

# Electroporation of the Lung Greatly Enhances Reporter Gene Expression Following Delivery of Plasmid DNA.



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http://users.ox.ac.uk/~genemed

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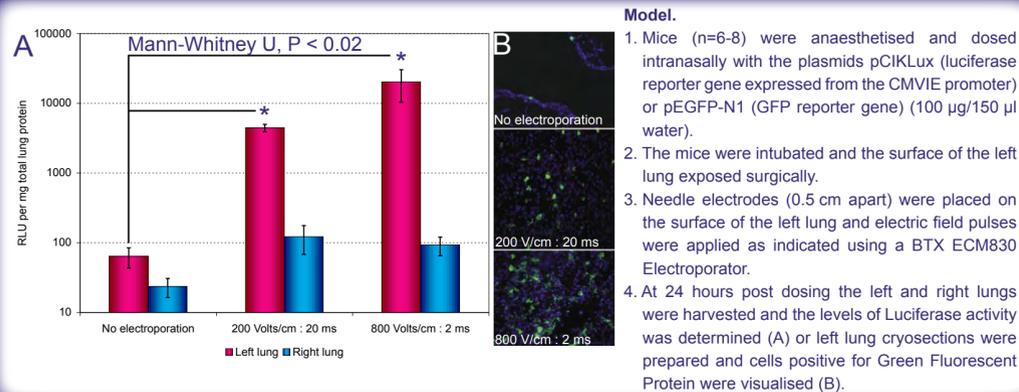
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## ► Overview of project.

- Existing non-viral gene therapy for CF lung disease is inefficient.
- Electroporation is an efficient method for increasing non-viral delivery.
- Lung access makes electroporation technically challenging.
- Electroporation enhances reporter gene expression in the mouse lung (Fig. 1).
- We studied the effect of electroporation on the duration of expression (Fig. 2).
- Wire electrodes were developed for electroporation in the sheep lung (Fig. 3).
- Electroporation increased Lux activity in the sheep lung (Fig. 4 & Fig. 5).

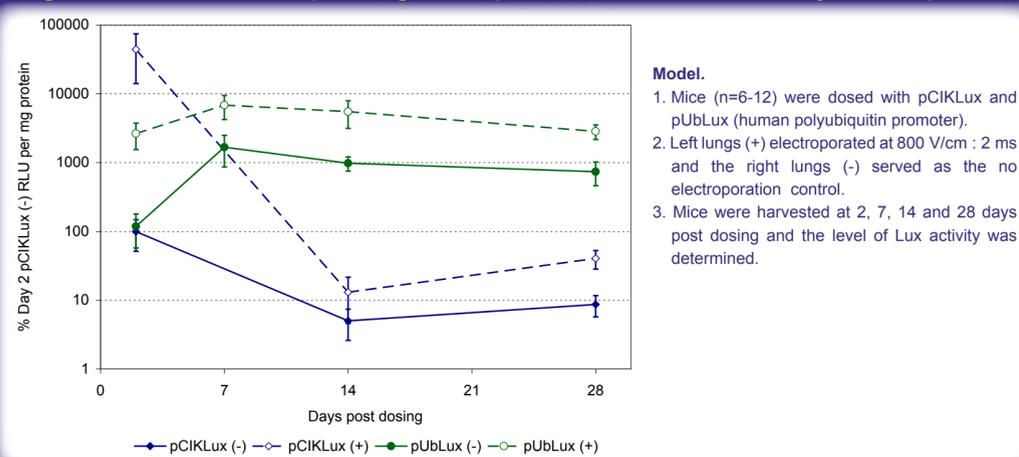
## ► Figure 1. Electroporation enhances reporter gene expression in the mouse lung.



## ► Results.

- Mean Lux activity (A) was increased by 100 and 500 fold compared to the no electroporation control when the left lungs were electroporated at 200 V/cm : 20 ms and 800 V/cm : 2 ms respectively.
- No increase in mean Lux activity (A) was observed in the right lungs at any condition.
- Many more cells were GFP+ following electroporation (B).
  - No electroporation: very few cells ever observed.
  - 200 V/cm : 20 ms: Localised patch of GFP+ cells observed.
  - 800 V/cm : 2 ms: Widespread GFP+ cells throughout section.

## ► Figure 2. Duration of reporter gene expression is not affected by electroporation.



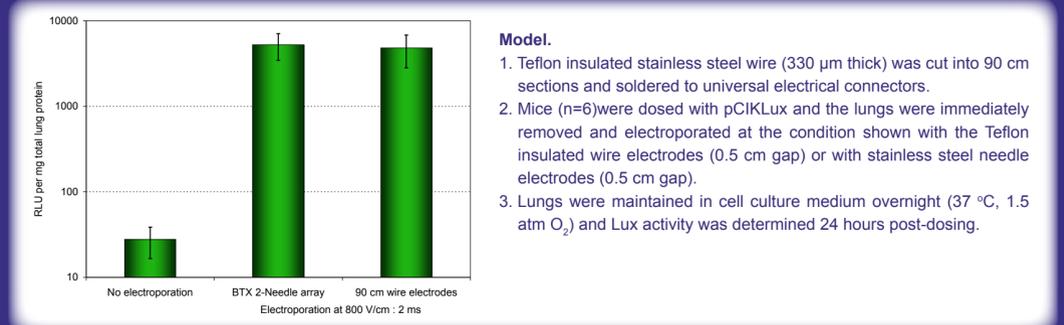
## ► Results.

- At day 2, Lux activity from electroporated (+) pCIKLux was 500 fold higher than the no electroporation (-) control, but by day 14 the activity from both conditions had fallen to background levels.
- The duration of expression from pCIKLux was not enhanced by electroporation.
- Electroporation increased the level of Lux activity from pUbLux at each time-point of the study. By 28 days post dosing, the Lux activity from pUbLux (+) was 30 fold higher than the pCIKLux (-) at 2 days post-dosing.
- While electroporation does not improve the limited duration of expression from the CMV promoter, when appropriate long duration constructs are used, activity may be maintained at elevated levels for at least 1 month.

## ► Conclusions from mouse studies.

- Electroporation increases Lux activity up to 500 fold.
- GFP positive cells were easily detected following electroporation.
- Electroporation increased Lux activity for 1 month in the mouse lung.

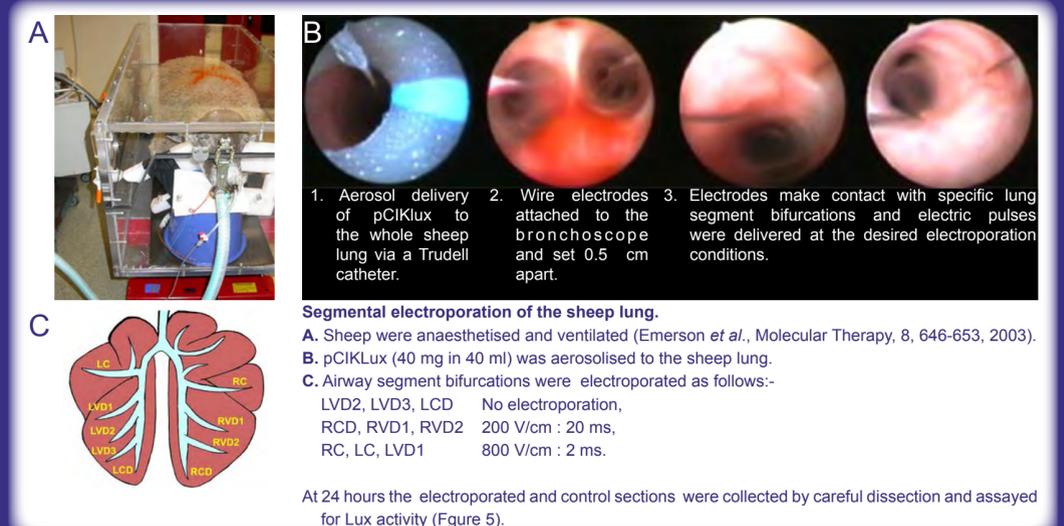
## ► Figure 3. Teflon insulated wires function as electroporation electrode devices.



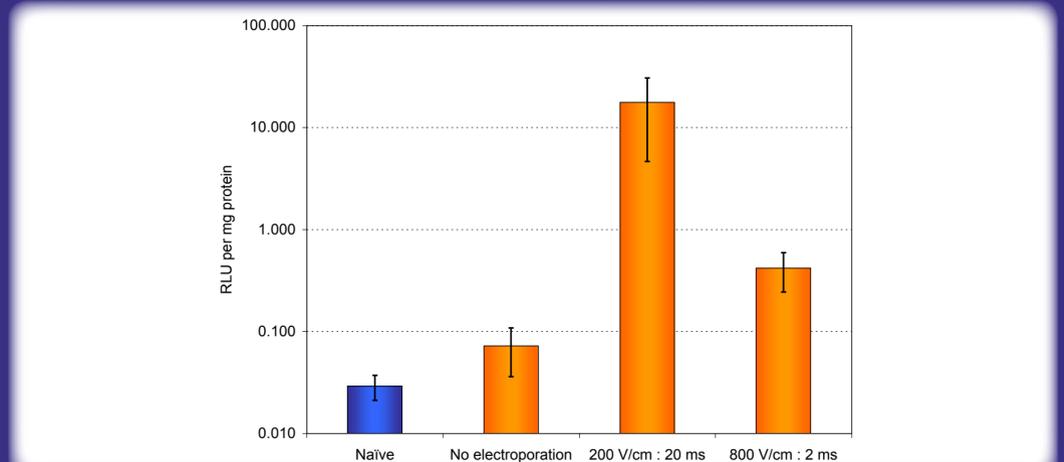
## ► Results.

- Both electrode designs resulted in approximately 200 fold higher levels of Lux activity compared to the no electroporation control.
- The Teflon insulated wires were easy to manoeuvre and safe to use.
- Wires could be used in conjunction with a bronchoscope to electroporate targeted areas of the lung.

## ► Figure 4. Electroporation of sheep lung segments with wire electrodes.



## ► Figure 5. Electroporation enhances reporter gene expression in the sheep lung.



## ► Results.

- The procedure was well tolerated by the sheep.
- Lux activity in the absence of electroporation was very low.
- Electroporation at 800 V/cm : 2 ms resulted in a modest 5 fold increase in Lux activity.
- Electroporation at 200 V/cm : 20 ms resulted in a 250 fold increase in Lux activity.

## ► Conclusions from sheep studies.

- Wire electrodes are safe and perform as well as needle electrodes.
- Electroporation increased Lux activity by 250 fold in the sheep lung.
- Successful translation of electroporation to a large animal model.