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-5 is completely cloning-ready. It has been linearized at either its unique BamHI 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.

Cloning Efficiency

Greater than 10^6 recombinants per microgram of pIndigoBAC-5 DNA are observed when pIndigoBAC-5 (BamHI I-Cloning Ready) or pIndigoBAC-5 (Hind III-Cloning Ready) vector is ligated with the appropriate control insert DNA and transformed into TransfornMax™ EC100™ Electropotent 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 preparations tested for the absence of contaminating E. coli DNA by ligating the vectors with and without added control insert DNA.

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 TransfornMax™ EC100™ cells, fewer than 5 X 10^4 blue colonies (containing non-linearized pIndigoBAC-5 DNA) per microgram of DNA are observed. With a cloning efficiency of >10^6 colonies/µg, 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 TransfornMax EC100 cells, fewer than 5 X 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.

References