**Amylase – from Molecules to Systems**

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### 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 r² 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 did 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?

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### 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.

- Questions:
  - If all organisms produce alpha amylase enzymes, how similar are they 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

- 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...

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### 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 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.

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### Module 1: Assessment

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

### 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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