Inflammation Free Human and Murine Promoters for Non-Viral CFTR Lung Gene Therapy

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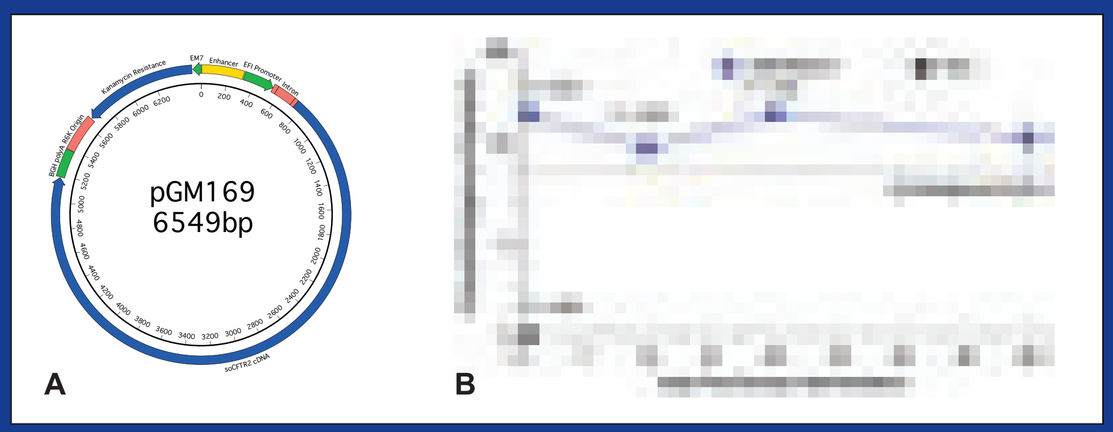
Introduction

The UK Cystic Fibrosis Gene Therapy Consortium are planning clinical studies with a non-viral gene transfer formulation consisting of a CFTR expression plasmid (pDNA) complexed with Genzyme lipid formulation GL67A. The pDNA/GL67A complexes will be delivered to the lungs and nose of subjects with CF by aerosol.

In order to minimise CpG-related inflammatory responses, the pDNA to be used is pGM169 (sometimes also termed pG4-hCEFI-soCFTR2), a CFTR expression pDNA that is devoid of any CG-dinucleotides (Figure 1A; Hyde *et al.*, 2008 *Nature Biotechnology* 26: 549).

Figure 1



A) pGM169 CpG-free CFTR expression vector contains the soCFTR2 cDNA under the transcriptional control of a CpG-free CMV enhancer/Elongation Factor 1-alpha promoter (termed hCEFI). B) Duration of pGM169/GL67A CFTR mRNA expression in the mouse lung.

Although pGM169 has demonstrated persistent high-level CFTR expression following aerosol delivery to the lungs of mice (Figure 1B) the limited number of CpG-free enhancers and promoters available severely restricted the design of these vectors.

Consequently, we have conducted a bioinformatics driven *in vivo* screening programme to identify novel CpG-free promoters.

Identification of Novel CpG-Free Promoters

To extend the available repertoire of CpG-free promoters we used the Eukaryotic Promoter Database (www.epd.isb-sib.ch) to identify human and mouse promoters that lacked CpGs in the region around their transcription start site (-49 to +10).

Subsequently, extended promoter sequences were obtained from Genbank and six promoters containing >500bp of CpG free sequence around their transcription start site were identified (Table 1). None of these promoter elements has, to our knowledge, been studied previously in the context of gene therapy.

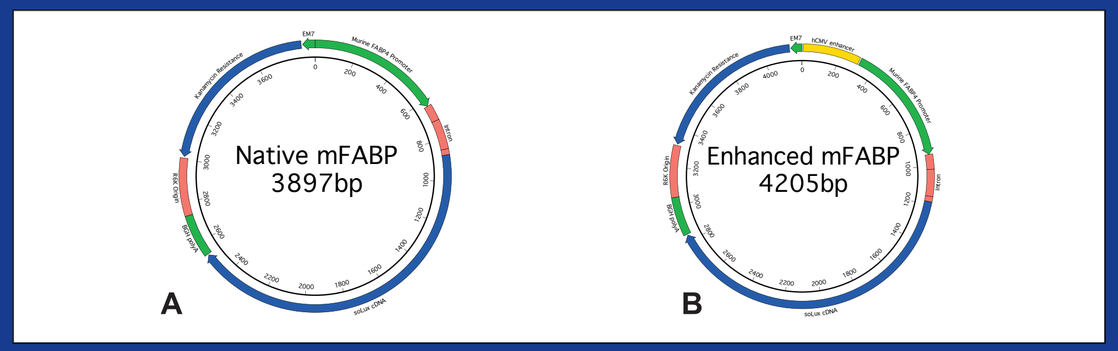
Table 1

Species	Gene	Abbr	-5' to +3' CpG-free
Human	Thyroid stimulating hormone-ß	hTSHB	-750 to +350
Human	Apolipoprotein A	hAPO2A	-513 to +26
Human	Carboxypeptidase B1	hCBOX	-630 to +16
Human	Regenerating islet-derived 1ß	hREG1B	-625 to +70
Human	Tryptophan 2,3 dioxygenase	hTDOX	-670 to +52
Mouse	Fatty acid binding protein 4	mFABP	-623 to +52

Construction Of Expression Vectors Harbouring Novel CpG-Free Promoters

Genomic BAC or PAC clones were obtained containing the promoter sequences listed in Table 1. The promoter sequences were amplified by PCR, cloned and sequenced to confirm their CpG-free status. Subsequently, the novel promoters were inserted into a CpG-free pDNA containing a luciferase (Lux) reporter gene (Native Plasmids, Figure 2A). The novel promoters were also inserted into a similar CpG-free pDNA that also contained the human CMV enhancer (Enhanced Plasmids, Figure 2B).

Figure 2



Examples of expression vectors harbouring novel CpG-free promoters

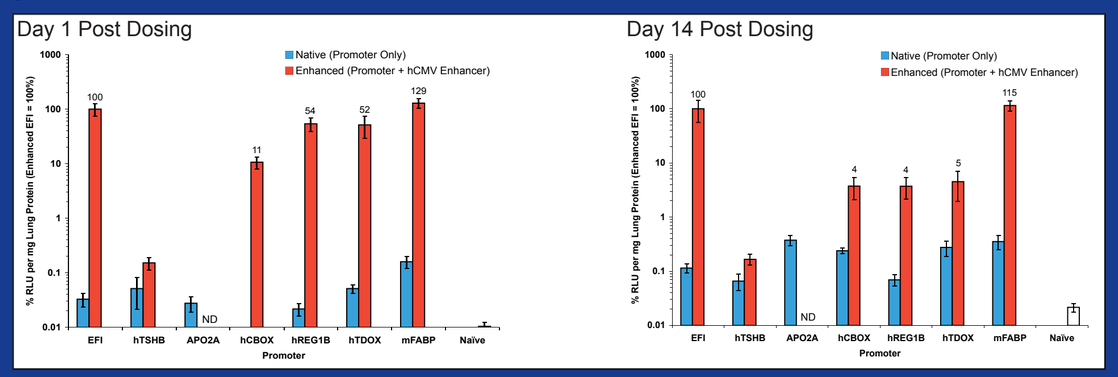
Lung Transgene Expression Directed By Novel CpG-Free Promoters

Lung expression from Native and Enhanced pDNAs complexed with the cationic lipid formulation GL67A was compared with expression from the hCEFI enhancer/promoter found in the plasmid pGM169 (Figure 3).

Native promoters at both d1 and d14 performed poorly, with Lux activity substantially lower than those observed with hCEFI ($P<0.001$), at levels only slightly higher than naïve animals.

Transgene expression mediated by Enhanced hREG1B, hTDOX and mFABP4 promoters was similar to hCEFI ($P>0.05$). In particular, the Enhanced mFABP4 promoter performed well, generating lung reporter gene activity of 129% and 115% hCEFI levels at d1 and d14 respectively.

Figure 3



CONCLUSIONS

- CpG-free promoters exist in the mouse and human genomes.
- Six CpG-free promoters were isolated and utilised to construct novel CpG-free expression pDNAs.
- The native CpG-free promoters directed low levels of lung transgene expression.
- Addition of a CpG-free CMV enhancer, significantly increased lung transgene expression directed by four of the CpG-free promoters.
- The CMV enhancer/mFABP promoter directed sustained high-level lung transgene expression.

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