pET11c

5,675 base pairs
Sequence file available at www.neb.com
Not currently available from NEB.

Feature	Coordinates	Source
T7 cassette:		
T7 ϕ10 promoter	446-429	T7
transcription start	428 (ccw)	T7
lac operator	427-403	*E. coli*
translation start	358 (ccw)	T7
Tϕ transc. term.	316-194	T7
lacI	833-1915	*E. coli*
rop	3339-3530	pMB1
origin	4436-3848	pMB1
bla (Ap^R)	5467-4607	*Tn3*

ori = origin of replication, Ap = ampicillin
(cw) = clockwise, (ccw) = counterclockwise

There are no restriction sites for the following enzymes: AarI(x), Acc65I, AflII, AgeI, AleI, AscI, AsiSI, AvrII, BaeI, BbvCI, BmgBI, BseRI, BsiWI, BsrGI, BstBI, Bsu36I, CspCI, DraIII, FseI, I-CeuI, I-SceI, KpnI, MfeI, NcoI, NotI, NsiI, PI-PspI, PI-SceI, PacI, PaeR7I, PmeI, PmlI, PsiI, PspXI, RsrII, SacI, SacII, SalI, SanDI(x), SbfI, SexAI, SfiI, SmaI, SnaBI, SpeI, SrfI(x), StuI, SwaI, TliI, TspMI, XhoI, XmaI.

(x) = enzyme not available from NEB

The pET (**p**lasmid for **E**xpression by **T**7 RNA polymerase) vectors were created as a component of a system for protein expression in *E. coli*. These vectors employ a T7 phage promoter that is recognized by the phage T7 RNA polymerase but not by the host *E. coli* RNA polymerase (1,2). Production of the cloned protein occurs after expression of T7 RNA polymerase, initiated either through phage infection (ICE6) or induction of strains containing integrated copies of the polymerase gene [e.g., BL21(DE3)] (2).

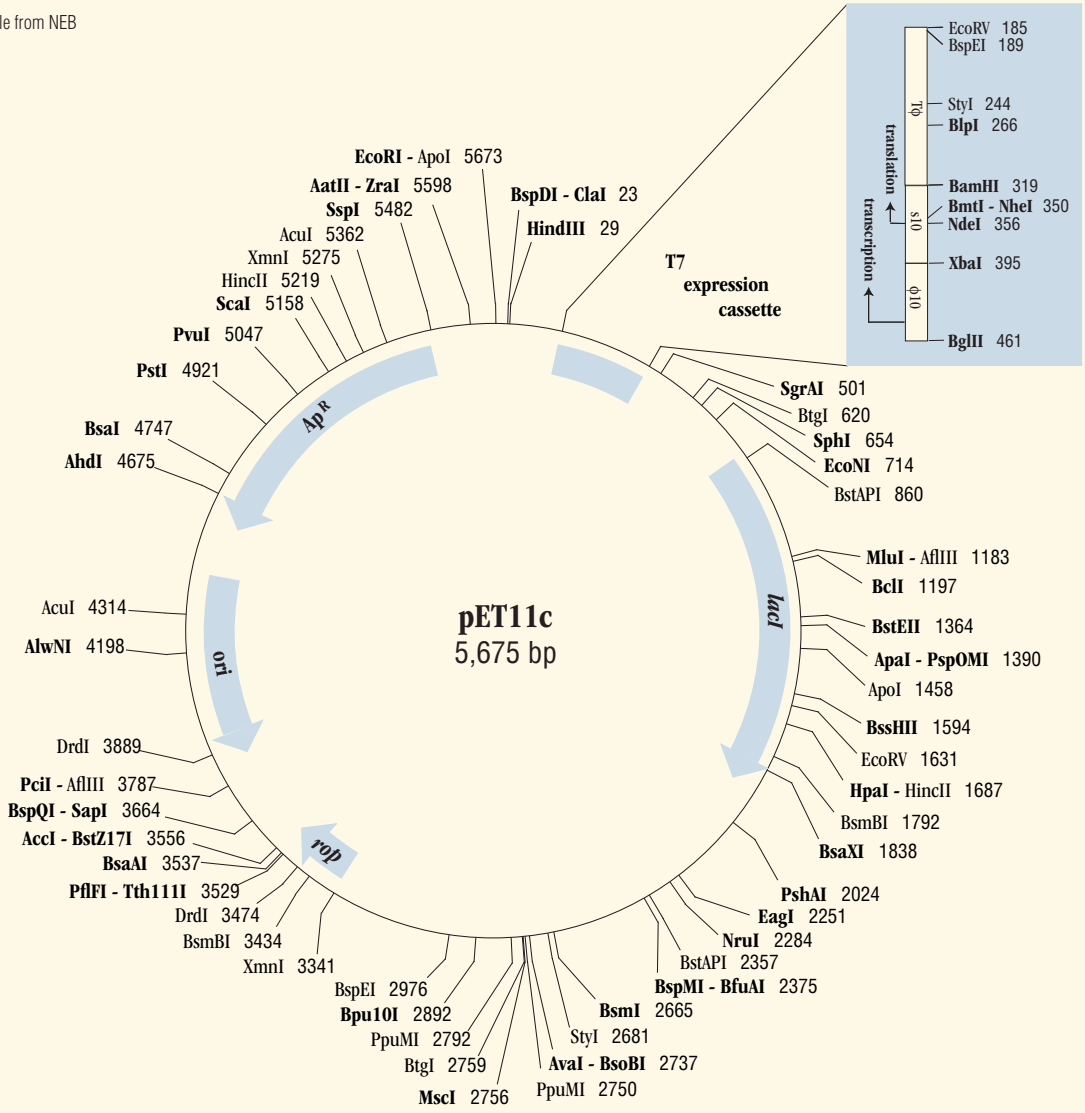
All plasmids in the pET series were derived from pBR322 and contain the pMB1 origin of replication. Transcription from the T7 promoter (ϕ10) is in the counterclockwise direction. Plasmids pET1-12 carry the ampicillin resistance gene, except for the pET9 series which carry that for kanamycin resistance. Vectors lacking a letter suffix are transcription vectors, and lack translation initiation signals. Vectors with suffixes a, b, c or d contain translation initiation signals (Shine Dalgarno sequence) for the strongly expressed T7 gene 10 protein (s10) (3). These translation vectors contain either an NdeI (a, b, c) or Nco I site (d) overlapping the initiating methionine codon. Vectors a, b and c differ in the spacing to a downstream BamHI site, allowing fusions to be made to the first 11 codons of the gene 10 protein in all three reading frames.

pET11c is a translation vector that contains the lac operator immediately downstream of the T7 promoter (4). This vector has the lowest basal level of expression of the pET series, a consequence of lac repressor binding to the operator. Despite this basal repression, induced expression is similar to constructs lacking the lac operator. The lac repressor is encoded by the *lacI* gene carried on pET11c.

Enzymes with unique restriction sites are shown in **bold** type and enzymes with two restriction sites are shown in regular type. Restriction site coordinates refer to the position of the 5´-most base on the top strand in each recognition sequence.

Open reading frame (ORF) coordinates are in the form "translational start – translational stop"; numbers refer to positions on the top (clockwise) strand, regardless of the direction of transcription and include the start and stop codons.

Origin of replication coordinates include the region from the -35 promoter sequence of the RNAII transcript to the RNA/DNA switch point. *bla* (Ap^R) gene coordinates include the signal sequence.



References

1. Studier, F.W., Rosenberg, A.H., Dunn, J.J. and Dubendorff, J.W. (1990) *Methods Enzymol.* 185, 60–89.
2. Studier, F.W. and Moffatt, B.A. (1986) *J. Mol. Biol.* 189, 113–130.
3. Rosenberg, A.H., Lade, B.N., Chui, D.-S., Lin, S.-W., Dunn, J.J. and Studier, F.W. (1987) *Gene* 56, 125–135.
4. Dubendorff, J.W. and Studier, F.W. (1991) *J. Mol. Biol.* 219, 45–59.