

# pMiniT 2.0

Sequence available at [www.neb.com](http://www.neb.com)  
See page 97 for more information.

Feature	Coordinates	Source
Constitutive promoter	1-214	pNK2138
SP6 promoter	479-496	SP6
Toxic minigene	541-549	—
Synthetic T7 promoter	619-602	T7
<i>bla</i> ( <i>Ap</i> <sup>R</sup> )	733-1593	<i>Tn3</i>
origin	1764-2352	pUC19

There are no restriction sites for the following enzymes: AbsI(x), Acc65I, AccI, AflII, AgeI, Ajul(x), AleI, Alol(x), Apal, Arsl(x), Ascl, AsI, AslI, Avrl, Bael, BanII, Barl(x), BbsI, BbvCI, BclI, BgIII, BplI(x), BmgBI, BmtI, BplI(x), Bpu10I, Bsal, BsaAI, BsaBI, BseRI, BsgI, BsiWI, BsmFI, BsmI, BspDI, BspEI, BsrGI, BssHII, BstAPI, BstBI, BstEI, BstXI, BstZ17I, BsU36I, BtgI, Clal, CspCI, DrallI, Eco53KI, EcoNI, EcoO109I, EcoRV, Fall(x), Fsel, FspAI(x), HinclI, HindIII, Hpal, Kasi, Kfil(x), KpnI, MauBII(x), MfeI, MluI, MreI(x), Mscl, Mtel(x), Nael, NarI, NcoI, NgoMIV, NheI, NsiI, PstI(x), PflI, PIMI, Prol(x), PluTI, PmlI, PpuMI, PshAI, PsiI, PspOMI, Psrl(x), Pvull, RsrI, SacI, SacII, Sall, SexAI, SfiI, SfoI, SgrAI, SgrDI(x), Smal, SnaBI, Spel, SpHI, SrfI, StuI, StyI, Swal, TspMI, Tth111I, XbaI, XcmI, XmaI

(x) = enzyme not available from NEB

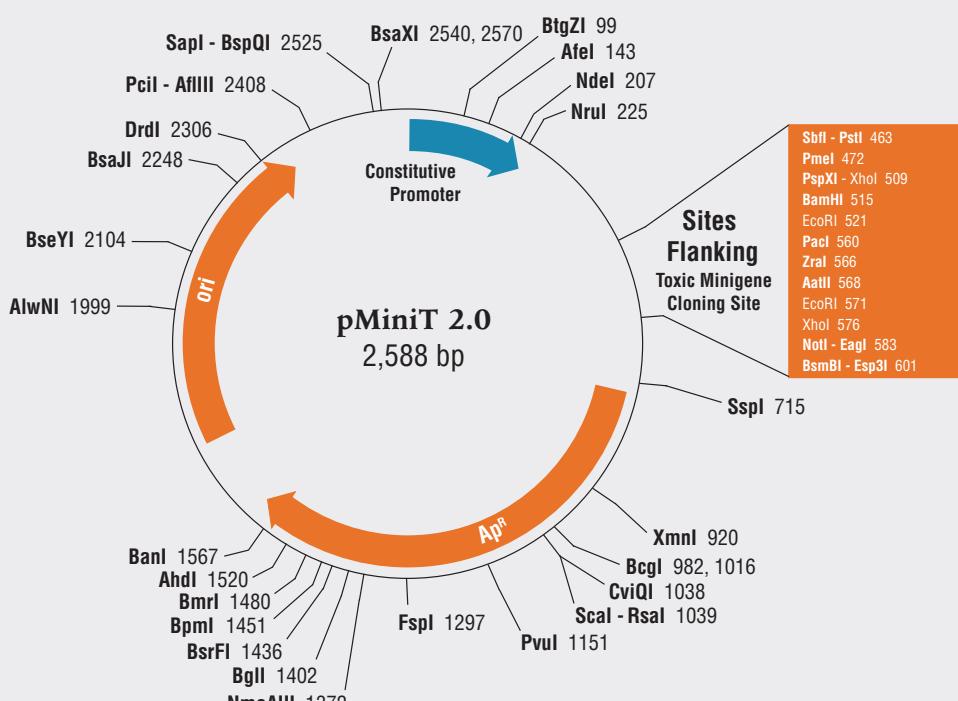


We recommend NEBCutter at [NEBCutter.com](http://NEBCutter.com) to identify the restriction sites within your DNA sequence. NEBCutter indicates cut frequency and methylation-state sensitivity.

pMiniT 2.0 is an *E. coli*/plasmid cloning vector designed for cloning blunt-ended or single-base overhang PCR products, or amplicons, using the NEB PCR Cloning Kit (NEB #E1202, #E1203). The pMiniT 2.0 also enables *in vitro* transcription using SP6 and T7 promoters. It is compatible with Golden Gate Assembly as the BsaI site has been removed from the Ampicillin resistance gene.

In *E. coli*, it replicates using the pMB1 origin of replication from pUC19 and carries the *bla* (*Ap*<sup>R</sup>) marker for selection with ampicillin. pMiniT 2.0 contains a toxic minigene that is under the control of a constitutive promoter. If the pMiniT 2.0 vector recircularizes without an insert, the toxic minigene will cause lethal inhibition of protein synthesis and no colony will result. If the pMiniT 2.0 Vector carries an insert, a colony will grow.

The map shown below displays the construct formed if no insert is present. Unique restriction sites are shown in **bold**. Additional restriction sites that can be used for subcloning are also shown. Expanded box below shows location of sequencing primers, restriction sites for subcloning or linearization for *in vitro* transcription, RNA Polymerase promoter sequences and placement of insertion site within the toxic minigene. **Coordinates indicate position of cutsites on the top strand. In previous catalogs, coordinates referred to the position of the 5' most base on the top strand, please make note of new numbering system.**

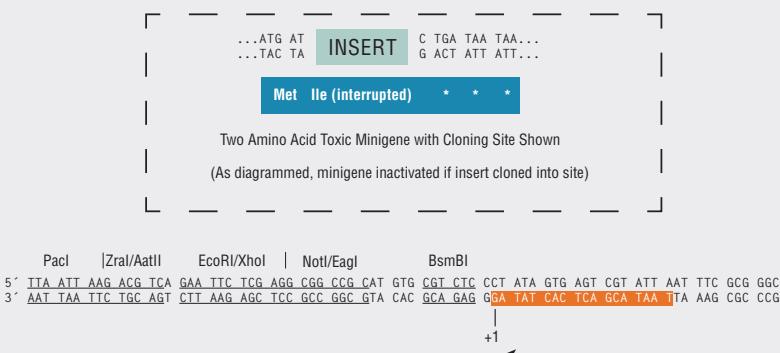


## Features within Sequence Flanking the Toxic Minigene/Cloning Site:

Cloning Analysis Forward Primer →

5' ACC TGC CAA CCA AAC CGA GAA CAA AAC ATA ACA TCA AAC GAA TCG ACC GAT TGT TAG GTA ATC GTC ACC TGC AGG AAG GTT  
3' TGG ACG GTT GGT TTC GCT CTT GTT TTG TAT TGT AGT TTG CTT AGC TGG CTA ACA ATC CAT TAG CAG TGG AGC TCC TTC CAA

+1  
Pmel      SP6 Promoter      ← |  
5' TAA ACG CAT TTA GGT GAC ACT ATA GAA GTG TGT ATC GCT CGA GGG ATC CGA ATT CAG GAG GTA AAA ACC  
3' ATT TGC GTA ATT CCA CTG TGA TAT CTT CAC ACA TAG CGA GCT CCC TAG GCT TAA GTC CTC CAT TTT TGG



+1  
← T7 Promoter  
Cloning Analysis Reverse Primer

5' GGA ACC CCT ATT TGT TTA TTT TTC TAA ATA CAT TCA AAT ATG TAT CCG CTC ATG AGA CAA TAA CCC TGA 3'  
3' CCT TGG GGA TAA ACA AAT AAA AAG ATT TAT GTA AGT TTA TAC ATA GGC GAG TAC TCT GTT ATT GGG ACT 5'