

PV92 PCR Informatics Kit: Where did you get those GENES?



Chromosome 16: PV92 PCR Informatics Kit

Instructors



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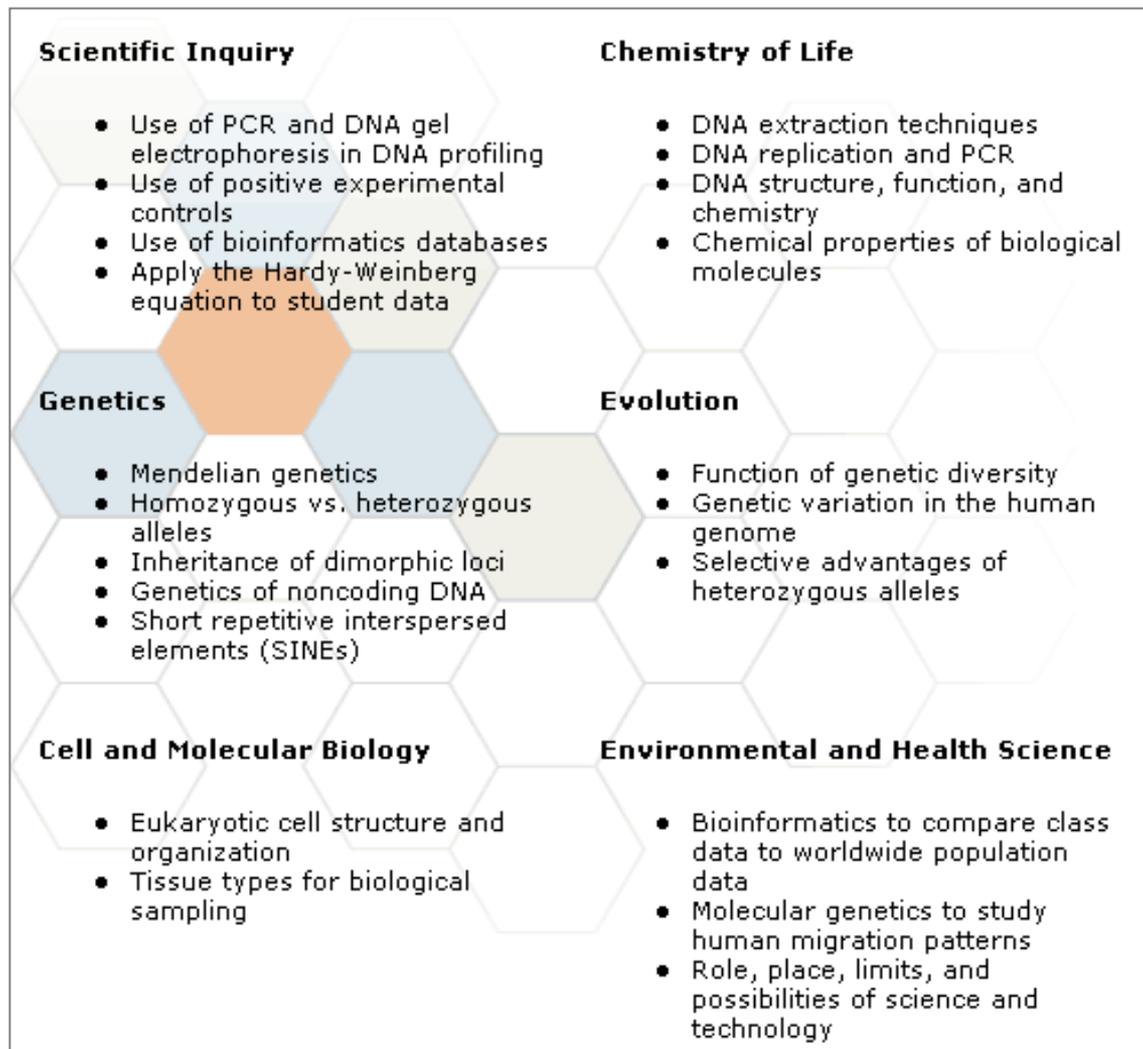
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Curriculum and Training Specialist
Bio-Rad Laboratories

Why Teach Polymerase Chain Reaction (PCR)?



- **Powerful teaching tool**
- **Real-world connections**
- **Link to careers and industry**
- **Tangible results**
- **Laboratory extensions**
- **Standards-based**



Chromosome 16: PV92 PCR Informatics Kit Advantages



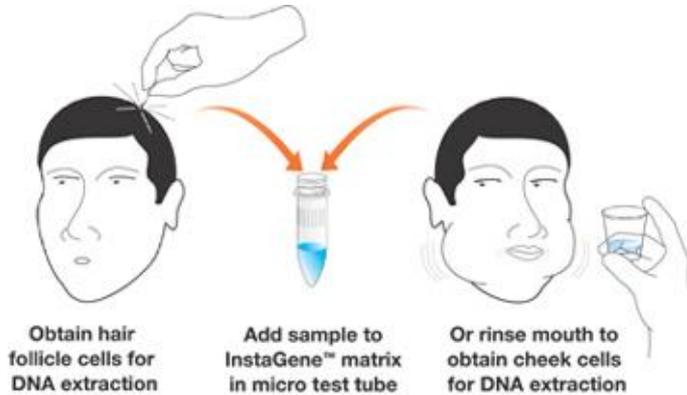
- Aligns with AP Biology **AP Lab 8**
- Extract genomic DNA and amplify student samples
- Introduce the polymerase chain reaction (PCR)
- Apply PCR to population genetics
- Directly measure human diversity at the molecular level
- Compare results to online data
- Sufficient materials for 8 student workstations
- Complete activity in three 45 minute sessions

Chromosome 16: PV92 PCR Informatics Kit Workshop Timeline

- Introduction
- Extract genomic DNA and prepare samples for PCR
- Cycle samples
- Agarose gel analysis
- Hardy-Weinberg analysis
- Bioinformatics

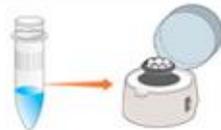


Chromosome 16 PV92 PCR Procedure Overview



Incubate at 56°C for 10 minutes, then vigorous agitation

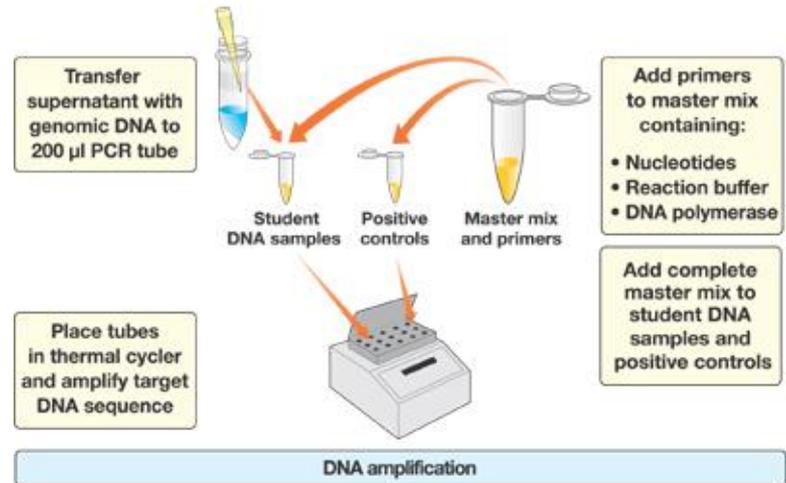
Incubate at 100°C for 6 minutes, then repeat vigorous agitation



Centrifuge samples for 5 minutes to pellet matrix

DNA template preparation

Day 1



Day 2

The positive controls supplied in the kit represent the three possible student outcomes.



1. DNA molecular mass ruler
2. Homozygous control (+/+)
3. Homozygous control (-/-)
4. Heterozygous control (+/-)
5. Student sample (-/-)
6. Student sample (+/+)
7. Student sample (+/-)
8. Student sample (-/-)

Electrophorese PCR samples on agarose gels at 100V for 30 minutes. Stain with Fast Blast™ DNA stain or ethidium bromide

Determine student genotypes for Alu insertion and perform Hardy-Weinberg analysis on class results

Extension: Web-based bioinformatics

Day 3

What Is PCR?



- **DNA replication gone crazy in a test tube!**
- **Makes millions of copies of a target sequence from template DNA**
- **Uses heat-resistant *Taq* polymerase from *Thermus aquaticus***

Laboratory Quick Guide

Quick Guide

Lesson 1 Cheek Cell DNA Template Preparation

1. Label one 1.5 ml micro test tube with your initials. Label one screwcap tube containing 200 μ l of InstaGene matrix with your initials.



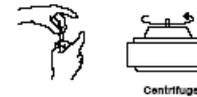
2. Obtain a cup containing saline solution from your instructor. Pour the saline into your mouth and rinse vigorously for 30 seconds. Expel the saline back into the cup.



3. Transfer 1 ml of your saline rinse into the micro test tube (NOT the screwcap tube) with your initials. If a P-1000 micropipette is not available, carefully pour ~1 ml of your saline rinse into your micro test tube (use the graduations on the side of the micro test tube to estimate 1 ml).



4. Spin your tube in a balanced centrifuge at full speed for 2 minutes. When the centrifuge has completely stopped, remove your tube. You should see a match-head sized pellet of whitish cells at the bottom of the tube. If you don't see a pellet of this size, decant the saline, refill your tube with more of your oral rinse, and repeat the spin.



Centrifuge

5. After pelleting your cells, pour off the saline. Being careful not to lose your pellet, blot your tube briefly on a paper towel or tissue. It's OK for a small amount of saline (< 50 μ l, about the same size as your pellet) to remain in the bottom of the tube.



6. Resuspend the pellet by vortexing or flicking the tube so that no clumps of cells remain.



7. Using a 2–20 μ l adjustable-volume micropipette set to 20 μ l, transfer all of your resuspended cells to the screwcap tube containing InstaGene.



8. Screw the cap tightly on the tube. Shake or vortex to mix the tube contents.

Micropipet Use



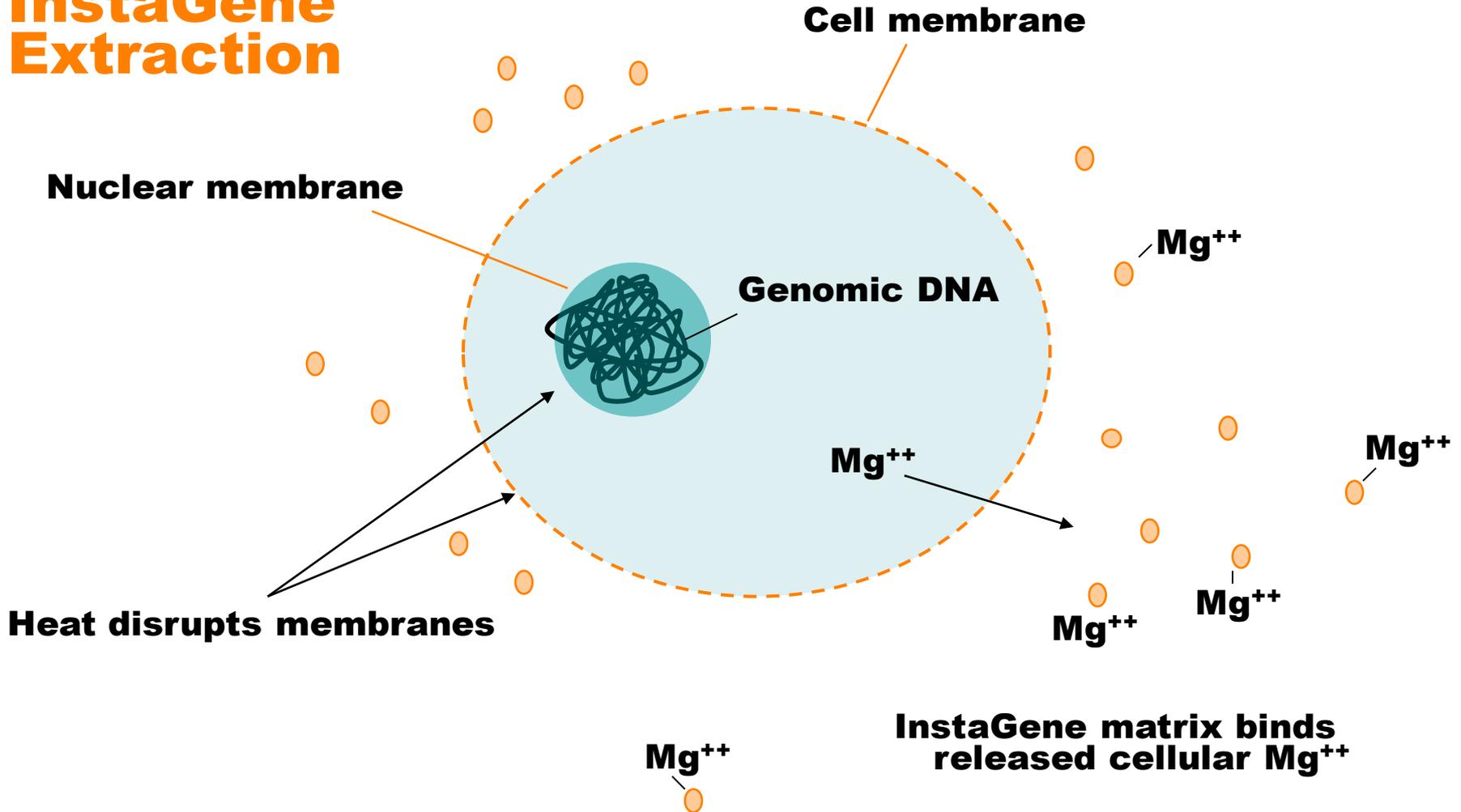
- 1. Twist dial to desired volume**
- 2. Pick up pipet tip**
- 3. Press plunger to first, soft stop**
- 4. Insert pipet tip into solution to be transferred**
- 5. Slowly release plunger to retrieve liquid**
- 6. Move pipet tip into desired tube**
- 7. Press plunger past first stop to second, hard stop to transfer liquid**

Protocol Highlights

Genomic DNA Extraction

- **InstaGene™ - Chelex® cation exchange resin; binds cellular magnesium ions**
- **56°C - loosens connective tissue and inactivates DNAses**
- **100°C - ruptures cell membranes and denatures proteins**

InstaGene Extraction



What Is Needed for PCR?

- **Template (the DNA you want to amplify for the study)**
- **Sequence-specific primers flanking the target sequence:**



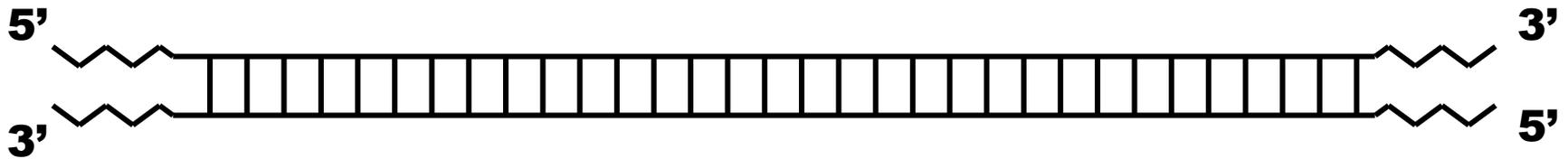
- **Nucleotides (dATP, dCTP, dGTP, dTTP)**
- **Magnesium ions (enzyme cofactor)**
- **Buffer, containing salt**
- ***Taq* polymerase**

How Does PCR Work?

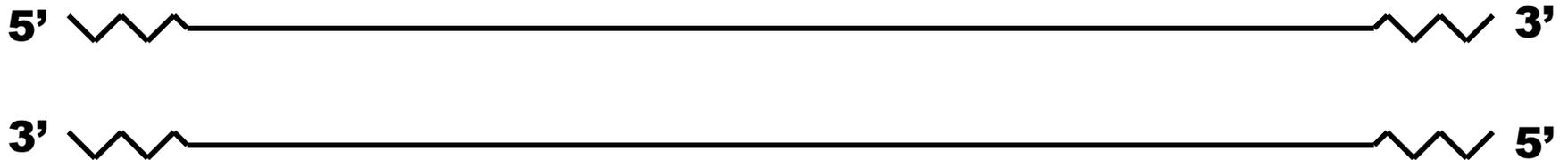
- **Heat (94°C) to denature DNA strands**
- **Cool (60°C) to anneal primers to template**
- **Warm (72°C) to activate *Taq* polymerase, which extends primers and replicates DNA**
- **Repeat multiple cycles**

Denaturing Template DNA

Heat causes DNA strands to separate

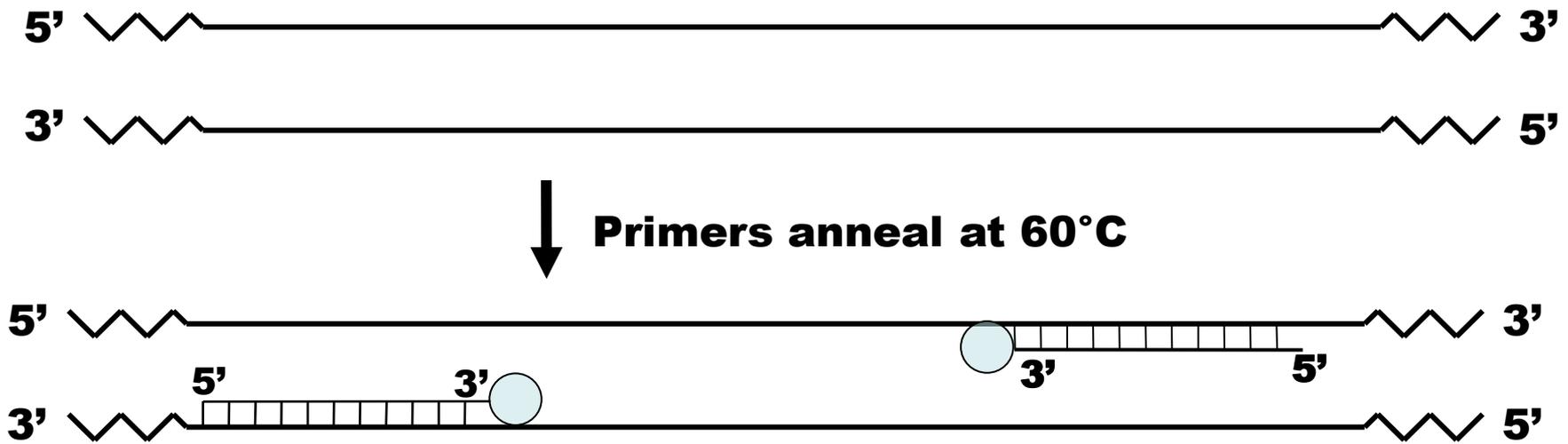


Denaturation of DNA at 94°C



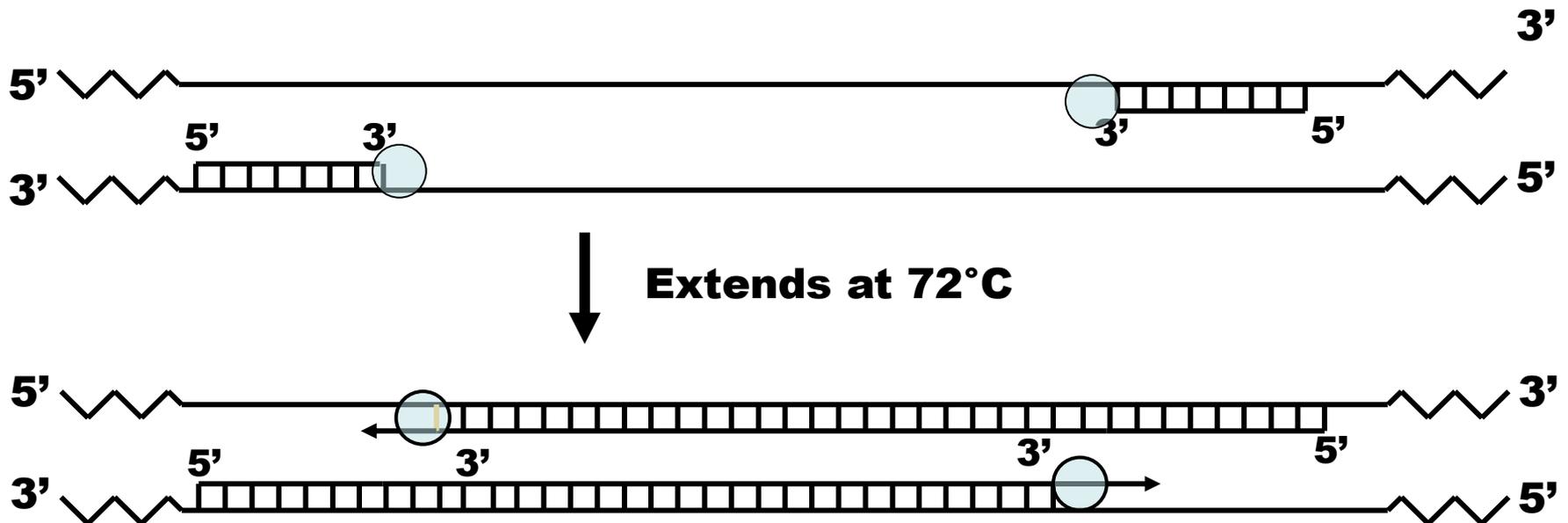
Annealing Primers

- Primers bind to the template sequence
- *Taq* polymerase binds to double-stranded substrate



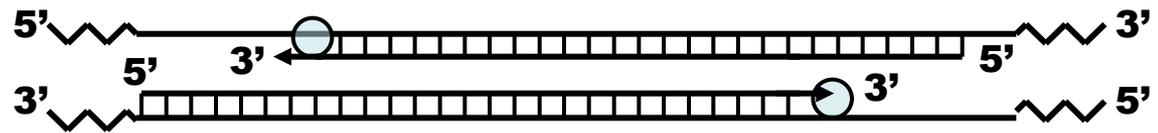
Taq Polymerase Extends...

- **Taq polymerase extends primer**
- **DNA is replicated**

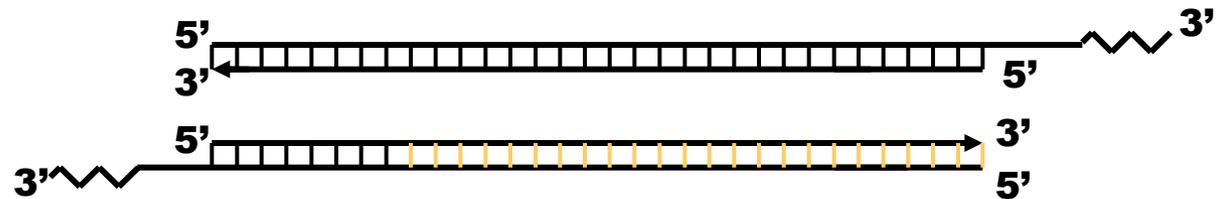


Exact-length Target Product is Made in the Third Cycle

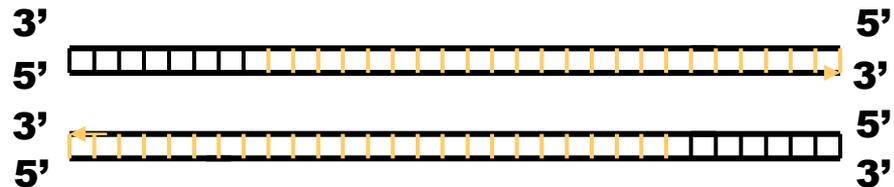
Cycle 1



Cycle 2

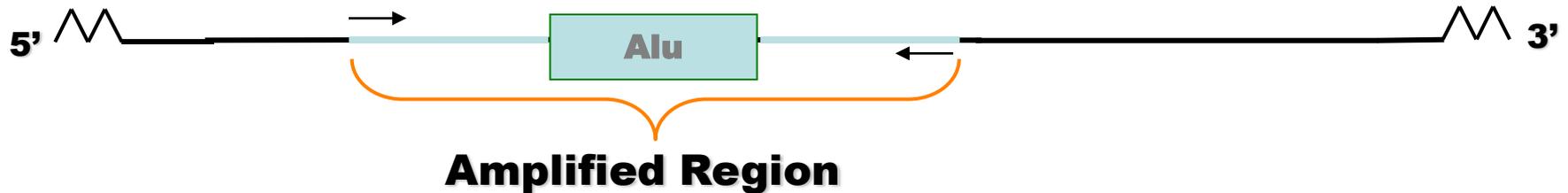


Cycle 3



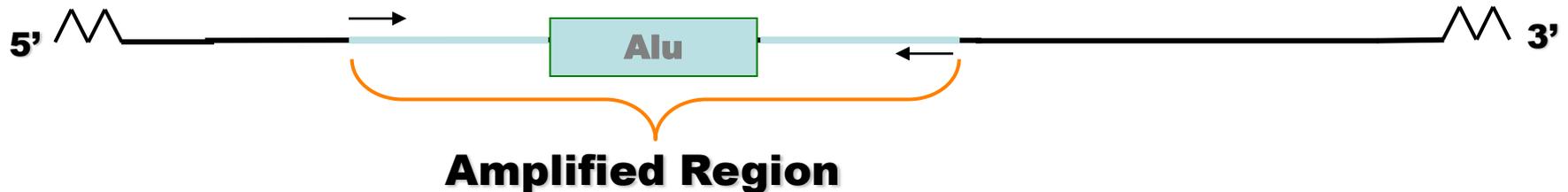
The Target Sequence

- **PV92 *Alu* insertion**
- **Located on Chromosome 16**



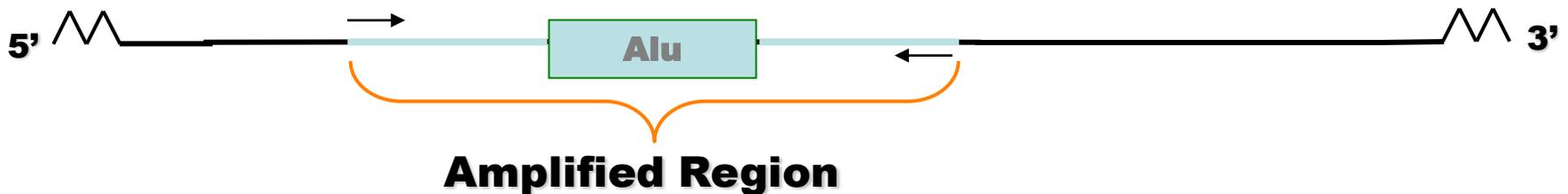
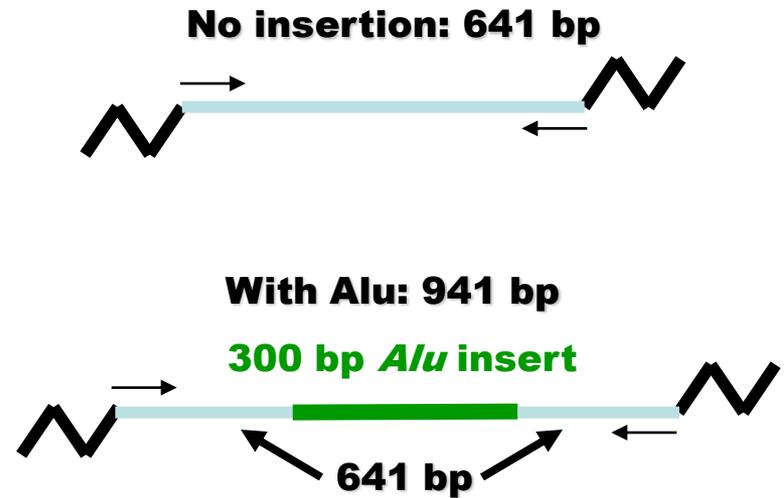
PV92 *Alu* Insertion

- A member of *Alu* repeat family
- Human-specific *Alu* insertion
- Found in a non-coding region of your DNA
- **Not** diagnostic for any disease or disorder

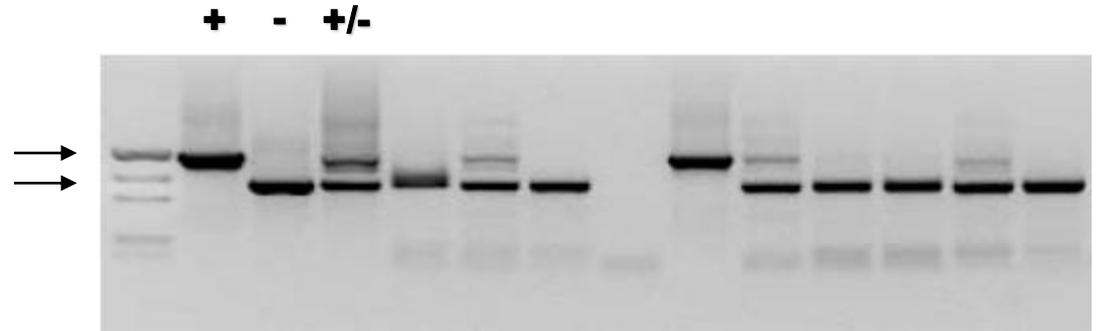
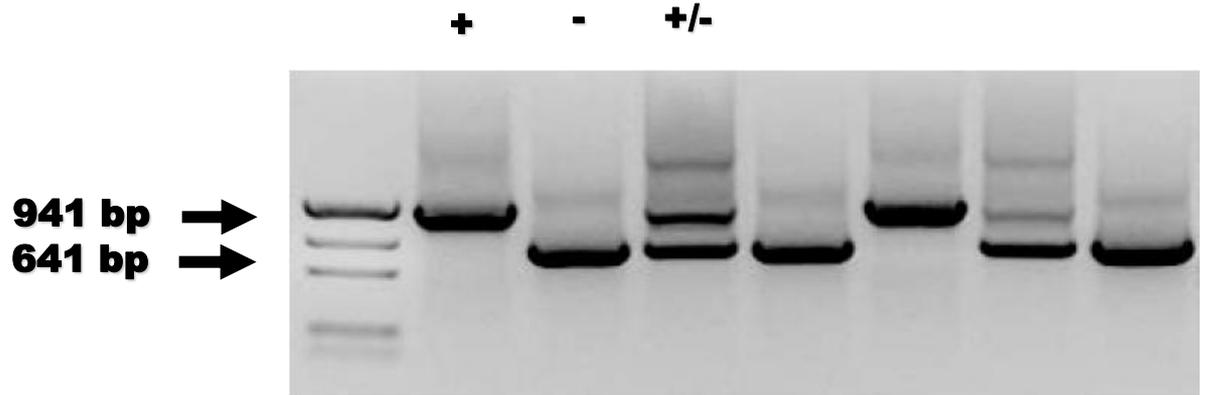


PCR Results

- The PV92 *Alu* is dimorphic so there are two possible PCR products: **641 bp** and **941 bp**



Actual Alu PCR Results



***Alu* Repeats**

- **Classified as SINEs (Short Interspersed Repetitive Element)**
- **Mobilized by an RNA polymerase-derived intermediate (retroposition)**
- **Approx. 500,000 *Alu* copies per haploid genome, representing about 5% of the genome**
- **Named for the *Alu* I restriction site within the element**

Evolutionary Significance of PV92 *Alu* Inserts

- **Highly conserved**
- **Inserted in the last 1,000,000 years**
- **Genotypes (+/+, +/-, -/-)**
- **Used in population genetics, paternity analysis, and forensics**

Determination and analysis of *Alu* Frequency in a population

- **Amplify *Alu* insert from representative sample population**
- **Calculate the expected allelic and genotypic frequencies**
- **Perform Chi-square test**

Calculating Observed Genotypic Frequencies

Genotype	+/+	+/-	-/-	Total (N)
# of People	25	5	8	38
Observed Frequency	0.66	0.13	0.21	1.00

$$\begin{aligned}
 \text{+/+ Genotypic frequency} &= \frac{\text{Number with genotype}}{\text{Population total (N)}} \\
 &= \frac{25}{38} \\
 &= .66
 \end{aligned}$$

Calculating Allelic Frequencies

Number of + alleles

25 individuals with two + alleles = 50 + alleles
5 individuals with one + allele = 5 + alleles
Total = 55 + alleles

Total number of alleles

$$2N = 2(38) = 76$$

$$p = \frac{\text{Frequency of + alleles}}{\text{Total number alleles}} = \frac{\text{Number of + alleles}}{\text{Total number alleles}} = \frac{55}{76} = 0.72$$

$p = 0.72$; therefore $q = 0.28$ since $p + q = 1.00$

***Alu* and Population Genetics**

	p	q
p	pp	pq
q	pq	qq

$$\begin{aligned} +/+ &= p^2 \\ +/- &= 2pq \\ -/- &= q^2 \end{aligned}$$

Hardy-Weinberg Equation

$$p^2 + 2pq + q^2 = 1$$

Using the Hardy-Weinberg Equation to Determine Expected Genotypic Frequencies

(p^2 , $2pq$, q^2 values)

$$p^2 + 2pq + q^2 = 1.00$$

$$(0.72)^2 + 2(0.72)(0.28) + (0.28)^2 = 1.00$$

$$0.52 + 0.40 + 0.08 = 1.00$$

$$p^2 = 0.52 \quad 2pq = 0.40 \quad q^2 = 0.08$$

Calculate Expected Numbers for Each Genotype

		Genotype frequency	X	Population total (N)	=	Expected number
Genotype	+/+ (p^2)	0.52	X	38	=	20
	+/- ($2pq$)	0.40	X	38	=	15
	-/- (q^2)	0.08	X	38	=	3

Chi-Square Test

χ^2 critical value (from statistics table) = 5.9

$$\chi^2 = \sum \frac{(\text{Observed} - \text{Expected})^2}{\text{Expected}}$$

16.25 is above 5.9 so the observed genotypic frequencies are not in genetic equilibrium

		Observed	Expected	$\frac{(O-E)^2}{E}$
Genotype	+/+	25	20	1.25
	+/-	5	15	6.67
	-/-	8	3	8.33

$$\chi^2 = 16.25$$

Allele Server

(1 of 17)

Cold Springs Harbor Laboratory
DNA Learning Center

Web site:
<http://www.dnalc.org/>

Allele Server

(2 of 17)

Scroll through DNALC internet sites until BioServers Link appears

The screenshot shows the DNALC website homepage. At the top left, it says "The source for timely information about genes in your life". The main heading is "GENE ALMANAC" with a yellow circle to its right. Below this is a section for "DNALC INTERNET SITES: Info...". A row of six thumbnails is displayed: "Inside Cancer", "DNA from the Beginning" (with text "NOW ALSO IN BRAZILIAN PORTUGUESE!"), "DNA Interactive" (with a photo of two men), "Dynamic Gene" (with a grid of faces), "myDNAi" (with a green apple), and "BioServers" (with a tuxedo icon). A red arrow points from the text above to the "BioServers" link, which is circled in red. To the right of the thumbnails is a "RESOURCES..." section with "Media Showcase" and "Media Search". Below the thumbnails is a "Classified Ads" section with a sign that says "CLICK for DNALC Employment Opportunities!". At the bottom left is a building illustration labeled "COLD SPRING HARBOR LABORATORY DOLAN DNA LEARNING CENTER". At the bottom right is a "GENE NEWS" section with a "FEATURE" titled "And a hush fell over the genome..." and a list of activities: "ABOUT THE DNALC STUDENT CAMPS", "EDUCATOR TRAINING", "FIELD TRIPS & MEMBERSHIP", "SATURDAY DNA!", "EXHIBITION & MORE DIRECTIONS".

Allele Server

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Click on Bioservers



The source for timely information about genes in your life

GENE ALMANAC

DNALC INTERNET SITES: info...

BIOSERVERS
Go to Bioservers

RESOURCES...
Media Showcase
Roll over thumbnails to see titles.
Media Search
Search our media database. **GO!**

DNA TODAY
Episode 5:
Genome Scans Pay Off **View!**

GENE NEWS more news...
Missing gene makes mice compulsive

FEATURE
And a hush fell over the genome...
Visit our newest site, *Silencing Genomes*, for laboratory and bioinformatics exercises on RNA interference in *C. elegans*.

Classified Ads
CLICK for DNALC Employment Opportunities!

COLD SPRING HARBOR LABORATORY
DOLAN DNA LEARNING CENTER

ABOUT THE DNALC
STUDENT CAMPS
EDUCATOR TRAINING
FIELD TRIPS & MEMBERSHIP
SATURDAY DNA!
EXHIBITION & MORE
DIRECTIONS

Allele Server

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Enter the Allele Server

BioServers - Microsoft Internet Explorer

File Edit View Favorites Tools Help

Back Forward Stop Home Search Favorites Media Print Mail Internet Options

Address <http://www.bioservers.org/bioserver/> Go Links

DOLAN DNA LEARNING CENTER

Custom Workspaces and Educational Databases for Bioinformatics

This site contains user-friendly tools to launch DNA database searches, statistical analyses, and population modeling from a centralized workspace. Educational databases support investigations of an *A/u* insertion polymorphism on human chromosome 16 and single nucleotide polymorphisms (SNPs) in the human mitochondrial control region. This site requires Java, Javascript, and cookies - [click here](#) to test that you have these features enabled.

SEQUENCE SERVER

Enter DNA sequences, perform multiple sequence alignments, generate phylogenetic trees, and search Genbank by BLAST and keywords. Includes mitochondrial reference data from world populations, ancient humans, and other organisms.

ALLELE SERVER

Enter data on *A/u* PV92, test Hardy-Weinberg equilibrium, and compare human populations by contingency chi-square, genetic drift, and genetic distance. Includes PV92 reference data from more than 40 world populations.

SIMULATION SERVER

Model genetic changes over time to study the effects of drift, selective pressure, and population bottlenecks. The Monte Carlo generator can test 100 or more populations at once and link experiments using different parameters.

ENTER **REGISTER**

Username:

ENTER **REGISTER**

Username:

ENTER

No login feature available.

ENTER: You have access to all tools and databases, but your work is not saved between sessions.

Done Internet

start BioServers - Microsof... Microsoft PowerPoint ... 3:44 PM

Allele Server Type of Data

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Select Group

The screenshot shows a web browser window titled "Student Allele Server - Microsoft Internet Explorer". The address bar shows "http://www.bioser...". The main content area displays a "MANAGE GROUPS" dialog box with a "Classes" dropdown menu circled in red. A red arrow points from the text "Select Group" to this dropdown. The dialog box contains a table with the following data:

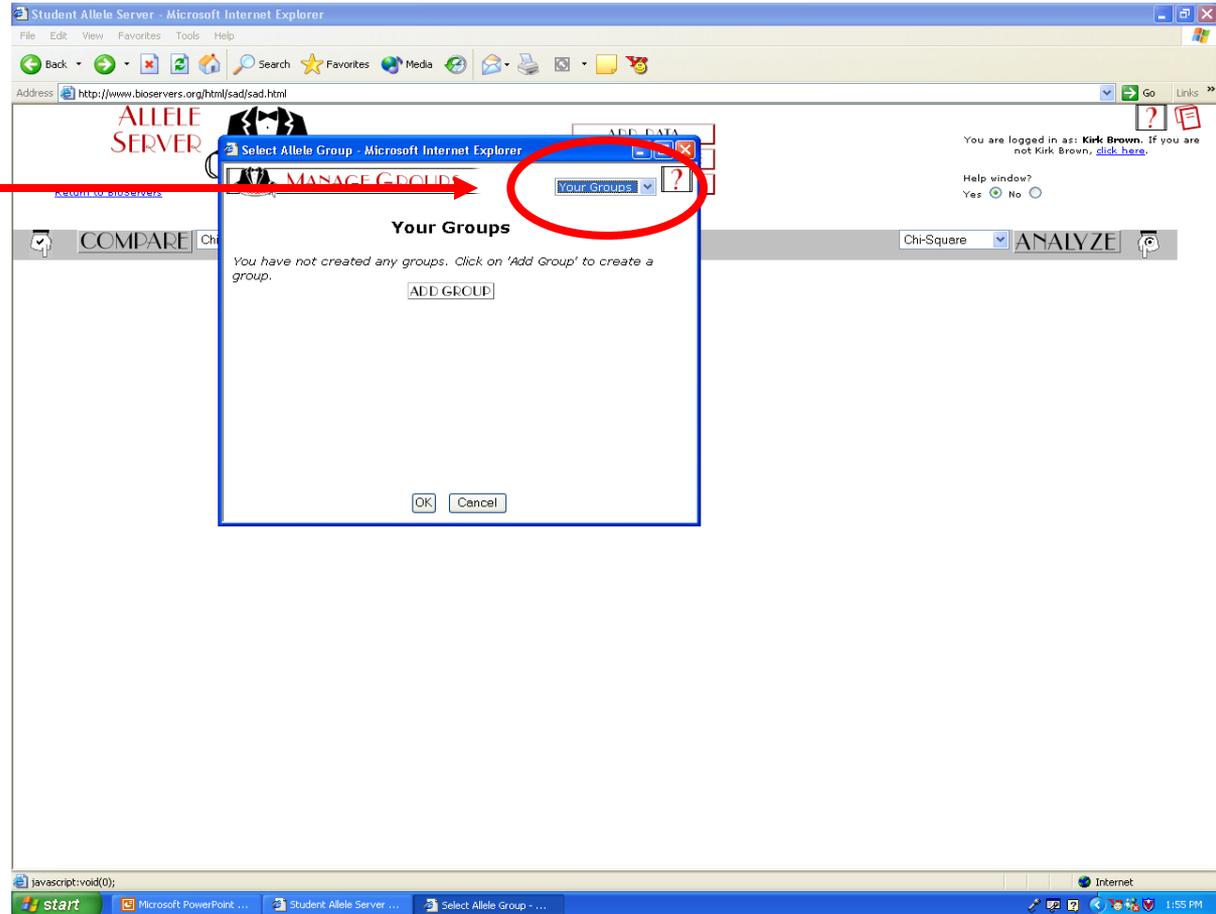
Date	Classes	Institution	Location
<input type="checkbox"/>	03/07/2002 AUM2001	Auburn University Montgomery	Montgomery, AL, USA
<input type="checkbox"/>	03/07/2002 AUM2002	Auburn University Montgomery	Montgomery, AL, USA
<input type="checkbox"/>	03/04/2002 biol 445		
<input type="checkbox"/>	03/04/2002 biol445b		
<input type="checkbox"/>	03/04/2002 Loyd Bio 02	St. Ignatius College Prep	San Francisco CA, USA
<input type="checkbox"/>	03/01/2002 Hong's Alu test	DNALC	NY, NY, USA
<input type="checkbox"/>	03/01/2002 Locust Valley HS	Locust Valley	NY, NY, USA

At the bottom of the dialog box are "OK" and "Cancel" buttons. The background web page shows a "Return to BioServers" link and a "COMPAR" button. The system tray at the bottom shows the time as 3:51 PM.

Allele Server

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Scroll Down to Select
"Your Group"



Allele Server

(9 of 17)

Fill Out Form



Student Allele Server - Microsoft Internet Explorer

File Edit View Favorites Tools Help

Address <http://www.bioservers.org/html/sad/sad.html>

ALLELE SERVER

Return to BioServers

COMPARE Chi

Chi-Square ANALYZE

You are logged in as: **Kirk Brown**. If you are not Kirk Brown, [click here](#).

Help window? Yes No

CREATE GROUP

New Group

You must give your group a name, and choose whether it is private (only for you to see) or public (will appear in the "Classes" section of the database):

Name

Type

The following fields are optional:

Institution

City

State

Country

Your

OK Cancel

Done

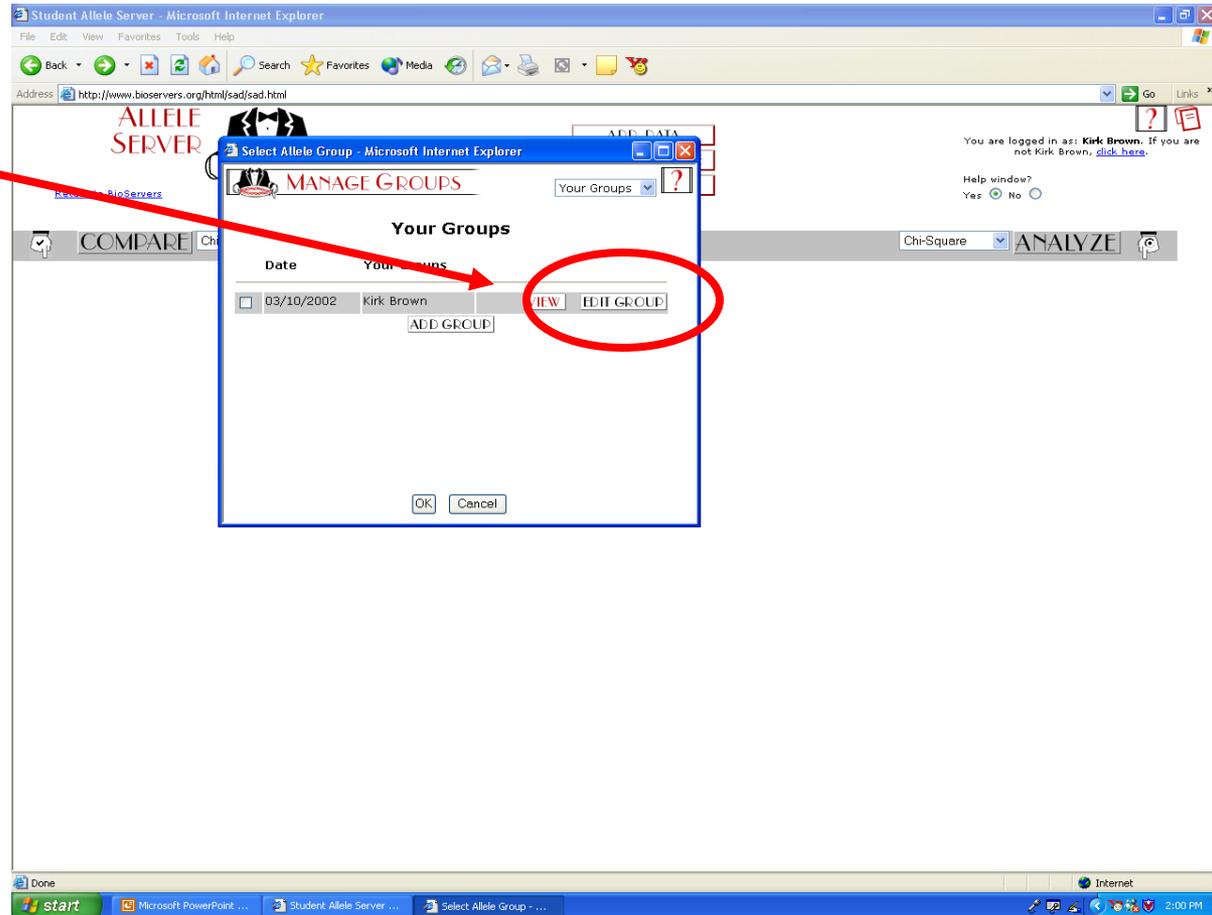
start Microsoft PowerPoint... Student Allele Server... Select Allele Group - ... Add Group - Microsof...

Internet 1:57 PM

Allele Server

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Click on Edit Group



Allele Server

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Edit Your Group Information



The screenshot shows the Allele Server interface in Microsoft Internet Explorer. The main page displays a list of groups, with 'Kirk Brown' selected. An 'EDIT GROUP' dialog box is open, allowing the user to modify group information. The dialog box contains the following fields and options:

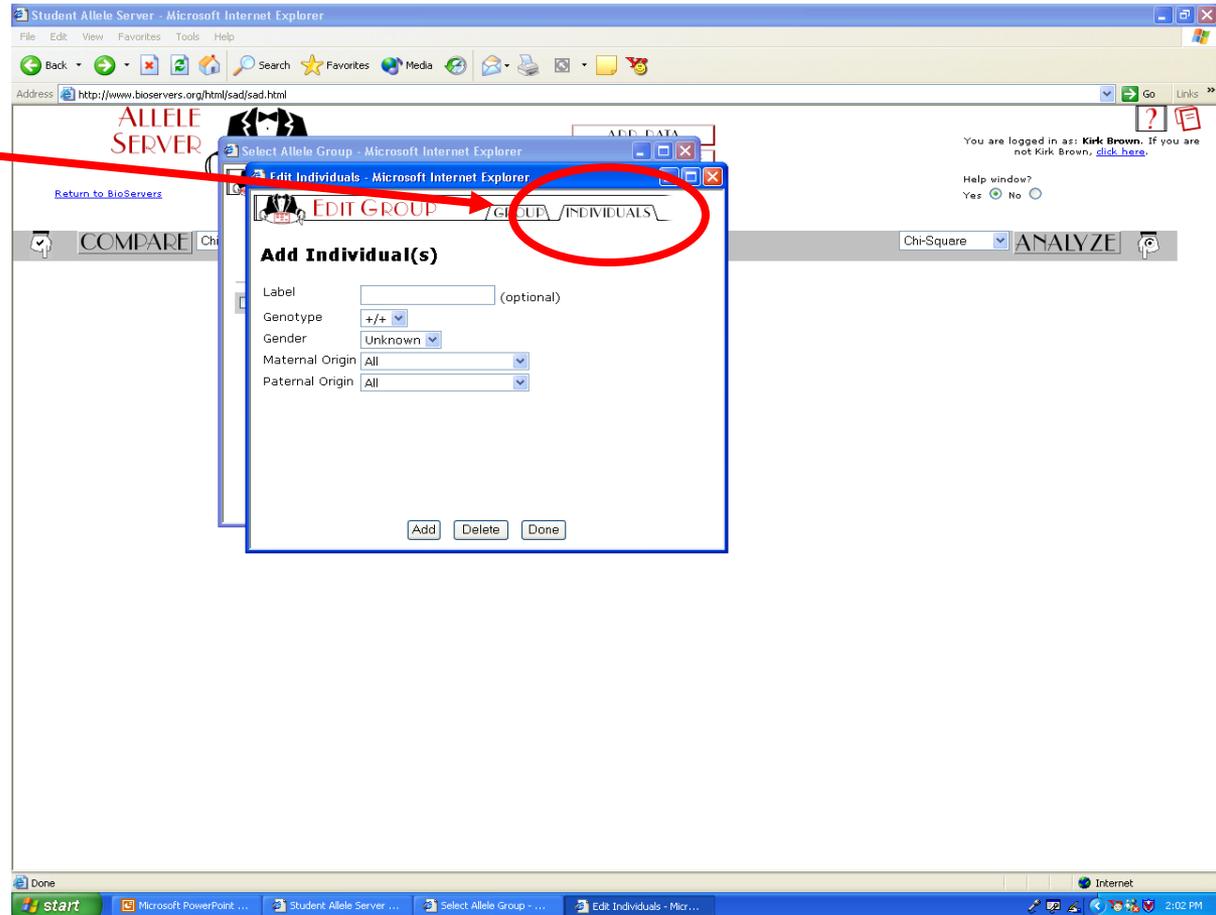
- Name:** Kirk Brown
- Type:** Private (dropdown menu)
- Institution:** Tracy High School
- City:** Tracy
- State:** CA
- Country:** USA
- Your Email:** kbrown@tUSD.net

Below these fields, there is a note: "If you want your students to be able to enter data using the 'Add Data' button, enter a password in the password field below. You will..." followed by an empty password field and 'OK', 'Delete', and 'Cancel' buttons.

Allele Server

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Click on Individuals Tab



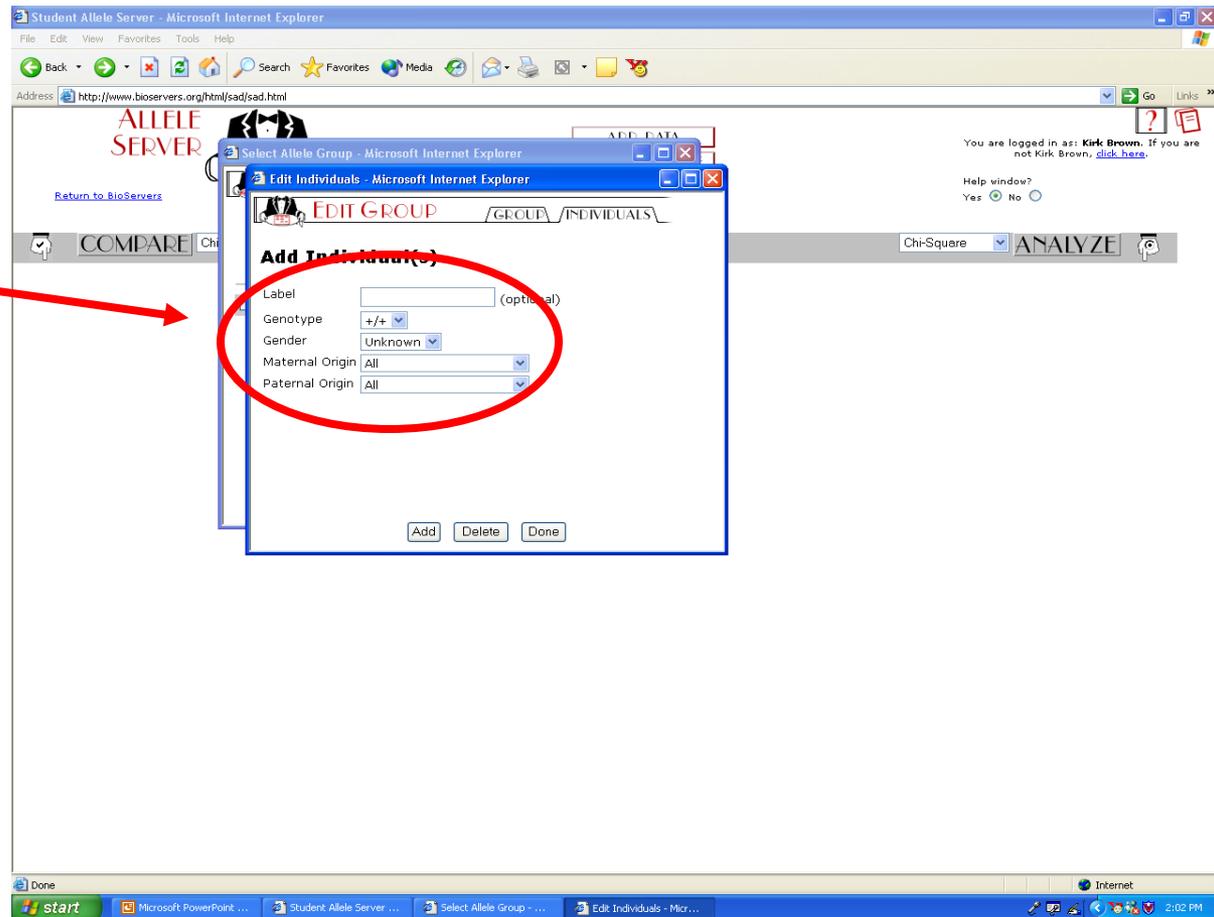
Allele Server

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Add Each Student's Information

Add as much information as possible:

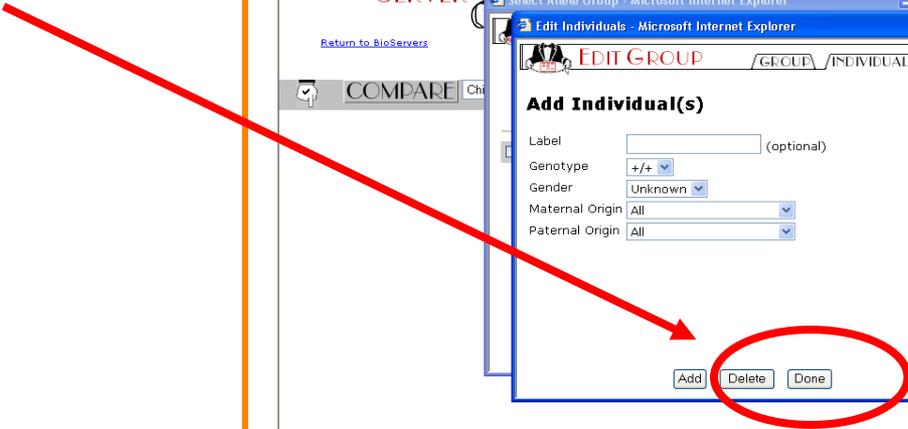
- Genotype (+/+, +/-, -/-)
- Gender
- Personal Information



Allele Server

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Click on Done

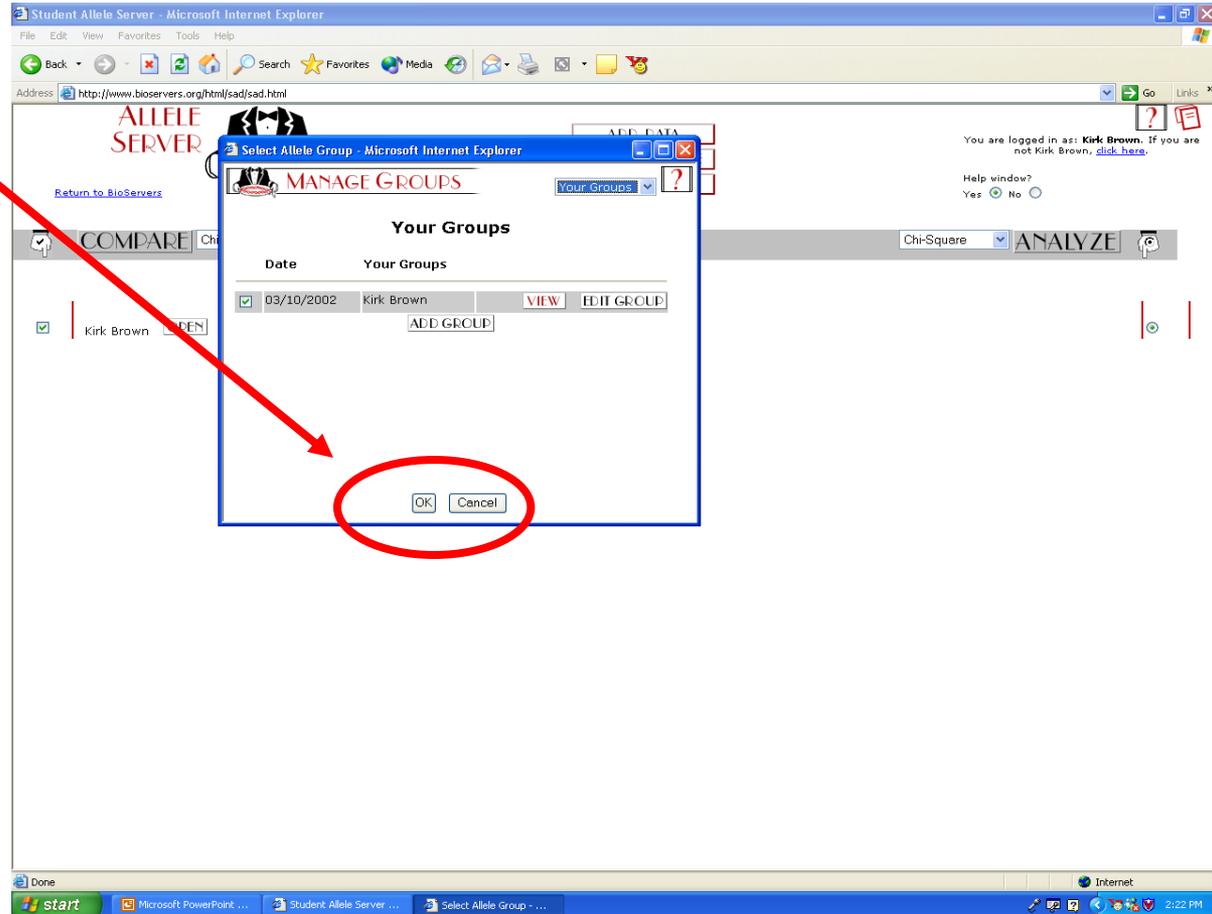


The screenshot shows the Allele Server web application in Microsoft Internet Explorer. The main window displays the 'ALLELE SERVER' logo and navigation options like 'COMPARE' and 'ANALYZE'. A modal window titled 'EDIT GROUP' is open, showing the 'Add Individual(s)' form. The form includes fields for Label (optional), Genotype (+/+), Gender (Unknown), Maternal Origin (All), and Paternal Origin (All). At the bottom of the form are 'Add', 'Delete', and 'Done' buttons. The 'Done' button is circled in red. The browser's address bar shows 'http://www.bioservers.org/html/sad/sad.html'. The system tray at the bottom indicates the time is 2:02 PM.

Allele Server

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Select and then Click OK



Allele Server

(16 of 17)

Analyze Data

Student Allele Server - Microsoft Internet Explorer

File Edit View Favorites Tools Help

Back Forward Stop Home Search Favorites Media Mail Print

Address http://www.bioservers.org/html/sad/sad.html

ALLELE SERVER

Return to BioServers

ADD DATA
QUERY DATABASE
MANAGE GROUPS

You are logged in as: Kirk Brown. If you are not Kirk Brown, [click here](#).

Help window?
Yes No

COMPARE Chi-Square CLEAR Chi-Square ANALYZE

Kirk Brown OPEN

2: Then Click Here

1: Click Here First

Done

start Microsoft PowerPoint ... Student Allele Server ... Select Allele Group ... Internet 2:23 PM

Allele Server

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Click on the Terse and Verbose Tabs to Review Data Results

The screenshot shows the Allele Server web application interface. A window titled "Chi Squared Test - Microsoft Internet Explorer" is open, displaying the results of a Chi-Square test. The window has two tabs: "TERSE" (selected) and "VERBOSE". The results are as follows:

Observed	Expected
Total samples: 7	Total samples: 7
+ alleles: 7	+ alleles: 7
- alleles: 7	- alleles: 7
+ / + 0.29;	0.25
+ / - 0.43;	0.50
- / - 0.29;	0.25

Below the table are two pie charts. The left chart is labeled "Observed" and the right chart is labeled "Expected". Both charts show the distribution of alleles: +/+ (red), +/- (green), and -/- (blue). The observed chart shows a higher proportion of +/- alleles compared to the expected chart.

The main window also shows a "COMPARE" button and a "Chi-Square" dropdown menu. The "ANALYZE" button is visible on the right. The user is logged in as "Kirk Brown".

Extensions

- **Add each class separately and compare to see if the classes differ from each other**
- **Compare your group to other existing groups**
- **Have students do manual calculations first and then compare to the computer generated version**