pADL-100 Phagemid

INSTRUCTION MANUAL

pADL™-100 Phagemid Vector for Phage Display

Catalog #: PD100

Version: A1.5 – June 2023

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Description

Introduction

The pADL™-100 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™-100 phagemid vector offers optimal characteristics to maximize display with a strong ribosome binding site, no tag to limit unwanted proteolysis and no amber codon, often only partially suppressed *in vivo*. The fusion protein is under the control of the lac promoter, allowing metabolic repression by glucose and induction by IPTG. A copy of the lambda t1 terminator located downstream gene III prevents leakiness of the transcription during induction, in particular preventing excessive expression of the beta-lactamase and rapid consumption of ampicillin.

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™-100	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™-100 phagemid vector is shipped on wet ice. Upon receipt, store the vector at -20ºC.

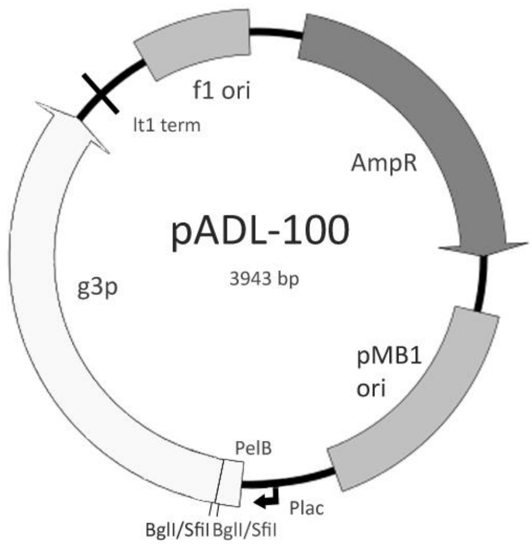
Limited Product Warranty

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™-100 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 3943 bp.



Feature Table

The features of pADL™-100 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.
CAP binding site	1911-1931	Mediate the catabolite repression of the *lac* operator in the presence of glucose >1% w/v.
-35 signal	1946-1951	Lac promoter -35 signal
-10 signal	1970-1975	Lac promoter -10 signal
PelB leader sequence	2025-2087	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	2150-3370	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).
lt1	3389-3493	Lambda t1 terminator
oriF1	3614-3920	Origin of replication for phage f1

Cloning Site

Following is an illustration of pADL™-100 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).

```
                                         O3 operator               CAP binding site
1870  GGTTTCCCGA CTGGAAAGCG GGCAGTGAGC GCAACGCAAT TAATGTGAGT
                                              Lac promoter
1920  TAGCTCACTC ATTAGGCACC CCAGGCTTTA CACTTTATGC TTCCGGCTCG
                       O1 operator                    EcoR I
                  +1
1970  TATGTTGTGT GGAATTGTGA GCGGATAACA ATTTGAATTC AAGGAGACAG
                                              Not I
           PelB leader peptide
          MetLy sTyrLeuLeu ProThrAlaA laAlaGlyLe uLeuLeuLeu
2020  TCATAATGAA ATACCTATTG CCTACGGCGG CCGCTGGATT GTTATTACTC
        Bgl I/Sfi I           Spe I      Bgl I/Sfi I
      AlaAlaGlnP roAla*  Thr SerGlyProG lyGlyGlnGl yGlyGlySer
2070  GCGGCCCAGC CGGCCTAACT AGTGGCCCGG GAGGCCAAGG CGGTGGTTCT
                         Abs I      Asc I       g3p
      GluGlyGlyG lySerLeuGl uGlyAlaPro AlaGluThrV alGluSerCys
2220  GAGGGTGGTG GCTCCCTCGA GGGCGCGCCA GCCGAAACTG TTGAAAGTTGT
```

Restriction Site Summary

Enzyme	Site	Nb	Position	Strand	Isoschizomers
AbsI	CC^TCGAGG	1	2134		
AflII	C^TTAAG	1	3366		BfrI BspTI BstAFI MspCI Vha464I
AloI	(7/12)GAACNNNNNNTCC(12/7)	1	3648		
AscI	GG^CGCGCC	1	2141		PalAI SgsI
BamHI	G^GATCC	1	2737		
BbsI	GAAGAC(2/6)	1	3451	-	BpiI BstV2I
BcgI	(10/12)CGANNNNNNTGC(12/10)	1	386	-	
BmrI	ACTGGG(5/4)	1	863		BmuI
BsePI	G^CGCGC	1	2142		BssHII PauI PteI
BseRI	GAGGAG(10/8)	1	2525		
BsmI	GAATGC(1/-1)	1	2263		Mva1269I PctI
BspMI	ACCTGC(4/8)	1	2775		Acc36I BfuAI BveI
BstXI	CCANNNNN^NTGG	1	2104		
BtgZI	GCGATG(10/14)	1	3692		
ClaI	AT^CGAT	1	3044		BsuTUI BspDI BseCI Bsa29I BshVI Bsu15I
DraIII	CACNNN^GTG	1	3701		AdeI
EagI	C^GGCCG	1	2047		BseX3I BstZI EclXI Eco52I
Eam1105I	GACNNN^NNGTC	1	908		AhdI BmeRI DriI
EarI	CTCTTC(1/4)	1	119	-	Bst6I Eam1104I

Eco31I	GGTCTC(1/5)	1	841		Bso31I BsaI BspTNI
EcoRI	G^AATTC	1	2004		
FalI	(8/13)AAGNNNNNCTT(13/8)	1	3003		
FspI	TGC^GCA	1	688		Acc16I NsbI
GsuI	CTGGAG(16/14)	1	823		BpmI
NdeI	CA^TATG	1	3240		FauNDI
NheI	G^CTAGC	1	3371		AsuNHI BmtI BspOI
NmeAIII	GCCGAG(21/19)	1	785	-	
NotI	GC^GGCCGC	1	2046		CciNI
PsiI	TTA^TAA	1	3576		AanI
PspXI	VC^TCGAGB	1	2134		
PvuI	CGAT^CG	1	541		Ple19I
PvuII	CAG^CTG	1	1856		
ScaI	AGT^ACT	1	430		BmcAI ZrmI
SmaI	CCC^GGG	1	2095		Cfr9I TspMI XmaI
SpeI	A^CTAGT	1	2087		AhlI BcuI
XhoI	C^TCGAG	1	2135		Sfr274I PaeR7I SlaI
AclI	AA^CGTT	2	310		Psp1406I
		2	683		
AlwNI	CAGNNN^CTG	2	1387		CaiI PstNI
		2	2704		
ApaLI	G^TGCAC	2	241		Alw44I VneI
		2	1487		
BaeI	(10/15)ACNNNNGTAYC(12/7)	2	2312	-	
		2	2654		
BciVI	GTATCC(6/5)	2	65		BfuI BsuI
		2	1592		
BglI	GCCNNNN^NGGC	2	2073		
		2	2094		
BseYI	CCCAGC(-5/-1)	2	1497		GsaI PspFI
		2	2074		
BspHI	T^CATGA	2	73		CciI PagI
		2	1081		
BsrDI	GCAATG(2/0)	2	672		Bse3DI BseMI
		2	854		
BssSI	CACGAG(-5/-1)	2	244		BauI Bst2BI
		2	1628		
DrdI	GACNNNN^NNGTC	2	1693		AasI DseDI
		2	3655		
Eco57I	CTGAAG(16/14)	2	226		AcuI
		2	1274		
KroI	G^CCGGC	2	2078		
		2	3807		
NaeI	GCC^GGC	2	2078		PdiI NgoMIV MroNI
		2	3807		
SfiI	GGCCNNNN^NGGCC	2	2072		
		2	2093		
XmnI	GAANN^NNTTC	2	309		Asp700I MroXI PdmI
		2	3163		

Absent Sites:
AarI, AatII, AgeI, AjuI, AlfI, ApaI, ArsI, AsuII, AvrII, BalI, BarI, BbvCI, BclI, BglII, BlpI, BplI, Bpu10I, BsaBI, BsgI, BsiWI, Bsp1407I, BspEI, BstAPI, BstEII, BstZ17I, Bsu36I, BtrI, CspCI, Eco47III, EcoNI, EcoRV, Esp3I, FseI, FspAI, HindIII, HpaI, I-CeuI, I-PpoI, I-SceI, KflI, KpnI, MauBI, MfeI, MluI, MreI, NarI, NcoI, NruI, NsiI, OliI, PI-PspI, PI-SceI, PacI, PasI, PciI, PflMI, PfoI, PmaCI, PmeI, PshAI, PsrI, PstI, RsrII, SacI, SacII, SalI, SapI, SexAI, SgfI, SgrAI, SgrDI, SnaBI, SphI, SrfI, Sse8387I, StuI, SwaI, Tth111I, XbaI, XcmI.

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-100. Practically we recommend SS320 bacterial strain. SS320 has been widely used for phage display and is 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. SS320 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)]

Helper Phage

Phagemid pADL-100 is compatible with most helper phage. We recommend CM13d3 helper phage to maximize display. CM13d3 is a protein pIII defective helper lacking a functional gene III. When used as a helper, the only source of protein pIII is the phagemid, thus driving the display of the fusion on the phage head (Dueñas (1995), Rondot (2001)). CM13d3 is made available by **Antibody Design Labs** under product number PH040L, which offers a highly concentrated virion preparation, eliminating the need to generate and characterize your own helper phage stocks. Alternatively, M13KO7 (product number PH010L) or CM13 (product number PH020L) can be used to modulate the display to a lower level.

Plasmid Maintenance

Propagation and maintenance of pADL-100 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-100 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-100 plasmid DNA can be isolated in large quantities.

Cloning into pADL-100

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 *SfiI* site to obtain a complete PelB leader encoding sequence; cleavage will occur on the C-terminal side of the terminal alanine (codon GCN).

```
                           BglI/Sfi I                                  Spe I    BglI/Sfi I
                           |                                           |        |
     yLeuLeuLeu LeuAlaAlaG lnProAlaMe tAla [Xxx] ThrSerGlyP roGlyGlyGl
3220 ATTGTTATTA CTCGCGGCCC AGCCGGCCAT GGCN [NNN] ACTAGTGGCC CGGGAGGCCA
```

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

Cloning in pADL-100 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-100 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 <u>we strongly recommend</u> opening pADL-100 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-100 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 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. Primer locations can be found in the corresponding GenBank sequence file.

Forward or Sense Primers

phiS2 5’-ATGAAATACCTATTGCCTACGG

phiS4 5’-GCGGATAACAATTTGAATTCAAGGAGACAG

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 phiS2 and psiR3 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 psiR2. 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-100-containing bacteria to produce virions. Please, consult the M13KO7d3 or M13KO7 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 M13KO7d3 or M13KO7 helper phage are used), no glucose or less than 0.1% w/v, and incubation from 8 h to overnight at 30ºC and 250 rpm. Supplementation with IPTG is not necessary to get display on the phage but is recommended to maximize display at the risk of an increased toxicity. We recommend adding the helper phage when the bacterial culture reaches an optical density at 600 nm between 0.4 OD and 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

- Shorter incubation times 6 to 8 h long will produce less virions; we have not seen improvement of display on shorter incubation times; 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 usually occurs on concentrated virions; always prepare virions quickly and on ice.
- Kanamycin 50 µg/ml is enough to ensure selection with derivatives of M13KO7d3 and M13KO7. Higher concentrations may be needed if your culture medium contains phosphate salts.

Induction Conditions & Control of Expression

Expression of the pIII fusion protein is under the control of the lac promoter. Because of the high copy number of the phagemid, there are not enough molecules of lacI repressor in the cell to bind to all O1/O3 operators. As a result, the lac promoter is in a induced state in TG1 and SS320. Control of the induction is done in those strains by binding of the CAP protein (catabolite activator protein) to the CAP binding site in the presence of cyclic AMP (catabolic repression). In the presence of glucose, the level of cAMP decreases, the CAP protein leaves the CAP binding site and transcription is activated. A higher level of transcription is achieved by further adding IPTG, a non-metabolizable analog of lactose.

CONDITION	STATE	NOTES
Glucose 1%	Repressed	Repression is strong but not complete. There is some leakage of the promoter. This is the recommended conditions for repression.
Glucose 2%	Repressed	A higher level of repression is achieved. Some protocols recommend this concentration (~100 mM glucose).
No Glucose	Induced	Withdrawal of the catabolic repression induces the lac promoter, resulting in expression and display.
No glucose + IPTG	Induced	A higher level of induction is obtained in the presence of IPTG (>200 µM, usually 0.5 mM). Higher levels of display may be achieved.
No Glucose + 30°C	Induced	This condition favors folding and is believed to decrease the toxicity of large proteins (e.g. Fab). 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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San Diego, CA 92117

Email: support@abdesignlabs.com
Phone: 1-877-223-3104 (TOLL-FREE)
(Monday – Friday 9:00 AM – 5:00 PM PST)

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