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PCR Perfection

PLTW SUMMIT

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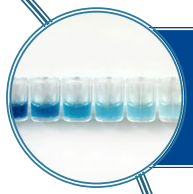

Experiments

Reagents

Equipment

Resources

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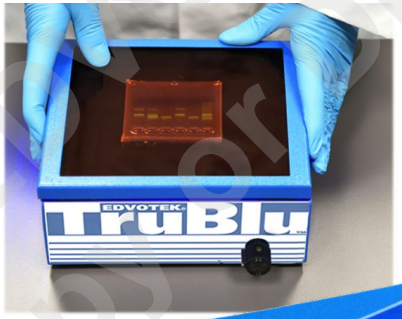
Edvotek PLTW Experiments

Kit	Description
118-PLTW → 418	Hypercholesterolemia Kit
225-PLTW → 425	DNA Detectives Kit
235-PLTW → 435	DNA Microarrays
268-PLTW → 468	Mystery Infections Kit
303 - PLTW → 403	Exploring Biotechnology with GFP
339 - PLTW → 439	Sequencing The Human Genome Kit
990-PLTW → 490	Morphology of Cancer Cells Kit
953	Multiplex PCR Testing of Water Contaminants
345	SNP Analysis of the PTC Gene
491 (1.1.4)	Forensic Blood Typing
401 (4.2.3)	Under the Sea
485	Preparation of Metaphase Chromosome Spread
430	DNA Fingerprinting

Edvotek PLTW Equipment

Kit	Description
504	M12 Complete™ Electrophoresis Apparatus
5010	TetraSource™ 300 Digital Electrophoresis PS
557	TruBlu™ Blue Light Transillumiantor
589 / 591 / 592-1	Edvotek Variable Micropipets, 0.5-10 µl, 2-20 µl, 10-100 µl, 100-1000 µl
540 / 541-542	Edvocycler Jr, Edvocycler 2



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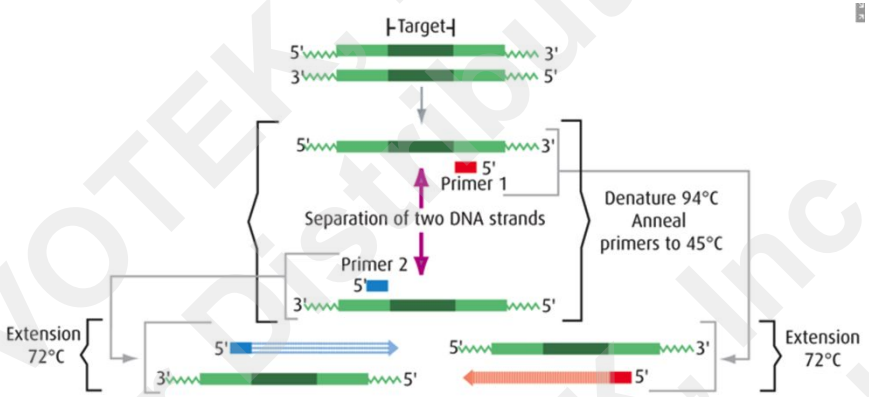
Today's Strategy

New water quality extraction - Tips & Tricks for PTC



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Water Quality Testing Extraction

- Updated the extraction protocol this summer
- Removed incubation steps
- Check literature link on freezer bag
- Will still work with old components

Water Quality Testing Extraction

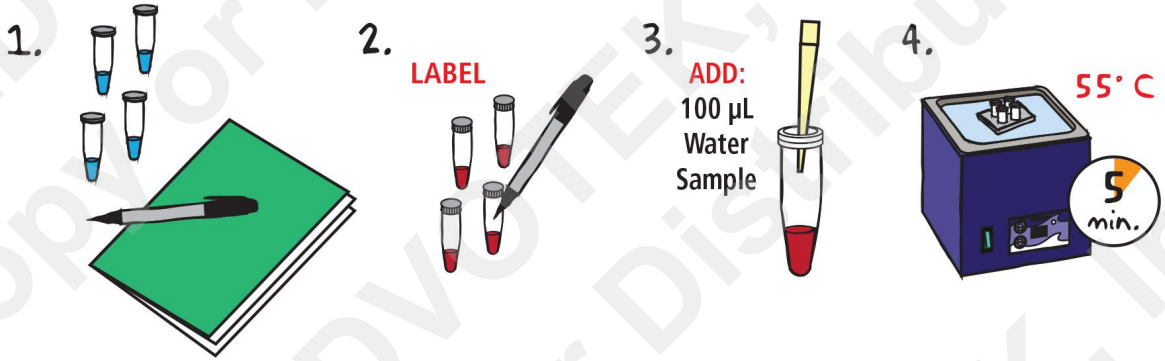
Preparation For:	What to do:	When:	Time Required:
Module I: Isolation of Bacterial DNA from Contaminated Water	Prepare contaminated water samples and DNA extraction solutions.	No more than one hour before class.	45 min.
	Prepare and aliquot Lysis Buffer.	Prepare on the day the students will be performing the experiment **OR** freeze for up to one week.	15 min.
	Preheat water baths.	Anytime before performing the experiment.	15 min.
Module II: Multiplex PCR Amplification of Water Contaminants	Program thermal cycler.	Anytime before performing the experiment.	15 min.
	Prepare and aliquot various reagents (Primer, DNA template, ladder, etc.)	One day to 30 minutes before performing the experiment.	30 min.
Module III: Separation of PCR Product by Electrophoresis	Prepare 1X Electrophoresis buffer and dilute SYBR® Safe.	Up to one week before performing the experiment.	45 min.
	Prepare molten agarose and pour gel.		
Module IV: Staining Agarose Gels with FlashBlue™ (OPTIONAL)	Prepare staining components.	The class period or overnight after the class period.	10 min.

Isolation of DNA from Bacteria

FOR MODULE I
Each Group should receive:

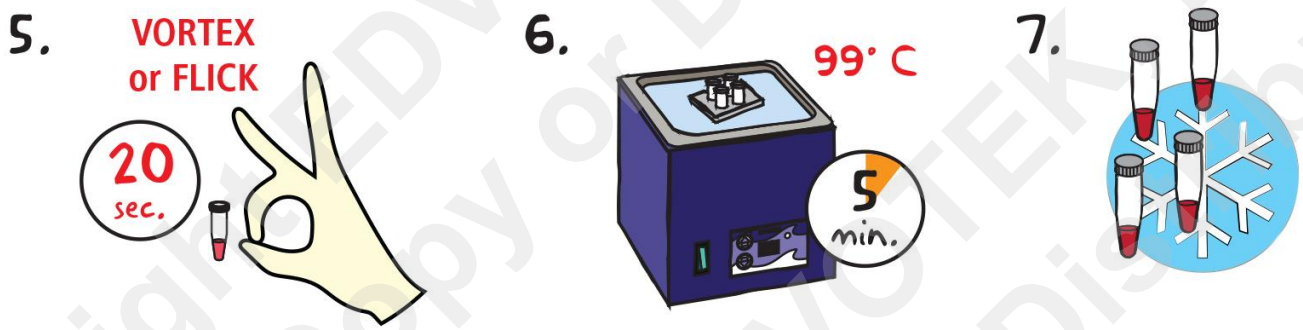
4 0.5 mL screw-cap tubes containing contaminated water samples (one of each)

4 1.5 mL screw-top tubes containing Lysis Buffer, on ice



- Step 1: Label tubes with bacteria
- Step 2: Add 100 uL water/bacteria sample to lysis buffer
- Step 3: Incubate at 55 deg. C for 5 min

Isolation of DNA from Bacteria



- Step 5: Vortex or flick to mix
- Step 6: Incubate at 99 deg. C for 5 min
- Step 7: Place on ice and proceed to PCR

Have the old freezer bag? No problem!

Components	Storage	Check (✓)
• PCR EdvoBeads™ *Each PCR EdvoBead™ contains dNTP mixture, Taq DNA polymerase buffer, Taq DNA polymerase, $MgCl_2$, and reaction buffer*	Room Temp., desiccated	❑
A Universal DNA Buffer	-20° C Freezer	❑
B TE buffer	-20° C Freezer	❑
C LyphoPrimer™ Mix *Contains E. coli-specific primers, Bacillus subtilis-specific primers, and Serratia marcescens-specific primers*	-20° C Freezer	❑
D LyphoControl™ (Complete PCR Control)	-20° C Freezer	❑
E EdvoQuick™ DNA ladder	-20° C Freezer	❑
F Proteinase K	-20° C Freezer	❑
~~G Potassium Acetate~~	~~-20° C Freezer~~	~~❑~~
~~H DNA Extraction Buffer~~	~~-20° C Freezer~~	~~❑~~
• *E. coli* BactoBeads™	4° C, desiccated	❑
• *Bacillus subtilis* BactoBeads™	4° C, desiccated	❑
• *Serratia marcescens* BactoBeads™	4° C, desiccated	❑

This experiment is designed for 6 groups of students.

LyphoPrimer™

LyphoControl™

IMPORTANT NOTE:

This protocol has been optimized to reduce time and improve results. Because of this, *components G and H will no longer be used.*

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Amplification of the bacteria

Teacher preparation:

Preparation For:	What to do:	When:	Time Required:
Module II: Amplification of the PTC Region	Prepare and aliquot various reagents (Primer, DNA template, ladder, etc.)	One day to 30 min. before performing the experiment.	30 min.
	Program Thermal Cycler	One hour before performing the experiment.	15 min.

- Student experiment should take 15-20 minutes.
- PCR <u>reactions</u> should be setup and run on same day.
- PCR <u>products</u> can be frozen and stored until needed.

DNA Amplification using PCR

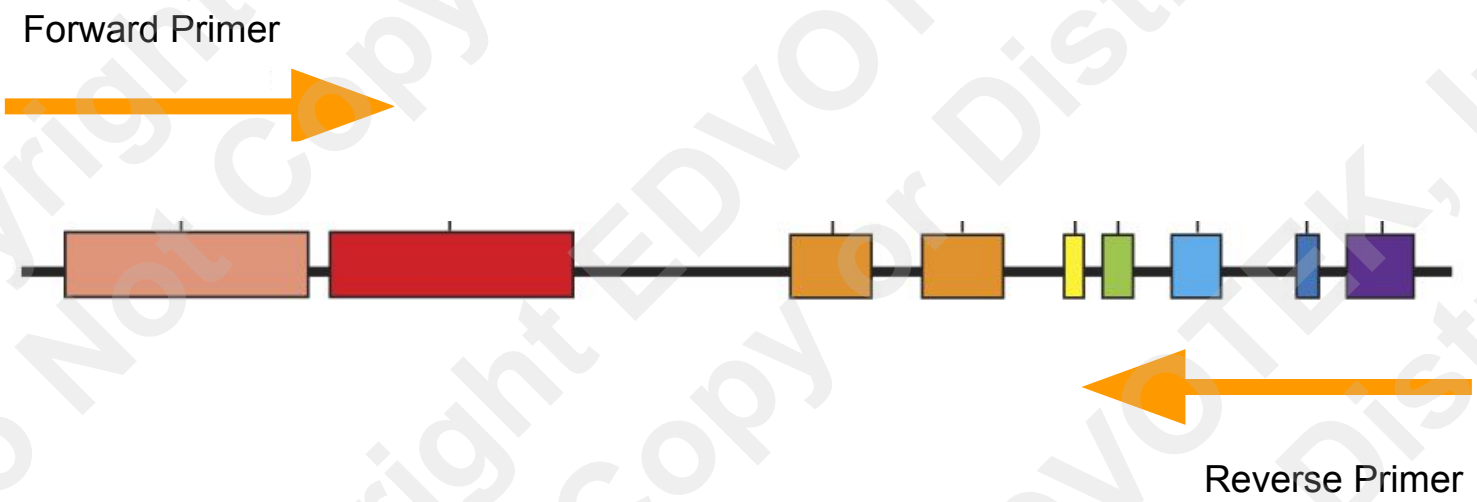
- DNA template
- Primers
  - Short piece of DNA that defines the area to amplify
- Thermostable DNA polymerase (*Taq*)
- Free nucleotides (dNTPs)
- Buffer

DNA Amplification using PCR

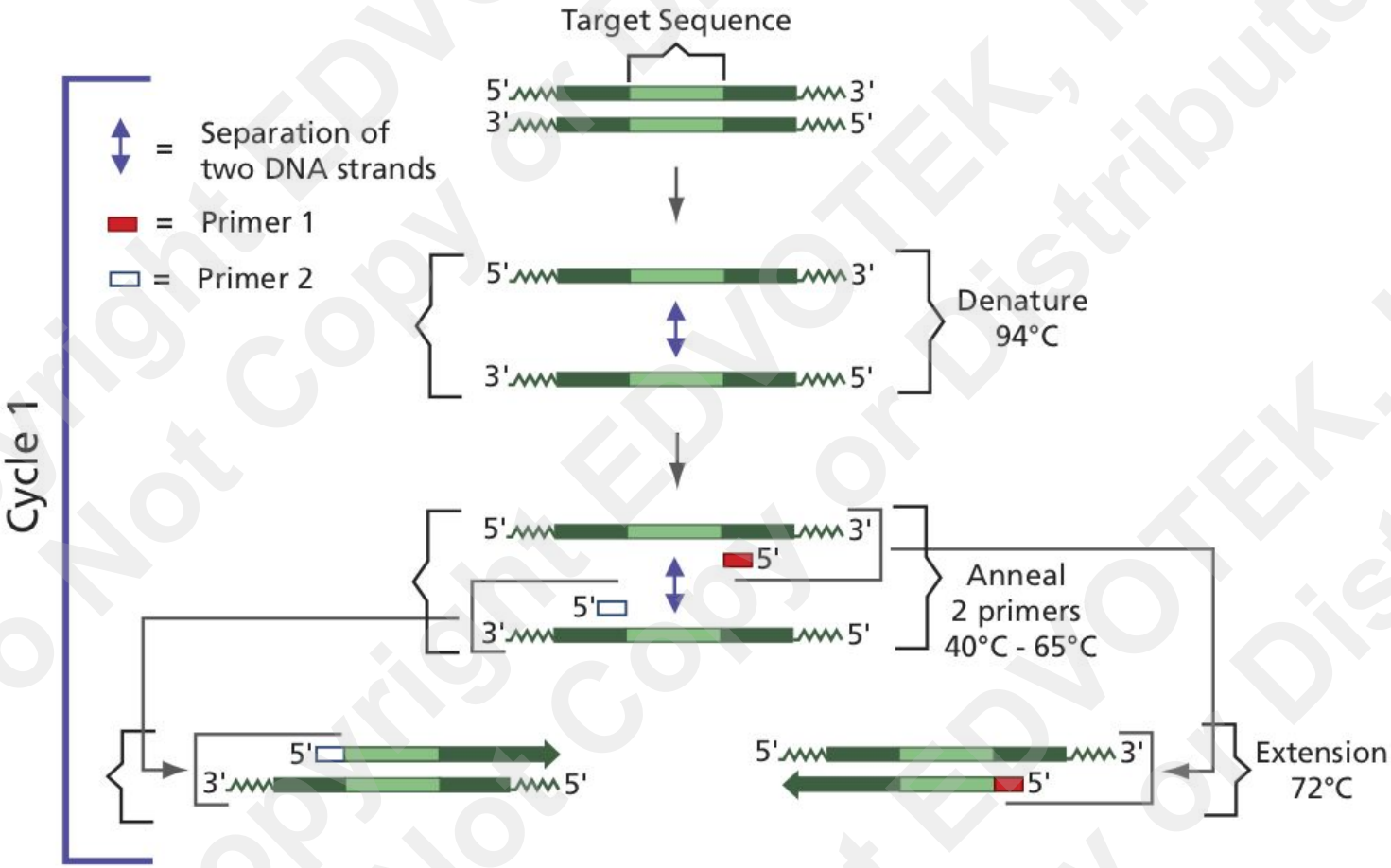
- DNA template
- Primers
  - Short piece of DNA that defines the area to amplify
- Thermostable DNA polymerase (*Taq*)
- Free nucleotides (dNTPs)
- Buffer

DNA Primers

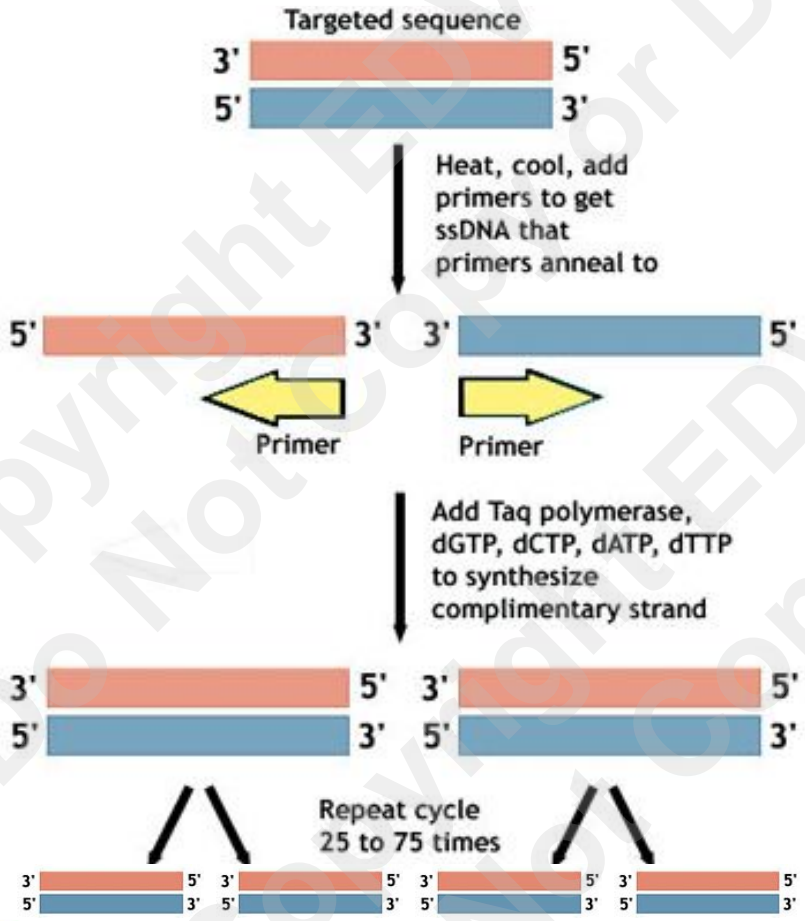
- 50 kb segment of Lambda Phage DNA



PCR Amplifies Specific DNA Sequences

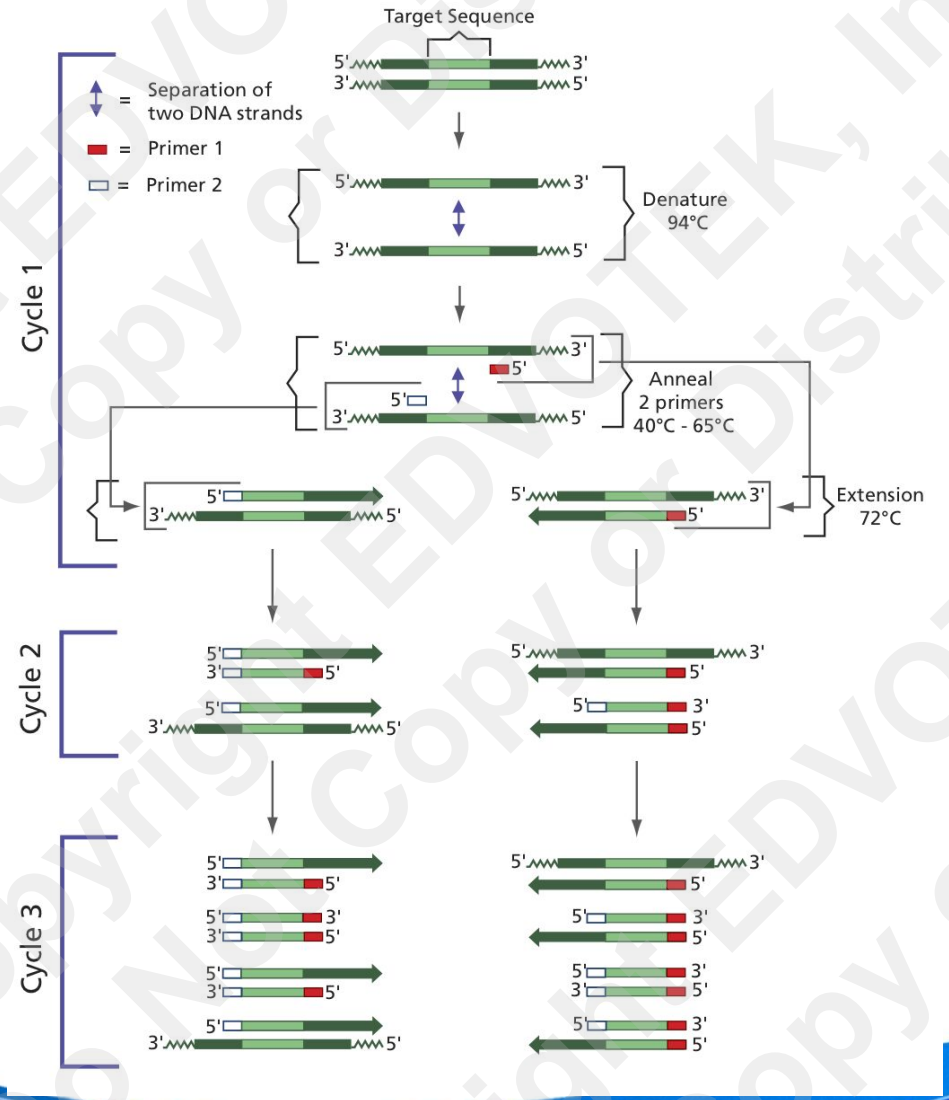


PCR Amplifies Specific DNA Sequences



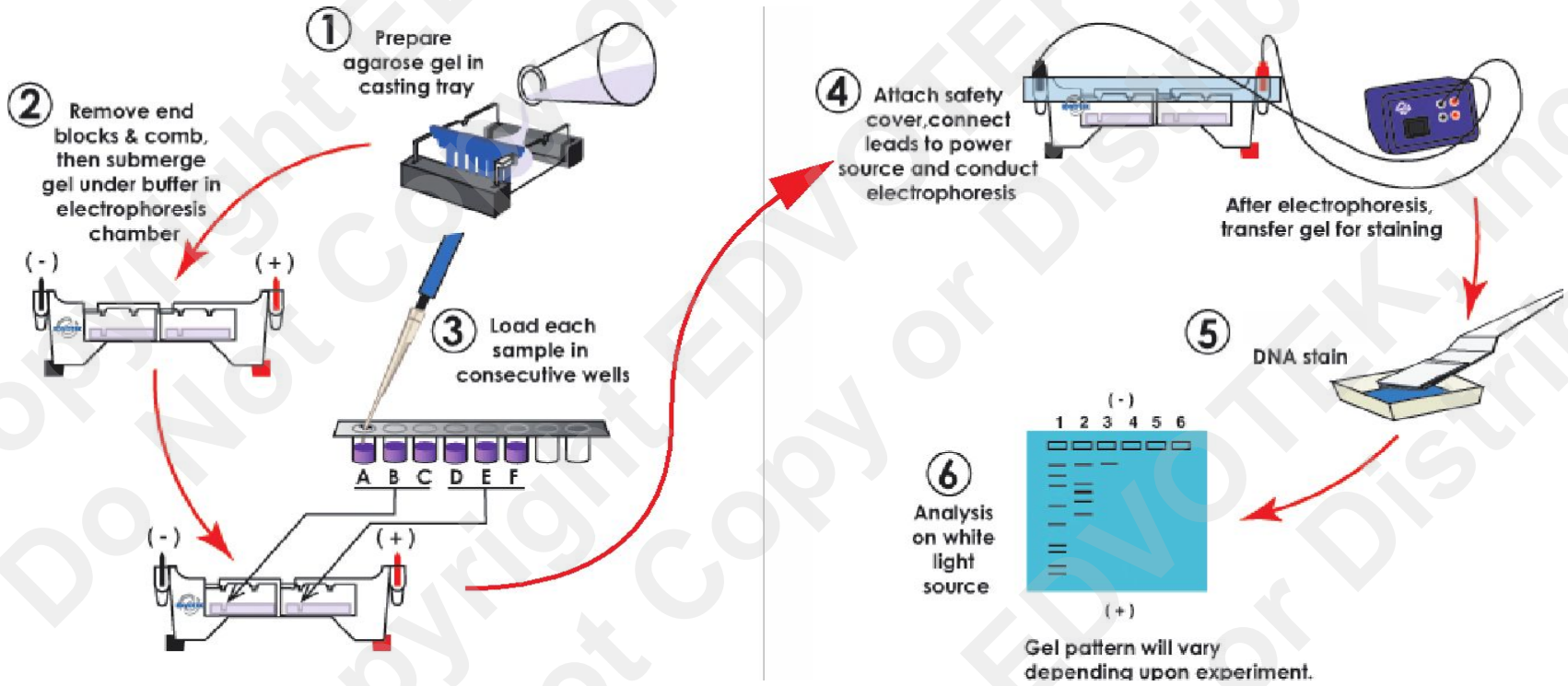
- **Denaturation (94°C)**
  Double-stranded DNA is "unzipped" into single strands.
- **Annealing (40-65°C)**
  Primers base pair with the target DNA sequence.
- **Extension (72°C)**
  Taq polymerase extends the primer and synthesizes a new strand of DNA.

PCR Amplifies DNA Exponentially



PCR Amplifies DNA Exponentially

Electrophoresis at a Glance



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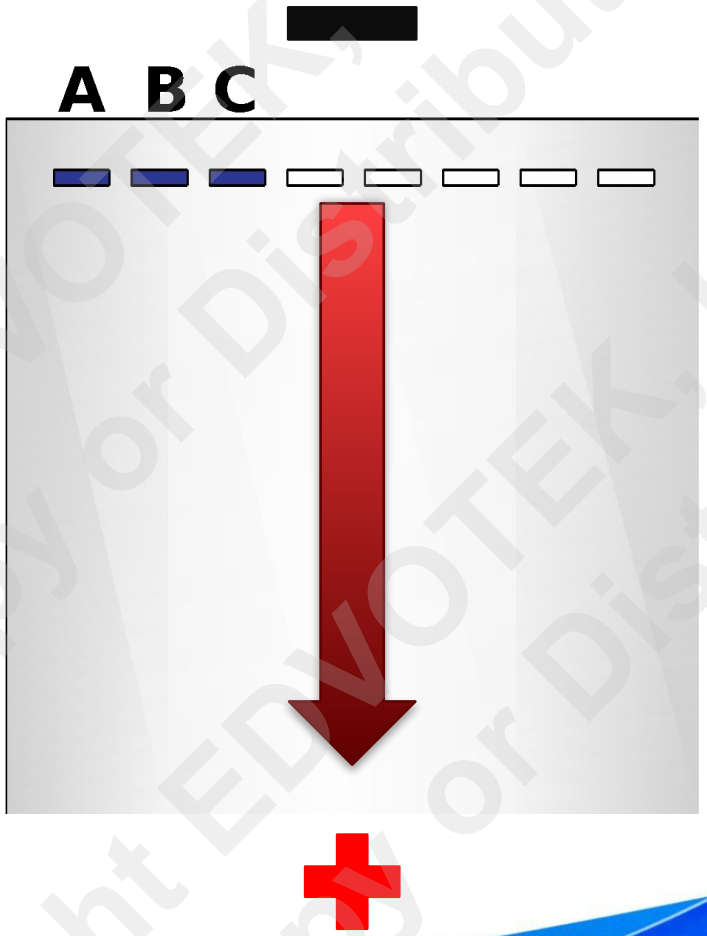
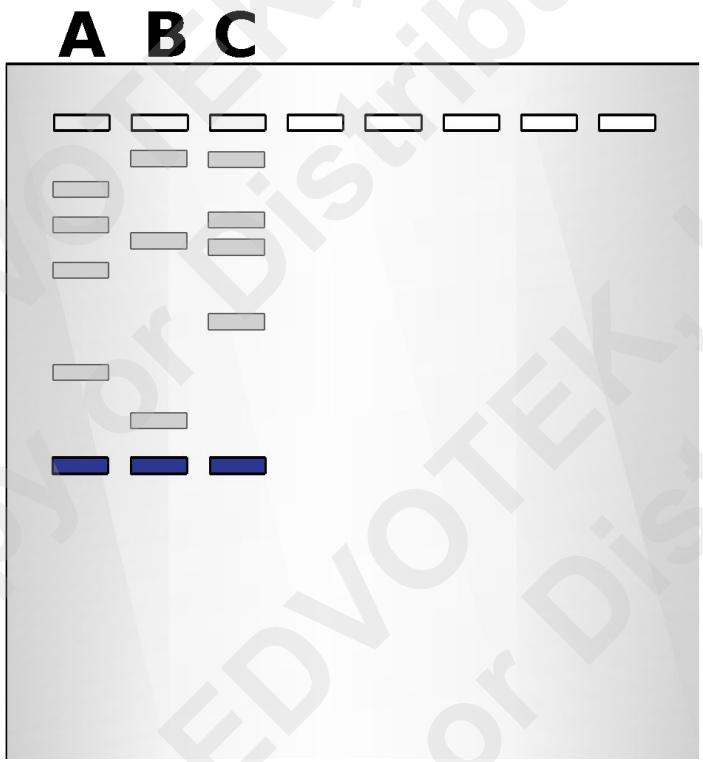
Electrophoresis Separates DNA Fragments By Size

- The sugar-phosphate backbone of DNA has a strong negative charge.
- When an electrical current is passed through the gel, the current drives the DNA fragments through the gel towards the positive electrode.



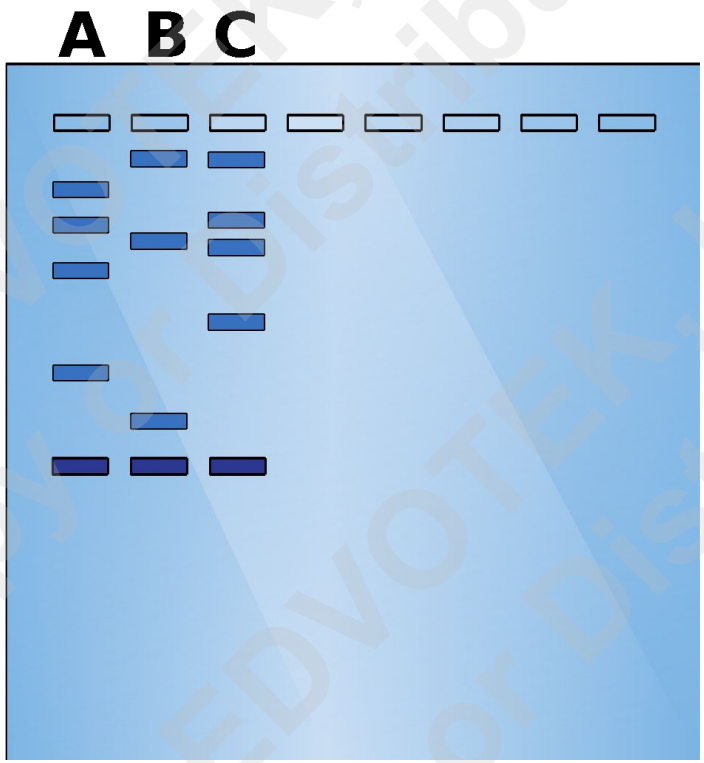
Electrophoresis Separates DNA Fragments By Size

- The gel contains small channels through which the DNA can pass.
- Small DNA fragments move through these holes easily, but large DNA fragments have a more difficult time squeezing through the tunnels.



Electrophoresis Separates DNA Fragments By Size

- Because molecules of different sizes travel at different speeds, discrete bands are formed.
- After the current is stopped, the bands can be visualized using a stain that sticks to DNA.
  - InstaStain ® EtBr
  - InstaStain ® Blue
  - FlashBlue™ Stain
  - Sybr® Safe stain



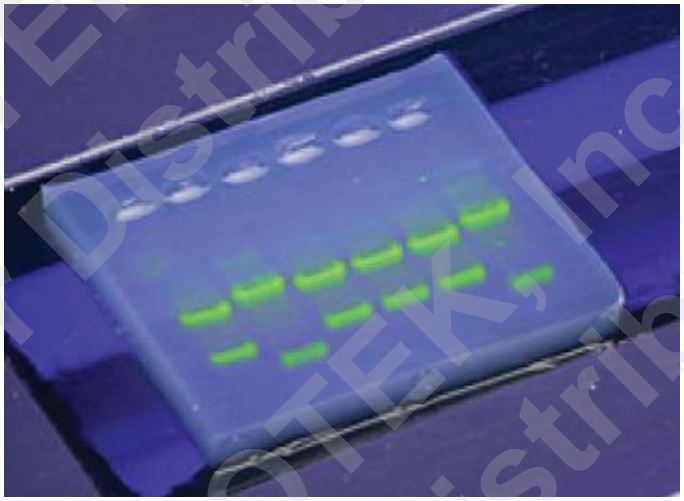
SYBR® Safe DNA Stain

In-gel Staining

1. Melt agarose and cool to 65°C.
2. Add concentrated Sybr® Safe stain to the molten gel at 1:10,000 dilution (5 µL per 50 mL agarose solution).
3. Run DNA samples through gel – no post staining or destaining necessary!

Post-electrophoresis Staining

1. Dilute concentrated stain to 1:20,000 (5 µL per 100 mL distilled water).
2. After electrophoresis, place gel in tray. Cover gel with diluted Sybr® Safe stain.
3. Stain gel for 10 – 15 minutes.



Kit #109
Transilluminator #558
SybrSafe® Stain #608

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TruBlu2™ Bluelight Transilluminator

• Optimized for **SYBR® Safe** stained gels • Option for blue or white light •
• Large viewing area • No harmful UV •

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Multiplex PCR Testing of Water Contaminants

Lane	Sample
1	EdvoQuick™ DNA Ladder
2	Extracted DNA - E. coli
3	Extracted DNA - Serratia
4	Extracted DNA - B. subtilis
5	Extracted DNA - Serratia & E. coli
6	Control DNA - E. coli

EDVO-Kit #953

REVISED & UPDATED

Edvo-Kit #

345

Edvo-Kit #345

Exploring the Genetics of Taste: SNP Analysis of the PTC Gene Using PCR

Experiment Objective:

In this experiment, students will isolate their own DNA and use PCR to amplify a segment of the *TAS2R38* gene, which is responsible for detecting the bitter taste of PTC. Digestion of the PCR products and analysis by agarose gel electrophoresis are used to differentiate tasters and non-tasters. Finally, students' genotype is linked to phenotype by tasting the PTC paper.

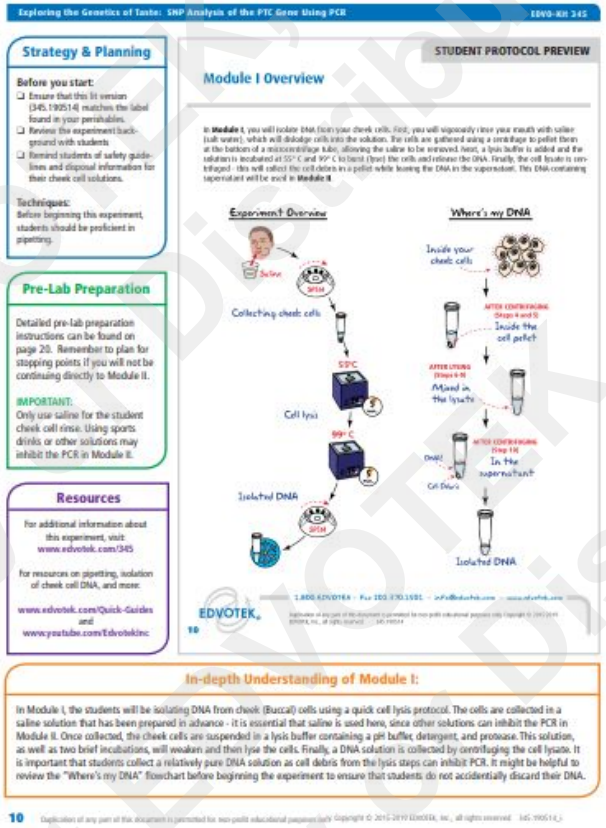
LyphoPrimer™

LyphoControl™

See page 3 for storage instructions.

PTC Improvements

- Lyophilized reagents increase stability
- Multiple stopping points
- Complete Control
- Larger volumes
- PCR cycling time reduced
- Instructor guide



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Instructor's Guide

EDVO-Kit 345 — Exploring the Genetics of Taste: SNP Analysis of the PTC Gene Using PCR

STUDENT PROTOCOL PREVIEW

Module I: Isolation of DNA from Human Cheek Cells

1. **LABEL** an empty 1.5 mL screw top microcentrifuge tube and a cup of saline with your lab group and/or initials.
2. **RINSE** your mouth vigorously for 60 seconds using 10 mL saline solution. **EXPEL** the solution back into the saline cup.
3. **SWIRL** the cup gently to resuspend the cells. **TRANSFER** 1.5 mL of the cell solution into the tube with your initials.
4. **CENTRIFUGE** the cell suspension for 2 minutes at full speed to pellet the cells. **POUR** off the supernatant (the liquid above the cell pellet) but **DO NOT DISTURB THE CELL PELLET!**
5. **REPEAT** steps 3 and 4 once more.
6. **RESUSPEND** the cheek cell pellet in 150 µL lysis buffer by pipetting up and down or by vortexing vigorously. *NOTE: Ensure that the cell pellet is fully resuspended and that no clumps of cells remain.*
7. **CAP** the tube and **PLACE** it in a water bath (hot). **INCUBATE** the sample in a 55° C water bath for 5 minutes.
8. **MIX** the sample by vortexing or by flicking the tube vigorously for 20 seconds.
9. **INCUBATE** the sample in a 99° C water bath for 5 minutes. *NOTE: Students MUST use screw-cap tubes when boiling samples.*
10. **CENTRIFUGE** the cellular lysate for 2 minutes at full speed.
11. **TRANSFER** 50 µL of the supernatant to a clean, labeled microcentrifuge tube. **PLACE** the tube in ice.

The extracted DNA is now ready for Module II: Amplification of the PTC Region. If you are ready to proceed, turn to page 12. Alternatively, the extracted DNA may be stored in the **FREEZER** (-20° C) until needed.

OPTIONAL STOPPING POINT: The extracted DNA may be stored in the freezer (-20° C) until needed.

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11

Safety

Human saliva may transmit disease. We recommend sterilizing all human samples with 10% bleach after completing the experiment.

Remind students to wear gloves and goggles while working in the lab, and to leave the lab area before eating or drinking (after the saline rinse).

Boiling water is hot, use caution!

Strategy & Planning

1) You must use saline for the rinse!

5) After centrifuging you should see at least a faint pellet in each student's tube. If not, ask the students to swirl their remaining saline solution to resuspend settled cells and repeat the steps 3 and 4 again.

6) Ensure cells are fully resuspended - no clumps!

9) This does not need to be a rolling boil, but we want the water as hot as possible.

11) Ensure that there is no debris transferred with the DNA supernatant. If you notice any dumps or cloudiness in the samples they can be centrifuged again to pellet any debris.

Vocabulary Review

Saline - A salt water solution.
Resuspend - Place a solid back into solution.
Supernatant - The liquid layer above a sediment or solid.
Incubate - To maintain a solution at an ideal condition or temperature.

Experimental Assessment

Ask the students to critically analyze each step of the protocol. For example: Why is the saline rinse centrifuged twice, or, why do we only collect 50 µL of the supernatant in step 11?

Stopping Point

DNA is very stable when frozen. Once the students have completed this module, their samples can be stored in the freezer (-20° C) until needed in Module II.

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Exploring the Genetics of Taste: SNP Analysis of the PTC Gene Using PCR — EDVO-Kit 345

Before You Start

Picking up where you left off:

- ❑ If the samples were frozen at the end of Module I, they can be thawed on ice, or briefly held between the student's fingers.
- ❑ Remind students that these are their extracted DNA samples which will be used for the PCR.
- ❑ Review the steps of the protocol, ensure that each student or pair of students has the necessary reagents.
- ❑ Ensure that the thermal cycler is programmed with the correct protocol and is functioning properly.

Pre-Lab Preparation

Detailed pre-lab preparation instructions can be found on page 21. Remember to plan for a stopping point if you will not be continuing directly to Module III.

IMPORTANT: Prepare the controls before beginning the student protocol (see page 21). Controls should be run alongside student samples if possible.

Strategy & Planning

A properly mixed PCR reaction will be a light orange color after step 4. This is a good checkpoint to ensure that students have added the template and primers in the correct volumes.

Once the reaction mixture has been prepared (after step 4) we recommend proceeding through the PCR before stopping the experiment at the end of the module.

PCR is incredibly sensitive - each student must use a fresh pipet tip when mixing their samples.

STUDENT PROTOCOL PREVIEW

Module II: Amplification of the PTC Region

Now that you have isolated your DNA, during Module I, the next step is to amplify a specific region of the DRD2/ANK gene. First, you will combine your DNA (red) with a mixture of PTC primers (yellow) and a PCR EdvoBead™ PLUS, creating a PCR sample. Once this sample has been prepared it will be placed into a thermal cycler and the DNA will be amplified by PCR.

1. **OBTAIN** the red extracted DNA from Module I.
2. **LABEL** a fresh 0.2 mL PCR tube with your initials.
3. **ADD** 48 µL PTC primer mix (yellow), 10 µL extracted DNA (red), and a PCR EdvoBead™ PLUS.
4. **MIX** the PCR sample. Make sure the PCR EdvoBead™ PLUS is completely dissolved. If mixed correctly, the final solution will be light orange.
5. **CENTRIFUGE** the tube for a few seconds to collect the sample at the bottom of the tube.
6. **AMPLIFY** the DNA using PCR.
   PCR cycling conditions:
   - Initial denaturation: 94° C for 4 minutes
   - 94° C for 30 seconds
   - 65° C for 30 seconds } 35 cycles
   - 72° C for 30 seconds
   - Final extension: 72° C for 5 minutes

The amplified DNA is now ready for Module III: Restriction Digest of the PTC PCR Product. If you are ready to proceed, turn to page 13. Alternatively, the amplified DNA may be stored in the **FREEZER** (-20° C) until needed.

NOTE: If your thermal cycler does not have a heated lid, it is necessary to overlay the PCR reaction with oil to aid to prevent evaporation. Visit our website for more information.

OPTIONAL STOPPING POINT: The PCR samples may be stored at -20° C for restriction digest at a later time.

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Preparing Students for PCR:

PCR is a ubiquitous technique that has become a cornerstone of modern biotechnology. Despite this, many of the details of the PCR process can be obscured behind the relatively simple protocol. Ensure that your students fully understand the science behind PCR by carefully reviewing the background information or incorporating supplemental lessons.

Additional PCR lesson plans can be found at:
www.edvotek.com/site/pdf/PCR_Lesson_Plan.pdf

12

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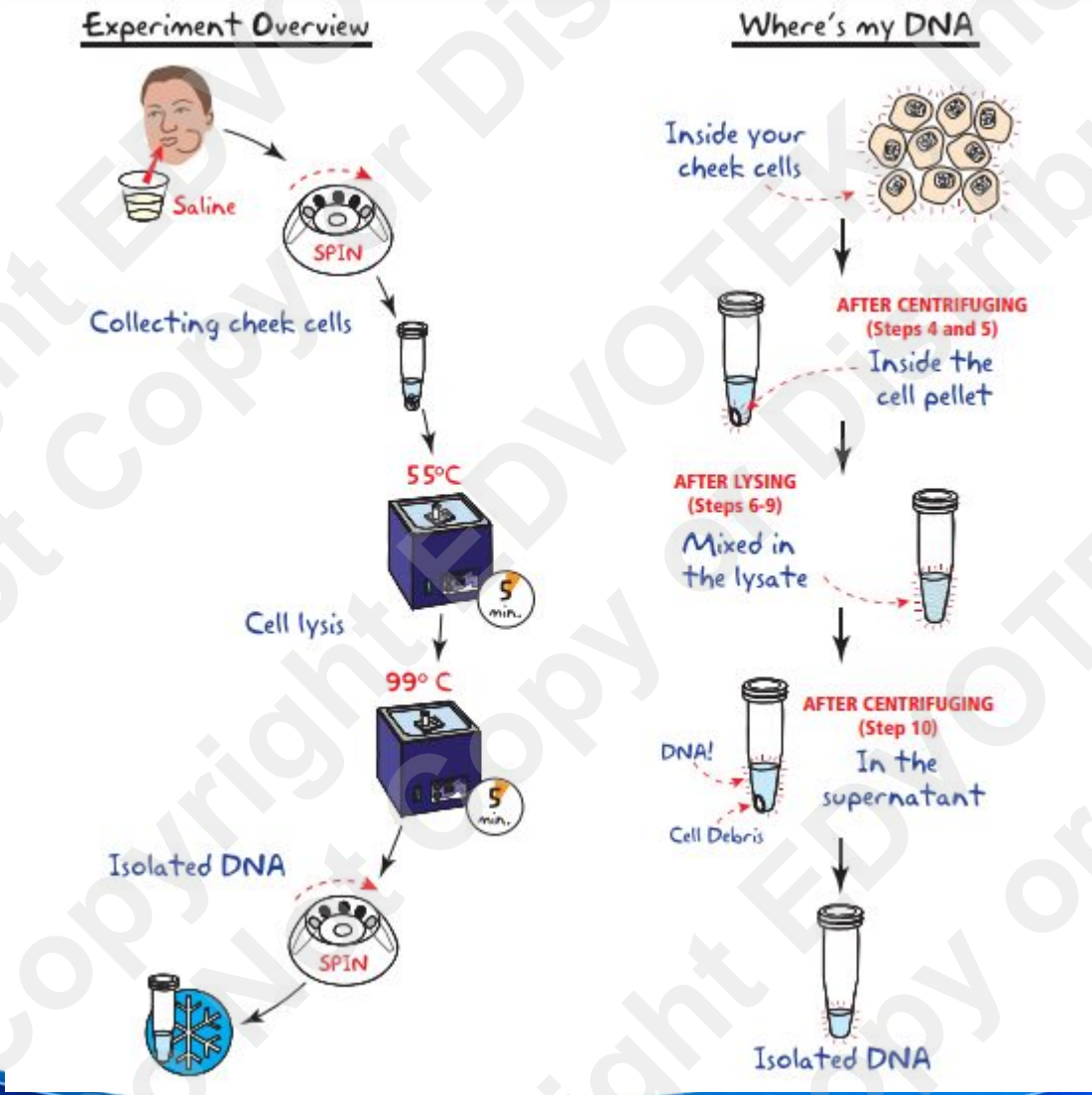
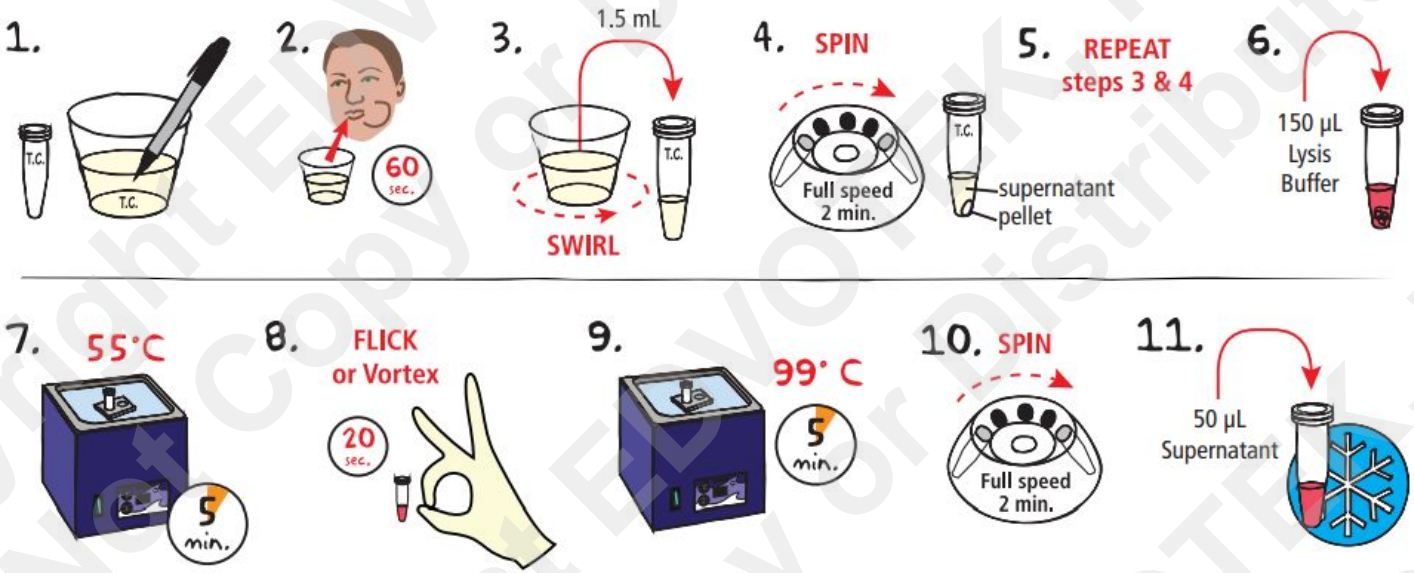
Where's my DNA?

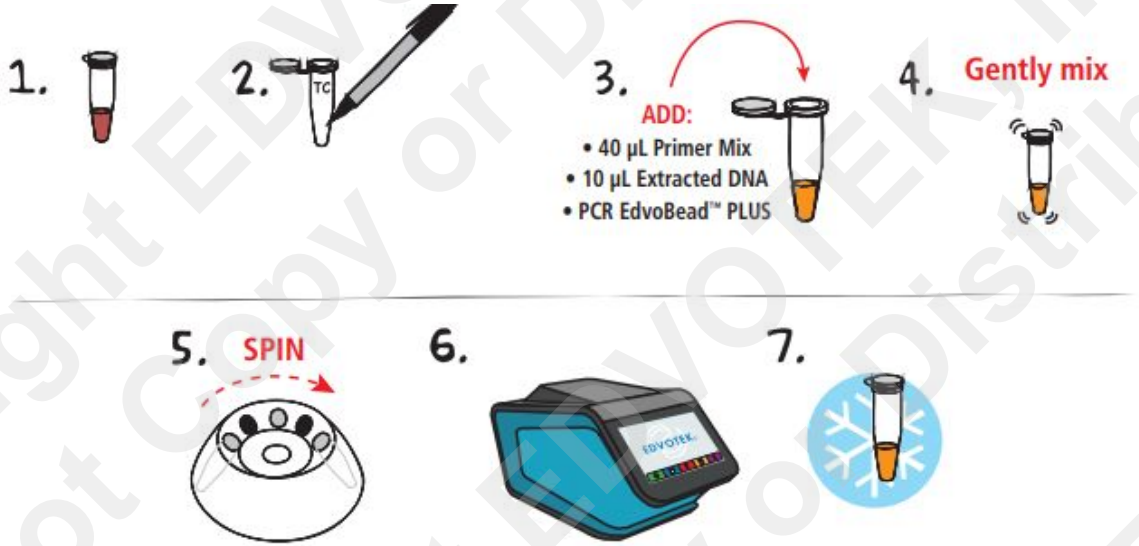


Isolation of DNA from Cheek Cells



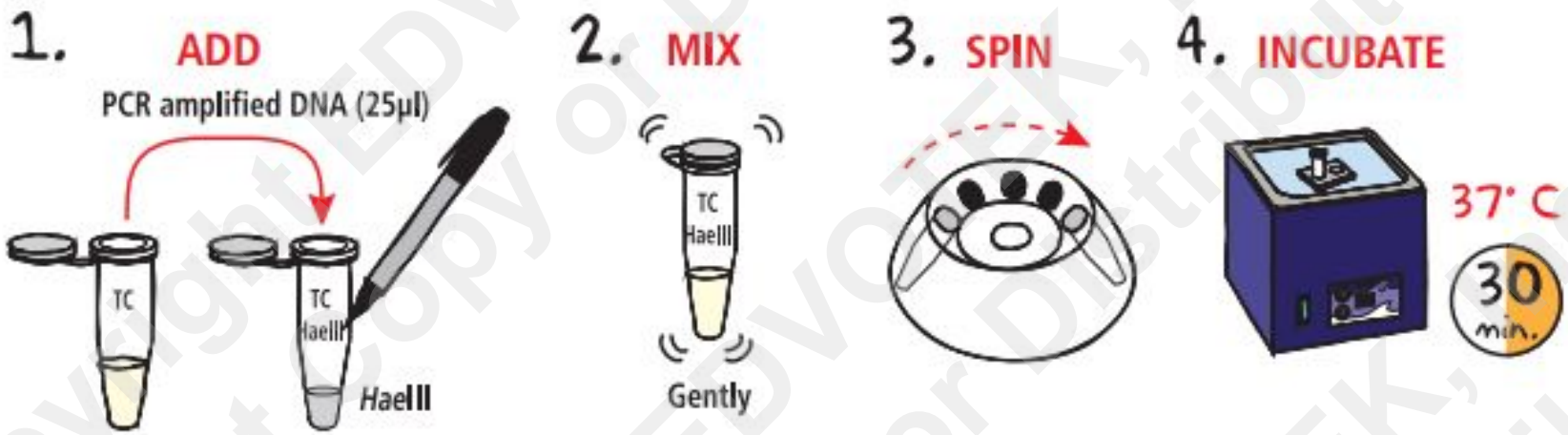
- Tip #1: Make sure the pellet is visible
- Tip #2: Resuspend VERY well in lysis buffer
- Tip #3: Water bath needs to be boiling

Isolation of DNA from Cheek Cells



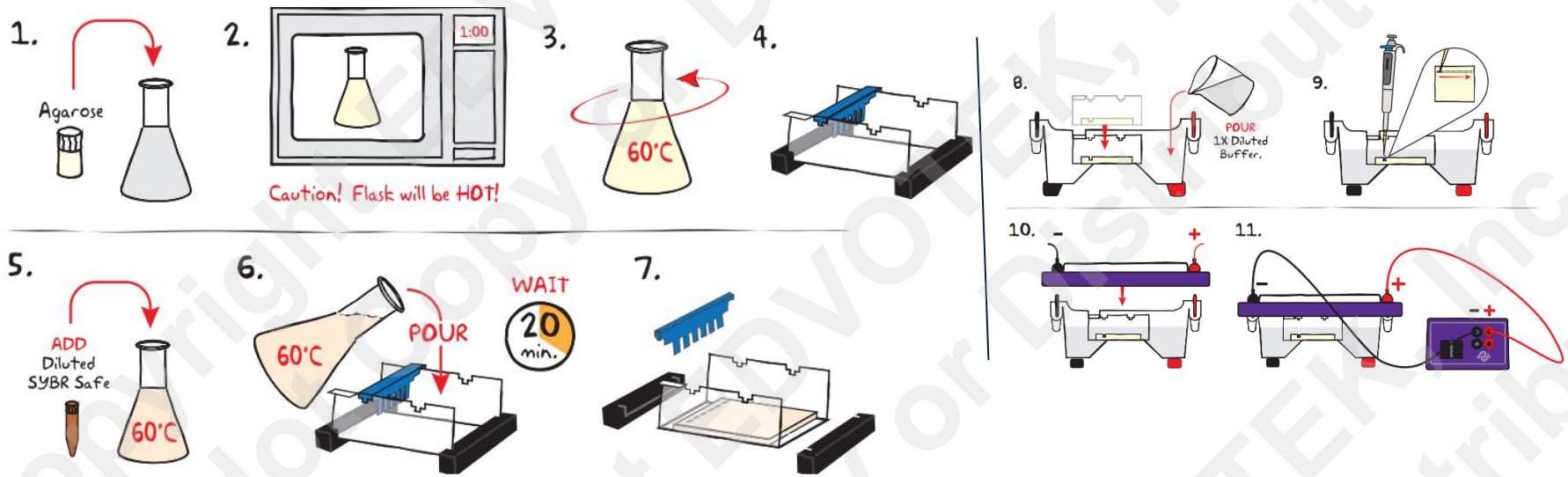
- Tip #1: Run the Complete Control
- Tip #2: Students can use gloves to transfer PCR beads
- Tip #3: PCR product should be light orange

Isolation of DNA from Cheek Cells



- Tip #1: Perform digestion with the control
- Tip #2: Incubate for the whole 30 minutes
- Tip #3: Tubes can be incubated for longer than 30 min

Isolation of DNA from Cheek Cells



- Tip #1: Use Sybr Safe
- Tip #2: Use the DNA with the kit
- Tip #3: Load the Complete Control in lane 2
- Tip #4: Watch the gel closely - bands are small

Edvotek PLTW Experiments

Kit	Description
118-PLTW	Hypercholesterolemia Kit
225-PLTW	
235-PLTW	
268-PLTW	
301-PLTW	
303 - PLTW	
339 - PLTW	
990-PLTW	
116	
330	
345	
951	
953	
956	Bioremediation by Oil-eating Bacteria Kit

Mistakes ~~Hurt!~~ Happen!

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