



pIndigoBAC-5 Cloning-Ready Vectors are Extensively Tested for High Cloning Efficiency & Low Background

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pIndigoBAC-5 is the first commercially available Bacterial Artificial Chromosome (BAC) vector for cloning and preparation of primary BAC libraries. The vector is derived from pBeloBAC11 and pIndigoBAC.¹ pIndigoBAC-5 is completely cloning-ready. It has been linearized at either its unique *Bam*H I or its unique *Hind* III site and is then completely dephosphorylated. All preparations of EPICENTRE's pIndigoBAC-5 Cloning-Ready vectors are extensively tested to ensure successful and efficient BAC library production.

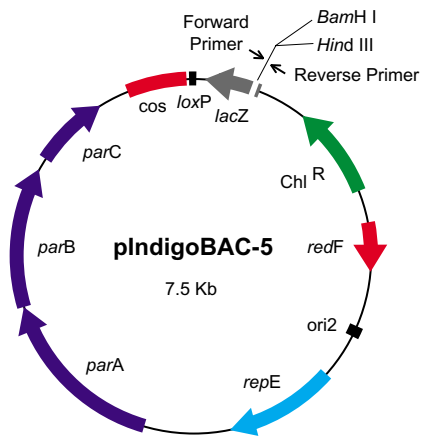


Figure 1. pIndigoBAC-5 Cloning-Ready vectors are provided linearized at either the *Bam*H I or *Hind* III site and completely dephosphorylated. pIndigoBAC-5 vectors feature enhanced blue/white screening of recombinants and very low backgrounds.

Cloning Efficiency

Greater than 10^6 recombinants per microgram of pIndigoBAC-5 DNA are observed when pIndigoBAC-5 (*Bam*H I-Cloning

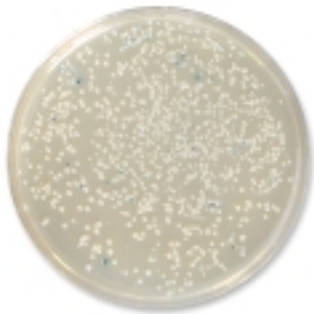


Figure 2. Cloning of *Hind* III-digested *E. coli* DNA into pIndigoBAC-5 (*Hind* III-Cloning Ready) vector produces a library containing >90% recombinants (white colonies) and > 10^6 clones/ μ g DNA. Less than 10% of the transformants are non-recombinant blue colonies.

Ready) or pIndigoBAC-5 (*Hind* III-Cloning Ready) vector is ligated with the appropriate control insert DNA and transformed into TransforMax™ EC100™ Electrocompetent *E. coli*. Greater than 90% of the recombinants contain an insert as analyzed using the Colony Fast-Screen™ Kit (p. 13).

No contaminating *E. coli* DNA

A major concern of BAC vector preparations is that they be free of contaminating *E. coli* chromosomal DNA fragments. Contaminating *E. coli* DNA competes with the genomic DNA fragments of interest during the ligation step and results in an unacceptably high number of clones containing *E. coli* DNA instead of the genomic DNA of interest. pIndigoBAC-5 Cloning-Ready preps are tested for the absence of contaminating *E. coli* DNA by ligating the vectors with and without added control insert DNA. pIndigoBAC-5 Cloning-Ready vector preparations produce 1000-fold more recombinant colonies when ligated with the control insert DNA. The EPICENTRE Quality Specification for this assay is that >90% of recombinants will contain inserts from the DNA of interest.

Complete Linearization

Unacceptably high backgrounds (observed as blue colonies on a plate) can result if the pIndigoBAC-5 is not completely linearized. When pIndigoBAC-5 Cloning-Ready vector preparations are used directly to transform TransforMax™ EC100™ cells, fewer than 5×10^4 blue colonies (containing non-linearized pIndigoBAC-5 DNA) per microgram of DNA are observed. With a cloning efficiency of > 10^6 colonies/ μ g, the background due to incomplete linearization of the pIndigoBAC-5 Cloning-Ready vector can be as low as 2%. The EPICENTRE Quality Specification for this test is that >90% of the transformants in a BAC library are recombinant (white) colonies.

Complete Dephosphorylation

Blue colonies can also result from incomplete dephosphorylation of the linearized pIndigoBAC-5 vector. When pIndigoBAC-5 Cloning-Ready vector preparations are treated with T4 DNA Ligase, without added insert DNA, and then used to

transform TransforMax EC100 cells, fewer than 5×10^4 blue colonies are observed per microgram of DNA. The EPICENTRE Quality Specification for this test is that >90% of the transformants in a BAC library are recombinant (white) colonies.

Integrity of the *Bam*H I and *Hind* III ends

Efficient ligation of genomic DNA requires that the *Bam*H I and *Hind* III cohesive ends of the pIndigoBAC-5 Cloning-Ready vectors are intact. To assay for the integrity of the cohesive ends, each batch of pIndigoBAC-5 Cloning-Ready vectors is 5'-phosphorylated using Polynucleotide Kinase and then self-ligated. Following transformation, >90% blue colonies are observed indicating that the *Bam*H I and *Hind* III ends of the vectors are fully intact.

References

- Birren, B., Mancino, V. and Shizuya, H., Bacterial Artificial Chromosomes in *Genome Analysis: A Laboratory Manual* v.3, 241-295, Cold Spring Harbor Press (1999).
- Hurowitz, E.H. et al., (2000) *DNA Research* 7:2,1.

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

BACB085H 500ng 25 ng/ μ l
Supplied linearized at *Bam* HI site and completely dephosphorylated.

pIndigoBAC-5 (*Hind* III-Cloning Ready)*

BACH095H 500ng 25 ng/ μ l
Supplied linearized at *Hind* III site and completely dephosphorylated.

BAC End-Sequencing Primers²

pIndigoBAC-5 Forward Sequencing Primer

BFP0701 1 nmole 50 μ M

pIndigoBAC-5 Reverse Sequencing Primer

BRP0801 1 nmole 50 μ M

TransforMax™ EC100™ Electrocompetent *E. coli*

Highest efficiency electrocompetent cells available. Ideal for preparing BAC libraries. See center insert for more information.

EC10005 5 X 100 μ l
(10 Electroporations)

EC10010 10 X 100 μ l
(20 Electroporations)

*pIndigoBAC-5 is exclusively licensed by EPICENTRE Technologies.