Antibody Design LABORATORIES

pADL-23Chlo Phagemid

INSTRUCTION MANUAL

pADL™-23Chlo Phagemid Vector for Phage Display

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Legal and Disclaimers

Description

Introduction

The pADL™-23Chlo 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. A HIS tag and a Myc tag followed by an amber codon are conveniently located before the gene III protein. Phage display will be done using bacterial strains that suppress the amber codon while growth on non-suppressive strains will result in the expression of free scFvs or Fab fragments in the periplasm space; a classical application of this vector is the production of free scFv and detection of its binding through the Myc tag.

The pADL™-2x phagemid vector series offers optimal characteristics for phage display with optimized expression of the fusion protein for strong display, suppressible amber codon to direct the fusion either as a gene III fusion or as a secreted free entity, 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 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™-23Chlo	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™-23Chlo phagemid vector is shipped on wet ice. Upon receipt, store the vector at -20ºC.

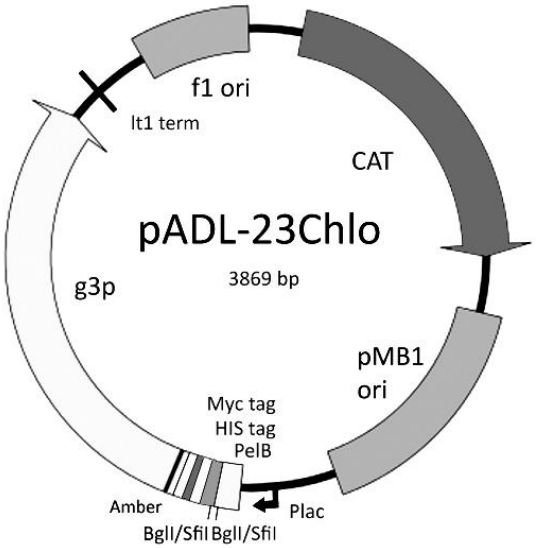
Limited Product Warranty

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™-23Chlo 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 3960 bp.



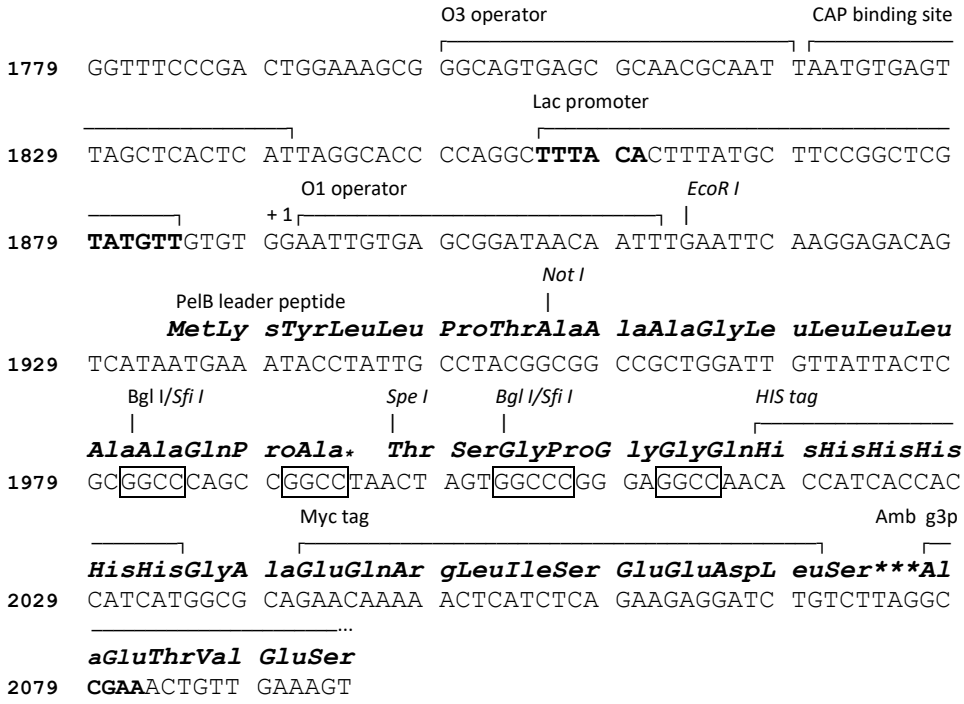
Feature Table

The features of pADL™-23Chlo phagemid vector are highlighted in the following table.

FEATURE	LOCATION	DESCRIPTION
Chloramphenicol acetyltransferase	236-895	Chloramphenicol resistance for selection in *E. coli*.
pMB1 origin	1050-1669	pBR322 origin for replication in *E. coli* with a high copy-number.
CAP binding site	1820-1840	Mediate the catabolite repression of the *lac* operator in the presence of glucose >1% w/v.
-35 signal	1855-1860	Lac promoter -35 signal
-10 signal	1879-1884	Lac promoter -10 signal
PelB leader sequence	1934-1996	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.
HIS tag	2017-2034	Peptide H H H H H H
Myc tag	2041-2070	Peptide E Q K L I S E E D L
Amber Stop codon	2074-2076	
g3p fusion coding sequence	2077-3297	Full-length gene III fusion protein coding sequence; the M13 g3p protein is fused on its N-terminal side to the HIS tag and the Myc tag; the exact final sequence of the fusion depends on the cloning strategy (see cloning site).
lt1	3307-3419	Lambda t1 terminator
oriF1	3540-3846	Origin of replication for phage f1

Cloning Site

Following is an illustration of pADL™-23Chlo 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).



Restriction Site Summary

Enzyme	Site	Nb	Position	Strand	Isoschizomers
AclI	AA^CGTT	1	535		Psp1406I
AflII	C^TTAAG	1	3293		BfrI BspTI BstAFI MspCI Vha464I
AloI	(7/12)GAACNNNNNNTCC(12/7)	1	3574		
ApaLI	G^TGCAC	1	1396		Alw44I VneI
ArsI	(8/13)GACNNNNNNTTYG(11/6)	1	725		
BalI	TGG^CCA	1	714		MlsI MluNI Mox20I MscI Msp20I
BamHI	G^GATCC	1	2664		
BbsI	GAAGAC(2/6)	1	3377	-	BpiI BstV2I
Bpu10I	CCTNAGC(-5/-2)	1	221	-	
BseRI	GAGGAG(10/8)	1	2452		
BspEI	T^CCGGA	1	445		Aor13HI Bsp13I Kpn2I BseAI MroI AccIII
BspHI	T^CATGA	1	990		CciI PagI
BspMI	ACCTGC(4/8)	1	2702		Acc36I BfuAI BveI
BsrDI	GCAATG(2/0)	1	461		Bse3DI BseMI
BssSI	CACGAG(-5/-1)	1	1537		BauI Bst2BI
BstXI	CCANNNNN^NTGG	1	2025		

BtsI	GCAGTG(2/0)	1	1800		
ClaI	AT^CGAT	1	2971		BsuTUI BspDI BseCI Bsa29I BshVI Bsu15I
DraIII	CACNNN^GTG	1	3627		AdeI
EagI	C^GGCCG	1	1956		BseX3I BstZI EclXI Eco52I
EarI	CTCTTC(1/4)	1	2059	-	Bst6I Eam1104I
Eco57I	CTGAAG(16/14)	1	1183	-	AcuI
EcoRI	G^AATTC	1	1913		
FalI	(8/13)AAGNNNNNCTT(13/8)	1	2930		
GsuI	CTGGAG(16/14)	1	550		BpmI
NcoI	C^CATGG	1	750		Bsp19I
NdeI	CA^TATG	1	3167		FauNDI
NheI	G^CTAGC	1	3298		AsuNHI BmtI BspOI
NotI	GC^GGCCGC	1	1955		CciNI
PasI	CC^CWGGG	1	680		
PflMI	CCANNNN^NTGG	1	675		AccB7I Van91I
PsiI	TTA^TAA	1	3502		AanI
ScaI	AGT^ACT	1	864		BmcAI ZrmI
SmaI	CCC^GGG	1	2004		Cfr9I TspMI XmaI
SpeI	A^CTAGT	1	1996		AhlI BcuI
XmnI	GAANN^NNTTC	1	3090		Asp700I MroXI PdmI
AlwNI	CAGNNN^CTG	2	1296		CaiI PstNI
		2	2631		
BaeI	(10/15)ACNNNNGTAYC(12/7)	2	2239	-	
		2	2581		
BciVI	GTATCC(6/5)	2	120		BfuI BsuI
		2	1501		
BglI	GCCNNNN^NGGC	2	1982		
		2	2003		
BseYI	CCCAGC(-5/-1)	2	1406		GsaI PspFI
		2	1983		
DrdI	GACNNNN^NNGTC	2	1602		AasI DseDI
		2	3581		
Esp3I	CGTCTC(1/5)	2	135	-	BsmBI
		2	667		
KroI	G^CCGGC	2	1987		
		2	3733		
NaeI	GCC^GGC	2	1987		PdiI NgoMIV MroNI
		2	3733		
PvuII	CAG^CTG	2	347		
		2	1765		
SfiI	GGCCNNNN^NGGCC	2	1981		
		2	2002		
TaqII	GACCGA(11/9)	2	2889		
		2	3520		
VspI	AT^TAAT	2	1758		AseI PshBI
		2	1817		

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 that suppresses the amber codon is suitable for phage display using pADL-23c. Practically we recommend TG1 bacterial strain; TG1 is widely used for phage display and is well documented in the literature. TG1 suppresses amber codons and can also be made highly competent for transformation by electroporation. TG1 phenotype is highlighted below:

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

SS320 is a non-amber suppressive strain that 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 can used for example to make free scFv or free Fab secreted in the culture supernatant for performing assay in free form. SS320 phenotype is highlighted below:

SS320 *hsdR2 mcrA0 araD139 Δ(araA-leu)*7697 Δ*lac*X74 *galK16 galE15(GalS) λe14$^-$ rpsL150(Str^R) spoT1 thi*
F'[proAB+lacIqlacZΔM15 Tn10 (tet^r)]

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.

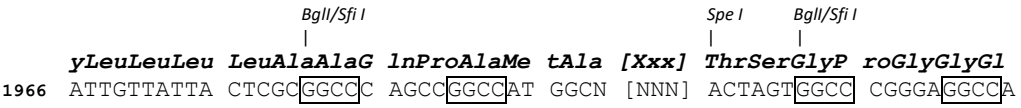
Plasmid Maintenance

Propagation and maintenance of pADL-23Chlo is obtained on any *recA1, endA1 E. coli* strain using LB or 2xYT medium supplemented with chloramphenicol 37 µg/ml as a selection marker, without glucose, and incubated at 37ºC with agitation. Phagemid pADL-23Chlo 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 DH5α, on which pADL-23Chlo plasmid DNA can be isolated in large quantities.

Cloning into pADL-23Chlo

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).



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

Cloning in pADL-23Chlo 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-23Chlo 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-23Chlo 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-23Chlo 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-23Chlo-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 chloramphenicol 37 µg/ml, kanamycin 50 µg/ml (when M13KO7 or CM13 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 achieve display on the phage with pADL-23c. 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

- Supplementation with IPTG is not necessary during virion production but is recommended for the expression of free antibodies in the periplasm with the non-amber suppressive SS320 bacterial strain.
- 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 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 + 30ºC	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

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Phone: 1-877-223-3104 (TOLL-FREE)
(Monday – Friday 9:00 AM – 5:00 PM PST)

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