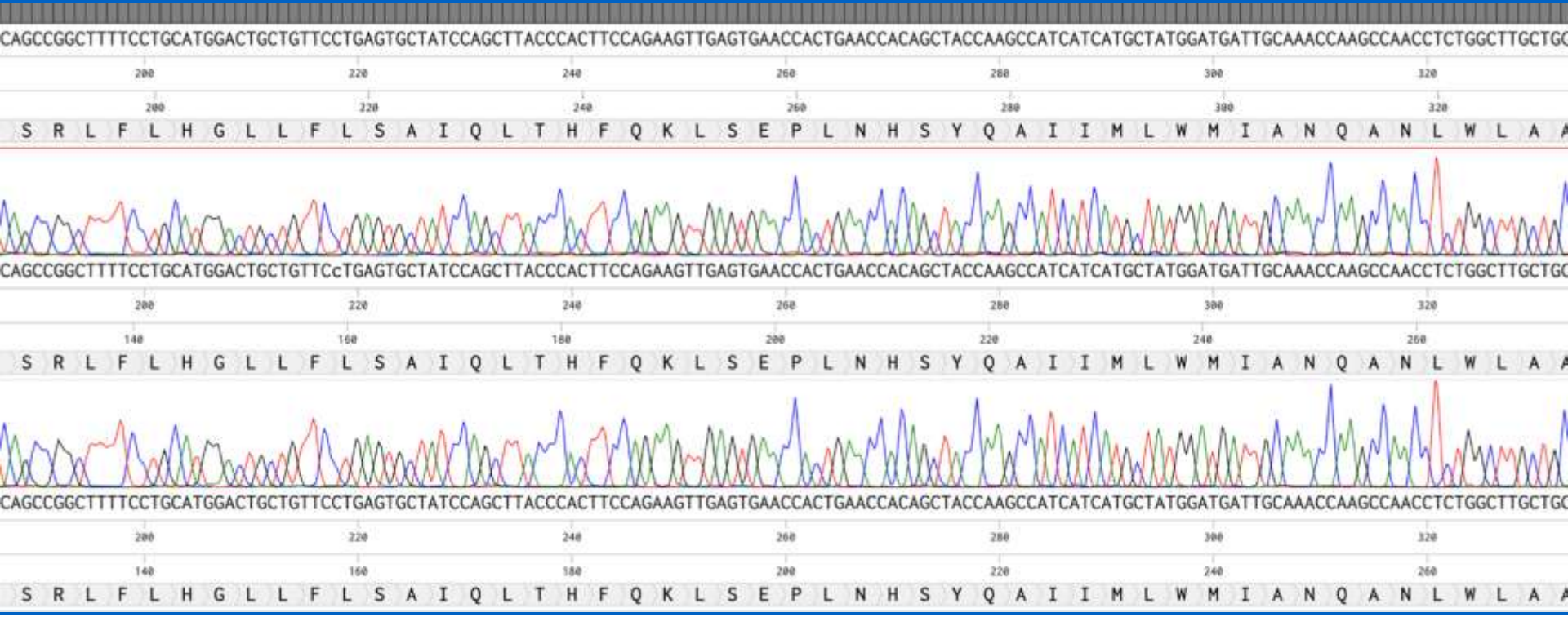
Exploring Precision Medicine

- Chapter 1: What's the Right Medicine?
- Chapter 2: Is My Sense of Taste Controlled by my Genes?
- Chapter 3: Exploring Our DNA
- Chapter 4: How Is DNA Sequenced, and What Can We Learn?
- Chapter 5: Restriction Enzyme Digestion of TAS2R38 PCR Products
- Chapter 6: Gel Electrophoresis and Genotyping
- Chapter 7: SNPs and Drug Metabolism

Chapter 4: How is DNA sequenced, and what can we learn?



Exploring Precision Medicine: Activities

AMGEN Biotech Experience
Scientific Discovery for the Classroom

Sample Our Own DNA

Take a sample of cheek cells, isolate DNA, and amplify a short sequence of the bitter taste gene.

Sequence Analysis

Use bioinformatics software to explore the bitter taste gene and how genotypes can be distinguished.

Is Taste Genetic?

Begin to explore a trait (taste) and investigate whether people experience it differently.

UNDERSTANDING PRECISION MEDICINE

In this module, we explore the genetics of the ability to taste bitter substances. It turns out that even small differences in our DNA—our genotype—can lead to major differences in traits—our phenotype. All of our genes have such individual differences, and some lead to changes in medically important traits. Advances in DNA sequencing and bioinformatics have made it much easier to discover these differences. Similarly, understanding how each of us metabolizes medications differently allows doctors to practice precision medicine—medicine based on each individual's genotype.

Restriction Enzyme Digest

Use restriction enzyme digestion to prepare to determine our bitter taste genotypes.

Medical Mystery

Explore the idea that people respond differently to medications and consider the possible reasons.

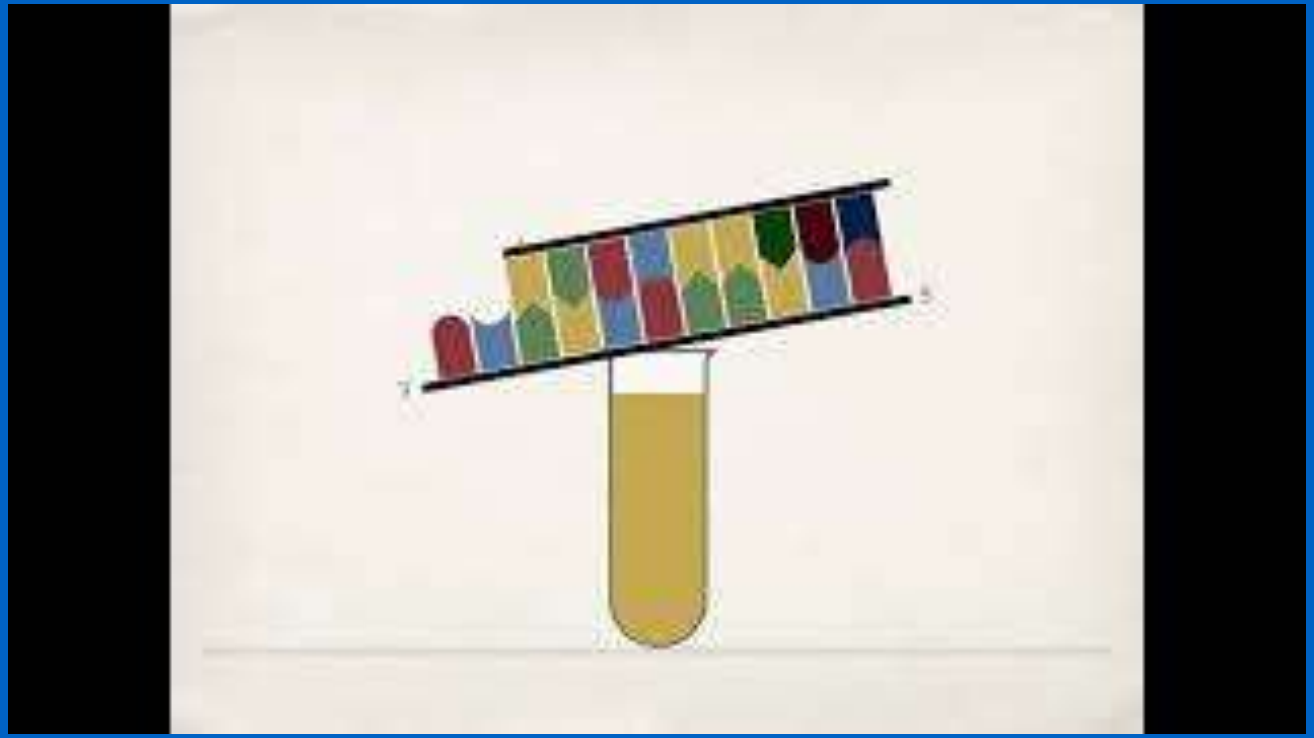
Investigate Genetics of Drug Metabolism

Investigate the genetics of drug metabolism and consider how genotyping can aid medical treatment.

Gel Electrophoresis

Use gel electrophoresis to determine our bitter taste genotypes.

Video: The Sanger Method of DNA Sequencing

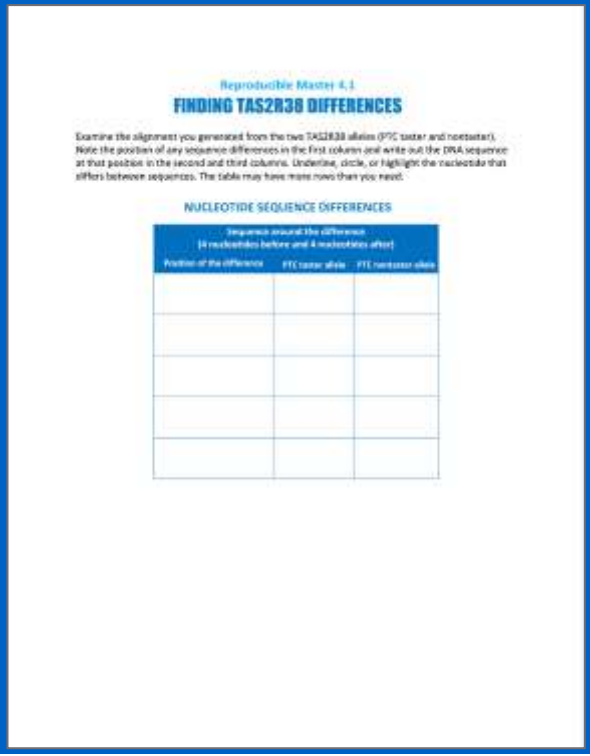


What underlies the ability to taste bitter things?

- What kind of genetic difference do you think separates bitter taster and nontaster alleles?
- We will use DNA analytic software to explore this difference.

Activity: Finding TAS2R38 differences

- **Learn** how to use NCBI BLAST
- **Align** a taster and nontaster **DNA sequence** of TAS2R38
- **Look** for single nucleotide variants
- **Record** differences on **RM 4.1**
- **Repeat,** this time with a taster and nontaster **amino acid sequence**



Reproducible Master 4.1

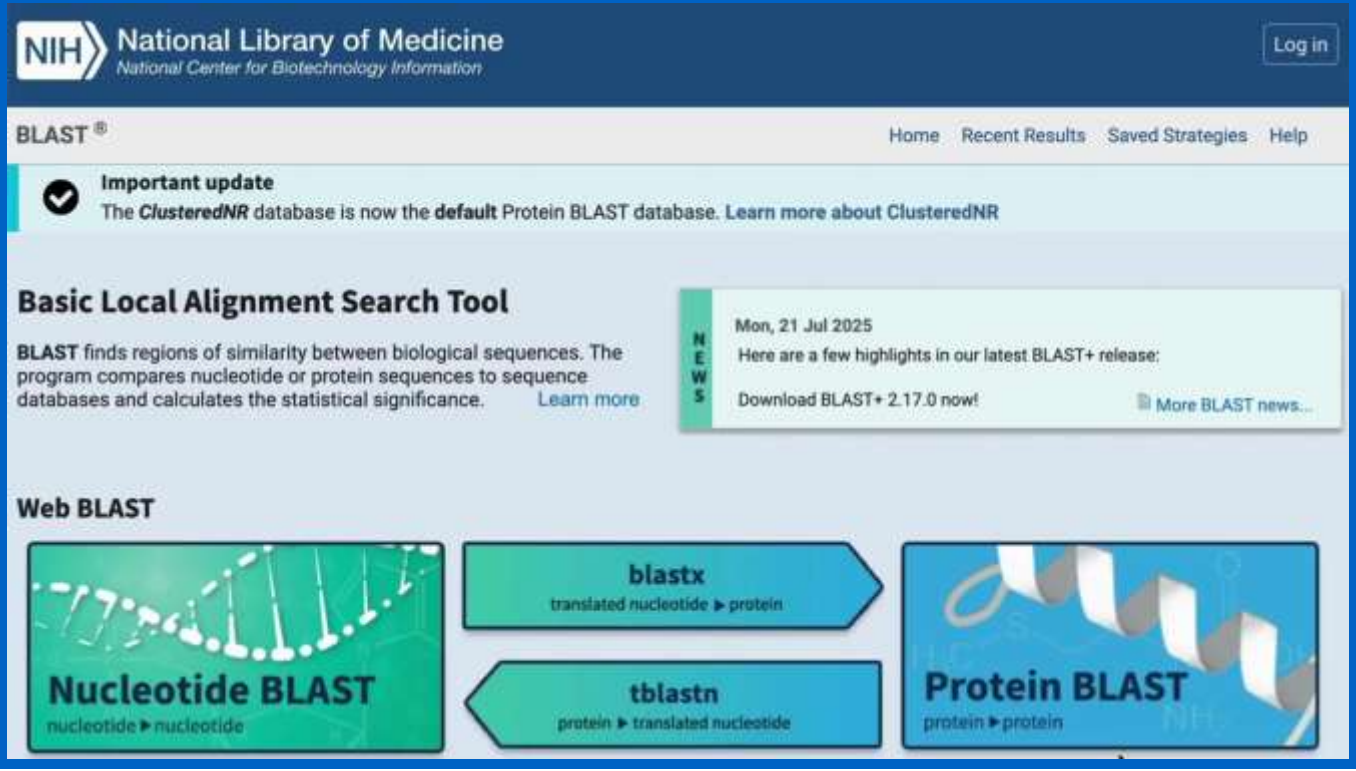
FINDING TAS2R38 DIFFERENCES

Examine the alignment you generated from the two TAS2R38 alleles (PTC taster and nontaster). Note the position of any sequence differences in the first column and write out the DNA sequence at that position in the second and third columns. Underline, circle, or highlight the nucleotide that differs between sequences. The table may have more rows than you need.

NUCLEOTIDE SEQUENCE DIFFERENCES

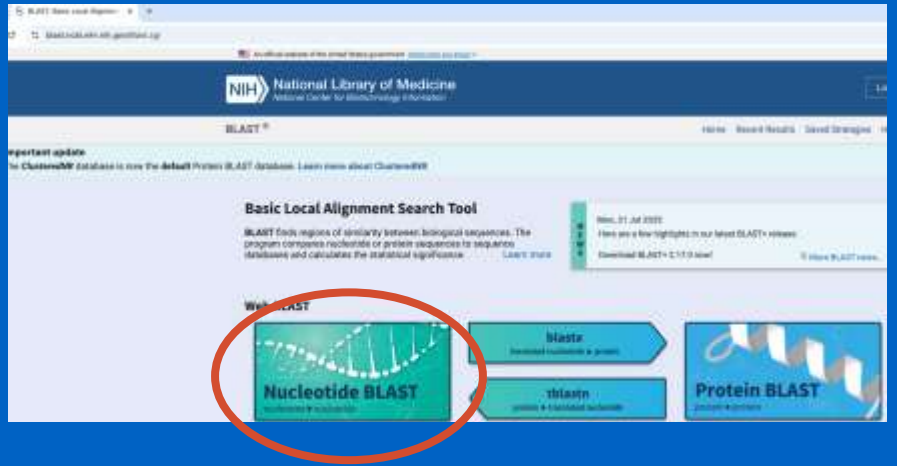
Position of the difference	Sequence around the difference (4 nucleotides before and 4 nucleotides after) PTC taster allele	PTC nontaster allele

Tutorial: How to use NCBI BLAST



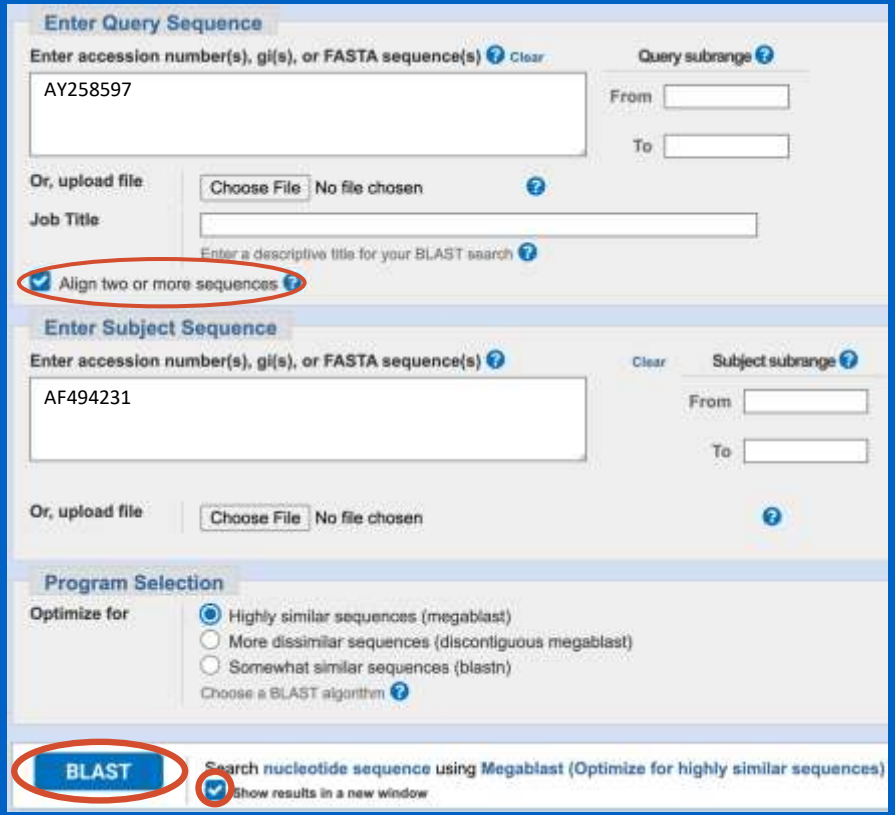
How to use BLASTn

- Navigate to blast.ncbi.nlm.nih.gov
- Click on “Nucleotide BLAST”



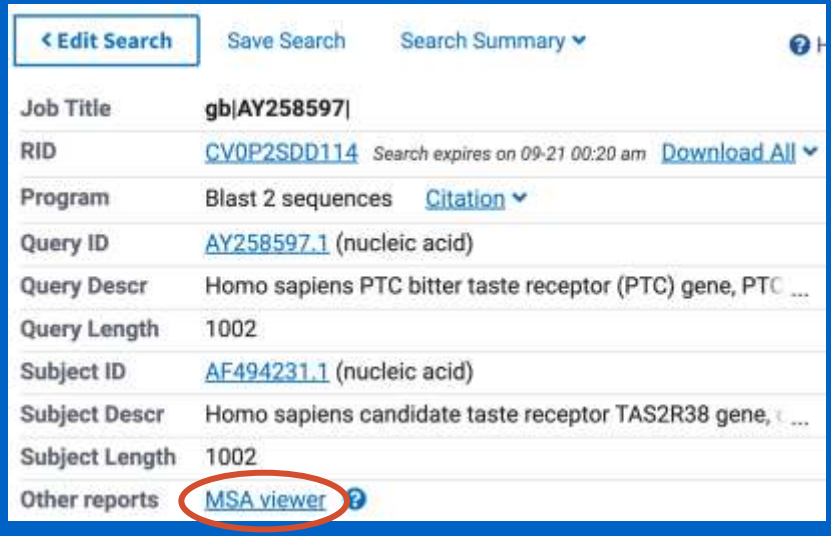
How to use BLASTn

- Click on “Align 2 or more sequences”
- Enter these GenBank DNA accession numbers:
  - PTC taster: AY258597
  - PTC nontaster: AF494231
- At the bottom of the page, select “Show results in a new window,” then click on the “BLAST” button



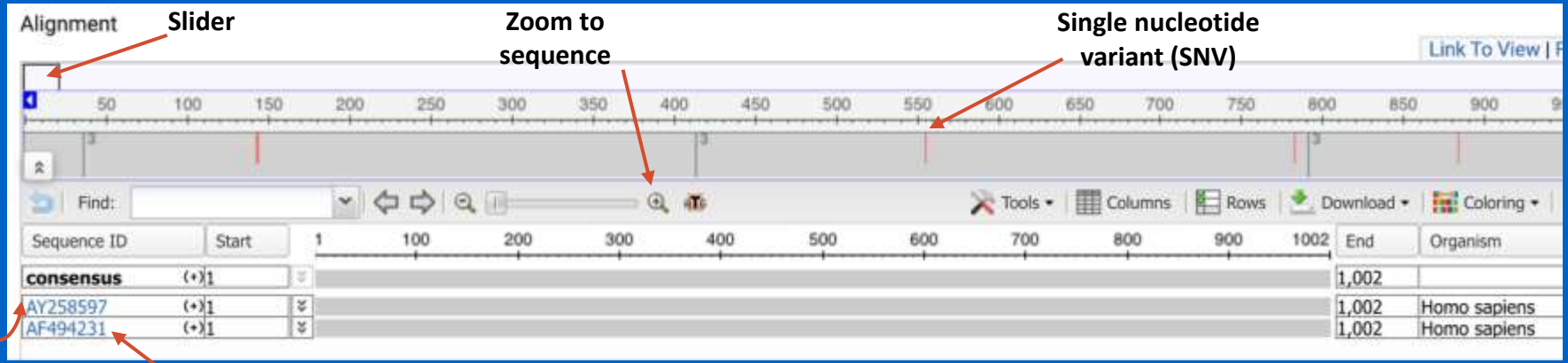
How to use BLASTn

- On the new page, click “MSA Viewer” next to “Other Reports”
- Your sequence alignment will appear in a new window



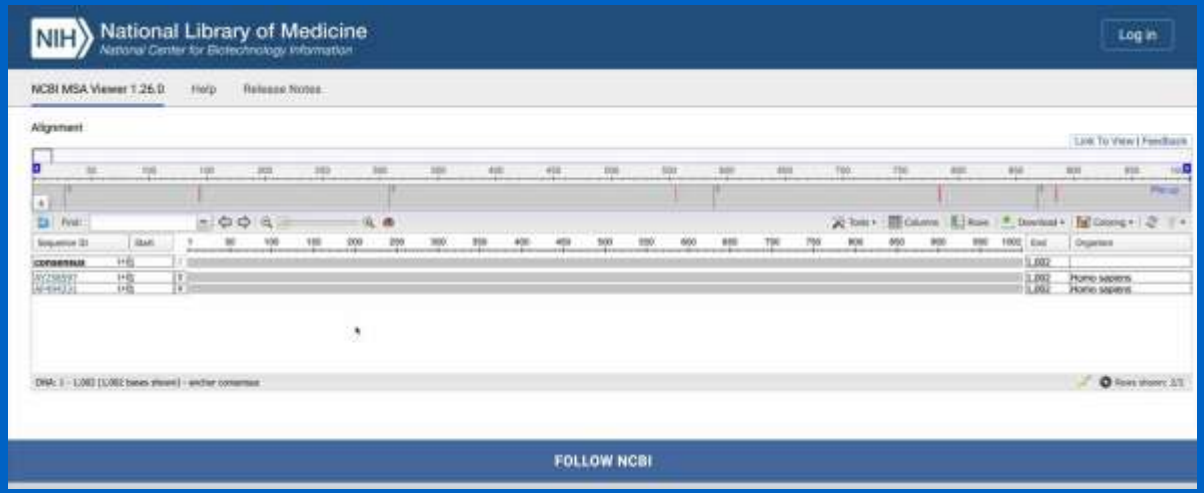
How to use BLASTn

Navigate through your nucleotide sequence alignment



How to use BLASTn

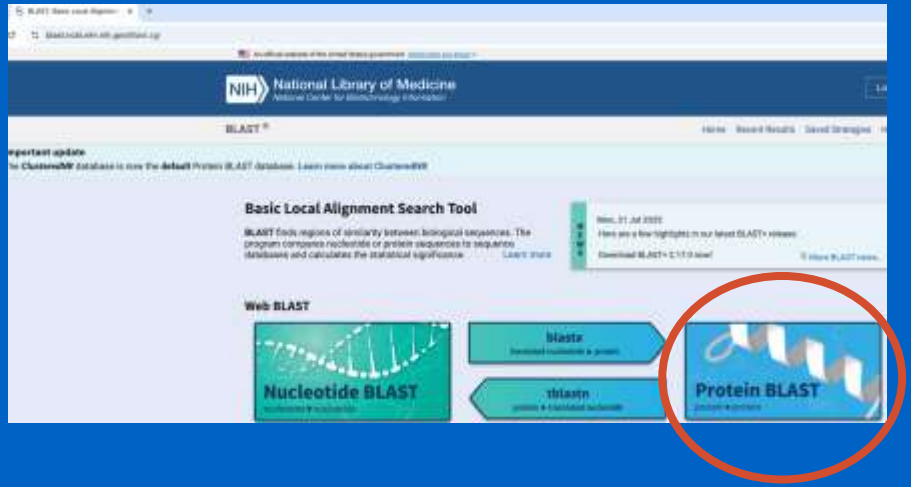
- Zoom to sequence and move the slider to identify the position and sequence of SNVs.



- Record your findings on **RM 4.1**.

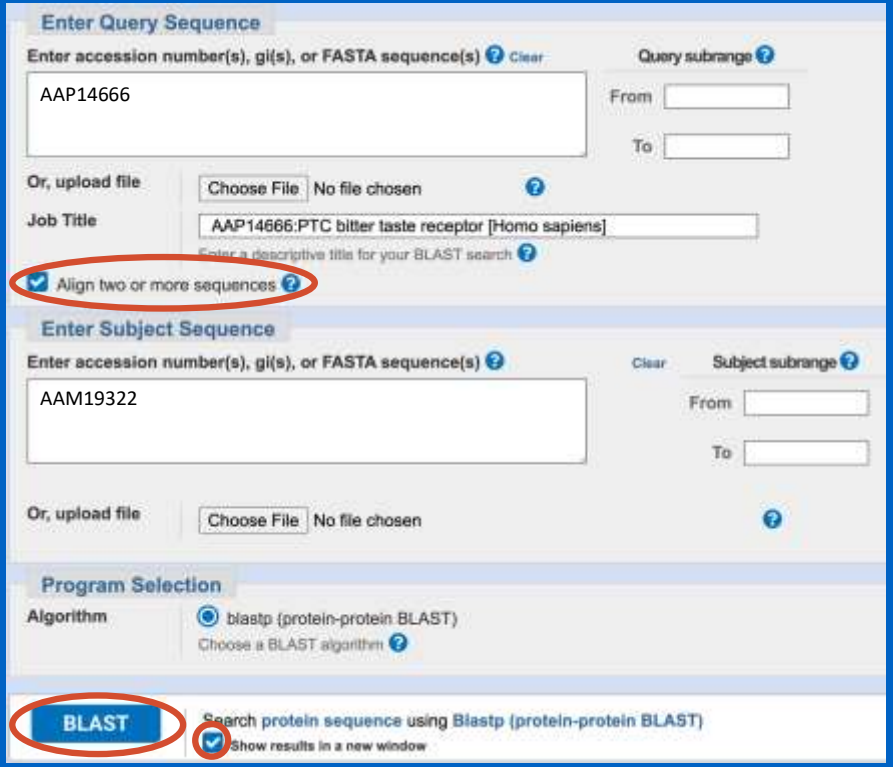
How to use BLASTp

- Navigate to blast.ncbi.nlm.nih.gov
- Click on “Protein BLAST”



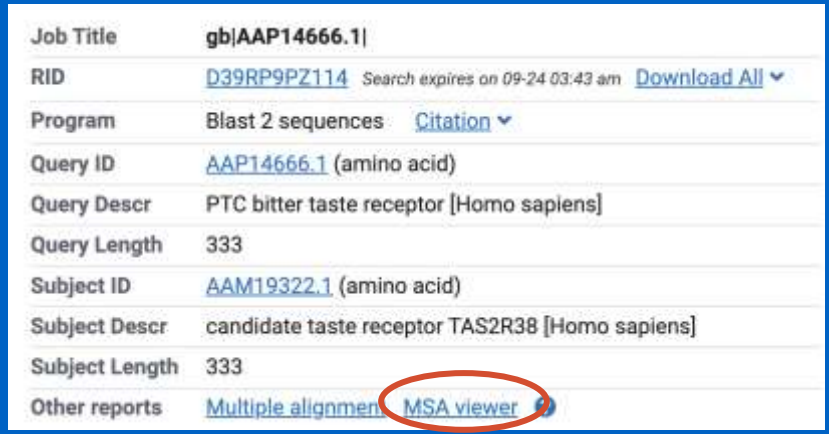
How to use BLASTp

- Click on “Align 2 or more sequences”
- This time, enter these GenBank amino acid accession numbers:
  - PTC taster: AAP14666
  - PTC nontaster: AAM19322
- At the bottom of the page, select “Show results in a new window,” then click on the “BLAST” button



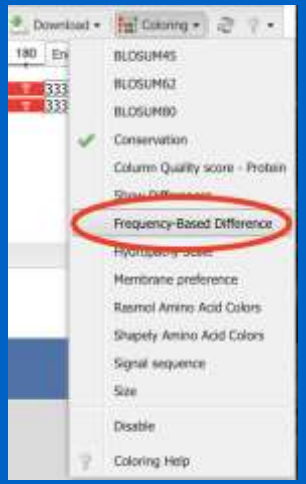
How to use BLASTp

- On the new page, click “MSA Viewer” next to “Other Reports”
- Your sequence alignment will appear in a new window



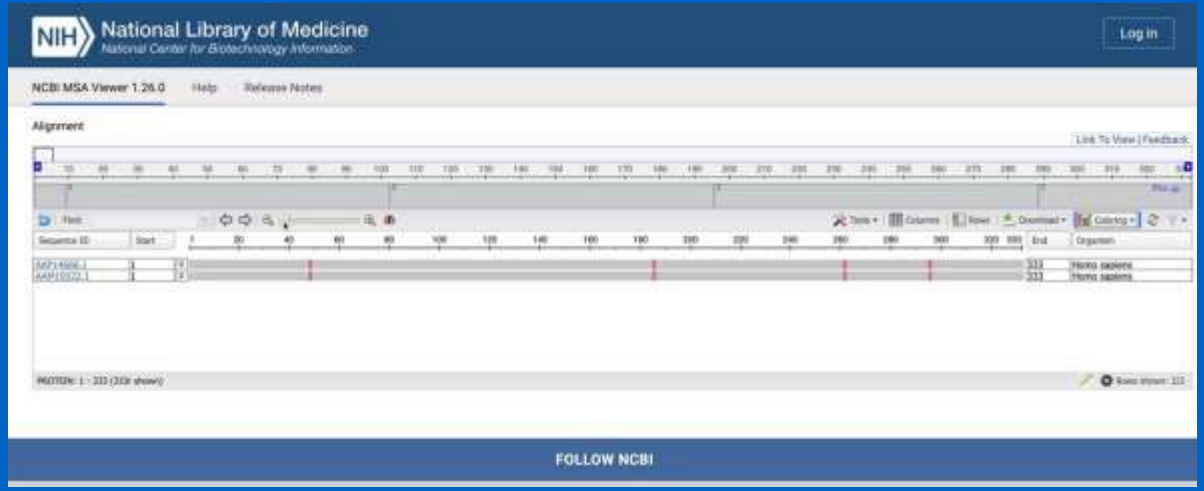
How to use BLASTp

- Under the “Coloring” menu, select “Frequency-Based Difference”
- Amino acid substitutions will be visible as vertical red lines



How to use BLASTp

- Zoom to sequence and move the slider to identify the position and sequence of amino acid substitutions.



- Record your findings on **RM 4.1.**

Review RM 4.1: What *nucleotide* differences did you find?

Sequence around the difference (4 nucleotides before and 4 nucleotides after)		
Position of the difference	**PTC taster allele**	**PTC nontaster allele**
145	GCAG**C**CACT	GCAG**G**CACT
557	CAGA**T**TAAA	CAGA**A**TAAA
785	TGTG**C**TGCC	TGTG**T**TGCC
886	AGCC**G**TCCT	AGCC**A**TCCT

Review RM 4.1: What *amino acid* differences did you find?

Sequence around the difference (1 amino acid before and 1 amino acid after)		
Position of the difference	**PTC taster allele**	**PTC nontaster allele**
49	Q**P**L	Q**A**L
186	Q**I**K	Q**N**K
262	C**A**A	C**V**A
296	A**V**L	A**I**L

What are single nucleotide variants (SNVs)?

- Changes in single nucleotides at the same position in a gene between individuals are known as **single nucleotide variants,** or **SNVs**
- SNVs arise due to errors in DNA replication during cell division, or from mutagenesis
- Some SNVs result in changes to a gene's amino acid sequence (missense) while others do not (synonymous)
- SNVs are only heritable if they occur in germ cells

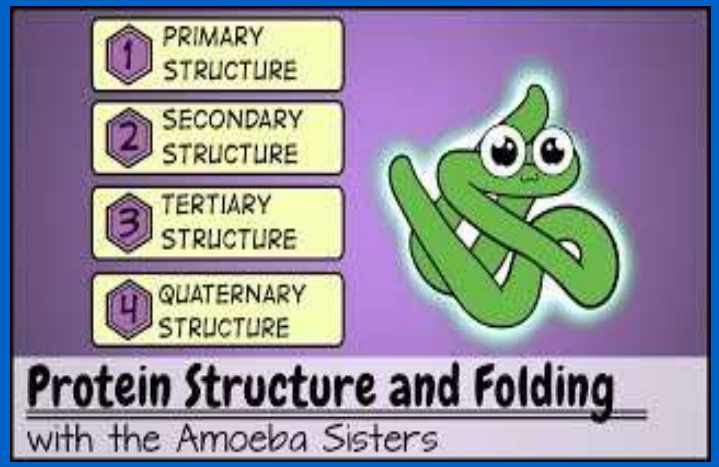
SNPs vs. SNVs: What's the difference?

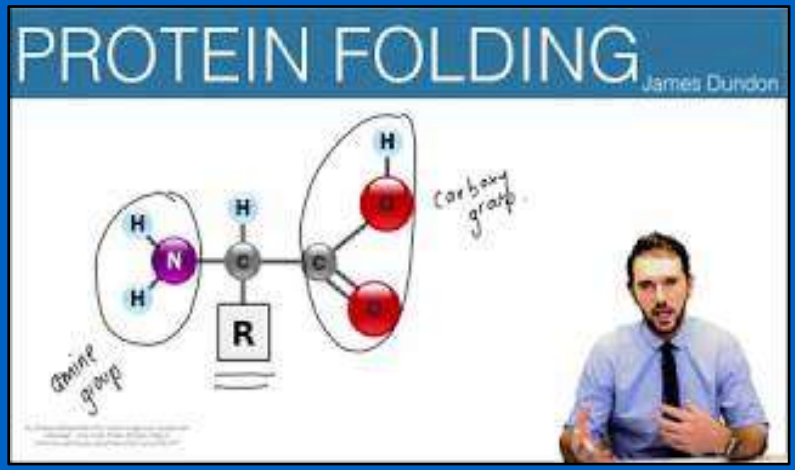
- **SNVs** = **S**ingle **N**ucleotide **V**ariants
- **SNPs = S**ingle **N**ucleotide **P**olymorphisms
- SNPs are SNVs that occur in a population at 1% frequency or greater
- If single nucleotide substitutions change amino acids, they can alter the shape and function of proteins

How do amino acid substitutions affect proteins?

To understand, you need to learn how proteins fold

Videos: How proteins fold





What is the relationship between certain SNPs and protein shape?

- A SNP that changes an amino acid may change protein folding
- Different R groups have different polarity and affinity for water
- This causes variant amino acids within the protein to bind differently to each other
- Result: potential change to both secondary and tertiary structure

What happens if a SNP alters a protein's folding?

- A protein's function depends on its 3D structure
- If the structure changes, its function may change
- For example, a mutant receptor may no longer accept a ligand

Are SNPs the only mutations that affect gene function?

- Other mutations can affect gene function:
  - Insertions
  - Deletions
- Larger chromosomal rearrangements may disable a gene:
  - Translocation
  - Duplication
  - Inversion
  - Deletion

Could SNPs have health consequences?

Does every SNP affect protein structure or function?

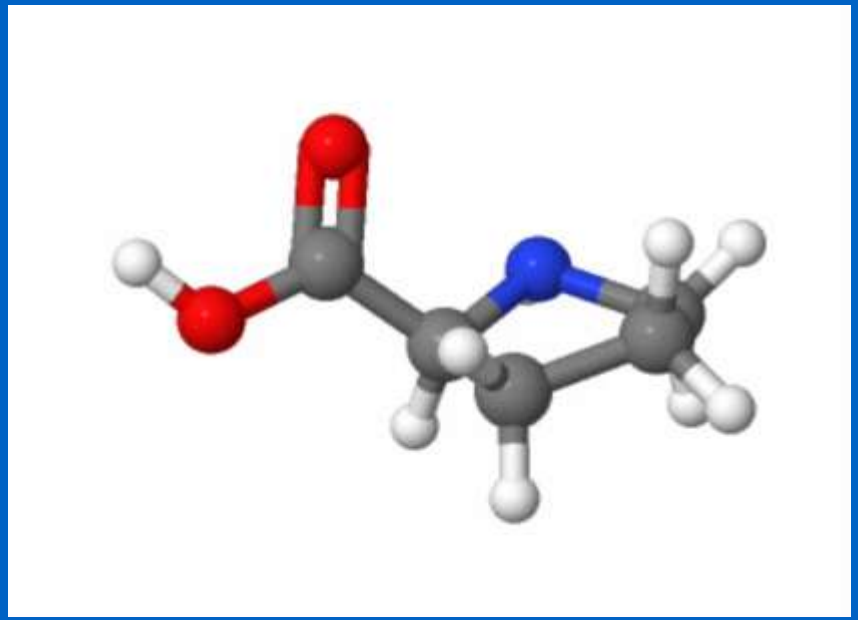
- Some (“silent”) mutations don’t change amino acid sequence
- Other SNPs may occur in noncoding, regulatory regions
  - These can affect level/location of protein expression

The TAS2R38 SNP at nucleotide 145

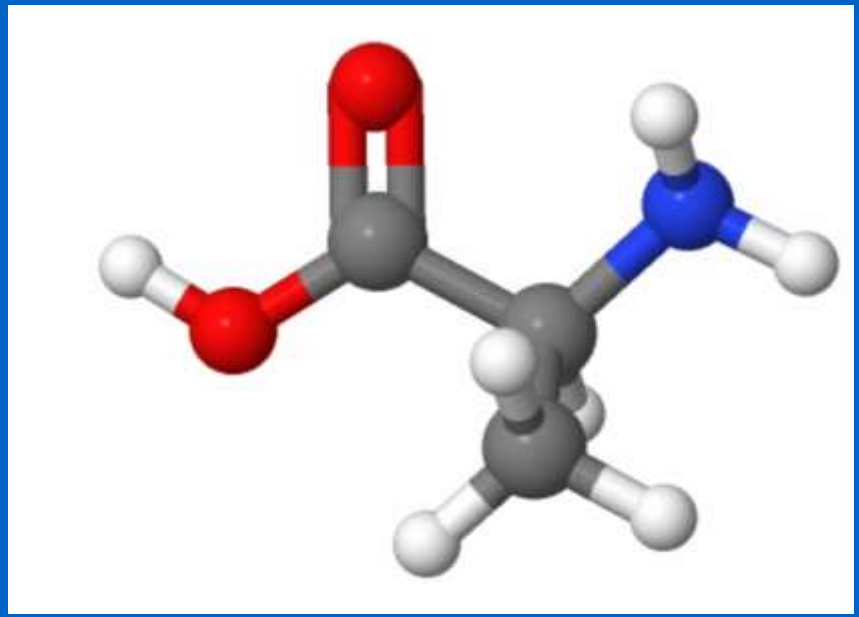
Sequence around the difference (4 nucleotides before and 4 nucleotides after)		
Position of the difference	**PTC taster allele**	**PTC nontaster allele**
145	GCAG**C**CACT	GCAG**G**CACT

Sequence around the difference (1 amino acid before and 1 amino acid after)		
Position of the difference	**PTC taster allele**	**PTC nontaster allele**
49	Q**P**L	Q**A**L

Side-by-side comparison of proline and alanine

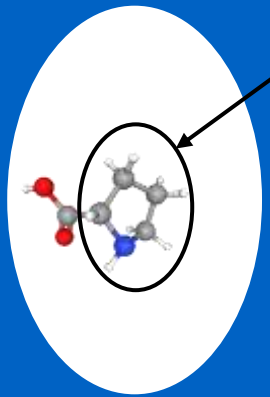


Proline



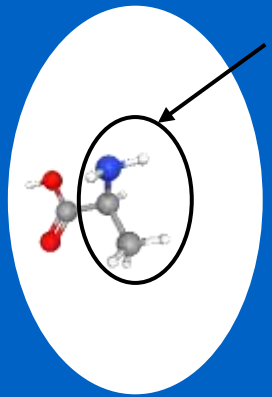
Alanine

How the R groups of proline and alanine differ



R group is a ring. Can't fit in an α helix

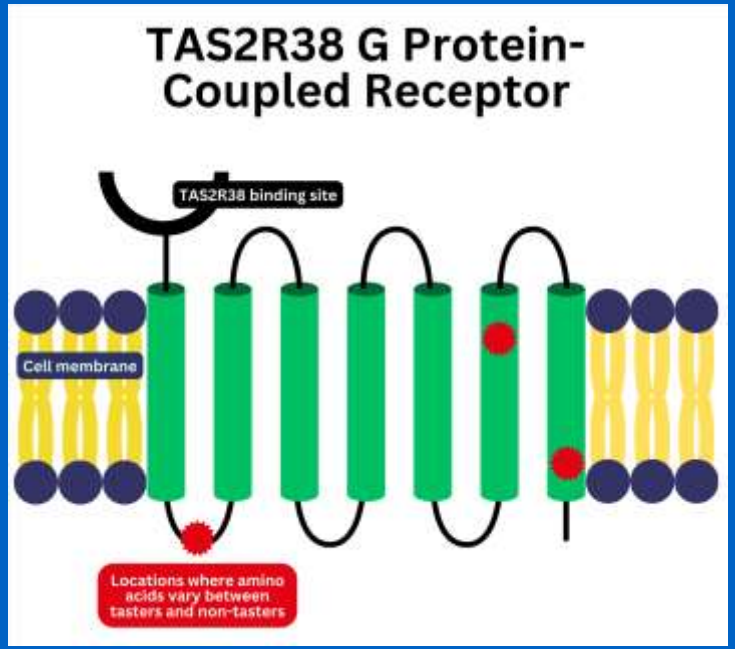
Proline

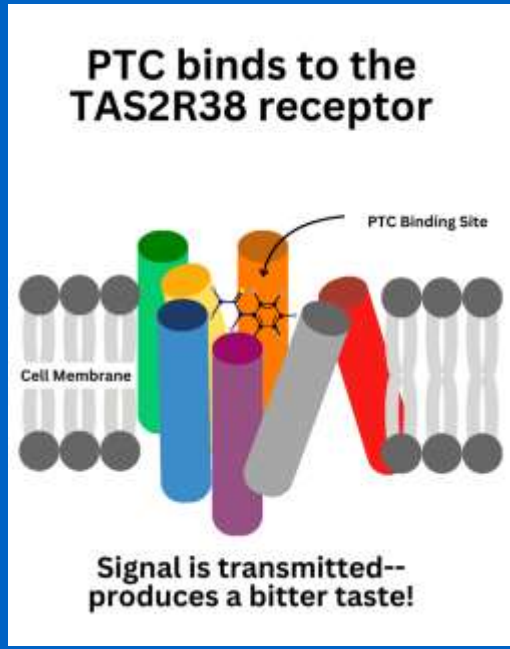


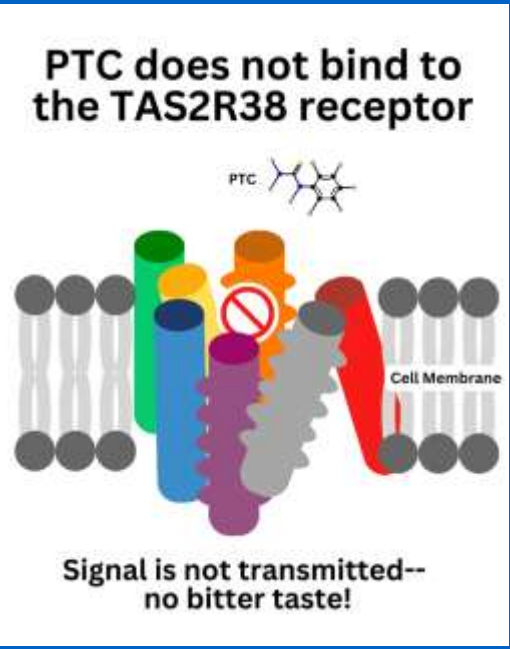
R group is a methyl group. Can appear in either an α helix or a β pleated sheet

Alanine

Location of the important SNPs of TAS2R38



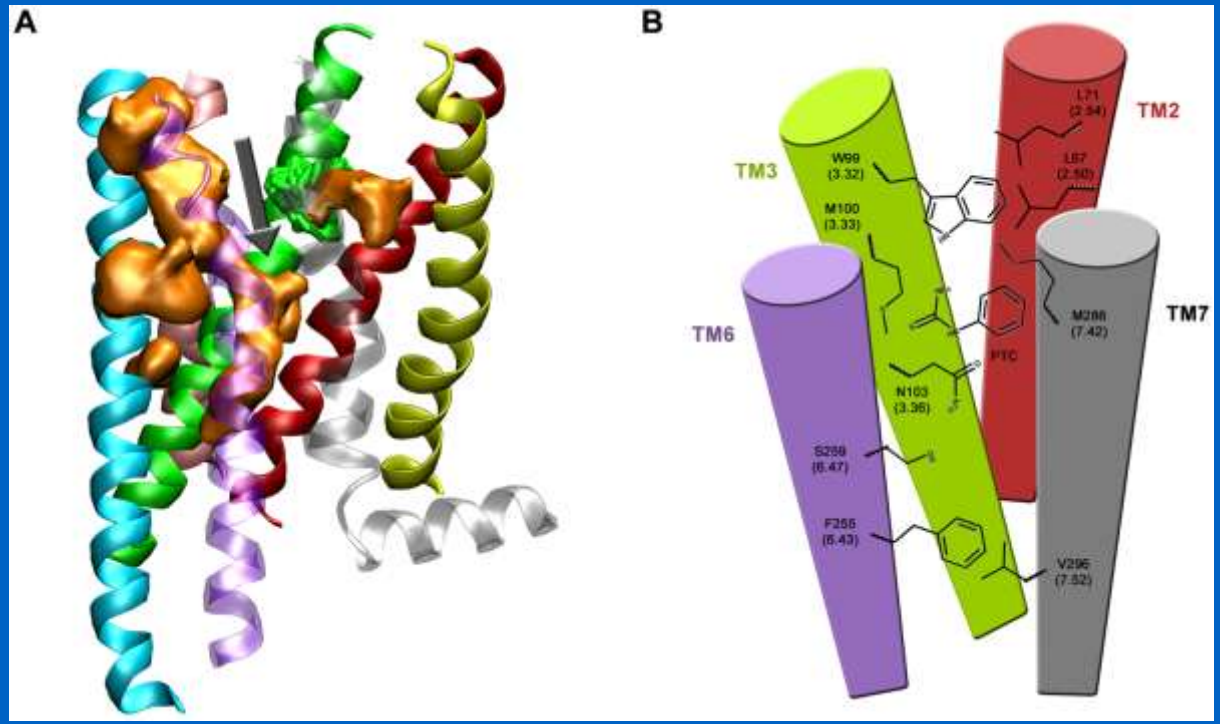




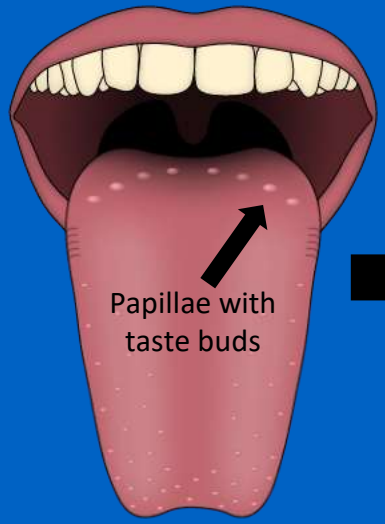
What about the single nucleotide variant at position 557?

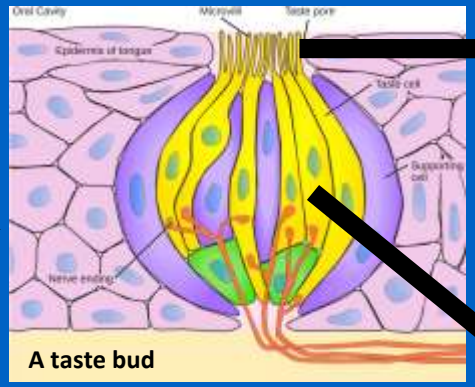
- Found at low frequency in populations
- Not associated with a change in taster phenotype
- Although there is an amino acid substitution, it must not affect the binding of PTC to the TAS2R38 receptor protein

How PTC might bind to the TAS2R38 bitter taste receptor

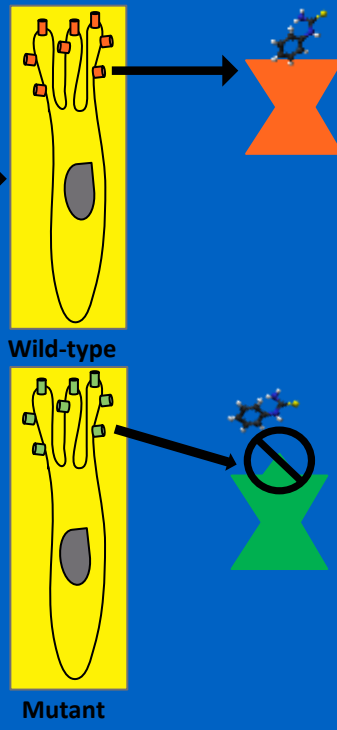


Two alleles of the TAS2R38 receptor have different structures





The TAS2R38 receptor protein is expressed on the surface of sensory cells within a taste bud



T

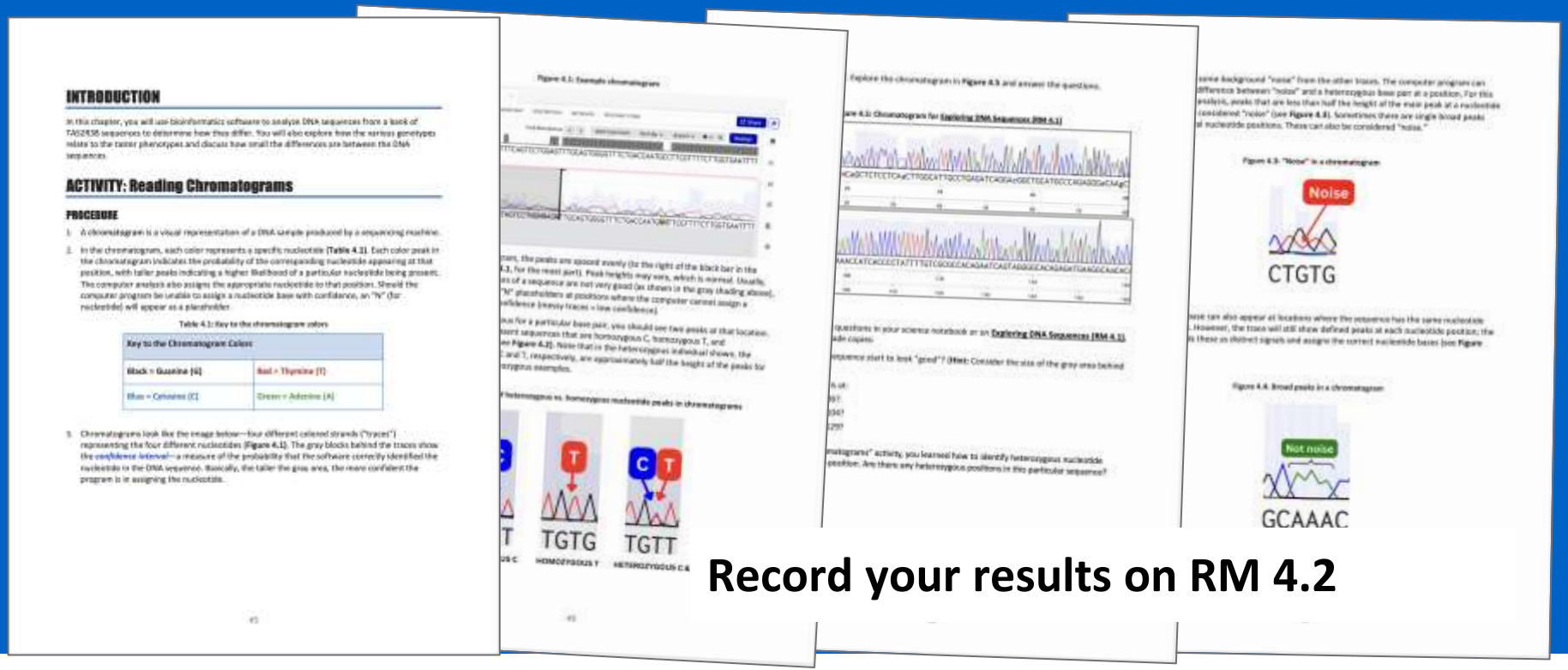
Dominant allele: Can taste PTC

(Receptor shape for schematic purposes only!)

t

Recessive allele: Cannot taste PTC

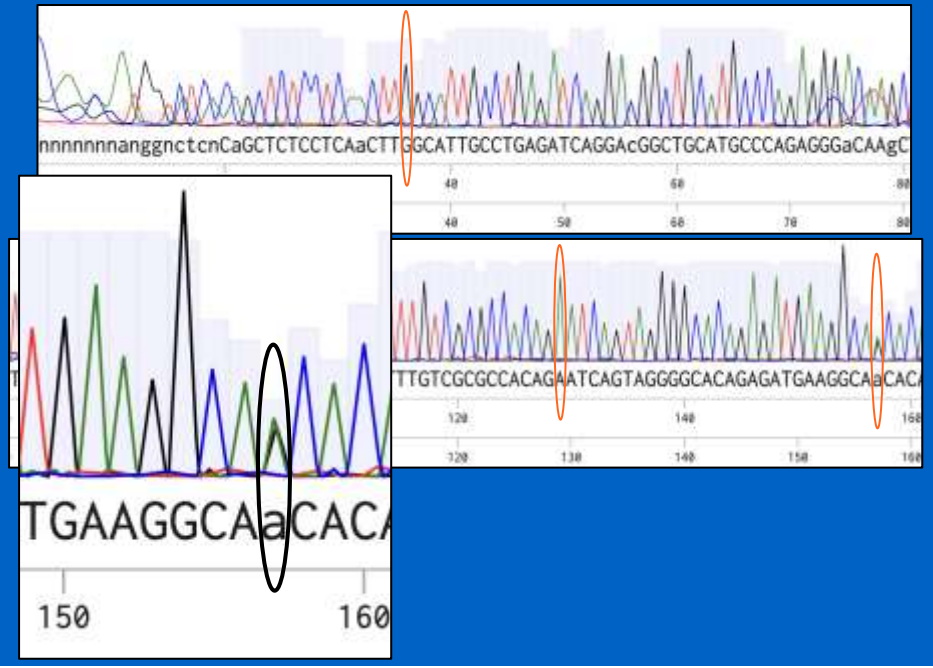
Activity: Reading DNA chromatograms



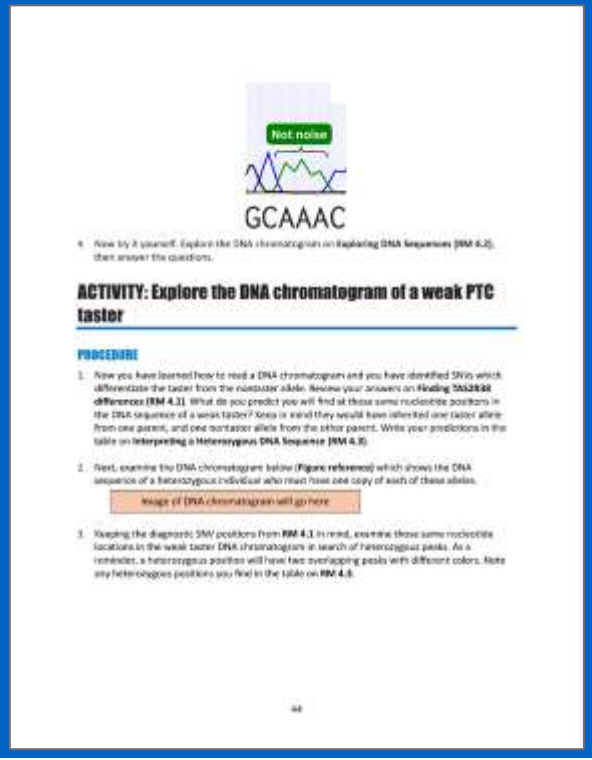
Record your results on RM 4.2

Review: RM 4.2

- **Where does the DNA sequence start to look "good?"**
  - Starting around nucleotide 22
- **What nucleotide is at position:**
  - **36?**
    - G
  - **104?**
    - T
  - **129?**
    - A
- **Are there any positions which might be heterozygous?**
  - Position 157



Activity: Explore the DNA chromatogram of a weak PTC taster



4. Now try it yourself. Explore the DNA chromatogram on **Exploring DNA Sequences (RM 4.2)**, then answer the questions.

ACTIVITY: Explore the DNA chromatogram of a weak PTC taster

PROCEDURE

1. Now you have learned how to read a DNA chromatogram and you have identified SNVs which differentiate the taster from the nontaster allele. Review your answers on **Finding TAS2R38 differences (RM 4.1)**. What do you predict you will find at those same nucleotide positions in the DNA sequence of a weak taster? Keep in mind they would have inherited one taster allele from one parent, and one nontaster allele from the other parent. Write your predictions in the table on **Interpreting a Heterozygous DNA Sequence (RM 4.3)**.

2. Next, examine the DNA chromatogram below (**Figure reference**) which shows the DNA sequence of a heterozygous individual who must have one copy of each of these alleles.

   Image of DNA chromatogram will go here

3. Keeping the diagnostic SNV positions from **RM 4.1** in mind, examine those same nucleotide locations in the weak taster DNA chromatogram in search of heterozygous peaks. As a reminder, a heterozygous position will have two overlapping peaks with different colors. Note any heterozygous positions you find in the table on **RM 4.3**.

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Reproducible Master 4.3

INTERPRETING A HETEROZYGOUS DNA SEQUENCE

1. TAS2R38 is on Chromosome 7. What must the genotype of a weak taster be? Fill in the blanks on this pair of Chromosome 7's. You can use T for a taster allele and t for a nontaster allele.

2. In **RM 4.1, Finding TAS2R38 Differences**, you observed single nucleotide variants (SNVs) that differ between PTC tasters and nontasters. What nucleotide sequence(s) do you expect to see at each of those same nucleotide positions in a weak taster? Fill in your predictions on the table below.

PREDICTED NUCLEOTIDE SEQUENCES AT VARIANT POSITIONS IN A WEAK TASTER

Position of single nucleotide variant	Predicted sequence

Review: RM 4.3

- **TAS2R38 is on Chromosome 7. What must the genotype of a weak taster be?**
  - Tt
- **What nucleotide sequence(s) do you expect to see at each of the 3 diagnostic SNPs in a weak taster?**

PREDICTED NUCLEOTIDE SEQUENCES AT SNPS IN A WEAK TASTER

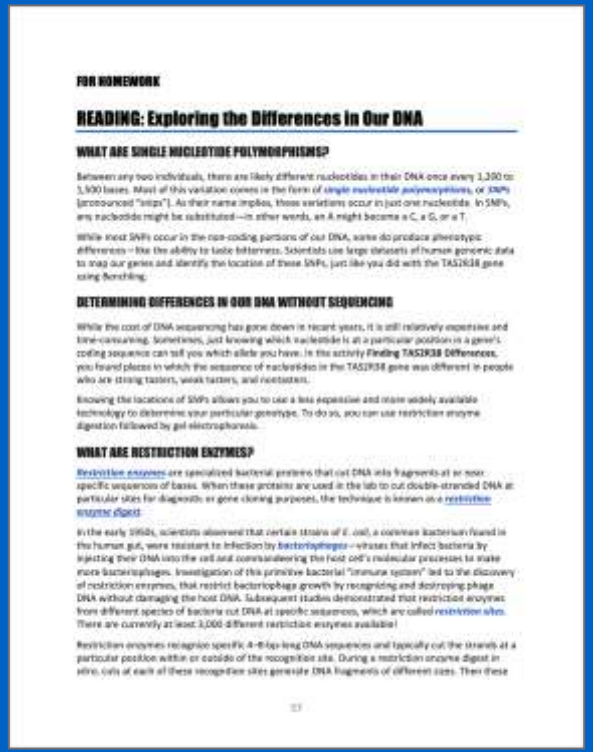
Position of SNP	Predicted sequence
145	C/G
557	T/A
785	C/T
886	G/A

Review: RM 4.3 (continued)

- **Did you observe the predicted heterozygous positions in the DNA chromatogram?**
- **If you could isolate and sequence the TAS2R38 protein from taste bud cells of a weak taster, what would you expect to see?**
  - Half of the protein will be the P-A-V haplotype
  - The other half will be the A-V-I haplotype
- **How do you explain an intermediate phenotype?**

Before the next lab

Read “Exploring the Differences in Our DNA” in your Student Guide



FOR HOMEWORK

READING: Exploring the Differences in Our DNA

WHAT ARE SINGLE NUCLEOTIDE POLYMORPHISMS?

Between any two individuals, there are likely different nucleotides in their DNA once every 1,100 to 1,500 bases. Most of this variation comes in the form of single nucleotide polymorphisms, or SNPs (pronounced “snips”). As their name implies, these variations occur in just one nucleotide. In SNPs, any nucleotide might be substituted—in other words, an A might become a C, a G, or a T.

While most SNPs occur in the non-coding portions of our DNA, some do produce phenotypic differences—like the ability to taste bitterness. Scientists use large datasets of human genomic data to map our genes and identify the location of these SNPs, just like you did with the TAS2R38 gene using BioHelling.

DETERMINING DIFFERENCES IN OUR DNA WITHOUT SEQUENCING

While the cost of DNA sequencing has gone down in recent years, it is still relatively expensive and time-consuming. Sometimes, just knowing which nucleotide is at a particular position in a gene’s coding sequence can tell you which allele you have. In the activity **Finding TAS2R38 Differences**, you found places in which the sequence of nucleotides in the TAS2R38 gene was different in people who are strong tasters, weak tasters, and nontasters.

Knowing the locations of SNPs allows you to use a less expensive and more widely available technology to determine your particular genotype. To do so, you can use restriction enzyme digestion followed by gel electrophoresis.

WHAT ARE RESTRICTION ENZYMES?

Restriction enzymes are specialized bacterial proteins that cut DNA into fragments at or near specific sequences of bases. When these proteins are used in the lab to cut double-stranded DNA at particular sites for diagnostic or gene cloning purposes, the technique is known as a restriction enzyme digest.

In the early 1950s, scientists observed that certain strains of *E. coli*, a common bacterium found in the human gut, were resistant to infection by bacteriophages—viruses that infect bacteria by injecting their DNA into the cell and commandeering the host cell’s molecular processes to make more bacteriophages. Investigation of this primitive bacterial “immune system” led to the discovery of restriction enzymes, that restrict bacteriophage growth by recognizing and destroying phage DNA without damaging the host DNA. Subsequent studies demonstrated that restriction enzymes from different species of bacteria cut DNA at specific sequences, which are called restriction sites. There are currently at least 3,000 different restriction enzymes available!

Restriction enzymes recognize specific 4–8-bp-long DNA sequences and typically cut the strands at a particular position within or outside of the recognition site. During a restriction enzyme digest in vitro, cuts at each of these recognition sites generate DNA fragments of different sizes. Then these