Appendix 4: Grading Guidelines for Mammalian Lab Report

Introduction

- Elbashir et al. 2001, size matters
- Describe all 4 constructs and purpose
    - Scrambled (scm): what it targets, no silencing
    - AmbGFP: what it targets, amount of mismatch w/ egfp, possibly no silencing
- CMV-EGFP stably expressed

Materials and Methods

- Plasmid prep Endotoxin free, quantification and gel
- Transient transfection (and cell count practice, don't need to mention)
    - mention Lipofectamine2000 and the conditions
    - purpose of no DNA and no Lipofectamine2000 reactions
- Visual assessment of transfected samples & microplate (qualitative and quantitative)
- Cell counts for transfected cells and cell/mL
- Volumes used for 20,000 and 40,000 cells
- Gel, transfer, and western blot

Results:

- Plasmids prep
    - Agarose gel picture, and amounts and purity
- Confluency
- Visual assessment of transfected samples- pictures
- Microplate reads: values, average, and %silencing or remaining
  - mention corrected for background and normalized to scrambled
  - Class data
- rainbow marker showed transfer worked
- Western blot image and quantification with Kodak MI software
    - was a correct sized band detected?

Discussion:

- Define silencing phenotype
- For each condition (siEGFP439 and 497, AmbGFP and scrambled)
    - Should mention if they expected no, little or lots of silencing
    - what they saw
    - did this match expectations? Why or why not?
    - which construct worked best
- Transient transfection silencing: visually correspond to microplate
- Quality of microplate data:
    - how were replicates?
    - 2X twice 1X?
    - Comparison to class
- Western blot

    - Could you tell a difference in protein levels for the samples?
    - How relate to microplate data?
- Lipofectamine2000 toxic? - comparison of OptiMEM only to No DNA sample

References: Class protocols and Elbashir et al. 2001 paper