LacZα initiation codon

M13 Reverse priming site

T3 priming site

```
201  CACACAGGAA ACAGCTATGA CCATGATTAC GCCAAGCTCA GAATTAACCC TCACTAAAGG
     GTGTGTCCTT TGTCGATACT GGTACTAATG CGGTTCGAGT CTTAATTGGG AGTGATTTCC
```

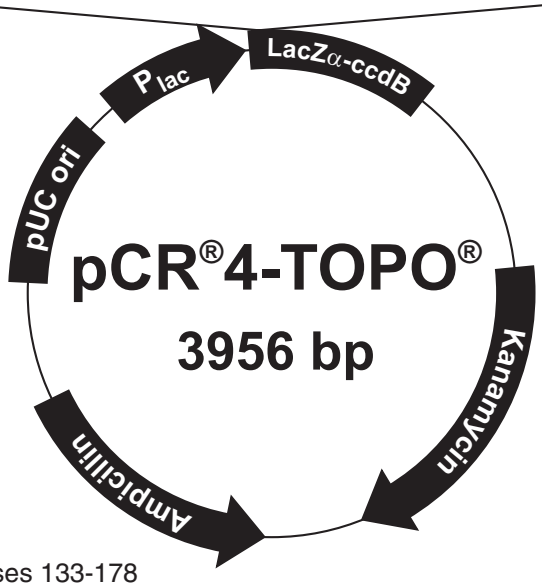
Spe I *Pst* I *Pme* I *Eco*R I *Eco*R I *Not* I

```
261  GACTAGTCCT GCAGGTTTAA ACGAATTCGC CCTT[PCR Product]AAGGGC GAATTCGCGG
     CTGATCAGGA CGTCCAAATT TGCTTAAGCG GGAA[PCR Product]TTCCCG CTTAAGCGCC
```

T7 priming site

M13 Forward (-20) priming site

```
311  CCGCTAAATT CAATTCGCCC TATAGTGAGT CGTATTACAA TTCACTGGCC GTCGTTTTAC
     GGCGATTTAA GTTAAGCGGG ATATCACTCA GCATAATGTT AAGTGACCGG CAGCAAAATG
```



Comments for pCR®4-TOPO®
3956 nucleotides

lac promoter region: bases 2-216
- CAP binding site: bases 95-132
- RNA polymerase binding site: bases 133-178
- Lac repressor binding site: bases 179-199

Start of transcription: base 179
M13 Reverse priming site: bases 205-221
LacZα-*ccdB* gene fusion: bases 217-810
- LacZα portion of fusion: bases 217-497
- *ccd*B portion of fusion: bases 508-810

T3 priming site: bases 243-262
TOPO® Cloning site: bases 294-295
T7 priming site: bases 328-347
M13 Forward (-20) priming site: bases 355-370
Kanamycin promoter: bases 1021-1070
Kanamycin resistance gene: bases 1159-1953
Ampicillin (*bla*) resistance gene: bases 2203-3063 (c)
Ampicillin (*bla*) promoter: bases 3064-3160 (c)
pUC origin: bases 3161-3834

(c) = complementary strand

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