Data Set:

This data set is from my single cell tracking experiment, which has more than 1500 cells tracked. As it has not been published yet, I will not put the raw data here.

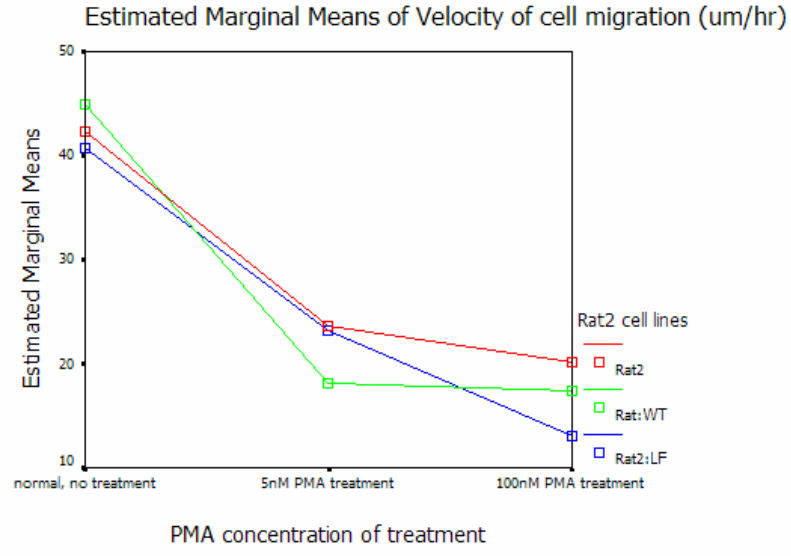
Set alpha=0.05. Use *SPSS® 11.5* to do two-way ANOVA:

**Tests of Between-Subjects Effects**

Dependent Variable: Velocity of cell migration (um/hr)

| Source | Type III Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|
| Corrected Model | 230404.438[a] | 8 | 28800.555 | 186.309 | .000 |
| Intercept | 1041555.708 | 1 | 1041555.708 | 6737.754 | .000 |
| CELL | 2228.976 | 2 | 1114.488 | 7.210 | .001 |
| PMA | 215975.499 | 2 | 107987.750 | 698.565 | .000 |
| CELL * PMA | 6296.110 | 4 | 1574.027 | 10.182 | .000 |
| Error | 237442.575 | 1536 | 154.585 | | |
| Total | 1850928.335 | 1545 | | | |
| Corrected Total | 467847.013 | 1544 | | | |

a. R Squared = .492 (Adjusted R Squared = .490)




a)

Rat2 is a fibroblast cell line suitable for single cell tracking. Our lab's interest is protein X's function in cell migration. Two stable Rat2 cell lines were constructed by retrovirus infection and FACS: WT means the wild-type protein tagged with EGFP was expressed in Rat2 cells, while LF means a phosphorylation mutant of protein X tagged with EGFP was expressed. From my biochemistry data, this mutant mimic the phosphorylation stage of protein X, but can not be regulated by phosphorylation. Here, the hypothesis I want to test is that the phosphorylation of protein X has an effect on cell migration and Rat2 cells infected with different mutant of the protein are affected differently. The drug PMA is used here to stimulate endogenous protein kinase C, which we think is the upstream of protein X. PMA treatment of 100nM is the classic concentration used, which is thought to

fully activate PKC but not other kinases. PMA treatment of 5nM is also used here, which will partially activate PKC.

b)

In this experiment, all the environment, like cell culture condition, genetic background of cell line (before retrovirus infection), cell-confluence during tracking experiments, parameter-setting of tracking program, *etc*, is the same. There is only one dependent variable: velocity of migration; two independent variables: PMA concentration (no treatment; 5nM treatment; 100nM treatment) and Rat2 cells infected with different constructs (Rat2 cell; Rat2 cell with extrinsic EGFP-WT protein X expressed; Rat2 cell with extrinsic EGFP-LF protein X expressed).

c)

I use two-way ANOVA to analyze the data, because I am interested in investigating the hypotheses that cell migration is affected by (1) different PMA treatment (2) Rat2 cells infected with different constructs (3) the interaction of PMA and cell lines. Two-way ANOVA is the best way to figure out the problem. As *Prism 4*® only can do two-way ANOVA for sample size less than 50, I borrowed one of my friend's laptop and run *SPSS 11.5*® to do the analysis.

d)

The required assumptions for two-way ANOVA are:

Independent observations -- According to the experimental design, only velocity depends on the tracking method I used. PMA treatment and different Rat2 cell lines are independent across the experiment. One cell only contributes one velocity value, and there are no redundancies to be dealt with.

Continuous interval -- The measurement of velocity is continuous.

Random sampling -- A 10-field-movie was taken randomly from Petri dishes. All cells in randomly picked fields are tracked without any bias.

Normal population distribution -- With large n (n>100 for each group), sampling distribution of means will be normal. I also check the values in each group by leaf-and-stem plot, which shows that the distribution is normal.

Homogeneity of variance among groups -- Based on the calculation within the groups, S.D. of each group are 14.32, 12.92, 12.16, 14.17, 9.100, 9.352, 13.49, 13.32, 8.269; $F=14.32/8.269=1.73 < 2.26$ (while n=60)[1]. Since the variances of groups are smaller than 2, according to a conservative rule of thumb I treat the data as "homogeneity of variance".

e)

**A. The main effect of PMA treatment:**

NULL - there is no difference among population velocity means of different

[1] *http://helios.bto.ed.ac.uk/bto/statistics/tress6.html*

PMA treatments;

ALTERNATIVE - PMA treatments change population means of cell migration speed;

**B. The main effect of cell lines:**

NULL - there is no difference among population velocity means of different cell lines with different protein X expressed;

ALTERNATIVE - expression of different protein X changes population means of migration speed;

**C. The interaction effect:**

NULL - there is no interaction between PMA treatment and cell lines;

ALTERNATIVE - PMA treatment and cell lines interact with each other and affect cell migration.

f)

Alpha level is 0.05.

g)

**Between subjects:**

Degree of freedom of PMA treatment is 2;

Degree of freedom of cell lines is 2;

Degree of freedom of the interaction is 4;

**Within subjects:**

Degree of freedom is 1536+1=1537. Since I have unequal number of observations among groups, SPSS did a correction and the corrected degree of freedom is 1536.

h)

p value of observed result from cell lines is 0.001;

p value of observed result from PMA treatment is <0.001;

p value of observed result from the interaction is <0.001;

i)

Reject all three NULL hypotheses.

j)

Data were analyzed with general linear model univariate two-way ANOVA, conducted with the PC-based program SPSS 11.5 (SPSS Inc, Chicago, Illinois). Alpha was set at 0.05. The results of this analysis indicated that the mean cell migration velocity varied significantly among different cell lines [$F(2,1536)=7.210$, $p=0.001$]. PMA treatment to Rat2 cells significantly decreases cell migration velocity [$F(2,1536)=698.565$, $p<0.001$]. And there is an interaction between PMA treatment and cell lines [$F(4,1536)=10.82$, $p<0.001$], which fits with the regulation mechanism of protein X in cell.