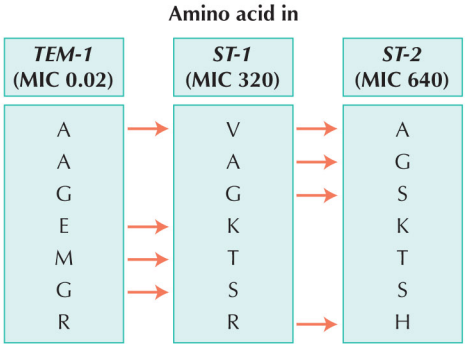


**FIGURE 23.21.** Recombination selects for much more efficient antibiotic resistance genes. In vitro the β-lactamase gene *TEM-1* in *E. coli* has poor activity against the antibiotic cefotaxime: The minimum inhibitory concentration (MIC) of antibiotic against *E. coli* carrying this gene is only 0.02 μg/ml. The gene was broken into fragments and amplified by **polymerase chain reaction**, which caused recombination between fragments and also introduced mutations. The recombined genes were inserted back into *E. coli*, and were then selected on increasing concentrations of antibiotic. This process was repeated three times and produced a gene *ST-1*, which conferred a 16,000-fold increase in resistance. This had four amino acid replacements, as well as four silent changes and a mutation that increased promoter strength. Two generations of recombination in the presence of excess wild-type DNA (a "backcross") eliminated mutations unnecessary for resistance, but also gave further amino acid substitutions that further doubled resistance. The final product was 64 times more resistant than any previously published *TEM-1*-derived gene; a similar experiment using mutation alone gave only a 16-fold increase in resistance. (*Letters* indicate amino acids at positions that evolved.)

**23.21,** modified from Smith G.P., *Nature* **370:** 324–325, © 1995 Macmillan, www.nature.com