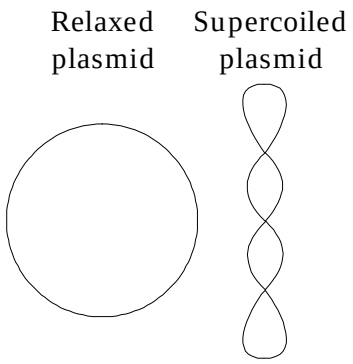
Biol 213 Genetics (Fall 2000)
Lab 2: Isolation and Characterization of Plasmid and Chromosomal DNA
An Important Clue

Imagine a bacterial cell, with a chromosome -- large and scrunched -- and a plasmid -- small and round. Actually, that view of a cell is not quite right. Plasmids are not round circles within the cell. If you go to your telephone, pick up the receiver, and rotate it many times you'll see why. After a while, the cord starts twisting around itself making loops. If you let the receiver dangle, it rotates by itself the other way, relieving the cord of its stress.



Plasmids are twisted in the same way (by the action of a topoisomerase working in the reverse direction, introducing twists). But since it is a circle, the twists have no way of untwisting by themselves. All the plasmids in a cell are in this twisted (or supercoiled) form. So is the chromosome. When you isolate DNA from a cell, the mayhem is enormous. The membrane to which the chromosome was attached is either broken or shattered, depending on which procedure you used. The chromosome is sheared to bits. Imagine trying to handle a 4.6 million base pair piece of uncooked spaghetti. You can't help breaking it, and as soon as it's released from its constraints, it relaxes, like the dangling telephone receiver. Plasmids are much smaller and more resistant to breakage, but some breakage inevitably occurs.

How can we distinguish the different forms of plasmid -- supercoiled and relaxed? As you are well aware by now, electrophoresis does not, strictly speaking, separate DNA on the basis of size but rather by frictional drag. Since linear DNA has the same shape, the larger pieces experience the greater amount of friction. But what about circular, relaxed DNA vs supercoiled? Supposing you compared two plasmids with the same number of bases -- one relaxed, one supercoiled? Which would run through the gel faster?