Expression of Transferase A and Fucosyltransferase in Balb/c Mice

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Introduction

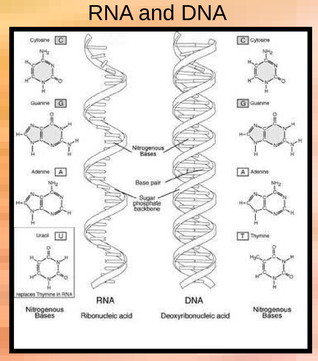
The purpose of my project was to test different tissues from a Balb/c mouse to look for the specific gene expression of *Transferase A* and *Fucosyltransferase.* Expression occurs when RNA of a specific gene is created, leaves the nucleus, and carries the genetic code to create a protein, therefore we must test the RNA rather then the DNA. I did this by using the process of RT-PCR (Reverse Transcriptase - Polymerase Chain Reaction). The steps in this process are:

1. Collect Mouse Tissues
2. Isolate the RNA
3. cDNA Synthesis (RT-PCR)
4. Perform PCR on the cDNA

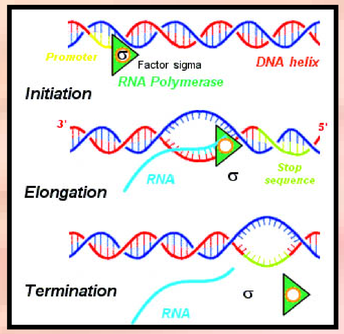
What is RNA?

RNA is the way in which the genetic code is transferred from the DNA in the cell nucleus to the cytoplasm to be translated into Proteins. DNA cannot leave the cell nucleus so instead it makes copies of the genes it wants to send out and make proteins, these copies are RNA. The making of the copy is called Transcription.

RNA and DNA



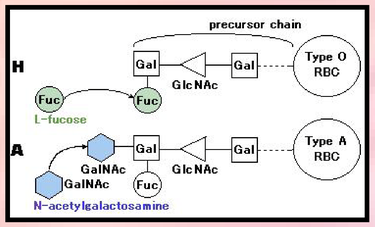
RNA Transcription



What are *Transferase A* and *Fucosyltransferase?*

The *Transferase A* and *Fucosyltransferase* genes code for the TRANSFERASE A and FUCOSYLTRANSFERASE proteins. These proteins help determine what your blood type will be by attaching certain carbohydrate groups onto the antigenic structure which is located on the outside of the red blood cells, and other cell types. FUCOSYLTRANSFERASE protein attaches an L-fucose to the precursor chain which results in type O red blood cells. If after the L-fucose is attached TRANSFERASE A protein attaches N-acetylgalactosamine to the precursor chain it changes the blood cells to type A.

Antigenic Structure



Tissue Extraction

I extracted 12 tissues each from one male and one female Balb/c mouse:
Heart, Bone Marrow, Kidney, Liver, Intestine, Colon, Lung, Spleen, Stomach, Testes, Uterus, Thymus.

RNA Isolation

I isolated the RNA using the following Protocol:
1) Homogenize the sample in Trizol (Invitrogen)
2) Separate the Phases using Chloroform
3) Precipitate the RNA by adding Isopropanol to the aqueous phase
4) Wash the RNA pellet with 75% Ethanol
5) Re-suspend the RNA pellet in DEPC treated water

I measured the concentration and purity of the RNA using a Spectrophotometer.

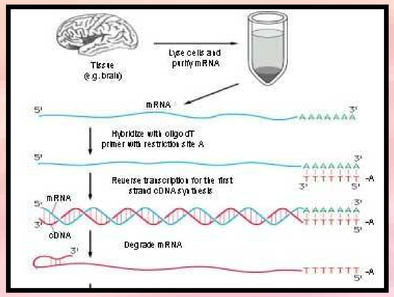
Collection of Samples with the best purity

Mouse Sample (m=male, f=female)	Concentration (ug/ul)	260/280 (Purity: best = 2.0)
Kidney (m)	4.47	1.63
Lung (m)	4.50	1.59
Testes (m)	4.55	1.50
Intestine (m)	4.29	1.74
Spleen (f)	4.64	1.36
Colon (f)	4.48	1.55
Stomach (m)	3.94	1.86
Heart (m)	0.55	1.91
Liver (m)	4.19	1.79
Uterus (f)	3.81	1.88
Thymus (m)	3.83	1.88
Bone Marrow (m)	1.38	1.98

RT-PCR (cDNA synthesis)

I created the complementary DNA from the RNA using the ThermoScript RT-PCR System (Invitrogen). Before leaving the nucleus, the RNA gets a poly A tail attached onto its 3' end. The Oligo (dT) Primer is composed entirely of Thymine (T) and during RT-PCR it attaches onto the Poly A tail of the RNA. Then the Thermoscript enzyme starts the reverse transcription of the RNA into cDNA. After creating the cDNA sequence the RNAse enzyme is added to degrade the RNA and leave only the cDNA. I made two tubes for each sample: one contains Thermoscript (positive), and the other doesn't (negative).

cDNA Synthesis

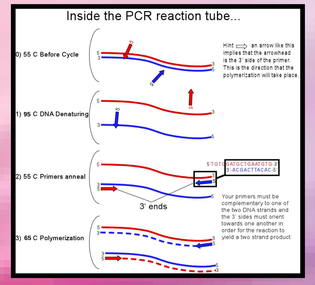


PCR (Polymerase Chain Reaction)

PCR amplifies the DNA so that it can be seen. I used the HotMaster Taq DNA Polymerase Kit (Eppendorf). After designing the primers for our specific sequence of cDNA we were able to start amplifying. In the PCR tube we add sterile water, the forward and reverse primers, the Taq DNA polymerase, the dNTP mix, the Taq Buffer, and the cDNA sample. What first happens is the forward primer binds to the single stranded cDNA and the Taq DNA Polymerase creates its complimentary strand making it into double stranded cDNA. We then increase the temperature to 95 degrees to denature the cDNA. When it cools to around 55 degrees both primers bind onto each strand and at around 65 degrees the Taq polymerase creates the complimentary strand for each sequence. We repeat these changes in temperature to keep multiplying the cDNA. The dNTP mix provides the amino acid ingredients to make up the new strands of cDNA, and the Taq buffer provides the optimal environment for the DNA polymerase to work.

Electrophoresis

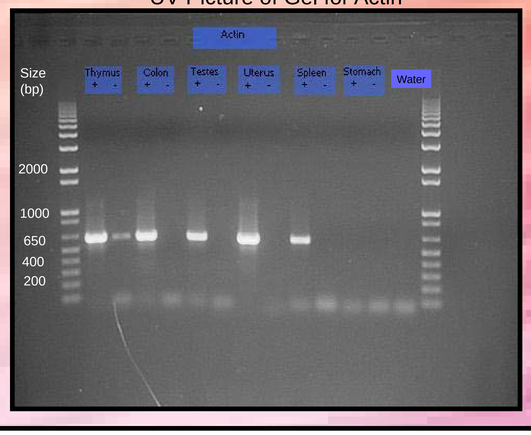
Before electrophoresis, a gel is created by heating Agarose in 1X TAE and adding Ethidium bromide as a stain. Wells are made in the gel while it is cooling to a solid and into those wells is where the amplified cDNA is placed. The gel is then placed in 1X TAE and run through an electrical current with a positive charge on one end of the gel and a negative charge on the other. The cDNA samples are placed in the negative end, and when the voltage is turned on the samples flow towards the positive end. This happens, because cDNA has a negative charge due to its phosphate groups. After the dye has run to the opposite end we take out the gel and take a picture of it under UV light.



PCR Control

Our control in the experiment to make sure that the PCR worked was the gene sequence for *Actin* which should be present in all the tissues we tested. ACTIN is a protein which combines into actin filament that helps form the cytoskeleton in cells. When I did the PCR I used the specific primers for *Actin*. On the picture of the gel a band of light under the positive lane (Polymerase added) means that the gene was expressed. Out of the 12 tissues tested 9 worked for the *actin* gene. We could then use the 9 that worked to test for *Transferase A* and *Fucosyltransferase.* The three with negative results will have to have cDNA synthesis or RNA isolation repeated and then have PCR done again and run through a gel. For every PCR I had a tube that contained no sample and so therefore should not have a band of light when put under UV light. If a band does appear it means that there has been contamination.

UV Picture of Gel for Actin



Results

After finding out which tissues worked for *Actin* I tested them for *Transferase A* and *Fucosyltransferase* by using the *Transferase A* and *Fucosyltransferase* Primers in the PCR process. Then I ran the samples through the gel and took a picture of the gel under UV light. Again if a band appeared under the positive lane it means that the gene is expressed in that tissue. I performed PCR for the 9 samples that had positive results on *Actin* with *Transferase A.* Out of the 9 only 3 tested positive for *Transferase A:* Heart, Colon, and Testes. The rest of the samples tested negative and did not show a band of light under UV light but to be sure the test needs to be repeated three times. When the 9 samples were tested for Fucosyltransferase only 2 samples tested positive, Thymus and Liver. These results must also be confirmed.

Summary of PCR Results

Mouse Tissue	*Actin (610bp)*	*Transferase A (360bp)*	*Fucosyltransferase (460bp)*
Kidney	Not Present	Not Tested	Not Tested
Intestine	Present	Not Present	Not Present
Heart	Present	Present	Not Present
Thymus	Present	Not Present	Present
Lung	Not Present	Not Tested	Not Tested
Liver	Present	Not Present	Present
Bone Marrow	Present	Not Present	Not Present
Colon	Present	Present	Not Present
Testes	Present	Present	Not Present
Uterus	Present	Not Present	Not Present
Spleen	Present	Not Present	Not Present
Stomach	Not Present	Not Tested	Not Tested

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