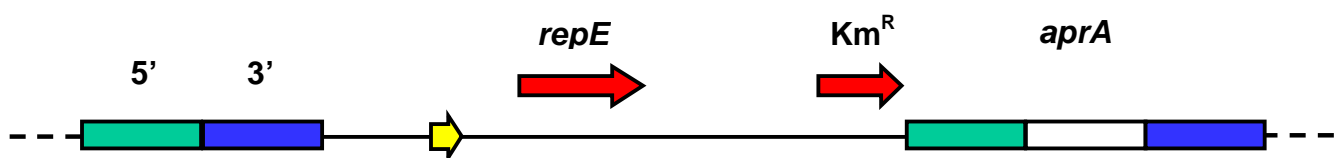


Shift to non-permissive temperature (48°C) → chromosomal integration



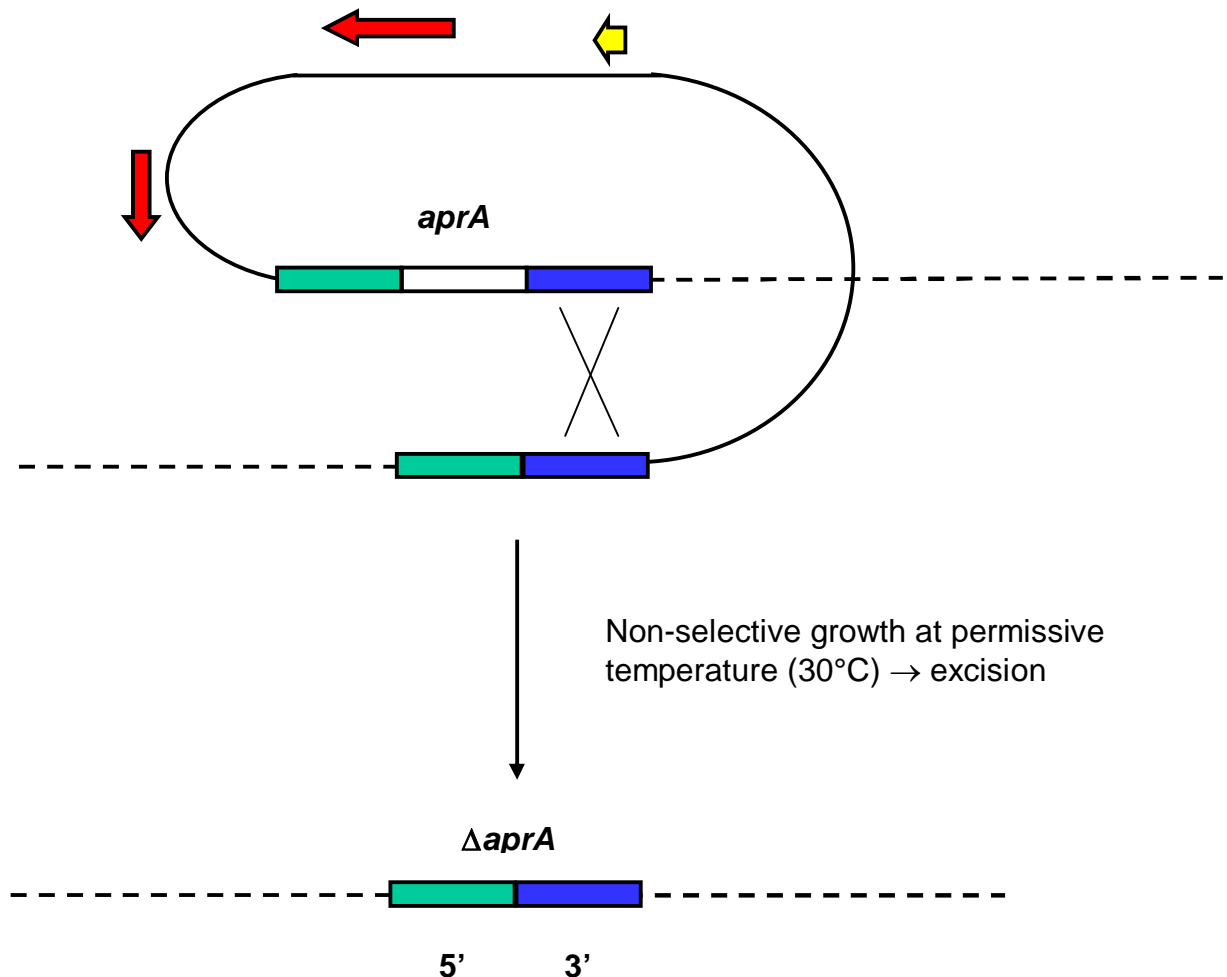


Figure 2. Schematic representation of the integration-excision procedure adopted for the construction of deletion mutants using the temperature sensitive origin of replication of pE194. (A) Integration of the *aprA* deletion vector pGBBdapr1. In this example integration occurs by a single cross-over recombination between the 5' flanking sequences. (B) Excision of the integrated vector via recombination between the 3' repeats results in deletion of the intervening sequences and, thus, deletion of the *aprA* gene. Chromosomal sequences are indicated by the dashed lines; vector sequences are marked by the solid lines.