Figure 1

BamHI digestion

Ligation

Sau3AI

1.0 kb SsoDNA fragment

Ligation

BamHI digestion

BamHI

XhoI

1.7 Kb His3-aadA products

Sau3AI

Sso7-4

4977bps

XcmI

1.2Kb XcmI Cassette

BamHI

pRpb-S7

5.0Kb

Digested by XcmI

pBXcm-T

6.2Kb

3756, XcmI

XcmI, 2524

Digested by XcmI
A routine of preparation of the XcmI 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 λcl 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 MCMΔ segment containing two XcmI sites in both its termini, the XcmI Cassette. The cassette was derived from the mini-chromosome maintenance 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 XcmI Cassette was inserted into BamHI-digested pRpb-S7 to produce a plasmid pBXcmT. When digested with XcmI, 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.