Testing the Effects of Transfection on Mammalian Cytokine RNA Expression

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

I. Introduction

This section introduces the topic and leads the reader on to the main part.

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.

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.

Interleukin-6 (IL-6) is a multifunctional cytokine that defends the host in response to immune and hematopoietic activities.

http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2143693/pdf/9144766.pdf http://www.molbiolcell.org/content/22/21/4047.full.pdf+html?with-ds=yes

Poly24 Av Poly24 Ts Just Cells No Wash

It is virtually impossible to completely eliminate all genomic DNA from RNA preparations. Therefore, if the assay is not cDNA-specific, it is important to include a minus-reverse transcriptase ("-RT") control in real-time RT-PCR experiments. Typically, the "-RT" control is a mock reverse transcription containing all the RT-PCR reagents, except the reverse transcriptase. The presence of an amplification product in the "-RT" control is indicative of contaminating DNA in the sample.

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.

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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.

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 by ¡insert¿ in the 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. 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.

TABLE I
QUBIT RESULTS

SD1	54.27
SD2	1057.52
Tk	8.75
DA	68.0
AV	487.0
Tk	28.0

Fig. 1. Agarose Gel

III. RESULTS

A. Qubit

B. QtPCR

C. Gel Electrophoresis

IV. DISCUSSION

For a future experiment, it would be suggested that the polyplex cells are treated in 3 hour increments from 3 hours to 24 hours.

V. CONCLUSION

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.

VI. FIGURES

- Columns 1-3 IL6
- Columns 4-6 INFα
- Columns 7-9 β-acting
- Columns 10-12 RPL13A

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						

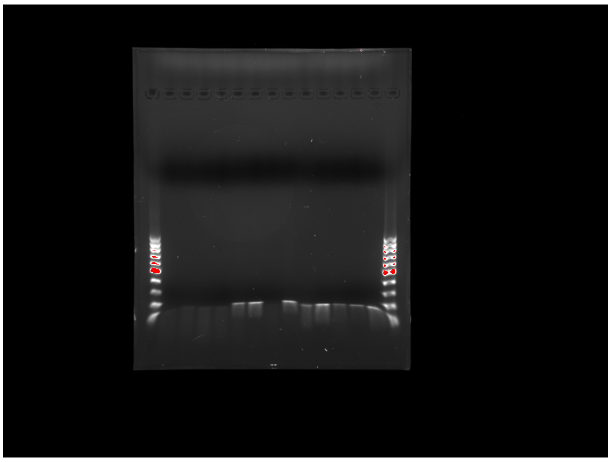

Fig. 2. Agarose Gel

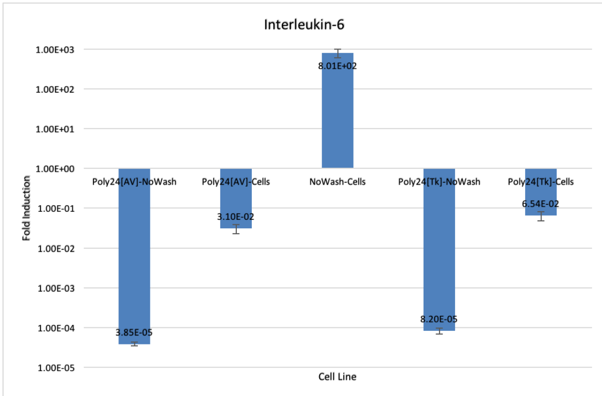

Fig. 3. Fold Inductions for

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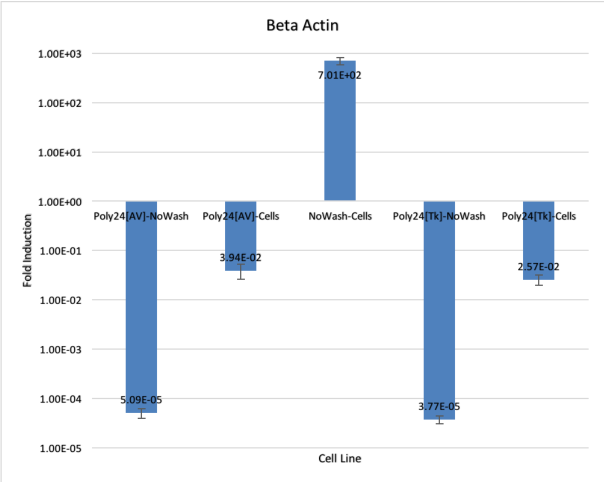

Fig. 4. Fold Inductions for

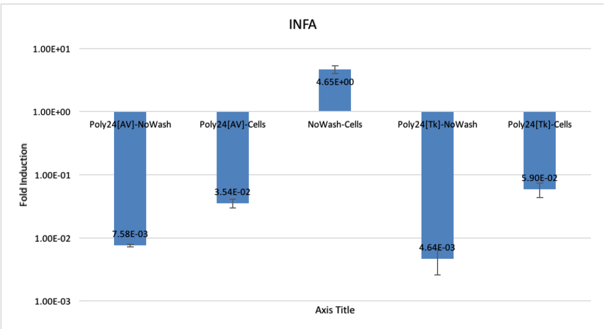

Fig. 5. Fold Inductions for

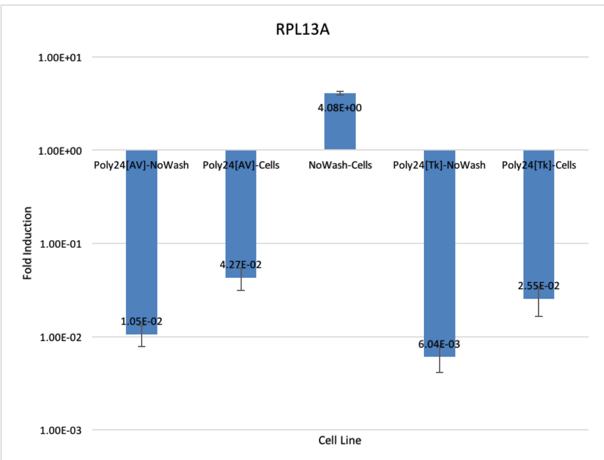

Fig. 6. Fold Inductions for