G0796 pacAd5TREtightmcsSV40pA

Plasmid Features: 6009bp

<table>
<thead>
<tr>
<th>Coordinates</th>
<th>Feature</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>ITR:</strong></td>
<td>16-118</td>
</tr>
<tr>
<td>Ad5 wt</td>
<td>16-365 and 1240-3698</td>
</tr>
<tr>
<td><strong>TRE-tight:</strong></td>
<td>402-717</td>
</tr>
<tr>
<td><strong>SV40:</strong></td>
<td>802-1239</td>
</tr>
<tr>
<td><strong>Ampicillin:</strong></td>
<td>5806-4946</td>
</tr>
</tbody>
</table>

Antibiotic Resistance: Ampicillin
Backbone: pBR322

Note: To check the integrity of the Ad5 backbone perform single restriction enzyme digestions with NheI or PacI, BssHII, SacII, and XmaI.
Multiple cloning site just downstream of the TRE tight

**G0796 pAd5TREtightmcspA 6009bp**

ATTTGCTCTAGGAGATCCGGTACCAGCG GCCGC CACCGCGGGGAGATCCAGACATGATAAGATACATTGATGAGTTTGGACAAACCACAACTAGAATGCAGTGA AAAAAAAAAATGCTTTATTTGTGAAATTTGTGATGCTATTGCA

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CACATTTCGGAAAAGTGCCACCTGACGTCTAAGAAACCATTATTATCATGACATTAAC
CTATAAAATAGGGCGTATCACGAGGCCCTTTCGTCTTC

Plasmid Digestion: pacAd5TRE-tightmcsSV40pA

VVC#: G0796
Prep date: May 2013
Concentration: 0.975ug/ul

Expected Fragments:
Pael or Nhel: Linear 6009
BssHII: 2 131 1256 4620
SacI: 860 2000 3150
SacII: 420 500 1654 3429
Xmal: 180 1076 4753

G0796 pAd5TREtightmcsApA 5/24/13 1ug/well
Introduction to the TRE tight promoter system

TRE tight is the name of the second generation Clontech promoter which can be induced by the tTA regulator in the Tet-Off system, or it can be induced by the rtTA regulator in the Tet-On system depending on your cloning needs. Both systems offer inducible expression of your gene of interest based on the presence or absence of Dox and/or Tet. Please note that the TRE promoter without rtTA or tTA yields extremely low expression of its gene of interest, and can fully minimize background expression in certain cell line types, making it ideal for genes that can be toxic or lethal to host cells.

The Tet-On system induces expression of the TREtight promoted gene of interest only in the presence of Doxicyclin (it is not affected by Tetracyclin). When both the rtTA regulator and TREtight cassette are present in a cell, gene expression will only be seen after the addition of Doxicyclin antibiotics.

The Tet-Off expression system induces expression of the TREtight promoted gene of interest only when both Dox and Tet are absent. When both the tTA regulator and TREtight cassette are present in a cell, gene expression will only be turned on in the absence of both Tetracyclin and Doxicyclin. If either antibiotic is present, gene expression is turned off.

Clontech designed these systems such that double-stable Tet cell lines could be constructed with the vectors or purchased direct from the company to save time. Technical notes about expression, binding mechanics and cloning recommendations can be found in the Clontech Manual.

In Adenovirus, your gene of interest should be cloned in behind TRE-tight, with or without a reporter as you see fit. During the viral packaging process, rtTA or tTA will be incorporated as part of the viral backbone so that the final virus will work according to the Tet-On or Tet-Off system.

For the Clontech Tet Systems Manual, visit this link:
http://www.google.com/url?sa=t&rct=j&q=&esrc=s&frm=1&source=web&cd=1&ved=0CDEQFjAA&url=http%3A%2F%2Fwww.clontech.com%2Fxxclt_ibcGetAttachment.jsp%3FcItemId%3D17553&ei=B8WTUf0hPzlAYKKgeAG&usg=AFQjCNHr1sfeponxfMFbYv1PS49qRSaEg&sig2=GuAovwifIcIn-cjmL9u5nQ&bvm=bv.46471029,d.aWc
Information and Cloning Suggestions for Working with pacAd5 plasmids:

Background:
Adenoviruses are very important tools in basic research. They are used to identify a protein's 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 VVC (For a 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. The vectors also have a partial E3 deletion: 720bp deleted in the sub360 backbone, 1.6Kb deleted in the dl309 backbone, and 3.1Kb deleted for the total E3 deleted backbone.

*m.u = Map units (1 m.u = 360bp)

Adenovirus 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.
- 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).

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.

Cloning Adenovirus Plasmids:
The RapAd™ System uses a two plasmid transfection system to generate recombinant adenovirus particles, the shuttle plasmid and the viral backbone plasmid:

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. Reporter genes such as eGFP may be cloned into this plasmid or may come from the viral backbone plasmid. See the advantages and disadvantages below. The VVC offers shuttle plasmids with a number of common promoters already prepared and are available free of charge. The VVC also offers limited cloning services for investigators.

2. The Viral Backbone Plasmid: This plasmid contains most of the adenoviral genome, including the partially (or fully) deleted E3 region. With a portion of the E3 deleted, an additional gene cassette can be added. The VVC offers several very convenient viral backbone plasmids with and without reporters for investigators to choose from. No cloning of these plasmids is necessary unless you have a custom design in mind. Simply choose the desired backbone when ordering the virus construction.
- Inserting a reporter gene in the shuttle plasmid allows for tracking of protein of interest expression and/or location, depending on cloning design, i.e. fused proteins, bicistronic elements, etc. It also allows tracking viral infection efficiency. The advantages of using a viral backbone plasmid expressing a reporter gene include reduced shuttle plasmid cloning time and complexity and it also offers very high expression of the reporter. The main disadvantages are that the expression of the reporter is not directly tied to the expression of the protein of interest. So, these backbones should only be used for tracking infection efficiency and not protein of interest expression. Also, due to the high
expression level of the reporter it can elicit a toxic effect depending on the reporter and the cell line used.

- We also offer the ability to express multiple proteins within a single viral particle with custom made backbones. See the available E3 shuttles for cloning. The combination of shuttle plasmids and viral backbones offer the investigators great versatility in gene expression. One example is our pacAd5(9.2-100)RSVrtTA-Advanced backbone used with the pacAd5TRE-tightmcspA plasmid. This method allows for the transactivator protein (rtTA) and the TRE driven gene of interest protein to be expressed in the same cell from a single viral particle. Previously, the transactivator and gene of interest expression cassettes had to be packaged in separate vectors and co-infected.

**Insert Size:**
Adenovirus vectors can accommodate inserts of up to 7.5kb, this includes promoters, gene(s) of interest, and other elements. Larger inserts can be accommodated though, provided that an equivalent part of the viral genome has been properly deleted. The VVC currently has two backbones with additional portions of the E3 region deleted (known as the G0685 pacAd5 (9.2-100)Δ1.6KbE3 and G0686 pacAd5 (9.2-100)Δ3.1KbE3).

**E. coli Competent Cell Recommendations:**
We recommend using DH5alha cells to grow the adenovirus plasmids.

**Required Plasmid Quality Control:**
As part of our standard quality control practices, we now require new constructs to provide a standard ITR digest on the shuttle plasmid, which should include a single digest with each SacII, XmaI, and PacI(or NheI). All wells and the ladder must be clearly labeled, as well as a list of expected fragments. We also require the full text sequence and map, and NCBI gene identification numbers for all inserted plasmid elements. It is imperative that we follow NIH guidelines for recombinant DNA technology. Therefore, all work must be approved by our Recombinant DNA Advisory Committee. Complete gene information must be completed on the Service Request Form. University of Iowa Investigators need a copy of the approved rDNA form.

**Recommended Plasmid Quality Control:**

<table>
<thead>
<tr>
<th>Sequencing:</th>
<th>The following primers have been deposited at the University of Iowa DNA facility to aid in sequencing pacAd5CMVmcspA plasmid.</th>
</tr>
</thead>
<tbody>
<tr>
<td>SV40 193 rev</td>
<td>CORE.polyArev 5'-TTA AAA AAC CTC CCA CAC CTC CCC-C-3'</td>
</tr>
<tr>
<td>CMV 544 forward</td>
<td>CORE.CMVpFor 5'-GTG GGA GGT CTA TAT AAG CAG AGC TCG-3'</td>
</tr>
<tr>
<td>Ad5 Forward</td>
<td>CORE.Ad5For 5'-ACT GAA TAA GAG GAA GTG AAA-3'</td>
</tr>
</tbody>
</table>

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:**
All of the adenoviral shuttle plasmids can act as expression plasmids and it is therefore highly recommended that shuttle plasmids are tested for protein expression prior to sending it to the VVC.

**Integrity Digest:**
We recommend that submitted plasmids be checked by digest as well as sequencing. We suggest single restriction enzyme digestions with PacI, BssHII, SacII and Xmal. As a required step during viral production, the shuttle and backbone plasmids need to be linearized. Generally, the PacI restriction enzyme is used for linearization. If PacI is no longer a unique site in your plasmid the unique NheI site will be used. We appreciate knowing if your final plasmid does not contain either a unique PacI or NheI site ahead of time.

**Virus Construction:**
The shuttle plasmid and viral backbone plasmids are linearized with PacI or NheI and transfected into HEK293 cells where homologous recombination occurs at the overlapping section of the Ad5 genome. See the diagram below.

**Recombination and Biosafety:**
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. It is still highly recommended that all work incorporating recombinant Adenovirus be done under biosafety level 2 conditions, including animal studies.
Available Shuttle vectors:
The map and sequence for these vectors are found on our webpage (http://www.medicine.uiowa.edu/vectorcore/ad/shuttles/). You can also login to our online ordering system iLabs using your account registration information, then go to “Request Services” Plasmid - Information and Request Forms.

Additional plasmids are always being constructed. Please inquire.

Viral Backbones Available for Use
Below are the current viral backbones that the VVC has available.

- G0431 \( \text{pacAd5(9.2-100)sub360 (Δ720bp in E3)*} \)
- G0186 \( \text{pacAd5(9.2-100)RSVeGFP in E3} \)
- G0940 \( \text{pacAd5(9.2-100)RSVntLacZ in E3} \)
- G0599 \( \text{pacAd5(9.2-100)RSVmCherry in E3} \)
- G0808 \( \text{pacAd5(9.2-100)RSVrtTA-Advanced (Tet-On)**} \)
- G0685  pacAd5(9.2-100)∆1.6Kb E3 (dl309)**
- G0686  pacAd5(9.2-100)∆3.1Kb E3***

* Default viral backbone, no additional genes inserted into E3 region. If no backbone is specified on the order, this is the backbone that will be used.

** This backbones express the transactivator for the 2nd generation Tet-On system.

***The ∆1.6Kb and ∆3.1Kb have additional deletions in the E3 region, but no genes inserted into the region. These backbones are useful when attempting to create vectors with large inserts in the viral shuttle plasmid.

** E3 Backbone Shuttle vectors**
- G0226  pAd5E3RSVKan

The pAd5E3RSVKan backbones shuttle plasmid is used to create new backbones. The gene that is intended for backbone expression is inserted into this plasmid by the investigator or core personnel. Then the backbone will be created by the VVC by E. coli recombination. For more information contact us at Vectors@uiowa.edu.

**Adenovirus Viral Production Services:**
The VVC offers two standard services for adenovirus production:
1. Amplification, purification and titer from viral particles
2. Construction, amplification, purification and titer from plasmid

1. **Amplification, purification & titer from viral particles**
   - **Sample required:** Viral Particles (either purified or crude cellular lysate)
   - **Time line:** 3-5 weeks from the time the virus particles are received.
   - **Service:**
     - 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 replication competent adenovirus (RCA) contamination.
   - **Material provided:** 2ml and a 100ul tester of ready to use virus at a concentration of 1E+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.

2. **Recombination, amplification, purification & titer**
   - **Shuttle:** A cloning/expression adenovirus shuttle vector (see list below) is provided free of charge except for shipping after a Material Transfer Agreement is signed.
   - **Sample required:** 40ug 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 recombination.
• Large-scale amplification.
• Purification by:
  - Double cesium chloride gradient or,
  - Adenopure™ filter purification method.
• 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 of 1E+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:


Contact Information:

Vectors@uiowa.edu

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Fax: (319) 335-6895

*version 1/23/15 AN