Firefly Luciferase Quantification Assay

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Background

- LNP-mediated delivery of mRNA towards specific cells is the current platform we are deeply interested in for effective targeted therapeutics
- When delivered, the mRNA utilizes cytosolic ribosomes to translate and produce a therapeutic biomolecule
- To test the efficiency of the mRNA delivery and translation, we will deliver firefly luciferase(fLUC) mRNA via LNPs intravenously (IV) and perform quantification assays on the product
- We selected fLUC as it is not endogenous to the system and it can be easily induced to luminesce

Assay Goals

- We will perform a full panel analysis of fLUC for the site of injection muscle, plasma, liver, kidney, heart, and brain
- Each organ will be homogenized and lysed, then centrifuged for supernatant
- fLUC mRNA and protein will be quantified to determine concentration
- Results will be recorded and delivered in meetings

Issues

- Unlike our previous biomarker assays, we cannot procure fLUC quantification kits as they are not available
- The current body of literature does not have an established fLUC quantification assay
- Developing immunoassays like ELISA or Western-Blot cannot be performed due to the lack of available commercial monoclonal or polyclonal antibodies
- The best available product is the Promega fLUC luminescence assay. However:
  - The assay only works for cell lines
  - And to perform the assay, we would need to purchase the Promega plat reader
- Our best bet is to perform the assay in-house

Developing the Assay

- Our main approach will be an ELISA-style induced luminescence assay, where we will quantify the firefly luciferase based on a standard curve
  - We can purchase fLUC commercially to help us build controls and a standard curve
- There are a few elements that can help us develop this assay in-house:
  - Our current plate reading templates and procedures in Excel are already optimized for fitting the standard curve
  - Promega kits do provide resuspended luciferin protein, which will activate fLUC and induce luminescence
  - The Variosakan-LUX plate reader can be modified to not just record luminescence in dark conditions(optimal for FLUC) but also dispense the resuspended Promega luciferin solution via dispensers, which we can procure and easily install
  - In addition, the Variosakan-LUX manual does provide detailed methods for how we can effectively dispense

Developing the Assay

- Since our main target is luminescence, we are going to focus on two elements
  - Determine optimal luminescence recording conditions
  - Develop a standard curve and controls for fLUC
- We determined that the optimal way of inducing luminescence via the fLUC and luciferin nitration would be to add equal parts in a 96-well plate
  - The plate cannot be translucent, or we risk luminescence crossing over
  - Solid plastic white or black plates do not seem to be different in measurements
- Samples of the same group must be pooled and tested with various dilutions
  - This is to determine the level of sensitivity we need to employ
- The optimal volume seems to be about 50 µL for recording
  - We decided that we can achieve this with 25 µL of tissue homogenate/plasma and 25 µL of resuspended luciferin
  - Therefore, we must account for the 1:2 dilution as well

Developing the Standard Curve

- Our standard curve needs to be a reflection of the level of sensitivity that we can achieve
  - We started out with a standard curve of: 250, 125, 75, 25, 10,0 µg/mL
  - This curve was a success in detection, so we decided to lower our sensitivity to about 10ng/mL, allowing us to achieve a dynamic sensitivity range
  - The ultimate standard curve we settled was: 250,125,62.5,31.25,15.625,7.813, 0 ng/mL
  - Controls were planned to be 50ng/mL and 25ng/mL and must be done by diluting the stock with untreated tissue homogenate
- Once we established a standard curve for use, we optimized the process by using a stock of 1 µg/mL fLUC, for which we performed a 1:4 dilution for the curve
  - Stock was saved in 2-8*C for short term and -20*C for long term storages

Results

- The standard curve and controls, alongside mock samples diluted with homogenate, seem to indicate that the assay and record luminescence
- Testing with samples did yield some level of record; however, the fLUC only seems to be measurable in samples that have a significant concentration
- Samples with lower concentrations are more complex to measure, and our assay lacks the sensitivity to measure the concentrations in the pg/mL range
- Overall, the assay seems to help detect and measure fLUC concentrations in samples with high translation efficiency
- However, further work and literature reviews on recent studies must be performed to be able to extend our sensitivity ranges