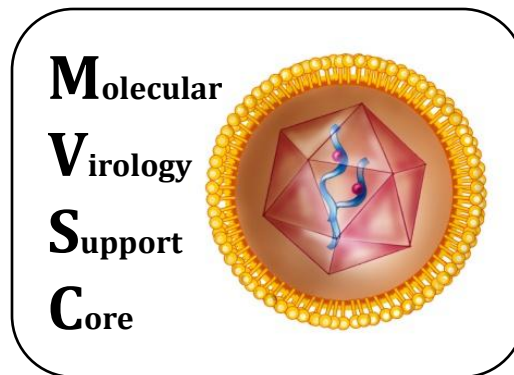


The Molecular Virology Support Core: Adenoviral Vectors and Beyond

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Viral Vector Workshop, May 5th, 2011

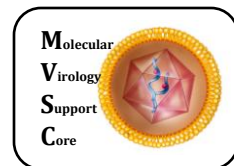


Outline

- 1) Overview of the MVSC
- 2) Adenoviral Vectors
- 3) Expertise and Services

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



Adenoviral Vectors

- General features:
 - Replication-defective (unless in complementing cells)
 - Large transgene carrying capacity (~7-8 kb in $\Delta E1/E3$ 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)

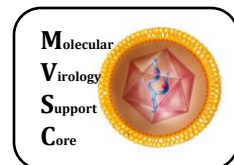
Adenoviral Vectors

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- α 2	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

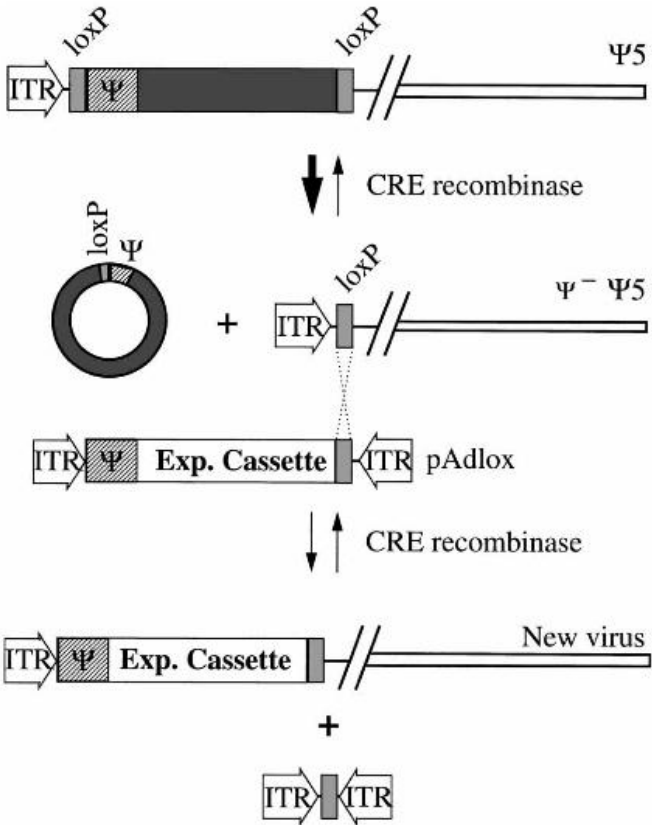
2. Vector generation

- Homologous recombination in mammalian cells
 - Ad ψ 5 system in 293 CRE cells (Hardy et al JVI 1997, Ad5 Δ E1/E3)
- Homologous recombination in bacteria
 - Shuttle plasmid with homologous end sequences (e.g. AdEasy)

3. Vector amplification and production

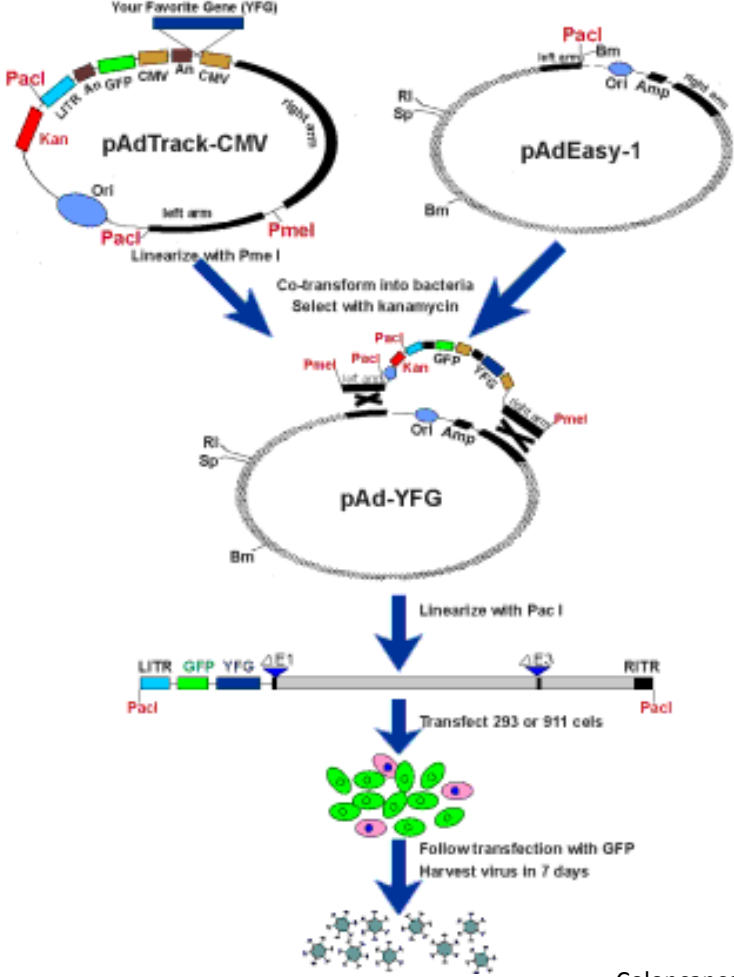
- Vector amplification and passaging
- Seed stock generation
- Production of purified high-titer vector stock
 - Scalable
 - Different purification methods
 - Can remake new vector stock from prior vector prep or lysate (no need for new transfection)

Mammalian Recombination

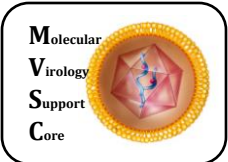


Hardy et al., JVI 1997

Bacterial Recombination



Coloncancer.org



Adenoviral Vectors

- QC Testing Methods:
 - 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 (TCID₅₀, 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:
 - Ad ψ 5 system (Δ E1/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*

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

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

Questions?

