

Detection of Viral Vector Sequences in Animal Excretions

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Outline

- Previous Study
- Current Study
 - Vector Systems
 - QPCR Assay
 - Virus Stocks
 - Animal Experiments
 - Results
 - Conclusion
- Future Direction

Original Research

Assessment of Hazard Risk Associated with the Intravenous Use of Viral Vectors in Rodents

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“...we conclude that commonly used replication-deficient viral vectors pose minimal risk by 72 hours after inoculation....Level 1 safety measures may be sufficient after cage changing and biosafety evaluation.”

Previous Shedding Study

- Mice, 8-wk-old females
 - Outbred strain
 - Immune deficient
- Vector Viruses
 - Lenti and Adeno
 - EGFP transgene driven by CMV promoter
 - $\sim 10^9$ IU/mouse
- Urine and Feces
 - Collected as excreted
 - Time points -1, 1, 3, 7

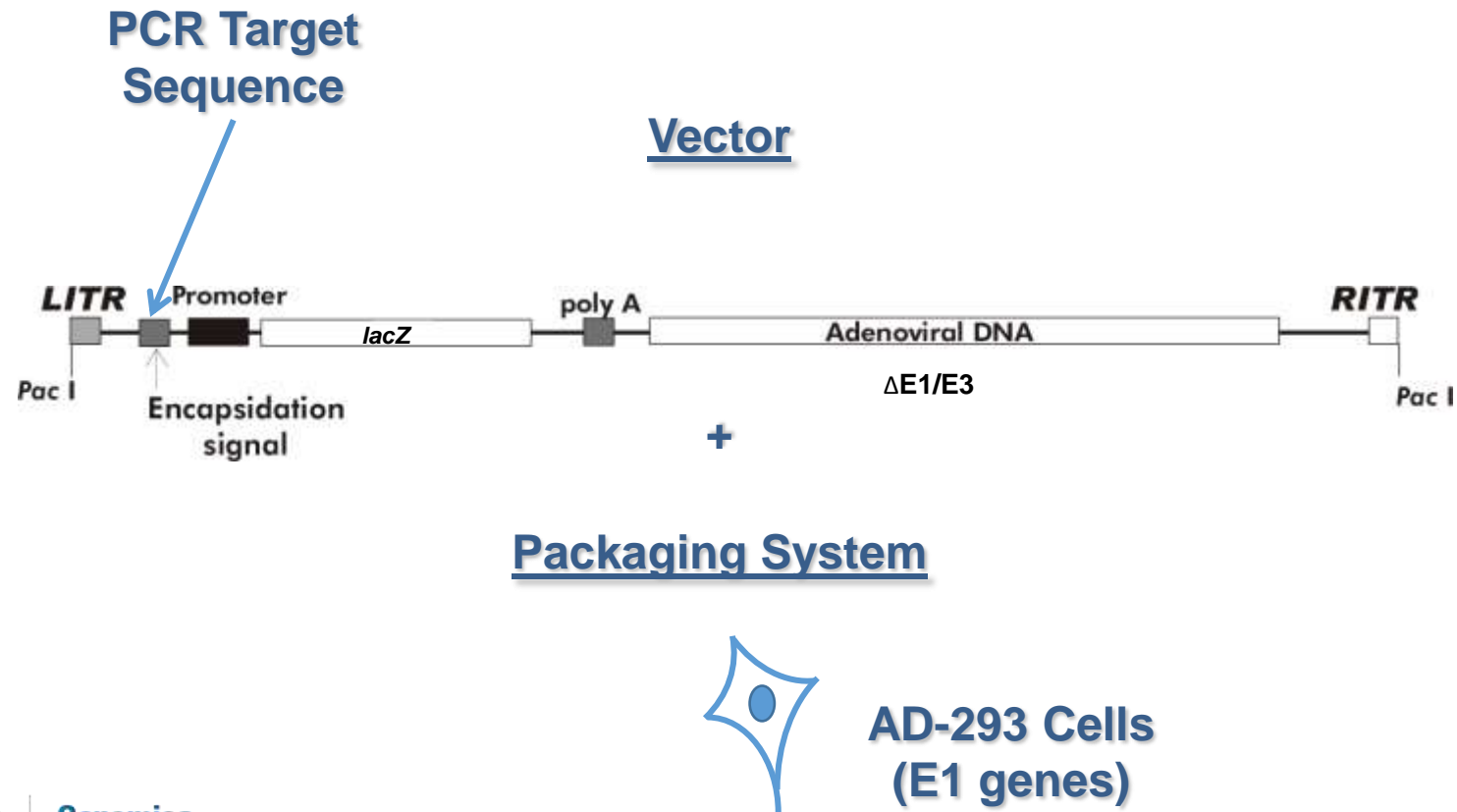
Previous Shedding Study (continued)

- Isolated nucleic acids
- Performed quantitative PCR
 - Primers against the transgene
 - No internal control
- Positive controls
 - Spiked whole blood with vector virus
 - Limit of detection was 200
 - Used as control for urine and fecal samples
- Result
 - No shedding detected for Lenti and Adeno

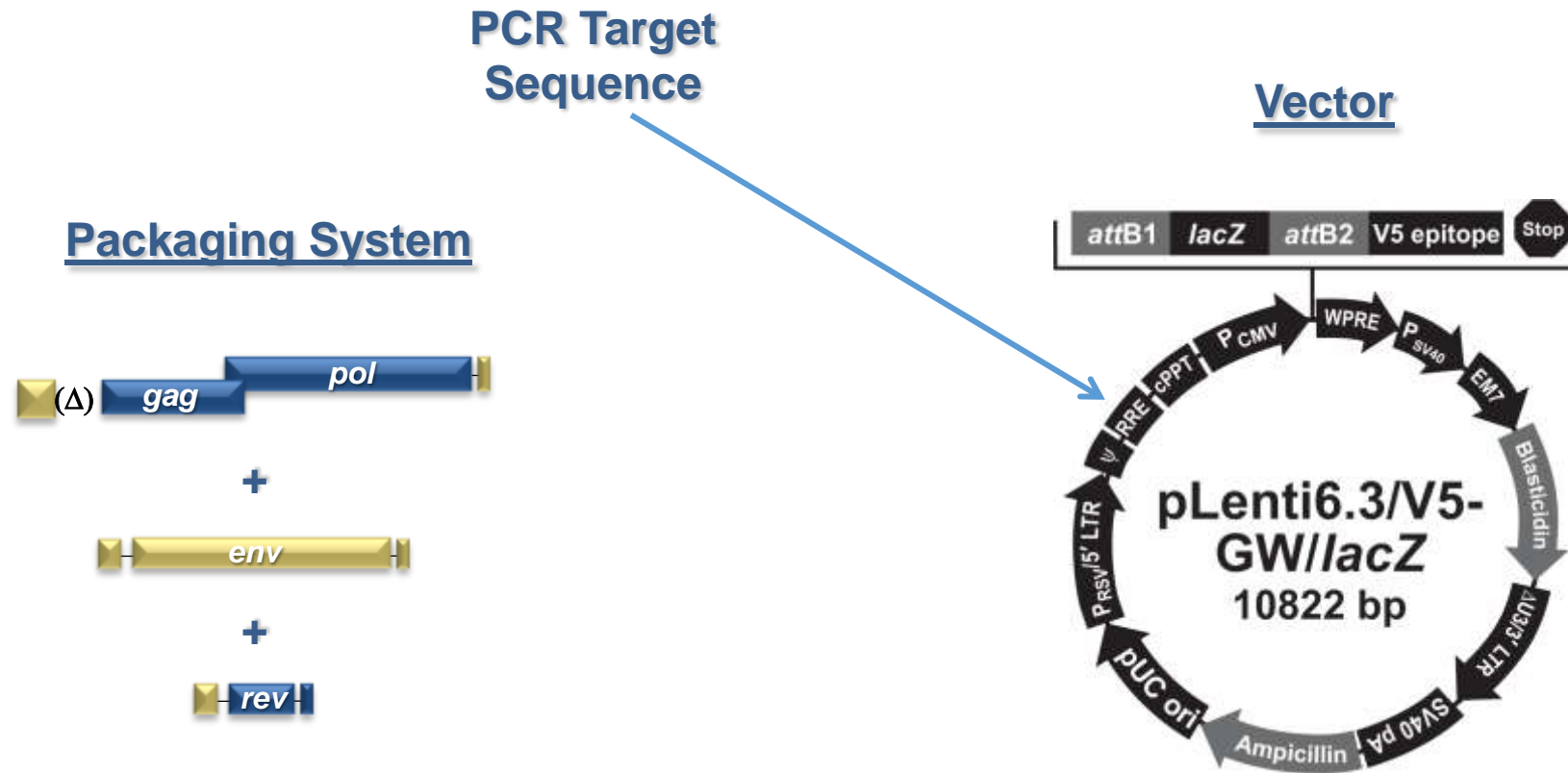


High Pure Viral Nucleic Acid Large Volume Kit, Roche

AdEasy™ Adenoviral Vector System

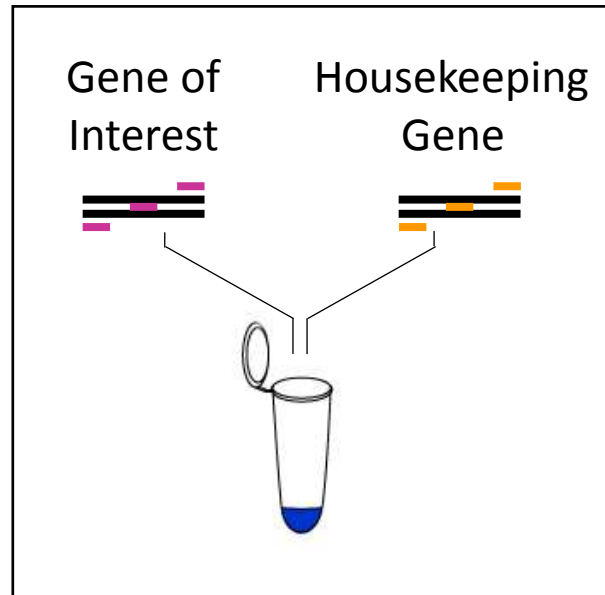


ViraPower™ Lentiviral Expression System (HIV)

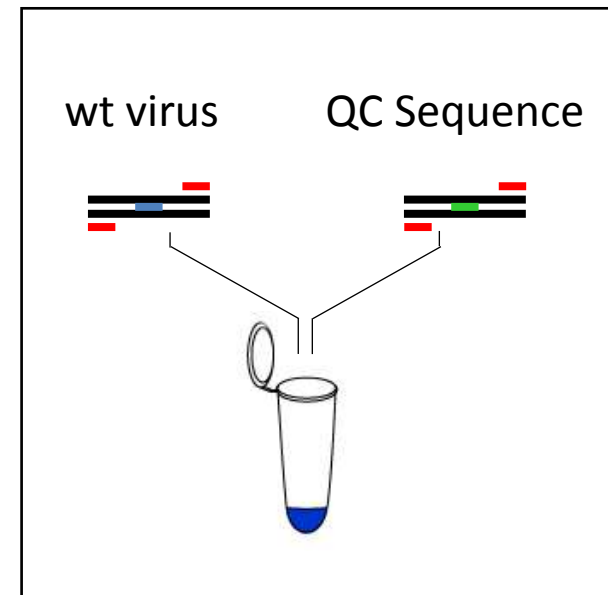


Multiplex PCR

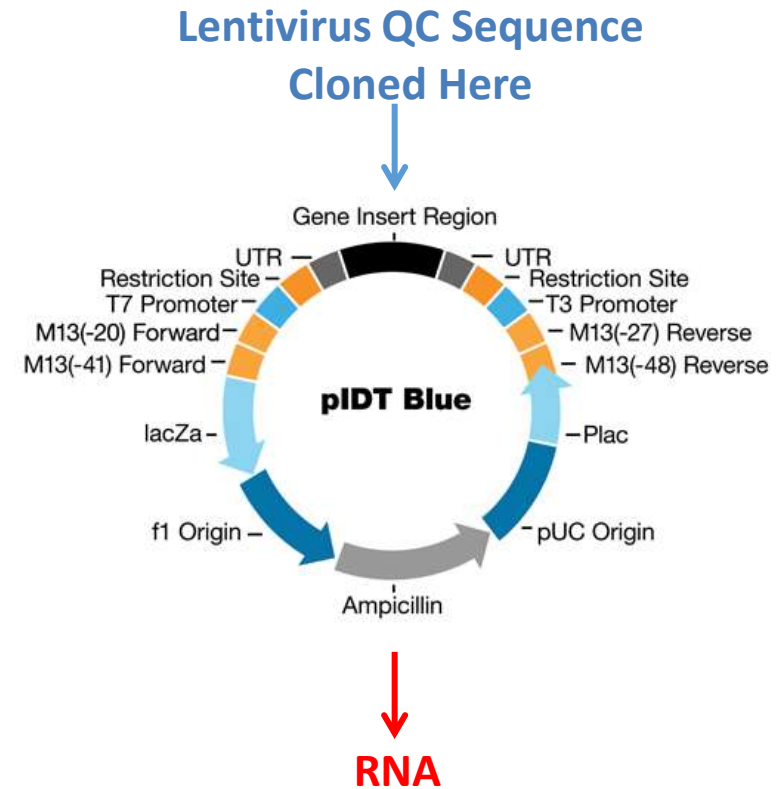
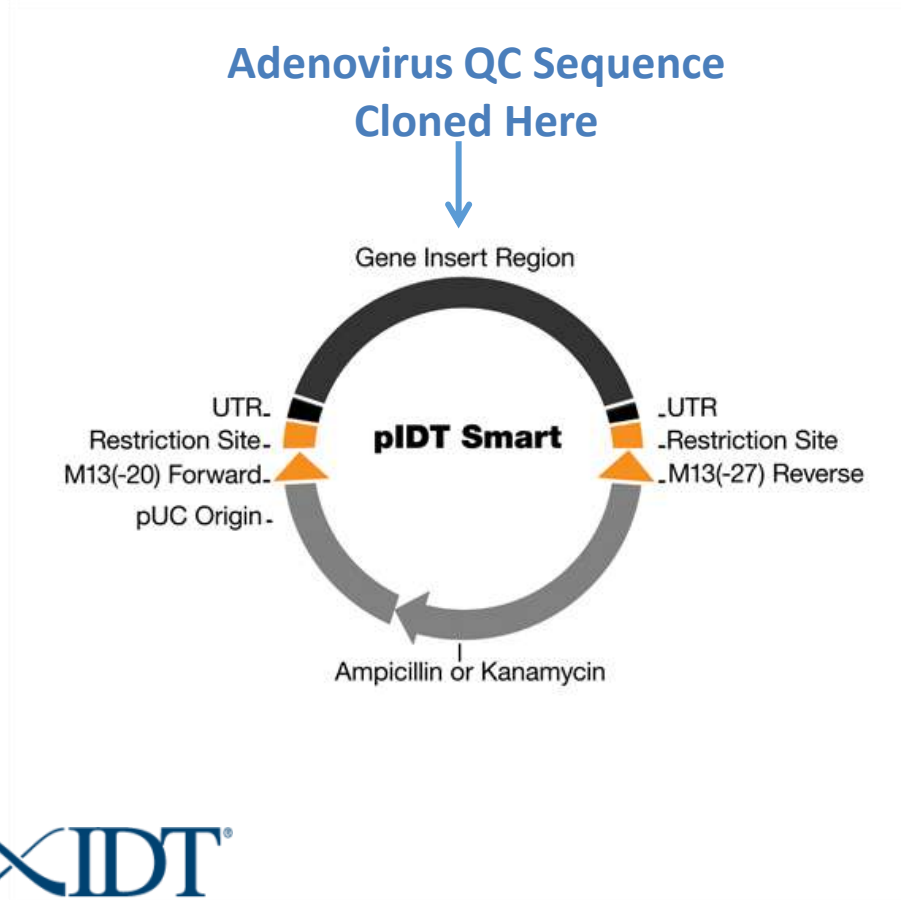
Traditional



Ours



Quality Control (QC) Sequences



Note: RT Efficiency
Is about 20%

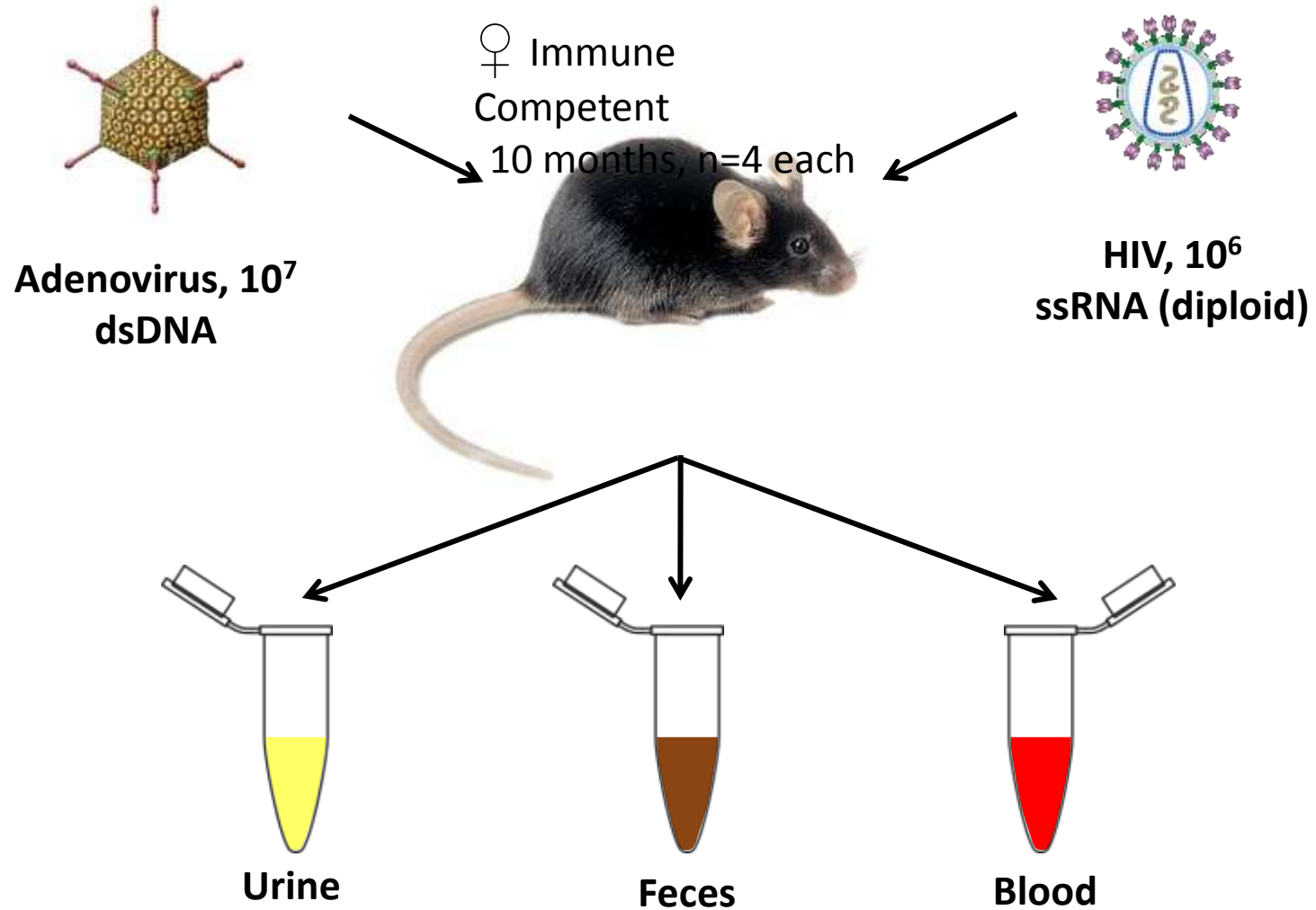
Virus Stock Characterization

Stock	Infectious Titer (pfu/ml or cfu/ml)	Total No. of Virus Particles (vg/ml)	Total Particle: Infectious Particle Ratio	p24 Protein Content ($\mu\text{g/ml}$)
Adeno	4×10^8	2×10^9	5	N/A
Lenti	1×10^7	3×10^{11}	3×10^4	13.3 ^a

N/A = Not Applicable

^aEquivalent to 2.7×10^{11} particles/ml

Experimental Scheme



Mouse Experimental Setup



Excretion Outputs from Infected Mice

Adeno Vector in Mice (Summer months)

- Urine output was 1.9 ml/day/mouse (0.75-3.8)
- Fecal output was 1.7 g/day/mouse (0.46-3.8)

Lenti Vector (Winter months)

- Urine output was 0.9 ml/day/mouse (0.11-2.1)
- Fecal output was 1.2 g/day/mouse (0.29-2.1)

Daily Fecal Excretions



$2.298 \text{ g} / 96 \text{ pellets} = 24 \text{ mg per pellet}$

DNA Isolations

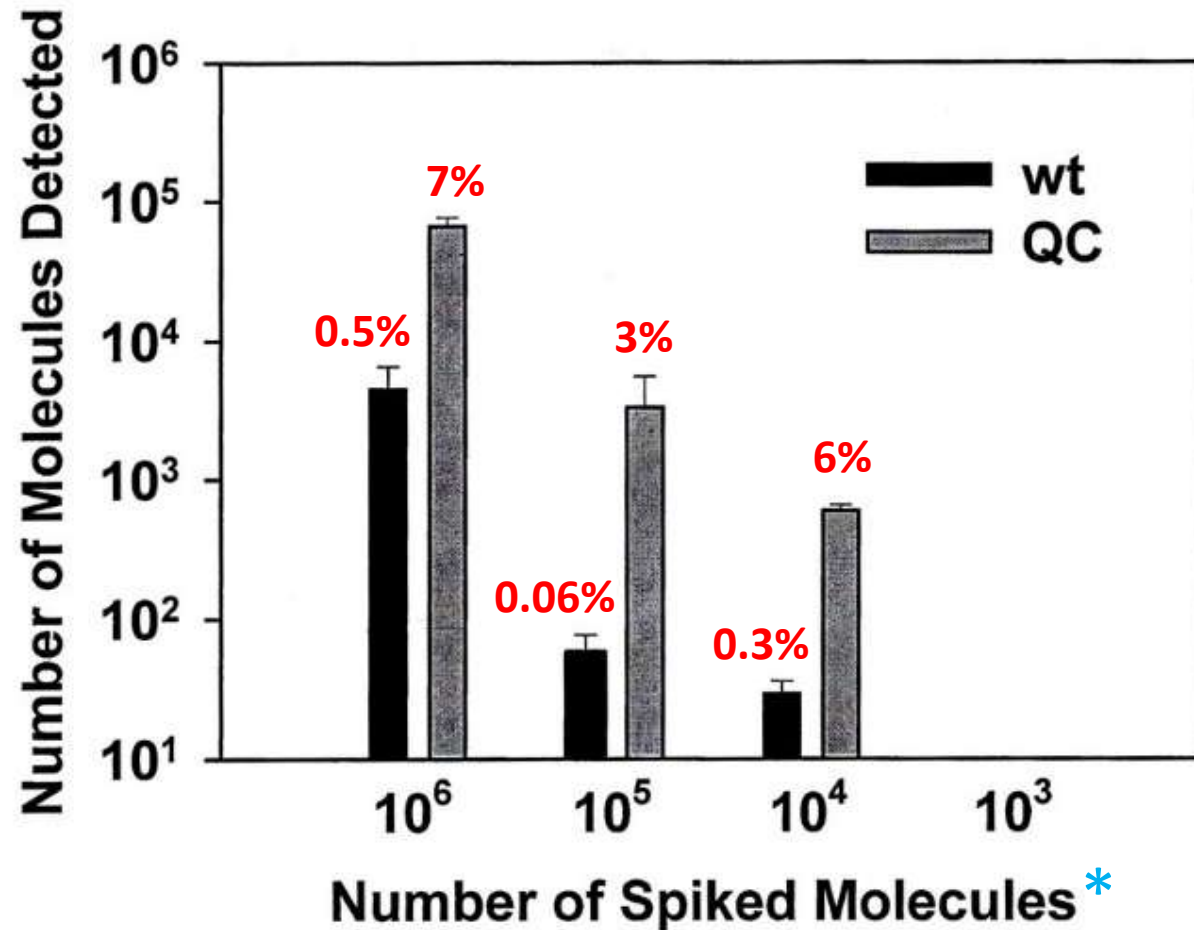
QIAamp MinElute Virus Spin Kit



QIAamp DNA Stool Mini Kit

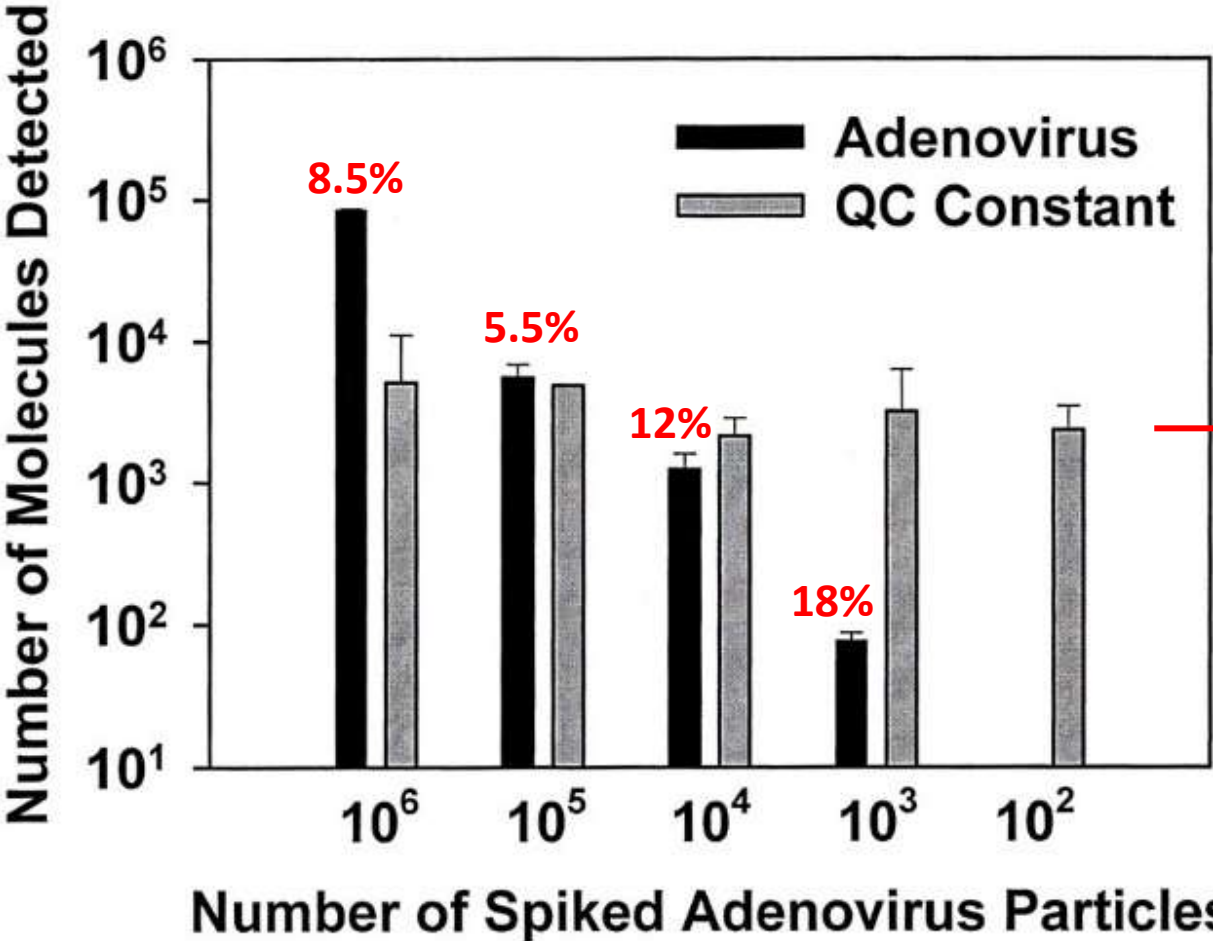


QPCR Experiment with Plasmid-Spiked Urine for Adenoviral Vector



* Note: Sample was spiked just after addition of lysis buffer and carrier RNA using Qiagen MinElute Kit

QPCR Experiment with Virus-Spiked Urine for Adenoviral Vector



QC average = 3.5%
(2.2-5.1%)

* Note: Sample was spiked at beginning with virus and QC plasmid added just after addition of lysis buffer and carrier

Conclusions

- Shedding studies are technically challenging, not a trivial undertaking
- Be cautious with interpreting negative results
- Robust positive controls must be designed
- Limits of detection must be accurately established
- Sample size must be considered
- Virus particles must be kept intact as long as possible
- Viral lysis should be performed as far downstream as possible.

Ongoing and Future Studies

- Develop a more sensitive way of processing the current samples
- Higher doses of vector viruses
- Different routes of administration
- Different Lentivirus and Adenovirus vector constructs
- Armored RNA and DNA controls

Acknowledgements

Wright State University

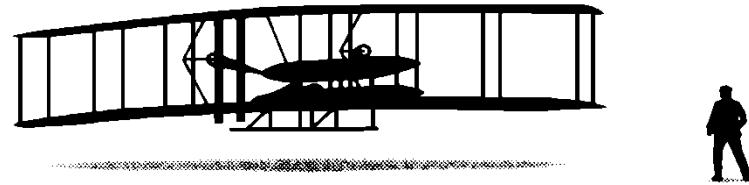
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Questions?



“A pessimist is simply an optimist in full possession of the facts.”

Edward Abbey