Development of an Epithelial Mesenchymal Transition Tracing Vector

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Introduction

- Epithelial mesenchymal transition (EMT) is a process whereby epithelial cells lose adhesion and gain the migratory properties of mesenchymal cells [1].
- EMT is involved in the repair of damaged epithelial tissue, and if left unchecked, can lead to fibrosis[2].
- EMT has primarily been observed *in vitro*, however detecting EMT *in vivo* is challenging, with current methods only able to observe a snapshot in time rather than cell changes over time.
- Here we have developed a simple cell lineage tracing technique that allows us to visually observe epithelial and mesenchymal cell transdifferentiation *ex vivo*.
- We designed a permanent GFP/mScarlet fluorescence switch that, when dual stained for specific cell markers, provides details on the current cell type, as well as whether the cell was previously of the epithelial or mesenchymal lineage.

EMT tracing vector synthesis

- A bicistronic vector was designed to contain A) Cre recombinase under the control of the epithelial-specific E-cadherin (*CDH1*) promoter, and B) a floxed GFP/mScarlet sequence driven by the constitutive SFFV promoter (Fig 1).
- Gene synthesis was performed by GeneArt (ThermoFisher) and InFusion cloning (Takara) was used to clone into a pLNT lentiviral backbone.

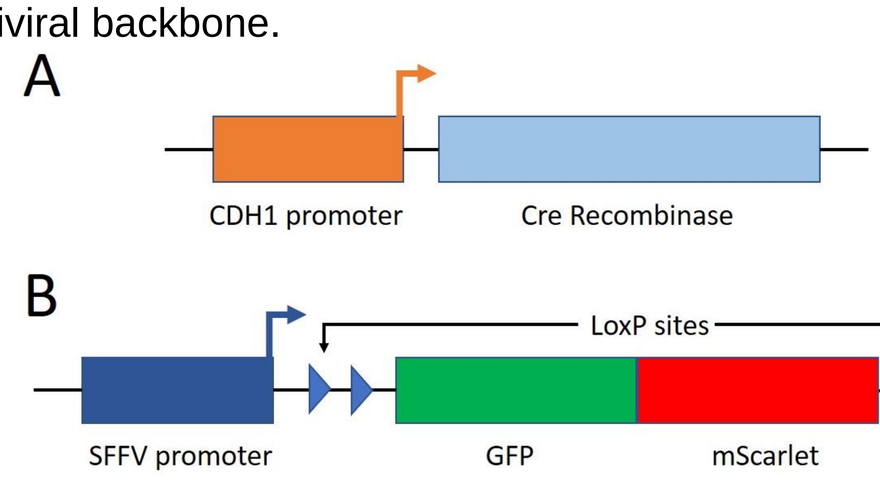

Figure 1: Graphical representation of the bicistronic EMT tracing vector

LoxP flipping is dependant on Cre recombinase

- To show that the LoxP sites do not spontaneously flip in the absence of Cre recombinase (Cre^-), the Cre recombinase sequence was disrupted.
- EMT-tracing plasmid was treated with exogenous Cre recombinase prior to transfection in HEK293T cells and compared to cells transfected with untreated EMT tracing plasmid containing the disrupted Cre recombinase sequence.

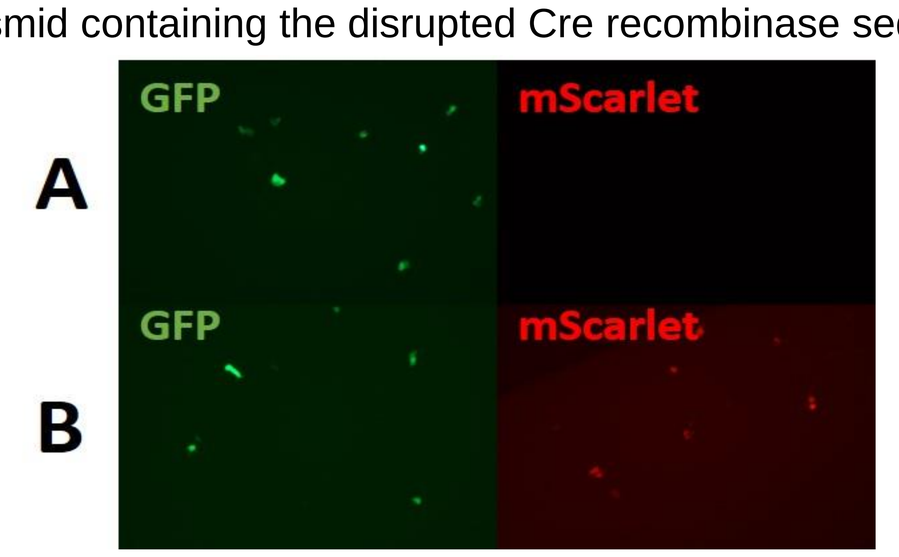

Figure 2: HEK293T cells transfected with A) Cre^- EMT tracing plasmid or B) recombinant Cre recombinase pre-treated Cre^- EMT tracing plasmid.

- Only GFP expressing cells were observed in Cre^- transfected cells (Fig 2A). Exogenous Cre recombinase treatment of plasmid resulted in cells expressing either GFP or mScarlet (Fig 2B).
- LoxP site flipping does not occur in the absence of Cre recombinase.

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Spontaneous LoxP site flipping occurs during viral production

- HEK293T cells were seeded in 6-well plates and transfected with the EMT tracing vector and helper plasmids (Tat, Gag-Pol, Rev and VSV-G).
- 48 hours post transfection, fluorescent images were taken to assess whether GFP was exclusively expressed.

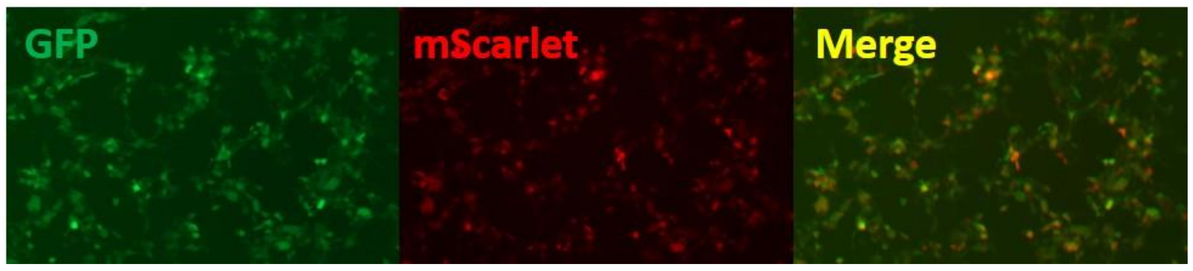

Figure 3: HEK293T 48 hours post transfection with EMT tracing vector and lentiviral helper plasmids.

- Dual GFP and mScarlet expressing cells were observed (Fig 3).
- GFP has a half life of 26 hrs. It is expected that GFP/mScarlet dual expression will persist until remaining GFP signal is lost after flipping at LoxP sites.

HEK293T cells express E-cadherin

- Since mScarlet positive HEK293T cells were present during viral production, E-cadherin expression in HEK293T cells was assessed.

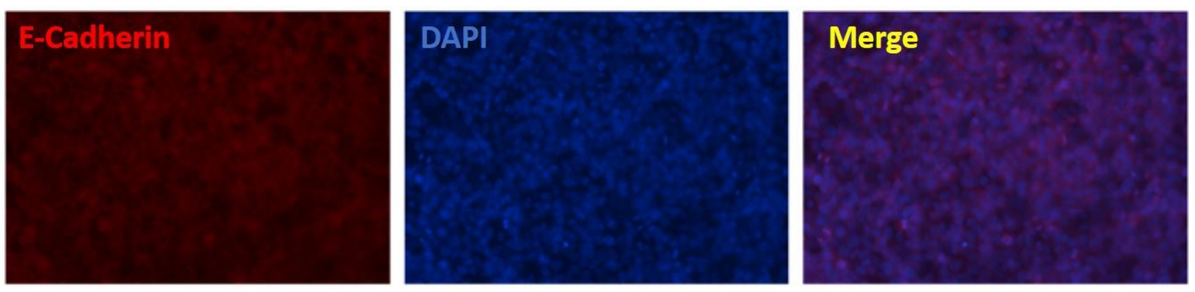

Figure 4: HEK293T cells were stained with E-cadherin antibody and counterstained with DAPI.

- HEK293T cells express E-cadherin (Fig 4). The active E-cadherin promoter region resulting in E-cadherin expression is likely responsible for spontaneously activating Cre recombinase expression and inducing LoxP site flipping.

Future directions

- Cre recombinase must remain inactive during lentiviral vector production to ensure no virions contain viral RNA containing flipped mScarlet sequence.
- An E-cadherin KO HEK293T cell line has been produced and is currently being validated.
- A vector, substituting the E-cadherin promoter for the mesenchymal N-cadherin promoter is currently in progress.
- Future *ex vivo* experiments will be performed using the EMT tracing vector to determine differences in EMT magnitude under physiological and disease states.

References

[1] Rout-Pitt, N., et al., *Epithelial mesenchymal transition (EMT): a universal process in lung diseases with implications for cystic fibrosis pathophysiology.* Respir Res, 2018. **19**(1): p. 136.

[2] Zhao, Y.L., et al., *Epithelial-mesenchymal transition in liver fibrosis.* Biomed Rep, 2016. **4**(3): p. 269-274.