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Identifying Hipk1 as a target of Mir-22-3p enhancing recombinant protein production from Hek 293 by using microarray and Htp sirna screen

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Identifying *HIPK1* as Target of miR-22-3p, Enhancing Recombinant Protein Production From HEK 293 Cells [1]

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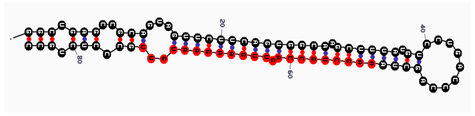
Introduction

- Mammalian cells are being used for producing proteins and antibodies for therapeutic, biochemical and structural studies. [2]
- Chinese Hamster Ovary (CHO) cells are commonly used for recombinant protein production but Human Embryonic Kidney (HEK-293) cells have the advantage of human post-translational modification.[3]
- microRNA (miRNA or miR) are small non-coding RNA that regulate gene expression. [4]



hsa-mir-22 secondary structure [4]

- Our previous high throughput screen identified several miRNA, which improve protein production in HEK cells with multiple protein types, from these we decided to concentrate on hsa-mir-22-3p. [6]

Objectives

A. To improve protein expression in HEK 293 cells
B. To identify genes involved with improving protein expression as a result of treatment with the identified hsa-mir-22-3p
C. To create cell lines with improved protein expression

Work Flow

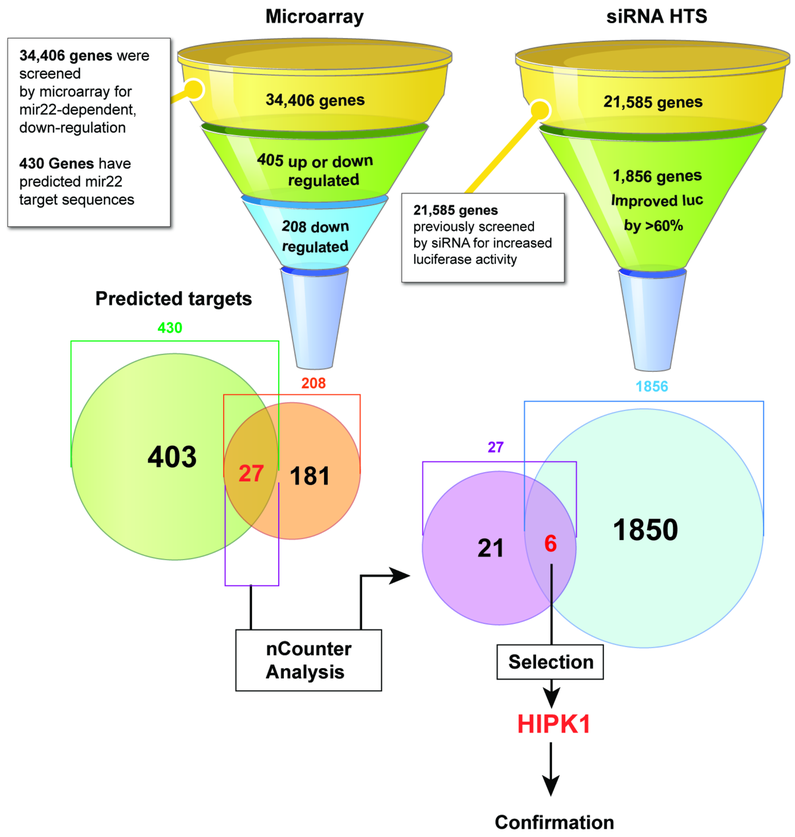


Fig. 1 Work flow of gene identification, selection and confirmation for improving protein production.
HTS = High throughput screen

Conclusions

- miR-22-3p improves recombinant protein expression
- Using microarray analysis along with an siRNA screen, [7] common seed analysis and nCounter analysis can identify genes involved in recombinant protein production
- *HIPK1* is connected with improving luciferase and GPC3 expression
- Validated with siRNA and RT-PCR

Future work

- Investigate the mechanism(s) leading to improved protein biogenesis
- Create high producing cell line by stable knock-out of top candidate genes
- Compare to a high producing cell line with stable over-expressing mir-22

References

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Acknowledgement

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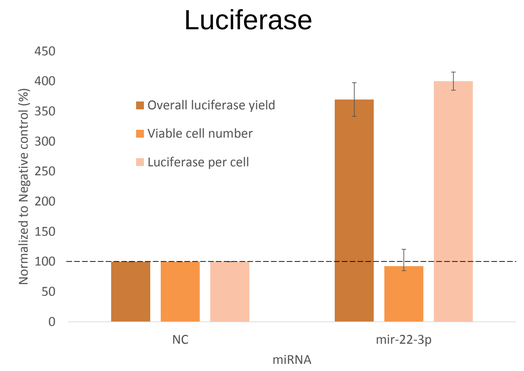
Results

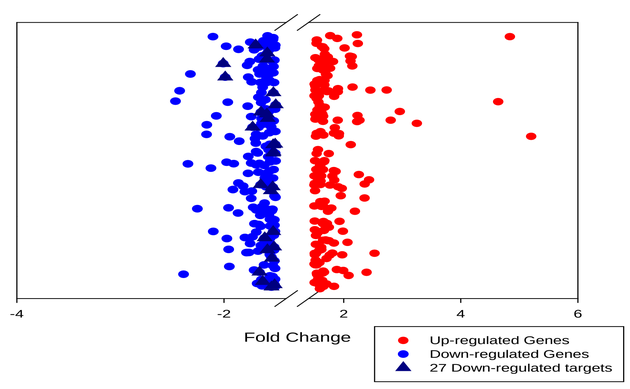
Gene Identification and Selection

(A)

Transient Transfection
In each well:
•miRNA (mir-22-3p, negative control (NC) or si-killer)
•RNAiMAX + serum free DMEM
•Cells in 20% FBS DMEM
After 48 hrs harvest, assay, extract RNA, microarray



(B)

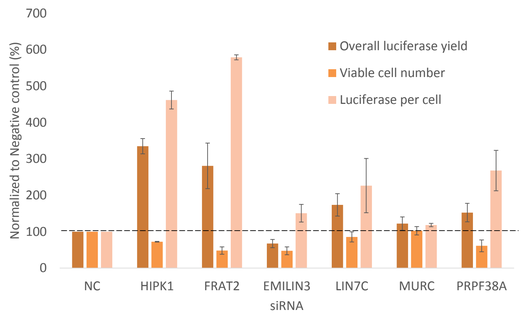


- 34,460 genes on microarray
- 405 differentially expressed
- 218 down regulated
- 27 down regulated targets of mir-22-3p

Fig. 2 (A) Relative luminescence and cell viability of luciferase expressing HEK cells transiently transfected with mir-22-3p
(B) Differentially expressed genes from microarray. NC=Negative Control

Gene Confirmation

(A)



(B)

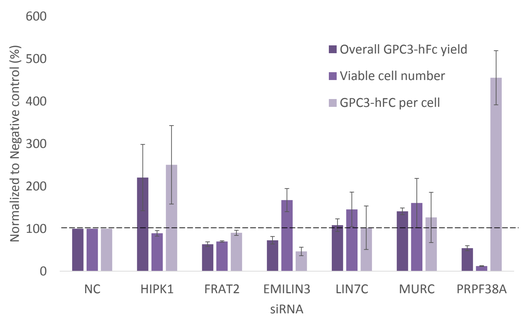


Fig. 3 (A) Relative luminescence and cell viability of luciferase expressing HEK 293 cells treated with siRNA
(B) Relative GPC3-hFc yield and cell viability of GPC3-hFC expressing HEK 293 cells treated with siRNA