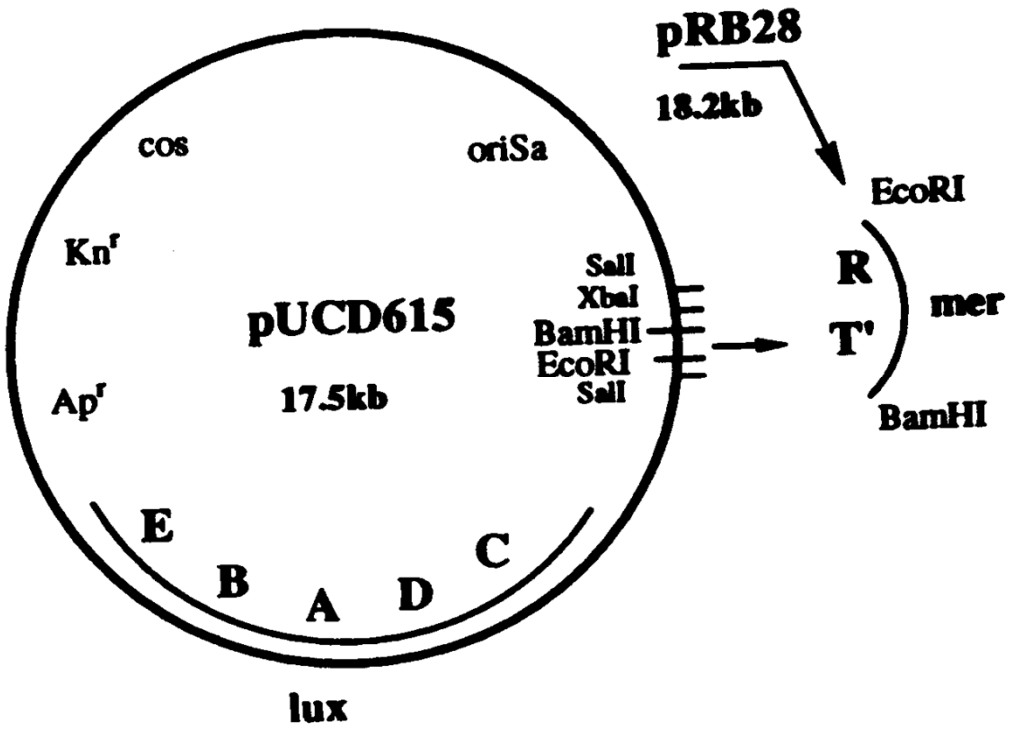
Preliminary Bioreporter Study

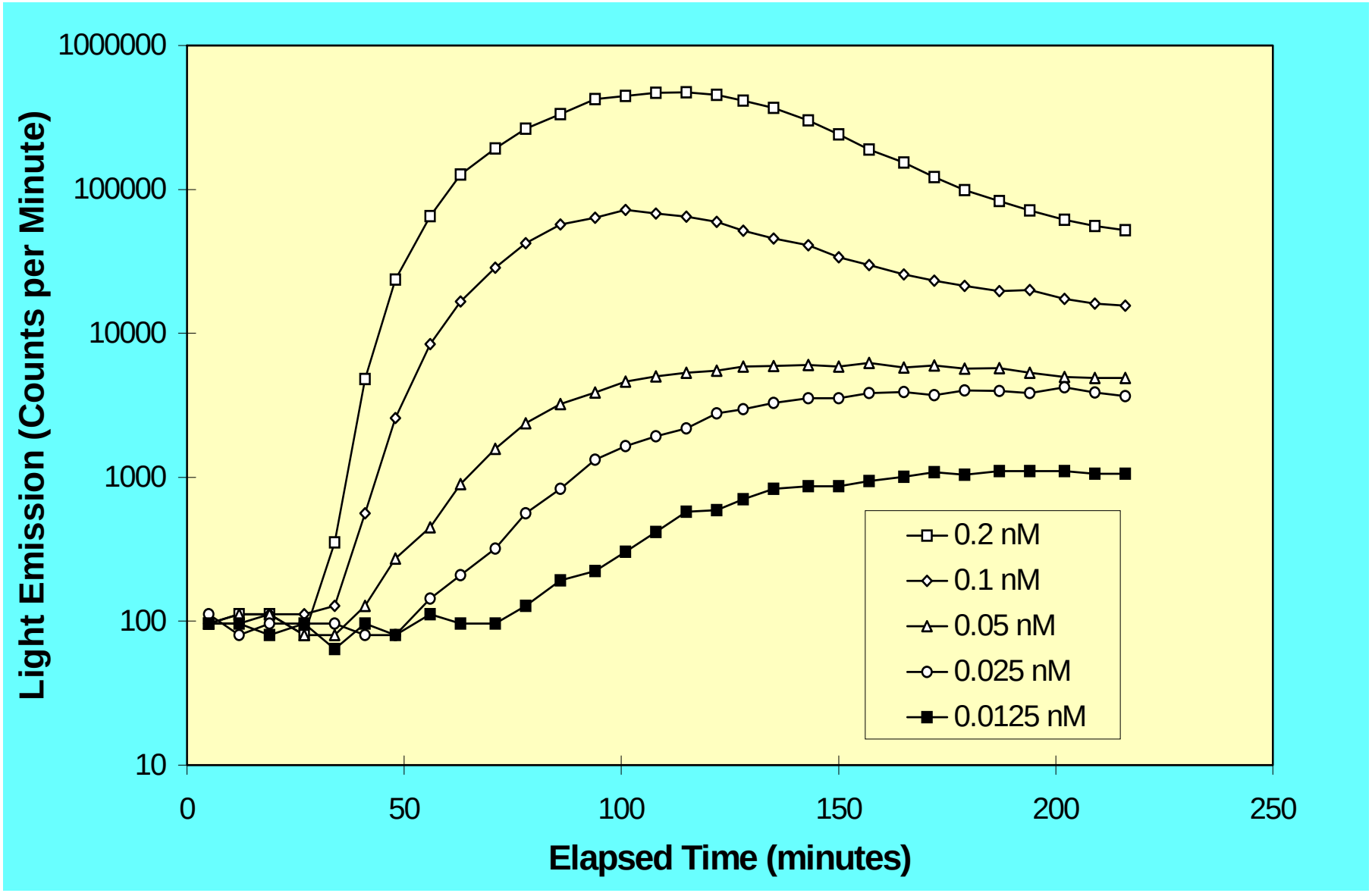
April 2008

What is a Mercury Bioreporter

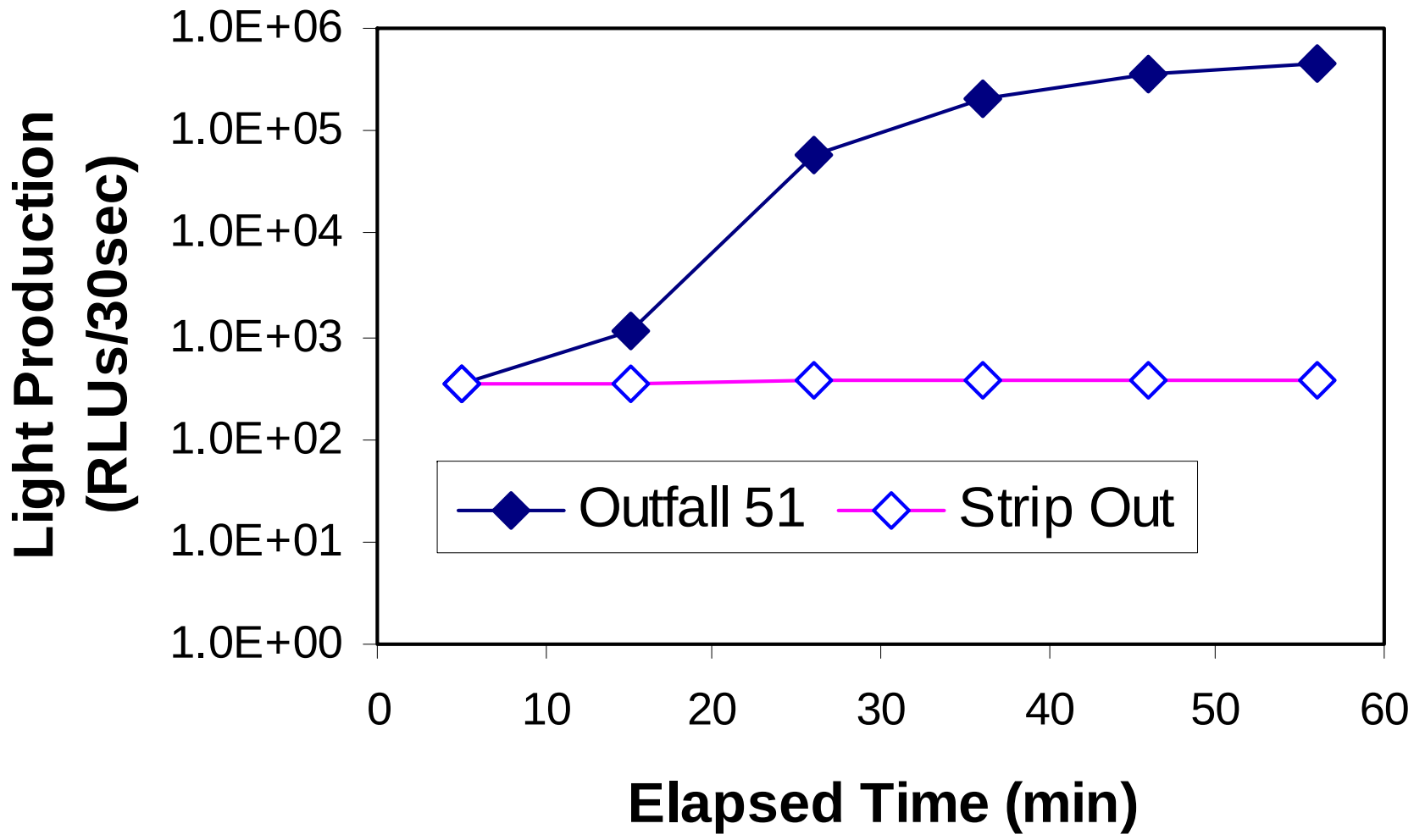
- **A bacterium (*E. coli*) that contains genetic information specifying the production of light when bioavailable Hg is present in the organism's environment. The *lux* genes from a marine bacterium (*V. fischeri*) were cloned downstream from a transcriptional promoter that is turned on by Hg^{2+}**



Bioreporter Calibration (1e5 cells/mL)

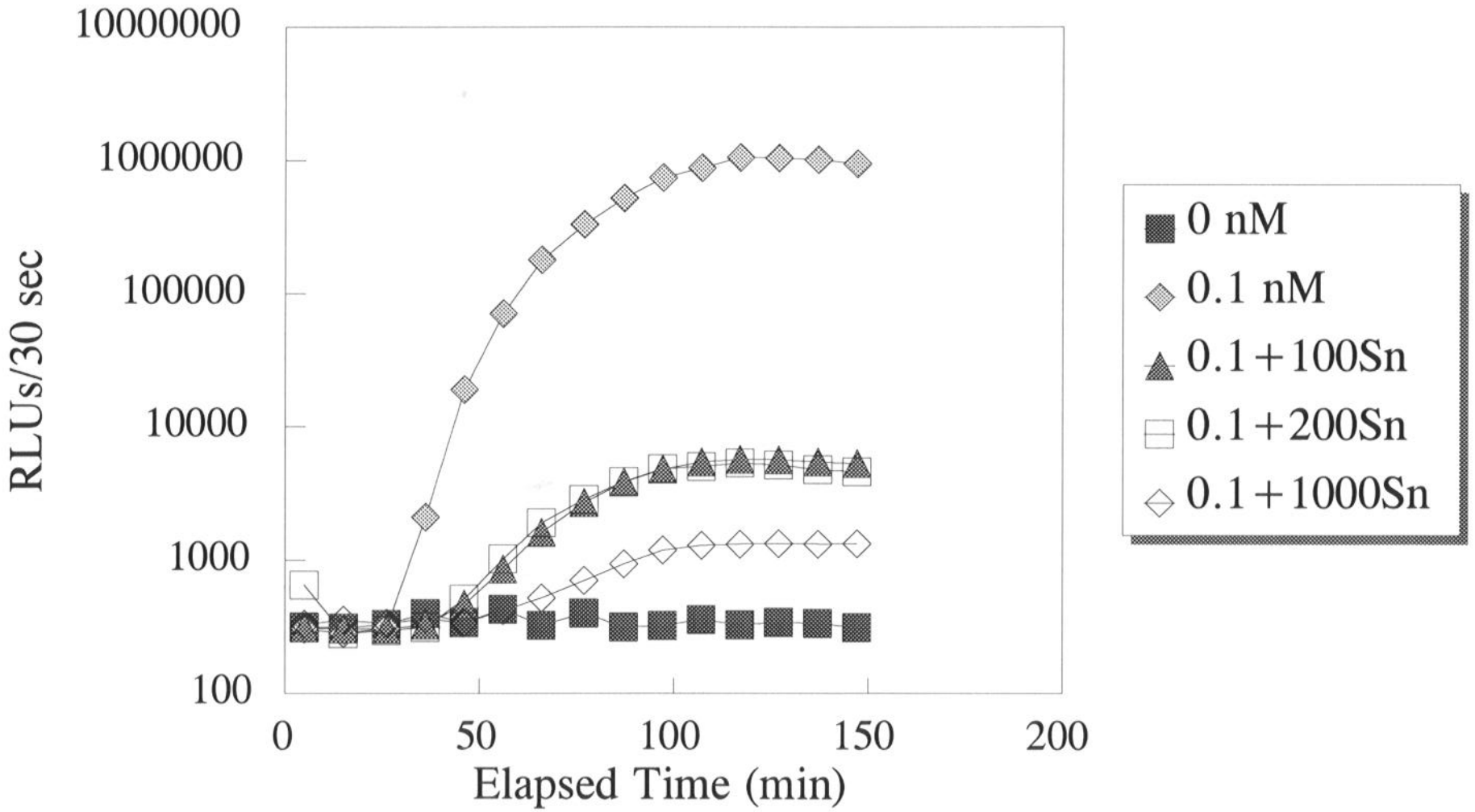


Effect of Tin Reduction and Air Stripping on *merlux* Bioreporter



Effect of Tin Additions on 0.1 nM Hg

7/21/96



merlux58.wk4

Objectives (for SR)

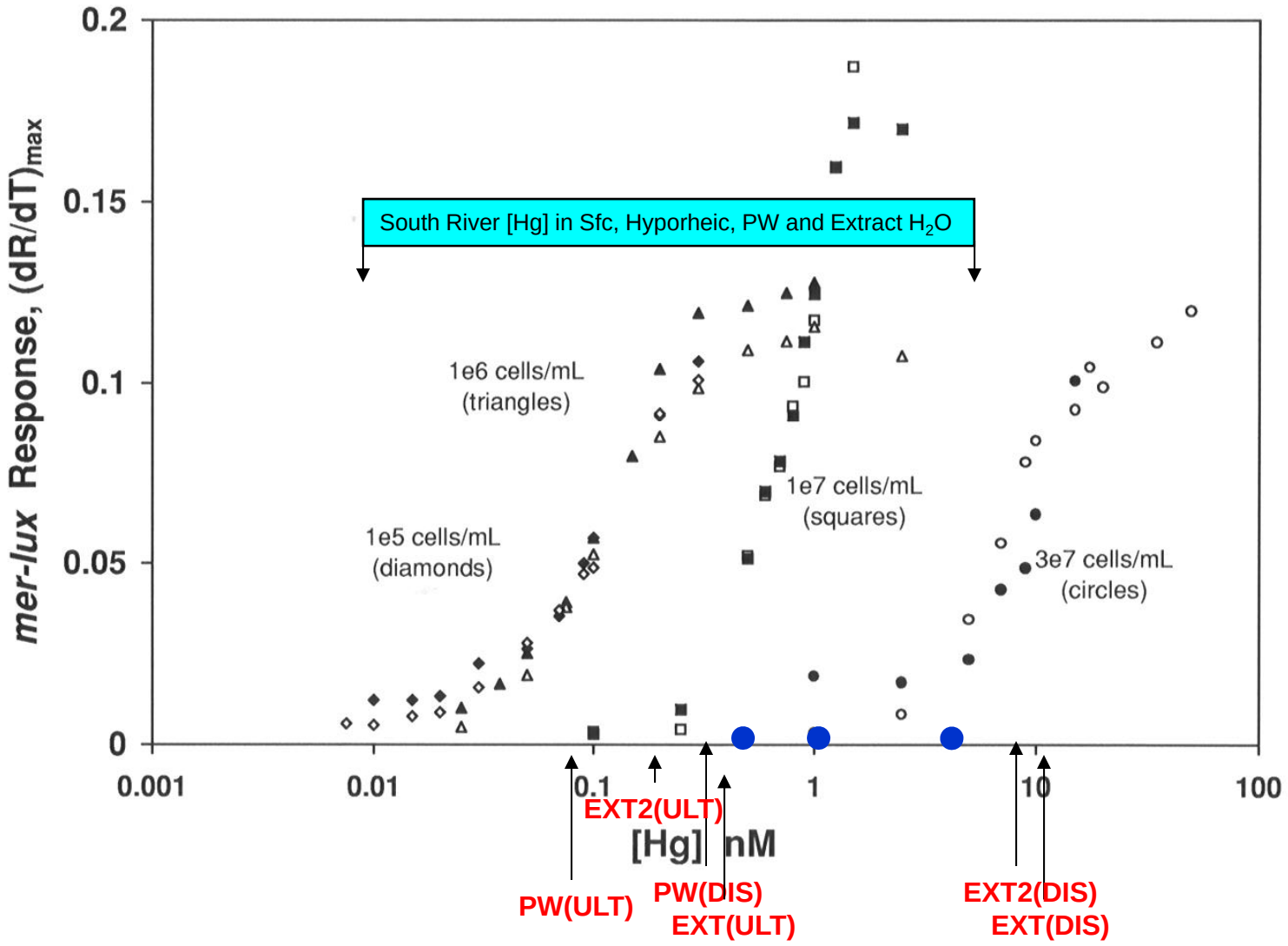
- Troubleshoot assay
- Compare chemistry and bioreporter outcomes for samples prepared in Waynesboro vs Rutgers
  - Porewater and Soil extracts only
- Compare bioreporter response to filter-passing (0.45 uM) Hg, Hg(II) and ultrafiltered Hg
- Samples used (from BPK): Sfc water, hyporheic water, porewater and soil extract

Sample Chemistry

(***** Prepared at Rutgers)

Sample ID	Description	Dissolved Hg (0.45 µ)	Hg(II)	<3000 MWCO Hg
		(ng/L)		
BP	Surface Water	2.06	0.62	0.60
HYP	Hyporheic Water	7.28	1.90	2.34
PW	Porewater (Sed THg= 17.6 ug/g)	70.3	11.3	34.5
PW2*	Porewater* (Sed THg= 17.6 ug/g)	76.6	3.08	19.4
EXT	Soil Extract (Soil THg = 25.7 ug/g)	2374	543	88.4
EXT2*	Soil Extract* (Soil THg = 25.7 ug/g)	1894	590	58.1

Effect of Cell Density

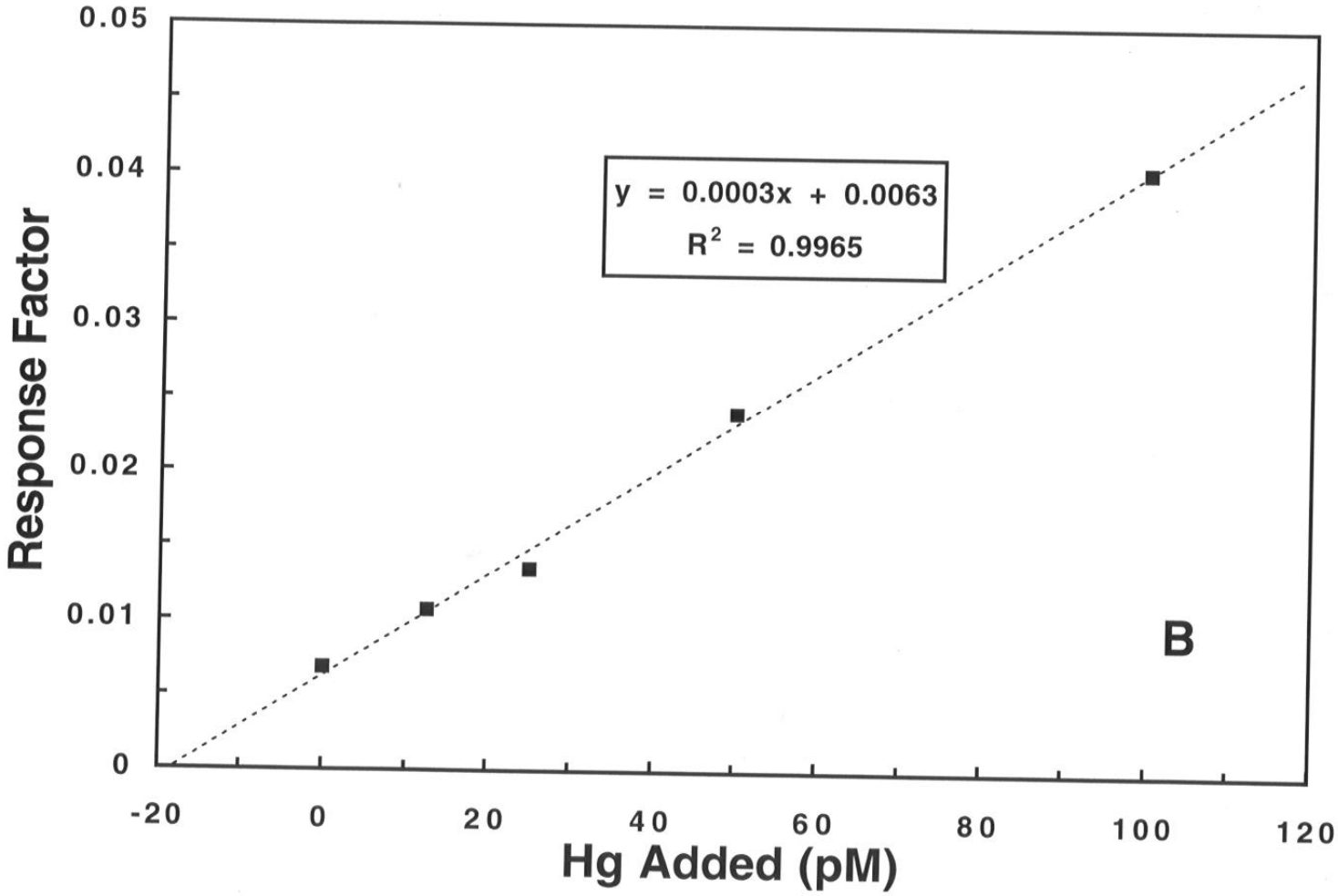


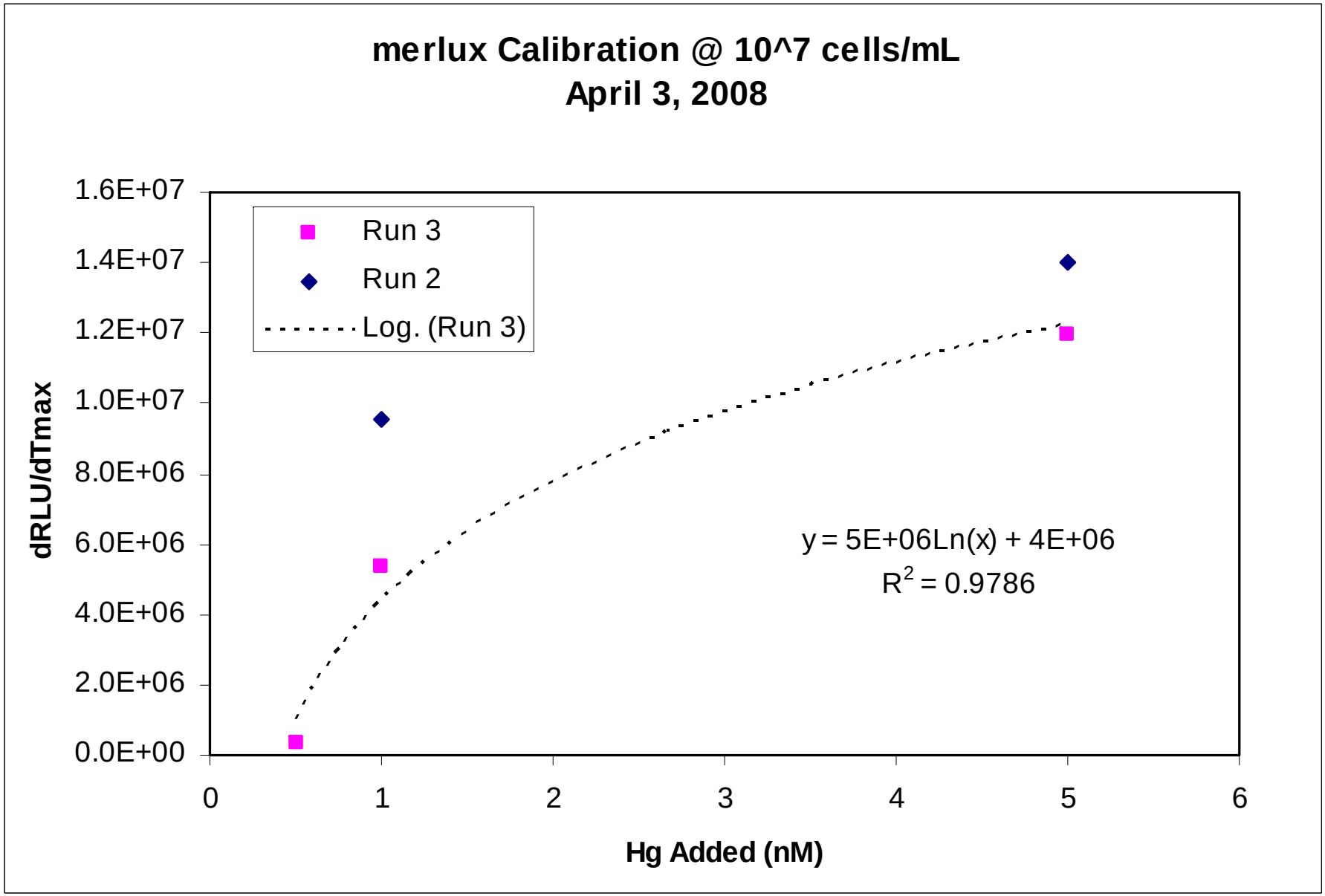
Bioreporter Data Processing

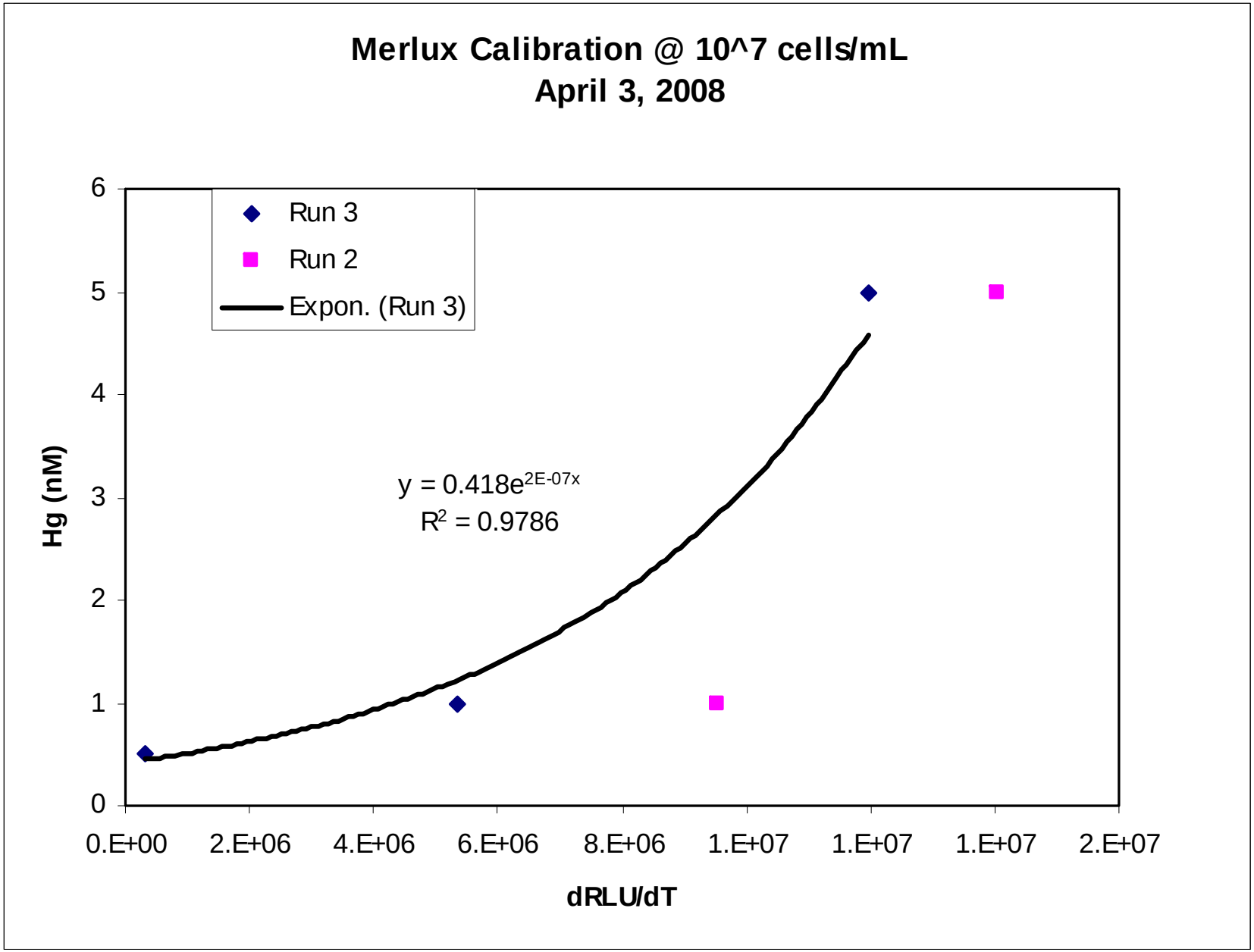
- Possible Figures of Merit for Calibration
  - Peak light value (RLU)
  - Integrated light production (ΣRLU)
  - Lag Time (minutes)
  - **Maximum rate of light production ($dRLU/dT_{max}$)**

“Typical” Linear Calibration

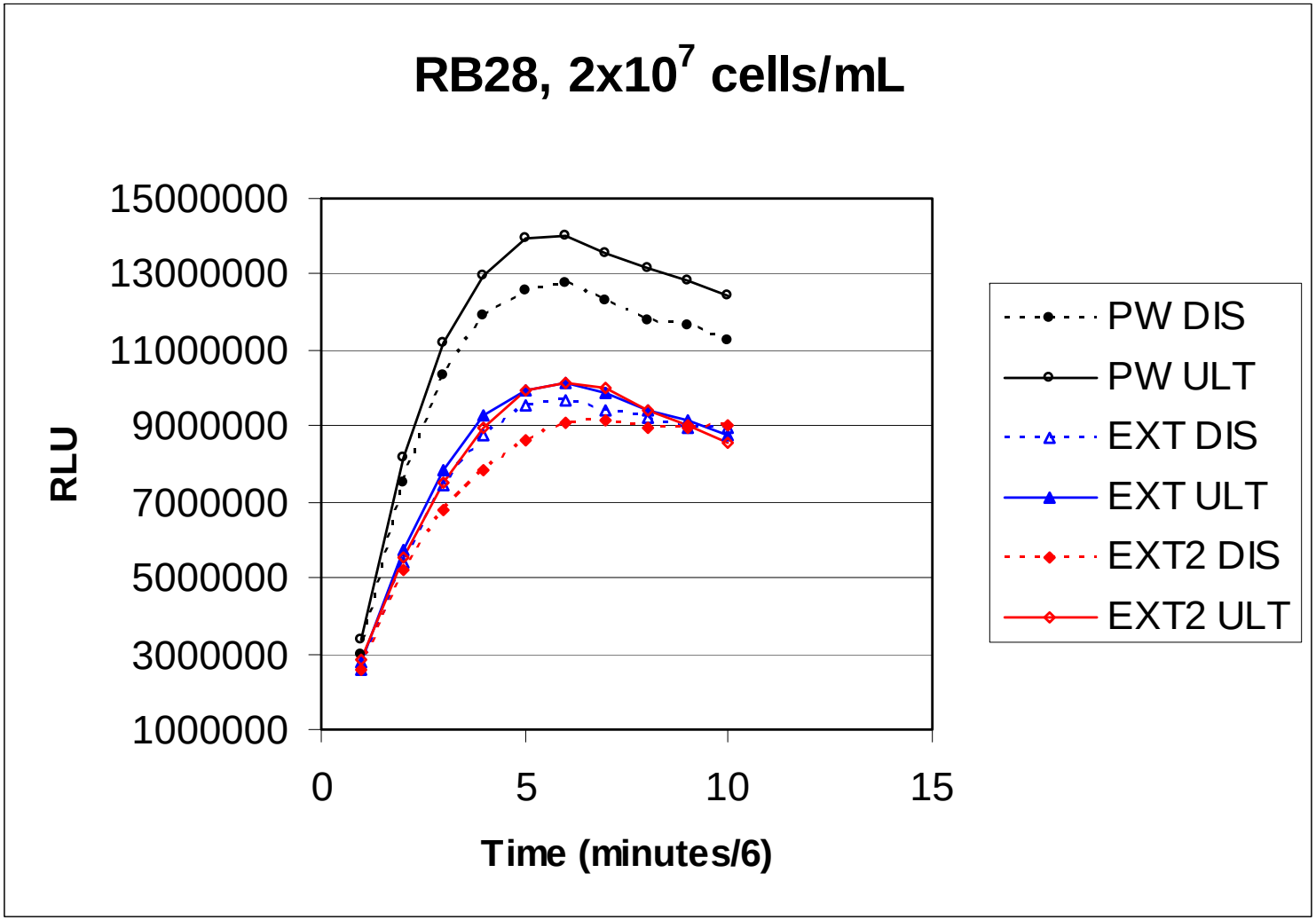
(from ORNL/EPA work)




merlux Calibration @ 10^7 cells/mL
April 3, 2008
dRLU/dTmax
1.6E+07
1.4E+07
1.2E+07
1.0E+07
8.0E+06
6.0E+06
4.0E+06
2.0E+06
0.0E+00
0
1
2
3
4
5
6
Hg Added (nM)
Run 3
Run 2
Log. (Run 3)
y = 5E+06Ln(x) + 4E+06
R2 = 0.9786


Merlux Calibration @ 10^7 cells/mL
April 3, 2008
Run 3
Run 2
Expon. (Run 3)
y = 0.418e2E-07x
R2 = 0.9786
Hg (nM)
0
1
2
3
4
5
6
0.E+00
2.E+06
4.E+06
6.E+06
8.E+06
1.E+07
1.E+07
1.E+07
2.E+07
dRLU/dT

Bioreporter Results



Trial Predictions

(y = 0.418e2E-07x)

Sample	Date Run	Response Factors		Predicted Bioavailable Hg (Analytical "total" value) (Analytical Hg(II) value)	
		Diss Hg (dRLU/dT)	<3000 MWCO (dRLU/dT)	Diss Hg (ng/L)	<3000 MWCO (ng/L)
BP	2-Apr-08	?	?	?	?
HYP	2-Apr-08	?	?	?	?
PW	3-Apr-08	7.55E+05	7.92E+05	97.2 (70.3) (11.3)	98.0 (34.5)
EXT	3-Apr-08	4.73E+05	4.92E+05	91.8 (2374) (543)	92.2 (88.4)
EXT2	3-Apr-08	4.38E+05	4.51E+05	91.2 (1894) (590)	91.4 (58.1)

Problems/Issues

- Possible labware/reagent Hg contamination in first runs with low Hg and low cell density runs.
  - No usable data for surface and hyporheic water samples
- Possible RB27 contamination with RB28
  - no constitutive controls for this set.
- Non-linear calibration
  - Cell density too low for EXT and PW samples??
- Porewater recovery
  - Needs to be done quickly in the field rather than shipped overnight for next day recovery.

Next Steps?

- Arrange for cell preparation at JMU
- Use Luminometer in Waynesboro to repeat calibration and field sample assays.
- Run some parallel “Std Addition” calibrations.
- Revisit usefulness of Hg(II) and ultrafiltration as surrogates for bioreporter.