

# Tuberculosis: Flava'd RNA Expression Analysis

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# Outline

- Background
- Data
- Statistical Analysis
- Biological Implication





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# Tuberculosis (TB)

- Second most deadly infectious disease
  - 8.7 million new cases (2011)
  - 1.4 million deaths (2011)
- Multi-drug resistance found in virtually all countries surveyed
- Infection with *Mycobacterium tuberculosis* corresponds to 10% lifetime risk of falling ill with TB



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# Data Set

- Triplicate cultures of wildtype CDC1551 *M. tuberculosis* strain and mutant with *whiB6* gene disrupted
- C Larsson, J Meyers, and T Mosbruger at Umeå Universitet, Sweden
- Data gathered using AB SOLID RNA sequencing



# Goal

- What is the biological impact on protein expression of knocking out a single gene?
- How many and which pathways might be affected?
- Are any pathways affected or just isolated cellular functions?



# Data Processing

- SRA files from the wild type and the mutants are downloaded then converted to \*.fastq files
- Galaxy:
  - FastQC
  - FastQ Groomer
  - Bowtie for SOLiD: map reads to genome





# Data Processing

- Further analysis:
  - Cufflinks
  - Cuffcompare
  - Cuffmerge
  - Cuffdiff

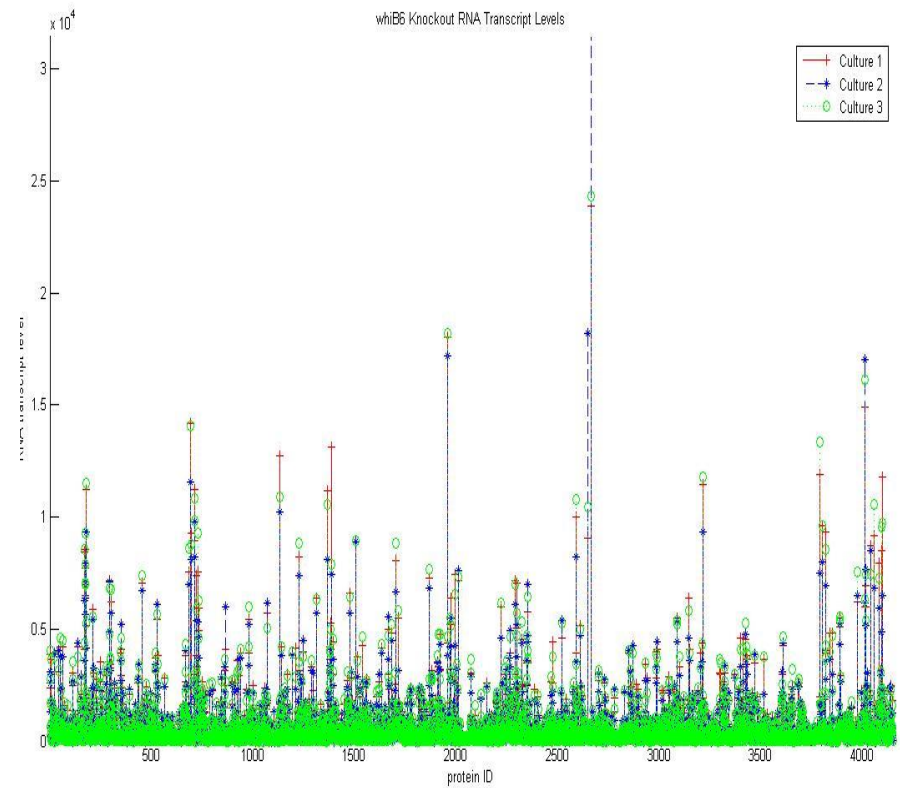
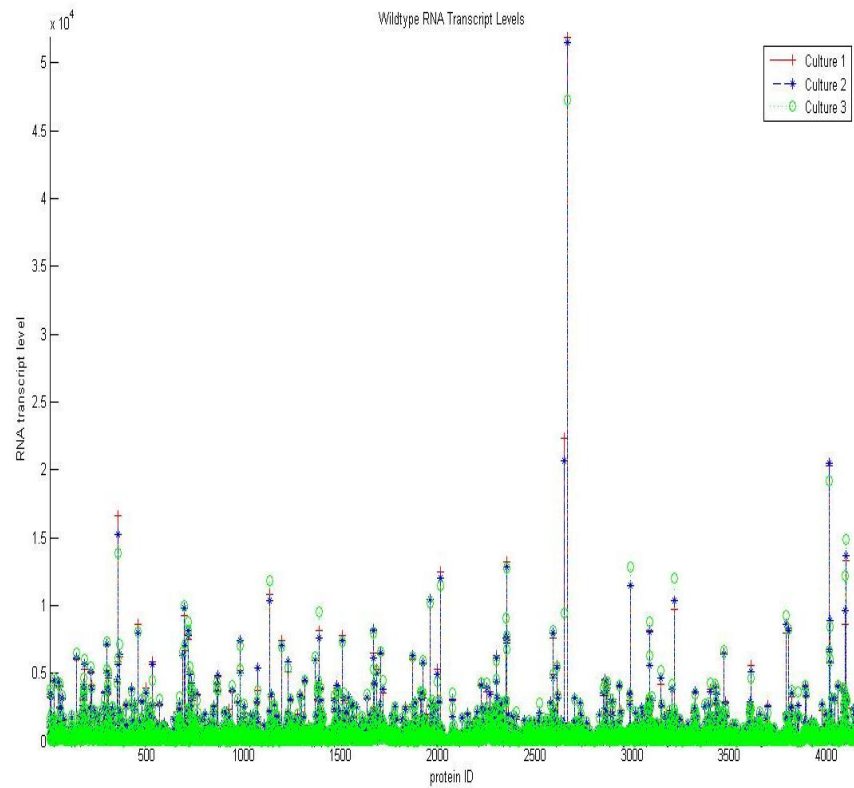


# Outline

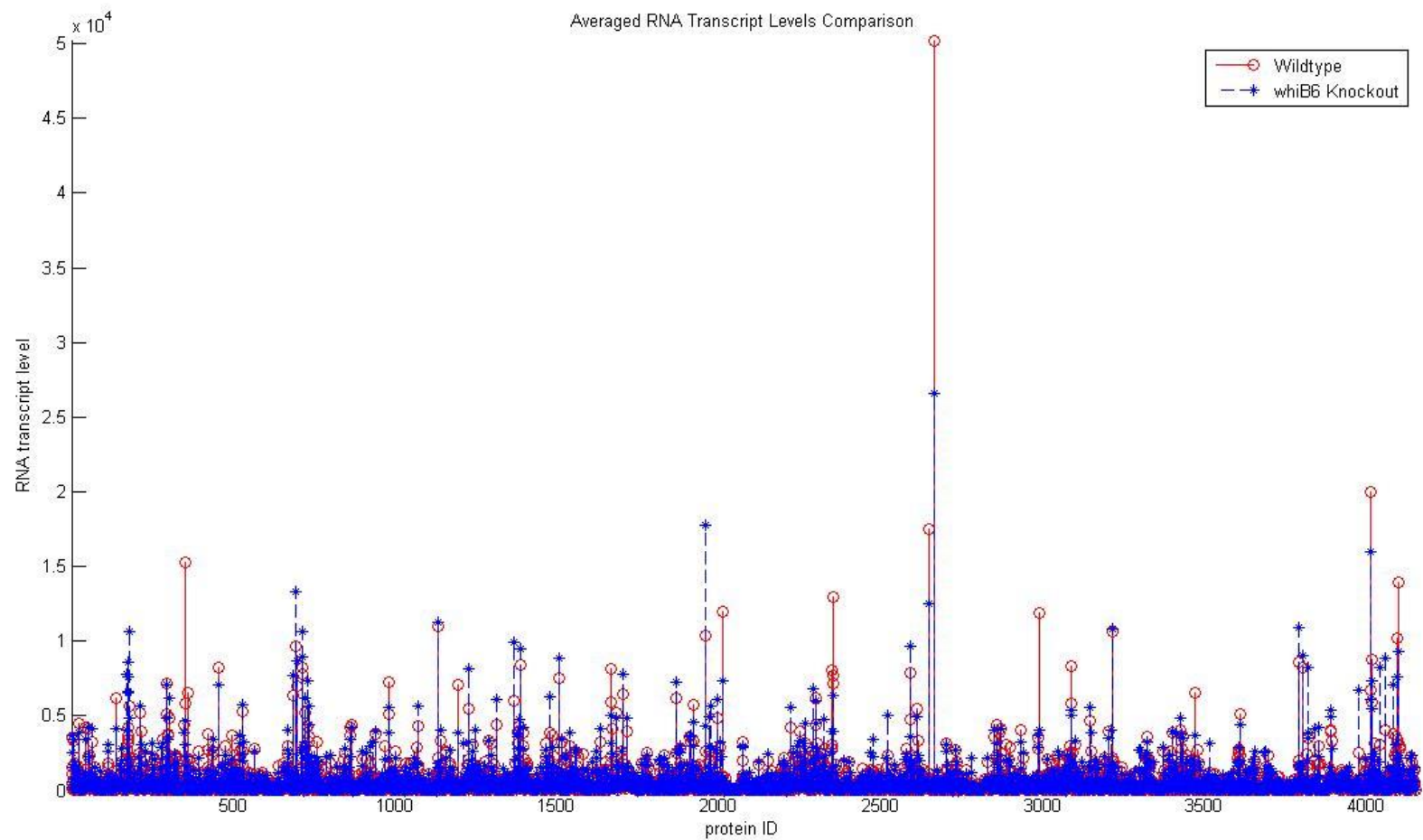
- Background
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# Raw Data

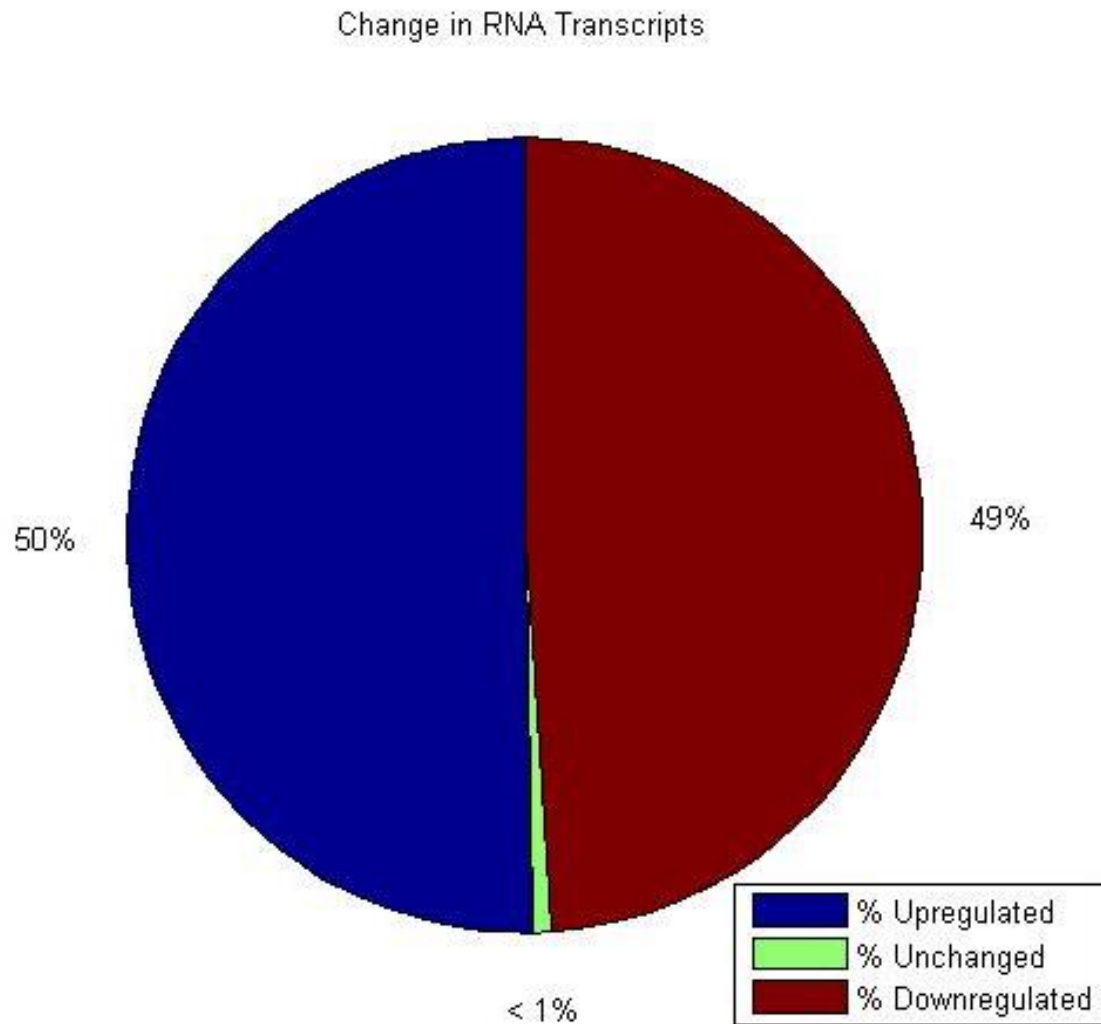


# Strain Comparison

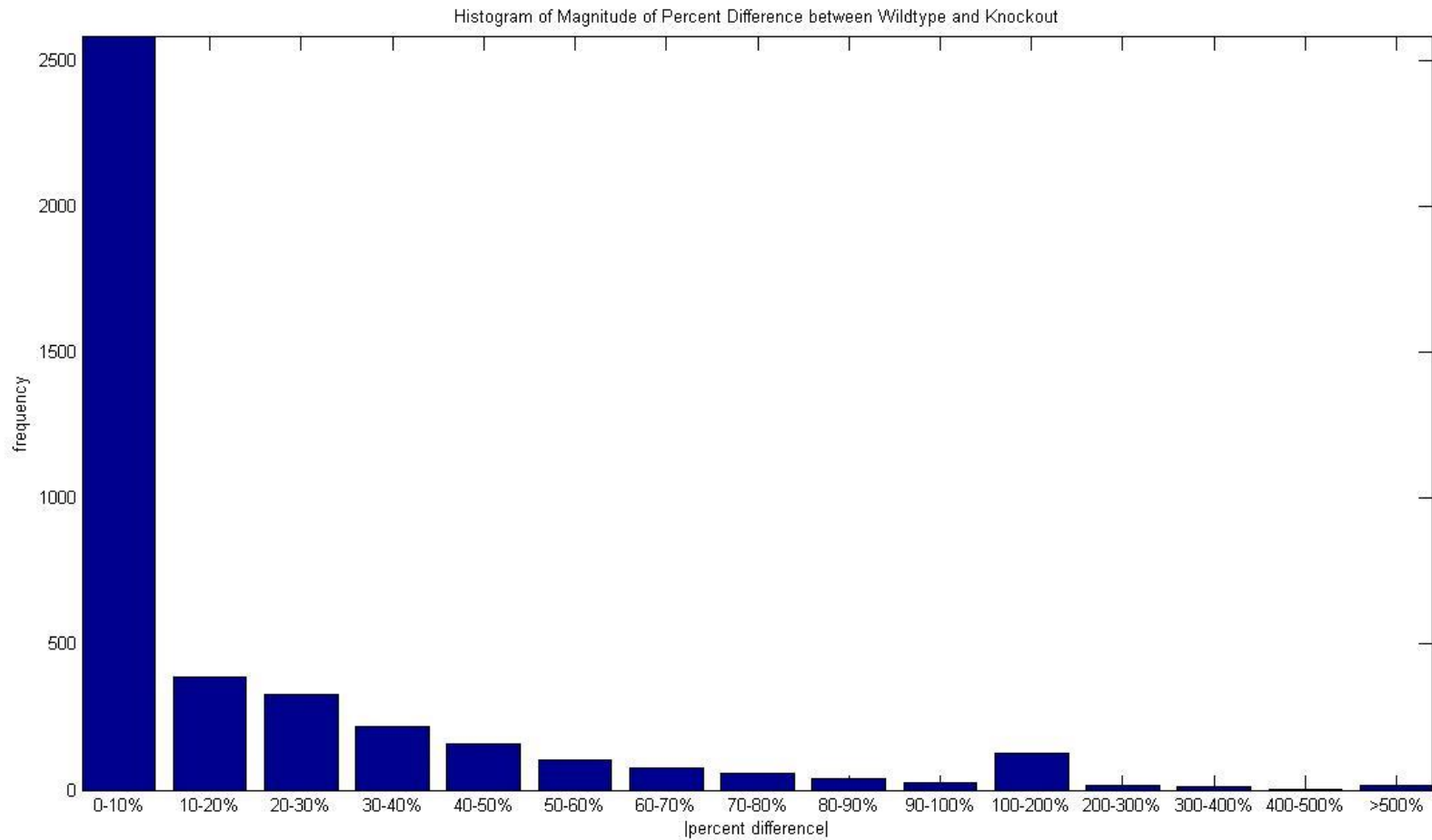




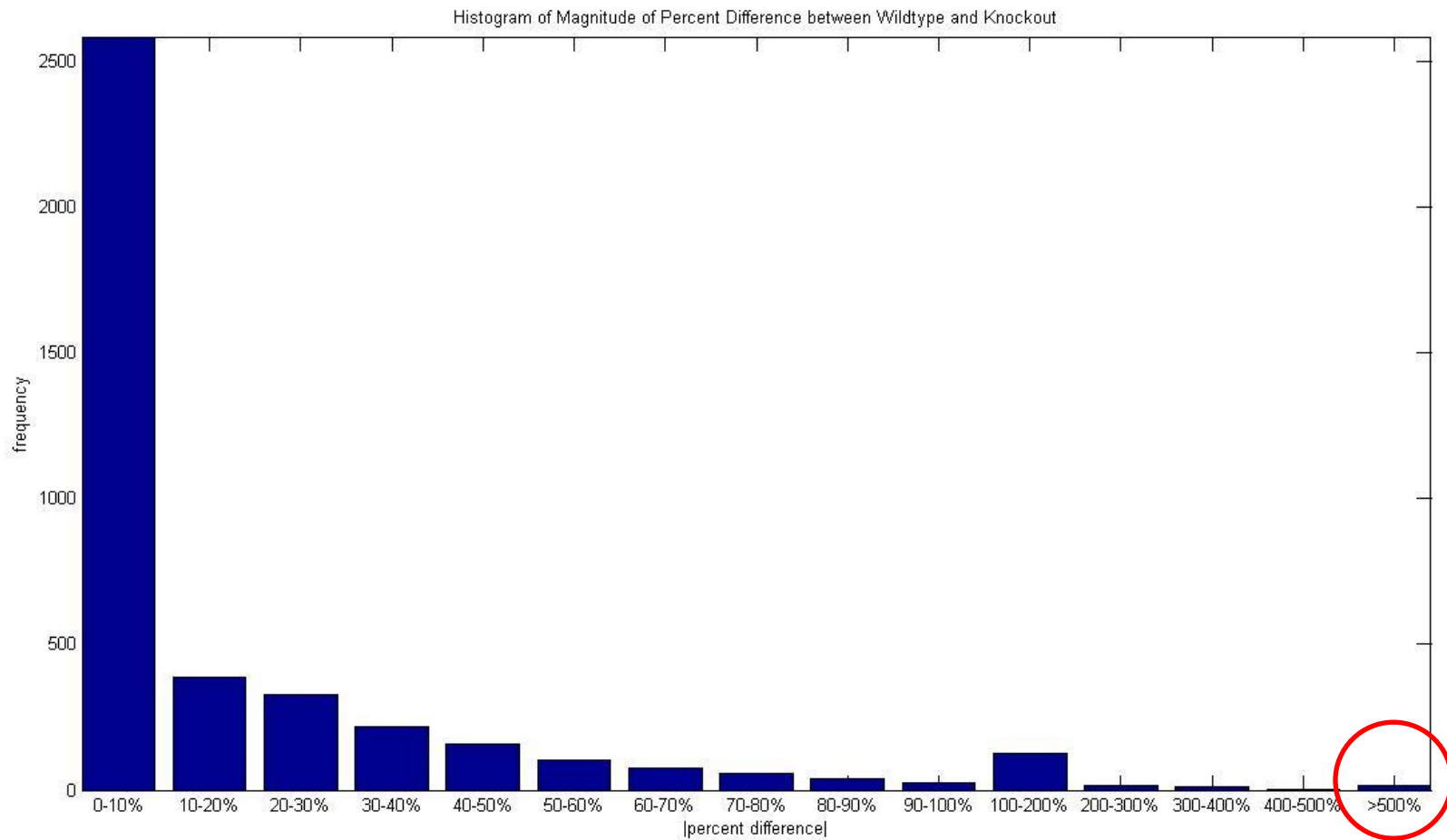
# First Glance



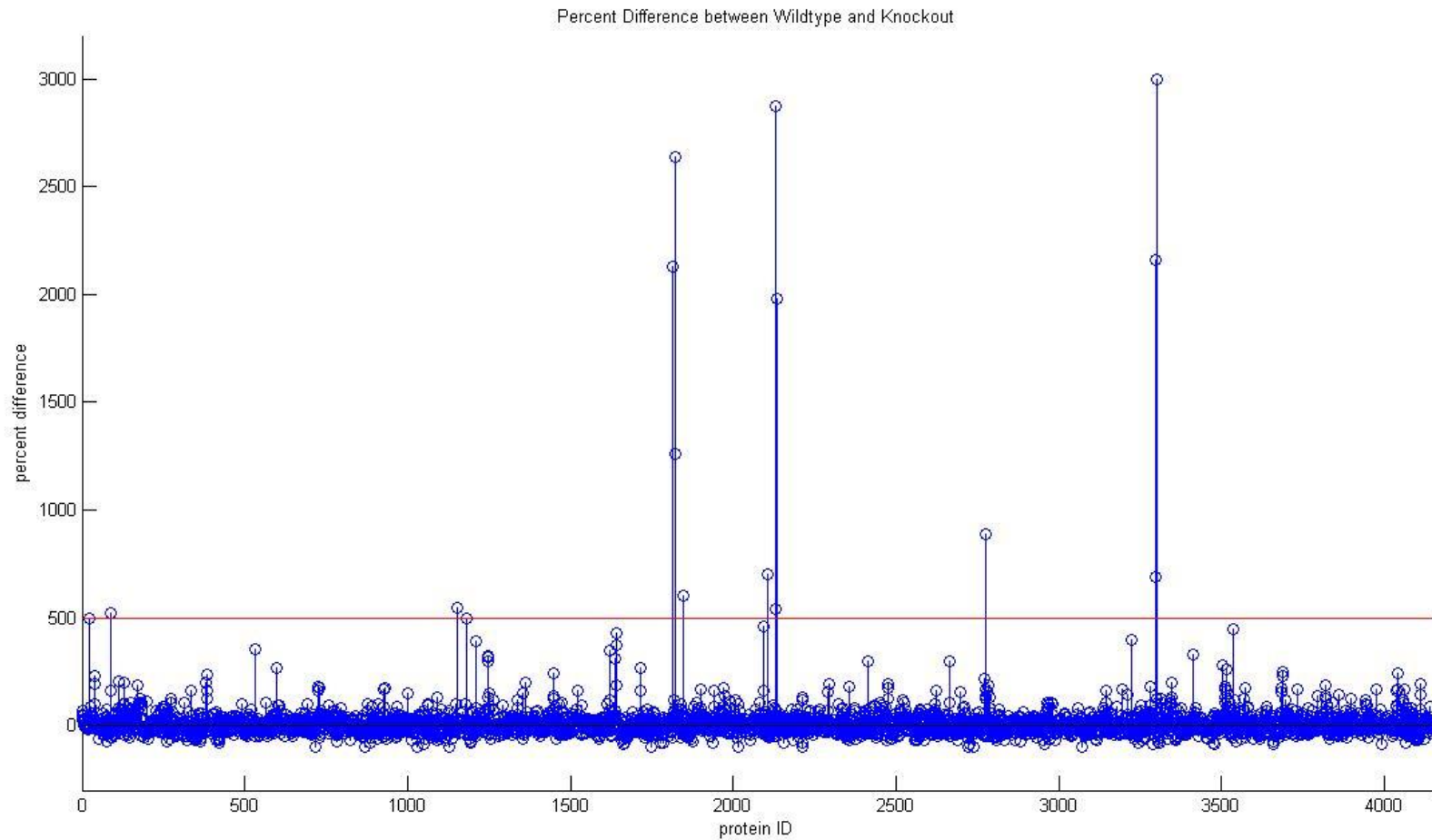
# Outliers



# Outliers



# Outliers





# Outliers

- Identified 16 proteins that show greater than 500% upregulation in mutant compared to wildtype
- Identified 22 proteins that show greater than 80% downregulation in mutant compared to wildtype
- Data gathered using AB SOLID RNA sequencing



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# MT1162

- Downregulated by 82% in mutant strain
- 526 amino acids
- Uncharacterized protein involved in propionate catabolism
- Downregulation could result in higher fatty acid production



# MT1741, MT3885

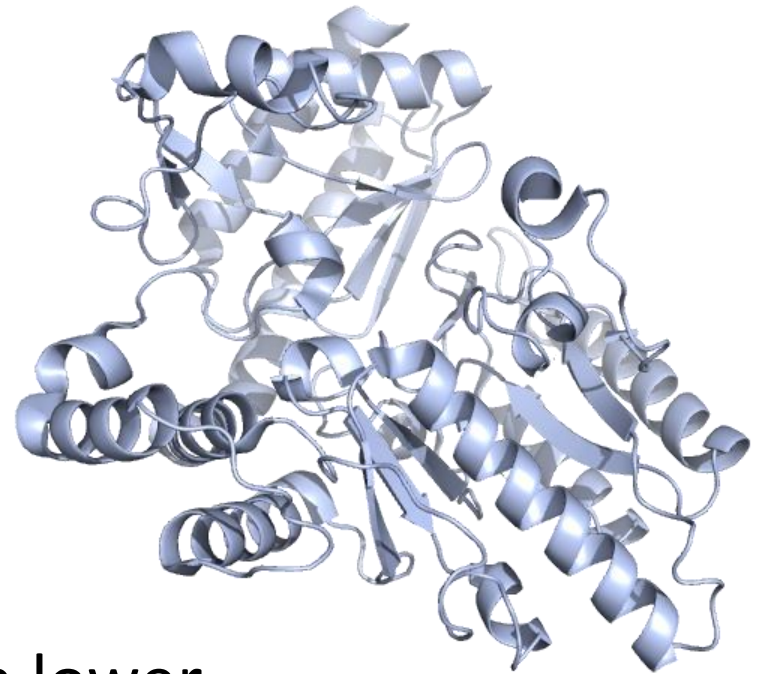
- MT1741:
  - Downregulated by 81% in mutant strain
  - 454 amino acids
- MT3885:
  - Downregulated by 85% in mutant strain
  - 542 amino acids
- Hypothetical proteins belonging to protein family that catalyzes hydrolysis of genomic DNA





# MT3389

- Downregulated by 85% in mutant strain
- 449 amino acids
- Protein belonging to aminotransferase family
- Downregulation could result in lower lysine and arginine biosynthesis



# Downregulation

- Mutant strain:
  - Could be prone to higher environmental stress due to increased fatty acid production
  - seems to contain a downregulated DNA hydrolysis pathway (two proteins affected) stemming from reduced HNH endonuclease function leading to reduced DNA repair of UV-damaged segments
  - could experience trouble synthesizing lysine and arginine, leading to potential junk proteins being produced





# MT3217, MT3212, MT2091

- MT3217:
  - Upregulated 3000%
  - 344 amino acids
- MT3212:
  - Upregulated 691%
  - 348 amino acids
- MT2091:
  - Upregulated 1980%
  - 333 amino acids
- Hypothetical proteins belonging to protein family that catalyzes flavin reduction



- Upregulated by 1260%
- Nitrate/nitrite transporter NarK, 397 aa
- Regulates nitrate reduction:
  - Transports nitrate to nitrate reductase
  - Inhibited by oxidizing conditions
- Nitrate reductase has respiratory function in the absence of oxygen





# MT3216

- Upregulated by 2161% in mutant strain
- Hypothetical protein, 463 amino acids
- Described as “wax ester synthase-like Acyl-CoA acyltransferase” on NCBI
- Known that MT produces wax esters when subjected to stress, which are required for entering dormancy (may be drug-resistant state)



- Upregulated by 600% in mutant strain
- Cutinase, 254 amino acids
- Catalyzes the cleavage of the ester bonds of cutin, a waxy polymer



# Upregulation

- Mutant strain:
  - could be reducing flavin which would lead to increased production of FADH, suggesting greater energy generation
  - may have a boosted wax ester metabolism, indicating greater stress from its environment



# Summary

- Mutant strain:
  - may be prone to higher levels of environmental stress
  - could have issues repairing damaged DNA and producing function proteins
  - could produce energy at an increased rate
  - upregulated proteins are related to dormancy

