

CHAPTER 18**HANDOUT****Cohen-Boyer Experiment****BLM 18.3.8**

1973 was the year in which genetic engineering was born. In this year, the American researchers Stanley Cohen and Herbert Boyer created the first genetically engineered organism. This organism, known as a chimera, consisted of a bacterium that contained DNA from an unrelated species, *Xenopus laevis*, the African clawed toad. To create this chimera, Cohen and Boyer first isolated a bacteria plasmid known as pSC101 (so named as it was the 101st plasmid isolated by Stanley Cohen). A plasmid is a double stranded, circular molecule of DNA that replicates on its own, independently of the bacterial chromosomal DNA. Plasmid pSC101 contains a derivative of an R factor, a factor that codes for antibiotic resistance in bacteria, in this case to tetracycline. In general, R factors are very large and can be cleaved at many sites by restriction enzymes. Plasmid pSC101 contains a derivative of an R factor, which has only 9000 base pairs, and is cleaved in only one site by the restriction endonuclease *EcoRI*. When cleaved, the plasmid opens at this specific site to form a linear piece of double stranded DNA with sticky ends on both ends. Another gene that is cut by restriction endonuclease *EcoRI* may then bind to these sticky ends and be added to the plasmid.

Over 30 years ago, Cohen and Boyer created their chimera in just this way. The scientists isolated a gene from *Xenopus laevis* that coded for the production of rRNA. This gene was cleaved with restriction endonuclease *EcoRI* and inserted into plasmid pSC101. The plasmid now contained the amphibian gene for rRNA production and the bacterial gene for tetracycline resistance. Bacteria were then exposed to both the recombinant plasmid and to tetracycline. Those bacteria that displayed tetracycline resistance had taken up the plasmid.

The following figure illustrates the Cohen-Boyer experiment. In the figure, the amphibian gene coding for the production of rRNA is shown in black and the bacterial gene, *tetR*, which confers resistance to the antibiotic tetracycline, is shown in white. The restriction endonuclease *EcoRI* and DNA ligase were used to splice (insert) a gene from the toad into the plasmid pSC101.

