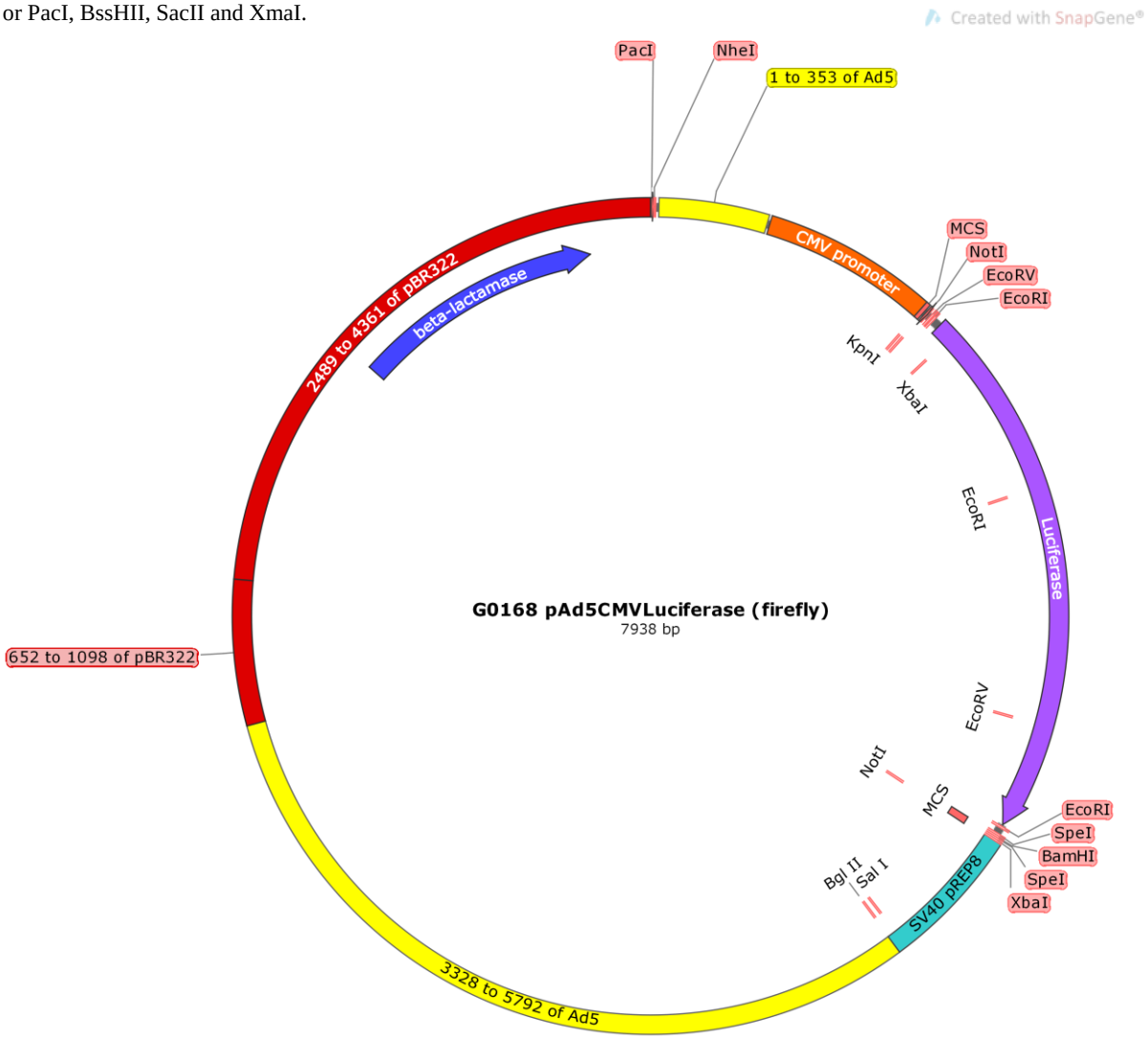
U of Iowa-538 Ad5CMVLuciferase (firefly)
Plasmid: G0168 pacAd5CMVLuciferase-SV40pA

UNIVERSITY of IOWA CARVER COLLEGE OF MEDICINE
VIRAL VECTOR CORE

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.



Features:

Ad5 ITR	**16-118 bp**
CMV promoter	**385-907 bp**
Luciferase	**990-2642 bp**
SV40 pA	**2724-3161 bp**
Ad5 BB map units 9.2-16	**3164-5618 bp**
Beta-lactamase Ampicillin (Complimentary)	**6868-7728 bp**

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Vector Bio-safety Information

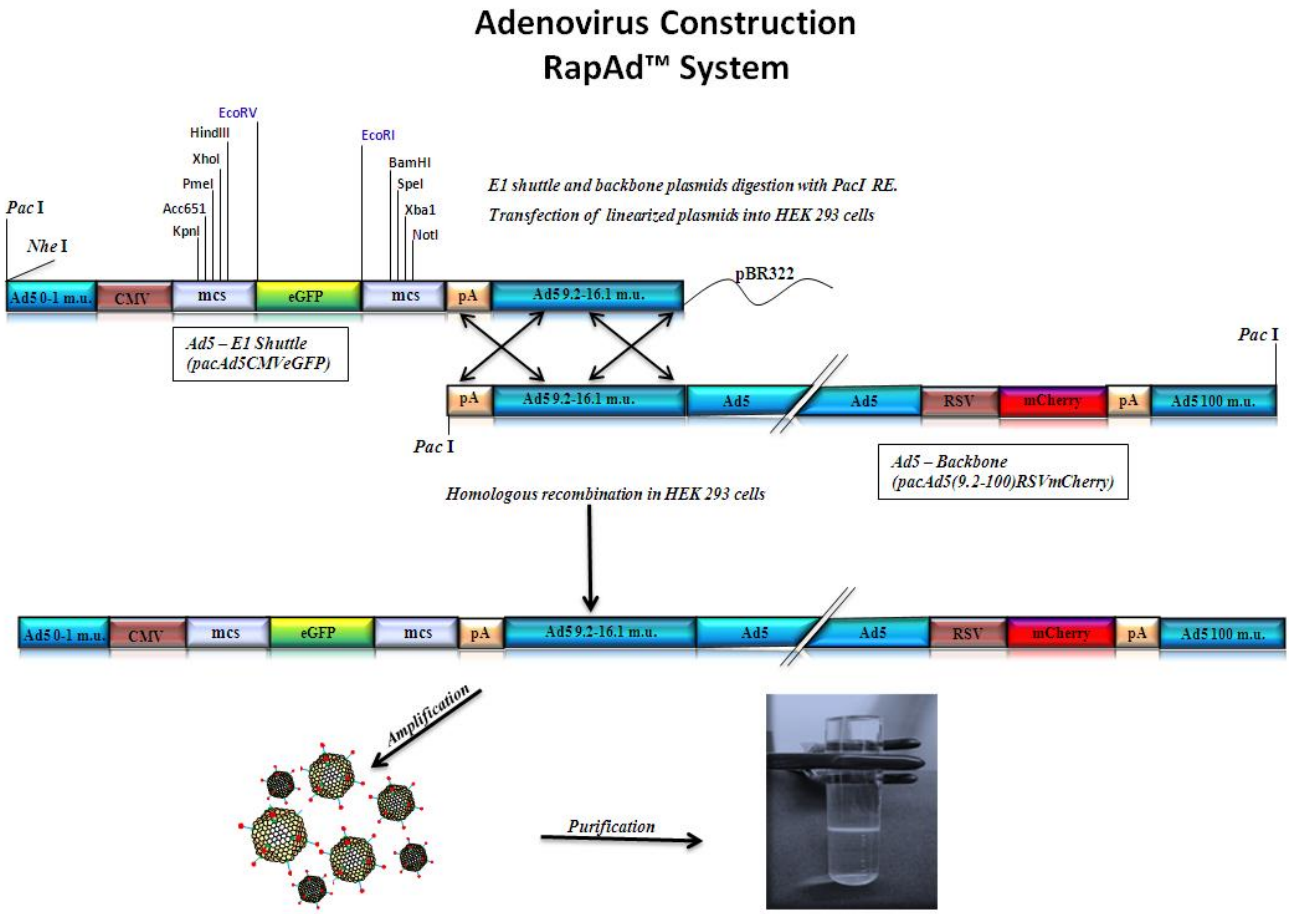
At the University of Iowa, all varieties of viral vectors produced at the Viral Vector Core are required to be handled at Biosafety Level 2 (BSL2). In animal studies, adenoviral vectors require ABL2 containment. Please check with your institution's Biosafety Officer to confirm local requirements

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

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