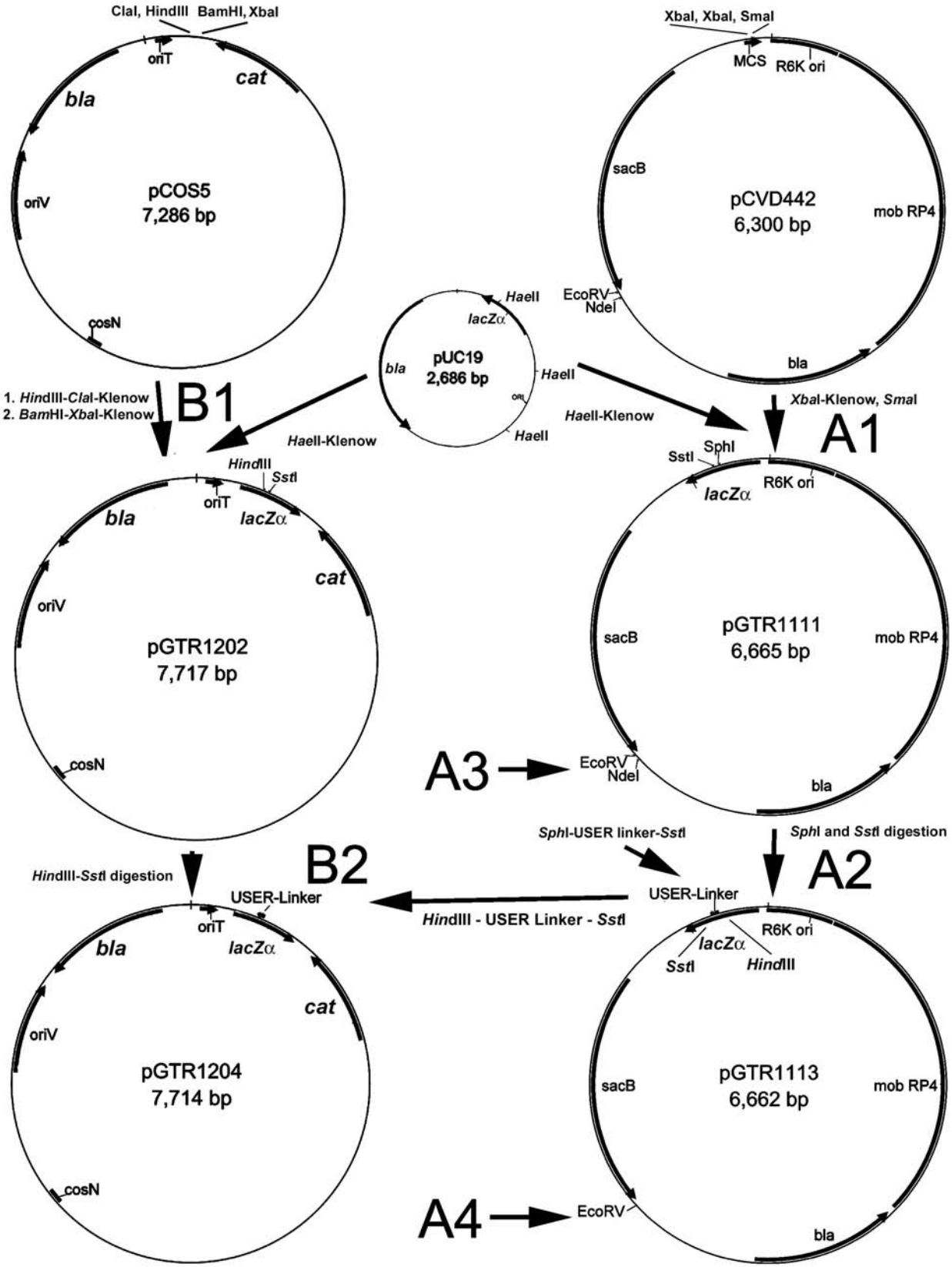
Supplemental Figure 1. Construction of pCVD442 and pCOS5 plasmid derivatives for USER friendly cloning. pCVD442-derived plasmids: Step A1 – the *lacZ*α gene was removed from pUC19 on a *Hae*II restriction enzyme fragment, made blunt-ended with the Klenow fragment of DNA polymerase I, and cloned into pCVD442 that had been digested with *Xba*I (made blunt-ended with the Klenow fragment of DNA polymerase I) and *Sma*I, yielding pGTR1111. Step A2 – The *lacZ*α gene was opened with *Sph*I and *SstI*, and a double stranded oligonucleotide with the USER friendly cloning sequences from pNEB206A with the same overhangs was inserted, yielding pGTR1113. Steps A3 and A4 – *aph*, *cat*, and *tetAR* gene cassettes were cloned into pGTR1111 and pGTR1113 at either *Eco*RV or *Nde*I made blunt-ended with Klenow. pCos5-derived plasmids: Step B1 - the *lacZ*α gene was removed from pUC19 on a *Hae*II restriction enzyme fragment, made blunt-ended with the Klenow fragment of DNA polymerase I, and cloned into pCOS5 that had been digested with *Hin*dIII and *Cla*I, made blunt-ended with Klenow and ligated (named pGTR1200, not shown) then digested with *Xba*I and *Bam*HI made blunt-ended with Klenow, yielding pGTR1202. Step B2 – The pNEB206A USER friendly cloning acceptor sequences were removed from pGTR1113 with *Sst*I and *Hin*dIII and cloned into pGTR1202 that had been digested with the same enzymes, yielding pGTR1204. Not shown is creation of pGTR1160, which is pRK437 with the USER friendly cloning acceptor site created using a double stranded oligonucleotide with *Bam*HI and *Hin*dIII ends inserted into the *lacZ*α gene that had been digested with *Bam*HI and *Hin*dIII.


ClaI, HindIII BamHI, XbaI
oriT
cat
bla
oriV
pCOS5
7,286 bp
cosN
XbaI, XbaI, SmaI
MCS
R6K ori
sacB
pCVD442
6,300 bp
mob RP4
EcoRV
NdeI
bla
HaeII
lacZα
pUC19
2,686 bp
ori
1. HindIII-ClaI-Klenow
2. BamHI-XbaI-Klenow
B1
HaeII-Klenow
HaeII-Klenow
XbaI-Klenow, SmaI
A1
HindIII
SstI
SstI
SphI
R6K ori
lacZα
pGTR1202
7,717 bp
pGTR1111
6,665 bp
A3
EcoRV
NdeI
SphI-USER linker-SstI
SphI and SstI digestion
HindIII-SstI digestion
B2
USER-Linker
A2
USER-Linker
HindIII - USER Linker - SstI
SstI
HindIII
pGTR1204
7,714 bp
pGTR1113
6,662 bp
A4
EcoRV