## M1D3:

## Evaluate cell loading results

1. Communication Lab workshop
2. Image CometChip
3. Benchwork

- Prep CometChip(s) for biochemical test

4. Improve homework due M1D3
5. Postlab discussion

## Overview of Mod1 experiments



1. Optimize comet chip assay

- Test loading variables


2. Use comet chip assay to measure DNA repair

- Measure effects of MMS and $\mathrm{H}_{2} \mathrm{O}_{2}$ on BER


3. Use immuno-fluorescence assay to measure DNA repair

- Examine effect of MMS and $\mathrm{H}_{2} \mathrm{O}_{2}$ on DSB abundance


## Follow-up from M1D2: lysis and staining

- Alkaline lysis
- $\mathrm{NaCl}, \mathrm{Na}_{2}$ EDTA, Tris
- Triton X-100
$-\mathrm{pH}=10$
- Neutralization
- Tris
$-\mathrm{pH}=7.5$
- Staining
- SYBR Gold
intercalating agent


Possible considerations when choosing a DNA stain?
signal, sensitivity, chemistry

## Exp1: Optimize CometChip loading

What cell number is best for loading?

|  | 'low' cell number (\% filled) | 'high' cell number (\% filled) |
| :---: | :--- | :--- |
| Red | $60 \mathrm{~K}(85 \%)$ | $120 \mathrm{~K}(95 \%)$ |
| Orange | $30 \mathrm{~K}(63 \%)$ | $120 \mathrm{~K}(75 \%)$ |
| Green | $20 \mathrm{~K}(85 \%)$ | $100 \mathrm{~K}(85 \%)$ |
| Blue | $25 \mathrm{~K}(55 \%)$ | $50 \mathrm{~K}(72 \%)$ |

## Exp2: Biochemical testing using CometChip

## M1D3:



M1D4:


M1D5:
Chip 1: no enzyme control

Chip 2: purified enzyme treatment


Load


Lyse



## How will we communicate these data?

Data summary draft due Mon., Oct. 9 at 10p

```
Summary content
1. Title
2. Abstract
3. Background \& Motivation
4. Figures, Results \& Interpretation
5. Implications \& Future Work
```

Homework due M1D4 is practice for Background \& Motivation section

## How to write the Background \& Motivation



## Write a single, consistent story

## Before you leave...

- Let us know if you did not finish preparing two CometChips
- Or if we need to finish the preparation
- Revised figure homework due by 10 p
- Email to Noreen (nllyell@) and Josephine (@joshaw)

