

Name: \_\_\_\_\_

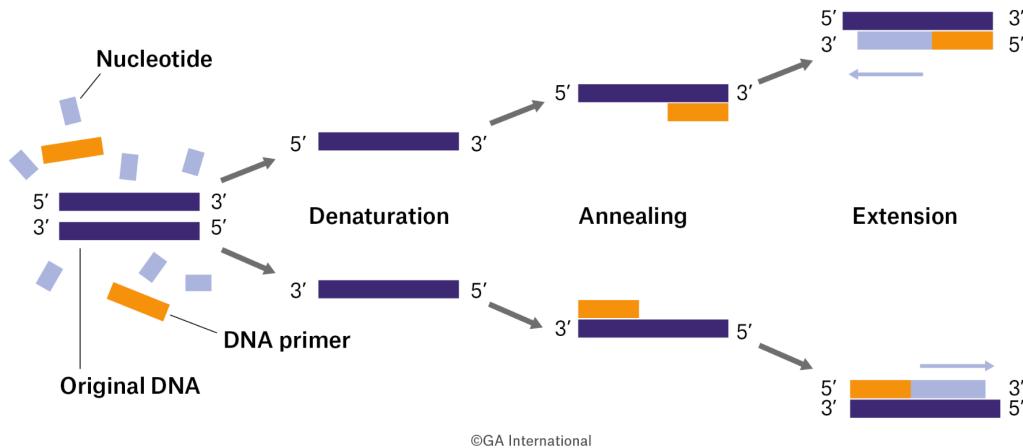
CWID: \_\_\_\_\_

**Pre-Lab:** Reverse transcription is the process by which RNA is converted into DNA. The most common adapter for reverse transcription is an oligo-DT primer, which binds to polyA tails. Should you expect to detect mitochondrial RNA (mtRNA), meaning that it is transcribed from the mitochondrial genome?

Is mitochondrial RNA polyadenylated? “*Polyadenylation of mitochondrial RNAs* in higher eukaryotic organisms have diverse effects on their function and metabolism” ([10.1016/j.bbagr.2011.10.012](https://doi.org/10.1016/j.bbagr.2011.10.012)) Other species do not have polyadenylated mitochondrial RNAs - such as yeast!

A few strategies to isolate mitochondrial RNA from yeast:

- 1.] use random hexamer primers instead of oligo-DT primers (which target polyA tails)
- 2.] perform mitochondrial isolation to remove other RNA from the cytoplasm / nucleus
- 3.] use custom oligos to anneal specifically target all 37 genes in the mitochondrial genome, either via a pulldown or PCR amplification



Polymerase chain reaction describes the amplification of a single product based on specific / unique DNA primers. How many base-pairs do you need to define a ‘unique’ primer? What is the statistical reason for this definition?

You need enough unique sequences to tile the entire genome. Strictly speaking,  $4^{16} = 4.2$  billion base pairs, which can tile a 3 billion base pair genome [e.g. human]. In practice, primers are generally designed to be between 18-22 bp.

A common polymerase for PCR the Taq polymerase, provided as a master-mix kit as described below by Promega:

GoTaq® Green Master Mix, 2X: GoTaq® DNA Polymerase is supplied in 2X Green GoTaq® Reaction Buffer (pH 8.5), 400 $\mu$ M dATP, 400 $\mu$ M dGTP, 400 $\mu$ M dCTP, 400 $\mu$ M dTTP and 3mM MgCl<sub>2</sub>. Green GoTaq® Reaction Buffer is a proprietary buffer containing a compound that increases sample density, and yellow and blue dyes, which function as loading dyes when reaction products are analyzed by agarose gel electrophoresis. The blue dye migrates at the same rate as 3–5kb DNA fragments, and the yellow dye migrates at a rate faster than primers (<50bp), in a 1% agarose gel.

- 1.] Write out all of the components below as ingredients.
- 2.] Describe the purpose of each component in the mixture.
- 3.] Describe why we utilize a Taq polymerase instead of a native human polymerase, based on your understanding of PCR.
- 4.] Describe when and why we utilize a ‘master-mix.’

1. & 2.]

GoTaq® DNA Polymerase: performs DNA synthesis in PCR reaction

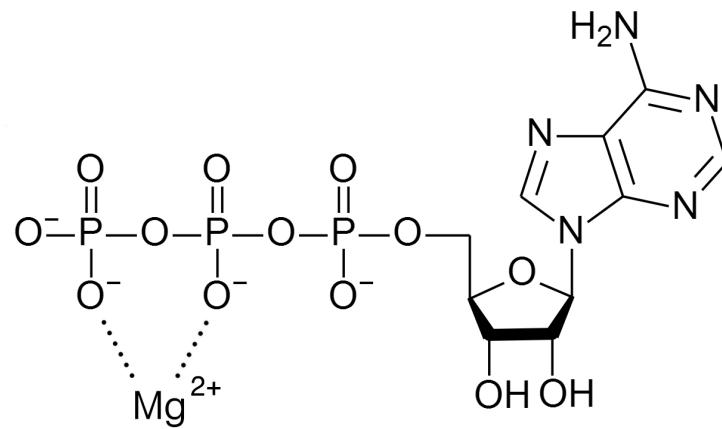
2X Green GoTaq® Reaction Buffer: “increases sample density” -> makes the PCR reaction energetically more favorable, “yellow and blue dyes” -> function described, combination of yellow + blue makes the mixture “GoTaq Green”

400 $\mu$ M dATP, 400 $\mu$ M dGTP, 400 $\mu$ M dCTP, 400 $\mu$ M dTTP: nucleotides to add in PCR reaction

3mM MgCl<sub>2</sub>: serves as a stabilization agent for the dNTPs + DNA template [see below]; cofactor for Taq polymerase; <https://biology.stackexchange.com/questions/51177/what-is-the-half-life-of-dntps-at-95-c>

3.] The Taq polymerase evolved in extreme environments and is capable of maintaining activity after exposure to 95°C denaturation temperatures. There is no equivalent mammalian polymerase with this potential activity.

4.] A master-mix is utilized to ensure that we have standardized buffer conditions across multiple replicates and samples.  
Master-mixes are prepared to a volume 20% greater than expected use.

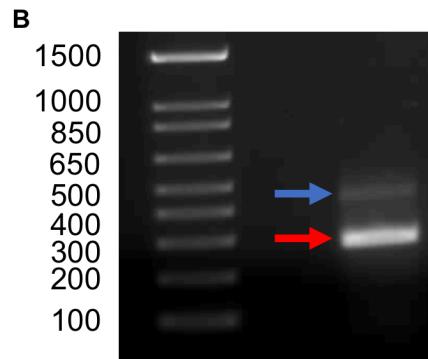


Q1: What is the size of the **blue** band in the gel?

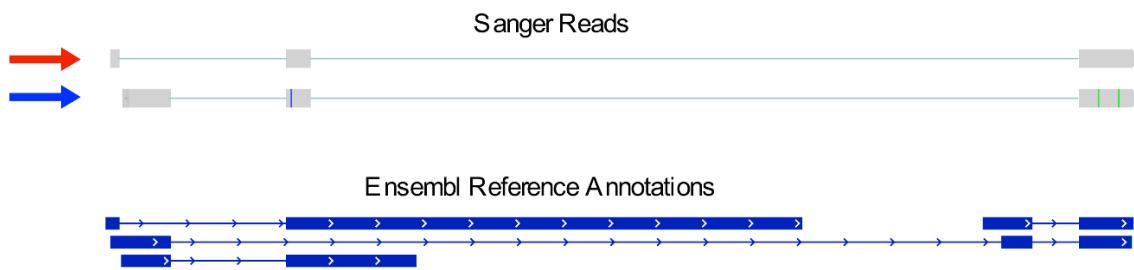
435 bp +- 50 bp

Q2: What is the size of the **red** band in the gel?

280 bp +- 50 bp



Q3:



What makes the **blue** Sanger read novel compared to the reference annotation?

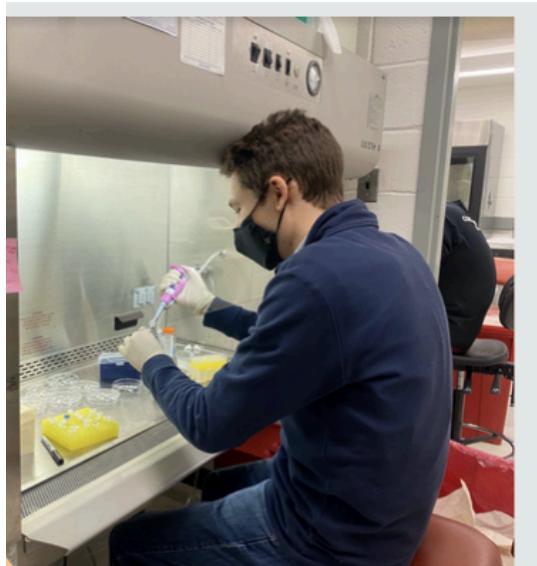
Has a novel second exon.

What makes the **red** Sanger read novel compared to the reference annotation?

Has a novel second exon.

Describe how this relates to size selection in sequencing processes:

In size selection, the entire library of DNA or RNA is run on a gel or gel-adjacent system, and only fragments of a certain length are selected for downstream library preparation and sequencing. As such, you can target super-long fragments, or exclude other kinds of fragments / transcripts. For example, if you selected all fragments > 500 bp, you would miss these two transcript variants: TN\_280 and TN\_435.



What's wrong with these lab techniques?

Lack of PPE [Gloves, Goggles, Lab Coat]