

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 $INF\alpha$.

I. INTRODUCTION

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

Real Time PCR Generate large quantity of DNA from cDNA templates. Can view amount of DNA in each cycle. Can be seen if immunological response was indicated.

In this experiment, β -actin and RPL13A will be used for direct controls against the immunological transcripts. $INF\beta$ 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.

Outline

polyplex polycationic treatments samples form an immune response The mRNA sample

Test the effects on

Each student was given a sample as follows

IL6 and $INF\alpha$ are used as the immunological transcripts DEPC water is the no template control

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 homogenized sample was incubated following a vigorous shake. The sample was then centrifuged before transferring 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 H2O 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 QPCR, any contaminating genomic DNA will be amplified.

C. QPCR

iTaq universal SYBR Green supermix
20 μ L Reaction 10 Permutation of Green supermix with four
ina2 : 1 ratio. 5 microliters and 10 microliters.

TABLE I
cDNA CONCENTRATIONS

INFA	(Ts, AV, Mi)	1.32
INFA	(Ni)	11.79
β -Actin	(Ts, AV, Mi)	2.00
β -Actin	(Ni)	2.9

Each well will get 6 μ L of 4ng/ μ L of dna as per. The concentrations of cDNA for Ts, Av and Mi were all around 100ng/ μ L. Therefore one set of calculations can be used for all three experiments. The concentrations of Ni, however, were reported to be 11.2ng/ μ L and thus, had to have a different set of calculations.

Adding supermix, following charts from above and water make mix.

The QPCR was ran for 40 cycles.

D. Gel Electrophoresis

The following was loaded in to the wells:

E. Analysis

Used $\Delta\Delta$ ct method was used in calculating fold inductions.

TABLE II
QUBIT RESULTS

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

III. RESULTS

A. Qubit

B. QrtPCR

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

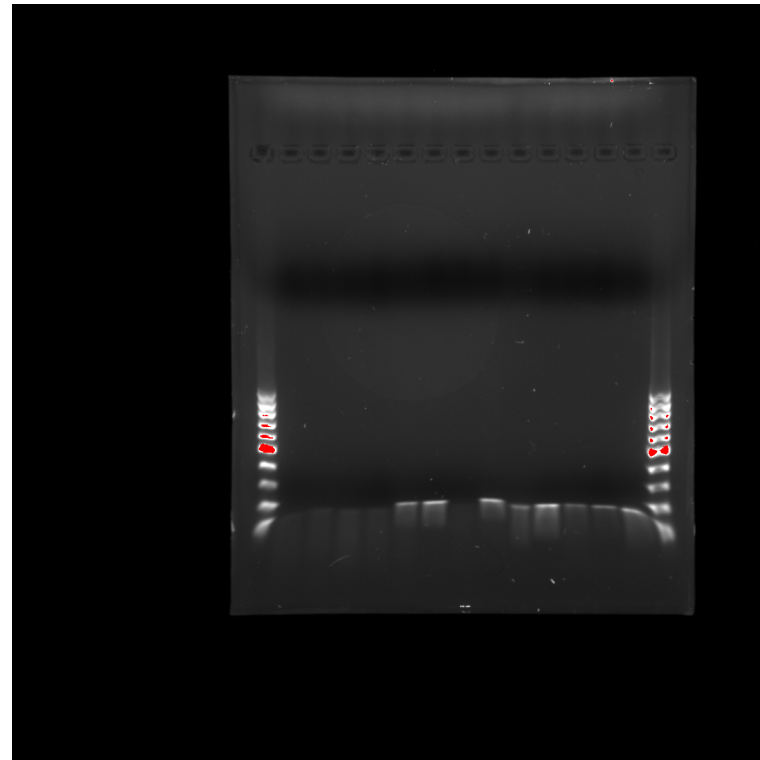
- Columns 1-3 IL6
- Columns 4-6 INF α
- Columns 7-9 β -actin
- Columns 10-12 RPL13A

TABLE III
WELLS FOR IMMUNOLOGICAL RESPONSES

	1	2	3	4	5	6
A	4ng/ μ L	4ng/ μ L	4ng/ μ L	4ng/ μ L	4ng/ μ L	4ng/ μ L
B	4ng/ μ L	4ng/ μ L	4ng/ μ L	4ng/ μ L	4ng/ μ L	4ng/ μ L
C	4ng/ μ L	4ng/ μ L	4ng/ μ L	4ng/ μ L	4ng/ μ L	4ng/ μ L
D	4ng/ μ L	4ng/ μ L	4ng/ μ L	4ng/ μ L	4ng/ μ L	4ng/ μ L
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 IV
WELLS FOR CONTROLS

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



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