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Lentivirus and AAV FAQ

1. What cells do we use for packaging Lentivirus and AAV?
   The most common cell line used for packaging both Lentivirus and AAV is HEK293T which is what is used at OriGene.
2. How do we determine Lentiviral titer?
   Lentiviral titer is determined with an ELISA (enzyme-linked immunosorbent assay) to quantify P24 protein, a core capsid protein of the lentivirus. This protein is directly proportional to the number of viral particles present, making it a reliable indicator of viral titer.
3. What are the limitations of using P24 ELISA for quantifying lentivirus titer?
   P24 ELISA measures total viral particles, including non-functional or defective particles. This may overestimate the infectious titer. To complement this, functional assays like transduction-based titration are often used alongside P24 ELISA to determine the infectious titer directly.
4. How do you determine functional viral titer (TU/ML) using the P24 assay?
   There are approximately 2000 molecules of p24 per physical particle (PP) of lentivirus:
   $(2 \times 10^3) \times (24 \times 10^3$ Da of p24 per PP), 48×10^6/Avogadro = $(48 \times 10^6) / (6 \times 10^{23}) = 8 \times 10^{-17}$ g of p24 per PP, approximately 1 PP per 1×10^{-16} g of p24, 1×10^4 PP per pg of p24
   A reasonably well packaged, VSV-G pseudo typed lentiviral vector will have an infectivity index in the range of 1 TU per 1000 physical particles (PP) to 1 TU per 100 PP (or less). Thus, the range is approximately 10 to 100 TU/pg of p24. It is through this conversion that TU/mL is obtained.
5. Do we provide protein location for customer?
   We perform QC for GFP and DDK expression to confirm our virus. However, we cannot determine protein location using our microscope.

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6. What is our lentiviral packaging system?

   Our lentiviral packaging system is the 3rd generation lentiviral packaging system that includes Transfer plasmid (LTR, long terminal repeat deleted), Envelope plasmid and Packaging plasmid which is divided into two plasmids, one encoding Gag and Pol and the other encoding Rev.

7. Does our lentiviral system have the viral replication defective?

   Yes, our Lenti vector is replication deficient as it contains SIN (Self Inactivation), a deletion in the 3′ LTR (ΔU3). This SIN deletion does not affect lentiviral packaging but results in “self-inactivation” after integration into the transduced cell. The integrated lentiviral genome is no longer capable of self-replication.

8. Storing Your Lentiviruses

   Lentiviral particles can be stored for up to a week at 4°C. However, for long-term storage, samples should be frozen as soon as possible at -80°C. Frozen lentiviruses are stable for at least one year. One important thing to note is that freeze-thaw cycles can decrease the titer drastically (sometimes as much as 10-fold!). It is recommended that you aliquot your lentiviruses into working volumes before you freeze them.

9. What’s the required biosafety level for using AAVs?

   Recombinant AAV constructs in which the transgene does not encode a potentially tumorigenic gene product or a toxin molecule and is produced in the absence of a helper virus can be handled in a Biosafety Level 1(BSL-1) facility. Otherwise, it should be handled as biohazardous material under Biosafety Level 2 (BSL-2) containment.

   For more information on biosafety levels, please read the NIH Biosafety Guidelines.

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10. Are recombinant AAVs replication deficient?

In their recombinant form, the genes necessary for viral replication (such as Rep and Cap) are removed from the viral genome and replaced with the gene of interest flanked by inverted terminal repeats (ITRs). This means that while rAAVs can efficiently deliver genes into target cells, they lack the machinery required to replicate on their own.

To produce rAAVs, helper plasmids or helper viruses (such as adenovirus or herpesvirus) are required during the packaging process in producer cells. These helpers provide the Rep and Cap proteins in trans (from separate plasmids), allowing for the assembly of viral particles. However, the packaged rAAV itself cannot replicate once it infects a target cell, enhancing its safety profile for gene therapy applications.

11. What's the cloning capacity for recombinant AAVs?

AAV has a packaging capacity of ~4.7Kb. Since the two ITRs of AAV are about 0.2-0.3Kb total, the foreign DNA that can be introduced between these 2 ITRs should be smaller than 4.4Kb. When the length of inserted DNA between the 2 ITRs is close to the maximum allowed (4-4.4Kb), the packaging efficiency decreases significantly.

For double-stranded AAV (dsAAV), the capacity is half that of the single-stranded AAV, or ssAAV.

12. What is the difference between transfection and transduction?

Transfection: the transfer of nucleic acid without using a virus. Transfection uses chemical as well as non-chemical methods like electroporation and microinjection to deliver genetic material into cells.

Transduction: the transfer of nucleic acid with the use of a viral vector. The virus infects the cells and releases the genetic material.

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13. Considerations for Using Transfection versus Transduction:

    Biosafety Level (BSL): BSL is an important consideration during planning of a study or when determining the capabilities of a facility. Working with viruses can require an increase in BSL due to their inherent infectivity, and some plasmids contain elements considered high risk. Be sure to take BSL into account when planning a transfection or transduction experiment.

    Target Cell Type: Transfection via electroporation or with chemical transfection reagents is quick and easy. However, some cell types are inherently refractory to transfection. In these cases, employing a virus to transduce the cells can be a work-around for delivery of genetic material.

    Use In Vivo: Transfection reagents typically work to deliver nucleic acid to any cell that encounters a transfection complex. On the other hand, many viruses are inherently capable of or can be pseudotyped for cell-type-specific infection. For experiments where a specific tissue or cell type is the target or in the case of a gene or cell therapy for a specific disease, use of a virus to transduce the target cells can be a more tailored approach.

    Stable Transgene Expression: Transduction with some viruses, like AAV, can lead to durable transgene expression in non-dividing cells. Lentiviruses can efficiently integrate genetic material into the genome of a transduced cell, which can be a timely way to generate stable cell lines. Transfection can also be used to generate stable cell lines. A common approach is to transfect a plasmid with a selectable marker or to transfect CRISPR/Cas-gRNA complexes.

14. What is multiplicity of infection?

    Multiplicity of Infection (MOI) is the ratio of infectious agents (such as viruses, bacteria, or plasmids) to target cells in an experiment. It indicates how many viral particles are added per cell during infection or transduction.

    Formula for MOI= Number of infectious Particles/Number of Target Cells

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15. What are factors that can affect your MOI?

- **the current state of your cell line:** dividing or non-dividing
- **the characteristics of the virus:** lentivirus, adenovirus, etc.
- **the transduction efficiency**
- **your application**: transducing a packaging cell line for virus production, generating a stable cell line for protein production, etc.

16. Why does MOI matter?

High MOI: Increases the likelihood that most cells get infected but can lead to cytotoxicity or multiple integrations.

Low MOI: Reduces toxicity but may result in fewer infected cells, leading to heterogeneous expression.

17. How long will it take to detect expression?

GFP expression is typically detectable 48 hours after infection with AAV. Expression of most genes is expected within 2-7 days after in vitro infection; however, protein expression levels may vary based on the protein being expressed, the promoter, and the cell type.

18. What are the advantages to using AAV for gene delivery?

There are three main advantages to using AAV:

- AAV has not been reported to cause any diseases. Together with its replication defective nature, AAV has a good safety profile to be used in gene transfer in vivo and as potential gene therapy vehicles.
- Recombinant AAV is capable of infecting a broad range of cell types including non-dividing cells and remaining as concatemers for long-term expression.
- Compared with other viral vectors such as adenovirus, AAV elicits a very mild immune response in animal models.

19. Can AAV be used to make stable cell lines?

    AAV can remain in cells as concatemers for long term expression through antibiotic selection; however permanent stable cell lines cannot be created with AAV because recombinant AAV does not integrate into the host cell genome.

20. What cells are suited for AAV-MyoAAV 1A serotype?

    MyoAAV1A is designed for efficient targeting and transduction of muscle cells. It likely incorporates specific modifications to the AAV1 capsid or uses muscle-specific promoters to enhance its affinity for skeletal and cardiac muscle tissues. This variant may be particularly useful for diseases where localized muscle gene therapy is required.