Manufacturing Viral Gene Therapy Vectors: General Approaches and Challenges

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Natural virus life cycle

Viral particle

Viraally infected cell

Naive cell

Production of injectable viral vectors

Viral vector particle

Vector producer cell

Patient’s cell

Cleanroom manufacturing facility

Airlock
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Production of viral vectors for *ex vivo* transduction

Vector producer cell → Viral vector particle → Patient's cell

Cleanroom manufacturing facility → Airlock

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Engineering vector producer cells

Introduce necessary genetic material into vector producer cell:
1) Helper components
2) Vector component

Vector producer cell → Viral vector particle

Cleanroom manufacturing facility → Airlock

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Stable vector producer cells

Vector producer cell → Viral vector particle

Cleanroom manufacturing facility → Airlock

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Summary of terminology

- Vector producer cells
- Helper components
- Vector components
- Stable producer cells
- Transient production systems
- Replicative production systems

Design strategies for engineering helper cassettes
Goals for the design of a replication incompetent viral vector production system

• Vector genome – minimal cis-acting elements
• Helper – only necessary trans-acting factors
• Optimization of viral ‘pseudoreplication’

Individual vector production systems

• First generation adenovirus
• Helper dependent adenovirus
• γ-retrovirus
• Lentivirus
• Adeno-associated virus

Adenoviral vectors

Wild type virus

Helper

Vector

ΔE1

HEK293

Haj-Ahmad and Graham, J Virol, 1986 v57 p267
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First generation adenovirus vector production

Minimally deleted adenovirus vectors

Helper dependent adenovirus vector production

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Helper dependent adenovirus vector production (2)

RJ Parks, et al., PNAS, 1996

Retroviral vectors

Mann, Mulligan, and Baltimore, Cell, 1983

Moloney Murine Leukemia Virus

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Stable cell lines
- Vector producer cell
- Packaging cell line
- Producer clone
- Cleanroom manufacturing facility
- Airlock

The challenge of self-inactivating (SIN) retroviral vector genomes
- Non-SIN Genomic RNA
- Proviral DNA
- Reverse transcription
- SIN Genomic RNA
- Proviral DNA

SIN retroviral vector production with transient transfection
- SIN-Vector
- gagpol Helper
- env Helper
- γ-retroviral vector particle
- Vector producer cell -- HEK293T

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Lentiviral stable cell lines

Induction

Vector particle

Vector producer cell

Cleanroom manufacturing facility

Airlock

Packaging cell line

Producer clone

Lentiviral stable cell lines

Vector producer cell

Vector particle

Vector genome

gagpol Helper

env Helper

rev Helper

Throm, et al., Blood, 2009

AAV vector & helper components

Adenovirus genome - 35,935 bp


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**Plasmid-based AAV vector production**


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**Dedicated AAV producer cells**

Production systems which are dependent upon stable introduction of some vector or helper components into a cell. Production is initiated in the cleanroom by infection with helper virus, which may or may not carry additional components. For example:

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**Baculovirus-based AAV vector production**

Transient delivery of AAV helper and vector genome sequences via baculovirus vectors into insect cells provides alternative system to mammalian cells for vector production

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34

Downstream processing

- Bulk harvest
  - Cell disruption for non-secreted virions
    - Detergent lysis
    - Physical disruption
  - Supernatant harvest for secreted products
    - Continuous perfusion
    - Batch harvest

35

Downstream processing (2)

- Purification
  - Early processes developed in academic laboratories:
    - Centrifugation
    - Density gradients
    - Dialysis
  - Modern scalable processes
    - Tangential flow filtration
    - Chromatography (ion exchange, affinity)
    - Single use, disposable units, closed systems

36
Cleanroom management

- Raw materials
- Personnel
- Air
- Equipment

Quality systems
Standard operating procedures
Document control
Personnel training
Production methodology
Quality control

- http://www.fda.gov/BiologicsBloodVaccines/

Release of product

Cleanroom manufacturing facility

Product testing

• Physical titer
  - Genome copy numbers
  - Real-time PCR or RT-PCR
  - Blot hybridization
  - Direct dye binding
  - Digital PCR
  - Particles
    - ELISA for capsid
    - UV spectrophotometry
    - Light scattering
    - HPLC
    - Mass spectrometry

Product testing (2)

• Other particle parameters
  - Infectious titer
  - Potency
  - Gene delivery
  - Gene expression
  - Enzymatic function
  - Identity
  - Homogeneity
• Contaminants
  - Anything anyone has ever seen, if possibly relevant
Example release testing for AAV vector product

Bulk purified material
- Titer
- Sterility
- Residual BSA
- Residual host cell protein
- Residual host cell DNA
- Residual benzonase
- E1A DNA
- SV40 DNA
- Plasmid backbone DNA
- Caprid DNA
- Rep-ITR DNA
- Vector genome sequencing

Final vialled product
- Restriction analysis
- Caprid Western blot
- In vitro adventitious virus assay
- Replication competent virus testing
- Container integrity
- Vial seal integrity
- Sterility
- pH
- Titer
- Endotoxins
- General safety
- Potency

Thank you