Promoter & PRE Triage for Gene Expression & Function Optimization

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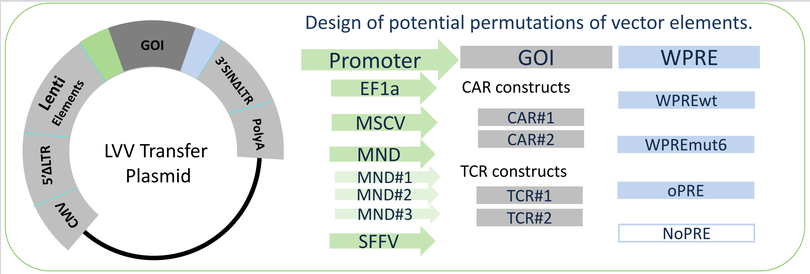
INTRODUCTION

Optimizing lentiviral vector designs to increase titer, decrease production costs, and increase potency is an active area of exploration especially for vectors entering large scale manufacture. Here, we tested the effect of different promoters and PREs (Post-Regulatory Element) on multiple CAR and transgenic TCR constructs on titer, integration, expression, and *in vitro* function. To achieve this, we developed a scaled down mid-throughput lentiviral vector production platform to produce multiple constructs in tandem with sufficient titers for functional screening. Experiments were separated into two stages. In the 1st stage we triaged three unique MND promoters, and four types of PRE elements. The lead constructs were moved to the 2nd stage triage comparing EF1a, MSCV, MND, and SFFV promoters, for titer, transduction, expression and function using 4 genes of interest (GOI).

Plasmid design of combinations for triage



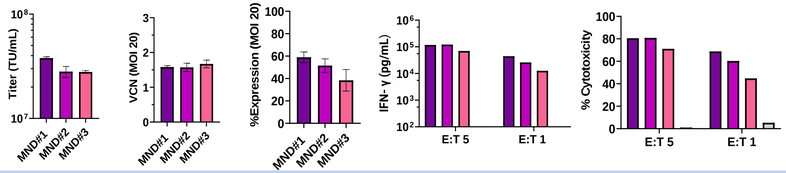
Design of potential permutations of vector elements.

Promoter	GOI	WPRE
EF1a	CAR constructs: CAR#1, CAR#2	WPREwt
MSCV		WPREmut6
MND: MND#1, MND#2, MND#3	TCR constructs: TCR#1, TCR#2	oPRE
SFFV		NoPRE

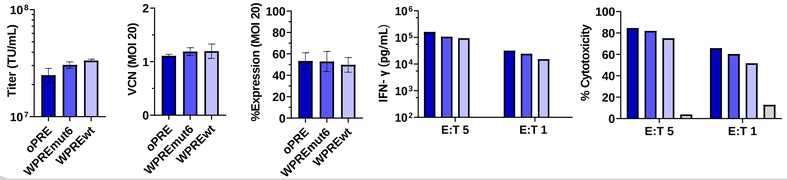
RESULTS - 1st stage triage

LVV Crude Titer | VCN | % Expression | Cytokine IFN-γ release | Cytotoxicity % Killing

MND promoter triage: MND#1 will be applied in the 2nd stage triage.



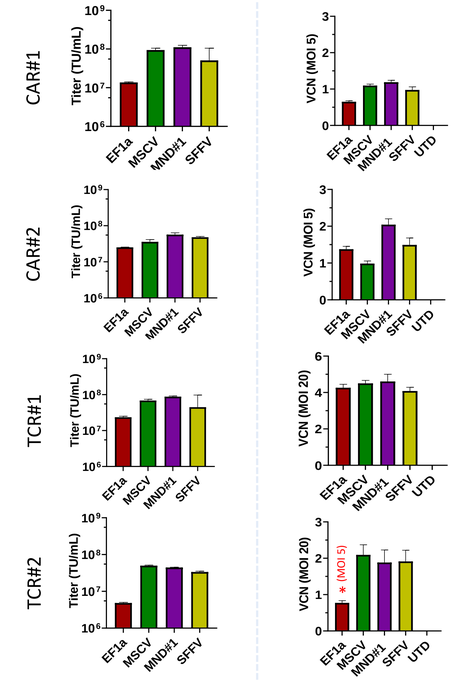
PREs triage: oPRE will be applied in the 2nd stage triage.



RESULTS – 2nd stage: Comparison of Four Promoters

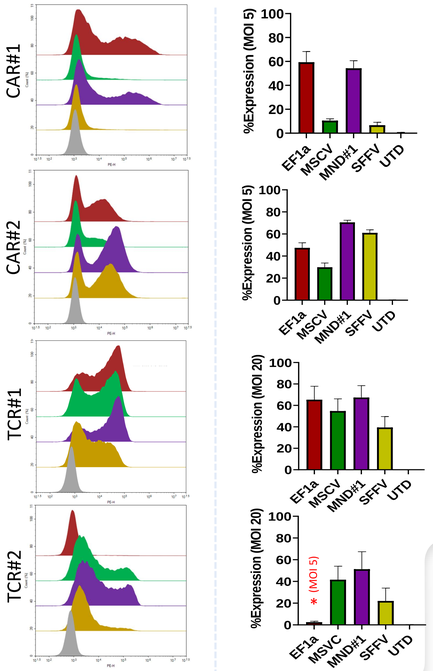
Lentiviral Vector Production

LVV Crude Titer | Virus Copy Number



T-Cell Engineering

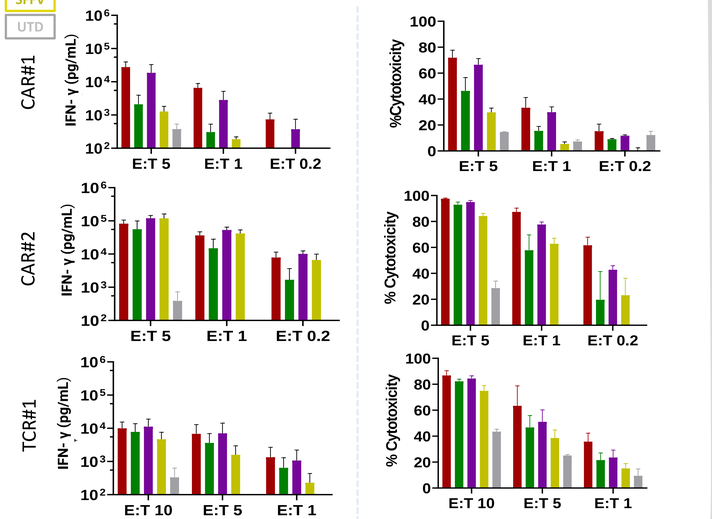
Flow cytometry | %Expression



EF1a | MSCV | MND | SFFV | UTD

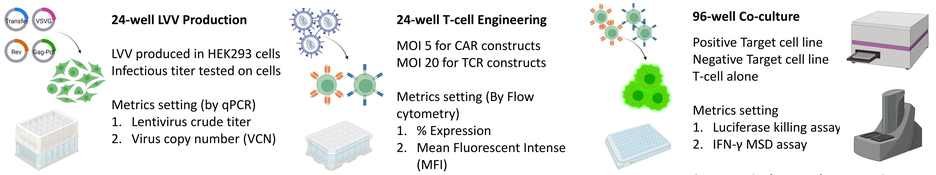
Functional Assays

Cytokine IFN-γ release | Cytotoxicity %Killing



Workflow and Readouts for Triage

Lentiviral Vector | Primary Human T-cells | Functional Assays



24-well LVV Production

LVV produced in HEK293 cells
Infectious titer tested on cells

Metrics setting (by qPCR)
1. Lentivirus crude titer
2. Virus copy number (VCN)

24-well T-cell Engineering

MOI 5 for CAR constructs
MOI 20 for TCR constructs

Metrics setting (By Flow cytometry)
1. % Expression
2. Mean Fluorescent Intense (MFI)

96-well Co-culture

Positive Target cell line
Negative Target cell line
T-cell alone

Metrics setting
1. Luciferase killing assay
2. IFN-γ MSD assay

*Figures in this frame are from BioRender.

CONCLUSION

- MND promoter was able to ubiquitously produce high titers of lentiviral vector and generated a high level of expression across all CAR and TCR constructs tested.
- EF1a consistently produced lower titers compared to MND based constructs, but when transduced at the same MOI produced high levels of expression in T cells.
- SFFV and MSCV promoters showed varying titers and expression dependent on the encoded gene of interest.

Comparing viral copy number and expression data, we were able to evaluate transduction efficiency and surface expression levels separately for each gene of interest. Unsurprising, cytotoxicity and function of each construct tested was highly correlated to the expression level on the cell surface in most cases. These data will help guide future constructs design and streamline development of clinical candidates and feasibility studies.

Contact Information

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