

Supplemental Materials

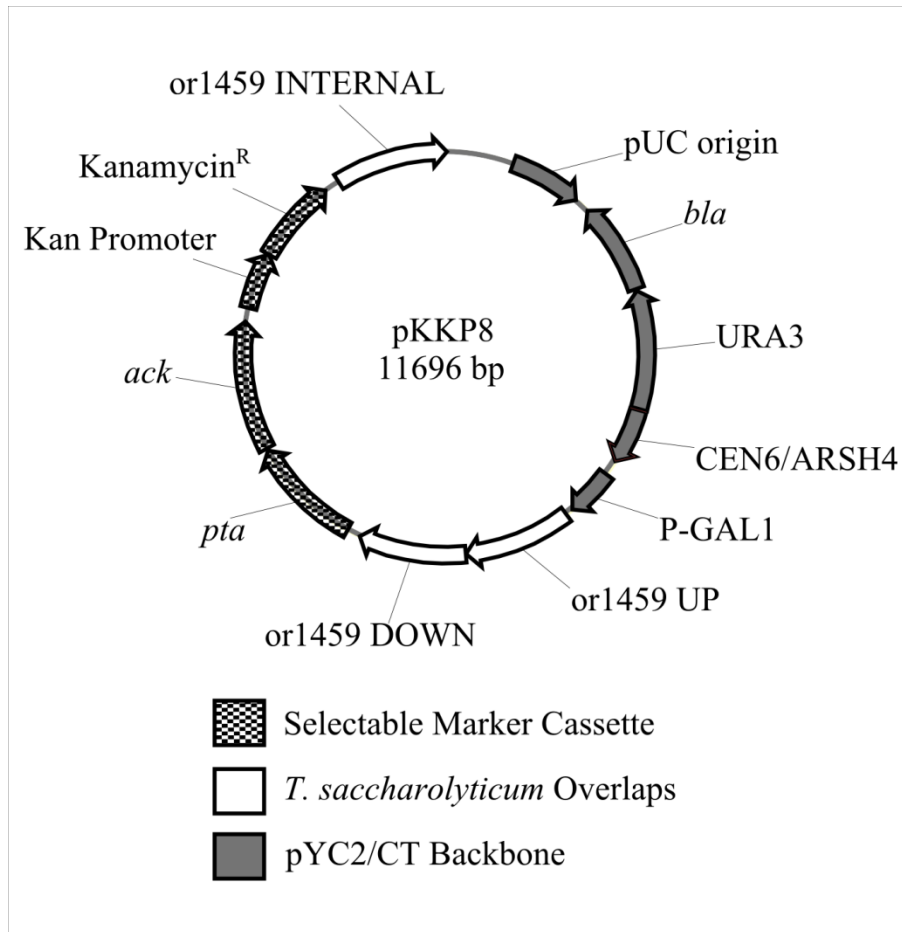


Figure S1: Map of plasmid used to delete *xynA* (*or1459*). Sections in white represent the regions of homology with *T. saccharolyticum*. Checkered sections indicate the selectable marker cassette, featuring both positive (kanamycin resistance) and negative (*pta-ack*) selectable markers. Grey regions indicate the pYC2/CT backbone. Similar vectors were constructed for each deletion strain using primers listed in Table 1.

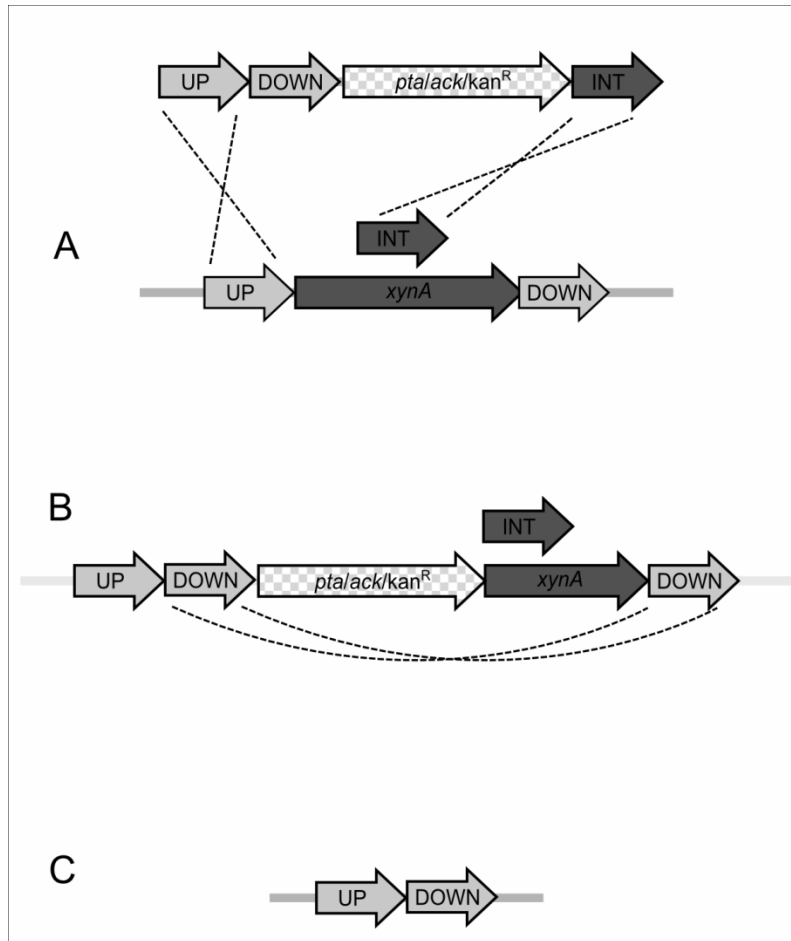


Figure S2: Transformation schematic for markerless deletions. A) Plating on kanamycin in the presence of the linear construct selects for vector recombination at the upstream and internal regions, removing a portion of the *xynA* gene and replacing it with *pta-ack-kan*. B) Growth in the presence of chloroacetate selects for strains that have lost *pta-ack* via a third recombination event between the repeated downstream regions, resulting in C) a markerless deletion of *xynA*.

Table S1: Annotation of open reading frames deleted in *T. saccharolyticum* M0355 background.

Target	Description	Source
or0897	Putative endoxylanase (<i>xynC</i>)	This work
or0271	Putative glucokinase	C. Herring, Mascoma annotation, GenBank # CP003184
or0272	Putative ABC transporter	C. Herring, Mascoma annotation, GenBank # CP003184
or0273	Putative monosaccharaide-transporting ATPase	C. Herring, Mascoma annotation, GenBank # CP003184
or0274	Putative D-xylose ABC transporter	C. Herring, Mascoma annotation, GenBank # CP003184
or0275	Putative D-xylose ABC transporter	C. Herring, Mascoma annotation, GenBank # CP003184
or0276	Putative xylulokinase	C. Herring, Mascoma annotation, GenBank # CP003184
or0277	Xylose isomerase	Liu et al 1996
or1451	Putative beta-xylosidase (<i>xyID</i>)	This work
or1452	Beta-xylosidase (<i>xyIB</i>)	Lorenz and Wiegel 1997
or1453	Acetyl transferase	Lorenz and Wiegel 1997
or1456	Putative extracellular solute binding protein	C. Herring, Mascoma annotation, GenBank # CP003184
or1457	Putative ABC transporter	C. Herring, Mascoma annotation, GenBank # CP003184
or1458	Putative ABC transporter	C. Herring, Mascoma annotation, GenBank # CP003184
or1459	Endoxylanase (<i>xynA</i>)	Shao et al 1995, Liu et al 1996