

**pKCC was obtained from pECFP-N1 (Clontech #6900-1) after insertion of a new mutiple cloning site in NheI-NotI and mutation of the 3' end of Xba I site to prevent methylation (T<sub>690</sub>→A, see \* in MCS below)**

- Human cytomegalovirus (CMV) immediate early promoter: 1–589  
Enhancer region: 59–465  
TATA box: 554–560  
Transcription start point: 583  
C→G mutation to remove *Sac* I site: 569
- MCS: 591–689
- SV40 early mRNA polyadenylation signal  
Polyadenylation signals: 825-830 & 854-859  
mRNA 3' ends: 863 & 875
- f1 single-strand DNA origin: 922-1377
- Bacterial promoter for expression of Kan<sup>r</sup> gene:  
–35 region: 1439-1444; –10 region: 1462-1467  
Transcription start point: 1474
- SV40 origin of replication: 1517-1853
- SV40 early promoter  
Enhancer (72-bp tandem repeats): 1551-1622 & 1623-1694  
21-bp repeats: 1698-1718, 1719-1739 & 1741-1761  
Early promoter element: 1774-1780  
Major transcription start points: 1770, 1808, 1814 & 1819
- Kanamycin/neomycin resistance gene  
Neomycin phosphotransferase coding sequence: 1902-2696  
G→A mutation to remove *Pst* I site: 2084  
C→A (Arg to Ser) mutation to remove *Bss*H II site: 2430
- Herpes simplex virus (HSV) thymidine kinase (TK) polyadenylation signal  
Polyadenylation signals: 2932-2937 & 2945-2950
- pUC plasmid replication origin: 3281-3924

