Utilizing Polymerase Chain Reaction and DNA Extraction to Genotype High and Low Fat Diet Mice

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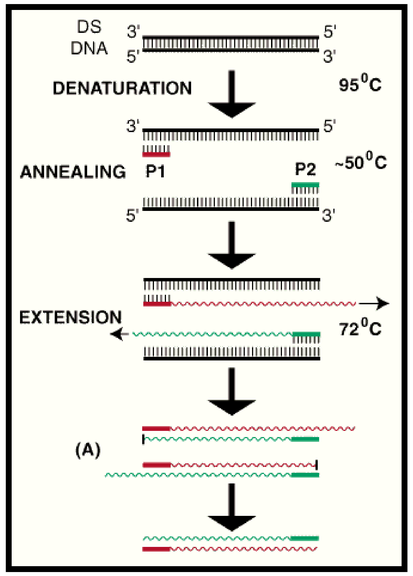
By utilizing polymerase chain reaction and DNA extraction, we were able to understand the genotypes of mice and the different effects of certain genes. We were able to identify a triple knockout mouse which had the AP2Cre, NCor and LDL-R genes, resulting in a mouse that exhibited atherosclerosis, yet had no inflammation. We were able to extract DNA from cecal and fecal contents, allowing us to compare the concentration of high fat and low fat specific bacteria.

Genotyping

Polymerase Chain Reaction



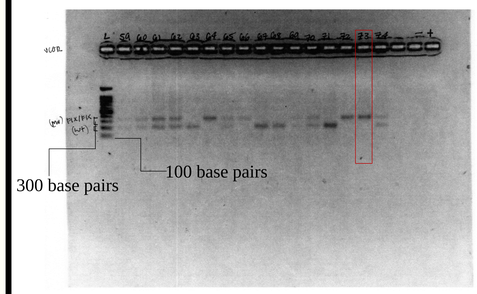
We began by genotyping mice #59-74 by using the process of PCR to amplify the NCoR-floxed, AP2-Cre, LDL-R mutant and LDL-R wildtype genes to determine the genotype of mice within the new litter. The results of our gel electrophoresis are on the right, displaying each mouse's genotype for the specific gene we amplified.

AP2Cre: AP2 is a gene expressed only in adipose tissue. The Cre serves as a recombinase and "cuts out" the **floxed Ncor gene**, resulting in no inflammation in adipose tissue.

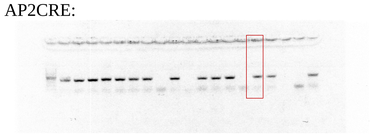
LDL-R (Low density lipoprotein receptors): **LDL receptors** are on the surface of liver cells where cholesterol from the body is collected. A deficiency in the LDL receptors causes your body to accumulate tremendous amounts of cholesterol. By knocking out the LDL receptors in the mouse, the cholesterol was not collected in the liver and instead accumulates in the walls of the arteries, modeling atherosclerosis.

Gel Electrophoresis

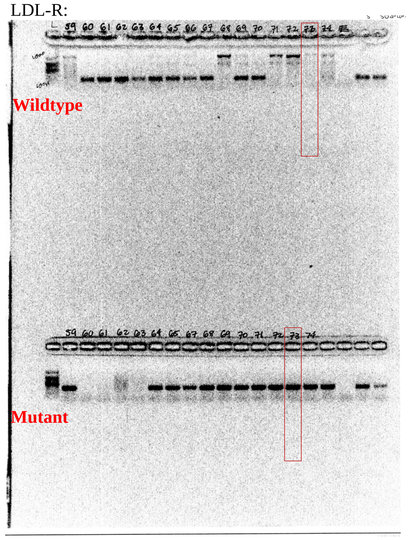
NCOR:



AP2CRE:



LDL-R:

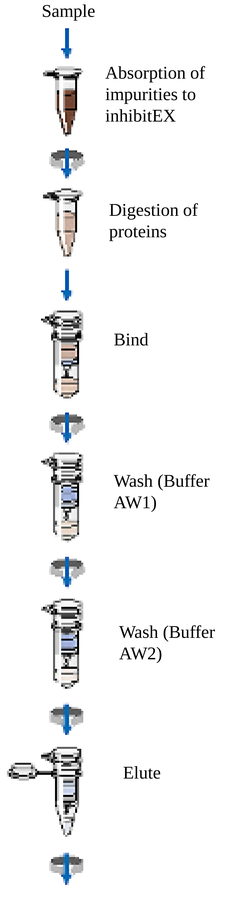


DNA Extraction

DNA isolation method (Qiagen)

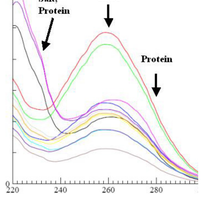
Process by which we extract DNA:

Sample



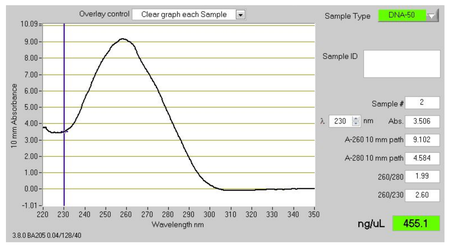
The aim of the second week was to isolate bacterial DNA from cecal contents and fecal pellets of high-fat and low-fat mice using the **Qiagen DNA isolation kit**. In addition, we used the **nanodrop spectrophotometer** to measure DNA concentrations and compare the results.

Nanodrop spectrophotometer



Generally, nucleic acid peaks at around 260 wavelength.

This is an example of a nanodrop spectrophotometer result



The results of the amount of DNA between the high-fat diet and low-fat diet mice were varied. I speculate that the high-fat diet mice generally have more DNA because of the increased number of bacteria which causes inflammation, which, in turn, reduces the insulin produced therefore causing diabetes.

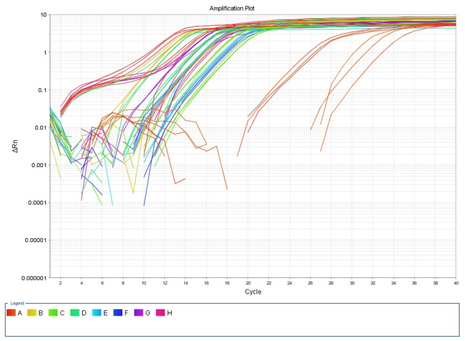
Results

High Fat Diet	Low Fat Diet	High Fat Diet + Ros	Fecal Pellet
CC 1: 21.3	CC 2: 48.9	CC 3: 11.8	FP 1: 29.5
CC 4: 42.5	CC 5: 31.2	CC 6: 7.5	FP 2: 4.8
CC 7: 60.2	CC 9: 51.3	CC 10: 39.5	FP 3: 7.6
CC 8: 51.9	CC 13: 72.0	CC 14: 50.2	FP 4: 9.9
CC 12: 31.9			
CC 15: 32.9 CC 16: 27.7			

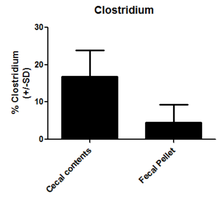
QPCR

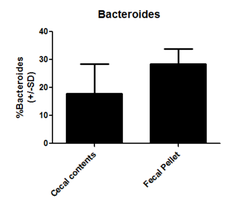
QPCR, or **quantitative real time polymerase chain reaction** is used to amplify and simultaneously quantify a targeted DNA molecule. The procedure follows the general principle of polymerase chain reaction, however its key feature is that the amplified DNA is detected as the reaction progress in real time (compared to standard PCR in which the product of the reaction is detected at its end). We utilized this specific method of PCR in order to quantify the total amount of bacterial DNA present in the DNA and then further quantify what percentage of that bacterial DNA were firmicutes and bacteriodes. Although firmicutes and bacteriodes are present throughout a body, firmucutes outnumber bacteriodes in high-fat diet mice. Both clostridium and lactobacilli are types of firmicutes. Firmicutes regulate how the energy in food is stored, meaning that if a high-fat diet mouse eats the same food as a low-fat diet mouse, the former's firmicutes will gather more energy and store it in the form of fat.

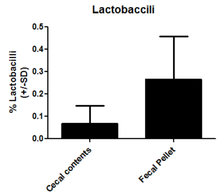
QPCR Results



The amount of DNA in a sample and the earlier cycles are directly proportional—because QPCR shows the amplification in real time, the lines that begin earlier indicate samples that have more DNA.







The disparity between the clostridium and the bacteroides graphs can be attributed to the positioning of where the DNA was isolated from.