

# 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  $\beta$ -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.

There are multiple controls used throughout this experiment. For controls that compare directly against the immunological transcripts  $\beta$ -actin and RPL13A will be used.  $INF\beta$  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. Purification

mRNA

### B. Reverse Transcription

### C. Q<sub>t</sub> PCR

iTaq universal SYBR Green supermix  
20 $\mu$ L Reaction 10 Permutation of Green supermix with four <>  
ina2 : 1 ratio 5 microliters and 10 microliters.

Each well will get 6 $\mu$ L of 4ng/ $\mu$ L of dna as per. The concentrations of cDNA for Ts, Av and Mi were all around

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TABLE I  
cDNA CONCENTRATIONS

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

TABLE II  
cDNA CONCENTRATIONS

+RT	-RT
4 $\mu$ L 5x Reaction Mix	4 $\mu$ L 5x Reaction Mix height 2 $\mu$ L 1
2 $\mu$ L DEPC H2O height 4 $\mu$ L Nuclease Free Water	4 $\mu$ L Nuclease Free Wa

TABLE III  
cDNA CONCENTRATIONS

INFA	(Ts, AV, Mi)	1.32
INFA	(Ni)	11.79
$\beta$ -Actin	(Ts, AV, Mi)	2.00
$\beta$ -Actin	(Ni)	2.9

100ng/  $\mu$  L. Therefore one set of calculations can be used for all three experiments. The concentrations of Ni, however, were reported to be 11.2ng/  $\mu$  L and thus, had to have a different set of calculations.

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

### D. Analysis

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

## III. RESULTS

### A. Qubit

TABLE IV  
QUBIT RESULTS

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

## 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/ $\mu$ L	4ng/ $\mu$ L	4ng/ $\mu$ L	4ng/ $\mu$ L	4ng/ $\mu$ L	4ng/ $\mu$ L
B	4ng/ $\mu$ L	4ng/ $\mu$ L	4ng/ $\mu$ L	4ng/ $\mu$ L	4ng/ $\mu$ L	4ng/ $\mu$ L
C	4ng/ $\mu$ L	4ng/ $\mu$ L	4ng/ $\mu$ L	4ng/ $\mu$ L	4ng/ $\mu$ L	4ng/ $\mu$ L
D	4ng/ $\mu$ L	4ng/ $\mu$ L	4ng/ $\mu$ L	4ng/ $\mu$ L	4ng/ $\mu$ L	4ng/ $\mu$ 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						

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/ $\mu$ L	4ng/ $\mu$ L	4ng/ $\mu$ L	4ng/ $\mu$ L	4ng/ $\mu$ L	4ng/ $\mu$ L
B	4ng/ $\mu$ L	4ng/ $\mu$ L	4ng/ $\mu$ L	4ng/ $\mu$ L	4ng/ $\mu$ L	4ng/ $\mu$ L
C	4ng/ $\mu$ L	4ng/ $\mu$ L	4ng/ $\mu$ L	4ng/ $\mu$ L	4ng/ $\mu$ L	4ng/ $\mu$ L
D	4ng/ $\mu$ L	4ng/ $\mu$ L	4ng/ $\mu$ L	4ng/ $\mu$ L	4ng/ $\mu$ L	4ng/ $\mu$ 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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