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

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.

There are multiple controls used throughout this experiment. For controls that compare directly against the immunological transcripts β-actin and RPL13A will be used. 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.

Outline

polyplex polycationic treatments samples form an immune response The mRNA sampl

Test the effects on

Each student was given a sample as follows

IL6 and IFNα are used as the immunilogical 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. Purification

1) Thaw poly treated cells in trizol on ice.
2) Phase separation
   a) Incubate polytreated cells + Trizol mixture for 5 min.
3) Add 0.2mL of chloroform per 1mL of Trizol reagent.

600

μL of Trizol * frac0.2 of choloform1μL of Trizol (1)

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

4)
5)
6)

B. Reverse Transcription

TABLE I
cDNA Concentrations

+RT	-RT
1μL 10x dsDNase Buffer	10x 1L 10x dsDNase Buffer
1μL dsDNase	1L dsDNase
1μL Template RNA	1L Template RNA
2μL Total RNA (Poly24)	2L Total RNA (Poly24)

TABLE II
cDNA Concentrations

+RT	-RT
4μL 5x Reaction Mix	4μL 5x Reaction Mix
2μL Maxima Enzyme Mix	2μL DEPC H20
4μL Nuclease Free Water	4μL Nuclease Free Water

C. Qt PCR

iTaq universal SYBR Green supermix $20\mu L Reaction 10 Permuation of Green supermix with four <> in a 2:1 ratio. 5 microliters and 10 microliters.$

TABLE III
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 6μL of 4ng/μL 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 ot be 11.2ng/ μ L and thus, had to have a different set of calculations.

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

The QPCR was ran for 40 cycles.

D. Analysis

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

TABLE IV
QUBIT RESULTS

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

III. RESULTS

A. Qubit

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

TABLE V
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						

Tasha (Ts) Micah (Mi) Nick (Ni) Austin (AV) -rt controls NTC

Columns 1-3 IL6 Columns 4-6 INF$\alpha Columns 7-9\beta - acting Columns 10-12 RPL13A$

TABLE VI
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						

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