LITMUS 28i

There are no restriction sites for the following enzymes: AarI(x), AccI, AfeI, AflII, AleI, ApaI, AscI, AsiSI, BbsI, BbvCI, BclI, BfuAI, BlpI, BmgBI, BmtI, Bpu10I, BsaBI, BseRI, BsgI, BsmFI, BspDI, BspEI, BspMI, BspQI, BsrGI, BstAPI, BstEII, BstXI, BstZ17I, Bsu36I, ClaI, CspCI, EagI, EcoNI, EcoO109I, FseI, FspAI(x), I-CeuI, I-SceI, KasI, MfeI, MluI, MscI, NaeI, NarI, NdeI, NgoMIV, NheI, NotI, NruI, PI-PspI, PI-SceI, PacI, PflFI, PflMI, PmeI, PmlI, PpuMI, PshAI, PspOMI, PspXI, RsrII, SacII, SalI, SanDI(x), SapI, SexAI, SfiI, SfoI, SgrAI, SmaI, SpeI, SphI, SrfI(x), TfiI, TspMI, Tth111I, XcmI, XmaI, XmnI

(x) = enzyme not available from NEB

LITMUS 28i is a small, high copy number *E. coli* plasmid vector designed for efficient transcription of double-stranded RNA using the HiScribe T7 *In Vitro* Transcription Kit (NEB #E2030). It contains the origin of replication from pUC19 and is maintained at a similar copy number to pUC19. In addition, it contains an ampicillin selectable marker, an M13 origin of replication, and wild type T7 promoters flanking the multiple cloning site (MCS) (1).

LITMUS 38i (next page) is identical to LITMUS 28i except for the MCS region between the SnaBI and StuI sites. The MCS of both vectors is in frame with the *lacZ*α gene, allowing screening for insertions using α-complementation (blue/white).

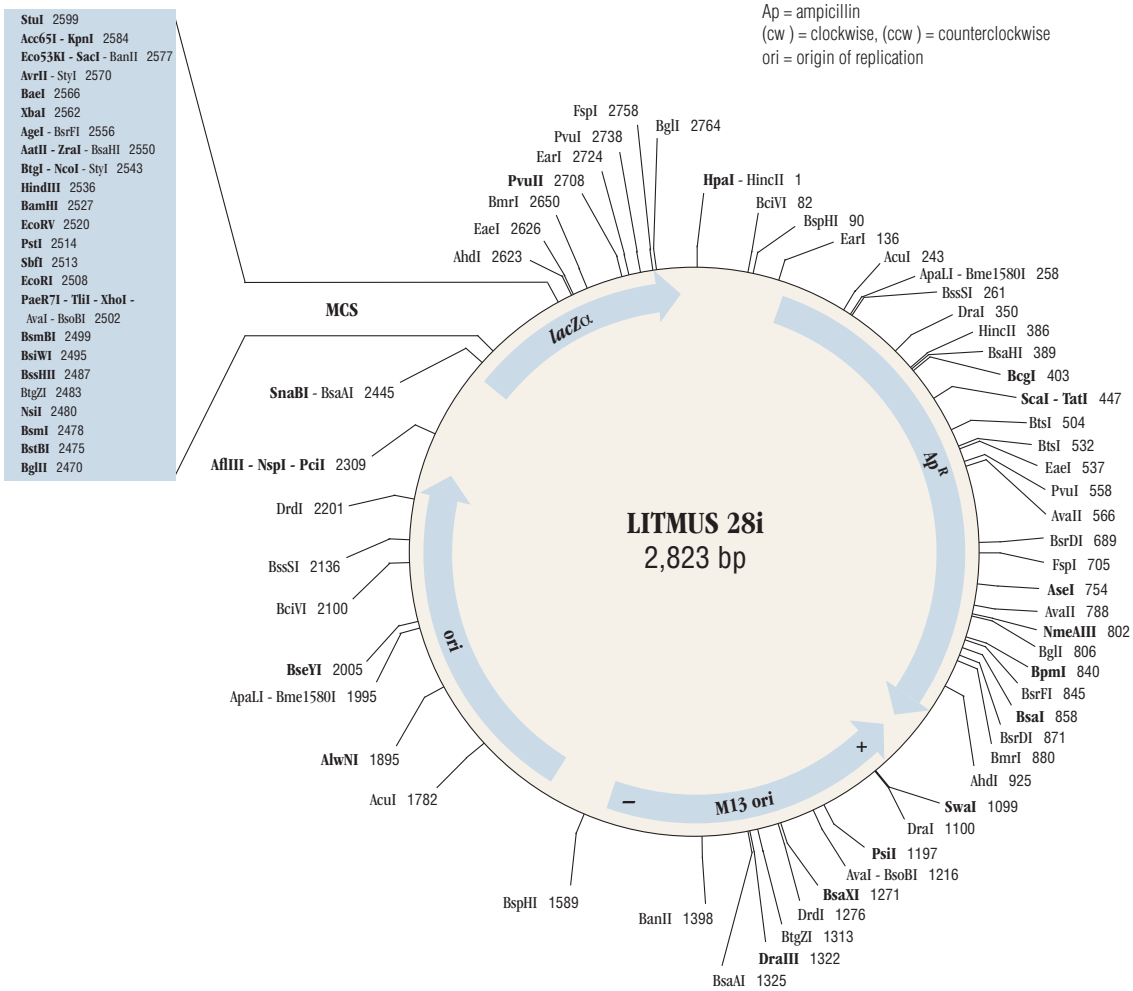
Enzymes with unique restriction sites are shown in **bold** type and enzymes with two restriction sites are shown in regular type. Location of sites of all NEB restriction enzymes can be found on the NEB web site (choose Technical Reference > DNA Sequences and Maps). Restriction site coordinates refer 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.

pUC19 origin of replication coordinates include the region from the -35 promoter sequence of the RNAII transcript to the RNA/DNA switch point. For the M13 origin, the arrow shows the direction of synthesis of the (+) strand, which gets packaged into phage particles. *bla* (Ap^R) gene coordinates include the signal sequence.

Feature	Coordinates	Source
bla (Ap^R)	143-1003	*Tn3*
M13 origin	1045-1554	M13
origin	1665-2253	pUC19
T7 promoter	2449-2466	T7
transc. start	2466 (cw)	–
MCS	2470-2589	–
T7 promoter	2620-2603	T7
transc. start	2603 (ccw)	–
*lacZ*α	2424-2795	–

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

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                T7 promoter
                ------------>
                                  BstBI                 BsmBI        SbfI
      SnaBI                     BglII     NsiI   BssHII  BsiWI  XhoI  EcoRI PstI  EcoRV  BamHI
...AGCTACGTAATACGACTCACTATAGGGCAGATCTTCGAATGCATCGCGCGCACCGTACGTCTCGAGGAATTCCTGCAGGATATCTGGATCCA
                        .    +1   .         .         .         .         .         .         .
                      2460        2480      2500      2520

          NcoI
  HindIII       AatII AgeI  XbaI    AvrII  SacI   KpnI          StuI
CGAAGCTTCCCATGGTGACGTCACCGGTTCTAGATACCTAGGTGAGCTCTGGTACCCTCTAGTCAAGGCCTATAGTGAGTCGTATTACGGACTG...
    .         .         .         .         .         .         .  +1    .         .
  2540      2560      2580      2600
                                                                  <------------
                                                                  T7 promoter
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Reference

(1) Evans, P.D. et al. (1995) *Biotechniques,* 19, 130–135.