OKR

Main objective: Confirm promoter via dual luciferase assay (before April 25th)

- **Key results**
  1.1. Determine the concentration of βCD and cholesterol to simulate high and low cholesterol levels in human cells
  1.2. Optimize the transfection procedure and specifically the amount of DNA and Lipofectamine 2000 needed
  1.3. Run Dual Luciferase assays(DLA) to confirm the function of the promoter

General Deliverables

1. Confirmed that the synthetic promotor is statistically sensitive to cholesterol levels running a DLA. (1.3 - Luis and Khaliq; April 25)
2. Determining what maximum and minimum conditions of cholesterol allow cells to grow via kill curve analysis. (1.1- Miriam, Raphael, Elias; April 7th)
3. Habituate wild type cells to the optimized BCD and cholesterol concentrations to prepare them for transfection process) (1.1- Miriam; April 4th and 11th)
4. Determine the Optimal Transfection DNA:Lipofectamine Conditions that result in the most transfected cells (1.2- Mehdi, Yari, Katana; April 2nd)
5. Comparative luminescence sensitivity analysis of the FLx800 and GloMax 20/20 (1.3- Luis, Khaliq; April 2nd)

Draft of timeline

Relevancy:

a. The promoter is part of a synthetic plasmid vector engineered, and itself is synthetic.

Introducing a new possible therapeutic solution for maintaining homeostatic cholesterol levels. We want to present our work to IGEM.

Timeline

Step one: due by April 16th

- Transfect cells with v2

    - Will be done on April 29
    - Checklist
        - Cells
            - Coordinate with Miriam
        - DNA
            - X amount needed
                - Coordinate with Yari and Katana
                - Dependent on pilot transfection – Use ratios that had highest transfection efficiency. Likely wells C3, C6, B6, which correlate to _*check well plate*_.
- Run dual luciferase assay (practice)
    - Will be done on April 30
    - Checklist
        - Assay kit
            - Coordinate with IPRO treasure
        - Prepared reagents
            - Coordinate with Luis
        - Transfected V2 cells
            - Coordinate with transfection team
        - Finalize protocol
            - Coordinate with Khaliq
- Growth curve to determine the max BCD and cholesterol concentrations for transfection in order to determine if the promoter is sensitive to varying cholesterol levels
    - Will be done with BCD and Cholesterol triplicates by April 18th
    - Coordinate with Growth curve team
    - Cholesterol
        - Last GC to be started on April 7th --> 30 µM and 50 µM again, 60 µM if current results show cells are viable
        - Input 60 µM Cholesterol Growth Curve
        - Update existing growth curves with new values
        - Will be finished by April 18th
    - BCD
        - Next BCD (trial 2) --> start March 31st
        - Last BCD GC (trial 3) to be started April 7th -- > with more narrowed down range
        - Input BCD Cell data

        - Produce new curves
        - Set new range of BCD concentrations
        - Will be finished by April 18th

Step two: Due April 22nd

- Transfection optimization
    - Due April 18th
    - Must be performed three times
    - DNA extraction takes 3 days
    - Check list
        - Cells
            - Coordinate with Miriam
                - Ensure cell flasks are passaged according to when cells are needed
        - DNA
            - X amount needed
                - Coordinate with Yari and Katana
- Kill Curve Chart
    - Input all X-ranges bar graph to find bell curve
    - Due April 19th
        - Coordinate with Elias and Raphael
- Preform final Transfection
    - Due April 22nd

Step three: due April 25rd

- Do the final run of the dual luciferase assay
    - Will be done on April 24th
    - Must be triplicated
    - Checklist
        - Cells
            - Coordinate with Miriam
        - DNA
            - X amount needed
                - Coordinate with Yari and Katana
- Determine whether or not the promotor is sensitive to cholesterol
    - Will be done by April 25

- Run a good dual luciferase assay
    - Requires transfection optimization
    - Must be triplicated
    - Needs to be practiced before it is run

- Run dual luciferase practice
    - Requires V2 transfection
    - Doesn't require optimization to be done
    - Requires cells

- Transfection optimization
    - Requires enough DNA to be isolated
    - Needs to be triplicated
    - Requires cells

- Growth curve
    - To determine maximum and minimum cholesterol levels cells can tolerate
    - For other experiments
    - Must be triplicated [cholesterol and BCD conditions]