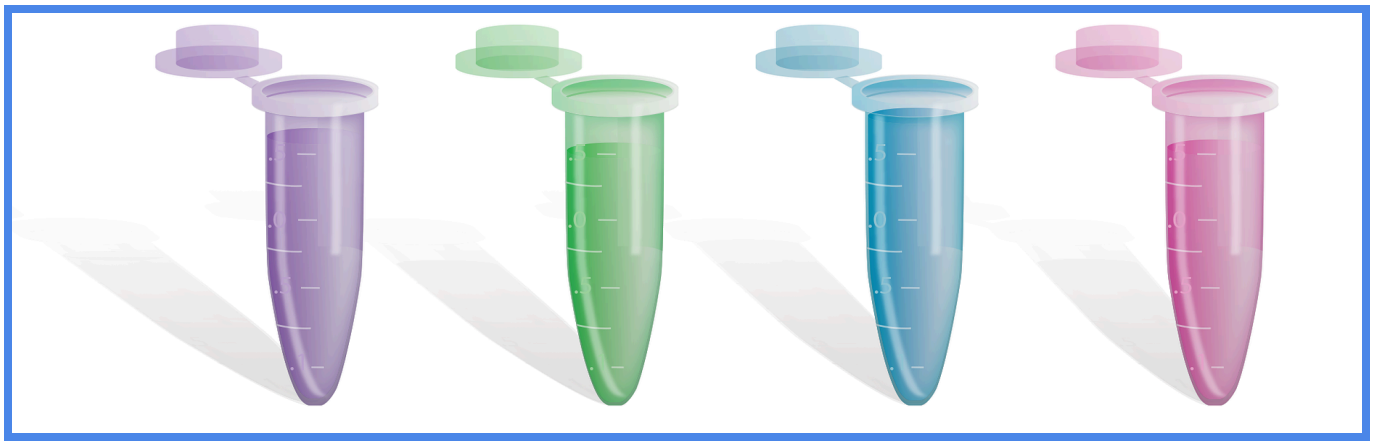
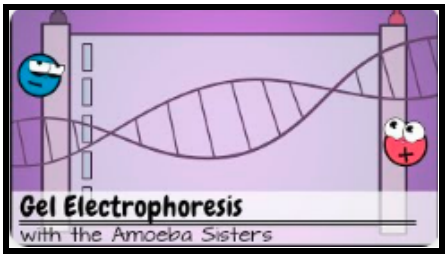


AMGEN: Biotech Experience *(ABE)*

Choose a **Lab Team** to complete this document - **rename document** to: P#AMGEN - Last Names….
Names: **TYPE HERE**

Prep-Work

Video: *Gel Electrophoresis*



Click image to view video

Watch Video: *Gel Electrophoresis (& Restriction Enzymes)*

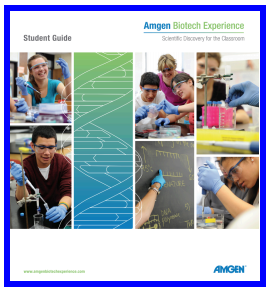
1. What is the purpose of gel electrophoresis? **TYPE**
2. What part of a nucleotide contributes a negative charge to the DNA molecule? **TYPE**
3. What is the gel made of, and where is it usually found? **TYPE**
4. When DNA travels through the agarose, does it travel toward the positive or negative side of the gel? **TYPE**
5. What is the purpose of restriction enzymes? **TYPE**
6. If you added the same restriction enzyme to the baby and mother guppy's DNA, would it necessarily cut fragments of the same length? Explain. **TYPE**
7. Who travels faster through the gel, longer or shorter pieces of DNA? **TYPE**
8. Will longer pieces of DNA be closer to the wells or positive side of the gel? **TYPE**
9. Which guppy is most likely the mother? **TYPE**
    a. What additional DNA sample would be needed to know for sure? **TYPE**
10. What is the purpose of a DNA ladder? **TYPE**
11. Why do we care about gel electrophoresis (what is it used for)? **TYPE**

Lab A/Day 1: Some Tools of the Trade *(Hint: look at Task 4 and add to it as you go along)*

AMGEN: Biotech Experience

Task 1: Begin Reading (Digital or Hard Copy) at A-1

1. Name and describe the two key discoveries made in the 1970's and '80s? **TYPE**
2. You will be carrying out some of the very same procedures that scientists used to produce insulin. What will you produce instead of insulin and how will you produce it? (Identify where the gene

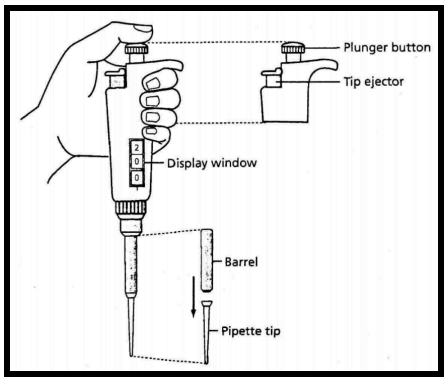


Digital Lab Book: click image above

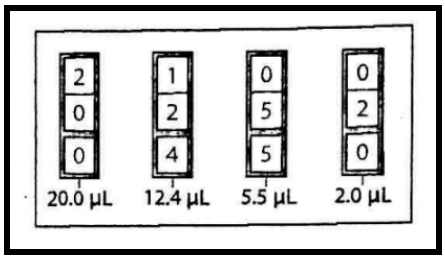
originated and how it was changed as well.) **TYPE**

3. Skip to p. A-22, What is a gene? **TYPE**
4. What is a plasmid? **TYPE**

Review of Pipette-ing



Parts of a Micropipette



Setting the correct volume

NOTE: When setting the volume please use the black knob, NOT the white push button.

INSERT IMAGE OF P20 SET TO 7.2µL HERE (use watermark - just the shot, not extra background)

INSERT IMAGE OF P20 SET TO 13.5µL HERE (use watermark)

INSERT IMAGE OF P20 SET TO 18µL HERE (use watermark)

ONE IMAGE OF C-2e HERE (all of your microfuge tubes - label your microfuge tubes with initials include watermark)

Task 2: Watch Using a Micropipette and Loading a Gel for Electrophoresis

A. Using a Micropipette

1. How are micropipettes named? **TYPE**
   a. When would you use a P20? **TYPE**
2. If the viewer shows 152 on a P20, what volume is set? **TYPE**
3. ***REMEMBER: Before you dip, put on a fresh tip!***
4. List the steps for drawing up a liquid into the pipette tip. **TYPE**
5. List the steps for dispensing a liquid. **TYPE**
6. How do you release a tip without touching it? **TYPE**
7. List 3 common things to avoid when using a micropipette.
   a. **TYPE**
   b. **TYPE**
   c. **TYPE**

B. Loading a Gel for Electrophoresis – *review*

1. How do you load a gel well? **TYPE**
2. ***NOTE: When loading your gel press only to the FIRST stop to avoid pushing your DNA sample out of the well. Either stop is technically correct, but the first stop will prevent more accidents.***

*C. Practice Pipetting Like a Pro

1. Practice setting the volume of a P20 pipette by turning the black knob - *need a watermark*.
   a. Set the P20 to 7.2µL. **Insert an image of your display window in the left column.**
   b. Set the P20 to 13.5µL. **Insert an image of your display window in the left column**.
   c. Set the p20 to 18µL. **Insert an image of your display window in the left column.**
2. ALL members of Lab Team: Practice loading & dispensing liquids.
   a. Obtain a microfuge tube. Label the cap with your initials.
   b. Add 6µL of distilled water (DI) to the tube. ***(REMEMBER: Before you dip, put on a fresh tip!)***
   c. Add 6µL of red loading dye (RL) to the tube.
   d. Gently flick the tube or tap it on your lab table to mix liquids - or centrifuge.
   e. Insert a picture of your team's microfuge tubes (all of your HyperDoc partner's) in the left column.

*This is a microfuge tube!

Task #2: Lab 1.2

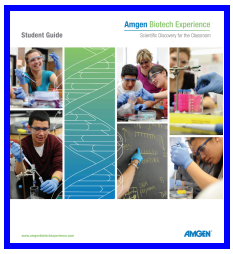


IMAGE OF LOADED Gel before you turn the MiniOne on. (use watermark)

IMAGE OF Completed GEL (use watermark)

Task 3: Begin Reading (Digital or Hard Copy) at A-24

Modifications or Additions Below

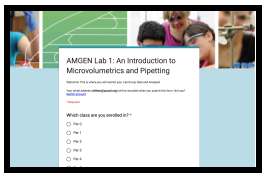
MATERIALS: all stock solutions are located in the small white box with a clear lid. Be careful to only dip with a fresh tip - do not contaminate stock solutions.

***Part A: Pipetting into Wells** (optional and is still optional) - use hard model of a gel in little petri dish, if you like with red dye and tap water

***Part B: Separating Dyes with Gel Electrophoresis - use real gel**

1. You will not share gels, each team has their own MiniOne and Microcentrifuge. Make sure the gray plate (not black) is at the base of the box chamber. Pick up premade gels from the gel stock box.
2. Ensure that well holes of the gel are upright & on the negative side - the current will draw them to the positive side of the gel.
3. Insert an image (with watermark) of the loaded gel in the column on the left - be sure to load gels in this order: S1, S2, S3 - start your loading in the top left corner with S1, then S2 etc.
4. Step 11: put on the lid, turn MiniOne on - large button.
5. Once the dyes have separated - take a photo and insert image (with watermark).
6. Throw away gel, drain/dry gel box. Be careful to return clear plastic mold back into the casting tray!!!

Google Form Amgen-LAB 1



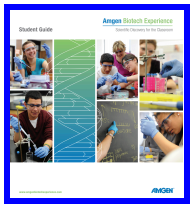
Task 4: **Identify** what each member of your team did **today** to **contribute** to your HyperDoc, Lab and Google Form. Everyone should be contributing, **working collaboratively** - do not divy the work. ***Write name and contributions:***

Member 1: TYPE
Member 2: TYPE
Member 3: TYPE
Member 4: TYPE

Driving Question: How can I verify that I have both cut and ligated DNA to form a recombinant plasmid? (Labs 2-4)

Lab B2/Day 2: Digesting pKAN-R and pARA

AMGEN: Biotech Experience



INSERT IMAGE OF pKAN-R Here

Begin Reading (Digital or Hard Copy) at B, 4-13

1. Explain what a plasmid is. **TYPE**
2. Explain what restriction enzymes can do and how they do it. **TYPE**
3. **Insert images** of pKAN-R and pARA from your digital lab book in the ***column on the left***.
4. Skip ahead to the directions of the lab, B12-13, What are the names of the two restriction enzymes you will be using in this Lab? (*AP Variation add to question: ...and why did we need two

INSERT IMAGE OF pARA Here

different restriction enzymes instead of one?) to **TYPE**

Table 2.2: Addition of reagents to the K+, K–, A+, and A– tubes

	K+ tube	K– tube	A+ tube	A– tube
Step 4a: Restriction buffer (2.5xB)	4.0 µL	4.0 µL	4.0 µL	4.0 µL
Step 4b: pKAN-R plasmid (K)	4.0 µL	4.0 µL		
Step 4c: pARA plasmid (A)			4.0 µL	4.0 µL
Step 4d: BamHI and HindIII (RE)	2.0 µL		2.0 µL	
Step 4e: Distilled water (dH_2O)		2.0 µL		2.0 µL

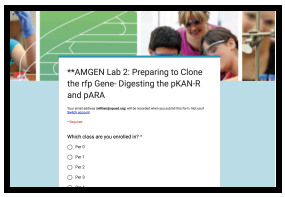
See Lab Book for Instructions, B14-16

Laboratory 2: Preparing to clone the rfp gene: Digesting the pKAN-R and pARA (p. B-14)

Modifications or Additions Below

***METHODS**: follow all instructions, be sure that you decide, as a class, at what time the microfuge tubes should be removed from the water bath (by me) and placed in the freezer. Indicate time here: **TYPE**

Google Form Amgen-LAB 2

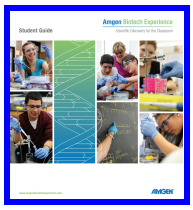


As before, Identify what each member of your team did **today** to **contribute** to your HyperDoc, Lab and Google Form.

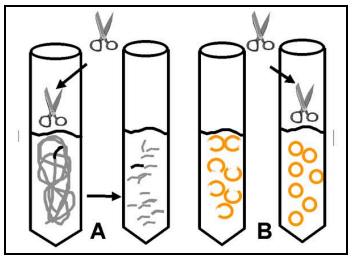
Member 1: TYPE
Member 2: TYPE
Member 3: TYPE
Member 4: TYPE

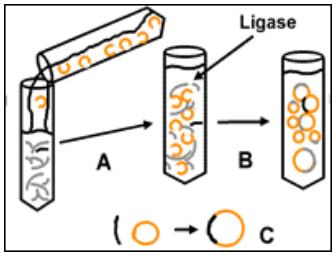
Lab B3/Day 3: Building the pARA-R plasmid

AMGEN: Biotech Experience



INSERT IMAGE OF pARA-R Here





Begin Reading (Digital or Hard Copy) at B, 23-27

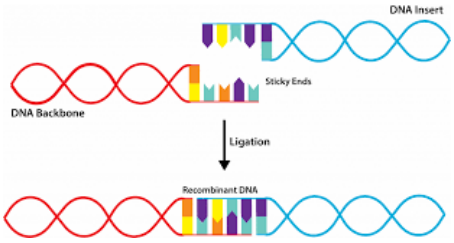
1. What is the role of ligases in genetic engineering? **TYPE**
2. What is necessary (in the restriction fragments) in order for ligase to work?. **TYPE**
3. In your Lab today you will ligate the DNA fragments you produced during Lab 2. The ligation process will generate many different plasmids (as you will be able to make any combinations of the four fragments generated in Lab 2), The desired recombinant is pARA-R (insert an image to the left), identify the role of each of the regions of this plasmid: (B-12 and B-27)
   a. pBAD: **TYPE**
   b. rfp: **TYPE**
   c. ampR: **TYPE**
   d. ori: **TYPE**
   e. araC: **TYPE**
4. *Skip ahead* to the directions of the lab, B28-29, Why is it important to inactivate (placing A+ and K+ tubes in 70°C water bath for 30 mins - Methods, Step 2) the BamHI and HindIII restriction enzymes before ligating the fragments? **TYPE**

Laboratory 3: Building the pARA-R Plasmid (p. B-28)

Modifications or Additions Below

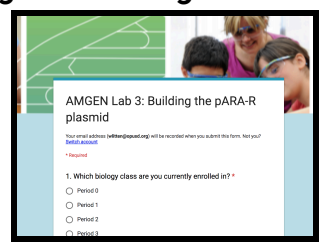
***METHODS**:

- ➔ *CRITICAL*: Put A+ and K+ tubes in 70°C water bath for 30 mins (step 2)
- ➔ I am giving you a "LIG" microfuge tube with 2ul of ligase



(enzyme) ***already*** in the tube (step 3) - you will add DNA from your A+ tube and K+ tube, some buffer (5xB) and water to bring the volume to 15ul. If it doesn't look like 15ul - you missed something. Make a list and check it twice! Follow instructions!!!

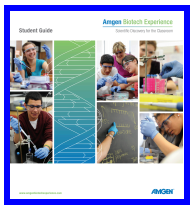
Google Form Amgen-LAB 3

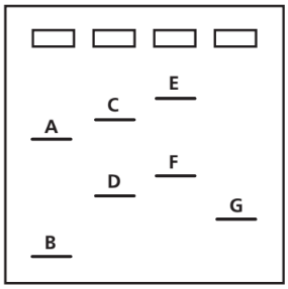


As before, Identify what each member of your team did **today** to **contribute** to your HyperDoc, Lab and Google Form.
Member 1: TYPE
Member 2: TYPE
Member 3: TYPE
Member 4: TYPE

Lab B4/Day 4: Gel Electrophoresis

AMGEN: Biotech Experience



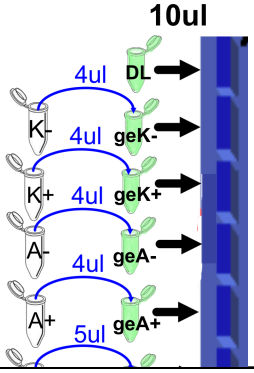


Begin Reading (Digital or Hard Copy) at B35-40

1. Why do DNA restriction fragments and plasmids separate when analyzed by gel electrophoresis? **TYPE**
2. Explain why whole plasmids will travel at different distances through a gel even though they may have the same number of base pairs. **TYPE**
3. List the fragment letters smallest to largest (use all 7 letters) from the diagram on the left: **TYPE**
4. What type of banding pattern do you expect to see from the gelLIG tube and why do you expect it? **TYPE**

Sequence	geK– tube	geK+ tube	geA– tube	geA+ tube	geLIG tube
Steps 4 and 5: Distilled water (dH_2O)	4.0 µL	4.0 µL	4.0 µL	4.0 µL	3.0 µL
Step 6: Loading dye (LD)	2.0 µL	2.0 µL	2.0 µL	2.0 µL	2.0 µL
Step 7: Nondigested pKAN-R (K–)	4.0 µL				
Step 7: Digested pKAN-R (K+)		4.0 µL			
Step 7: Nondigested pARA (A–)			4.0 µL		
Step 7: Digested pARA (A+)				4.0 µL	
Step 8: Ligated plasmid (LIG)					5.0 µL

See Lab Book for Instructions, B42-45
Add Water, **then** DNA source (clear tubes) to your colored

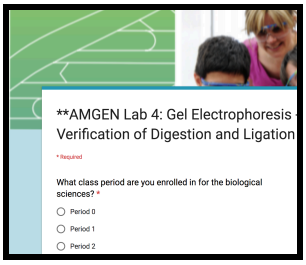


Laboratory 4: Verification of Restriction and Ligation Using Gel Electrophoresis (p. B41)

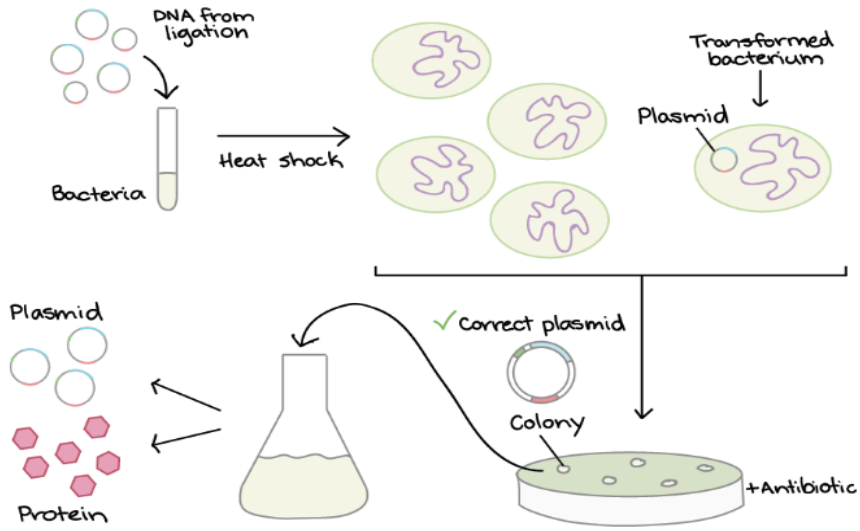
Modifications or Additions Below

***METHODS**:

- ➔ Use designated colored tubes for ge tubes (step 2)
- ➔ Have a system (ie moving tubes from one side of the rack to the other) after each item is loaded into the ge tubes (Steps 3-8)
- ➔ Your "DL" tube does not need anything added to it - except LD (it contains the DNA Ladder used for comparison)
- ➔ Pick up a gel from stock table, make sure wells are right side up and on the negative side of the MiniOne.
- ➔ With your gel in the MiniOne and the words MiniOne in the top left corner - you should load your gels in this well order top/bottom:
  - ◆ Well 1: DL
  - ◆ Well 2: geK-
  - ◆ Well 3: geK+

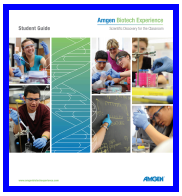
ge tubes, **lastly** LD	◆ Well 4: geA- ◆ Well 5: geA+ ◆ Well 6: geLIG If you did it differently change the descriptions above
INSERT IMAGE OF completed Gel - crop it so that you only see the gel - the assumption will be that the wells contain the DNA in the order listed above. Arrange photo so that Well 1 is in the top left hand corner of this box	***ANALYSIS:** 1. What evidence do you have that your plasmids were cut in your A+ and K+ tubes and not in your A- and K- tubes? **TYPE** 2. What evidence do you have that your A+ and K+ plasmids were cut in the right places? **TYPE** 3. What evidence do you have that your DNA was ligated? (geLIG)? **TYPE**
Google Form Amgen-LAB 4 	**As before, Identify** what each member of your team did **today** to **contribute** to your HyperDoc, Lab and Google Form. **Member 1: TYPE** **Member 2: TYPE** **Member 3: TYPE** **Member 4: TYPE**

Prep-Work: Picture Analysis

In order to perform transformation name/describe what had to happen previously: 1. **First: TYPE** 2. **TYPE (Hint: hot!)** 3. **TYPE** 4. ***(This fourth step is not necessary, but we did this to check our work so far):* TYPE** 5. **Adding DNA from ligation to bacteria that are competent (ready to absorb DNA)** 6. **TYPE (Hint: briefly warm)** 7. **Feed and Plate bacteria - put in incubator and allow those bacteria that can survive on the medium (type of agar) to multiply.**	**Discuss this image from Khan Academy:** Here is a typical procedure for transforming and selecting bacteria: 

Lab B/Day 5 Background: Getting Recombinant Plasmids in Bacteria

AMGEN: Biotech Experience	**Begin Reading (Digital or Hard Copy) at B, 51-55** 1. What is transformation and what purpose does it serve? **TYPE**



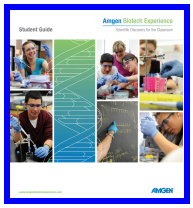
Digital Lab Book: click image above

2. Why is transformation difficult in nature? **TYPE**
3. In what two ways has the bacteria in our lab been treated in order to increase the efficiency of DNA uptake? **TYPE**

Driving Question: What role does bacteria and its growth play in recombinant DNA procedures? - or why are bacteria necessary for this lab? (Lab 5)

Lab B/Day 5: Transforming Bacteria with the Ligation Products

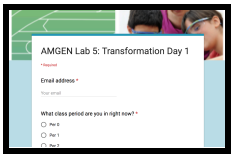
AMGEN: Biotech Experience



Begin Reading (Digital or Hard Copy) at B, 56-63. Read through all the *steps of the lab* so you are familiar with the procedures and then answer the following questions:

1. What type of bacteria can grow on a Luria Broth (LB) plate? **TYPE**
2. Why are both the control (P-) and the treatment (P+) bacteria groups spread onto the LB plates? (Think: What would it provide evidence for if there was no growth?) **TYPE**
3. Why are both the control (P-) and the treatment (P+) bacteria groups spread onto the LB plates that are infused with ampicillin? (Think: evidence) **TYPE**
4. Why is the treatment group (P+) spread onto the LB plates that are infused with both ampicillin and arabinose? (Think: evidence) **TYPE**
5. Proceed to Lab 5B - and *complete the predictions*

Google Form Amgen-LAB 5A



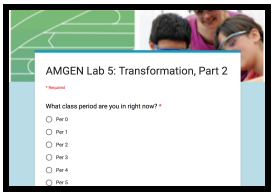
As before, Identify what each member of your team did **today** to **contribute** to your HyperDoc, Lab and Google Form.
Member 1: TYPE
Member 2: TYPE
Member 3: TYPE
Member 4: TYPE

Lab B/Day 6: RESULTS

INSERT an IMAGE of your THREE plates here - best view of colony growth as possible

BACTERIAL COLONIES						
Plates	P- control bacteria with white colonies		P+ treatment bacteria with white colonies		P+ treatment bacteria with red colonies	
	Predicted (Y/N)	*Actual (#)	Predicted (Y/N)	*Actual (#)	Predicted (Y/N)	*Actual (#)

LB ***(can put lawn)***	**TYPE**	**TYPE**	**TYPE**	**TYPE**	**TYPE**	**TYPE**
LB + amp	**TYPE**	**TYPE**	**TYPE**	**TYPE**	**TYPE**	**TYPE**
LB + amp + arabinose			**TYPE**	**TYPE**	**TYPE**	**TYPE**

Google Form Amgen-LAB 5B  	**As before, Identify** what each member of your team did **today** to **contribute** to your HyperDoc, Lab and Google Form. **Member 1:** **TYPE** **Member 2:** **TYPE** **Member 3:** **TYPE** **Member 4:** **TYPE**
***ANALYSIS**	
MAKE A CLAIM: Did you make a recombinant plasmid?	**TYPE**
GIVE EVIDENCE TO SUPPORT YOUR CLAIM: Use your data table above	**TYPE**
REASON: Make a logical connection between your evidence and your claim - your reason should come from comparing your treatment group traits with the control group traits.	**TYPE**