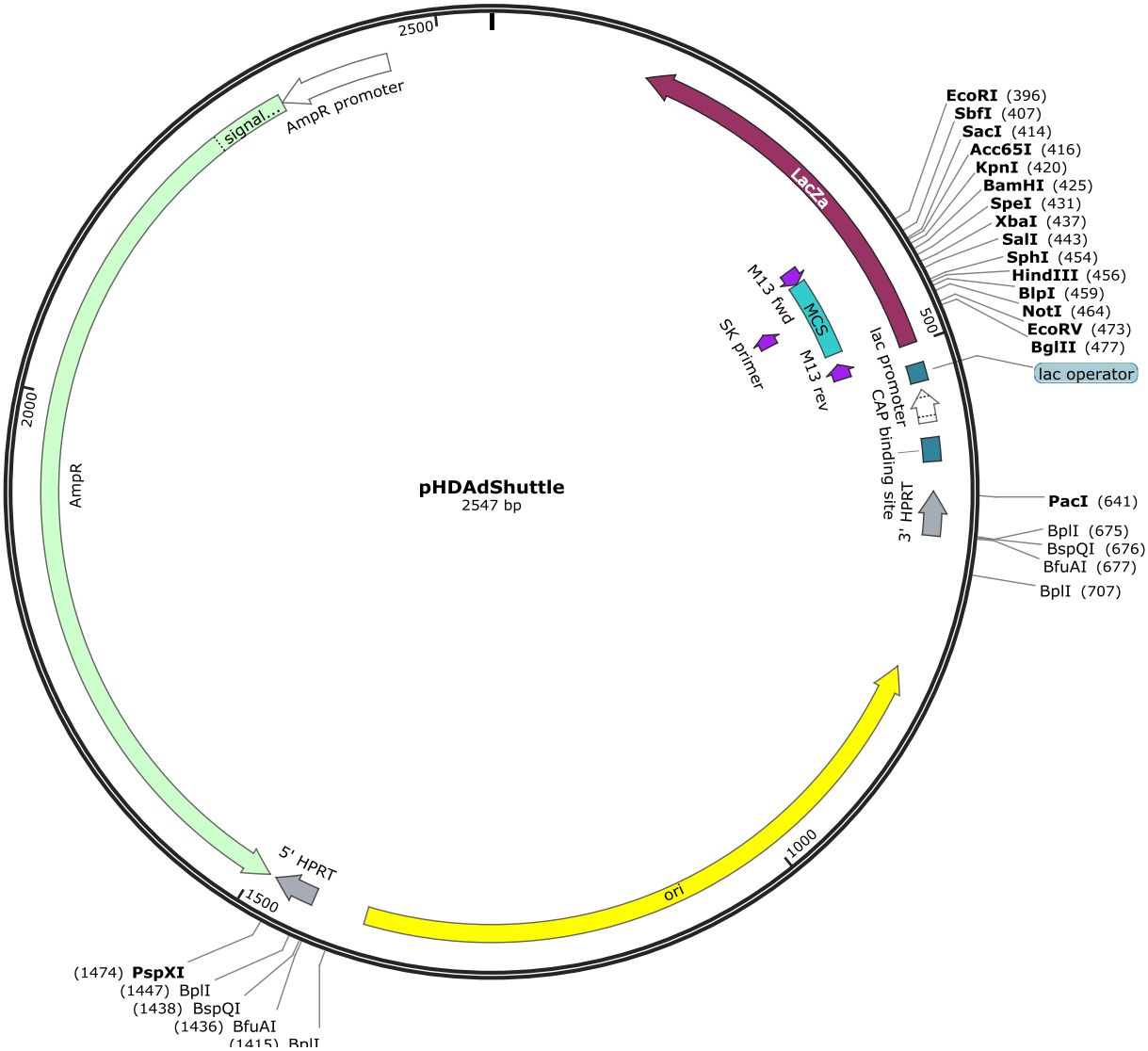
G1092 pHDAdShuttle1-PacI-PspXI

UNIVERSITY of IOWA CARVER COLLEGE OF MEDICINE
VIRAL VECTOR CORE

Plasmid Features:	**2574bp**
Coordinates	**Feature**
LacZa	**146-499**
MCS:	**386-482**
3' HPRT	**637-677**
Ampicillin:	**1487-2452**
5' HPRT	**1441-1480**

Antibiotic Resistance: Ampicillin
Backbone: pUC57

Note: A double digest with PacI and PspXI is recommended to assure the integrity of the plasmid.



G1092 pHDShuttle 2547bp

TCGCGCGTTTCGGTGATGACGGTGAAAACCTCTGACACATGCAGCTCCCGGA
GACGGTCACAGCTTGTCTGTAAGCGGATGCCGGGAGCAGACAAGCCCGTCAG
GGCGCGTCAGCGGGTGTTGGCGGGTGTCGGGGCTGGCTTAACTATGCGGCAT
CAGAGCAGATTGTACTGAGAGTGCACCATATGCGGTGTGAAATACCGCACAG
ATGCGTAAGGAGAAAATACCGCATCAGGCGCCATTCGCCATTCAGGCTGCGC
AACTGTTGGGAAGGGCGATCGGTGCGGGCCTCTTCGCTATTACGCCAGCTGG
CGAAAGGGGGATGTGCTGCAAGGCGATTAAGTTGGGTAACGCCAGGGTTTTC
CCAGTCACGACGTTGTAAAACGACGGCCAGTGAATTCCCTGCAGGGAGCTCG
GTACCAGGGGATCCACTAGTTCTAGAGTCGACGGCATGCAAGCTTAGCGGCC
GCGATATCAGATCTGGCGTAATCATGGTCATAGCTGTTTCCTGTGTGAAATTG
TTATCCGCTCACAATTCCACACAACATACGAGCCGGAAGCATAAAGTGTAAA
GCCTGGGGTGCCTAATGAGTGAGCTAACTCACATTAATTGCGTTGCGCTCACT
GCCCGCTTTCTTAATTAACCCCATCTAATATGGATTAGTTAACAATCCAGTCA
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AAAAATCGACGCTCAAGTCAGAGGTGGCGAAACCCGACAGGACTATAAAGA
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GCCGCTTACCGGATACCTGTCCGCCTTTCTCCCTTCGGGAAGCGTGGCGCTTT
CTCATAGCTCACGCTGTAGGTATCTCAGTTCGGTGTAGGTCGTTCGCTCCAAG
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TAACTATCGTCTTGAGTCCAACCCGGTAAGACACGACTTATCGCCACTGGCA
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ACGCTCAGTGCCGAGACCTGCTCTTCAAACCAGCACTTTGGGAGGCCAAGGC
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AGATAACTACGATACGGGAGGGCTTACCATCTGGCCCCAGTGCTGCAATGAT
ACCGCGAGACCCACGCTCACCGGCTCCAGATTTATCAGCAATAAACCAGCCA
GCCGGAAGGGCCGAGCGCAGAAGTGGTCCTGCAACTTTATCCGCCTCCATCC
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LacZ
CTATGCGGCATCAGAGCAGATTGTACTGAGAGTGCACCATATGCGGTGTGAAATACCGCACA
GATGCGTAAGGAGAAAATACCGCATCAGGCGCCATTCGCCATTCAGGCTGCGCAACTGTTGG
GAAGGGCGATCGGTGCGGGCCTCTTCGCTATTACGCCAGCTGGCGAAAGGGGGATGTGCTGC
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GTGAATTCCCTGCAGGGAGCTCGGTACCAGGGGATCCACTAGTTCTAGAGTCGACGGCATGCA
AGCTTAGCGGCCGCGATATCAGATCTGGCGTAATCATGGTCAT

MCS
GAATTCCCTGCAGGGAGCTCGGTACCAGGGGATCCACTAGTTCTAGAGTCGACGGCATGCAA
GCTTAGCGGCCGCGATATCAGATCT

3’ HPRT
TTAATTAACCCCATCTAATATGGATTAGTTAACAATCCAGT

5’ HPRT
CCAGCACTTTGGGAGGCCAAGGCAAGTTGATCGCTCGAGG

Ampicillin (ORF and promoter):
TTACCAATGCTTAATCAGTGAGGCACCTATCTCAGCGATCTGTCTATTTCGTTCATCCATAGTT
GCCTGACTCCCCGTCGTGTAGATAACTACGATACGGGAGGGCTTACCATCTGGCCCCAGTGCT
GCAATGATACCGCGAGACCCACGCTCACCGGCTCCAGATTTATCAGCAATAAACCAGCCAGC
CGGAAGGGCCGAGCGCAGAAGTGGTCCTGCAACTTTATCCGCCTCCATCCAGTCTATTAATTG
TTGCCGGGAAGCTAGAGTAAGTAGTTCGCCAGTTAATAGTTTGCGCAACGTTGTTGCCATTGC
TACAGGCATCGTGGTGTCACGCTCGTCGTTTGGTATGGCTTCATTCAGCTCCGGTTCCCAACGA
TCAAGGCGAGTTACATGATCCCCCATGTTGTGCAAAAAAGCGGTTAGCTCCTTCGGTCCTCCG
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TCTCTTACTGTCATGCCATCCGTAAGATGCTTTTCTGTGACTGGTGAGTACTCAACCAAGTCAT
TCTGAGAATAGTGTATGCGGCGACCGAGTTGCTCTTGCCCGGCGTCAATACGGGATAATACCG
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CAGCATCTTTTACTTTCACCAGCGTTTCTGGGTGAGCAAAAACAGGAAGGCAAAATGCCGCAA
AAAAGGGAATAAGGGCGACACGGAAATGTTGAATACTCATACTCTTCCTTTTTCAATATTATT
GAAGCATTTATCAGGGTTATTGTCTCATGAGCGGATACATATTTGAATGTATTTAGAAAAATA
AACAAATAGGGGTTCCGCG

Plasmid Digestion: pHDAdShuttle

GTVC#:

Information and Cloning Suggestions for Working with pHDAdShuttle:

Background:

Adenoviruses are very important tools in basic research. They are used to identify a proteins role in different biological processes both *in vivo* and in *vitro*. Adenoviral vectors were first generated by deleting portions of the E1 and E3 genes. Helper dependent or "gutless" adenoviral vectors have been generated as the final generation of adenoviral vectors. Helper dependent adenoviral vectors have had all expressed viral proteins removed. The viral "genome" only contains the adenoviral packaging signal, an untranslated portion of the E4 gene, and stuffer sequences. The pHDAdShuttle is a simple plasmid to prepare a gene cassette for insertion into one of the helper dependent adenoviral backbones. The viral backbone that will be used for viral construction will depend on the size of the inserted gene cassette.

Backbone	Insert size available
pΔ28E4	0-9kb
pΔ25E4	3-12kb
pΔ21E4	7-16kb
pΔ17E4	11-20kb
pΔ12E4	16-25kb
pΔ8E4	20-29kb

Helper Dependent Adenoviral Vectors Characteristics:

- Episomal gene expression.
- Infects dividing and non-dividing cells.
- Transient high-level protein expression.
- Accommodates inserts of up to 30kb.
- Preps can be produced either as: Regular concentration preps: 1E+10 to 5E+10pfu/ml (1E+12pt/ml) or High concentration preps: 8E+10 to 1E+11/ml (1E+13pt/ml).
- Limited host immune response based on the lack of viral protein products.

Disadvantages and adverse effects:

- Immune responses against the transgene can still illicit an immune response.
- 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.

Cloning Helper Dependent Adenovirus Plasmids:

The helper dependent adenovirus system uses two cloning steps to generate the helper dependent adenoviral genome:

1. The Shuttle Plasmid: The shuttle plasmid is usually cloned by the investigator and contains the gene of interest driven by the desired promoter and any other elements needed for a single or bicistronic construct. The gene cassette must not contain a PmeI site and must be free of either BfuAI, BplI, or BspQI sites. BfuAI, BplII, or BspQI is used to cut out the gene cassette in the shuttle prior to cloning into the viral backbone and PmeI is used to linearize the viral backbone prior to the initial transfection. The shuttle vector contains two small sections of stuffer sequences that can be used for Gibson Assembly into the helper dependent viral backbones. The MCS of the shuttle plasmid is inserted into a LacZ ORF. This allows for simple white-blue screening using X-gal. The VVC also offers limited cloning services for investigators. Once the shuttle plasmid is received by the VVC, then the gene cassette will be cloned into the appropriate backbone plasmid.

2. The Viral Backbone Plasmid: This plasmid contains the adenoviral packaging signal, an untranslated portion of the E4 gene, and stuffer sequences. The investigator's gene cassette is moved from the pHDAdShuttle into the appropriate backbone by restriction digest and then gibson assembly. Multiple gene cassettes can be transferred into a single viral backbone as long as the final backbone size is between 28 and 37kb in length.

Insert Size:
Helper dependent Adenovirus vectors can accommodate inserts of up to 30kb, this includes promoters, gene(s) of interest, and other elements. Multiple viral backbones are available with varying sizes of stuffer sequences. Choosing which backbone to use depends on the size of the gene cassettes that will be inserted into them.

E. coli Competent Cell Recommendations:
We recommend using DH5alpha cells to grow the plasmids.

Recommended Plasmid Quality Control:

Sequencing:
The Multiple Cloning Sequence in the HDAdShuttle plasmid is flanked by M13Forward and M13 Reverse. As there are multiple versions of these sites, the primers that should be used are below. Depending on the size of the insert cloned into the shuttle, multiple internal primer sequences may need to be used as well to fully sequence the insert.

M13 For	**5'-GTA AAA CGA CGG CCA GT-3'**
M13 Reverse	**5'-CAG GAA ACA GCT ATG AC-3'**

It is highly recommended to sequence all genes inserted into the viral shuttle plasmid before it is sent to us for virus construction.

Protein Expression:
The HDAd5shuttle plasmids can be used as expression vectors if the gene cassette includes a promoter, the gene of interest, and a suitable polyA signal.

Integrity Digest:
We recommend that submitted plasmids be checked by digest as well as sequencing. For this plasmid we suggest cutting with PacI and PspXI. This should result in two bands. One band should be around 830bp and the other band should be around 1700bp plus the size of your inserted gene cassette.

Virus Construction:
Once the shuttle plasmid is received by the VVC, we will first digest it using PacI and PspXI to remove the ori site, linearize the plasmid, and prepare it for Gibson assembly into the chosen HDAd backbone plasmid. The HDAd backbone plasmid with the inserted gene cassette will then be prepared, digested, and transfected into 116 cells (HEK293 cells that express the Cre recombinase protein). After this transfection, a helper virus will then be added to the cells. This helper virus is an E1A deleted adenoviral vector that only expresses viral proteins. The proteins expressed by the helper virus then self-assemble and packaged the HDAd backbones that have been transfected into the cells.

Wild Type Recombination:
The recombinant adenoviruses (from the helper virus) 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 or qPCR.

Available Shuttle vectors:

The map and sequence for these vectors are found in our webpage (http://www.medicine.uiowa.edu/vectorcore/). Requests can be submitted through our iLabs system.

HDAd Shuttle vectors

- G1092 pHDAdShuttle1-PacI-PspXI
- G1236 pHDAdShuttle6-BsiWI

Viral Backbones Available for Use

- pΔ28E4
- pΔ25E4
- pΔ21E4
- pΔ17E4
- pΔ12E4
- pΔ8E4

The choice of which vector to use is dependent on the size of the inserted gene cassette. Additional plasmids are always being constructed. Please inquire.

Helper Dependent Adenovirus Viral Production Services:

The standard service for HDAd includes construction, amplification, purification and titer for vector from a shuttle plasmid.

1. Transfection, amplification, purification & titer

- **Shuttle:** One of the above HDAd shuttle vectors is provided free of charge except for shipping.
- **Sample required**: 20ug of adenovirus plasmid expressing the gene of interest at a concentration greater than 0.25ug/ul.
- **Time line**: 5-6 weeks from the time the plasmid is received.

- **Service:**
  - Transfection and rescue of Helper Dependent Adenovirus
  - Large-scale amplification.
  - Purification by double cesium chloride gradient
  - With every new lot an infectious titer (pfu/ml) is provided.
  - Each lot is checked for wild type virus contamination (RCA).

- **Material provided:** 2ml and a 100ul tester at a concentration of1E+10 to 5E+10pfu/ml (1E+12pt/ml) or 1mL of High Titer virus at a concentration of 8E+10 to 1E+11pfu/ml (1E+13pt/ml).**
- **Vehicle:** Adenovirus vectors are re-suspended in A195 buffer (See reference below)
- **Stock:** The vector is kept in stock. Additional 1ml aliquots can be purchased.

**Additional charges apply to High Titer preparations

References:

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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