Establishment of an inducible bioluminescent reporter system to label extracellular vesicle (EV) secretion

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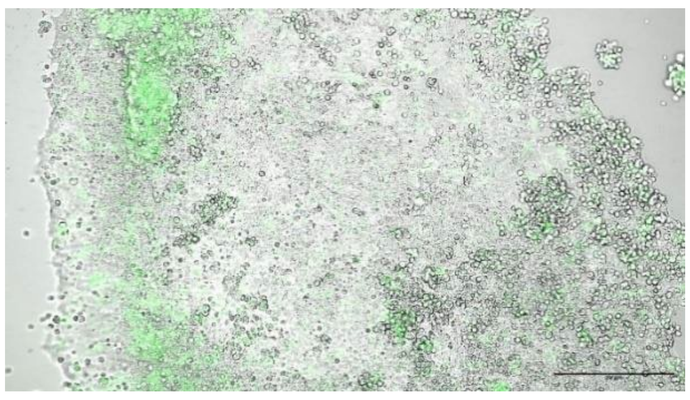
1. Summary

The planned inducible CD63–Luciferase–FLAG reporter for extracellular vesicle (EV) secretion remained unvalidated because of cloning delays. Practical work focused on GFP-based transfection optimization in T-REx-293 cells. Omitting penicillin/streptomycin (Pen/Strep) for the first 24 h improved survival. Approximately 75% confluency balanced cell number and transfection yield. With 100 ng DNA and 0.50 µL Lipofectamine 3000 per well, robust GFP expression with minimal cytotoxicity was observed. [1]



Initial challenge

GFP signal alone did not indicate a viable culture: cells rounded up and detached. [1]

Fig. 1 Initial lipofection, 24 h. GFP expression was visible despite cell stress and mortality. Original image from thesis Fig. 11. [1]

2. Introduction

EVs mediate intercellular communication. CD63 is a membrane-associated EV marker and was selected as an anchor for a luciferase reporter. [1–3]

The planned pcDNA5/FRT/TO system was designed for inducible reporter expression. Efficient plasmid delivery and viable host cells were prerequisites. GFP expression served as the transfection readout here, not as validation of the EV reporter. [1]

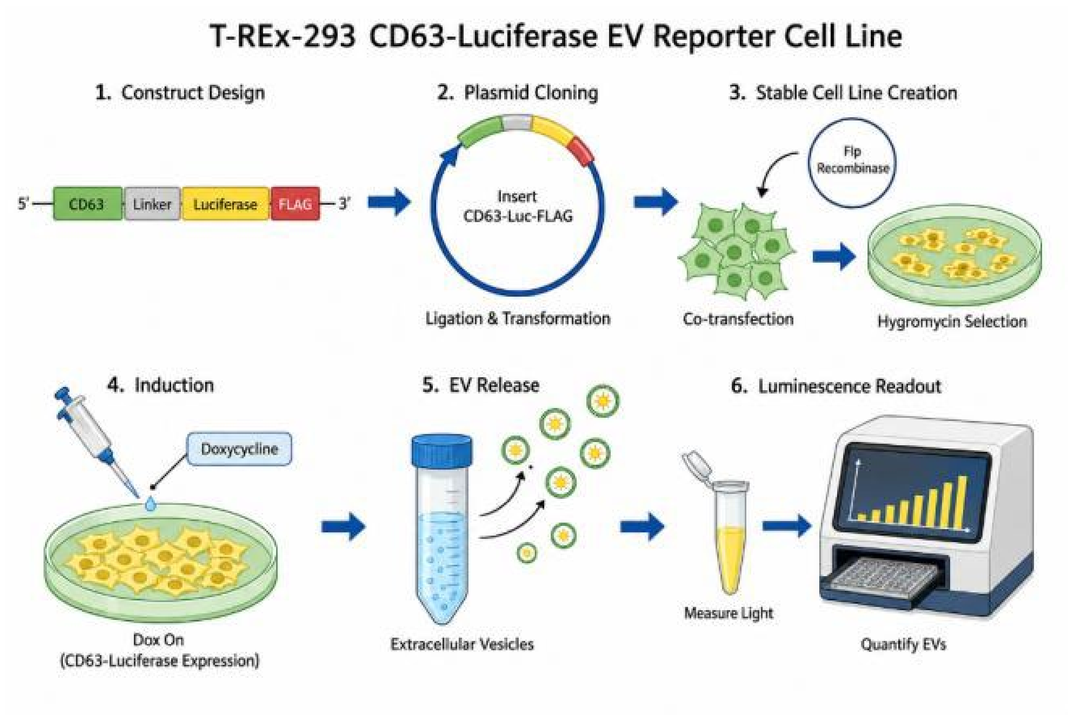


Fig. 2 Planned generation and readout of the inducible EV reporter. This workflow was not fully validated. Original schematic from thesis Fig. 3. [1]

3. Aims and leading questions

Selected focus: transfection optimization (Aim 3)

How do cell confluency, Lipofectamine volume and the exclusion of Pen/Strep affect transfection efficiency in T-REx cells? [1]

Objective: improve GFP delivery while minimizing cytotoxicity.

4. Material, methodology and approach

GFP plasmid-based optimization in T-REx cells (MH02).

Cell system	T-REx-293 cells; 96-well plate
DNA per well	100 ng GFP plasmid DNA
Confluency	60%, 75% or 90%
Lipofectamine 3000	0.15, 0.20 or 0.50 µL per well*
Antibiotics	Pen/Strep omitted for the first 24 h
Readout	GFP microscopy, ImageJ and cell morphology; 24 h

*Doses shown in thesis Fig. 12 and the 96-well protocol. [1]

5. Results

Transfection without Pen/Strep during the first 24 h

100 ng DNA/well | Left: brightfield/GFP overlay | Right: GFP

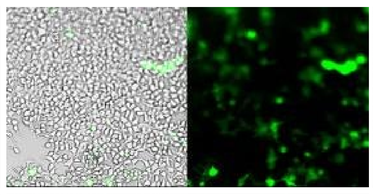
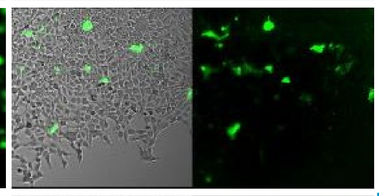
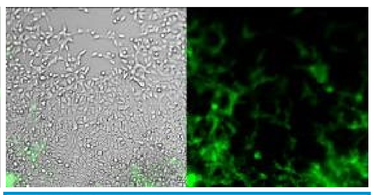
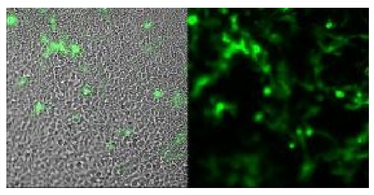
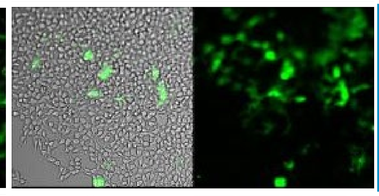
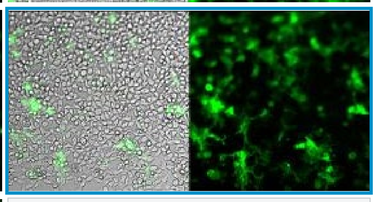
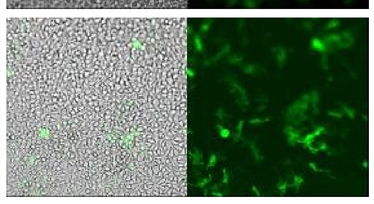
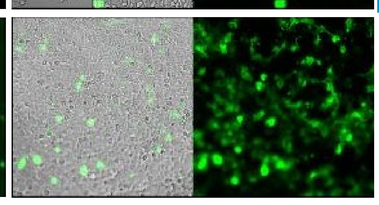
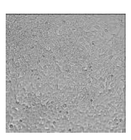
Cell confluency	0.15 µL Lipofectamine 3000	0.20 µL Lipofectamine 3000	0.50 µL Lipofectamine 3000
60%			
75%			
90%			Not completed Reagent depletion

Fig. 3 Transfection optimization at 24 h. Original images from thesis Fig. 12, rearranged by confluency and dose. The 90% / 0.50 µL condition was not completed. [1]



Untransfected negative control: no specific GFP fluorescence was reported. Brightfield image from thesis Fig. 12. [1]

Working condition identified in this study

~75% confluency + 100 ng DNA
0.50 µL Lipofectamine 3000 per well
No Pen/Strep during the first 24 h. [1]

6. Discussion and conclusion

Transfection success depended on culture conditions as well as GFP expression. Low confluency limited absolute cell yield; 90% confluency reduced the fluorescent-cell yield. Approximately 75% provided a practical balance. [1]

These parameters support further reporter development but do not establish a functional EV reporter. Construct assembly and functional validation still need to be completed. [1]

References

[1] Holzer M. Establishment of an inducible bioluminescent reporter system to label extracellular vesicle (EV) secretion. Diploma thesis. medi / DBMR, University of Berne; 2026.

[2] Raposo G, Stoorvogel W. Extracellular vesicles: Exosomes, microvesicles, and friends. J Cell Biol. 2013;200:373–383.

[3] van Niel G, D'Angelo G, Raposo G. Shedding light on the cell biology of extracellular vesicles. Nat Rev Mol Cell Biol. 2018;19:213–228.