pDNAVACultra Expression Vectors
Instruction Manual

Catalog Numbers
NTC-DVU1
NTC-DVU2
NTC-DVU3
NTC-DVU4
NTC-DVU5
NTC-DVU6
NTC-DVU7
NTC-DVU5-EGFP
NTC-DVU1-5
NTC-DVU4-7

Version 8
April 2013
General Information

Contents: 20 ug each of pDNAVACCultra vector shipped in 1x TE buffer.

Storage: Plasmids should be stored at -20°C
pDNAVACCultra vector family

Introduction

Nature Technology Corporation’s (NTC’s) pDNAVACCultra DNA Vaccine plasmids (e.g. Figure 1; pDNAVACCultra 3) were specifically designed as safe minimalized vectors for the production of neutralizing immune responses by genetic immunization. The vectors combine minimal prokaryotic sequences, kanamycin selectable marker, and highest-level expression with the ability to target individual compartments (secreted, endosomal, membrane-anchored, proteosome, or native) (Williams et al. 2006). The ability to control antigen trafficking provides investigators with a rapid, rational approach to antigen development for cancer therapy and emerging infectious diseases.

The plasmids were designed to be responsive to Food and Drug Administration (FDA) regulatory guidance’s regarding DNA Vaccine vector composition (FDA 1996, FDA 2007; reviewed in Williams et al. 2009). All sequences that were not essential for Escherichia coli plasmid replication or mammalian cell expression of the target gene were eliminated. Synthetic eukaryotic mRNA leader and terminators were utilized in the vector design to limit DNA sequence homology with the human genome to reduce the possibility of chromosomal integration.

Target gene expression is driven from an optimized CMV enhancer-promoter-synthetic intron. The vectors encode an consensus Kozak translation initiation sequence and ATG start codon. The CMV promoter utilized in the pDNAVACCultra vectors achieves significantly higher expression levels than traditional human cytomegalovirus (CMV) promoter based vectors (Figure 2).

In summary, the seven pDNAVACCultra vectors offer the following advantages

• High level expression in a wide range of mammalian cells using an optimized CMV promoter-synthetic intron
• Optional N- or C-terminal fusion peptide tags for intracellular targeting protein products
• High throughput cloning into multiple vectors through use of universal precision cloning cassettes
• Small vectors for more efficient transfection
• Compliance with regulatory guidance (i.e. Reduced size, elimination of homology to human genomic DNA)
pDNAVACCultra vector construction

NTC used Gene Self-Assembly (GENSA) technology to create a series of validated modular elements for vector assembly. Each modular element was assigned a position in a circular array, providing a promoter, 5-leader/splice-site, target gene or high throughput cloning site, terminators, and prokaryotic origin/selection/terminator sites. Individual modules were assigned a position and directionality by means of 4bp unique, non-palindromic address tags. Modules were constructed using a novel cloning process that generates unique, non-palindromic address tags. This allowed precise minimal vector design to eliminate all extraneous sequences (Williams et al. 2006).

The pDNAVACCultra vectors were assembled from GENSA modules consisting of products representing:

- Optimized inducible high copy number pMB1-derived pUC prokaryotic replication origin;
- Prokaryotic selectable marker gene (kanamycin);
- Eukaryotic enhancer-promoter (CMV)
- Optimized synthetic eukaryotic untranslated leader-intron-translational initiation sequence (Kozak sequence) cassette
- Seamless targeting gene leader cassette (including TPA, ubiquitin or LAMP peptide leaders and C terminal membrane anchoring tags, as necessary) containing the high throughput cloning site;
- Optimized synthetic eukaryotic transcriptional terminator.

**pDNAVACCultra vector features**

<table>
<thead>
<tr>
<th>Feature</th>
<th>Function</th>
</tr>
</thead>
<tbody>
<tr>
<td>Optimized human cytomegalovirus (CMV)</td>
<td>High-level mammalian cell expression in vitro and in vivo</td>
</tr>
<tr>
<td>Immediate-early enhancer promoter</td>
<td></td>
</tr>
<tr>
<td>Synthetic Intron</td>
<td>Increase mRNA nuclear export</td>
</tr>
<tr>
<td>Precision cloning cassette with targeting tags</td>
<td>For cloning antigen gene into the vector with targeted intracellular trafficking</td>
</tr>
<tr>
<td>Synthetic eukaryotic polyadenylation signal-eukaryotic terminator</td>
<td>Effective mRNA transcriptional termination and polyadenylation</td>
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<tr>
<td>trpA prokaryotic terminator</td>
<td>Protection of replication origin from insert initiated transcription</td>
</tr>
<tr>
<td>pUC replication origin</td>
<td>High copy number plasmid production in Escherichia coli cells</td>
</tr>
<tr>
<td>Kanamycin resistance (kanR) gene*</td>
<td>Plasmid selection in Escherichia coli cells</td>
</tr>
<tr>
<td>tonB and Fd gene VIII prokaryotic terminators</td>
<td>Protection of replication origin from insert initiated transcription</td>
</tr>
</tbody>
</table>

* The kanR gene is not expressed in mammalian cells
pDNAVACCultra: Intracellular targeting

<table>
<thead>
<tr>
<th>Protein destination</th>
<th>Targeting Tag</th>
</tr>
</thead>
<tbody>
<tr>
<td>secreted</td>
<td>human tissue plasminogen activator (TPA)</td>
</tr>
<tr>
<td>proteasome</td>
<td>murine Ubiquitin A76</td>
</tr>
<tr>
<td>membrane-anchored</td>
<td>human alkaline phosphatase (PLAP)</td>
</tr>
<tr>
<td></td>
<td>glycosylphosphatidylinositol (GPI)-anchor</td>
</tr>
<tr>
<td>endosome</td>
<td>human lysosomal-associated membrane protein 1 (Lamp1)</td>
</tr>
<tr>
<td>native</td>
<td>ATG*</td>
</tr>
</tbody>
</table>

* Native vectors express the target gene from a vector encoded ATG start codon immediately downstream of an optimized kozak sequence. Protein targeting (*e.g.* cytoplasmic or nuclear) will be determined by protein-intrinsic factors.

Each of these targeting peptides have been demonstrated to efficiently target heterologous proteins to the indicated intracellular destination (*Wu et al.*, 1995; *August et al.* 1997; *Zhongming et al.* 1999; *Weiss et al.* 2000; *Gerber et al.* 1992; *Rodriguez et al.* 1998; *Delogu et al.* 2000). The pDNAVACCultra vector family has been utilized to correctly target Anthrax PA63 (*Midha and Bhatnagar, 2009ab*) and rabies virus RV-G (*Kaur et al.* 2009) antigens to the indicated intracellular destinations.

The destabilizing ubiquitin molecule (UbiquitinA76 versus native UbiquitinG76) is utilized to enhance entry into proteosomal degradation pathway and MHC class I presentation, and shifts host response towards TH1 type immunity (*Rodriguez et al.* 1998; *Delogu et al.* 2000).

**RAPID-VACC™ CONTRACT CLONING** is an optional service whereby NTC clones investigator-specified genes into the desired DNA vaccine vectors, providing sequence-validated clones, or purified plasmid DNA ready for animal testing (see [www.natx.com](http://www.natx.com)).

**RAPID-VACC™**

- Seamless cloning gene of interest into any vector $400.00
- Seamless cloning gene of interest into five vectors $1600.00
- 1mg endotoxin-free plasmid preparations, each $335.00
### Plasmid Vectors

<table>
<thead>
<tr>
<th>Vector</th>
<th>Targeting</th>
<th>Quantity</th>
<th>Catalog Number</th>
<th>Price</th>
</tr>
</thead>
<tbody>
<tr>
<td>pDNAVACCultra1</td>
<td>secreted-endsome</td>
<td>20 µg</td>
<td>NTC-DVU1</td>
<td>$299.00</td>
</tr>
<tr>
<td>pDNAVACCultra2</td>
<td>secreted</td>
<td>20 µg</td>
<td>NTC-DVU2</td>
<td>$299.00</td>
</tr>
<tr>
<td>pDNAVACCultra3</td>
<td>secreted-GPI</td>
<td>20 µg</td>
<td>NTC-DVU3</td>
<td>$299.00</td>
</tr>
<tr>
<td>pDNAVACCultra4</td>
<td>proteasome</td>
<td>20 µg</td>
<td>NTC-DVU4</td>
<td>$299.00</td>
</tr>
<tr>
<td>pDNAVACCultra5</td>
<td>native</td>
<td>20 µg</td>
<td>NTC-DVU5</td>
<td>$299.00</td>
</tr>
<tr>
<td>pDNAVACCultra6</td>
<td>GPI membrane-anchored</td>
<td>20 µg</td>
<td>NTC-DVU6</td>
<td>$299.00</td>
</tr>
<tr>
<td>pDNAVACCultra7</td>
<td>endosome</td>
<td>20 µg</td>
<td>NTC-DVU7</td>
<td>$299.00</td>
</tr>
<tr>
<td>pDNAVACCultra5-EGFP*</td>
<td>cytoplasmic EGFP</td>
<td>20 µg</td>
<td>NTC-DVU5-EGFP</td>
<td>$299.00</td>
</tr>
<tr>
<td>pDNAVACCultra7 kit (vectors 1-5)</td>
<td>All destinations kit‡</td>
<td>20 µg each</td>
<td>NTC-DVU1-5</td>
<td>$1,196.00</td>
</tr>
<tr>
<td>pDNAVACCultra7 kit (vectors 4-7)</td>
<td>All destinations kit‡</td>
<td>20 µg each</td>
<td>NTC-DVU4-7</td>
<td>$957.00</td>
</tr>
</tbody>
</table>

* The pDNAVACCultra5-EGFP control plasmid is available for use as a transfection control for expression in a cell line of interest.
‡ Vector kit 1-5 allows targeting of an antigen to secreted, endosomal, membrane-anchored, proteasome, or native destinations using, where necessary, a vector encoded TPA secretion sequence (pDNAVACCultra1-3). Vector kit 4-7 allows targeting of an antigen to secreted, endosomal, membrane-anchored, or proteasome destinations using, where necessary, an investigator specified secretion sequence (e.g. IgK).

### Linearized Vectors‡

<table>
<thead>
<tr>
<th>Vector</th>
<th>Targeting</th>
<th>Quantity</th>
<th>Catalog Number</th>
<th>Price</th>
</tr>
</thead>
<tbody>
<tr>
<td>pDNAVACCultra1</td>
<td>secreted-endsome</td>
<td>1 µg</td>
<td>NTC-DVU1-LV</td>
<td>$344.00</td>
</tr>
<tr>
<td>pDNAVACCultra2</td>
<td>secreted</td>
<td>1 µg</td>
<td>NTC-DVU2-LV</td>
<td>$344.00</td>
</tr>
<tr>
<td>pDNAVACCultra3</td>
<td>secreted-GPI</td>
<td>1 µg</td>
<td>NTC-DVU3-LV</td>
<td>$344.00</td>
</tr>
<tr>
<td>pDNAVACCultra4</td>
<td>proteasome</td>
<td>1 µg</td>
<td>NTC-DVU4-LV</td>
<td>$344.00</td>
</tr>
<tr>
<td>pDNAVACCultra5</td>
<td>native</td>
<td>1 µg</td>
<td>NTC-DVU5-LV</td>
<td>$344.00</td>
</tr>
<tr>
<td>pDNAVACCultra6</td>
<td>GPI membrane-anchored</td>
<td>1 µg</td>
<td>NTC-DVU6-LV</td>
<td>$344.00</td>
</tr>
<tr>
<td>pDNAVACCultra7</td>
<td>endosome</td>
<td>1 µg</td>
<td>NTC-DVU7-LV</td>
<td>$344.00</td>
</tr>
<tr>
<td>pDNAVACCultra7 kit (vectors 1-5)</td>
<td>All destinations kit‡</td>
<td>1 µg each</td>
<td>NTC-DVU1-5-LV</td>
<td>$1,376.00</td>
</tr>
<tr>
<td>pDNAVACCultra7 kit (vectors 4-7)</td>
<td>All destinations kit‡</td>
<td>1 µg each</td>
<td>NTC-DVU4-7-LV</td>
<td>$1099.00</td>
</tr>
</tbody>
</table>

‡ SapI linearized vector sufficient for 20 cloning reactions
NTC offers the following products for use cloning with the pDNAVACCultra vectors

### Accessory Products

<table>
<thead>
<tr>
<th>Product</th>
<th>Description</th>
<th>Quantity</th>
<th>Catalog Number</th>
<th>Price</th>
</tr>
</thead>
<tbody>
<tr>
<td>pVAC5’</td>
<td>Forward sequencing primer</td>
<td>500 pmol</td>
<td>NTC-DVU-SP1</td>
<td>$30.00</td>
</tr>
<tr>
<td>pVAC3’</td>
<td>Reverse sequencing primer</td>
<td>500 pmol</td>
<td>NTC-DVU-SP2</td>
<td>$30.00</td>
</tr>
</tbody>
</table>

### Cloning into pDNAVACCultra vectors

**Overview:** An example strategy for cloning into the pDNAVACCultra vectors is outlined in Figure 3. Genes are copied by PCR amplification from clones or genomic DNA using primers with generic address tags, and unique-sequence specific sequences. Internal SapI sites in the target gene are generally not detrimental since there is only a 1/16 chance that an internal SapI site would match one of the address tags. This method is superior to recombination mediated cloning for this application, since with class IIS cloning a single primer pair facilitates cloning into all three vectors, whereas sets of longer primers with vector specific sequences would be needed for seamless recombination cloning into trafficking vectors.

**Protocol:** Genes are PCR amplified with primers incorporating SapI sites into termini to generate 5’ ATG and 3’ TAA (Ubiquitin or secreted) or 5’ ATG and 3’ GGC (Endosomal or secreted endosomal) 3 bp sticky ends upon digestion with SapI. SapI is commercially available from New England Biolabs.

Example primers, for cloning a target gene of interest into the secreted, proteosome and native vectors (pDNAVACCultra 2, 4 and 5, respectively) are diagramed below.

\[
\text{Sticky} \quad \text{SapI end} \\
\text{GCGGATGCTCTTCCATG-GENE OF INTEREST 5’ END} \\
\text{GCAGAAGCTCTTGTTA-GENE OF INTEREST (REVERSE COMPLEMENT) 3’ END}
\]

These stuffers do not have a C terminal extension (e.g. Ubiquitin, native and secreted) so the address tags correspond precisely to the start (ATG) and stop (TAA) codons of the gene. The PCR product is cleaved with SapI and purified.

For endosome and GPI-membrane anchored targeted vectors (pDNAVACCultra 1 and 3, respectively), the 3’ END primer incorporates a GGC glycine linker is used instead of
TAA stop, to facilitate the C terminal extensions needed for trafficking (i.e. GPI or endosomal targeting).

\[
\text{Sticky SapI end}
\]

GCAGAAAGCTCTTTCCGGC–GENE OF INTEREST (REVERSE COMPLEMENT) 3’ END

The same 5’END PCR product is used with this primer in the PCR reaction. The PCR product is cleaved with SapI and purified.

Cleavage of the vector with SapI generates sticky ends compatible with the cleaved PCR product. The insert is thus directionally and precisely cloned into the vector. The majority of recovered colonies are recombinant, since the SapI generated sticky ends in the parental vector are not compatible. The vector and PCR product SapI sites are removed and are not incorporated into the final vector. This allows for addition of SapI to the ligation reaction, to eliminate uncut or singly cut parental vector, selectively enriching for recombinant transformation colonies (this strategy can be used only for inserts that do not contain internal SapI sites).

Recombinant clones can be identified by restriction digestion. NcoI and BglII to release the gene insert with an additional CMV vector diagnostic 421 bp fragment (pDNAVACCultra1-3, 5-7). BglII alone is utilized to release the insert in proteosome vector pDNAVACCultra4.

The sequence of the gene insert can be determined using the following cloning cassette flanking sequencing primers.

pVAC5’: GCTTTTCTGCCAGGTGCTGA
(hybridizes to intron and sequences from 5’ end of gene)

pVAC3’: GCCAGAAAGTCAGATGCTCAA
(hybridizes to terminator and sequences from 3’ end of gene)

If desired, high-throughput cloning operations can be performed using SapI enzyme to produce unique, non-palindromic address labels on PCR amplified inserts. Typically, an 8-96 well (PCR [96-well gradient block]) format can be used for high throughput applications (PCR, purification, ligation to SapI digested vector).
References


FDA. (1996) Points to consider on plasmid DNA vaccines for preventive infectious disease indications. US Food and Drug Administration


Patent and Licensing information

Limited License
Nature Technology Corporation (NTC) grants the end user (purchaser) of the pDNAVACCultra Vectors a nontransferable, non-exclusive license to use the plasmids for non-commercial research purposes only. These vectors are intended for research use only by the purchaser.

The purchaser cannot sell or otherwise transfer (a) this product (b) its components or (c) materials made using this product or its components to a third party or otherwise use this product or its components or materials made using this product or its components for commercial purposes.

Separate licenses are available from NTC for the express purpose of non-research use or applications of the pDNAVACCultra Vectors.

Product Use Limitations
The pDNAVACCultra Vectors are sold for research use only. They are not to be used for human diagnostic or included/used in any drug intended for human use.

Patent Information

The CMV promoter is covered under U.S. Patents 5,168,062 and 5,385,839 and its use is permitted for research purposes only. Any other use of the CMV promoter requires a license from the University of Iowa Research Foundation, 214 Technology Innovation Center, Iowa City, IA 52242.

The LAMP sequence, and related endosomal targeting sequences, is covered under U.S. Patents 5,633,234 and its use is permitted for research purposes only. Any other use of the LAMP technology requires a license from Immunomic Therapeutics Inc, 9290 Gaither Road Gaithersburg, MD 20877.

The polymerase chain reaction (PCR) process is covered by patents owned by Roche and requires a license for use.

NTC makes no representations that the use of the pDNAVACCultra vectors will not infringe any patent, copyright, trademark, or other proprietary rights.

For more information, please contact:
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Fax: (402) 323-6292
Email: natx@natx.com
**Vector Features**

- trpA prokaryotic terminator: 3308-16
- pUC replication origin: 23-1037
- Kanamycin resistance marker: 1045-1965
- tonB bidirectional terminator: 2023-2060
- CMV enhancer: 2078-2635
- CMV promoter: 2636-2755
- Untranslated leader (exon 1): 2756-2807
- Intron: 2808-2933
- Kozak sequence (exon 2): 2934-2942
- TPA N-terminal targeting tag: 2943-3011
- SapI cloning cassette: 3012-3048
- GPI C-terminal targeting tag: 3049-3147
- Eukaryotic terminator: 3158-3297
Figure 2: expression of GFP driven by pDNAVACCultra5-EGFP vector (top) versus gWiz-GFP (bottom)
**Figure 3.** Method for directional amplification and cloning of cDNA sequences into pDNAVACCultra vectors. A) Plasmid containing two unique address tags, created by class IIS enzyme recognition signal (SapI), at least one intervening nucleotide, and an overlapping region with a unique, non-palindromic sequence (GGG, the address tag in this example).

A.

![Diagram of plasmid with class IIS enzyme sites, tags, and cDNA](image)

B.

5'-'GCGCTTC N^GGG^NNNNNNNNNNNN-3'

*SapI*  -  *tag*  -  *primer*
VECTOR INFORMATION: Targeting gene leader-cloning cassettes

See the NTC website www.natx.com for complete vector sequences of pDNAVACCultra1, 2, 3, 4, 5, 6 and 7

pDNAVACCultra1 (secreted-endosome)

See the NTC website www.natx.com for complete vector sequences of pDNAVACCultra1, 2, 3, 4, 5, 6 and 7

pDNAVACCultra1 (secreted-endosome)
pDNAVACCultra Expression Vectors

pDNAVACCultra2 (secreted)

SapI stuffer; tPA 1-23 signal leader (no pro)

18 bp

\[
\text{NcoI} \\
\text{MetAspAla MetLysArg GlyLeuCysCys ValLeuLeu···}
\]

1 CAGGCCGCCA CCATGGATGC AATGAAGAGA GGGCTCTGCT GTGTGCTGCT
GTCCGGCGGT GGTACCTACG TTACTTCTCT CCCGAGACGA CACACGACGA
SapI
~~~~~~~
·LeuCysGly AlaValPheVal SerProSer Met
51 GCTGTGTGGA GCAGTCTTCG TTTCGCCCAG CATGGGAAGA GCGTTCCATG
CGACACACCT CGTCAGAAGC AAAGCGGGTC GTACCCTTCT CGCAAGGTAC
SapI
~~~~~~~ XhoI BglII
101 CATCCTAGCT CTTGCTAACT CGAGCCGACAGATCT
GTAGGATCGA GAAGCATTGA GCTCGGCTCTAGA

ATG TAA = address tags
pDNAVACCultra3 (secreted-membrane anchored)

NcoI

MetAspAla MetLysArg GlyLeuCysCys ValLeuLeuLeu·

1  CAGGCCGCCA  CCATGGATGC  AATGAAGAGA  GGGCTCTGCT  GTGTGCTGCT
GTCCGGCCGT  GGTACCTACG  TTACTTCTCT  CCCGAGACGA  CACACGACGA
SapI

·LeuCysGly AlaValPheVal SerProSer Met

51  GCTGTGTGGA  GCAGTCTTCG  TTTCGCCCAG  CATGGGAAGA  GCGTTCCATG
CGACACACCT  CGTCAGAAGC  AAAGCGGGTC  GTACCCTTCT  CGCAAGGTAC
SapI

101  CATCCTAGGC  TCTTCGGGCA  CCACCGACGC  CGCGCACCCG  GGGCGGTCCG
GTAGGATCCG  AGAAGCCCGT  GGTGGCTGCG  GCGCGTGGGC  CCCGCCAGGC

151  TGGTCCCCGC  GTTGCTTCCT  CTGCTGGCCG  GGACCCTGCT  GCTGCTGGAG
ACCAGGGGCG  CAACGAAGGA  GACGACCGGC  CCTGGGACGA  CGACGACCTC
XhoI  BglII

201  ACGGCCACTG  CTCCCTGACT  CGAGCCGCAG  ATCT
TGCCGGTGAC  GAGGGACTGA  GCTCGGCGTC  TAGA

ATG GGC = address tags
pDNAVACCultra4 (proteosome)

**SapI stuffer; UbiquitinA76 (mouse) leader**

290 bp

<table>
<thead>
<tr>
<th>BglII</th>
<th>MetGlnIle PheValLys ThrLeuThrGly LysThrThr</th>
</tr>
</thead>
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<td>GTCCGGCGGT GGTACGTCTA GAAGCACTTC TGGGACTGCC CGTTCTGGTG</td>
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<tr>
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</tr>
<tr>
<td>51</td>
<td>CACTCTTGGG GTGAGGCCCA GTGACACCAC CGAGATGTTA AAGGCCAAGAG</td>
</tr>
<tr>
<td></td>
<td>GTGGAACCCC CACCTCGGGT CACTGTTGTA GCTCTTACAG TTCCGTTCT</td>
</tr>
<tr>
<td>•IGlnAspLys GluGlyIle ProProAspGln GlnArgLeu IlePheAla</td>
<td></td>
</tr>
<tr>
<td>101</td>
<td>TCCAAGACAA GGAAGGCCATC CACCTGCCAC CGCAGAGGCT GCTATAGCT</td>
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<td>GlyLysGlnLeu GluAspGly ArgThrLeu SerAspTyrAsn IleGlnLys•</td>
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<tr>
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<tr>
<td></td>
<td>CCGTTGTCG ACCCTCTACC GGCCTGGAG ACCTCTTAGT TGTTAGCTCTT</td>
</tr>
<tr>
<td></td>
<td>SapI ~~~</td>
</tr>
<tr>
<td></td>
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</tr>
<tr>
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<td>AGAGTCCACC TGGACCTGG TGCTCGTGCT GCGCGGTGCC GCTATAGAAGA</td>
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<tr>
<td></td>
<td>TCTCAGTGGG AACGTGGGCC ACCGACCCAGC CGCAGCCGCG CGCAGGACTTT</td>
</tr>
<tr>
<td></td>
<td>SapI SapI ~~~~</td>
</tr>
<tr>
<td></td>
<td>~~~~~ XhoI BglII</td>
</tr>
<tr>
<td>251</td>
<td>GAGCGTTCCA TGCATCCTAG AGCTTCCCTGTA ACGTGGCCAGCAGCTCGGTG</td>
</tr>
<tr>
<td></td>
<td>CTCGCAAGGGTC GTACCGTCGC GCCACCAGCTCAGTCGAGTG</td>
</tr>
</tbody>
</table>

**Ala = A76.** There is a single amino acid spacer prior to the fusion.

**ATG TAA = address tags**
pDNAVACCultra Expression Vectors

pDNAVACCultra5 (native)

address tag 1 (ATG)
ATGstart
SapI (13)
kozak consensus
splice acceptor

SapI stuffer Native
59 bp

NcoI SapI SapI
~~~~~~~~~~~~~~

1 CAGGCGCGCA CCATGGGAAG AGCGTTCCAT GCATCCTAGCTCTTCGTAACGTCCGGCGGT GGTACCCTTC TCGCAAGGTA CGTAGGATCG AGAAGCATTG
XhoI BglII

51 TCGAGCCGCA GATCT
AGCTCGCG

ATG TAA = address tags
pDNAVACCultra6 (membrane anchored)

<table>
<thead>
<tr>
<th>Address Tag 1 (ATG)</th>
<th>SspI (16)</th>
<th>ATG Start</th>
<th>Address Tag 2 (GCC)</th>
<th>Gly Spacer</th>
<th>SspI (51)</th>
<th>GPI Anchor</th>
</tr>
</thead>
<tbody>
<tr>
<td>NcoI</td>
<td>~~~~~~~~~</td>
<td>SspI</td>
<td>~~~~~~~~~</td>
<td>SspI</td>
<td>Gly</td>
<td></td>
</tr>
</tbody>
</table>

1  CAGGCCGCCA CCATGGGAAG AGCGTTCCAT GCATCTAGG CTCTTCGGGC GTCCGGCGGT GGTACCCTTC TCGCAAGGTA CGTAGGATCC GAGAAGCCCG
   ThrThr**Asp**Ala AlaHisPro GlyArgSer ValValProAla LeuLeuPro·

51 ACCACCGACG CCGCGACCCCC GGGCGGTCCC GTGGTCCCCG GTGGTCTTCC TGGTGGCTGC GGCACGTGGG CCCGCCAGG CACCAGGGGC GCAACGAAGG
   LeuLeuAla GlyThrLeuLeu LeuLeuGlu ThrAlaThr AlaProm

101 TCTGCTGGCC GGGACCCTGC TGCTGCTGGA GACGGCCACT GCCGCCCTGA CACGAGCCAGG CCGACGACCT CTGCCGGGTGA CGAGGGACTG
   XhoI       BglII         ~~~~

151 TCGAGCCGCCA GATCT
   AGCTCGGCGT CTAGA

**Asp** = omega residue, site of GPI uptake
**ATG** **GCC** = address tags
pDNAVACCultra7 (endosome)

SapI stuffer LAMP-1 endo anchor
181 bp

NcoI  SapI  SapI

1 CAGGCCGCCA CCATGGGAAG AGCGTTCCAT GCATCCTAGG CTCTTCGGGC
GTCCGGCGGT GGTACCCTTC TCGCAAGGTA CGTAGGATCC GAGAAGCCCG
LeuAsnAsnMet LeuIlePro IleAlaVal GlyGlyAlaLeu AlaGlyLeu·

51 CTTAACAACA TGTTGATCCC CATTGCTGTG GGCGGTGCCC TGGCAGGGCT
GAATTGTTGT ACAACTAGGG GTAACGACAC CCGCCACGGG ACCGTCCCGA
·ValLeuIle ValLeuIleAla TyrLeuIle GlyArgLys ArgSerHisAla·

101 GGTCCTCATC GTCCTCATTG CCTACCTCAT TGCCAGGAAG AGGAGCTCACG
CCAGGAGTAG CAGGAGTAAC GGATGGAGTA ACCGTCCTTC TCCTCAGTG
·AGlyTyrGln ThrIle***  XhoI  BglII

151 CCGGCTATCA GACCATCTAA CTCACTGCGC AGATCT
GCCGATAGT CTGGTAGATT GAGCTCGGCC TCTAGA

ATG GGC = address tags