Title: The Impact of Understanding of Current Biotechnology Research and Protocols on Student Biotechnology Attitudes, Knowledge, and Skills

Deborah Vasconi

vasconid@pcsb.org

Teacher, Department Chair

Tarpon Springs High School

Bench to Bedside Action Research 2011-2012

SC.912.L.16.10
Evaluate the impact of biotechnology on the individual, society and the environment, including medical and ethical issues.

SC.912.L.14.52
Explain the basic functions of the human immune system, including specific and nonspecific immune response, vaccines, and antibiotics. Also assesses SC.912.L.14.6, HE.912.C.1.4, and HE.912.C.1.8.

Abstract:

The purpose of this research process was to assess and study student's preconceived understanding and attitudes about biotechnology without the benefit of the most current knowledge sources and laboratory applications then measure if their attitude, knowledge and skills changed after participating in a rigorous course of study including case studies, lab activities with biotechnological protocols, interactive PowerPoint presentations, videos and lab simulations. After measuring knowledge by pre and post tests and student attitude change by repeat of a modified Likert scale forms, students did demonstrate an increase in knowledge, skills, and a positive attitude shift.

Rationale:

Biotechnology, as a topic in preAICE Biology IH and AP Biology has never been thoroughly investigated in our science curriculum at Tarpon Springs High School. The resources and equipment have been limited for many public high schools and our science teachers have not had the most modern science pedagogy and skills to maintain a rigorous biotechnology study. Many students have preformed opinions and attitudes which have come from their cultural and family influences. In addition to supporting the highest levels of scientific literacy for our students', it is also important to prepare our students for their role as citizens, to make well informed, ethical decisions.

To successfully introduce biotechnology to students, it is important to uncover their pre-conceived or inherent understanding and attitudes. Globally studied in Australia, Turkey, UK and Taiwan, these studies acknowledged that students don't come to a course in genetics and biotechnology as a blank slate. Evidence of students' misinformation has been documented in Australia where " students also appeared to be confused about the difference between cloning and genetic engineering. Almost half (48.4%) of the students gave Dolly (the sheep) as an example of cloning. However, more than a quarter (28.7%) of students also gave cloning/ Dolly the sheep as an example of genetic engineering." (Dawson & Schibeci,2003, p 7). A form of a Likert scale can be used to anonymously measure a student's attitude with other forms of assessment measuring the student's level of scientific literacy. In addition the CRISS strategy, KWL, were used in a more open ended assessment of attitude to determine what the student knows and what do they want to learn about biotechnology.

The research in other countries indicates attitudes depended upon the type of organism involved and the end product. For example, most pupils in both countries (83 per cent in Taiwan, 80 per cent in UK) agree or strongly agree with genetic engineering of plants (Chen & Raffan, 1999). However, when animals and humans are indicated as research organisms approval drops. This acceptance is helpful the development of future crops and improvement

of current food sources; it is in the area of biomedical technology that attitudes need to be more positive. When students were asked whether inserting genes from animal cells into yeast used to make bread is acceptable or not, only 35 per cent (Taiwan) and 23 per cent (UK) found this acceptable. About half the students in both countries (55 per cent in Taiwan, and 50 per cent in the UK) thought it acceptable to change yeast genetically to enhance growth. Acceptance of transgenic biotechnology was later investigated by asking whether the transfer of animal genes was acceptable or not. Students' attitudes towards this were even less positive, with 33 per cent in Taiwan and 29 per cent in the UK rating it as acceptable. The acceptance level was least in both countries when considering the transfer of virus genes, at 26 percent in Taiwan, and 23 percent in the UK. (Chen & Raffan, 1999). With an extensive exposure to the correct and modern research, case studies and applications of current biotechnology, students included animal and human study protocols.

With the improved knowledge level and attitude found after the biotechnology course of study, students will make better informed decision in the future. The best guarantee of responsible and ethical use of biotechnology or any use of science and technology is a well-educated public. (Armstrong & Weber, 1991)

The purpose of this study was to monitor and measure student's prior and acquired knowledge, skills and attitudes as they enter and leave a rigorous biotechnology unit.

Action Research Intervention:

The primary objective of this Action Research Intervention is assess student's attitude and preconceived knowledge towards biotechnology and then measure attitude and knowledge change after a comprehensive biotechnology unit of study and numerous hands on activities. The preAICE Biology students were the main study group, primarily because their course must document those standards tested on the Florida Biology EOC Exam and which will be 30 percent of their final grade. The standards supported by the action research process are: **SC.912.L.16.10** Evaluate the impact of biotechnology on the individual, society and the environment, including medical and ethical issues. **SC.912.L.14.52** Explain the basic functions of the human immune system, including specific and nonspecific immune response, vaccines, and antibiotics. Also assesses SC.912.L.14.6, HE.912.C.1.4, and HE.912.C.1.8.

The AP Biology class was also included in the action research to document the twenty five percent portion of the Advanced Placement Biology Course outline Heredity and Evolution: Molecular Genetics and recommended Lab 6 Molecular Biology.

- Become knowledgeable with terms and common biotechnological procedures, i.e. PCR , gel electrophoresis, and micro-pipetting techniques using the Designer plates in the equipment locker.

- Discuss various case studies of current research- Golden Rice, An Intimate Debate Case (done as a team activity, see Appendix C) , Power Point developed with information/slides from the lecture series Biochemical Explorations: Bench to Bedside Course
- Investigation of current biotechnological procedures by laboratory activities and simulations.
    - Pre-AICE Biology : Science Take-Out Diagnosing Diabetes& Stem Cells/ Creating a Stem Cell Line, Ed heads/ Create a Stem Cell line, Click and Clone, Designer Plates, Modes of Inheritance, Strawberry DNA Extraction
    - AP Biology: Dr. Lawrence's Citrus Microarray Simulation/ AP Biology Lab #6 pGLO Bacterial Transformation by Bio-Rad/ Designer Plates/ Modes of Inheritance/ Science Take-Out Diagnosing Diabetes, Stem Cells, Flower Forensics & Genetic Testing for Huntington's Disease

Connection to Bench to Bedside summer institute:

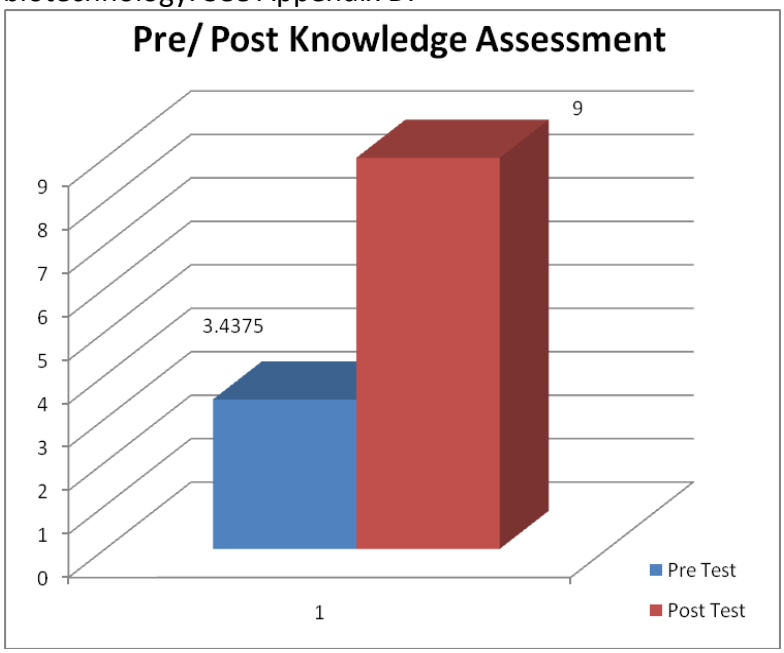
The equipment loaned by UF CPET Equipment Locker program with the $200 grant for consumable materials made this biotechnological investigation accessible to our students and this would not have been possible without the Bench to Bedside summer institute. The lecture series Biochemical Explorations: Bench to Bedside Course provided the context in which to motivate and engage students. Students were fascinated by the Glycogen Storage Disease of Dr. Weinstein, which was the first discussion to engage them, later they investigated the Stem Cells Showing Their Potential for Correction of Type I Diabetes of Dr Petersen after the Science Take-out activity. Dr. Darwiche's presentation, Current Topics in Stem Cell Research, was essential in our discussion of stem cells because there is not a lot of material that is so well organized and understandable for students

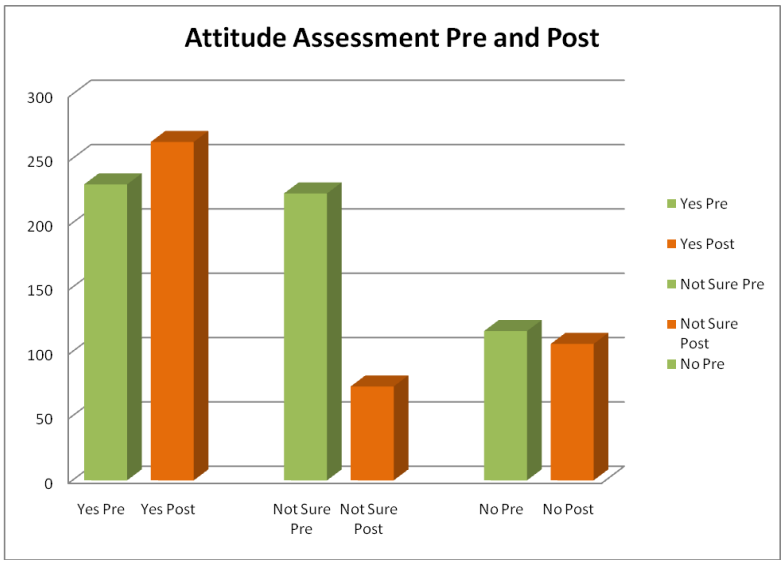
Data Collection and Analysis:

Data, both quantitative and qualitative will be collected in many forms: We began with a Likert scale to assess student's attitude at the beginning as well as the end of the biotechnology unit. A form of a Likert scale can be used to anonymously measure a student's attitude with other forms of assessment measuring the student's level of scientific literacy. This collected data will be visually represented by a graph. Next, the students took a Pre-Test to assess their prior knowledge along with the same test at the end of the unit as the Post-test to assess and visually represent learning gains. Student artifacts representing their efforts in the classroom to synthesize all knowledge they receive from the Golden Rice debate can be seen in Appendix C. Another type could be an interactive notebook/journal, or a brochure/ portfolio that may contain open- ended questions. Measure of central tendency, such as mean, was applied to this collected pre and post test data and visually represented by graph, however, attitude pre

and post Lickert scale assessment did not make sense to use mean and the difference, positive and negative was more applicable.

Our data demonstrated a definite increase in knowledge about biotechnology from the twenty multiple choice Pre/Post Assessment. Attitude change is more difficult to measure. Informal observations told me the students were engaged, interested and stimulated by the lab activities. The data from the Likert scale shows a positive trend, more answered yes, fewer were not sure and much fewer were negative in their attitude towards stem cells, and biotechnology. See Appendix D.





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NSF, National Center for Case Study Teaching in Science, University of Buffalo, State University of New York

Permissions:

Tarpon Springs High School administrator for curriculum provided permission for the knowledge and attitude assessment of students with the understanding the data would be unpublished, anonymous, and only used for personal analysis to determine the level of success of this action research project.

Budget and budget justification:

$200 Mini Grant from UF/ consumable materials for labs was used to purchase Science Take-Out products for the involved biology classes. "Diagnosing Diabetes and Stem Cell" Kits were purchased in bulk and assembled by the teacher to cost cut and allow the purchase of additional kits "Flower Forensics and Genetic Testing for Huntington's Disease" . These kits are self contained and easy to use in freshman and sophomore level biology classes however, AP Biology level indicated there just wasn't enough information or investigation for them to be highly interested. Bio-Rad pGLO Transformation was purchased with Advance Placement funds from Pinellas County and was only used in AP Biology class. UF CPET equipment locker loan of

invaluable equipment, priceless and this unit of study would not have been as successful without it.

Modifications from original proposal:
Due to the timing of this biotechnology unit, the proposed intervention did change from that listed below to the action research intervention completed in January 2012, before the end of Term 1, as described in the action research intervention.
Original intervention:

- Become knowledgeable with terms and common biotechnological procedures, i.e. PCR and microarray using vocabulary building activities
- Discuss various case studies of current research- PowerPoint
- Investigation of current biotechnological procedures by laboratory activities and simulations.
    - Pre-AICE Biology : Diagnosing Diabetes/ Creating a Stem Cell Line, Ed heads/ Create a Stem Cell line, Click and Clone, Virtual PCR, Virtual Microarray, The Stem Cell Controversy, Elisa Allergy Array, etc.
    - AP Biology: Southern Blot- The High Rise Killer, Microarray simulation AP Biology Lab #6, Elisa Allergy Array, Pipetting by Design, PCR/ Transformation Lab Sequence, GMO / Thermal Cycler, etc.
- AP Biology will study case study "The Case of Eric, Lou Gehrig's Disease, and Stem Cell Research" (McCain, 2005)
- Students will discuss ethics of biotechnology (the Stem Cell Controversy) by role play/debate of an assigned opinion after opportunity to discuss/research/ within their role group.

The modifications to the action research intervention were done to accomplish the student's hand on lab activities in the time allotted because this was the most important component of the intervention- students learn best and remember what they are actively engaged in. After exams and the end of Term 1 and start of Term 2 at Tarpon Springs High School in Pinellas County, the Post Test and Attitude Likert scales were administered.

Learnings from your Action Research:

All lab activities went smoothly with very good results, but perhaps the extraction of DNA from strawberries was the most dramatic for students. The missing step was to then manipulate the extracted DNA in some way because students did indicate an interest. Also the pGLO Bacterial Transformation was very successful.

Trying to find enough time, at the end of Term 1 and beginning of Term 2 , scheduling around exams, was the largest challenge and I would try to schedule this unit at a different time when

it is repeated. The timing problems was made worse by a very limiting pacing guide we are asked to follow in biology classes.

The action research process was flexible to allow changes as we progressed. Knowledge assessment is easier than attitude assessment and I would not attempt this type of assessment without more training on Likert scales and the design to collect data that will not be unclear.

Dissemination:

As Science Department chair and Biology Team leader, I had several opportunities to share with our staff what we were doing in class and the results of the action research. I spoke with the county wide Biology Team, a group of biology teachers disseminating information and motivating the biology teachers, about UF/CPET Summer Institute, along our District High School Science Staff Developer. Once students took the post test, the data in the form of two charts was discussed with students, and staff.

Appendix A

Biotechnology Questionnaire

This questionnaire consists of 2 open ended questions and 12 statements about implications of genetic engineering and biotechnology. Please give a brief description for open-ended questions.

1. What is genetic engineering?

2. Can you give an example about the studies of biotechnology?

Please circle a response for each of the following statements.

1. Biotechnology makes human life easier.

YES	NOT SURE	NO

2. Biotechnology can provide opportunities for new discoveries.

YES	NOT SURE	NO

3. The biotechnology/genetic engineering studies with animals are beneficial to people.

YES	NOT SURE	NO

4. Gene transfers from animals to plants can cause plants to improve features like animals.

YES	NOT SURE	NO

5. Stem cells can only be extracted from embryos.

YES	NOT SURE	NO

6. Cloning is an acceptable therapy.

YES	NOT SURE	NO

7. The animal meats obtained with genetic manipulations can be sold without giving any information to the consumer.

YES	NOT SURE	NO

8. It is acceptable to produce plants with enrichment proteins.

YES	NOT SURE	NO

9.It is acceptable to produce plants that synthesize substances with effective medical importance.

YES	NOT SURE	NO

10.Transgenic organisms contain risks for nature.

YES	NOT SURE	NO

11. It is acceptable to provide lipase from bacteria to use in detergent.

YES	NOT SURE	NO

12.Releasing GM (genetically modified) organisms to nature without control contains risks.

YES	NOT SURE	NO

Appendix B

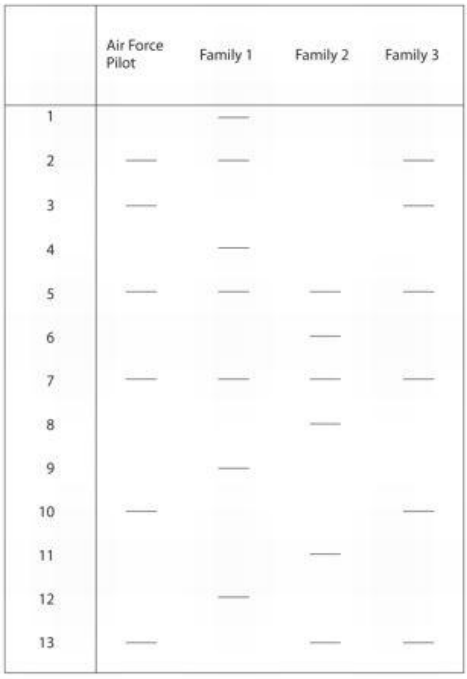
Pre/Post Assessment Biotechnology

Name_____

MULTIPLE CHOICE. Choose the one alternative that best completes the statement or answers the question.

After reading the following paragraph, answer the question(s) below.

Four decades after the end of the Vietnam War, the remains of an Air Force pilot were discovered and returned to the United States. A search of Air Force records identified three families to which the remains might possibly belong. Each family had a surviving twin of a missing service member. The following STR profiles were obtained from the remains of the pilot and the surviving twins from the three families.



	Air Force Pilot	Family 1	Family 2	Family 3
1		—		
2	—	—		—
3	—			—
4		—		
5	—	—	—	—
6			—	
7	—	—	—	—
8			—	
9		—		
10	—			—
11			—	
12		—		
13	—		—	—

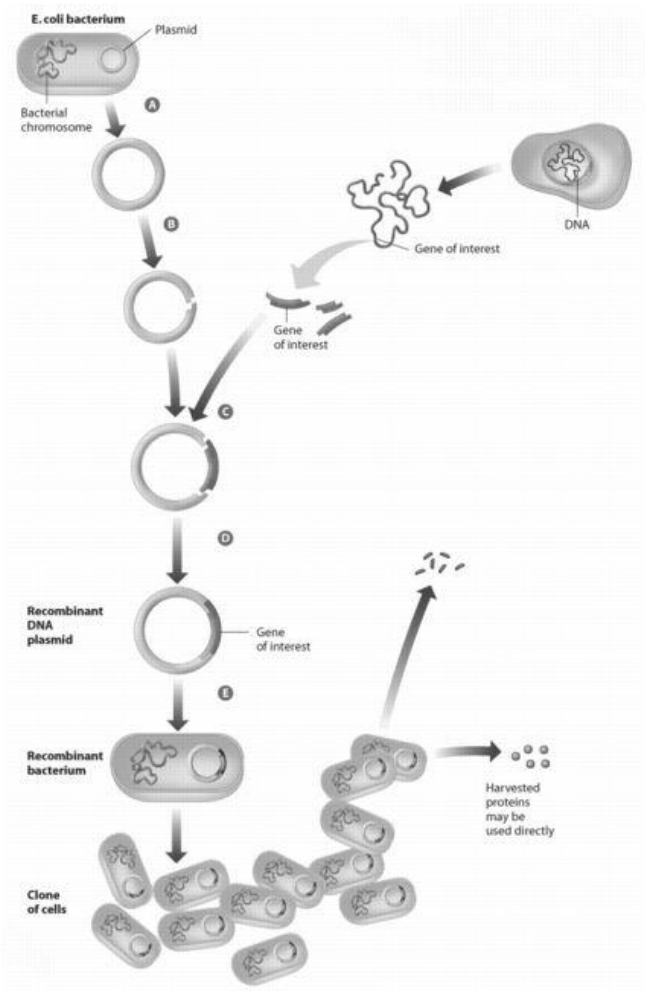
1) Based on analysis of the STR sites shown, does the missing pilot belong to any of these three families? 1) _____

A) No, none of the families match.
B) Yes, family 3 matches.
C) Yes, family 2 matches.
D) Yes, family 1 matches.
E) The information provided is not sufficient to determine whether or not there is a match.

2) In order to match the pilot's remains to the correct family using DNA profiling, 2) _____

A) 50% of the STR bands must match.
B) bands 5 and 7 must match.
C) each of the 13 STR bands must match.
D) the bands for site 13 must match.
E) the majority of the STR bands must match.

3) 3) _____



Which step in this process requires use of restriction enzymes?

A) step A B) step B C) step C D) step D E) step E

4) The production of multiple identical copies of gene-sized pieces of DNA defines 4) _____
A) plasmid transformation.
B) clonal selection.
C) plasmolysis.
D) tissue culturing.
E) gene cloning.

5) Which of the following statements about the problems created by cloning is false? 5) _____
A) Cloning leads to malfunctions in gene regulation.
B) Cloned animals are less healthy than animals created by natural methods.
C) Cloning endangered species may de-emphasize the need to preserve critical natural habitats.
D) Cloning does not increase genetic diversity in the cloned species.
E) Cloned animals live longer compared to naturally bred animals.

6) The type of recombinant bacteria most often used to mass-produce genes is 6) _____
A) Agrobacterium tumefaciens.
B) Escherichia coli.
C) Pseudomonas aeruginosa.
D) Saccharomyces cerevisiae.
E) Rhizobium.

7) The advantage of being able to clone the gene for human insulin is that 7) _____
A) human insulin is more variable than other sources of insulin, so cloning provides a greater chance of obtaining a form that can be used by the diabetic's muscles.
B) using human insulin increases the probability that, in the future, the person suffering from diabetes can be weaned from a dependence on insulin.
C) human insulin is less likely to provoke an allergic reaction than cow, pig, or horse insulin.
D) there are too few cows, pigs, and horses to provide an adequate supply of their insulin.
E) cow, pig, or horse insulin cannot keep a diabetic alive for more than three months.

8) The term "gene expression" refers to the 8) _____
A) fact that each individual of a species has a unique set of genes.
B) flow of information from parent to offspring.
C) fact that individuals of the same species have different phenotypes.
D) fact that certain genes are visible as dark stripes on a chromosome.
E) process by which genetic information flows from genes to proteins.

9) Golden rice is golden in color because it is rich in 9) _____
A) beta-carotene.
B) vitamin A.
C) protein.
D) vitamin C.
E) chromium picolinate.

10) In order for gene therapy to be permanent, 10) _____
A) the defective gene must undergo restriction enzyme analysis first.
B) the normal gene must be transferred to somatic cells that can continuously multiply.
C) the defective gene must first be removed from all somatic cells.
D) the normal gene must first be treated with UV radiation to ensure noninfectivity.
E) the normal gene must be added to the germ line cells.

11) Adult stem cells have limited therapeutic potential 11) _____

A) because they lack a complete set of genes.
B) because scientists have no reliable method of identification.
C) because they are fully differentiated.
D) because their developmental potential is limited to certain tissues.
E) due to their excessive numbers in tissues.

After reading the following paragraph, answer the question(s) below.

All apples in the United States, regardless of variety or where they're purchased, are produced by cloning. For more than 2,000 years, apple growers around the world have used a type of cloning called grafting to produce larger, better-tasting apples. Why has cloning become the primary method of apple growing? Apples grown from seeds usually don't produce apples with the same taste and appearance as that of the parent tree because there's a high degree of genetic variability among the seeds. Making identical genetic copies of the preferred fruit is the only way to get reliable apple quality.

In grafting, the shoots and branches of the desired fruit, called a scion, are attached onto the trunk and root system of a previously existing tree, called the rootstock. Both components of the graft are needed. The rootstock controls gene expression in scion, triggering production of apples that match the cloned scion.

Grafting research can be used to produce some interesting tree combinations that are beneficial for intensive agriculture. For example, if you graft the root of a small tree variety, such as the crab apple, onto the shoot from a larger apple tree, such as the Gala, you can produce Gala apples on a much smaller tree. Other scientists are trying to create disease-resistant varieties that would need less pesticides. This is beneficial for the environment and also lowers the price of apples in the grocery store.

12) Why don't the grafted hybrids produce apples with a blend of traits from the scion and the rootstock? 12) _____

A) Transplanted nuclei from scion cells regulate gene expression in the rootstock.
B) The rootstock regulates gene expression in the scion, but contributes no genetic information for fruit production.
C) The rootstock is unable to perform photosynthesis and so can't produce fruit.
D) The rootstock suppresses activation of the scion genes, which alters fruit production.
E) The lac operon in the scion is the only regulator of gene expression in the hybrid.

13) Half the trees in an orchard were derived from rootstock "A" and half from rootstock "B," but all the trees had the same scion. If the trees grafted onto rootstock "A" were infected by a parasite that causes blossom rot, the trees grafted onto rootstock B 13) _____

A) would be more likely to become infected, since the pathogen would spread through the soil to the roots of other trees.
B) would be very likely to become infected, because the remaining scions are genetically identical to those that are already infected.
C) would be less likely to become infected because they're grafted onto different rootstocks.
D) Only half of the remaining trees are likely to become infected because they're in a different location in the orchard.
E) There's no way to determine the likelihood of infection, since genetic variability gives all the trees different characteristics.

14) Which of the following statements regarding stem cells is false? 14) _____

A) Embryonic stem cells can give rise to all the different specialized cells in the body.
B) Embryonic stem cells can be induced to differentiate.
C) Adult stem cells are present in adult tissues.
D) Adult, but not embryonic, stem cells can be grown in laboratory culture.
E) Adult stem cells are partway along the road to differentiation.

15) The cloning of Dolly the sheep 15) _____
A) demonstrated that differentiated cells contain only a fraction of their full genetic potential.
B) revealed that cloned mammals most resemble the sperm donor.
C) revealed that cloned mammals most resemble the egg donor.
D) demonstrated that the nuclei from differentiated mammalian cells can retain their full genetic potential.
E) demonstrated, for the first time, that eggs are haploid and body cells are diploid.

16) Which of the following possible uses of reproductive cloning is still considered by most to be an unresolved ethical issue? 16) _____
A) the production of organs in pigs for transplant into humans
B) the production of genetically identical animals for experimentation
C) the production of potentially valuable drugs
D) the production of genetically identical humans for therapeutic purposes.
E) the improvement of the quality of farm animals

17) Cloning to produce embryonic stem cells is called 17) _____
A) therapeutic cloning.
B) reproductive cloning.
C) transplantational cloning.
D) regenerative cloning.
E) dedifferentiation.

18) Which of the following statements about microarrays is false? 18) _____
A) Microarrays are used to determine which genes are active in different tissues or in tissues of different states of health.
B) Microarrays use fluorescently labeled cDNA molecules to identify particular genes expressed at a particular time.
C) Microarrays help scientists understand how genes interact, particularly during embryonic development.
D) Microarrays use tiny portions of double-stranded RNA fragments from a large number of genes.
E) Microarrays enable scientists to determine the activity of thousands of genes at once.

19) The fact that the nucleus from an adult somatic cell can be used to create all of the cell types in a new organism demonstrates that development depends upon 19) _____
A) the timing of mitosis and meiosis.
B) the timing of meiosis and cell migrations.
C) the deposition of materials in the extracellular matrix.
D) the position of cells within an embryo.
E) the control of gene expression.

20) If you commit a crime, you need to make sure that you do not leave even the smallest speck of blood, hair, or other organic matter from your body. If you do, the DNA in this material can be amplified by _____, subjected to genetic analysis, and used to identify you as the perpetrator of the crime. 20) _____
A) PCR
B) reverse transcriptase
C) ATP
D) blotting
E) RFLP

21) Gel electrophoresis sorts DNA molecules on the basis of their 21) _____
A) nucleotide sequence.
B) solubility in the gel.
C) ability to bind to mRNA.
D) solubility in water.
E) size.

22) During the process of electrophoresis, the _____ functions like a thick filter, separating the samples according to their size. 22) _____
A) sample well
B) positively charged electrode
C) gel
D) sample mixture
E) negatively charged electrode

23) DNA fragments that have matching sticky ends are joined by covalent bonds formed by the action of 23) _____
A) DNA ligase.
B) DNA helicase.
C) covalentase.
D) DNA polymerase.
E) a restriction enzyme.

24) In the process of human gene cloning using recombinant plasmids, the bacterial plasmid 24) _____
A) is used to insert the human gene into the bacterial chromosome.
B) is the source of the gene to be cloned.
C) is cultured inside the human cell, which contains the gene to be cloned.
D) functions as a vector.
E) comes from the same organism as the gene of interest.

25) When DNA from two sources is combined into one single piece of DNA, it is known as 25) _____
A) a vector.
B) recombinant DNA.
C) cloned DNA.
D) a DNA library.
E) a plasmid.

Appendix C

Alexis Curd
Olivia Albertson
Santiago Benitez
Shaw Covington

Golden rice position paper

My group's position on Golden Rice is that it is beneficial. Millions of children die each year due to vitamin A deficiency, causing childhood blindness. Golden rice, genetically engineered with b-carotene and vitamin A, could helps to reduce the childhood deaths in developing countries. Non wealthier families do not always have access to vitamin A rich foods and Golden Rice can help to fix that problem. Researchers cannot seem to come up with a harmful scenario by GR Rice is a staple in some countries so that it is easier for them to obtain and grow it. No specific harm emanates from transgenic crops, while clear life-threatening conditions arise from the lack of micronutrients

Appendix D

Pre Test	Post Test
2	8
1	7
3	12
4	15
2	12
2	14
3	9
5	8
7	14
6	13
4	11
4	17
9	10
1	16
0	7
2	13
3	15
6	14
7	12
8	13
4	15
7	12
3	10
2	7
5	16
4	11
8	17
1	8
3	14
5	16
4	8
1	12
2	9
4	3
8	17
9	18
12	17
11	19
9	17
8	15
10	19
11	20
8	18
6	13
Sum & Mean	
55	**27**
3.4375	**9**

Statement	Yes/Pre	Yes/Post	Difference	Not Sure/ Pre	Not Sure/Post	Difference	No/Pre	No/Post	Difference	
Biotechnology makes life easier.	28	31	3	16	4	-12	3	2	-1	
Biotechnology can provide opportunities for new discoveries.	40	36	-4	7	1	-6	0	0	0	
The biotechnology/genetic engineering studies with animals are beneficial to people.(humans)	26	26	0	19	7	-12	3	4	1	
Gene transfers from animals to plants can cause plants to obtain features like animals.	12	7	-5	18	8	-10	18	23	5	
Stem cells can only be extracted from embryos	10	8	-2	23	4	-19	14	24	10	
Cloning is an acceptable therapy/ practice.	7	15	8	15	8	-7	25	14	-11	
The animal meats obtained with genetic manipulations can be sold without giving any information to the consumer.	15	12	-3	10	4	-6	22	21	-1	
It is acceptable to produce plants with enriched proteins.	30	23	-7	15	8	-7	1	5	4	
There are two types of stem cells.	9	33	24	32	4	-28	6	0	-6	
Transgenic organisms contain risks for nature.	10	23	13	30	9	-21	6	4	-2	
Stem cells are unspecialized.	8	16	8	24	13	-11	15	8	-7	
Releasing GM (genetically modified) organisms to nature without controls contains risks.	35	33	-2	14	3	-11	3	1	-2	
	230	263		223	73		116	106		
			2.75			**-12.5**			**-0.833333**	Mean