pDNAVACCultra 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

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General Information

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

Storage: Plasmids should be stored at -20˚C

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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

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

* The kanR gene is not expressed in mammalian cells

pDNAVACCultra: Intracellular targeting

Protein destination	Targeting Tag
secreted	human tissue plasminogen activator (TPA)
proteasome	murine Ubiquitin A76
membrane-anchored	human alkaline phosphatase (PLAP) glycosylphosphatidylinositol (GPI)-anchor
endosome	human lysosomal-associated membrane protein 1 (Lamp1)
native	ATG*****

***** 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 (UbiquitinA76versus native UbiquitinG76) is utilized to enhance entry into proteosomal degradation pathway and MHC class I presentation, and shifts host response towards T_H1 type immunity (Rodriguez *et al.*1998; Delogu *et al.*2000).

RAPID-VACC^{tm} 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*).

RAPID-VACC^{tm}

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

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

* 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, proteosome, 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 proteosome destinations using, where necessary, an investigator specified secretion sequence (e.g. IgK).

Linearized Vectors‡

Vector	Targeting	Quantity	Catalog Number	Price
pDNAVACCultra1	secreted-endosome	1 µg	NTC-DVU1-LV	$344.00
pDNAVACCultra2	secreted	1 µg	NTC-DVU2-LV	$344.00
pDNAVACCultra3	secreted –GPI membrane-anchored	1 µg	NTC-DVU3-LV	$344.00
pDNAVACCultra4	proteasome	1 µg	NTC-DVU4-LV	$344.00
pDNAVACCultra5	native	1 µg	NTC-DVU5-LV	$344.00
pDNAVACCultra6	GPI membrane-anchored	1 µg	NTC-DVU6-LV	$344.00
pDNAVACCultra7	endosome	1 µg	NTC-DVU7-LV	$344.00
pDNAVACCultra kit (vectors 1- 5)	All destinations kit	1 µg each	NTC-DVU1-5-LV	$1,376.00
pDNAVACCultra kit (vectors 4-7)	All destinations kit	1 µg each	NTC-DVU4-7-LV	$1099.00

‡ SapI linearized vector sufficient for 20 cloning reactions

NTC offers the following products for use cloning with the pDNAVACCUltra vectors

Accessory Products

Product	Description	Quantity	Catalog Number	Price
pVAC5'	Forward sequencing primer	500 pmol (10 pmol/µL)	NTC-DVU-SP1	$30.00
pVAC3'	Reverse sequencing primer	500 pmol (10 pmol/µL)	NTC-DVU-SP2	$30.00

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 *Sap*I sites in the target gene are generally not detrimental since there is only a 1/16 chance that an internal *Sap*I 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 *Sap*I 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 *Sap*I. *Sap*I 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.

Sticky
*Sap*I end
GCGGATGCTCTTCCATG-GENE OF INTEREST 5' END

GCAGAAGCTCTTCGTTA-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 *Sap*I 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).

Sticky
*Sap*I end
GCAGAAGCTCTTCGGCC-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 *Sap*I and purified.

Cleavage of the vector with *Sap*I 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 *Sap*I generated sticky ends in the parental vector are not compatible. The vector and PCR product *Sap*I sites are removed and are not incorporated into the final vector. This allows for addition of *Sap*I 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 *Sap*I sites).

Recombinant clones can be identified by restriction digestion. *Nco*I and *Bgl*II to release the gene insert with an additional CMV vector diagnostic 421 bp fragment (pDNAVACCultra1-3, 5-7). *Bgl*II 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': GCCAGAAGTCAGATGCTCAA
(hybridizes to terminator and sequences from 3' end of gene)

If desired, high-throughput cloning operations can be performed using *Sap*I 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 *Sap*I digested vector).

References

August JT, Pardoll DM, and Guarnieri FG. (1997) Lysosomal targeting of immunogens. US patent 5,633,234

Delogu G, Howard A, Collins FM, and Morris SL. (2000) DNA vaccination against tuberculosis: Expression of a Ubiquitin-conjugated tuberculosis protein enhances antimycobacterial immunity. *Infect. Immun*. 68: 3097-3102

Gerber L, Kodukula K, and Udenfriend S. (1992) Phosphatidylinositol Glycan (PI-G) anchored Membrane Proteins. *J Biol Chem* 267: 12168-12173

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

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Kaur M, Rai A, and Bhatnagar R. (2009) Rabies DNA vaccine: no impact of MHC Class I and Class II targeting sequences on immune response and protection against lethal challenge. *Vaccine* 27:2128-2137

Midha S, and Bhatnagar R. (2009) Anthrax protective antigen administered by DNA vaccination to distinct subcellular locations potentiates humoral and cellular immune responses. *Eur. J. Immunol*. 39: 159-177

Midha S, and Bhatnagar R. (2009) Genetic immunization with GPI-anchored anthrax protective antigen raises combined CD1d- and MHC II-restricted antibody responses by natural killer T cell-mediated help. *Vaccine* 27:1700-1709

Rodriguez F, An LL, Harkins S, Zhang J, Yokoyama M, Widera G, Fuller JT, Kincaid C, Campbell IL, and Whitton JL. (1998) DNA immunization with Minigenes: Low frequency of memory cytotoxic T lymphocytes and inefficient antiviral protection are rectified by Ubiquitination. *J Virology* 72: 5174-5181

Weiss R, Durnberger J, Mostbock S, Scheiblhofer S, Hartl A, Breitenback M, Strasser P, Dorner F, Livey I, Crowe B, and Thalhamer J. (2000) Improvement of the immune response against plasmid DNA encoding *OspC* of Borrelia by an ER-targeting leader sequence. *Vaccine* 18: 815-824

Williams JA, Carnes AE, and Hodgson CP. (2009) Plasmid DNA vector design; impact on efficacy, safety and upstream production. *Biotechnology Advances* 27:353-370

Williams JA, Luke J, Johnson L, and Hodgson CP. (2006) pDNAVACCultra vector family: high throughput intracellular targeting DNA vaccine plasmids. *Vaccine* 24:4671-4676

Wu T, Guarnieri FG, Staveley-O'Carroll KF, Viscidi RP, Levitsky HI, Hedrick L, Cho KR, August JT, and Pardoll DM. (1995) Engineering an intracellular pathway for major histocompatibility complex class II presentation of antigens. *PNAS* 92: 11671-11675

Zhongming L, Howard A, Kelley C, Delogu G, Collins F, and Morris S. (1999) Immunogenicity of DNA vaccines expressing tuberculosis proteins fused to tissue plasminogen activator signal sequences. *Infect Immun*. 67: 4780-4786

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.
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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.

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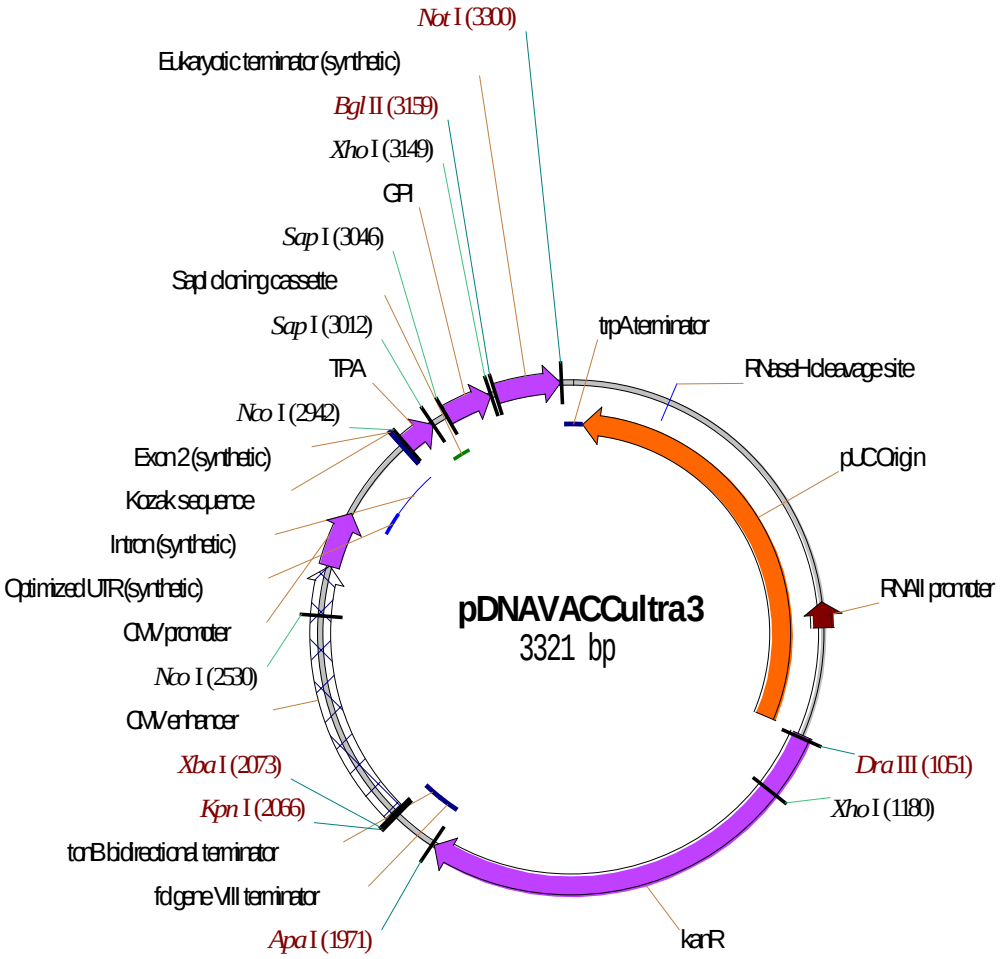
Figure 1: pDNAVACCultra3



Vector Features
trpA prokaryotic terminator: 3308-16
pUC replication origin: 23-1037
Kanamycin resistance marker: 1045-1965
Fd gene VIII prokaryotic terminator:1972-2015
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)

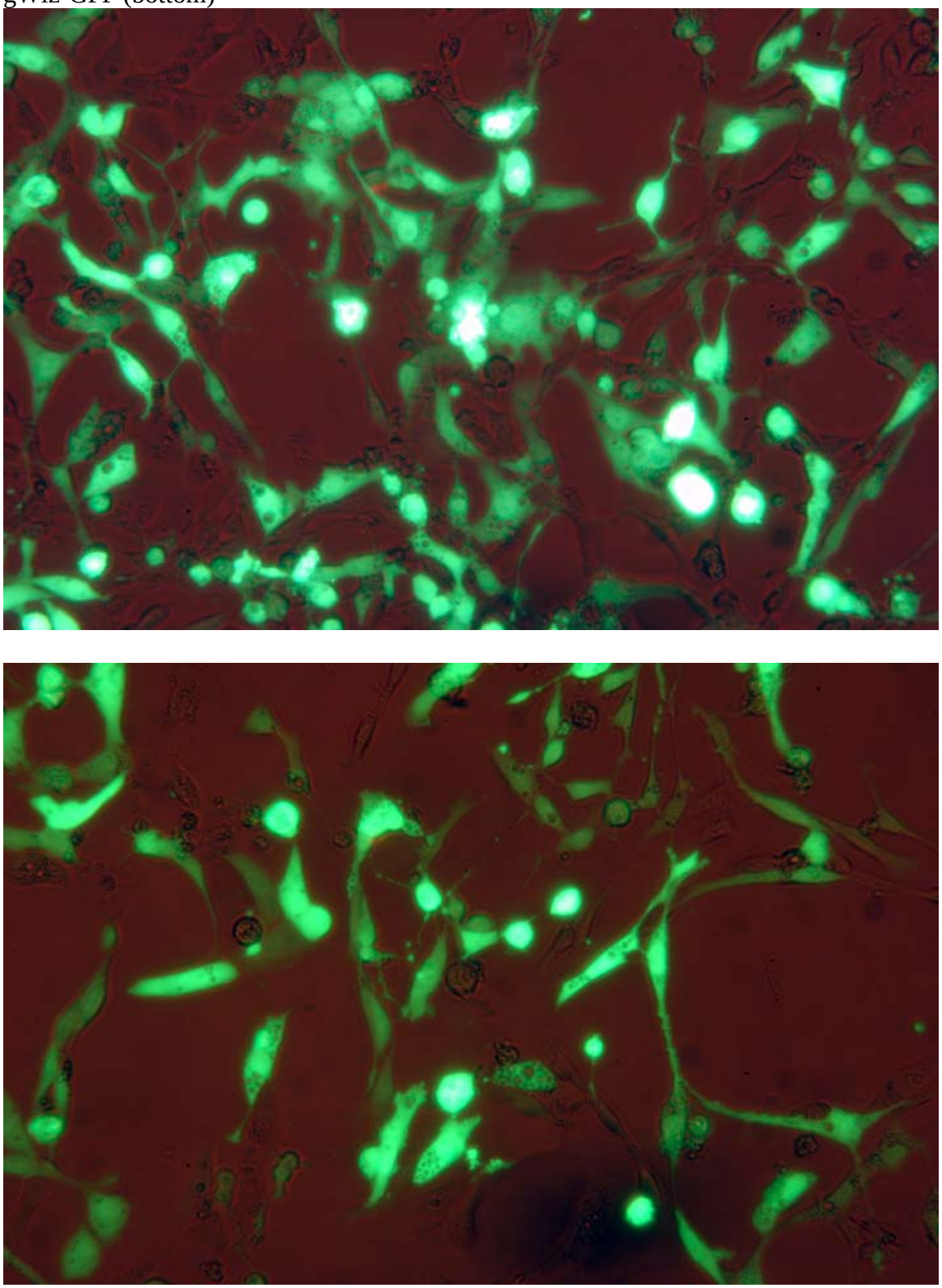
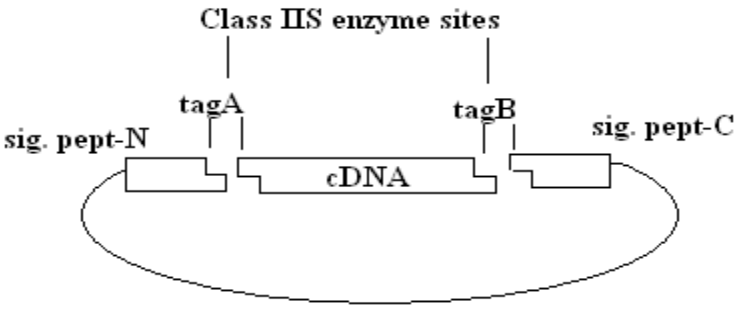


Figure 3. Method for directional amplification and cloning of cDNA sequences into pDNAVACCultra vectors. A) Plasmid containing two unique address tags, created by class IIS enzyme recognition signal (*Sap*I), at least one intervening nucleotide, and an overlapping region with a unique, non-palindromic sequence (GGG, the address tag in this example).

A.



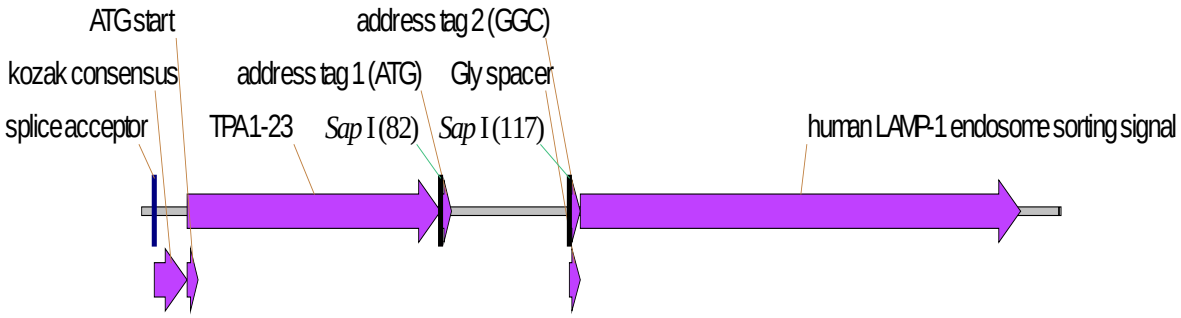
B.

5'-GCGCTTC N ^GGG^NNNNNNNNNNN-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)



SapI stuffer TPA secretion- LAMP-1 endo anchor

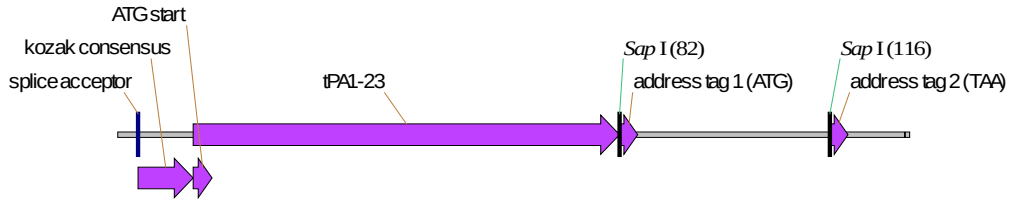
250 bp

```
                      NcoI
   1   CAGGCCGCCA CCATGGATGC AATGAAGAGA GGGCTCTGCT GTGTGCTGCT
       GTCCGGCGGT GGTACCTACG TTACTTCTCT CCCGAGACGA CACACGACGA
                                                  SapI
                                                ~~~~~~~~
  51   GCTGTGTGGA GCAGTCTTCG TTTCGCCCAG CATGGGAAGA GCGTTCCATG
       CGACACACCT CGTCAGAAGC AAAGCGGGTC GTACCCTTCT CGCAAGGTAC
                 SapI
               ~~~~~~~~
 101   CATCCTAGGC TCTTCGGGCC TTAACAACAT GTTGATCCCC ATTGCTGTGG
       GTAGGATCCG AGAAGCCCGG AATTGTTGTA CAACTAGGGG TAACGACACC

 151   GCGGTGCCCT GGCAGGGCTG GTCCTCATCG TCCTCATTGC CTACCTCATT
       CGCCACGGGA CCGTCCCGAC CAGGAGTAGC AGGAGTAACG GATGGAGTAA
                                                  XhoI
 201   GGCAGGAAGA GGAGTCACGC CGGCTATCAG ACCATCTAAC TCGAGCCGCA
       CCGTCCTTCT CCTCAGTGCG GCCGATAGTC TGGTAGATTG AGCTCGGCGT
        BglII
 251   GATCT
       CTAGA

ATG GGC = address tags
```

pDNAVACCultra2 (secreted)



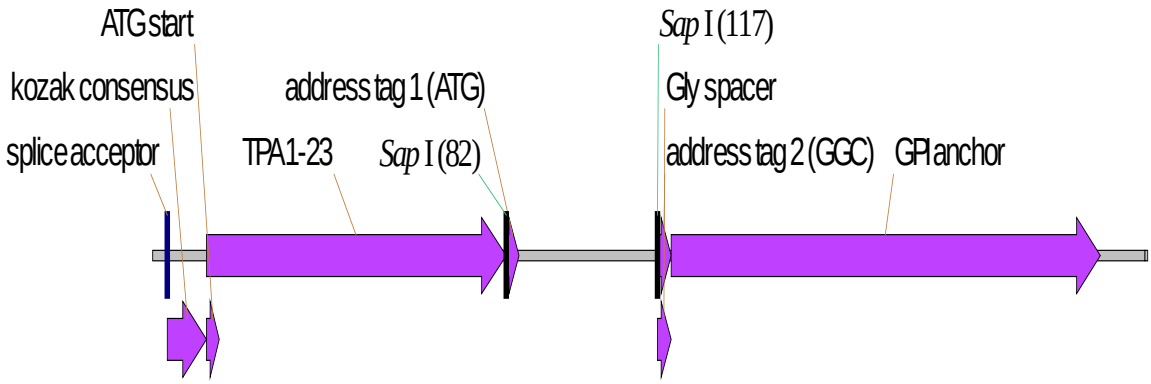
SapI stuffer; tPA 1-23 signal leader (no pro)

128 bp

```
                    NcoI
                     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 CTTCGTAACT CGAGCCGCAGATCT
       GTAGGATCGA GAAGCATTGA GCTCGGCGTCTAGA
```

ATG TAA = address tags

pDNAVACCultra3 (secreted-membrane anchored)



SapI stuffer TPA secretion- GPI anchor

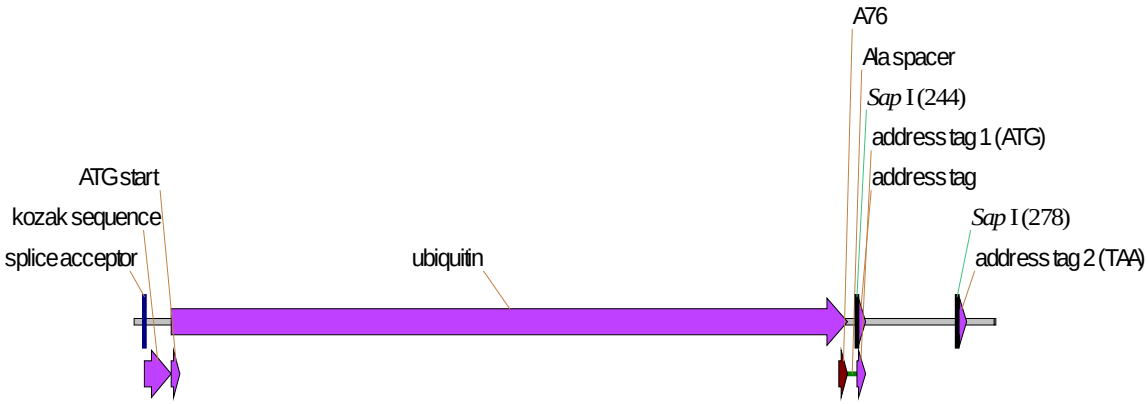
229 bp

```
                  NcoI
                   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
                ~~~~~~~~
  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

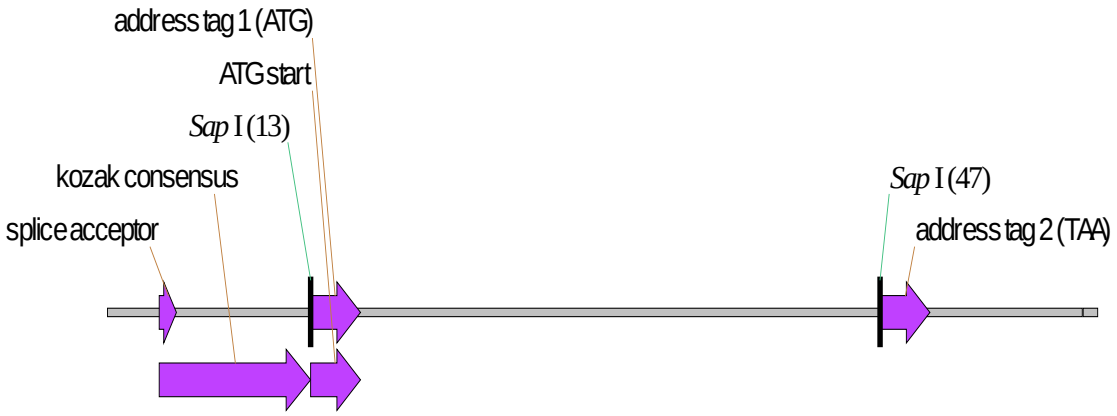
290 bp

```
                      BglII
                  MetGlnIle PheValLys ThrLeuThrGly LysThrThr·
    1   CAGGCCGCCA CCATGCAGAT CTTCGTGAAG ACCCTGACGG GCAAGACCAC
        GTCCGGCGGT GGTACGTCTA GAAGCACTTC TGGGACTGCC CGTTCTGGTG
        ·ThrLeuGly ValGluProSer AspThrIle GluAsnVal LysAlaLysIle·
   51   CACTCTTGGG GTCGAGCCCA GTGACACCAT CGAGAATGTC AAGGCCAAGA
        GTGAGAACCC CAGCTCGGGT CACTGTGGTA GCTCTTACAG TTCCGGTTCT
        ·IGlnAspLys GluGlyIle ProProAspGln GlnArgLeu IlePheAla
  101   TCCAAGACAA GGAAGGCATC CCACCTGACC AGCAGAGGCT GATATTCGCG
        AGGTTCTGTT CCTTCCGTAG GGTGGACTGG TCGTCTCCGA CTATAAGCGC
        GlyLysGlnLeu GluAspGly ArgThrLeu SerAspTyrAsn IleGlnLys·
  151   GGCAAACAGC TGGAGGATGG CCGCACCCTG TCCGACTACA ACATCCAGAA
        CCGTTTGTCG ACCTCCTACC GGCGTGGGAC AGGCTGATGT TGTAGGTCTT
                                                        SapI
                                                        ~~~
        ·GluSerThr LeuHisLeuVal LeuArgLeu ArgGlyAla AlaMet
  201   AGAGTCCACC TTGCACCTGG TGCTGCGTCT GCGCGGTGCC GCTATGAGAA
        TCTCAGGTGG AACGTGGACC ACGACGCAGA CGCGCCACGG CGATACTCTT
        SapI                SapI
        ~~~~                ~~~~~~~~      XhoI       BglII
  251   GAGCGTTCCA TGCATCCTAG CTCTTCGTAA CTCGAGCCGC AGATCT
        CTCGCAAGGT ACGTAGGATC GAGAAGCATT GAGCTCGGCG TCTAGA
```

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

ATG TAA = address tags

pDNAVACCultra5 (native)



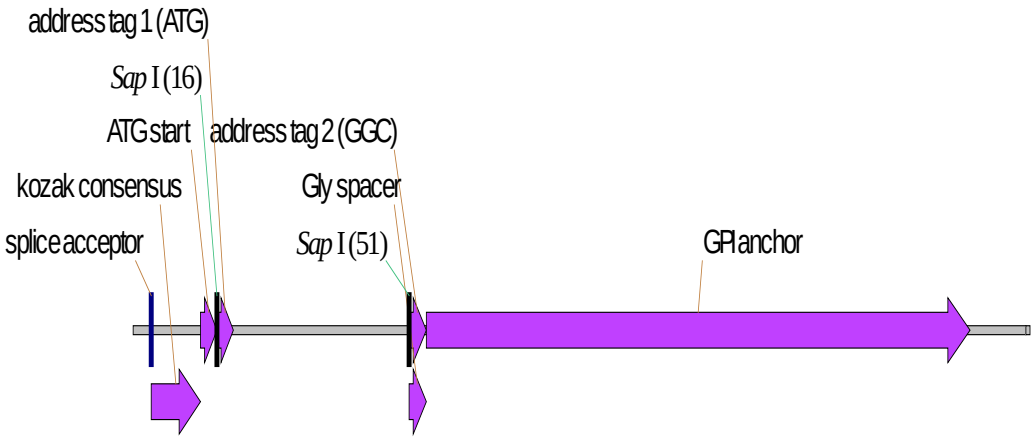
SapI stuffer Native

59 bp

```
                    NcoI  SapI                    SapI
                       ~~~~~~~~                ~~~~~~~~
   1   CAGGCCGCCA CCATGGGAAG AGCGTTCCAT GCATCCTAGC TCTTCGTAAC
       GTCCGGCGGT GGTACCCTTC TCGCAAGGTA CGTAGGATCG AGAAGCATTG
       XhoI     BglII
  51   TCGAGCCGCA GATCT
       AGCTCGGCG
```

ATG TAA = address tags

pDNAVACCultra6 (membrane anchored)



SapI stuffer GPI anchor

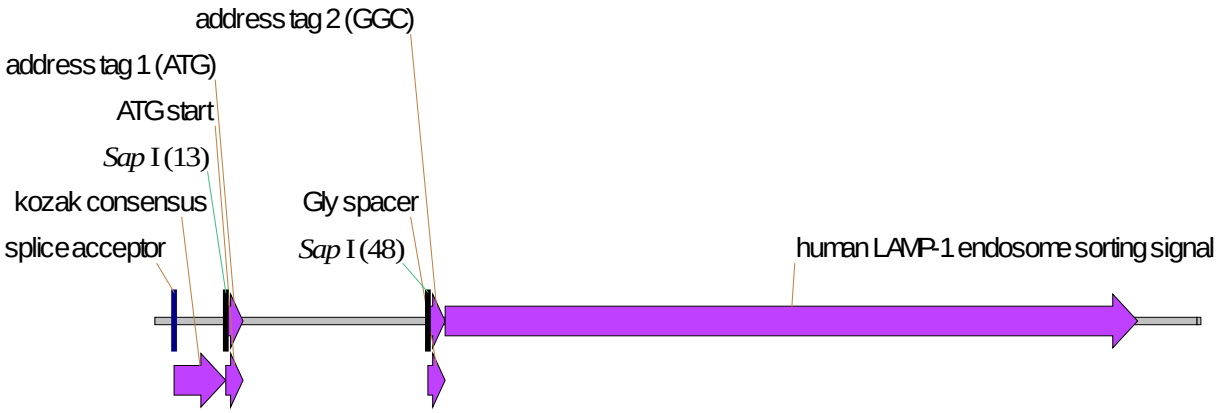
163 bp

```
                    NcoI
                   ~~~~~~
                          SapI                      SapI
                         ~~~~~~~~                  ~~~~~~~~
                                                          Gly
    1   CAGGCCGCCA CCATGGGAAG AGCGTTCCAT GCATCCTAGG CTCTTCGGGC
        GTCCGGCGGT GGTACCCTTC TCGCAAGGTA CGTAGGATCC GAGAAGCCCG

        ThrThrAspAla AlaHisPro GlyArgSer ValValProAla LeuLeuPro·
   51   ACCACCGACG CCGCGCACCC GGGGCGGTCC GTGGTCCCCG CGTTGCTTCC
        TGGTGGCTGC GGCGCGTGGG CCCCGCCAGG CACCAGGGGC GCAACGAAGG
                                                          XhoI
                                                          ~
        ·LeuLeuAla GlyThrLeuLeu LeuLeuGlu ThrAlaThr AlaPro***
  101   TCTGCTGGCC GGGACCCTGC TGCTGCTGGA GACGGCCACT GCTCCCTGAC
        AGACGACCGG CCCTGGGACG ACGACGACCT CTGCCGGTGA CGAGGGACTG
        XhoI     BglII
        ~~~~~    ~~~~~~~
  151   TCGAGCCGCA GATCT
        AGCTCGGCGT CTAGA
```

Asp = omega residue, site of GPI uptake
ATG GGC = 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 TGGCAGGAAG AGGAGTCACG
       CCAGGAGTAG CAGGAGTAAC GGATGGAGTA ACCGTCCTTC TCCTCAGTGC
       ·AGlyTyrGln ThrIle***  XhoI      BglII
 151   CCGGCTATCA GACCATCTAA CTCGAGCCGC AGATCT
       GGCCGATAGT CTGGTAGATT GAGCTCGGCG TCTAGA
```

ATG GGC = address tags