
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

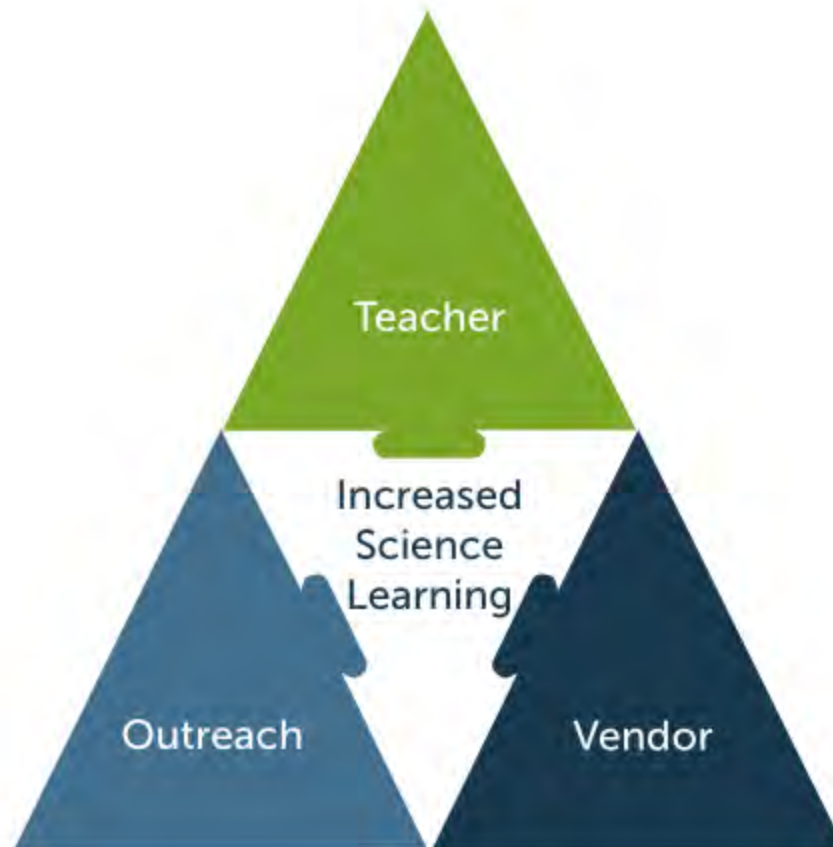
Lab focus teaching increases learning and test scores

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

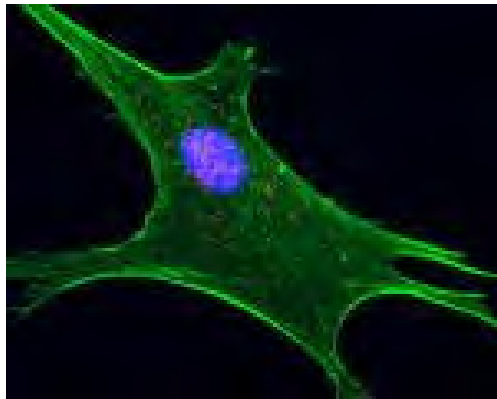
Science Education Partnership- Fred Hutch-PTC PCR

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*

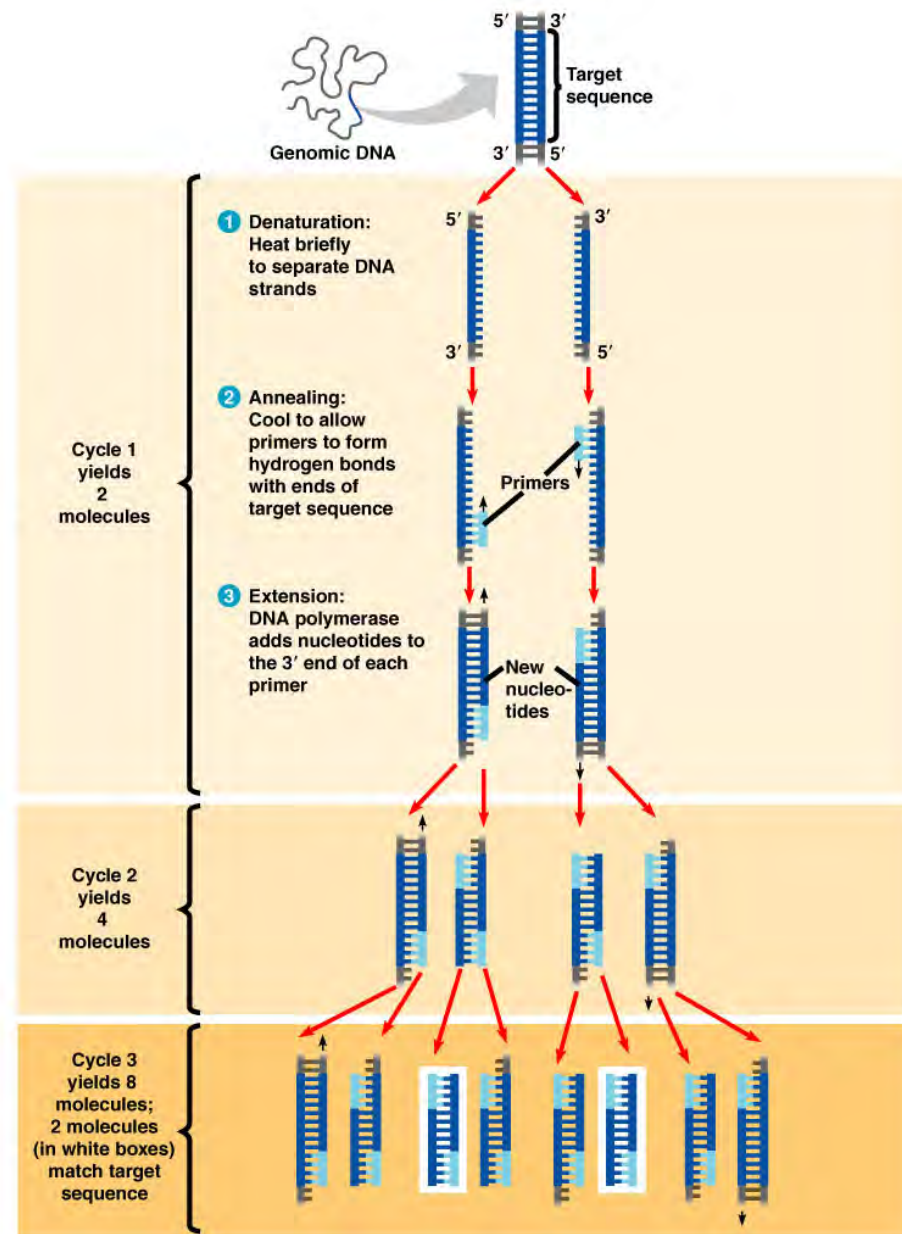
Applications

-Forensics (CSI)

-Evolutionary Relationships

-Cloning (Jurassic Park)

- Genetic Testing



The Power of PCR

Number of PCR Cycles (n)	Copies of DNA (2^n)
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)

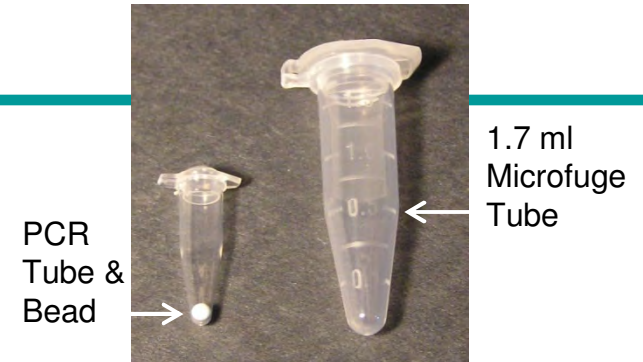
Building blocks of DNA

4. Primers

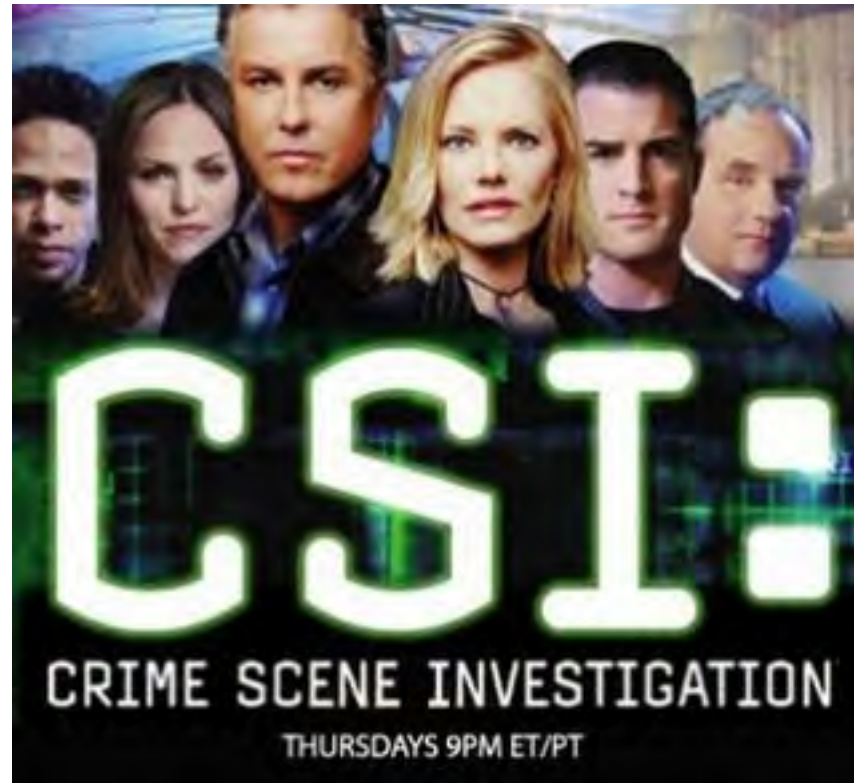
Small pieces of DNA bind to your gene

5. Buffer and water

Maintain pH of reaction



What is PCR and What it is Not?



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

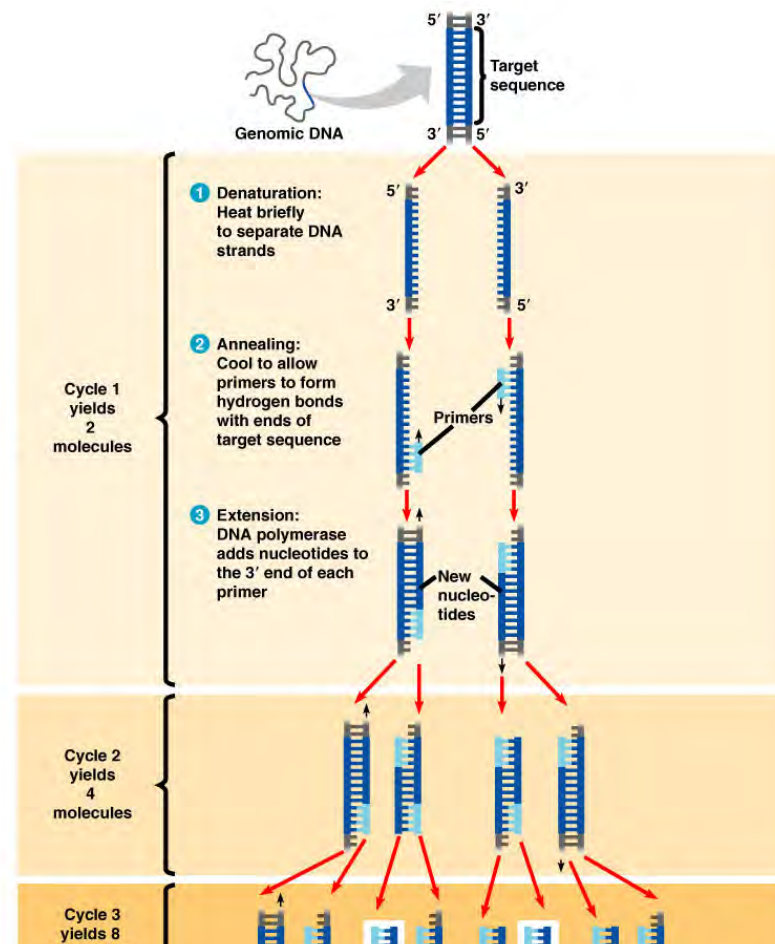
What Do You Need to Carry Out PCR?

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°C) used for denaturing DNA.



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

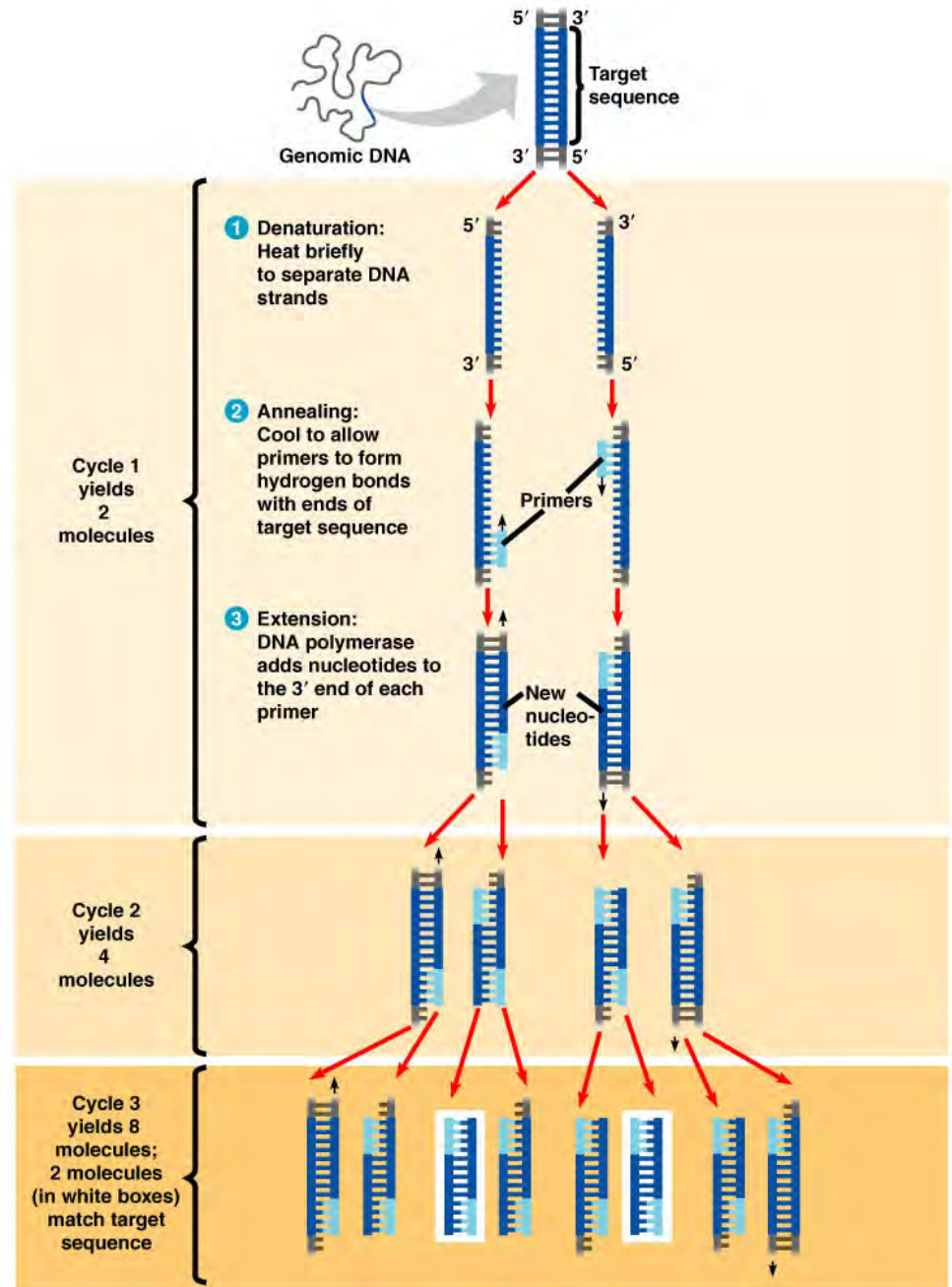
More cycles = more

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>
-



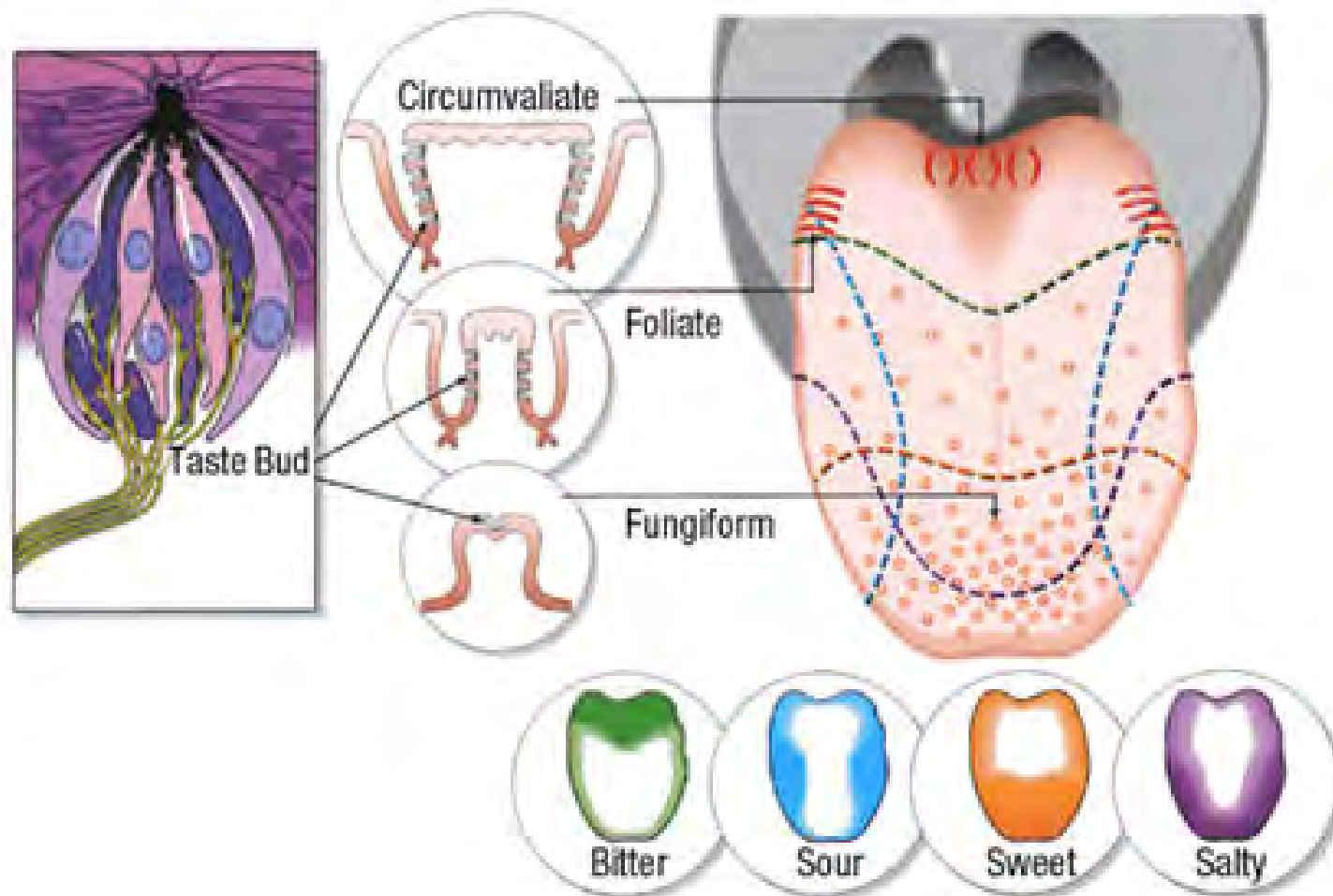
**Analyzing the PTC Taster Gene (tas2r38)
through PCR Amplification
ABE-WA PCR Lab**



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)

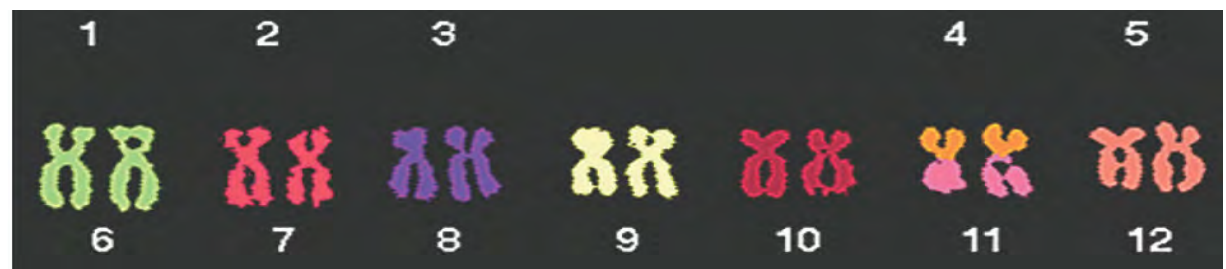
- Arther Fox in the late 20's used this chemical in a lab at DuPont.
 - His colleague complained that he could taste the chemical in the air, but Fox was not experiencing the same taste sensation.
 - This was tested with many co-workers and friends and genetics was thought to play a role.
 - It is said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

Position	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:

CCTTCGTTTTCTTGGTGAATTTTTGGGATGTAGTGAAGAGGCCGG
AGGTTGGCTTGGTTTGCAATCATC



Amplified Region is 221 bp with the 145 position SNP

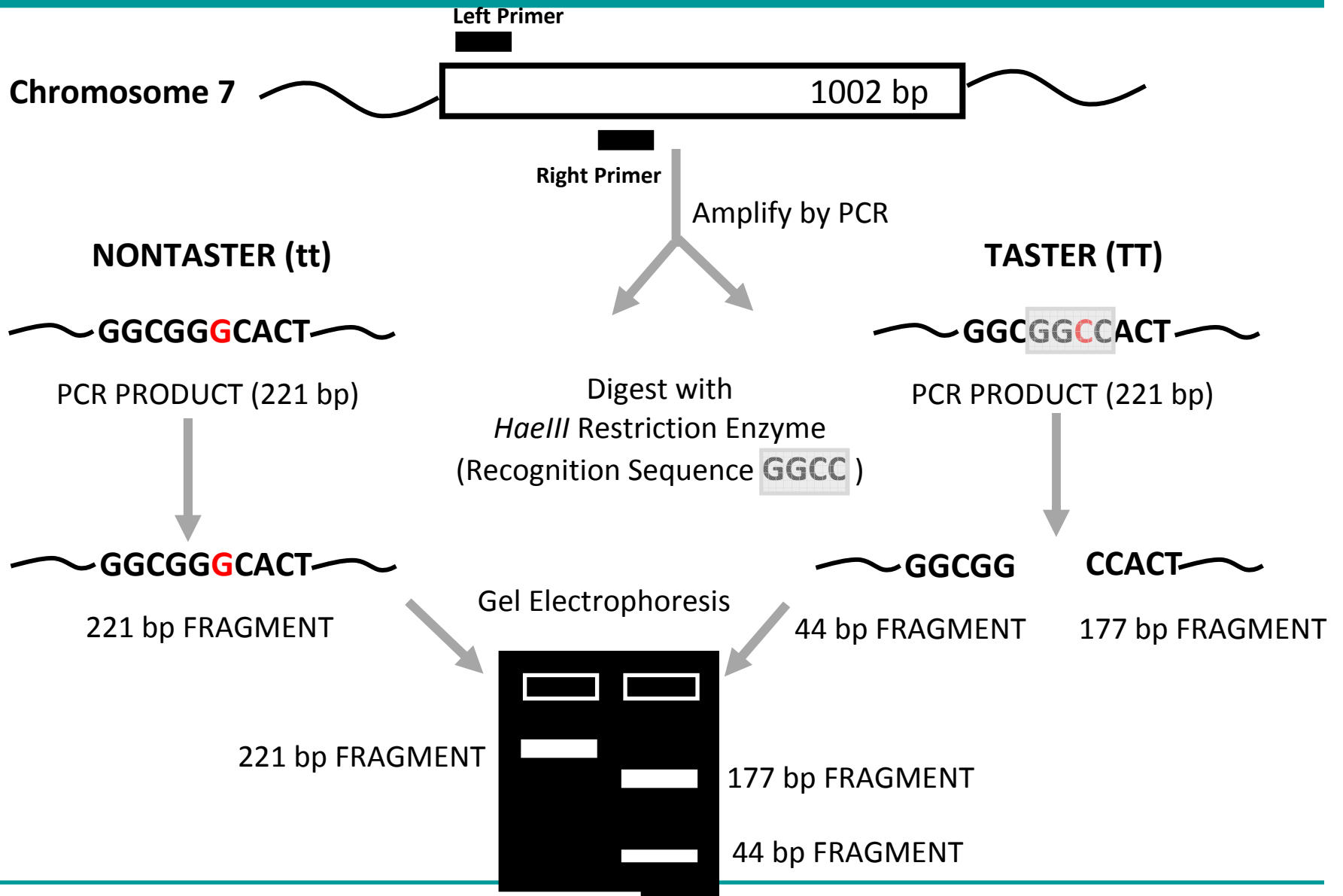
Predicting Alleles and Trait

- 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
-

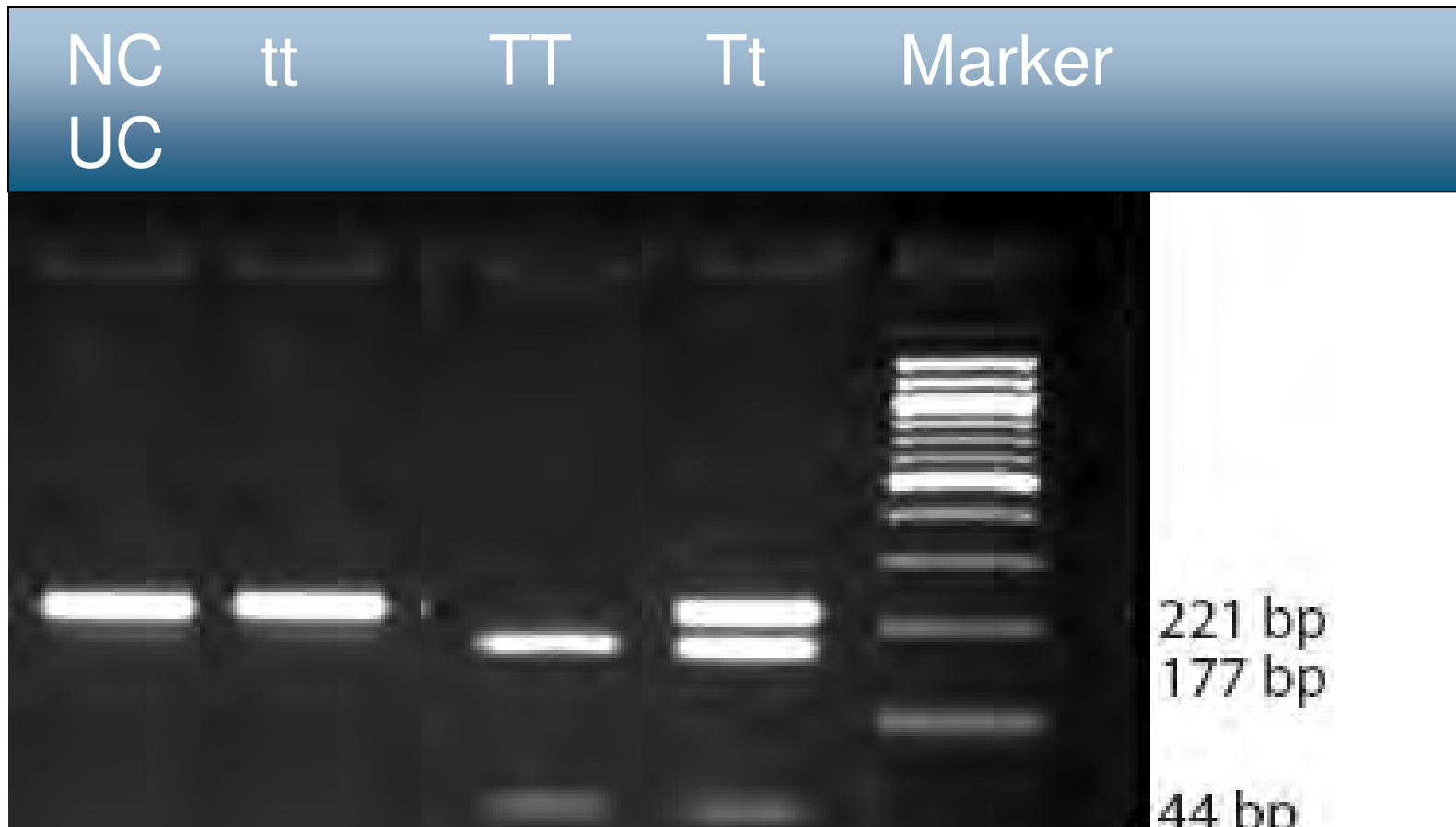
Predicting Alleles and Trait

- *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



Non-Taster

Taster

Taster

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

The screenshot shows the NCBI BLAST website. At the top, there is a navigation bar with the BLAST logo and the text "Basic Local Alignment Search Tool". The navigation bar includes links for "Home", "Recent Results", "Saved Strategies", and "Help". On the right side of the navigation bar, there are links for "My NCBI" and "Sign In | Register".

Below the navigation bar, there is a main content area. On the left, there is a section titled "NCBI/BLAST Home" with a description: "BLAST finds regions of similarity between biological sequences. [more...](#)". Below this, there is a "New" banner for "DELTA-BLAST, a more sensitive protein-protein search" with a "Go" button.

The main content area is divided into two columns. The left column has a section titled "BLAST Assembled RefSeq Genomes" with the instruction "Choose a species genome to search, or [list all genomic BLAST databases](#)". Below this, there is a list of species genomes with checkboxes: Human, Mouse, Rat, Cow, Pig, Dog, Rabbit, Chimp, Guinea pig, Sheep, Fruit fly, Honey bee, Chicken, Zebrafish, Clawed frog, Arabidopsis, Rice, Yeast, Neurospora crassa, and Microbes.

The right column has a section titled "Your Recent Results" with a "New!" indicator and a link for "All Recent results...". Below this, there is a "News" section with a link for "Custom BLAST databases" and a message: "Create custom BLAST databases with entrez. Mon, 14 Apr 2014 08:00:00 EST" with a link for "More BLAST news...". At the bottom of the right column, there is a "Tip of the Day" section with a link for "More tips...".

Below the main content area, there is a section titled "Basic BLAST" with the instruction "Choose a BLAST program to run.". Below this, there is a list of BLAST programs with descriptions and algorithms:

- [nucleotide blast](#): Search a **nucleotide** database using a **nucleotide** query
Algorithms: blastn, megablast, discontinuous megablast
- [protein blast](#): Search **protein** database using a **protein** query
Algorithms: blastp, psi-blast, phi-blast, delta-blast
- [blastx](#): Search **protein** database using a **translated nucleotide** query
- [tblastn](#): Search **translated nucleotide** database using a **protein** query
- [tblastx](#): Search **translated nucleotide** database using a **translated nucleotide** query

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

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

BLAST Assembled Genomes

Find Genomic BLAST pages:

Enter organism name or id-completions will be suggested

GO

- [Human](#)
- [Mouse](#)
- [Rat](#)
- [Cow](#)
- [Pig](#)
- [Dog](#)
- [Rabbit](#)
- [Chimp](#)
- [Guinea pig](#)
- [Fruit fly](#)
- [Honey bee](#)
- [Chicken](#)
- [Zebrafish](#)
- [Clawed frog](#)
- [Arabidopsis](#)
- [Rice](#)
- [Yeast](#)
- [Microbes](#)

The screenshot shows the BLAST Basic Local Alignment Search Tool interface. The top navigation bar includes 'Home', 'Recent Results', 'Saved Strategies', and 'Help'. The main content area is titled 'Standard Nucleotide BLAST'. A dropdown menu is open, listing various databases and search options. The 'Nucleotide collection (nr/nt)' option is highlighted in blue. Other options include 'Genomic plus Transcript', 'Reference RNA sequences (refseq_ma)', 'Reference genomic sequences (refseq_genomic)', 'RefSeq Representative genomes (refseq_representative_genomes)', 'NCBI Genomes (chromosome)', 'Expressed sequence tags (est)', 'Genomic survey sequences (gss)', 'High throughput genomic sequences (HTGS)', 'Patent sequences(pat)', 'Protein Data Bank (pdb)', 'Human ALU repeat elements (alu_repeats)', 'Sequence tagged sites (dbsts)', 'Whole-genome shotgun contigs (wgs)', 'Transcriptome Shotgun Assembly (TSA)', '16S ribosomal RNA sequences (Bacteria and Archaea)', and 'Sequence Read Archive (SRA)'. The background shows the 'Enter Query Sequence' and 'Enter accession number' fields.

How to BLAST

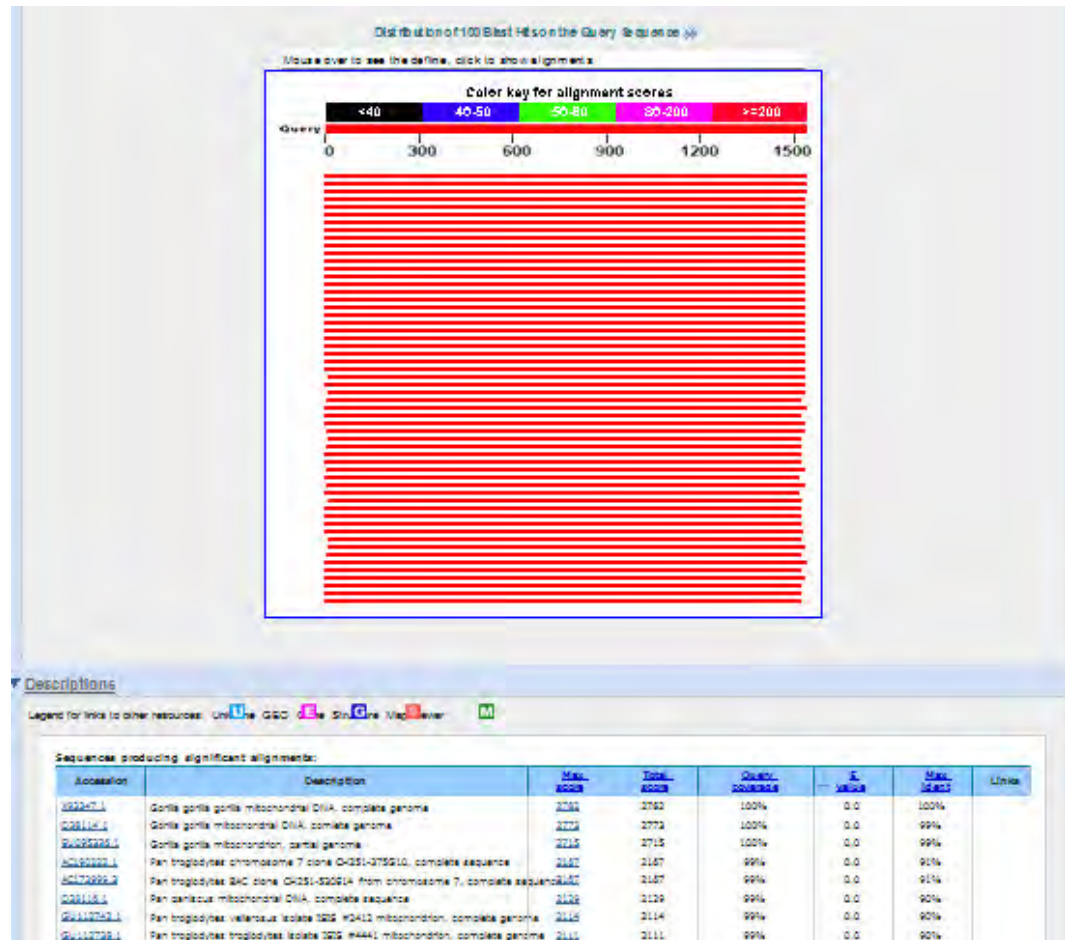
Comparing Two or More Sequences to One Another

The screenshot shows the 'Align Sequences Nucleotide BLAST' interface. It features two input fields for 'Enter Query Sequence' and 'Enter Subject Sequence', each with a 'Browse...' button and a 'Job Title' field. The 'Query subrange' and 'Subject subrange' options are visible. The 'Program Selection' section is set to 'Optimize for' with 'Highly similar sequences (megablast)' selected. A 'BLAST' button is at the bottom left, and a 'Show results in a new window' checkbox is at the bottom right.



Compare a Sequence “Query” to an NCBI Database

The screenshot shows the 'Basic Local Alignment Search Tool' interface. The 'Enter Query Sequence' field contains the sequence 'ATGCCGCA'. The 'Choose Search Set' section is expanded, showing a list of databases. The 'Nucleotide collection (nr/nt)' database is selected, and a dropdown menu is open, showing options like 'Genomic plus Transcript', 'Reference mRNA sequences (refseq_ma)', and 'Reference genomic sequences (refseq_genomic)'. The 'Database' section is set to 'Human genomic + transcript', 'Mouse genomic + transcript', and 'Others (nr etc.)'. The 'Organism' section is set to 'Human genomic plus transcript (Human G+T)' and 'Mouse genomic plus transcript (Mouse G+T)'. The 'Exclude' and 'Entrez Query' sections are also visible.

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 ATG TTC ACC GAC CGC TGA TTATTCTCTACAAACCATAAAGATATTGGAACACTATATCTA 60  
|||||  
Sbjct 5326 ATG TTC ACC GAC CGC TGA TTATTCTCTACAAACCATAAAGATATTGGAACACTATATCTA 5385  
  
Query 61 CTATTCGGCGCATGAGCTGGAGTCCTAGGCACAGCCCTAAGTCTCCTTATTCGAGCAGAA 120  
|||||  
Sbjct 5386 CTATTCGGCGCATGAGCTGGAGTCCTAGGCACAGCCCTAAGTCTCCTTATTCGAGCAGAA 5445  
  
Query 121 CTTGGTCAACCAGGCAACCTTCTAGGTAACGATCACATCTATAATGTTATCGTCACAGCC 180  
|||||
```

Reformatting Results Permits Custom Views

The screenshot displays the BLAST web interface for a search titled "NCBI/ BLAST/ blastn suite-2sequences/ Formatting Results - RZM06UEG11N". The top navigation bar includes "Home", "Recent Results", "Saved Strategies", and "Help". The main content area shows the "Formatting options" panel, which is used to customize the output of the search. The panel includes the following sections:

- Show:** Alignment as: HTML (dropdown), Old View (checkbox), Reset form to defaults (button).
- Alignment View:** Query-anchored with dots for identities (dropdown).
- Display:** Graphical Overview (checked), Sequence Retrieval (checked), NCBI-gi (checkbox).
- Masking:** Character: Lower Case (dropdown), Color: Grey (dropdown).
- Limit results:** Descriptions: 100 (dropdown), Graphical overview: 100 (dropdown), Alignments: 100 (dropdown), Line length: 60 (dropdown).
- Expect Min:** (input field), **Expect Max:** (input field).
- Percent Identity Min:** (input field), **Percent Identity Max:** (input field).

Below the formatting options, the search results are displayed under the heading "Blast 2 sequences". The first result is "BRCA1_Reference_DNA_Sequence (600 letters)".

Three black arrows with white text indicate specific steps in the process:

- Step 11:** Points to the "Formatting options" link in the top navigation bar.
- Step 12:** Points to the "Alignment View" dropdown menu.
- Step 13:** Points to the "Reformat" button.

Query-Anchored with Dots for Identities

Alignments			
Query	1	ATGTTCCGCCGACCGCTGACTATTCTCTACAAACCACAAAGATATTGGAACACTATACCTA	60
19247	1A.....T.....T.....T.....	60
19247	821	812
19248	1G.....C..G.....G.....G	60
19249	4CT.....T..	44
19249	78	70
19250	13C..T..A.....T.....C.....C..G..TT..	60
19250	252	255
19251	13T.....A..C.....T.....C..C.....T...	60
Query	61	CTATTCCGGCGCATGAGCTGGAGTCCCTGGGCACAGCCCTAAGTCTCCTTATTCGGGCTGAA	120
19247	61A.....A.....A..A...	120
19248	61	T.G.....T.....A.....T.....C.....C.....T.....	120
19248	371	379
19249	45T..T.....AC.G.A..T.T..TA.....A..C...	104
19249	69	.	69
19250	61T..T.....A..A.A.....T.....C.....C.....A.....	120
19250	256	A..A.....	266
19251	61T.....A..G.CAG.A..A.....A.....A..G...	120
Query	121	CTAGGCCAACCAGGCAACCTTCTAGGTAATGACCACATCTACAATGTCATCGTCACAGCC	180
19247	121	..T..T.....C..T.....T.....T.....	180
19248	121C.....C.....T..T.....	180
19248	380	.	380
19249	105	..G..T.....T..T..T..GT...C.....T.....T.....T.....	164
19250	121C.....A.....C..C.....T..C..T..T..A..G...	180
19250	440T.A.....	426
19251	121	..C..T.....G.GT..AA...AAG.C.....TG.T..T.....A.....T..CT.T	180
19251	744	.	744
Query	181	CATGCATTCGTAATAATCTTCTTCATAGTAATGCCTATTATAATCGGAGGCTTTGGCAAC	240
19247	181G.....T.....C.....	240
19247	751G.....C.CA..T....	775
19247	1396T.....AAT.T.....T...	1420
19248	181T..T.....G..C.....C..A.....T.....	240
19248	751GC.....C.CA..C....	775
19249	165T..C.....T.....T..G..T..A..C..C.....T.....T..C..A...	224
19249	1147	1163
19250	181	T..C..T..T..C..A..C.....T.....C..C..C	240

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		
<input type="checkbox"/>	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

Sequences producing significant alignments:

Select: [All](#) [None](#) Selected:0

Alignments		Download	Graphics				Settings
	Description	Max score	Total score	Query cover	E value	Ident	Accession
<input type="checkbox"/>	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**T**T****G**

A**G****G****C****A****A****C****A****G**

Predicting PTC tasting of non-human primates



Resources- Biotech 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](#)
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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?
