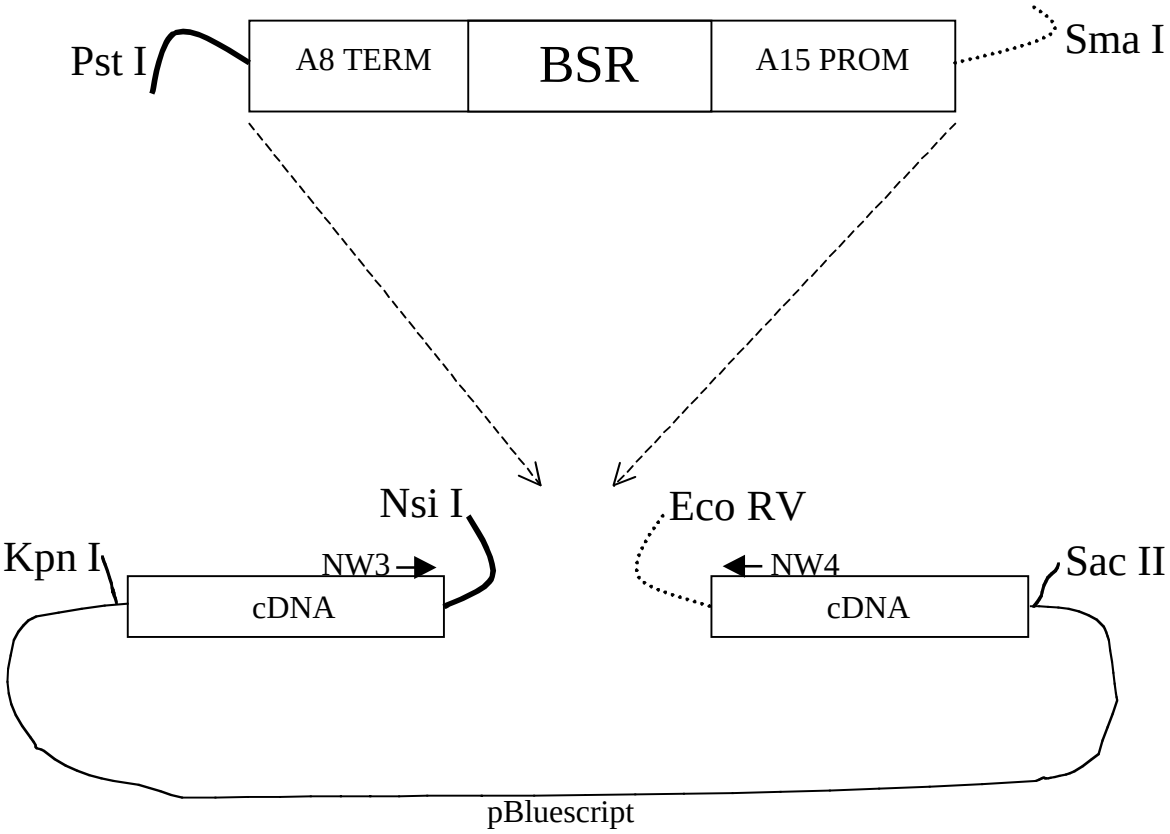
Pst I
A8 TERM
BSR
A15 PROM
Sma I
Nsi I
Eco RV
Kpn I
NW3
NW4
Sac II
cDNA
cDNA
pBluescript

Figure 17. Generation of TF2 Knock-out Construct.

The blasticidin resistance gene (BSR) was digested out from the pBSR19 plasmid with *Pst* I and *Sma* I and cloned into the SSL494 vector cleaved with *Nsi* I and *Eco* RV. This positioned the BSR gene within the TF2 cDNA sequence in SSL494. Compatibility between restriction enzyme ends is represented schematically (bold line for *Pst* I and *Nsi* I and dotted line for *Sma* I and *Eco* RV). The BSR plus cDNA cassette was cut out with *Kpn* I and *Sac* II and used for transformation of *Dictyostelium* cells by electroporation. Following transformation, clones were screened by PCR to identify desired clones (see Fig. 18).