CpG DEPLETION RESULTS IN INCREASED DURATION OF GENE EXPRESSION FROM PLASMID DNA VECTORS *IN VIVO*

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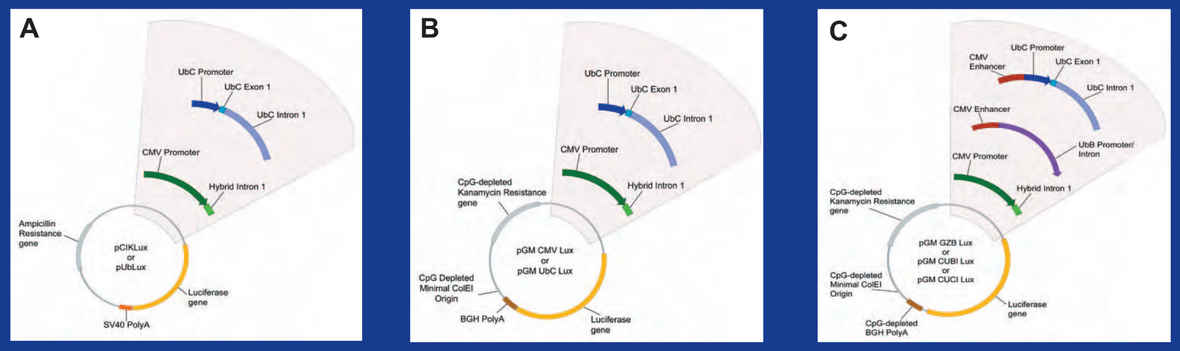
Introduction.

- Gene therapy is being developed as a treatment for Cystic Fibrosis.
- Treatments will likely require long-term gene expression and/or repeated administration.
- We are developing plasmid-based vectors which may be less immunogenic than viral vectors.
- CpG motifs in plasmids can cause a host inflammatory response when complexed with liposomes.
- The host imflammatory response may result in transient gene expression.
- We investigated the effects of CpG depletion on *in vivo* gene expression.

Aims.

- To investigate reporter gene expression from plasmids with a reduced CpG content.
- To test the persistence of gene expression following aerosol delivery.

Construction of Plasmid Vectors

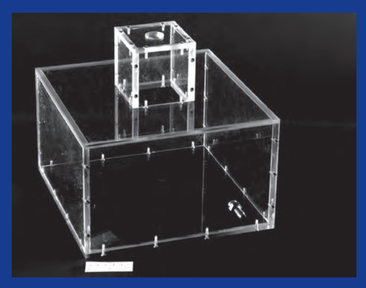


A: pCIKLux and pUbLux share a CpG-rich backbone and Luciferase gene. pCIKLux contains the CMV promoter, pUbLux contains the UbC promoter.
B: pGM CMV Lux and pGM UbC Lux share a CpG-depleted backbone (Kanamycin resistance gene, BGH PolyA, ColEI origin) and a CpG-rich Luciferase gene.
C: These plasmids share a CpG-depleted backbone. pGM GZB Lux contains a CpG-depleted CMV enhancer-promoter, pGM CUBI/CUCI Lux plasmids contain the CMV enhancer and UbB/UbC promoter respectively.

CpGs Per Plasmid

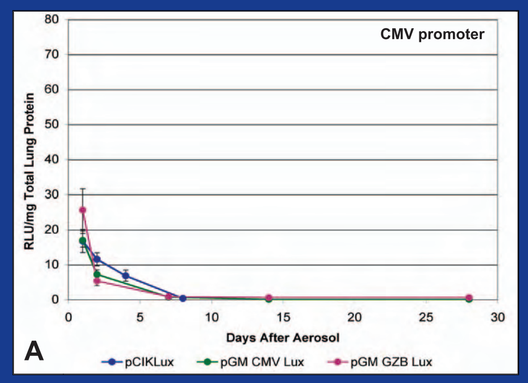
pCIKLux	pUbLux	pGM CMV Lux	pGM UbC Lux	pGM GZB Lux	pGM CUBI Lux	pGM CUCI Lux
317	369	193	245	146	231	262

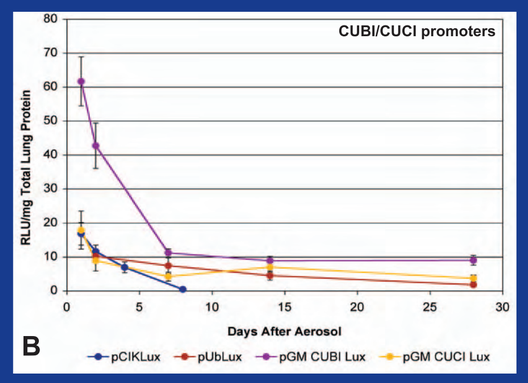
Aerosol Delivery of Plasmids to Mice

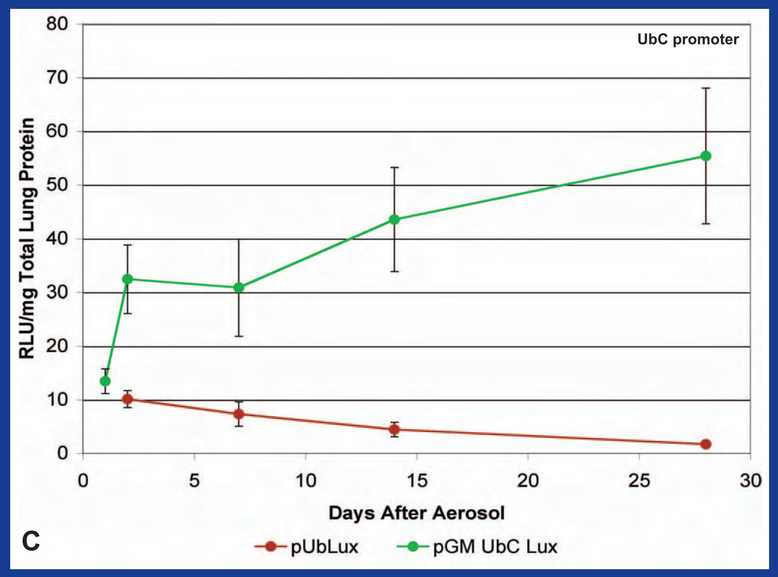


Plasmid DNA (2mg) was complexed with polyethyleneimine (PEI) at an N:P ratio of 10:1 in water for injection to give 10ml total dose per experiment. The aerosol was delivered to female BALB/c mice housed in in a polycarbonate box (left) using an Aerotech II nebuliser. Whole lungs were harvested and homogenised and the homogenates assayed for Luciferase expression at the timepoints shown.

Luciferase Expression in Whole Lung Lysates of Aerosol Dosed Mice







A: pGM CMV Lux and pGM GZB Lux give a similar expression profile to pCIKLux, peaking at Day 1 and dropping to background levels by Day 7.
B: pGM CUBI Lux and pGM CUCI Lux follow a similar expression profile to pUbLux, peaking at Day 1 then falling to a low level which persists to Day 28. pGM CUBI Lux generally gives increased levels of expression compared to pGM CUCI Lux.
C: Overall pGM UbC Lux generally gives increased levels and duration of expression compared to the other plasmids.

Conclusions.

- CpG depletion of the backbone does not have a significant effect on the level or duration of Luciferase expression from plasmids which contain the CMV Promoter.
- Incorporation of the CMV enhancer into a plasmid containing the UbB promoter to generate pGM CUBI Lux results in increased gene expression at Day 1 and expression which is still detectable at Day 28.
- For persistent high-level gene expression following aerosol delivery, a plasmid containing the UbC promoter in a CpG-depleted backbone is optimal.

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