Supplemental Material

CBE—Life Sciences Education

Abdullah *et al.*

Supplementary Materials

Supplementary Figure Legends

Supplementary Figure S1: Changes in self-efficacy ratings within and outside of the students' thesis topic field (N = 28 students). A. Students' self-efficacy ratings on proposing an experiment within (top) and outside of (bottom) their field. B. Students' self-efficacy ratings on independently drawing conclusions from data within (top) and outside of (bottom) their field. C. Students' self-efficacy ratings on proposing an experiment within (top) and outside of (bottom) their field.

Supplementary Figure S2: Breakdown of inference components and evaluation components of science process test. A. Pre- and post-test scores in questions pertaining to drawing conclusions or proposing a hypothesis. B. Pre- and post-test scores where students were asked to evaluate a hypothesis based on either 1 or 2 related data sets.

Supplementary Figure S3: Breakdown of experimental design question. A. Pre- and post-test average percent scores for each of the scoring categories. Only the Appropriateness category showed a statistically significant increase in the post-test score ($p = 0.0049$).

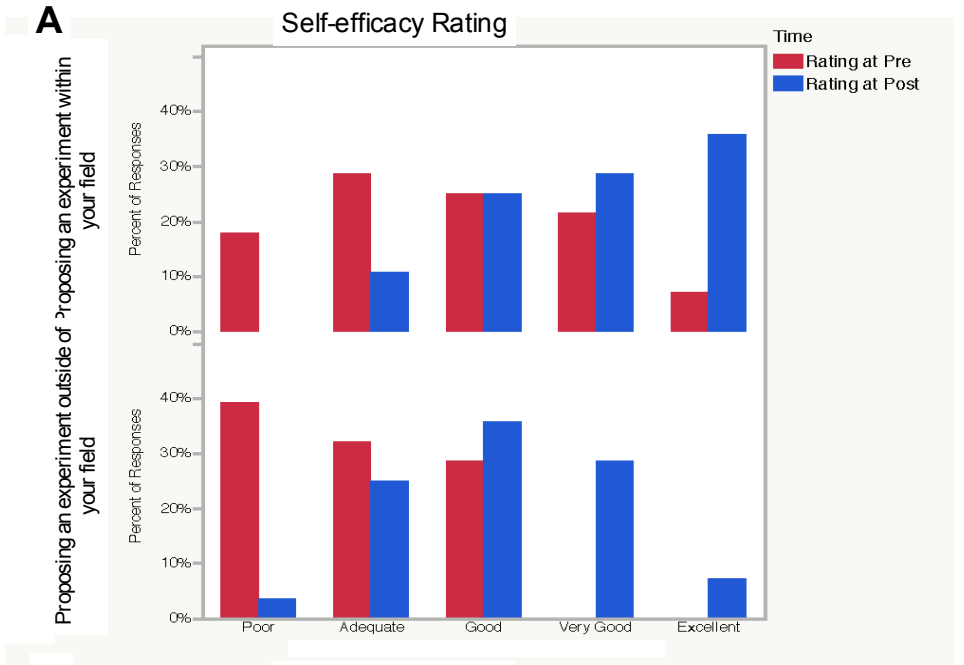
Supplementary Figure S4: Analysis of the science process tests, Fall 2012 and Winter 2013. 41 pairs of pre- and post-tests were compared. Error bars in A. and B are Standard Error of the Mean Difference. A. Comparison between students' performance in the categories of Analysis, Synthesis, and Evaluation. Small, but statistically significant gains were observed in the Synthesis category: ability to design a follow-up experiment ($p = 0.0096$). B. Average quantitative data evaluation scores of the post-tests were significantly higher than the scores of the pre-tests ($p = 0.015$). Quantitative data evaluation scores were determined by frequency and quality of students' comparison between experimental and control data that contained the magnitude of the difference, in percentage or fold difference. We observed 18.5% increase in the average score in this category in the post-tests, comparing to the pre-tests ($t(40)=2.535$, $p=0.015$, Cohen's $d=0.556$). However, there is still a large room for improvement in this category: the average post-test score was only 35% of the maximal possible score.

Supplementary Figure S5: Breakdown of experimental design question from Fall 2012 and Winter 2013 which used the prompt: "Suggest a follow-up experiment to any aspect of this study. Do not use either of the experiments previously described. Propose a hypothesis and include all relevant components of the experimental design in your experiment." Pre- and post-test average percent scores for each of the scoring categories. Only the Appropriateness category showed a statistically significant increase in the post-test score. Experimental System ($p=0.0042$), Independent variable/Treatment ($p=0.0131$), and Quantity Measured ($p=0.0136$) were statistically significant.

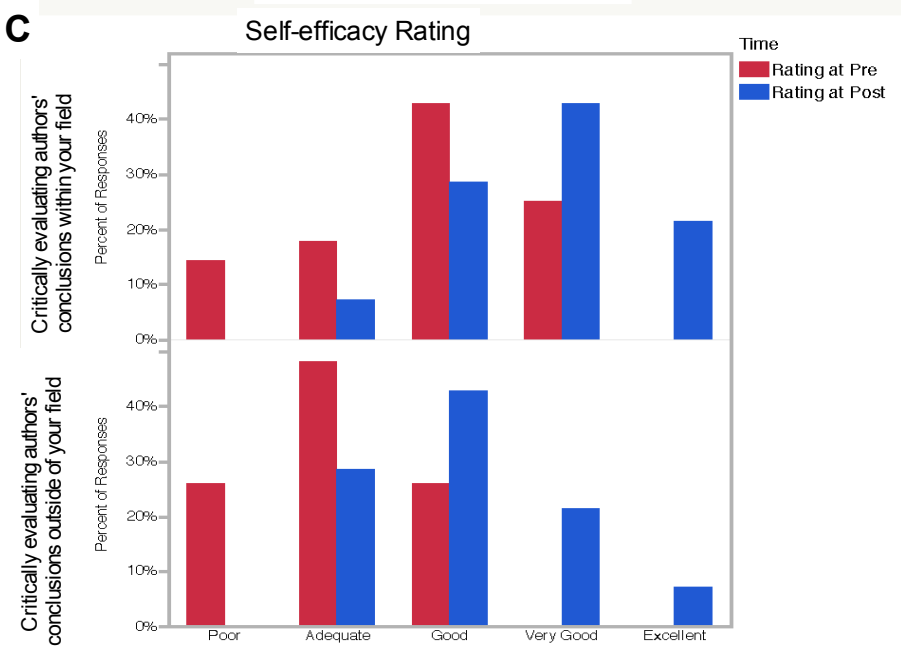
Supplementary Table S1: Focus papers for the Fall 2013 and Winter 2014 quarters.
Supplementary Table S2: Expert validation of science process test questions and alignment of primary and secondary core critical thinking skills.
Supplementary Table S3: Alignment of the criteria applied to evaluate students' experimental proposals (Q4-2 in the Science Process test) with the established experimental design difficulties (as summarized in Dasgupta et al., 2014) and Areas of difficulties from the Rubric for experimental design (RED, Dasgupta et al., 2014).
Supplementary Table S4: Full numerical list of students' self-efficacy survey results
Supplemental Table S5: Full experimental design responses from the samples given in Table 2.

Appendix A- Guidelines for the analysis of three experiments individual assignment
Appendix B- Guidelines for the experimental proposal group assignment
Appendix C- Science Process test Version A
Appendix D- Science Process test Version B
Appendix E- Grading rubric for FA13/WI14 science process test. Rubric used to score the pre- and post-science process tests. Each row consists of the question, value of the question, and scoring scheme used by the raters.

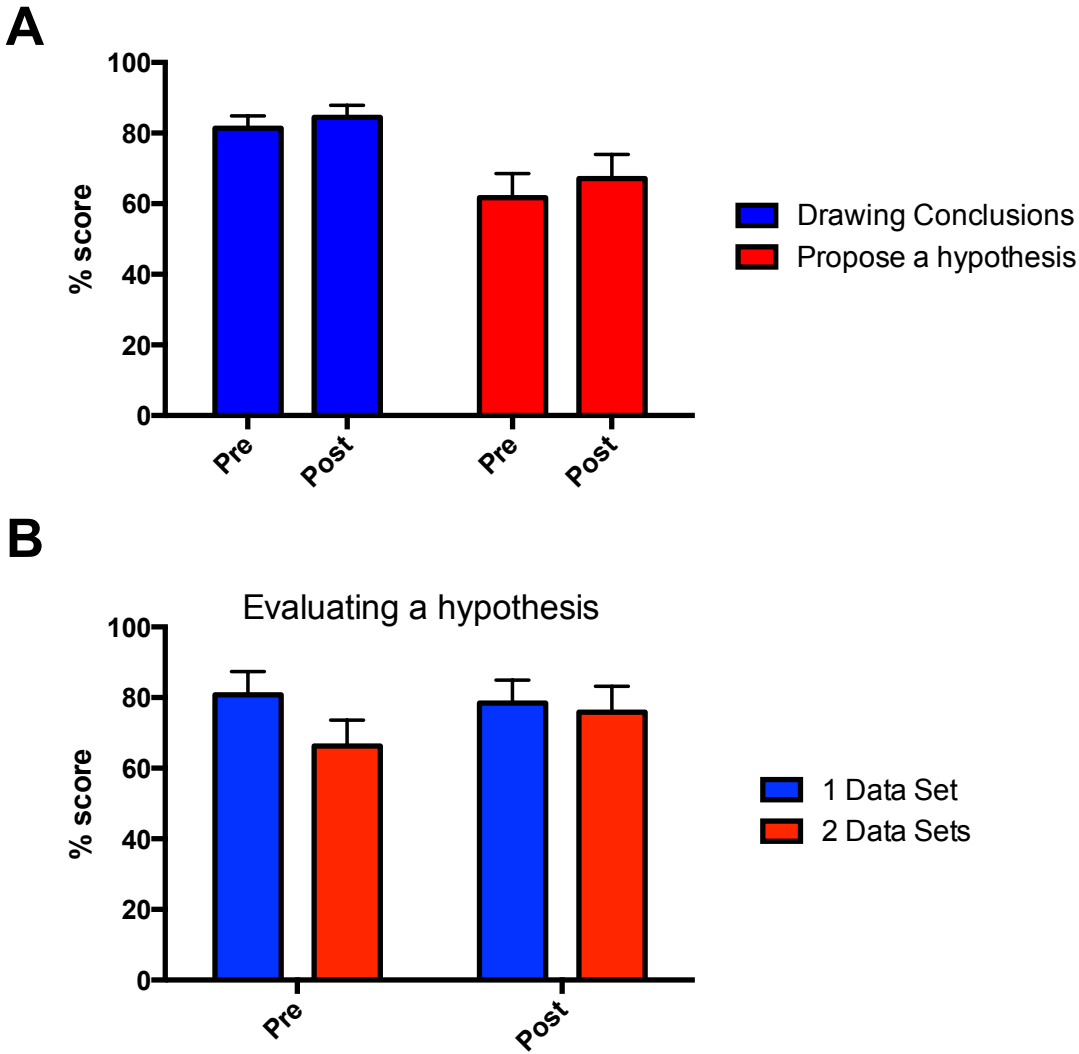
Figure S1


A
Self-efficacy Rating
Time
Rating at Pre
Rating at Post
Proposing an experiment outside of your field
Proposing an experiment within your field
Percent of Responses
0%
10%
20%
30%
40%
Poor
Adequate
Good
Very Good
Excellent


B
Self-efficacy Rating
Time
Rating at Pre
Rating at Post
Independelty drawing conclusions from data oustide of your field
Independelty drawing conclusion from data within your field
Percent of Responses
0%
10%
20%
30%
40%
Poor
Adequate
Good
Very Good
Excellent


C
Self-efficacy Rating
Time
Rating at Pre
Rating at Post
Critically evaluating authors' conclusions outside of your field
Critically evaluating authors' conclusions within your field
Percent of Responses
0%
10%
20%
30%
40%
Poor
Adequate
Good
Very Good
Excellent

Supp. Figure S2



Supp. Figure S3

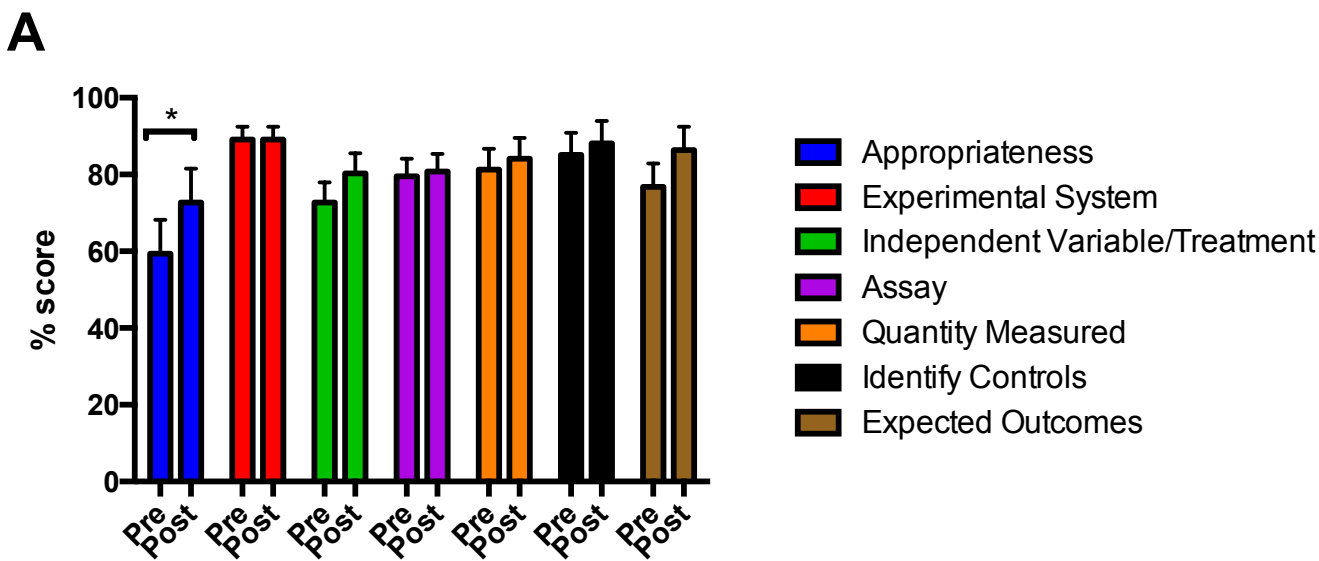


Figure S4

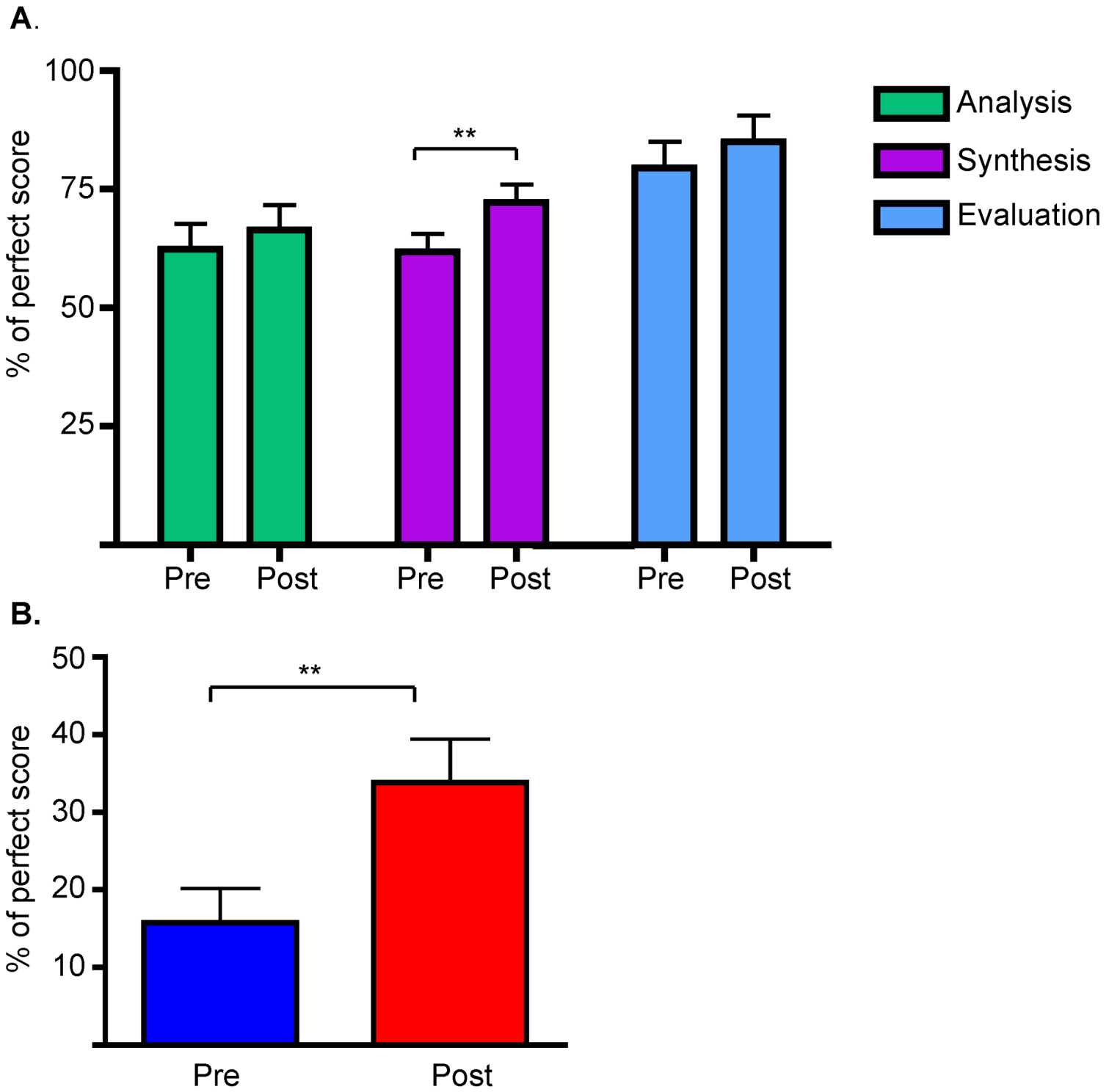

A.
100
75
50
25
% of perfect score
**
Pre
Post
Pre
Post
Pre
Post
Analysis
Synthesis
Evaluation
B.
50
40
30
20
10
% of perfect score
**
Pre
Post

Figure S5

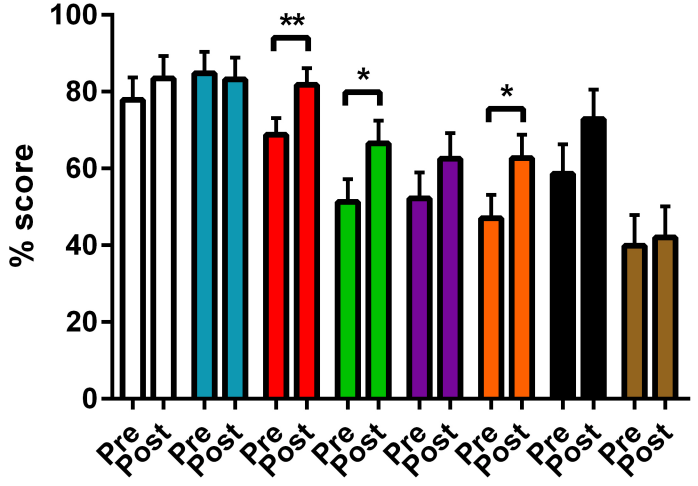

100
80
60
40
20
0
% score
**
*
*
Pre
Post
Stated Hypothesis/Experimental Question
Relevant/Consistent with Previous Data
Experimental System
Independent Variable/Treatment
Assay
Quantity Measured
Identify Controls
Anticipated Outcomes

Table S1

Fall 2013 Papers		Focus Paper(s)
	Paper 1	Nat Genet. 1996 Jun;13(2):233-7. Graded activation of fibroblast growth factor receptor 3 by mutations causing achondroplasia and thanatophoric dysplasia. Naski MC[1], Wang Q, Xu J, Ornitz DM.
	Paper 2	Nature. 2012 Jul 5;487(7405):57-63. doi: 10.1038/nature11244. Embryonic stem cell potency fluctuates with endogenous retrovirus activity. Macfarlan TS[1], Gifford WD, Driscoll S, Lettieri K, Rowe HM, Bonanomi D, Firth A, Singer O, Trono D, Pfaff SL.
	Paper 3 & 4	Neuron. 2010 Jun 10;66(5):663-70. doi: 10.1016/j.neuron.2010.05.002. Assessing spinal axon regeneration and sprouting in Nogo-, MAG-, and OMgp-deficient mice. Lee JK[1], Geoffroy CG, Chan AF, Tolentino KE, Crawford MJ, Leal MA, Kang B, Zheng B. J Neurosci. 2010 May 19;30(20):6825-37. doi: 10.1523/JNEUROSCI.6239-09.2010. MAG and OMgp synergize with Nogo-A to restrict axonal growth and neurological recovery after spinal cord trauma. Cafferty WB[1], Duffy P, Huebner E, Strittmatter SM.
Winter 2014 Papers		Focus Paper(s)
	Paper 1	Nat Genet. 1996 Jun;13(2):233-7. Graded activation of fibroblast growth factor receptor 3 by mutations causing achondroplasia and thanatophoric dysplasia. Naski MC[1], Wang Q, Xu J, Ornitz DM.
	Paper 2	Neuron. 2010 Jun 10;66(5):663-70. doi: 10.1016/j.neuron.2010.05.002. Assessing spinal axon regeneration and sprouting in Nogo-, MAG-, and OMgp-deficient mice. Lee JK[1], Geoffroy CG, Chan AF, Tolentino KE, Crawford MJ, Leal MA, Kang B, Zheng B.
	Paper 3 &4	Nature. 2002 Feb 21;415(6874):914-7. Epub 2002 Feb 6. Hox protein mutation and macroevolution of the insect body plan. Ronshaugen M[1], McGinnis N, McGinnis W. Nature. 2002 Feb 21;415(6874):910-3. Epub 2002 Feb 6. Evolution of a transcriptional repression domain in an insect Hox protein. Galant R[1], Carroll SB.

Table S2

	Core critical thinking skill	
Questions	**Primary alignment**	**Secondary alignment**
1-1A	Interpretation (80%), Explanation (80%)	
1-1B	Interpretation (73%)	Explanation (67%)
1-1C	Inference (100%)	Analysis (60%), Interpretation (60%)
1-2	Evaluation (93%)	Inference (80%)
1-3	Inference (80%)	Analysis (67%), Explanation (67%)
2-1A	Interpretation (77%), Explanation (77%)	
2-1B	Interpretation (62%), Explanation (62%), Analysis (62%)	
2-1C	Inference (100%)	Analysis (54%), Explanation (54%), Interpretation (54%)
2-2	Evaluation (85%), Analysis (85%)	Inference (77%)
3-1	Inference (100%)	Explanation (85%)
4-1	Inference (77%), Analysis (77%)	

Table S3

Q4-2 Experimental Design categories	**Experimental design difficulties (Dasgupta et. al. 2014)**	**RED, areas of difficulties (Dasgupta et. al. 2014)**
Appropriateness: does the proposed experiment answer the question posed?	II. Variables C) Treatment (independent) variable	2. Manipulation of variables, d) Treatment conditions … inappropriate according to the goal of investigation g) Variables unrelated to the research question. 3. Measurement of outcome: h. There is a mismatch between that the investigation claims to test and the outcome variable.
Experimental System	I. Identifying the experimental subject	1. Variable property of an experimental subject (experimental subject or units)
Treatment	II. Variables: Treatment (independent variable)	2. Manipulation of variables
Control	II. Variables: Control (comparison) group	2. Manipulation of variables: Control groups
Assay	III. Measurement of results	3. Measurement of outcome
Quantity measured	III. Measurement of results	3. Measurement of outcome
Expected Outcomes	II. Outcome (dependent) variable	3. Measurement of outcome

Table S4

Please evaluate your current skills in:		Self-efficacy Rating									
		Poor		Adequate		Good		Very Good		Excellent	
Category	Time	Row %	N	Row %	N	Row %	N	Row %	N	Row %	N
Critically evaluating authors' conclusions in a paper in your area of research	Pre	14.29%	4	17.86%	5	42.86%	12	25.00%	7	0.00%	0
	Post	0.00%	0	7.14%	2	28.57%	8	42.86%	12	21.43%	6
Critically evaluating authors' conclusions in a paper outside of your area of research	Pre	25.93%	7	48.15%	13	25.93%	7	0.00%	0	0.00%	0
	Post	0.00%	0	28.57%	8	42.86%	12	21.43%	6	7.14%	2
Generate a hypothesis based on data observations	Pre	0.00%	0	33.33%	4	33.33%	4	33.33%	4	0.00%	0
	Post	0.00%	0	8.33%	1	25.00%	3	41.67%	5	25.00%	3
Independently drawing conclusions from data presented in a paper in your area of research	Pre	3.57%	1	17.86%	5	42.86%	12	25.00%	7	10.71%	3
	Post	0.00%	0	3.57%	1	25.00%	7	35.71%	10	35.71%	10
Independently drawing conclusions from data presented in a paper outside of your area of research	Pre	21.43%	6	46.43%	13	25.00%	7	7.14%	2	0.00%	0
	Post	0.00%	0	25.00%	7	32.14%	9	35.71%	10	7.14%	2
Interpreting data in a paper outside of my area of research	Pre	7.14%	2	50.00%	14	35.71%	10	7.14%	2	0.00%	0
	Post	0.00%	0	28.57%	8	28.57%	8	39.29%	11	3.57%	1
Interpreting data in a paper within my area of research	Pre	0.00%	0	21.43%	6	25.00%	7	35.71%	10	17.86%	5
	Post	0.00%	0	0.00%	0	28.57%	8	35.71%	10	35.71%	10
Proposing an experiment, with the appropriate controls, that would follow up on a paper in your area of research	Pre	17.86%	5	28.57%	8	25.00%	7	21.43%	6	7.14%	2
	Post	0.00%	0	10.71%	3	25.00%	7	28.57%	8	35.71%	10
Proposing an experiment, with the appropriate controls, that would follow up on a paper outside of your area of research	Pre	39.29%	11	32.14%	9	28.57%	8	0.00%	0	0.00%	0
	Post	3.57%	1	25.00%	7	35.71%	10	28.57%	8	7.14%	2
Talking about scientific ideas NOT related to your research in public	Pre	32.14%	9	28.57%	8	21.43%	6	14.29%	4	3.57%	1
	Post	3.57%	1	17.86%	5	21.43%	6	53.57%	15	3.57%	1
Talking about scientific ideas related to your research in public	Pre	15.38%	4	23.08%	6	30.77%	8	15.38%	4	15.38%	4
	Post	0.00%	0	10.71%	3	17.86%	5	39.29%	11	32.14%	9
Understanding why a particular control is being used in an experiment	Pre	0.00%	0	25.00%	3	33.33%	4	33.33%	4	8.33%	1
	Post	0.00%	0	0.00%	0	25.00%	3	41.67%	5	33.33%	4
When encountering a result obtained using a method you know little about, being able to independently figure out	Pre	10.71%	3	50.00%	14	32.14%	9	7.14%	2	0.00%	0

how the method works											
	Post	0.00%	0	17.86%	5	28.57%	8	46.43%	13	7.14%	2
When encountering terminology or concept you don't understand, being able to figure out what it means on your own	Pre	0.00%	0	28.57%	8	57.14%	16	7.14%	2	7.14%	2
	Post	0.00%	0	7.14%	2	28.57%	8	50.00%	14	14.29%	4
Writing about science in general	Pre	14.29%	4	17.86%	5	32.14%	9	35.71%	10	0.00%	0
	Post	0.00%	0	17.86%	5	17.86%	5	42.86%	12	21.43%	6
Writing about topics related to your research thesis	Pre	14.29%	4	17.86%	5	28.57%	8	25.00%	7	14.29%	4
	Post	0.00%	0	7.14%	2	21.43%	6	28.57%	8	42.86%	12

Table S5

Test Number	Student Response
1272	in vivo assay testing the proliferation effects of Gene Y mutation on tumor formation. Gene Y mutation containing colon cancer cells will be injected subcutaneously in different amounts into immunodeficient mice and monitored biweekly for tumor formation. Controls: inject healthy colon cells without gene Y mutation into mice at some place. Monitor tumor formation, measure size, and analyze the tumor genome for gene Y mutation to show that gene Y can cause tumor formation. Gene Y mutation containing cells are expected to promote colon cancer and thus promote tumor formation in inmmunodeficient mice.
8529	Using a rat model we could test to see whether gene Y contributes to colon cancer. The assay would be whether or not the rats develop colon cancer. There would be a minimum size of the tumor required to be considered cancer. We would be observing the appearance of colon cancer in rats that are just past middle aged. Controls would include: healthy rats with no gene Y mutation; gene Y mutation rats. After rats reach the equivalence of 65 yrs old, they will be killed and examined for a tumor of a certain weight. If there is a correlation it is expected that healthy rats will not have tumors and rats w/ the gene Y mutation will have tumors.
6582	Use cultured cells to induce the same Gene Y mutation as seen in humans. Use non-mutated, wildtype cultures as controls. Perform a proliferation assay to measure the # of proliferated cells. Those with Gene Y mutation should have more cells in the culture than wild-type cells.
3064	I would perform an experiment on cultured cells to overexpress Gene X and look at its effects by transfecting cells with an expression vector that contains Gene X and a strong promoter. Untreated cells would be used as a control to detect proliferation in the original cell line. Cells transfected with a vector without Gene X would be used as a control to ensure the vector itself (as well as the transfection process) did not produce additional effects. A proliferation assay would be performed on all the controls and the Gene X overexpression cells. I would expect untreated and vector control cells to have similar levels of cell proliferation while cells overexpressing Gene X would have significantly higher rates of cell proliferation.
1524	-Transfection of Gene Y into non-gene Y expressing cells to look for increasing proliferation. -Untreated cells will be grown with treated cells and the overall # of cells after 4-6 days will be compared. -If gene Y expression promotes cell growth then the gene Y treated cells will have higher numbers than the non-gene Y cells. -This increase in proliferation would be consistent with the previous experiments data and the finding of a gene Y mutation in colon cancer.
7803	mutation in gene X→ cancer in young adults. Use mice….? Or clinical study w/ human patients to see if there are any individuals w/o gene X mutant, but w/ cancer.

Appendix A

Guidelines for analysis of 3 Key experiments

Submit this paper as an electronic copy on Tunritin (through Ted) and bring a hard copy to class. The assignment is due on Turnitin before 9:30AM on the day it is due in class (except for Paper 1 analysis, due at 11:59PM on that day).

Your paper critique should be a stand-alone piece of writing, that is, an educated biologist (but not a specialist in paper's field) should understand what you are talking about without having to read the paper. Format: the critique should be **no longer than 1 page (**longer write-ups will result in deduction of points). Font size should be at least 11, single-spaced or double spaced.

Your critique should have the following:
- Your name, course, quarter, year
A. Paper to be discussed: authors, title, year of publication, journal

B. Introduction (one paragraph):

In this paragraph, you will introduce the **important biological processes** and **any crucial terminology** your reader, who is an educated biologist, but not a specialist in the field, needs to know to understand **the main topics of paper**. Why is it important to understand these processes ("why should we care about them"). What is/are the **over-arching question/s** the authors wanted to answer in this paper? Imagine that you are talking to a fellow MS student in the elevator. In no more than 4 sentences, try to explain what this paper is about (without talking about the results yet).

C. Results. You will be describing three KEY experiments, in most cases from different figures. Many figures contain more than one experiment, so you don't need to present the entire figure in this situation, just the relevant panel/s. For each experiment, address the following questions, however, be sure that your paragraph is a flowing piece of writing, not a list of answers. **Always indicate which panel** and which figure you are describing. One paragraph per experiment.

Why is this experiment being performed: what specific question/s does it ask? Why did you select it as one of the **key experiments** of this paper?

Describe the experimental design, including:

A. the system, (in vitro, in vivo, cells, animals)

B. the components critical for the experiment (genetic or molecular modifications).

C. What is the assay (e.g. Western blot, immunofluorescence) and what is being measured or observed (e.g., protein levels, protein localization)?

D. Identify the experiment and the controls, explain the purpose of each of the controls. Are there any other controls that should have been included?

E. Describe the experimental results and how they compare to the controls. Include quantitative evaluation: to what extent does the experimental condition differ from the control (2 fold? 10%?)

F. What is your interpretation from the results (do you agree with the authors' interpretation of the results?) Are there any problems or limitations that you notice (this might require expert knowledge, but use your common sense: can you see what the authors claim you should be seeing? Are you impressed with the difference between the experiment and the control, etc.). You don't have to explicitly say" yes, I agree", but provide your evaluation as part of your description of the results, for example: "The three-fold difference in the response to X comparing

to Y strongly supports the idea that…." "The authors claim that this experiment demonstrate X, but the difference between the experimental and the negative control is not very impressive"

Appendix B

Guidelines for the experimental follow-ups

This is a group assignment, each member of the group will receive the same grade.
Format: this assignment should be no longer than 1 page, font size no smaller than 10, the margins should be at least 1 inch wide. You should submit it to Turnitin through Ted (look for Turnitin assignments). In addition, please send me (as a image file or as a slide) a schematic drawing (a cartoon) of your experiment the way you would explain it on a blackboard. Please include your names or your group number on the drawing.

Experimental follow-up: this is an original experiment of your own design that is inspired by the paper or directly follows up on the study. It can be a question that remained unanswered by the authors, or an idea on how the research described in the article can be advanced. It can also be a question that aims to test your own, alternative interpretation of the authors' results. Your experiment will be evaluated based on the clarity of the question you are aiming to answer/hypothesis you are testing, clearly explained experimental design, inclusion of the necessary controls, and a prediction about what results will support or refute your hypothesis. The originality and feasibility of your experiment will be also evaluated.
Your experimental design should contain the following elements:

A. State one specific question your experiment aims to answer or a hypothesis you want to test. Briefly (in 1-2 sentences) explain why is it an important question to answer.
B. Describe the experimental design, including:
   - The system, (in vitro, in vivo, cells, animals) and the components critical for the experiment (genetic or molecular modifications).
   -
   - What is the assay (e.g. Western blot, immunofluorescence) and what is being measured or observed (e.g., protein levels, protein localization)?

   - Identify the experiment and the controls, explain the purpose of each of the controls.
   - Expected outcomes. Refer to your original question in (A). If you are testing a hypothesis, address what outcomes will support your hypothesis and which outcomes will refute it.
   - Address the feasibility of your experiment. Are you using a well-established method or will your method need to be developed? In 1-2 sentences, briefly describe and cite at least one study in which this method was used to investigate a similar problem. If a new method needs to be developed, explain why do you think it could be developed within a reasonable period of time and without a big budget.

Finally, draw a cartoon or a schematic of your experiment, including all major steps of the experiment, as well as the controls. Your cartoon should be clear enough for somebody who have not read your proposal to understand the experiment. You can also draw it on a sheet of paper, take a picture of it and send it to me.

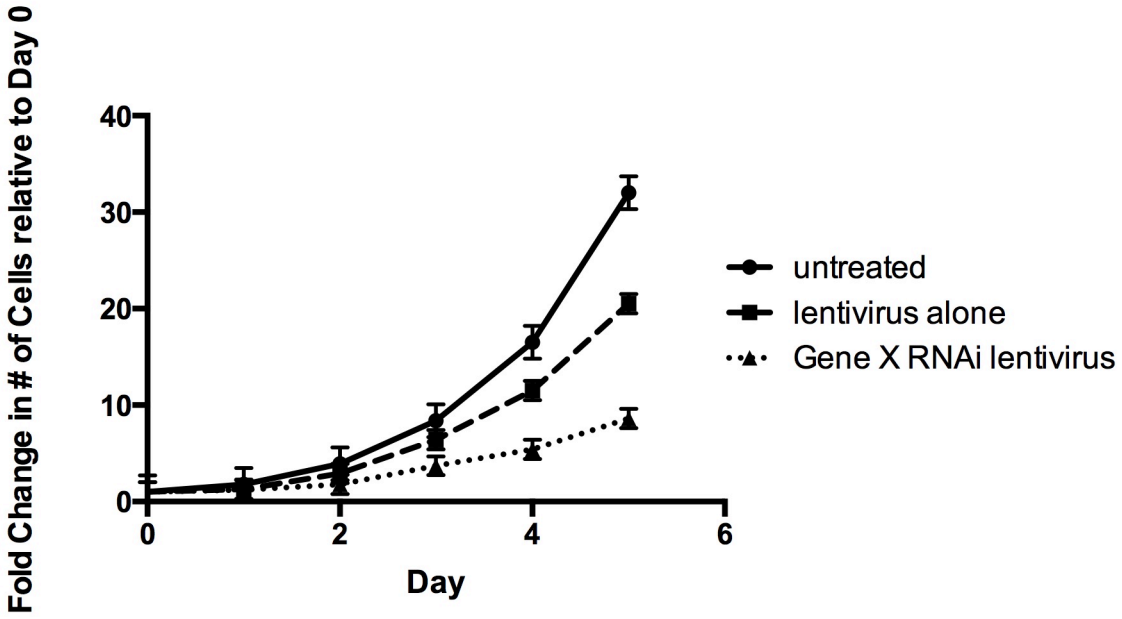
Appendix C

Your name:_____

Version A

An experiment was performed to test the effects of decreased expression of Gene X. To decrease the expression of gene X, a cell line was infected with a lentivirus that expresses short hairpin RNA (shRNA) targeting the mRNA of gene X to degradation via the process of RNA interference, or RNAi. This leads to decreased expression of Gene X in these cells. Additional conditions included untreated cells and cells infected with the lentivirus alone (lentivirus not carrying shRNA). The lentivirus alone does not affect levels of Gene X. Cells in each condition were counted daily. The results are shown in Graph 1.

Graph 1:



Q1-1. Analyze the data shown in Graph 1 by addressing the following questions.

Q1-1A – List any and all controls and why were they included.

Q1-1B - Describe the effects seen in each condition.

Q1-1C – What conclusions can you draw from these data?

Q1-2. Based on these data, evaluate the following hypothesis: “The product of gene X promotes cell proliferation”

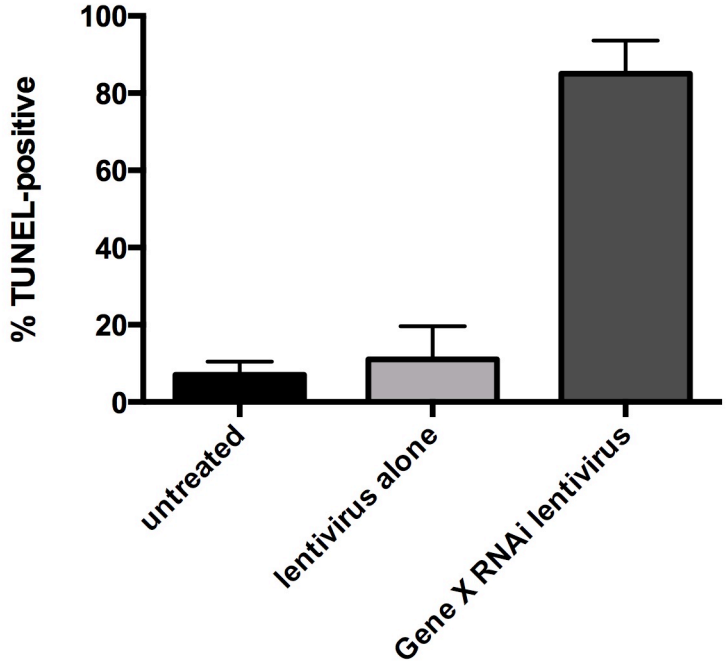
A) consistent with the above data
B) inconsistent with the above data
C) I don’t understand the data

Briefly explain your reasoning behind your choice:

Q1-3 Are there any alternative hypotheses you can propose?

A second experiment was done in the same cell line using the same conditions as the first assay. This time, on Day 5 of the experiment, the researchers looked at the number of cells that were undergoing programmed cell death (apoptosis), using TUNEL assay. The TUNEL assay labels fragmented DNA resulting from apoptosis. In Graph 2, the data from this assay are presented as the percentage of total cells being counted which are positive for TUNEL staining.

Graph 2:



Q2-1. Analyze the data shown in Graph 2 by addressing the following questions.

Q2-1A - List any and all controls and why were they included.

Q2-1B - Describe the effects seen in each condition.

Q2-1C – What conclusions can you draw from these data?

Q2-2. Based on **both** pieces of data (Graph 1 and Graph 2), evaluate the following hypothesis: “The product of gene X promotes cell proliferation”

A) consistent with the above data
B) inconsistent with the above data
C) I don’t understand the data

Briefly explain your reasoning behind your choice, addressing the results of both experiments (Graph 1 and Graph 2):

Q3-1 Propose a new hypothesis for the effects of the **lentivirus alone**, using the two data sets above, including what data support this hypothesis.

Q4: Experimental proposal:

A large family was found that has high incidence of brain cancer that develops in young adults (ages 20-30). In an attempt to find out what mutations predisposed members of this family to cancer, scientists sequenced the genomes of the sick and the healthy adults from this family. One particular mutation in gene X was found in the genomes of the family members who had cancer, but not in the genomes of the healthy family members.

Q4-1. Based on the results from the previous experiments, do you expect this mutation to be inactivating (causing loss of function of the product of gene X) or over-activating (causing increased function of the product of gene X)? Circle one:

A. Inactivating mutation

B. Over-activating mutation

Q4-2. The sequencing data described above point to a correlation between having the mutation in gene X and developing brain cancer. Propose **one experiment** that would test whether the mutation in gene X indeed contributes to brain cancer development. Your experiment can be conducted in any type of experimental system (cultured cells, in animals, human populations, test tube, etc.). The description of your experiment should include the **experimental system**, the **assay** you will be using**, what** will you be measuring or observing, and what **controls** will you use. State the **expected outcomes** of your proposed experiment.

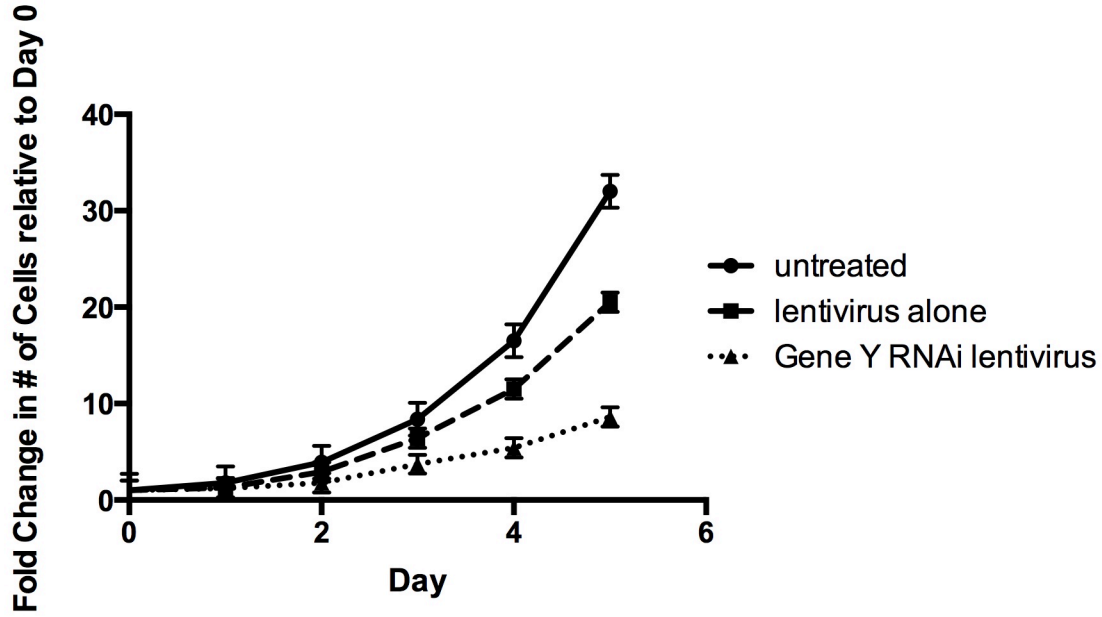
Appendix D

Your name:_____

Version B

An experiment was performed to test the effects of decreased expression of Gene Y. To decrease the expression of gene Y, a cell line was infected with a lentivirus that expresses short hairpin RNA (shRNA) targeting the mRNA of gene Y to degradation via the process of RNA interference, or RNAi. This leads to decreased expression of Gene Y in these cells. Additional conditions included untreated cells and cells infected with the lentivirus alone (lentivirus not carrying shRNA). The lentivirus alone does not affect levels of Gene Y. Cells in each condition were counted daily. The results are shown in Graph 1.

Graph 1:



Q1-1. Analyze the data shown in Graph 1 by addressing the following questions.

Q1-1A – List any and all controls and why were they included.

Q1-1B - Describe the effects seen in each condition.

Q1-1C – What conclusions can you draw from these data?

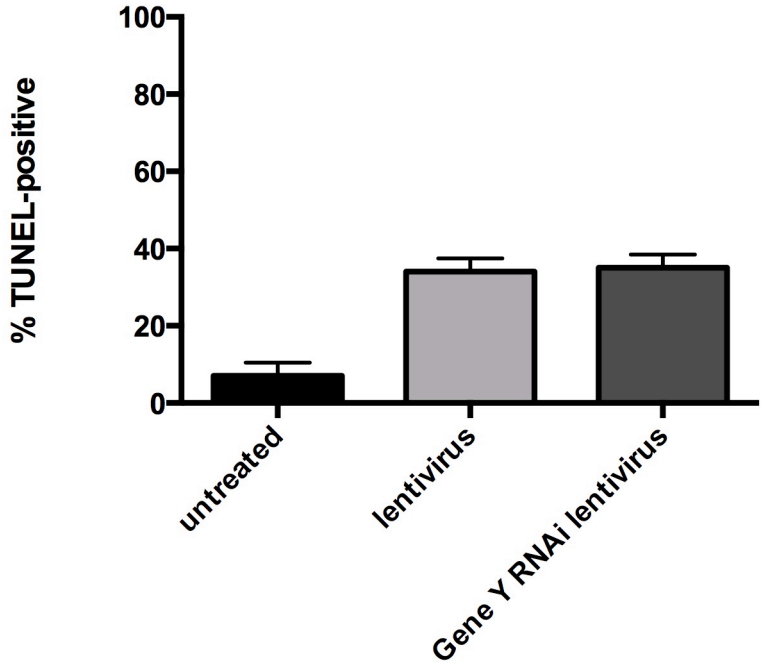
Q1-2. Based on these data, evaluate the following hypothesis: “The product of Gene Y promotes cell proliferation”

A) consistent with the above data
B) inconsistent with the above data
C) I don’t understand the data

Briefly explain your reasoning behind your choice:

Q1-3 Are there any alternative hypotheses you can propose?

A second experiment was done in the same cell line using the same conditions as the first assay. This time, on Day 5 of the experiment, the researchers looked at the number of cells that were undergoing programmed cell death (apoptosis), using TUNEL assay. The TUNEL assay labels fragmented DNA resulting from apoptosis. In Graph 2, the data from this assay are presented as the percentage of total cells being counted which are positive for TUNEL staining.



Graph 2

Q2-1. Analyze the data shown in Graph 2 by addressing the following questions.

Q2-1A - List any and all controls and why were they included.

Q2-1B - Describe the effects seen in each condition.

Q2-1C – What conclusions can you draw from these data?

Q2-3. Based on both pieces of data, evaluate the following hypothesis: “The product of Gene Y promotes cell proliferation”

A) consistent with the above data
B) inconsistent with the above data
C) I don’t understand the data

Briefly explain your reasoning behind your choice, addressing the results of both experiments (Graph 1 and Graph 2):

Q3-1 Propose a new hypothesis for the effects of the **lentivirus alone**, using the two data sets above, including what data support this hypothesis.

Q4: Experimental proposal:

A large family was found that has high incidence of colon cancer in middle-age individual (40-50 year olds). In an attempt to find out what mutations predisposed members of this family to cancer, scientists sequenced the genomes of the sick and the healthy 55-65 year old individuals from this family. One particular mutation in gene Y was found in the genomes of the family members who had cancer, but not in the genomes of the healthy family members.

Q4-1. Based on the results from the previous experiments, do you expect this mutation to be inactivating (causing loss of function of the product of gene Y) or over-activating (causing increased function of the product of gene Y)? Circle one:

A. Inactivating mutation

B. Over-activating mutation

Q4-2. The sequencing data described above point to a correlation between having the mutation in gene Y and developing colon cancer. Propose **one experiment** that would test whether the mutation in gene Y indeed contributes to colon cancer development. Your experiment can be conducted in any type of experimental system (cultured cells, in animals, human populations, test tube, etc.). The description of your experiment should include the **experimental system**, the **assay** you will be using**, what** will you be measuring or observing, and what **controls** will you use. State the **expected outcomes** of your proposed experiment.

Appendix E

Question	Max. Points	Scoring Rubric
Q1-1A-Identify Controls (Interpretation/Explanation and Understanding Controls)	4	0 = no/incorrect response; 1 = one control with no reasoning; 2 = one control with correct reasoning OR two controls with no/incorrect reasoning; 3 = two controls with one correct reasoning; 4 = two controls with correct reasoning for both
Q1-1B-Describe the Effects in Each Condition (Interpretation)	3	0 = no/incorrect response; 1 = comparison of experimental condition to either control; 2 = comparison of experimental condition to each control without comparison of controls to each other; 3 = comparison of experimental condition to both controls and comparison of controls.
Quantitative comparison Q1	2	0 = No quantitative comparison (% or fold difference) between experimental and control is present; 1 = Quantitative comparison between experimental and both control conditions is present, but incorrectly calculated OR Only quantitative comparison between the experimental and one control condition is present and correctly calculated; 2 = Quantitative comparison between experimental and both control conditions is present. The quantitative difference is correctly calculated.
Q1-1C-Draw Conclusions (Inference-Drawing conclusions)	2	0 = No response or the effect of the treatment is incorrectly interpreted; 1 = Correct interpretation of the effect of the treatment: decrease in cell proliferation; 2 = Statement that connects the effect of the treatment on the expression of gene X/Y AND the decrease in cell proliferation.
Q1-2 - Evaluate hypothesis (Evaluation)	3	Multiple Choice: 0 = incorrect choice; 1 = correct choice; Reasoning: 0 = no/incorrect or irrelevant response; 1 = partially correct explanation; 2 = correct explanation
Q1-3 - Alternative hypothesis (Inference-Propose a hypothesis)	1	0 = no/infeasible hypothesis; 1 = feasible hypothesis based on the data
Q2-1A-Identify Controls (Interpretation/Explanation and Understanding Controls)	4	0 = no/incorrect response; 1 = one control with no reasoning; 2 = one control with correct reasoning OR two controls with no/incorrect reasoning; 3 = 2 controls with one correct reasoning; 4 = 2 controls with correct reasoning for both

Appendix E

Q2-1B-Describe the Effects in Each Condition (Interpretation/Explanation/Analysis)	2	0 = no/incorrect response; 1 = comparison of experimental condition to either control; 2 = comparison of experimental condition to each control with or without comparison of controls to each other;
Quantitative comparison Q2	1	0 = No quantitative comparison (% or fold difference) between experimental and control is present; 1 = Quantitative comparison between experimental and both control conditions is present. The quantitative difference is correctly calculated.
Q2-1C-Draw Conclusions (Inference-Drawing Conclusions)	2	0 = No response or the effect of the treatment is incorrectly interpreted; 1 = Version A: Correct interpretation of the effect of the treatment on apoptosis, but no explicit connection between the treatment (decrease in gene X expression) and increased cell death. Version B: realization that there is no difference between "lentivirus alone" and experimental condition, but lack of conclusion that experimental condition had no effect (e.g. "I cannot make a conclusion…Lentivirus alone (negative control) did not function as expected"). 2 = Version A: Statement that connects the effect of the treatment on the expression of gene X/Y AND the increase in apoptosis. Version B: Statement that lack of difference between "lentivirus alone" and experimental condition, means that experimental condition had no effect
Q2-2 - Evaluate hypothesis (Evaluation)	4	Multiple Choice: 0 = incorrect answer; 1 = correct answer; Reasoning: 0 = no/incorrect response; 1 = Correct conclusion from Exp 1 OR Correct conclusion from Exp 2 OR Correct integrated conclusion without integration of data; 2 = Correct conclusion of Exp 1 and Exp 2 without integrated conclusion OR Correct integrated conclusion with either Exp 1 or Exp2 explanation; 3 = Correct Exp 1 and Exp 2 conclusions plus an integration of the two data sets.
Q3-1-Propose New Hypothesis (Inference-Propose a hypothesis)	2	0 = no/incorrect interpretation of the effects of the lentivirus alone control (e.g., "The lentivirus alone was successfully used to perform RNAi of gene X…"; 1 = Correct analysis of the effect of the lentivirus on cell proliferation and death, but no hypothesis of how the lentivirus might cause this effect is proposed. 2 = correct integrated analysis of the effects of lentivirus in

Appendix E

		Experiments 1 and 2 and a reasonable hypothesis of how the lentivirus might cause this effect is proposed.
Q4-1-Function of Gene (Inference/Analysis)	1	Multiple Choice: 0 = incorrect answer; 1 = correct answer
Q4-2 Experimental Design		
Appropriateness	2	0 = completely irrelevant experiment to hypothesis; 0.5= experiment is based on similar concepts, but lacks any connection to hypothesis/data provided; 1 = experiment has some basis in provided hypothesis, but question is already answered or incorrect experiment is being developed; 1.5 = Experiment is mostly relevant to previous data but details are lacking; 2 = Experiment tests the hypothesis based on the data provided
Experimental System	1	0 = no experimental system described; 0.25 = vague description of system OR indirect discussion of system; 0.5 = experimental system described, but inappropriate for the question or assay OR incorrect description of system required; 0.75 = System is described but lacks a necessary component or description required OR well explained but only somewhat appropriate to test the hypothesis; 1 = experimental system clearly described and is appropriate to test the hypothesis.
Independent Variable/Treatment	1	0 = lacks critical components required for hypothesis/experimental system; 0.25 = vague, mostly incomplete mention of components required for system/assay; 0.5 = components well described, but incorrect for system OR correct components described but explained incorrectly or poorly OR only some components are described adequately; 0.75 =Most components are described, but lacking at least 1 components required for experiment; 1 = complete description of components required for experiment/assay to test hypothesis.
Assay/How the dependent variable will be measured	1	0 = no mention of the assay needed to test hypothesis; 0.25 = merely mentions name without reasoning as to what it tests OR name of assay without any description of how it works; 0.5 = describes the assay incompletely, but generally explains what is being tested or why it is being used; 0.75 = describes assay including why it is used but lacks a connection

Appendix E

		to the question or why it needs to be utilized; 1 = complete description of assay and what is required for it as well as how it tests the hypothesis.
Quantity measured/Dependent variable to be measured	1	0 = no description of what is being measured with the assay or why it is needed to test the hypothesis; 0.25 = incompletely or incorrectly describes some aspect of what is being measured but doesn't provide explanation of what it tests or how it tests the question; 0.5 = describes what is being measured mostly, but does not explain how it tests the hypothesis OR describes what is being measured but is inappropriate for the question/system/assay; 0.75 = mostly describes the measurement lacking small details or minor incorrect aspects OR describes fully but lacks connection to assay or hypothesis; 1 = complete description of measurement, relation to hypothesis, and how they relate.
Identify Controls	1	0 = no controls described; 0.25 = inadequate control OR inappropriate control; 0.5 = mention of control but no description of what it controls for OR multiple controls without descriptions; 0.75 = control somewhat described and appropriate but missing another control OR multiple controls described but missing minor descriptions; 1 = all controls described as well as what they control for.
Anticipated Outcomes	1	0 = no description of expected outcomes; 0.25 = discussion of general trends of outcomes without specific details; 0.5 = prediction of either control or experiment without discussion of the other; 0.75 = Adequate description lacking specific `1details or full expectations; 1 = all controls and experimental outcomes are described and compared.
Feasibility	1	0 = infeasible experiment; 0.25 = some aspects are feasible, but majorly flawed; 0.5 = Aspects are somewhat feasible but description does not fully ascertain feasiblity; 0.75 = Feasible experiment, but aspects are missing to fully describe experiment; 1 = fully described and feasible experiment.