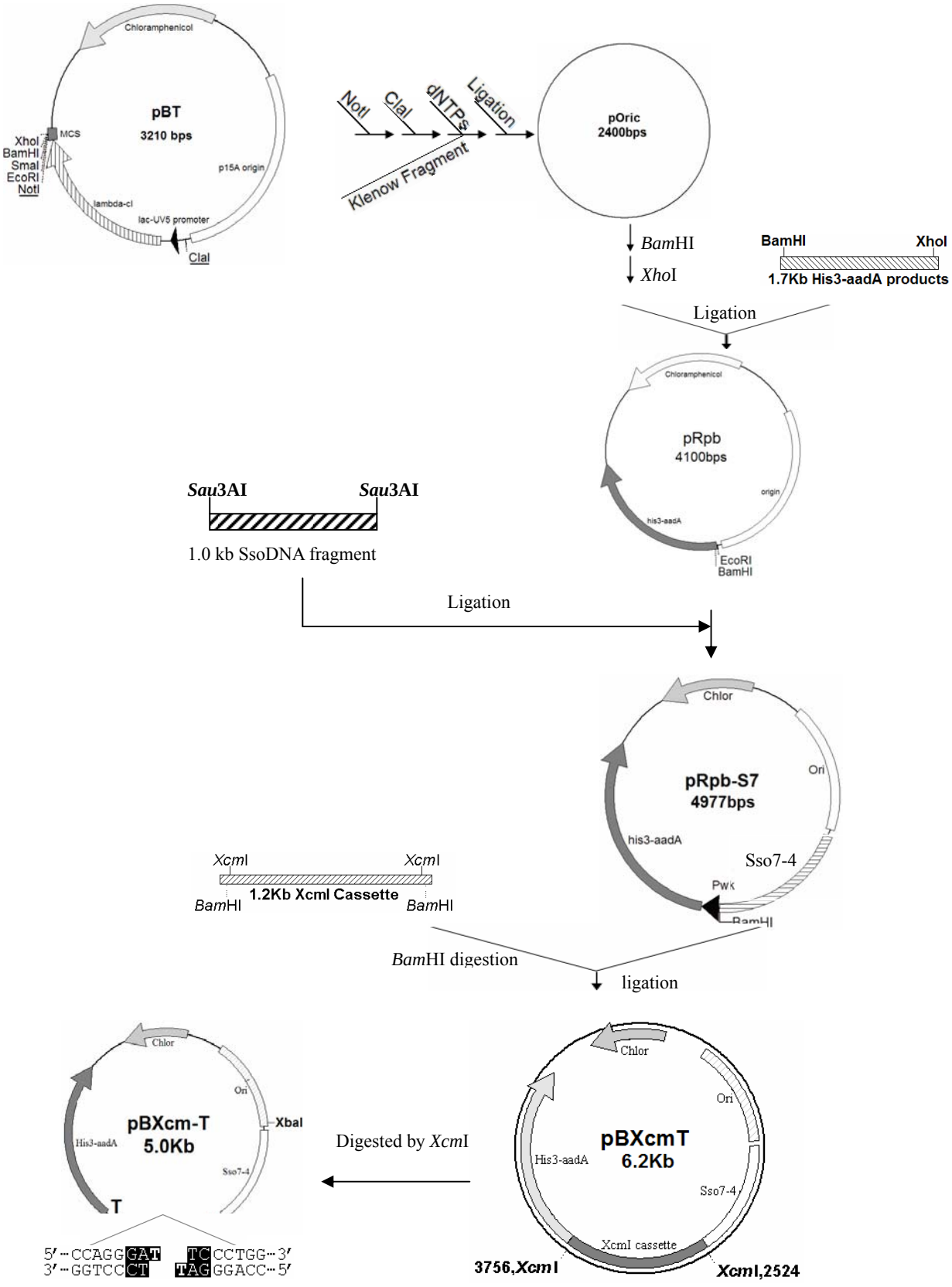
Figure 1


Chloramphenicol
pBT
3210 bps
MCS
XhoI
BamHI
SmaI
EcoRI
NotI
p15A origin
lambda-cI
lac-UV5 promoter
ClaI
NotI
ClaI
dNTPs
Ligation
Klenow Fragment
pOric
2400bps
BamHI
XhoI
BamHI
XhoI
1.7Kb His3-aadA products
Ligation
Chloramphenicol
pRpb
4100bps
origin
his3-aadA
EcoRI
BamHI
Sau3AI
Sau3AI
1.0 kb SsoDNA fragment
Ligation
Chlor
Ori
pRpb-S7
4977bps
his3-aadA
Sso7-4
PWK
BamHI
XcmI
XcmI
BamHI
1.2Kb XcmI Cassette
BamHI
BamHI digestion
ligation
Chlor
Ori
pBXcm-T
5.0Kb
His3-aadA
XbaI
Sso7-4
T
5′···CCAGGGAT
3′···GGTCCCT
TC CCTGG···3′
TAG GGACC···5′
Digested by XcmI
Chlor
Ori
pBXcmT
6.2Kb
His3-aadA
Sso7-4
XcmI cassette
3756,XcmI
XcmI,2524

A routine of preparation of the *Xcm*I cassette and plasmid pBXcmT. The reporter vector pBXcmT was derived from the bait plasmid pBT of BacterioMatch@ II two-hybrid system (Stratagene). For developing a new bacterial one-hybrid (B1H) reporter vector, an approximately 1.7 kb DNA fragment containing the *His3-aadA* reporter cassette was cloned from the BacterioMatch@ II two-hybrid reporter system (Stratagene) using a pair of unique primers (Suppl. Table S1). This reporter cassette was further used to replace λcI and lac-UV5 promoters from pBT to produce a preliminary vector named pRpb. In order to restrain the self-activation and reduce screening background, a mediator DNA fragment was successfully screened from the genomic library of the archaeon *S. solfataricus* and the fragment was inserted into the right upstream of the *His3-aadA* reporter cassette. After further modifications, a derivative reporter vector of pRpb-S7 was produced, in which a BamHI restriction enzyme site was integrated into the upstream of the His3-aadA reporter cassette. For the convenient and rapid cloning of the short promoter DNA fragment into the one-hybrid system, we further engineered a 1.2 kb MCM△ segment containing two *Xcm*I sites in both its termini, the *Xcm*I Cassette. The cassette was derived from the mini-chromosome maintainance gene (MCM) of the archaeon *S. solfataricus* which was amplified by using a pair of specific primers containing the *XcmI* site (Suppl. Table 1). The *Xcm*I Cassette was inserted into *Bam*HI-digested pRpb-S7 to produce a plasmid pBXcmT. When digested with *Xcm*I, the recombinant plasmid pBXcmT resulted in a vector with a single deoxythymidine (dT) overhang at its 3'-end. This linearized T-vector could then be used for the rapid cloning of PCR products.