

M13 Reverse Primer *lacZα*ATG

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CAG GAA ACA GCT ATG ACC ATG ATT ACG CCA AGC TTG GTA CCG AGC TCG GAT CCA CTA GTA ACG GCC
GTC CTT TGT CGA TAC TGG TAC TAA TGC GGT TCG AAC CAT GGC TCG AGC CTA GGT GAT CAT TGC CCG

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          BstXI EcoRI                      Bpi I                      Bpi I
          |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |
GCC AGT GTG CTG GAA TTC GCC CTT ATT GAA GAC TTA AGC << YOUR GENE >> ACC TTT GTC TTC AAT
CGG TCA CAC GAC CTT AAG CGG GAA TAA CTT CTG AAT TCG << YOUR GENE >> TGG AAA CAG AAG TTA

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          EcoRI          EcoRV          BstXI          NotI          XhoI          NsiI XbaI          ApaI
          |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |
AAG GGC GAA TTC TGC AGA TAT CCA TCA CAC TGG CGG CCG CTC GAG CAT GCA TCT AGA GGG CCC AAT
TTC CCG CTT AAG ACG TCT ATA GGT AGT GTG ACC GCC GGC GAG CTC GTA CGT AGA TCT CCC GGG TTA

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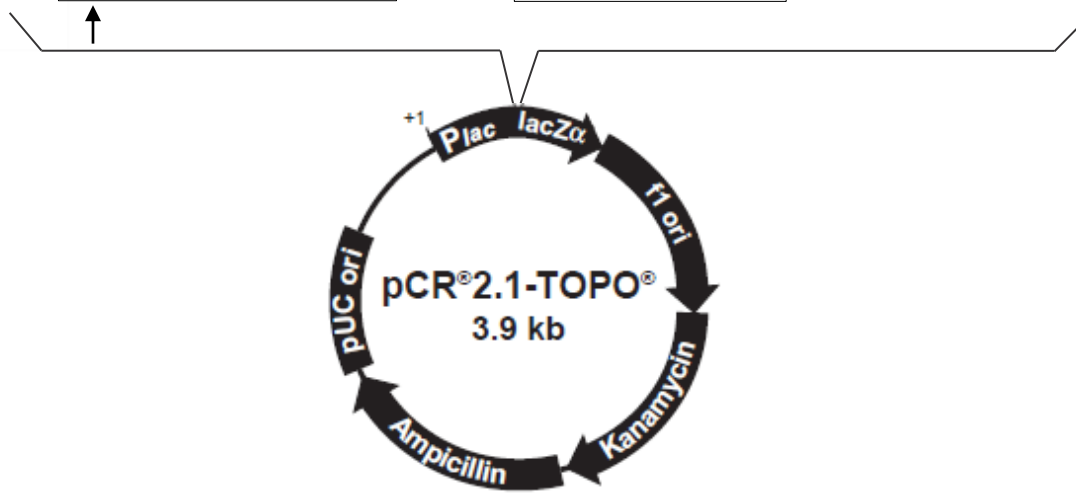
T7 Promoter

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TCG CCC TAT AGT GAG TCG TAT TAC AAT TCA CTG GCC GTC GTT TTA CAA CGT CGT GAC TGG GAA AAC
AGC GGG ATA TCA CTC AGC ATA ATG TTA AGT GAC CGG CAG CAA AAT GTT GCA GCA CTG ACC CTT TTG

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M13 Forward (-20) Primer



**Comments for pCR<sup>®</sup>2.1-TOPO<sup>®</sup>**  
**3931 nucleotides**

- LacZ* fragment: bases 1-547
- M13 reverse priming site: bases 205-221
- Multiple cloning site: bases 234-357
- T7 promoter/priming site: bases 364-383
- M13 Forward (-20) priming site: bases 391-406
- f1 origin: bases 548-985
- Kanamycin resistance ORF: bases 1319-2113
- Ampicillin resistance ORF: bases 2131-2991
- pUC origin: bases 3136-3809