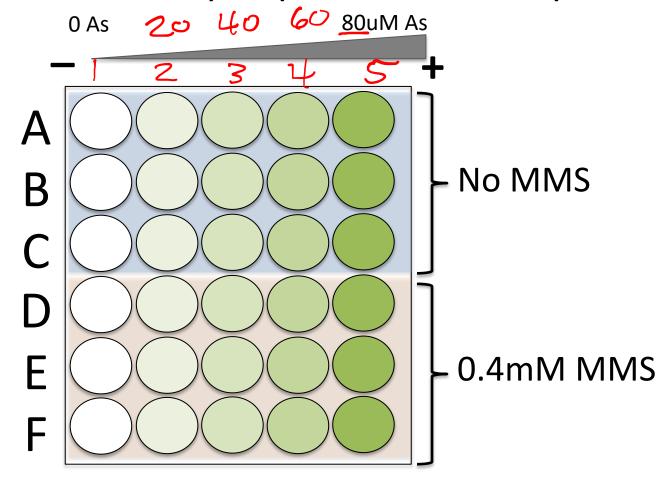
M1D6: Image and Analyze Comet Chip

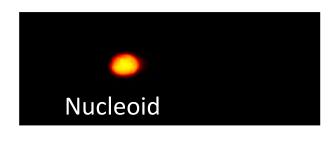
10/01/19

- 1. Communication workshop
- 2. Prelab
- 3. Comet Chip analysis
- 4. Paper discussion
- 5. Resubmit modified figure hw before 10pm tonight

Reminder: CometChip experimental setup

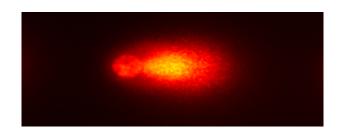


Output of Alkaline CometChip Assay



No Damage

- Supercoiled nucleoid
- Little or no migration

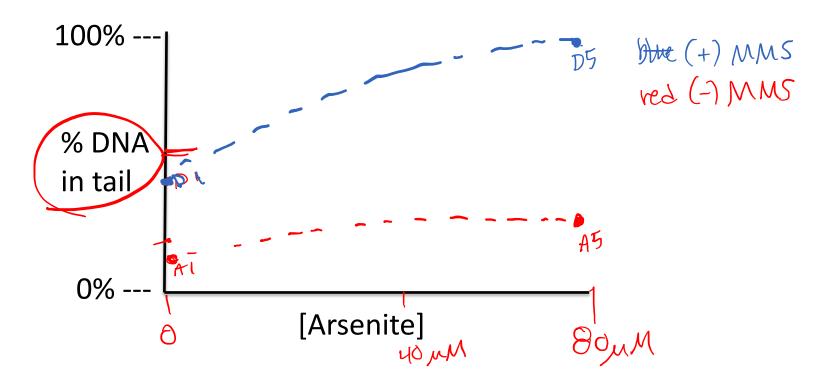


High Damage

- SSBs, abasic sites, alkali labile sites
- forms a "Comet tail"

Genomic damage from direct strand breaks and REPAIR INTERMEDIATES

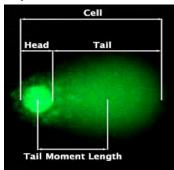
What result do we expect from the CometChip Analysis?



What's in the final Trevigen Excel file?

Region	#Found	#Counted	Moment_Mean	%DNA_in_TailMean	_ength_Mean	Area_Mean	IntensityMean	Moment_StDev
A1	64	64	2.35	33.19	16.69	849	5.77E+06	0.81
A2	120	120	2.48	32.25	19.13	982.72	3.22E+06	1.5
A3	96	96	4.25	48.44	20.82	1045.16	4.32E+06	1.53
A4	86	86	4.11	47.15	19.7	1011.69	5.82E+06	1.64
A5	63	63	5.14	54.17	21.49	1107.44	5.22E+06	1.9

- Region: 96 well plate macrowell letter/number
- #Counted: how many comets were used for calculation in each macrowell
- %DNA in Head Mean= 100 * HeadFluorescence / (HeadFluorescence + TailFluorescence)
- %DNA in Tail Mean= 100 * TailFluorescence / (HeadFluorescence + TailFluorescence)
- Moment Mean= (%TailDNA / 100) * (TailCenterOfMass HeadCenterOfMass)



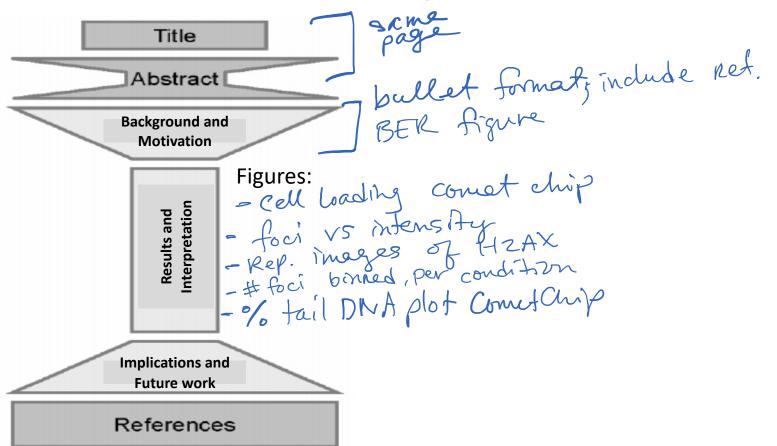
Major assignments for Mod1

- Data summary draft
 - due by 10pm on Mon., October 14
 - revision due by 10pm on Sat., October 26

Summary content

- 1. Title
- 2. Abstract
- 3. Background & Motivation
- 4. Figures, Results & Interpretation
- 5. Implications & Future Work

M1 Data summary Architecture



Reminder: Example Results slide

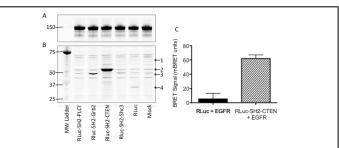


Figure 1: Development of BRET assay to monitor EGFR and SH2 domain interactions. CHO-K1 cells were transfected with Citrine-EGFR (A) and renilla luciferase (RLuc)-tagged SH2 domains from PLCg, Grb2, CTEN, and Shc3 (B). Western blots of CHO-K1 lysates were probed with anti-EGFR (A) or anti-RLuc (B) antibodies. Arrowheads indicate the expected molecular weight of the RLuc-tagged proteins; (1) RLuc-SH2-PLCg, (2) RLuc-SH2-CTEN, (3) RLuc-SH2-Grb2 and RLuc-SH2-Sh2-Sh3, and (4) RLuc alone. Mock indicates no cDNA was utilized during transfection. (C) For CTEN only, BRET signal was quantified using a luminometer after stimulation of CHO-K1 with 100 ng/mL EGF for 15 min.

BRET system effectively measures EGFR activation:

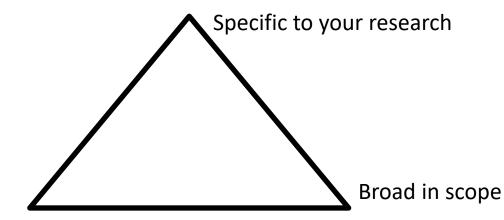
- To determine if the BRET system could be used to monitor EGFR activation, CHO-K1 cells were transfected with fluorescent EGFR and luciferase-tagged SH2 domains and a BRET assay was performed after growth factor stimulation.
- CHO-K1 were transfected with Citrine-EGFR in all conditions as indicated by correct molecular weight band at 150 kDa (Figure 1A).
- Several protein bands are present in Mock transfection lane suggesting off-target binding of the RLuc antibody (Figure 1B).
- RLuc alone, RLuc-SH2-Grb2, and RLuc-SH2-CTEN were successfully transfected as indicated by correct molecular weight bands (Figure 1B).
- RLuc-SH2-PLCg and RLuc-SH2-Shc3 did not appear by Western blot analysis -bands different from those in the Mock lane are not identifiable. This outcome could be due to protein expression levels below the detection limit by Western blot or to unsuccessful transfection of cDNA.
- BRET signal increased in cells transfected with Citrine-EGFR and RLuc-SH2-CTEN versus Citrine-EGFR and RLuc alone after EGF stimulation. This difference suggests that the BRET signal is specific for an SH2-EGFR interaction versus randomly localized RLuc.
- In sum, these data suggest that the RLuc-SH2 constructs can be utilized to monitor EGFR phosphorylation, as SH2 domain-EGFR association occurs only at sites of EGFR tyrosine phosphorylation. Next, we determined the dynamic range of the BRET assay.

- PowerPoint format
- Limit figure size (1/3 of page)
- Caption describes image or graph
- Results text (2/3 of page) in bullet points
 - Look ahead to M1D7 for results text guidelines

HW M1D7: Implications & Future Works

Implications and Future Work: potential topics [edit]

- Topic: Did your results match your expectations?
 - If no, provide a putative explanation. If yes, how can you further test if your hypothesis is correct?
- Topic: Based on the results, whether they matched your expectations or not, what experiments might you recommend next?
 - Follow-up experiments could distinguish between competing explanations of a given outcome or broaden the sample set for a question you already asked, to give just two examples.
- Topic: How might this assay be improved?
- Topic: How might this assay be used as a research tool? in the clinic? in industry?



In your Data summary tie together (and mirror) your background and motivation topics in your implications and future work section

Organization of Implications & Future Work

- Start with a very similar paragraph to the last paragraph in your Background/Motivation (restate major results and broad implications)
- Follow same order as in Figures/Results
 - Describe your conclusions from your data
 - If necessary describe caveats of experiment and suggest improvements
 - Identify unknowns and speculate (within reason)
 - Don't make huge generalizations or overreach
- Propose future experiments, identify new questions that arise
- Come back to the big picture/impact statement topic introduced in background

In lab today

- 1. Start Matlab analysis of Comet Chip images, Part 3
- 2. Groups of 2 will be taken to the Engelward microscope throughout the afternoon
- 3. Work on modifying your figure during downtime
- 4. Half the class will go to 56 302 to discuss Weingeist et al.

HW due M1D7 (individual)

Name homework file: LeslieM1D5hw futureworks

1. Outline the future works section (bullet format!) Include references.