

- **Announcements**

- **Pre-lab Lecture**

- ❖ About the robot
- ❖ Mechanism of the CTG assay
- ❖ Visualizing CTG data
- ❖ Potential comparisons
- ❖ Today in Lab: M2D7

after
SKH
slides

Announcements

- M2D6 FNT due Monday, Nov 4th at 5 pm
 - to be returned M2D8 in class
- Quiz next time!
- Journal club: 16-336, starting 1:30 sharp *1:15 for presenters*
- OH plan next week
 - Monday by appt (journal club folks)
 - Tuesday 4:15-4:30, M2D5 FNT pick-up only
 - Friday lab open 1:30-4:30 pm (no official class)
 - Sunday in lunchroom w/Shannon, time TBD *→ + dog :)*
- Report due Monday, 11/11 at 5 pm

Robot(s)!

Ambistore: "home base"



B-191
incubation

LidValet



+100mL CTG reagent

EVO: liquid handling



BioTek plate washer/shaker



A-1'



Tecan plate reader

★ collect light 1sec
★ in black walled,
white-well plates
★ transfer 100mL for
consistency

CellTiter-Glo Assay (by Promega)

Very popular: easy, fast, sensitive ★
 "add-mix-measure" 10-20' protocol

Standard, esp. for HTS

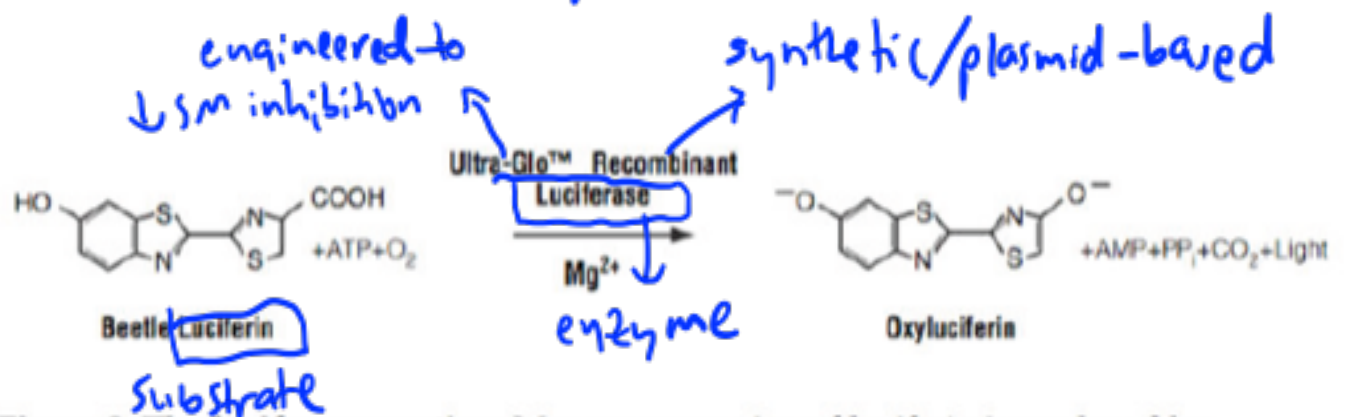
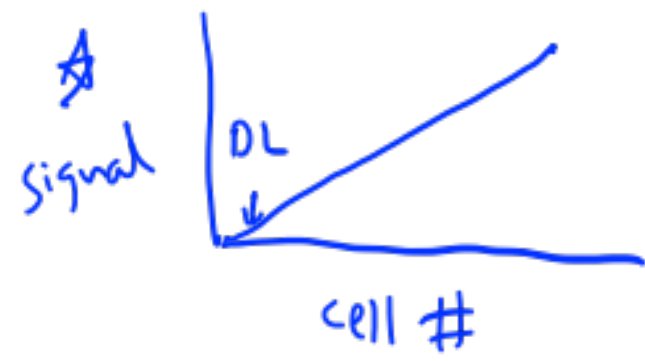


Figure 3. The luciferase reaction. Mono-oxygenation of luciferin is catalyzed by luciferase in the presence of Mg²⁺, ATP and molecular oxygen.

→ α live "metabolically active" cells



- wide linear range
- DL ~ 15 cells!!
- pro + con → errors amplified

cf MTT in 1995

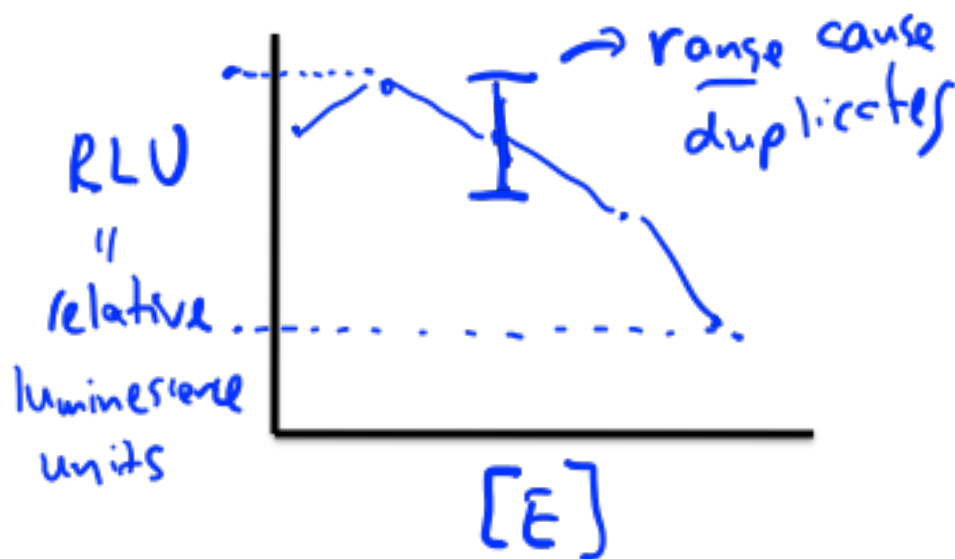
- 1500 (ATP)
- 25,000 (MTT)

Visualizing CTG data and error

std. curve [ATP]
needed

- RLU vs ALU; error bars; cross-plate comparisons

① at a given [x]



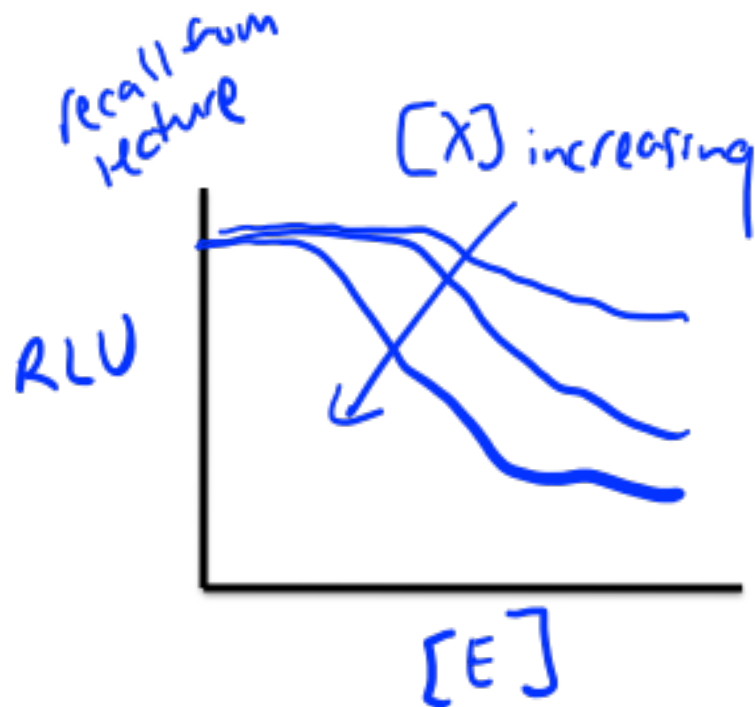
② sample plot



- across plates, to compare ① directly, normalize $\frac{\text{MAX} - Y}{\text{MAX} - \text{MIN}}$
- pool for errors and/or fit

Potential comparisons/analyses

What is our question? how does $[X]$ affect E-based IC_{50} ?



transform \Rightarrow IC_{50}



2 options for error bars

- (1) own data \rightarrow 95% CI of fit
(note: using average duplicates)
- (2) own + wider data \rightarrow error of mean

$[X]$ many WB/CTG analyses: "do at least one really well!"

Today in Lab: M2D7

- Reminder for future (non-109) work: Pasteur pipets must be treated as sharps! → in mayo jars
- Two shifts for CTG assay
 - Group 1: Red, White, Green, Blue
 - Group 2: Orange, Yellow, Pink, Purple
- CTG assay
 - plates to room temp 30-45 min
 - 4 plates take up to 1 hr – stay to watch *one* full one *shRNA*
 - Chat w/Ian Tay! S13 109 TA; HTS Comet assay in BPE lab
- When not assaying, work on analysis: WB and/or CTG

And... your toddler inspiration!

Day 1 Who Knows the 5 senses?

Senses is dentist-Feliks
Senses is senses- X

left field
tautology

Day 2

Discussion:
What are your 5
senses?

See, hear, mouth, fingers- X

Eyes to see- Y

close-

See, talk with my mouth, I put food in my mouth- Z

Throw up with my mouth, hear with my ears, see Spider-
man with my eyes- Feliks

Day 3

Discussion:
What are your 5
senses?

Eyes, ears, nose, fingers, mouth- A

Eyes, fingers- B

Eyes, ears, nose, mouth, hands- C

Eyes, ears, mouth, legs, shoes- D

Ears-listen, nose-smell, hands-clap and touch, eyes-see,
mouth-talk and eat- Feliks

Mouth, ears, shoes, feet, toes, socks- E

very
close!