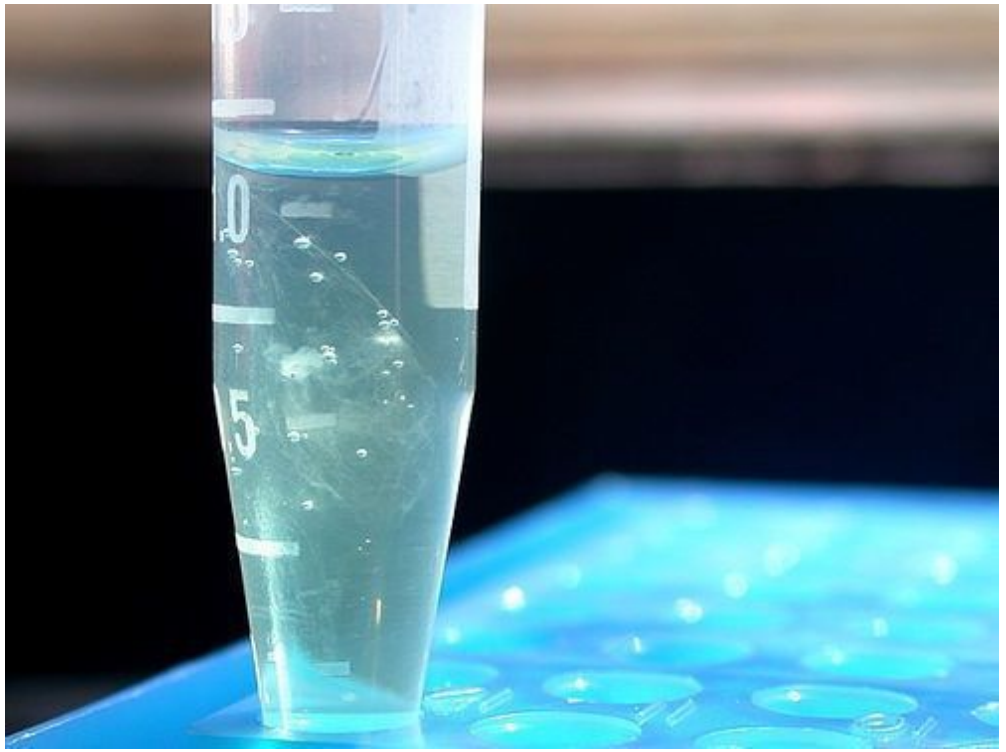
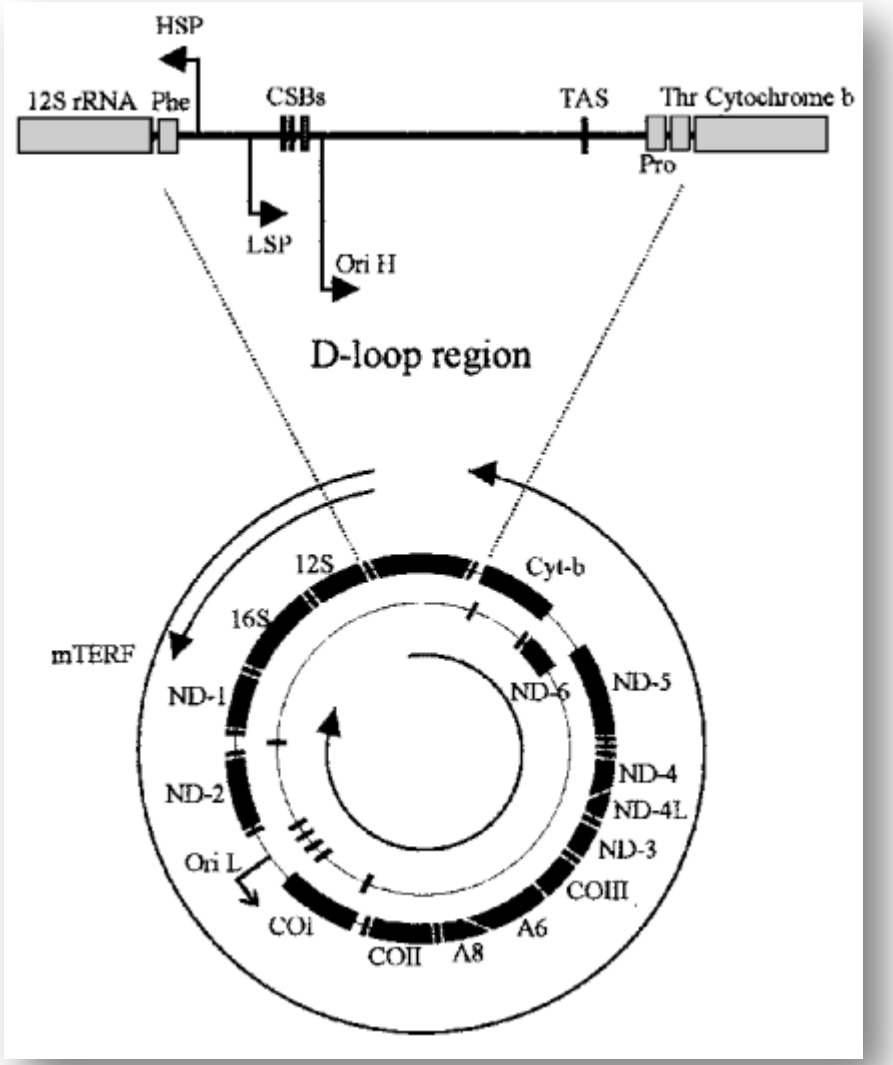
Electrophoresis and Sequencing Basics

Review: Mitochondrial lab

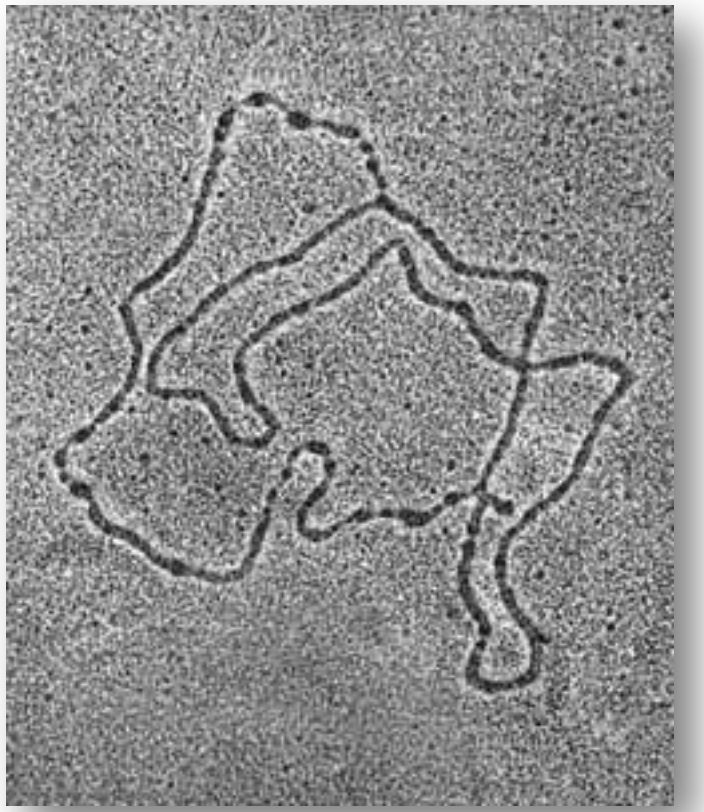


DNA Extraction from buccal cheek cells

Review: Mitochondrial DNA Structure



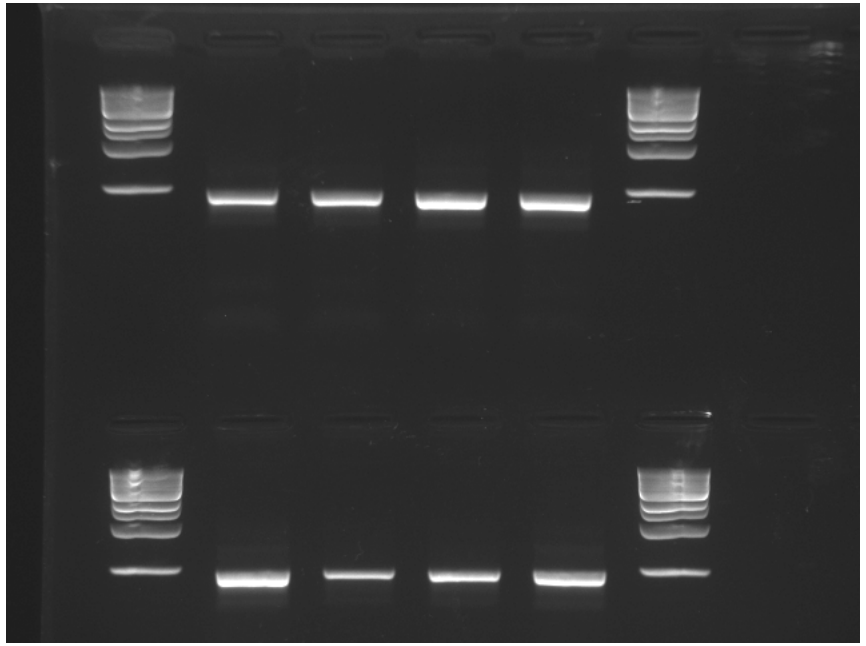
Human Mitochondrial DNA
16569 bp



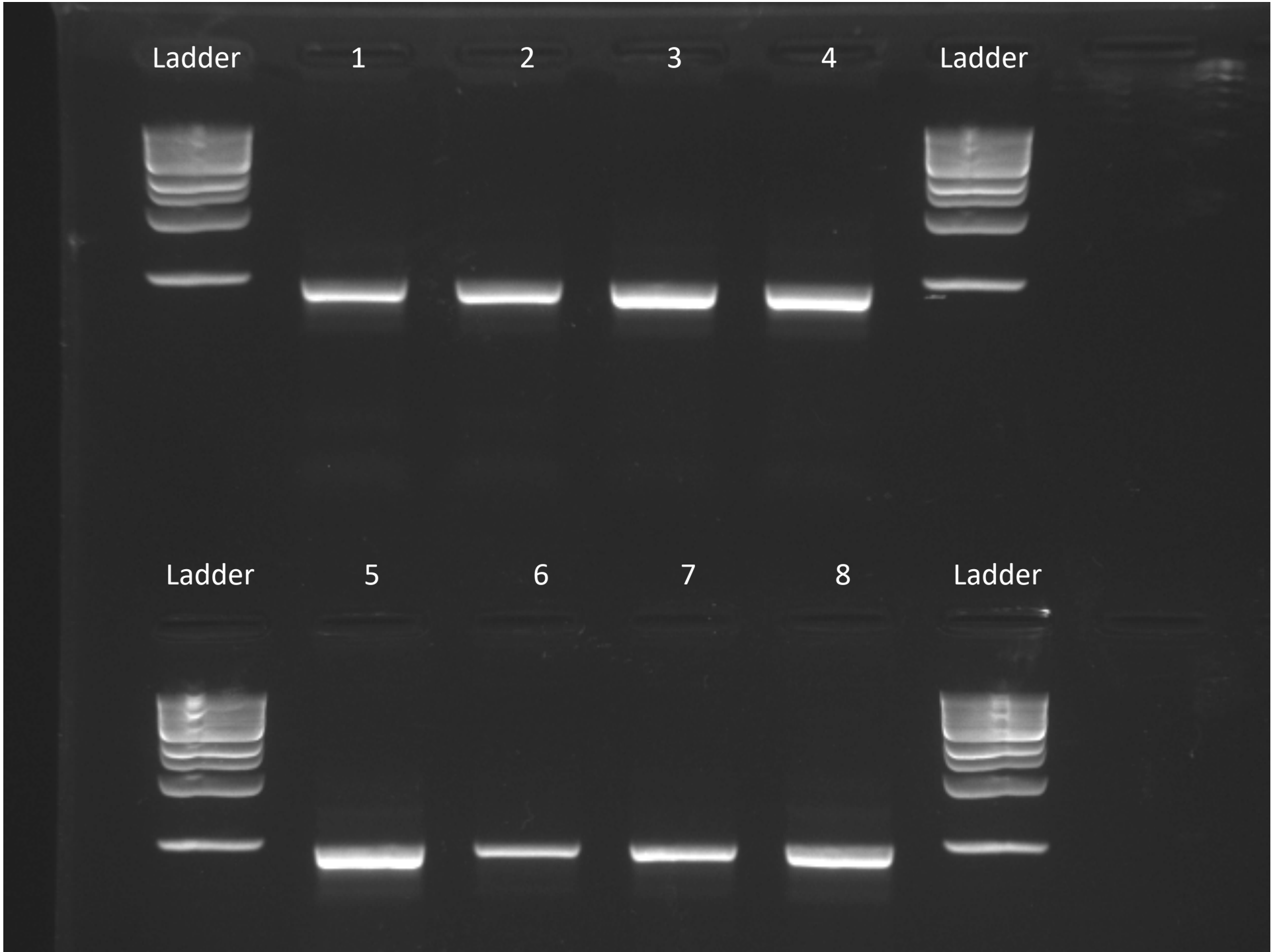
Review: Mitochondrial lab

DNA Amplification by PCR

Review: Mitochondrial lab



Electrophoresis


Ladder
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Ladder

Electrophoresis – movement by electricity

- Allows us to visualize DNA (PCR product)
- We can estimate the size (distance a “band” travels)
- We can estimate the amount (brightness of the band

Electrophoresis – Components

- Gel tank: Holds electrolytic buffer and has electrodes where electricity is applied
- Buffer: A salt solution that conducts electricity
- Gel: Made of agar; holds the DNA and separates DNA according to size
- Loading Dye: Makes it easier to load the PCR sample and keeps the DNA in the gel.
- UV Dye: Binds to DNA. Can be in the gel or the loading dye. (We use Syber Green. Ethidium Bromide can also be used)
- We can estimate the amount (brightness of the band

Electrophoresis – Setup

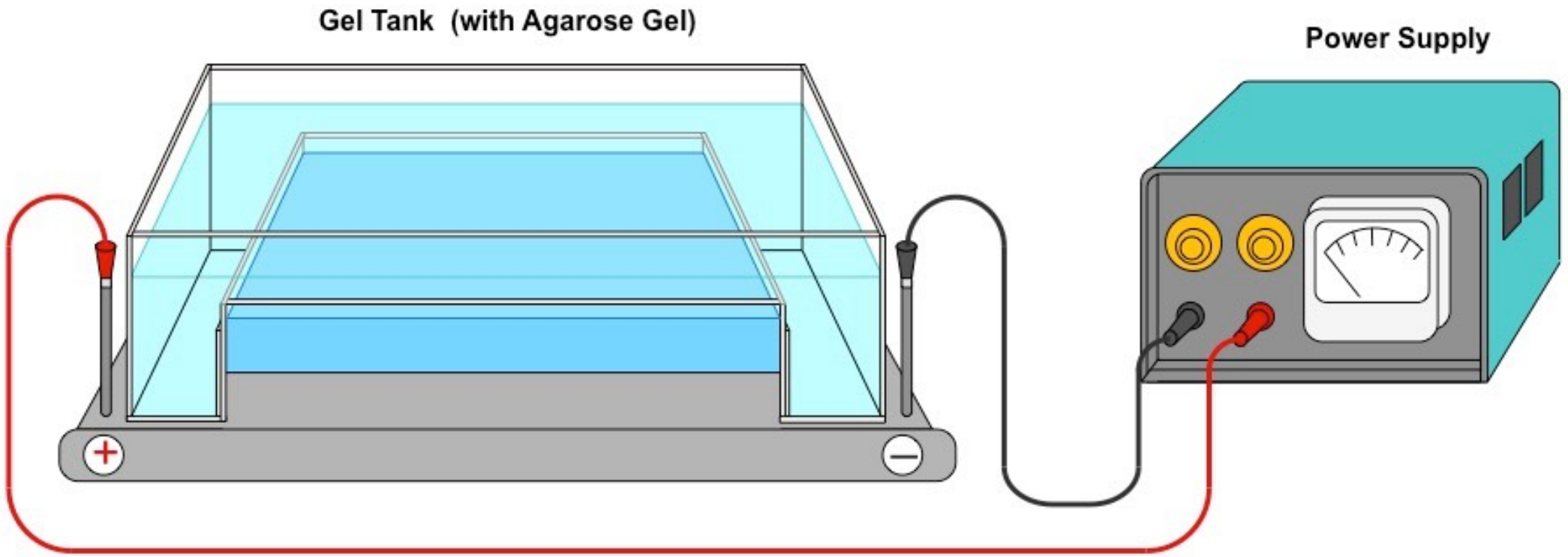


Photo: https://ib.bioninja.com.au/_Media/electrophoresis-dna_med.jpeg

Electrophoresis – Setup

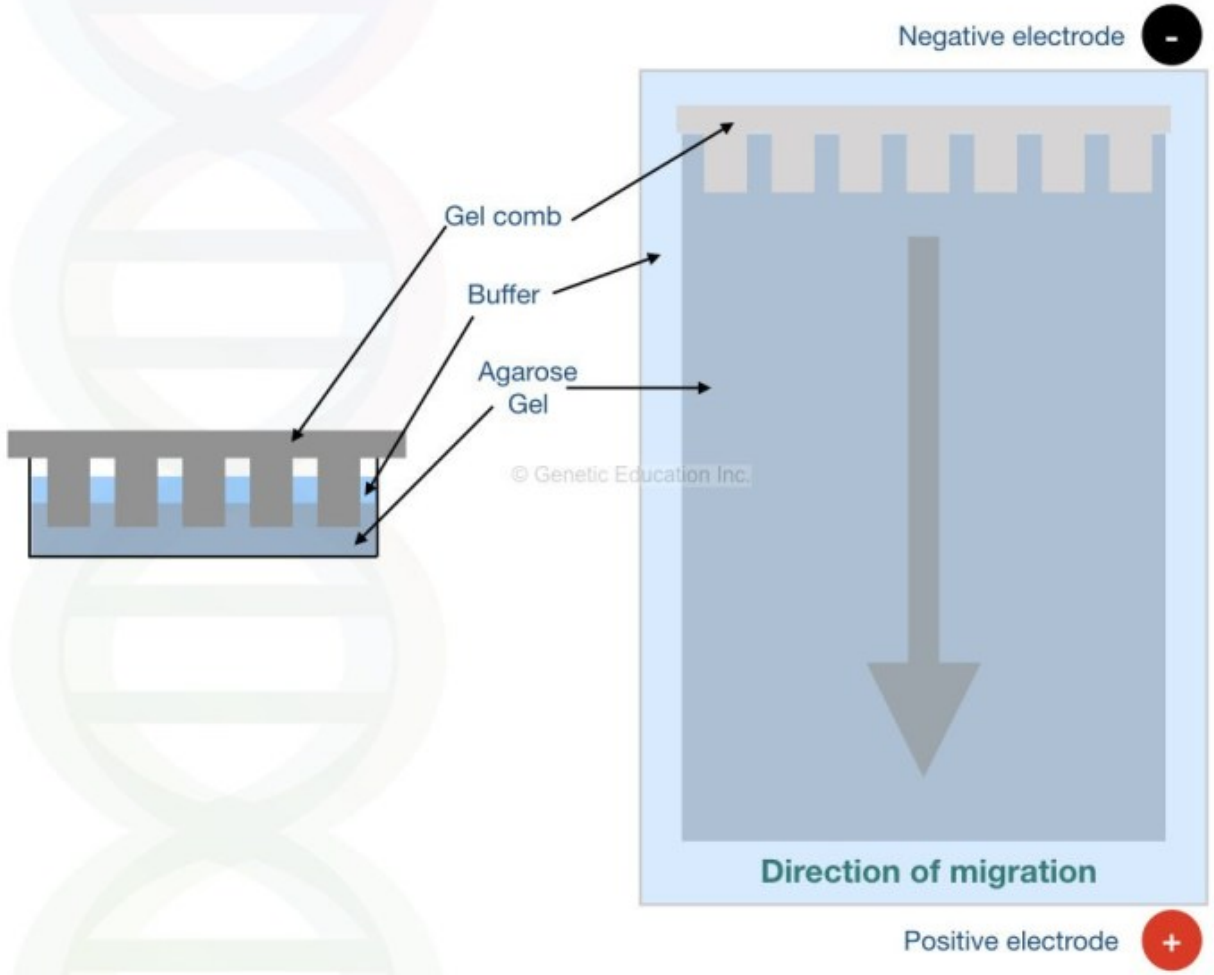
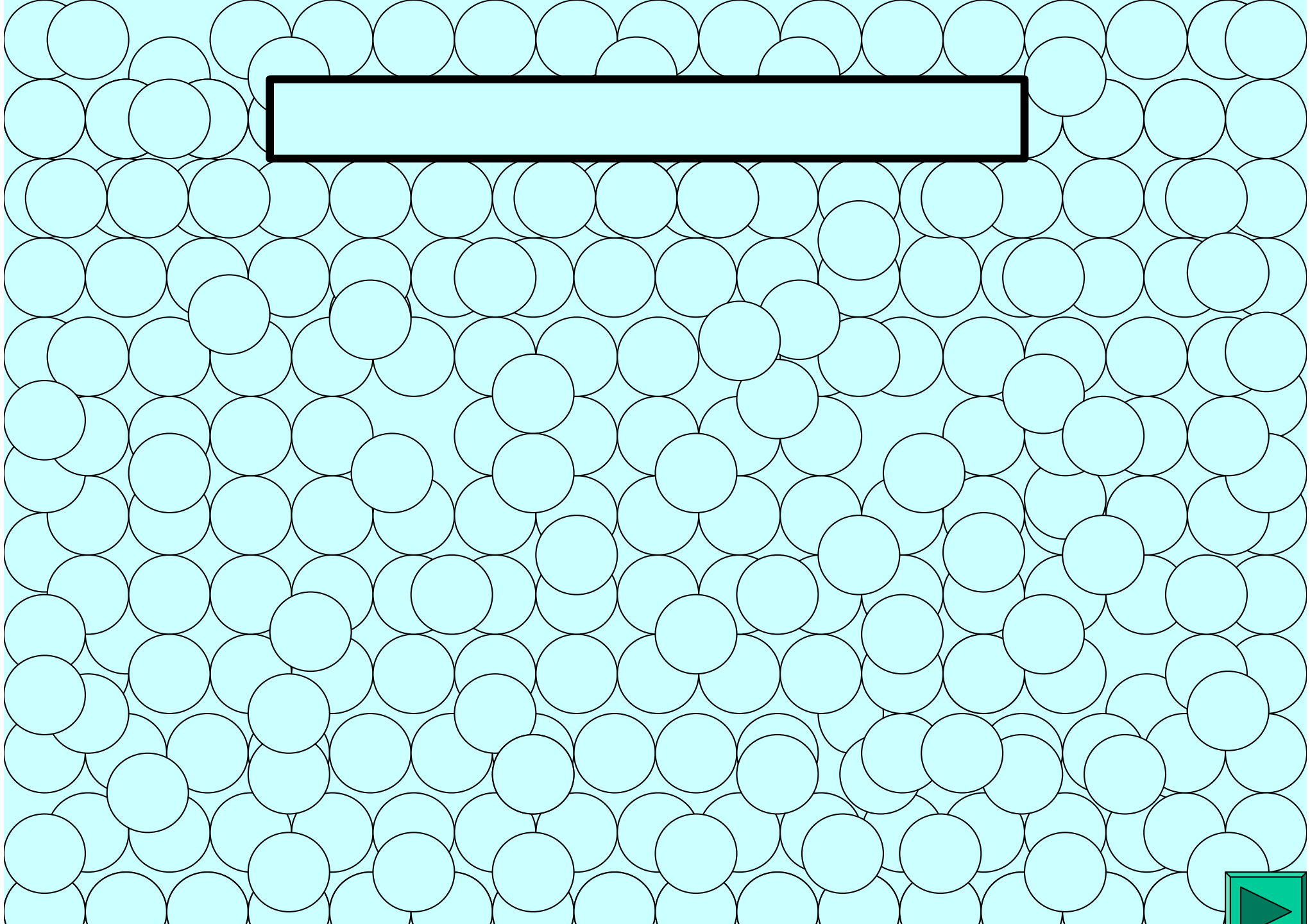
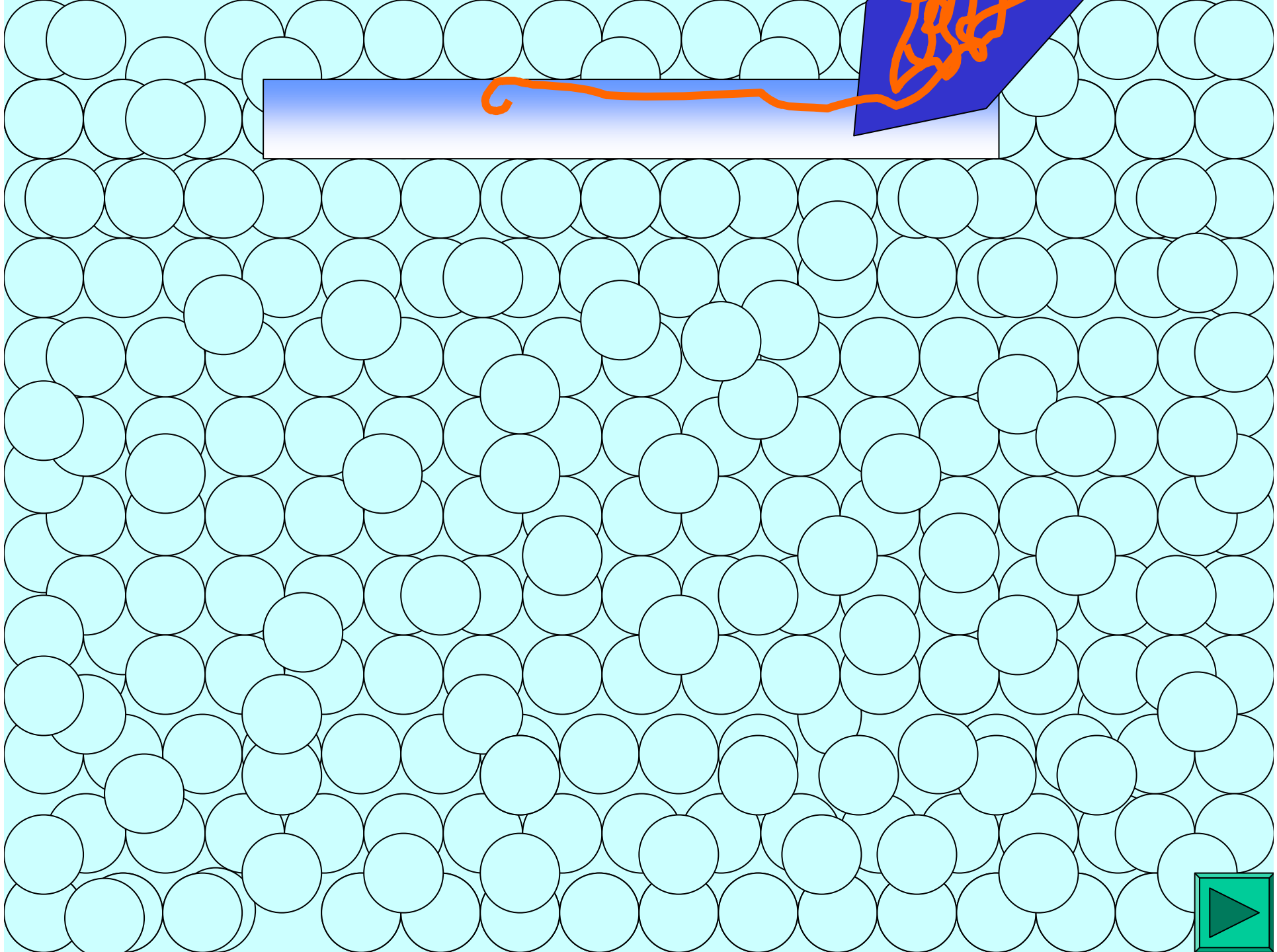
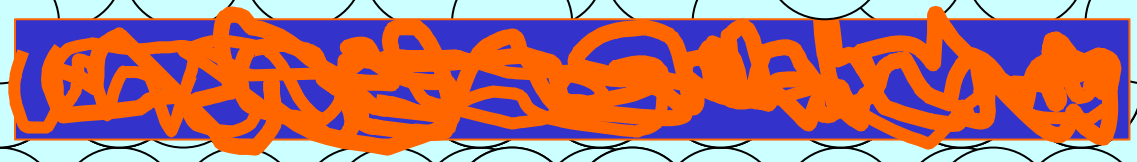


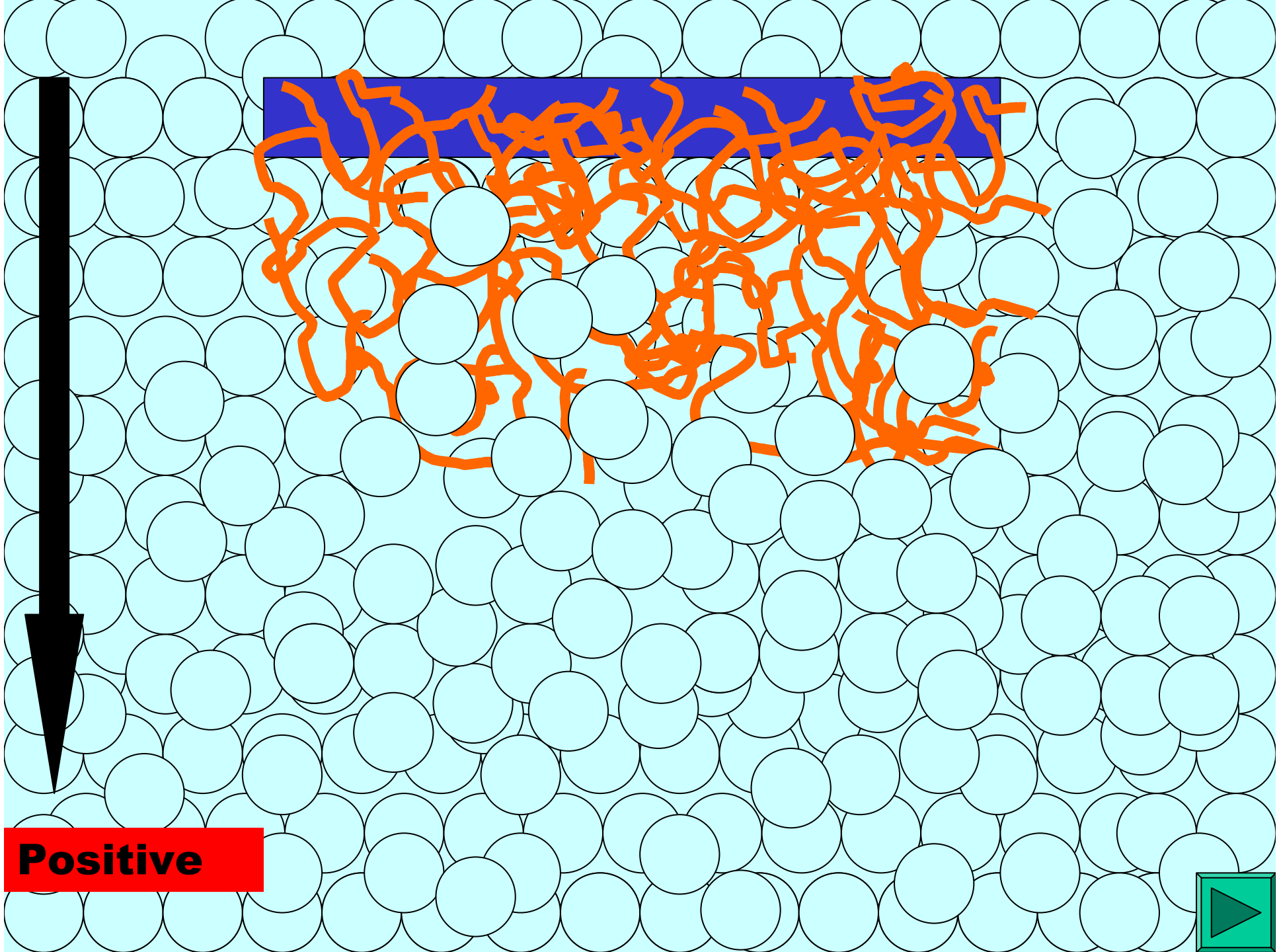
Photo: https://i2.wp.com/geneticeducation.co.in/wp-content/uploads/2018/09/electrophoresis-.001-e1558614261130.jpeg?resize=719%2C566&ssl=1

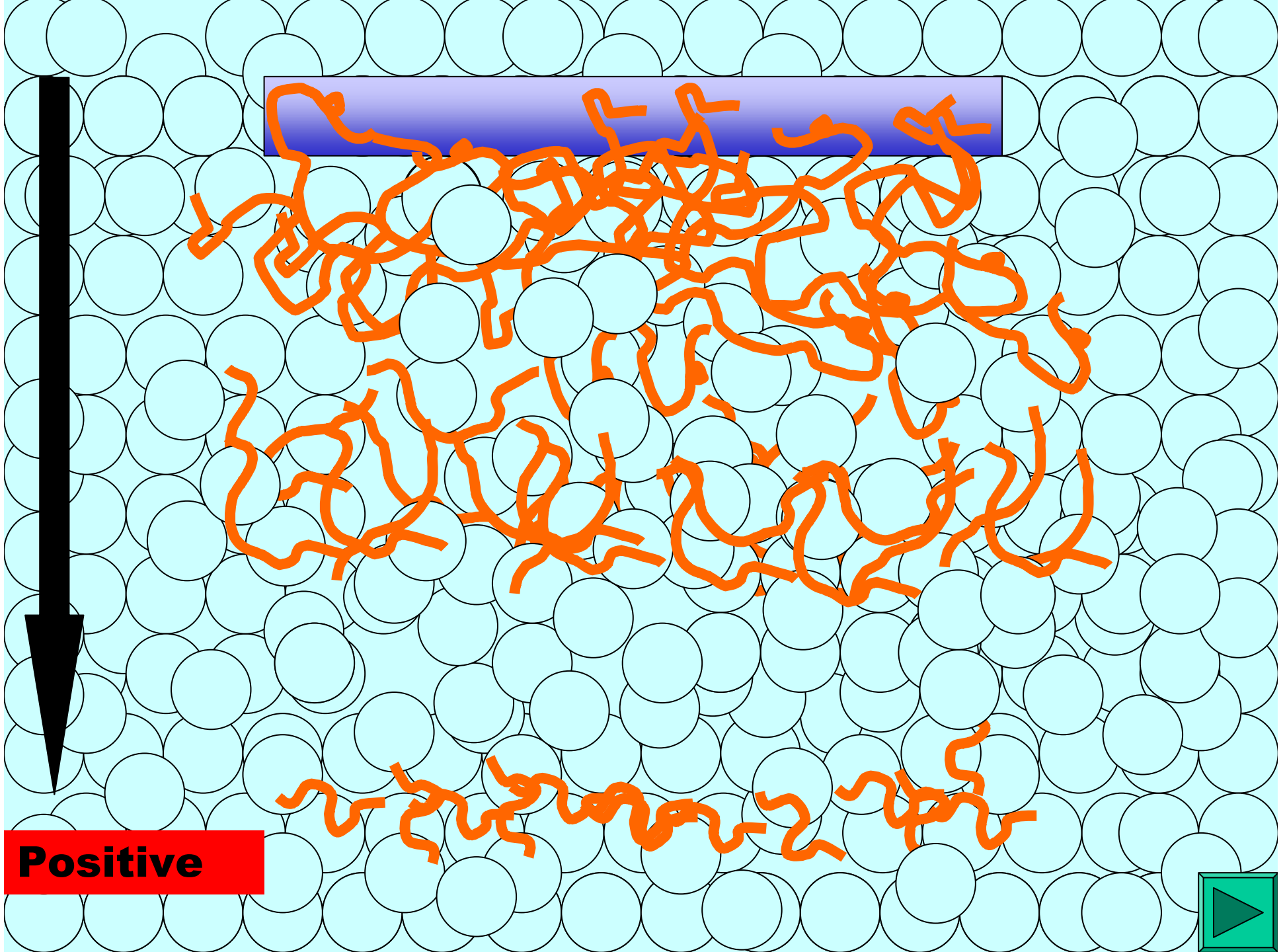


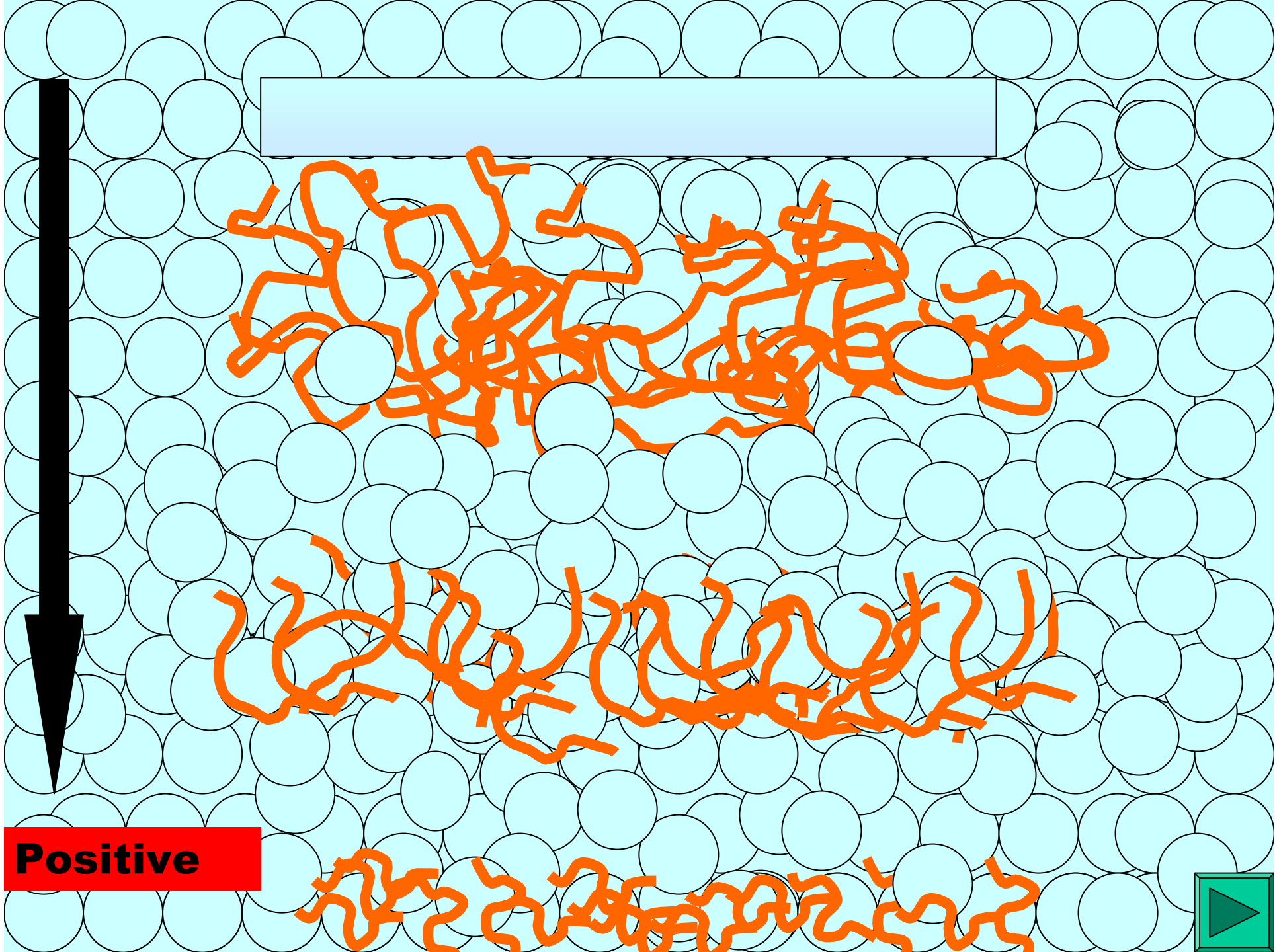




Positive


Positive


Positive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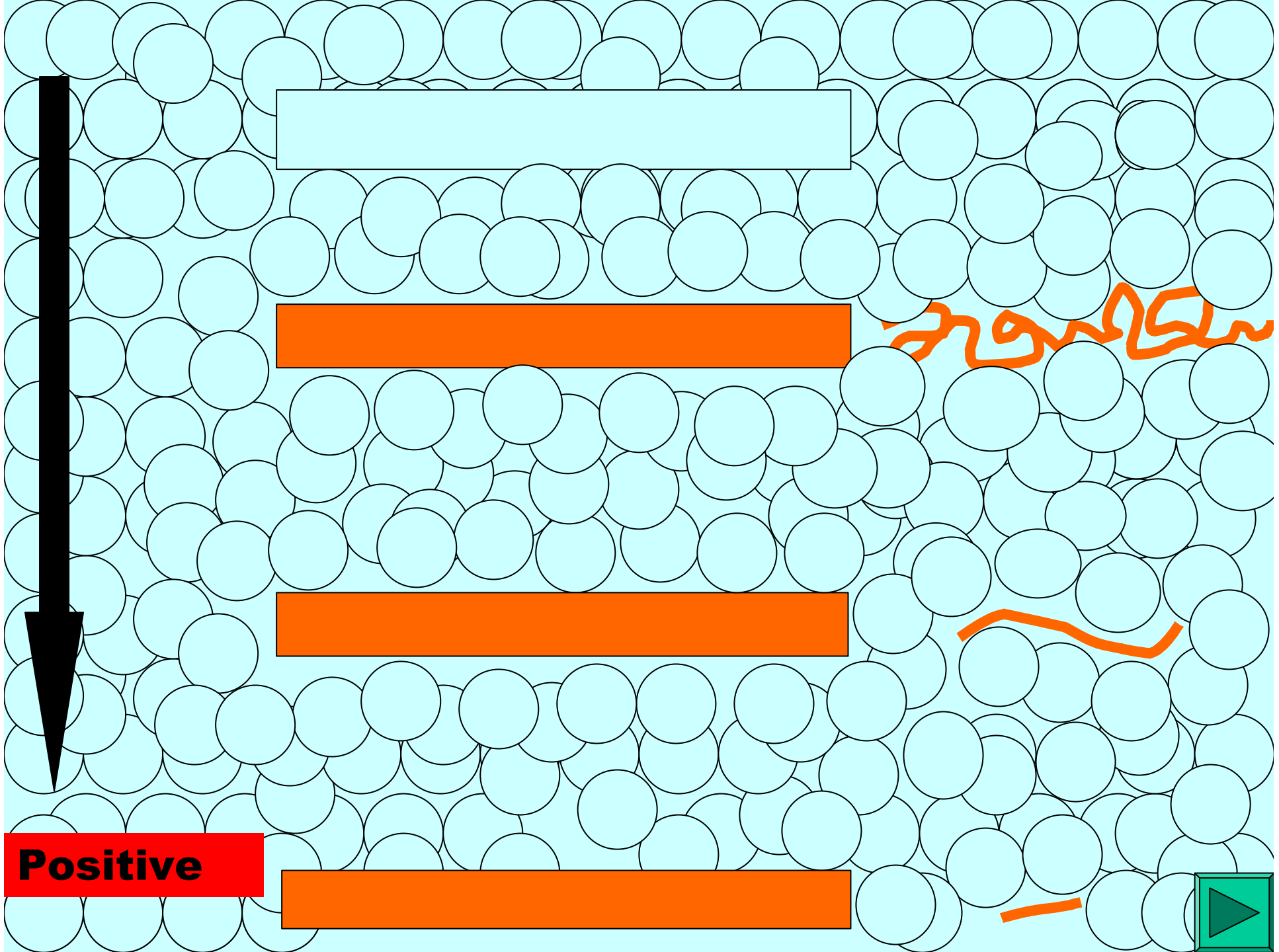
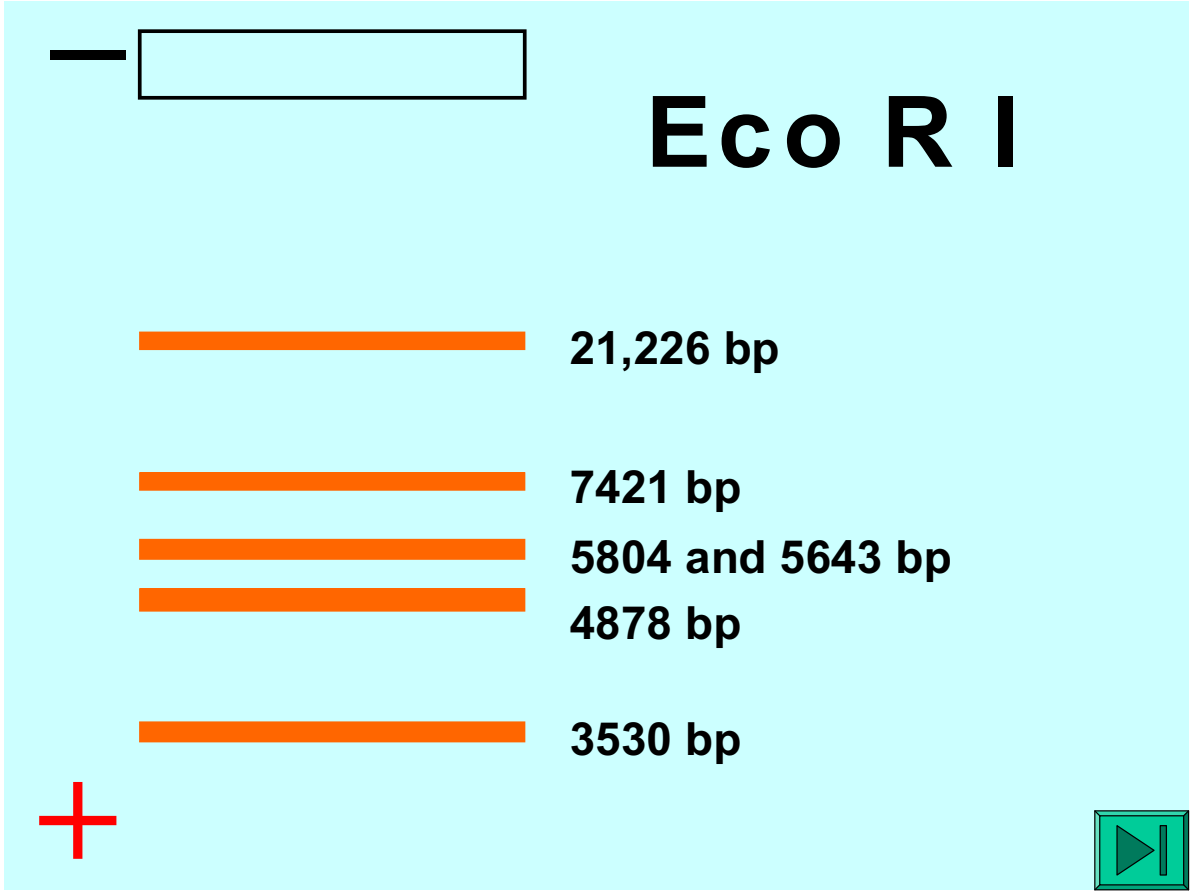
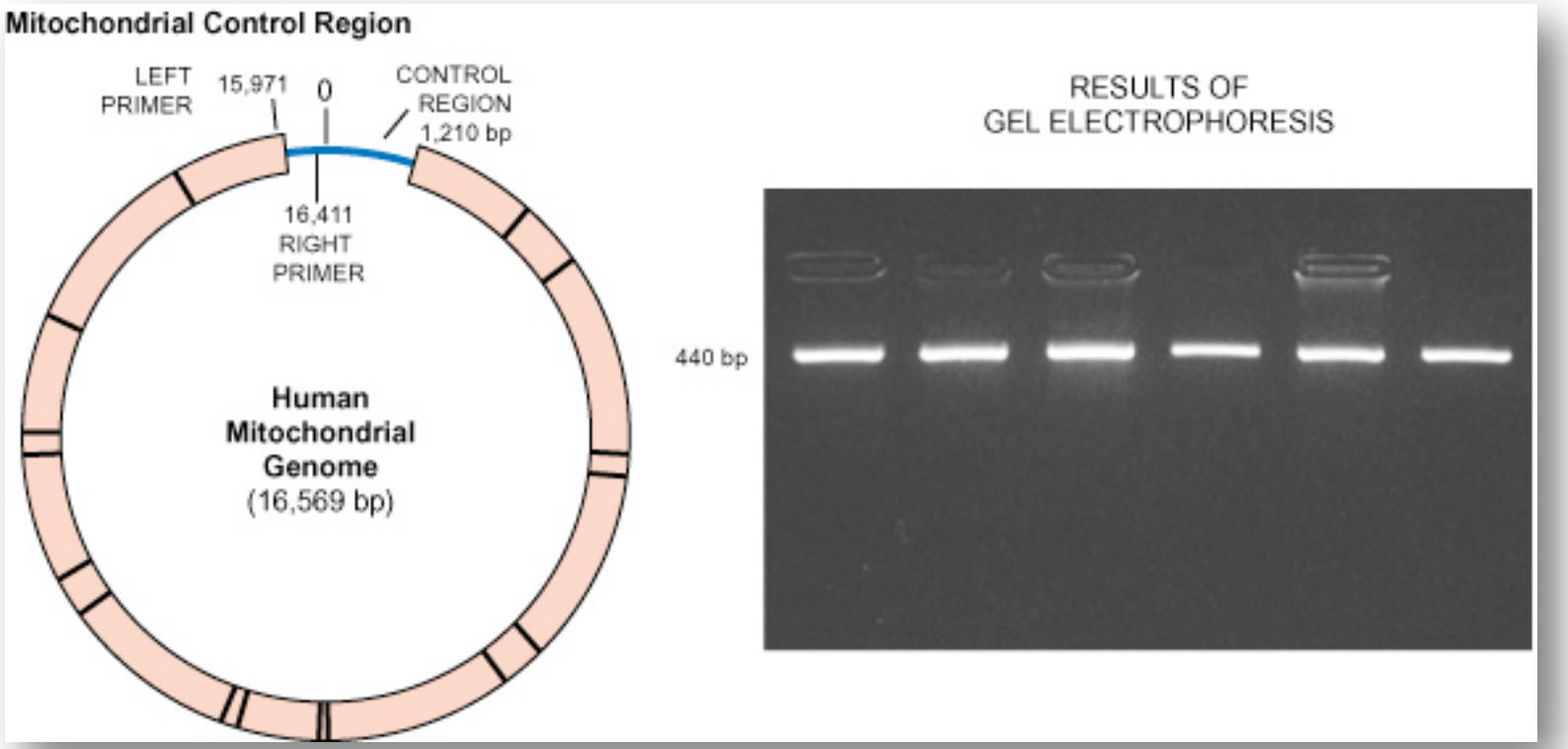
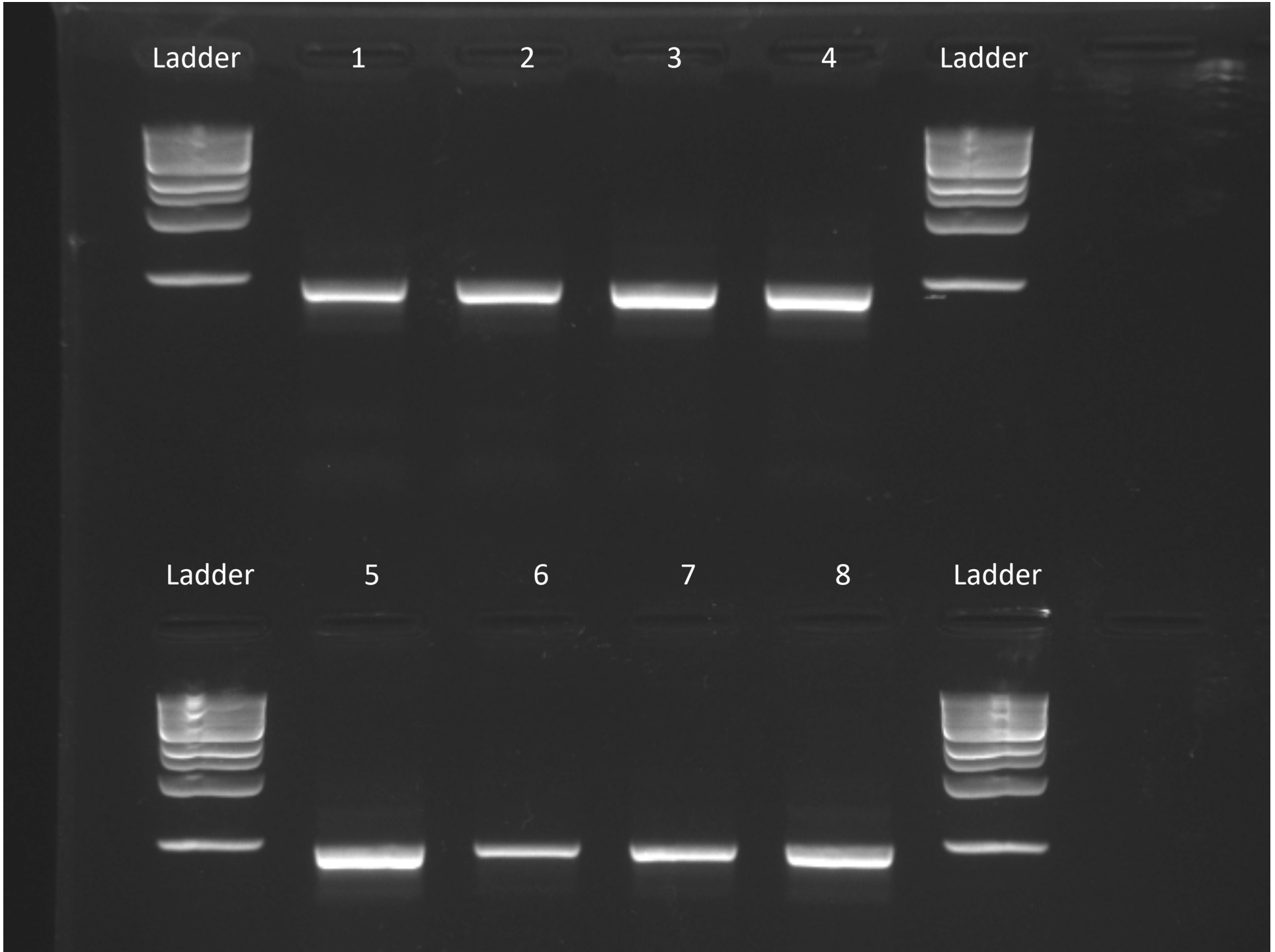

Positive

Electrophoresis – Cut DNA example



Mitochondrial DNA – replicated by PCR

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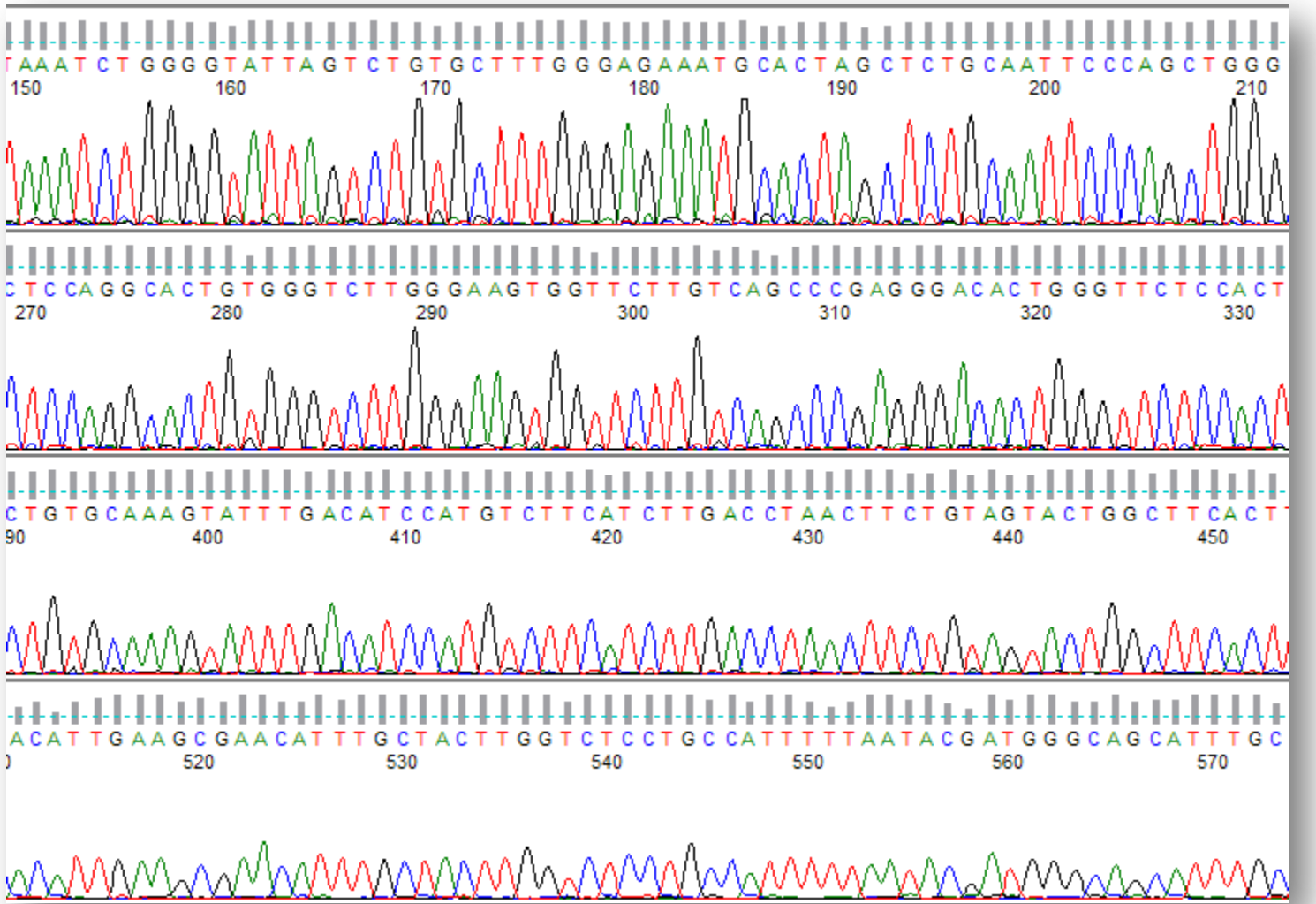
DNA Sequencing



DNA Sequencing

- Allows us to determine the nucleotides and their order on a segment of DNA
- There are many types of DNA sequencing. For PCR products we use “Sanger” (cycle) sequencing (Fredrick Sanger)
- In this method we usually can sequence up to 1000bp of DNA
- Many components in common with PCR so be careful not to confuse them!

DNA Sequencing

Cycle Sequencing

To sequence a piece of DNA, you need:

the DNA you want to sequence (template DNA),

a short DNA "primer" that is complementary to the DNA you want to sequence,

an enzyme called DNA polymerase,

four nucleotides.