Modified from Question 4, Problem Set 7, Spring 2011

4. You are studying the cell division cycle of a mammalian cell line grown in culture. You would like to determine how long the cells spend in each stage of the cell cycle.

a) Using the reagents provided to you, design an experiment to test

Your goal is to determine how long cells spend in each part of the cell cycle. The culture is not synchronized: each cell in the culture is going through a cell cycle independent of the other cells. Therefore, at any point in time, a range of cell cycle states is present.

You pulse the cells for 30 minutes with BrdU, wash it out, and then add an excess of cold thymidine (in what is called a pulse-­‐chase experiment). Following the Pulse-­‐ Chase treatment, you remove an aliquot of cells every hour and perform the following types of analysis.

Analysis 1: First you count the number of cells in each aliquot and calculate the total number cells in the culture flask at various times. Your results are shown in figure 1.

Analysis 2: Next, you stain the cells with fluorescent anti-­‐tubulin antibodies and (using a  microscope) determine the percentage of cells that are undergoing mitosis. At each time point, you consistently find that approximately 5% of the cells are undergoing mitosis.

Analysis 3: From the hourly aliquots you isolate the mitotic cells. You use antibody staining to determine what fraction have been labeled with BrdU. Your results are shown in Figure 2.

(4a) Draw a simple representation of the cell cycle showing the relationship of G1, mitosis, S-­phase and G2. Indicate the position of START (the restriction point).

(4b) What is the division time for this cell line? What data lead you to this conclusion?

(4c) What is the duration of mitosis in the cell line used in analyses 1-­3? What data lead you to this conclusion?

(4d) What is the duration of S phase, G1 and G2 in the cell line in analyses 1-­3? What data lead you to this conclusion?