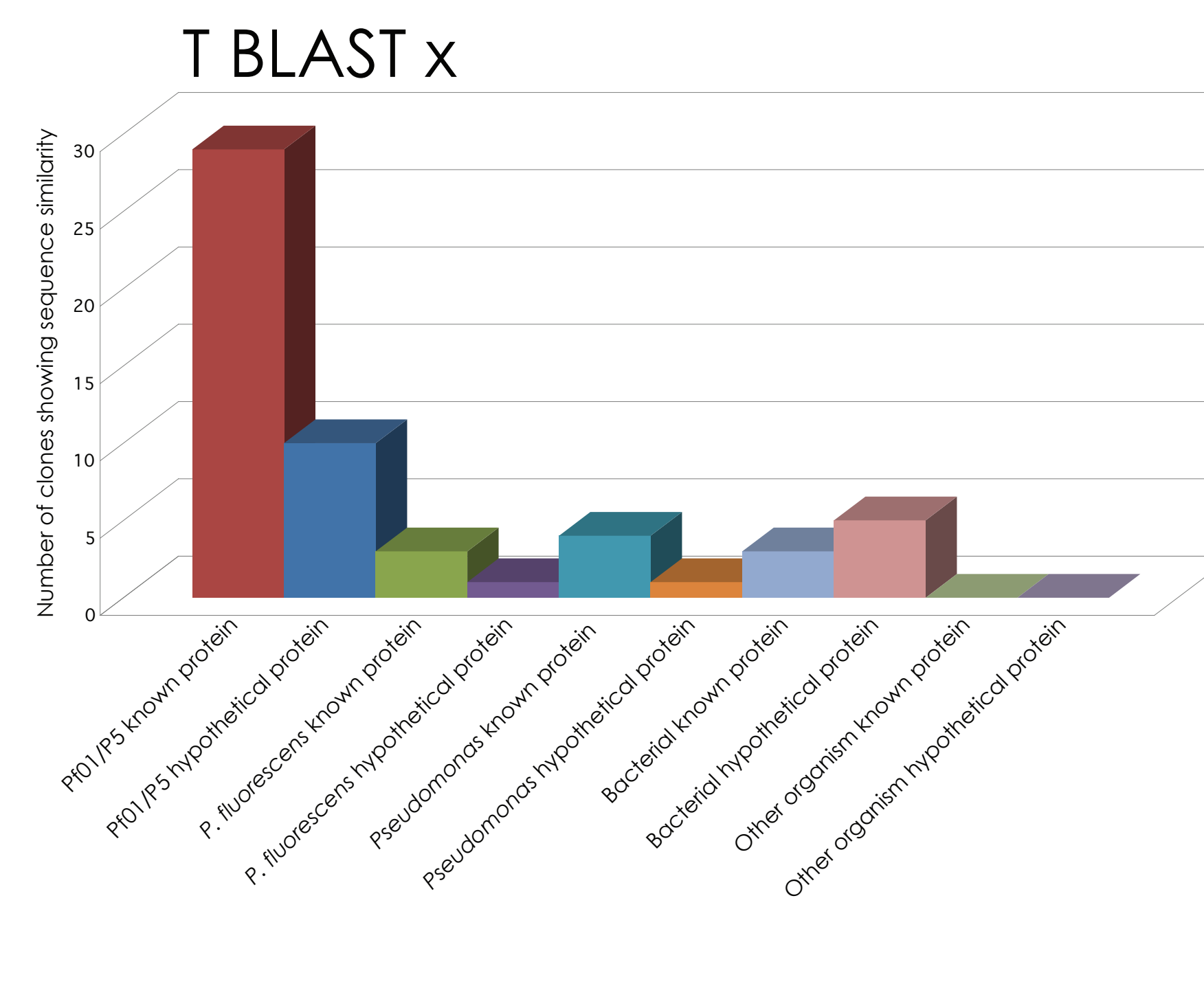
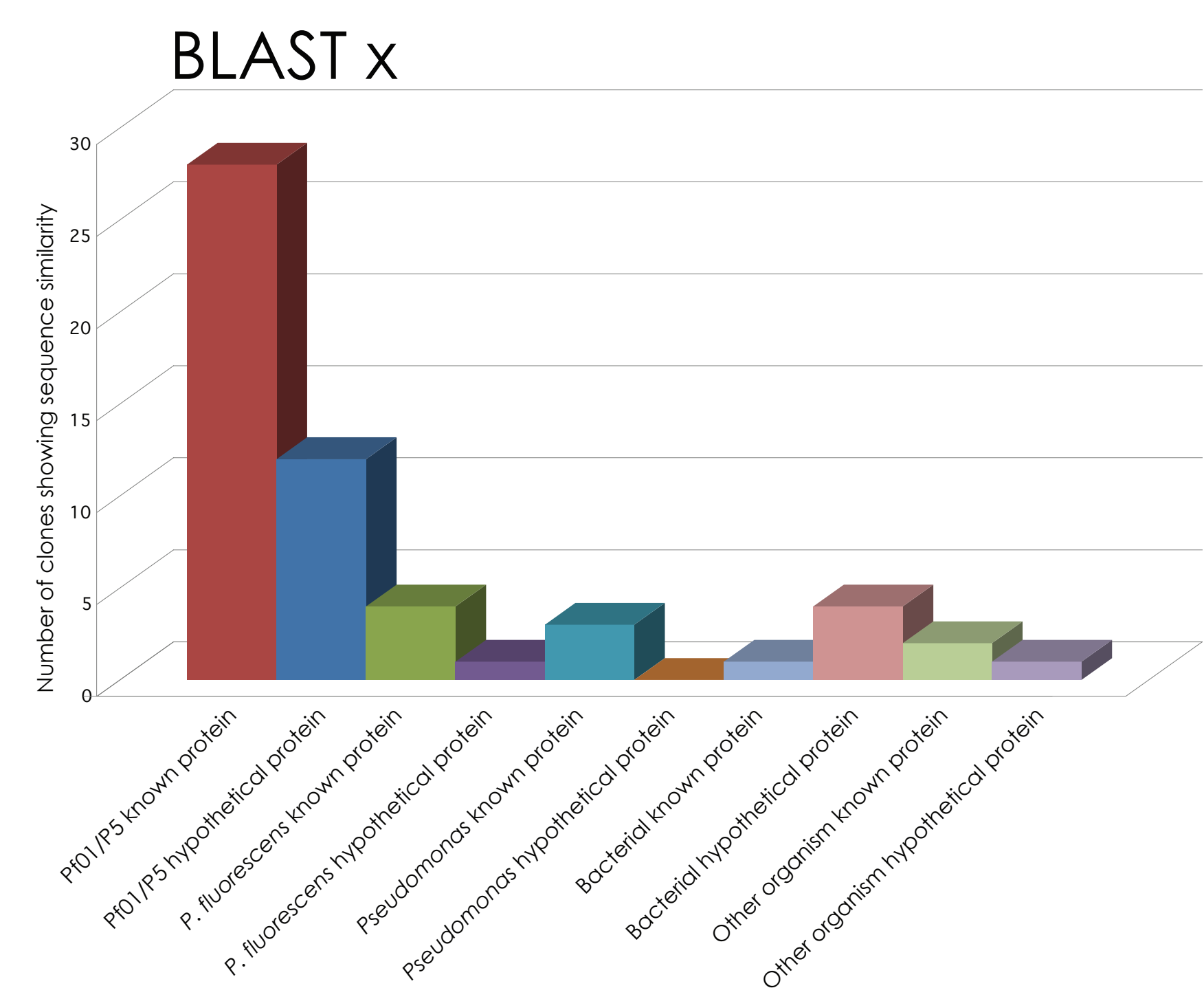
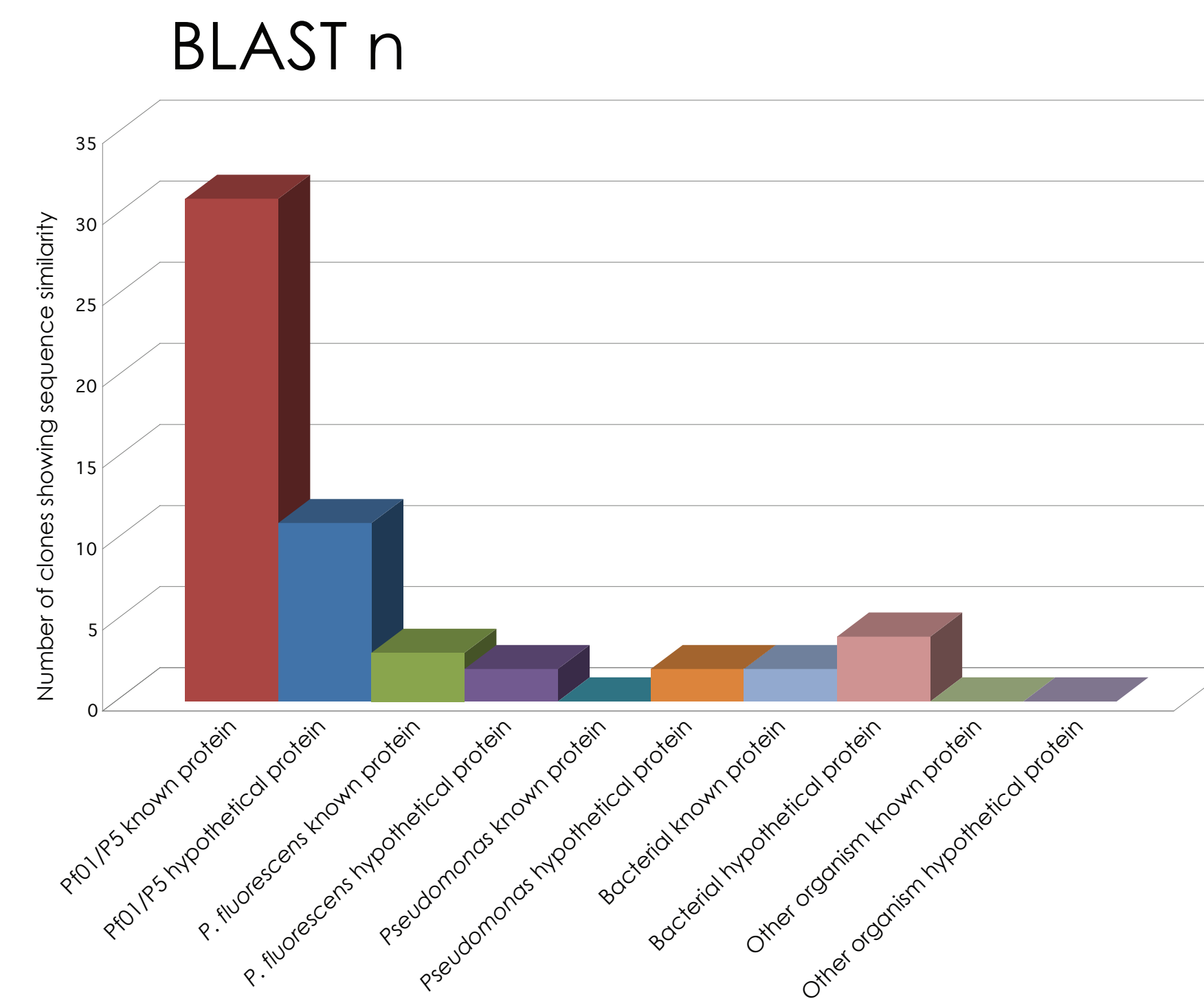
Sequence Analysis

BLASTn
BLASTx
TBLASTx



Pf-5

Results



Attempted: 90
Successful: 56
Likely Sources of error: Cycle sequencing PCR clean up
Plasmid isolation
Contamination

Students spoke of suddenly being able to understand specialized vocabulary of guest speakers -- something that makes them feel like members of the community of scientists

Discussion

Funded by a grant from NSF and in collaboration with the Root Disease and Biological Control Unit of the USDA-ARS, Bellevue College students with minimal prior experience with molecular biology techniques and bioinformatics successfully sequenced a portion of the *P. fluorescens* L5.1-96 genome and analyzed the sequence data.

Genes identified so far represent transport proteins, anabolic/catabolic pathway enzymes, a transcriptional regulator and a putative chemotaxis protein. Sequence analysis identified genes not currently found in the Pf-5/Pf01 genomes.

Students going through this program show increased ability to visualize themselves as scientists. (See highlighted quotes).

"I just started thinking about [molecules] from a much more questioning, scientific way."

"I couldn't see myself... in a research role, and being in a class like this it's a little more comfortable for me."

"I never thought frustration could be fun."

"I've got a clearer view of what happens in research."

Support

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