pADL-10b Phagemid

INSTRUCTION MANUAL

pADL-10b Phagemid Vector for Phage Display

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Description

Introduction

The pADL-10b phagemid is a type 3+3 phage display vector with a cloning site for display on the N-terminal side of the full-length gene III protein. Secretion in the periplasm of the fusion protein is driven by the PelB leader peptide.

The pADL-10 phagemid vector series offers optimal characteristics for phage display with tight control of the fusion protein expression, cloning site amenable to multiple cloning strategies and varied linker and display options. The fusion protein is under the control of the lac promoter, allowing metabolic repression by glucose and induction by IPTG; a copy of the tHP terminator located upstream to the promoter limits transcription leakiness in absence of induction, thus preventing elimination of clones bearing toxic proteins during library amplification (Krebber 1996). Additionally a copy of the lacI transcriptional repressor insures complete saturation of the operator binding site of this high-copy-number plasmid. The vector contains two origins of replication, the f1 origin, which packages the single-stranded phagemid DNA into nascent virions, and the pMB1 origin of replication derived from pBR322, which results in a high-copy-number phagemid. The pMB1 sequence lacks the *rop* gene and carries a point mutation in the RNAII transcript (G 2975 in pBR322 to T 1304 on the reverse complement strand responsible for a temperature-sensitive very high copy number phenotype (Lin-Chao 1992).

Content, Shipping & Storage

Content

VECTOR	COMPOSITION	AMOUNT
pADL-10b	20 µl at 0.5 µg/µl of DNA vector in DNA Conservation Buffer (Tris-HCL 5 mM, EDTA 0.1 mM, pH 8.5)	10 µg

Shipping & Storage

pADL-10b phagemid vector is shipped on wet ice. Upon receipt, store the vector at -20ºC.

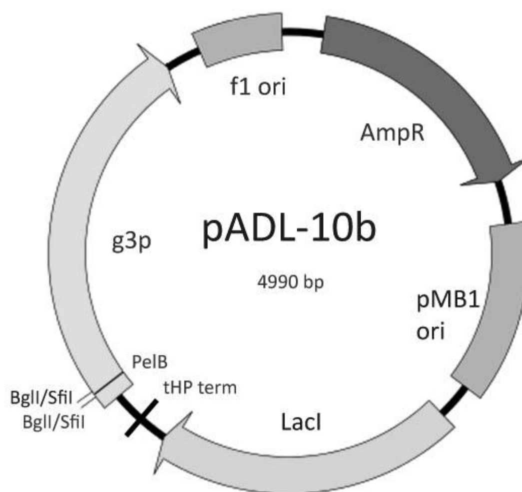
Limited Product Warranty

This warranty limits our liability to the replacement of this product. No other warranties of any kind express or implied, including, without limitation, implied warranties of merchantability or fitness for a particular purpose, are provided by Antibody Design Labs. Antibody Design Labs shall have no liability for any direct, indirect, consequential, or incidental damages arising out of the use, the results of use, or the inability to use this product.

For research use only; not intended for any animal or human therapeutic or diagnostic use.

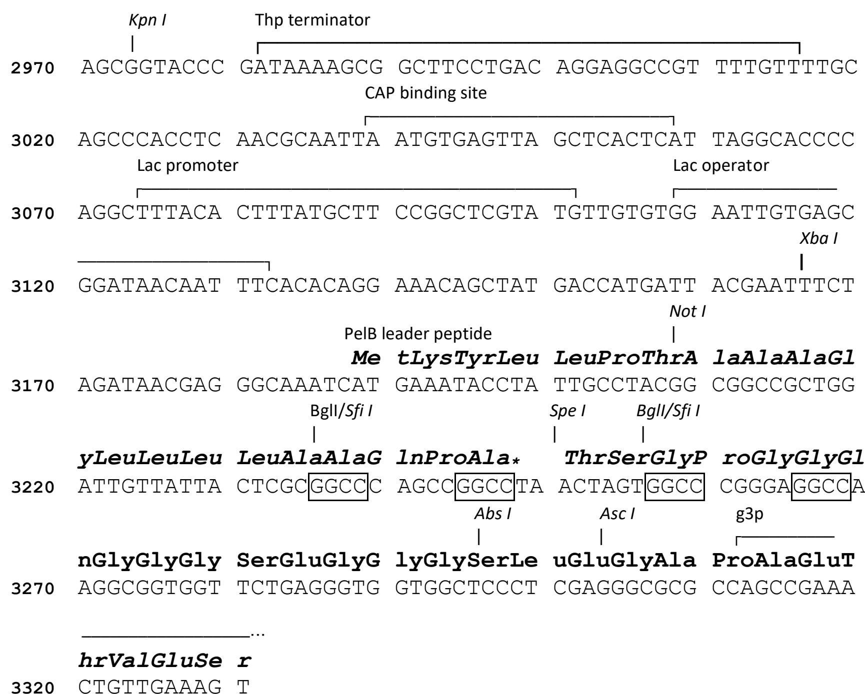
Vector Map

The figure below illustrates the main features of pADL-10b phagemid vector. The full vector sequence is available online for download in varied formats on the product web page; the total length of the vector is 4990 bp.



Cloning Site

Following is an illustration of pADL-10b cloning site from the regulatory region to the g3p sequence. The PelB peptide leader sequence (translation *MKYLLPTAAAGLLLLAAQPAMA*) is interrupted by a stop codon and the two last amino acids MA will have to be re-introduced during cloning to insure proper reading frame and cleavage (on the C-terminal side of the terminal Alanine).



Feature Table

The features of pADL-10b phagemid vector are highlighted in the following table.

FEATURE	LOCATION	DESCRIPTION
TEM1 beta-lactamase	126-986	Ampicillin resistance for selection in *E. coli*.
pMB1 origin	1141-1760	pBR322 origin for replication in *E. coli* with a high copy-number.
LacI	1888-2970	LacI transcriptional repressor for controlled expression of gene III fusion protein in *E. coli* strains lacking LacI gene.
tHP terminator	2981-3017	Transcriptional terminator (taken from the glutamine permease operon) to prevent transcriptional leakage into the lac operon.
CAP binding site	3039-3059	Mediate the catabolite repression of the *lac* operator in the presence of glucose >1% w/v.
-35 signal	3074-3079	Lac promoter -35 signal
-10 signal	3098-3103	Lac promoter -10 signal
PelB leader sequence	3188-3250	PelB leader sequence for export in the periplasm of the host bacteria. The missing terminal methionine and alanine will have to be added during the cloning to obtain a complete leader peptide (MKYLLPTAAAGLLLLAAQPAMA) necessary for proper removal of the leader during the export process.
g3p fusion coding sequence	3250-4533	Full-length gene III fusion protein coding sequence; the M13 g3p protein is fused on its N-terminal side to the linker GPGGQGGGSEGGGSLEGAP; the exact final sequence of the fusion depends on the cloning strategy (see cloning site).
oriF1	4661..4967	Origin of replication for phage f1

Restriction Site Summary

Enzyme	Site	Nb	Position	Strand	Isoschizomers
AbsI	CC^TCGAGG	1	3297		
AloI	(7/12)GAACNNNNNNTCC(12/7)	1	4695		
ApaI	GGGCC^C	1	2445		Bsp120I PspOMI
AscI	GG^CGCGCC	1	3304		PalAI SgsI
AviII	TGC^GCA	1	688		Acc16I FspI NsbI
BamHI	G^GATCC	1	3900		
BclI	T^GATCA	1	2252		FbaI Ksp22I
BseRI	GAGGAG(10/8)	1	3688		
BsmI	GAATGC(1/-1)	1	3426		BsaMI Mva1269I PctI
BspMI	ACCTGC(4/8)	1	3938		Acc36I BfuAI BveI
BstAPI	GCANNNN^NTGC	1	1915		
BstEII	G^GTNACC	1	2419		BstPI Eco91I EcoO65I PspEI
ClaI	AT^CGAT	1	4207		BsuTUI BspDI BseCI Bsa29I BanIII BshVI Bsu15I
DraIII	CACNNN^GTG	1	4748		AdeI
EagI	C^GGCCG	1	3210		BseX3I BstZI EclXI Eco52I
Eam1105I	GACNNN^NNGTC	1	908		AhdI AspEI BmeRI DriI
Eco31I	GGTCTC(1/5)	1	841		Bso31I BsaI
Esp3I	CGTCTC(1/5)	1	2847		BsmBI
FalI	(8/13)AAGNNNNNCTT(13/8)	1	4166		
HindIII	A^AGCTT	1	4536		
HpaI	GTT^AAC	1	2742		KspAI
KpnI	GGTAC^C	1	2973		Acc65I Asp718I
MluI	A^CGCGT	1	2238		
NarI	GG^CGCC	1	2877		DinI EgeI EheI KasI Mly113I SfoI

```
                                                              SspDI
NdeI       CA^TATG                     1          4403        FauNDI
NotI       GC^GGCCGC                   1          3209        CciNI
PflMI      CCANNNN^NTGG                1          1814        AccB7I BasI Van91I
PsiI       TTA^TAA                     1          4623        AanI
PspXI      VC^TCGAGB                   1          3297
PvuI       CGAT^CG                     1           541        Ple19I BpvUI MvrI
ScaI       AGT^ACT                     1           430        AssI BmcAI ZrmI
SmaI       CCC^GGG                     1          3258        Cfr9I TspMI XmaI
SpeI       A^CTAGT                     1          3250        AhlI BcuI
TstI       (8/13)CACNNNNNNTCC(12/7)    1          2307
XbaI       T^CTAGA                     1          3167
XhoI       C^TCGAG                     1          3298        StrI TliI Sfr274I PaeR7I SlaI
AlwNI      CAGNNN^CTG                  2          1387        CaiI PstNI
                                       2          3867
BaeI       (10/15)ACNNNNGTAYC(12/7)    2          3475   -
                                       2          3817
BauI       CACGAG(-5/-1)               2           244        BssSI Bst2BI
                                       2          1628
BcgI       (10/12)CGANNNNNNTGC(12/10)2             386   -
                                       2          2541
BglI       GCCNNNN^NGGC                2          3236
                                       2          3257
BpiI       GAAGAC(2/6)                 2          2377        BbsI BpuAI BstV2I
                                       2          2716
BsePI      G^CGCGC                     2          2649        BssHII PauI PteI
                                       2          3305
BsgI       GTGCAG(16/14)               2          2104   -
                                       2          2304
DrdI       GACNNNN^NNGTC               2          1693        AasI DseDI
                                       2          4702
Eam1104I   CTCTTC(1/4)                 2           119   -    Bst6I EarI
                                       2          1861
Eco57I     CTGAAG(16/14)               2           226        AcuI
                                       2          1274
NaeI       GCC^GGC                     2          3241        PdiI NgoMIV MroNI
                                       2          4854
NmeAIII    GCCGAG(21/19)               2           785   -
                                       2          2463
PvuII      CAG^CTG                     2          2836
                                       2          2929
SfiI       GGCCNNNN^NGGCC              2          3235
                                       2          3256
XmnI       GAANN^NNTTC                 2           309        Asp700I MroXI PdmI
                                       2          4326
```

Absent Sites:
AarI, AatII, AflII, AgeI, AjuI, AlfI, ArsI, AsuII, AvrII, BalI, BarI, BbvCI, BglII, BplI, Bpu10I, BsaBI, BsiWI, Bsp1407I, Bsp1720I, BssNAI, Bsu36I, BtrI, CspCI, Eco47III, EcoNI, EcoRI, EcoRV, FseI, FspAI, KflI, MauBI, MfeI, MreI, MroI, NcoI, NheI, NruI, OliI, PacI, PasI, PfoI, PmaCI, PmeI, PscI, PshAI, PsrI, PstI, RsrII, SacI, SacII, SalI, SapI, SexAI, SgfI, SgrAI, SgrDI, SnaBI, SphI, SrfI, Sse8387I, StuI, SwaI, Tth111I, Zsp2I.

Experimental Procedures

General Molecular Biology Techniques

Molecular cloning and phage display should be conducted under the supervision of a qualified instructor trained to standard safety practice in a molecular biology laboratory environment. Standard molecular biology procedures can be found in a general molecular biology handbook such as Sambrook (1989).

Working with Filamentous Phage

Keep the bench clean and regularly wiped with 2% bleach to limit phage cross-contamination and only use filtered tips to prevent aerosol contaminations. Phages are known to survive standard autoclaving conditions and are not removed by 0.22 µm filtration. Phages are either killed by heat-treating dry, autoclaved materials in an oven for 4 hours at 105ºC (Phage Display (2001)) or by incubation in 2% bleach for at least 1 hour. We recommend to extensively wash with hot water all glass and plastic-ware, then submerge (tubes) or incubate (flasks) with a 2% solution of bleach for at least one hour. Heat-resistant glassware can then be autoclaved in an autoclave that is never used for biological waste while sensitive plastic-ware can be used directly or at best heat-treated as described above.

Bacterial Strains and Helper Phage

Bacterial Strains

In theory, any K12 F^+ *E. coli* strain is suitable for phage display using pADL-10b. Practically we recommend either SS320 or TG1 bacterial strains; both of them have been widely used for phage display and are well documented in the literature. SS320 derives from MC1061 by introduction of the F' episome (Sidhu 2000). Like most derivatives of MC1061, SS320 can be made highly competent for transformation by electroporation. TG1 suppresses amber codons and can also be made highly competent for transformation by electroporation. Their respective phenotype is highlighted below:

SS320 *hsdR2 mcrA0 araD139 Δ(araA-leu)*7697 Δ*lac*X74 *galK16 galE15(GalS)* $\lambda e14^-$ *rpsL150*(Str^R) *spoT1 thi*
F'[proAB+lacIqlacZΔM15 Tn10 (tet^r)*]*

TG1 *supE thi-1 Δ(lac-proAB) Δ(mcrB-hsdSM)5,* ($r_K^- m_K^-$)
F' [traD36 $proAB^+$ $lacI^q$ *lacZΔM15]*

Helper Phage

We recommend CM13 helper phage. CM13 is made available by **Antibody Design Labs** under product number PH020L, which offers a highly concentrated virion preparation, eliminating the need to generate and characterize your own helper phage stocks. CM13 derived from M13KO7 by a single point mutation and produces on average twice more virions.

Plasmid Maintenance

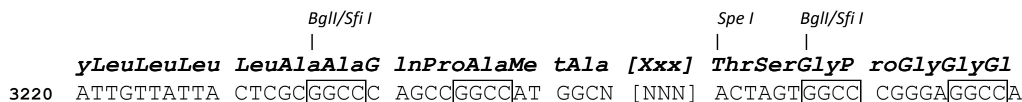
Propagation and maintenance of pADL-10b is obtained on any *recA1, endA1 E. coli* strain using LB or 2xYT medium supplemented with ampicillin 100 µg/ml as a selection marker, without glucose, and incubated at 37ºC with agitation. Phagemid pADL-10b is a derivative of pBR322 with a high copy number origin of replication and usually gives high yields of plasmid DNA with most standard laboratory strains such as XL1-blue or DH5α. Some DNA stabilizing strains are known to

produce smaller amounts of plasmid DNA. In case of issues, we recommend using XL10-Gold® from Agilent Technologies, Inc., on which pADL-10b plasmid DNA can be isolated in large quantities.

Cloning into pADL-10b

Primer Design and PelB Leader Sequence

A complete PelB leader sequence *MKYLLPTAAAGLLLLAAQPAMA* is necessary for export in the periplasm and proper removal of the leader peptide by host proteases. In the following schema, where [NNN] represents the insert sequence and [Xxx] the translated amino acid sequence, the short hexanucleotide ATGGCN must be appended immediately to the first *BglI/SfiI* site to obtain a complete PelB leader encoding sequence; cleavage will occur on the C-terminal side of the terminal alanine (codon GCN).



Retention of the *SpeI* site is optional during cloning and the encoded dipeptide *ThrSer* is not known to interfere with display.

Cloning in pADL-10b Using *BglI/SfiI* Sites

Large libraries in the 1 x 10^9 range and above can easily be constructed using the double BglI/SfiI cloning site.

WORKING WITH BGL I/SFI I SITES

The *SfiI* restriction enzyme recognizes rare 8-base-long interrupted palindromes GGCCNNNN/NGGCC and leaves 3-nucleotide-long overhangs after digestion. The pADL-10b cloning site contains one *SfiI* site close to the end of the PelB leader sequence and a second *SfiI* site 8 nucleotides apart from the first site. The PelB sequence of the empty vector has an early termination by an ochre stop codon and no gene III protein is produced by the vector alone.

The *SfiI* restriction enzyme requires two copies of its recognition sequence for cleavage to occur; cleavage of the two sites happens simultaneously through interaction of two *SfiI* tetramers (Wertzell 1995). Vectors bearing two sites very close to each other are cut in trans and digestion might not complete. Therefore we strongly recommend opening pADL-10b with the alternative *BglI* restriction enzyme, which cuts the shorter 6-base-long interrupted palindromes GCCNNNN/NGGC and generates identical overhangs.

Sites open with BglI will re-ligate with sites open with SfiI as long as overhangs are complementary. Practically, the pentanucleotide NNNNN must be identical to the original vector sequence to handle both ligation of the complementary overhangs and conservation of the amino acid sequence (PelB sequence and linker to protein III). Since the overhang of the two BglI/SfiI sites are non-palidromic and different, a cut empty vector cannot ligate onto itself; it is therefore possible to follow a ligation reaction by minigel analysis since remaining unligated vector or unligated insert will migrate unchanged at their expected size.

PREPARATION OF VECTOR DNA FOR CLONING

1. On ice add successively water, *BglI* buffer (1x final), pADL-10b vector and *BglI* enzyme 5 units/µg DNA; make sure the enzyme volume does not to exceed 1/10 of the total reaction volume.

2. Incubate 4 h to overnight at 37ºC.

3. Inactivate for 20 min at 70ºC.

4. Confirm the digestion by DNA analysis on a minigel.
5. Purify the cut vector.

For routine cloning, a standard DNA purification kit can be used directly after the digestion to remove the excess of buffer, the small DNA stuffer and leftover restriction enzyme. For library construction, phenol/chloroform extraction and/or gel purification may be required.

PREPARATION OF INSERTS

SfiI digestion should be rapid and complete in 4 hours especially for fragments longer or equal to 200 bp where sites are cut in *cis*. *BglI* may be used when the insert sequence is known to be free of *BglI* site and therefore is not recommended for building antibody libraries.

Cloning using *NotI-SpeI* sites

The *NotI* site located in the first half of the PelB leader encoding sequence may be used in conjunction with the *SpeI* site to clone inserts. This strategy has been applied in some early phage display vectors. Consult your restriction enzyme distributor resources to identify a buffer compatible with both enzymes and follow the concentration schema given above to conduct the digestion. *NotI* and *SpeI* can be inactivated by heat before DNA purification.

Sequencing of Inserts

The following primers give both strong PCR amplification and sequencing traces.

Forward or Sense Primers

phiS3 5'-CACAGGAAACAGCTATGACCATGATTA

phiS2 5'-ATGAAATACCTATTGCCTACGG

Backward, Antisense or Reverse Primers

psiR2 5'-CGTTAGTAAATGAATTTTCTGTATGAGG

psiR3 5'-GCGTAACGATCTAAAGTTTTGTCG

Nested Sequencing

Often it is easier to sequence an insert by PCR on the bacterial culture supernatant or directly from a colony rather than on tediously isolated plasmids. Use the outward primers phiS3 and betaR3 together with a DNA polymerase not inhibited by bacterial cultures such as TAQ polymerase for the PCR and sequence the insert with the nested reverse primer betaR2. Use less than 1 µl of bacterial culture supernatant per 50 µl-PCR reaction or the touch of a toothpick on a colony as DNA template.

Phagemid Virion Production

A superinfection by a helper phage is necessary for phagemid pADL-10b-containing bacteria to produce virions. Please, consult the M13KO7 or CM13 helper phage manual for optimal conditions of superinfection. We recommend a rich medium such as 2xYT medium supplemented with ampicillin 100 µg/ml, kanamycin 50 µg/ml (when M13KO7 or CM13 helper phages are used), IPTG 200 µM, no glucose or less than 0.1% w/v, and incubation from 8 h to overnight at 30ºC and 250 rpm. We recommend adding the helper phage when the bacterial culture reaches an optical density at 600 nm of 0.5 OD; large amounts of non-superinfected cells due to immunity to superinfection will decrease virion production above 0.5

OD while disparities caused by differences in phage growth rates will be amplified at a lower OD. Immunity to superinfection refers to the difficulty to transduce bacteria when protein III is expressed, as it is the case when with phagemids expressing a full-length pIII fusion protein.

Notes

- Supplementation with IPTG 200 µM is necessary to achieve display on the phage with pADL-10b. Virions produced in absence of IPTG will not bear any antibodies and subsequent selection will not work.
- Shorter incubation times 6 to 8 h will produce less virions; we have not seen improvement of display on shorter incubation times or, inversely, we have not seen sign of proteolysis of the linker after overnight incubation. Always use freshly prepared buffers from commercial concentrates during virion preparations to limit sources of proteolysis. Proteolysis sometimes occurs on concentrated virions; always prepare virions quickly and on ice.
- Kanamycin 50 µg is enough to ensure selection with derivatives of M13KO7. Higher concentration may be needed on very rich culture media or if your culture medium contains phosphate salts.
- Higher IPTG concentrations will give higher levels of display and is recommended for making initial libraries or preparing single phages.

Induction Conditions & Control of Expression

The expression of the pIII fusion protein is regulated by the lac promoter. Due to the high copy number of the phagemid, the chromosome-encoded lacI repressor molecules are insufficient to bind to all O1/O3 operators. However, pADL-10b carries its own copy of the lacI repressor gene, ensuring tight control of pIII expression. Consequently, the lac promoter remains in a repressed state in TG1 and SS320 cells. IPTG, a non-metabolizable lactose analog, is essential for induction. The level of induction varies from 20 µM to 1 mM IPTG. Typically, 20 µM IPTG is used for low-level induction, and 200 µM for medium-level induction. Glucose is not needed for using pADL-10b.

CONDITION	STATE	NOTES
No Glucose	Repressed	Repression is strong but not complete. There is some leakage of the promoter. This is the recommended conditions for repression.
No glucose + IPTG	Induced	IPTG is required for induction (10-1000 µM, usually 20 µM or 200 µM).
No Glucose + IPTG + 30ºC	Induced	This condition favors folding and is believed to decrease the toxicity of large proteins (e.g. Fab). Also, the phagemid copy number is lower. This is the recommended condition for induction.

Appendix

MSDS Information

MSDSs (Material Safety Data Sheets) are available on the **Antibody Design Labs** website at the corresponding product page.

Quality Control

Specifications and quality control are detailed on the online product page. **Antibody Design Labs** certifies that the product will perform according to these specifications.

Technical Support

Visit **Antibody Design Labs'** website at **www.abdesignlabs.com** for technical resources, including manuals, vector maps and sequences, application notes, FAQs, etc.

FOR MORE INFORMATION OR TECHNICAL ASSISTANCE, CALL, WRITE, FAX, OR EMAIL US AT:

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(Monday – Friday 9:00 AM – 5:00 PM PST)

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