Splice / overlap PCR to create site-specific deletions

A   B   target gene   C   D

1) 1st PCR; primer tails of B & C can anneal

A   B   C   D

2) Anneal, elongate, then 2nd PCR

cat   kn sac

A   D

B   C

3) ligate into suicide vector

4) mobilize into target strain

A   B   target gene   C   D

5) Plate on sucrose; forces marker-exchange and loss of suicide vector

A   B C   D