Amylase – from Molecules to Systems

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Introduction

This pair of Introduction to Biology modules examines starch-degrading amylase enzymes from various organisms. The two modules take students from starch detection assays, through enzyme assays to simple bioinformatics.

In **Module 1** students learn to use pipettes and spectrophotometers to measure starch concentrations. They perform assays using amylase enzymes form different organisms and finally devise their own experiments.

In **Module 2,** students use PCR to amplify alpha amylase from their own DNA and then use simple bioinformatics tools (NCBI and DNA subway) to explore differences in gene structure and sequences among amylase enzymes from different organisms.

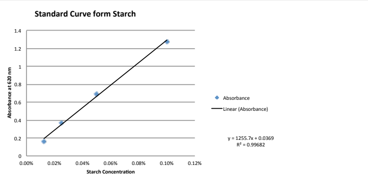

Figure 1. Standard curve for starch concentration using starch/iodine mixtures

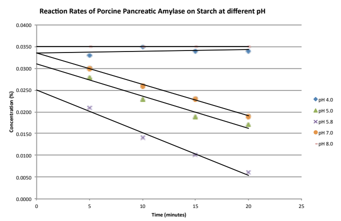

Figure 2. Reaction rates for Porcine Pancreatic Amylase under varying pH conditions

Module 1

Week 1: Pipettes & Specs

Correct use of pipettes and micropipettes
Using a spectrophotometer to measure starch concentration
Exercises:

- Which pipette?
- How's my pipetting?
- Prepare dilutions of starch/iodine solution
- Relationship between absorbance and color
- Relationship between absorbance and concentration

Week 2: Measuring Starch Concentrations

- Making standard curves for starch/iodine
- Using the curve to determine starch concentration
- Devise a dilution series for starch/iodine that can be measured on class spectrophotometers
- Prepare and measure solutions
- Graph absorbance and concentration using Excel. (Figure 1).
- Practice until r^2 of linear trend line is 0.99
- Use equation of the line to find unknown starch concentrations.

Weeks 3 & 4: Enzyme assays

- Amylases break down starch to disaccharides
- Measure the amount of starch remaining over time.
- Compare different amylase enzymes:
  - Porcine pancreatic amylase
  - Alpha amylase from *Aspergillus oryzae*
  - Alpha amylase from *Bacillus sp*.
- Assays conducted at room temperature
- Vary pH conditions: Figure 2
- Determine rate of reaction from slope of best fit line.
- **Questions:**
- How do pH optima for different amylase enzymes differ?
- Why do you think the optimum pH conditions differ?

Week 5: Team experiment

- Using the tools and concepts learned so far, devise an experiment to answer a question about amylase enzymes.
- Propose a reasoned hypothesis for your experiment.
- Plan and conduct your experiment with suitable controls.

Examples:

- Can cellulase break down starch?
- What is the pH optimum for human salivary enzyme?
- Determine the temperature optimum for one or more of the amylase enzymes available.
- Can we extract amylase enzymes from plants or mushrooms?
- Can amylase break down glycogen?
- Can amylase break down cellulose?

Module 2

Weeks 1 & 2: Molecular Biology

Concepts:

Central Dogma
Introduce PCR
Introduce NCBI and alignment tools
Introduce molecular evolution

- Extract DNA from (student) cheek cells
- Amplify part of human salivary amylase gene by PCR

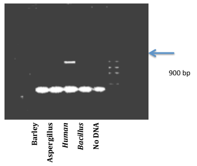

Figure 3: PCR amplification of a 900 bp amplicon from genomic DNA from barley, *Aspergillus*, human, and *Bacillus sp* with primers designed to the human salivary alpha amylase sequence.

Q: If these enzymes all do the same job, are they similar at the nucleotide level?

- Use NCBI to find primer sequences in humans, plants, fungi and bacteria
- Use these results to explain the PCR results

Week 3: Bioinformatics extension

Q: If all organisms produce alpha amylase enzymes, how similar are they at the nucleotide and amino acid level?

Species	gDNA, bp	mRNA, bp	Protein, aa
Homo sapiens	9033	1781	511
Hordeum vulgare	2625	1461	427
Aspergillus oryzae	3366	1907	499
Bacillus cereus	1761	1761	586

Align and compare mRNA and amino acid sequences

- Students download sequences from NCBI
- We used DNA subway (iPlant) to do this. Students prepared a 'similarity matrix' for the sequences they choose. They can add in sequences from chimp, tiger, other mammals….

Module 1: Learning Goals

- Use pipettes, micropipettes and spectrophotometers proficiently.
- Know how to plot simple graphs in Microsoft Excel
- Use standard curves to determine concentration of unknown solutions using measured absorbance readings
- Work as a team to perform enzyme assays
- Work as a team to conduct a Team experiment
- Work as a team on a Team Lab Report

Module 1: Assessment

- Laboratory notebook entries (individual)
- Google Docs summaries (individual and team)
- Laboratory report (team)

Module 2: Learning Goals

- Summarize the central dogma of molecular biology
- Explore NCBI and become familiar with nucleotide and amino acid sequences
- Summarize and explain the Polymerase Chain Reaction
- Discuss the differences and similarities between alpha amylase sequences in different organisms.

Module 2: Assessment

- Laboratory notebook entries (individual)
- Annotated lab protocol describing PCR for a High School teacher (team)
- 3 minute video describing a question or application about alpha amylase enzymes (team).

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