CH-313 (Autumn 2023: Instructor: Prof. Yimon Aye; PhD-student teaching fellow: Song Chen)
In-class Problem Set (Lecture 5-6) **Students may do only one problem (either Q1 or Q2) out of the two. If you attempt them both, well done as usual, and we will grade them all, but we will only take the *grade from the problem with a better score*.**

Q1 (18 points). You discover a new microorganism living deep in ocean vents in Europa, a moon of Jupiter. You are tasked with studying this unusual organism. This organism shows a traditional cell cycle, and with a doubling time of 34 hours. It has a double helical DNA structure made up of A, T, C and G, but the proteins it uses are very different from those we find on earth, with several amino acids that do not exist on earth either. Fortunately, you can culture this organism and transfect it efficiently.

(i) In a flow cytometry analysis, show how the data should look like when growing cells from this microorganism are stained with propidium iodide to measure amount of DNA per cell. Label the different phases and axes of the plot. **(2 points)**

(ii) You are asked to examine the response of these cells to DNA damage. You thus stress the cells with one of the viable DNA-damaging conditions for 2 hours, and immediately afterwards, execute standard work flow for propidium iodide staining-based flow-cytometry analysis. You found that this does not change the cell cycle distribution significantly. Given what you are told in the introduction in question, is this expected? Circle the correct answer **(1 point)**

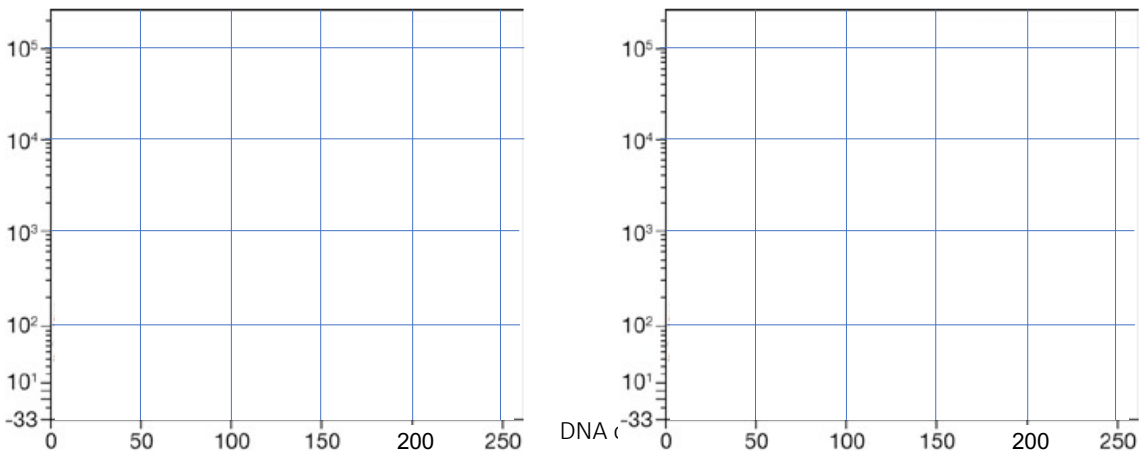
YES NO

(iii) Design an experiment (i.e., show a schematic workflow) to test whether or not DNA synthesis, specifically in S-phase, is affected in cells treated with DNA-damaging conditions for 2 hours. For full credit, include in your workflow the time parameter and consider choice of reagents carefully. There is more than one correct answer/set of reagents **(3 points)**

(iv) In the two 2-D plots below, show the anticipated data output if DNA-synthesis were inhibited (right) against control (left). For full credit, first fill out the missing Y-axis label, and label the phases of cell cycles, against relevant cell populations **(4 points)**

Note: Absolute values on the axes do not matter but relative positioning of cell populations within a given plot and across the two plots do matter.

Control sample: no treatment Experiment sample: S-phase inhibited



(v) By searching the genome, you notice that this organism indeed has no protein similar to canonical DNA damage proteins that are known in eukaryotes, archaea, or prokaryotes. You use a chemical biology method to ID DNA damage recruitment proteins, i.e., iPOND. Using this technique, you discover 4 proteins, called A, B, C and D that are recruited to replicating DNA, following replication stress. You want to measure kinetics of A, B, C and D recruitment to sites of DNA damage in these alien cells. One classic way to do this uses BrdU-incorporation into DNA, followed by shining a fine laser across a specific stretch of the nucleus to create localized DNA damage (by laser-induced fragmentation of the carbon-bromine bond in BrdU), and measuring recruitment of the specific proteins to the sites of damage in real time. To do this, you would need to generate fluorescent protein tagged A, B, C, and D. Outline (1) how you would do this, including expected outcomes, and (2) what you would see if DNA damage is repaid by the cell; and (3) controls you could use.

Please limit your answer to space below. Answers written elsewhere will *not* be graded **(5 points)**

Experimental steps/workflow:

Expected observation if recruitment occurs:

Expected observation should the DNA-damage be repaired post recruitment:

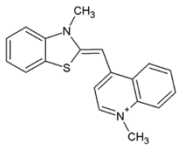
Two most relevant/important functional controls:

(vi) Other researchers working on this organism have suggested that a specific RNA (endogenous to this organism) is involved in DNA damage response in this organism, being recruited to damaged DNA. Outline how you could develop a similar live cell reporter assay for recruitment of the specific RNA to DNA damage in these cells. **(3 points)**

Name of technology:

Workflow:

Q2 (18 points). We came across in class RNA Spinach aptamers, the GFP mimic of RNA. This problem concerns application of another "RNA Mango" aptamer, which fluoresces upon binding to **TO** (thiazole orange, see structure below, instead of DHFBI), under 644 nm excitation wavelength (instead of 488 nm in the case of GFP/DHFBI), resulting in orange/red fluorescence, as opposed to green.



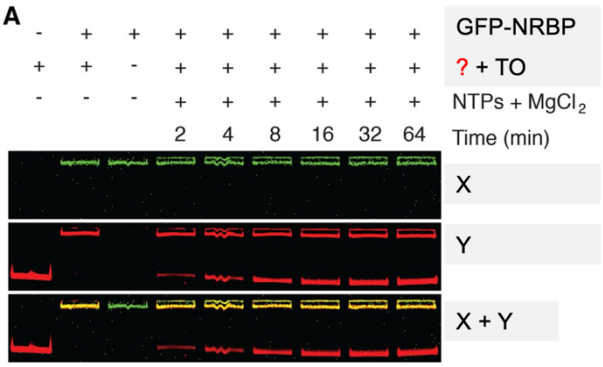
Thiazole orange (TO)

(i) You are tasked to characterize novel intercellular protein complexes in humans that can bind and recognize a specific viral RNA of interest, known as ROI, that originates from a new flu virus strain. Design an experiment that will enable you to identify these novel human proteins that target this viral ROI. **Illustrate** (i.e., please kindly sketch out, instead of writing out loads of texts) your proposed experimental workflow schematically in the space below (instead of a long answer in text). **Hints:** Assume that you have ready access to human cell culture/virus infection model, i.e., you have a means to infect human cells with relevant viruses encoding any engineered RNAs and the latter will be ultimately present inside your cultured infected human cells. You also have access to beads coated with Streptavidin and all the reagents/modern instruments necessary. **Key Hint:** this experiment needs you to *map proteins' identities*. **(8 points)**

In the space below, state one major concern in using this approach above to identify the ROI-interacting proteins: **(2 points)**

_____

(ii) From a bunch of newly-identified targets, one top-ranked protein target that consistently appears in multiple replicate experiments that you perform above, is a protein known as NRBP (Novel RNA-binding protein). You set about validating the binding between NRBP and ROI using an in-gel fluorescence analysis and obtain the following data (see figure labeled **A**). Answer the questions below:



(a) What is "**?**" ? **(2 points)**

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(b) Which molecular species do **X** and **Y** report, respectively? Write out corresponding excitation wavelengths. **(2 points)**

X: _____ (excitation at _____ nm)

Y: _____ (excitation at _____ nm)

(c) In one single phrase, explain the observed gel shift between 1st and 2nd lane from the left, for Y. **(2 points)**

_____

(d) NTPs = nucleotide triphosphates. In one sentence, interpret the effect(s) of addition of NTPs and Magnesium Chloride, based on the gel data. **(2 points)**