BioPharmaceutical Technology Center
INSTITUTE

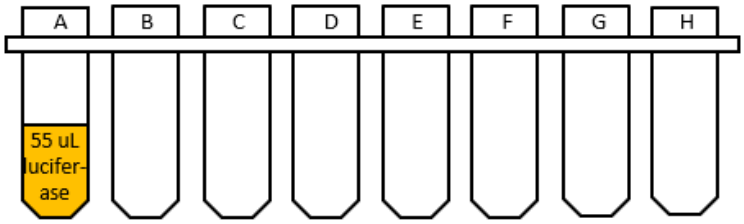
Bioluminescence II – Serial Dilution with Luciferase

Protocol

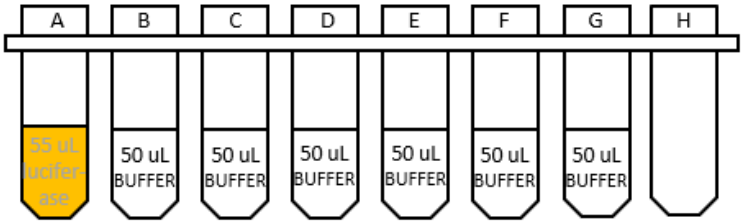
Reaction Set-up

1. Prepare a serial dilution of luciferase in your individual strip tubes. A serial dilution involves diluting a compound many times by taking a known concentration of a substance and moving a standard volume from one tube to the next in order to further dilute it.

   A. Pipette 55 µl from the tube labeled "luciferase" into the first tube (A) of your strip tube.



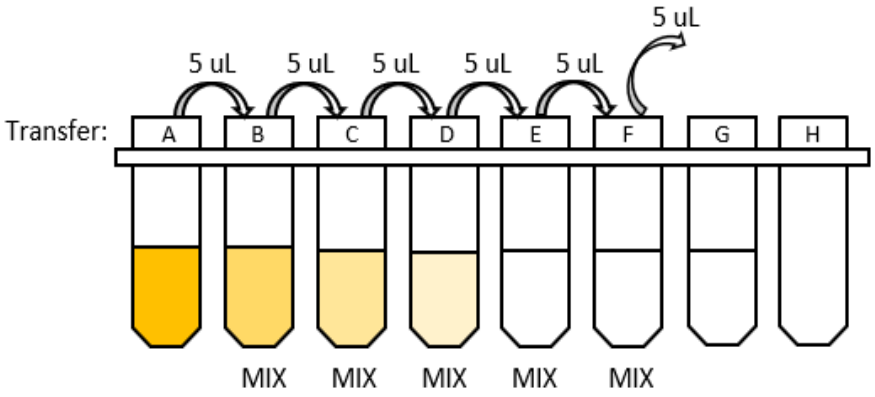
   B. Pipette 50 µL from the tube labeled "BUFFER" into tubes B - G of your strip tube.



   C. Pipette 5 µl of the luciferase solution from tube A and transfer it to tube B, pipetting up and down several times to mix.

   D. Repeat step C, but instead transfer 5 µL from tube B and transfer it to tube C, carefully pipetting up and down several times to mix. Continue transferring across the tubes (C→D, D→E, E→F) using the same technique.

   E. After you mix 5 µl into tube F, discard 5 µl from tube F into the waste container so that each tube now contains 50 µl liquid. **Do not transfer anything into tube G or H.**



2. Discuss with your group such that each of you will claim column 1, 2, 3, or 4 of your 96-well plate. Pick your columns in order and do not skip a column if your group is less than 4.

    a. Write which column is yours here: _____

3. Using a multichannel pipette, move 40 µl of each of your dilutions from your strip tube into your chosen column, rows A through G. Align your transfer such that tube A is transferred to row A and tube G is transferred to row G. Make sure each member of your group has added their dilution to the plate.

4. Using a multichannel pipette, move 40 µl of Bright Glo reagent into Rows A→H, Columns 1→4.
    a. Pour solution labeled "Bright Glo" into a plastic trough. Label your trough.
    b. Use a multichannel pipette to add Bright Glo to rows A→H of columns 1→4.

	1	2	3	4	5	6	7	8	9	10	11	12
Row A	**40 µl Bright Glo**	**40 µl Bright Glo**	**40 µl Bright Glo**	**40 µl Bright Glo**								
Row B	**40 µl Bright Glo**	**40 µl Bright Glo**	**40 µl Bright Glo**	**40 µl Bright Glo**								
Row C	**40 µl Bright Glo**	**40 µl Bright Glo**	**40 µl Bright Glo**	**40 µl Bright Glo**								
Row D	**40 µl Bright Glo**	**40 µl Bright Glo**	**40 µl Bright Glo**	**40 µl Bright Glo**								
Row E	**40 µl Bright Glo**	**40 µl Bright Glo**	**40 µl Bright Glo**	**40 µl Bright Glo**								
Row F	**40 µl Bright Glo**	**40 µl Bright Glo**	**40 µl Bright Glo**	**40 µl Bright Glo**								
Row G	**40 µl Bright Glo**	**40 µl Bright Glo**	**40 µl Bright Glo**	**40 µl Bright Glo**								
Row H	**40 µl Bright Glo**	**40 µl Bright Glo**	**40 µl Bright Glo**	**40 µl Bright Glo**								

View Results

After Bright Glo reagent is added, read your plate on a plate reader. Observe your data and write down any trends in amount of light output.

Questions

A. Why did you perform your experiment in replicate (doing the same thing multiple times)?

B. As you move from the top of the plate (Row A) to the bottom (Row H), does the amount of luciferase increase or decrease? Does is change by a similar amount or is it very different each time?

C. As you move from the top of the plate (Row A) to the bottom (Row H), does the amount of light increase or decrease? Does is change by a similar amount or is it very different each time?

D. When the data you generated was graphed so that the amount of luciferase in each well is on the x-axis and the corresponding amount of light produced on the y-axis, what was the shape of the data points?

E. We already knew the concentrations of luciferase in each well after the serial dilution. Explain the use in creating a graph like the one you have generated.