Laboratory 3: Got Lactase? Determination of your Lactase Genotype

Background Reading and Links for Laboratory 2:

2. *DNA to Darwin: Humans: Lactose tolerance*
   [http://www.dnadarwin.org/casestudies/5/](http://www.dnadarwin.org/casestudies/5/)

3. *2011 Holiday Lectures Bones, Stones, and Genes: The Origin of Modern Humans*
   Sarah Tishkoff’s lecture is relevant to mt, genetic origins, lactase
   [http://www.hhmi.org/biointeractive/evolution/lectures.html](http://www.hhmi.org/biointeractive/evolution/lectures.html)

Objectives of Laboratory 3:

1. Set up a PCR reaction with your buccal cell DNA for amplification of the regulatory region of the lactase gene
2. Characterize the PCR product using agarose gel electrophoresis
3. Digest your PCR product with BsmFI to determine genotype
4. Separate digest products on an agarose gel to determine sizes
5. Analyze whether your genotype is C/C, C/T, or T/T

Flow Chart for Laboratory 3:
Introduction

Lactose is the main carbohydrate in milk and is normally broken down in the intestine of mammals into galactose and glucose. The enzyme that performs this hydrolysis is lactase. Production of lactase is high in infancy when milk is the only source of nutrition. In most mammals, the production of lactase decreases after weaning. However, in some people the production of lactase persists at a high level throughout adulthood. This trait is known as lactase persistence, and people with persistent production of lactase are able to drink large quantities of milk without any complications. In humans who are lactase nonpersistent, they are not able to process the lactose in the milk, and usually show symptoms of lactose intolerance after consuming milk or milk products.

Scientists estimate that approximately 65-75% of the human population has a decreased ability to digest lactose as adults (they have lactose intolerance). Interestingly, this varies by population. The frequency of lactase persistence is very high (over 80%) in Northern European populations and some East African populations. It is very low in other populations such as West Africans, East Asians, and some Arab populations. The practice of farming and in particular raising cattle and dairy animals resulted in people drinking fresh milk beyond childhood. The advantage conferred to lactose-digesting individuals is thought to have been quite considerable. The mutations that allow the persistent production of lactase are calculated to have arisen between 3000-5000 years ago. Interestingly, different populations have different mutations that result in lactase persistence, which is a striking example of convergent evolution.

The precise mechanism that allows the continued production of lactase in is still not fully understood. The polymorphisms identified that are associated with lactase persistence do not fall in the lactase gene (LCT) coding region, but instead are located about 14,000 bases upstream of the gene in a region that is required for transcriptional activation. Lactase persistence is inherited as a dominant trait. The polymorphism identified in European populations is located at position 13910. A C at this position results in lactase non-persistence while a T at this position is associated with lactase persistence in about 90% of Europeans.

Materials

Buccal cell DNA prep
Ready-To-Go PCR bead tubes
Lactase DNA primer mix
Aerosol resistant tips (ART tips)
**Experimental Procedures**

Setting up a PCR Reaction for Your Lactase DNA: Each person will set up their own PCR reaction specific for the lactase regulatory region. Please change your gloves now before working with your DNA solution.

1. Label a clear 0.5 ml Ready-To-Go PCR tube with your photo ID number on the top and side of the tube using a fine-tipped black marker.

2. Using aerosol resistant tips and the “PCR ONLY” P200 Pipetman, add 23 µl of the prepared PCR primer mix to the Ready-To-Go tube. Aerosol resistant tips (ARTs) are used to minimize contamination.

3. Vortex or flick your Ready-To-Go tube to mix contents well. Please keep this tube on ice.

4. Obtain the tube of your buccal cell DNA from the front bench.

5. Using your P20 with a fresh 20 µl ART, transfer 2 µl of your buccal cell DNA to the Ready-To-Go clear PCR tube.

6. Flick the PCR tube gently with your fingers or gently vortex until the PCR bead is dissolved.

7. Pulse spin your tube in the microfuge.

8. Store your buccal cell DNA in your storage box in the freezer at -20 °C.

9. Put your PCR tube into the Techne automated thermal cycler that has been programmed for:
   - 1 minute 94°C denaturing
   - 30 cycles of the following PCR program:
     - Denaturing time and temperature: 30 sec at 94°C
     - Annealing time and temperature: 30 sec at 58°C
     - Extending time and temperature: 30 sec at 72 °C
   - 10 minutes at 72 °C final extension
INTRODUCTION

To confirm that you have successfully amplified your lactase regulatory DNA, you will run a sample of the PCR product in a 2% agarose e-gel. Following electrophoresis, you should be able to visualize a DNA band 448 base pairs in size. You will then digest this PCR product to determine if you are the C/C, C/T, or T/T genotype.

Materials

Ice and isotherm
Microfuge tubes
TE buffer (Tris-EDTA) or dH2O
10X DNA loading dye
2% agarose E-gel
100bp DNA ladder

Procedure

Preparing your sample for E-gel analysis
Please wear gloves for this experiment.

1. Obtain a small grey ice bucket and ice at the back of the lab.

2. Obtain the tube containing your amplified lactase PCR product and keep this tube on ice at all times.

3. Obtain a clean 0.65 ml tube from your Rainbow Pack and transfer 5 µl of your lactase PCR product to it.
   Remember to label this tube with your photo ID number.

4. Add 13 µl of TE and 2 µl of loading dye to this tube.

5. Finger flick to mix the contents and pulse-spin to bring the sample to the bottom of the tube.
Loading your sample and Electrophoresis

E-gels are pre-cast bufferless gels. The E-Gels that we are using contain ethidium bromide to stain the DNA. While the E-gel is contained within a cassette, it is best to wear gloves when you are working with E-Gels.

The class will share 2 E-Gels. 11 people can load their sample on each gel.

1. **One person from each gel group should follow the directions in the Appendix to correctly snap the E-gel into the base unit. Remove the comb that is in the wells.**

2. **One person from each gel group should load 20 µl of a 100 bp ladder into one lane of that groups’ gel.**

3. **Each person should load 20 µl of the sample they just prepared into one well in the order of your photo ID.**
   
   Be careful not to introduce bubbles into the wells as this can affect how the gel runs. Also be sure to record the lane number into which you loaded your sample.

4. **Add 20 µl of water to all empty wells.**

5. **Start the electrophoresis by pressing the 30-minute button.**

6. **When the electrophoresis is complete, remove the gel cassette from the apparatus.**

7. **Place the cassette on top of the UV transilluminator and photograph your gel.** Check to make sure the camera is set to 4.5 (f stop) and 2 (an exposure of ½ second). Take 10 photographs and three extra for a total of 13 for each let.
   
   If you want a longer exposure, set the camera to 4.5(f stop) and 1 (for an exposure time of 1 second).

8. **You should be able to identify a band that is 448 base pairs. If no such band is visible in your lane, please speak with Dr. Sliski immediately.**

9. **If you have a 448 bp product proceed to set up the digest of your PCR.** Based on the intensity of the band, you may need to add more or less of your PCR into the restriction enzyme digest.
1. Please wear gloves for this part of the lab.

2. Using a 0.65 ml rainbow pak tube add the following components:

   DNA - 10 µl of lactase PCR product
   water -7 µl
   10x Buffer -2 µl
   BsmFI - 1 µl (enzymes are stored in stratacoolers at the front of the lab)

   **when adding the buffer and enzyme to your digest dispense the small volume onto the side wall of the tube. This gives you visual confirmation that you have added the component. You can cap the tube and “wrist flick” the tube to bring the contents to the bottom.

   FastDigest BsmFI
   5’...G G A C (N)₁₀↓...3’
   3’...C C T G (N)₁₄↑...5’

   Thermo Scientific # FD1814

3. Pulse-spin the tube.

4. Place your tube in the 40 °C water bath for 20 minutes.

5. The class will again share gels to analyze the products of the digestion.

6. One person from each gel group should prepare an E-Gel and one person should load a 100 bp ladder onto the gel.

7. When your digest is finished add 2 µl of loading dye directly to the digest.

8. Mix and pulse spin this sample.

9. Load 20 µl of your digest onto the E-gel. Be sure to record which lane you have loaded your sample into.

10. Once everyone has loaded their samples fill any empty wells with 20 µl of water.

11. Start the electrophoresis by pressing the 30-minute button and run until the dye is 2/3 of the way down the gel.

12. Wearing gloves, remove the gel cassette and visualize the DNA by placing on a UV light box. Please take enough photographs for each person and 3 additional photos.
Compare your results with the results below.

Genotype C/C will have bands sized 351 and 97 bp

Genotype C/T will have 351, 253 and 97 bp bands

Genotype T/T will have 253 and 97 bp bands

LABORATORY 3 ANALYSES AND DISCUSSION QUESTIONS

1. Explain briefly why there are 30 cycles in the PCR protocol.

2. What is the purpose of the 10 minute incubation at 72 °C?

3. What is the exact DNA sequence to which each primer anneals for amplification of the lactase control region? Be sure to mark the 5’ and 3’ ends of the target sequence. (Be careful in answering this question. It may be helpful to draw a diagram of the locus, the location of the primers, and the three reactions in a typical PCR cycle before answering.)
   Primer sequences:
   Primer 1: 5’ GGATGCACTGCTGRGATGAG 3’
   Primer 2: 5’ CCCACTGACCTATCCTCGTG 3’

4. The percentage of GC base pairs is an important factor when designing PCR primers. Should the percentage of GC base pairs be high or low? Explain your answer briefly. What is the percentage of GC base pairs in Lactase primer 1? What is the percentage of G/C base pairs in Lactase primer 2?