Testing the Effects of Transfection on Mammalian Cytokine RNA Expression

Austin Mitchell Vecchio

***Abstract*—Abstract outline**
This experiment aims to study the effect of genetic expression on polyplex treated cells against β-actin and INFα.

I. Introduction

Gene therapy is an experimental method for treating disease by introducing a healthy copy of a defective gene into the patient's cells to alter the patients genetic material. The alternative is a non viral gene therapy method which can often have simple and large scale production with low host immunogenicity. However, non viral gene therapy can often yield low transfection efficiencies. New advances in technologies have imporoved transfection efficiency by leveraging cytokines. Cytokines are small protiens that are involved in various types of cell signaling. Classifications of these signalings include interferos, chemokines and interleukins.

In this experiment, β-actin and RPL13A will be used for direct controls against the immunological transcripts. INFβ will not be used as it will only appear after cycle 40 in the experiment producing undesirable results. The No Template Control will consist of DEPC water.

Beta actin is a type of actin isoform which is highly involved in cell motility, structure and integrity. RPL13A is a gene that codes for the 60S ribosomal L13a protein. Interleukin-6 (IL-6) is a multifunctional cytokine that defends the host in response to immune and hematopoietic activities. Interferon-α is a part of a large subgroup of interferon protiens that help regulate the activity of the immune system.

In this experiment, there are two devices used. The first device, the Qubit, is an electronic device that quantifies a substance through fluorescence. The second device is qPCR. Reverse Transcriptase (RT)qPCR is a technique used to describe the level of genetic expression is occuring in vitro by measuring the amount of mRNA within a sample. In this technique, mRNA is reverse transcribed in to complementary DNA by an enzyme labeled reverse transcriptase. For the Real Time PCR experiment, since it is impossible to eliminate all genomic DNA from the cDNA synthesis, two solutions will be prepared. The first solution will be a +RT which will include reverse transcriptase and thus, will have cDNA. The second solution will be a -RT solution which will lack reverse transcriptase. When QtPCR is performed on the -RT solution, genomic DNA will be amplified and will be seen in the resulting data.

Advisor: Dipl.–Ing. Michelle Ammerman, Lehrstuhl für Nachrichtentechnik, TUM, WS 2050/2051.

II. Methods

A. RNA Purification

The cells in trizol were thawed before phase separation. During phase separation, the poly treated cells in trizol were incubated after which an addition of chloroform was added. The homoginized sample was incubated following a vigorous shake. The sample was then centrifuged before transfering the aqueous phase to a second tube. Isopropanol was added to the aqueous phase. The solution underwent a series of centrifuges in between removing ethanol resulting in an RNA pellet. The pellet was incubated following a resuspension in RNase free water.

B. cDNA Synthesis

For the first step of cDNA Synthesis, two solutions (+RT/-RT) were formed from the following compounds: 10x dsDNase Buffer, dsDNase, Template RNA, polyplex 24hour RNA and nuclease free water. Both solutions were incubated after being centrifuged. The solutions were then chilled on ice, centrifuged and placed back on ice. For both solutions, 5X Reaction mix and nuclease free water was added. In the +RT solution, Maxima Enzyme mix (reverse transcriptase) was added to the mixture. The -RT solution used DEPC H20 instead of Maxima Enzyme mix so that the -RT solution can simulate the +RT solution without synthesizing RNA into cDNA. When the -RT solution undergoes QtPCR, any contaminating genomic DNA will be amplified. The two solutions will be then mixed gently and centrifuged. An incubation period will take place at 25°C for 10 min followed by a second incubation session at 50°C for 15 min. The reaction will be terminated by heating the solutions at 85°C for 5 min. This process was conducted using Maxima First Strand cDNA Synthesis Kit with dsDNase (Thermo Scientific, Cat: K1671)

C. Qt PCR

Each well will contain 2X iTaq universal SYBR Green Supermix, one of the four primers (IL6, INFα, β-actin or RPL13A), and the respective cDNA. The cDNA needs to be diluted to 100ng/μL before it is added to the wells. The concentrations of cDNA for both sets of Poly24 and no washed cells were all around 100ng/ μ L. Therefore only one set of calculations were needed for determining the proper dilution of cDNA into water for either INFα and IL6 or β-actin andRPL13A and the respective -RT wells. For just cells, a different set of calculations were needed since the original cDNA concentrations were around 11 ng/ μ L. All wells contained the Green Supermix. The columns were sorted in the

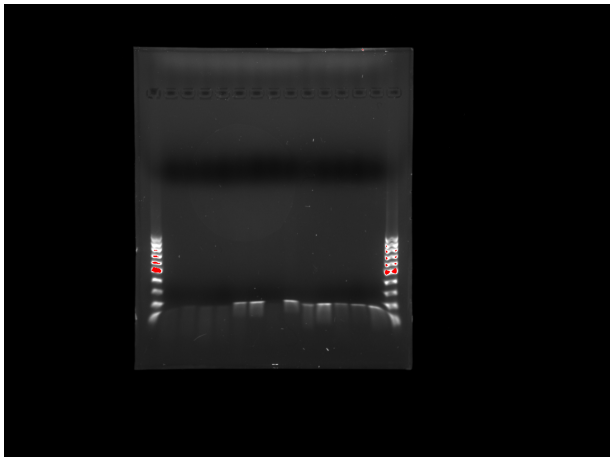

Fig. 1. Agarose Gel

following manner: 1-3 contained IL6, 4-6 contained INFα, 7-9 contained β-acting and 10-12 contained RPL13A. The QtPCR was ran for 40 cycles.

D. Gel Electrophoresis

In order to further understand the results gained from the QtPCR, an agarose gel was ran from the resulting amplified DNA from the Real Time PCR. The wells were constructed in the following manner: (1) Ladder, (2) E1, (3) E3, (4) G1, (5) E2, (6) B1, (7) C1, (8) A5, (9) C4, (10) A8, (11) D10, (12) E11, (13) E12, (14) A8, (15) Ladder

E. Analysis

The results from the QtPCR produced Ct values from each well. A Ct value is a numeric inverse correlation to the quantity of nucleic acid detected by the aparatus. The Ct values can be used in combination with the $\Delta\Delta$ct formula to produce fold inductions.

III. Results

A. Qubit

Poly24

B. QtPCR

TABLE I
WELLS FOR IMMUNOLOGICAL RESPONSES

	IL6	INFα	β-actin	RPL13A
Poly24[AV]-NoWash	3.85E-05	7.58E-03	5.09E-05	1.05E-02
Poly24[AV]-Cells	3.10E-02	3.54E-02	3.94E-02	4.27E-02
NoWash-Cells	8.01E+02	4.65E+00	7.01E+02	4.08E+00
Poly24[Tk]-NoWash	8.20E-05	4.64E-03	3.77E-05	6.04E-03
Poly24[Tk]-Cells	6.54E-02	5.90E-02	2.57E-02	2.55E-02
Poly6	500.25	421.07	25.83	22.29

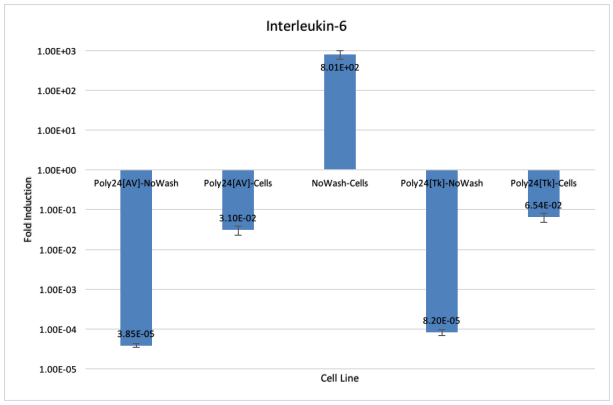

Fig. 2. Fold Inductions for Interleukin-6.

C. Gel Electrophoresis

IV. Discussion

The mRNA was purified and converted to cDNA. THe resulting concentration was relatively high compared to peers. This eludes that the treatment for these cells of polycationic DNA for 24 hours could result in higher transcription rates.

V. Conclusion

To futher understand the nature polyplex treated cells in response to a specific duration, it would be suggested that the polyplex cells are treated in 3 hour increments from 3 hours to 24 hours.

VI. Figures

TABLE II
WELLS FOR IMMUNOLOGICAL RESPONSES

	1	2	3	4	5	6
A	4ng/μL	4ng/μL	4ng/μL	4ng/μL	4ng/μL	4ng/μL
B	4ng/μL	4ng/μL	4ng/μL	4ng/μL	4ng/μL	4ng/μL
C	4ng/μL	4ng/μL	4ng/μL	4ng/μL	4ng/μL	4ng/μL
D	4ng/μL	4ng/μL	4ng/μL	4ng/μL	4ng/μL	4ng/μL
E	Ts	Ni	AV	Ts	Ni	AV
E	Ts	Ni	AV	Ts	Ni	AV
G	Mi -RT	Mi NTC		Mi -RT	Mi NTC	
H						

TABLE III
WELLS FOR CONTROLS

	7	8	9	10	11	12
A	4ng/μL	4ng/μL	4ng/μL	4ng/μL	4ng/μL	4ng/μL
B	4ng/μL	4ng/μL	4ng/μL	4ng/μL	4ng/μL	4ng/μL
C	4ng/μL	4ng/μL	4ng/μL	4ng/μL	4ng/μL	4ng/μL
D	4ng/μL	4ng/μL	4ng/μL	4ng/μL	4ng/μL	4ng/μL
E	Ts	Ni	AV	Ts	Ni	AV
E	Ts	Ni	AV	Ts	Ni	AV
G	Mi -RT	Mi NTC		Mi -RT	Mi NTC	
H						

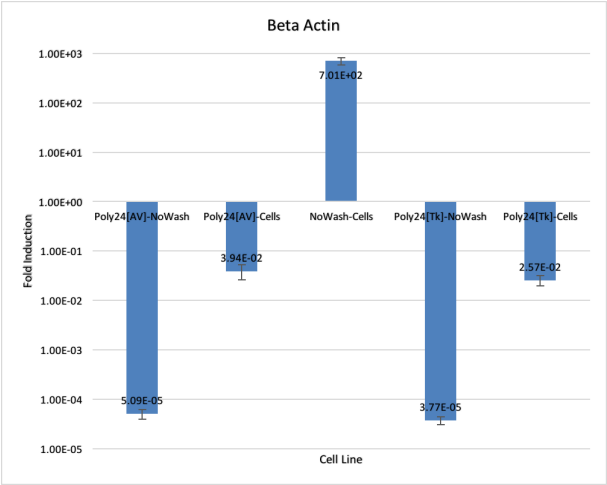

Fig. 3. Fold Inductions for β-actin.

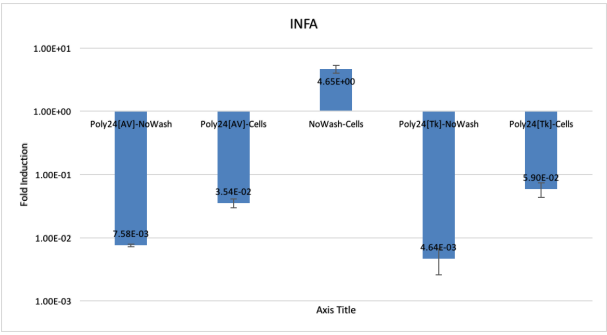

Fig. 4. Fold Inductions for Interferon-α.

Fig. 5. Fold Inductions for

REFERENCES

[1] J. Hagenauer, E. Offer, and L. Papke. Iterative decoding of binary block and convolutional codes. *IEEE Trans. Inform. Theory*, vol. 42, no. 2, pp. 429-445, Mar. 1996.

[2] T. Mayer, H. Jenkac, and J. Hagenauer. Turbo base-station cooperation for intercell interference cancellation. *IEEE Int. Conf. Commun. (ICC)*, Istanbul, Turkey, pp. 356–361, June 2006.

[3] J. G. Proakis. *Digital Communications*. McGraw-Hill Book Co., New York, USA, 3rd edition, 1995.

[4] F. R. Kschischang. Giving a talk: Guidelines for the Preparation and Presentation of Technical Seminars. http://www.comm.toronto.edu/frank/guide/guide.pdf.

[5] IEEE Transactions LaTeXand Microsoft Word Style Files. http://www.ieee.org/web/publications/authors/transjnl/index.html