Biol 213L: Genetics Lab (Fall 2000)

Isolation and Characterization of Plasmid DNA. . . (Sample Report)

Introduction

Purple Tongue Syndrome is one of the major plagues on humanity. X% of all males are afflicted and inevitably suffer severe embarrassment, often leading to premature death. *[Is there a cure?]* While there is no known cure, the oppressive symptoms may be relieved by daily injections of enzymes that reduce the purple coloration. *[That sounds horrible. Isn't there an alternative?]* This inconvenient procedure might be avoided if it were possible to indroduce into patients' cells a gene encoding a pigment-degrading enzyme. *[What gene?]* The enzyme ß-galactodepurplease, encoded by *lacT*, degrades most purple pigments. If this bacterial gene is appropriately engineered, it might be expressed in tongue cells of affected patients. The first step in this project is to clone the gene.

[How do you go about doing this?] *lacT* has been reported to lie on the plasmid pUR47 along with several other genes of no interest to our project [Elhai et al., 2000]. *[What are plasmids?]* Plasmids are small circular molecules of DNA found in some bacteria. We obtained what was claimed to be pUR47 in a strain of *E. coli* but were concerned that the strain might not actually contain the plasmid. *[What a predicament! How can you tell?]* Restriction analysis provides a means of distinguishing plasmid DNA from chromosomal DNA and one plasmid from another. *[What's restriction analysis?]* This is possible by the ability of restriction enzymes to recognize specific short DNA sequences and cut within them. The fragments produced by restriction digestion therefore are reproducible and provide a fingerprint of a plasmid that can be used for identification. The available sequence of pUR47 [Elhai et al., 2000] made it possible to locate restriction recognition sites that flank *lacT*.

We used the restriction digestion and the sequence of pUR47 to identify the DNA from the strain purported to possess pUR47. With the identity of the plasmid established, the plasmid was digested in such a way as to separate *lacT* from the rest of the plasmid as a first step towards cloning the gene.

Results

Before isolating the *lacT* gene from pUR47, it was necessary to determine whether the plasmid was accurately identified. To this end, I purified plasmid and total DNA from *E. coli* nominally carrying pUR47. These DNA samples were run on a gel, with and without digestion by *Eco*RIII (Fig. 1). This enzyme was chosen because it flanks *lacT*, producing a fragment with a predicted length of 1029 bp. The sizes of the fragments were determined (Table 1) by comparison with DNA fragments of known size (Fig. 2)….