I. Project Start Date: *Oct 7 ,2013*

II. Project leader: *Bryan Linggi*

III. Data analysis leader:*Bryan Linggi*

III. Location of project files: *afp://we25473/Documents/R\_onAir/EGF\_gage analysis*

Experimental Design

V. Experiment Information

a. Experiment type:

New Data Analyses: generated in house, but not “new”

Public Data Analyses

Gene Expression

Microarray

RNA-Seq

RT-PCR

Other:

Epigenetic analysis

Methyl-Seq

ChIP

Protein expression

Mass-spectrometry

Other:

b. Experiment layout

Experimental Hypothesis: *EGF affects different cell processes depending on concentration of ligand*

Output result (check all required):

Powerpoint presentation of highlights

Excel table of raw data

Excel table of normalized data

Excel table with p-values and fold changes

p-value cutoff:

multiple hypothesis correction? y/n

fold change cutoff:

Graph of data summary

Details:

All analyses files (will be archived even if not returned to user)

Cell Type(s): *human mammary epithelial cells 184 A1 (hmec)*

Conditions:  *EGF at concentrations 0 to 100ng/ml*

*Inhibitors, mek, U0126*

Controls

Positive: *EGF at 1hr*

Expected result: *dusp6 expression increases*

Negative: *no EGF, 4 hr*

Expected result: *no change in dusp6 expression*

c. Power analysis

Replicates needed for desired power: *experiment already performed \*\*\**

Type of replicate: *duplicate, from separate culture dishes treated independently, I think*

*\*note: there is no ‘inhibitor alone’ control*

1. VI. Preprocessing Steps:

(Mod from Faraway 2002)

Set up Git locally and on GitHub. Record details of Github locally): *afp://we25473/Documents/R\_onAir/EGF\_gage analysis*

Github address: *https://github.com/bedward1/EGF\_gage.git*

Data collection

how are data collected (random sampling?): *affymetric microarray. Sample prep randomization methods unknown (were preformed other investigators before I arrived.)*

Run preprocessing code in R

Output is

1) html file in

*we25473/Documents/R\_onAir/EGF\_gage analysis/report/preproc\_report.html*

2)R.data for input to next steps

*we25473/Documents/R\_onAir/EGF\_gage analysis/diagnostics/Preproc.R.data*

1. Initial analysis
   1. Correlation calculations and plots
      1. Save Rdata for next steps in :
         1. *afp://we25473/Documents/R\_onAir/EGF\_gage analysis/tests/*
      2. Save html report in
         1. *afp://we25473/Documents/R\_onAir/EGF\_gage analysis/reports/*

3) Pathway analysis

a) perform Gage analysis

use Kegg pathways

1. output table to /tests folder

comparing gene pathways at different densities and inhibitor presence

note changes in pathways and level in pathways.