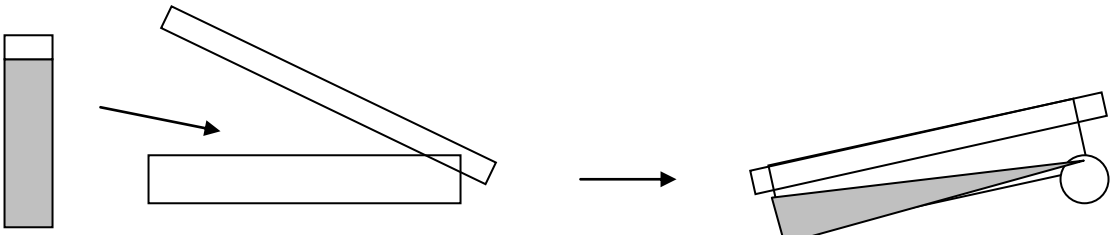
Gradient Plate Substitute

As you have already learned, random chance mutations occur in bacteria that enable them to resist antibiotics. In any given population of bacteria, there will be a few bacteria with naturally occurring mutations that provide some resistance to a particular antibiotic. We can select for those naturally occurring mutants using the gradient plate technique.

Two layers of agar are prepared: one layer is plain agar, the other layer contains streptomycin along with green food coloring so we can tell which layer is which. The plain agar is poured into a sterile petri plate and allowed to solidify at an angle. The streptomycin layer is poured on top. A concentration gradient of streptomycin will form. The darker green side has the highest concentration of streptomycin. The concentration decreases as you move across the plate to the low/no concentration side.

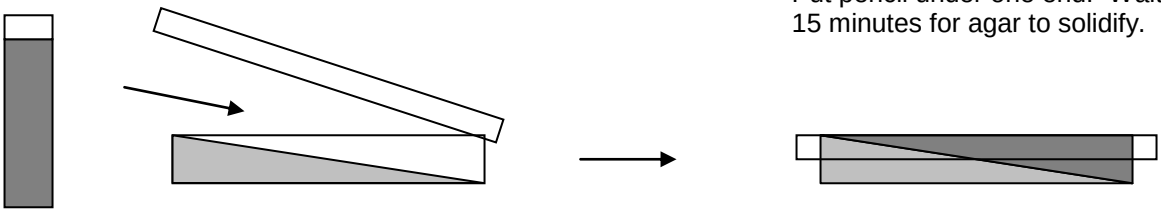
E. coli is spread all over the agar surface. After incubation, we see colonies growing in the high streptomycin concentration side of the plate.

Take Home Lesson: Why would you expect to find any streptomycin resistant mutants? Random chance, spontaneous mutations. What is the purpose of the streptomycin? To select for resistant mutants. Does streptomycin induce (cause) mutations? No What are some of the possible mechanisms a mutant might use to circumvent the streptomycin? Review the lecture on antibiotics.



First agar deep

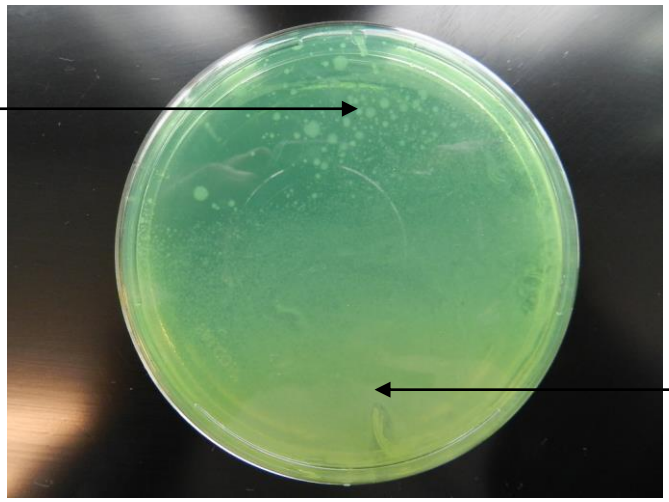
Put pencil under one end. Wait 15 minutes for agar to solidify.



Second agar deep + 1.0 mL streptomycin

Wait 15 minutes then spread 2 drops of *E. coli* on surface.

Mutant colonies in high streptomycin concentration



Lots of *E. coli* growing in low/no streptomycin concentration