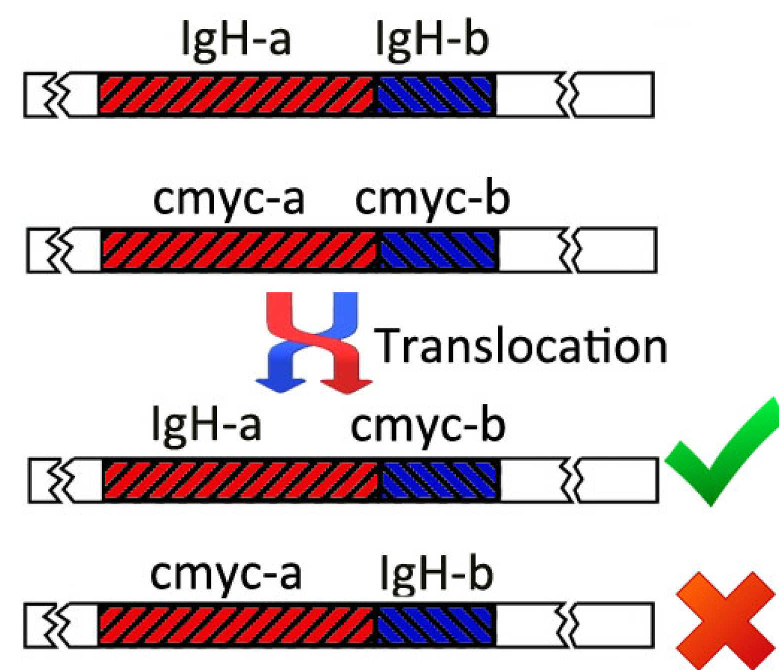
Isolating a Gene from Bacterial Plasmid DNA

Danny Srole, Shuwen Chen, and Cornelis Murre

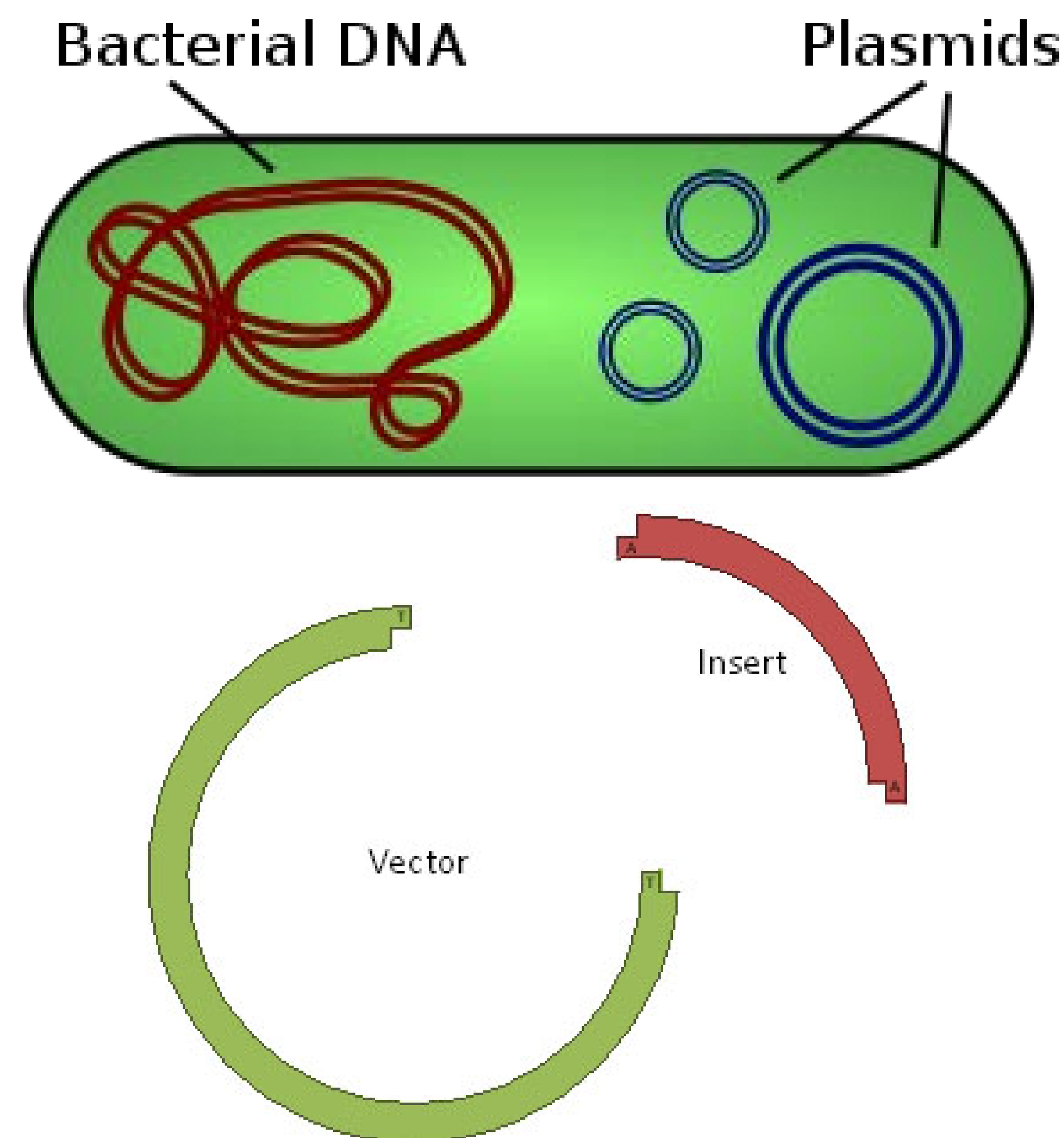
Department of Biological Sciences, University of California, San Diego, 9500 Gilman Drive, La Jolla, California

The Problem

Gene expression is very strictly regulated under normal circumstances. However, in some pathological cases such as tumors, the expression of some genes gets out of hand, especially oncogenic genes and tumor suppressor genes. Translocation is one of many processes that can lead to this. As shown below, when a gene translocation occurs between the Igh locus and the c-myc locus, the expression of c-myc is under the control of the Igh promoter, thus inducing oncogenic c-myc expression, which may cause Burkitt lymphoma.



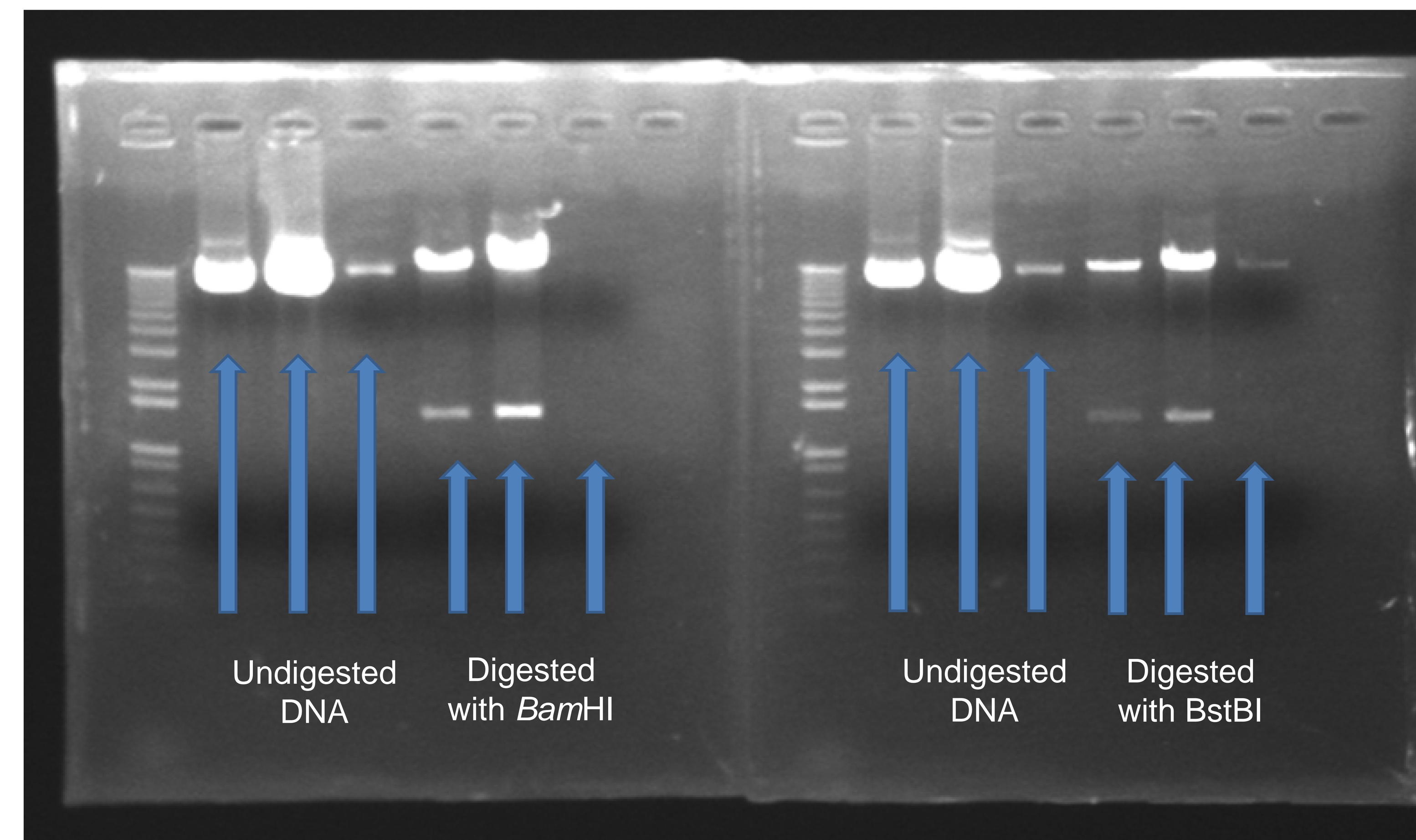
Plasmid DNA in Bacterial Cells



Plasmid DNA is a small molecule which can replicate independently of chromosomal DNA within a bacterial cell. It's very widely used in molecular biology because a gene of interest (insert) can be cloned into certain vectors. By transforming bacteria with these plasmids, we are able to isolate a huge amount of target DNA in later processes.

In our experiment, we transformed a plasmid, which is composed of a retroviral vector and the c-myc gene insert, into competent DH5α bacteria. As there is ampicillin resistance fragment(amp^R) within the plasmid, only ampicillin-resistant bacteria will grow on an LB agar plate containing ampicillin. This enables us to pick up single colonies and prepare for later maxi prep.

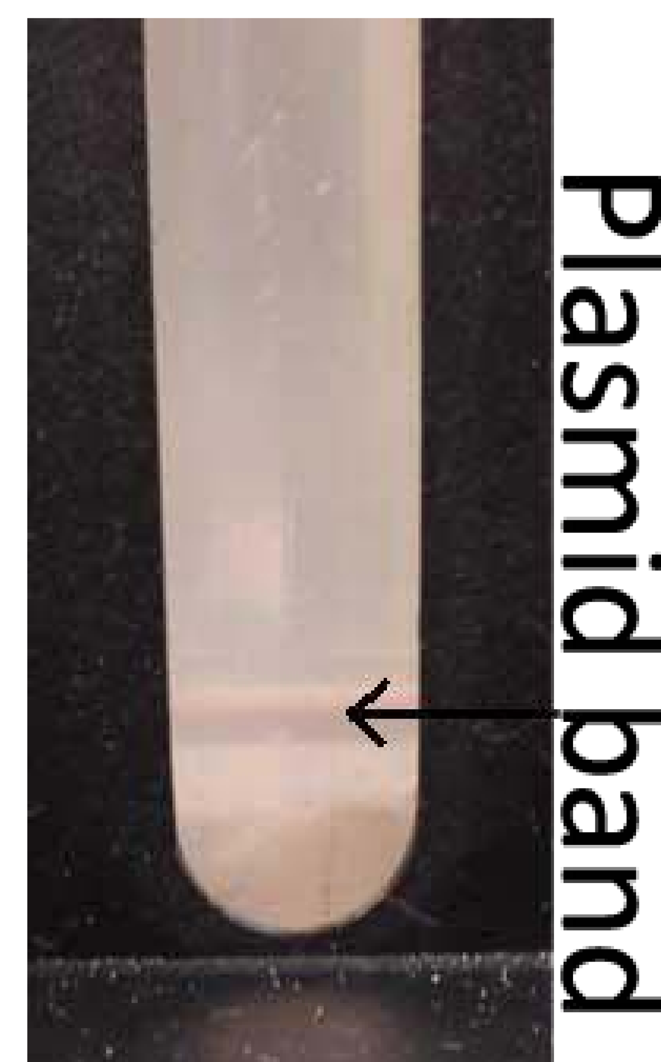
Enzyme Digestion



After mini prep, it is necessary to ensure that the colonies we picked are all exact clones. To achieve this goal, we used restricted endonucleases *Bam*HI and BstBI to digest the plasmid DNA. Then a gel electrophoresis was performed to compare undigested DNA to digested DNA.

The bands on the far left side of each gel represent the key while the next three are the undigested DNA samples. The next three are digested samples. The left gel was digested with *Bam*HI and the right with BstBI.

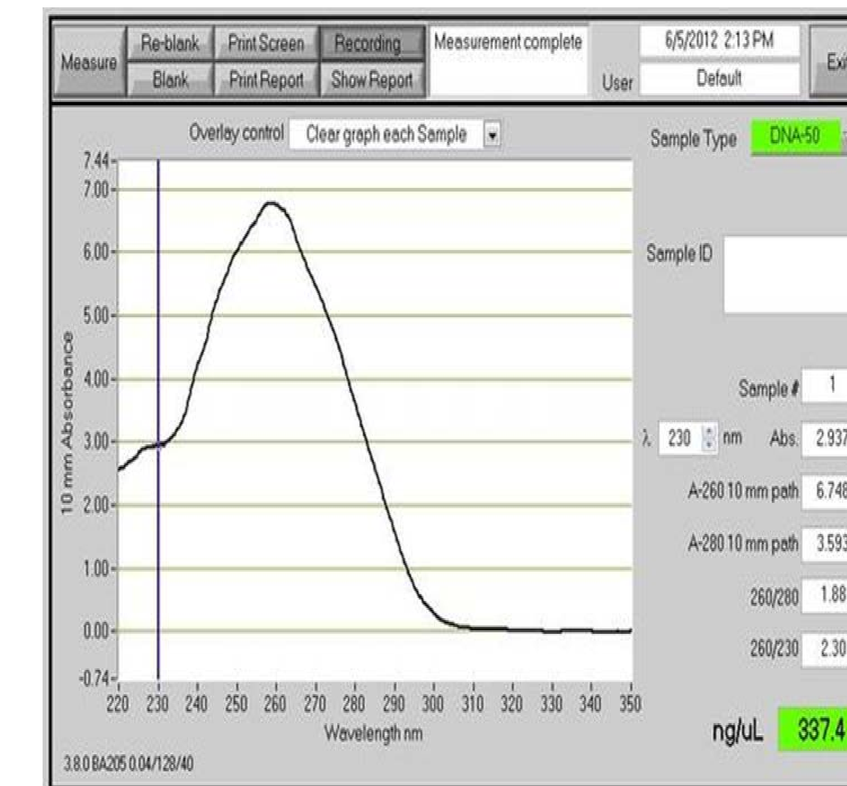
Enriching Plasmid DNA via Maxi Prep



To isolate plasmid DNA from the bacteria, we used an alkaline solution to lyse the bacterial cells and denature the double-stranded (ds) DNA into single strands (ss). Then, we renatured the ssDNA into dsDNA (this process will only renature the plasmid DNA; chromosomal DNA is too big to get renatured) and precipitated protein by SDS. After we roughly obtained DNA through isopropanol precipitation, we dissolved them in a CsCl solution. The CsCl dissociates in the water into Cs^+ and Cl^-. Being more dense than water, the Cs^+ will sink, thus creating a concentration gradient throughout the centrifuge tube. After high speed centrifugation, DNA will finally concentrate at the place where its density equals the local Cs^+ density. As we added ethidium bromide (EB) to DNA, it gave an orange color to the plasmid band.

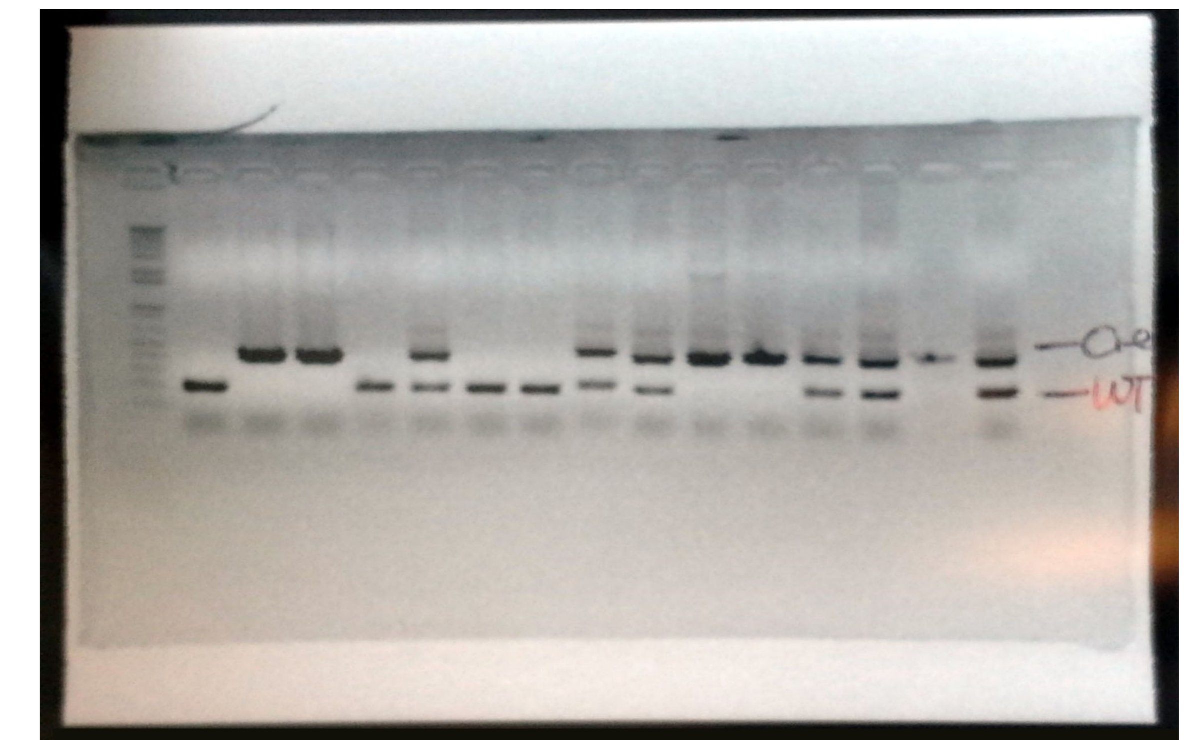
After being centrifuged for hours at a very high speed (around 60,000 RPM), the band of DNA can be extracted using a syringe. At this point the ethidium bromide (EB) must be removed from the solution so we are left with only pure plasmid DNA. Water-saturated butanol is added which removes the ethidium bromide. We then precipitated the DNA by adding 100% ethanol. After being centrifuged, the pellet of DNA can be isolated and resuspended in H_2O.

Testing the Concentration and Purity of DNA



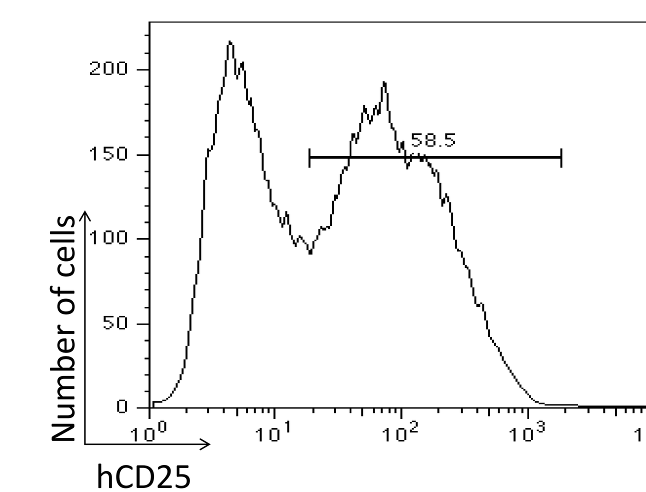
Once a sample consists of only the DNA and H_2O, a spectrophotometer can be used to measure the quality and concentration of the DNA while disregarding the aqua solution. The spectrophotometer measures the amount of light absorption at a wavelength of 260 nm vs 280 nm. For pure DNA, the ratio of light absorption ($A_{260/280}$) should be above 1.8.

Genotyping



Genotyping was used to determine which sample of mouse tail DNA had the Cre recombinase allele of a certain gene, which had the wildtype allele, and which had both. Agarose gel electrophoresis can be used to distinguish between different DNA fragments based on length. Longer fragments move more slowly on agarose gels than shorter ones. Here, the wildtype allele is around 200 base pairs while the Cre allele is around 400 base pairs. Notice that some samples carry both alleles, so they are heterozygous.

INFECTIVITY OF THE RETROVIRUS



The graph to the left shows the results of a spin infection. It visually demonstrates the number of cells infected with the retrovirus. By the second round of infection, 58.5% of the cells had been infected with hCD25 which is a human cell surface reporter molecule.

Acknowledgements

I would like to thank Dr. Murre for allowing me the experience of working in his lab. Everyone on the Murre Lab team was incredibly warm and welcoming to me, a hospitality that I am truly thankful for. I especially want to thank my mentor, Shuwen Chen, for her patience and willingness to reexplain concepts and procedures. Without all of these wonderful people and particularly Dr. Elizabeth Komives, my experience would not have been as amazing as it was. Thank you, everyone.