The Effect of Plasmid DNA Quality on Gene Transfer Outcome *in vivo*

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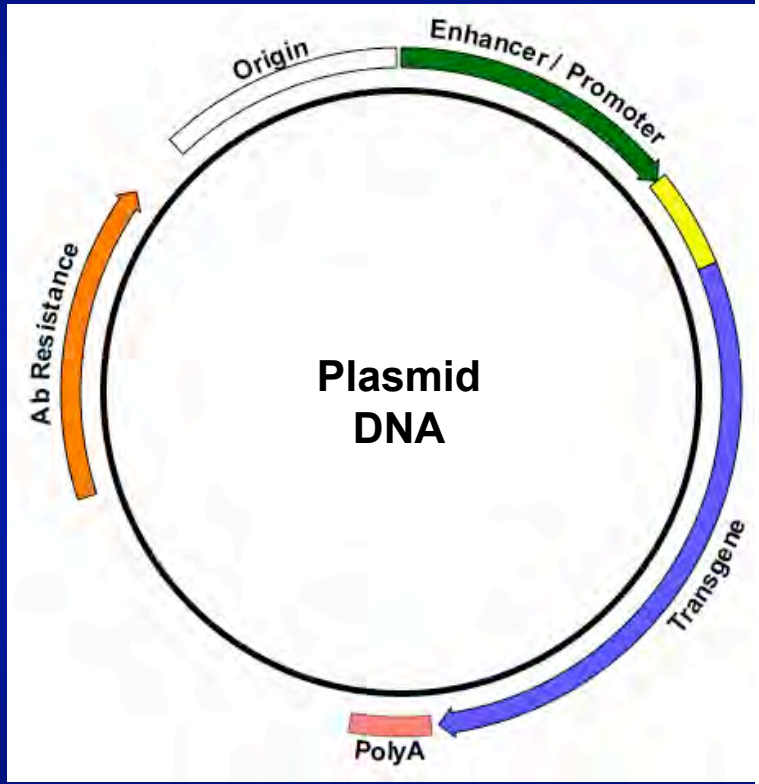
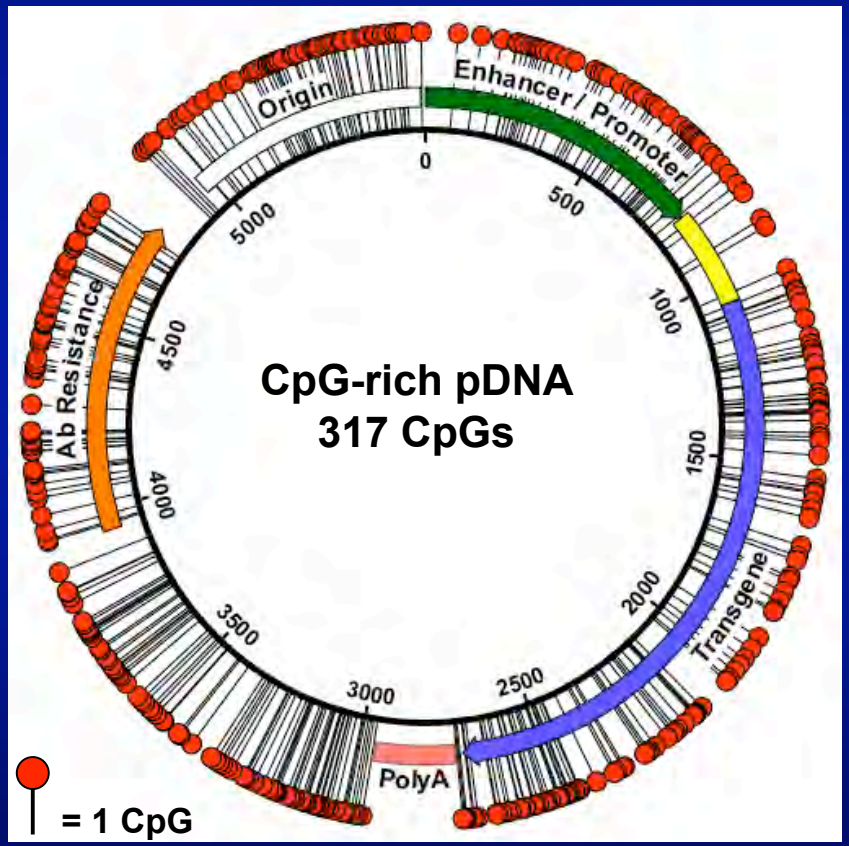
Non-Viral Gene Transfer to Lung

- Non-viral gene therapy for Cystic Fibrosis
  - Plasmid DNA complexed with cationic lipid
  - Plasmid DNA is:
    - Circular, bacterial, double stranded DNA
    - Carrier of gene of interest
    - Manufactured in bacteria
- UK Cystic Fibrosis Gene Therapy Consortium
  - 5 clinical trials
  - DNA-Lipid (GL67A) complex applied to the lung
  - Patients showed flu-like symptoms
  - Unmethylated CG dinucleotides (CpGs)

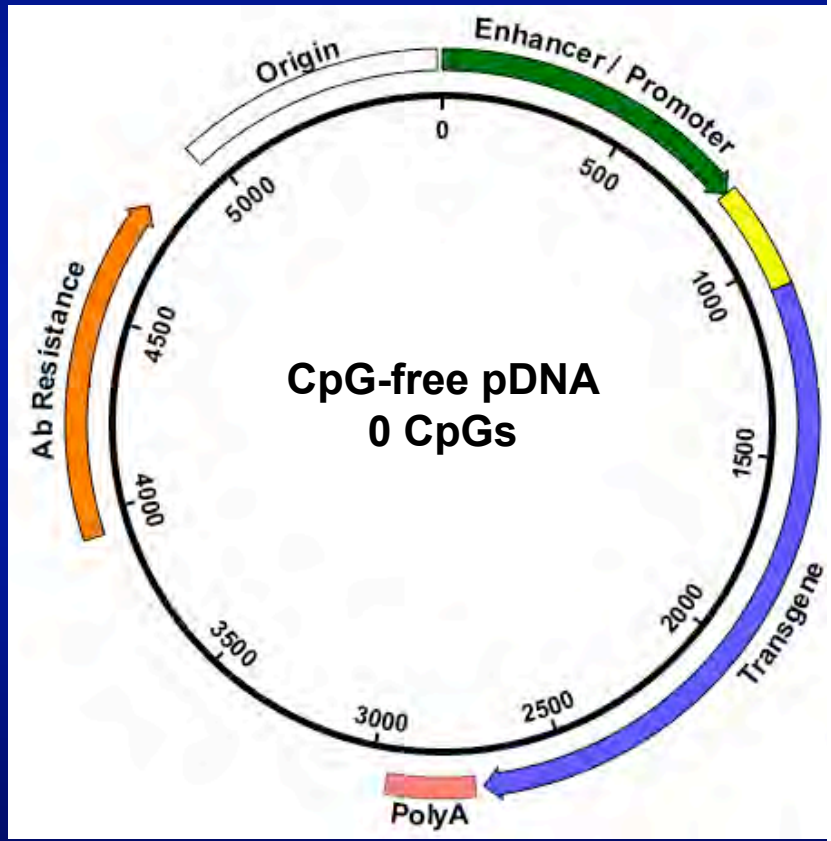


Development of CpG-Free Plasmid DNA

Previous plasmid in clinical trial



Current plasmid in clinical trial

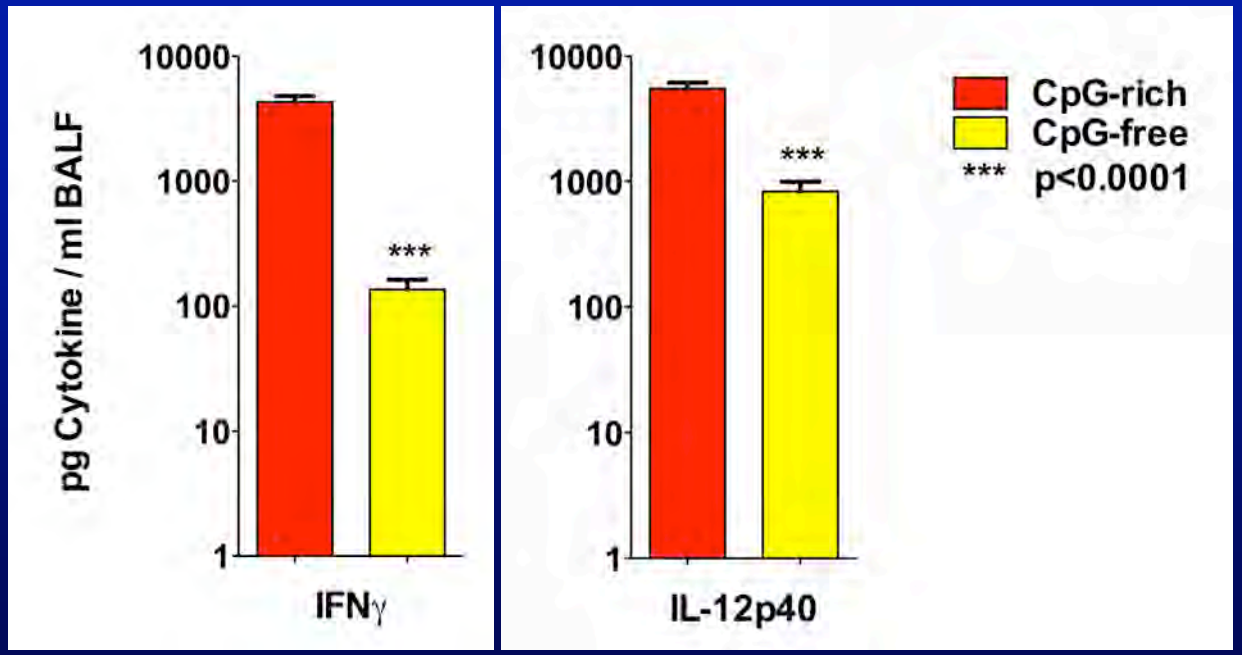


Hyde *et al.* (2008) Nat Biotech 26:54

Successful deletion of all CG dinucleotides on the plasmid DNA

Test Plasmids in Mouse Lung Model

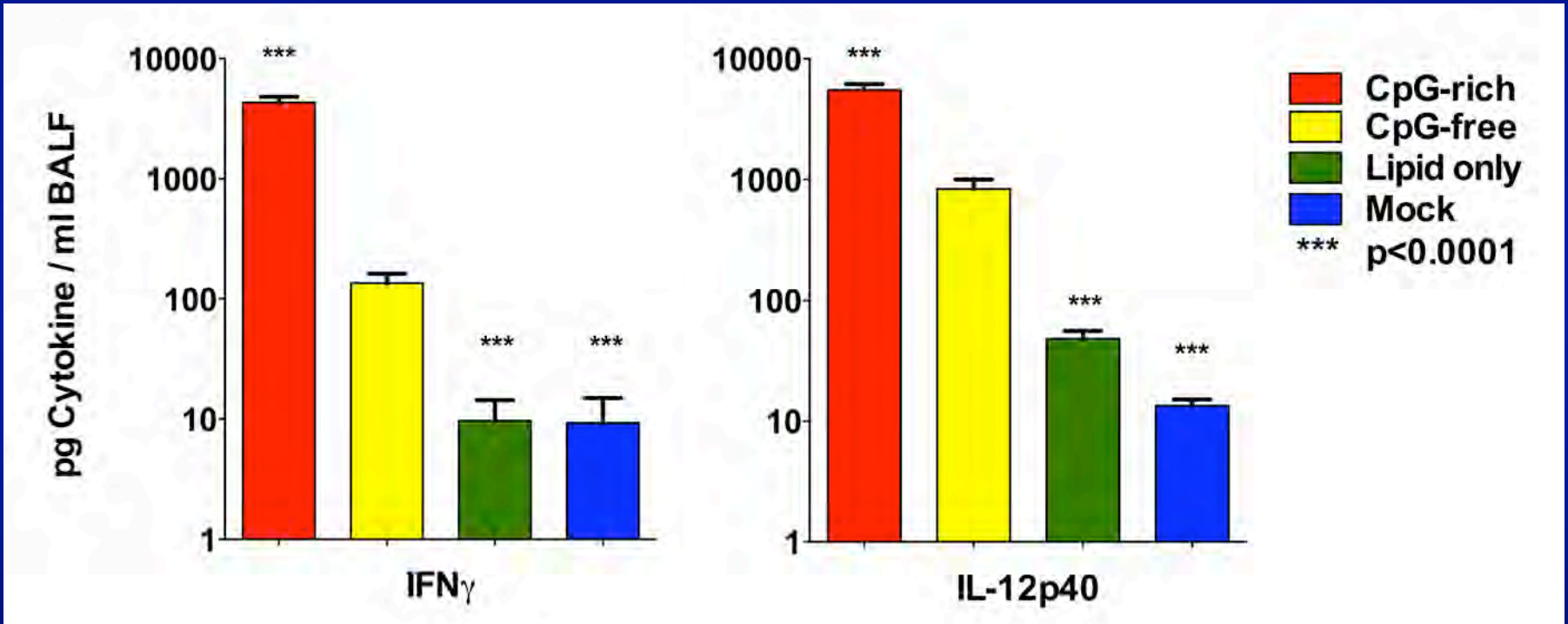
- Plasmid DNA/Lipid (GL67A) complexes (80 μg plasmid DNA in 100 μl)
- Delivered by intranasal instillation under general anaesthesia
- Female BALB/c mice (n=6 per group)
- At day 1 post delivery collection of bronchoalveolar lavage fluid (BALF)
- Analysis for pro-inflammatory cytokines
- Water (Mock) treated group as negative control



Significant decrease in CpG-associated pro-inflammatory cytokines

Residual levels of pro-inflammatory cytokines

- Significant increase of IFNγ and IL-12p40 after CpG-free plasmid DNA delivery



***: sig different from CpG-free group
One way ANOVA/Dunnett

- Additional component in plasmid DNA preparations may contribute to residual inflammatory cytokines

GMP Specifications for Clinical Plasmid DNA

- Is a component of the plasmid DNA preparation contributing to residual inflammatory cytokines?

• DNA concentration	≥5.3 mg/mL
• Circular forms	≥80%
• % host-cell DNA	≤2%
• % host-cell RNA	≤2%
• % host-cell protein	≤2%
• Endotoxin levels	≤5 EU/mg
• Total aerobic microbial, yeast and mold count	<10 CFU/mL
• Specific detection of *S. aureus*, *P. aeruginosa*, *Salmonella* sp & *E. coli*	<10 CFU/mL

Hypothesis

- Plasmid DNA preparations contain residual host bacterial chromosomal DNA that can act as a source of unmethylated CpGs contributing to low-levels of pro-inflammatory cytokines in the lung

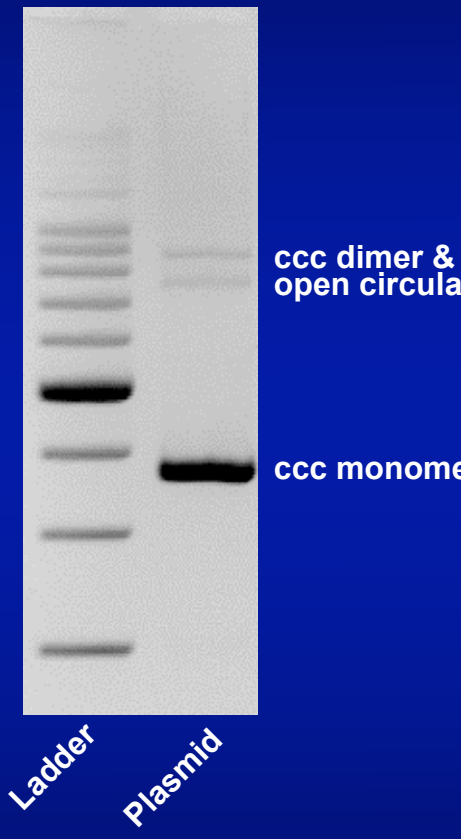
Aims

- Plasmid forms in different preparations - capillary gel electrophoresis
- Quantify chromosomal DNA in preparations - Q-PCR
- Analyse plasmid performance in mouse lung model
  - Quantify a range of pro-inflammatory cytokines - Multiplex assay (Bio-Plex)
  - Transgene expression - vector mRNA quantification by TaqMan

Characteristics of different pDNA preparations

- 5 different batches of pGM169 plasmid DNA tested
- Multiple manufacturers/processes
  - Similar levels of plasmid integrity & forms

Plasmid	ccc monomer [%]	ccc dimer [%]	oc forms [%]
pGM169-A	91.2	3.3	1.4
pGM169-B	91.1	1.9	0.3
pGM169-C	93.0	2.8	3.8
pGM169-D	96.7	2.7	0.4
pGM169-E	96.85	2.2	0.9



Characteristics of different pDNA preparations

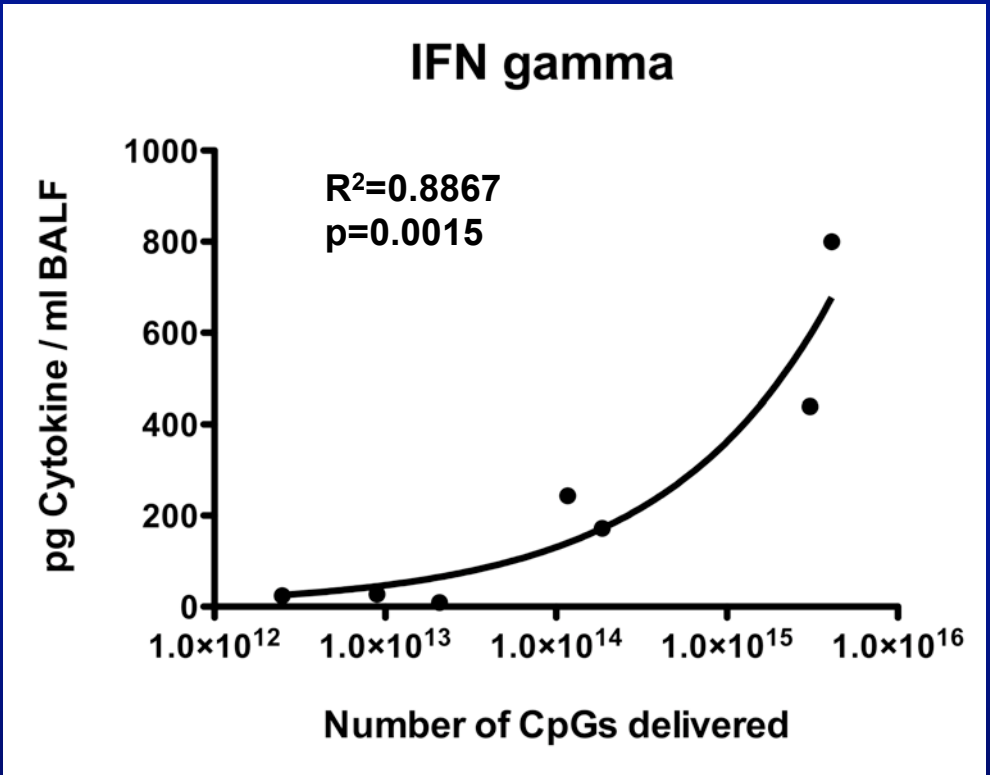
- 5 different batches of pGM169 plasmid DNA tested
- Multiple manufacturers/processes
  - Similar levels of plasmid integrity & forms

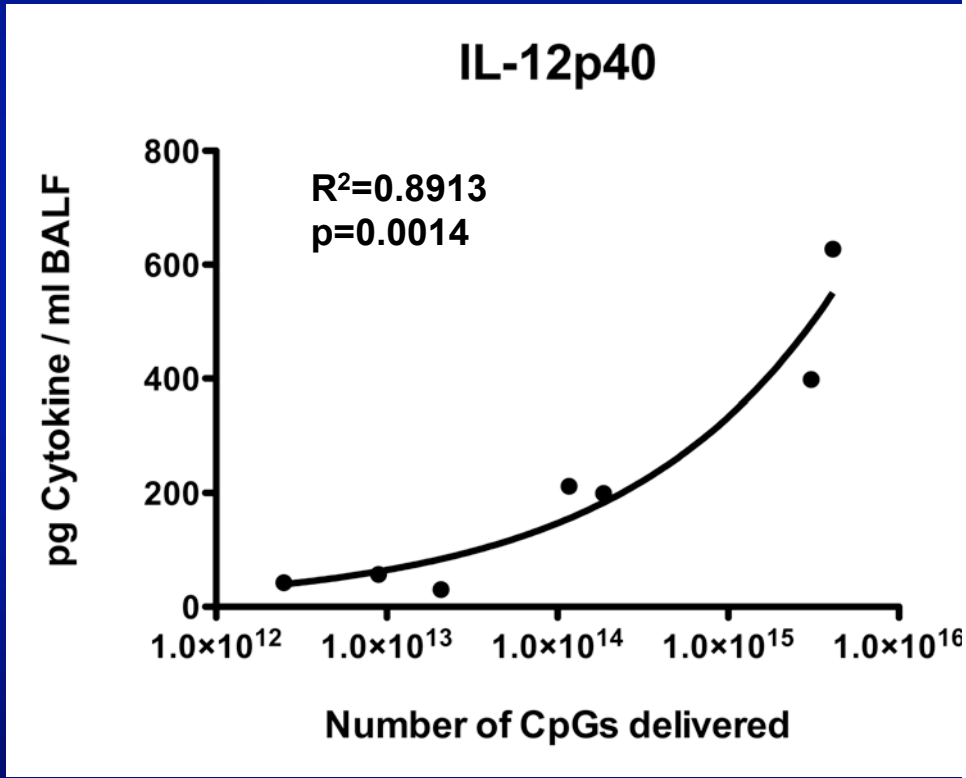
Plasmid	ccc monomer [%]	ccc dimer [%]	oc forms [%]	chrDNA [%]
pGM169-A	91.2	3.3	1.4	4.1
pGM169-B	91.1	1.9	0.3	2.6
pGM169-C	93.0	2.8	3.8	0.5
pGM169-D	96.7	2.7	0.4	0.2
pGM169-E	96.85	2.2	0.9	0.05

- Considerable variation in level of bacterial chromosomal DNA

Effect of chrDNA on pro-inflammatory cytokines

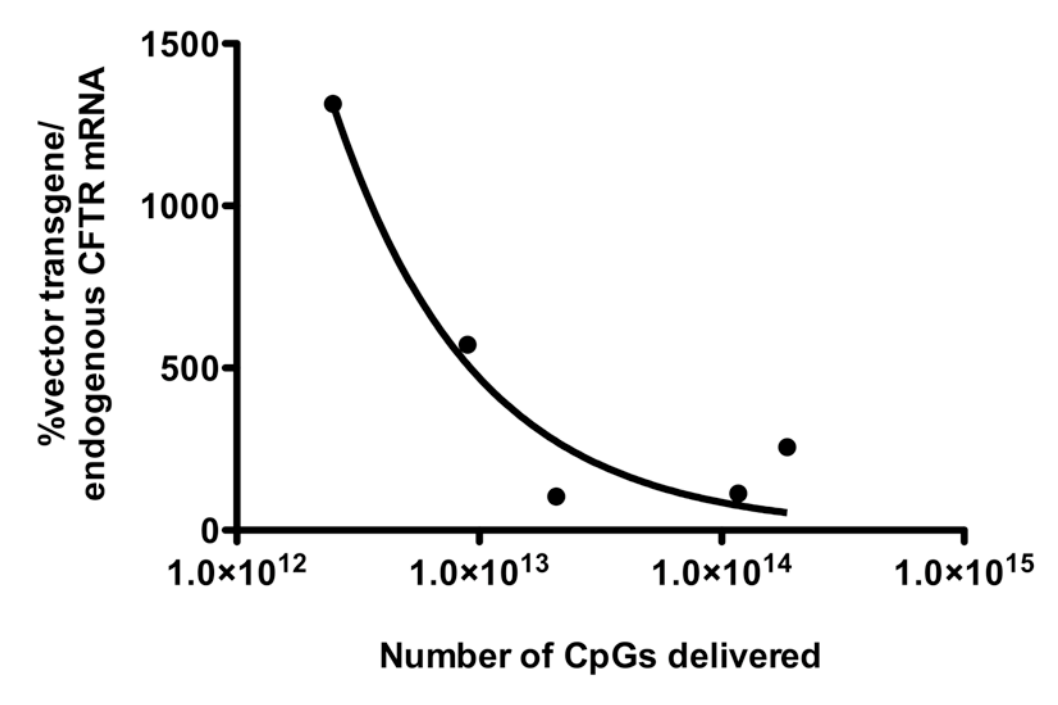
- Different plasmid DNA preparations were tested in the mouse lung model





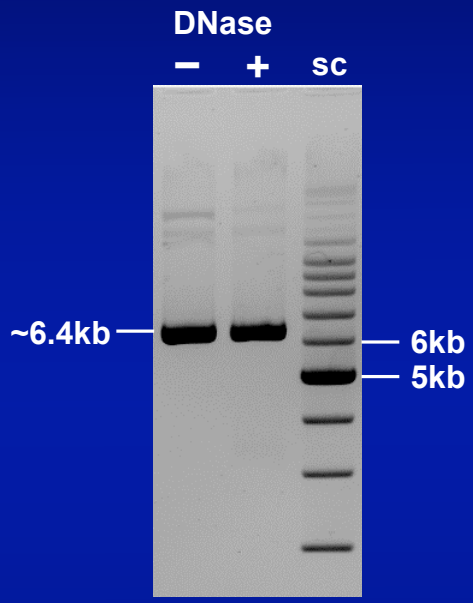
- Significant correlation between levels of residual bacterial chromosomal DNA & cytokines

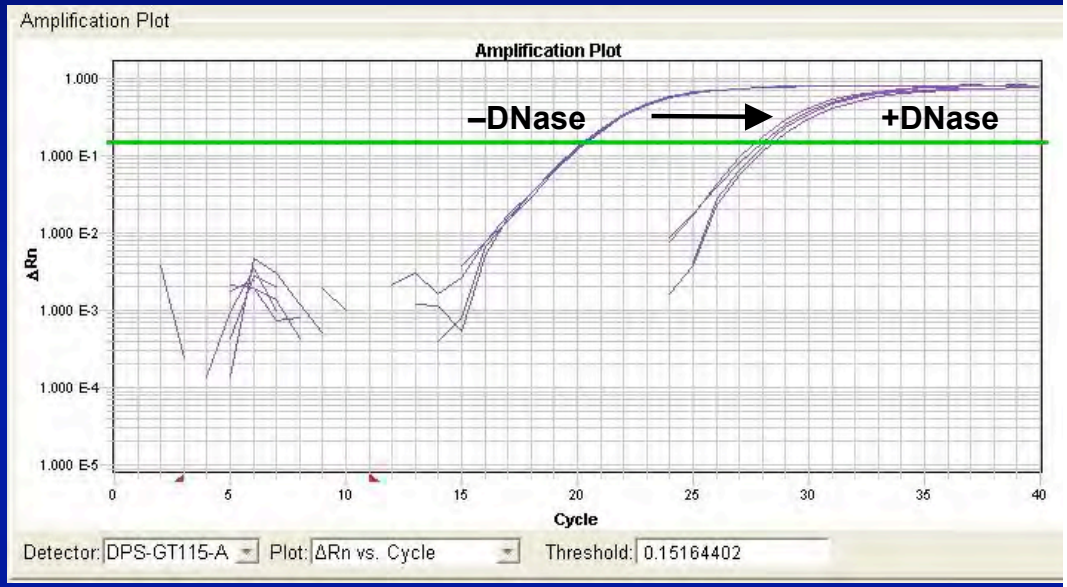
Effect of chrDNA on Vector mRNA Expression



Trend of higher vector mRNA expressio levels with preparations containing low levels of chromosomal DNA

Further Reduction of chromosomal DNA level





Treatment of plasmid DNA sample with 'Plasmid-Safe' DNase without altering plasmid DNA forms

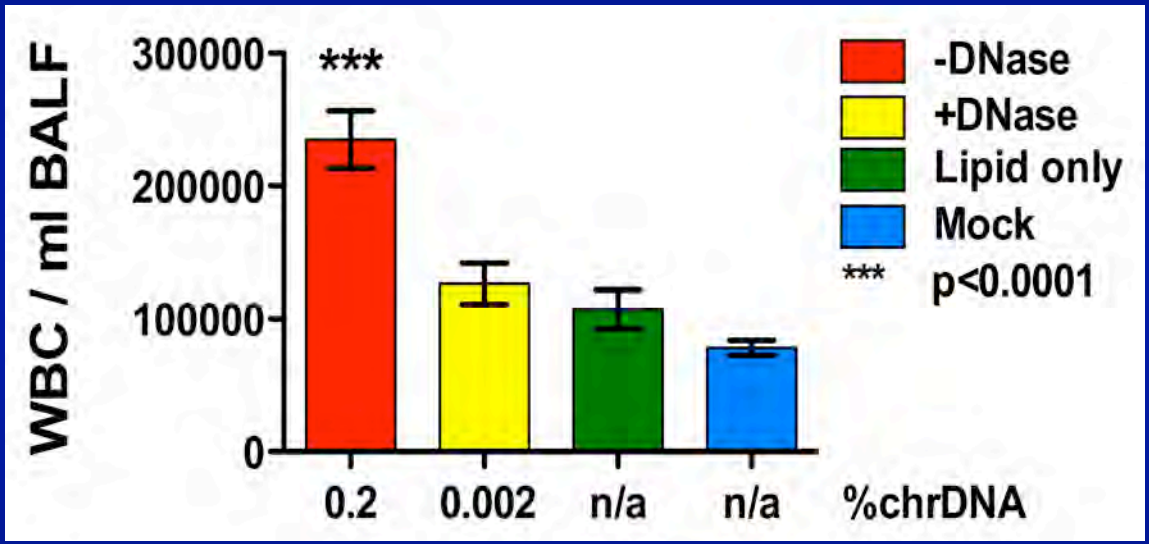
Reduced bacterial chromosomal DNA after DNase treatment:

pre-treatment: 0.2%

post-treatment: 0.002%

Measuring effect of reduction of chrDNA *in vivo*

- DNase treated sample tested in mouse lung model



- Significant decrease in total number of white blood cells after delivery of DNase treated plasmid DNA preparations

- Preliminary results of an ongoing study

Conclusion

- CpG-free plasmid DNA preparations contain low level bacterial chromosomal DNA
  - Level depends on manufacturer & process
  - Potential source of unmethylated CpGs
- Performance *in vivo*:
  - CpG-free plasmid DNA preparations containing low level bacterial chromosomal DNA can result in low levels of pro-inflammatory cytokines
  - May lead to reduced transgene expression
- Development of manufacturing processes to further reduce or eliminate bacterial chromosomal DNA is desirable

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