Plant Biology 316
Experiments in Plant Biology

Laboratory Manual
Molecular and Cellular Section

Spring 2011

Tuesdays 9:10 - 1:00
Thursdays 9:10 - noon
Room 255 Plant Biology Building
Room 147 Plant Biology Building

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The British physicist Lord Kelvin (1824-1907) said:

> "When you can measure what you are speaking about and express it in numbers you know something about it; but when you cannot express it in numbers your knowledge is a meager and unsatisfactory kind: it may be the beginning of knowledge but you have scarcely, in your thoughts, advanced to the stage of science."

***TENTATIVE* SCHEDULE OF LABORATORY EXERCISES:** see extra file.

INTRODUCTION TO PLB-316 EXPERIMENTS IN PLANT BIOLOGY Page

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COURSE OBJECTIVES

Knowledge and application of tools in plant molecular biology and biochemistry

The students will gain a thorough understanding of the basic laboratory techniques in plant molecular biology. They will also learn their use to address scientific questions in different fields of plant biology ranging from ecology to biochemistry. These techniques include the tools of DNA technology as well as the basic analysis of proteins.

Laboratory skills in plant molecular biology

The students will plan and carry out molecular biology experiments. They will learn how to keep a laboratory notebook and to gain the confidence and skills necessary to be able to attempt new laboratory procedures. The course will make students competitive for employment in an introductory laboratory research position.

Communication in the field of molecular biology

The students will learn how to communicate scientific information in writing. You will be required to illustrate your understanding of molecular biology by submitting abstracts and reports in the format of research articles.

DUE DATES

NOTE: Put the date of submission on all materials you turn in!

Photosynthesis report	March 15
Photosynthesis worksheet	March 17
Turn in lab notebooks (M&C) I	April 5
Pipettor accuracy summary	March 22
ga5-1 complementation report	April 7
Alpha-amylase worksheet	April 28
Turn in lab notebooks II	at Final Exam
Final Exam	May 6 (7:45-9:45am) May 6 (10:00-12:00 noon)

REQUIREMENTS AND PHYLOSOPHY

Things to bring to class:

- Notebook
- Scientific calculator
- Pen

1) Be prepared for each experiment! The key to getting the most from this course is PREPARATION! The more time you spend preparing yourself BEFORE each experiment, the more you will understand it, the more you will learn, and the less time you will waste.

2) To ensure you understand each experiment:

Each day, at the start of class, turn in:

A) A copy of your protocol(s) for the day (not necessary for the photosynthesis section).

B) A short summary that explains the objectives of your experiments (see below).

C) The answers to the questions in the lab manual due that day.

D) We will have 5 "surprise" quizzes about the current experiments. So, be ready everyday!

3) You should always have a written "working protocol" BEFORE you start each experiment! A "working protocol" is an abbreviated step-by-step outline of the experiment, to be used as a working reference at the bench.

4) You must THINK in this course rather than simply follow the written instructions. You should not carry out a procedure unless you understand why it is included in the experiment. During class, the instructors and TA's may ask you why

you are doing a particular experimental manipulation.

5) Your notebook and reports must demonstrate that you understand the experiment and that you can **interpret** the results.

6) This is a laboratory course. You should note that reports, abstracts, protocols and notebooks determine more of your grade than exams. Spend your time accordingly!

7) Make sure read and understand the sections "Useful Information and Background; Dilutions" "Safety Information" and "Other useful practical information" before starting this class.

8) There will be several reference books in class, feel free to make use of them during class hours.

9) Remember, if you do not know something, please realize that you do not know it, this is the first step of learning. Do not just make something up. Do some research of your own and then come and ask. Part of the goal of this class is to learn how to get information.

Attendance:

This is a laboratory class and attendance is mandatory. If you have a valid reason for missing class such as a University-sponsored activity, religious observances, illness, or family emergency, the instructor or TA will assist you in obtaining information and materials you may have missed. Students who skip class without a valid excuse should not expect the instructor or TA to supply class notes or provide special help. The official university policy, see: https://www.msu.edu/unit/ombud/attendance.html

Schedule Note:

Although we try to adjust the experiments to fit the scheduled time, this doesn't always work. Some experiments take less time than allocated, some take more. Therefore, some days you will be done early, and this will be a good time to work on your notebook and protocols for the next experiment. There will be days when you will need to stay later than the scheduled class. If this is a problem for you, speak to the instructors.

Grading: Molecular and Cellular Section

NOTE: You must include the submission date on everything you turn in!

Protocols and Lab Notebook	10%
Summaries and Questions	10%
Quizzes	10%
Worksheets	10%
Photosynthesis Report	15%
GA5-1 Report	15%
Alpha-Amylase Worksheet	15%
Final Exam	15%
Total M and C	*100%*

Total: *Molecular and Cellular Section* *100%*

Late Penalty for Abstracts, Protocols and Reports:

25% for first day, 10% per day for days 2 to 7, and no credit if more than7 days late.

Quizzes:

Will be given occasionally at the beginning of a lab period to test your comprehension of the experiment before starting it.

Final:

The final will cover all the material presented in class as well as in your lab manuals.

Lab Notebook:

This should be the major repository of raw data, working protocols, analysis of data, and conclusions. Lab notebooks will be examined at midterm and graded at end of course. Use a three ring notebook. Divide it into sections for 1) Lecture notes, and 2) Experiments, keep each experiment in a divided section, which includes your protocol. Lab notebooks need to include all the necessary information for you to repeat the experiments, a copy of the results and any comments you have about the results.

Summaries:

Summaries (about 5 sentences) should be handed in before starting each day's experiments. These should summarize, without any technical detail, what you are going to do that day, why you are doing it and how it relates to the previous and next part of your experiment (if there are any). You should write one even for the classes for which we don't have any "wet" experiments. It needs to show that you understand what you will be doing. Follow the instructions below from *Writing Abstracts*.

Questions:

In the Lab Manual there are occasional questions **(in green)** to be answered by the students as homework, answers should be handed in to the TAs. You might not know the answers to some of these questions, if you don't, look it up (the web is an easy and most of the time good resource, but as always it is good to have more than one source of information).

Lab Report:

Experiments should be summarized by a report of ~600-1000 words (longer is not always better), which are **due as stated above.** Follow the instructions below from *Writing Abstracts* (next page) and *Guide for Preparation of Reports*. If the experiment failed, explain what results were expected and why you might not have achieved these results.

The Lab Manual:

We are always trying to improve this course and so some of the experiments are new. Some experiments may even change or evolve *during* the course. We think this makes the course more up to date and better overall. But, sometimes the protocols you use for an experiment may not be the same as in the manual. Please be patient if we make some last minute changes. In the lab manual the instructions specific for the TAs are in blue.

Writing Abstracts

An abstract is a short summary of your experiment. Like a paper (or a lab report) it should contain an introduction, methods, results and conclusions. Every scientific paper, has an abstract at the beginning to summarize the study. Follow the rules below and you will have an abstract that works.

With the method outlined below, you should be able to produce a good abstract in about an hour. If you haven't clearly and carefully thought through what you did in the experiment, writing the abstract should help you do so. If you have thought about the lab exercise, writing the abstract should be easy and fast. It is shorter than a lab report, but includes the most important points. Correct English helps, because it allows you to convey exactly what you mean, and it indicates to the reader that you are careful in your thinking. Be specific! Fuzzy thinking leads to fuzzy writing; fuzzy writing can never convey clear thinking. Keep your abstract between 200-300 words. When a part of a report or scientific paper write the abstract last.

Guidelines for Writing Abstracts

1. Make up one or two sentences to answer each of the questions under Parts of the Abstract.

2. For the first round, do not try to make a neat sentence. Just jot down the idea you want to get across.

3. Keep the sentences separate until you are satisfied with all of them, then combine them.

4. THEN read through the whole abstract and change the sentences to make them flow more smoothly.

5. Style hints: Use plain English wherever you can, and use simple and concise sentences. Do not refer to any literature and state only your most important conclusion(s). You do not have to write a lot, but it must be compactly written.

Parts of the Abstract

1. Title: should indicate the question you investigated, or the method, if that is important. (Example: Effect of mechanical stimulation on the stem growth of soybean seedlings.)

2. Author(s) and some sort of address. (Example: George Smith, 2008 PLB-316 Class, Michigan State University, **and date submitted**)

3. What is the general topic you were investigating and why is it important? One sentence only. (Example: In nature plants are frequently stimulated by mechanical factors such as wind and rain.)

4. What is the specific question you are addressing with this experiment? This is not your methods, which come later. Sometimes you need two sentences here, but one is better if you can manage it. (Example: We investigated the effect of mechanical stimulation on stem growth of soybean seedlings.)

5. How did you do this? Three or four sentences are needed here. You are not trying to be complete, just to give a general idea of how you did it. (Example: Stems of soybean seedlings were stimulated by rubbing up and down three times daily with a force of approximately 10 g cm^{-2}. Stem diameter growth and length growth were measured daily for one week, and were compared with similar growth from unstimulated plants.)

6. What did you find out? **Be as specific as possible and use numbers to describe results whenever possible**. Two or three sentences ought to be enough; state only your main point(s). (Example: Stem diameter growth of stimulated plants was 30% greater than that of control plants. Stem length growth was reduced by 40% for mechanically stimulated plants.)

7. What did you find out about the general topic or question (see #3 above)? Use one or two sentences. (Example: Mechanical stimulation modified the growth of soybean stems.) That's it: 1-2 sentences, but there can be no waste.

8. If appropriate you can add a brief summary of your conclusions and/or interpretations (1-2) sentences.

Useful sites on scientific writing
http://www.unc.edu/depts/wcweb/handouts/sciences.html

Plagiarism:

Both published and unpublished work must always be properly credited. Reporting the work of others, without credit, as if it were one's own is plagiarism. See MSU's policy concerning plagiarism at this website. http://www.msu.edu/unit/ombud/plagiarism.html

Guide for Preparation of the Reports

The report should be structured like a scientific publication in a journal and according to the following outline. Write in simple, direct style, in THIRD person, PAST tense. At the top of the first page, write your name and departmental affiliation and date handed in. In the Methods, Results and Discussion sections, use topic headings. This will help the reader understand the organization of your report.

Title:

The title indicates precisely the subject of the experiment.

Abstract:

The body of the paper should be preceded by an abstract, summarizing your results as concisely as possible. Follow the guidelines in the previous section on Abstracts. *Use numbers to describe results whenever possible*.

Introduction (Purpose/Objective):

It should explain the objectives of the experiment, the importance of the experiment and give an overall background for understanding why the experiment was performed.

Material and Methods:

The complete procedure from the laboratory "handout" need not be reproduced here. Don't make a list of materials. Describe tests performed and number of samples used. List any variations that were made in a given procedure. Do not use "command" language.

Results: Be Specific! Use numbers to describe results whenever possible.

Data should be presented in an organized and easy to understand form. Average values from repeated trials should be used when available. Tables and figures (graphs, diagrams, sketches) are very useful here. All tables and figures should be numbered and given a descriptive title. Titles of tables should be placed above the table, whereas titles of figures should be given in the figure legend. The graph axes' descriptions should include units. Describe your the results shown in your figures in writing. Your report will be easier to read if you insert tables and figures into the text where possible. Don't present the same data figures.

Discussion:

Refer to literature pertaining to the subject of your experiment. Interpret your results for each variable under study. Your interpretations should be based on facts not your opinion. A SHORT paragraph of tentative trends as well as specific recommendations for further study is appropriate here.

You should also discuss the implications of your results. The Results and Discussion sections may be combined to avoid unnecessary repetition.

Literature Cited:

List only those sources that have actually been used in your report. These may represent only a small part of your total reading. Use the format of the journal Plant Physiology.

http://www.plantphysiol.org/misc/ifora.shtml

Example: Journal articles Author AB, Author BB (2006) Title of Article. Plant Physiol 59: 45-59

USEFUL INFORMATION AND BACKGROUND

You need to be familiar with the following terms and how they relate to each other:
Gene, DNA, RNA, protein, transcription, translation, promoter, terminator, intron, exon, splicing, and what are the main differences between bacteria and eukaryotic cells.

Dilutions:
You MUST know and understand how to do this

In this class and in any laboratory, you will frequently need to dilute a more concentrated to a less concentrated solution. Because the number of moles or equivalents is not changed by dilution, the following equation allows us to calculate the amount of more concentrated "stock" solution needed.

$$C_1V_1 = C_2V_2$$

where C_1V_1 refers to the concentrated "stock" solution and C_2V_2 refers to the dilute solution.

C1 = concentration before dilution
V1 = volume of solution before dilution
C2 = concentration of solution after dilution
V2 = Volume of solution after dilution

Example: What volume of 12 M HCl is needed to prepare 100 ml of 1.5 M HCl?

$$C_1V_1 = C_2V_2$$

we solve this for the volume of concentrated HCl needed:

$$V_1 = C_2V_2/C_1$$

$$= (1.5M)(0.100\ L)/12M$$

$$=0.0125\ L \text{ or } 12.5\ mL$$

Therefore, you would add 12.5 ml of 12 M HCl and dilute this to 100 ml.

Note that 1 nM is 1 million-fold less than 1 mM. You should become familiar with working with these types of numbers, calculations and dilutions! This is the nature of experimental biology.

Remember:
1 mM = 10^{-3}M
1 μM = 10^{-6}M
1 nM = 10^{-9}M
1 pM = 10^{-12}M

CHECK this website for more examples on making dilutions
http://abacus.bates.edu/~ganderso/biology/resources/dilutions.html

Note: because concentration represents a proportional relationship, you can select from a variety of units. For example, 1 milligram/milliliter is the same as 1 gram/liter, or 1 microgram/microliter, or 1 nanogram/nanoliter. Similarly 1 mole/liter is the same as 1 millimole/ml etc.

Other units for concentration:
% (v/v): percent volume per volume (for example 1 % ethanol is 1 ml ethanol + 99 ml water)
% (w/v): percent weight per volume (for example 1 % sucrose is 1 g sucrose in a total volume of 100 ml)

Other useful terminology when working with solutions
10x = ten times the final/working concentration (for example if you need a solution of 10 mM NaCl a stock of 100 mM would be 10x and a 50 mM stock would be 5x.)
1:5 = dilute 1 part in five. If you dilute a 50 mM stock 1:5 you will add 1 part of your stock to 4 parts of solvent (for example 1 ml of stock + 4 ml of solvent).

A few principles:

It is NOT a good idea to measure less than 2 μL. The pipettors are less accurate, and there are other pipetting problems with such low volumes.

You do not want to use large volumes of solvents to make the dilutions; this is wasteful and less convenient. Therefore, if you need to make a large dilution, it is best to make this in two or three steps.

Plastic microfuge tubes are convenient and disposable and easily sterilized are so are often useful for making dilutions.

SOME USEFUL UNITS AND CONVERSIONS
Average polypeptide has size of approx. 40,000 daltons with approx 350 amino acids
1 kb = 1 kilobase = 1000 bases or base pairs (bp) of single or double-stranded nucleic acid
1 kb = 660,000 daltons double stranded DNA
1 kb = 330,000 daltons single stranded DNA
1 kb = 340,000 daltons single-stranded RNA
1 kb = 333 amino acid coding capacity (approx. 37,000 dalton protein)
10,000 dalton protein = 270 bp DNA
30,000 dalton protein = 810 bp DNA
50,000 dalton protein = 1.35 kb DNA
100,000 dalton protein = 2.7 kb DNA
1 A260 unit double stranded DNA = 50 μg/ml
1 A260 unit single-stranded RNA = 40 μg/ml
1 A260 unit single stranded oligonucleotide = 33 μg/ml

Arabidopsis Genome

115,000,000 bp total
approx. 80% is coding
30,000 genes estimated
Average gene is 3,000 bp in length
Average primary transcript is 2400 bp
Average gene has 3 introns
Average intron size is 215 bp
Average 5' untranslated region is 161 bp
Average 3' untranslated region is 232 bp

Some websites with useful background info on molecular biology:

http://www.accessexcellence.org/AB/GG/
http://www.dnalc.org
http://www.dnai.org/index.htm
(Has interactive gene demonstration program called GeneBoy)
http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=Books
(*Scroll down to Molecular Biology of the Cell. You may also find insights in The Cell— A Molecular Approach, and Molecular Cell Biology [some of the figures in Molecular Cell Biology are unavailable at this time.])*
http://4e.plantphys.net/
(This is Plant Physiology Online—excellent resource)

SAFETY CONSIDERATIONS

LAMINAR FLOW HOOD

The sterile transfer hoods we will use are designed to filter the air coming into the hood and to continually blow `sterile' air over the work surface. Therefore, the hood helps protect your samples from contamination *by room air*. It does nothing to protect your samples from you or you from your samples!

SAFETY GLASSES

Although much effort has been made to avoid dangerous chemicals in this lab, in some cases you will use acids, bases, or solvents that could damage your eyes if accidentally splashed into them. **Therefore, safety glasses must be worn when you are actually working at the bench.** You may bring your own or use those provided. Always make sure you know the chemicals you are working with.

OTHER USEFUL PRACTICAL INFORMATION

USING A LAMINAR FLOW HOOD: STERILE TECHNIQUES

- Turn on the laminar flow hood 10 minutes before using and clean the working area with 70% ethanol using a spray bottle.
- Spray everything going into the sterile area.
- Have only the necessary items in sterile work area. Remove items that are no longer needed as quickly as possible. Act out each step before beginning so that you understand what you are about to do.

- Long hair should be tied back or covered. Hands should be washed, not scrubbed (scrubbing dries hands and creates flakes of skin that have bacteria) and sprayed with 70 % ethyl or isopropyl alcohol or coated with isopropyl alcohol gel. Do not talk while performing sterile operations.
- Do not lean over your work. Keep your back against the backrest of your chair. Try to work with your arms straight: this position may feel awkward, but it will reduce contamination. Do not pass nonsterile items over sterile areas or items. Reach around rather than over. Make your movements smooth and graceful so that you do not disturb the air more than is necessary.

CENTRIFUGES

Remember to **always** balance the tubes in the centrifuge with tubes with the same weight.

WHEN DOING EXPERIMENTS

a. Always make sure you read and understand the **whole** protocol before you start.

b. When reproducing somebody else's protocol write your own version.

c. Have all the materials needed before you start.

d. Be aware of dangerous chemicals and make sure you have the appropriate equipment before you start.

d. Make sure you write down any modifications of the protocol you do along the way.

GLOSSARY

Aleurone	Aleurone (from Greek aleuron, flour) is the outermost layer of the seed coat in some grains. Once triggered by hormones released from the embryo, the aleurone synthesizes enzymes to break down the starchy endosperm supplying sugars to drive the growth of the embryo.
Aliquot	An aliquot is usually a portion of a total amount of a solution.
Alpha-amylase	An enzyme that catalyses the hydrolysis of 1,4-alpha-glycosidic linkages in starch, glycogen, and related polysaccharides and oligosaccharides. (Biology-online.org)
Axenic	Not contaminated by or associated with any other living organisms. Usually used in reference to pure cultures of microorganisms that are completely free of the presence of other organisms. (http://www.thefreedictionary.com)
Benzylaminopurine	6-Benzylaminopurine, aka Benzyl adenine or BAP is a synthetic cytokinin which elicits plant growth and development responses such as setting blossoms and stimulating fruit richness by stimulating cell division.
Bradford Protein Assay	The Bradford assay, a colorimetric protein assay, is based on an absorbance shift in the dye Coomassie when bound to arginine and hydrophobic amino acid residues present in protein. The anionic (bound) form of the dye is blue and has an absorption spectrum maximum at 595 nm. The increase of absorbance at 595 nm is proportional to the amount of bound dye, and thus to the amount (concentration) of protein present in the sample. (Wikipedia)
chloramphenicol	A bacteriostatic antibiotic originally derived from the bacterium *Streptomyces venezuelae*. It was the first antibiotic to be manufactured synthetically on a large scale.
CDS	CoDing Sequence, region of nucleotides that corresponds to the sequence of amino acids in the predicted protein. The CDS includes start and stop codons, therefore coding sequences begin with an "ATG" and end with a stop codon. Note that the CDS does not correspond to the actual mRNA sequence. (http://www.yeastgenome.org/help/glossary.html#cds)
cDNA	complementary DNA- single-stranded DNA that is complementary to messenger RNA or DNA that has been synthesized from messenger RNA by reverse transcriptase. (http://www.thefreedictionary.com/DNA)
Coomassie	Coomassie blue dye is named after an African locale, in this case the city of Kumasi, now in Ghana. It was developed as an acid wool dye and was named after the Ashanti capital, then known as Coomassie.
Cyclohexamide	An inhibitor of protein biosynthesis in eukaryotic organisms, produced by the bacterium *Streptomyces griseus*. Cycloheximide exerts its effect by interfering with peptidyl transferase activity of the 60S ribosome, thus blocking translational elongation.

Dalton	Dalton, Da also called unified atomic mass unit (u), or), is a unit of mass used to express atomic and molecular masses. It is defined to be one twelfth of the mass of an unbound atom of the carbon-12 nuclide, at rest and in its ground state. (Wikipedia)
Enzyme activity	Moles of substrate converted per unit time = rate × reaction volume. Enzyme activity is a measure of the quantity of active enzyme present and is thus dependent on conditions, which should be specified. The SI unit is the katal, 1 katal = 1 mol s-1, but this is an excessively large unit. A more practical and commonly-used value is 1 enzyme unit (U) = 1 µmol min-1. 1 U corresponds to 16.67 nanokatals. Note that some enzyme manufactures might have a different definition of U, for some 1 U = 1nmol min^{-1}
Fusarium	A large genus of filamentous fungi widely distributed in soil and in association with plants.
Gibberellin	One of the classical plant hormones (over 112 forms discovered) responsible for stem and leaf elongation and loss of seed dormancy and mobilization of endosperm reserves.

Nitrocellulose membrane is a membrane used for immobilizing protein, RNA or DNA that have been separated by gel electrophoresis.

Plasmid	A Plasmid is an extra chromosomal DNA molecule separate from the chromosomal DNA, which is capable of replicating independently from the chromosomal DNA.
Restriction enzyme	A restriction enzyme (or restriction endonuclease) is an enzyme that cuts double-stranded or single stranded DNA at specific recognition nucleotide sequences known as restriction sites. Such enzymes, found in bacteria and archaea, are thought to have evolved to provide a defense mechanism against invading viruses. Inside a bacterial host, the restriction enzymes selectively cut up foreign DNA in a process called restriction; host DNA is methylated by a modification enzyme (a methylase) to protect it from the restriction enzyme's activity.
Specific activity	This is the activity of an enzyme per milligram of total protein (expressed in µmol min-1mg^{-1}). Specific activity gives a measurement of the purity of the enzyme. It is the amount of product formed by an enzyme in a given amount of time under given conditions per milligram of enzyme. Specific activity is equal to the rate of reaction multiplied by the volume of reaction divided by the mass of enzyme. The SI unit is katal kg-1, but a more practical unit is µmol mg-1 min^{-1}. Specific activity is a measure of enzyme processivity, usually constant for a pure enzyme.

Vector (molecular biology), vehicle used to transfer genetic material to a target cell

* Plasmid vector
* Binary vector, a cloning vector used to generate transgenic plants
* Cloning vector
* Expression vector, a plasmid specifically used for protein expression in the target
* Shuttle vector, a vector (usually a plasmid) constructed so that it can propagate in two different host species
* Viral vector, a virus modified to deliver foreign genetic material into a cell

Western blot

A western blot (a.k.a immunoblot) is a method to detect proteins in a sample of tissue homogenate or extract. It uses gel electrophoresis to separate denatured proteins by mass. The proteins are then transferred out of the gel and onto a membrane (typically nitrocellulose), where they are "probed" using antibodies specific to the protein. (Wikipedia)

Beer-Lambert law

(from http://www.chemistry.wustl.edu/~courses/genchem/Labs/Dyes/Beer.htm)

The ratio of the intensity of the transmitted light, I, to that of the incident light, I_0, is the transmittance, T, of the sample:

$$T = \frac{I_{measured}}{I_0}$$

It is useful to express the intensity of light absorbed in terms of absorbance, A :

$$A = \log\left(\frac{1}{T}\right) = -\log\left(\frac{I_{measured}}{I_0}\right) = \log\left(\frac{I_0}{I_{measured}}\right)$$

Absorbance is useful since it is proportional to the concentration of the absorbing species (c) and the path length that the light has to travel (b) :

$$A = \varepsilon\, b\, c$$

The extinction coefficient (ε) is called the extinction coefficient or absorptivity. It has units of M^{-1} cm^{-1} (M = molarity). The variation of ε with wavelength is characteristic of the substance. If you know the extinction coefficient of a species, you can measure the absorbance and the Beer-Lambert Law to calculate its concentration.

In case of DNA we know that at a wave length of 260 nm a DNA solution of 50 μg ml^{-1} will give an absorption of 1. That means that its extinction coefficient is (1/50) ml μg^{-1} cm^{-1}. Since we are using 1cm cuvettes, our path length is 1 cm. We can determine the concentration of our DNA solution by substituting these values in the above (green) equation:

A = **1/50** ml μg^{-1} cm^{-1} x **1** cm x **c** μg ml^{-1}

A 50 μg ml^{-1} = **c** μg ml^{-1}

In the alpha-amylase section we will also use the linear relationship between absorbance and concentration to determine the protein concentration of our extracts.