Increasing percentage of Auxotroph in Wild type population by penicillin and 5-BU

If a type of bacterial mutant being sought is rare, finding it by negative selection can be very laborious, even with replica plating. No more than about 500 bacteria can be spread on a plate and still give discrete colonies. So, for example, if the mutant occurs at a frequency of 1 in 1 million, more than 2,000 plates might have to be replicated to find a mutant!

Many fewer colonies need to be screened if the frequency of mutants is first increased through mutant enrichment. This method depends on the use of antibiotics such as ampicillin and 5-bromouracil (5-BU) that kill growing but not nongrowing cells. Ampicillin inhibits cell wall synthesis and causes a growing bacterial cell literally to grow out of its skin and lyse. A mutant cell that was not growing while the ampicillin was present will not grow out of its skin and so will not be killed. 5-BU also kills only growing cells but by a very different mechanism. DNA containing 5-BU (an analog of thymine) is much more sensitive to UV light than is normal DNA containing only thymine. Cells replicate their DNA only while they are growing, so they will take 5-BU into their DNA and become more UV sensitive only if they were growing while 5-BU was present in the medium.

ref.
Molecular Genetics of Bacteria
(Snyder & Champness)

To enrich for mutants that cannot grow under a particular set of selective conditions, the population of mutagenized cells is placed under the selective conditions in which the desired mutants stop growing. Meanwhile, the nonmutant wild-type cells will continue to multiply. The antibiotic—either ampicillin or 5-BU—is then added to kill any multiplying cells. The cells are then filtered or centrifuged to remove the antibiotic and transferred to nonselective conditions. The mutant cells will have survived preferentially because they were not growing in the presence of the antibiotic; therefore, they will have become a higher percentage of the population. No enrichment is 100% effective; however, even if the enrichment makes the mutant only 100 times more frequent, only 1/100 as many colonies and therefore 1/100 as many plates must be replicated to find a mutant after an enrichment. In the example given above, after an enrichment we would need to replicate only 20 plates instead of 2,000 to find a mutant.

Unfortunately, enrichments cannot be applied to all types of mutants. Some mutants are killed by the selective conditions and so cannot be enriched by these procedures. To be enriched, the mutant must still be alive and resume multiplying after it is removed from the selective conditions.