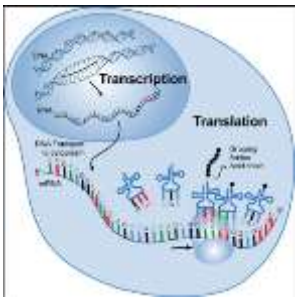
Name: _____ Date:_____



Gen Bio 1 Lab #10: PCR & Transcription/Translation Lab

Complete Pre-Lab vocabulary and Pre-Lab Questions before attending Lab.
Pre-Lab Reading: Handout text below; Pages 285-291 8th Ed. or pages 288-296 9th Ed.

Pre-Lab Vocabulary:

Define 1-4 as they relate to the Polymerase Chain Reaction [PCR]

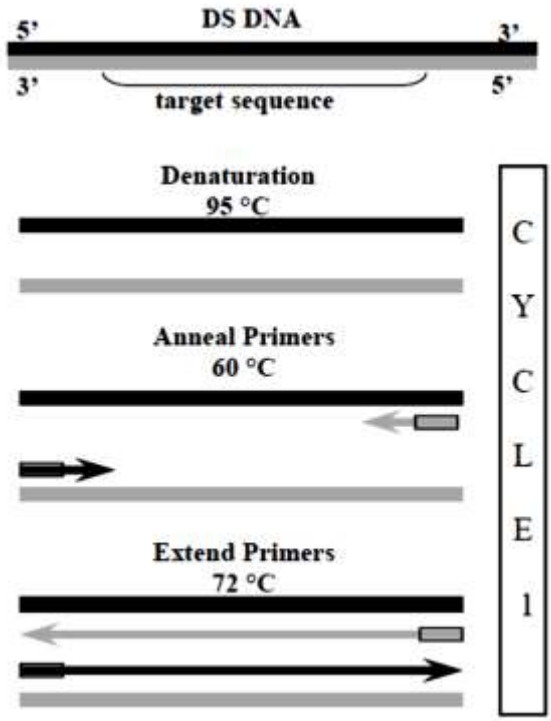
1. anneal-
2. denature-
3. primers-
4. *Taq* DNA polymerase-
5. gene-
6. codon-
7. mutation -
8. mRNA -
9. Genetically-modified organisms –

Pre-Lab Video: http://www.youtube.com/watch?v=JRAA4C2OPwg

Pre-Lab Reading: The Polymerase Chain Reaction and Genetically-Modified Organisms

The Polymerase Chain Reaction (PCR), invented by Kary Mullis of the Cetus Corporation in 1985, is essentially a system for cell-free DNA replication. In PCR, a solution of DNA and reagents are subjected to a repeated cycle - usually 30 to 40 times - of three different temperatures. A very high temperature (94°C-96°C) is used for DNA denaturation — separating double-stranded DNA into single strands of DNA. A lower temperature (50°C-65°C) is used for annealing (binding) primers to their complementary sequences (A with T, G with C) on the single-stranded DNA. And a high temperature (72°C) is used for extension — involving DNA polymerase adding new nucleotides and making a new copy. Each copy serves as a template for the next cycle, so that the amount of DNA located between the primers doubles with each cycle (see **Figure**). This amplifies the DNA of interest from miniscule quantities to levels sufficient for direct detection or analysis, such as with agarose gel electrophoresis. Thirty cycles provides a theoretical maximum of **2^{30} amplifications**; in other

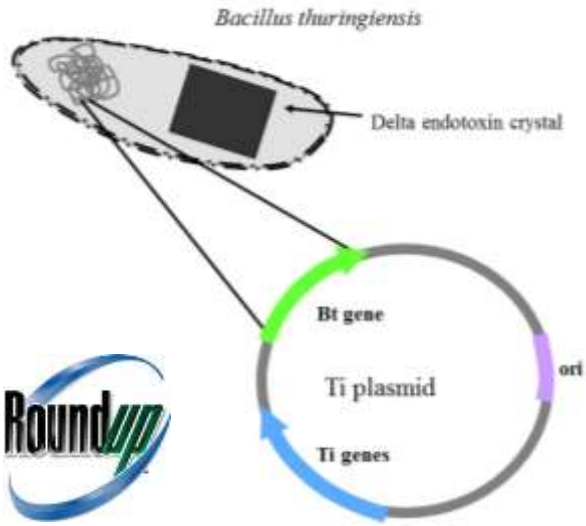
words, **one DNA molecule becomes a billion DNA copies after 30 cycles**. The use of specific primers that flank a certain sequence of DNA accounts for the "magic" of PCR amplifying just the DNA sequence of interest, called the target DNA. Virtually all of the final products will be just target DNA. PCR became practical when *Taq* DNA polymerase was purified from *Thermus aquaticus*, a bacterium isolated from the hot springs of Yellowstone National Park. Because PCR requires denaturation temperatures around 95°C, *Taq* polymerase was, fortunately, well suited for the reaction, surviving over 40 minutes at 95°C. *Taq* has an optimum reaction temperature of 72°C which is the temperature used for extending the primers and copying the template DNA.



Applications of PCR Because PCR relies on the hybridization of single-stranded DNA primers, it is very specific and can distinguish unique DNA samples from complicated mixtures of sequences. PCR is also very sensitive, able to detect very small amounts of the target DNA in a sample. These two characteristics make PCR extremely useful in scientific research, clinical medicine, and forensics. Infectious diseases can be diagnosed faster and more accurately by PCR than traditional methods. Diagnostics companies and clinical laboratories are developing commercial kits for the detection of many human and animal diseases, such as HIV, and viruses and fungi that infect plants and food crops. PCR can also be used to detect inherited disorders like cystic fibrosis or Huntington's disease, even in unborn fetuses or *in vitro* fertilized eggs ("test tube babies") before they are implanted in the mother's womb.

Genetically-Modified Organisms (GMO): an organism (plant or animal) in which the genetic material has been altered in a way that does not occur naturally by mating and/or natural recombination. The US currently mass-produces plants that are GMO, including **corn**, **soy**, potatoes, rice, squash, sugar beets, & tomatoes. Why would we approve and allow industrial agricultural companies to grow and sell GMO plants? The primary reasons include: a growing human population, loss of farmable land, remediation of soil, and to enrich the nutrient content of supermarket foods. What types of genes do we modify into our plant crops? The most prevalent genes added to our plant crops come from bacteria or yeast genes that code for **pest resistance, herbicide tolerance, plant virus resistance, & drought resistance**. However, scientists have also isolated plant genes from non-crop (non-domesticated) plants that give **increased nutritional value, improved fruit taste or appearance, and altered or faster ripening**. Current Federal legislation in the US says that food can be labeled "GMO-Free" only if its ingredients contain <5% GMO-products or by-products.

Are GM crops a good thing? Many people who object to the use of GM crops argue that there is a potential for "super weeds" to arise through cross-pollination with herbicide resistant crops, or that "superbugs" will evolve to be resistant to the toxins in pest-resistant crops. Many are concerned about potential allergic reactions to novel proteins, antibiotic resistance arising from the selectable markers used to develop the crops, or other unforeseen effects on public health. Some individuals believe that we have not done enough research to fully understand the implications of altering the planet's plant diversity. Proponents of GM crops and foods argue that these crops are beneficial for the environment because they reduce the use of herbicides and pesticides that are toxic to the environment and human health. In addition, GM crops may preserve arable land by reducing stresses on the land, improve the nutritional value of food for developing countries, and allow crops to be grown on previously non-arable land.



Regardless of where you might stand on the GM debate, would you be interested to know how much of the corn- or soy-based foods we eat have been genetically modified? Good, I'm glad you're interested in this because today we'll be using PCR to test whether corn- or soy-based foods from the grocery store are Genetically-Modified Organisms, and then we'll analyze our results in next week's lab.

We will be testing for 2 genes commonly added to GMO crops like corn and potatoes: "Bt" & "Round-Up Ready" genes. The "Bt" gene is added to crops for pest resistance. The gene codes for a *Bacillus thuringiensis* protein, which is a delta endotoxin that kills nasty and destructive insects called corn borers (see **Figure**). The "Round-Up Ready" gene is added to crops to help them tolerate the herbicide Round-Up. The gene codes for an *Agrobacterium tumifaciens* protein, which breaks down the Round Up herbicide (glyphosate) and allows the crop plant to live when the field is sprayed with Round-Up to kill weeds.

Pre-Lab Questions:

1. What are the **2 GMO genes** we are testing for?

2. Briefly, how does PCR work? What do we need it for?

3. What do we use primers for during PCR? What would happen if we left them out?

Pre-Lab Reading: Transcription & Translation

Transcription is the process by which RNA is synthesized from DNA. It occurs in the nucleus. RNA (RiboNucleic Acid) is very similar to DNA (DeoxyriboNucleic Acid). RNA normally exists as a single strand (and not the double stranded double helix of DNA). It contains the nucleosides adenine, guanine and cytosine. However, there is no thymine found in RNA, and in its place is a similar base called uracil.

Translation occurs in the cytoplasm, specifically on the ribosomes. The mRNA made in the nucleus travels out to the ribosome to carry the "message" of the DNA, in order to be translated into an amino acid sequence. Important to the process of translation is another type of RNA called transfer RNA (tRNA) which functions to carry the amino acids to the site of protein synthesis on the ribosome. A tRNA has two important areas. These are the anticodon, which matches the codon on the RNA strand, and the corresponding amino acid. Remember that codons are sets of three bases that code for a single amino acid. At the top of the tRNA is the amino acid.

There are twenty amino acids that can combine together to form proteins of all kinds, these are the proteins that are used in life processes. When you digest your food for instance, you are using enzymes that were originally proteins that were assembled from amino acids. Each tRNA has a different amino acid which link together like box cars on a train.

Pre-Lab Questions:

1. How many different kinds of bases can be found on DNA _____

2. What base is found on RNA but not on DNA? _____

3. How many bases are in a codon? _____ In an anticodon? _____

4. How many amino acids are attached to a single transfer RNA? _____

5. Transcription occurs in the _____; the process of assembling a protein from RNA is called _____ and occurs in the _____.

Procedure 1: DNA extraction from food and PCR Setup

Materials Needed per Lab

12 Screwcap tubes, 1.5 ml
36 PCR tubes, 0.2 ml
6 tube holders for boiling
6 colored plastic microtube racks
6 200 µl Eppendorf micropipetters and 6 boxes 200 µl tips
1 1000 µl Eppendorf micropipetters and 1 box 1000 µl tips
4-6 grocery store foods including: *Doritos, Protein drink powder, Corn bread mix, Boca burger, strawberries, & bananas*
Micro-Centrifuges
Bench weight scale
PCR machine, use program called "GMO"

From Prep room fridge: InstaGene matrix bottle, 20 ml
From Prep room freezer: *Boca burger*

From HS-220 freezer: (in an Isotemp cold box)

Bio-Rad certified non-GMO food control (1 bag oats)
PCR Master mix with GMO primers (red) 500-1000 µl
PCR Master mix with Plant PSII primers (green) µl
1 tube GMO positive control DNA

Materials Needed per Table

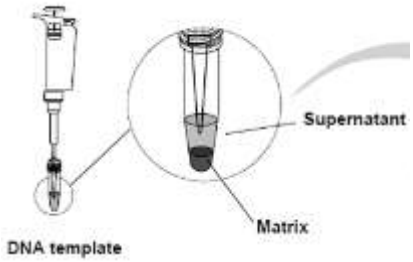
Sharpie of a unique color (pack up front)
200 µl Eppendorf micropipetters
250mL Beaker (for boiling)

Procedure 1 Part A: Grinding and Isolating DNA from food samples

Before starting, fill your beaker ½ way with tap water, and put it on your boiling plate set to 95

1. Find your screwcap tubes and label one "**non-GMO**" and one "**test food**".
2. Weigh out **2g** of certified non-GMO food (oats) and put it into the mortar.
3. Add **5 ml** of distilled water, and grind with pestle for at least **2 min** to form slurry.
4. Add **5 ml** of water again and mix or grind further with pestle until smooth enough to pipet.
5. Then pipet **50 µl** of ground slurry in and recap tube.
6. Add **500 µl of InstaGene** to a screwcap tube you have labeled **"non-GMO"**.
7. Repeat steps 2–5 to prepare 1 test food sample (your group's choice).
8. Add **500 µl of InstaGene** to a screwcap tube you have labeled **"Test food"**. Then pipet **50 µl** of ground slurry in and recap tube.
9. Make sure all liquid is at the base of each tube.
10. Place the InstaGene tubes in **95°C water using the boiling float in a beaker for 5 min**
11. Place tubes in micro-centrifuge and centrifuge for 5 min at max speed to pellet. Your instructor will give you a demonstration of how to do this.

Procedure 1 Part B: Setting up PCR tubes and running PCR



Number PCR tubes 1–6, and then add your group Greek letter (α, β, γ, δ, or ε) which is on your timer. Referring to the table, and using a fresh tip for each addition, add 20 µl of the indicated solution to each PCR tube, and then cap tubes. **Be sure to only pipette the supernatant, and avoid the InstaGene pellet at the bottom of the tubes.**

The tube numbers should correspond to the following tube contents:

Tube number	Master Mix	DNA
1	20 µl Plant MM (green)	20 µl Non-GMO food control DNA
2	20 µl GMO MM (red)	20 µl Non-GMO food control DNA
3	20 µl Plant MM (green)	20 µl Test food DNA
4	20 µl GMO MM (red)	20 µl Test food DNA
5	20 µl Plant MM (green)	20 µl GMO positive control DNA
6	20 µl GMO MM (red)	20 µl GMO positive control DNA

1. ***Thaw out*** the **"Plant" green**, **"GMO" red** and **yellow-label tubes** using your hands (***rolling between your palms***), then place back in your cold Isotemp box.
2. After pipetting, ***spin down all liquid droplets to the bottom of the inside of your tubes***. Spin for 20 sec.
3. **Keep your reactions in your Isotemp cold box** until every lab group is done and ready for starting the thermal cycler machine.
4. Place the reaction tubes in the **thermal cycler**. Handle the tubes **gently** to avoid shaking the contents.
5. Your instructor will start the thermal cycler machine **once everyone is finished** with their 4 tubes, and it will run our reactions for us, **allowing us to analyze the results next week.** Stay tuned!

Procedure 2: Transcription & Translation Cut & Paste

Materials needed for lab:
~260 copies (for 11 sections) of 3 handouts

Activity:

Obtain the three colored papers from your instructor and follow directions on them to complete this activity.
Staple completed part to the back of this lab handout.

	U	C	A	G
U	UUU = phe UUC = phe UUA = leu UUG = leu	UCU = ser UCC = ser UCA = ser UCG = ser	UAU = tyr UAC = tyr UAA = stop UAG = stop	UGU = cys UGC = cys UGA = stop UGG = trp
C	CUU = leu CUC = leu CUA = leu CUG = leu	CCU = pro CCC = pro CCA = pro CCG = pro	CAU = his CAC = his CAA = gln CAG = gln	CGU = arg CGC = arg CGA = arg CGG = arg
A	AUU = ile AUC = ile AUA = ile AUG = met	ACU = thr ACC = thr ACA = thr ACG = thr	AAU = asn AAC = asn AAA = lys AAG = lys	AGU = ser AGC = ser AGA = arg AGG = arg
G	GUU = val GUC = val GUA = val GUG = val	GCU = ala GCC = ala GCA = ala GCG = ala	GAU = asp GAC = asp GAA = glu GAG = glu	GGU = gly GGC = gly GGA = gly GGG = gly

The genetic code chart represents the sequence on the **mRNA** codon. Use this chart as information to fill in the next page.

For each of the following sequences, cross-convert it into the sequences that have been left blank for: DNA, mRNA codon, tRNA anticodon, or the amino acid sequence (AA). If several sequences might work, just choose any one.

1. DNA T A C C G C T C C G C C G T C G A C A A T A C C A C T

   mRNA _____

   tRNA _____

   AA _____

2. DNA _____

   mRNA A U G A C U A G C U G G G G G U A U U A C U U U U A G

   tRNA _____

   AA _____

3. DNA _____

mRNA _____

tRNA U A C C A C C C C G U A U G G C U G G G A A U A U C

AA _____

4. DNA _____

mRNA _____

tRNA _____

AA MET ARG GLY PHE PHE MET VAL GLY (STOP)

Questions to e x p a n d your mind.

1. What are the pros and cons of using GMO for food? What makes it necessary?

2. Now that you are more familiar with 2 examples of genes added to crop plants, what do you think of the process of transferring genes to plants, and of eating GMO foods?

3. Why do you think it's important that our cells rely on mRNA to communicate the "Genetic code" to our ribosomes? What would happen if we instead relied only on tRNA?

4. **INTERNET SEARCH OPPORTUNITY:** Aside from detection of non-plant genes in GMO plants, what are some of the other applications of the PCR process? Research an application of PCR and briefly describe it. You may include a diagram from a website, printed and attached to your lab.