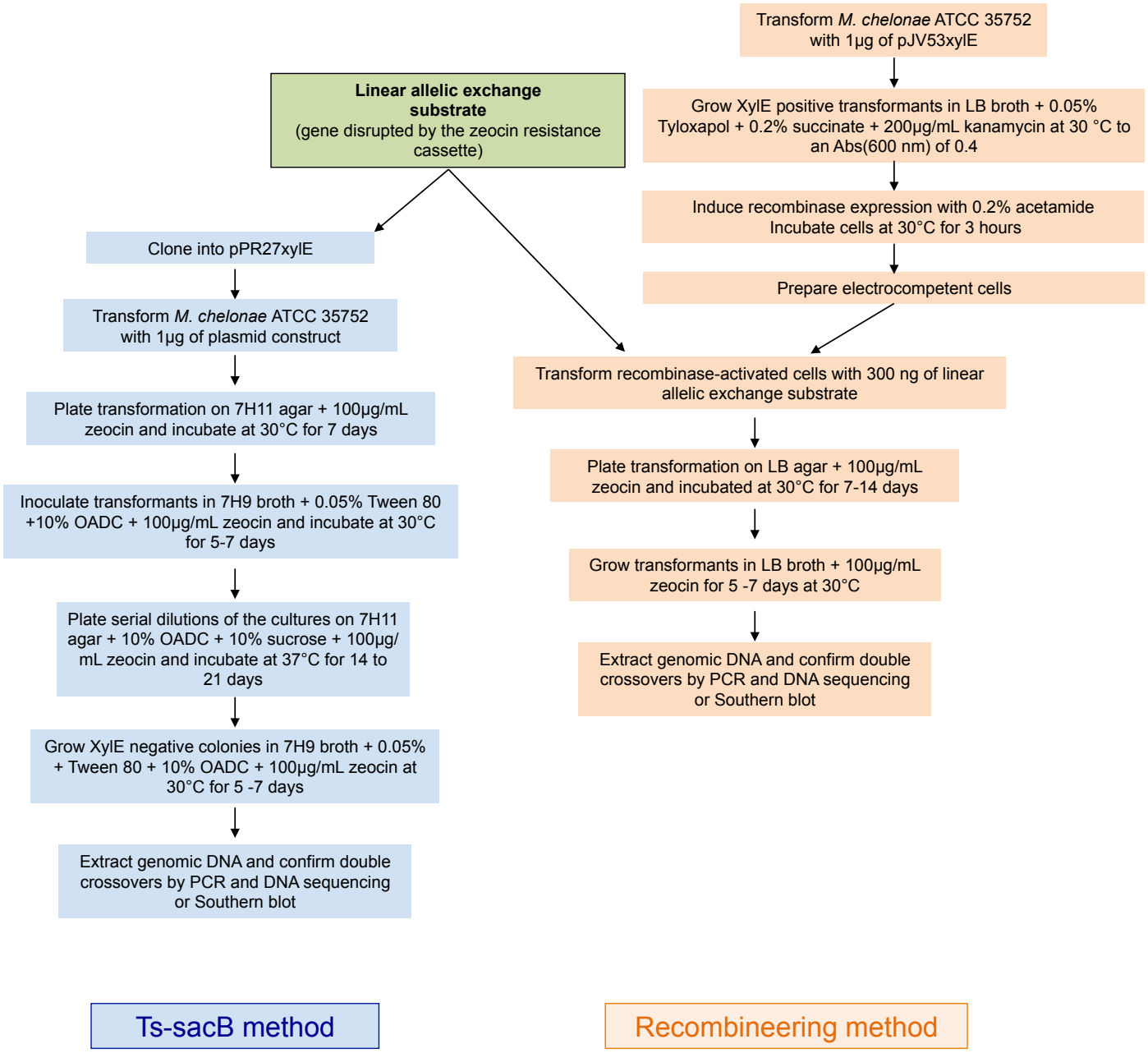
Linear allelic exchange substrate
(gene disrupted by the zeocin resistance cassette)
Clone into pPR27xylE
Transform M. chelonae ATCC 35752 with 1µg of plasmid construct
Plate transformation on 7H11 agar + 100µg/mL zeocin and incubate at 30°C for 7 days
Inoculate transformants in 7H9 broth + 0.05% Tween 80 +10% OADC + 100µg/mL zeocin and incubate at 30°C for 5-7 days
Plate serial dilutions of the cultures on 7H11 agar + 10% OADC + 10% sucrose + 100µg/mL zeocin and incubate at 37°C for 14 to 21 days
Grow XylE negative colonies in 7H9 broth + 0.05% + Tween 80 + 10% OADC + 100µg/mL zeocin at 30°C for 5 -7 days
Extract genomic DNA and confirm double crossovers by PCR and DNA sequencing or Southern blot
Transform M. chelonae ATCC 35752 with 1µg of pJV53xylE
Grow XylE positive transformants in LB broth + 0.05% Tyloxapol + 0.2% succinate + 200µg/mL kanamycin at 30 °C to an Abs(600 nm) of 0.4
Induce recombinase expression with 0.2% acetamide Incubate cells at 30°C for 3 hours
Prepare electrocompetent cells
Transform recombinase-activated cells with 300 ng of linear allelic exchange substrate
Plate transformation on LB agar + 100µg/mL zeocin and incubated at 30°C for 7-14 days
Grow transformants in LB broth + 100µg/mL zeocin for 5 -7 days at 30°C
Extract genomic DNA and confirm double crossovers by PCR and DNA sequencing or Southern blot
Ts-sacB method
Recombineering method

Figure S1