U of Iowa-1174 Ad5CMVCre-eGFP Plasmid: G0169 pacAd5CMVCre -eGFPSV40pA

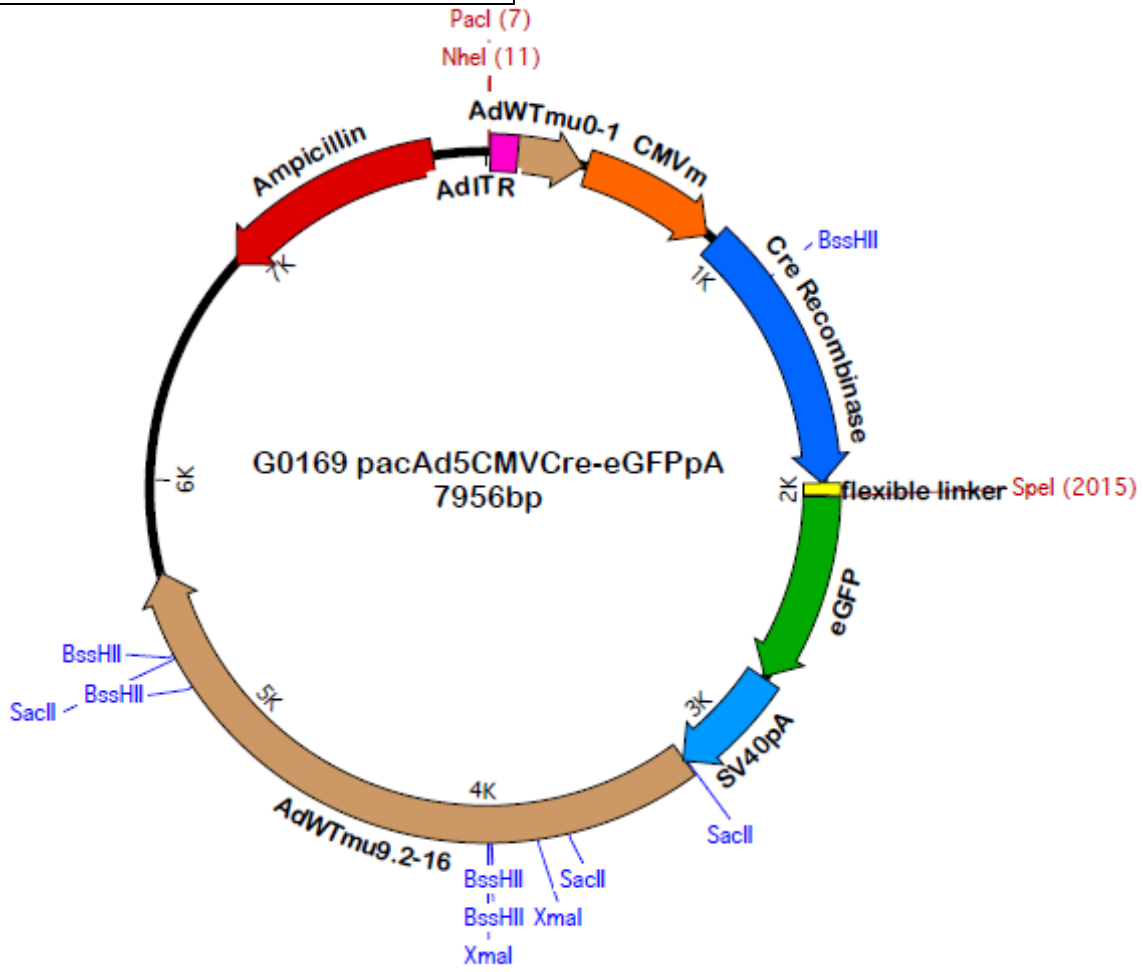
UNIVERSITY of IOWA CARVER COLLEGE OF MEDICINE
VIRAL VECTOR CORE

Plasmid Features: **7956bp**

Coordinates	Feature
Ad5:	16-368 and 3181-5645
mCMV:	385-907
Cre Recombinase:	941-1969
flexible linker:	1970-2014
eGFP	2021-2740
SV40:	2749-3186
Ampicillin:	6893-7753

Antibiotic Resistance: Ampicillin.
Backbone: pBR322

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



G0169 pacAd5CMVCre-eGFP SV40pA 7956 bp

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Vector Bio-safety Information

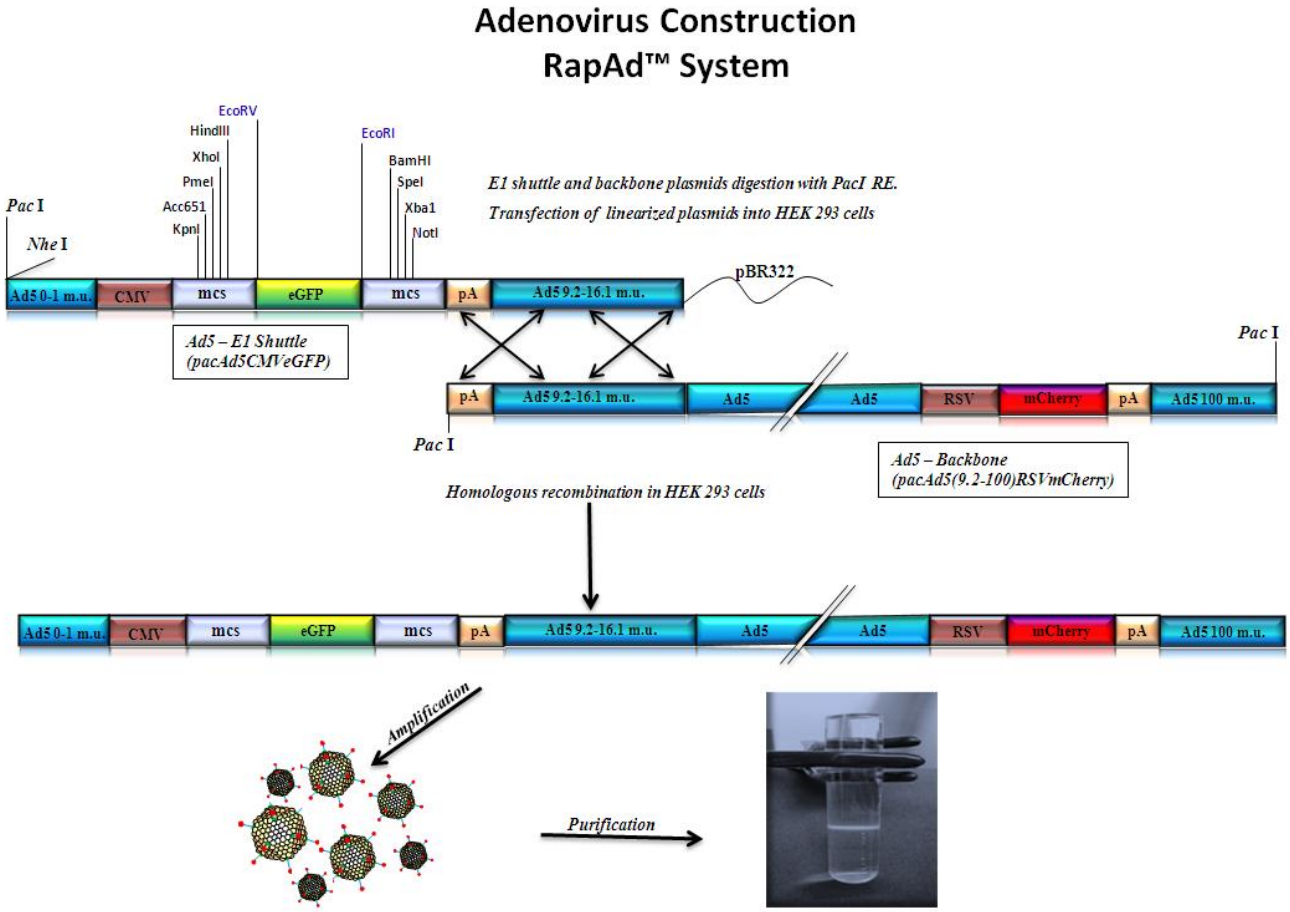
At the University of Iowa, all varieties of viral vectors produced at the Viral Vector Core are required to be handled at Biosafety Level 2 (BSL2). In animal studies, adenoviral vectors require ABL2 containment. Please check with your institution's Biosafety Officer to confirm local requirements

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™ 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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vectors@uiowa.edu

Specific Information on VVC-U of Iowa-1174 Ad5CMVCre-eGFP vector and plasmid G0169 pAd5CMVCre-eGFPSV40pA

Background on Cre Recombinase

This plasmid and vector express the Cre recombinase protein. This protein is derived from the P1 bacteriophage and belongs to the integrase family of site-specific recombinases. It recognizes 34bp sequences known as *loxp* sites, shown below.

13bp 8bp 13bp

ATAACTTCGTATA - NNNTANNN -TATACGAAGTTAT

Variations of the "N" base pairs allows multiple, specific *loxp* sites to be used at the same time. Depending on how the *loxp* sites are set up, the gene of interest can be turned off, turned on, or integrated into other sections of DNA.

The VVC tests for the presence and activity of Cre recombinase using the Flex-reporter system. This system uses a reporter in the reverse orientation flanked by two separate and different pairs of loxp sites. When infected or transfected with virus or plasmid with this cassette will not express the reporter gene under normal circumstances, but will express when exposed to Cre recombinase.

For more information on Cre recombinase please see *Cre recombinase: the universal reagent for genome tailoring*.

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. Ad5CMVCre-eGFP vector preparations are 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). All preparations are also tested for activity and presence of Cre recombinase protein on A549 cells.

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.