BIOLOGY 1200

VANCOUVER COMMUNITY COLLEGE

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Lab demonstrator: Robyn Wood

February 2021– hybrid course

Lab: DNA lab – The Genetics of Taste

Outline

- Objectives
- Overview
- Background
- Materials for the lab
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- Demonstrations
- Gel electrophoresis
- Gels

Objectives

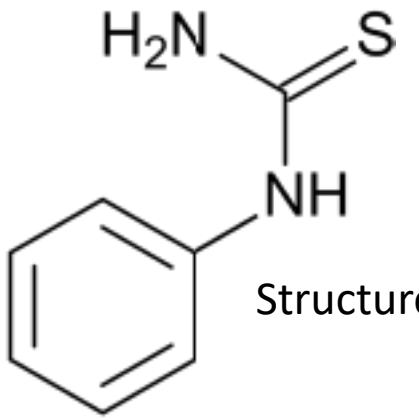
1. Learn the techniques of DNA extraction and analysis.
2. Describe the use of PCR.
3. Describe the use of gel electrophoresis.
4. Conduct a DNA experiment and analyze results

Overview

- In this experiment – students isolate their own DNA and use PCR to amplify a segment of the *TAS2R38* gene, which is responsible for detecting the biter taste of PTC. Digestion of the PCR products and analysis by gel electrophoresis are used to differentiate tasters and non-tasters. Finally, genotype is linked to phenotype by tasting the PTC paper.
- In lab work: 3 students, the instructor and lab demonstrator extracted their DNA, separated it from cells, and prepared it for PCR.
- Robyn will load the gels with everyone's DNA (digested and undigested), a ladder and control on Wednesday.

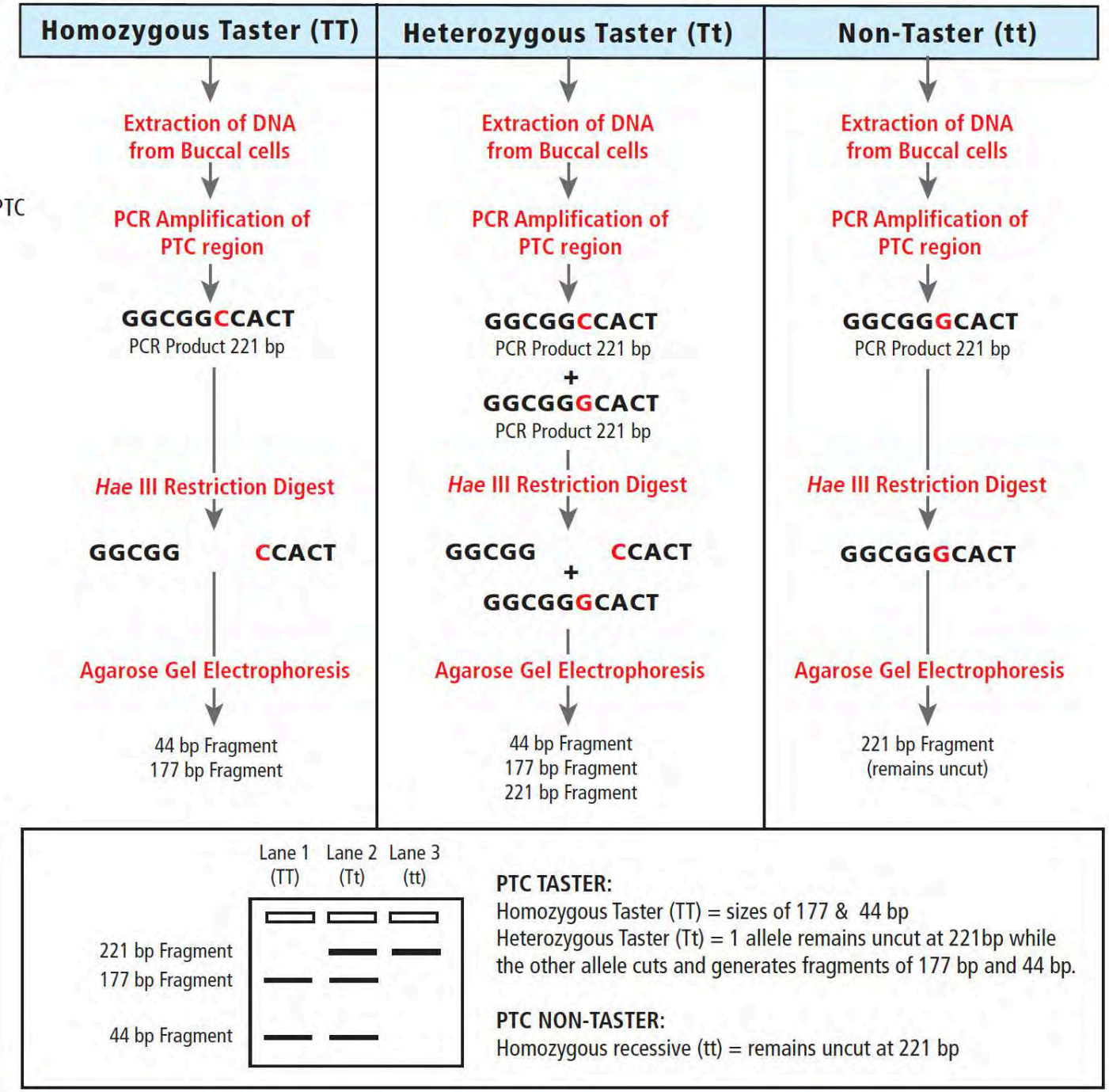
Background

- A SNP is a single nucleotide polymorphism, when nucleotides differ at a particular site on a strand of DNA. A G, instead of an A for example.
- A SNP occurs at position 145 on the Phenylthiocarbamide (PTC) sensitivity gene *TAS2R38.* The dominant allele T confers the ability to taste PTC, the non-taster allele t is recessive. In the population, 70% of people tested can taste PTC.
- The two alleles differ in SNPs at 3 locations – 145, 785, and 886.
- A restriction enzyme, *HAE*111, will be used to cut The Taster T allele.



Structure of PTC

Figure 3:
Determining PTC Genotype



Background 2

- PCR – polymerase chain reaction – will generate 1000's to 1000,000's of copies of a particular DNA sequence, by
  - using 2 primers to target the sequence. They correspond to the start and end of the targeted sequence.
  - In addition adding four deoxynucleotides, and a thermally-stable DNA polymerase. (*Taq* polymerase is purified from the thermophilic bacterium *Thermus aquaticus* that inhabits hot springs – stable at near-boiling temps.)
  - Putting all ingredients into a thermal cycler which will
    - Heat and cool in cycles, sequentially disrupting H bonds at 94^{o}Cand separating DNA strands, cool to attach the primer, then rise to 72^{o}C so Taq polymerase can add nucleotides to a growing complementary strand.

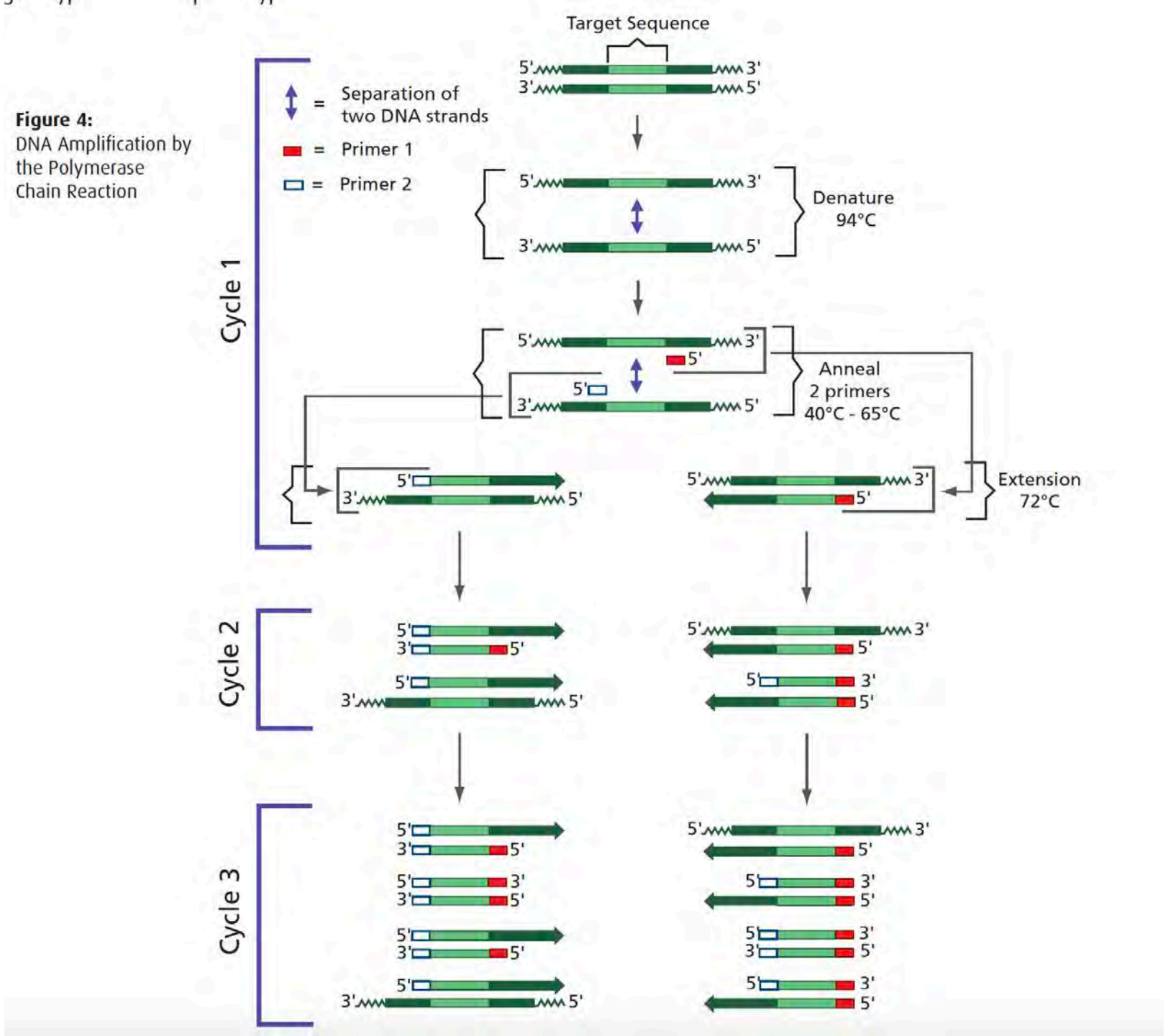


Figure 4:
DNA Amplification by the Polymerase Chain Reaction

Materials for the lab

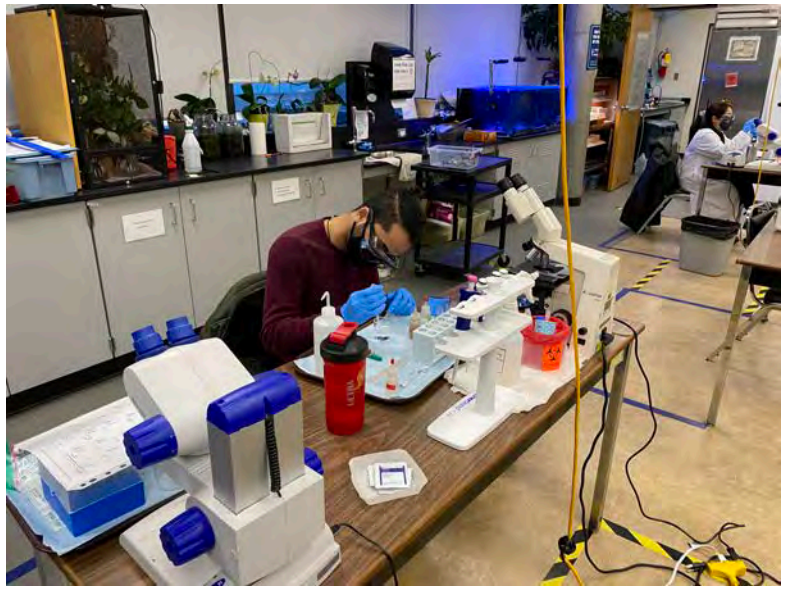
- Lab manual handout. All the steps are outlined in the handout.
- Robyn gave each student a diagrammed series of steps for the experiment.
- Each student had a separate station including:
  - 1.5 mL tubes, PCR tubes
  - Four automatic pipettors with different ranges of volumes
  - Biohazard container
  - Centrifuge
  - Loading dye
  - Bead in a small tube


Finnpipette® II
100-1000 µl
Finnpipette® II
10-100 µl
FINNPIPETTE® II
2-20 µl
Finnpipette® II
0.2-2 µl
Kimwipes
BIOHAZ


Fisherbrand
Fisherbrand Elite
KIMTECH
Fisherbrand
Specialty Tips - Micro
0.1-10µL
Cat. No. 21-277-2B
Fisher Scientific
1.5 mL tubes
ISOLATE DNA BY SALINE MOUTHWASH
Supplies and Equipment
Permanent marker
Micropipet and tips (1–100 µL)
Microcentrifuge tube rack
Thermal cycler
Container with cracked or crushed ice
Waste
Sterile Cotton Tipped Applicator
Puritan
SHARPIE permanent


RWV


SAVANNA
DESERT
TUNDRA
TAIGA
GAS SHUT OFF



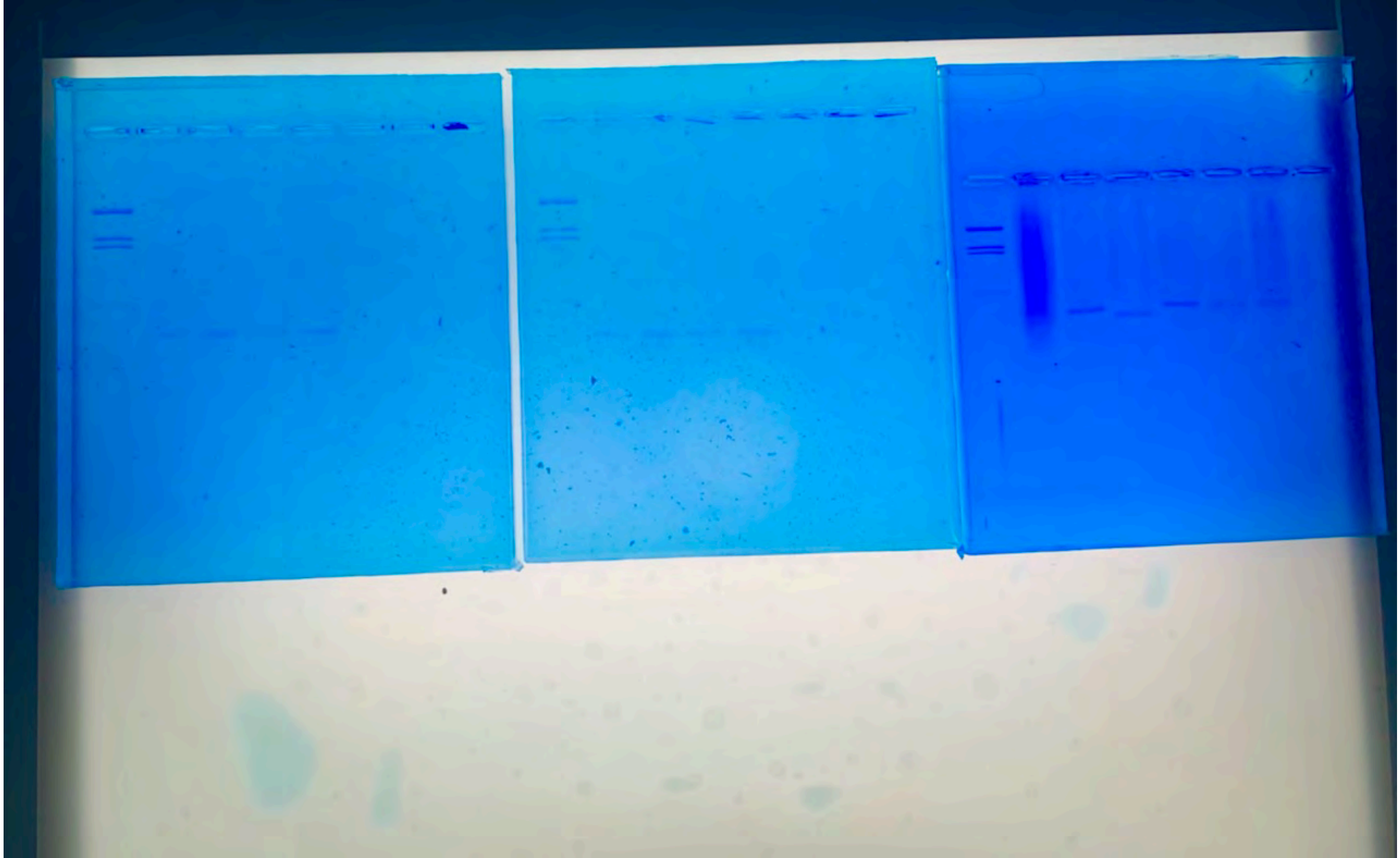

PRECISION

Demonstrations

- Robyn demonstrated:
  - DNA removal from mouth with saline solution swish and swab.
  - Removing and centrifuging a sample to isolated only cells.
  - Mixing the cells and heating to break the membranes, centrifuging again to isolate DNA in the supernatant.
  - Combining DNA with loading dye, and bead with all necessary ingredients for PCR.

PCR and Gel electrophoresis

- Robyn demonstrated:
  - The PCR process for the students' DNA (review PCR in the lab handout)
  - Setting up gel electrophoresis – adding DNA (review the process in the lab handout)
  - The result of electrophoresis including past gels (photos next slide)



Use this diagram to draw your graph of distance versus DNA size in base pairs, and use your graph to find the size of the undigested DNA , and the taster and non taster fragments.