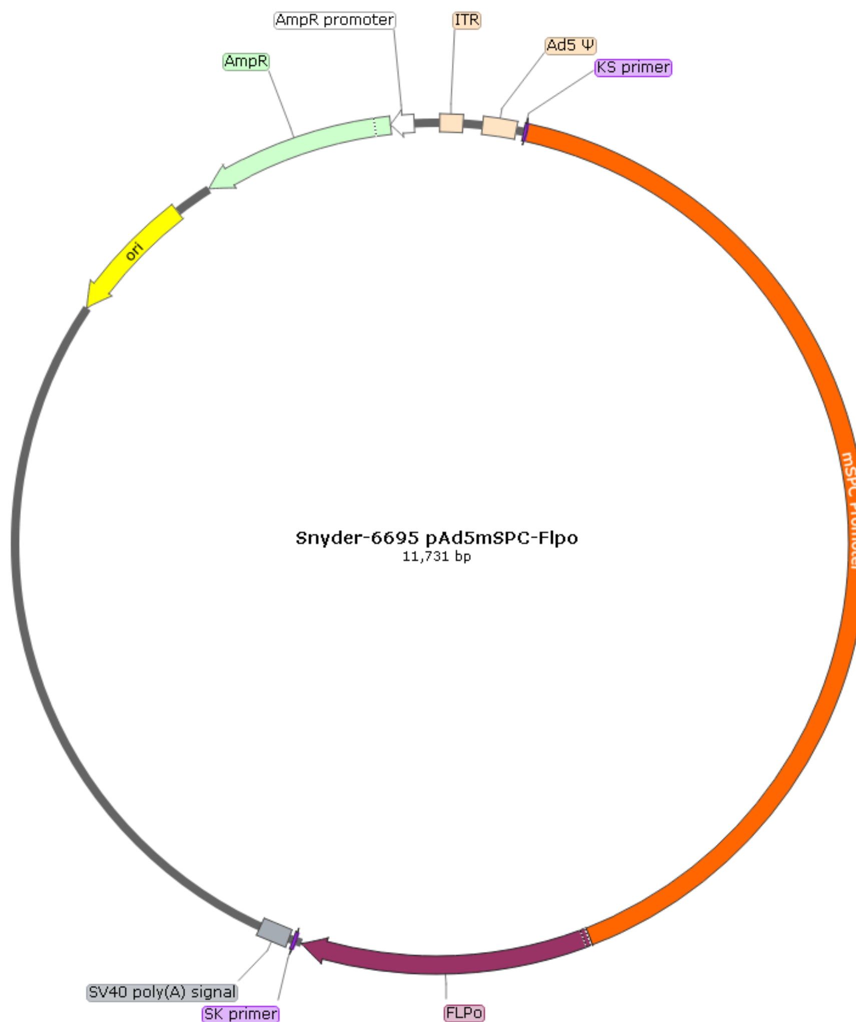
Snyder-6695 Ad5mSPC-FLPo
Plasmid: pAd5mSPC-FLPo-SV40pA

Adenoviral vector with Surfactant Protein C promoter driving the expression of Flpo recombinase protein, published from the lab of Dr. Philippe Soriano. A material transfer agreement is required for use of Flpo from Fred Hutchinson. Please email MTA@fredhutch.org to initiate this process. Once an MTA has been secured, please send a copy to vectors@uiowa.edu.

The mSPC promoter in this vector is specific to type II pneumocytes in the lung. Grace Orstad from the lab of Dr. Eric Snyder cloned the vector. Please acknowledge Dr. Eric Snyder from the Huntsman Cancer Institute in any publications using this virus. eric.snyder@hci.utah.edu.

Antibiotic Resistance: Ampicillin
Backbone: pBR322

Note: To check the integrity of the Ad5 plasmid, perform single restriction enzyme digestions with NheI, BssHII, SacII and XmaI.



Mouse sequence: chr14:70,524,050-70,528,805 (GRCm38/mm10 Assembly)
4756 bp. Includes part of 5' UTR of "surfactant associated protein C" (NM_011359) – stops 6 bp from ATG.

<u>pAd5mSPC-FLPo-SV40pA</u>

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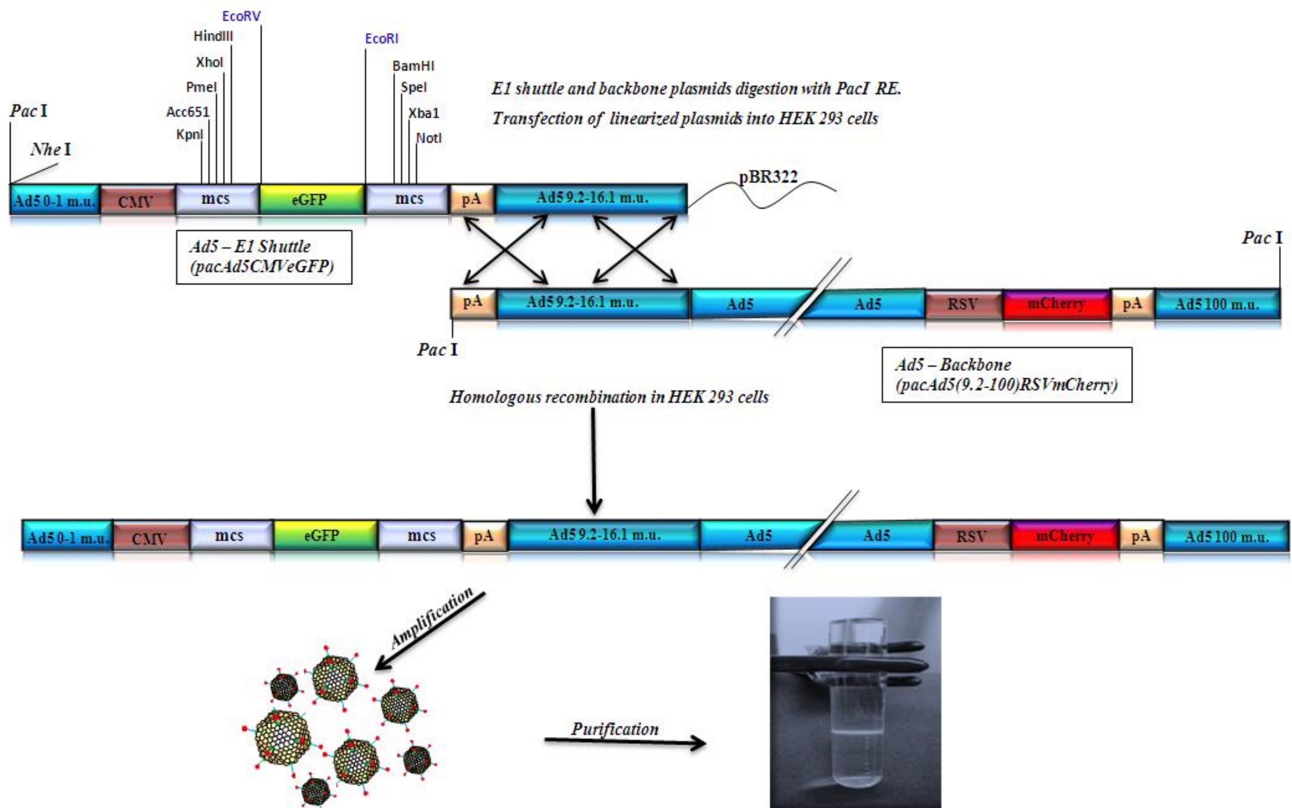
Vector Bio-safety Information
At the University of Iowa, all varieties of viral vectors produced at the Viral Vector Core are required to be handled at Biosafety Level 2 (BSL2). In animal studies, adenoviral vectors require ABL2 containment. Please check with your institution's Biosafety Officer to confirm local requirements

Adenovirus Background:
Adenoviruses are very important tool in basic research. They are used to identify proteins role in different biological processes both *in vivo* and in *vitro*. Virus construction is performed using the RapAd^{TM} System developed by the University of Iowa GTVC (For description, refer to the article "*A simple method for the rapid generation of recombinant adenovirus vectors*" published in Gene Therapy 7:1034-1038, 2000). Adenovirus vectors prepared in the core are E1 and E3 deleted. They have a total E1a deletion (*m.u 1.4 to 4.5) plus a partial E1b deletion (*m.u. 4.7 to 9.2). These deletions are what make the vector replication deficient. They also have a partial E3 deletion, 720bp for the sub360 backbone, a 1.6Kb deletion for the dl309 backbone and a 3.1Kb deletion for the total E3 deleted backbone.
*m.u = Map units (1 m.u = 360bp)

Characteristics:
- Episomal gene expression.
- Infects dividing and non-dividing cells.
- Transient high-level protein expression.
- Accommodates inserts of up to 7.5kb. Larger inserts can be added, provided that an equivalent part of the viral genome has been properly deleted.
- High viral titer can be produced, 1E+10 to 5E+10pfu/ml (1E+12pt/ml) to 8E+10 to 1E+11/ml (1E+13pt/ml).



Disadvantages and adverse effects:

- Elicits host immune response, thus depleting the number of transduced cells *in-vivo.*
- Viral particles can be neutralized by the host immune response.
- Short-term expression of the transgene due to lack of integration into the host genome.

Recombination:

The recombinant adenoviruses can revert to wild type during virus production, thus packaging replication competent particles (RCA). For this reason, each new lot produced at the core is tested for the presence of RCA by immuno-staining.

References:

- **RapAd™ System:** Anderson RD, Haskell RE, Xia H, Roessler BJ, Davidson BL. *"A simple method for the rapid generation of recombinant adenovirus vectors".* Gene Ther. 2000 Jun;7(12):1034-8

- **A195 Buffer**: Evans RK, Nawrocki DK, Isopi LA, Williams DM, Casimiro DR, Chin S, Chen M, Zhu DM, Shiver JW, Volkin DB. *Development of stable liquid formulations for adenovirus-based vaccines.* J Pharm Sci. 2004 Oct;93(10):2458-7

Contact Information:

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Tel: (319) 335-6726
vectors@uiowa.edu

Background on Virus production

The virus was made with our pacAd5(9.2-100)sub360 viral backbone. This backbone has a fully deleted E1a protein, a partially deleted E1b protein, and a partially deleted E3 protein to make the virus replication deficient. All of our Ad5mSPCFLPO vector preparations infected in HEK293 cells, purified by double CsCl protocol, and dialyzed and stored in our A-195 buffer. All preparations are titered on HEK 293 cells using the Clonetech Adeno-X titer kits and also tested for replication competent particles (RCA).

Bacterial Backbone:

The bacterial backbone is derived from pBR322 plasmid.

Antibiotic Resistance:

The adenovirus plasmids are ampicillin resistant. We recommend using an ampicillin concentration of100ug/ml of media.

E. coli Competent Cell Recommendations:

We recommend using DH5a cells to grow the adenovirus plasmids.

1/9/19 SJS