M1D5: Analyze Gamma-H2AX images and CometChip electrophoresis

09/26/19

Overview of CometChip Assay: Chemically treating cells and visualization

Treat captured cells in comet chip with MMS then As



Lysis, electrophoresis & staining CometChips

- Alkaline lysis solution (pH 10)
 - 2.5 M NaCl, 100 mM Na₂EDTA, 10 mM Tris
 - Triton X-100
- Unwinding/ electrophoresis buffer (pH 13.5)
 - 0.3M NaOH, 1mM Na₂EDTA
- Neutralize (pH 7.5)
 - 0.4M Tris
- Florescent stain for DNA (dye)
 - SYBR Gold in PBS



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 <u>REPAIR INTERMEDIATES</u>

HW M1D6:

- (1) Revise methods (*with partner*) M1D1-M1D5(2) Mini Presentation **Outline** (*individual*)
- Follow time and content guidelines
- Introduce yourself and your research
- Clearly identify main question(s) and state your hypothesis
- Be quantitative when stating your findings (<u>NOT</u> "This was more/less than...")
- For this HW assignment:
 - outline should be in bullet format, not script
 - put placeholder statements for key findings (example, H2AX foci increased by XX%)

(3) Prepare for in-class paper discussion

- Consider discussion guidelines on wiki while reading the paper
- Contributing to the discussion is impt. for your participation score

REPORT

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Single-cell microarray enables high-throughput evaluation of DNA double-strand breaks and DNA repair inhibitors

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