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Visualization of Growth Curve Data from Phenotype Microarray Experiments

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ESPP Environmental Stress Pathway Project

Outline of Presentation

- Background
- Growth curve basics
- Description of phenotype microarray experiments
- Goals of software development
- Color map approach to displaying growth curves
- PMColorMap Web interface
- Use of the interfaces to assess data quality, compare replicates, and compare phenotypes
- Future plans
- Summary

ESPP
Environmental Stress Pathway Project

Background

- The data are being generated as part of the Environmental Stress and Survival Project (ESPP), which is funded by the U.S. Department of Energy (DOE) Genomics:GTL Program.
- ESPP has been studying the response of *Desulfovibrio vulgaris* Hildenborough (DvH), an anaerobic bacterium found in sediments, to environmental stressors such as oxygen, sodium chloride, nitrate, nitrite, and low/high pH.
- These environmental stressors are typical of environmental conditions found in DOE waste sites contaminated with metals and radionuclides.
- The DOE Genomics:GTL Program is funding ESPP (and similar projects) as part of its overall strategy of supporting basic research in environmental (bio)remediation, energy production, and carbon cycling.

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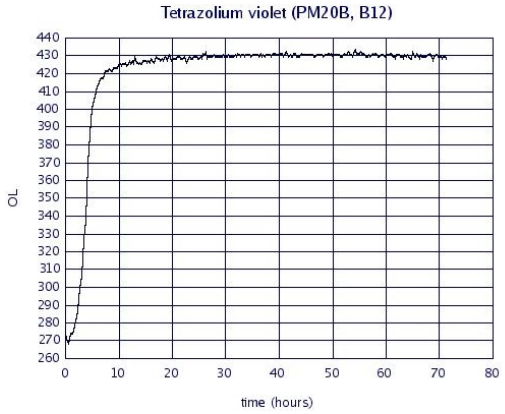
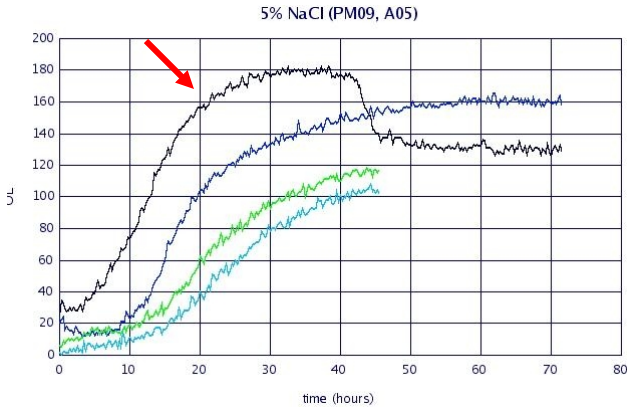
Growth Curve Basics

Growth curves tell us

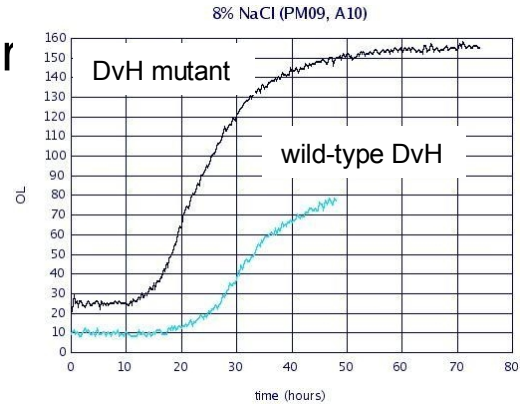
- how well an organism is growing,
- whether there are data quality problems,
- whether the data are reproducible,
- whether the growth or a microbe is inhibited and/or delayed when subjected to a stressor,
- how the growth of one organism compares to another.



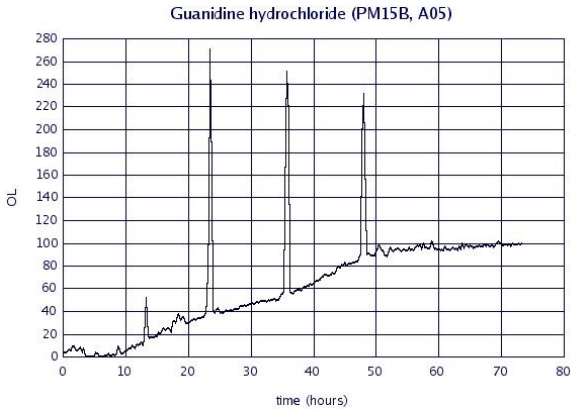
Chemical reaction



Two pairs of technical replicates



Phenotype comparison



Non-biological anomalies

phenotype: the observable physical or biochemical characteristics of an organism, as determined by both genetic makeup and environmental influences

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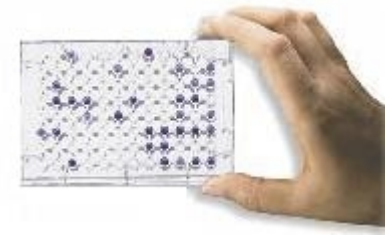
Phenotype Microarray Experiments

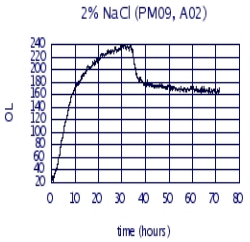
- Chemical treatments are provided as substrates in microwells on 96-well plates that are inoculated with the organism(s) of interest.
- The Omnilog® instrument provides a digital readout of the growth in each well and can scan fifty 96-well plates at one time, so that replicate data can be produced, or more than one organism can be studied.
- With current PM technology, it is possible to screen the response of a microbe to as many as 1,920 chemical treatments in a few days.
- The result is that in one to four days, data for 4,800 growth curves can be generated.

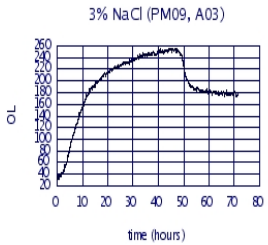
Plate	Mode of Action
PM01-PM02	carbon source
PM03	nitrogen source
PM04	sulfur, phosphorus sources
PM05	nutritional supplement
PM05-PM08	nitrogen utilization
PM09	osmotic sensitivity, toxicity
PM10	pH
PM11-PM20	chemical inhibitors, chemical sensitivity

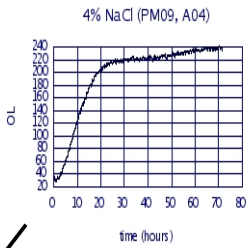


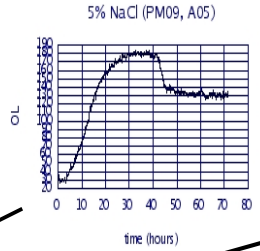
Each plate has eight rows and 12 columns.

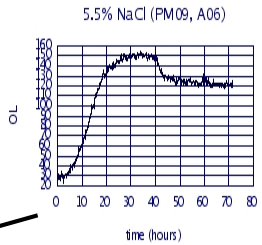
Growth Curves from PM Experiments

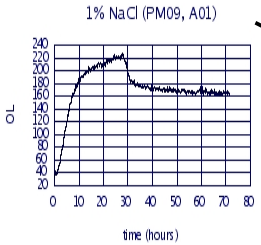








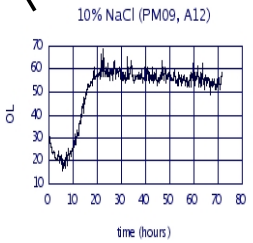


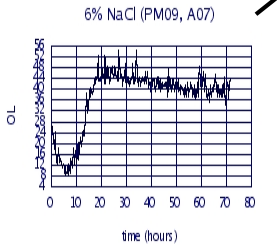


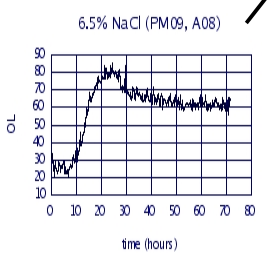
	1	2	3	4	5	6	7	8	9	10	11	12
A												
B												
C												
D												
E												
F												
G												
H												

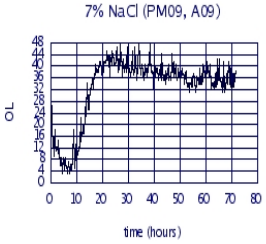
Plate columns are numbered 1 to 12.

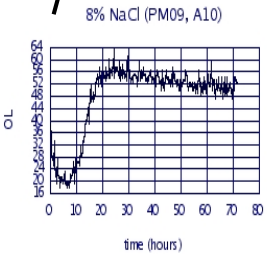
Plate rows are lettered A to H.

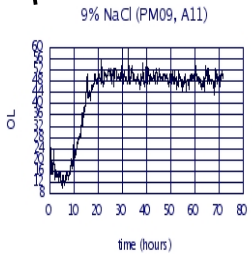












Goals of Software Development

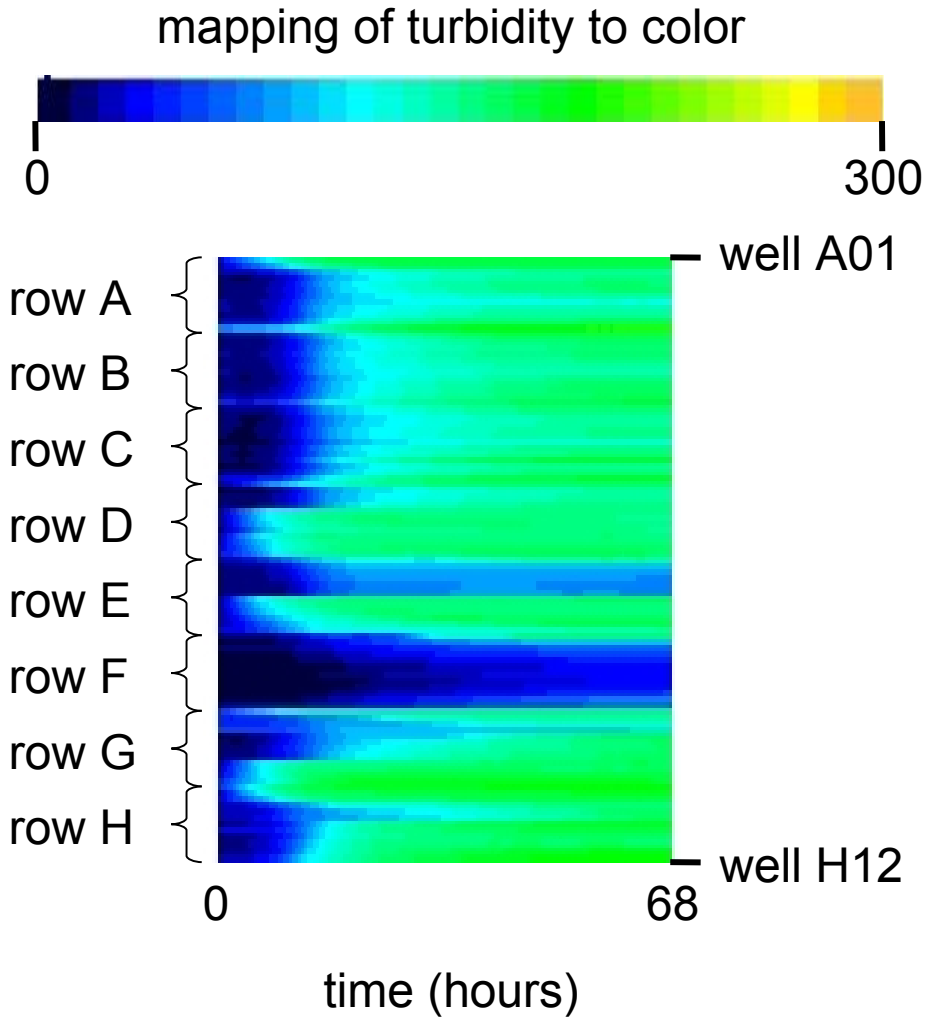
Our design goals were to

- easily display 1000's of growth curves,
- be able to assess data quality,
- facilitate comparison of replicates,
- highlight significant phenotypes,
- display data at different levels of detail,
- make data and display of data Web accessible.

phenotype: the observable physical or biochemical characteristics of an organism, as determined by both genetic makeup and environmental influences

The software tools were developed in response to the needs and desires of the experimentalists generating the data.

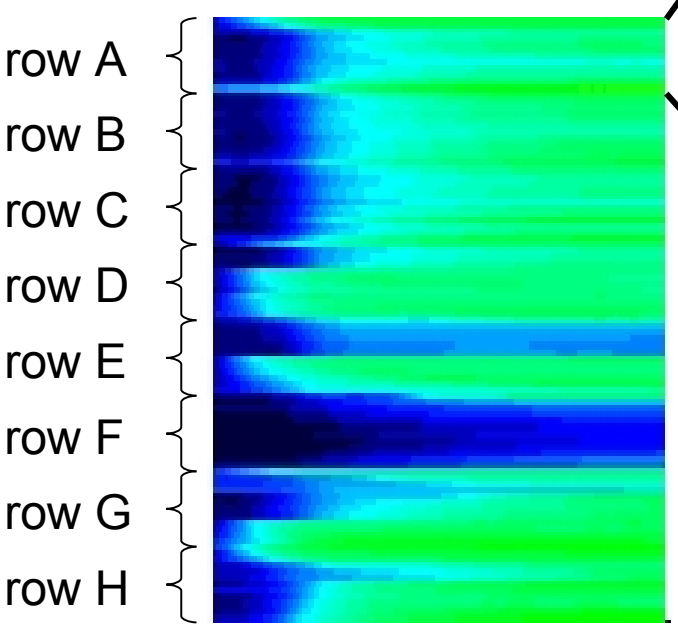
Solution: Use a Color Map

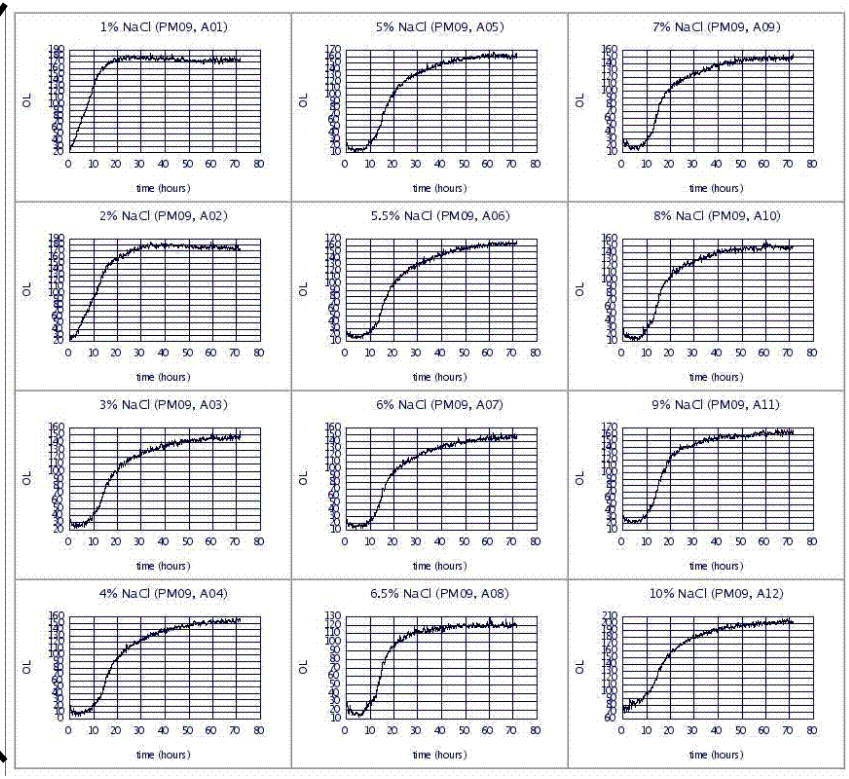


- Values of turbidity measured by the Omnilog® mapped to color.
- Growth curve data are smoothed before mapping to eliminate rapid variations in color (data are noisy).
- Spikes in data <u>not</u> eliminated.

Growth Curve → Color Image

Color images are about 3.75 x 5 cm on the Web interface.



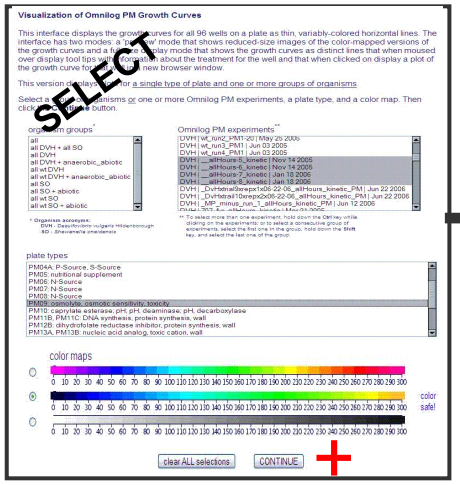


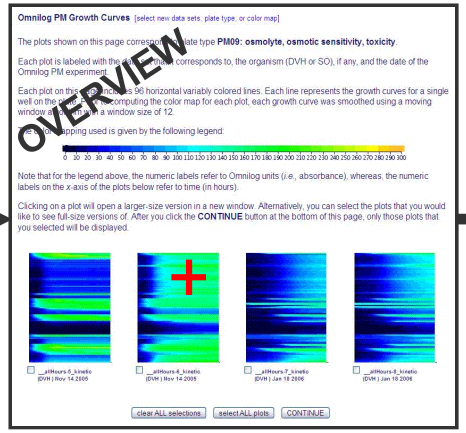
A single row of growth curves on the PMRowView (shown above) Web interface is about 25 x 20 cm.
Ratio: ~200 to 1 for 96 wells

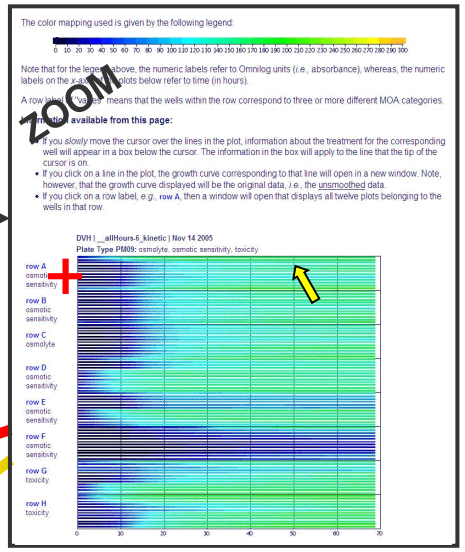
Select data, plate, color map.

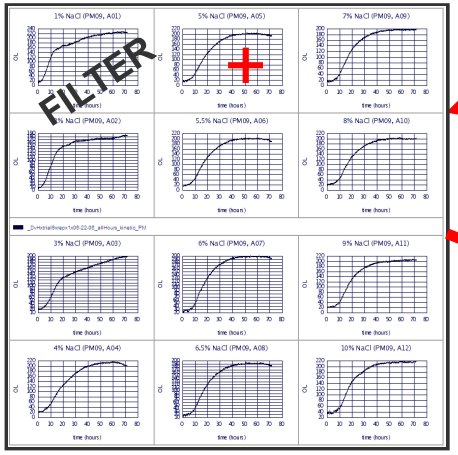
Preview data for 4 x 96 wells.

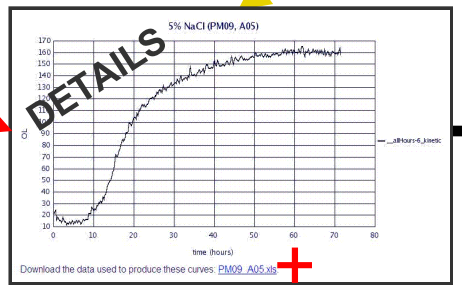
Full-size view of data for 96 wells (one plate).

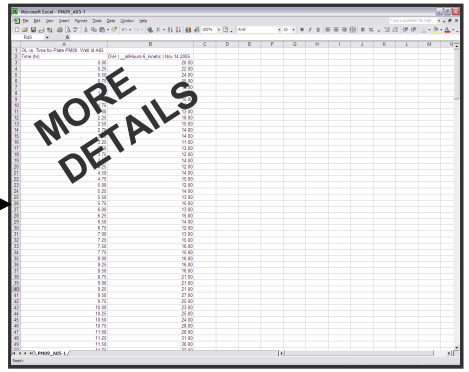












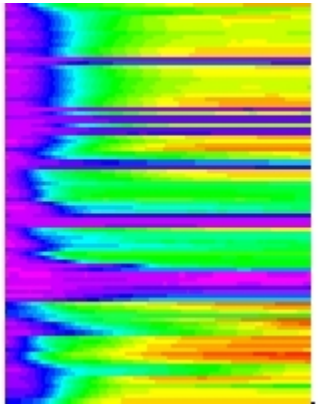
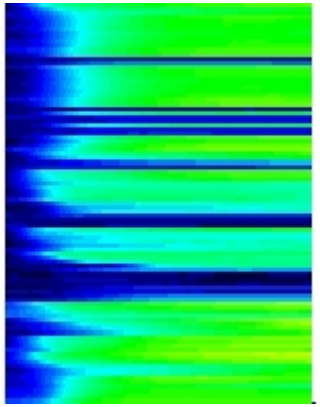
Display wells in one row.

Plot for one well.

Download data for one well as a tab-delimted file.

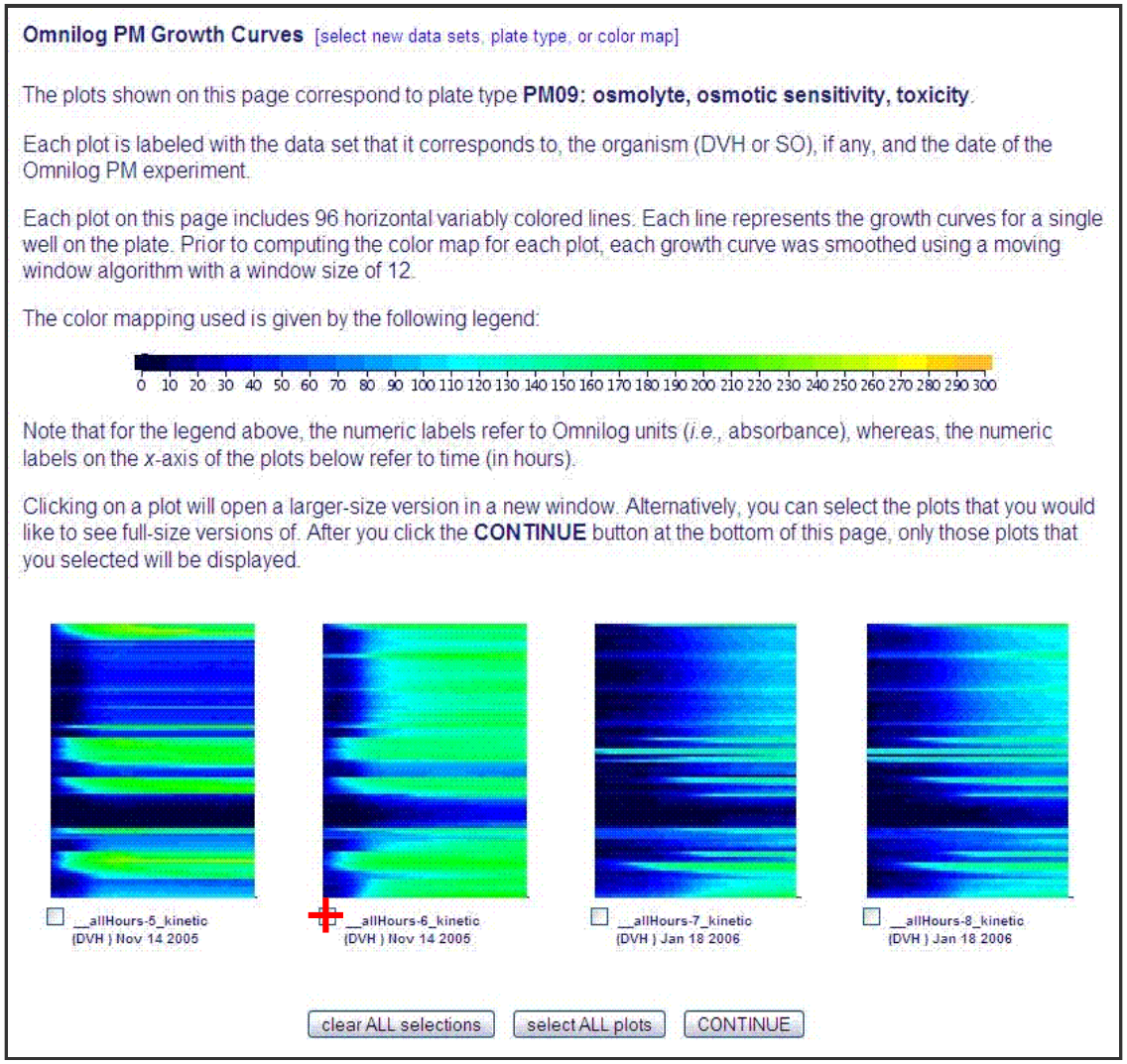
PMColorMap Interface

Color Maps

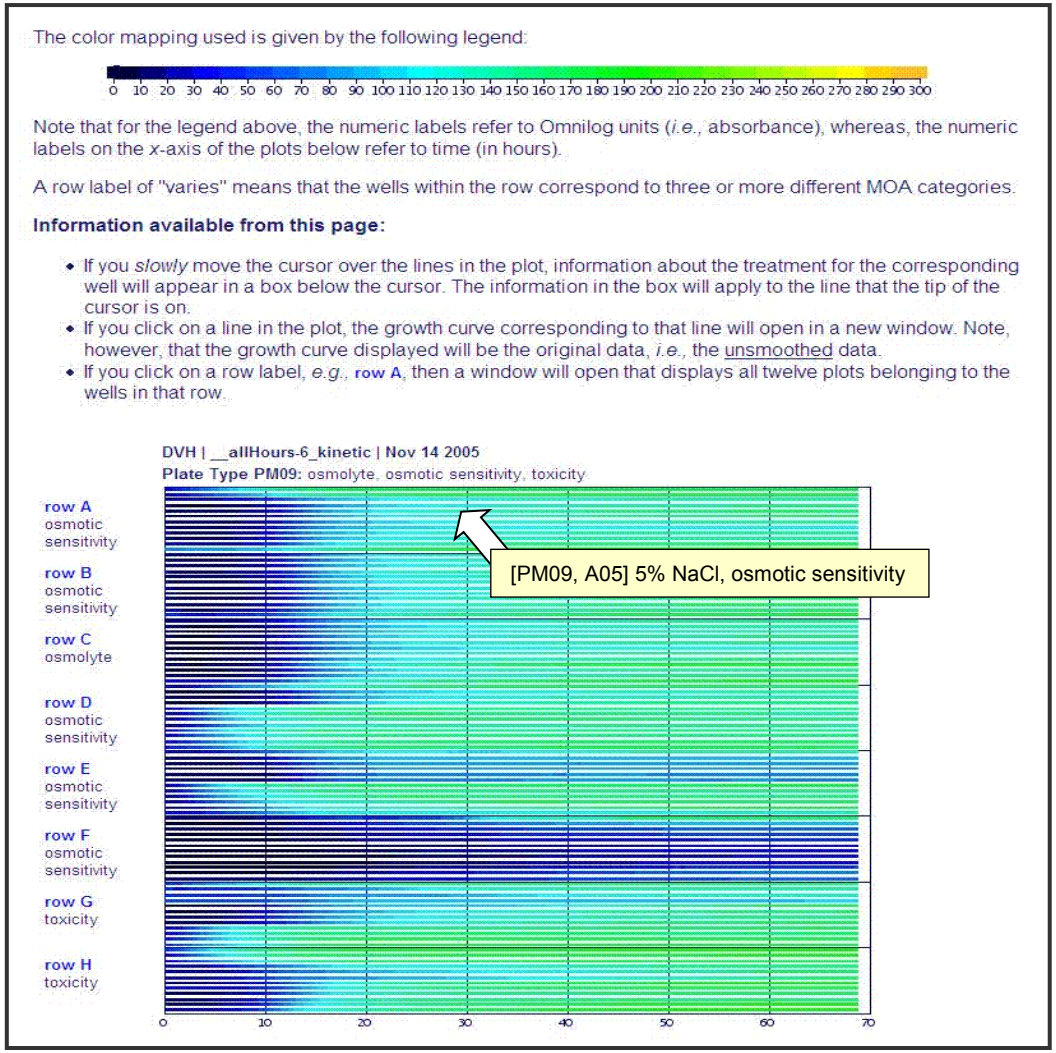

- On the PMColorMap home page, a user may select one of three color maps to use for color coding the growth curve data. The three images above show the same growth curve data from a single PM plate colored using each of the available color maps.
- The colors in the color map of the middle image were selected from a 'color safe' palette, that excludes colors that a person with colorblindness cannot easily distinguish. Both co-directors of ESPP are colorblind!

PMColorMap Preview Mode



- This is the 'preview' mode of the PMColorMap interface.
- The user selected plate PM09 and four datasets.
- On a computer screen, each image is about 3.75 x 5 cm.
- The user may select one or more of the color images to show in a larger size (next slide).

PMColorMap Full-Size Mode



- There is one pixel of space between each color line to make it easier for the user to select the line/well of interest.
- To provide detailed information about the well (chemical and mode of action), the yellow label appears when the cursor is positioned over (in this example) the 5th color line.

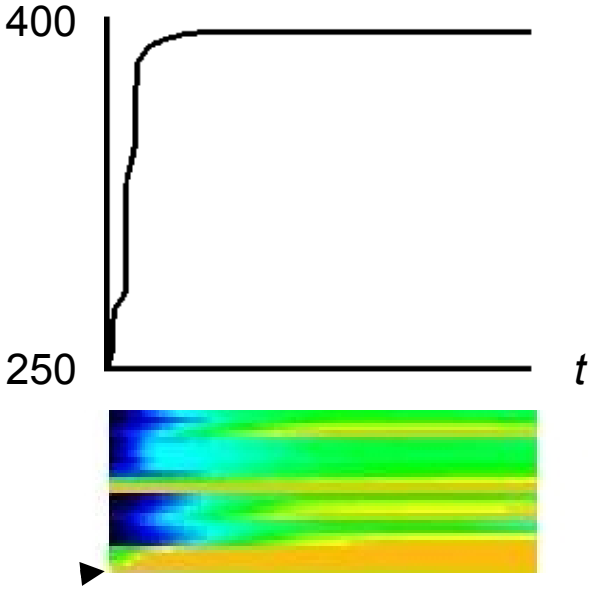
Use of the PMColorMap Interface

The PMColorMap interface provides a quick and easy way to

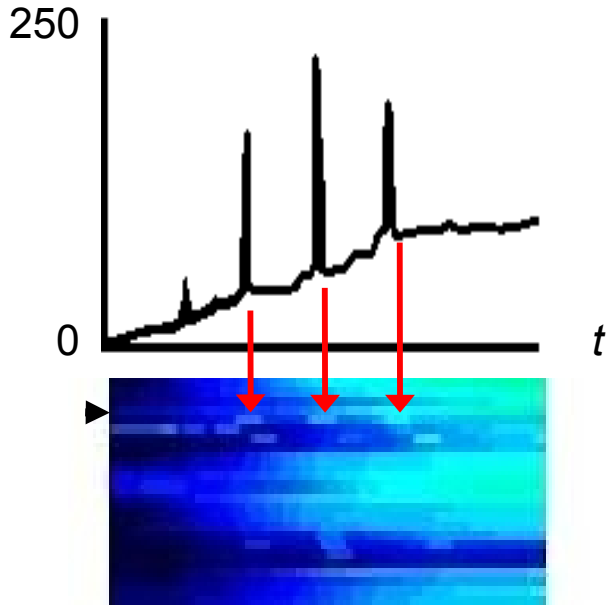
- assess data quality and identify anomalies on single plates,
- compare technical replicates and biological replicates,
- compare phenotypic responses of different organisms.

Examples in each category follow.

Assess Data Quality

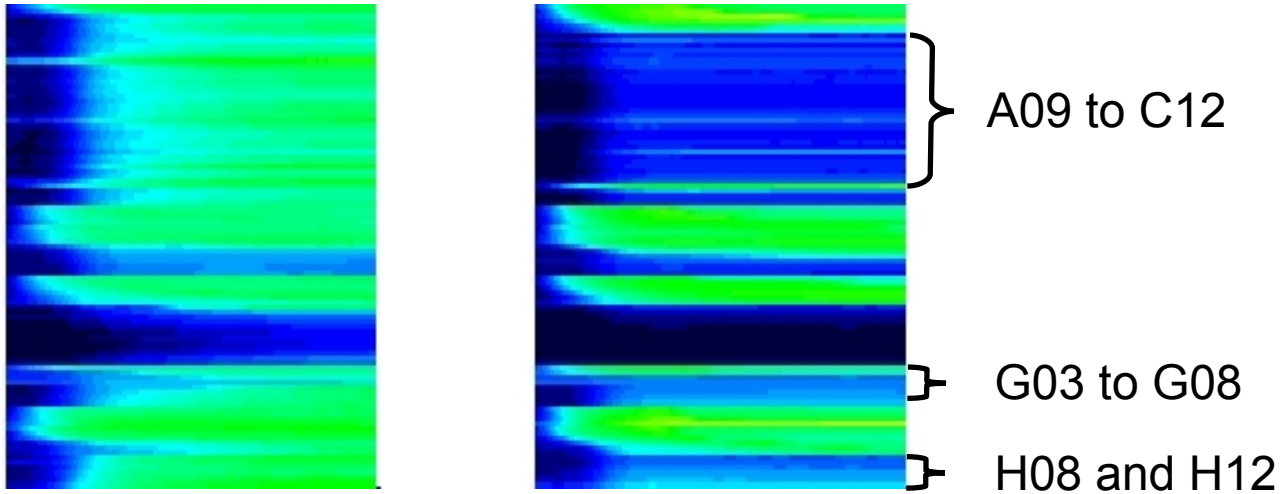


In this case, a chemical reaction between the substrate in the well and the growth medium occurred, resulting in very high values of turbidity. Note the large value at $t = 0$.



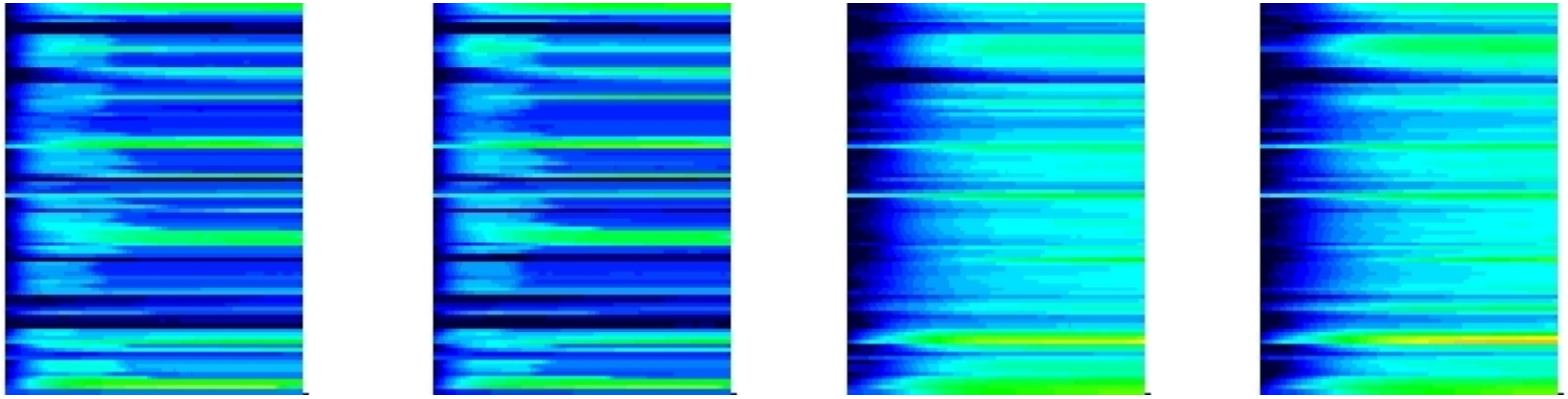
Instrument malfunctions may result in large spikes in turbidity measurements. Here we see vertical banding for many wells on the plate.

Compare Replicates



- These two images are technical (same inoculum) replicates of the same plate.
- Comparison of the two color images suggests that rows A09 to C12, G03 to G08, and rows H08 to H12 of the plate on the right were not properly inoculated.
- This example shows the need to perform PM experiments in replicate.

Compare Phenotypes



wild-type DvH, two technical replicates

DvH **mutant**, two technical replicates

- For this experiment, the two pairs of replicates show good reproducibility, *i.e.*, the two images on the left are similar to each other; the two images one the right are similar to each other.
- Comparing the images for the wild-type DvH with those for the mutant shows that the growth of the mutant was more robust (showed more vigorous growth, *i.e.*, higher values) than that of the wild-type DvH suggesting that knocking out the DVU3335 gene conferred an advantage on the mutant in the presence of the chemicals on plate PM15.

A. Mukhopadhyay, D.C. Joyner, J.S. Jacobsen, T.C. Hazen, J.D. Keasling. Phenotypic characterization of a *Desulfovibrio vulgaris* high affinity K+ uptake mutant. *Submitted to* ***Applied and Environmental Microbiology***.

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Future Work

- We are in the process of automating the processing pipeline.
- Due to the amount of data being generated, we plan to use additional computational methods to assess the quality of and to facilitate comparing datasets.
- In general, two approaches are used to characterize growth curves: growth curve models and calculation of growth indices.
- We will evaluate the latter method first and will link the display of the growth curves with the calculated growth indices in order to be able to easily evaluate how well the growth indices reflect the actual growth.

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Summary

We used a color map technique to create space-efficient representations of growth curves. We combined this technique with an information visualization approach (overview, zoom, filter, details) to met our design goals of being able to

- display large amounts of data (51 PM experiments to date),
- assess data quality and facilitate comparison of replicates,
- highlight significant phenotypes,
- display data at different levels of detail,
- make data and display of data Web accessible.

These software tools **are** being used by the experimentalists who helped to design them.

D.C. Joyner, J.S. Jacobsen, A. Mukhopadhyay, and T.C. Hazen. Assessment of Nitrogen Utilization in *Desulfovibrio vulgaris* using Phenotype Microarrays. *Annual Meeting of the **American Society for Microbiology***, Toronto, Canada, May 25, 2007.

A. Mukhopadhyay, D.C. Joyner, J.S. Jacobsen, T.C. Hazen, J.D. Keasling. Phenotypic characterization of a *Desulfovibrio vulgaris* high affinity K+ uptake mutant. *Submitted to **Applied and Environmental Microbiology**.*

S.E. Borglin, D.C. Joyner, J.S. Jacobsen, and T.C. Hazen. Anaerobic growth of *Desulfovibrio vulgaris* Hildenborough in microwell plates. *In preparation.*

Dome of Advanced Light Source
Lawrence Berkeley National Lab

Campanile on the University of California campus. The Bay Bridge and San Francisco are to the left, and the Golden Gate Bridge to the right.