A Symbiotic Relationship in Science Education
Teacher-Outreach-Supplier

Can you taste that?
Extending beyond the PTC tasting strip

Why invest in lab science education?

Doing science early and often breaks down students' perceptions that science is hard

Building an engaging interactive learning environment builds student confidence

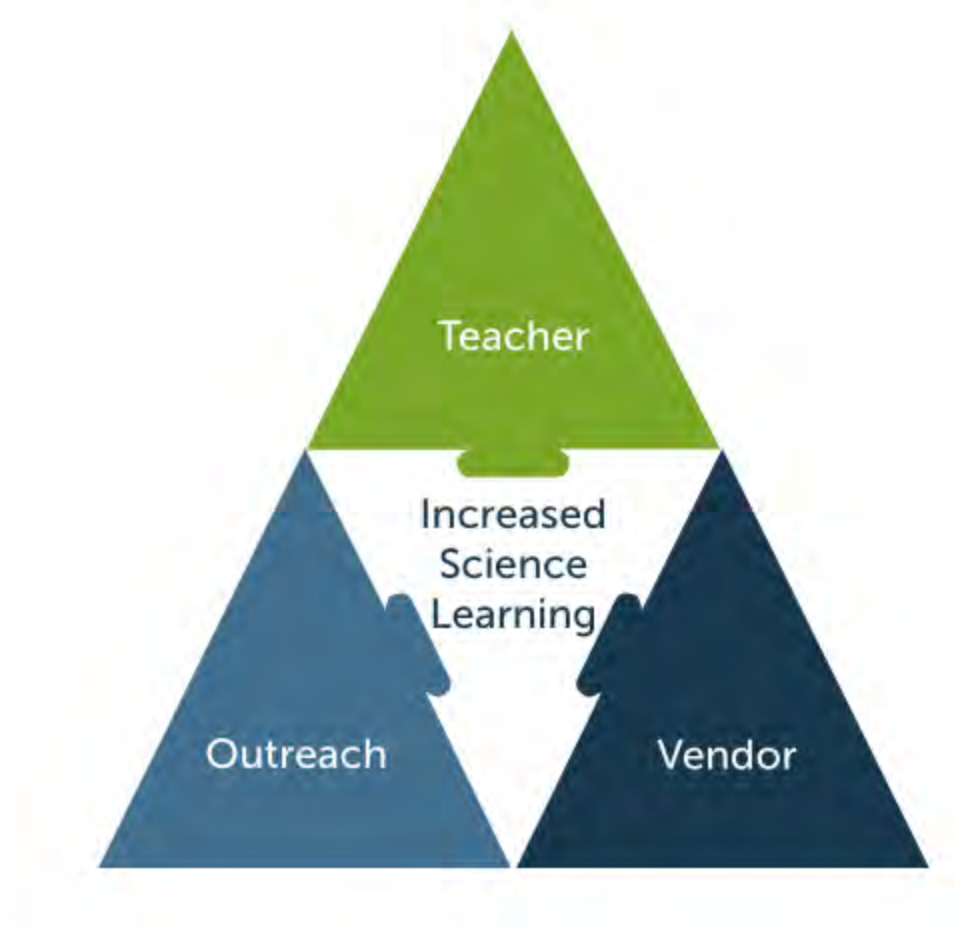
Lab focus teaching increases learning and test scores

Middle and low achieving students tend to participate more often when teachers show interest in their ability to gain these skills

Introducing the tools and techniques of science opens potential career opportunities

Utilizing partnerships with outreach and science vendors supplements your budget, time and skills.

A True Symbiotic Relationship



The true symbiotic relationship (notes)

Teacher- Gives-time, effort, and a real understanding of their constraints within the classroom. Shares their own best practices to other participating teachers

Gains-resources from other teachers, supplies, vetted real life curriculum and Biotech/lab skills with support. also connections for my students for future mentorships/internships/informational interviews/guest speakers/field trips. Access to more grants, administration support and potential of parent or rotary funders because these relationships shows commitment

Outreach – Gives time and effort, real lab skills, curriculum, sometimes equipment loaning, lab supplies, support for the teacher and a place/time for great teachers to collaborate with other great teachers

Gains data and proof of principal to apply to more grants, if higher education-the students that gain these skills (better prepared students) and work with key players that are changing administrations point of view towards the STEM classroom

Vendor- Gives time and effort to understand the American classroom, innovated products that engage students and are robust to handle the learning of the beginning student, cost vs. outcome = effective learning.

Gains informed clients, ones willing to help with more innovation and future clients in the students that move into STEM jobs who know of the equipment and what works.

Can you taste that?
Extending beyond PTC tasting paper

MiniLab- PTC PCR Simulation Kit

ABE-WA support for PTC PCR and Bioinformatics activity

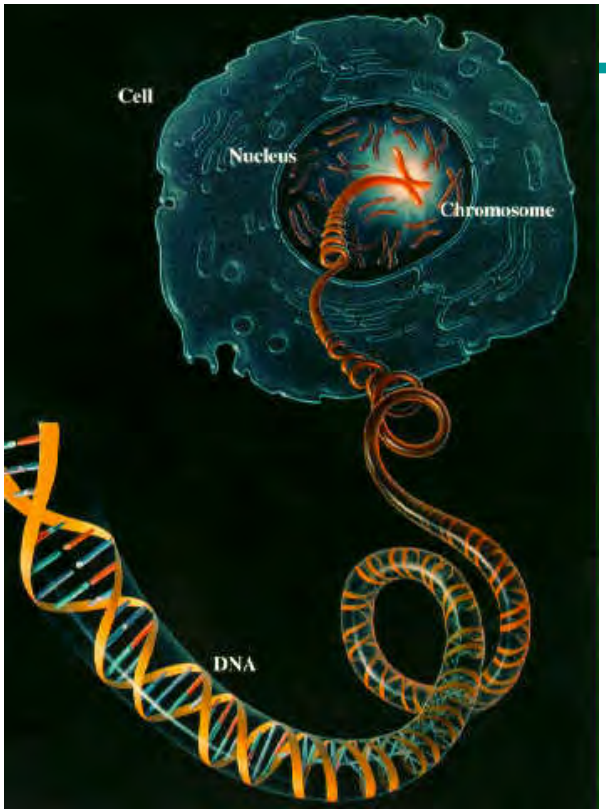
Science Education Partnership- Fred Hutch-PTC PCR

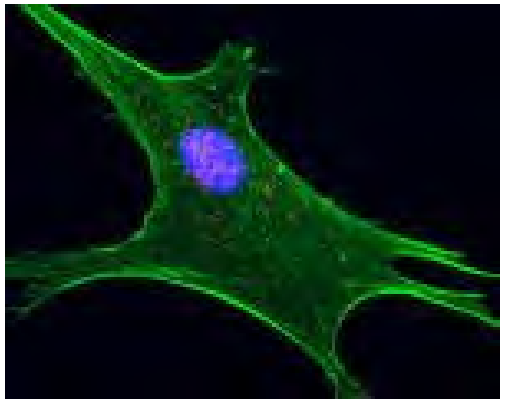
NEB- For reagents-TaqOne, DNA Marker(100bp), loading dye

Carolina Kit

PCR - Important Concepts

Central Dogma





Nucleus - Contains DNA - the blue print for all genetic information

Chromosomes = much longer sequences of DNA that contain many genes

Genes = sequence of DNA that tells the cell how to make a single protein

Protein=A compound molecule made from a gene which coded the specific amino acids for a specific job.

DNA---RNA---Protein

Polymerase Chain Reaction - PCR

Major Breakthrough in the early 1980s

Kerry Mullis – 1993 Nobel Prize

Short stretches of DNA could be copied very quickly and easily – *DNA synthesis in a tube*

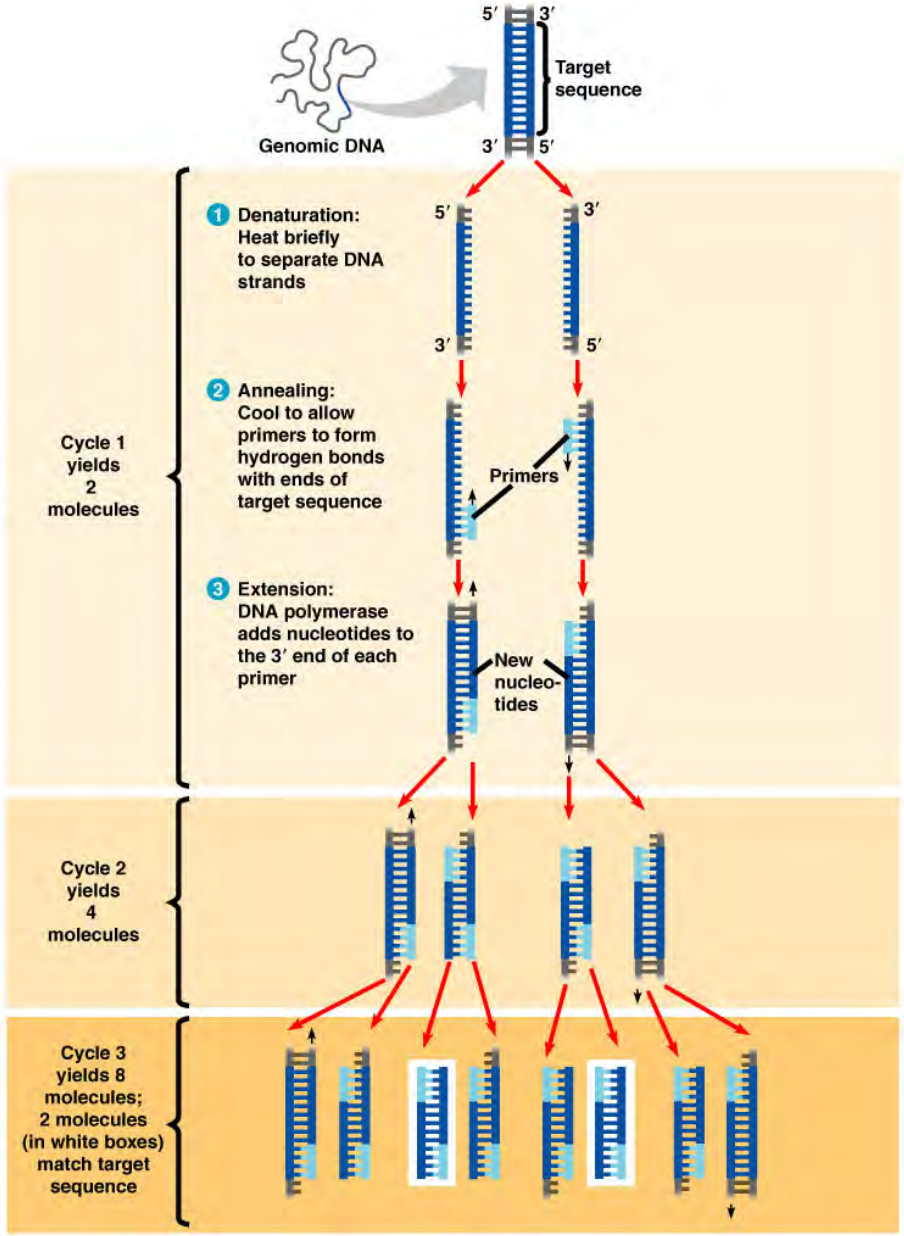
Applications

-Forensics (CSI)

-Evolutionary Relationships

-Cloning (Jurassic Park)

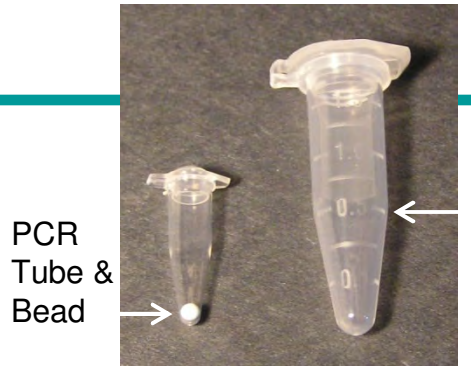
- Genetic Testing



The Power of PCR

Number of PCR Cycles (n)	Copies of DNA (2n)
0	1
1	2
2	4
3	8
4	16
5	32
6	64
7	128
8	256
9	512
10	1024
20	1,048,576
30	1,072,741,824

PCR Ingredients



1. DNA "template"

Your purified DNA sample

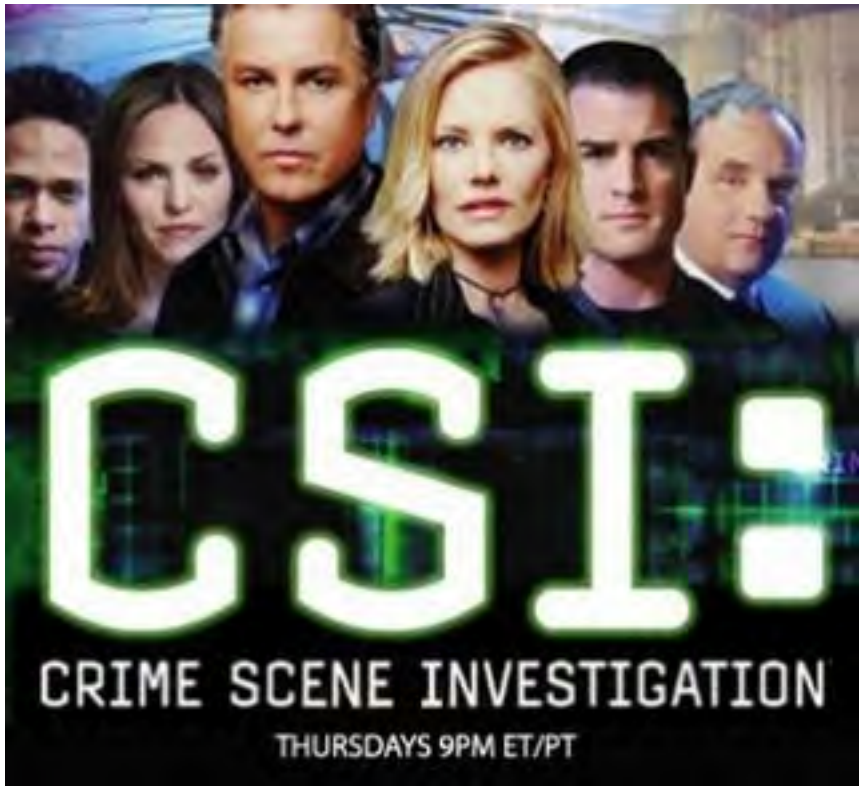
2. *Taq* Polymerase — *Heat-stable DNA polymerase*

3. Deoxynucleotides (dNTPs) — *Building blocks of DNA*

4. Primers — *Small pieces of DNA bind to your gene*

5. Buffer and water — *Maintain pH of reaction*

What is PCR and What it is Not?



http://www.youtube.com/watch?v=6iFDphWXjw4

What Do You Need to Carry Out PCR?

1. Source of DNA – template

2.Ingredients (DNA polymerase, dNTPs, buffer, Taq)

3. An understanding of the target DNA sequence to design primers

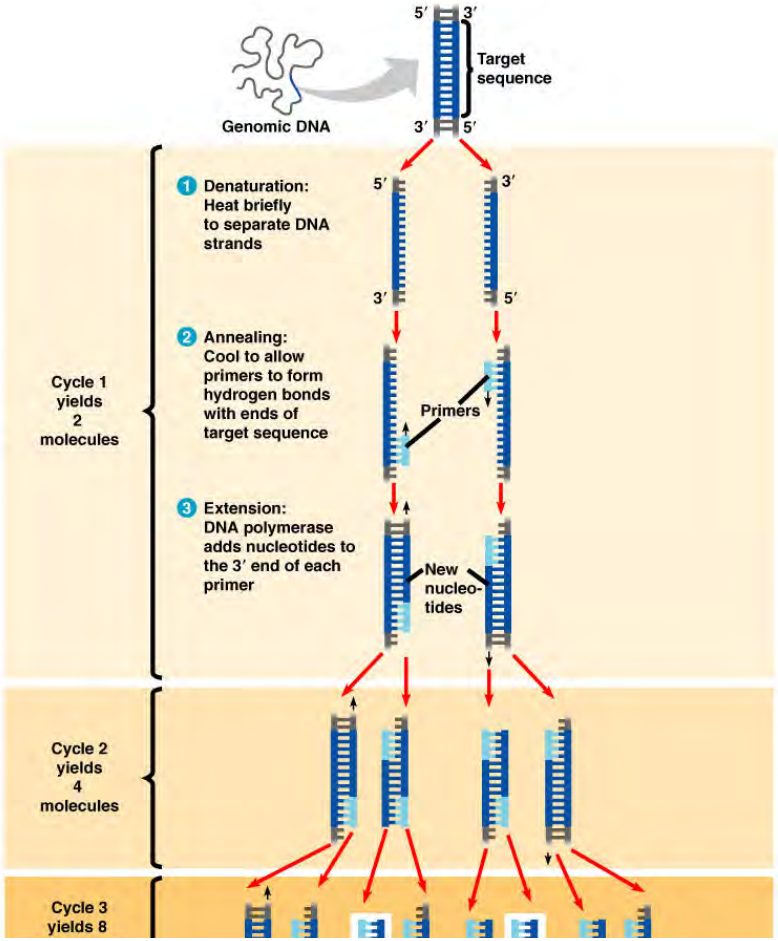
4. Thermocycler

5. Method to visualize DNA and see differences.

PCR – First Cycle

3 Steps

1) **Denature** template DNA – *94 degrees*
2) **Anneal** – Primer binds to complimentary site *45-72 degrees*
3) **Extension** - Taq polymerase synthesizes new strand – *68-72 degrees*
4) **Return** to denature



Breakthrough - Taq Polymerase Was the Key

- *Taq* DNA polymerase was isolated from the bacterium *Thermus aquaticus.*

- *Taq* polymerase is stable at the high temperatures (~95^{o}C) used for denaturing DNA.

Now researchers could add DNA polymerase once and it would work for 30 cycles

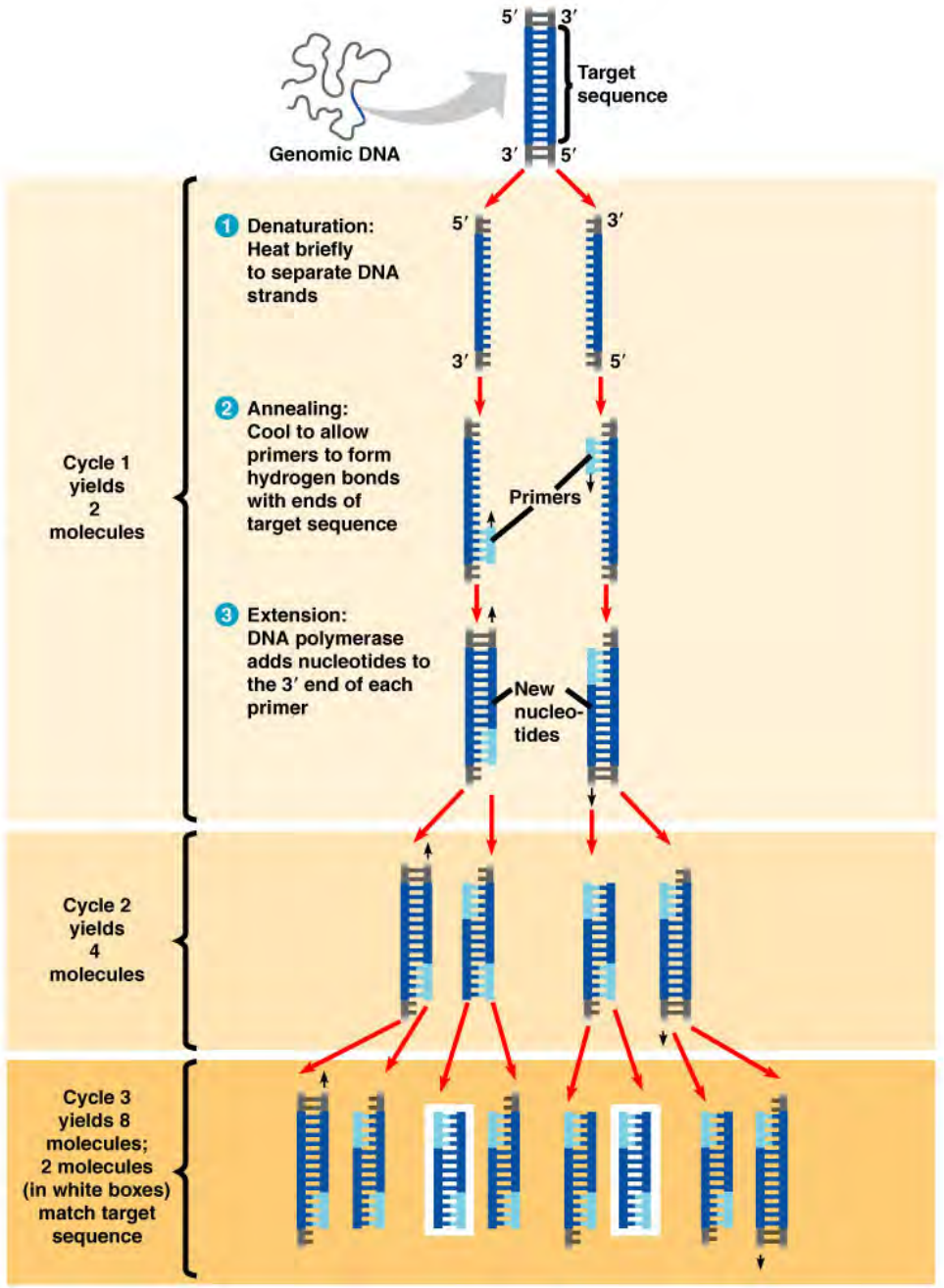
More cycles = more

DNA

Each cycle DOUBLES the amount of target DNA

Cycle 3 is the first cycle where a double stranded molecule is produced that is the EXACT size of the target DNA

TARGET DNA IS DEFINED BY THE DISTANCE BETWEEN TWO PRIMERS



PCR Animations

1) http://www.dnalc.org/ddnalc/resources/pcr.html
2) http://www.sumanasinc.com/webcontent/anisamples/molecularbiology/pcr.html
3) http://www.youtube.com/watch?v=x5yPkxCLads
4) http://www.hhmi.org/biointeractive/polymerase-chain-reaction-pcr

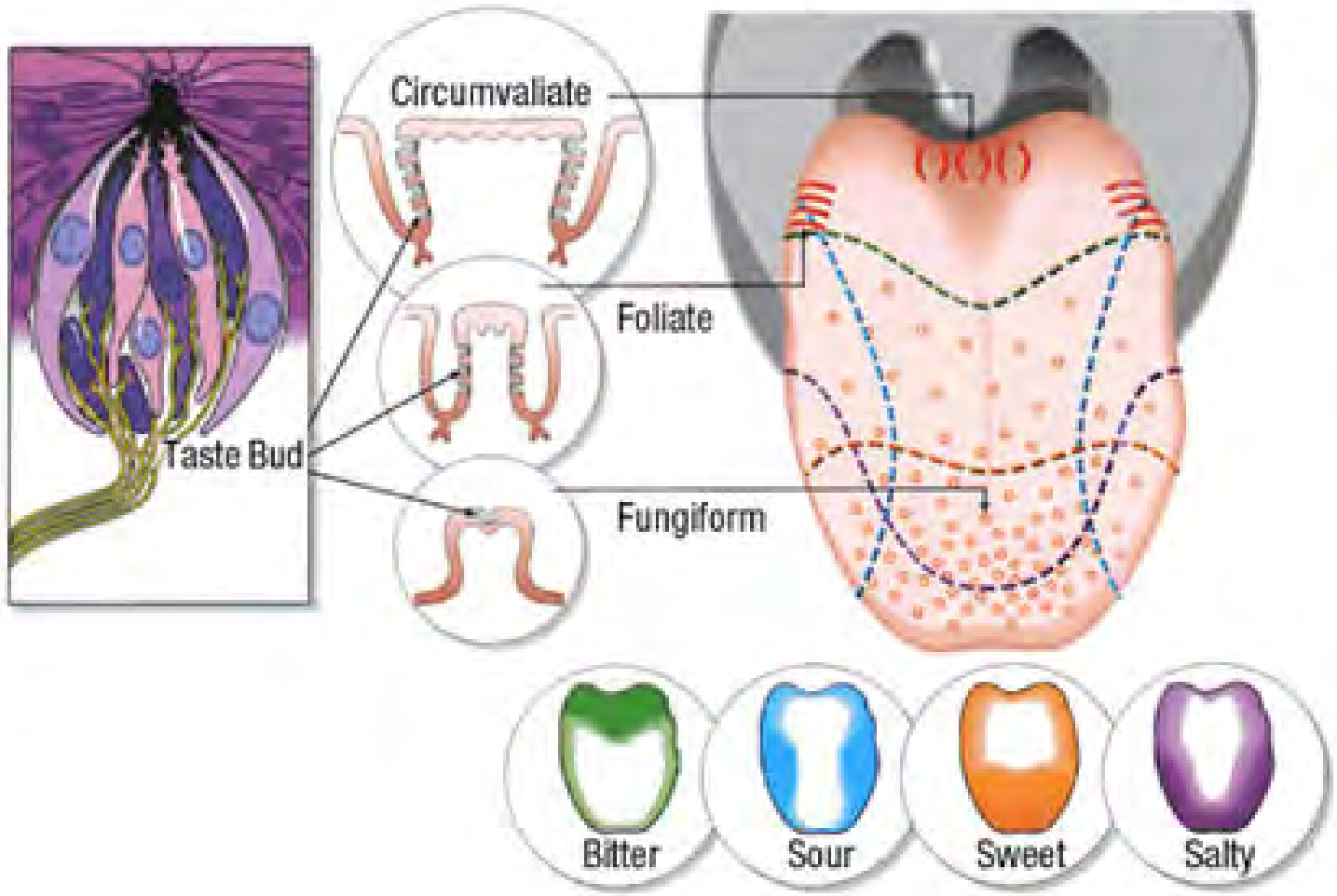
Analyzing the PTC Taster Gene (tas2r38) through PCR Amplification

ABE-WA PCR Lab

The human taste process

- Food is recognized by a taste receptor where the protein binds to the receptor most closely related to the 5 tastes: Sweet, Bitter, Sour, Salty, and Umami
- The shapes of the protein closely matches the shape of the related receptor.
- The receptor sends a nerve impulse to your brain which interprets it as one of those tastes.
- The receptors, neuron messages and interpretation are all determined by your genetics, though can be altered by environment or injury.

The human taste process



Bitter Tasting Chemical PTC (Phenylthiocarbamide)

- Arther Fox in the late 20's used this chemical in a lab at DuPont.
- His colleague complained that he could taste the chemical in the air, but Fox was not experiencing the same taste sensation.
- This was tested with many co-workers and friends and genetics was thought to play a role.
- It is said that paternity was even tested by this early on.

Bitter Tasting Chemical PTC

- Albert Blakeslee, in 1932, determined that the ability to taste this chemical must be a dominant trait when most test subjects could taste the chemical.*

*In 2004, the gene responsible was located on chromosome 7. We get one allele from our mother and one from our father.

Protocol for PTC PCR - Overview

- Day 1: Isolating your DNA
  Extract your own DNA using Chelex
- Day 2: Performing PCR
  Use Polymerase Chain Reaction (PCR) to amplify a portion of your own *TAS2R38* gene
- Day 3: Restriction Digest of PCR Product
  Use a restriction enzyme to potentially cut your *TAS2R38* genes
- Day 4: Run Product Samples on Gel to Analyze Results
  Use gel electrophoresis to separate any fragments produced by the restriction enzyme activity

Protocol for Today: Analyzing Student results

- You should have 6 samples: DNA Marker, PTC PCR Product Uncut, Student 1, Student 2, Student 3 and Student 4 (Cut with Restriction Digest)
- Your gel box should have TBE buffer in in the tank and a gel. The carriage should be plugged in. You shouldn't move the carriage after loading samples.
- Set your pipette to 12ul and make sure a tip is on the barrel of the pipette
- Pipette each sample into a well. Change tip each time you pipette a new sample.
- When samples are added place the orange viewing box on the top of unit with it in the correct position and push run.
- Make sure samples are running by turning on LED light.
- Results should be determined after about 25 mins. Check to see that the marker is spread out and you can determine the samples results. If necessary run another 5 minutes.

The gene is called TAST2R38

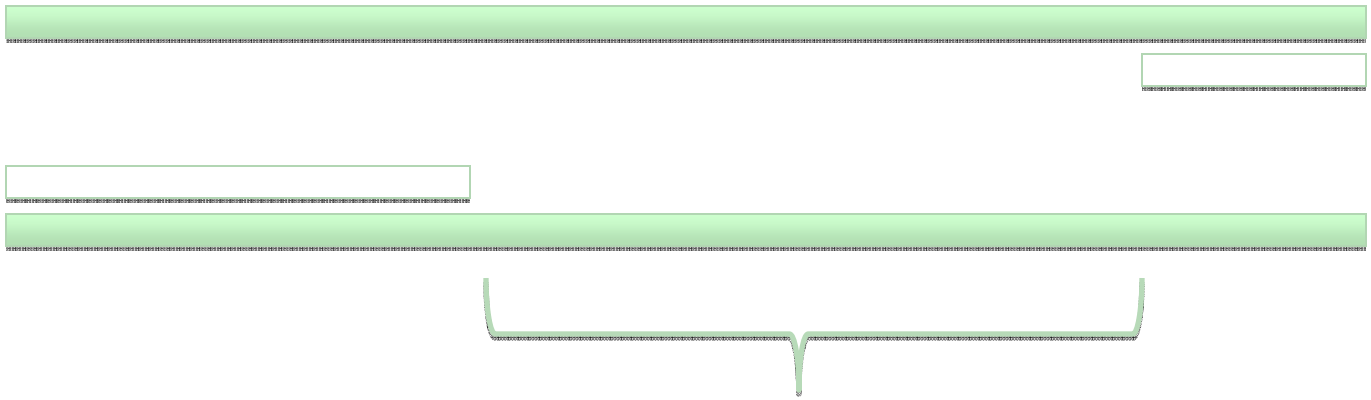
- The gene is just over 1000bp in length
- There are three areas of variance that causes the taster/nontaster forms or 3 SNPS-Single Nucleotide polymorphism.

Postition	Taster	Nontaster
145	C (proline)	G (alanine)
785	C (alanine)	T (valine)
886	G (valine)	A (isoleucine)

Amplifying TAST2R38 with PCR

*Primers used in the experiment:

CCTTCGTTTTCTTGGTGAATTTTTGGGATGTAGTGAAGAGGCGG
AGGTTGGCTTGGTTTGCAATCATC



Amplified Region is 221 bp with the 145 position SNP

Predicting Alleles and Trait

- If PCR was done correctly, everyone will have a very large amount of 221 bp PCR product
- To predict the alleles, you have to separate the dominant from recessive
- This is where the 145 SNP comes in

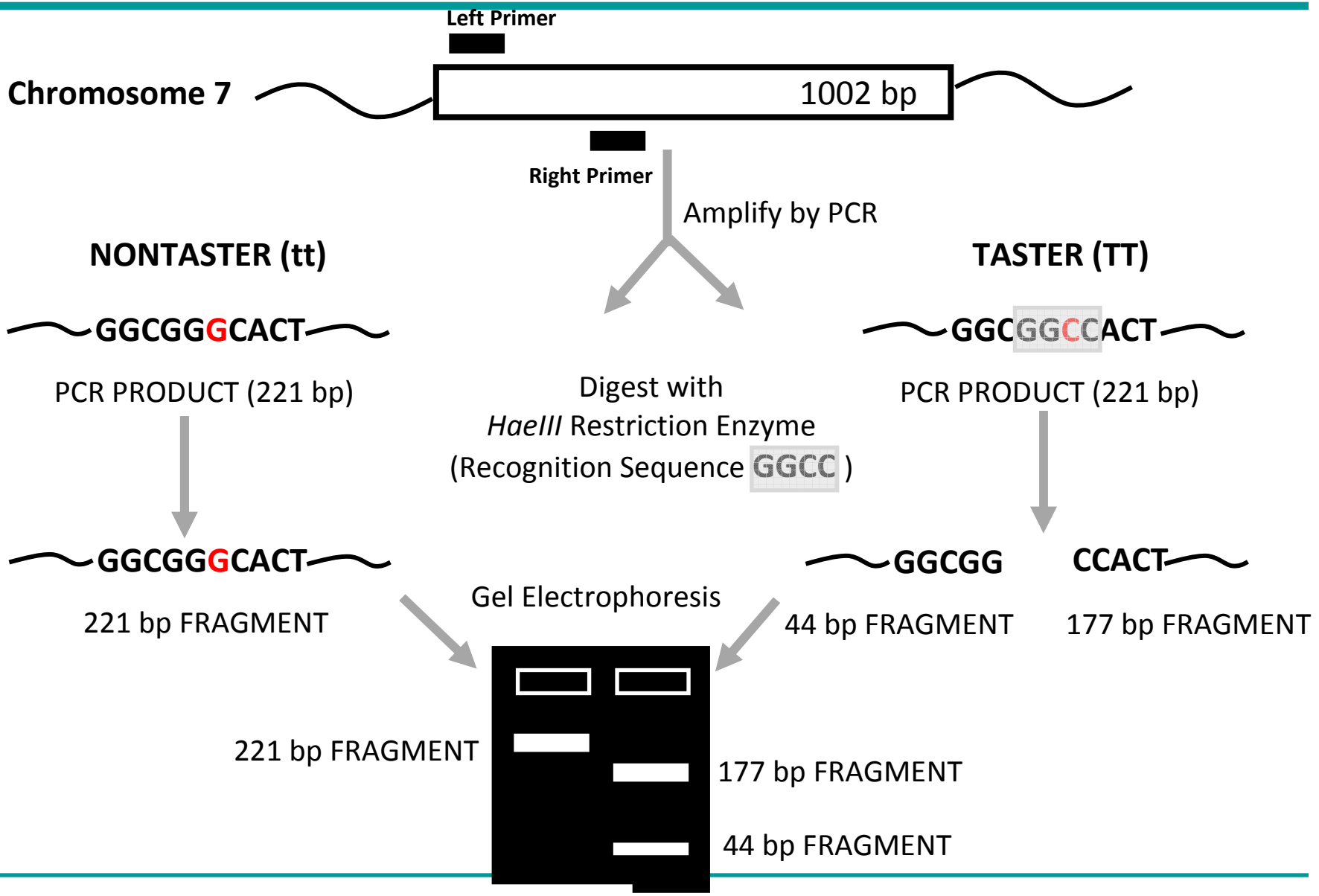
Predicting Alleles and Trait

*Using HAEiii enzyme, a restriction digest can be done at this SNP

*HAEiii restriction site is GGCC

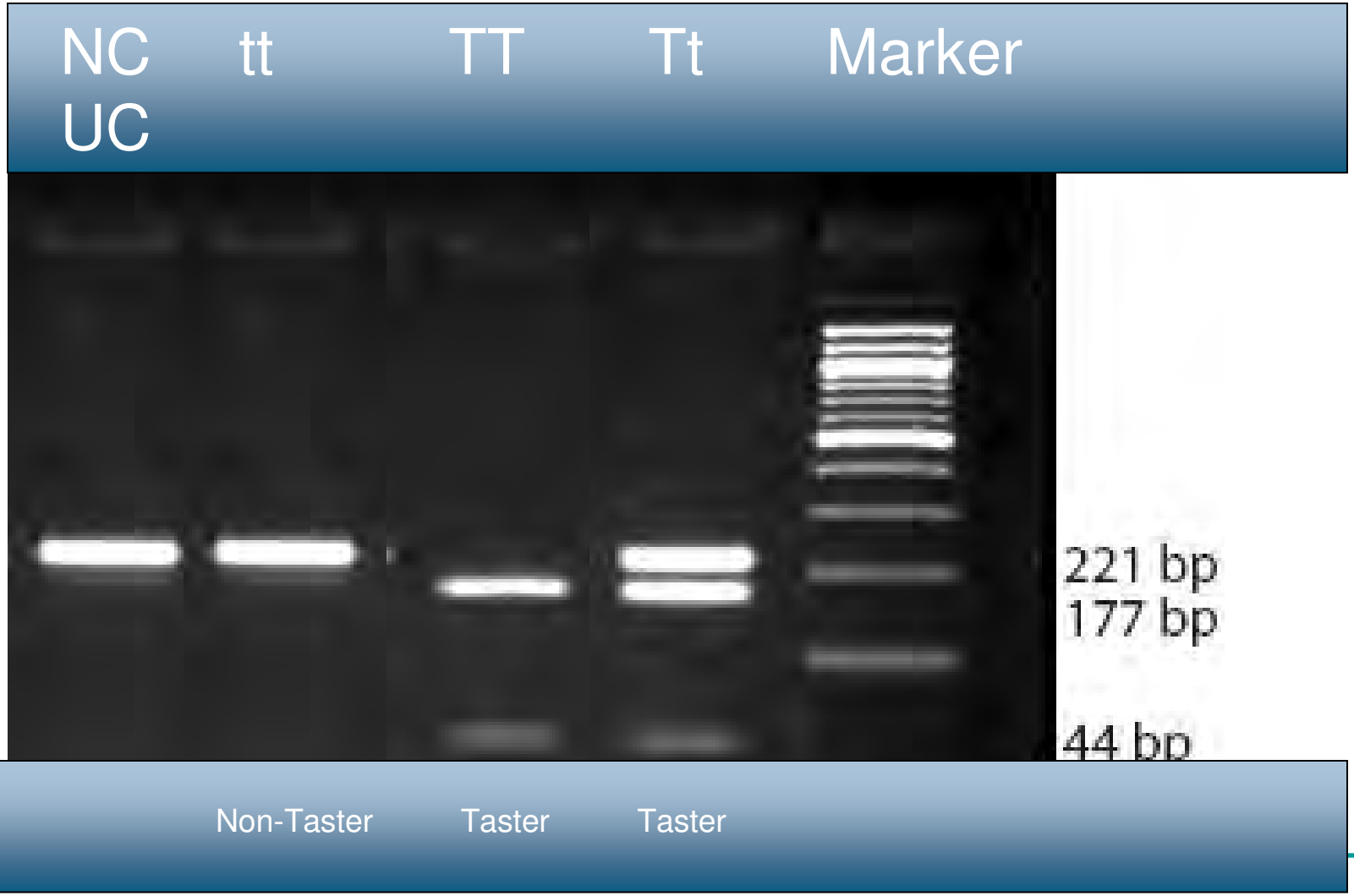
- The pcr product that has GGCC will be cut into two pieces
- The pcr product that has GGGC will not cut
- Those that are heterozygous will have a mixture of both. Some product with GGCC and some product with GGGC

TAS2R38 Bitter Taste Receptor ["PTC"] Gene



Visualization of DNA results

using gel electrophoresis



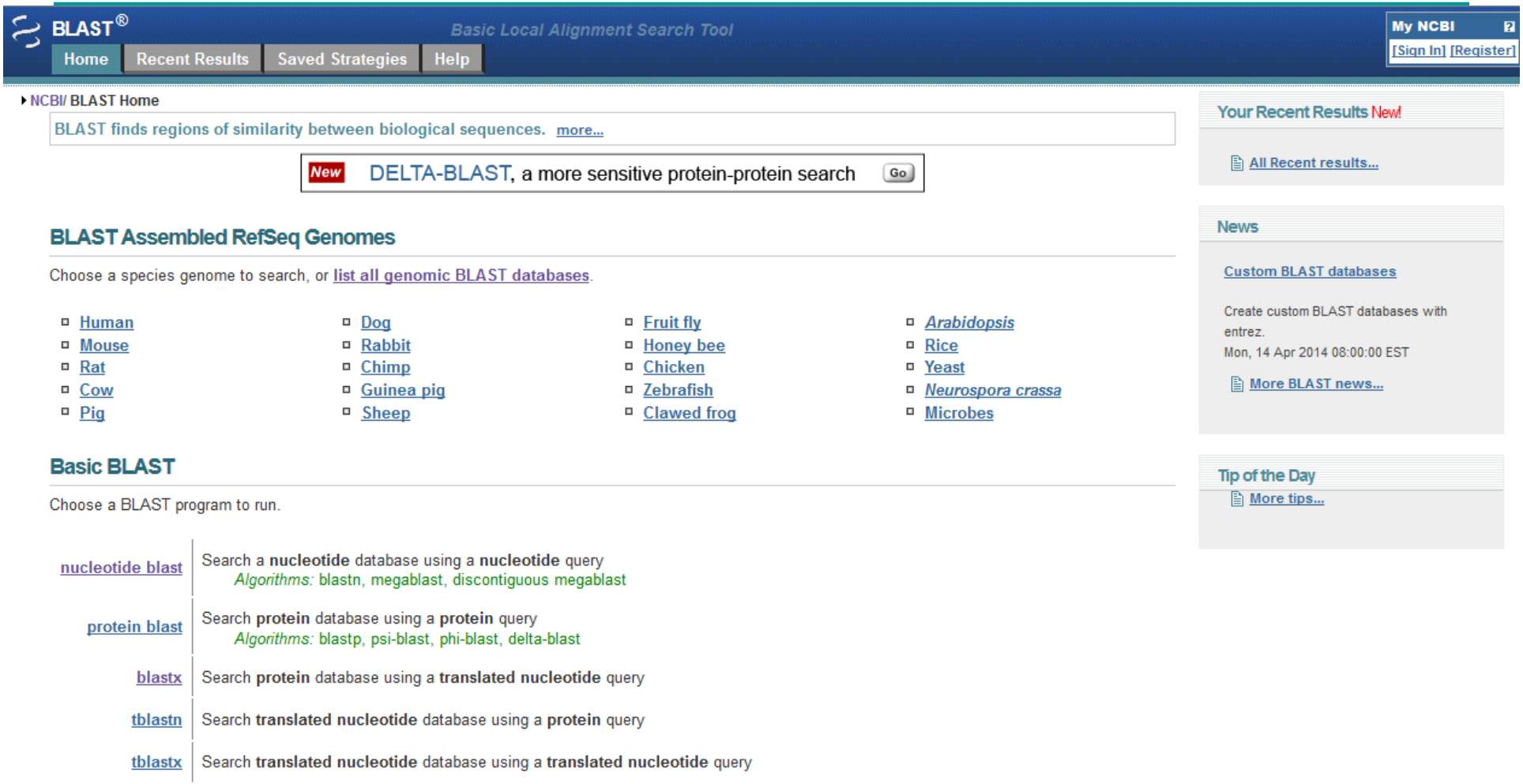
10 Minute Break

Using BLAST to Compare DNA and Protein Sequences

BLAST is One of the Most-Used Programs in Biology Today

- Determine probability two sequences share a common ancestor
- Determine where sequences match one another
- View relationship between mRNA and genomic DNA (ex: exons versus introns)
- Design and test PCR primers
- Distinguish or identify different species (ex: unknown samples, contamination)
- Build phylogenetic trees or cladograms

Basic Local Alignment Search Tool

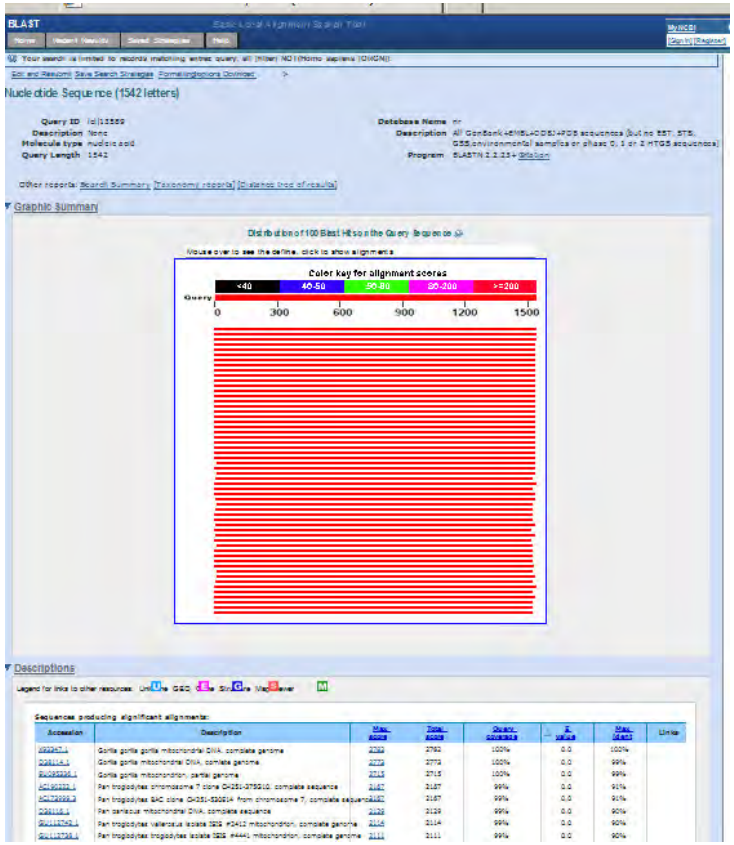


http://blast.ncbi.nlm.nih.gov/Blast.cgi

A Tool for Comparing Sequences

Compare Two or More Sequences to One Another

Compare a Sequence "Query" to an NCBI Database



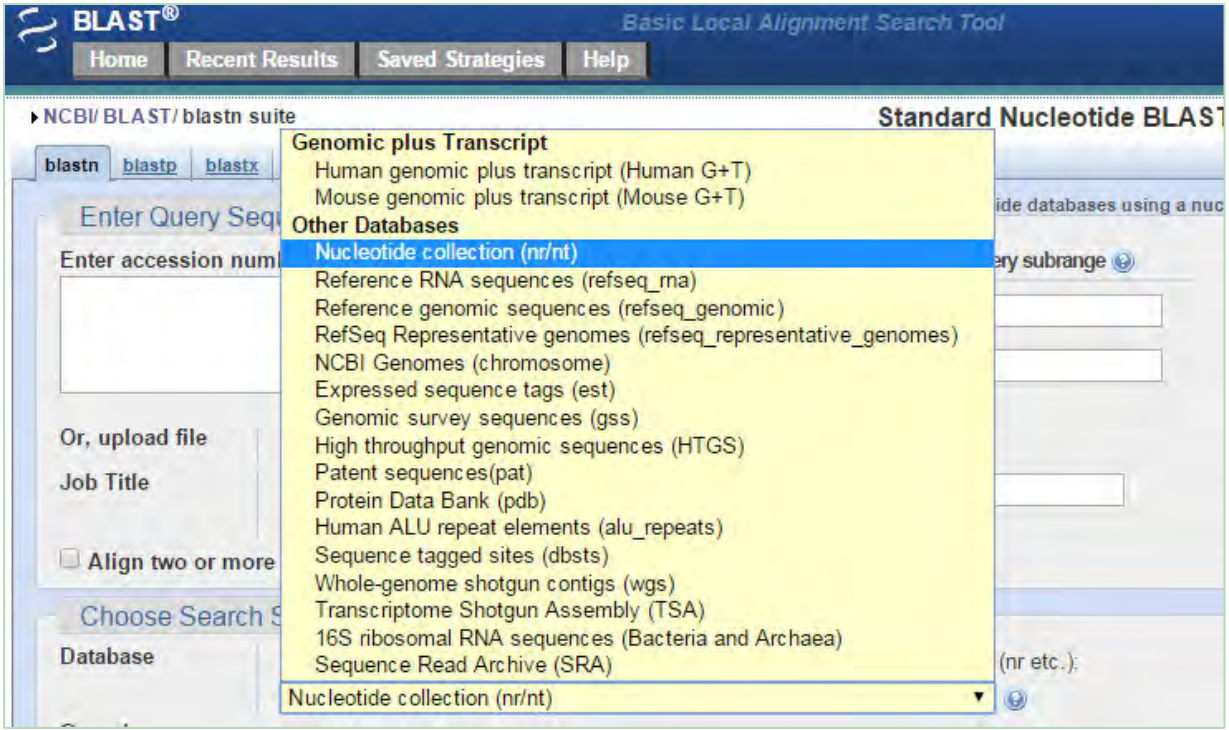
Terminology

- **Query Sequence**:
  - Same root as “question”
  - Sometimes called a “reference sequence”
  - the sequence to which other sequences are compared
  - independent or control variable.
- **Subject Sequence**:
  - the sequence being compared
  - dependent or experimental variable
- **BLAST Scores**
  - **Max Score, Total Score**
  - **Query Coverage**
  - **Percent Identity**

Different Types of BLAST Searches

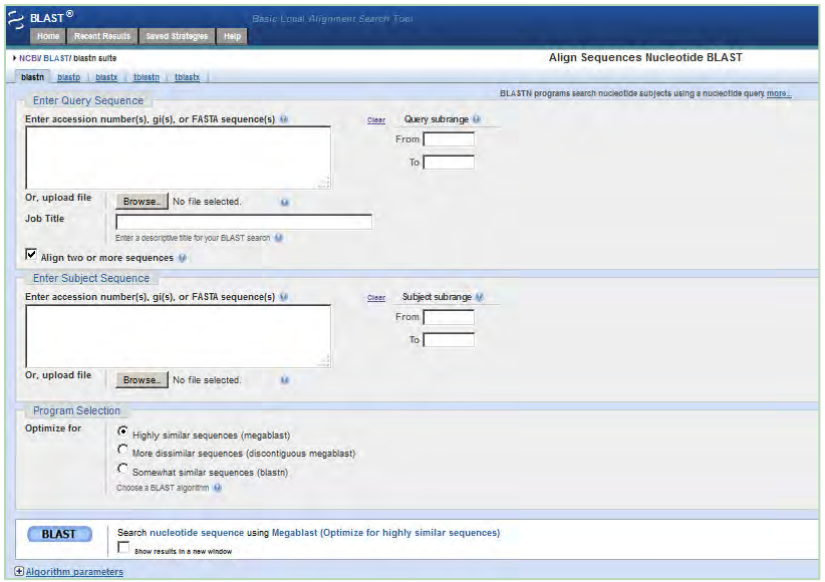
- **blastn**: Compares Nucleotide sequences
- **blastp**: Compares Protein sequences
- **blastx**: Translates a Nucleotide sequences into all 6 reading frames, searches against Protein database
- **tblast**: Compares a Protein sequence to the translated Nucleotide database
- **tblastx**: Translates both the Nucleotide query and the Nucleotide database, then compares

Available Genomes & Databases

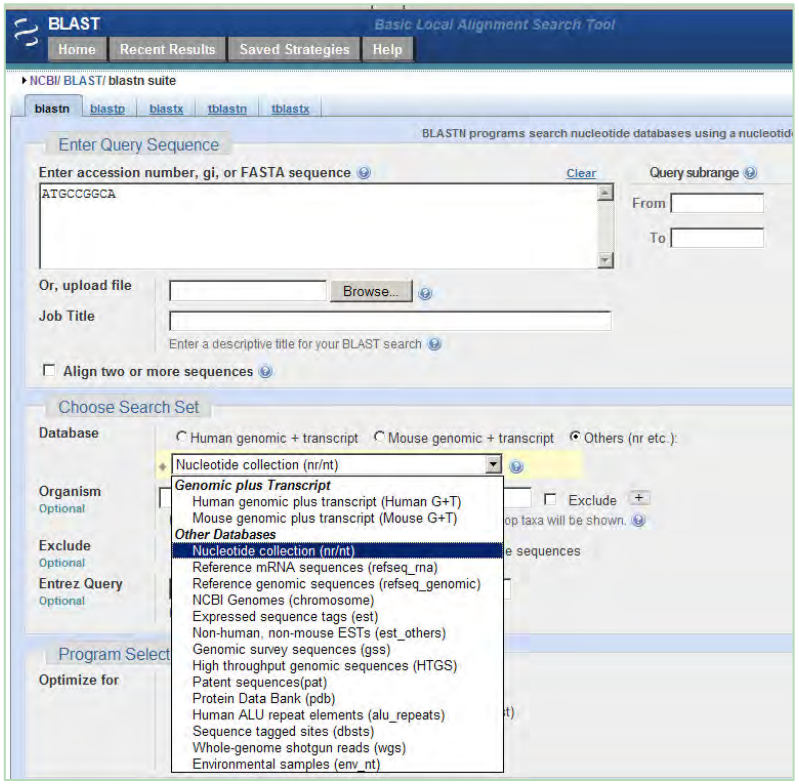


How to BLAST

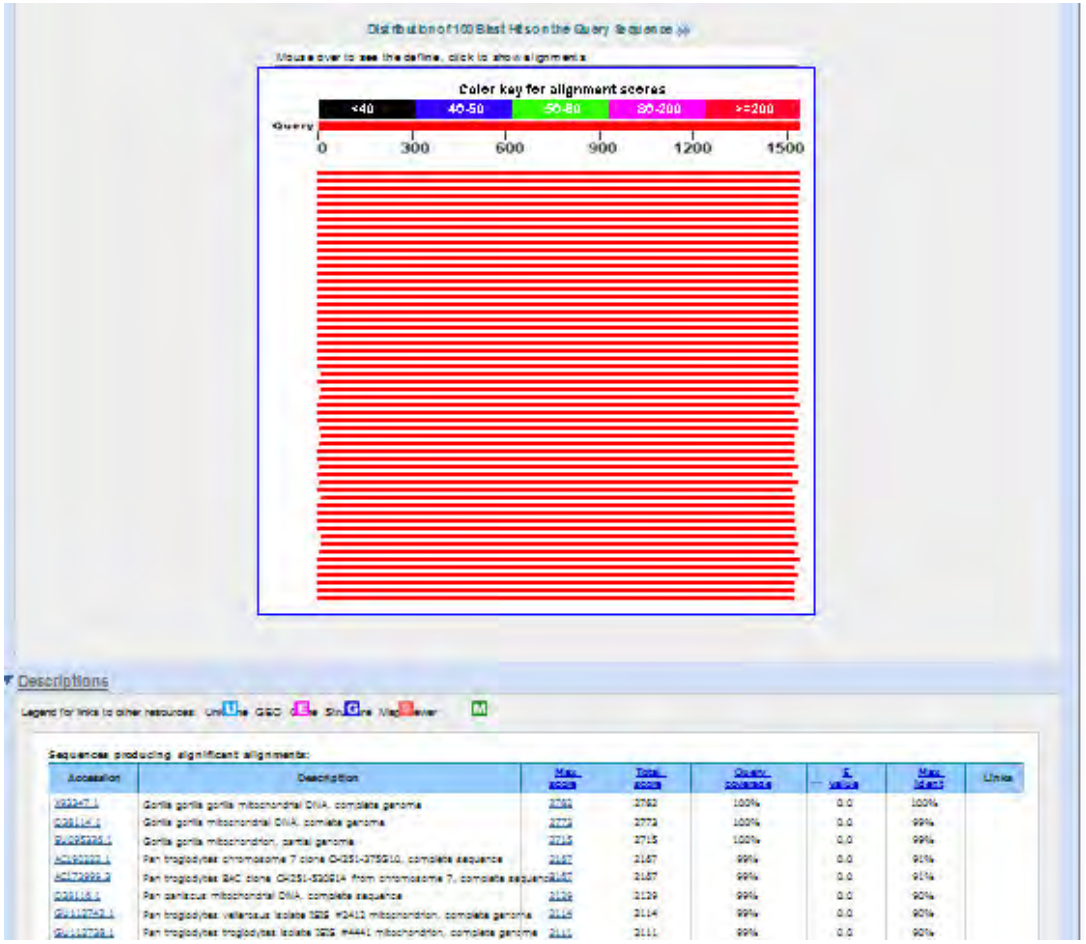
Comparing Two or More Sequences to One Another



Compare a Sequence "Query" to an NCBI Database



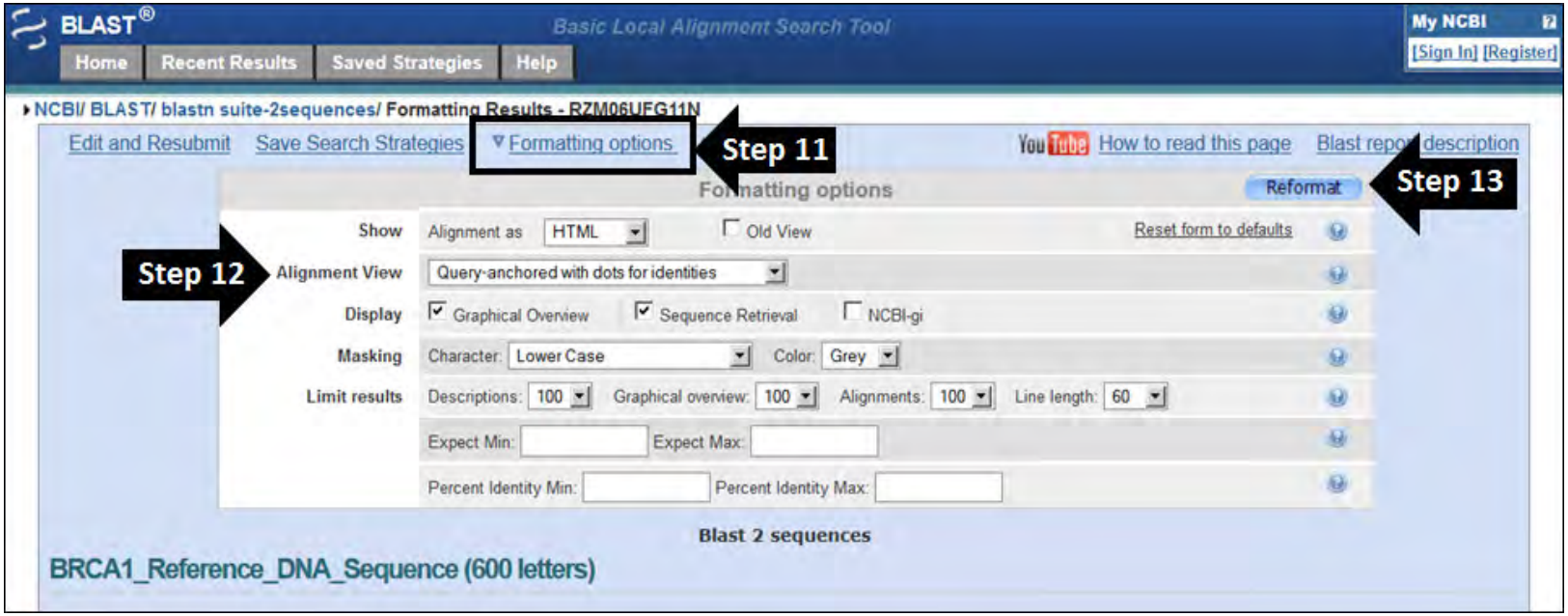
Sample Results



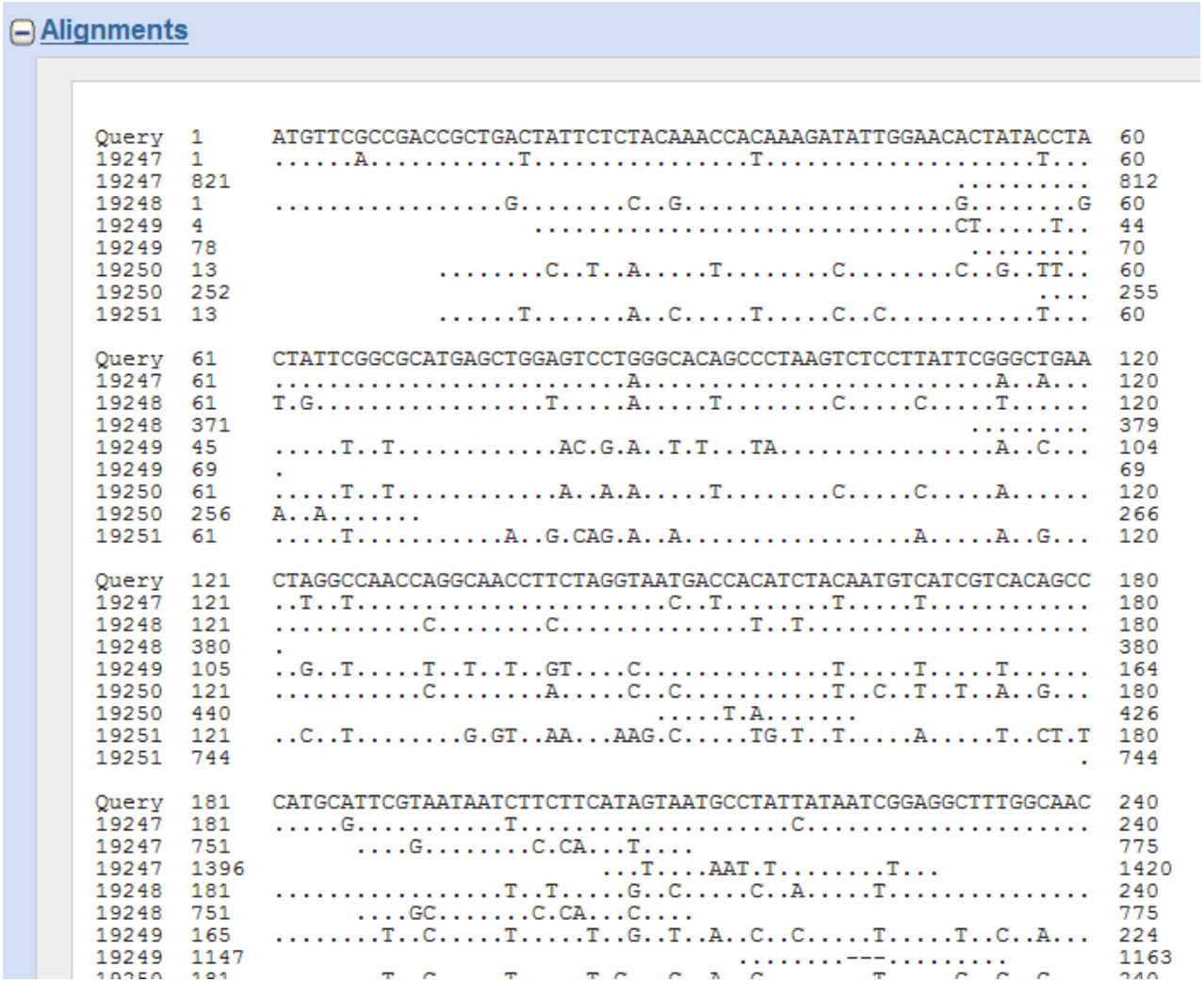
Pairwise Comparisons & Default Alignment Format

```
>emb|X93347.1|  Gorilla gorilla gorilla mitochondrial DNA. complete genome
Length=16412

 Score = 2782 bits (3084),  Expect = 0.0
 Identities = 1542/1542 (100%), Gaps = 0/1542 (0%)
 Strand=Plus/Plus

Query  1     ATGTTCACCGACCGCTGATTATTCTCTACAAACCATAAAGATATTGGAACACTATATCTA  60
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  5326  ATGTTCACCGACCGCTGATTATTCTCTACAAACCATAAAGATATTGGAACACTATATCTA  5385

Query  61    CTATTCGGCGCATGAGCTGGAGTCCTAGGCACAGCCCTAAGTCTCCTTATTCGAGCAGAA  120
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  5386  CTATTCGGCGCATGAGCTGGAGTCCTAGGCACAGCCCTAAGTCTCCTTATTCGAGCAGAA  5445

Query  121   CTTGGTCAACCAGGCAACCTTCTAGGTAACGATCACATCTATAATGTTATCGTCACAGCC  180
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
```

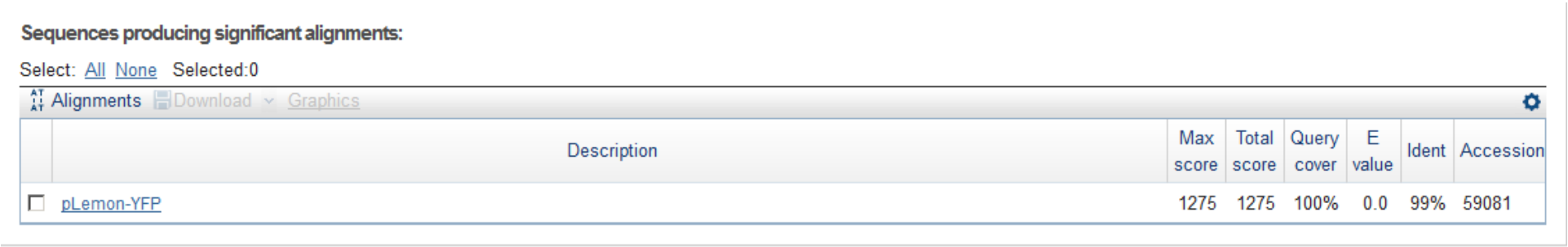
Reformatting Results Permits Custom Views



Query-Anchored with Dots for Identities



BLAST Scores: Defined



Sequences producing significant alignments:

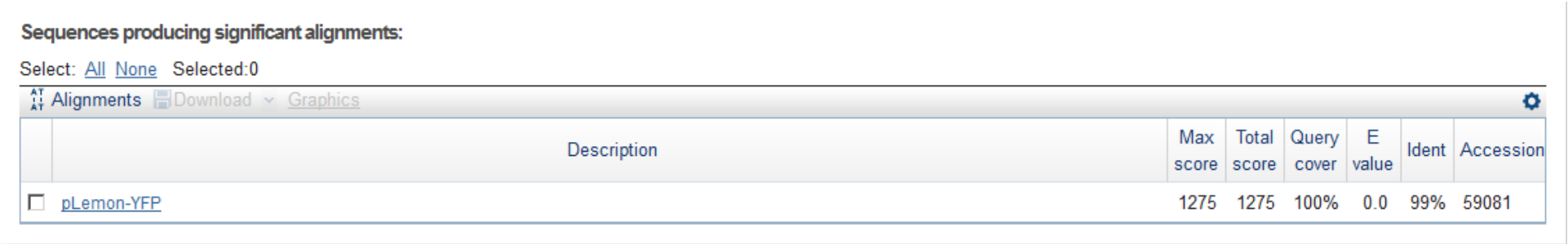
Select: All None Selected:0

Alignments Download Graphics

	Description	Max score	Total score	Query cover	E value	Ident	Accession
☐	pLemon-YFP	1275	1275	100%	0.0	99%	59081

- **Max Score** / **Total Score**: Algorithm specific
- **Query Coverage**: What % of the query and subject sequence match?
- **Percent Identity**: How well does the covered region match?
- **E** or **Expect Value**: What is the probability that the match is by chance?

BLAST Scores: Example



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Alignments Download Graphics

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☐	pLemon-YFP	1275	1275	100%	0.0	99%	59081

30% Query Coverage, 100% Identity

3/10 bases (30%) match perfectly (100%)

ATG**GAT**ACGT

TGA**GAT**GATC

100% Query Coverage, 50% Identity

All 10 bases (100%) align,

but only 5/10 (50%) match

AT**G**C**C**G**A**TT**G**

AG**G**G**C**A**A**CA**G**

Predicting PTC tasting of non-human primates

Resources- Bioteach Outreach Support

- **Shoreline Community College-Biotechnology Outreach**
- **Amgen Biotechnology Experience**
- **Science Education Partnership- Fred Hutch**
- **Genome Sciences Education Outreach**
- **Institute of System Biology - Baliga Lab- Systems Education Experiences**
  Logan Center
- **Center for Infectious Disease**
- **Northwest Association of Biotechnology Research**
- **Digital World Biology**
- **NOAA Fisheries and Seattle Aquarium**
- **LASER Leadership and Assistance for Science Education Reform**
- **Washington Alliance for Better schools**
- **Washington STEM**
- **Pacific Science Center-Middle**
- International Arctic Research Center
- Reed College Science Outreach
- Bay Area Biotechnology Education Consortium-BABEC
- MassBioEd
  - PTC PCR
- ASHG-American Society of Human Genetics
  - **Model PCR- paper model**

Resources- Equipment, supplies and labs

- MiniOne by Embi Tec **Pauline Cheng**
- New England Biolabs (NEB) Reagent support
- BioRad Damon Tighe
- Biotium
- Phenix
- Carolina
- Edvotek

How to get involved?
What if there is no outreach in your area?

Travel if you can

ABE-WA will support teachers as much as possible

We are looking at webinars as a way to reach others

Reach out to your community colleges and universities

Many have grant deliverables that require community outreach.

Many do not have a lot of time, but willing. Maybe it is just space for you to run a science collaboration meeting

Online resources

How can we help you vet the internet? What's Tried and True?