Interpreting Cytometry Data in CytoFlow

Introduction

You have completed all your experiments, yay! Now it is time to actually interpret the results

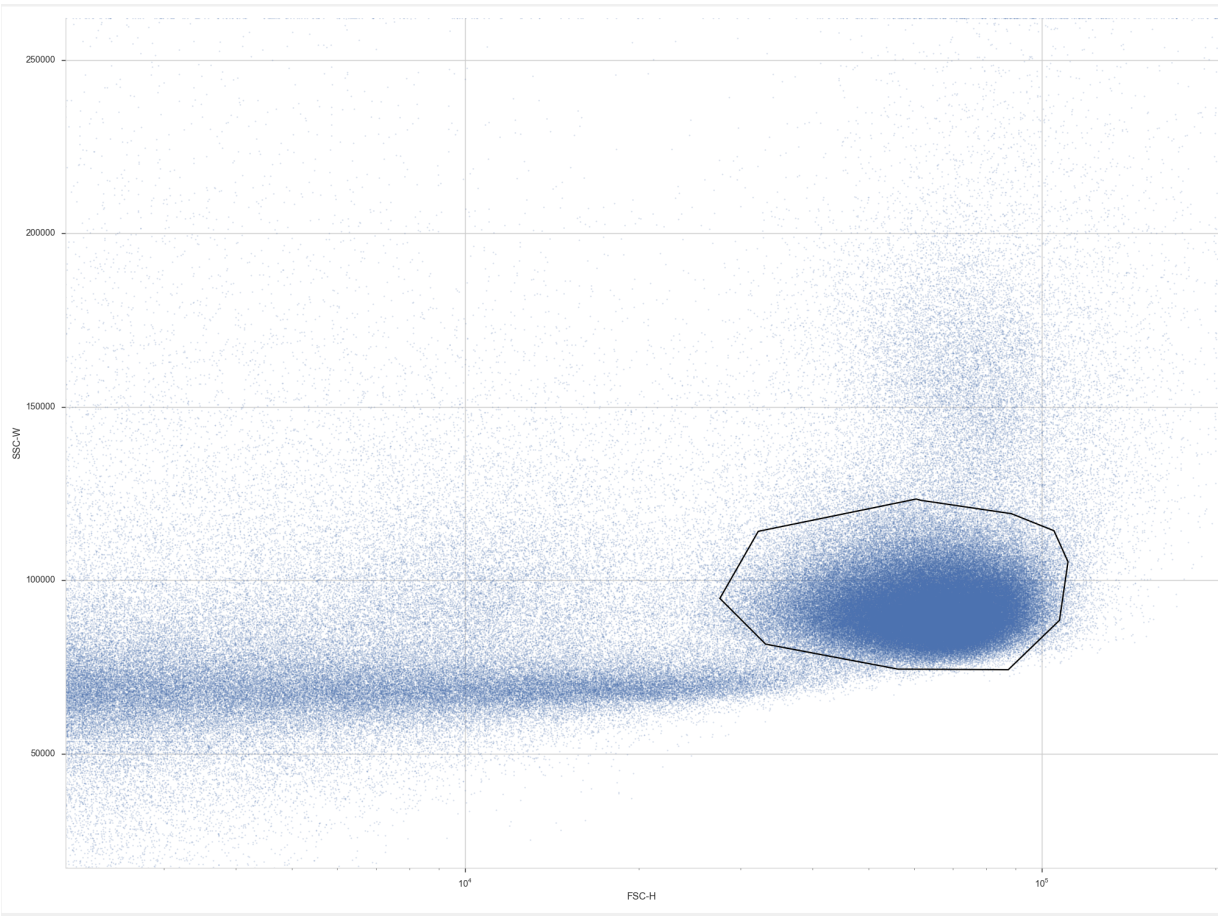
Materials

- Cytometry data
  - .fcs files
- CytoFlow Software

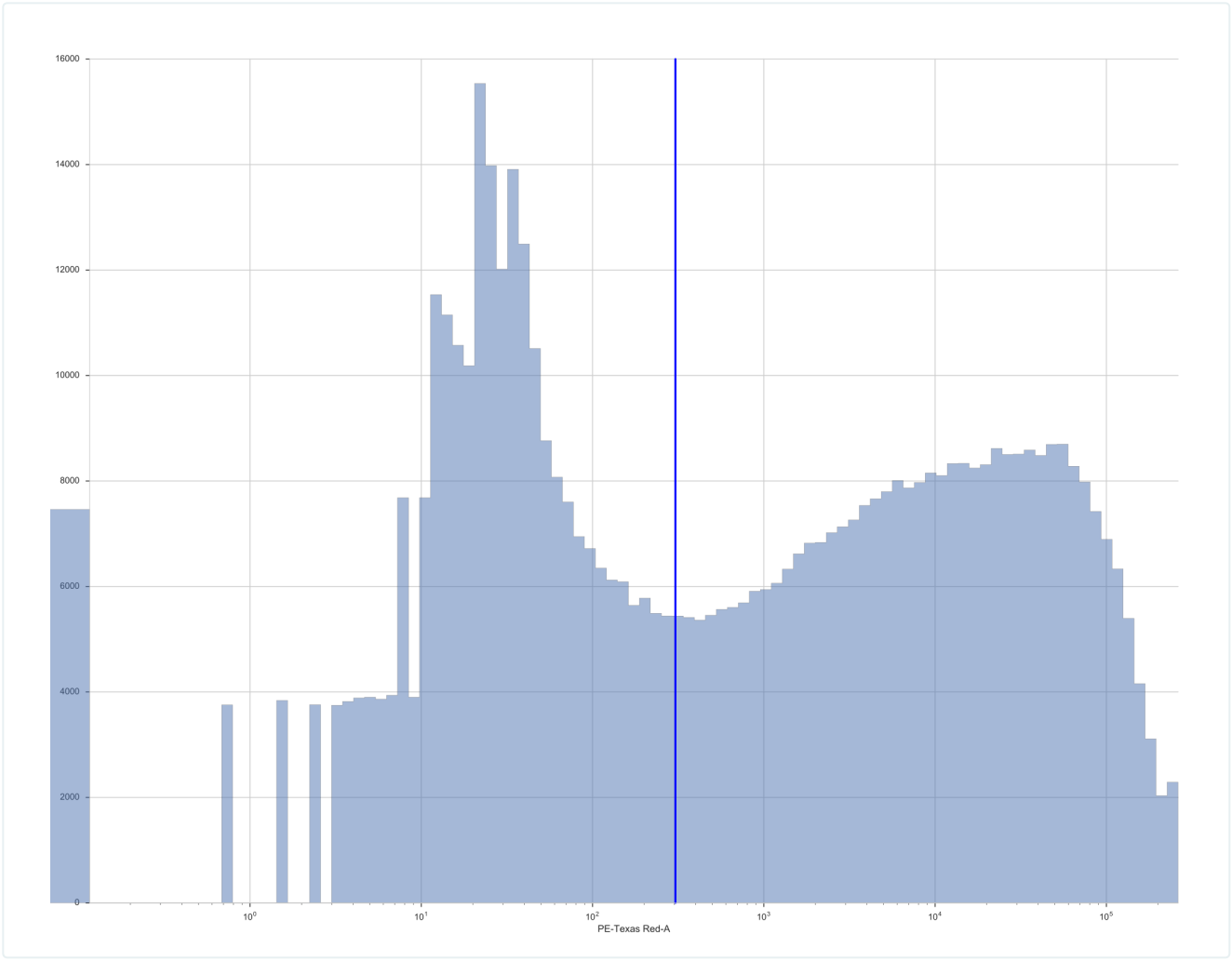
Procedure

Cytoflow Workflow

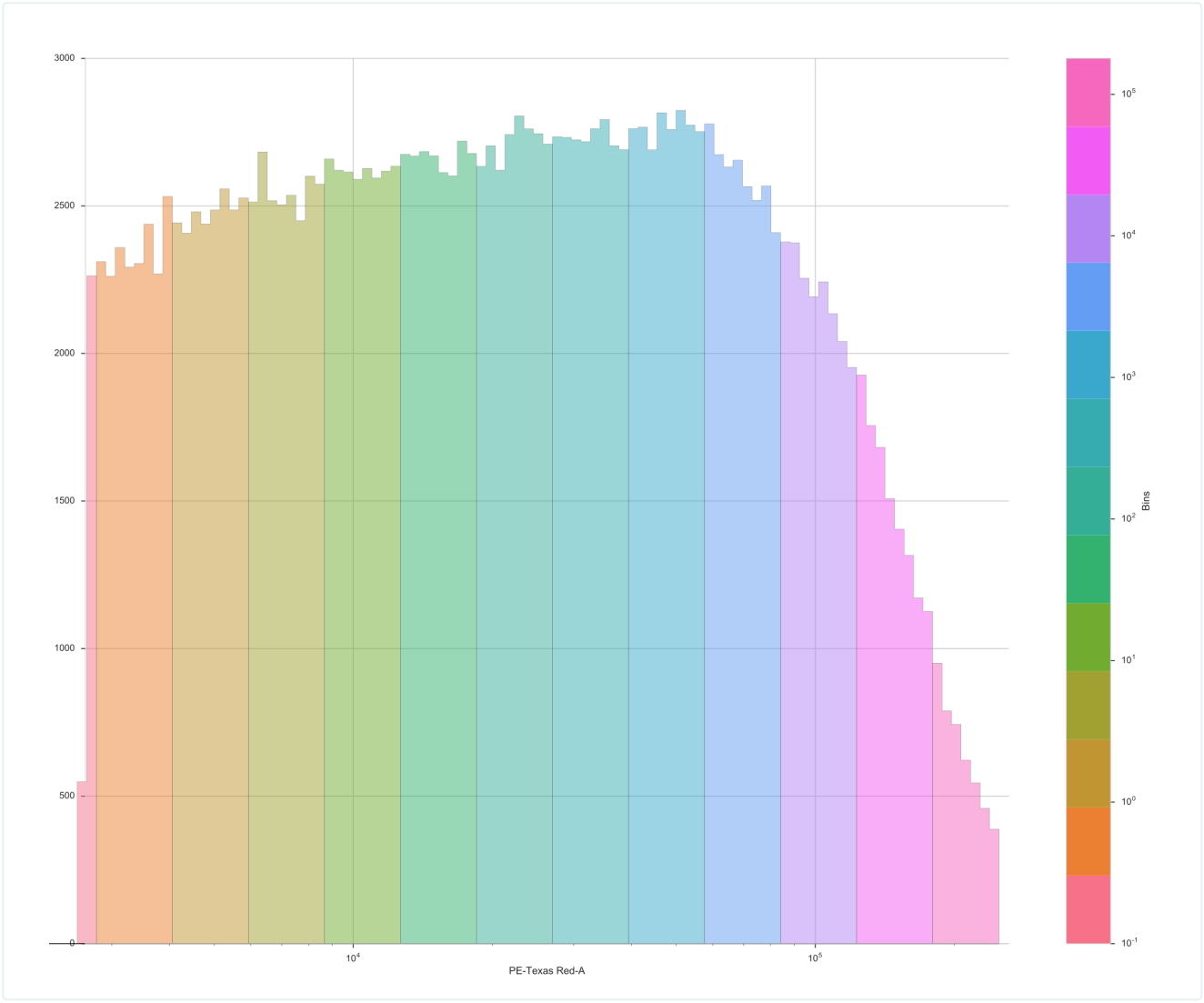
1. We used CytoFlow, a software developed by our iGEM advisor, Brian Teague, to analyze the results of our HEK transfections.
2. Import samples from file generated by the flow cytometer.
3. Assign each sample a unique set of conditions, which could be Booleans, Categories or Numbers.
4. These distinctions can be taken from your transfection planning document.
5. Name carefully, as these variable will be what you use to process your data later
6. Gate the live cells based off their forward scatter height (FSC-H) and side scatter width (SSC-W). These are the laser scatters caused by the cells passing through the cytometer. You want to select a population of cells that have a high FSC-H and SSC-W.



7. Set your transfection threshold, by measuring the amount of fluorescence outputted by your transfection marker. Transfection efficiencies typically range from approximately 10-85%.



8. Bin your cells by the amount of plasmid in each cell. Binning is a method of standardizing your output across different cell plasmid concentrations. If a cell receives more plasmid, we would expect it to have a higher fluorescence output across all colors. We divided our population into 40 transfection bins.



9. Based on your variable and output fluorescence, you want to group your cells by variables and compare the fluorescence in every case.

10.