Polymerase Chain Reaction: teaching PCR in one class period

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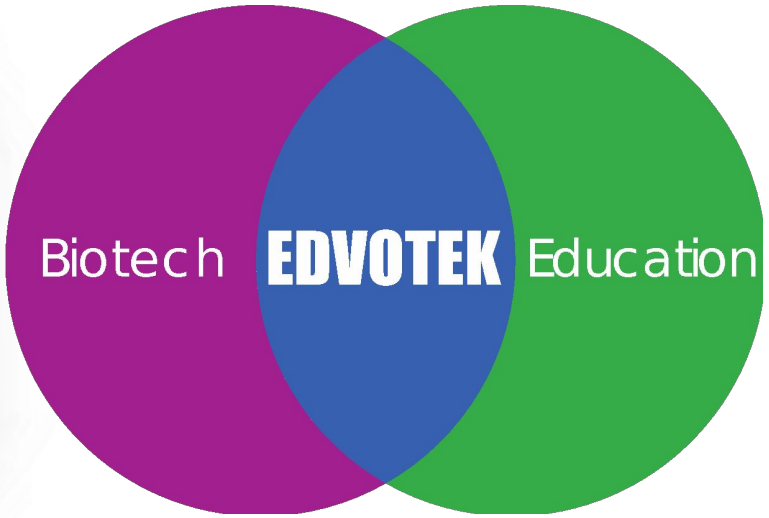

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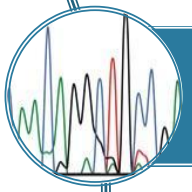
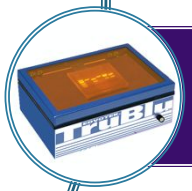
Experiments


Reagents


Equipment


Resources

DNA carries genetic information

- DNA is a double-stranded molecule carrying all genetic information
- Every cell contains DNA, but not enough for scientists to analyze by itself
- For scientists to examine DNA, it must be amplified using the Polymerase Chain Reaction (PCR)

Today's Workshop

1. Run a PCR on lambda DNA

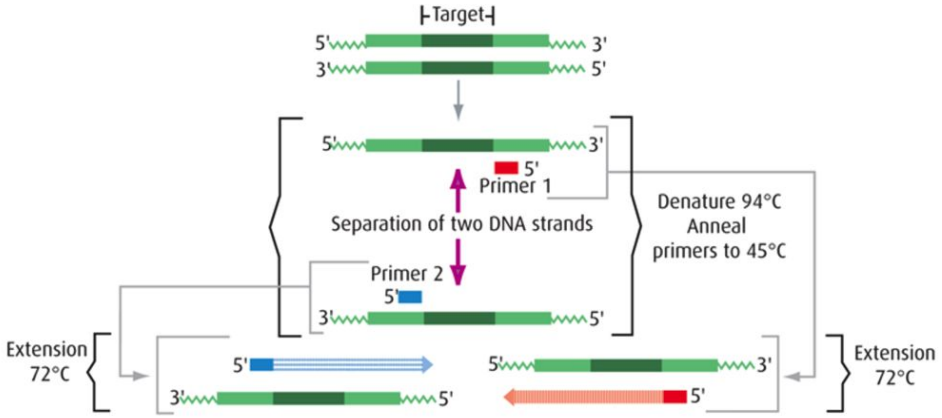
2. Discuss PCR

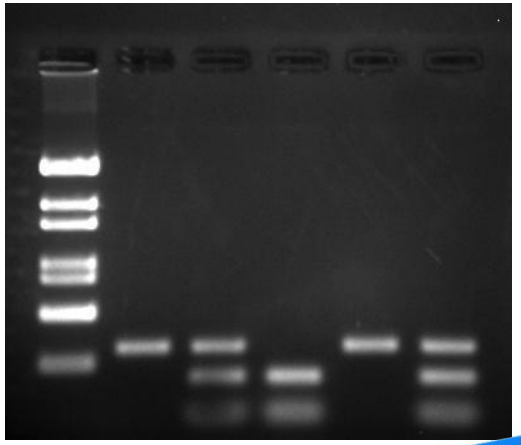
3. Go over agarose gel electrophoresis

4. Run gels

5. Extract DNA

6. Visualize samples





Running the PCR

- For a successful PCR you need:
  - DNA

5 uL Lambda DNA (blue)

  - Primers

20 uL Primers (yellow)

  - dNTPs
  - Taq
  - MgCl

PCR Edvobead

Green PCR:
Mix ready-to-run

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

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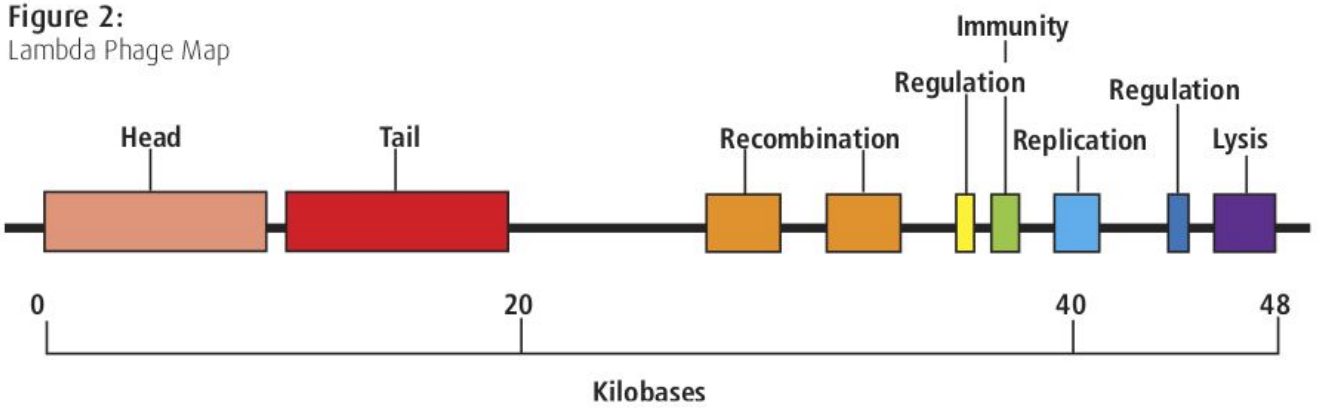
DNA Templates

- Lambda Phage DNA



- Extracted DNA from cheek cells: PTC taste (345), mitochondrial DNA (332), Alu (333), VNTR (334)
- Bacterial DNA: water contamination (953), transformation (321)
- GMO food (962)

DNA Amplification using PCR

- DNA template
- Primers
  - Short piece of DNA that defines the area to amplify
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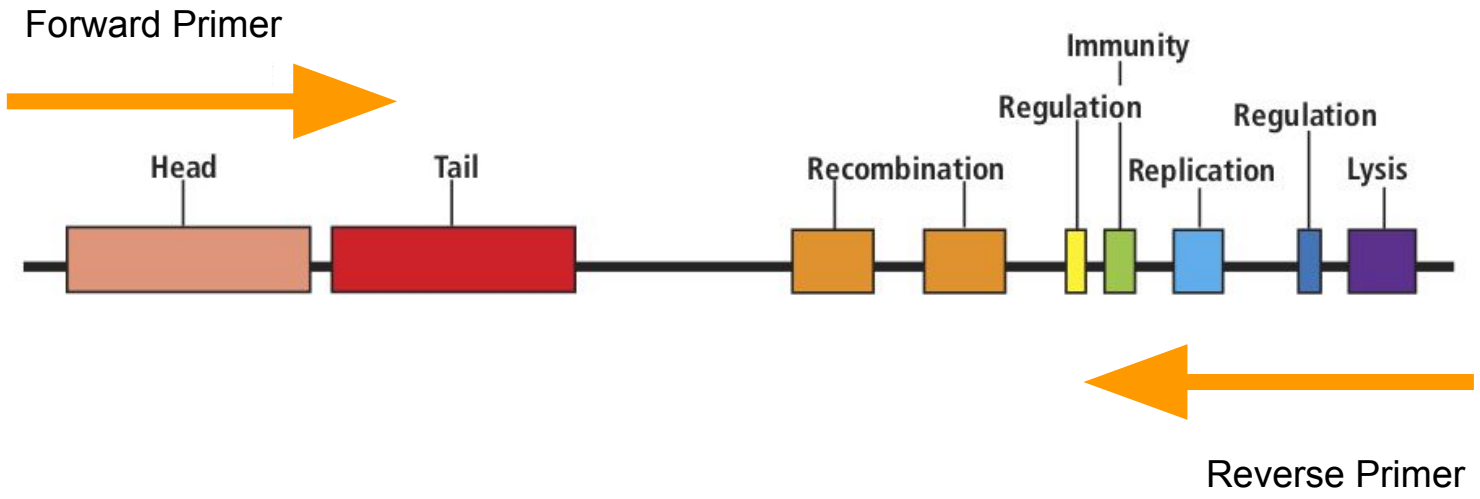
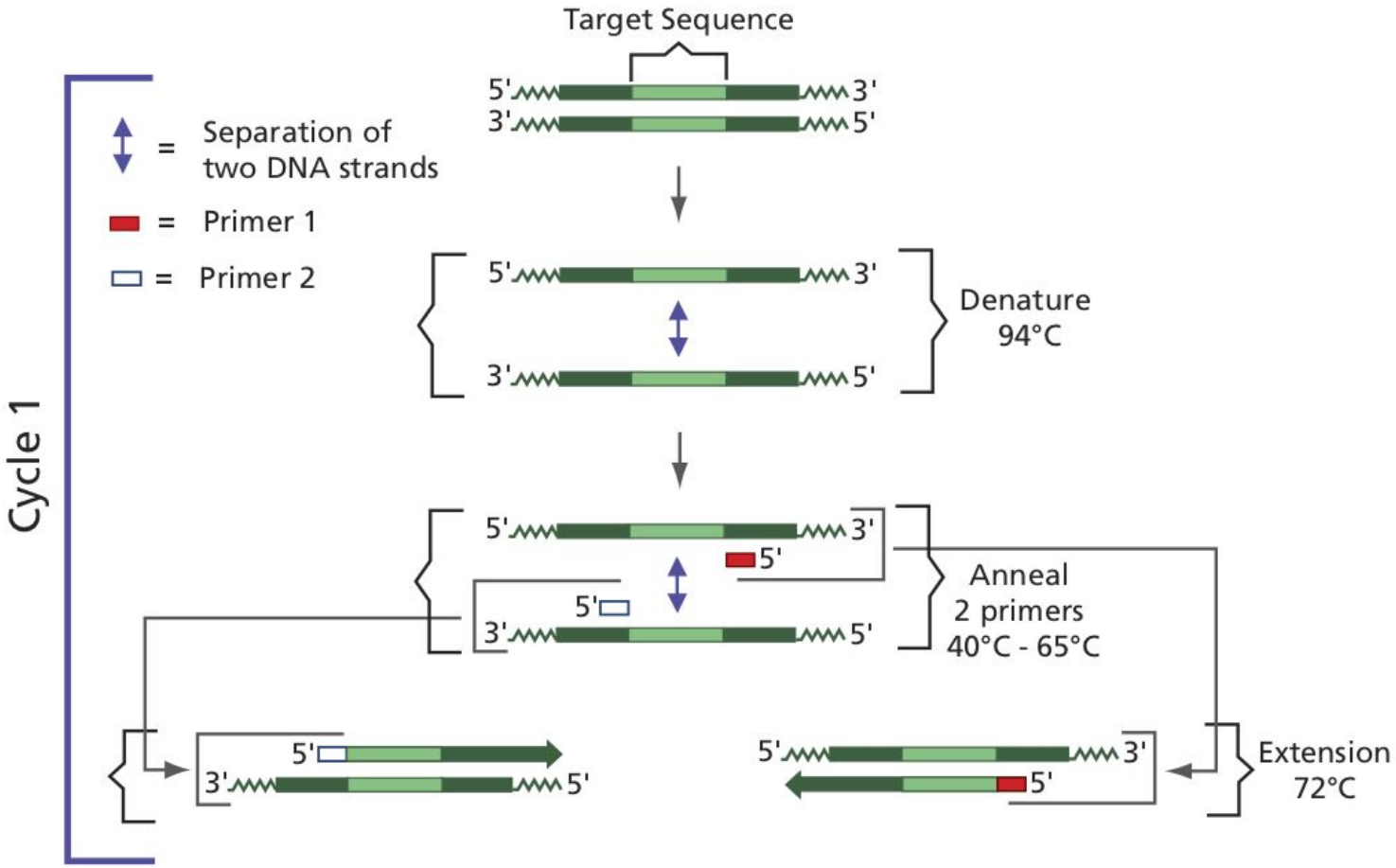
DNA Primers

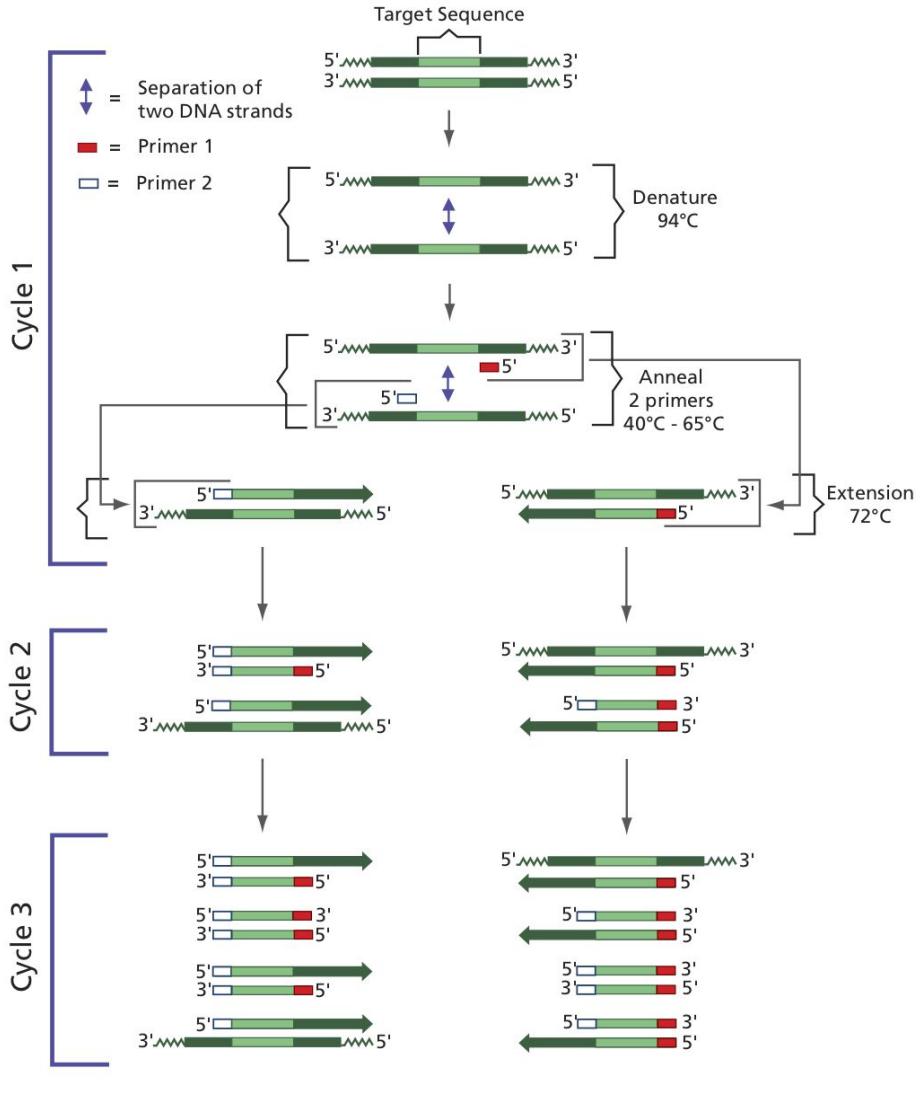
- 50 kb segment of Lambda Phage DNA



PCR Amplifies Specific DNA Sequences



PCR Amplifies DNA Exponentially



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Any questions so far?

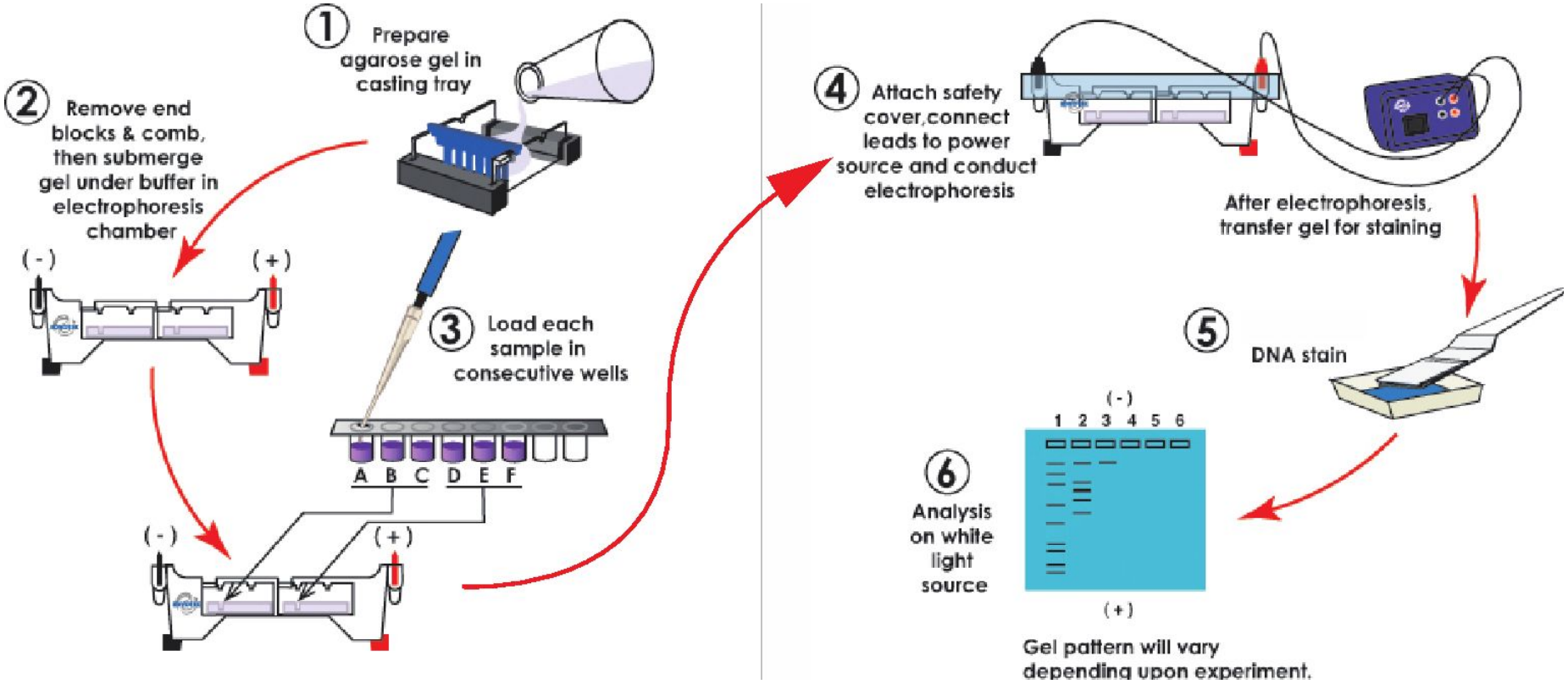
PCR Edvo Beads

Fast. Easy. Refreshing.

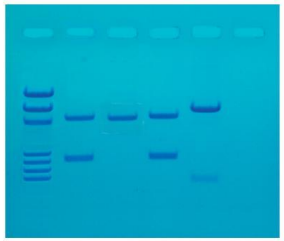
Amplifying and visualizing DNA

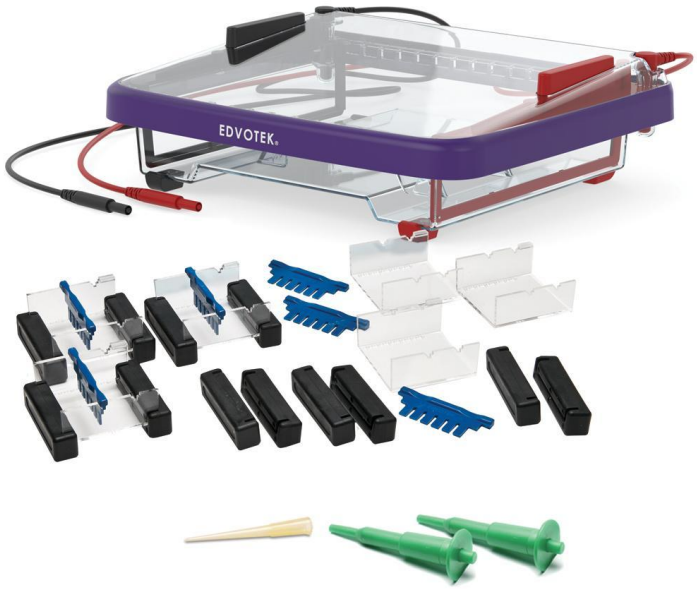
- Step 1: DNA is extracted from a cell
- Step 2: DNA is amplified using PCR
- Step 3: DNA is run on an agarose gel

Summary of Electrophoresis



What Do I Need to Perform Electrophoresis Experiments?





LabStation™ #5062

- Horizontal electrophoresis apparatus
- D.C. power source
- Micropipet or transfer pipet
- Agarose
- Electrophoresis Buffer
- Samples – dye, DNA, RNA
  - PCR products
- A way to visualize samples

Casting the Agarose Gel

1. Prepare the tray for gel casting by sealing the ends with rubber end caps.

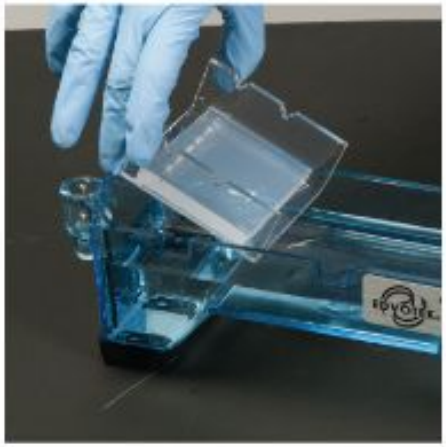
2. Place a comb in the appropriate notches.

3. Prepare the agarose gel solution. Cool to 60°C and then pour the gel.

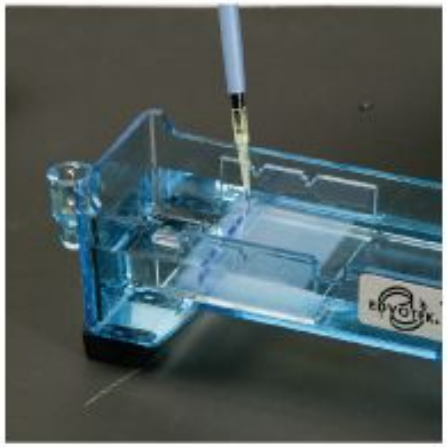
4. After approx. 20 min. the gel will solidify. Remove the comb from the gel tray.

Performing Electrophoresis

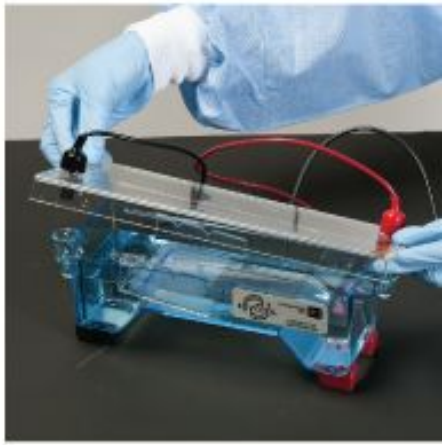
5. Slowly remove the rubber end caps. Be very careful not to damage or tear the gel!



6. Place the gel (on its tray) into the electrophoresis chamber. The gel should be completely submerged under electrophoresis buffer.

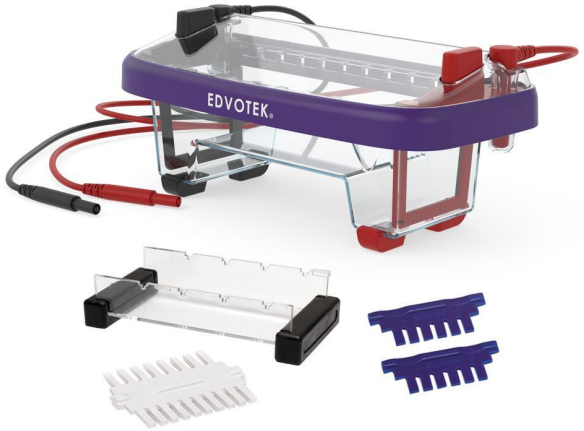


7. Load samples into wells in consecutive order, starting with the first well on the left.

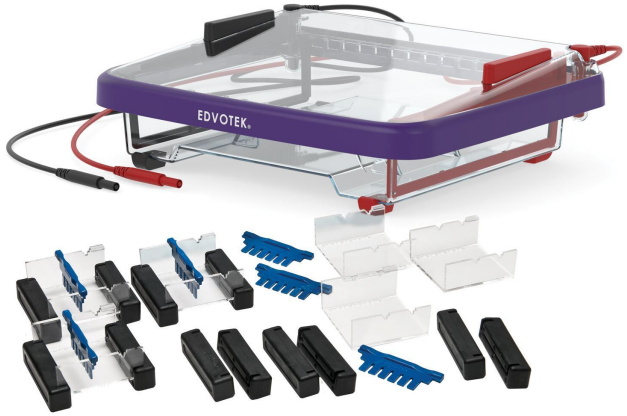


8. After samples are loaded, attach the safety cover, connect the leads to the D.C power source and set the power source at the required voltage.

Electrophoresis Chambers for Classrooms of all Sizes



Cat. # 502
Model M12
Two gels



Cat. # 515
Model M36
Six gels

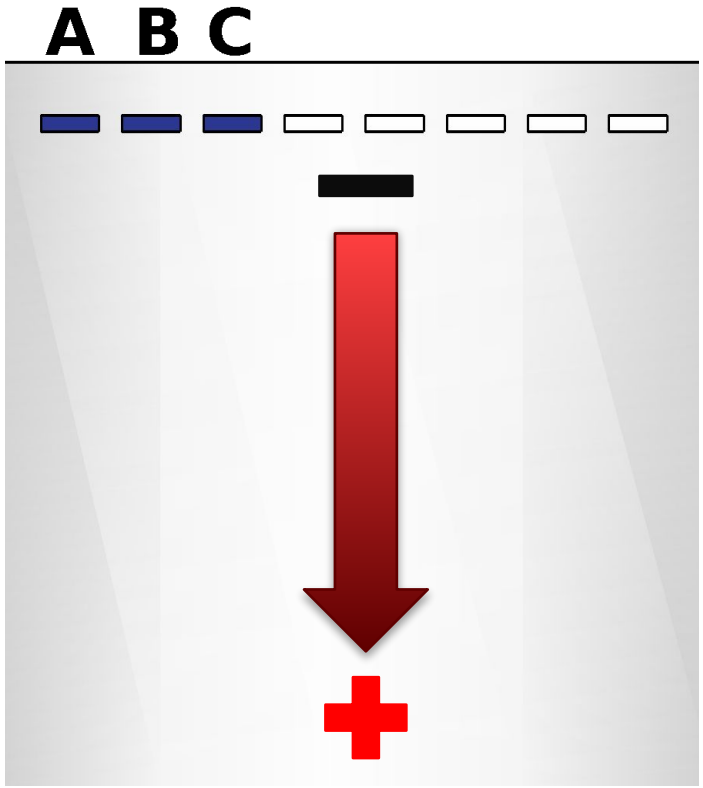
Power Supplies Provide Current for Electrophoresis

Cat. #509
DuoSource™ 150
(75/150 V)

Cat. #5010
TetraSource
(10-300V)

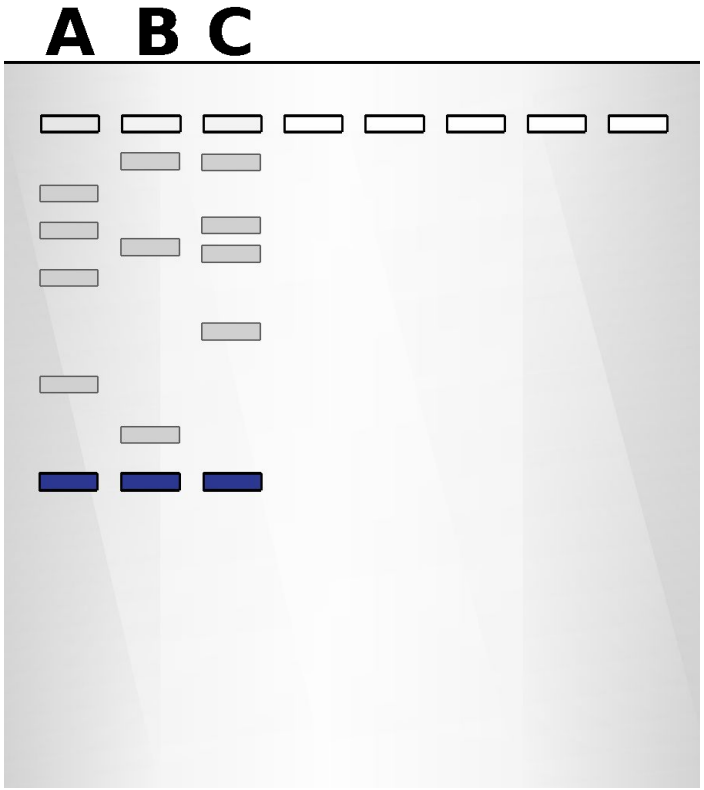
Electrophoresis Separates DNA Fragments By Size

- 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.



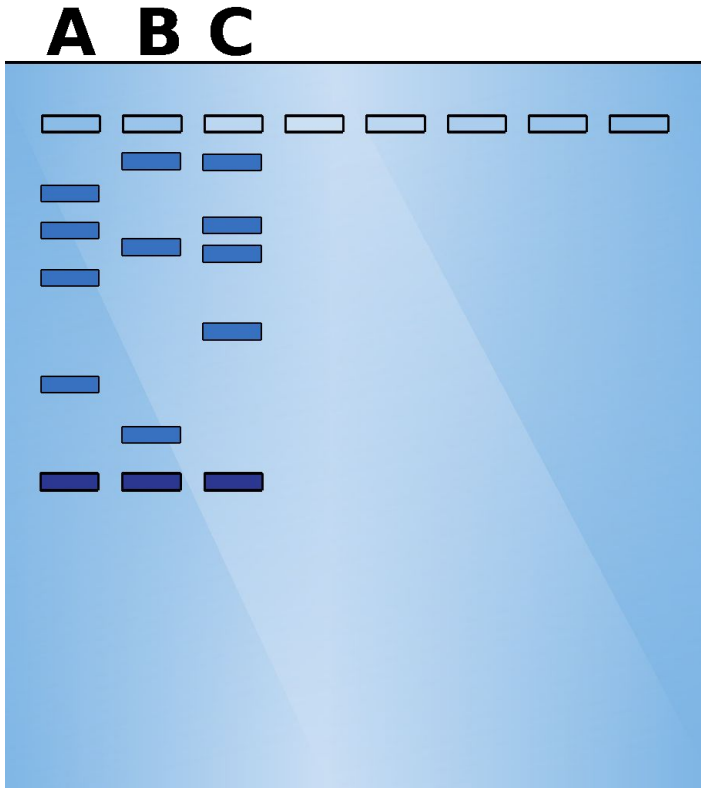
Electrophoresis Separates DNA Fragments By Size

- 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

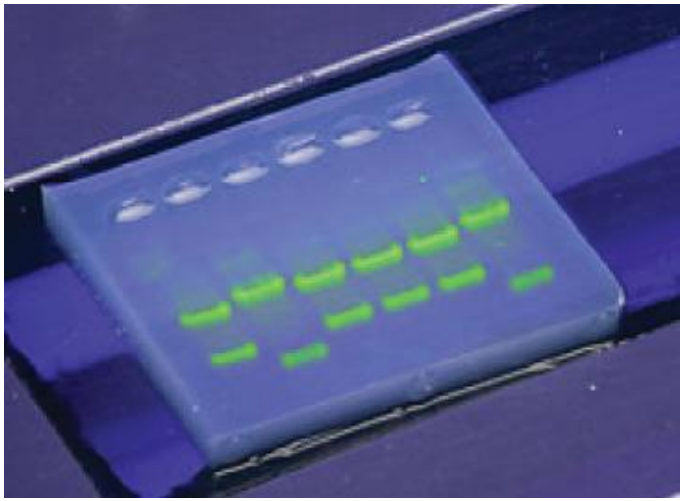
- Because molecules of different sizes travel at different speeds, discrete bands are formed.
- After the current is stopped, the bands can be visualized using a stain that sticks to DNA.
- UV-reactive dyes simulate DNA fragments, eliminating post-staining time.



SYBR® Safe DNA Stain

In-gel Staining

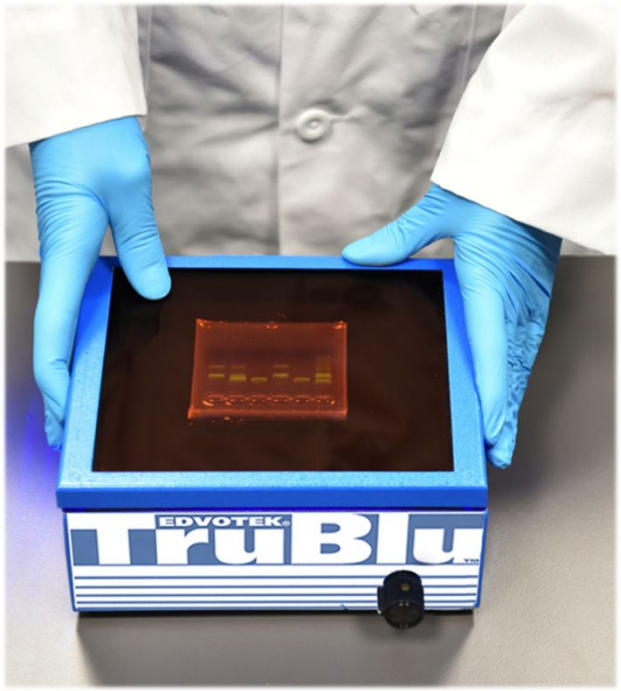
- Melt agarose and cool to 65°C.
- Add concentrated Sybr® Safe stain to the molten gel at 1:10,000 dilution (5 µL per 50 mL agarose solution).
- Run DNA samples through gel – no post staining or destaining necessary!

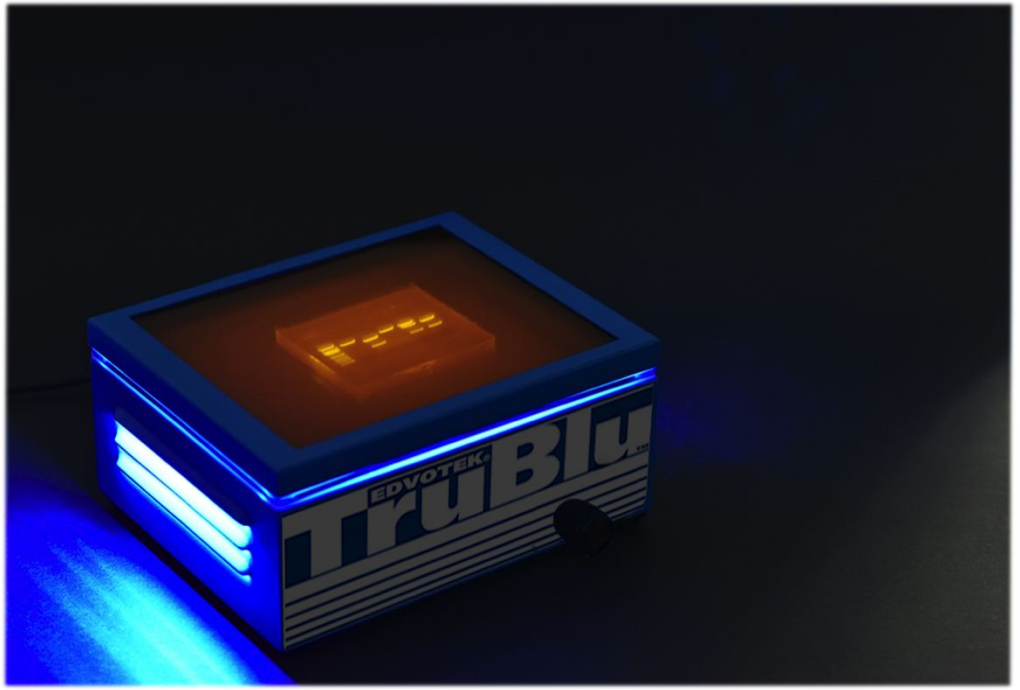


Kit #109
Transilluminator #558
SybrSafe® Stain #608

TruBlu™ Bluelight Transilluminator

Optimized for **SYBR® Safe** stained gels • Large viewing area • No harmful UV





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Let's run our gels!

Load 35 microliters of sample per well.

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

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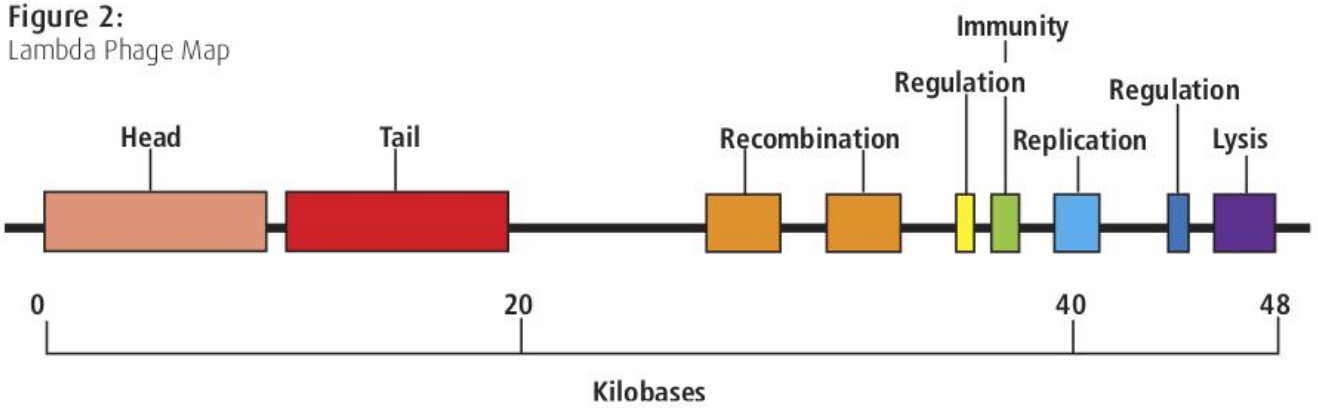
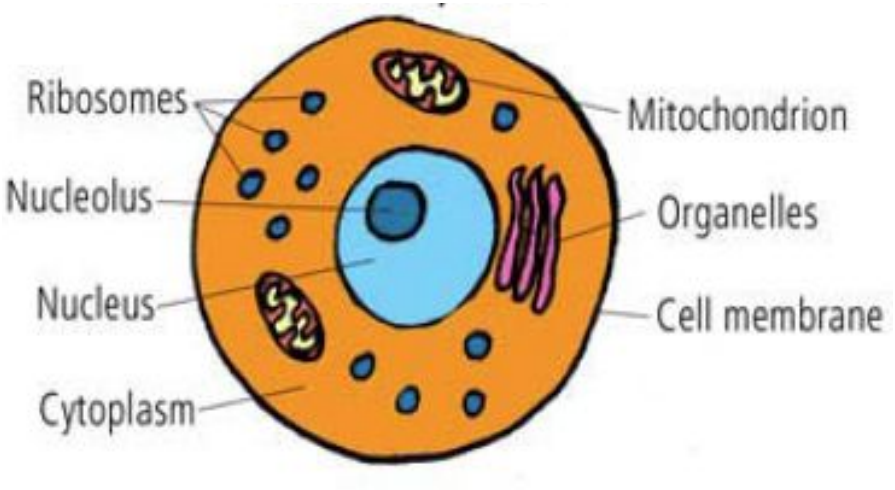
DNA Templates

- Lambda Phage DNA



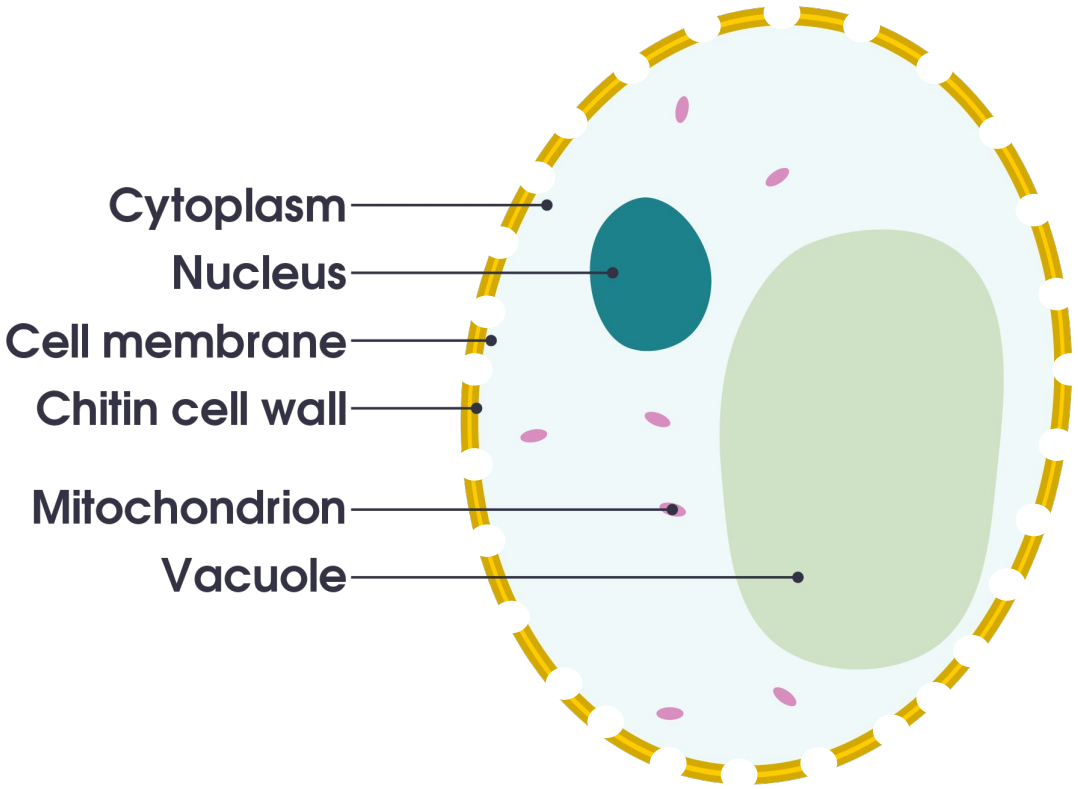
- Extracted DNA from cheek cells: PTC taste (345), mitochondrial DNA (332), Alu (333), VNTR (334)
- Bacterial DNA: water contamination (953), transformation (321)
- GMO food (962)

DNA is Isolated from Cellular Lysates



- Cell membranes are disrupted to release its intracellular components.
- The resulting "lysate" consists of cytoplasm, metabolites, DNA, RNA, proteins and organelles.
- DNA can be isolated from the lysate.

Lysing a cell



- Mechanical
- Freeze/Thaw
- Liquid Homogenization
- Sonication
- Manual Grinding

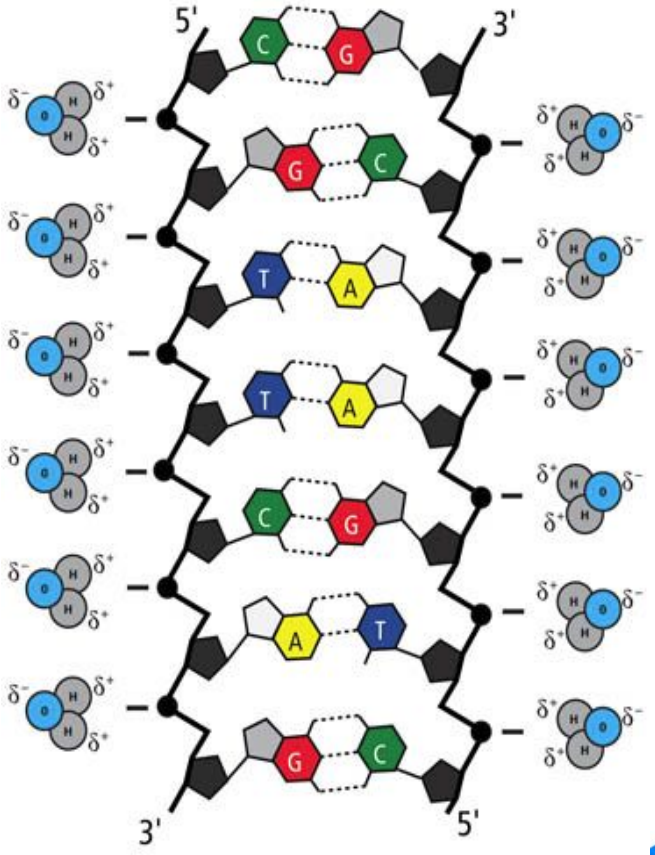
Cell Lysate

- Protein
- RNA
- Organelles
- Lipids
- Membranes
- DNA

The purity of the DNA affects the specificity of the PCR

DNA Isolation by Ethanol Precipitation

- DNA is composed of nucleotides that have three basic parts:
  - a phosphate group
  - a deoxyribose sugar
  - a nitrogenous base (A, C, G, T)
- Sugar of one nucleotide is covalently bonded to another phosphate
- DNA backbone is negatively charged
- When alcohol and salt are added to cellular lysate, interactions between the water molecules and DNA are disrupted.



DNA Isolation by Ethanol Precipitation

1. POUR the DNA solution into the beaker.
2. With a transfer pipet, STREAM the alcohol down the side of the beaker. Avoid Mixing.
3. PLACE the spooling rod just below the interface between the DNA and alcohol.
4. Quickly TWIRL the rod in a circulate motion.
5. REPEAT step 5 (spooling) about 10 times or until white fibers of DNA are visible.
6. TOUCH the rod to the bottom of the beaker. TWIRL several more times while keeping the rod touching the bottom.
7. REMOVE the rod and allow excess alcohol to drip onto the paper towel.
8. INCUBATE for 5 minutes.

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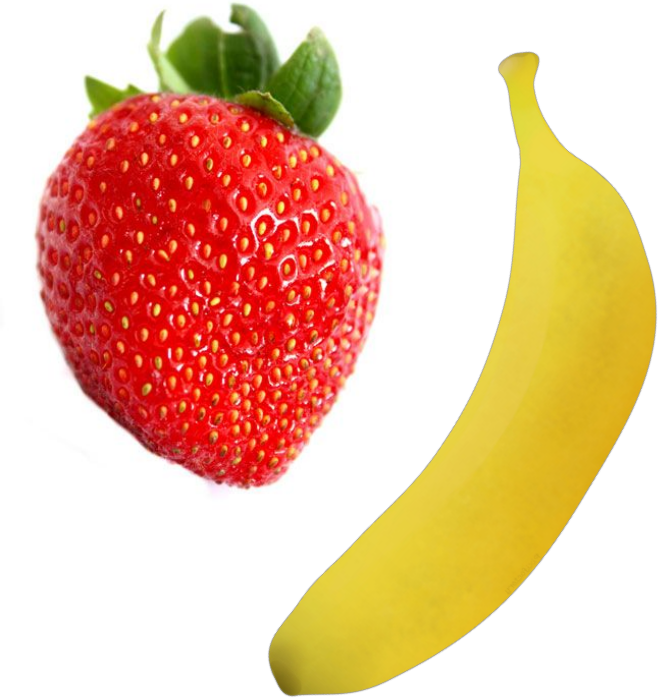
DNA Isolation from fruit

- Requires additional components to lyse the cells
  - Salt
  - Detergent
- Can also be spooled in the classroom
- DNA would not be pure enough to run PCR with



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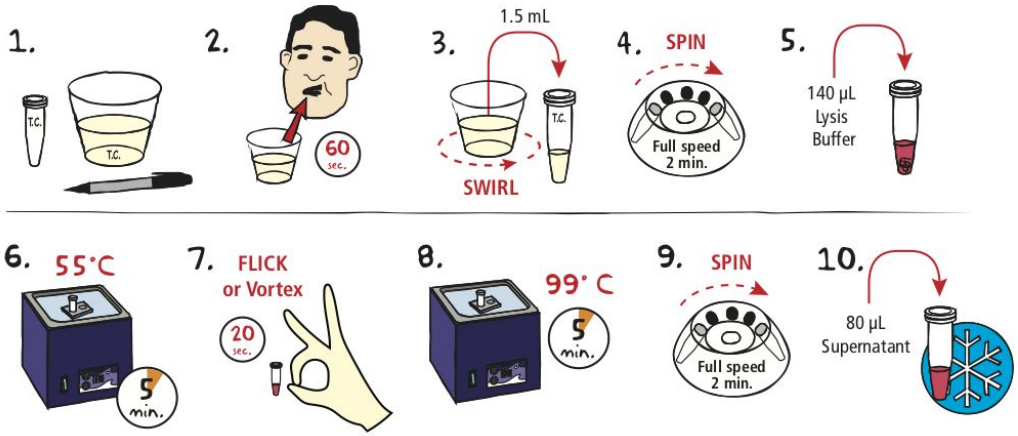
Teaching PCR

- Want pure DNA, or a kit where DNA can be extracted to a more pure form
- Show how additional cycles affect the amount of PCR product



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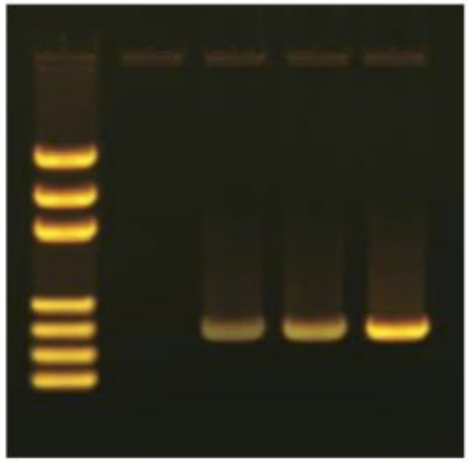

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Mistakes ~~Hurt!~~ Happen!

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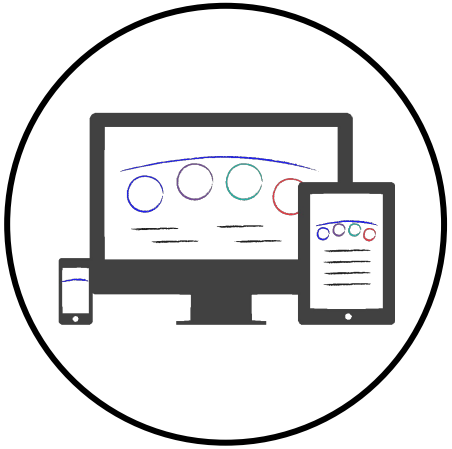

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