

Design of Hybrid Viral Vectors via association of Murine Leukemia Viruses with synthetic agents

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Real-Time PCR for Quantification of Moloney Murine Leukemia Viruses

Design of Hybrid/Viral Gene Therapy Vectors

Research Objective

Gene Therapy

- Mutation/Error in gene leads to malfunctioned or insufficient production of protein
- Gene Therapy is the correction of erroneous gene via external transfer of correct gene
- Cure for inherited and acquired diseases
- Side effects of incorrect delivery and trafficking is the biggest obstacle to gene therapy

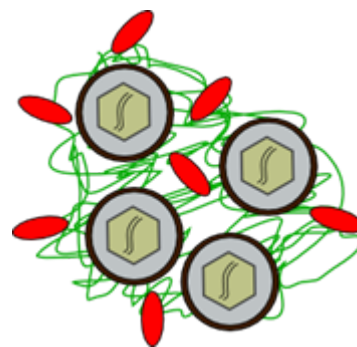
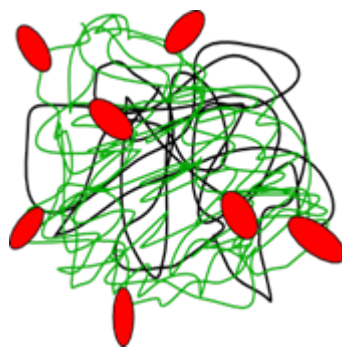
- Types of vectors
 - Viral Vectors (adenoviruses, retroviruses etc.)
 - Non-viral vectors (PEI, PLL, PEG, Lipofectamine)

Research Objective

Aim

To design a hybrid-retroviral gene therapy vector based on the Moloney Murine Leukemia Virus for enhanced cell targeting and physical stability with optimal gene transfection levels

DNA (black) complexed with targeted (red) polycation (green)

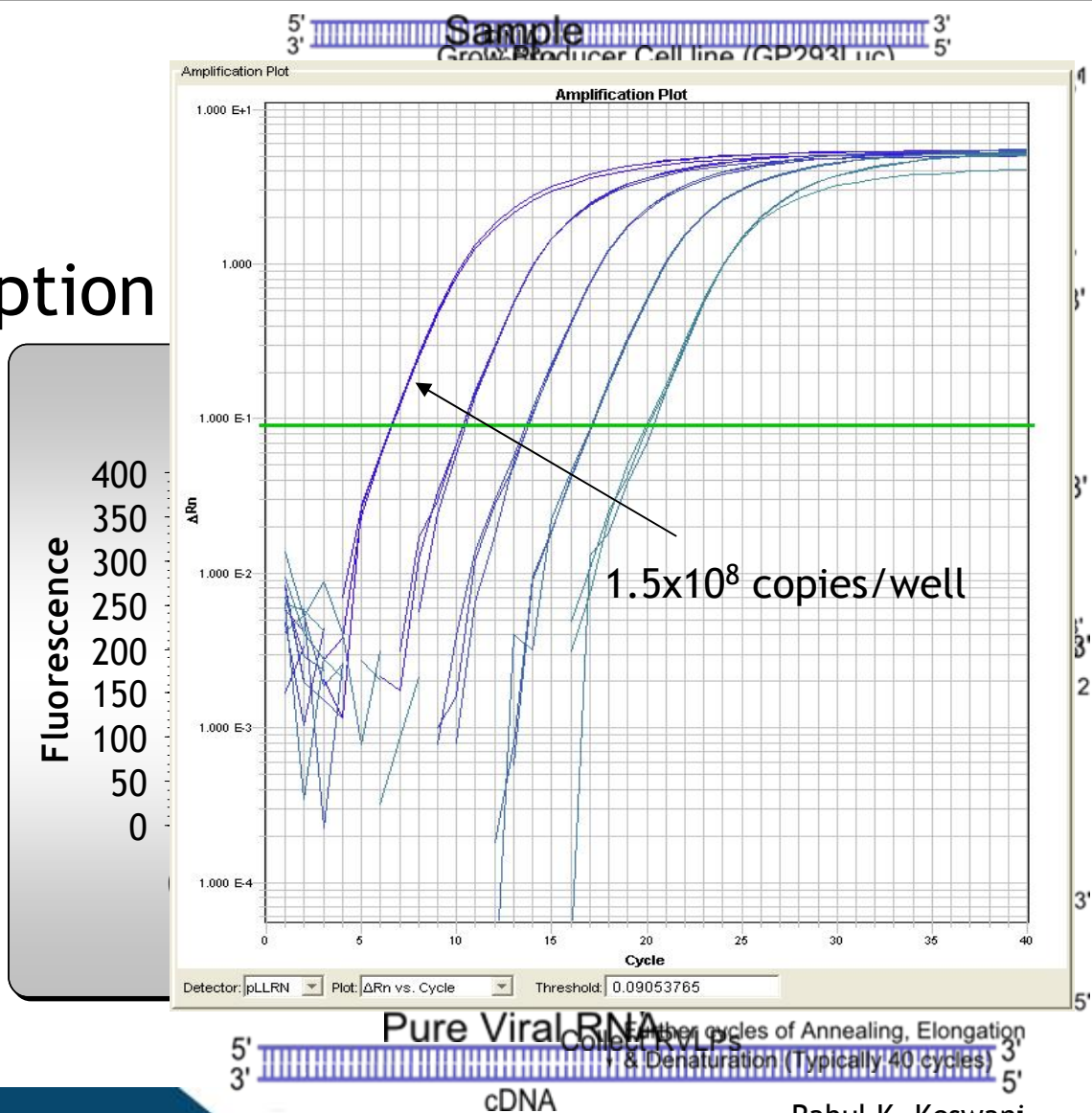


Retroviruses (RVLPs) complexed with targeted (red) polycation (green)

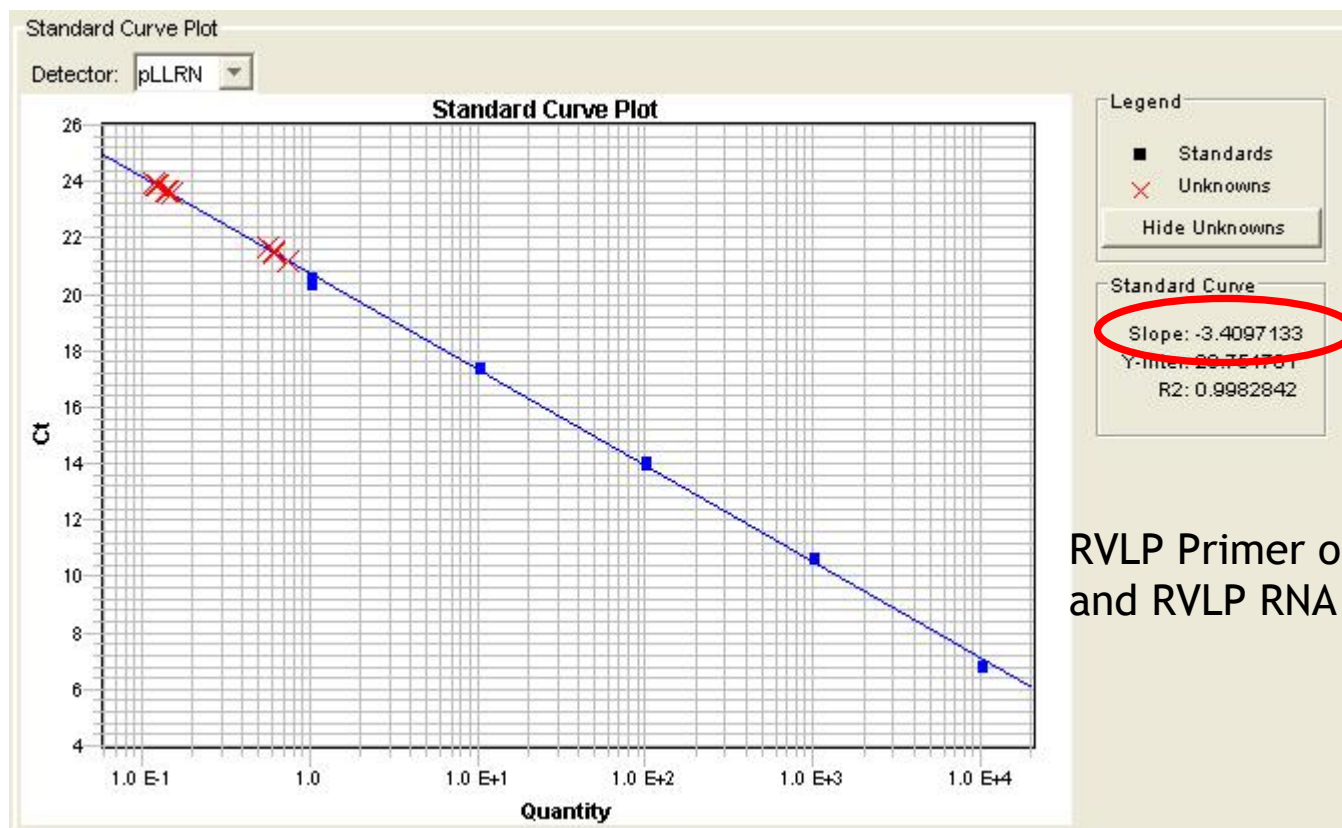
RT-PCR Methodology

Protocol

- Virus Collection
- RNA Isolation
- Reverse Transcription
- PCR (Standards)
- DNA Purification
- PicoGreen Assay
- Real-time PCR



Standard Curve



■ - Standard
× - Unknown

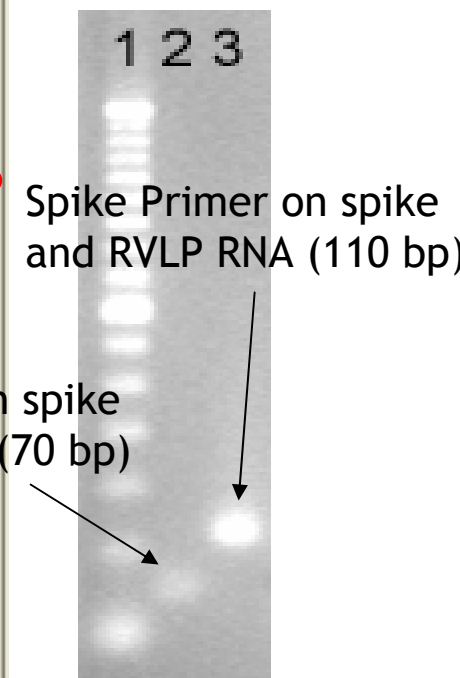


Figure 1: - (Left) Calibration Curve for RVLV cDNA standards (C_t vs. Quantity) (Y-axis scale : - 1= 1.5×10^4 copies/well); (Right) Gel Electrophoresis of PCR Products

Transfection Results

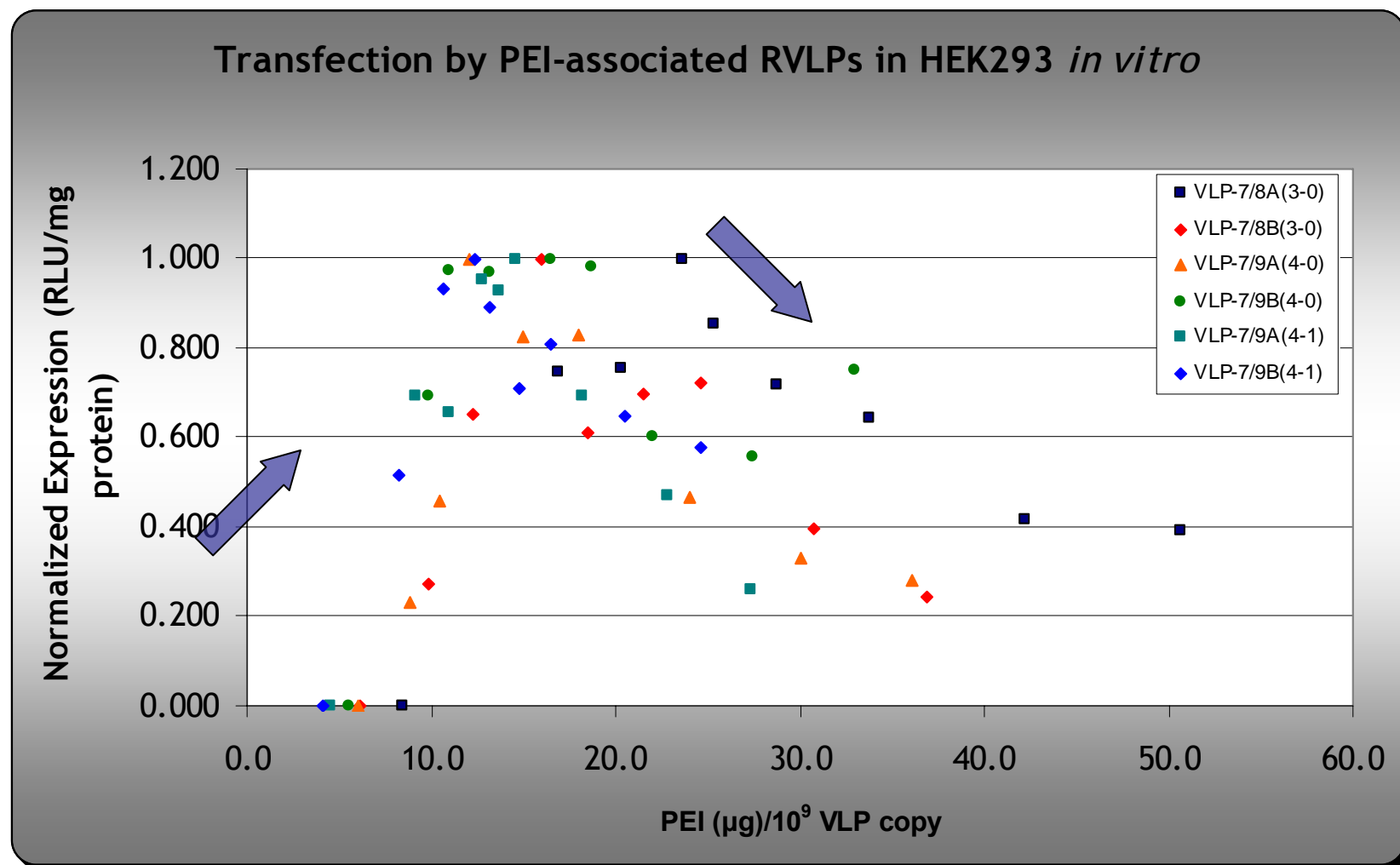


Figure 2: - Transfection of RVLPs grown in DMEM + 10% FBS

Transfection Results

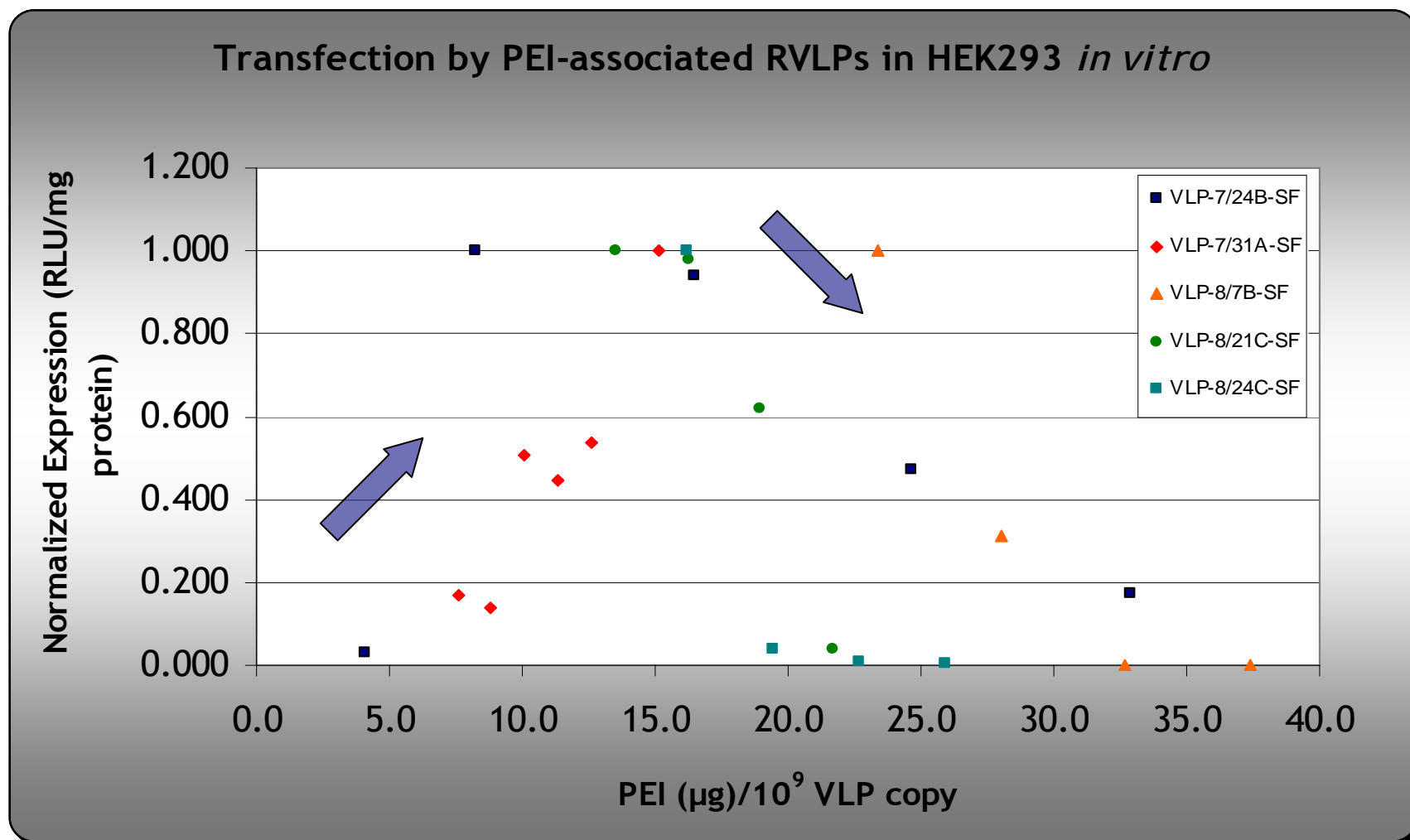


Figure 3: - Transfection of RVLPs grown in serum-free DMEM

Transfection Results

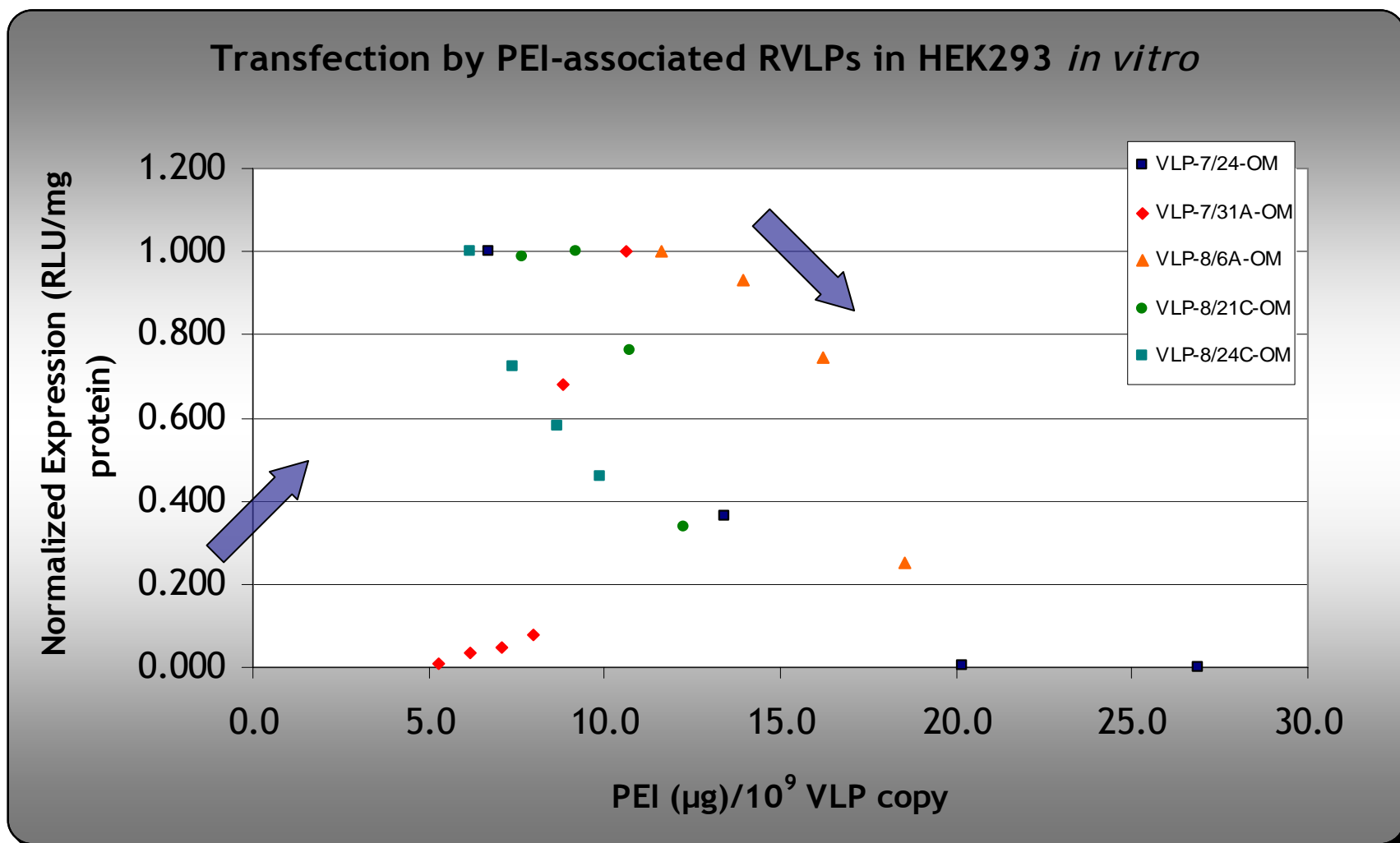


Figure 4: - Transfection of RVLPs grown in OptiMEM

Stealth Virus

Idea

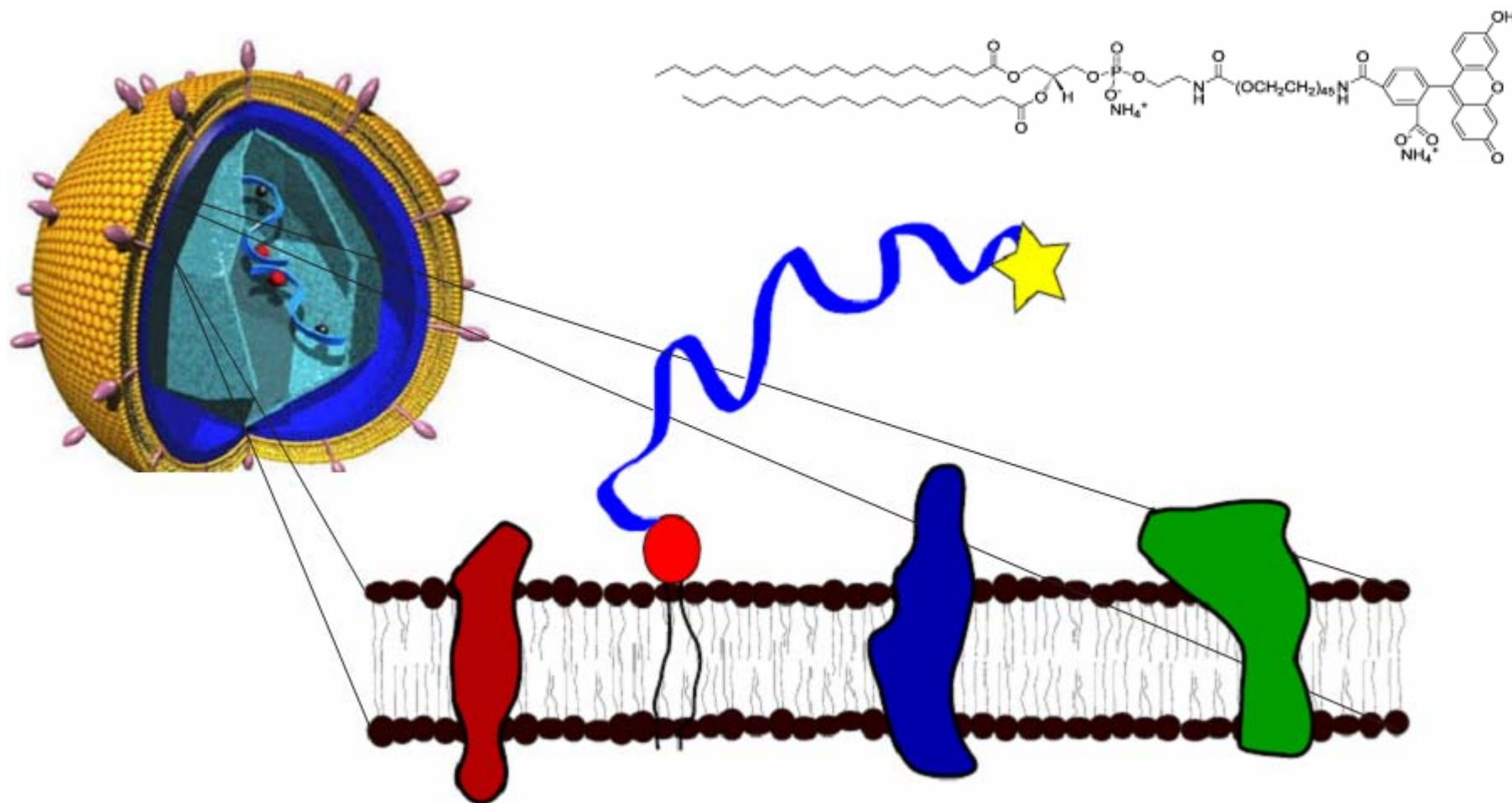


Figure 5: - Design of DSPE-PEG2000-CF/RVLP complexes via post-insertion

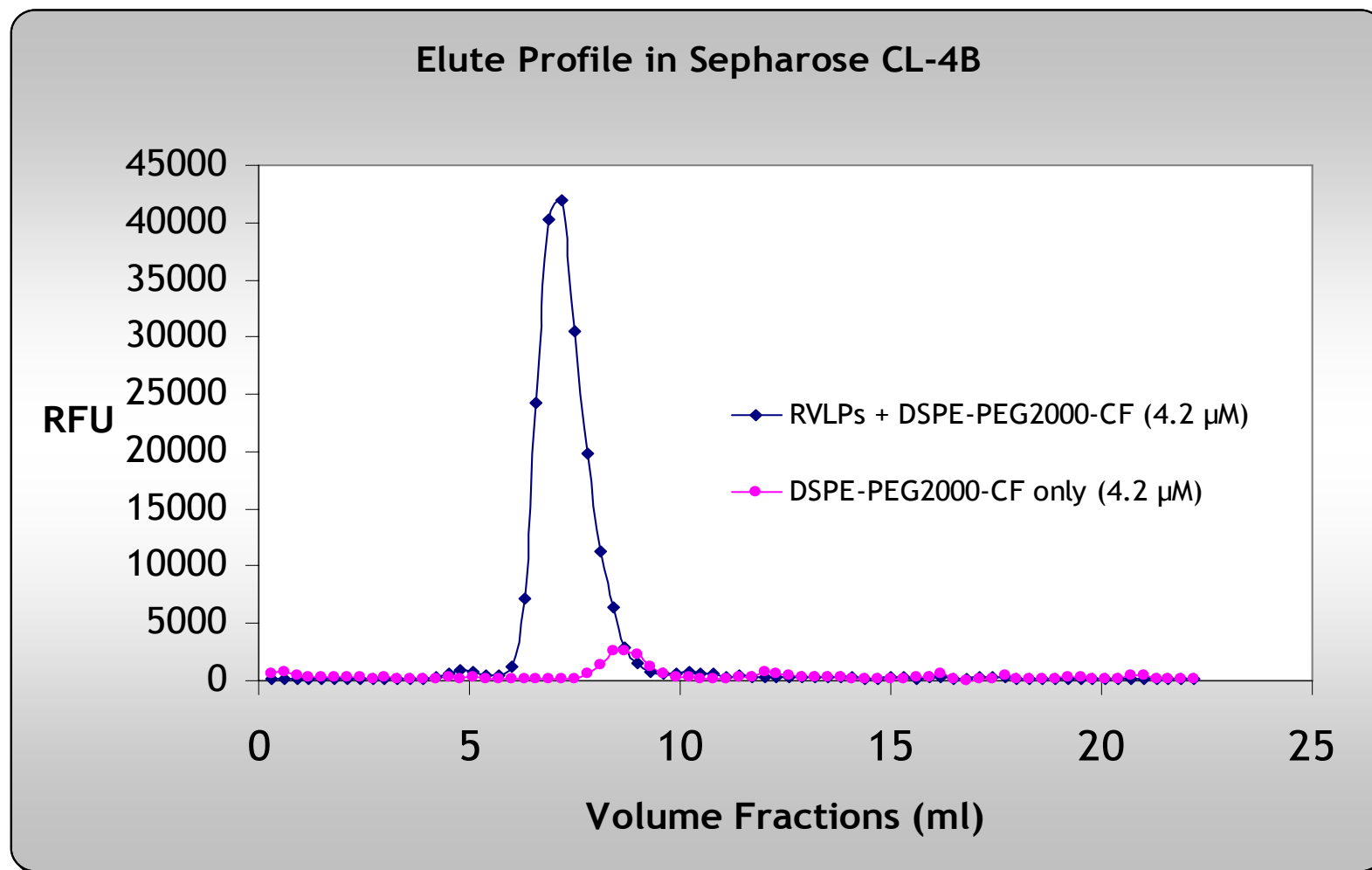


Figure 6: - Elution Profile of complexes through a Sepharose CL-4B bed

- Real-time PCR Assay developed successfully allowing for quantification of the retroviral population in cell supernatant
- Polymer/RVLP & lipid/RVLP complexes have been synthesized
- Transfection of Polymer/RVLP further optimized by using exact viral population
 - Previous studies used constant viral volumes
- Serum proteins have a significant effect on complex formation and transfection
- Further studies
 - Long term stability of gene transfection
 - Cytotoxicity studies
 - Complex characterization studies (TEM & DLS)
 - Further optimization of the lipid/RVLP complex formation

Thank You....

Appendix

RT-PCR

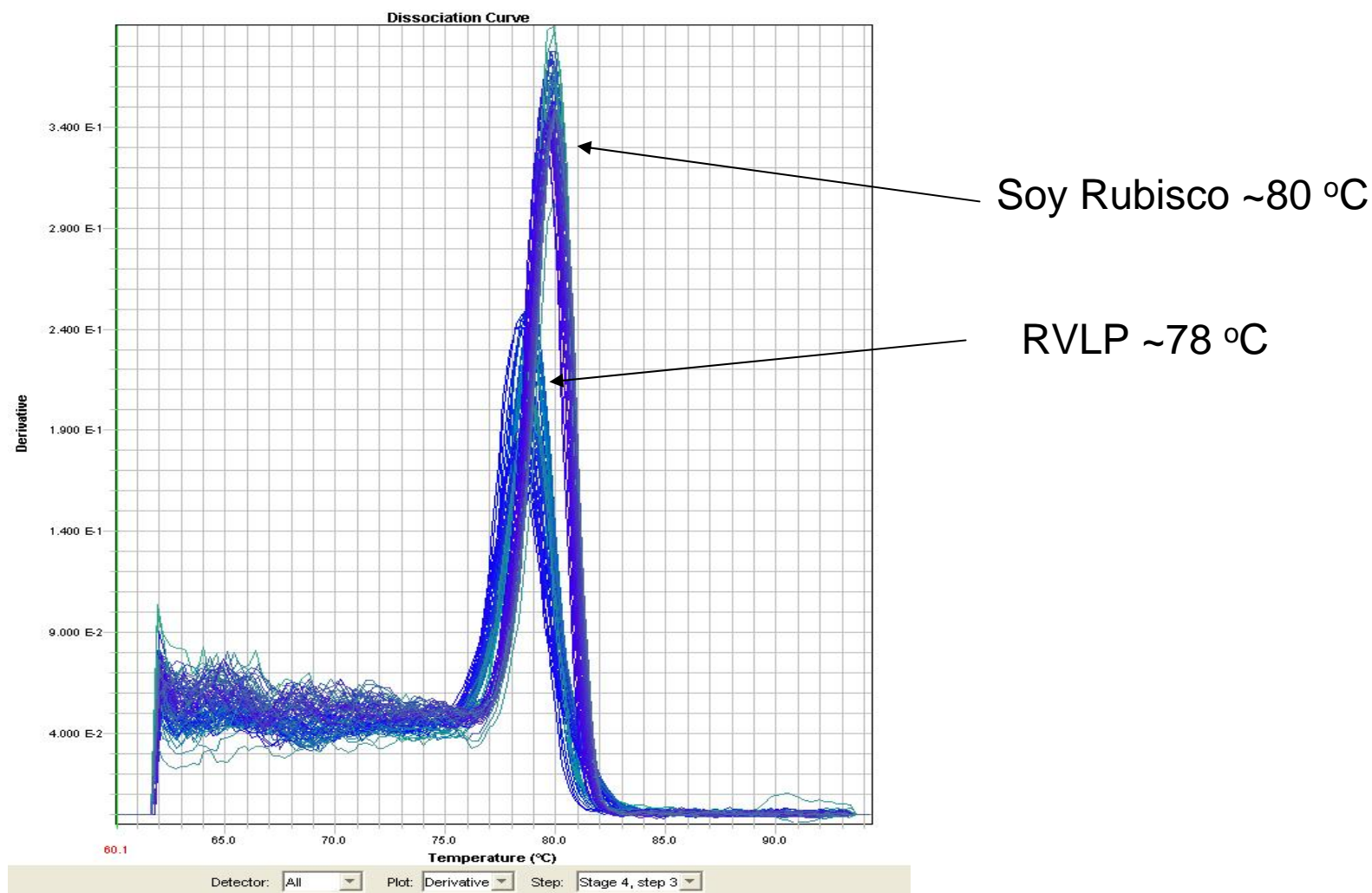


Figure A1: - Dissociation peaks of PCR products after amplification

Appendix

RT-PCR

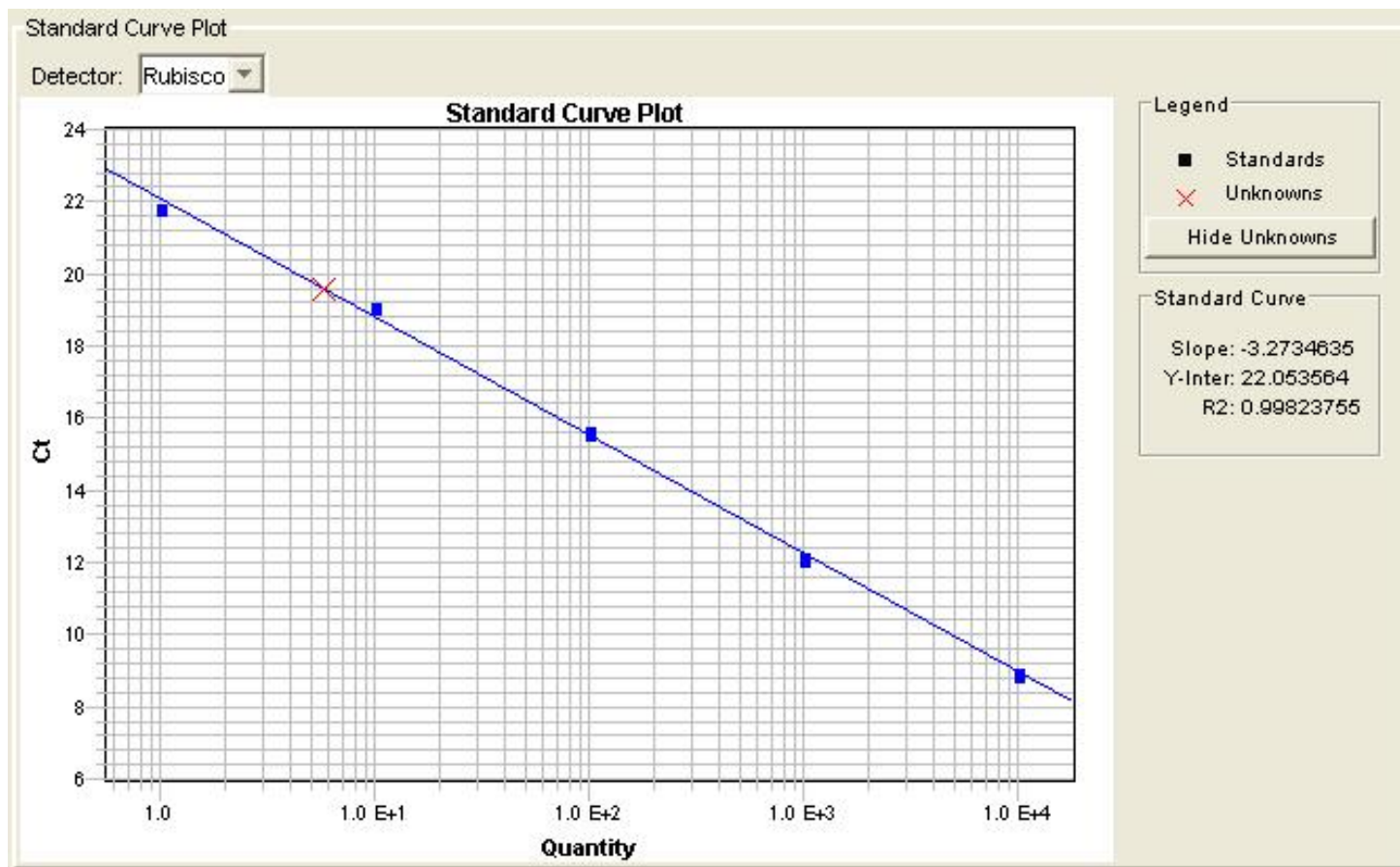


Figure A2: - Calibration Curve for SoyRubisco cDNA standards (Ct vs. Quantity)
(Y-axis scale : -)

Appendix

RT-PCR

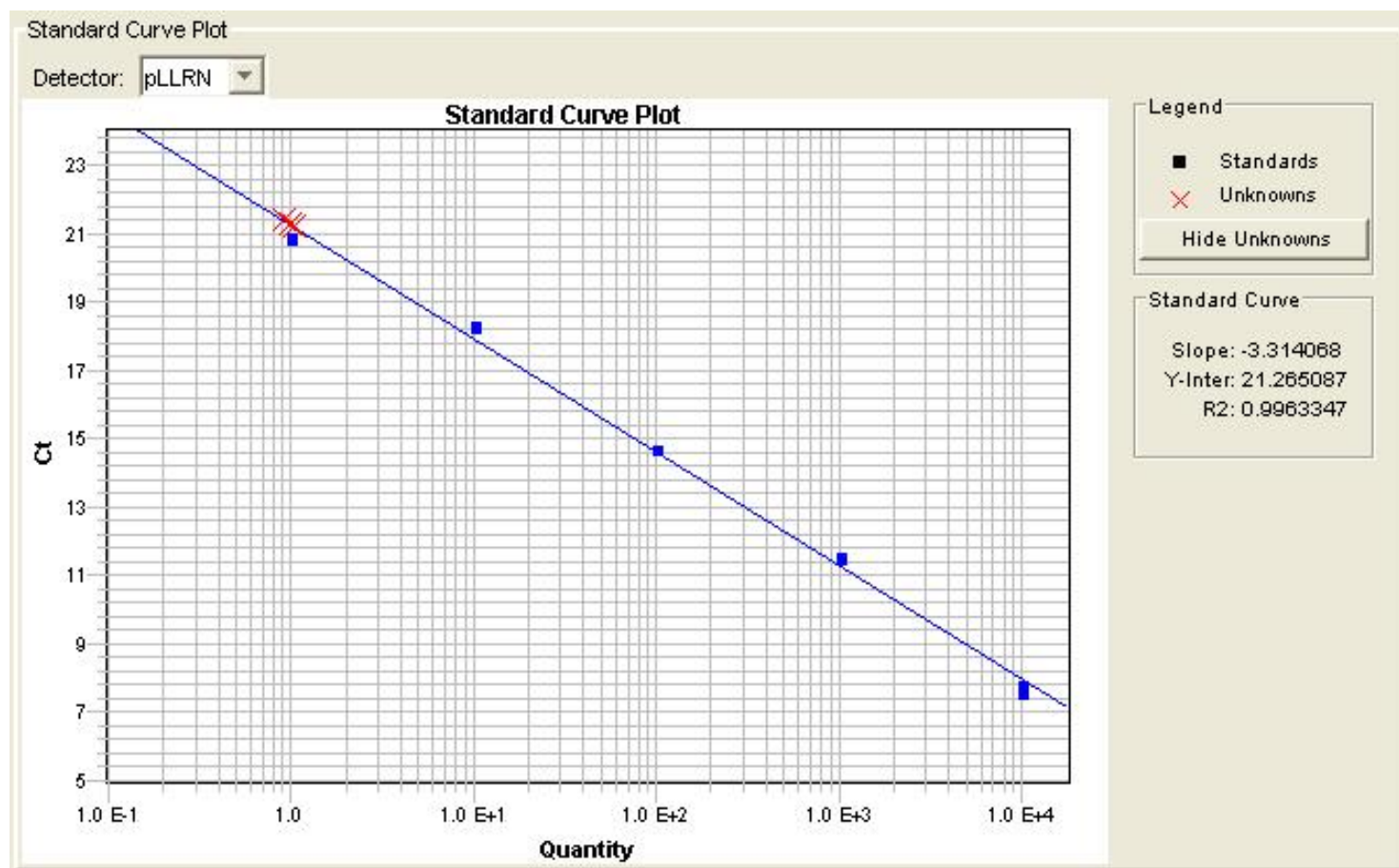


Figure A3: - Alternate Calibration Curve for RVLP cDNA standards (Ct vs. Quantity) (Y-axis scale : -)

Appendix

Transfection Results

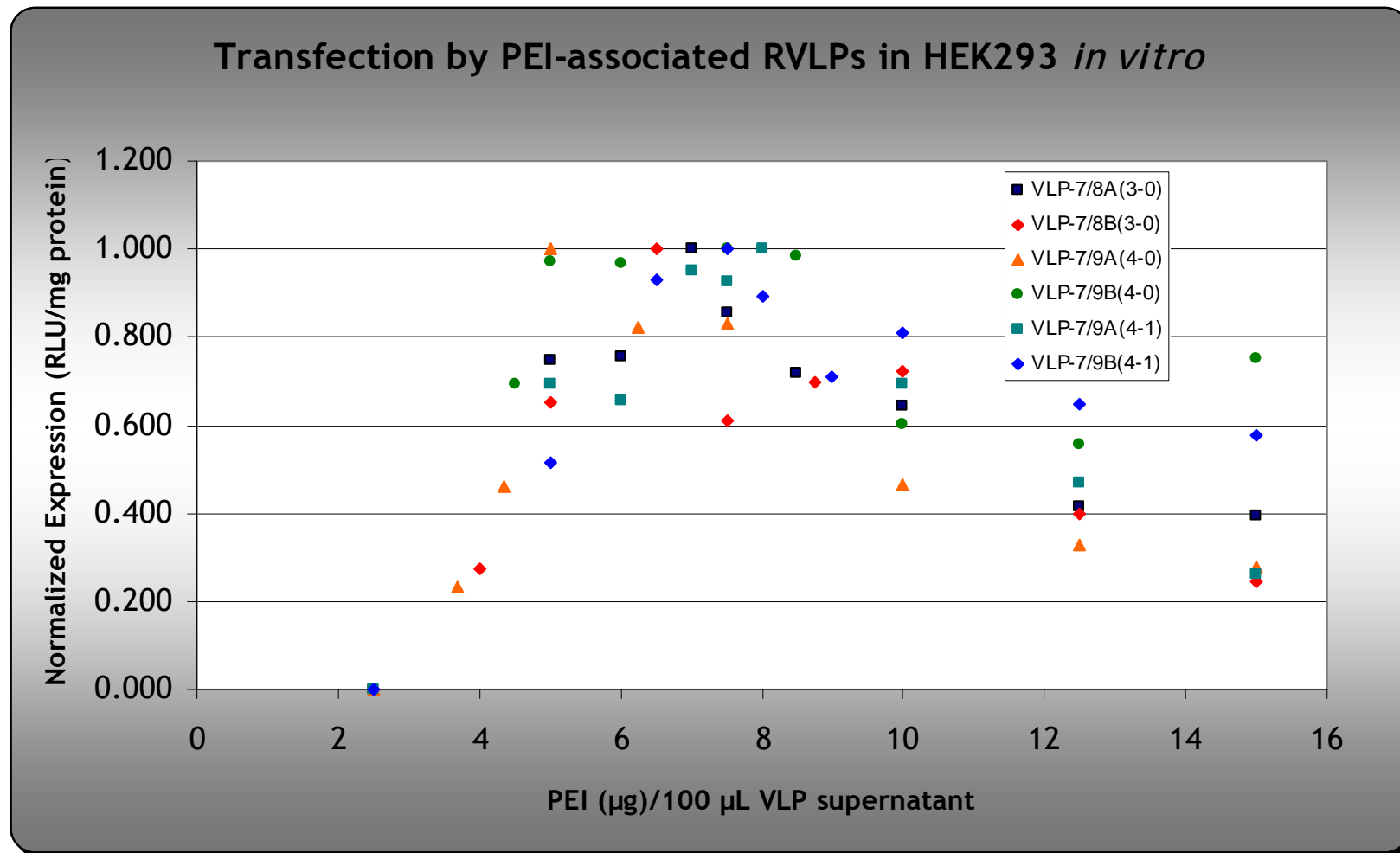


Figure A4: - Transfection of RVLPs grown in DMEM + 10% FBS

Appendix

Transfection Results

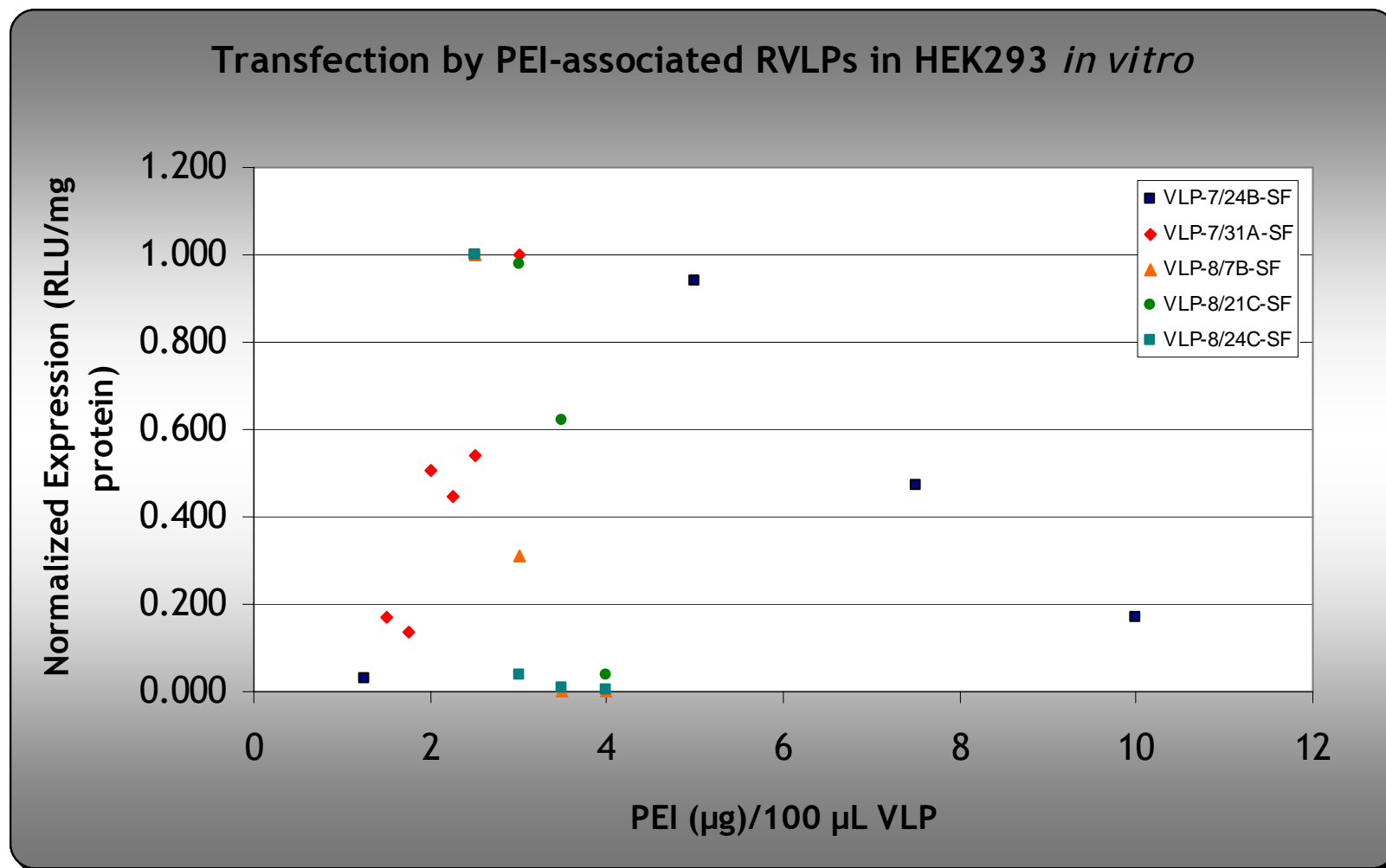


Figure A5: - Transfection of RVLPs grown in serum-free DMEM

Appendix

Transfection Results

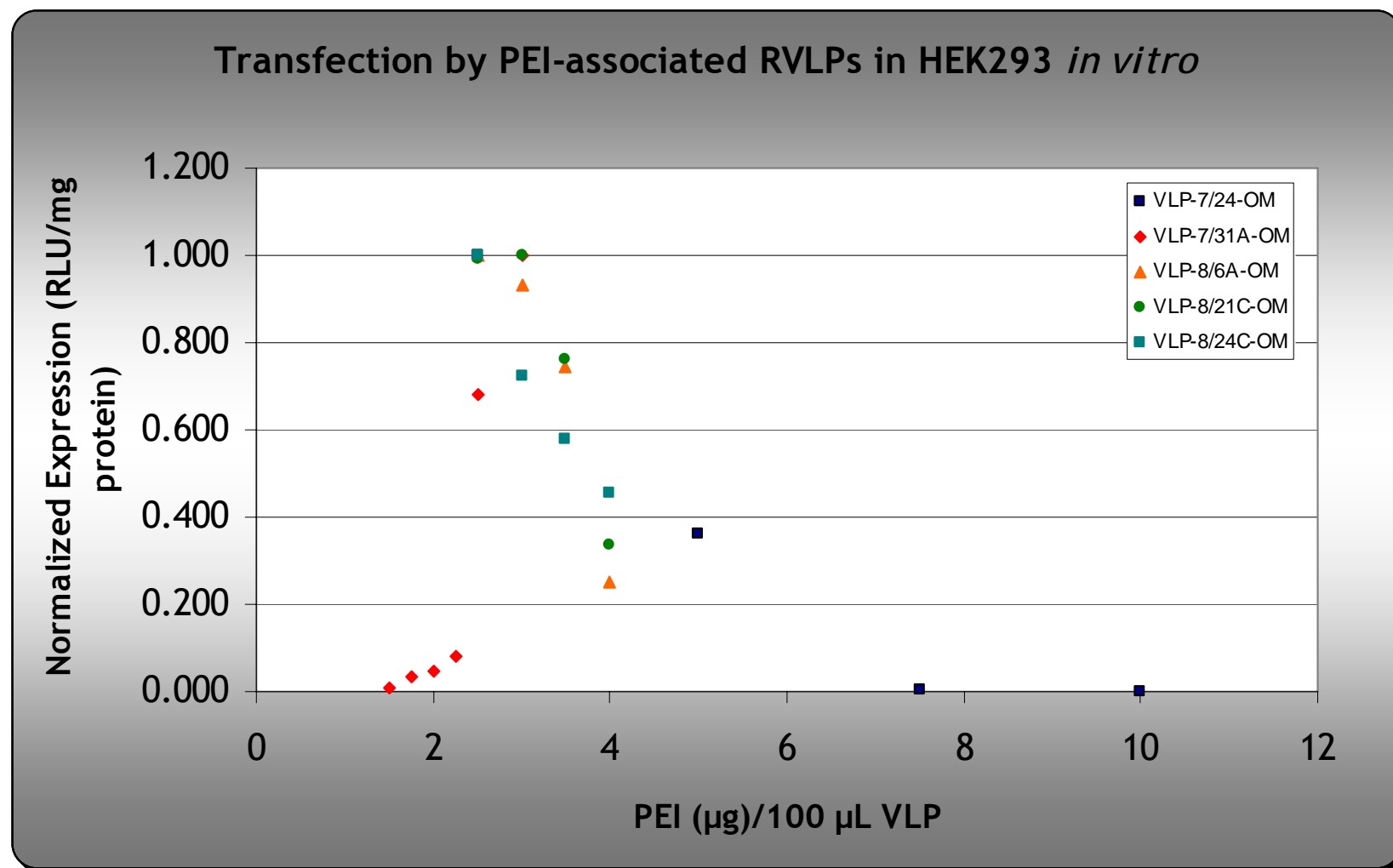


Figure A6: - Transfection of RVLPs grown in OptiMEM

Appendix

Protocol for lipid/RVLP complex

Ultracentrifugation (20,000 rpm, 4 °C, 2 hrs)



Resuspension and Co-incubation with lipid



Separation through Size Exclusion
Chromatography



Fluorescence Measurements

Appendix

Mechanism of Post-Insertion

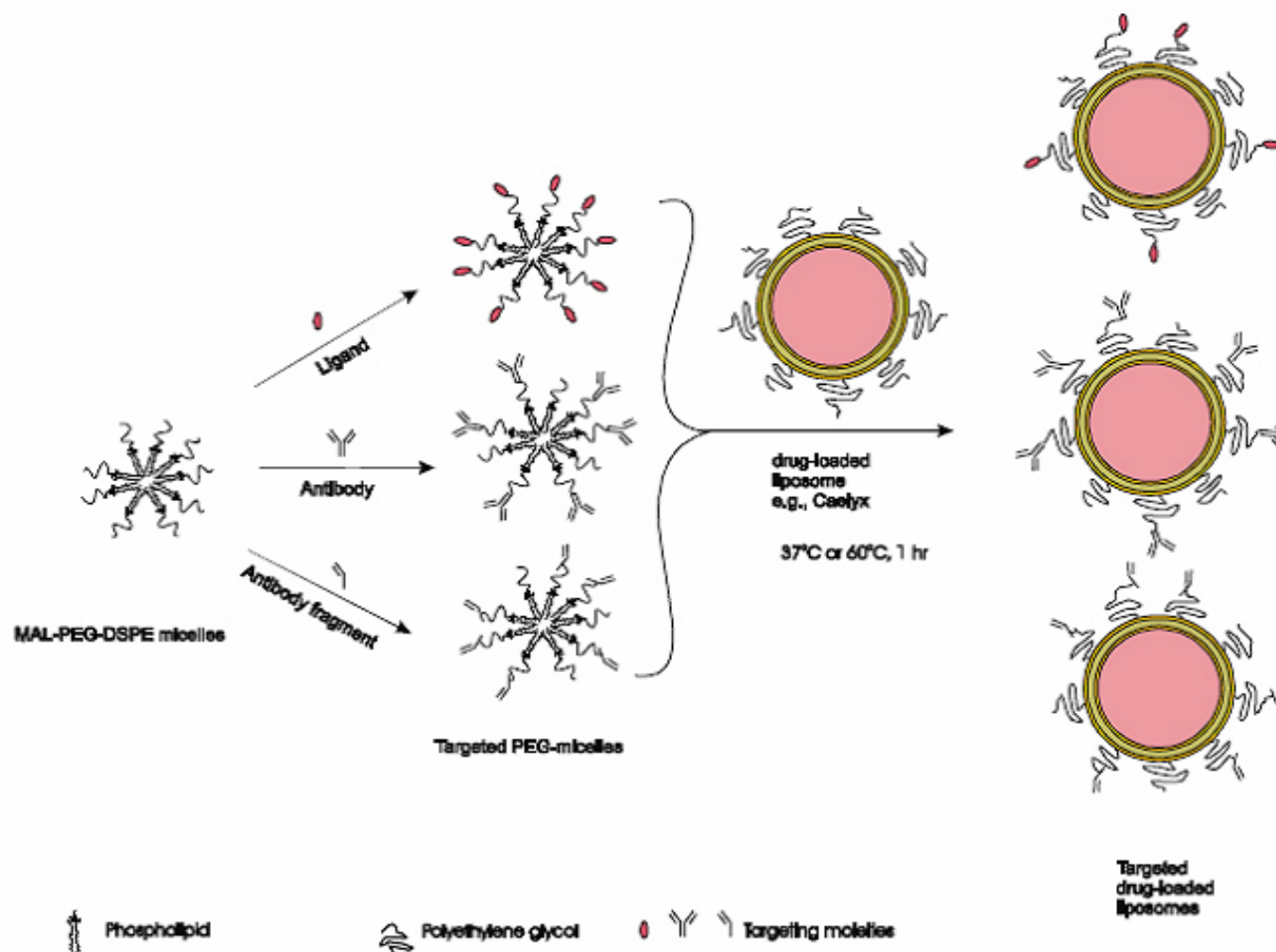


Figure A7: - Post-Insertion Mechanism

Appendix

Comparison of Viral and Non-Viral Vectors

Table A1: - Comparison of Viral and synthetic vectors

	Retroviral vectors	Synthetic vectors
Efficiency	Excellent	Poor
Gene Expression	Stable	Transient
Flexible Targeting	No	Yes
Pathogenicity/Toxicity	Insertional Mutagenesis	Possible Toxicity
Immunogenicity	Yes	Little
Production & Purification	Expensive	Inexpensive
Stability	Poor	Robust



Appendix

Polymer/RVLP complex synthesis protocol

