

*S. aureus*  
ATCC 29213

Streak on TSA  
blood agar plate

Incubate  
overnight at 37°C

Randomly pick  
isolated colonies  
to establish 18  
replicate  
populations

Inoculate the replicate  
cultures into broth  
microdilution plates

Increasing vancomycin  
concentrations

Transfer cells from  
the highest  
concentrations with  
visible growth into  
fresh growth  
medium with  
increased  
vancomycin  
concentrations

Continue until  
vancomycin-  
intermediate  
resistance is  
achieved for each  
replicate line

Sequence the  
complete  
genomes of the  
18 evolved VISA  
populations

Subject the 18 evolved VISA  
populations to 7 antibiotics and  
estimate collateral responses

