

# Transforming your classroom into a Neuroscience laboratory

Kelly Barford, Ph.D.  
Edvotek®

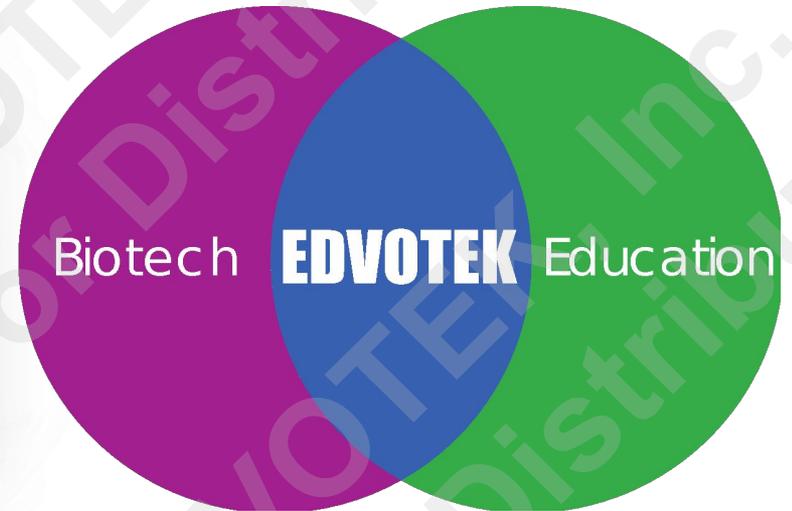
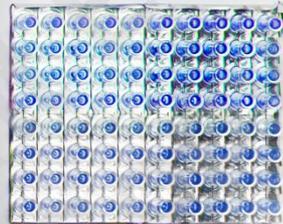
[www.EDVOTEK.com](http://www.EDVOTEK.com)

 Follow @Edvotek



# EDVOTEK

The **Biotechnology Education** Company



Celebrating 30 years of science  
education!

# EDVOTEK

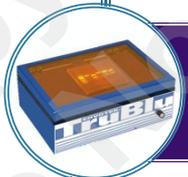
The **Biotechnology Education** Company



Experiments



Reagents

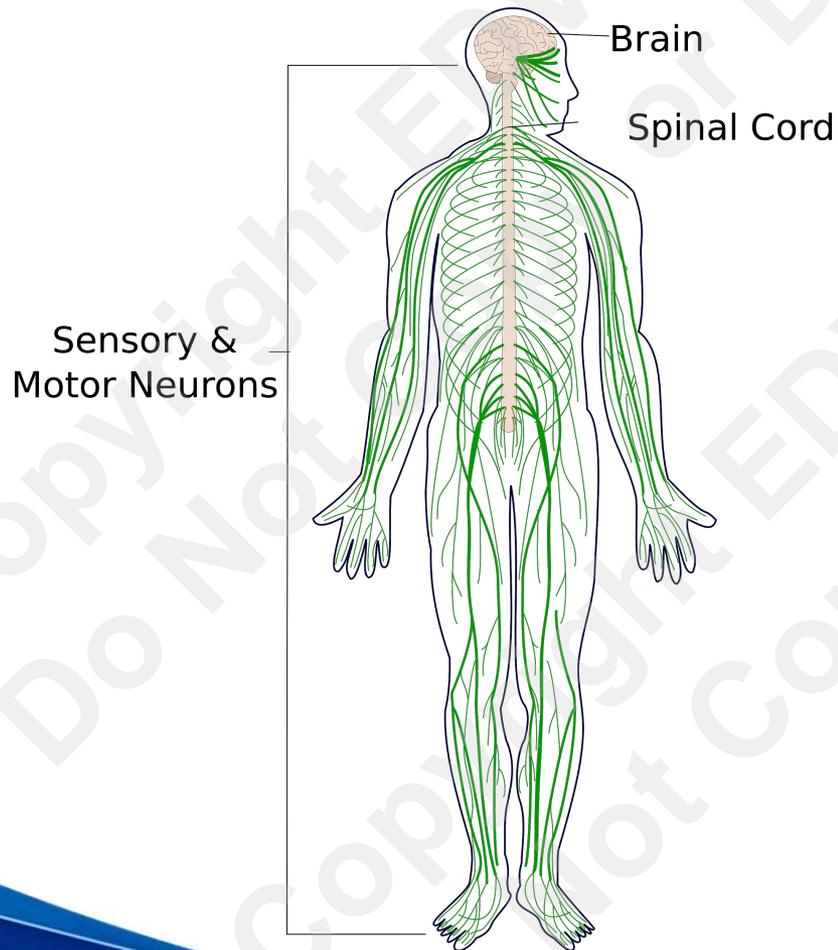


Equipment

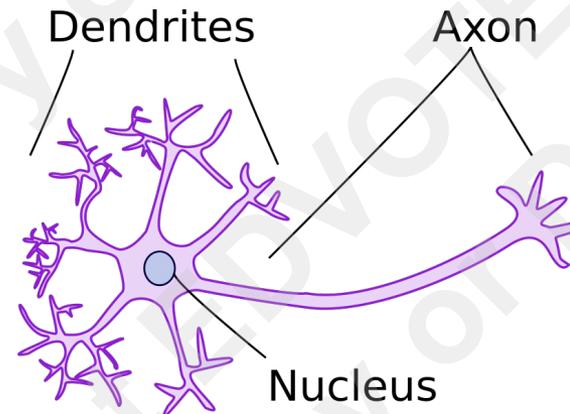


Resources

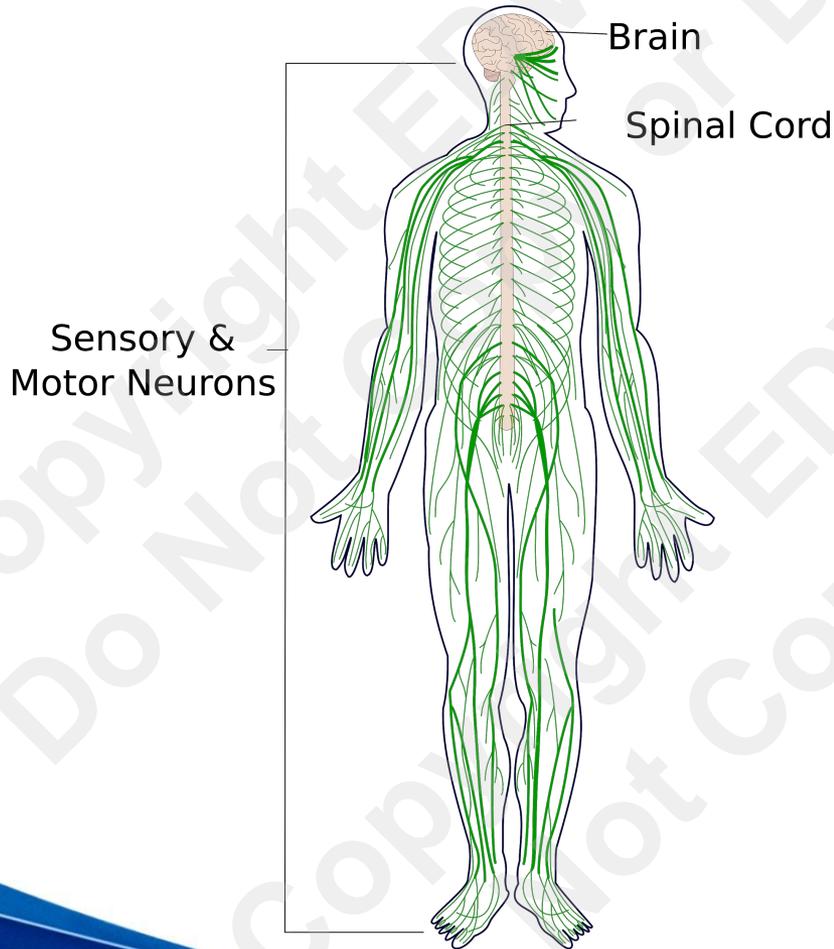
# Neuroscience: The Study of the Nervous System



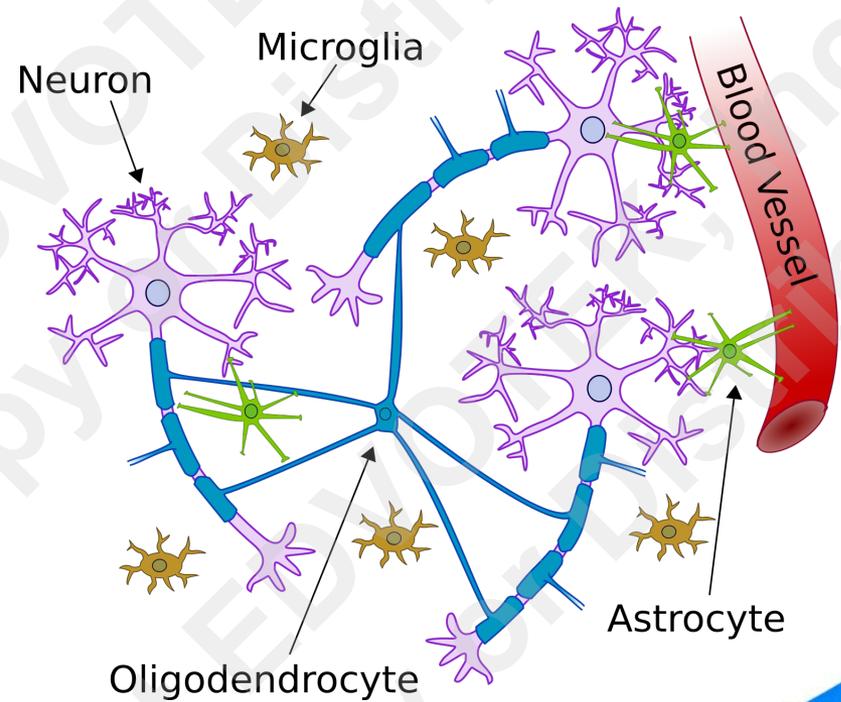
- Central Nervous System (CNS)
- Peripheral Nervous System (PNS)
- Major cell type: neuron



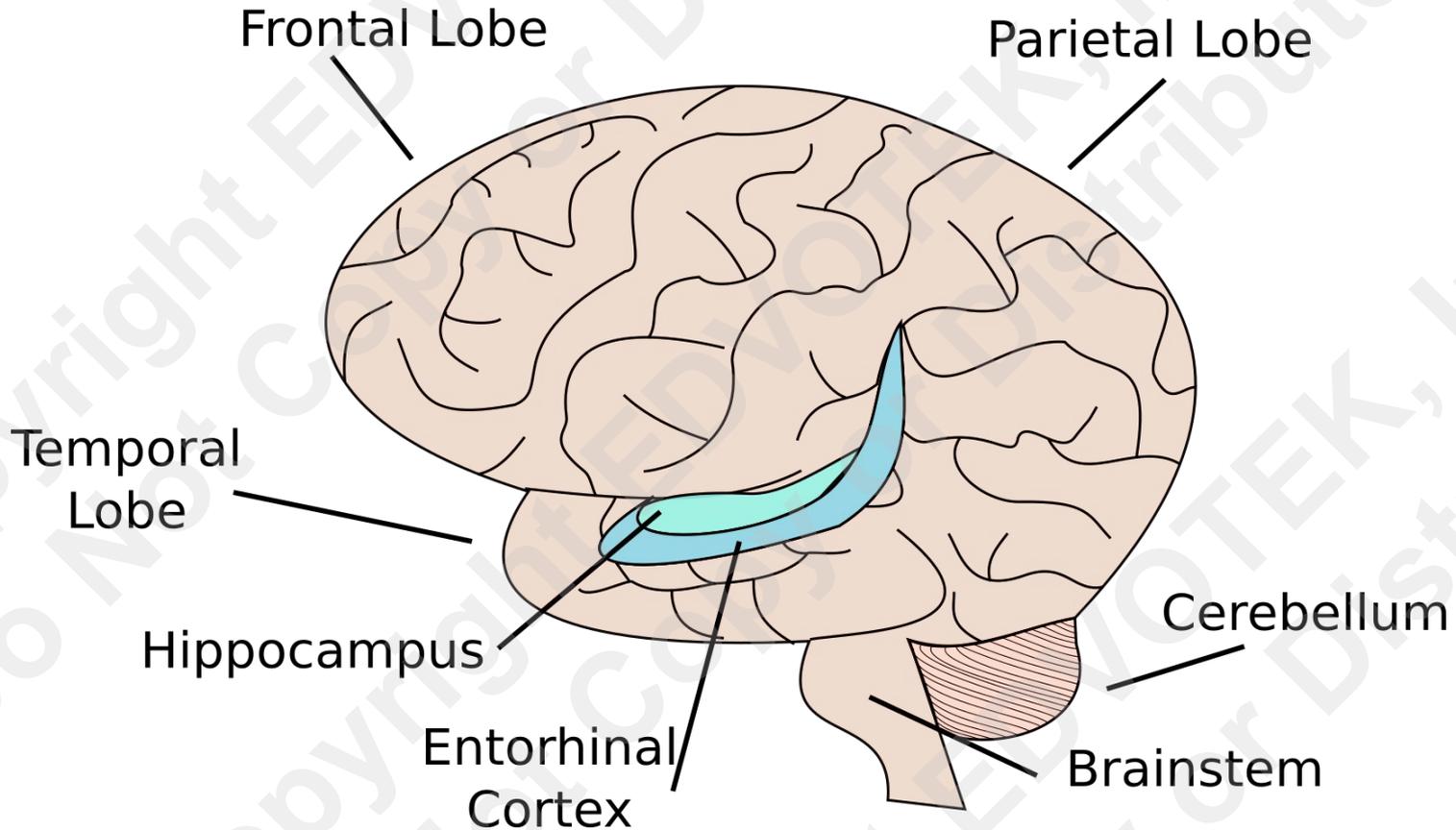
# Neuroscience: The Study of the Nervous System



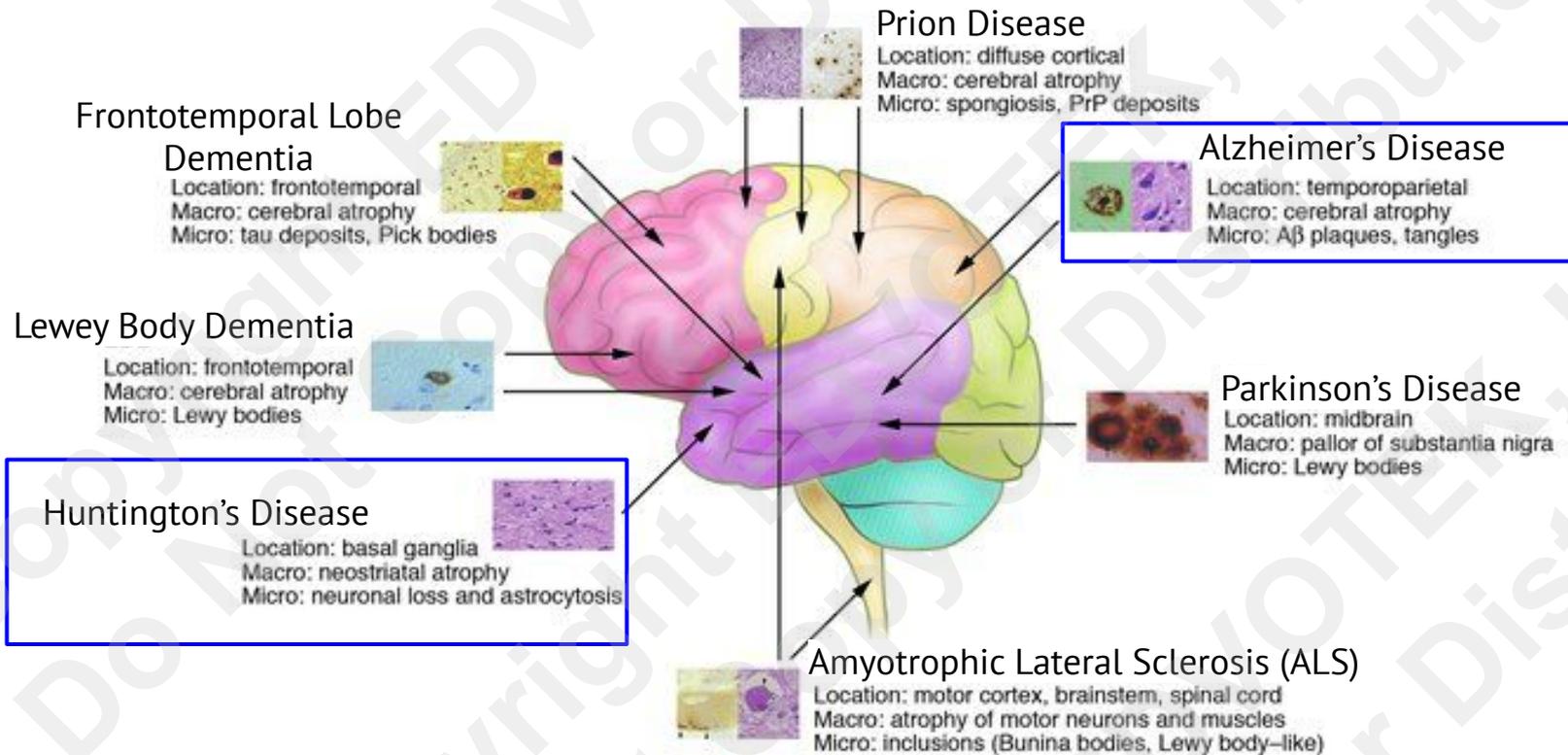
## Cell types in the brain:



# Parts of the Brain



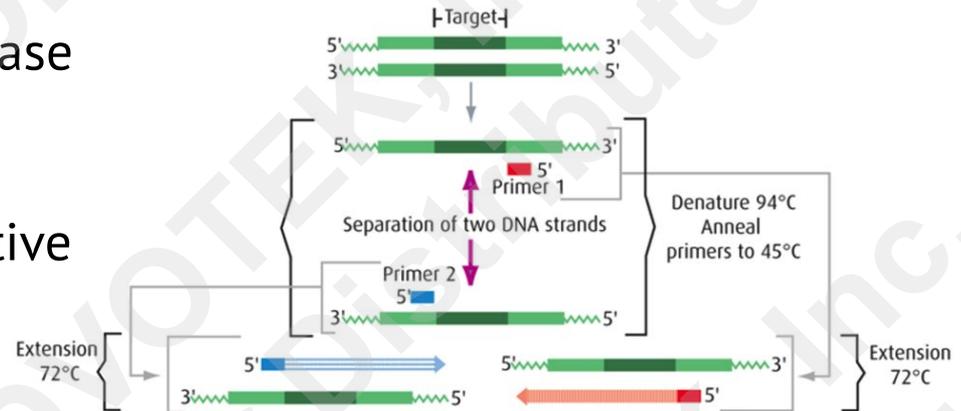
# Neurodegenerative Diseases



*J Clin Invest.* 2005;115(6).

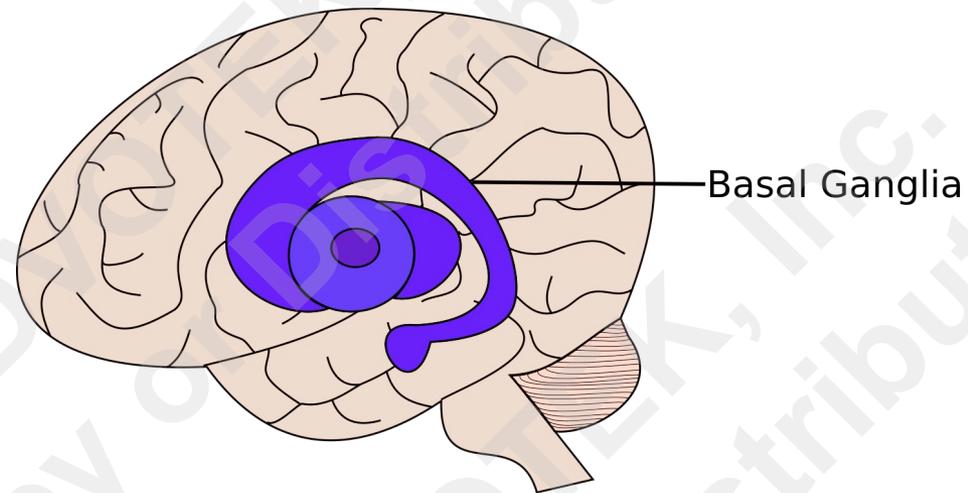
# Today's Workshop

1. Load gels with Huntington's disease samples post-PCR
2. Discuss PCR and neurodegenerative diseases
3. ELISA to research Alzheimer's disease
4. Examine gels



# Huntington's Disease

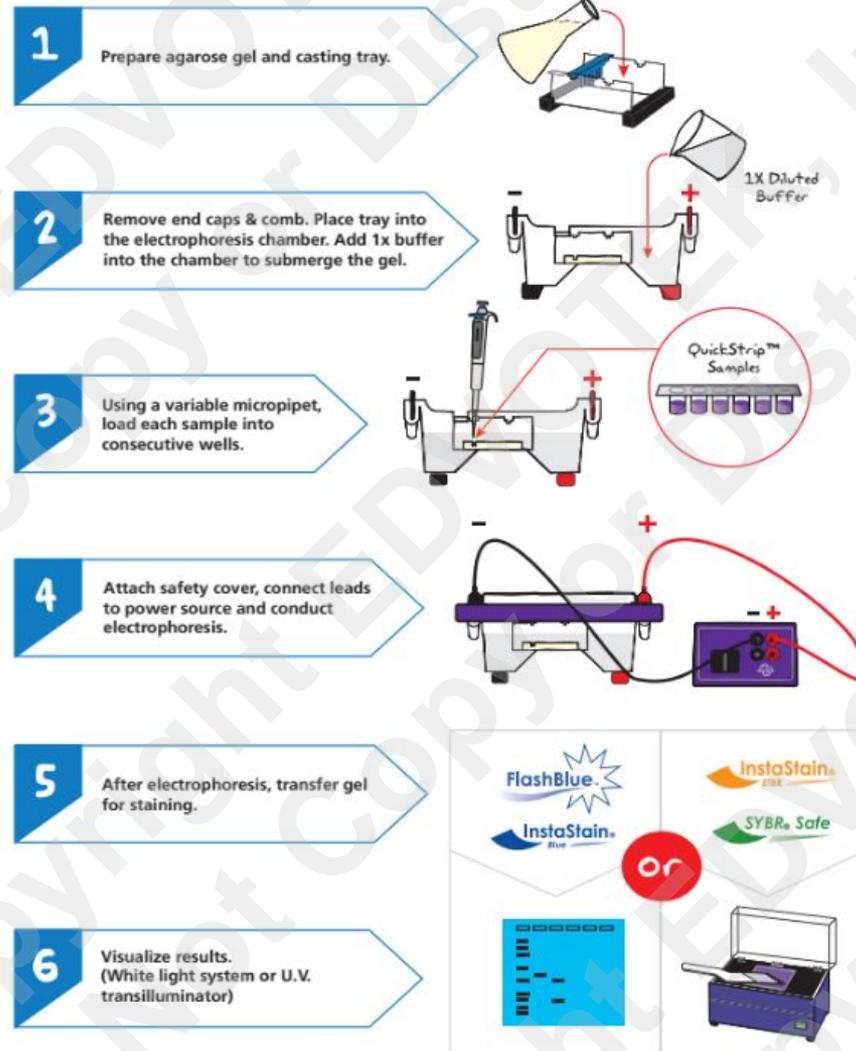
- Neurodegenerative disease
  - Symptoms develop in 30s-40s
  - Involuntary jerking and movement: chorea
  - Cognitive decline
- Brain area affected: basal ganglia
  - Normal role: limiting movement



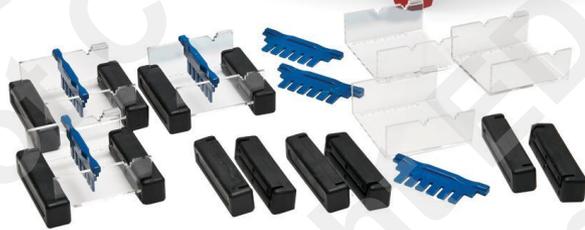




# Overview of Agarose Gel Electrophoresis



# 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

# Let's run our gels!



Load 25 microliters of sample per well.

Lane	Sample
1	EdvoQuick™ DNA Ladder
2	Positive Control
3	Father
4	Mother
5	Daughter
6	Son

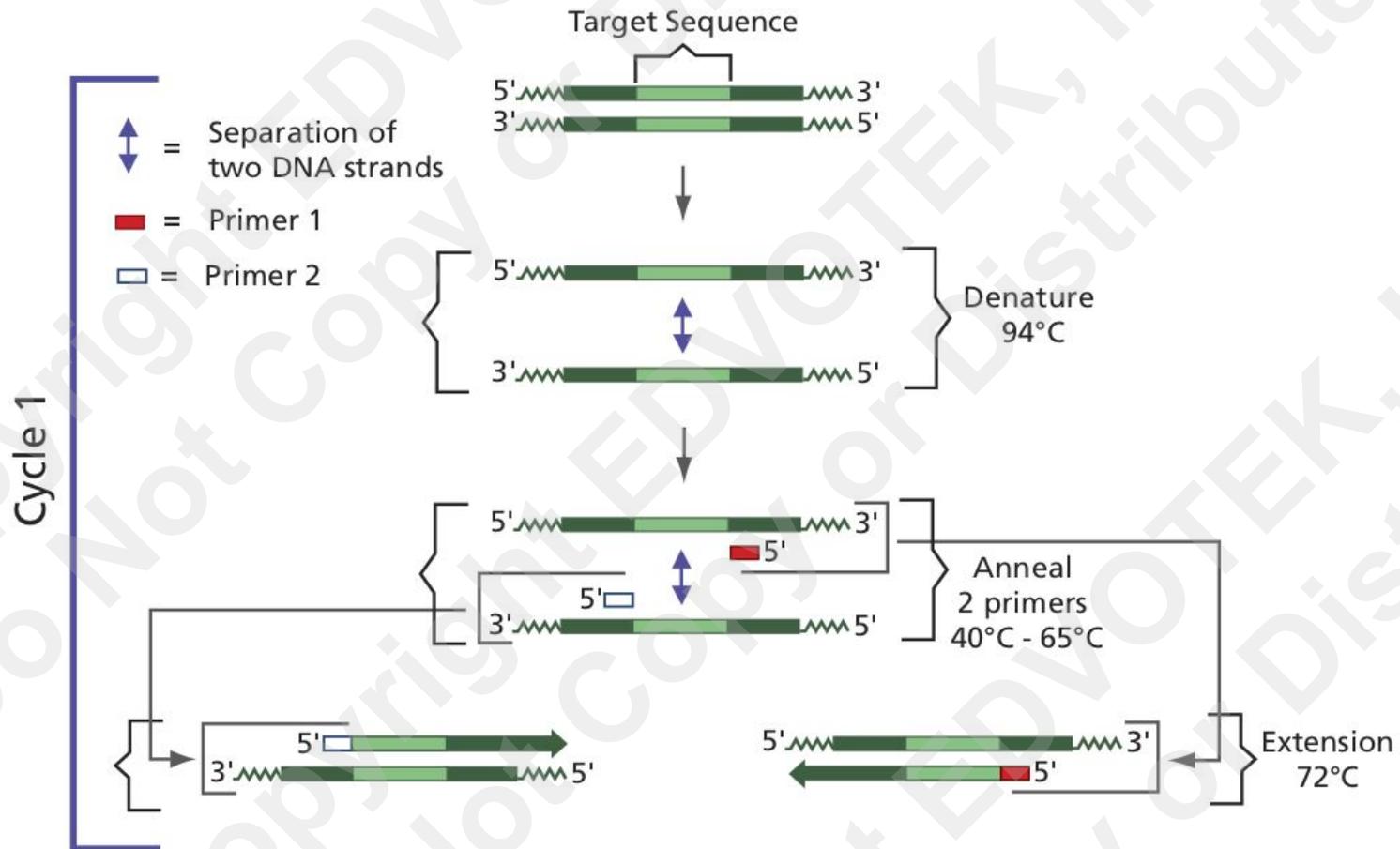
**New to electrophoresis? Try practicing loading gels at one of the DuraGel stations!**

# DNA Amplification using PCR

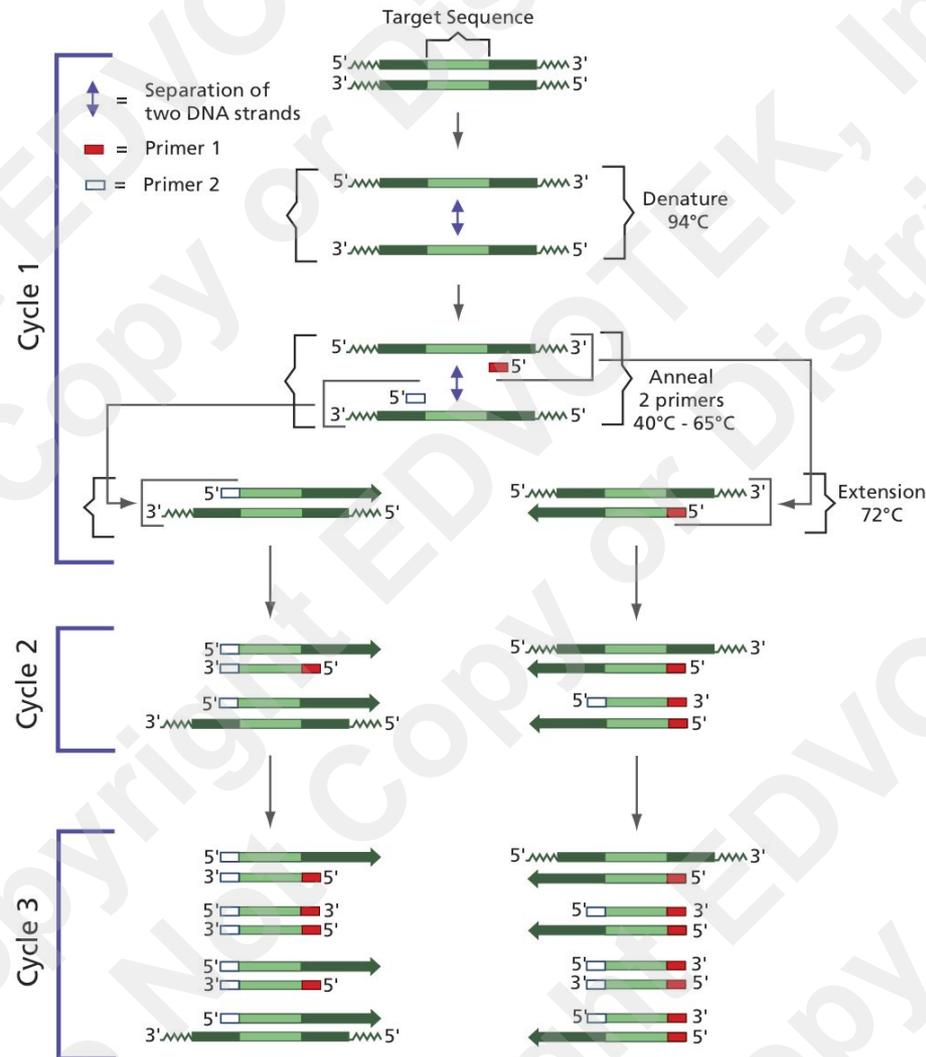
- DNA template
- Primers
  - Short piece of DNA that defines the area to amplify
- Thermostable DNA polymerase (*Taq*)
- Free nucleotides (dNTPs)
- Buffer



# PCR Amplifies Specific DNA Sequences



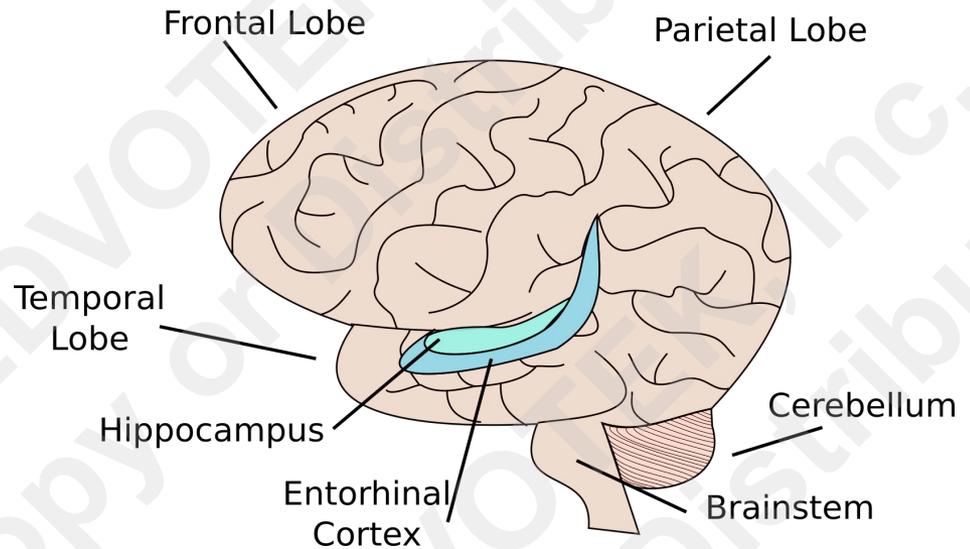
# PCR Amplifies DNA Exponentially



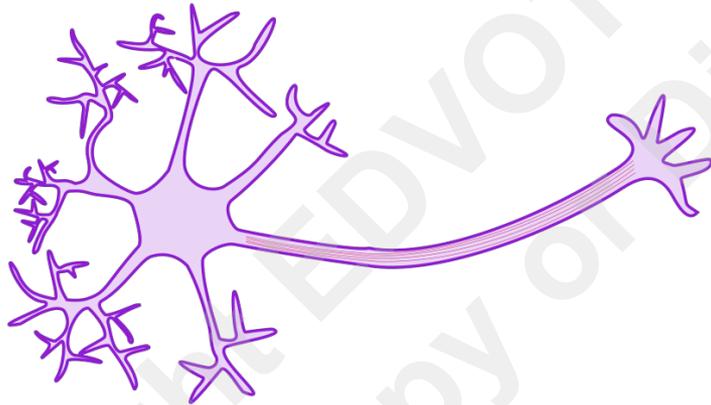
Any questions so far?

# Alzheimer's Disease

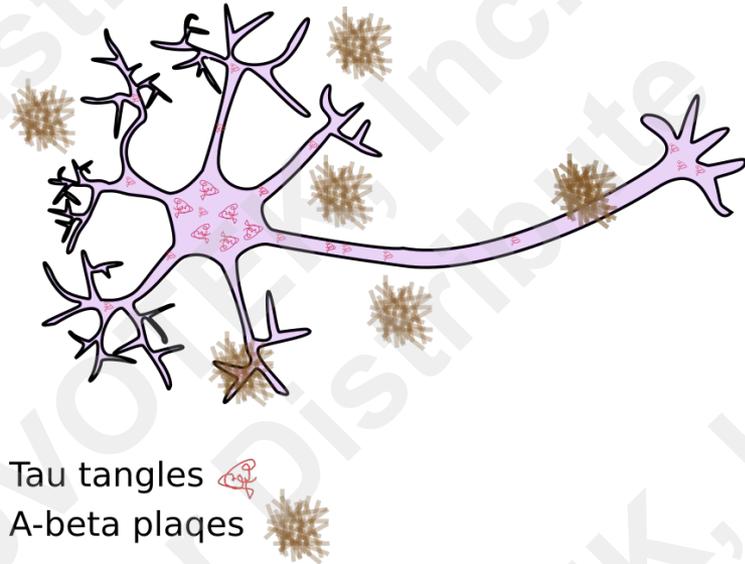
- Neurodegenerative disease
  - Symptoms develop in mid 60's
  - Destroys memory and thinking skills
  - Most common cause of dementia
- Brain area affected: begins in entorhinal cortex
  - Progressively spreads through the brain



## Healthy Neuron



## Alzheimer's Disease



Normal Tau (axonal)

Tau tangles

A-beta plaques

- Buildup of misfolded proteins in the brain
  - A $\beta$
  - Tau
- A $\beta$ : small peptide cleaved from larger protein (APP)
  - Creates plaques
- Tau: microtubule stabilizing protein
  - Dysregulated and forms tangles

# Barriers to Treatment

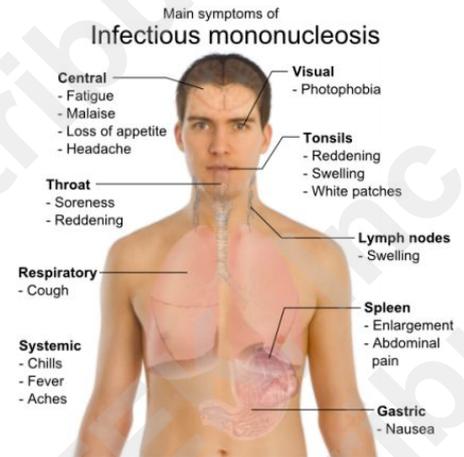
- Symptoms /diagnosis don't occur until ~ 20 years after plaque and tangle buildup
  - Many neurons have already died
- Only recently (10-15 years) started serious research
- Clinical trials can't start until diagnosis
  - Too late?

Can we find a biomarker that allows us to detect Alzheimer's disease earlier and start clinical trials before broad neuronal death?

# Researching Alzheimer's Disease by ELISA



- Scientists use the ELISA to quantitatively detect the presence of molecules within a sample.
  - Allergen detection – milk, peanut, walnut, egg proteins
  - Hormones – Pregnancy tests
  - Toxicology – drug tests



Cat. #279

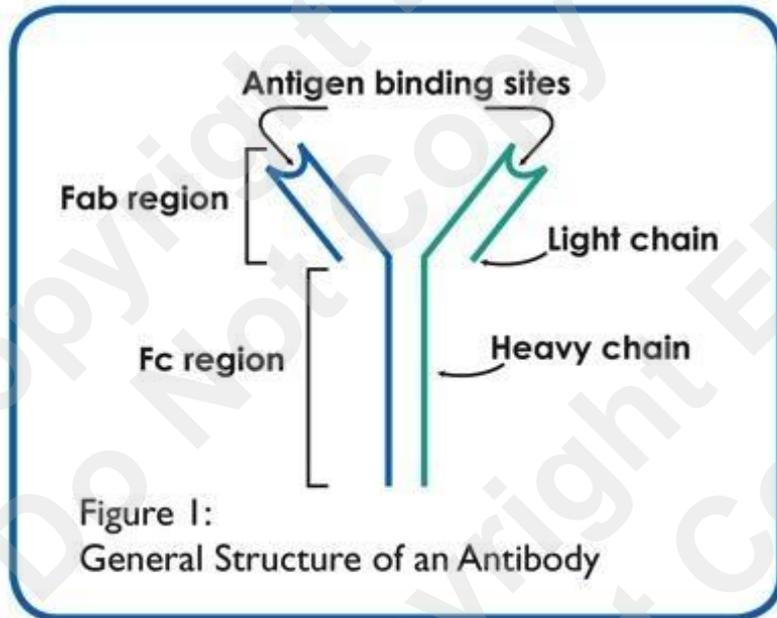
- The ELISA is commonly used for medical diagnostics, as it is can be used to identify antigens in blood and other biological samples.
  - Disease detection – WNV, HIV, TB, Infectious Mononucleosis
  - **Analyzing control and patient samples for amount of A $\beta$  in CSF**



Cat. #274



# Antibodies Distinguish Between “Self” and “Non-self”



- Antibody molecules comprise four linked polypeptide chains: two “heavy chains” and two “light chains”
- The amino acid sequence of the antigen binding site is variable
- Allows each antibody to recognize a unique **epitope** (a particular location within an antigen).

# What Do I Need to Perform ELISA Experiments?



- Paper towels
- Distilled water
- Lab glassware
- Disposable lab gloves
- Safety goggles
- Automatic micropipets & tips
  - 50 $\mu$ L recommended

# Performing the ELISA

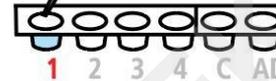
1.



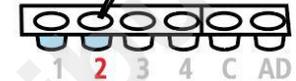
2. LABEL the strip of wells.



3a. ADD 100  $\mu$ L  $A\beta$  solution.



3b. ADD 100  $\mu$ L 1:4 solution.



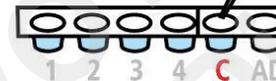
3c. ADD 100  $\mu$ L 1:8 solution.



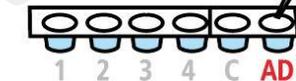
3d. ADD 100  $\mu$ L 0 solution.



4a. ADD 50  $\mu$ L "C" solution.



4b. ADD 50  $\mu$ L "AD" solution.



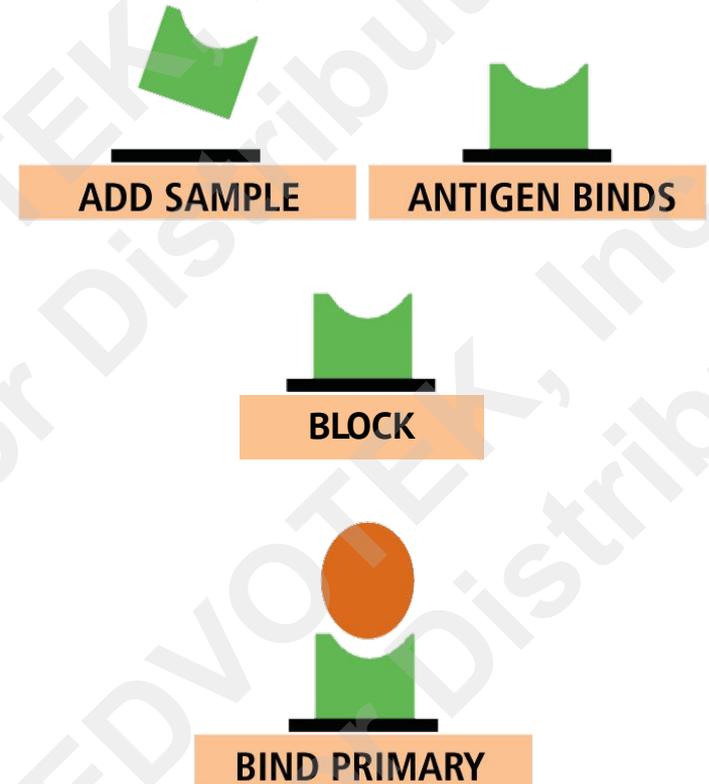
1. **ORIENTATE** your strip so that the longer box of wells is on the left.
2. **LABEL** the left wells 1 through 4. **LABEL** well 5 as "C" and well 6 as "AD".
3. **PIPET** the provided standard curve.
  - a. **PIPET** 100  $\mu$ L of  $A\beta$  solution to well 1 (provided in a starting concentration of 20  $\mu$ g/ml).
  - b. With a new pipet tip, **PIPET** 100  $\mu$ L of 1:4 solution to well 2.
  - c. With a new pipet tip, **PIPET** 100  $\mu$ L of 1:8 solution to well 3.
  - d. With a new pipet tip, **PIPET** 100  $\mu$ L of 0 solution to well 4.
4. **PIPET** the simulated patient samples.
  - a. With a new pipet tip, **ADD** 100  $\mu$ L of Control sample to well 5
  - b. With a new pipet tip, **ADD** 100  $\mu$ L of AD sample to well 6.
5. **INCUBATE** for 5 minutes at room temperature.

# ELISAs Detect Specific Antigens in Complex Mixtures

**Step 1:** The sample (CSF) is added to the wells of the microtiter plate, where it adheres to the plastic through hydrophobic and electrostatic interactions.

**Step 2:** After washing away any unadsorbed sample, the wells are “blocked” with a protein-containing buffer to prevent non-specific interactions.

**Step 3:** The **primary** antibody (detects A $\beta$ ) is added to the wells, where it recognizes the antigen and binds through electrostatic interactions. Excess antibody is washed out of the wells.

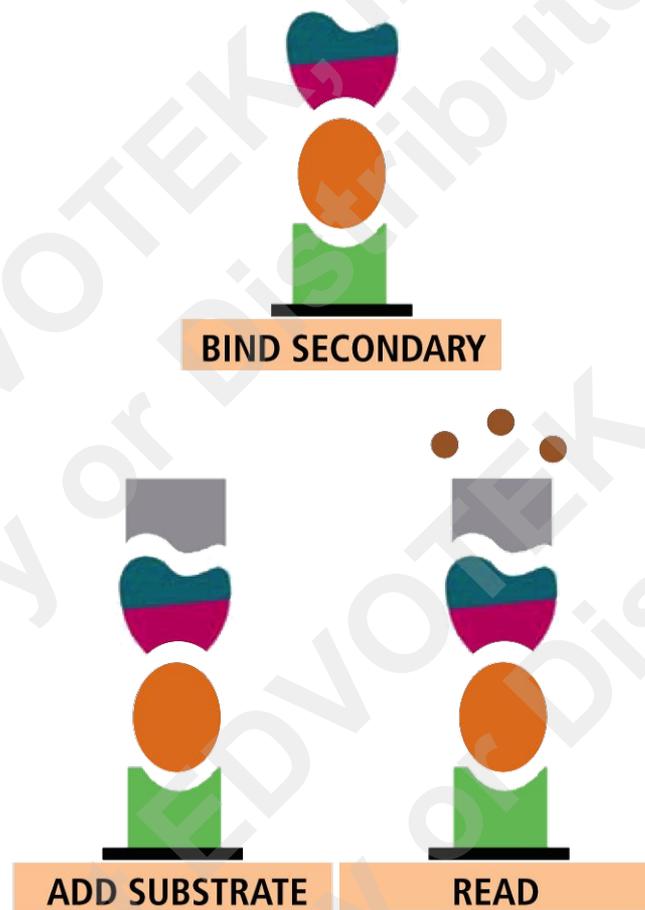


# ELISAs Detect Specific Antigens in Complex Mixtures

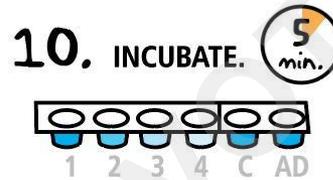
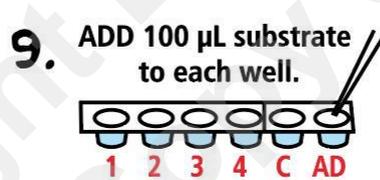
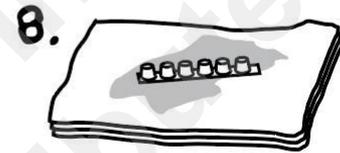
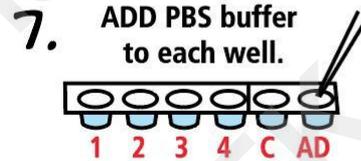
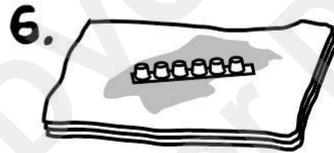
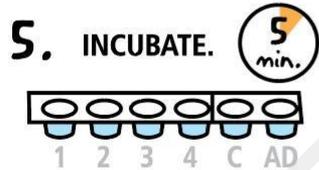
**Step 4:** The **secondary** antibody (recognizes the primary antibody) is added to the wells. If the antibody can bind with the antigen, it will remain in the well after the wells are washed.

**Step 5:** The enzyme-linked secondary antibody allows us detect the presence of the antibody-antigen complex when the substrate is added to all the wells.

- Chromogenic detection
- Fluorogenic detection



# Performing the ELISA

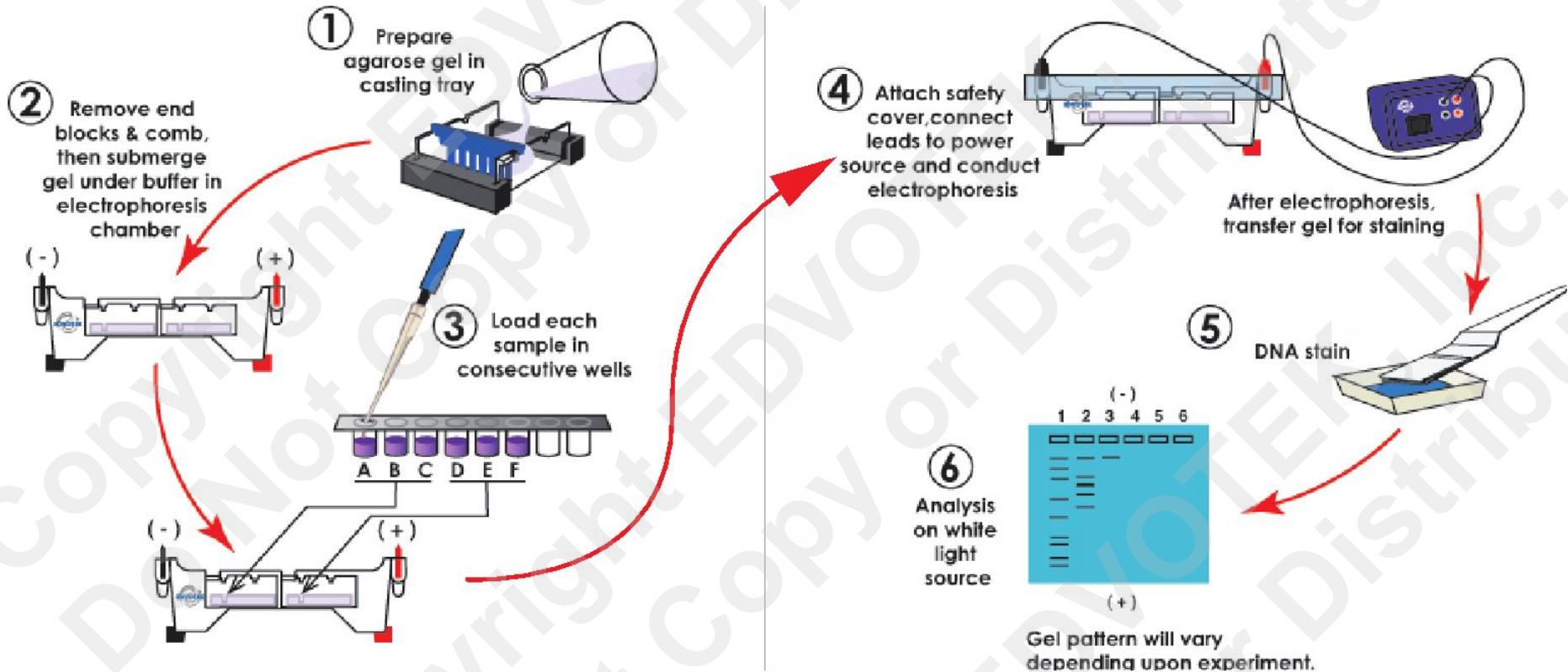


11. ANALYZE color changes.

6. **INVERT** the strips over the sink or a stack of paper towels to remove the samples. Gently **TAP** the strips 4-5 times onto a fresh paper towel. **DISCARD** the wet paper towels.
7. Using a transfer pipet, **ADD** PBS buffer to each well until it is almost full – around 9 drops.
8. **INVERT** the strips over the sink or a stack of paper towels to remove the samples. Gently **TAP** the strips 4-5 times onto a fresh paper towel. **DISCARD** the wet paper towels.
9. Using a new transfer pipet, **ADD** 100  $\mu$ L (2 drops) of substrate solution to all wells.
10. **INCUBATE** for 5 minutes at room temperature.
11. **ANALYZE** the strip.

Any questions so far?

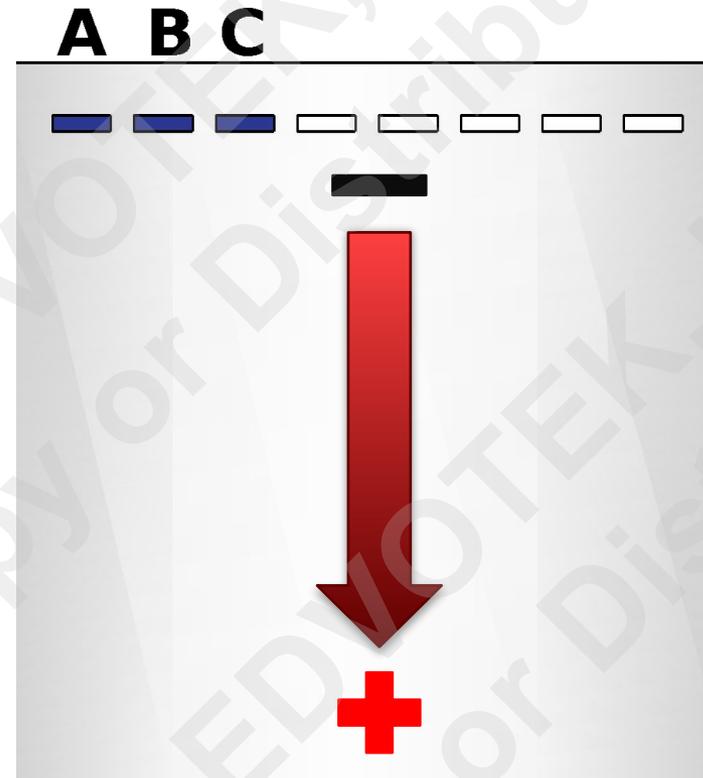
# Summary of Electrophoresis





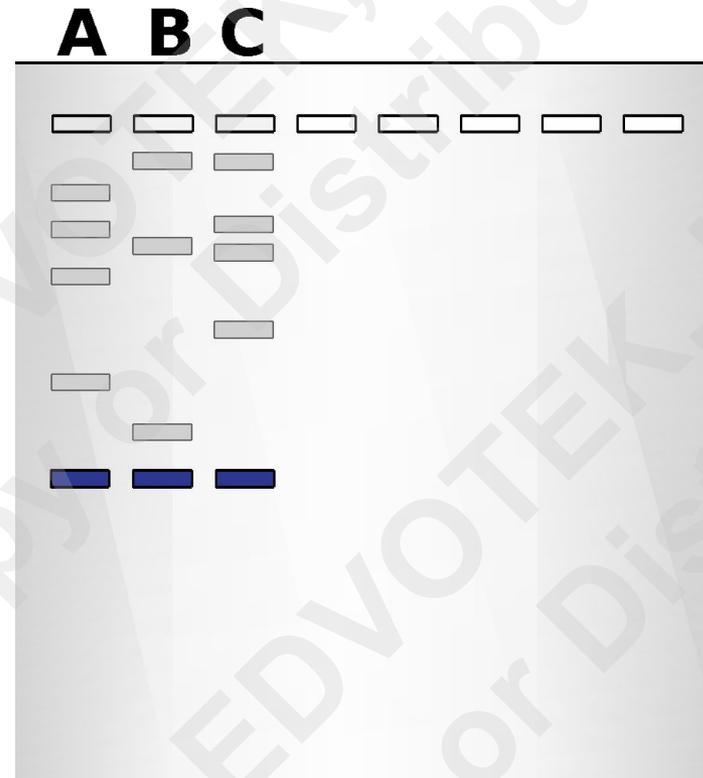
# Electrophoresis Separates DNA Fragments By Size

- 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.



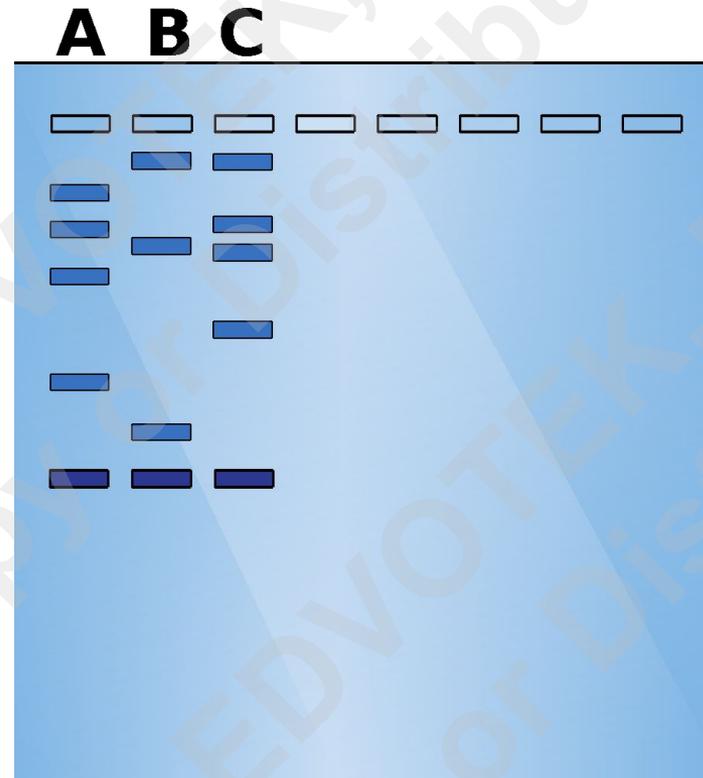
# Electrophoresis Separates DNA Fragments By Size

- 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

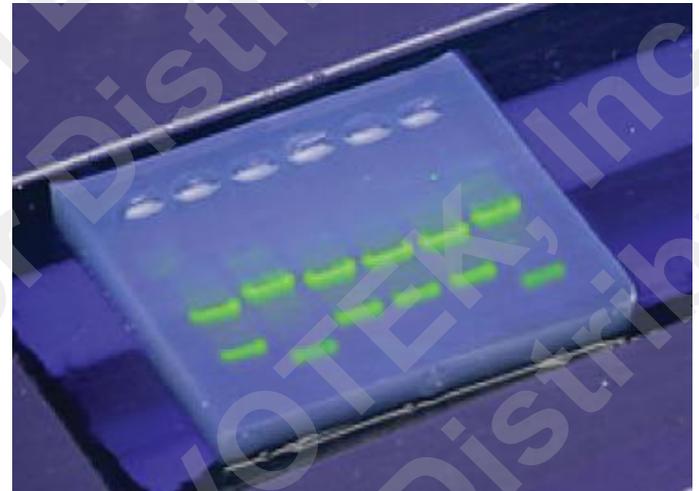
- Because molecules of different sizes travel at different speeds, discrete bands are formed.
- After the current is stopped, the bands can be visualized using a stain that sticks to DNA.
- UV-reactive dyes simulate DNA fragments, eliminating post-staining time.



# SYBR<sup>®</sup> Safe DNA Stain

## In-gel Staining

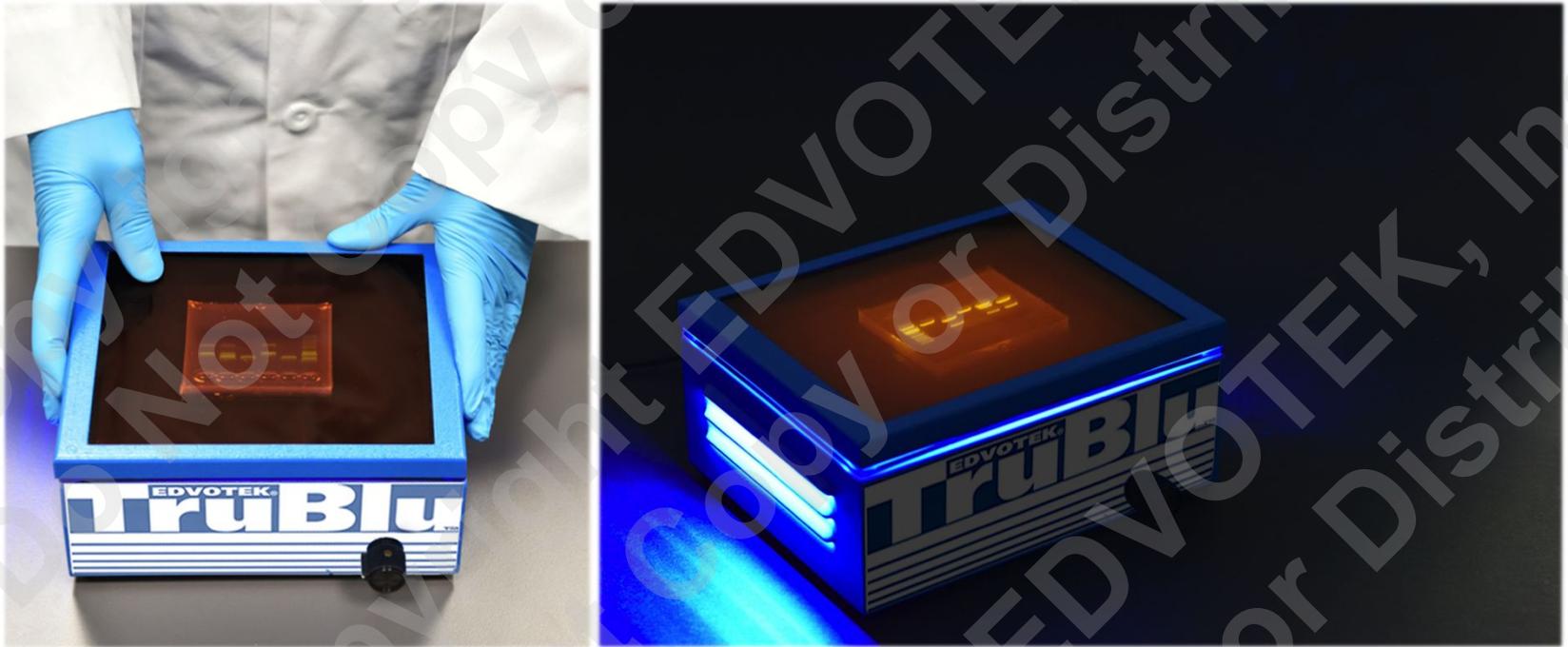
- Melt agarose and cool to 65°C.
- Add concentrated Sybr<sup>®</sup> Safe stain to the molten gel at 1:10,000 dilution (5 µL per 50 mL agarose solution).
- Run DNA samples through gel – no post staining or destaining necessary!



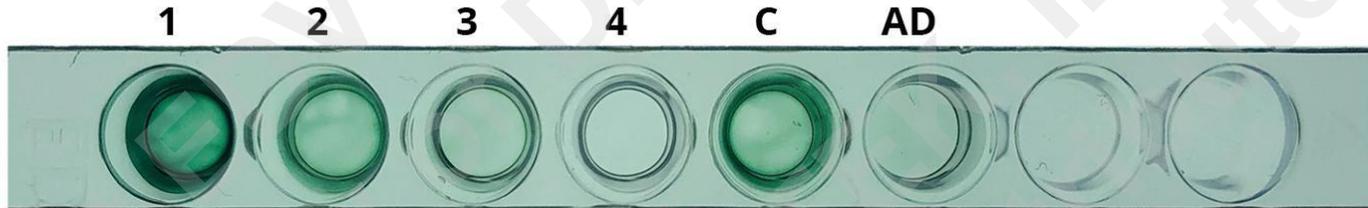
**Kit #109**  
**Transilluminator #558**  
**SybrSafe<sup>®</sup> Stain #608**

# TruBlu™ Bluelight Transilluminator

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



# Alzheimer's Disease ELISA Results



Well	1	2	3	4
Dilution	---	1:4	1:8	0
Concentration	20 $\mu\text{g/mL}$	5 $\mu\text{g/mL}$	2.5 $\mu\text{g/mL}$	0 $\mu\text{g/mL}$

The most reliable biomarkers for AD are decreased  $A\beta$  and increased Tau in the CSF. Researchers are actively looking into this and trying to discover new biomarkers.

Molinuevo JL. *Alzheimer's Dement.* 2014 Nov;10(6):808-16

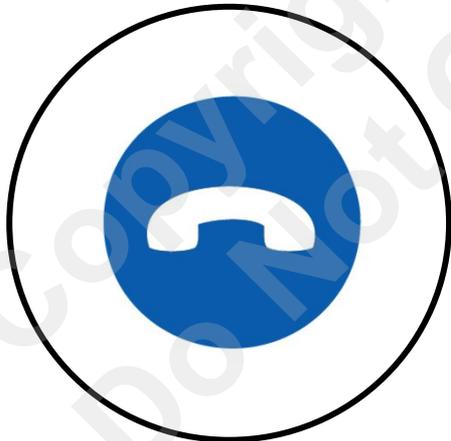
# Huntington's Disease Gel Results



Lane	Sample	Genotype
1	EdvoQuick™ DNA Ladder	-----
2	Positive DNA Control	45 CAG repeats: homozygous dominant
3	Father DNA	Heterozygote
4	Mother DNA	WT (Wild-Type): homozygous recessive
5	Daughter DNA	Heterozygote
6	Son DNA	WT (Wild-Type): homozygous recessive

# Mistakes ~~Hurt!~~ Happen!

## Call



1-800-EDVOTEK

## Online



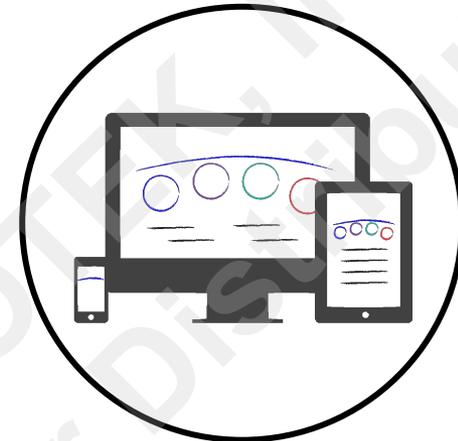
YouTube  
Facebook  
Twitter  
Pinterest

## Email



info@Edvotek.com

## Website

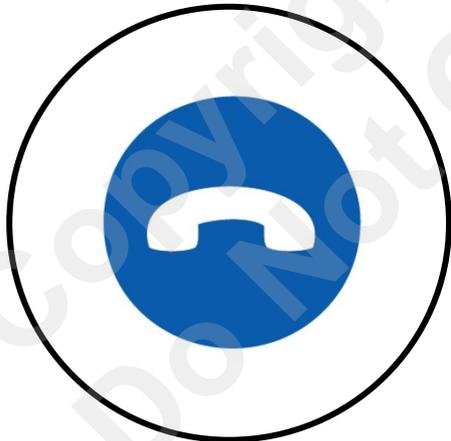


www.edvotek.com

## We are ready to help!

# Mistakes ~~Hurt!~~ Happen!

Call



1-800-EDVOTEK



Monday – Friday  
8AM-5:30PM EST

We are ready to help!



# EDVOTEK, Inc.

## The Biotechnology Education Company

**Come visit us at Booth #312!**

**Presentation and literature available at:**

<http://www.edvotek.com/NSTA-Workshops>

**For Orders & Technical Service:**

- Phone: 1-800-EDVOTEK (1-800-338-6538)
- Web site: [www.edvotek.com](http://www.edvotek.com)
- Email: [info@edvotek.com](mailto:info@edvotek.com)

**Check out our YouTube Channel:**

[www.youtube.com/EdvotekInc](http://www.youtube.com/EdvotekInc)

**Like us on Facebook for offers & updates:**

[www.facebook.com/edvotek](http://www.facebook.com/edvotek)

