



pNK2884 plasmid in *E. coli*

77340TM

Description

This construct is useful for general transposon mutagenesis in *Escherichia coli*. It contains the *ats1* *ats2* transposase gene that permits relaxed insertion specificity (altered target specificity, ATS). Expression is regulated by the Ptac promoter and inducible by IPTG. The transposase segment extends from IS10R to the EcoRI site at nt 3140 of Tn10, with a deletion of nucleotides 1329-1942 to remove the transposase binding site. It also contains a 1.4 kb mini-Tn10 cassette conferring chloramphenicol resistance, bounded by inverted repeats of the outermost 70 bp of IS10R and embedded in 40 bp of λcl terminating in HindIII sites. This cassette is inserted into the HindIII site (original nt 2272) of the ATS transposase-containing EcoRI fragment of pNK2881, ATCC 77351. Transcription from the Ptac promoter is in a direction opposite to that of amp. The pBR322 vector was modified by deleting bp 75-2352 and destroying (by filling in) the HindIII site. The order of major features in this plasmid is EcoRI ? Ptac ? ATS transposase ? mini-Tn10 cam ? EcoRI ? pMB ori ? ampR. - Methods Enzymol. 204: 139-180, 1991.

Organism: *Escherichia coli* (Migula) Castellani and Chalmers

Clone type: Clone

Shipping information: *Escherichia coli* containing the plasmid

Storage Conditions

Product format: Freeze-dried

Storage conditions: 2°C to 8°C

Intended Use

This product is intended for laboratory research use only. It is not intended for any



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animal or human therapeutic use, any human or animal consumption, or any diagnostic use.

BSL 1

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Certificate of Analysis

For batch-specific test results, refer to the applicable certificate of analysis that can be found at www.atcc.org.

Insert Information

Insert size (kb): 4

Insert information:

Gene: transposase, Tn10, ats1 ats2 (ATS)

Source: *Escherichia coli*

Insert ends: EcoRI

Target gene: transposase; Tn10; ats1 ats2 (ATS)

Gene product: transposase, Tn10, ats1 ats2 (ATS)

Insert end: EcoRI

Vector Information



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Construct size (kb): 6.1

Intact vector size: 2.1

Vector name: pBR322 derivative

Type of vector: plasmid

Vector end: EcoRI

Markers: cmlR; ampR

Growth Conditions

Medium:

ATCC Medium 1227: LB Medium (ATCC medium 1065) with 50 mcg/ml ampicillin

Temperature: 37°C

Handling Procedures

1. Open vial according to instructions.
2. Aseptically add 0.3 to 0.4 ml of liquid medium to the freeze-dried pellet and mix well. Transfer 100 uL to a test tube containing 5 mL LB + ampicillin (50-100 ug/ml). A loopful of culture can also be streaked on an agar plate of the same. Incubate cultures at 37°C.
3. Isolate DNA using standard plasmid preparation procedures.

Notes

Restriction digests of the clone gave the following sizes (in kb): HindIII 4.7, 1.5 ; EcoRI 2.5, 2.1, 1.7 ; PstI 6.2 -ATCC Staff

Material Citation

If use of this material results in a scientific publication, please cite the material in the following manner: pNK2884 plasmid in E. coli (ATCC 77340)



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References

References and other information relating to this material are available at www.atcc.org.

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