Quantitative PCR Assay for Detecting Viral Vector Shedding from Animals

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ABSA Griffin Grant Program

Overall objective: To quantify the amount of viral vector shedding from animals infected with adenovirus and HIV vectors.

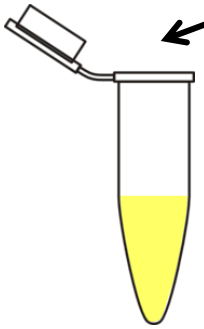
Outline

- Challenges
- Viral Systems
- Quantitative PCR System
- Assay Validation
- Summary
- Ongoing Studies

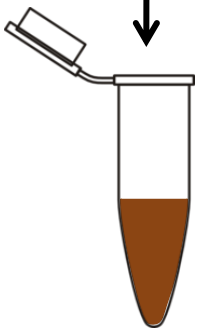
Challenges

Adenovirus
dsDNA

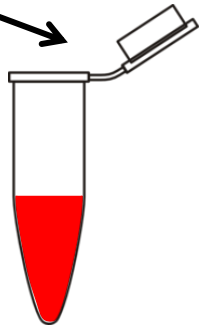
HIV
ssRNA (diploid)



Urine



Feces

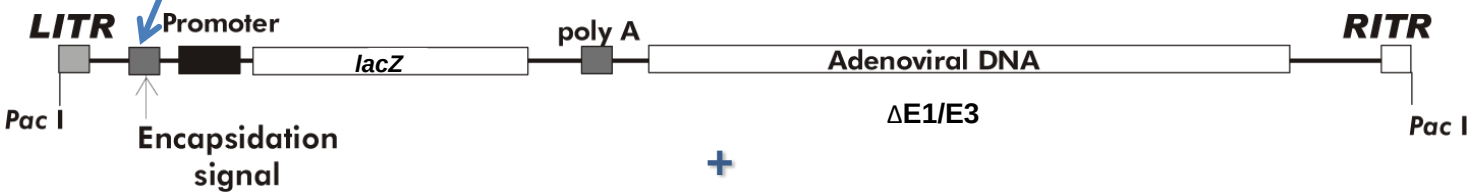


Blood

AdEasy™ Adenoviral Vector System

PCR Target Sequence

Vector

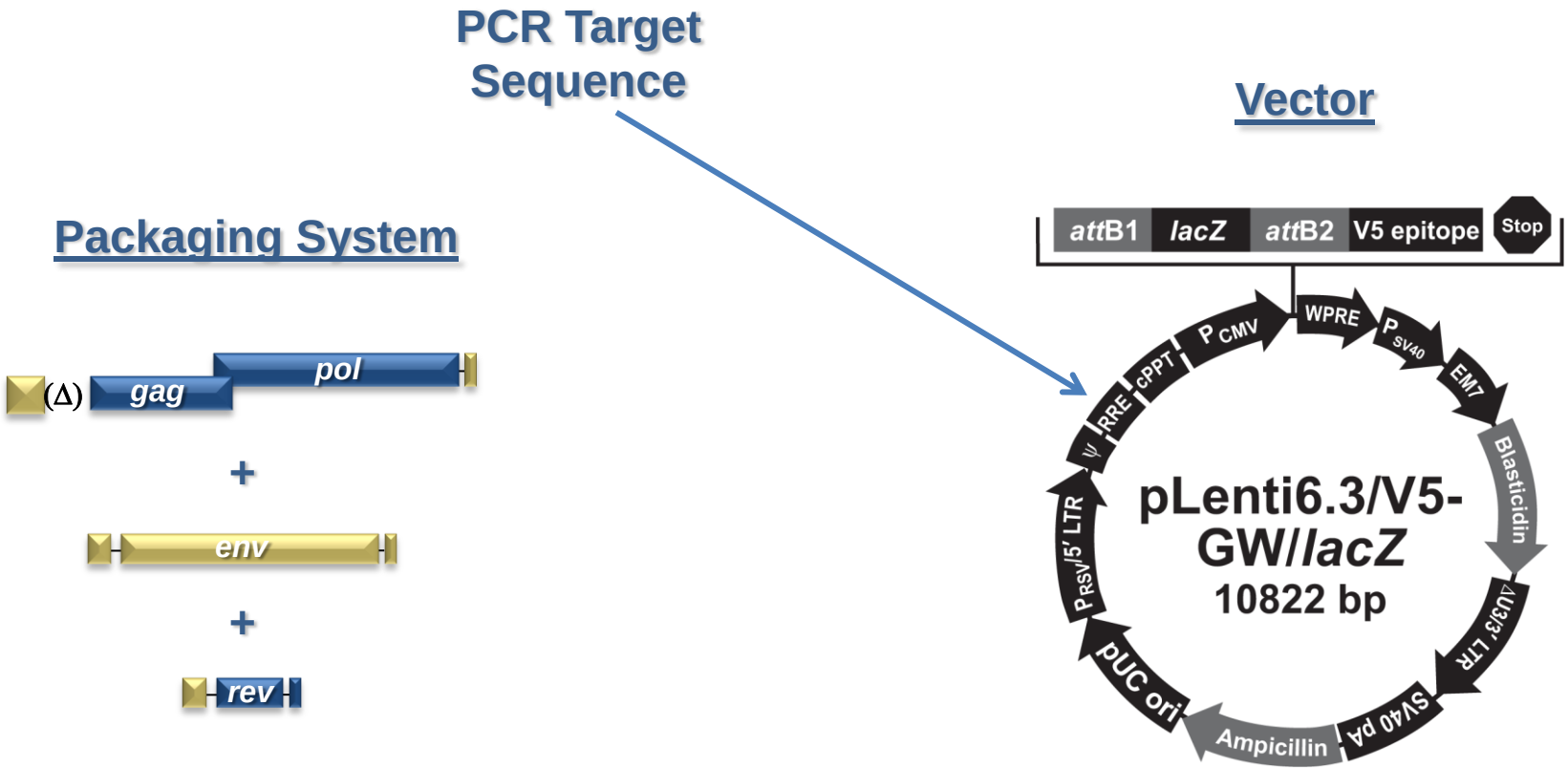


+

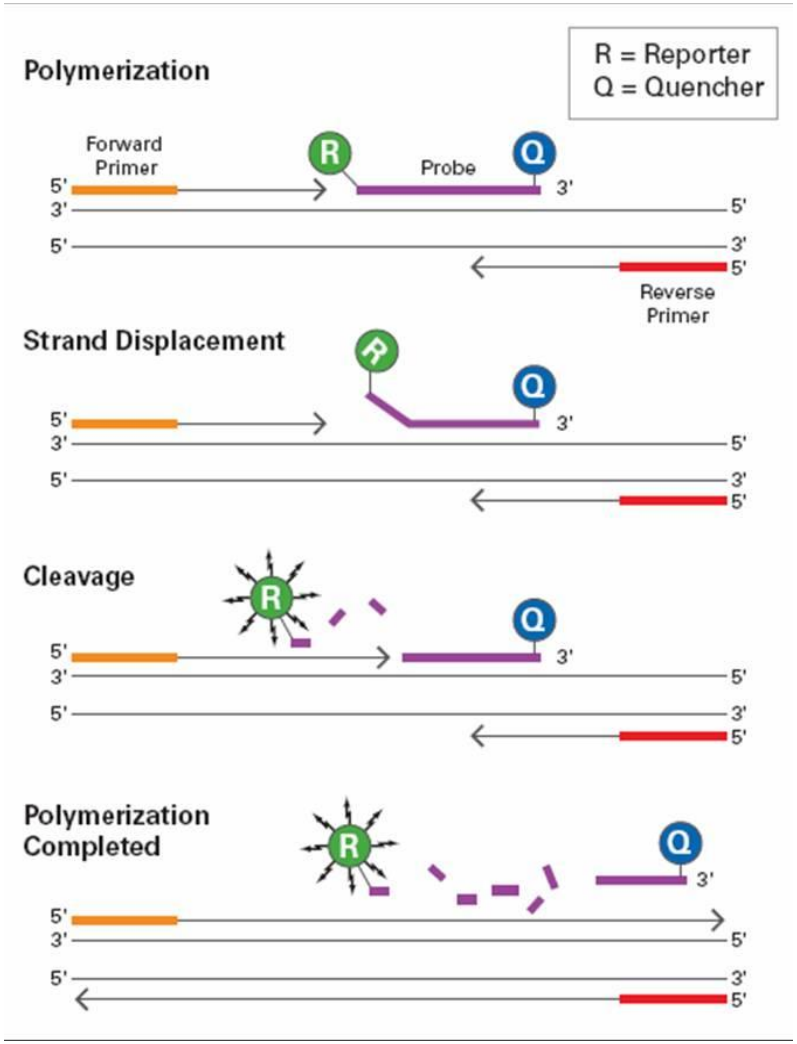
Packaging System

AD-293 Cells (E1 genes)

ViraPower™ Lentiviral Expression System (HIV)



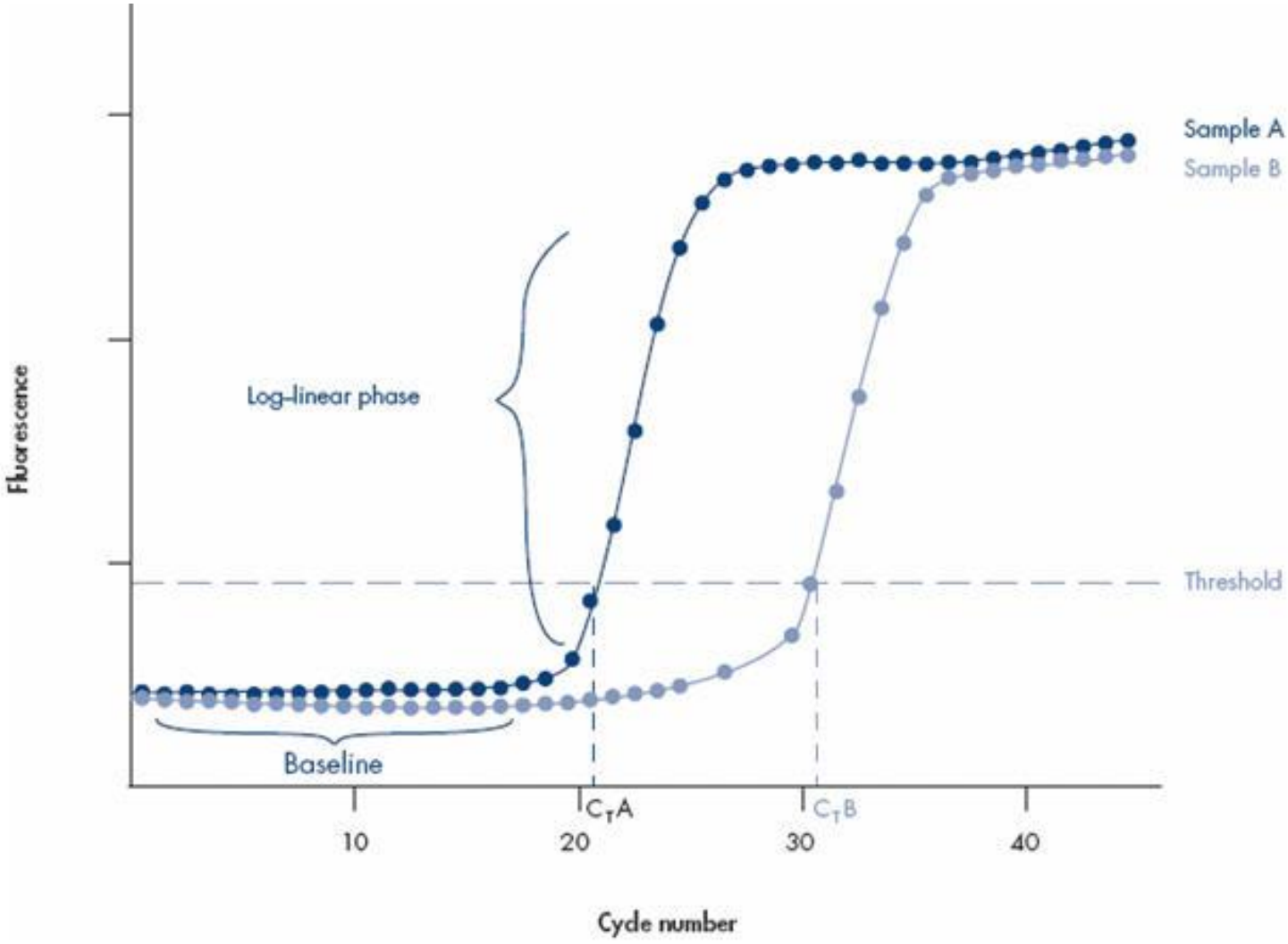
TaqMan® Chemistry



http://www.asuragen.com/Services/services/gene_expression/ab_taqman.aspx

applied biosystems

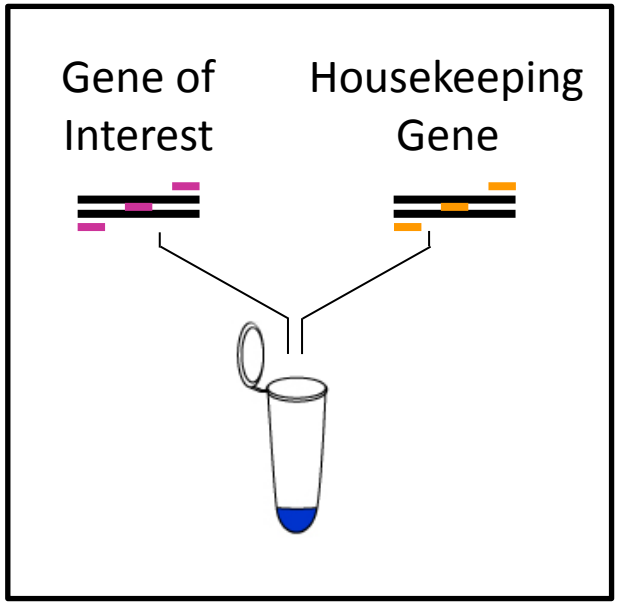
Quantitative PCR Profile



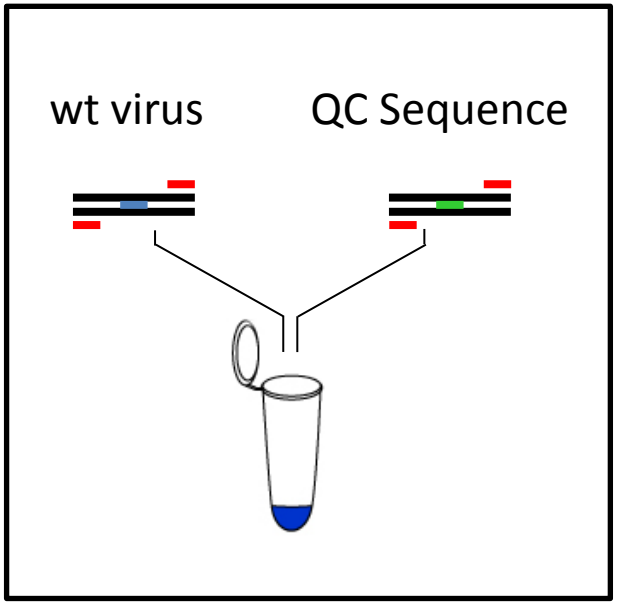
http://www.qiagen.com/resources/info/guidelines_rtpcr/dataanalysis_probe.aspx#fig1

Multiplex PCR

Common Example

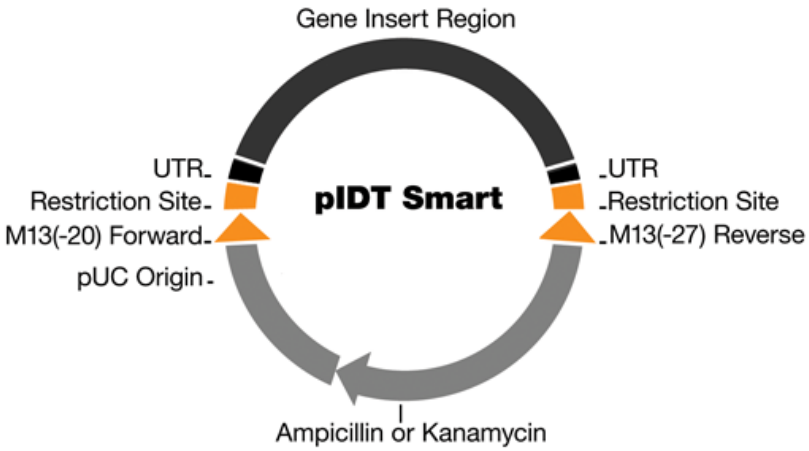


Shedding Assay

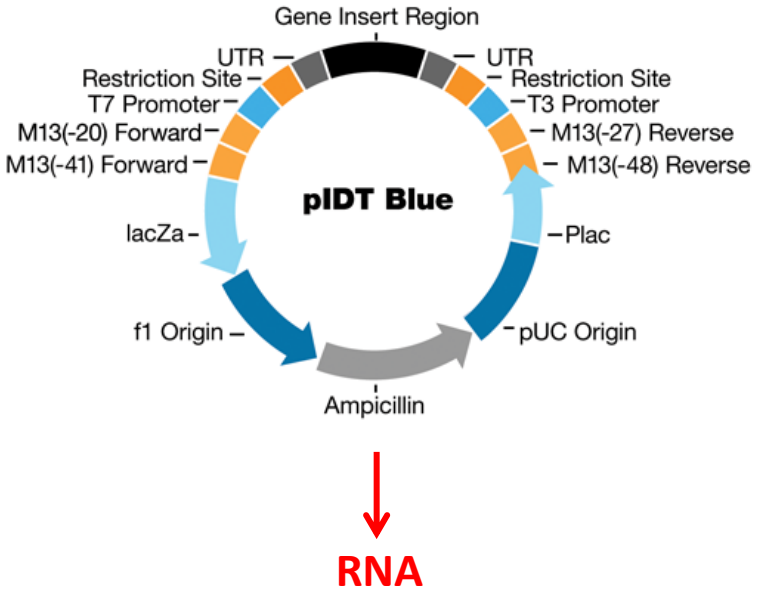


Quality Control (QC) Sequences

Adenovirus QC Sequence Cloned Here



Lentivirus QC Sequence Cloned Here



RNA

IDT
INTEGRATED DNA TECHNOLOGIES

Fluorophores and Quenchers

Nickname	Full Name	Excitation λ	Emission λ	Quenching λ
6-FAM	6-carboxyfluorescein	495 nm	520 nm	N/A
JOE	6-carboxyfluorescein-4',5'-dichloro-2',7'-dimethoxyfluorescein	529 nm	555 nm	N/A
BHQ-1	Black Hole Quencher-1	N/A	N/A	520-583 nm
BHQ-2	Black Hole Quencher-2	N/A	N/A	550-668 nm

IDT
INTEGRATED DNA TECHNOLOGIES

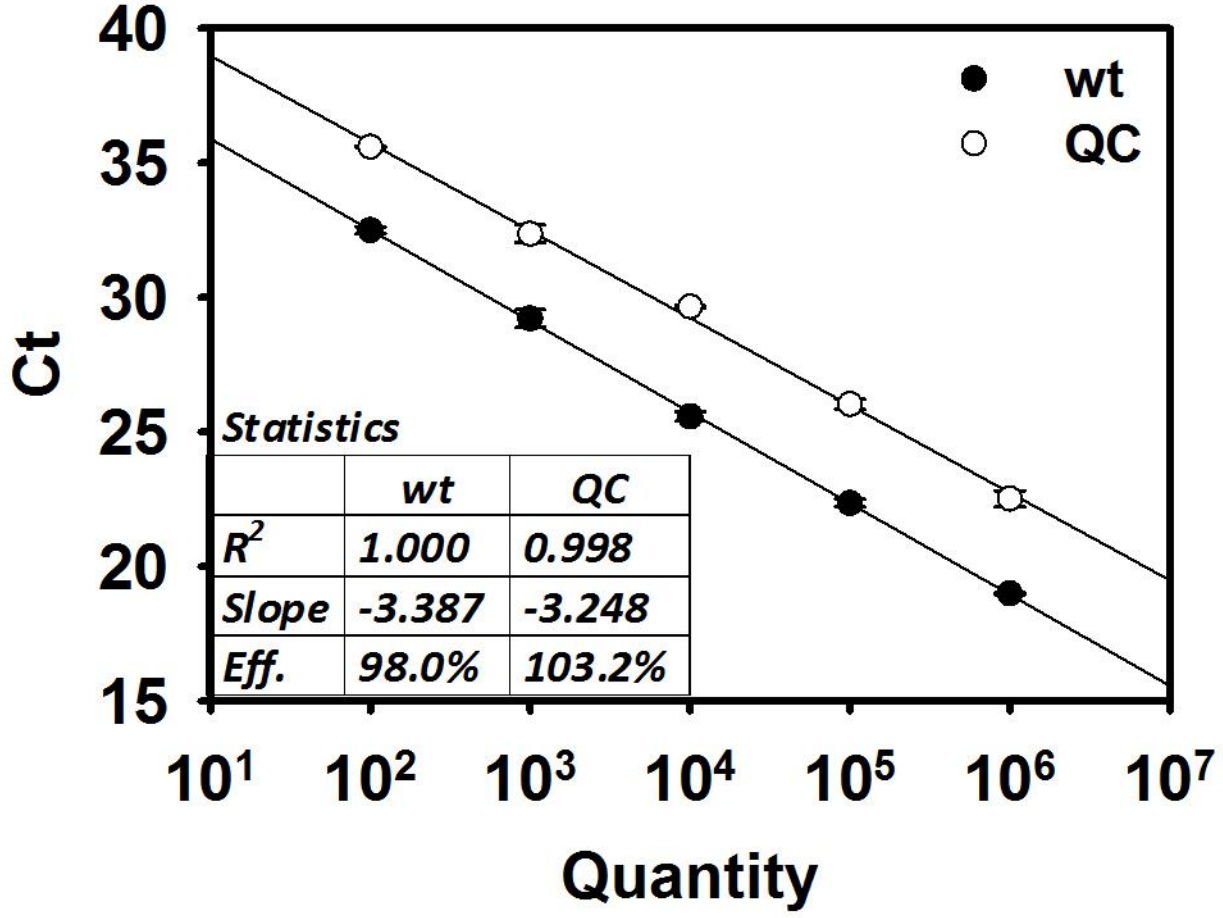
PCR Profile

- Stage 1
  - UNG Activation = 50°C, 2 min.
- Stage 2
  - Hot Start = 95°C, 10 min.
- Stage 3
  - Denaturation = 95°C, 15 sec.
  - Annealing/Extension = 60°C, 1 min.
  - Cycle Number = 40

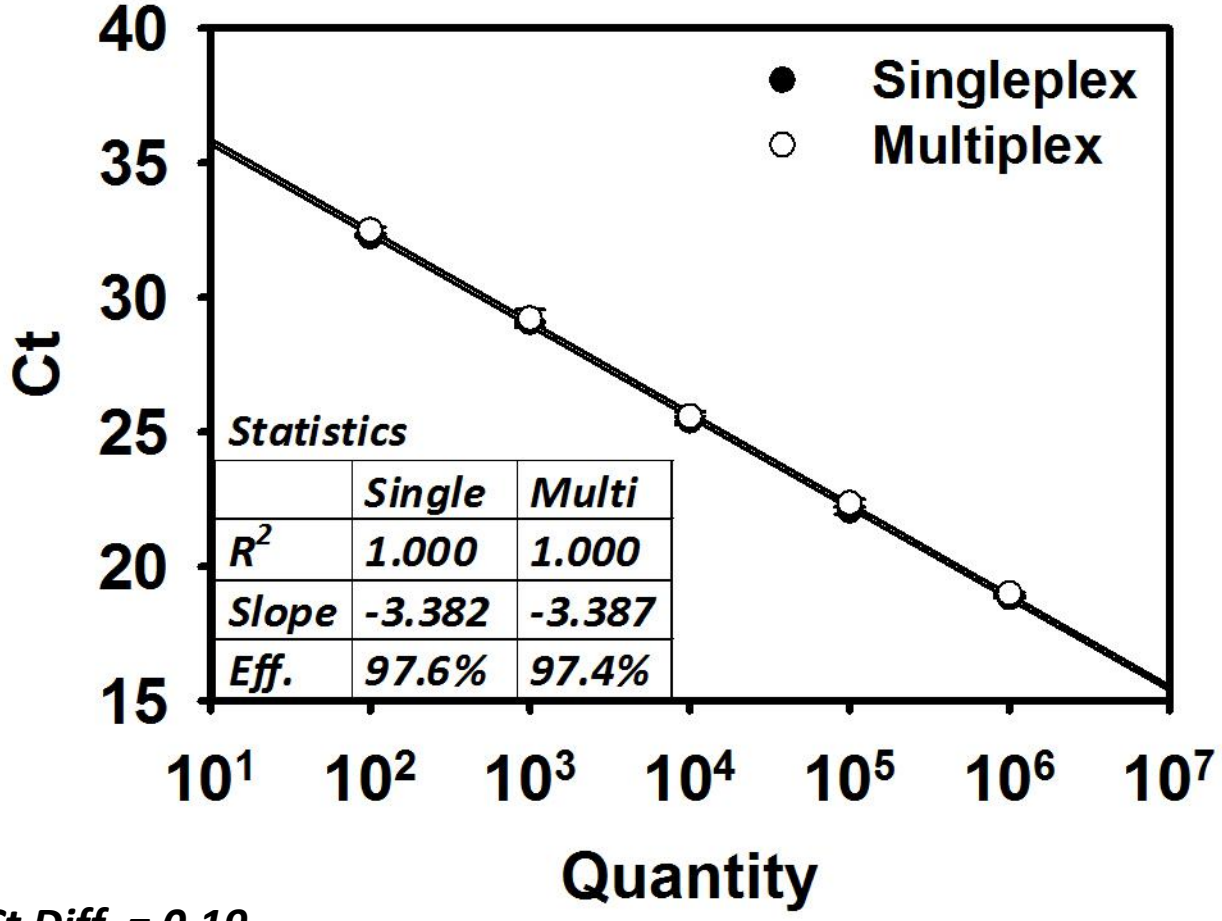
TaqMan® Universal PCR Mix

- AmpliTaq Gold® DNA Polymerase
- AmpErase® UNG
- dNTPs with dUTP
- Passive Reference 1
- Proprietary Buffer Components

Adenovirus Multiplex

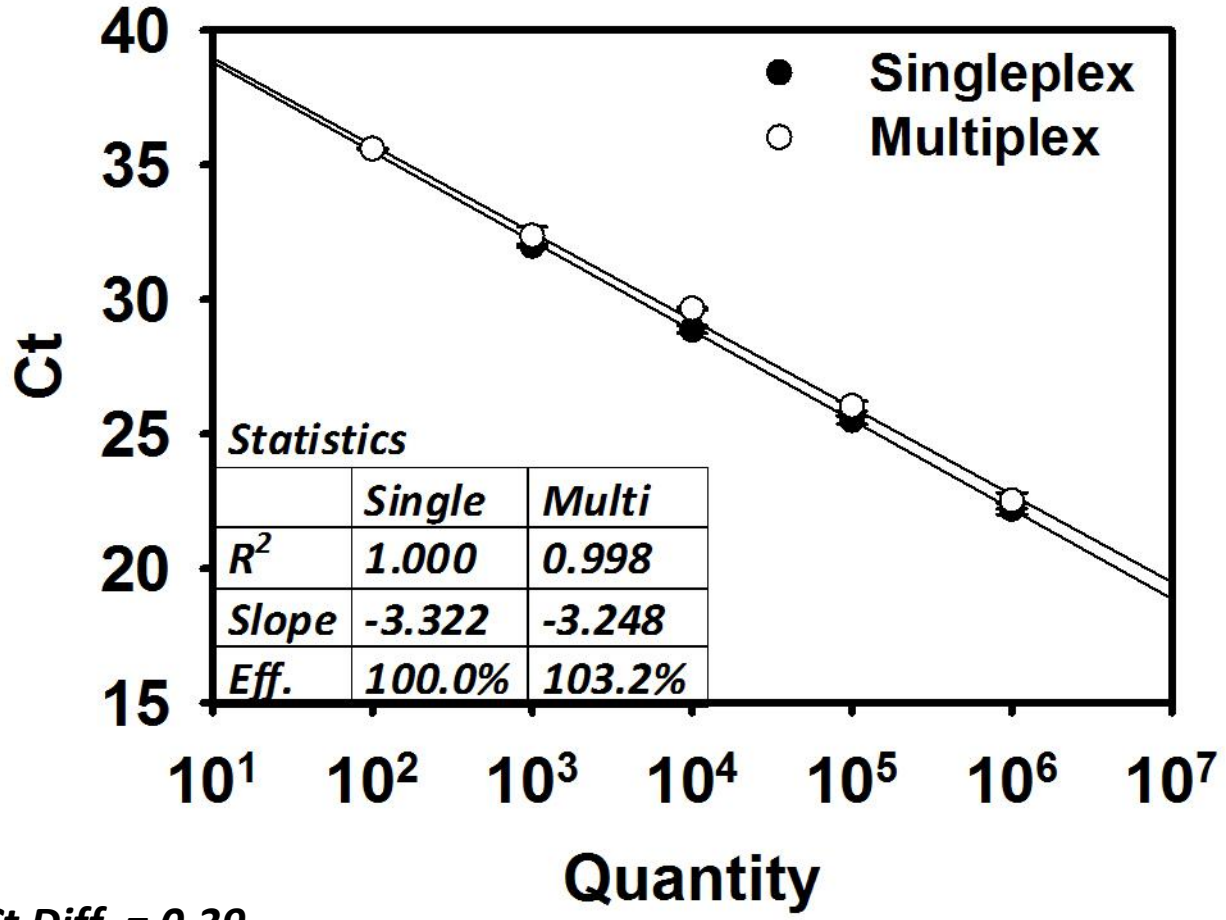


Adenovirus Wild-type Sequence



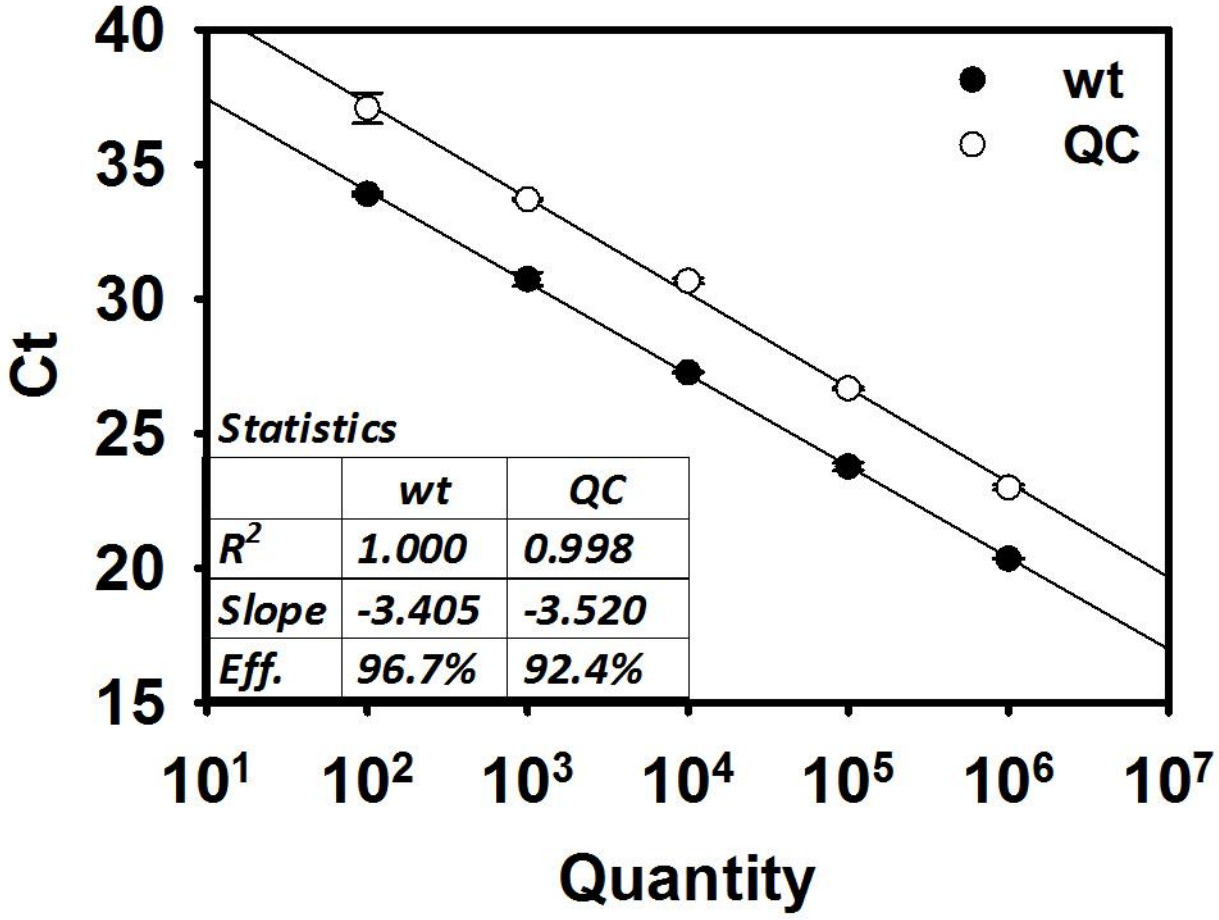
Avg. Ct Diff. = 0.19

Adenovirus QC Sequence

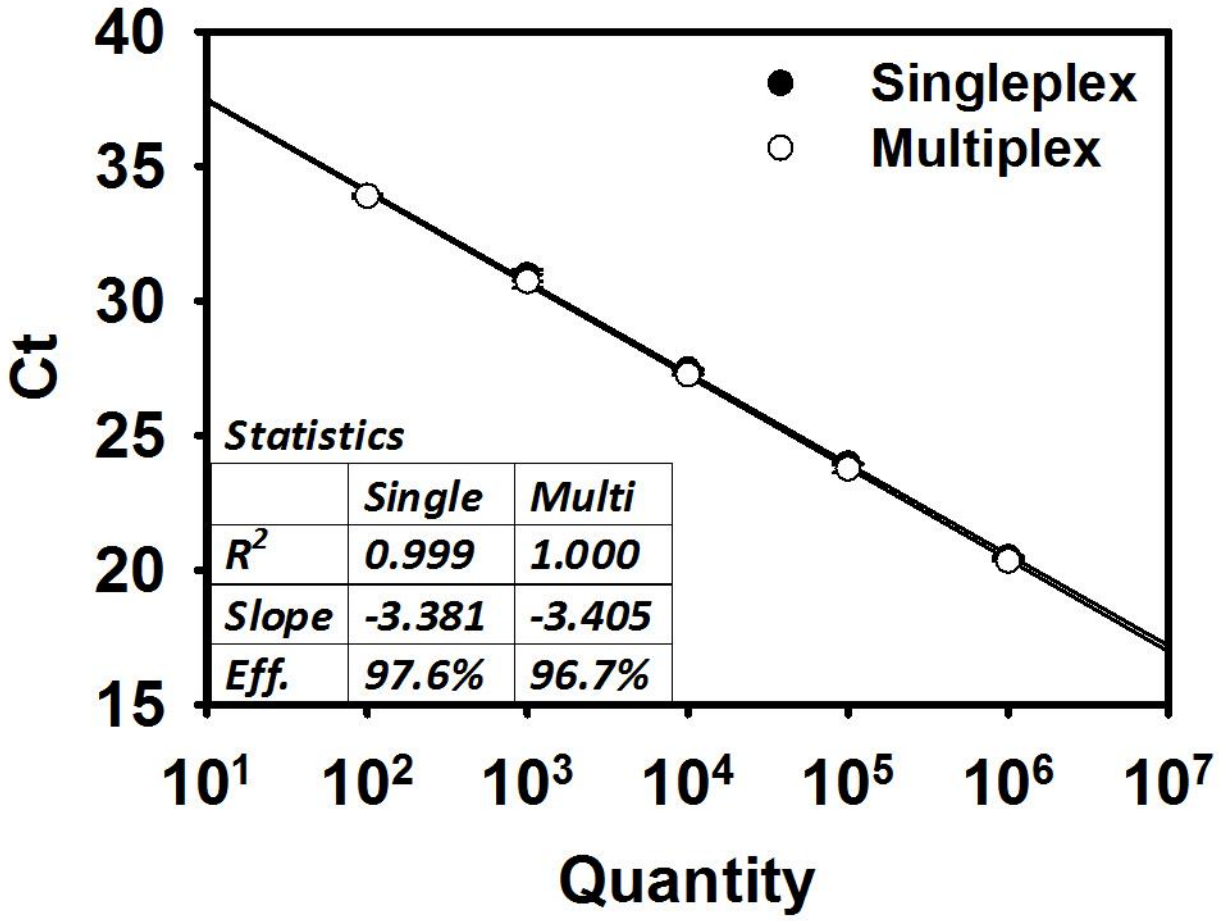


Avg. Ct Diff. = 0.39

Lentivirus Multiplex

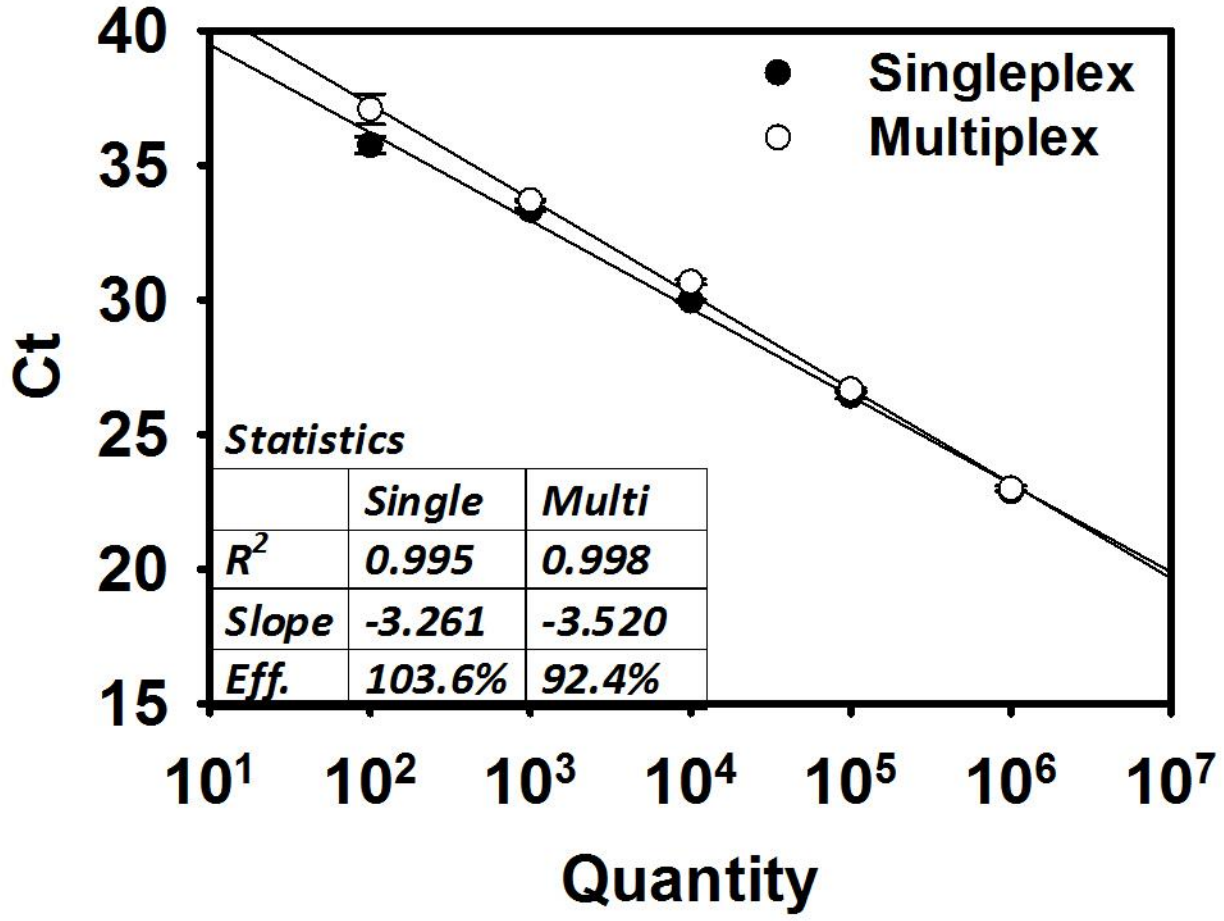


Lentivirus Wild-type Sequence



Avg. Ct Diff. = 0.14

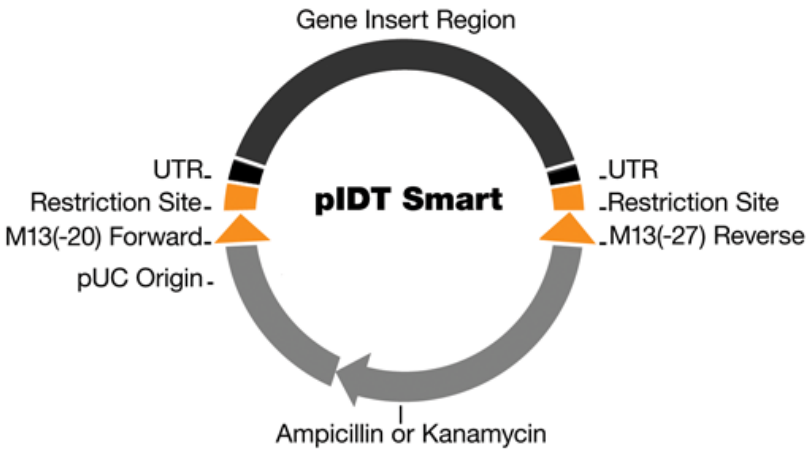
Lentivirus QC Sequence



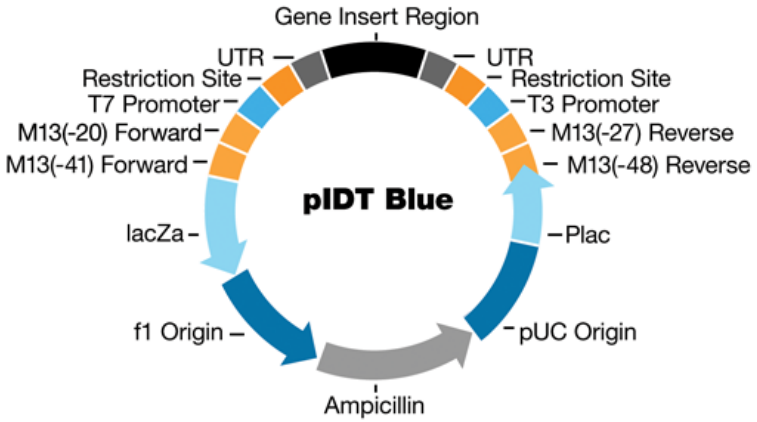
Avg. Ct Diff. = 0.54

Quality Control (QC) Sequences

Adenovirus QC Sequence Cloned Here



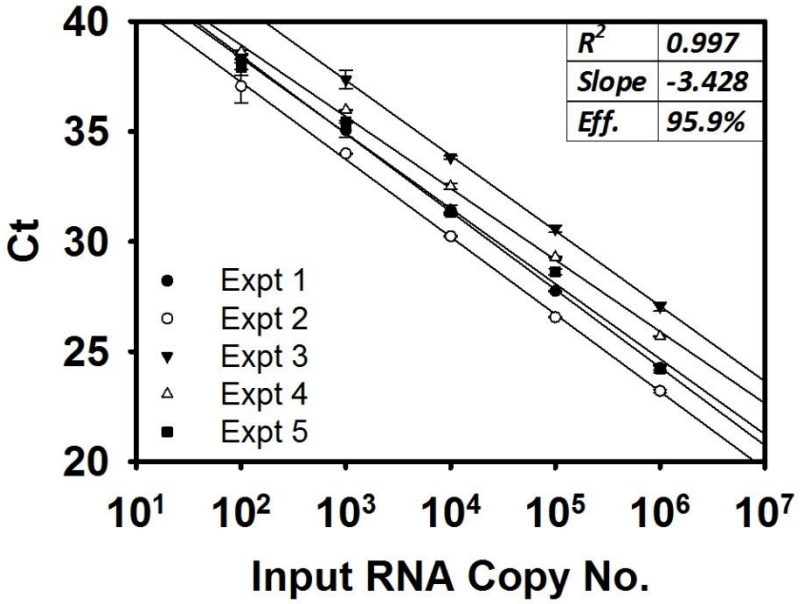
Lentivirus QC Sequence Cloned Here

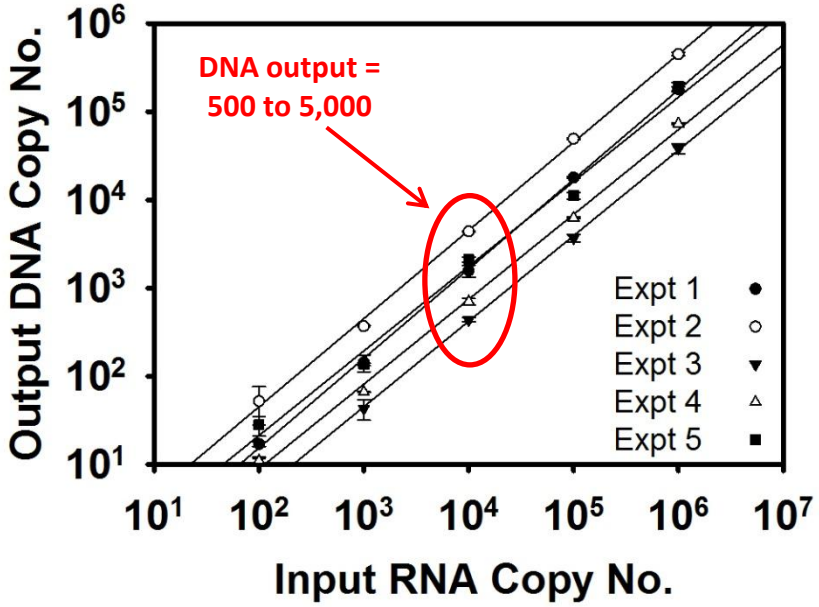


RNA

High Capacity cDNA Kit

Quality Control pLenti RNA Reverse Transcription Q-PCR





Average reverse transcription efficiency ~ 20%
(Range 5 to 50%)

Virus Stock Characterization

Stock	Infectious Titer (pfu/ml or cfu/ml)	Total No. of Virus Particles (vg/ml)	Total Particle: Infectious Particle Ratio
Adeno Stock #1	6×10^7	7×10^8	12
Adeno Stock #2	4×10^8	2×10^9	5
Lenti Stock	1×10^7	3×10^{11}	3×10^4

Summary

- A highly sensitive and specific Q-PCR assay of broad utility has been successfully developed to detect the shedding of viral vectors in excretions of experimentally infected animals.
- The inclusion of quality control sequences will ensure the avoidance of false negatives and will allow us to determine the limits of detection.
- Due to the reverse transcription step for the lentiviral (HIV) vector, the limit of detection will be higher than for the adenoviral vector.

Ongoing Studies

- Spiked Controls
- Infected Animal Experiments

Acknowledgements

- ABSA Griffin Grant Program
- Ms. Swathee Chinnasamy
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  - M.S. Program in Anatomy
- Dr. Adrian Corbett
  - Department of Neuroscience, Cell Biology, and Physiology

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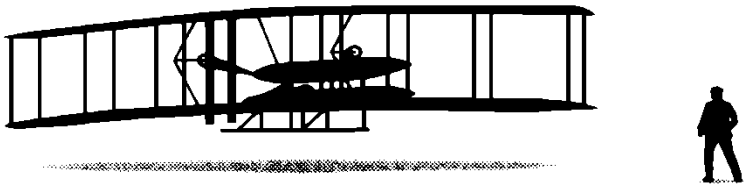
Questions?



“The difficulty lies, not in the new ideas, but in escaping from the old ones, which ramify, for those brought up as most of us have been, into every corner of our minds.”

John Maynard Keynes (1936)