DAY 1
Clone genes of interest between homologous arms and transform into competent pir+ strain.
OR construct insertion cassette by Gibson assembly (go to Day 3 step 2).

DAY 2
1. Test colonies from ligation by colony PCR. Select positives for plasmid preparation and sequencing. Streak out to purify colonies.

DAY 3
1. Inoculate culture from single colony for plasmid preparation.
2. Inoculate strain of interest harbouring pKD46 for preparation of competent cells.

DAY 4
1. Prepare plasmid and excise insertion cassette (Gibson-assembled fragments are ready to use). Sequence plasmid for QC.
2. Prepare competent cells using strain of interest (induce recombinase during preparation).
3. Transform insertion cassette into induced competent cells.

DAY 5
1. Screen colonies by colony PCR (target both integration junctions).
2. Select positives and run QC. Streak out to purify.

DAY 6
Inoculate broths for glycerol stocks and competent cell preparation.

DAY 7
Prepare competent cells and transform with pCP20 for flippase-mediated removal of resistance gene.

DAY 8
Screen for loss of antibiotic resistance cassette (negative selection and colony PCR).