



Figure S1. Extending the duration of the Cas9 expression promotes introduction of donor-less mutation by a gRNA-Cas9 vector.

A. The introduction of the *ade6* mutations with two vectors. The transformants were directly streaked on YE plates to obtain single colonies. All single colonies (#1–7) showed red color. Wild-type cell were used as a control (white colony, cnt).

B. The introduction of the *ade6* mutations by the *ade6* gRNA-*nmt41* promoter-Cas9 vector (pAH251, *LEU2* marker). Transformants were directly streaked on YE plate, and 7/8 transformants contained red/white color cells.

C. The same transformants shown in Figure S1B were incubated on EMM5S – leucine medium plate for 3 days and streaked on YE plate to obtain single colonies. All single colonies showed red color.