M1D2: Chemically treat cells for H2AX assay and Prepare CometChip

09/12/19

- 1. Pre-lab Discussion
- 2. Start H2AX assay treatments
- 3. Prepare CometChip during incubations

Overview of Module 1: Measuring Genomic Instability

Aim: Evaluate effect of Arsenic exposure on methylation induced base excision repair (BER)









γH2AX assay: Immuno fluorescence of cellular response to DNA damage CometChip assay: single cell gel electrophoresis measuring single strand breaks



Research Question: Does the presence of Arsenite affect Base Excision Repair (BER) following MMS treatment?

Controls Condition(s):

> O. 4 mM MMS alone > 80mm As alone

Experimental Condition:

7 O.4 mM MMS + 80 mM As

Blue: DNA Green: γH2AX staining

Additional Question: Which cells are in S phase at the time when Arsenite is added?



Timeline of CHO cell treatments: SFM = serm free media Α NO treatment 0.4 mM MMS В 1° Ab 2° Ab 80 uM As 1st hour 3rd hour 2nd hour 0.4 mM MMS, С 5m 80 uM As **FIX** +Edw' ~> Notreatment A1,2 SFM SFM SFM+ SFM 201. O. Frun MMS -> 0.4mm MMS A3,4 Edu As + Edu 84MAS -> 80mm As B3,4 SFM 0.4mm MMS Ast Fdu 80mm As -> 6.4mm MMS, 80mM As

Overview of CometChip Assay: stamping microwells and loading cells



Overview of CometChip Assay: chemically treating cells and visualization

Treat captured cells in comet chip with MMS and As



Today, make a CometChip



What goes into a background/motivation section?



- Your research is anchored in a general topic. Motivate the topic in a way that draws your audience into your study.
- Specific background connects your project with the general background.
 - Minimum essential information
 - References current work in the field
 - Introduce specific technologies necessary for understanding the project
- Clearly state the unknown or problem your research is addressing.
 - The question you address is clearly articulated, connected to the background, and has appropriate scope for the project
 - Give evidence of incompleteness of current understanding, therefore motivating the investigation
- The hypothesis specifically addresses the problem and has a measurable outcome.
 - <u>Draft a hypothesis (this does not need to be the hypothesis you use in your data summary)</u>
 - A preview of your findings and their implications
 - Light on Methods
 - include a space holder for this section in your homework

What goes into your background/motivation?



Choose one narrative chunical exposure/public health • measuring DNA dankge • MMS/AS, metholation danage • BER · Comet Chip How chem treatment affect DOVA / BER "We hypothe are ... "Here we show ... "

In lab today

- 1. Blue, Pink and Purple go to TC to start MMS treatment. Groups rotate through TC as needed for the afternoon.
- 2. During incubation times prepare comet chip
- 3. Stay aware of incubation time and prepared for next steps in the treatment of CHO cells!

HW due M1D3 mdiv.

- 1. Write topic sentences (1st sentence) for each paragraph that would be in your Data Summary's Background and Motivation section
 - Remember to include references with 1-2 sentences why you chose it
- 2. Visit Comm Lab before M1D5. Make your appt. today!