BTEC 2200 Module 1 Blueprint

Key

Curriculum Map

Module	Learning Outcomes	Learning Objectives	Assessments	Topics/Readings/ Learning Experiences
	The student conducts a scientific experiment using gene quantitation methods.	Describe quantitative PCR. Describe reverse transcriptase quantitative PCR. Explain how gene quantitation is used to answer scientific questions. Identify appropriate controls for quantitative PCR experiments Design primers for a quantitative PCR experiment. Implement appropriate controls for a quantitative PCR experiment. Quantify DNA via quantitative PCR. Analyze data from a gene quantitation experiment.		Lecture material in learning platform - How it works - Controls - What the data looks like - How to analyze the data - Examples of how it's used Do the meat lab

Media Tables

Images

Source (URL, file name, etc.)	Can we legally use it? Do we need to give credit? If being created, provide description	Final file name	Alt-text (if not decorative)

Videos

Source (URL, My Media, file name, etc.)	Title (what we'll call it)	Can we legally use it? Download? Embed? Give credit? Edit it?	Captioning

Module 1 Outline

Module Title: Module 1: PA - Quantitative PCR
Page: Learn: Introduction to Quantitative PCR
Page: Learn: Melting Curves
Page: Learn: Taqman
Page: Learn: Thresholds in qPCR
Page: Learn: ROX - A Passive Dye
Page: Learn: Getting Reliable Data in qPCR
Quiz: [Name of quiz]
Page: Learn: Examples of How Quantitative PCR Is Used
Lab: Meat Lab

Module 1 Content

Page: [Module 1 icon] Learn: Quantitative PCR

[Blue Introduction icon] Introduction to Quantitative PCR
Images

Source (URL, file name, etc.)	Can we legally use it? Do we need to give credit? If being created, provide description	Final file name	Alt-text (if not decorative)
Add image			

You've carried out PCR - Polymerase Chain Reaction - several times already. In BTEC 1000, you amplified the TAS2R gene from your own DNA in order to determine which PTC-tasting alleles you carry. In BTEC 1100, you used PCR to amplify the BglA gene, which you cloned into a plasmid so you could overexpress BglA from *E. coli*. Now, you will be performing a variation on PCR that allows you "see" how much DNA is in the tube after each cycle, and to use that information to determine how much template DNA was present in the original sample.

Quantitative PCR (qPCR) is also referred to as Real Time PCR, because of that ability to "watch" the progression in real time. Local company BioFire Diagnostics, formerly known as Idaho Technology, was an early leader in qPCR with their Light Cycler instrument. Today, qPCR is considered the "gold standard" for quantification of nucleic acids, and it is used in both diagnostic tests and basic research.

In this module, you will perform an experiment that models how qPCR could be used to determine the presence and level of pathogenic DNA in food. You will be given a sample of bacteria "grown from a meat sample", and asked to determine the degree of contamination by the "pathogenic bacterium O157:H7." (The quotes indicate that we are actually using safe samples rather than real pathogens for teaching purposes.)

Watch this video for an overview of qPCR. On the following pages, you'll learn about different ways to "see" the DNA as it's being made, and about important controls.

[Watch icon] Overview of qPCR
https://www.youtube.com/watch?v=1kvy17ugl4w&t=27s

Videos

Source (URL, My Media, file name, etc.)	Title (what we'll call it)	Can we legally use it? Download? Embed? Give credit? Edit it?	Captioning
https://www.youtube.com/watch?v=1kvy17ugl4w&t=27s	Overview of qPCR (New England Biolabs - 2:44)	**Embed** **Citation:** New England Biolabs. (2016, Dec. 12). *Overview of qPCR.* [Video file] Retrieved from https://www.youtube.com/watch?v=1kvy17ugl4w&t=27s	Already captioned

[Objectives icon] Objectives

- Describe quantitative PCR.
- Describe reverse transcriptase quantitative PCR.

- Explain how gene quantitation is used to answer scientific questions.
- Identify appropriate controls for quantitative PCR experiments
- Design primers for a quantitative PCR experiment.
- Implement appropriate controls for a quantitative PCR experiment.
- Quantify DNA via quantitative PCR.
- Analyze data from a gene quantitation experiment.
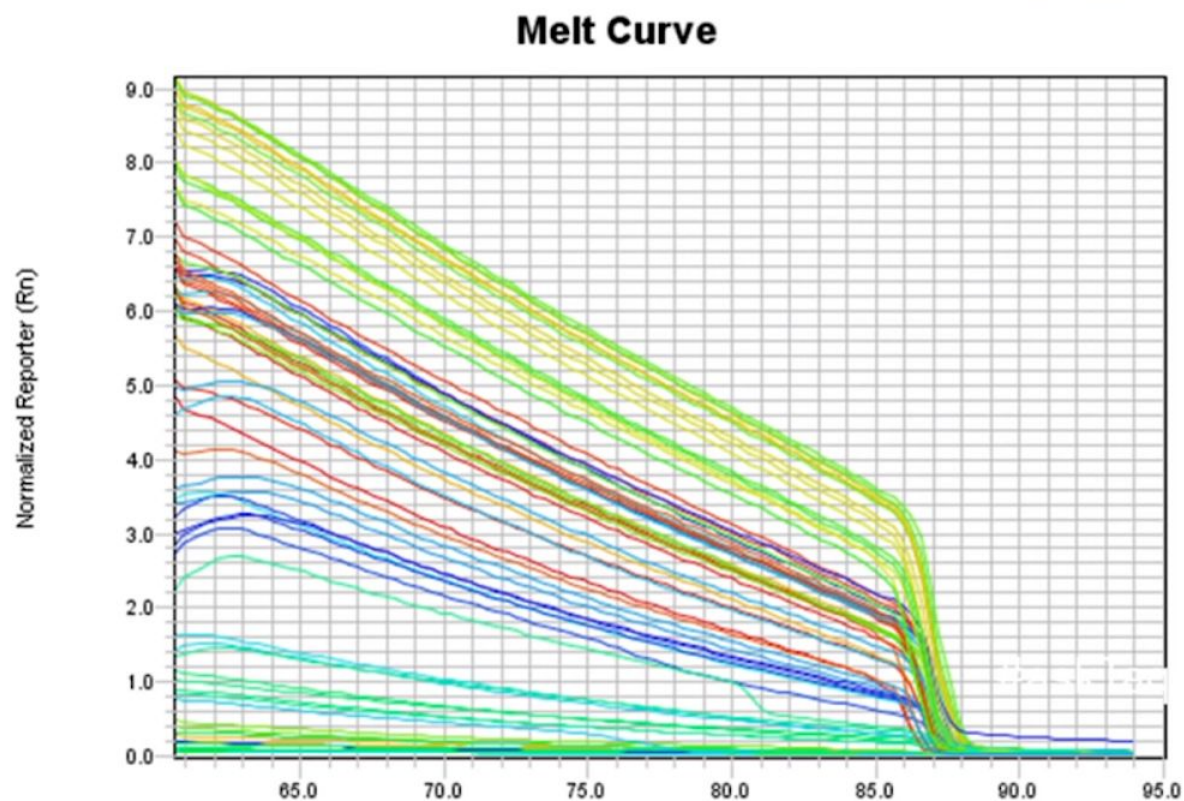

Page: Melting Curves

As you saw in the overview of qPCR video, a common way of carrying out qPCR (and the method you will use) is with SYBR green. SYBR green binds double stranded DNA, and fluoresces more brightly when it is bound to dsDNA. However, SYBR green binds to all dsDNA indiscriminately - it doesn't care whether it's newly created PCR product of your target gene, or some off-target product, or primers that are binding to each other.

Since we generally want to quantify only a particular target sequence rather than all double-stranded DNA, qPCR with SYBR green includes an additional step after cycling has finished, called the melting curve. In this step, the machine slowly raises the temperature from about 60 to 95 degrees. Recall that DNA melting temperature (the temperature at which the two strands come apart) depends on the length of the DNA molecule, and the proportion of GC base pairs. (GC has three hydrogen bonds, while AT has only two, so it takes more heat to melt GC.) When the DNA melts, SYBR fluorescence drops off sharply. This can be seen on the Melt Curve graphs below; both reflect the same data, but in different forms. Each colored line represents one well on the qPCR plate. The first graph simply shows normalized fluorescence as a function of temperature. The second shows the derivative of the normalized fluorescence - meaning the rate of change of fluorescence - as a function of temperature. The derivative graph isn't as intuitive, but shows the information a bit more clearly. In the derivative graph, you hope to see only one peak for each amplicon (or PCR product). Multiple peaks indicate that there are multiple temperatures at which DNA melts, which means there are multiple species of double stranded DNA. That indicates that you're quantifying not just the desired target sequence, but something else as well that melts at a different temperature.

Images

Source (URL, file name, etc.)	Can we legally use it? Do we need to give credit? If being created, provide description	Final file name	Alt-text (if not decorative)
Screenshot https://www.youtube.com/watch?v=4QPyVcpbvNw&index=36&list=PL4AACABAB6F402E9B	Screen shot from video - fair use **Credit:** Thermo Fisher Scientific. (2012, July 26). *Finding Multiple Melt-Curve Peaks When Using SYBR® Green in Real-Time PCR* -- Ask TaqMan®: Ep. 8. Retrieved from	*Finding Multiple Melt-Curve Peaks When Using SYBR® Green in Real-Time PCR* -- Ask TaqMan®: Ep. 8	A melt curve graph has temperature, from 60 to 95 degrees, on the X-axis, and normalized reporter (fluorescence) on the Y-axis. Many colored lines are on

	https://www.youtube.com/watch?v=4QPyVcpbvNw&index=36&list=PL4AACABAB6F402E9B		the graph, each representing one well on the qPCR plate. Nearly all of the lines drop precipitously at 87 degrees.

Melt Curve

[Image caption] A melt curve in SYBR-based Real-Time PCR, showing normalized reporter vs. cycle number. Source: Ask TaqMan®: Ep. 8.

Images

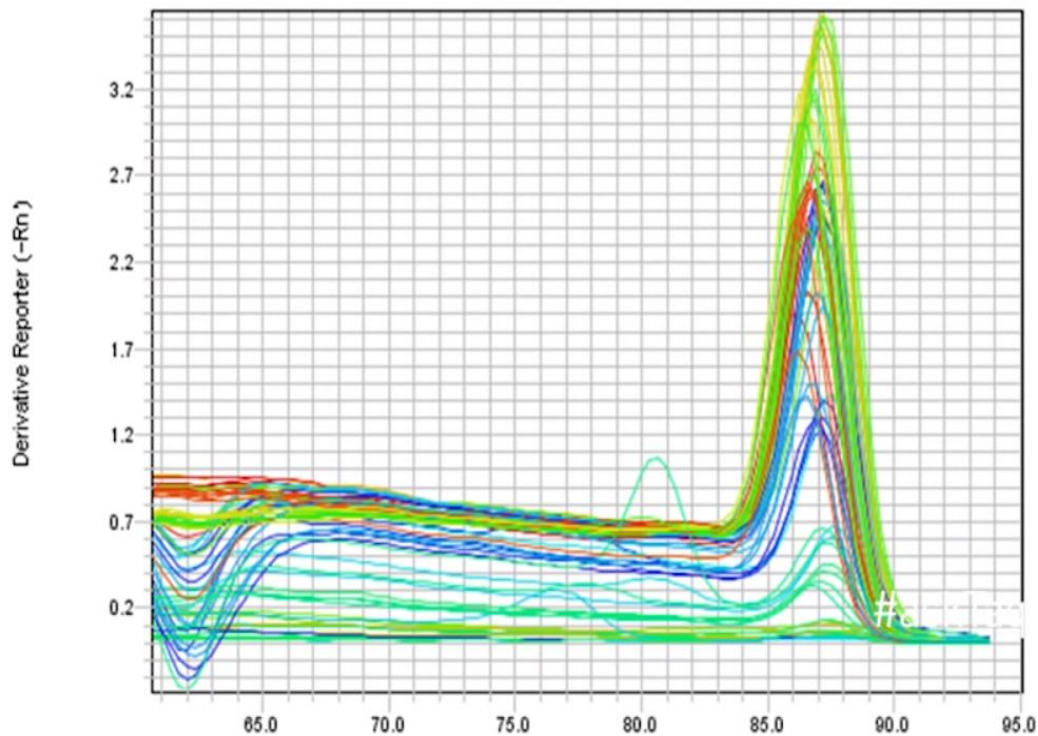| Source (URL, file name, etc.)	Can we legally use it? Do we need to give credit? If being created, provide description	Final file name	Alt-text (if not decorative)
Screenshot https://www.youtube.com/watch	Screen shot from video - fair use	*Finding Multiple Melt-Curve Peaks When*	A melt curve graph has temperature, from 60 to 95

Melt Curve

[Image caption] A melt curve in SYBR-based Real-Time PCR, showing the derivative (rate of change of) normalized reporter vs. cycle number. Generally, we hope to see just one peak for each line, which suggests that there is only a single amplicon (PCR product). Source: Thermo Fisher Scientific. (2012, July 26). *Finding Multiple Melt-Curve Peaks When Using SYBR® Green in Real-Time PCR -- Ask TaqMan®: Ep. 8*
 Retrieved from https://www.youtube.com/watch?v=4QPyVcpbvNw&index=36&list=PL4AACABAB6F402E9B

Watch the video below to learn more about melting curves.

Videos

Source (URL, My Media, file name, etc.)	Title (what we'll call it)	Can we legally use it? Download? Embed? Give credit? Edit it?	Captioning
https://www.youtube.com/watch?v=4QPyVcpbvNw&index=36&list=PL4AACABAB6F402E9B	Finding Multiple Melt-Curve Peaks When Using SYBR® Green in Real-Time PCR - Ask TaqMan®: Ep. 8 (3:28)	**Embed** **Credit:** Thermo Fisher Scientific. (2012, July 26). *Finding Multiple Melt-Curve Peaks When Using SYBR® Green in Real-Time PCR - Ask TaqMan®: Ep. 8.* [Video file]. Retrieved from https://www.youtube.com/watch?v=4QPyVcpbvNw&index=36&list=PL4AACABAB6F402E9B	Already captioned

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Page: [Module 1 icon] TaqMan

In TaqMan qPCR, the signal is provided by a sequence-specific probe. It still uses two primers flanking the desired sequence, but also another oligonucleotide, the probe, that binds between the primers. The probe has a reporter - which makes fluorescence - and a quencher - which absorbs the fluorescence if it is close enough to the reporter. When Taq polymerase starts extending a primer in PCR, it will "chew up" the probe as it passes through. Breaking up the probe separates the reporter and quencher, so that the fluorescence is now measurable by the instrument. In this way, fluorescence is tied directly to amplification of the probe sequence, and is likely to be truly target-specific.

Watch the video below to understand TaqMan even better. (Also, as you watch the video, admire the creativity of whoever came up with the name TaqMan. Doesn't it look like the video game PacMan?)

Videos

Source (URL, My Media, file name, etc.)	Title (what we'll call it)	Can we legally use it? Download? Embed? Give credit? Edit it?	Captioning
https://www.youtube.com/watch?v=fkUDu042xic&list=PL4AACABAB6F402E9B&t=0s&index=32	How TaqMan Works -- Ask TaqMan® Ep. 13 by Life Technologies (3:59)	**Embed** **Credit:** Thermo Fisher Scientific. (2013, February 25). *How TaqMan Works -- Ask TaqMan® Ep. 13 by Life Technologies.* [Video file]. Retrieved from https://www.youtube.com/watch?v=fkUDu042xic&list=PL4AACABAB6F402E9B&t=0s&index=32	Already captioned

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Page: [Module 1 icon] Thresholds in qPCR

The threshold in qPCR is a level of fluorescence detection that is above background noise. The threshold can be automatically identified by qPCR software, or manually adjusted. In the image below, the threshold is the red horizontal line. Note that the line is placed at a point about midway through the exponential phase of amplification in the wells.

In qPCR, we measure how many cycles of replication it takes for a sample's fluorescence reach the threshold. Recall that fluorescence is generated by double stranded DNA with SYBR green, or by destruction of the probe by Taq in TaqMan. So, effectively, the threshold is an amount of target DNA. If a sample started with lots of the target DNA, it won't take many cycles to reach the threshold. A sample that started with less target DNA will take more cycles of replication to reach the threshold.
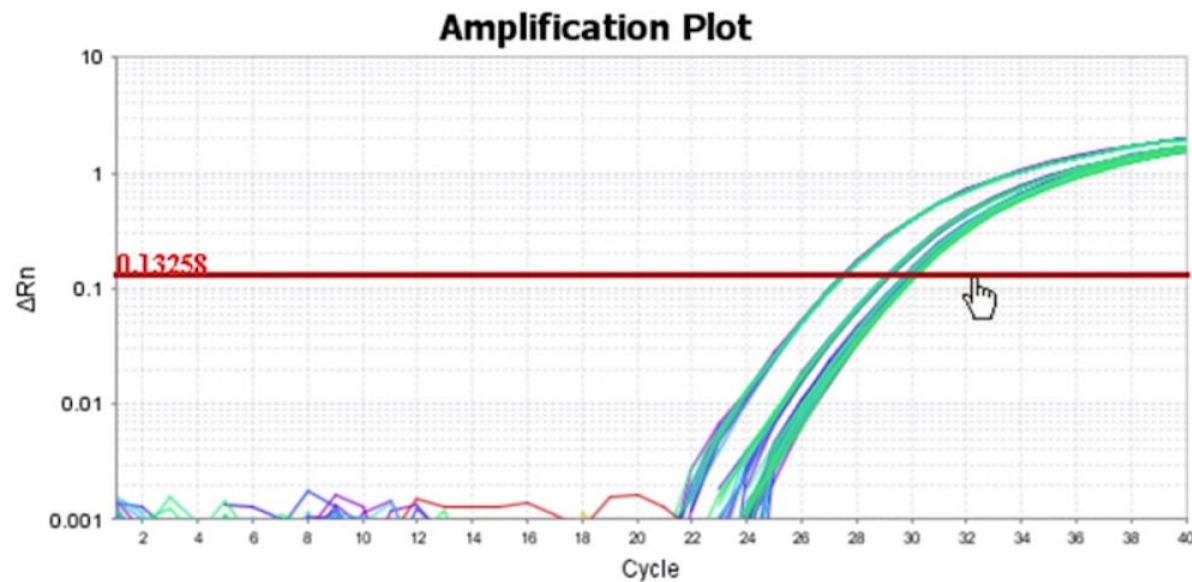
The number of cycles it takes to reach the threshold is called the Ct (cycle threshold) or Cq (cycle of quantitation). It is determined by the point at which the amplification curve crosses the threshold. Watch the video below to learn about considerations for threshold placement.

Images

Source (URL, file name, etc.)	Can we legally use it? Do we need to give credit? If being created, provide description	Final file name	Alt-text (if not decorative)
Screenshot https://www.youtube.com/watch?v=H_xsuRQIM9M&index=39&list=PL4AACABAB6F402E9B&t=0s	Screenshot from video - fair use Credit: Thermo Fisher Scientific. (2012, June 26). *Real-Time PCR Thresholds and Where to Place*	Amplification Plot with Threshold	An amplification plot with cycle number on the X-axis and delta Rn (the change in normalized fluorescence) on

	Them -- Ask TaqMan®: Ep. 6. Retrieved from https://www.youtube.com/watch?v=H_xsuRQIM 9M&index=39&list=PL4AACABAB6F402E9B&t=0s		the Y-axis.Each curve on the graph represents one well of the qPCR reaction; these curves all stay very low (with some noise) until cycle 22 or more, then they curve upwards until starting to level off at cycle 30 and later. A horizontal red line slightly higher than the middle of the curves represents the threshold.

[Image caption] A qPCR amplification plot showing the threshold (in red). Source: Real-Time PCR Thresholds and Where to Place Them -- Ask TaqMan®: Ep. 6

Videos

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https://www.youtube.com/watch?v=H_xsuRQIM9M&index=39&list=PL4AACABAB6F402E9B&t=0s	Real-Time PCR Thresholds and Where to Place Them -- Ask TaqMan®: Ep. 6 (2:50)	**Embed** **Credit:** Thermo Fisher Scientific. (2012, June 26). *Real-Time PCR Thresholds and Where to Place Them -- Ask TaqMan®: Ep. 6.* [Video file]. Retrieved from https://www.youtube.com/watch?v=H_xsuRQIM9M&index=39&list=PL4AACABAB6F402E9B&t=0s	Already captioned

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Page: [Module 1 icon] ROX - A Passive Dye

You may have noticed that the Y-axis of all the amplification plots you've been seeing is labeled "Rn". "Rn" refers to normalized reporter, where the reporter is fluorescence. How is the reporter data normalized?

Most qPCR systems incorporate a passive dye, which is a dye that does not change fluorescence as a direct result of amplification. Watch the video below to learn how ROX allows normalization of the data, correcting for well-to-well variability.

Videos

Source (URL, My Media, file name, etc.)	Title (what we'll call it)	Can we legally use it? Download? Embed? Give credit? Edit it?	Captioning
https://www.youtube.com/watch?v=qq0l_wyhFZo&index=38&list=PL4AACABAB6F402E9B&t=0s	The Purpose of ROX™ in Real-Time PCR -- Ask TaqMan®: Ep. 7 (3:29)	**Embed** **Credit:** Thermo Fisher Scientific. (2012, June 26). *The Purpose of ROX™ in Real-Time PCR -- Ask TaqMan®: Ep. 7.* [Video file]. Retrieved from https://www.youtube.com/watch?v=qq0l_wyhFZo&index=38&list=PL4AACABAB6F402E9B&t=0s	Already captioned

The two most common applications for qPCR are measuring changes in gene expression and quantifying nucleic acids. At the end of this module, you'll use qPCR to quantify the amount of a particular gene sequence in a sample. Watch the video below to learn more about these two main applications.

Videos

Source (URL, My Media, file name, etc.)	Title (what we'll call it)	Can we legally use it? Download? Embed? Give credit? Edit it?	Captioning
https://www.youtube.com/watch?v=Dh9qSMKFlEU&list=PL4AACABAB6F402E9B&t=0s&index=28	Real-time PCR Applications -- Ask TaqMan® Ep. 17 by Life Technologies	**Embed**	Already captioned

"Gene expression" refers to the process in which genetic information (genotype) affects and determines an organism's characteristics and traits (phenotype). Gene expression starts with transcription - making an RNA "copy" of the gene. For genes that encode proteins, translation - the making of a protein from RNA instructions - is also part of the expression process.

As you saw above, measurement of gene expression is a very common use of qPCR. In this application, it's the amount of RNA that's being quantified. To use qPCR to measure RNA, you first have to create cDNA (complementary DNA) from the RNA: this is accomplished by the enzyme reverse transcriptase. (The reverse transcriptase enzyme is from retroviruses like HIV, which have an RNA genome, but make cDNA for insertion into the host genome.) Once cDNA is made, it can be used as the template for quantitative PCR; this is known as RT-PCR, or reverse transcription PCR.

When qPCR is used to monitor gene expression, it's important to also measure an internal (also known as endogenous or housekeeping) control gene. The endogenous control should be a gene that does not change expression in response to the treatment being studied. Watch the video below to learn more about endogenous controls help normalize gene expression data.

Videos

Source (URL, My Media, file name, etc.)	Title (what we'll call it)	Can we legally use it? Download? Embed? Give credit? Edit it?	Captioning

https://www.youtube.com/watch?v=jst-3hD_xFQ&list=PL4AACABAB6F402E9B&index=28	How to Normalize cDNA Concentrations -- Ask TaqMan® Ep. 15 by Life Technologies	**Embed**	Already captioned

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Page: [Module 1 icon] Getting Reliable Data in qPCR

When performing qPCR, it's recommended that all reactions are performed at least in triplicate. This allows greater confidence that the Ct values obtained are reflective of the actual starting amount of nucleic acid, rather than being influenced by pipetting errors, well variability, or other anomalies. Watch the video below to learn how to evaluate your results and determine whether or not there is reason to exclude particular data points.

Videos

Source (URL, My Media, file name, etc.)	Title (what we'll call it)	Can we legally use it? Download? Embed? Give credit? Edit it?	Captioning
https://www.youtube.com/watch?v=eIaPGhOjBQo&list=PL4AACABAB6F402E9B&t=0s&index=23	Getting Reliable Data in qPCR - Ask TaqMan #22	**Embed**	Already captioned

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Summary - terms, relationships, a scaffold

Assignment: Meat Lab
[Lab icon]   Lab
Lab instructions and submittal Instructions
[Objective icon] Objectives
- Identify how much of a pathogen DNA is in a meat sample