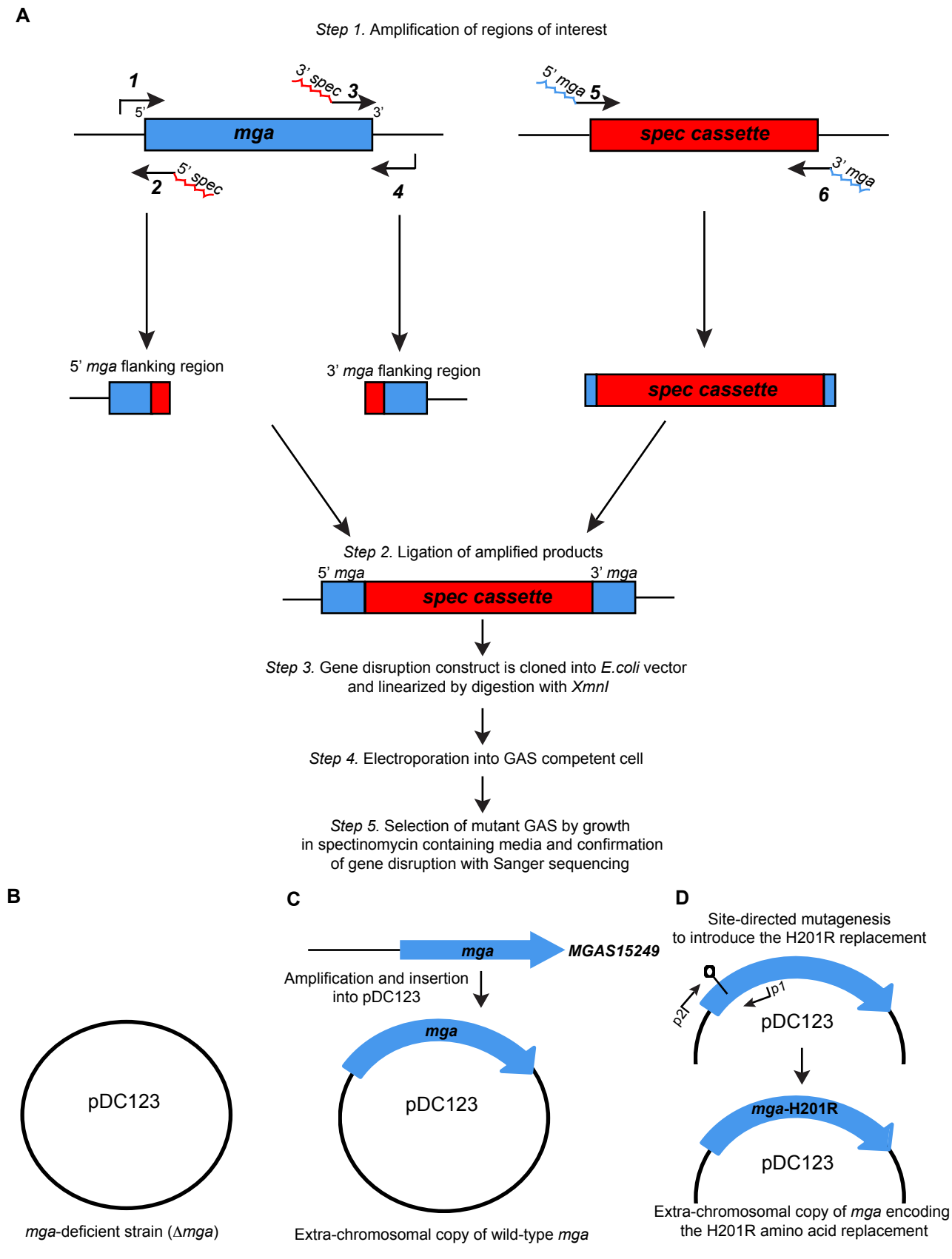
A
Step 1. Amplification of regions of interest
1
5'
mga
3' spec 3
3'
2
5' spec
4
5' mga 5
spec cassette
3' mga
6
5' mga flanking region
3' mga flanking region
spec cassette
Step 2. Ligation of amplified products
5' mga
spec cassette
3' mga
Step 3. Gene disruption construct is cloned into E.coli vector and linearized by digestion with XmnI
Step 4. Electroporation into GAS competent cell
Step 5. Selection of mutant GAS by growth in spectinomycin containing media and confirmation of gene disruption with Sanger sequencing
B
pDC123
mga-deficient strain (Δmga)
C
mga
MGAS15249
Amplification and insertion into pDC123
mga
pDC123
Extra-chromosomal copy of wild-type mga
D
Site-directed mutagenesis to introduce the H201R replacement
p2f
p1
pDC123
mga-H201R
pDC123
Extra-chromosomal copy of mga encoding the H201R amino acid replacement