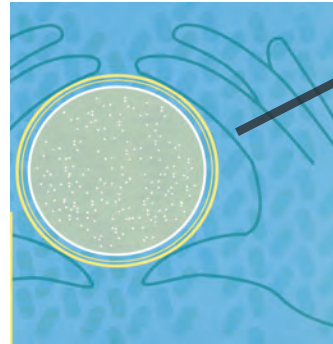


Select for rifamycin resistant mutations (mutant colonies). Measure frequency of rif<sup>S</sup> --> rif<sup>R</sup> mutations

Screen for lac<sup>-</sup> mutations (mutant colonies) on MacConkey plates Measure frequency of lac<sup>+</sup> ---> lac<sup>-</sup> mutations

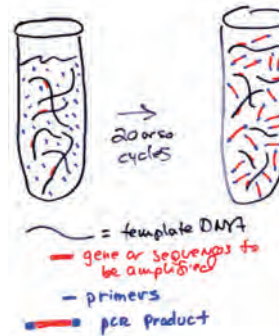
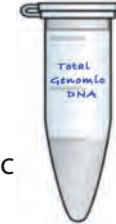


Pick individual colonies streak on rifamycin plates to confirm phenotype and isolate pure colonies



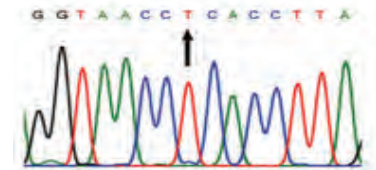
set up liquid cultures of rif<sup>R</sup> clones

Prepare genomic DNA

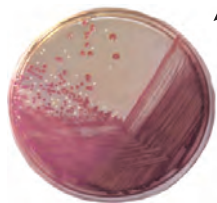
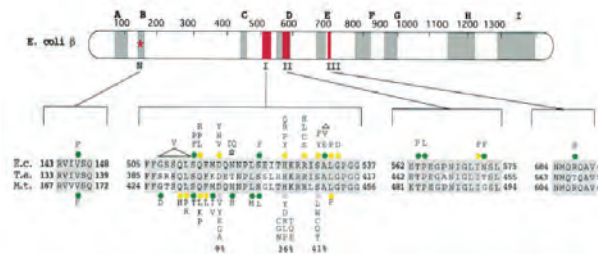


Use PCR to amplify a portion of the gene coding for RNA polymerase (target of rifamycin)

Send PCR products out for sequencing

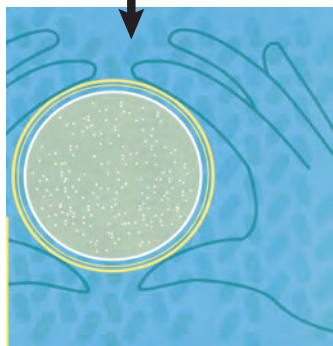


Determine site of resistance mutation



Pick individual lac<sup>-</sup> candidates and streak on MacConkey plates to confirm phenotype and isolate pure colonies

Set up liquid cultures of lac<sup>-</sup> clones



Measure frequency of lac<sup>-</sup> --> lac<sup>+</sup> reverse mutations by plating on selective media