pN-Z-TK$_2$ 2-step, nlacZ targeting vector

Polylinker

3'- CGAACCATGGGCCCCTA
ATCGATAAGCTAGCTTGGTACCCGGGGAT
ClaI **NheI*** **KpnI*** BamHI

GGAGAT-5'
CCTCTAGAGTCGACGGATCCGGGGAATTC
XbaI **SalI*** BamH1 EcoRI

CCCAGTCTCAGGATCCACC**ATG**G
BamH1 Nco1

* Note: AvrII, XbaI, and SpeI have ends that are cohesive with NheI
Asp718 and Acc65 also recognize this sequence;
BsiW1 & BsrG1 are cohesive with Acc65
XhoI is cohesive with SalI

The ATG of the NcoI site is the start of nuclear LacZ

An anti-sense PCR primer (~ 70° annealing temperature) is shown in italics #582

Suggestions: Clone promoter fragment of convenient PCR length (~1 kb) into polylinker in correct orientation. Clone largest piece possible into Xho, Spe, Not sites. Plan to linearize construct before electroporation with SstII or AscI (if your DNA has both of these sites, convert them to something unique first). If you don't want the lacZ gene, clone one piece of homologous DNA between NheI, KpnI, SalI and HindIII sites.

All restriction sites shown on the map are unique