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pIndigoBAC-5 (*Bam*H I-Cloning Ready)

pIndigoBAC-5 (*Hin*d III-Cloning Ready)

Cat. Nos. BACB085H and BACH095H

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1. Introduction

pIndigoBAC-5* is a 7506-bp bacterial artificial chromosome (BAC) cloning vector which is provided in a "ready-to-use" state for researcher convenience. The vector has been linearized at a unique restriction enzyme recognition site (*Bam*H I or *Hin*d III), dephosphorylated and rigorously tested for purity and recombinant cloning efficiency (Cloning-Ready).

pIndigoBAC-5 is derived from pIndigoBAC[1] which was in turn derived from pBeloBAC11[2]. Features of the vector include:

1) Enhanced color intensity production in X-gal-based "blue-white" screening as compared to pBeloBAC11.
2) Chloramphenicol-resistance as an antibiotic selectable marker.
3) *E. coli* F factor-based partitioning and copy number regulation system.
4) Bacteriophage lambda *cos* site for lambda packaging or lambda-terminase cleavage.
5) Bacteriophage P1 *lox*P site for Cre-recombinase cleavage.
6) Bacteriophage T7 RNA polymerase promoter flanking the cloning site.
7) BAC-end sequencing primer binding sites.[3]

BACs are widely used as cloning vectors for high molecular weight (HMW) DNA insert libraries. BACs allow for the cloning of inserts up to 300 kb in size.[4] The stability of such large constructs *in vivo* is facilitated by the single copy per cell regulation provided by the BAC vector. Cloning, restricting, mapping, isolating, and other common molecular techniques are achieved by standard laboratory procedures. For a review of BACs and related techniques see Birren, B. *et al.*, (1999), Bacterial Artificial Chromosomes in *Genome Analysis: A Laboratory Manual* **v. 3**, 241. For optimal yields, purify pIndigoBAC-5 DNA using Epicentre's BACMAX™ DNA Purification Kit.

Often achieved through a "shot-gun cloning approach", a BAC subclone library is commonly produced as an initial step in genomic sequencing projects. Genome-spanning representative clones can be screened, mapped, and used subsequently for the production of lower molecular weight DNA insert sublibraries resulting in the eventual production of sequencing templates.

Quality Control: A major concern with BAC vector preparations is that they be free of contaminating DNA, which if clonable, results in unacceptably high levels of background colony formation. High background levels can also be caused by incomplete linearization of the vector, since supercoiled DNA transforms bacteria at much higher efficiencies than linear DNA, and also by incomplete dephosphorylation of the vector thus leaving self-ligatable vector present for recircularization when treated with DNA ligase. Complete dephosphorylation of BAC preparations also renders any copurifying contaminating DNA (i.e., *E. coli* genomic DNA) unclonable and thus it would not contribute to background colony formation. pIndigoBAC-5 (*Bam*H I and *Hin*d III-Cloning-Ready) preparations in recircularization assays (+ligase, –insert) show a 10^5 reduction in background colony formation (transformation efficiencies in colonies per µg of DNA) as compared to supercoiled forms of the BACs. In cloning assays (+ligase, +insert) the preparations show a 10^3 increase in signal versus background colony formation. pIndigoBAC-5 (*Bam*H I and

*Hin*d III-Cloning-Ready) preparations must yield greater than 90% recombinant clones when used to clone *Bam*H I and *Hin*d III DNA fragments respectively in order to pass acceptance criteria.

2. Related Products

The following products are also available:

– BACMAX™ DNA Purification Kit
– TransforMax™ EC100™ Electrocompetent *E. coli*
– Fast-Link™ DNA Ligation Kits
– Colony Fast-Screen™ Kits
– Fast-Link™ DNA Ligation and Screening Kits
– GELase™ Gel-Digesting Preparation
– Lambda-Terminase
– EZ-Tn5™ Insertion Kits
– T7 RNA Polymerase
– MasterPure™ DNA Purification Kits
– Plasmid-Safe™ ATP-Dependent DNase

3. Product Specifications

Storage: Store at –20°C.

Size: 500 ng @ 25 ng/µl/20 µl in TE Buffer (10 mM Tris-HCl [pH 7.5], 1 mM EDTA).

Protocols: For protocols on BAC cloning and working with BAC clones, see the following references:

– Birren, B. *et al.*, (1999) Bacterial Artificial Chromosomes in *Genome Analysis: A Laboratory Manual* **v. 3**, 241.
– http://www.genome.clemson.edu

4. Primers

pIndigoBAC-5 Sequencing Primers (Primers are available separately)

pIndigoBAC-5 Forward Sequencing Primer ..... Cat. No. BFP0701

5′ GGATGTGCTGCAAGGCGATTAAGTTGG 3′ .....1 nmol supplied in TE Buffer at 50 µM

pIndigoBAC-5 Reverse Sequencing Primer ..... Cat. No. BRP0801

5′ CTCGTATGTTGTGTGGAATTGTGAGC 3′.....1 nmol supplied in TE Buffer at 50 µM

pIndigoBAC-5 Forward Sequencing Primer

Temperatures of Dissociation & Melting:

T_d: 79°C (nearest neighbor method)

T_m: 78°C (% G+C method)

T_m: 82°C ([2 (A+T) + 4 (G+C)] method)

T_m: 68°C ((81.5 + 16.6 (log [Na^+])) + ([41 (#G+C) - 500] / length) method)
where [Na^+] = 0.1 M

pIndigoBAC-5 Reverse Sequencing Primer

Temperatures of Dissociation & Melting:

T_d: 71°C (nearest neighbor method)

T_m: 75°C (% G+C method)

T_m: 76°C ([2 (A+T) + 4 (G+C)] method)

T_m: 65°C ((81.5 + 16.6 (log [Na^+])) + ([41 (#G+C) - 500] / length) method)
where [Na^+] = 0.1 M

Note: *The sequence of the pIndigoBAC-5 Forward and Reverse Primers do not function well as IRD800-labeled sequencing primers. We recommend using the T7 and pIndigoBAC-5 RP-2 Primers instead of the pIndigoBAC-5 Forward and Reverse Primers respectively, for this purpose.*

pIndigoBAC-5 RP-2 Reverse Sequencing Primer

5′ TACGCCAAGCTATTTAGGTGAGA 3′

Orientation for BAC End-Sequencing

The following is the nucleotide sequence of pIndigoBAC-5 (bases 230-489) from the pIndigoBAC-5 Forward Sequencing Primer (230-256) to the pIndigoBAC-5 Reverse Sequencing Primer (489-464) encompassing the T7 RNA polymerase promoter (311-330), the *Bam*H I site (353-358) and the *Hin*d III site (383-388).

```
230   GGATGTGCTG  CAAGGCGATT  AAGTTGGGTA  ACGCCAGGGT  TTTCCCAGTC
280   ACGACGTTGT  AAAACGACGG  CCAGTGAATT  GTAATACGAC  TCACTATAGG
330   GCGAATTCGA  GCTCGGTACC  CGGGGATCCT  CTAGAGTCGA  CCTGCAGGCA
380   TGCAAGCTTG  AGTATTCTAT  AGTCTCACCT  AAATAGCTTG  GCGTAATCAT
430   GGTCATAGCT  GTTTCCTGTG  TGAAATTGTT  ATCCGCTCAC  AATTCCACAC
480   AACATACGAG
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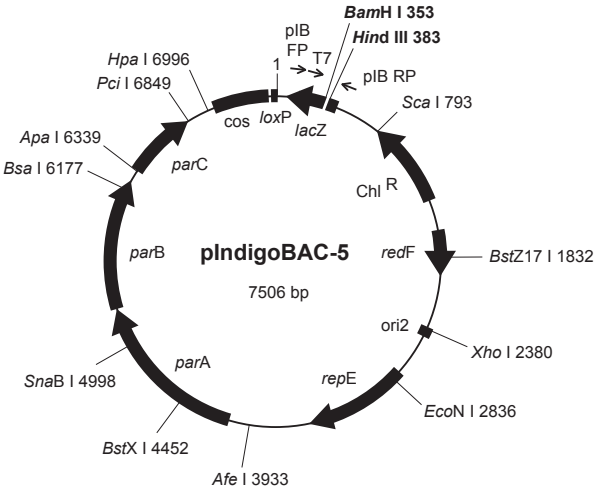
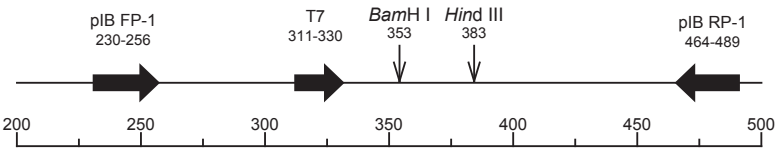
pIndigoBAC-5 (*Bam*H I-Cloning Ready) and *(Hin*d III-Cloning Ready*)* Bacterial Artificial Chromosome Cloning Vectors (7506 bp) differ in sequence by one nucleotide at two distinct loci.

An electronic copy of these sequences are available for downloading at our Web site at the URL: http://www.epibio.com/sequences.asp, or can be requested via e-mail (techhelp@epicentre.com) or by calling Technical Service.



Note: Not all restriction enzymes that cut only once are indicated above.
See the following page for further information.
Primers are not drawn to scale.



pIB FP = pIndigoBAC-5 Foward Sequencing Primer	5' GGATGTGCTGCAAGGCGATTAAGTTGG 3'
pIB RP = pIndigoBAC-5 Reverse Sequencing Primer	5' CTCGTATGTTGTGTGGAATTGTGAGC 3'
T7 = T7 Promoter Primer	5' TAATACGACTCACTATAGGG 3'

Figure 1. pIndigoBAC-5 BAC Vector.

Restriction Enzymes that cut pIndigoBAC-5 1 to 3 times:

Enzyme	Sites	Location	Enzyme	Sites	Location
Acc65 I	2	344, 4574	EcoR V	2	3495, 3724
Acl I	2	1121, 4966	Fsp I	3	167, 3119, 6945
Afe I	1	3933	**Hind III**	**1**	**383**
Afl II	2	5975, 6215	Hpa I	1	6996
Afl III	3	4340, 4514, 6849	Kpn I	2	348, 4578
Age I	3	3194, 4424, 5317	Mfe I	1	4354
Ahd I	1	6853	Msc I	2	943, 4785
Ale I	1	5910	Nae I	2	3072, 6988
Apa I	1	6339	Nar I	1	146
ApaB I	3	96, 1934, 7013	Nco I	2	905, 6554
ApaL I	1	87	Nde I	2	94, 4372
BamH I	**1**	**353**	NgoM IV	2	3070, 6986
Bbs I	3	4417, 4606, 5483	Not I	2	2, 631
Bcl I	1	5165	Nru I	2	1632, 7041
BfuA I	3	378, 3381, 7148	Nsp I	3	381, 1819, 6853
Bgl I	3	639, 2538, 6987	PaeR7 I	1	2380
Bgl II	2	2513, 4580	Pci I	1	6849
Blp I	1	3846	PflF I	1	4638
Bme1580 I	3	91, 743, 6339	PpuM I	2	1716, 7225
BmgB I	2	4404, 7164	Psi I	1	2489
Bmr I	3	268, 6385, 6514	PspOM I	1	6335
Bpu10 I	3	1434, 3294, 4489	Pst I	3	375, 3392, 4933
Bsa I	1	6177	Pvu I	2	188, 5240
BsaB I	2	7121, 7205	Sal I	3	365, 645, 7029
BsaH I	1	146	Sap I	2	3970, 5180
BseY I	2	5257, 6014	Sbf I	2	375, 3392
Bsm I	2	812, 1219	Sca I	1	793
BsmB I	3	982, 1535, 3309	SexA I	1	6967
BspE I	2	1210, 5134	Sfi I	1	639
BspLU11 I	1	6849	Sfo I	1	147
BspM I	3	378, 3381, 7148	SgrA I	2	4424, 5581
BsrB I	3	464, 1648, 2270	Sim I	2	4538, 7225
BsrG I	1	3147	Sma I	3	350, 639, 2860
BssH II	2	4831, 5375	SnaB I	1	4998
BssS I	3	4524, 6174, 6737	Spe I	1	6089
BstAP I	3	95, 1933, 7012	Sph I	1	381
BstE II	1	6971	Srf I	1	639
BstX I	1	4452	Sse8647 I	1	1716
BstZ17 I	1	1832	Stu I	1	2541
Bts I	2	558, 4926	Tat I	3	77, 791, 3147
Dra III	2	1933, 7190	Tli I	1	2380
Eag I	2	2, 631	Tth111 I	1	4638
Eco47 III	1	3933	Xba I	2	359, 2559
EcoN I	1	2836	Xho I	1	2380
EcoO109 I	2	1716, 7225	Xma I	3	348, 637, 2858
EcoR I	1	332			

Restriction Enzymes that cut pIndigoBAC-5 four or more times:

Acc I	Bsp1286 I	Eae I	Mae II	Rsa I
Aci I	BspH I	Ear I	Mae III	Sac I
Alu I	Bsr I	Fau I	Mbo I	Sau3A I
Alw I	BsrD I	Fnu4H I	Mbo II	Sau96 I
AlwN I	BsrF I	Gdi II	Mly I	ScrF I
Apo I	BssK I	Hae I	Mnl I	SfaN I
Ase I	BstDS I	Hae II	Mse I	Sfc I
Ava I	BstF5 I	Hae III	Msl I	Sml I
Ava II	BstN I	Hha I	Msp I	Ssp I
Ban I	BstU I	Hinc II	MspA1 I	Sty I
Ban II	BstY I	Hinf I	Mwo I	Taq I
Bfa I	Btg I	HinP I	Nci I	Tfi I
BsaA I	Cac8 I	Hpa II	Nla III	Tse I
BsaJ I	CviJ I	Hph I	Nla IV	Tsp45 I
BsaW I	Dde I	Hpy188 I	PflM I	Tsp4C I
BsiE I	Dpn I	Hpy99 I	Ple I	Tsp509 I
BsiHKA I	Dra I	HpyCH4 III	PshA I	TspR I
Bsl I	Drd I	HpyCH4 IV	PspG I	Xmn I
BsmA I	Dsa I	HpyCH4 V	Pvu II	

Restriction Enzymes that do not cut pIndigoBAC-5:

Aat II	BciV I	Bsu36 I	Nsi I	Sac II
Asc I	BfrB I	Cla I	Pac I	SanD I
AsiS I	BsiW I	Fse I	Pme I	Swa I
Avr II	BspD I	Mlu I	Pml I	Xcm I
BbvC I	BstB I	Nhe I	Rsr II	

5. References

1. Shizuya, H. California Institute of Technology, Pasadena, CA.
2. Shizuya, H. *et al.*, (1992) *Proc. Natl. Acad. Sci. USA* **89**, 8794.
3. Hurowitz, E.H. *et al.*, (2000) *DNA Research* **7** (2), 1.
4. Birren, B. *et al.*, (1999) Bacterial Artificial Chromosomes in *Genome Analysis: A Laboratory Manual, CSH Press*, New York, **v. 3**, 241.

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