Berns-1166 Ad5CC10-Cre
Plasmid Origin: Dr. Anton Berns and
Kate Sutherland
pAdPL-DEST-mCC10-Cre

UNIVERSITY *of* IOWA
CARVER COLLEGE
OF MEDICINE

VIRAL VECTOR CORE

VVC-Berns-1166 Ad5mCC10-Cre
36,849 bp
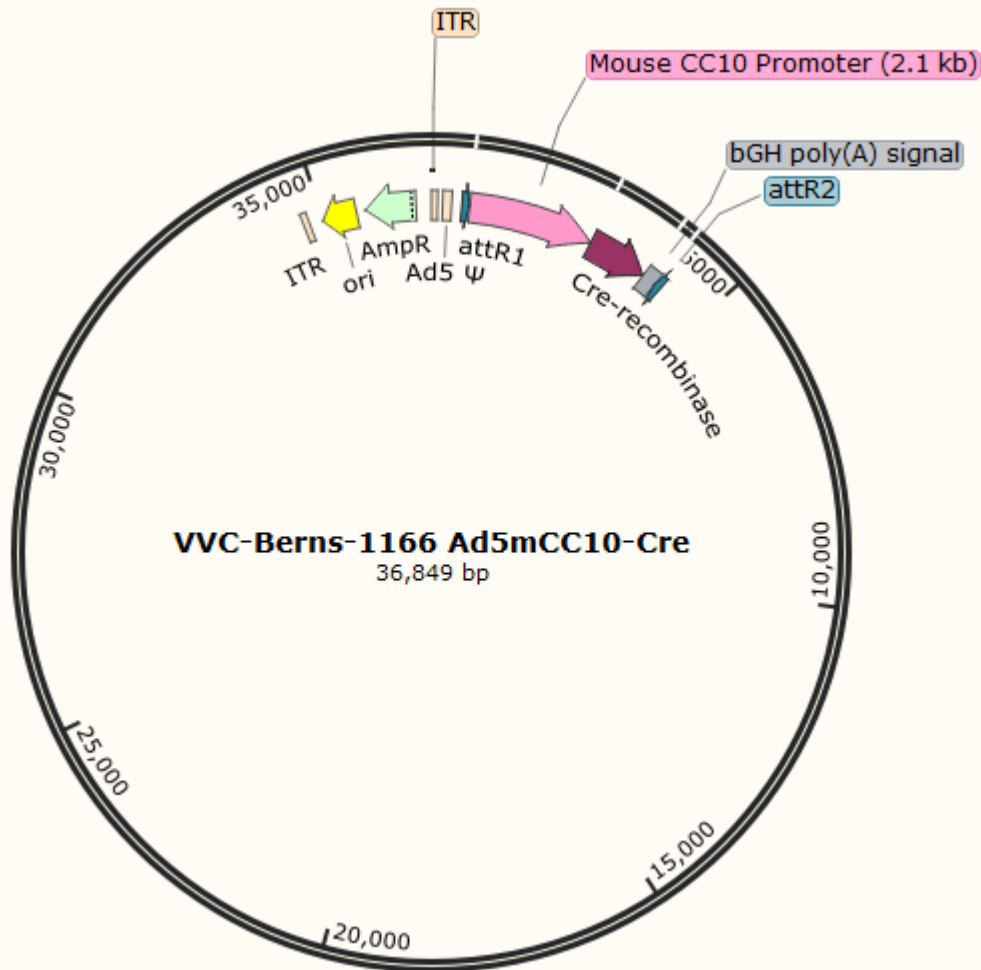
The investigator used a replication deficient Adenovirus pAd PL-DEST from Invitrogen Life Technologies/Thermofisher. Please see below information.

Please acknowledge Dr. Anton Berns from the Netherlands Cancer Institute in any publications using this virus (a.berns2@nki.nl). Publication: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5065004/

Inserts:
Lung specific Mus musculus gene CC10 Promoter, 2.1kb.
Cre-recombinase coding sequence, 1.5kb.

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

The ViraPower™ Adenoviral Expression System
https://www.thermofisher.com/us/en/home/references/protocols/proteins-expression-isolation-and-analysis/adenovirus-protocol/virapower-adenoviral-expression-system.html

The ViraPower™ Adenoviral Expression System facilitates highly efficient, in vitro or in vivo delivery of a target gene to dividing and non-dividing mammalian cells using a replication-incompetent adenovirus. Based on the secondgeneration vectors developed by Bett et al., 1994, the ViraPower™ Adenoviral Expression System takes advantage of the Gateway® Technology to simplify and greatly enhance the efficiency of generating high-titer, recombinant adenovirus.

The plasmid, pAd-DEST, is an E1 and E3-deleted expression vector into which the gene of interest will be cloned. Expression of the gene of interest is controlled by the human cytomegalovirus (CMV) promoter (in pAd/CMV/V5-DEST) or the promoter of choice (in pAd/PL-DEST). The vector , an "all in one" adenoviral plasmid, contains the elements required to allow packaging of the expression construct into virions (e.g. 5′ and 3′ ITRs, encapsidation signal, adenoviral late genes). For more information about the pAd-DEST expression vectors, refer to the pAd/CMV/V5-DEST and pAd/PL-DEST Gateway® Vector manual.

Adenovirus enters target cells by binding to the Coxsackie/Adenovirus Receptor (CAR) (Bergelson et al., 1997). After binding to the CAR, the adenovirus is internalized via integrin-mediated endocytosis (Russell, 2000) followed by active transport to the nucleus. Once in the nucleus, the early events are initiated (e.g. transcription and translation of E1 proteins), followed by expression of the adenoviral late genes and viral replication. Note that expression of the late genes is dependent upon E1. In the ViraPower™ Adenoviral Expression System, E1 is supplied by the 293A producer cells. The viral life cycle spans approximately 3 days. For more information about the adenovirus life cycle and adenovirus biology, refer to published reviews (Russell, 2000).

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.*

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.

Storage Buffer:
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

Background on Virus production
All of our adenoviral vector preparations are made 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).

Contact Information:
Viral Vector Core
University of Iowa
500 Newton Road

221 Eckstein Medical Research Building
Iowa City, IA 52242
Tel: (319) 335-6726
vectors@uiowa.edu

Hypothetical Plasmid Sequence. Sequence not provided by Dr. Berns and not confirmed by the Viral Vector Core. Particles were provided for amplification to the Viral Vector Core. Unknown cloning sites are represented with N.

pAd5PL-DEST-mCC10Cre

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