BIOLOGY 2230 WA – CELL BIOLOGY
DR. L. MALEK

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Useful website(s):
General intro to cell biology: http://en.wikiversity.org/wiki/Cell_Biology,
Christopher Gillen’s website on reading scientific articles:
http://biology.kenyon.edu/Bio_InfoLit/index.html,

Preamble:

The course historically suffered from the varied background of entering students. This ranges from no background in biology to good high school and subsequent university exposure to the introductory topics such as genetics and chemistry. Since this course is a second year BIOLOGY course, it will be taught at a level suitable to second year Biology students and assume introductory basic knowledge.

Serious attempt will be made to help those with deficiencies. This will be through the tutorials, but those students will also need to do EXTRA READING to fill in the potential gaps. Some students opt for free additional tutoring. I highly recommend developing a small social group which meets regularly for coffee/beer/whatever, to review the course material. Isolated students tend to fall through the cracks. I sympathize with those of you holding jobs, or if you have personal problems, but in the final analysis I have to be fair to everyone and cannot use dual marking scales.

Introductory lecture (L0)
Slide 2 GOALS OF THE COURSE
a) Quickly review the small molecules and macromolecules from which cells are built.
b) Discuss some methods used in cell biology – but Biology 2910 is designed to complement this course
c) Dissect the cell into cellular sub-compartments (organelles) and study organellar function in greater depth than in previous courses.
d) Review the genetic systems of the cell at the level of gene replication and expression. DNA technology will be introduced in one lecture.
e) Whenever possible, examples of societal relevance of specific issues will be brought up.
f) Emphasis will be placed on developing the basic vocabulary and concepts associated with cell and molecular biology. Reading and writing exercises are used to remind students of the importance of written communication and critical thinking in science.

Slide 3 TWO TEXTBOOKS

This book is particularly valuable to those continuing in the field of biology and is also used in Biology 2910 and 3330). The lectures will be your study guide and will give you a feel for what is important and what is being emphasized in the course. You clearly will not be able to, or expected to, learn all the details in the pertinent textbook chapters of “FAT ALBERTS”. However, you are particularly responsible for material covered in the lectures and material assigned as READING ONLY MATERIAL. The tutorials will be of primarily remedial type: i.e. the review of basic concepts of genetics and chemistry. The enhancement material WILL BE on the tests, but you will have to take your own notes. The main lectures (Powerpoint presentations) will be made available for purchase or posted on the Internet, but make sure you study from the textbook if you want to do better than a B grade.

2. Textbook for weekly writing exercises:
   Christopher M. Gillen, Reading Primary Literature (A practical guide to evaluating research articles in Biology), Pearson - Benjamin Cummins, San Francisco, 2007

Slide 4 QUIZZES
To ensure that continuous attention is given to the course, there will be five unannounced quizzes given throughout the course worth 13% each, for 65% of the total mark. Sample multiple choice questions will be given to you, but short answer and fill-in blanks types of questions may be included. I may also incorporate MCAT and GRE general questions, since cell biology comprises a good part of these tests. There is no final exam in this course (the last quiz is scheduled during the exam period, though). This may imply to you that you don’t have to pay attention to material from the early part of the course. THIS IS NOT THE CASE, the course builds on information acquired earlier and your ability to answer questions later in the course depends on the TOTAL SYNTHESIS of the body of knowledge acquired.

Slide 5 MARKING
To “practice” some science, you will an exercise to make you think about how a particular problem (single cell toxicology) may be approached (5%). A portion of this course is dedicated to reading and writing scientific articles. With 100 or so students it is impossible to mark essays. At least some attempt is made to improve your writing skills. Scientific writing, reading and graphical data presentation are practised in Bio 2910 Lab biology in much greater detail.  

Course marking:
Quizzes (5 x 13%)                                   65%
Writing and reading assignments 1, 2, 3, 4, 6, 7, 8 (7 x 3%) 21%
Experimental design assignment # 5                  5%
Specialized cell - writing assignment # 9            10%
TOTAL                                              101%

SUPPLEMENTAL EXAMS POLICY: There will be ONE SUPPLEMENTAL exam given for any ONE of the quizzes missed for medical or compassionate reasons (please, provide evidence) during the course. The one supplemental test will be scheduled as a short final exam, in the final exam period at the end of term.

OFFICE HOURS:
Tuesday 1:30-2:30 or APPOINTMENT BY E-MAIL. Marks, exam answers, assignment answers and additional course information will be posted in a glassed-in cabinet outside CB 3008/3009.
Completed assignments are to be placed in a wooden box outside CB 3010A AT THE LATEST by the morning of the day after the due date. 20% of assignment value will be deducted per each late day.

Slides 7-11 Course curriculum and textbook chapters to read. Sections of FAT ALBERTS (5th edition - previous edition references are crossed out) covered (attendance of lectures will ensure that you know what was actually dealt with). READ THIS MATERIAL BEFORE THE LECTURES!!

INTRODUCTORY (REVIEW) LECTURES AND TUTORIALS: Microscopy, basic chemistry of small molecules and macromolecules, introductory thermodynamics, energy relations, proteins as enzymes. READ, by January 16th or so:

Chapter 1, Chapter 2 (except p. 91 to 109 p.88-103), Chapter 3 (except details of self-assembly, mathematics of enzyme kinetics, NO reaction mechanisms). Chapter 9 on microscopy; portions of Chapters 10 and 11 on membranes, only as covered in lectures.

Lectures will be presented in the indicated order, however, due to a change in schedule to 1.5 hr lectures, the 33 lectures will be variously combined into 22 lectures. Highlighted lectures are new introductions and changes from last year.

Lecture/tutorial #
1 Light and electron microscopy
2 Scanning electron microscopy, freeze fracturing
3 Slides - review of images of cells
4 Chemical composition of cells, protein structure
5 Diversity of proteins
6 Lipids and membranes
6a Alexa – membrane function
7 Metabolic role of enzymes, intro to thermodynamics
8 Energy and metabolic intermediates
9 Specific enzyme examples
10 Regulation of enzymes and metabolic pathways
T1 Library orientation
T2 Importance of carbon, water, chemical bonds, small molecules in living cells
T3 Polymeric molecules - polysaccharides, proteins, nucleic acids

INFORMATION FLOW IN CELLS: from nucleus/DNA to proteins and structure/function of cells. READ:

Fat Alberts 5th edition:
Chapter 4, (but many details of nucleosome structure and chromosome structure are not dealt with), Chapter 5 (to page 294, NOT DNA repair, recombination), Chapter 6 (no details of RNA splicing/processing, i.e. only material covered in lectures p.309-335 p.329-348); no RNA world – pages 355-372 400-408 ), Chapter 7 all to page 401 440 (Prokaryotes only) and selected details from text dealing with Eukaryotes.
11 Discovery of DNA as genetic material (other textbooks, handouts)
12 Cell cycle, replication of DNA
13 Details at the replication fork, topoisomerase, helicase
14 Conversion of DNA to RNA, co-linearity, mRNA (tRNA, rRNA) synthesis, physical structure of a gene
15 RNA polymerase reactions
16 Breaking the genetic code, RNA to protein co-linearity
17 Transfer RNA as the adapter, RNA synthetases
18 Polyribosomes, rRNA and translation
19 Nucleus - RNA processing, macromolecular movement in and out of the nucleus
20 Histones and gene regulation
21 Elaboration on gene regulation in Eukaryotes TBA
22 Post-translational gene expression regulation by proteolysis
T4 Introduction to DNA, Central dogma
T5 The operon concept and gene regulation
T6 Review, questions & answers
T7 Intro to mitochondrion, glycolysis, TCA cycle
T8 Review session, discussion of graduate work, financing research, careers

GENETICALLY SEMI-AUTONOMOUS "energy" ORGANELLES
YOU NEED TO KNOW MATERIAL FROM THE FIRST PART OF THE COURSE TO UNDERSTAND THESE CHAPTERS!!

Fat Alberts 5th edition:
(Chapter 14, nearly the whole chapter, but not evolution issues and some of the details of techniques)

23 Mitochondrion – electron transport and ATP synthesis
24 Mitochondrion as a genetically semi-autonomous organelle
25 Photosynthetic reaction centers and electron transport
26 Dark reactions and chloroplast genome
27 Chlamydomonas as an experimental system
T9 Chloroplast structure
T10 -11 DNA transgenic technology

INTRACELLULAR COMPARTMENTS, PROTEIN SORTING, DNA TECHNOLOGY, CYTOSKELETON

Chapter 12 (Except details of nuclear pores, insertion of multiple pass proteins, but be aware of glycosylation mechanisms). Chapter 13 as covered in lectures. Chapter 8 only on DNA technology (no study of proteins). Chapter 16 – only introduction – Panel 16-1 and whatever detail will be given in the last lecture.
28 Import of proteins into chloroplasts and mitochondria
29 Peroxisomes
30 Golgi Body and exocytosis
31 Endocytosis and lysosomes
32 Cytoskeleton
33 Slime moulds – cell to cell communication, multicellularity

EXERCISES IN EXPERIMENTATION, WRITING AND READING primary scientific literature (i.e. scientific articles, NOT reviews, books and dictionaries). Completed assignments are to be placed in the box outside CB 3010A. Due to the size of the class, simplicity of the assignments, late returns (beyond 2 days, i.e. after 40% grade has been deducted) will not
be accepted except for medical or compassionate reasons. Electronic submissions WILL NOT BE ACCEPTED - make arrangements with a friend or the Learning Assistance Center to get a hard copy into the assignment box or to my Biology mailbox IF LATE.

Exercise 1. LIBRARY ORIENTATION and Gillen pages 6-7, Section 2 exercises 1 to 7. (January 6th, due Jan 11th) NOTE: The Sunday deadlines imply that I have to be able to find the report in the assignment box (outside CB 3010) sometimes on Monday morning – usually by 11AM or later.

 Attend a library orientation session on Tuesday, January 6th at 9:30, 10:30 or 11:30 AM in the room right next to the elevator door on the 5th floor of the library. You will be guided through some of the features of our library system. This will help you in dealing with some of the assignments below and in finding information for assignments in other Biology courses.

Links:

Biology Subject Guide - http://library.lakeheadu.ca/?pg=102 (this includes links to our databases, reference sources, websites and ejournals)

Biological Abstracts:

Just to let you know that in January Biological Abstracts will be changing its interface from Webspirs to OvidSP.


RefWorks - http://library.lakeheadu.ca/?pg=359

RACER - http://library.lakeheadu.ca/?pg=365

Answer Section 2 exercises in Gillen. If you can't think of any topic of interest to you, you may use these as a guide: fluorescence microscopy, essential fatty acids, RNA polymerase, histones.

Exercise 2. Read Gillen Section 3: “The Anatomy of a paper” (page 7 onwards) and answer Section 3 Exercises (on pages 14-15). WRITING A “LITERATURE CITED” SECTION. (DUE January 18th)

Examine the structure of the last section of scientific articles in several journals, variously entitled: References, Literature Cited, Bibliography, etc. This section lists the primary articles, as well as reviews and book chapters the authors have used to: A) introduce the topic of their research in the Introduction section of their article and B) evaluate their own results against in the Discussion section.

As an ADDITIONAL exercise, pretend you are preparing an article on one of the topics listed below, go to the library or use computer to search for PRIMARY literature on ONE of the topics and prepare a Literature Cited section in the current style of the journal Plant Physiology (http://www.plantphysiol.org/misc/ifora.shtml) containing five to six (6) references only (i.e.
make sure you use an article from a recent printed issue of Plant Physiology as your model (or pdf format if you use electronic version of the article, HTML format looks DIFFERENT!), watch for: capitalization, bolding of Author names and Journal volume, punctuation and of course spelling - this is critical, particularly in the computer age. Incorrect spelling will not later retrieve appropriate information from computer databases).

TOPICS: 1) Cell cycle regulation; 2) protein degradation via the ubiquitin and proteasome system; 3) DNA replication (NOT repair); 4) protein structure - any protein.

The following is an example of the ludicrous variety of ways to format the citation of THE SAME RESEARCH PAPER in different scientific journals (name of journal requiring this format is in brackets at the end of each citation). The example is based on a letter from S. Sperti et al., Nature 393:301, 1998. The present and future generation of scientists will have to work on standardization of bibliographic citations. For the moment you will have to conform, accept the system and recognize the subtle differences in preparing your own reports. The operative word in writing report bibliographies is CONSISTENCY required by the specific journal you are submitting an article to. The following example is as inconsistent as can be. If you look at each citation closely, you will see it is set up differently from all the others (order of items, punctuation, bolding, italics, placement of initials, etc.):


Exercise 3. "The Introduction" (Gillen, pages 15 - 20, answer questions on page 20, due January 25th)

Exercise 4. "The Materials and methods" (Gillen, pages 20 to 28, answer questions on pages 28 - 29, due February 1st)
Exercise 5. DESIGNING A MINI-EXPERIMENT IN TOXICOLOGY (due Feb 13th, the Friday immediately before the reading week)

University lab exercises at the first and second year are “pre-cooked” for you, but you should start learning about designing your own experiments. The following list is a general outline of issues you may have to consider before coming up with an experiment (at this stage even an experiment which was done many times already).
Things to consider when being asked to DESIGN AN EXPERIMENT:

First of all, THINK SIMPLE EXPERIMENTS. ANSWER QUESTIONS ONE AT A TIME!! Don’t try to learn everything at once in one experiment. You are just starting out, so “Science fair” level experiments are OK, provided they are well thought out (and executed - should you have the good fortune to actually do the work!).

What model system (bacteria; protozoa; fungi - yeast, Aspergillus, Dictyostelium; invertebrates such as Drosophila melanogaster, Caenorhabditis elegans; plant such as Arabidopsis thaliana; or vertebrates?) is the best to study the problem?
What am I expected to learn about (“discover”)? IDENTIFY TOPIC!
Learn something about the “effector” you are expected to study to come up with possible ideas for experiments. For example, if asked to study the effects of temperature, consider high, low temperature, duration of exposure, etc; magnetism, consider strength of the field, polarity, duration of exposure - continuous, intermittent, in relation to a specific stage of life cycle (vegetative, reproductive, etc.); gravitational force, consider no gravity, increased gravity, changing polarity, etc.; chemical substance, consider concentration, duration of exposure, exposing particular developmental stages of the organism, method of delivering the substance (tissue targeting), etc.
What constraints do I have (equipment, supplies)? For example, do I have access to the space shuttle to do microgravity experiments?
Do I work at the whole organism level, physiology, biochemistry, molecular biology, or do I use a genetic approach?
Is it a field problem or do I imitate natural conditions under controlled lab conditions?
Is the lab experiment going to miss some critical environmental factor which cannot be reasonably controlled or even provided in the lab (for example UV light in natural daylight or spectral qualities of moonlight)? Conversely, is a field experiment going to be meaningless due to uncontrollable factors?
Consider all you know about the issue and READ PRIMARY LITERATURE to get more ideas for possible experiments.
Do I have to satisfy any regulatory bodies - animal care, patient ethics, radioisotope use, toxic substance disposal?
What is my hypothesis (i.e. presumed outcome of the experiment)?
What am I actually going to do to perform the experiment? What will be my control and how many controls do I need in relation to the experiment? (NOT ONE FOR EACH CONCENTRATION OF A TEST CHEMICAL!!!)
How do I ensure repeatability - proper design to allow some statistical analysis. Bare minimum in cell biology is three repeats of the experiment with similar outcome. With treatments run at least in duplicate! TABLES OR FIGURES EVENTUALLY HAVE TO SHOW CONFIDENCE LIMITS, ERROR BARS, etc.! All or nothing experiments may be possible - genetic transformation, for example. Either you obtain the transformant, or not!
How do I ensure that the material I use is genetically uniform - use inbred or cloned individuals (conversely, if diversity is of interest, how do I ensure adequate population size?)

How do I ensure that biological material is not “contaminated” with other species (axenic cultures - sterility) or is this important?

If using chemicals, are they soluble in my delivery medium? (Merck Index, Handbook of Chemistry, Sigma Chemical Tech Support). What dilution series do I use - some hint from published literature (no point in testing one concentration at a time!!!)?

IF YOU ARE AWARE OF ANY DRAWBACK OF YOUR APPROACH, MENTION IT AND SUGGEST HOW YOU COULD DEAL WITH IT (including ignoring it, or making assumptions).

General suggestions: use future tense where appropriate, you are proposing FUTURE work; don’t forget to *italicize* foreign words, particularly Latin genus and species names - the convention is capital for genus name, lower case letter for species name, eq. *Drosophila melanogaster*. Use active voice (I, we will do such and such...), not passive voice (IT will be done)! This is a departure from what the old fashioned way you were taught to write lab reports in high school and perhaps in other courses. The modern way is to take responsibility for your own work and use ACTIVE VOICE!

Assignment:

Fish, amphibians, single celled organisms and cultured mammalian cells are frequently used to test chemical toxicities. Search the literature and the WEB for sites dealing with this issue in FREE LIVING SINGLE CELLED ORGANISMS (not multi-cellular organisms or cultured animal/plant cells). Based on the information you gather, in THREE short paragraphs propose a MINI project (essentially one set of experiments using a dilution set of one chemical), attempting to test the toxicity of a specific environmental contaminant on the model system you selected. I.e. in the first paragraph say why you chose a particular chemical and organism, in the second paragraph outline what will be done and why (controls, molar, millimolar or micromolar concentration range, type of vessels used, culture conditions, solubility of your test chemical, etc.). In the last paragraph, state what it is you expect to find. Address the issue of “lethal concentration 50” or “LC50” of your particular chemical under the conditions used. (A good starting point might be the library – books and review articles). Make sure you understand the difference between A) concentration of a toxin in the environment – expressed as amount per volume of water or air and B) lethal dose expressed as amount per body weight. The two measures are NOT freely interchangeable.

COMMON PITFALLS with this exercise:

Do NOT copy toxicological experiments published on the internet - these are invariably complex and clearly not your design

Stay away from standardized environmental protection agency (EPA) or other tests, although you can use these as a learning tool.

Stay away from the effects of pH or volatile substances, as these are independent variables difficult to control.
Seriously consider solubility issues for your substance - elemental mercury for instance is volatile in the air or slightly soluble in water - but determining the concentration in either the air or water is a difficult task. Choose soluble substances.

Exercise 6. “The Results” Gillen Section 6, pages 29 to 37, answer questions on page 37, due March 1)

Exercise 7. “The Discussion” Gillen section 7, pages 38-41, answer Section 7 exercises, due March 8).

Exercise 8. APPLICATIONS OF SINGLE CELL or SMALL COLLONIAL ORGANISMS. (Due March 15)
Perform a primary literature search (NOT WEB SEARCH – see library orientation, Exercise 1) for any single-celled free living organism, trying to find out commercial, i.e. biotechnological application; disease this organism causes, or any other interesting feature/use of this organism which grabbed your attention. You may use the following list as a guide:

Protozoans - Calcarina sp., Giardia lamblia, Plasmodium sp.


Algae/Diatoms – spp. Ulva , Botryococcus, Scenedesmus, Asterionella, Cyclotella, Chlamydomonas reinhardtii, Peridinium

Prokaryotes – Spirulina sp., Agrobacterium tumefaciens, Thiomargarita namibiensis

Write up to three sentences describing ONE commercial application of one of the organisms. If the application is only a potential one, i.e. yet to be realized, make sure you mention this. Include a photocopy or computer print out of the front page of the article you worked with.

Exercise 9. CELL TYPES and DIVERSITY - (due March 29th)

Part 1. IMAGE AND CAPTION (Figure legend). Perform a library / web search for images and information on a cell type of your choice (use Fat Alberts as your initial inspiration), selected from any multicellular organism. Use scanned images from library books or journals. Or use the image search capability of Google on the Internet. MAKE SURE YOU SELECT LARGE IMAGE to work with, so it prints at a decent quality level. Print an image of the cell type at nearly full page size at a minimum of 600 dpi resolution, use colour if you have access to a colour printer and the original picture is in colour. On a separate page or the back of the image, write a descriptive figure caption suitable for publication. i.e. in a minimalist way tell the reader what the subject is, what the magnification is and by what method the image was prepared.

Part 2. Write a short statement dealing with the function of the cell type you selected. Tell me why you found the cell interesting, and what further information you would like to obtain about the cell type if you were to do research on it.
Work individually or in teams on the structure and technical aspects of the project, but make sure you submit a scientifically unique essay based on YOUR individual work. Slight modifications of the same paper will be considered plagiarized and both authors will be penalized.

The purpose of the exercise is to introduce you to the variety of higher organism cell types, and to teach you how to communicate scientific information. Your target audience is not a group of scientists and graduate students, but your own peers, instructors and the general public, (i.e. aim the language level accordingly, define terms as needed, etc.). Your goal is to teach others enthusiastically about the cell type you selected and its functions within the organism.

Hints:
How does the structure of this cell differ from “typical” undifferentiated cell? What is interesting and unique about the cell type in the context of the entire organism? How is the cell type used in scientific research? Does it have any commercial applications or diseases associated with it? Less is often more!! Do not include pages of text with everything that is known about the cell!!!! Do NOT plagiarize, especially from the Internet. The faculty is implementing TurnitIn software to detect plagiarism.

1. Title / your name / affiliation - address, followed by Introduction: Why did you choose this cell type / organism? What is unique and interesting about this organism / cell type? What can we look forward to in the rest of the poster?

2. Main body built around the image and caption from part ONE. You MUST acknowledge sources!

3. Summary/Conclusions (main “take-home” message)

4. Literature cited.

SUBMISSION: Submit a pdf or Word version of the essay to me electronically by the 29th of March (lada.malek@gmail.com). Standard rules for late submissions apply.

Evaluation:
Ability to follow instructions
5/5
Overall neatness and image quality
3/3
Content – factual information, logical organization, discussion, conclusion
10/10
Grammar
2/2
TOTAL 20pts

ADDITIONAL USEFUL MATERIALS.
Bibliography and library call numbers of useful guides to effective scientific writing:


Hughes, K. and J. Vinall-Cox, The report writer’s manual, Mosaic Press, Oakville, ON, 1985 (PE 1478 H84)


TTHIS BOOK IS NOW A REQUIRED STANDARD IN THE BIOLOGY DEPARTMENT:
Moore, R., Writing to learn biology, Saunders College Publishing, 1992. It is a required text in Biology 3450, Fungi, and possibly other courses. You may consider buying it and reading it early.

As a future professional biologist, you should make it a habit to read regularly some scientific journals. The weeklies Nature or Science are good sources of information on science policy and international science news. They also contain top quality research reports in various scientific disciplines (you can only scan the abstracts of these very specialized articles - Nature has a section which summarizes in a popular writing form the contents of the detailed reports). Regularly scan a few journals of immediate interest to you.

UNNECESSARY WORDS (FYI)

Student writers are notorious for using piles of unnecessary verbiage (perhaps in an attempt to sound “scientific”?). Good scientific writing is quite simple, making its point in a forceful language limited in the use of technical terms.

The following pages give samples of two or more word sequences in which at least one word can be eliminated. Go through this list and CROSS OUT NEEDLESS WORDS. The edited word list will be posted so you can verify your choices. Keep this list when writing papers and reports in the future.

Unnecessary words which can be deleted:

<table>
<thead>
<tr>
<th>separate entities</th>
<th>entirely eliminated</th>
</tr>
</thead>
<tbody>
<tr>
<td>currently being</td>
<td>few in number</td>
</tr>
<tr>
<td>had done previously</td>
<td>personal opinion</td>
</tr>
<tr>
<td>none at all</td>
<td>reduce down</td>
</tr>
<tr>
<td>joint cooperation</td>
<td>separate out</td>
</tr>
<tr>
<td>any and all</td>
<td>disappear from sight</td>
</tr>
<tr>
<td>completely finish</td>
<td>come to an end</td>
</tr>
<tr>
<td>future plans</td>
<td>for the purpose of</td>
</tr>
<tr>
<td>unexpected surprise</td>
<td>duly noted</td>
</tr>
<tr>
<td>bisect into two parts</td>
<td>mutual cooperation</td>
</tr>
<tr>
<td>one and the same</td>
<td>red in colour</td>
</tr>
<tr>
<td>blame it on</td>
<td>already existing</td>
</tr>
<tr>
<td>actual facts</td>
<td>currently underway</td>
</tr>
<tr>
<td>basic essentials</td>
<td>never before</td>
</tr>
<tr>
<td>definite decision</td>
<td>continue to remain</td>
</tr>
<tr>
<td>usual custom</td>
<td>first began</td>
</tr>
<tr>
<td>subject matter</td>
<td>mix together</td>
</tr>
<tr>
<td>equally as effective</td>
<td>private industry</td>
</tr>
</tbody>
</table>
two equal halves
have need for
any and all
full and complete
various different
near the vicinity of
unusual in nature
active consideration
baffling enigma
conclusive proof
advance reservation
close proximity
brief in duration
merge together
repeat the same
until such time as
the actual number
conclusive proof
stunted in growth
hard evidence
assemble together
during the course of
revert back
advance plan
current status
repeat again
balance against one another
during the course of
because of the fact that
absolutely essential
quite impossible
past experience
combine into one
first and foremost
all of
necessary requisite
in between
file away
close scrutiny
enclosed herewith
by means of
equal halves
at a later date
join/bond together
for the purpose of
new initiatives
close proximity
the question as to whether
basic and fundamental
various differences
each individual
if at all possible
perform a study
join together
slow up
hectares of land
all throughout
large in size
exactly identical
underlying purpose
viable solution
past experience
joint partnership
while at the same time
at the time when
and so on and so forth
completely finish
each individual
initial preparation
absolute necessity
basic fundamentals/essentials
honest truth
this particular instance
range all the way from
excess verbiage
nominated for the position of
completely surround
wholly new
rarely ever
final outcome
refer back to
uniformly consistent
debate about
still remain
protrude out
consequent results
entirely eliminate
serious crisis
end result
smaller in size
is defined as
continue on
mix together
completely eliminate
personal friend
combine together
consensus of opinion
end product
hurry up
by means of
advance planning
previously found
total of 30 people
ask the question
period of time
authentic replica
young juvenile
past medical history
ultimate outcome
viable alternative
introduce a new
most unique
wish to thank
plan ahead for the future
science of biology
spherical in shape
in connection with
resume again
overall plan
whether or not
by means of
refer back
make a study of
deliberately chose
customary practice
any and all
one and the same
different species
cancel out
repeat again
circulate around
write up
definite proof
in conjunction with
subject matter
if it is assumed that
in between
completely full
early beginnings
recur again