A Novel Dual Promoter
for an
Improved HIV-1C Vaccine Plasmid

Fiona Tanzer, Enid Shephard, Anna-Lise Williamson, Edward Rybicki

Institute for Infectious Disease and Molecular Medicine
University of Cape Town
South Africa

- As part of the South African AIDS Vaccine Initiative (SAAVI), we have developed at UCT an HIV-1C candidate DNA vaccine encoding the GrttnC polyantigen (Gag-RT-Tat-Nef).

- Our DNA vaccine has shown good immunogenicity in primates as part of a DNA prime – heterologous boost regimen, and is scheduled for Phase I trial in humans.

- However, large doses of DNA vaccine are needed to prime an immune response.

- We want to increase DNA vaccine efficiency through improved plasmid design in order to achieve DNA vaccine dose reduction

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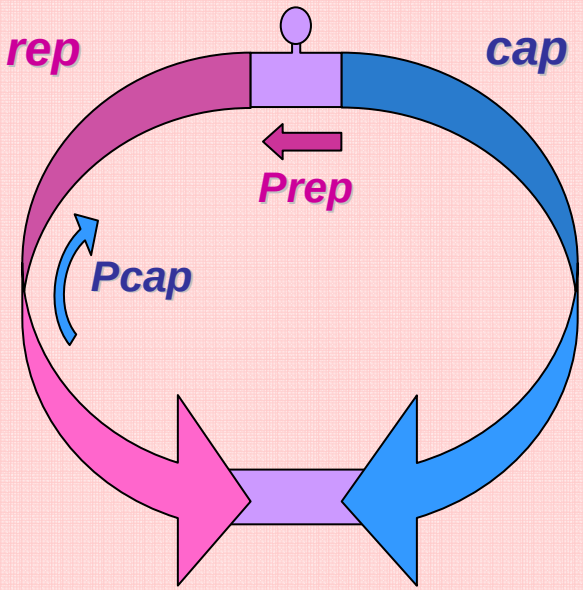
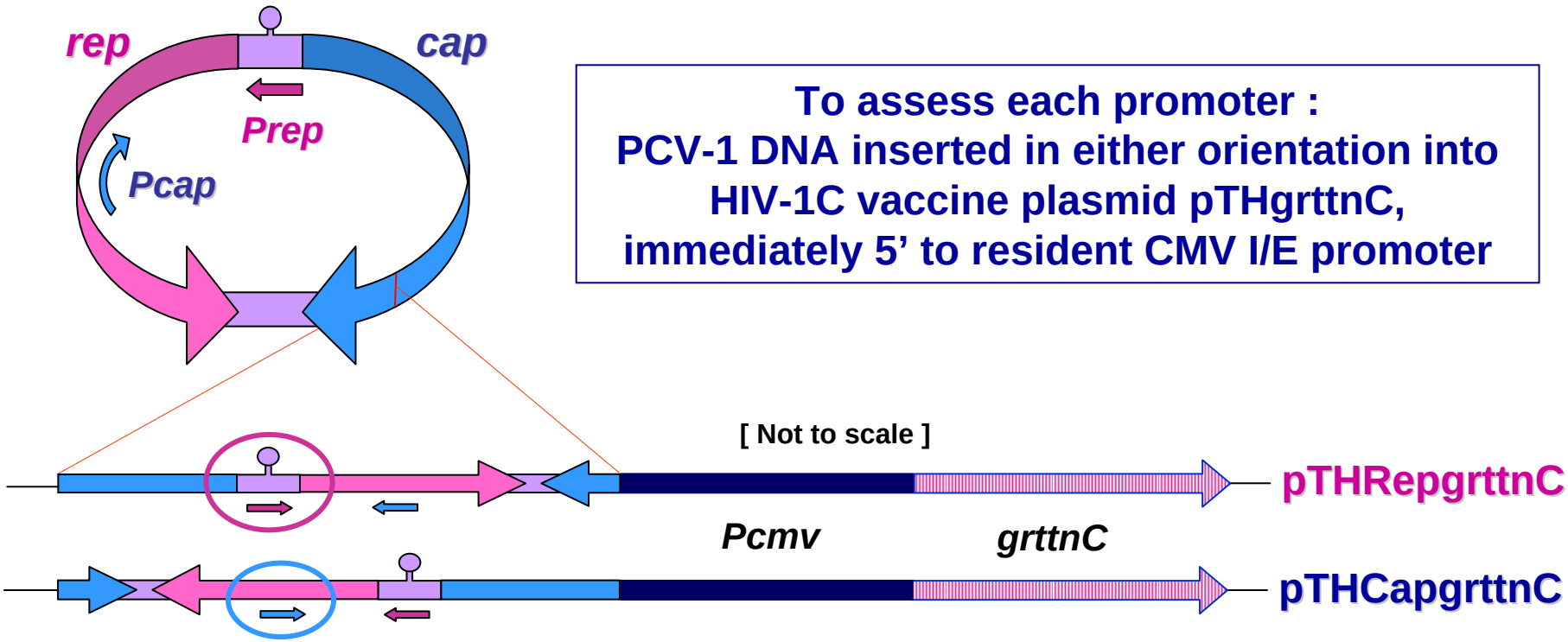
- We can reduce dose if more antigen is expressed per plasmid molecule
- Add novel gene regulatory elements that condition high level gene expression in mammalian cells
- viruses have compact elements for plasmid use
- Porcine circovirus type 1 (PCV-1) = good candidate source of elements

Porcine circovirus type 1 (PCV-1)



- Small mammalian DNA virus – 1759 bp
- highly compact bi-directionally transcribed genome - 2 ORFs

- **Apathogenic in all species, including humans**
- **Host range is restricted to cultured porcine kidney cells**

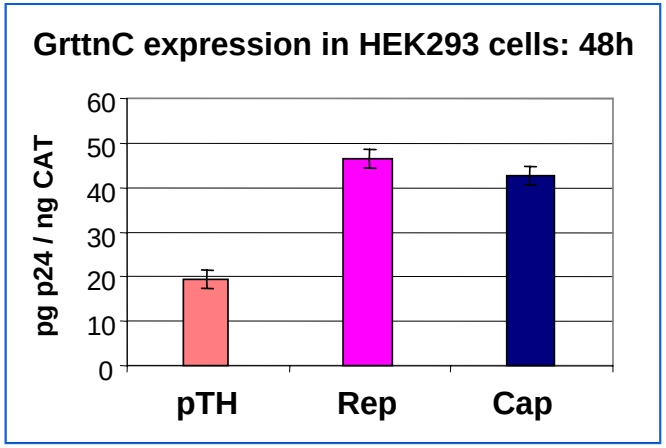


To assess each promoter :
PCV-1 DNA inserted in either orientation into HIV-1C vaccine plasmid pTHgrttnC, immediately 5' to resident CMV I/E promoter

Tested GrttnC polyantigen expression in HEK293 - **p24 antigen ELISA**

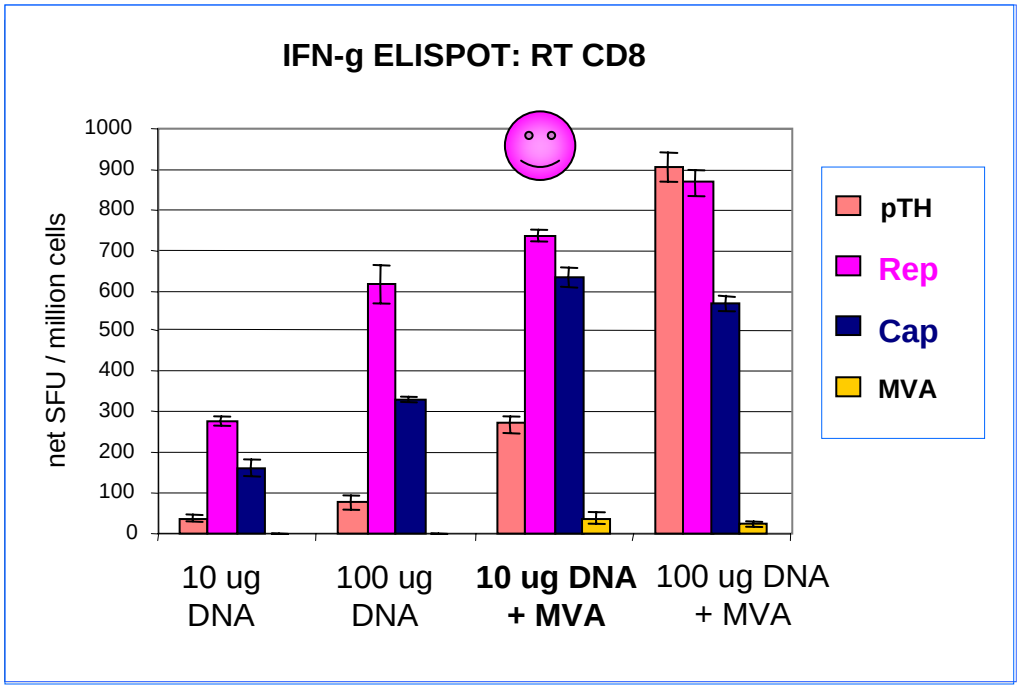
Tested vaccine plasmid immunogenicity in Balb/C mice:

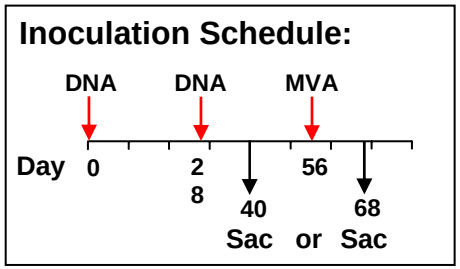
- **CTL response to our benchmark immunodominant RT CD8+ epitope of GrttnC via IFN- g ELISPOT.**



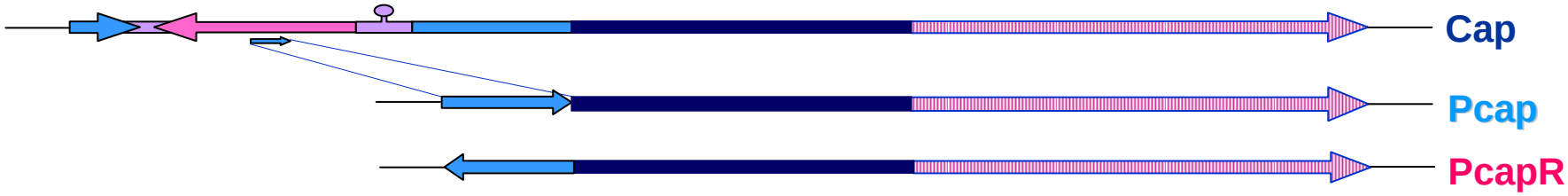
PCV-1 improves immunogenicity – effective at 1/10 priming dose

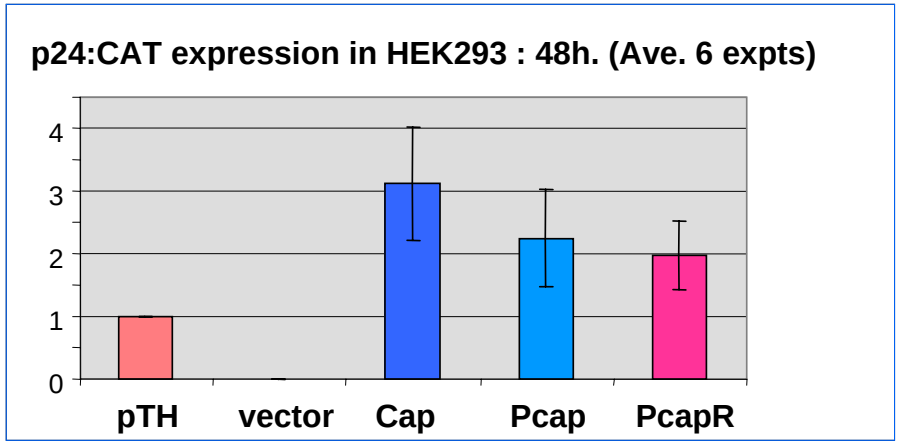
PCV-1 increases GrttnC expression 2-fold

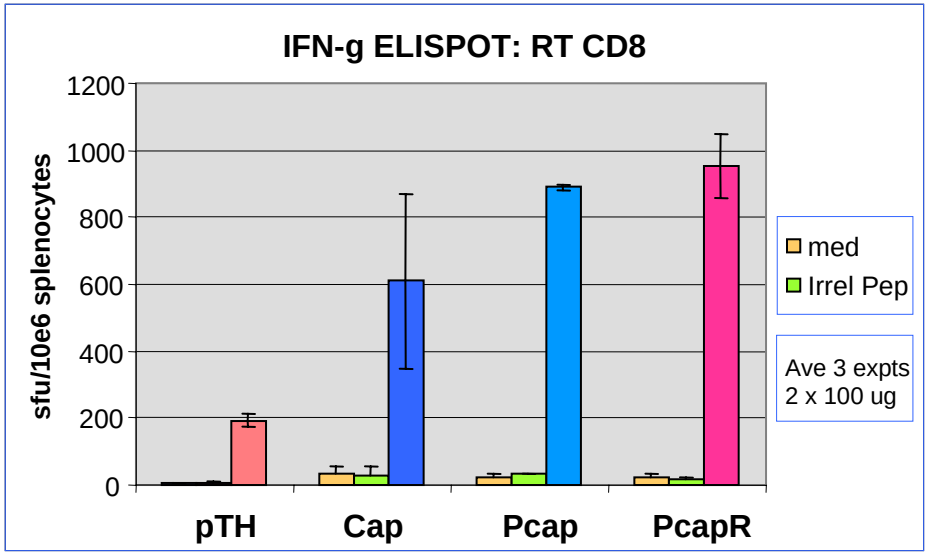


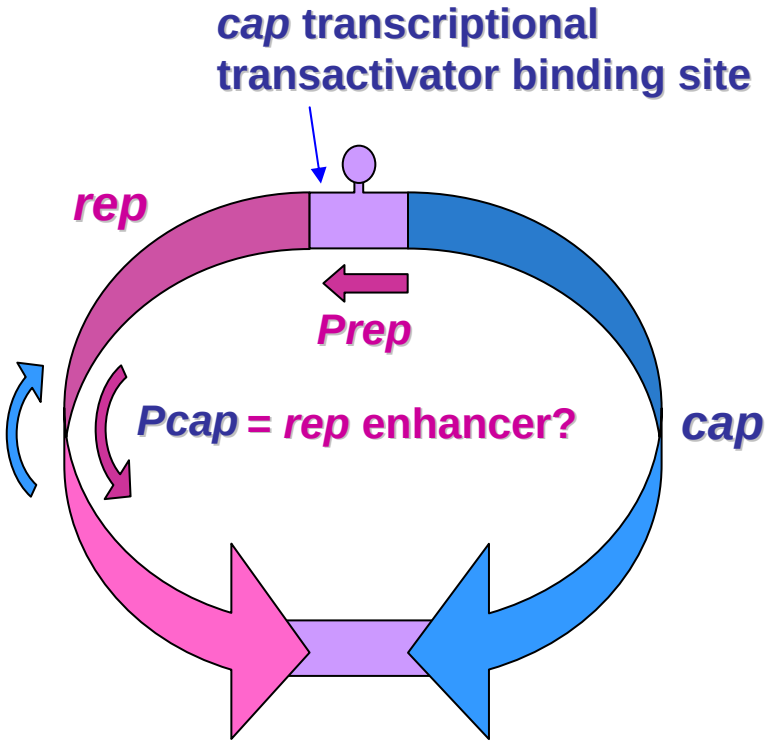


Inserted 172 bp PCV-1 capsid promoter (Pcap) only – either orientation.

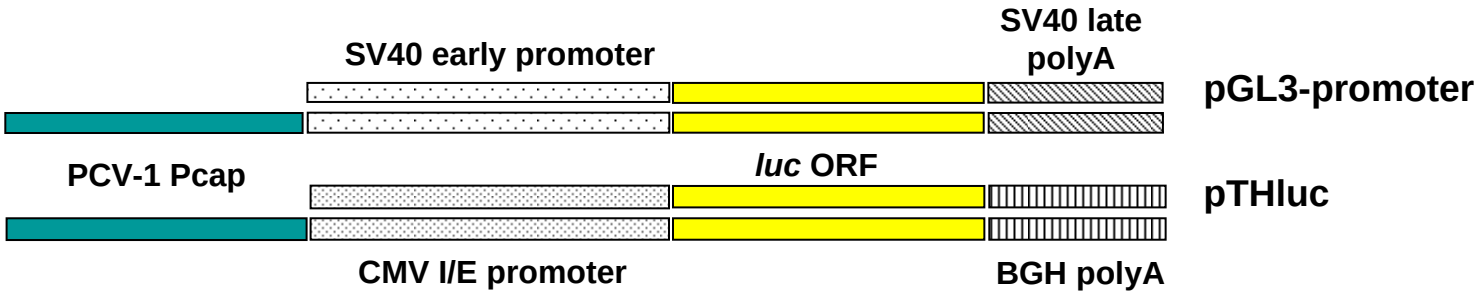




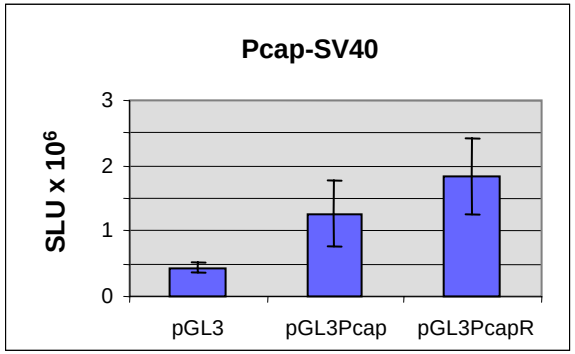


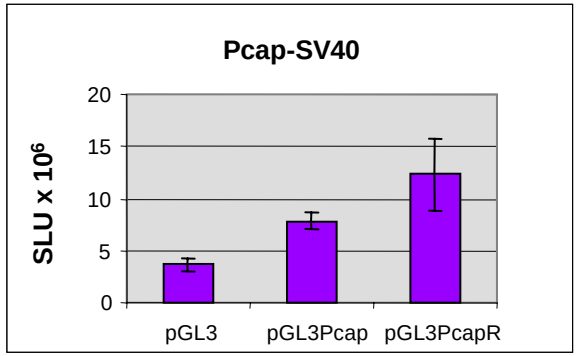


Generality of action: Pcap also operates in tandem with SV40 promoter



Pcap-SV40

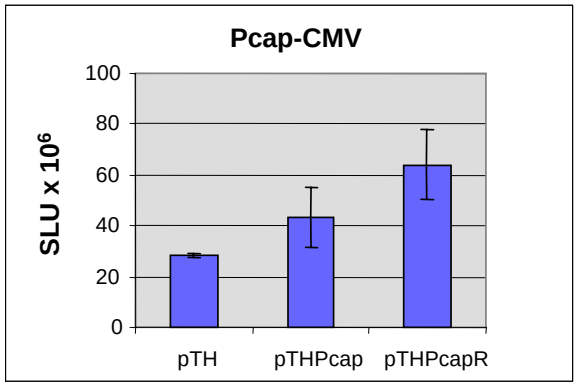


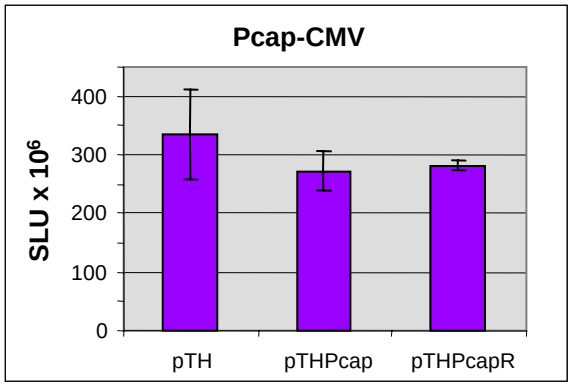


24h

48h

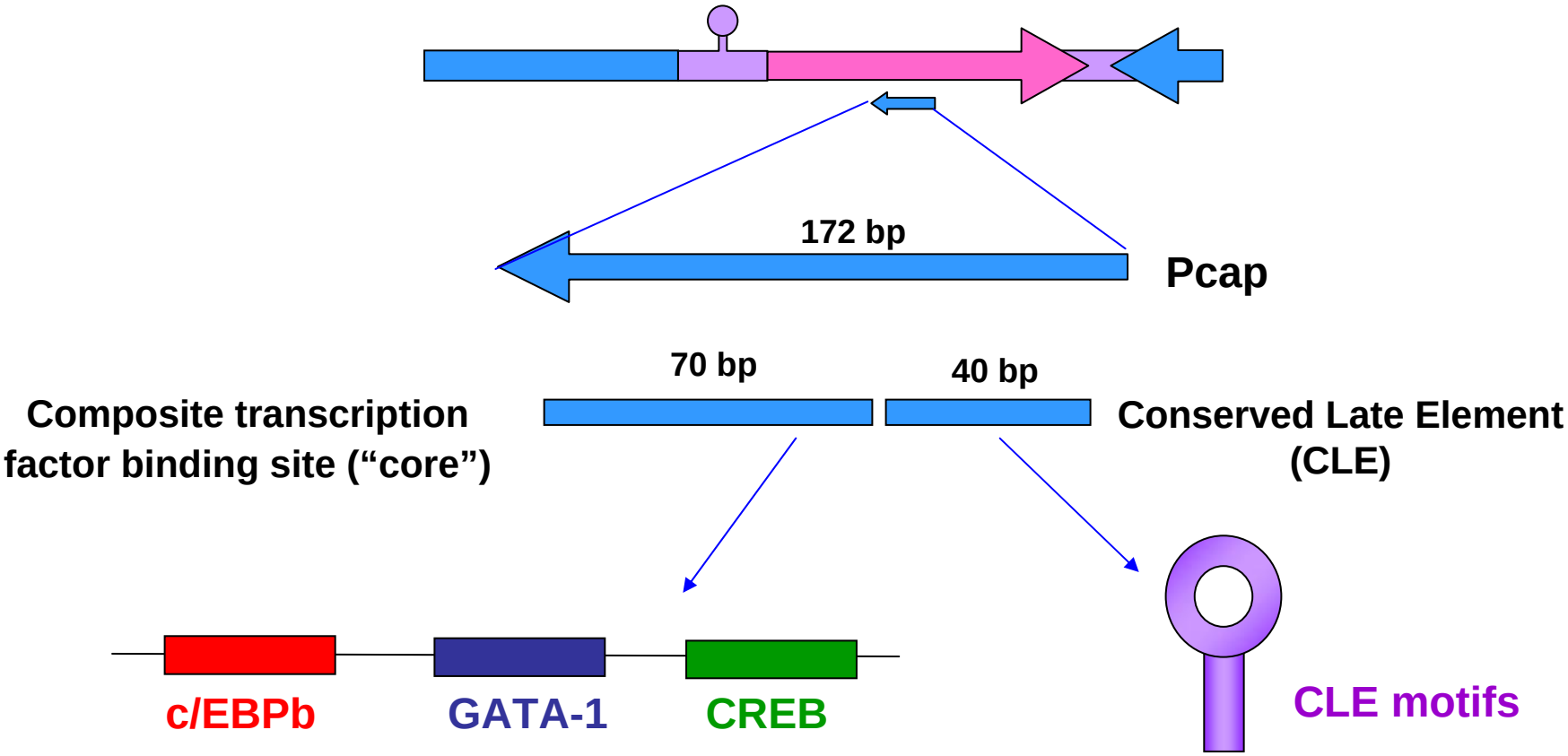
Pcap-Pcmv





Luciferase expression in HEK293 over 48h

Where does Pcap activity reside?



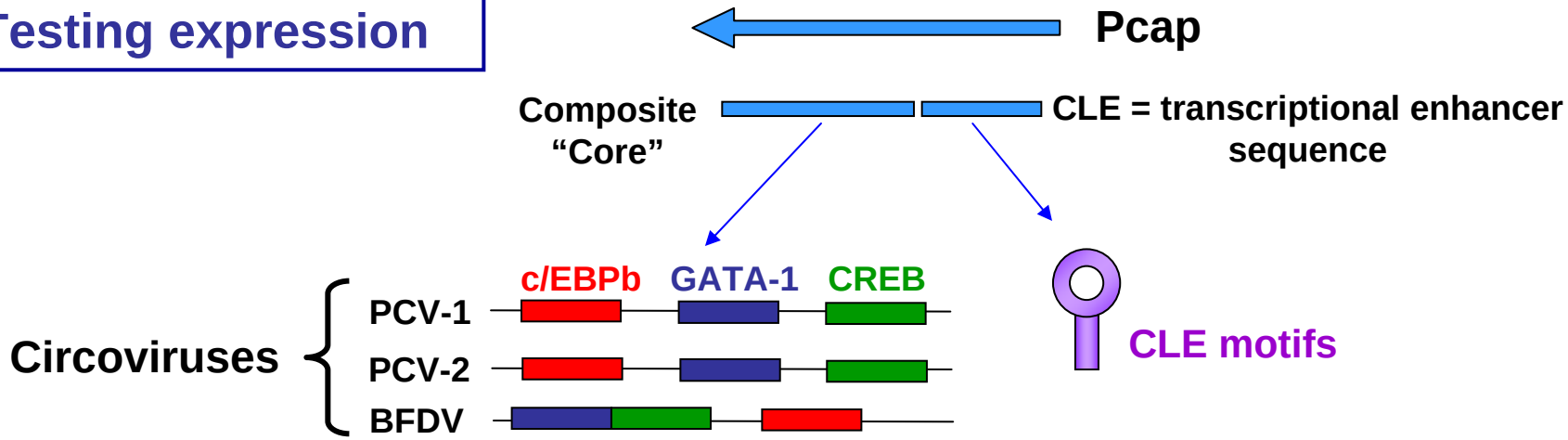
We identified a putative Composite TF binding site & Conserved Late Element

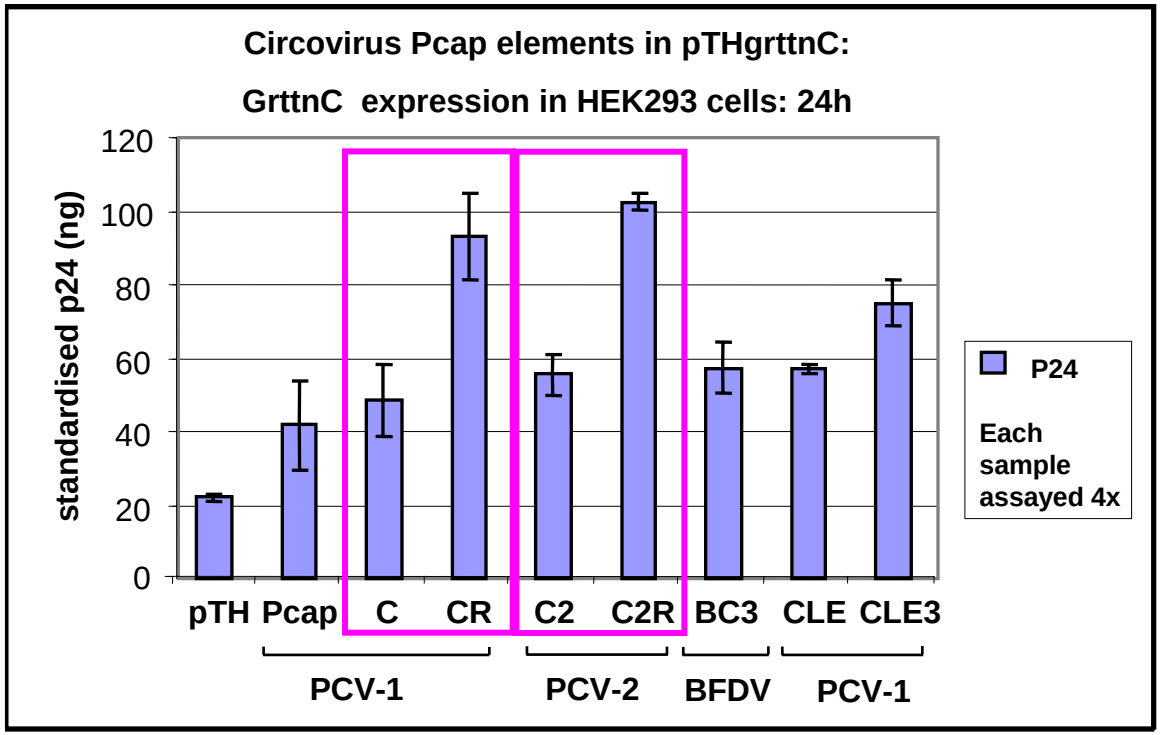
PCV-1 P*cap* core GCTG**AACTTTTGAAAGTG**AGCGGGAAGATGCAGCAG**CGTGATTGGA**AGACAGCTGT**ACACGTCA**T AGTGG

c/EBPb GATA-1 CREB

PCV-1 CLE T**AGTGGGCCCG**CCCGGTTGTGGGAAGAGCC**AGTGGGCCCG**TAATTTT **Direct repeat CLE motifs**

Testing expression





Pcap activity traced to - Composite core (sequence + position of sites important) & CLE (works additively)

pTH	pTHgrttnC
C	Pcap core
CR	**Pcap core reversed = rep enhancer**
BC3	BFDV core trimer
CLE3	CLE trimer

Conclusions

- HIV-1 DNA vaccine dosage can be significantly reduced 10-fold by addition of whole-genome PCV-1 derived DNA.
- Small sequence additions are also effective:
  - 172 bp Pcap enhances antigen expression and DNA vaccine immunogenicity 3-fold.
  - 70 bp Pcap Core (reversed) enhances antigen expression 4-fold.
- Ongoing work
  - test immunogenicity of plasmid with reversed Pcap core
  - Test dose response.

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