

# Zero CpG plasmids eliminate the inflammatory response associated with lung non-viral gene transfer

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<http://www.cfgenetherapy.org.uk>



## ▶ Introduction



- ▶ **Cystic Fibrosis:-**
  - ▶ **Common fatal monogenic disorder**
  - ▶ **Disease affects many organs**
  - ▶ **Chronic lung disease is the primary cause of mortality**
  
- ▶ **Multiple phase I clinical trials have been conducted**
  - ▶ **Repeat administration is required**
  - ▶ **Viral vectors - Lack of successful repeat administration**
  - ▶ **Non-viral - Limited duration of expression**
  
- ▶ **UK CF Gene Therapy Consortium**
  - ▶ **Genzyme lipid 67 (GL67) is our lead candidate for lung trials**

## ▶ GL67/pDNA Lung Trial

- ▶ GL67/plasmid DNA aerosol delivery to the lungs
  - ▶ 25 % correction of CF ion transport defect
  - ▶ Mild 'flu-like' symptoms and inflammation
- ▶ Inflammation attributed to CG dinucleotides (CpGs) in plasmid
- ▶ Research hypothesis/challenge:-
  1. Reduction of CpGs in plasmid will reduce inflammation
  2. Eliminate CpGs without compromising expression

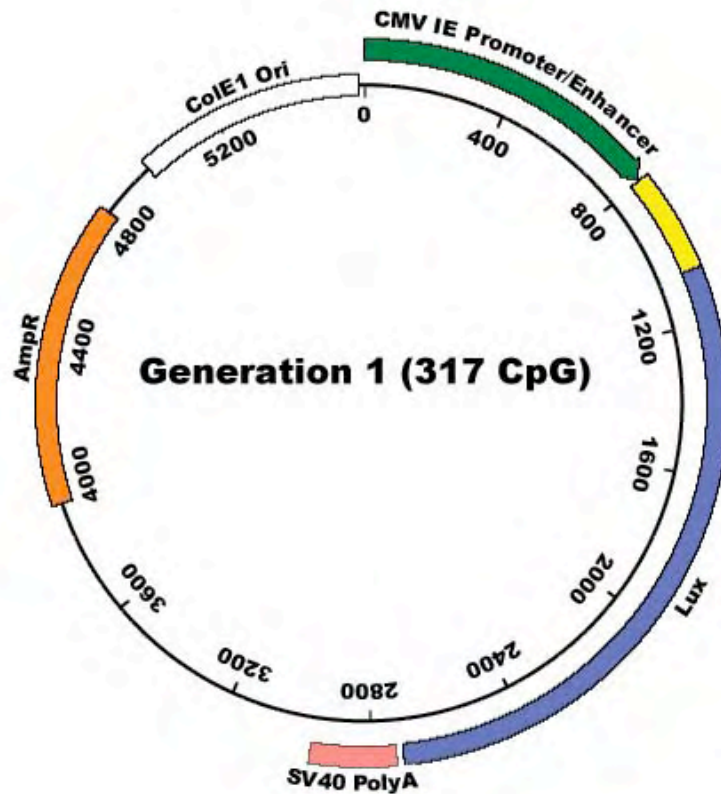
**Alton *et al.*, 1999. Lancet, 353, 947-953.**

**Cationic lipid-mediated CFTR gene transfer to the lungs and nose of patients with Cystic Fibrosis: a double-blind placebo controlled trial.**

## ▶ Mouse Lung Model

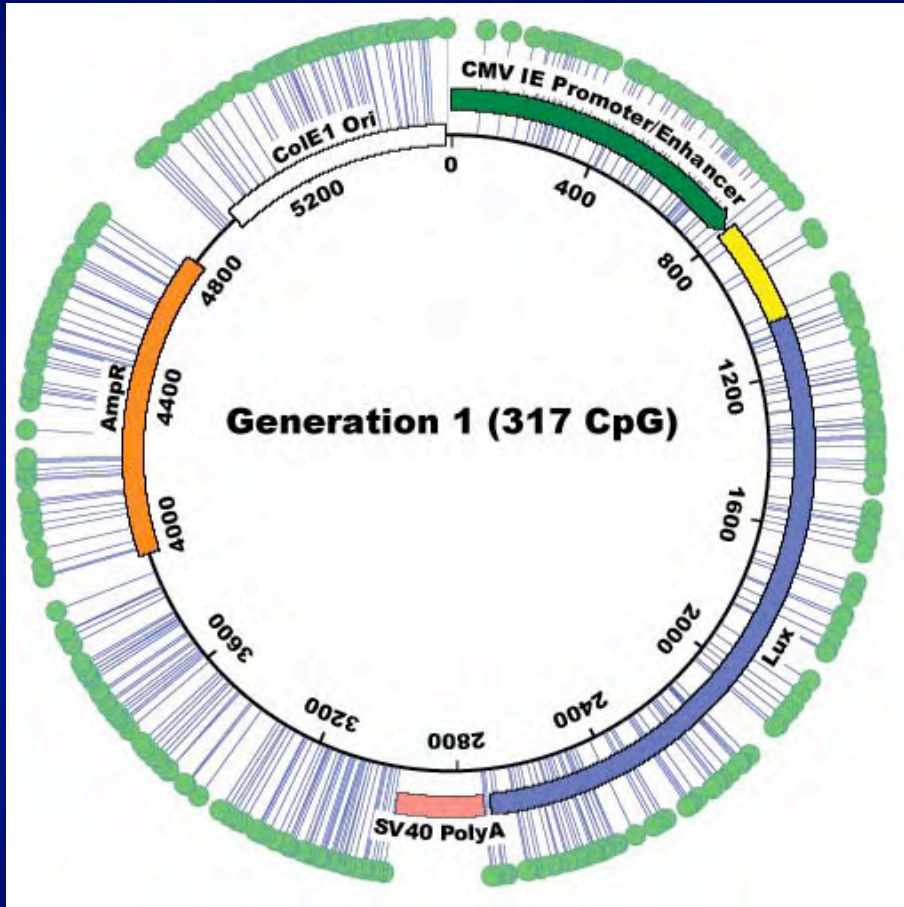
1. Produced plasmids with varying CpG content
2. Plasmid DNA complexed with GL67
3. Lung instillation to BALB/c (n=10) (100  $\mu$ l/80  $\mu$ g)
4. Harvest lungs at 24 hours post-dosing
  - ▶ Reporter gene expression
5. Inflammatory markers in bronchoalveolar lavage fluid (BALF)
  - ▶ Total cells per ml BALF (predominantly neutrophils)
  - ▶ Inflammatory cytokines IFN- $\gamma$ , IL-12 & TNF- $\alpha$

## ▶ Reducing CpGs to Reduce Inflammation?



**Backbone** - Similar To That Used  
In Multiple Phase I Trials In Mid 1990's

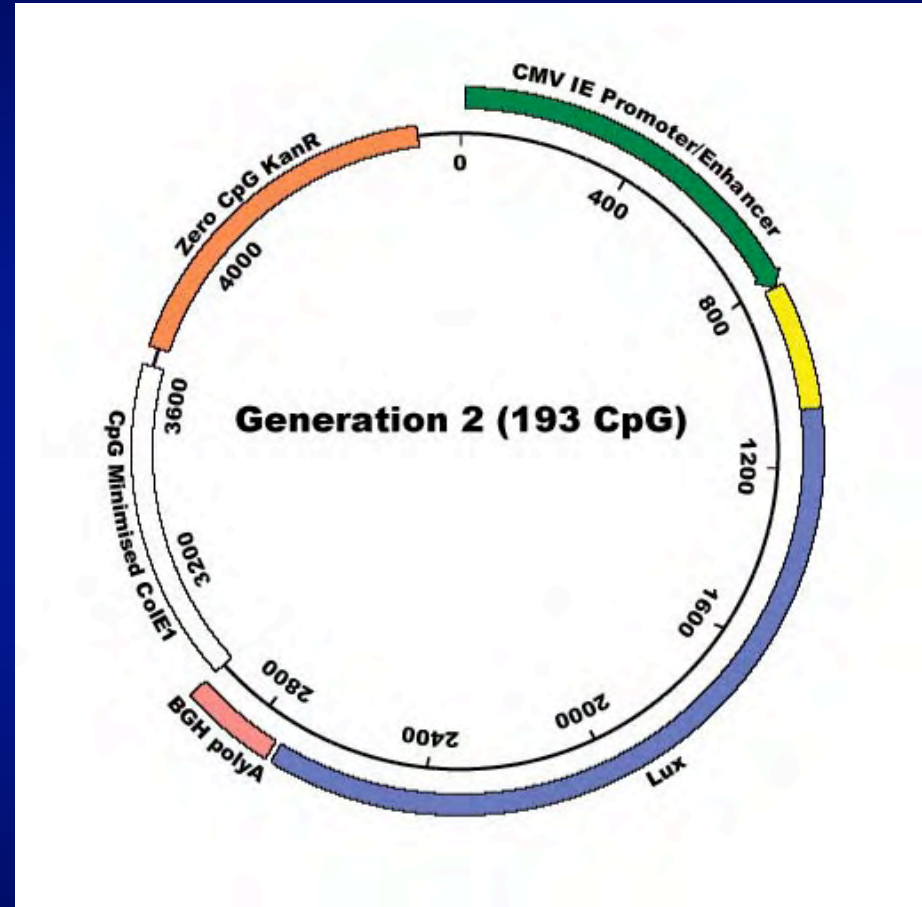
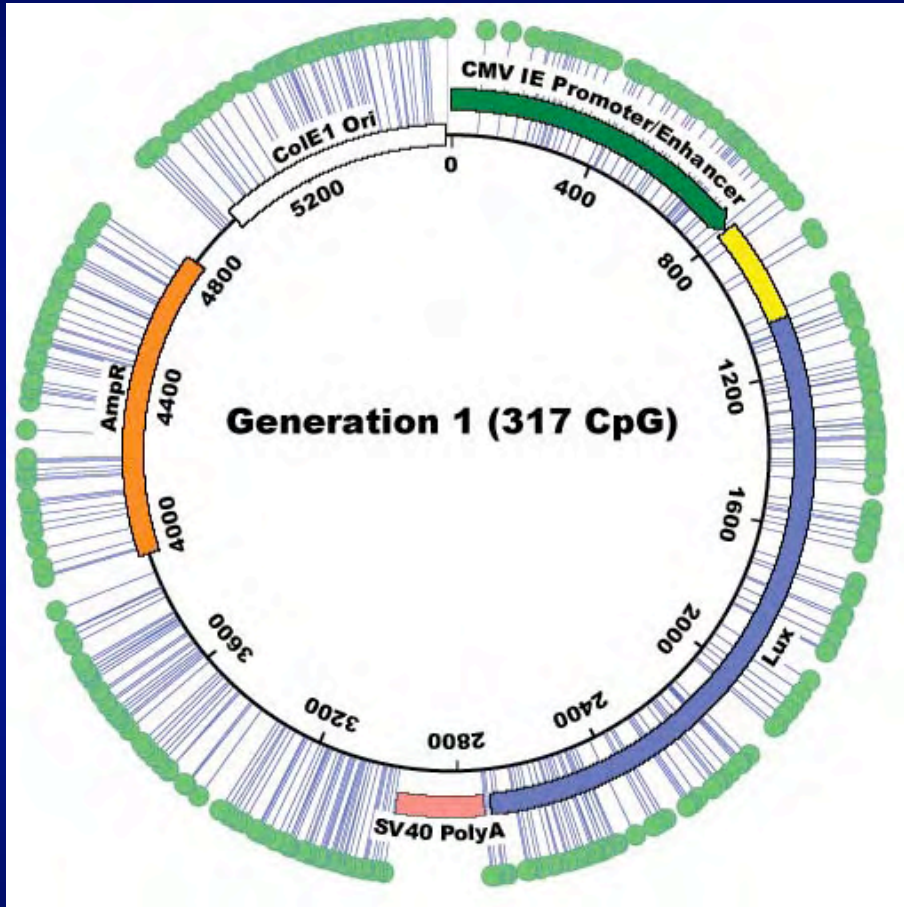
# ▶ Reducing CpGs to Reduce Inflammation?



 = 1 CpG Motif

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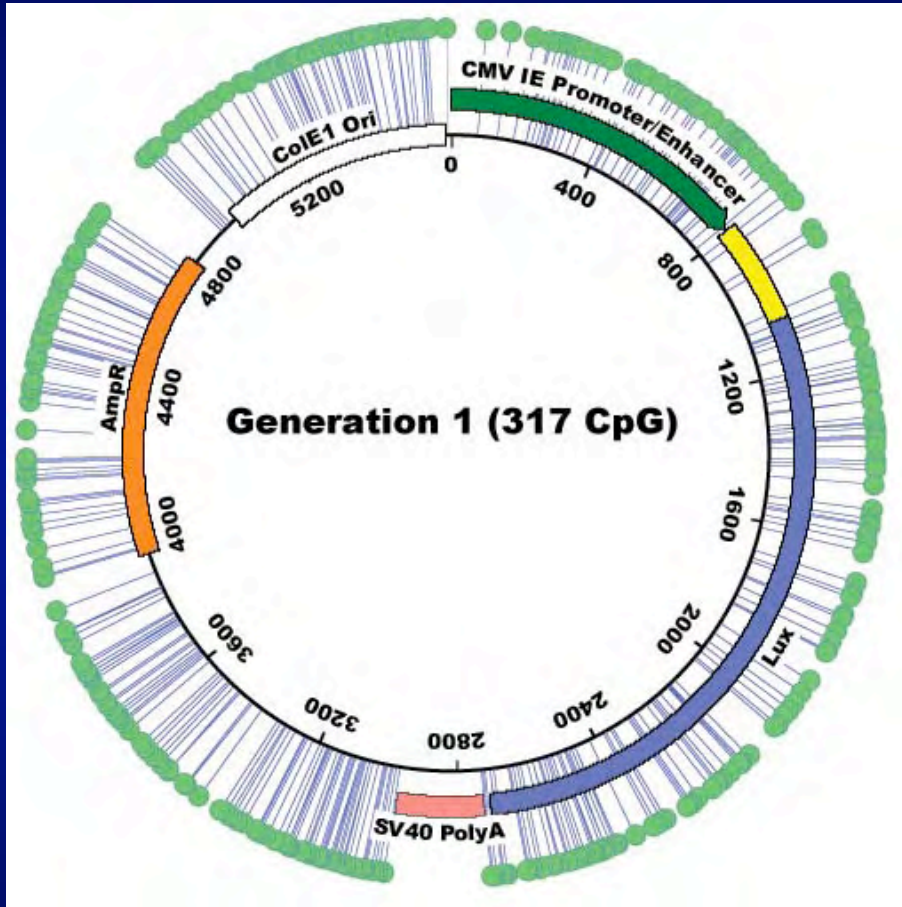


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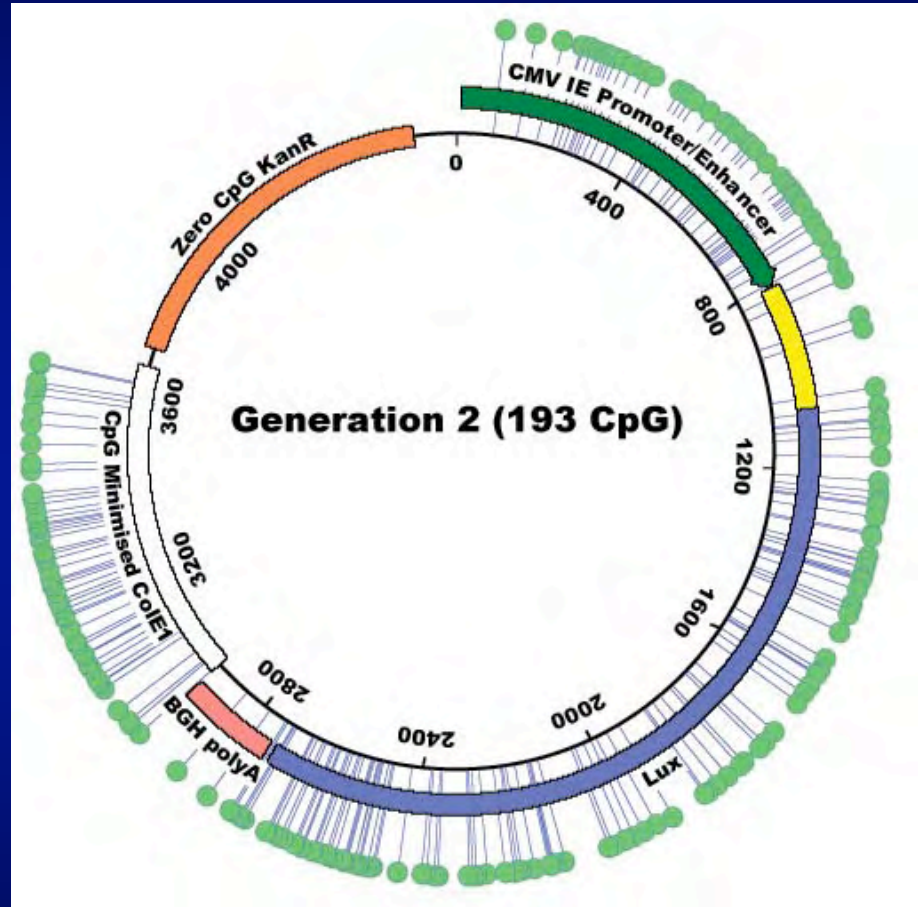
**Backbone** - Reduced CpG Content  
Overall **40 %** fewer CpGs



# ▶ Reducing CpGs to Reduce Inflammation?



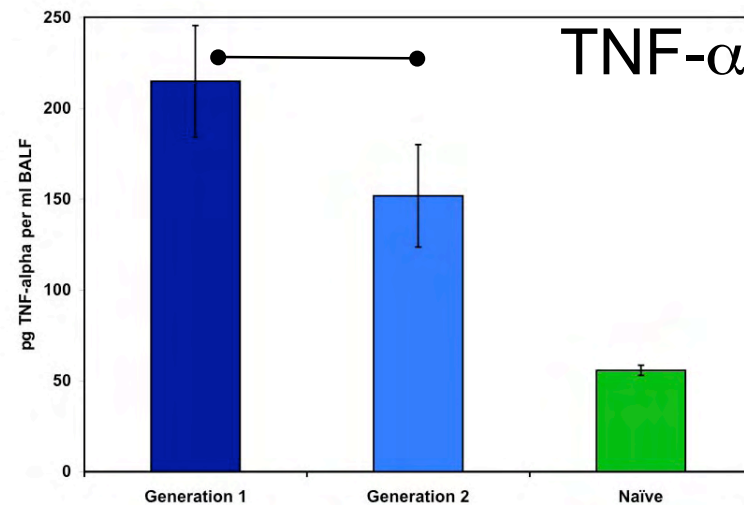
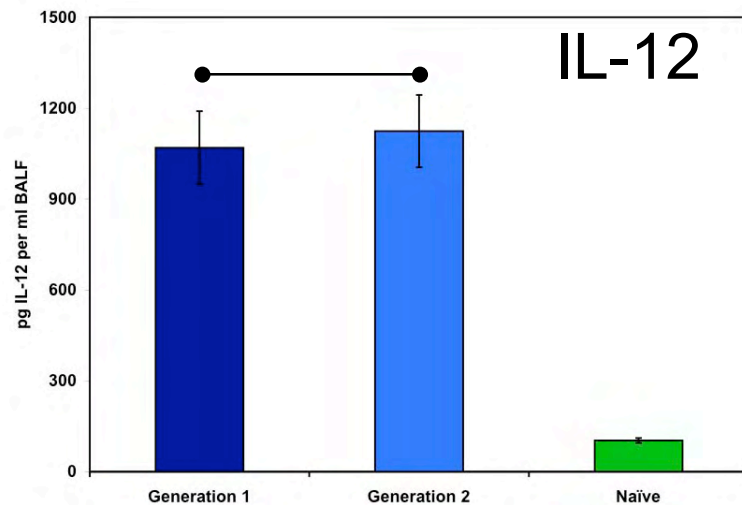
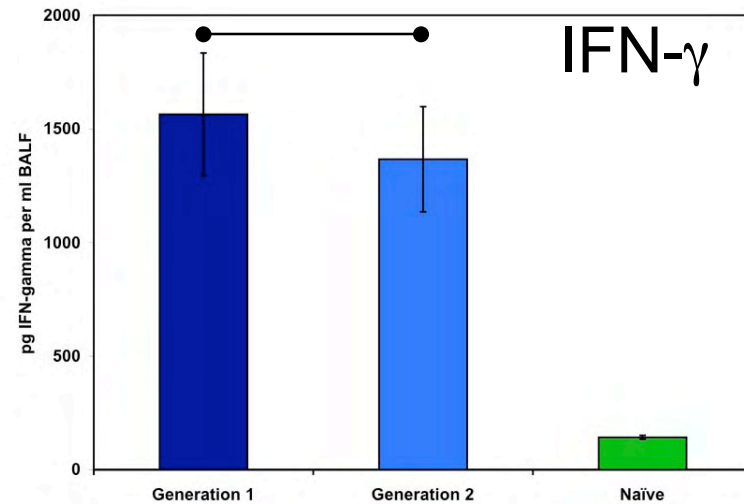
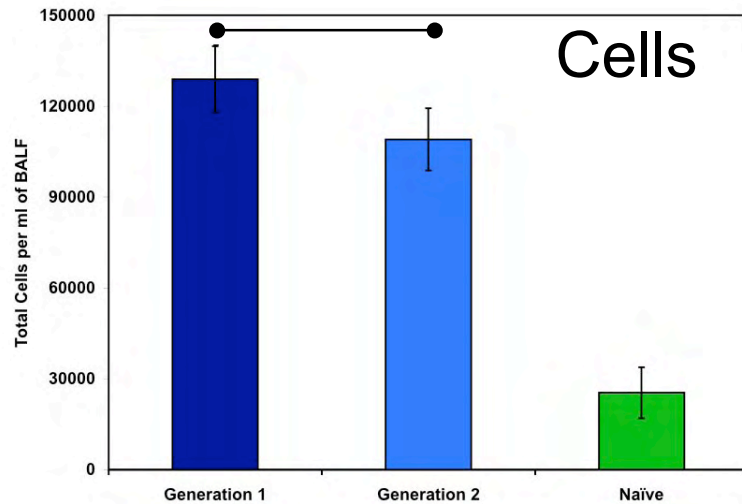
**Backbone** - Similar To That Used In Multiple Phase I Trials In Mid 1990's



**Backbone** - Reduced CpG Content Overall **40 %** fewer CpGs



# ► Inflammation from Generation 2 Plasmids

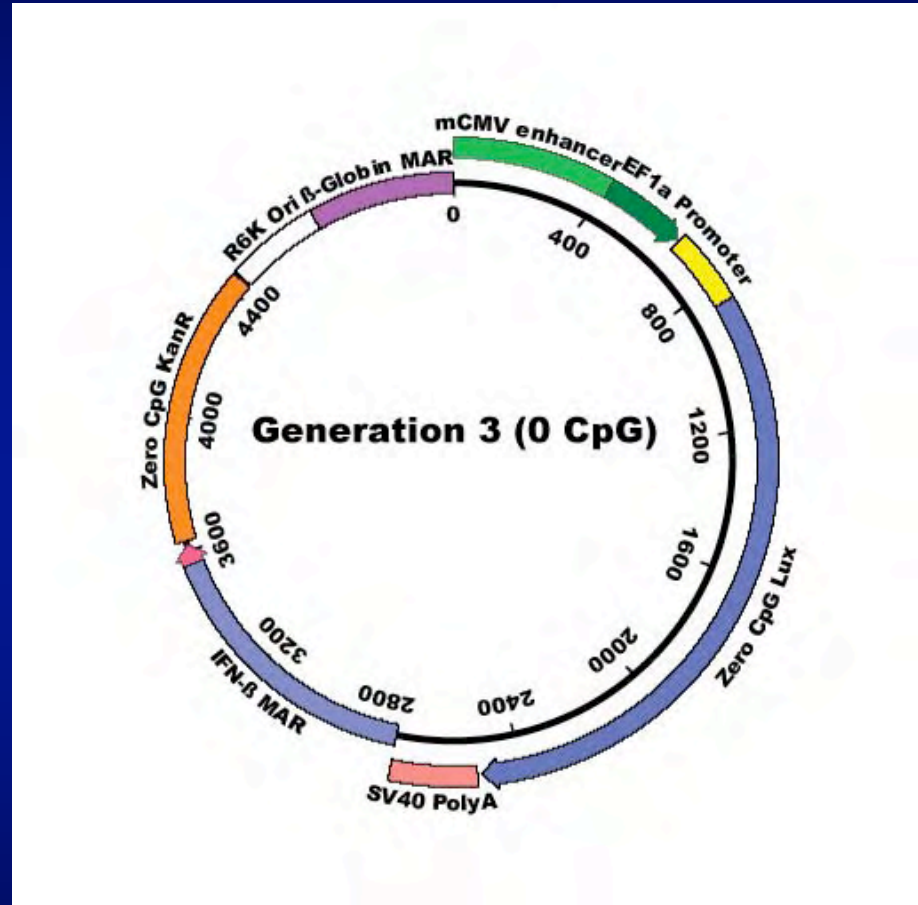
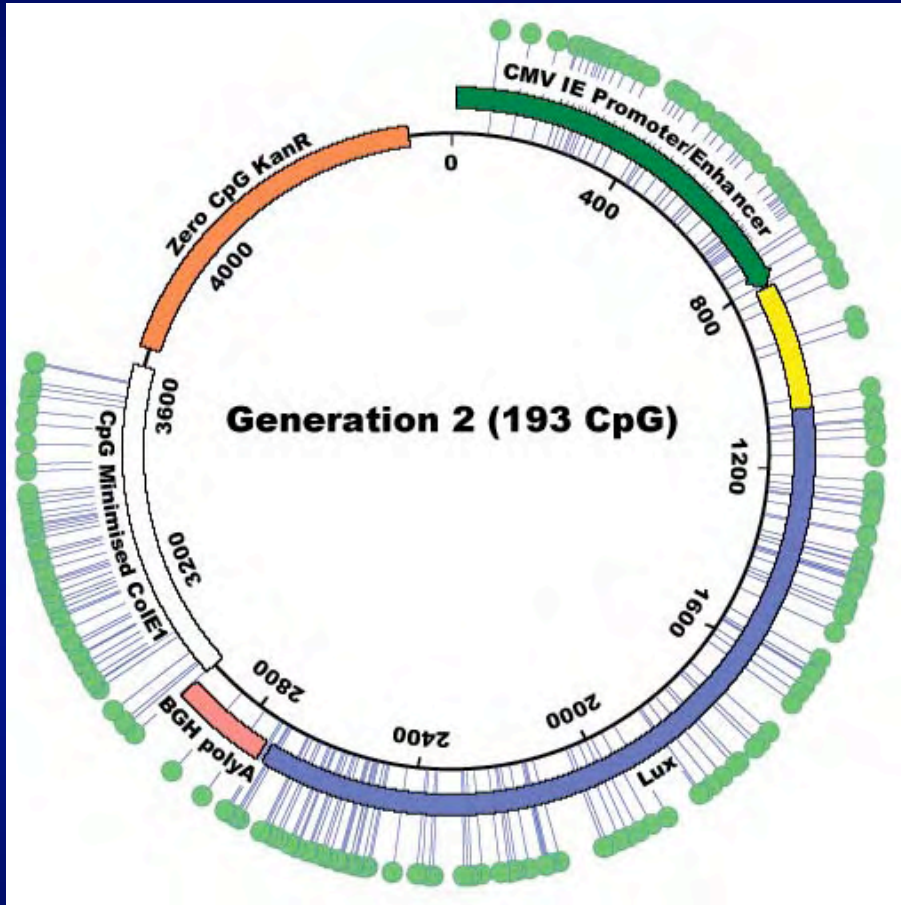


BALB/c  
n=10  
GL67  
Instillation

Mann  
Whitney  
P>0.05

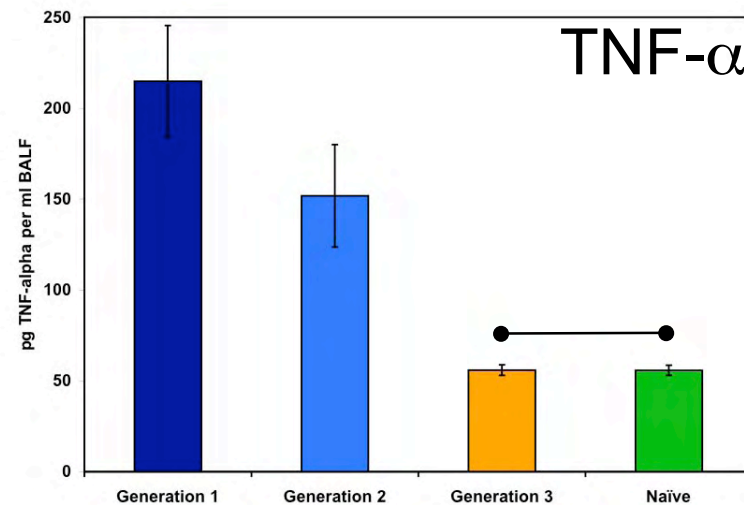
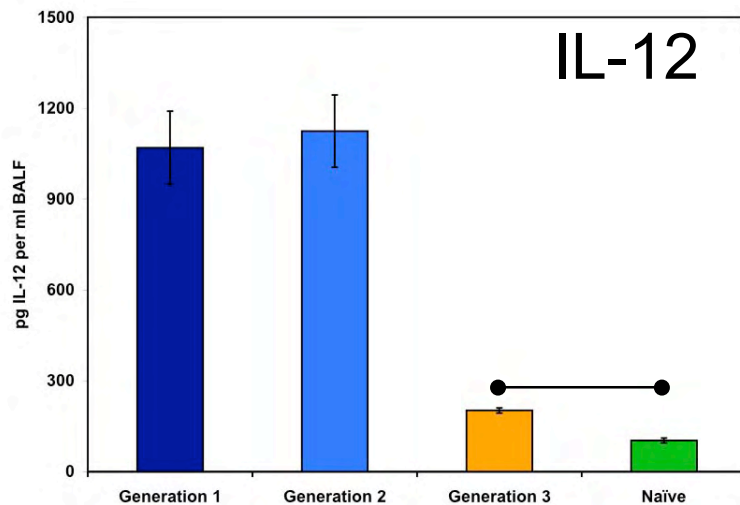
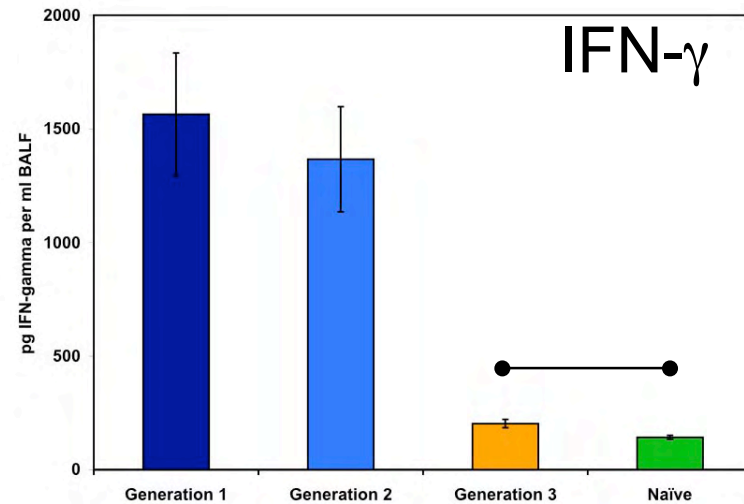
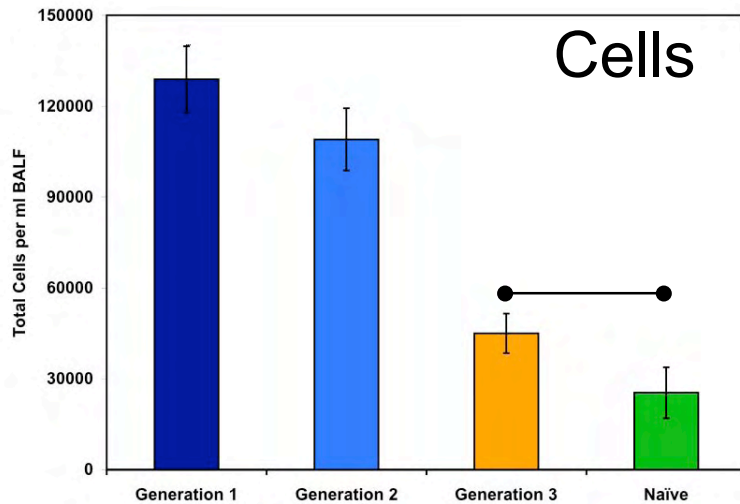
► 40 % reduction of CpGs had no effect on inflammation.

# ▶ Develop Zero CpG Generation 3 Plasmids



- ▶ R6K origin
- ▶ Extensive codon alteration
- ▶ Zero CpG promoters

# ► Effect of Zero CpG on Inflammation

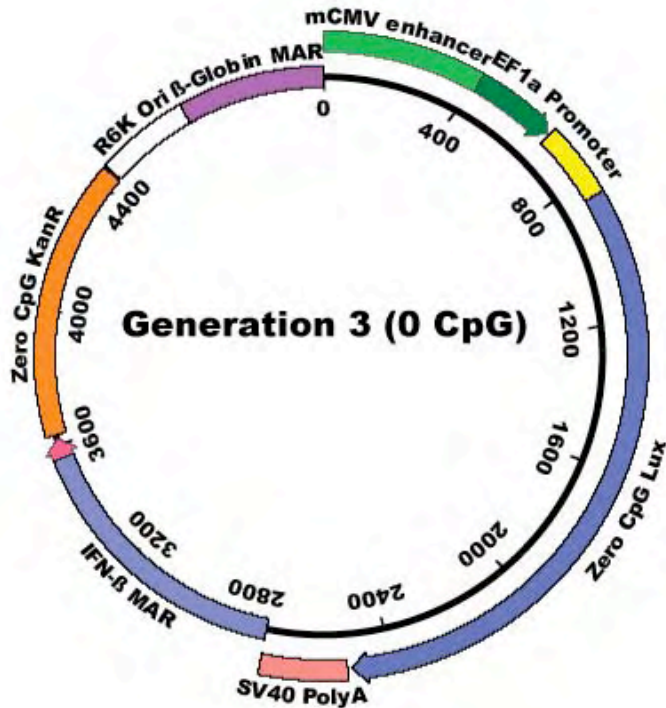


BALB/c  
n=10  
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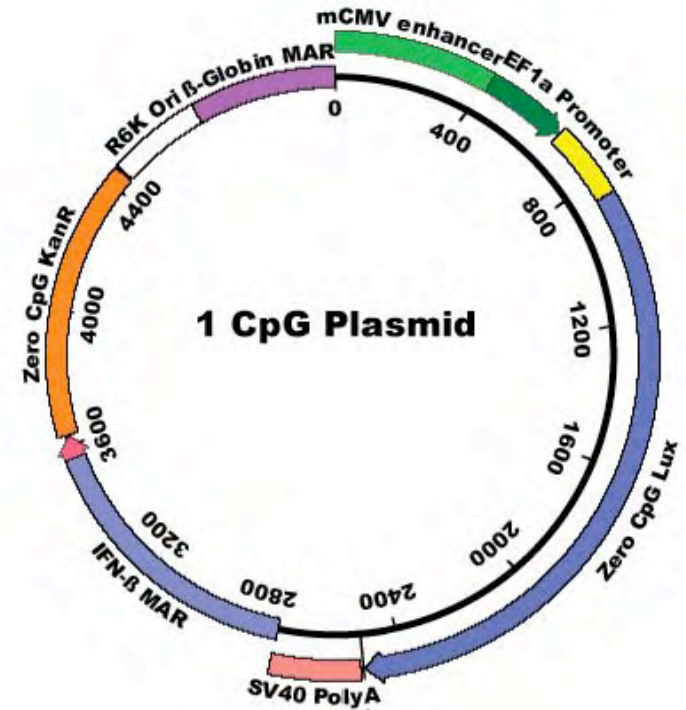
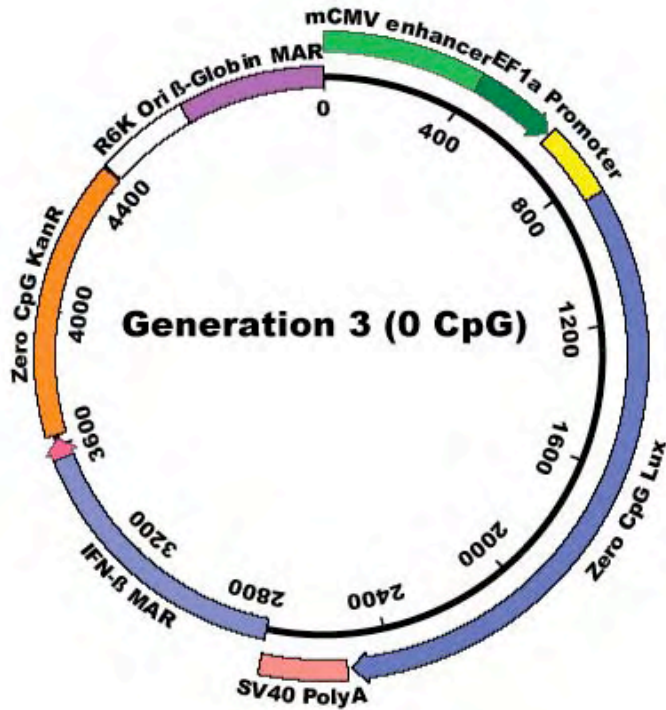
► Inflammation reduced to background levels by zero CpG plasmids

## ▶ Do we really need Zero CpGs?



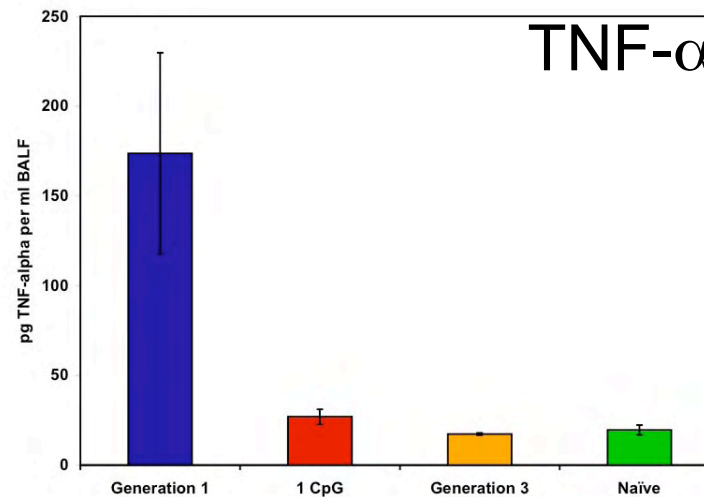
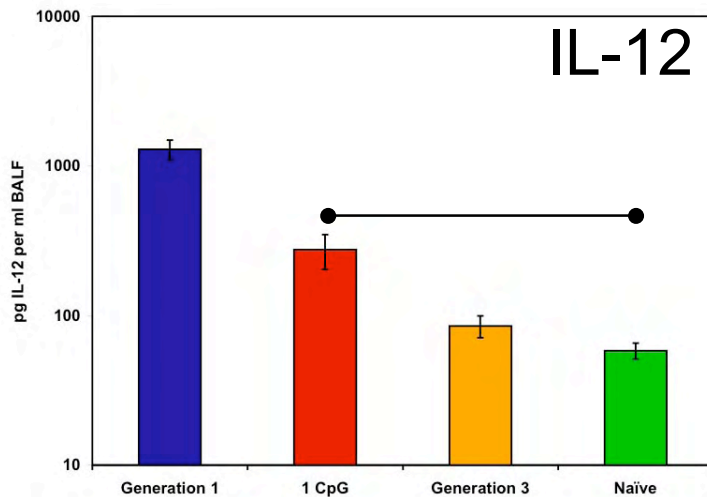
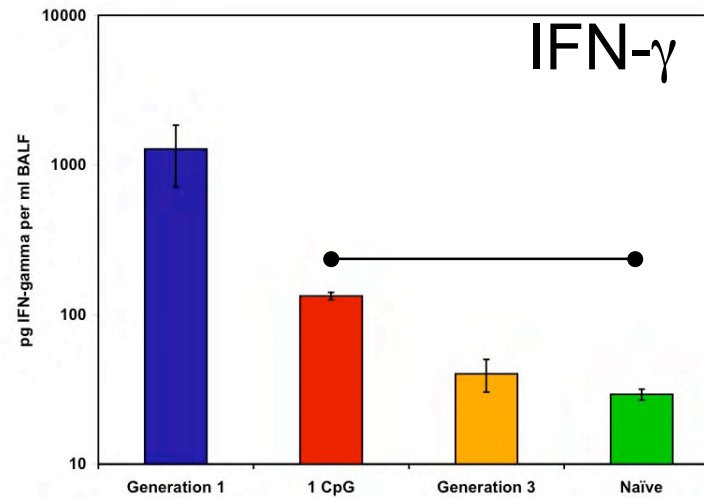
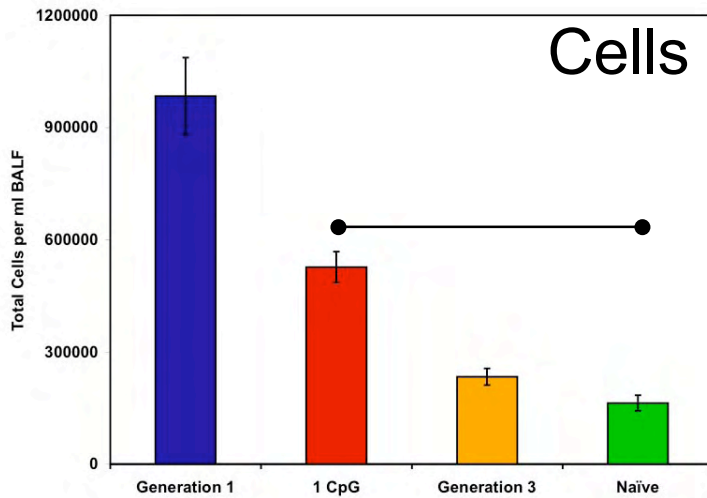
- ▶ Limits choice of promoter
- ▶ All genes have to be remade
- ▶ Less options for *E. coli* strain

# ▶ Do we really need zero CpGs?





# ▶ Effect of Single CpG Motif in Mouse Lung



BALB/c  
n=10  
GL67  
Instillation

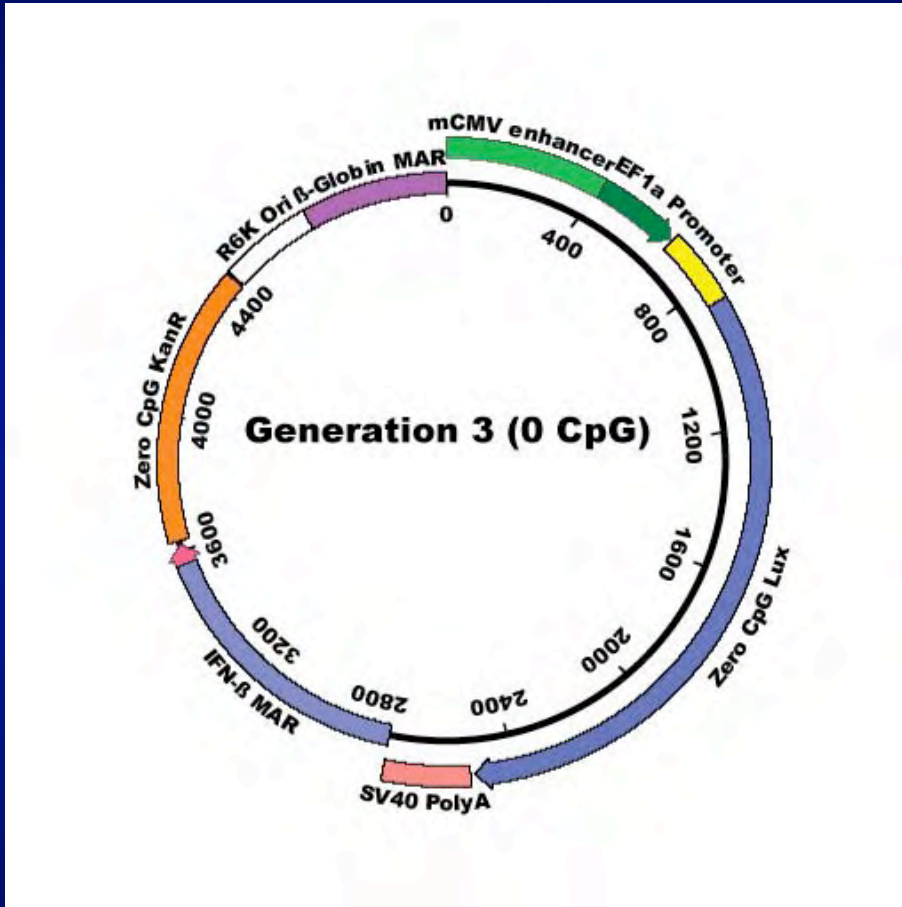
Mann  
Whitney  
P<0.05

▶ 1 CpG sufficient to induce an inflammatory response in mouse lung

## ▶ 1 CpG Causes inflammation

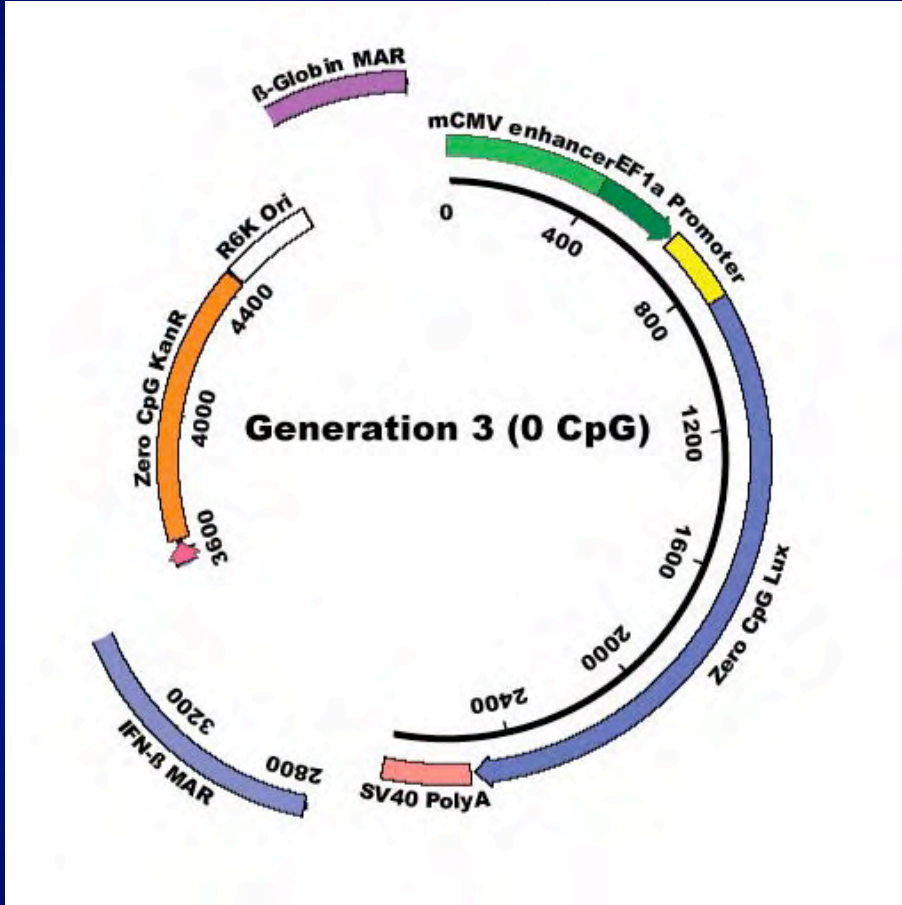
- ▶ Generation 1 Plasmid =  $8.0 \times 10^{13}$  CpG/100  $\mu$ g
  - ▶ Generation 2 Plasmid =  $4.0 \times 10^{13}$  CpG/100  $\mu$ g
  - ▶ 1 CpG Plasmid =  $2.5 \times 10^{11}$  CpG/100  $\mu$ g
- 
- ▶ Can we use zero CpG plasmids in the clinic?
  - ▶ Will zero CpG compromise expression?

# ▶ Develop Zero CpG Plasmids for the Clinic



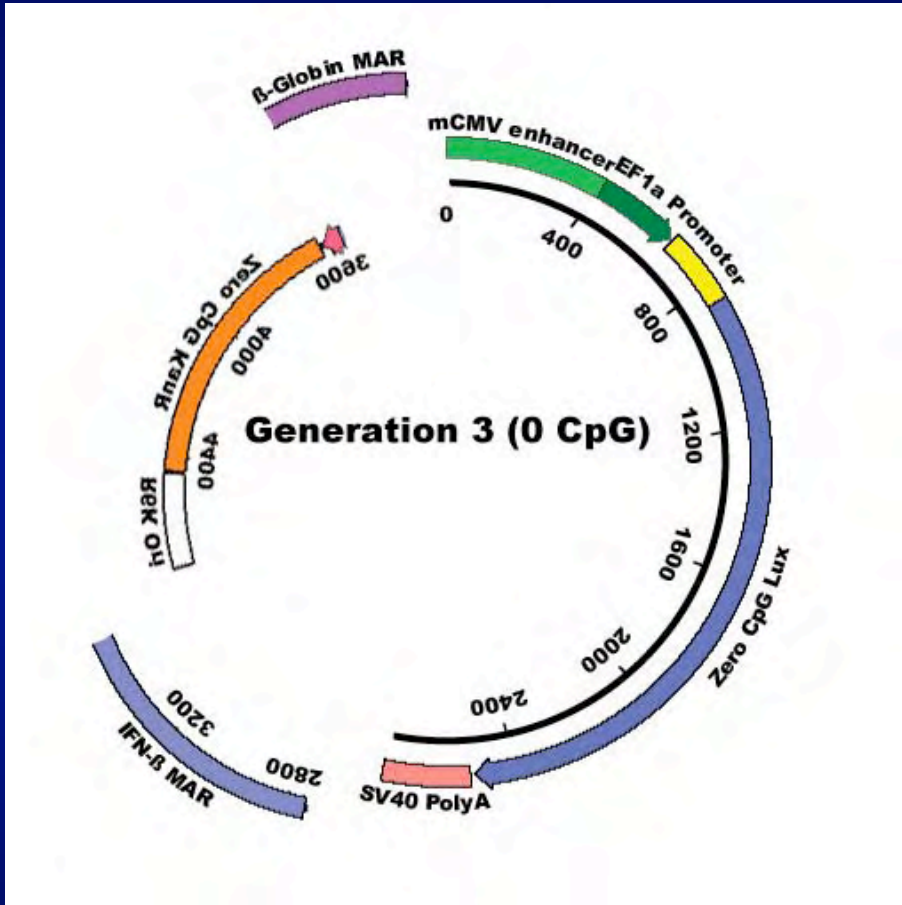
- ▶ Not suitable for clinical trials
  - ▶ Removed unnecessary DNA
  - ▶ Inverted the backbone

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## ▶ Develop Zero CpG Plasmids for the Clinic

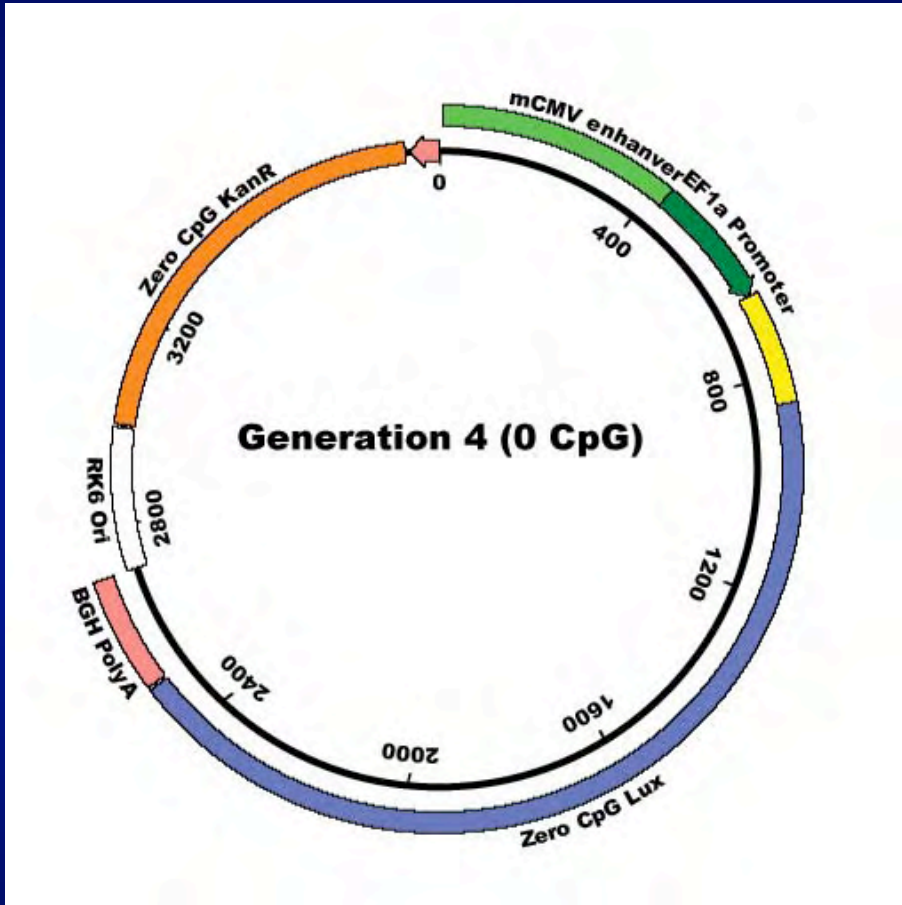


- ▶ Not suitable for clinical trials
  - ▶ Removed unnecessary DNA
  - ▶ Inverted the backbone

US FDA Docket No 96N-0400. Points to consider on Plasmid DNA Vaccines for Preventative Infectious Disease Indications

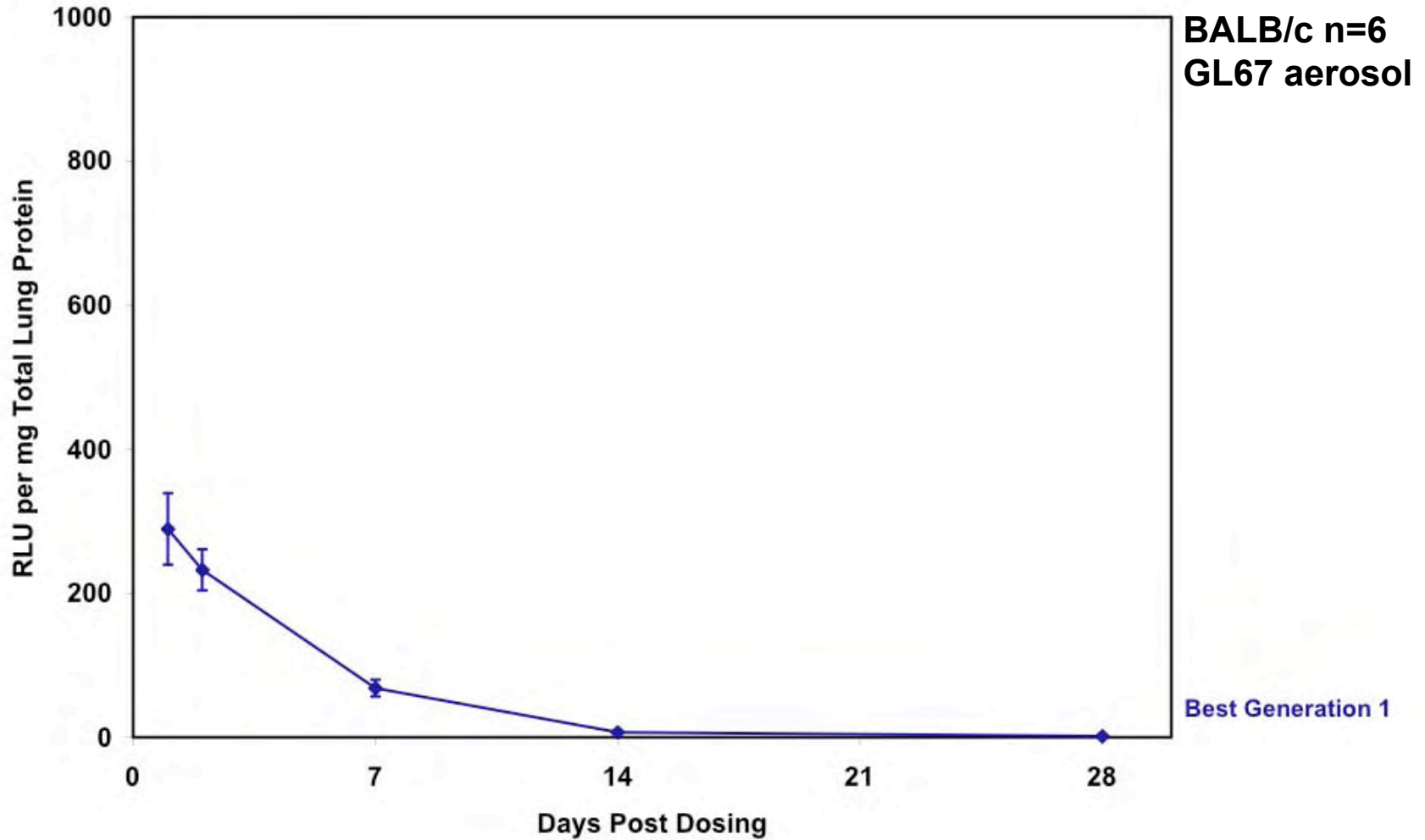


# ▶ Generation 4 Clinical Plasmids

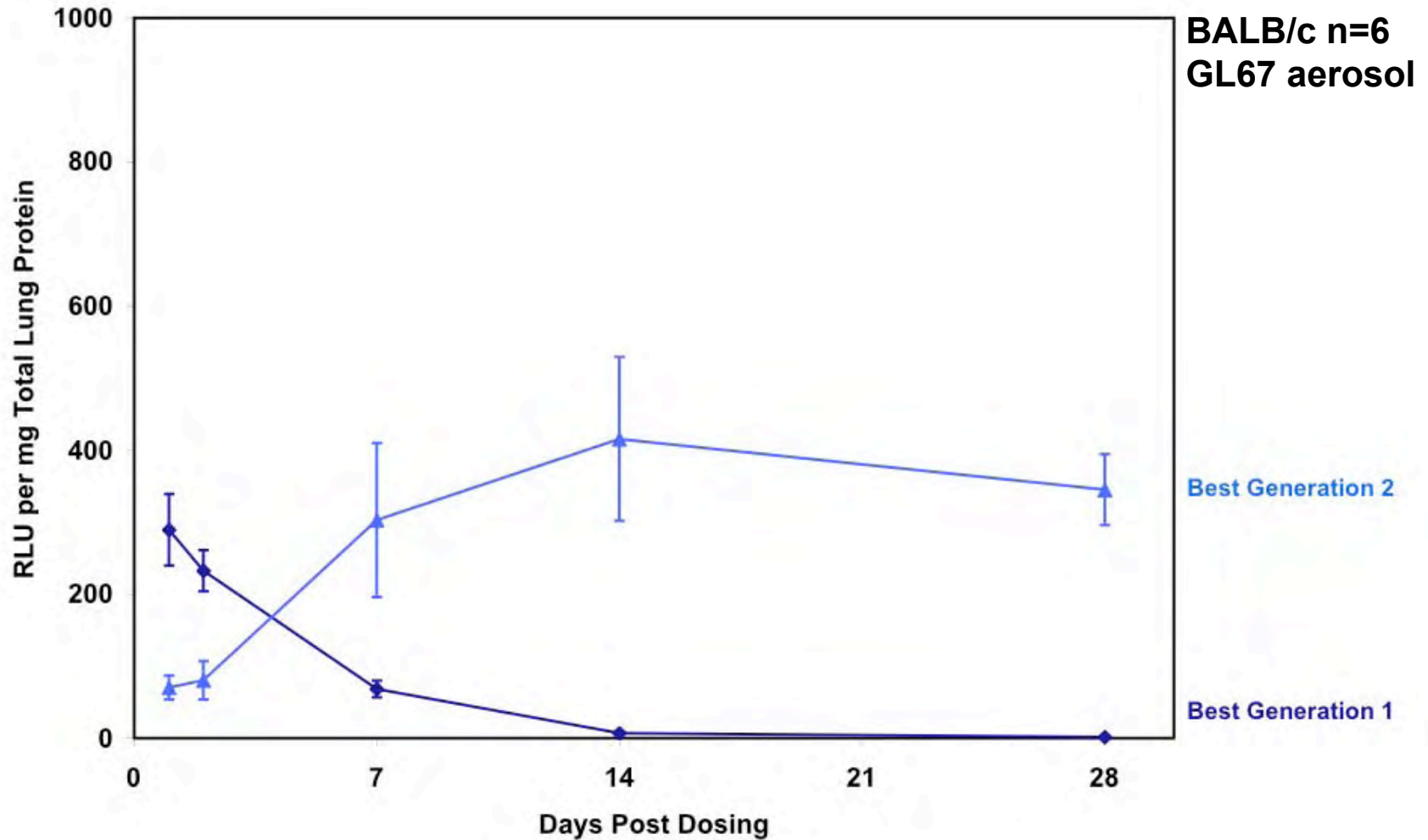


- ▶ Minimal backbone sequence
- ▶ High yields still possible
- ▶ Expression in **aerosol** studies
- ▶ Range of different promoters
  - ▶ EFI
  - ▶ mCEFI
  - ▶ hCEFI
  - ▶ GZB

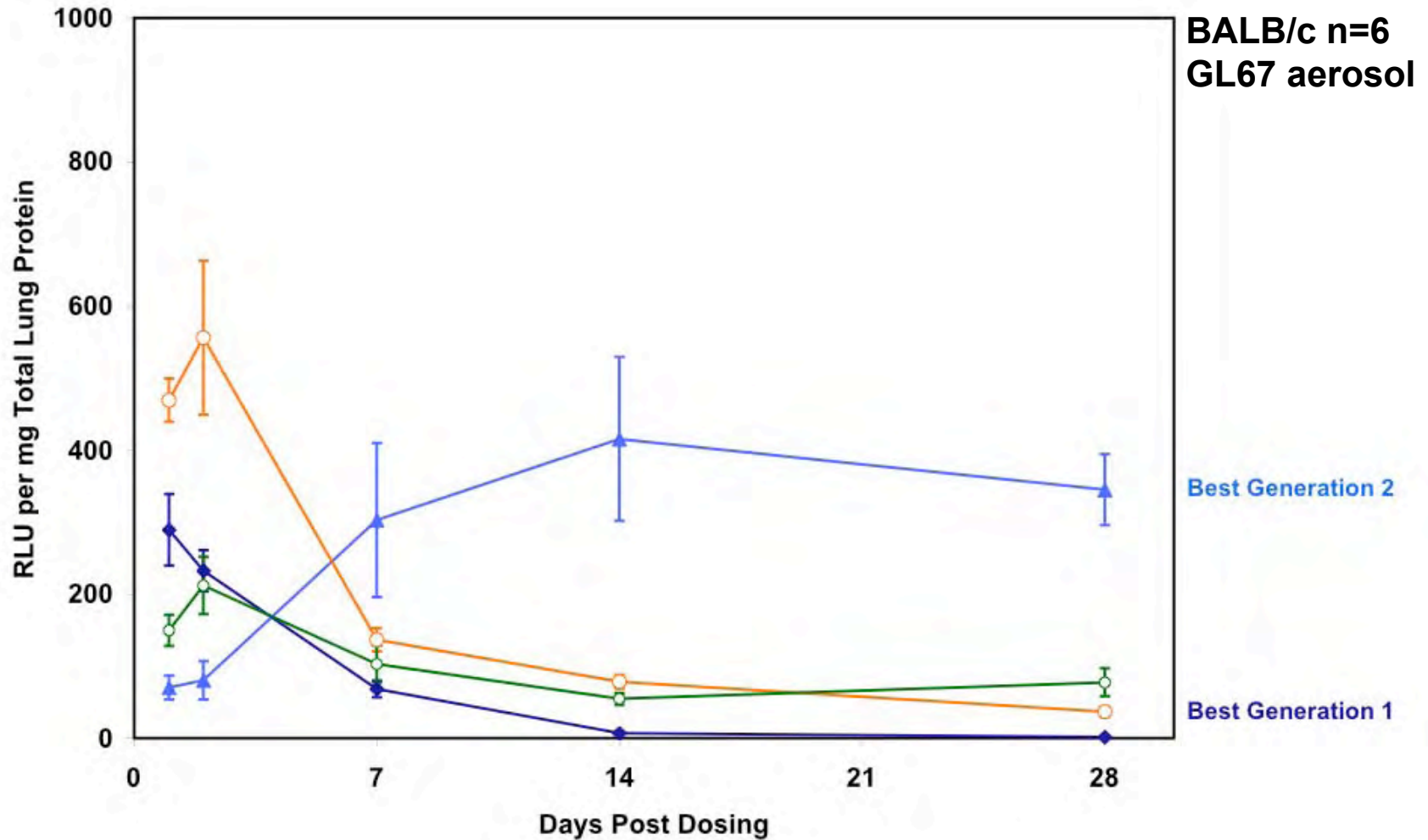
# ► Expression from Best Generation 1 Plasmid



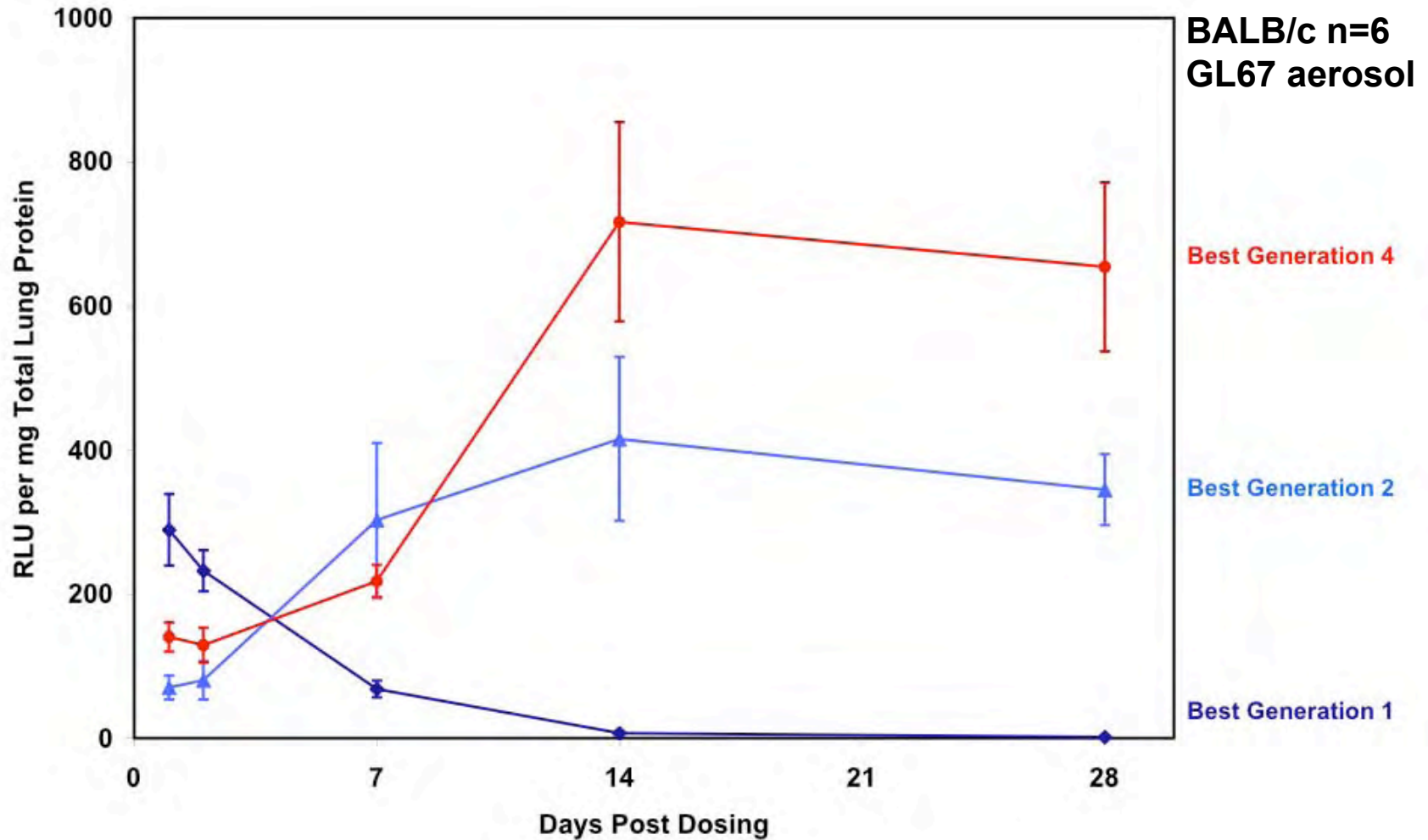
# ► Expression from Best Generation 2 Plasmid



# ► Expression from Generation 4 Plasmids



# ► Expression from Best Generation 4 Plasmid





## ▶ Summary & Future Work

- ▶ Developed plasmids with zero CpG motifs
- ▶ Eliminated CpG response to GL67/pDNA in mouse lung
- ▶ Improve level and duration of gene expression
  
- ▶ Planned clinical studies in CF patients
  - ▶ Single dose pilot study Spring 2007
  - ▶ Multi-dose study 2008

**Choice of plasmid has major impact of on overall performance of non-viral vectors**

## ▶ Acknowledgements

### ▶ Oxford University, UKCFGTC

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