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Choosing Between Lentivirus and Adeno-associated Virus For DNA Delivery

Presenter:

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April 12, 2017

Outline

- Introduction to GeneCopoeia
- Lentiviral technology
- GeneCopoeia Lentiviral products & services
- AAV technology
- GeneCopoeia AAV products & services
- Which should I choose?

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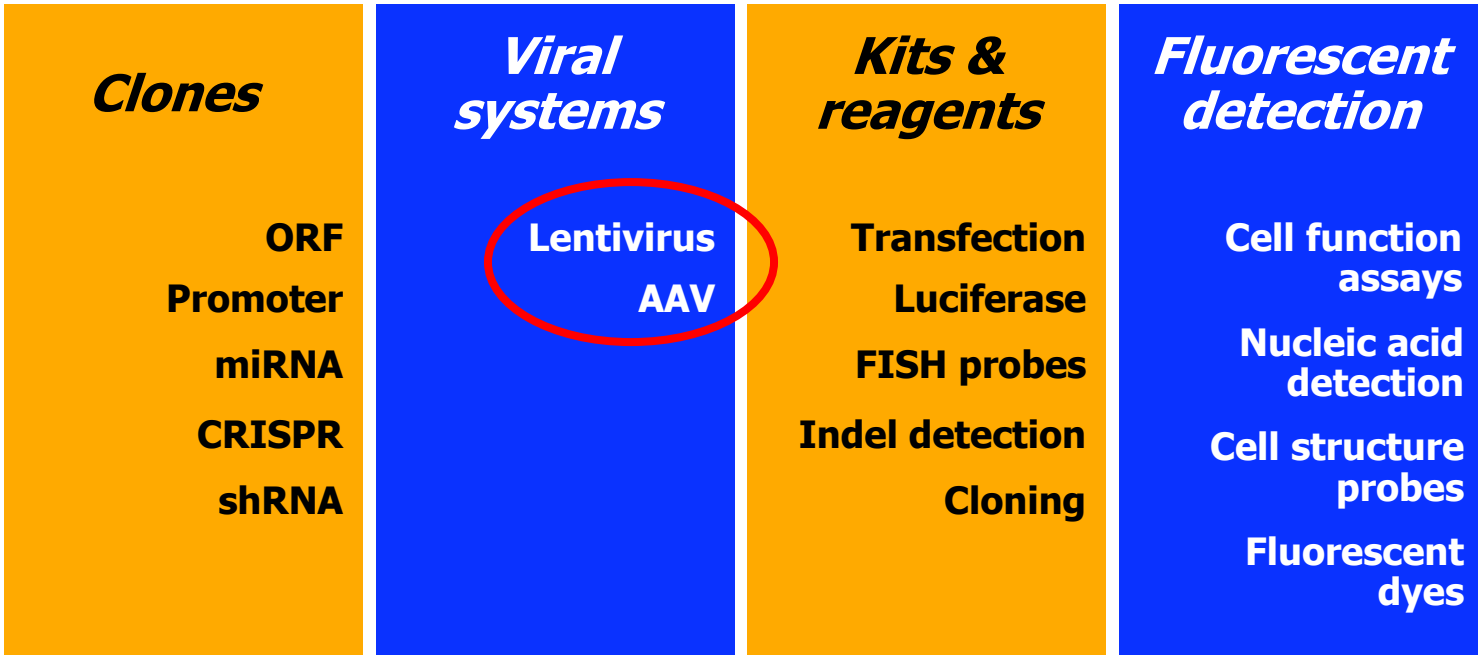
GeneCopoeia products & services

Functional Genomics & Cell Biology



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Why use viruses for DNA delivery?

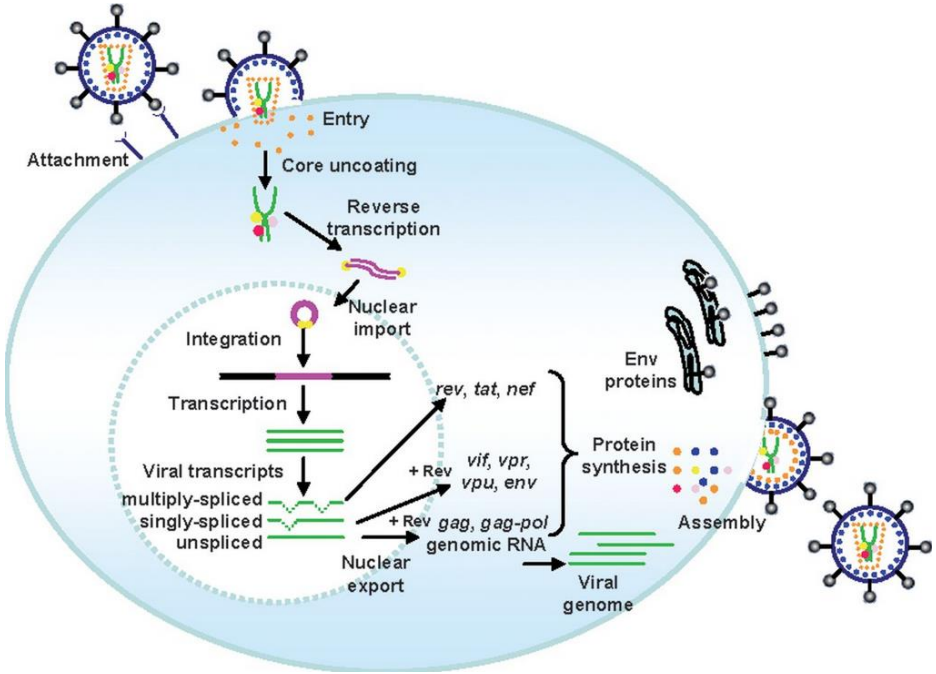
- DNA transfection not always possible or practical. Some cell lines difficult or impossible to transfect.
- Necessary for *in vivo*/gene therapy applications
- Most cells support viral infection

Applications for viral DNA delivery

- Protein expression via cDNA or open reading frame (ORF) clones
- Gene knockdown via RNA interference (RNAi)
- Reporter assays (GFP, luciferase)
- Gene editing via clustered, regularly-interspaced, short palindromic repeats-Cas9 (CRISPR-Cas9)

Have been successfully carried out in cultured immortalized mammalian cell lines, primary cell culture, animal models, and in gene therapy on human patients

Lentivirus technology

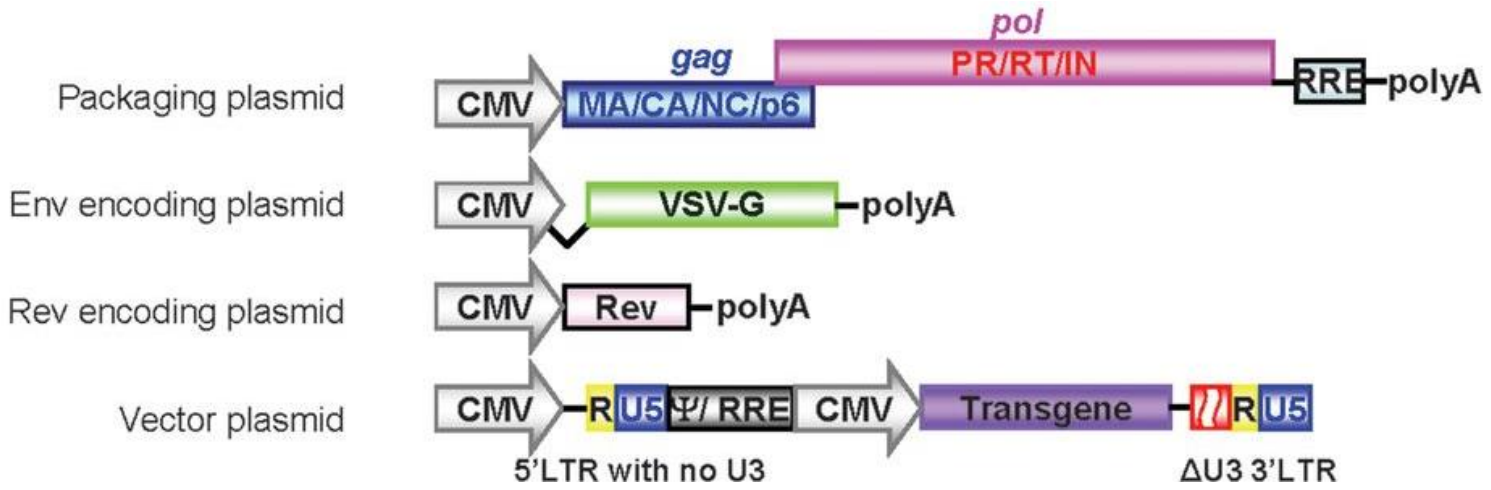


Samulski & Muzyczka (2014). Annu. Rev. Virol. 1, 427.

- Class of retroviruses that includes human immunodeficiency virus (HIV)
- Single stranded RNA genome of 9.7 kb
- Integrates into genomic DNA
- Infect dividing & non-dividing cells

Lentivirus technology

3rd generation lentivirus



Sakuma, et al. (2012). Biochem. J. 443, 603.

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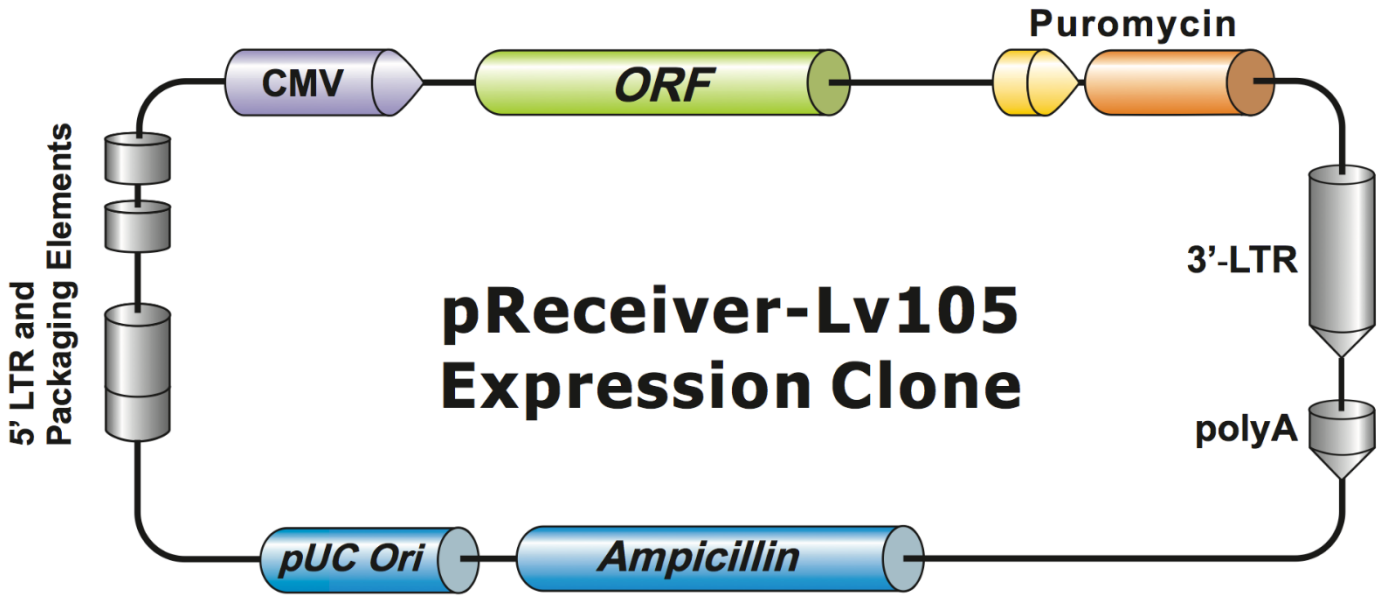
Lentivirus technology

GeneCopoeia lentiviral plasmids



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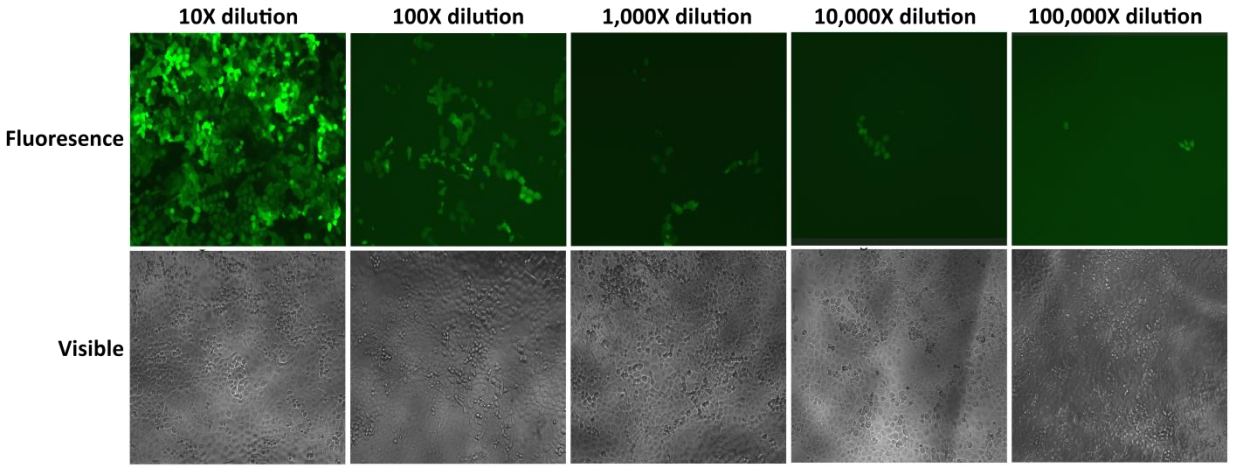
Lentivirus technology

GeneCopoeia lentiviral plasmids



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GeneCopoeia Lentiviral products and services

Product/service	Description
Lentiviral clones and cloning vectors	Pre-made and custom clones carrying ORFs, promoters, shRNAs, miRNA 3' UTRs, precursors, and inhibitors, sgRNAs, and more. Available with multiple promoters, tags and reporters. Vectors for do-it-yourself cloning of sequences of interest.
Lentifect™ lentiviral particles	Pre-made and custom-packaged, ready to use lentiviral particles. Produced from GeneCopoeia's extensive, genome-wide clone collections or from customer-submitted clones.
Lenti-Pac™ Lentiviral Packaging Reagents	Complete system of reagents for do-it-yourself lentiviral particle production. Includes packaging plasmids, packaging cell line, particle concentration solution, and titration kit.

GeneCopoeia Lentiviral products and services

Features

- Infect nearly all mammalian cell types
- Can be used to deliver relatively large DNA sequences-up to about 5-6 kb in length
- Can be used to generate stable cell lines, or drive stable gene expression in organs and tissues *in vivo*, due to integration of the transgene at random locations in the genome

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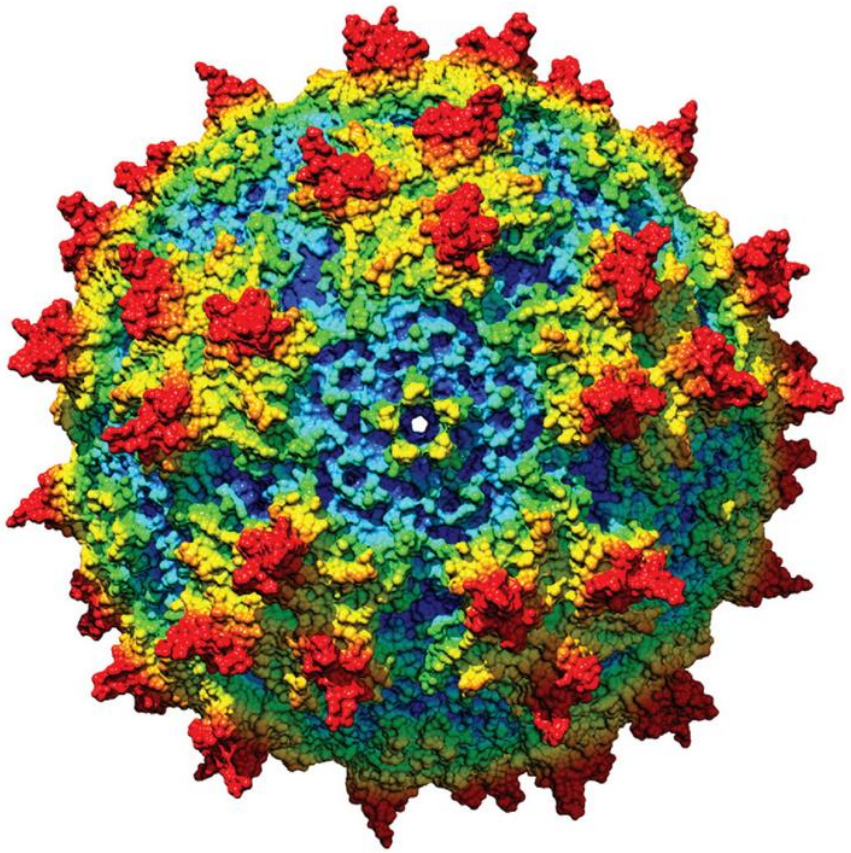
AAV technology

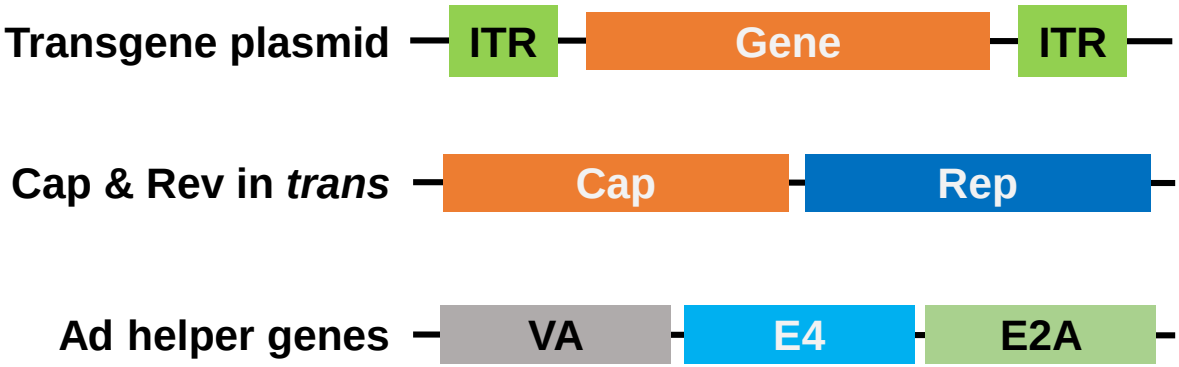


- Identified as contaminant of adenovirus (Ad)
- Single stranded DNA genome of 4.7 kb
- Requires helper virus for infection
- Integrates into genomic DNA
- Infects dividing & non-dividing cells

Ran, et al. (2013). Nature Protocols 8, 2281

AAV technology

AAV modifications



- Moved Cap & Rep genes to different plasmid-can accommodate inserts up to 4 kb (≤3 kb is ideal)

AAV technology

Serotypes

- Determined by proteins in capsid
- Influences tissue infectivity. 13 serotypes currently in use.
- Most common serotype is AAV-2, due to broad tissue infectivity
- Provides ability to restrict infection to specific tissues or cell types, if desired

AAV technology: Serotypes

Serotype	Primary target tissue	Description
AAV-1	Muscle	Best for cardiac muscle, skeletal muscle, neuronal and glial tissue.
AAV-2	Muscle, Liver, Retina	Most commonly-used serotype. Best for neurons, muscle, liver, and brain.
AAV-3	Megakaryocytes	Best for megakaryocytes, muscle, liver, lung, and retina.
AAV-4	Retina	Best for neurons, muscle, brain, and retina.
AAV-5	Lung	Best for lung, neurons, synovial joint, retina, and pancreas.
AAV-6	Muscle, Lung	Best for lung, liver, and heart.
AAV-7	Muscle, Retina, Neurons	Best for muscle, neurons, and liver.
AAV-8	Liver	Best for muscle, brain, liver, and retina.
AAV-9	Various	Best for muscle, heart, liver, lung, and brain.
AAV-10	Pleura, CNS	Cloned from Cynomolgus, almost identical with AAVrh10 except for 12 amino acids in VP1. Best for lung, muscle, heart, NCS and liver.
AAV-DJ	Various	A mixture of 8 naturally-occurring serotypes. Efficiently transduces a wide variety of cell types in vitro.
AAV-DJ/8	Various	A variant of AAV-DJ with a heparin binding domain (HBD) mutation, which permits infection of liver as well as other tissues in vivo.

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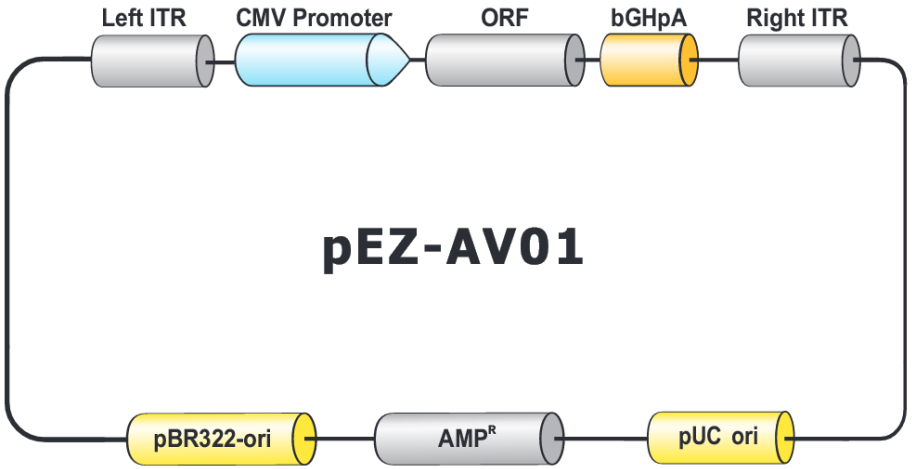
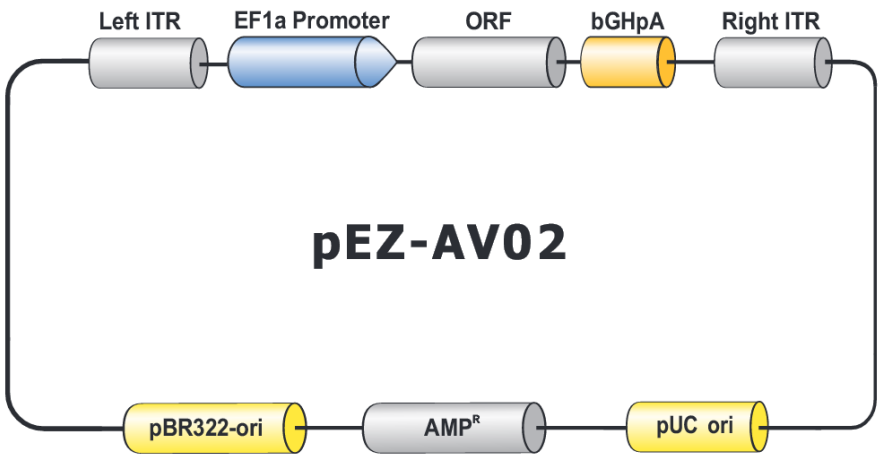
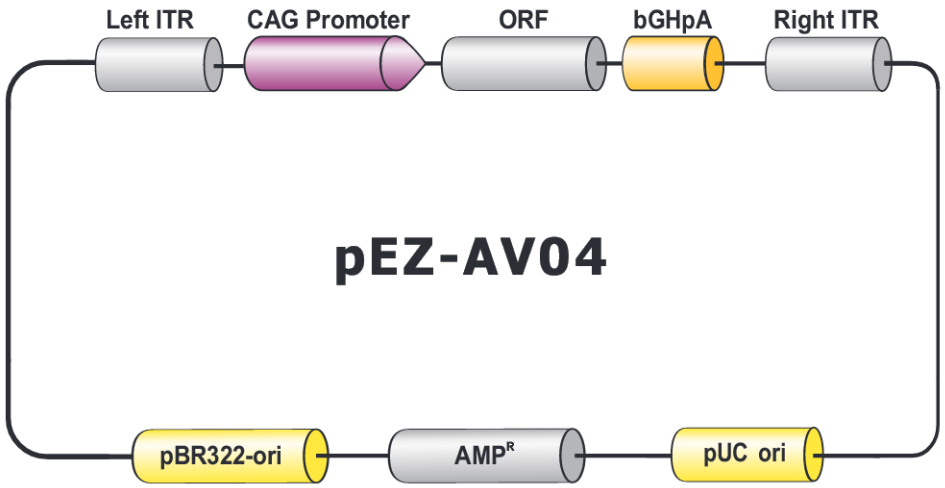
GeneCopoeia AAV products and services

Vector types







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GeneCopoeia AAV products and services

Packaging service

- Available in multiple vector types (CMV, CAG, or EF1α promoter), or you can submit your own vector
- Available for multiple serotypes.
- Available in standard purity (for *in vitro* use only; ≥ 1x10^11 GC/ml) or purified (for *in vivo* use; ≥ 5x10^12 GC/ml)
- Insert sizes must be ≤ 3 kb

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GeneCopoeia AAV products and services

Features

- High titers. Titer of purified particles can be up to 10^14 GC/ml (genome copies/ml)
- Versatile. Usable in a broad range of host cell types
- Low toxicity. Does not integrate into the host genome.
- Low immunogenicity. Minimal host immune response.
- Safe. Not associated with any human disease

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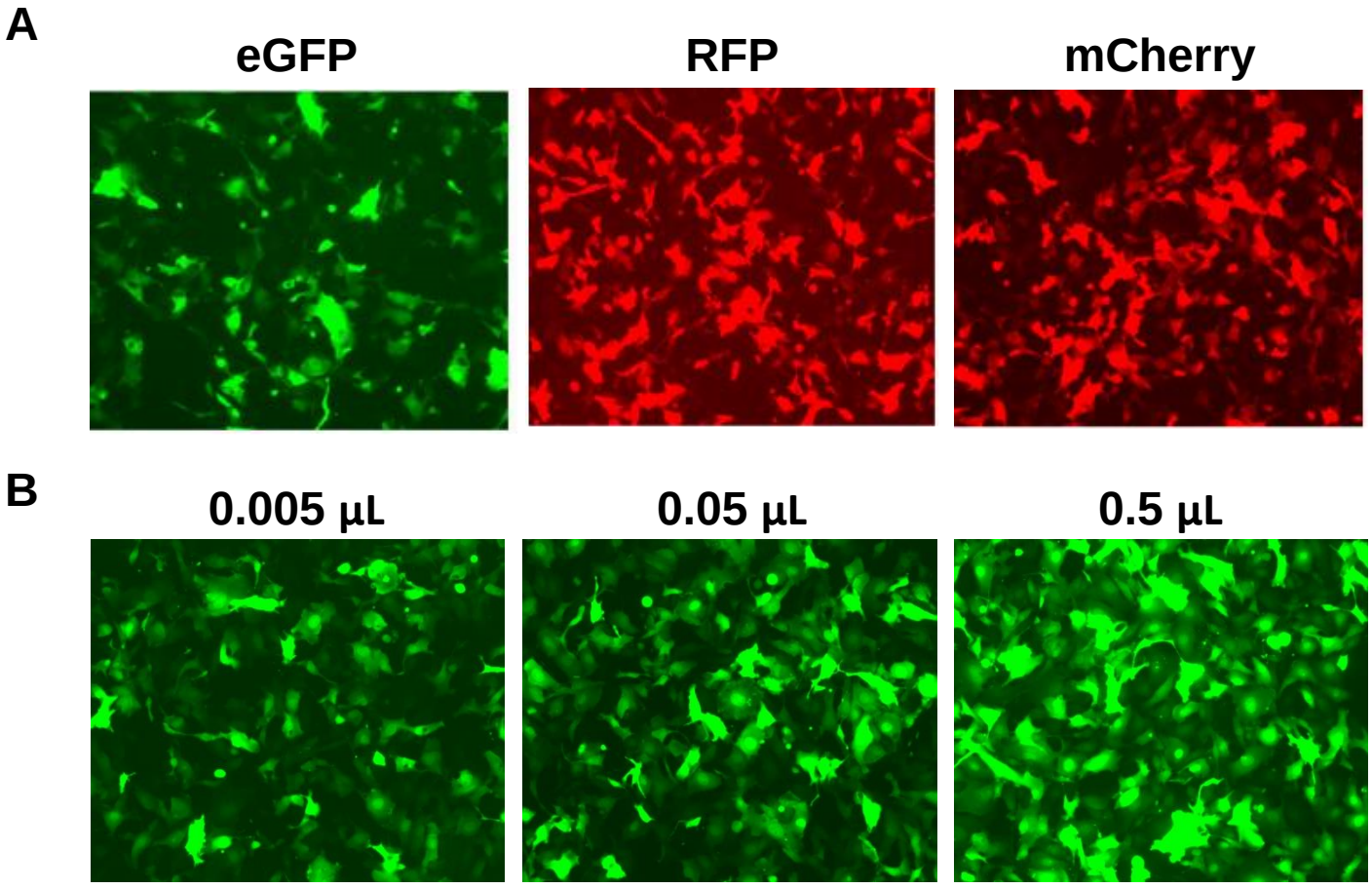
GeneCopoeia AAV products and services

Performance of standard & purified particles



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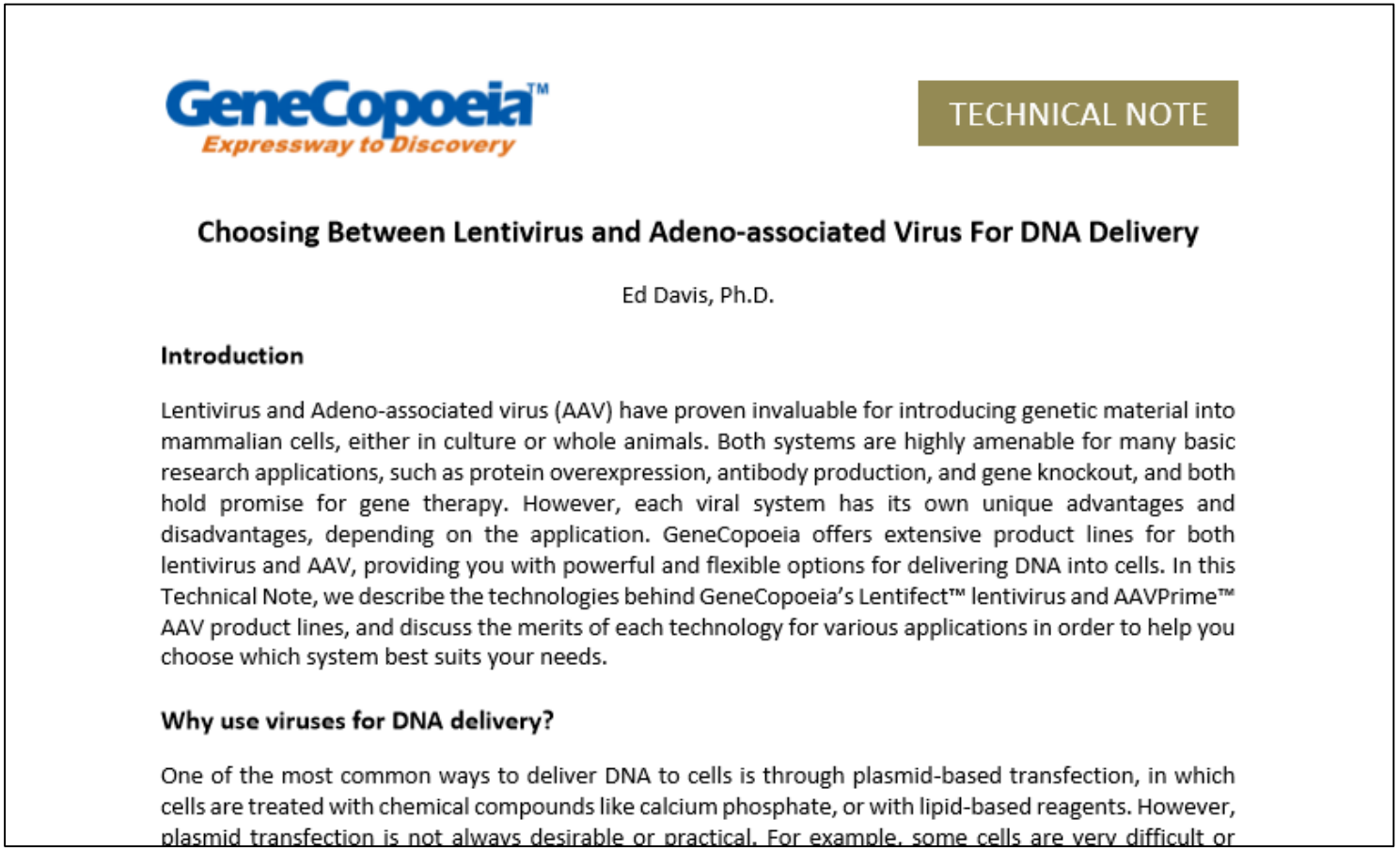
Which should I choose?

	Larger inserts	Stable integration	No integration	Cell/tissue specificity	*in vivo* safety
Lentivirus	✔	✔			
AAV			✔	✔	✔

GeneCopoeia AAV products and services

GeneCopoeia Technical Note: Lentivirus or AAV?



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TECHNICAL NOTE

Choosing Between Lentivirus and Adeno-associated Virus For DNA Delivery

Ed Davis, Ph.D.

Introduction

Lentivirus and Adeno-associated virus (AAV) have proven invaluable for introducing genetic material into mammalian cells, either in culture or whole animals. Both systems are highly amenable for many basic research applications, such as protein overexpression, antibody production, and gene knockout, and both hold promise for gene therapy. However, each viral system has its own unique advantages and disadvantages, depending on the application. GeneCopoeia offers extensive product lines for both lentivirus and AAV, providing you with powerful and flexible options for delivering DNA into cells. In this Technical Note, we describe the technologies behind GeneCopoeia's Lentifect™ lentivirus and AAVPrime™ AAV product lines, and discuss the merits of each technology for various applications in order to help you choose which system best suits your needs.

Why use viruses for DNA delivery?

One of the most common ways to deliver DNA to cells is through plasmid-based transfection, in which cells are treated with chemical compounds like calcium phosphate, or with lipid-based reagents. However, plasmid transfection is not always desirable or practical. For example, some cells are very difficult or

Download from:

http://www.genecopoeia.com/wp-content/uploads/2017/04/GeneCopoeia-Technical-Note-Lentivirus-vs.-AAV-03-2017.pdf

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Upcoming webinar!

Applications For Safe Harbor Transgenesis in Genome Editing

Wednesday, April 19, 2017 12:00 pm ET

Register here:

https://attendee.gotowebinar.com/register/7388044519385274881

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Summary

- Lentivirus & AAV are invaluable for introducing genetic material into mammalian cells, either in culture, in whole animals, or in gene therapy
- Each viral system has its own unique advantages and disadvantages, depending on the application
- Lentivirus is most useful for its broad tissue tropism and ability to carry larger inserts
- AAV is most useful for its lack of toxicity and immunogenicity, and its natural ability to limit tissue type infection
- GeneCopoeia offers extensive products and services for lentivirus and AAV, including custom viral particle production.

Thank you!

If you have any additional questions, please call

1-866-360-9531 x227

Email: edavis@genecopoeia.com

Or visit us on the web:

www.genecopoeia.com

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