The Molecular Virology Support Core: Adenoviral Vectors and Beyond

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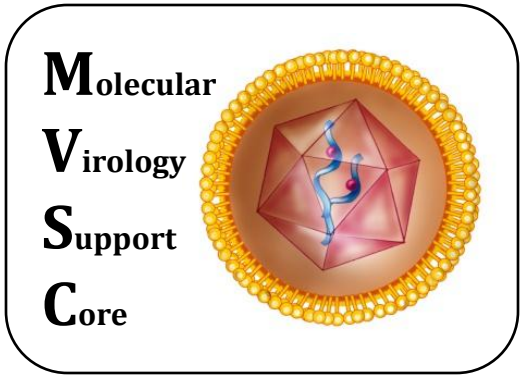
Viral Vector Workshop, May 5th, 2011



Outline

1) Overview of the MVSC

2) Adenoviral Vectors

3) Expertise and Services

Molecular Virology Support Core

Overview

- What does the MVSC offer?
  - Comprehensive and broad array of virology services
  - Broad virology expertise, particularly in non-human primate (NHP) virology
    - Viruses and virus-derived products (viral vectors, antigens, wild-type strains)
    - Viral diagnostics (tissue viral loads)
    - Virus serology (antibodies)

- Virology Core Director
    - Christoph Kahl, newly recruited in March 2010
    - Background
      - Gene therapy and recombinant vaccines
      - Lentiviral and adenoviral vectors

- How to find the MVSC:
  - Physical location:
    - OHSU West Campus (ONPRC), Research Building: Room 46 (lab), Room 163 (office)
  - Online:
    - OHSU Website: **Research > Research Cores & Shared Resources > Virology**
    - ONPRC Website: **Research Services > Research Support Cores > Virology**
    - VGTI Website: **VGTI > Core Services > Virology Core**
    - “eagle-i consortium” Website

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Adenoviral Vectors

- General features:
  - Replication-defective (unless in complementing cells)
  - Large transgene carrying capacity (~7-8 kb in ΔE1/E3 vector)
  - High titer production possible (up to 10^{13} vp at research scale)

- Common uses:
  - Transient gene expression
  - *in* vitro protein expression studies (high level expression)
  - *in vivo* vaccination and cancer therapy
    - Strong innate immune activation and immunogenicity

- Ad5
  - Most common vector serotype
  - Very broad tropism
    - Dividing and non-dividing cells
    - For transduction of HSC, DC, Synovio, VEC, smooth muscle need other serotype fibers
    - Targets liver upon system injection (can ablate by triple mutation in CAR, RGD, and KKTK)

Adenoviral Vectors

Species	Serotype	Receptor(s)	Tropism:	Seroprevalence (%)	Fibre shaft repeats
A	12, 18, 31	CAR, fIX, fX	Cryptic (enteric, respiratory)	35–70	23
B1	3, 7, 16, 21, 50	CD46, 'X', fX CD80, CD86	Respiratory, ocular	2–15 (Ad16, 21, 50) 35–70 (Ad3, 7)	6
B2	11, 14, 34, 35	CD46, 'X', fX CD80, CD86	Renal, ocular, respiratory	1–3 (Ad11, 34, 35) 18 (Ad14)	6
C	1, 2, 5, 6	CAR, fIX, fX, Lf, DPPC, VCAM-1, HS, MHC1-α2	Respiratory, ocular lymphoid	40–80	22
D	8–10, 13, 15, 17 19, 20, 22–30, 32, 33, 36–39, 42–49, 51	SA, CD46, CAR fx	Ocular (enteric)	3–44	8
E	4	CAR	Ocular, respiratory	45	12
F	40, 41	CAR	Enteric	41 (together)*	12 (short fibre) and 21/22 (long fibre)
G	52	ND	Enteric	ND	9 or 17

ND: not determined.
*Serotypes 40 and 41 are very closely related antigenically.

Arnberg, Rev.Med.Virol. 2009

Adenoviral Vectors

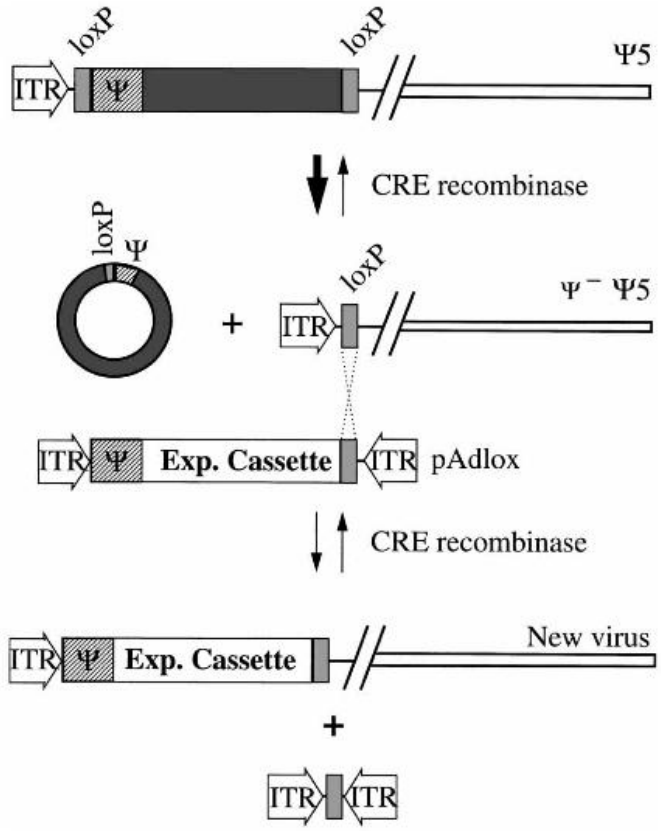
- Essential steps:

1. Vector design
   - Clone transgene or expression cassette into appropriate adenovirus shuttle plasmid
   - Need serotype-specific shuttle and vector genome backbone plasmids

2. Vector generation
   - Homologous recombination in mammalian cells
     - Adψ5 system in 293 CRE cells (Hardy et al JVI 1997, Ad5 ΔE1/E3)
   - Homologous recombination in bacteria
     - Shuttle plasmid with homologous end sequences (e.g. AdEasy)

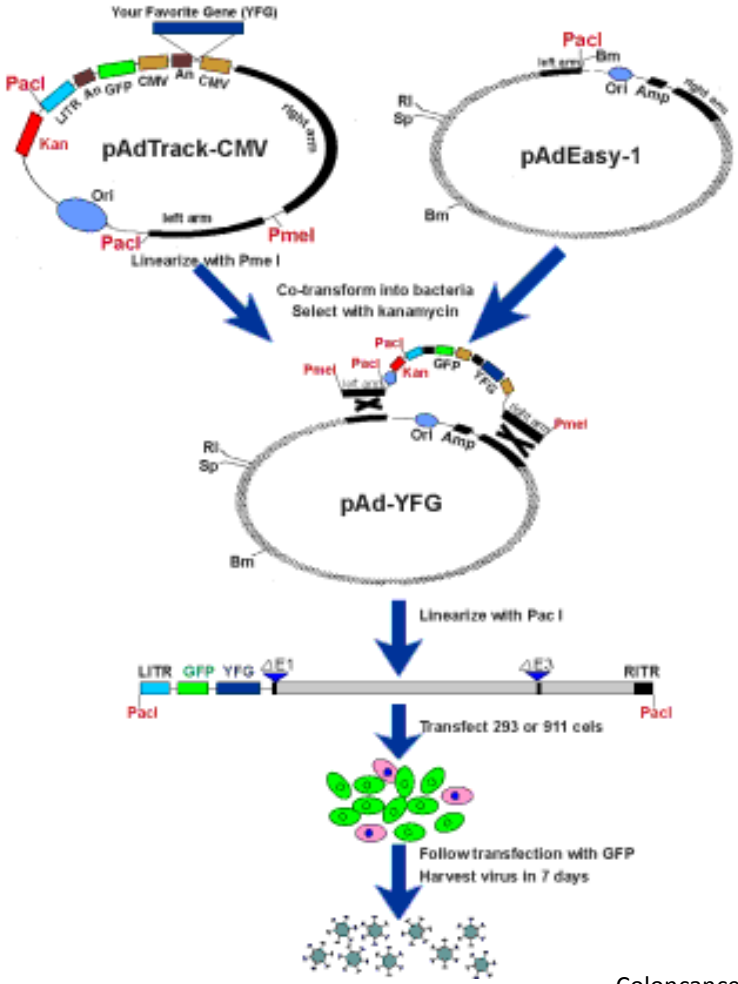
3. Vector amplification and production
   - Vector amplification and passaging
   - Seed stock generation
   - Production of purified high-titer vector stock
     - Scaleable
     - Different purification methods
     - Can remake new vector stock from prior vector prep or lysate (no need for new transfection)

Mammalian Recombination



Hardy et al., JVI 1997

Bacterial Recombination



Coloncancer.org

Adenoviral Vectors

- <u>QC Testing Methods:</u>
  - Vector stock titration
    - **Physical titer = total # of viral particles (vp)**
      - DNA dye detection assay
      - Spectrophotometric reading of absorbance at 260 nm
    - **Infectious titer = biologically active virus only**
      - Limiting dilution assays ($TCID_{50}$, plaque forming unit assay)
      - Immunofluorescence assays (focus-forming unit assay)
  - Vector Function and Integrity
    - **Transgene expression**
      - Appropriate assay for transgene (Western Blot, ELISA, IFA)
    - E1-region integrity
      - Transgene and E1-spanning PCR
      - Transgene cassette sequencing
    - Vector genome integrity
      - Restriction enzyme analysis
  - Biosafety
    - Replication-competent adenovirus (RCA) assay

Expertise and Services

- Viral stocks, vectors and antigens
  - Adenovirus
    - Adenoviral Vectors:
      - Adψ5 system (ΔE1/E3)
      - Inducible CMV Promoter by using Ad(Transgene) vector + Ad(Tet TA) vector
      - Other Ad vector systems and serotypes upon request
    - Adenovirus Antigen
  - Adeno-associated Virus (AAV) *In planning*
    - AAV Vectors:
      - MVSC planning to offer custom AAV production for NHP AAV studies
      - Targeted gene expression in different tissues *in vivo*

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Expertise and Services

- Viral stocks, vectors and antigens (continued)
  - RhCMV Vectors
    - WT virus and vector
      - Production, titration, plaque purification, growth curves
      - Persistent gene expression (replicating vector)
      - Immunogenic *in vivo* (SIV T cell vaccines)
    - RhCMV antigen
  - Lentivirus
    - HIV, SIV, and SHIV:
      - WT Stock production and titration
      - Virus susceptibility assays
    - Lentiviral Vectors:
      - Currently provided by the MCB Core at ONPRC (Eliot Spindel)
      - Contact Greg Dissen if interested
  - Vaccinia Virus
    - MVA Vectors:
      - Virus Stock production and titration

Expertise and Services

- Virus Diagnostics (Serology)
  - Qualitative
    - Co-Culture assays for SIV, RhCMV
  - Quantitative
    - qPCR assays for SIV, RhCMV, *VZV/SVV (future)*
  - Viral Antibodies
    - ELISA for SIV, RhCMV, *VZV/SVV (future)*
    - Consult with ONPRC SPF lab for RM screening
- Resources
  - Critical reagents (cell lines, virus strains, antisera etc.)
- Consulting and Research Support
  - Virology techniques and procedures
  - Virology studies in NHP
  - Working with viral biohazardous agents
  - IBC protocols

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Expertise and Services

- Information needed from user:

1. Initial service request
2. IBC and IACUC approval for infectious agent
3. Alias account information for billing

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Questions?