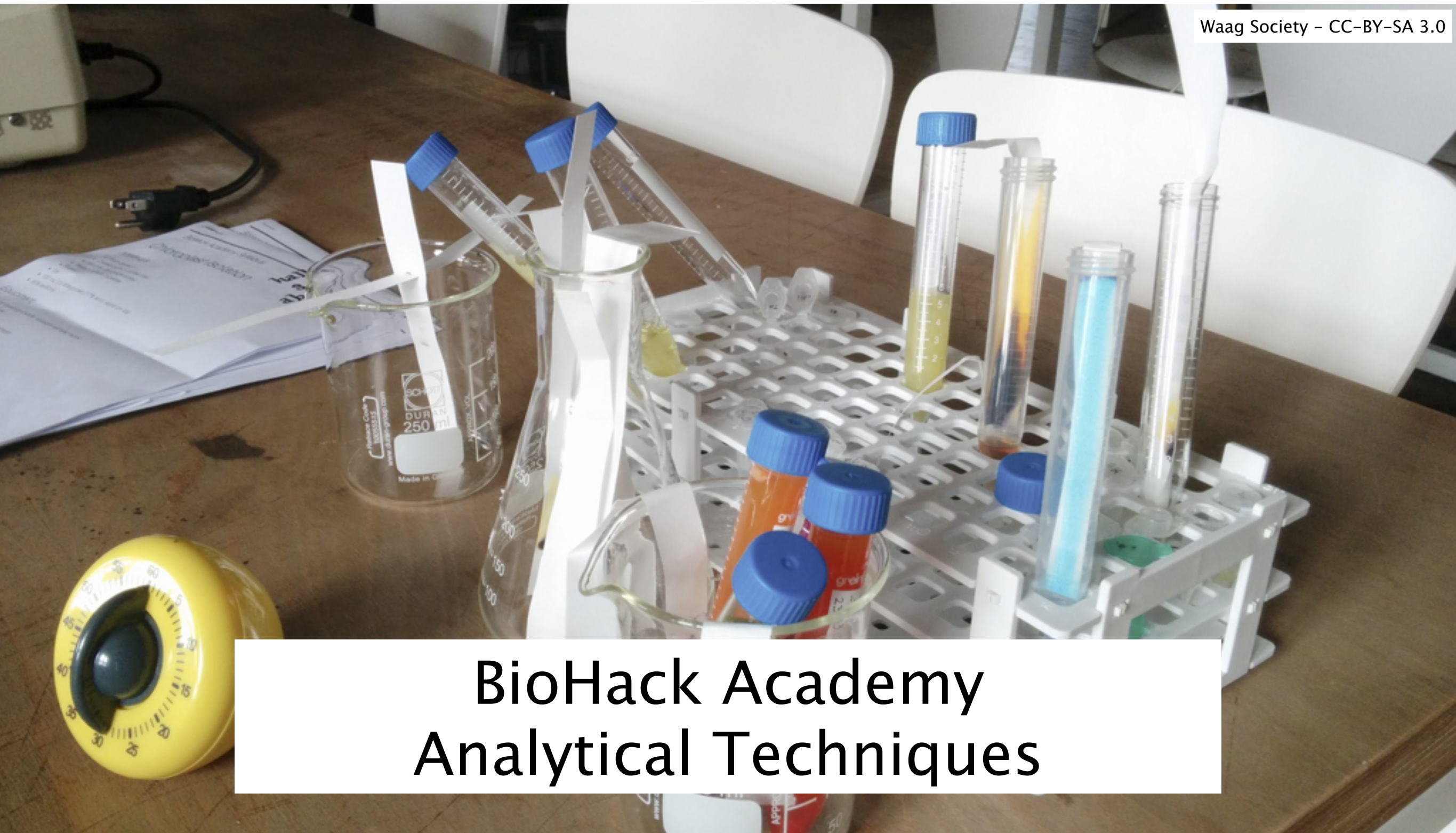




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BioHack Academy Analytical Techniques



Biological Analytical Techniques

- Spectrometry
 - Photo
 - Raman
 - Mass
- Chromatography
 - HPLC
- Electrophoresis
- Nuclear magnetic resonance
- DNA analytics
 - Sequencing
 - CHIP
 - DNA array
- DNA editing
 - Restriction ligation
 - Gateway
 - Gibson
 - CRISPR



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Spectrometry



Tibicos – kefir





Refraction

Normal

Incident
ray

θ_i

Air

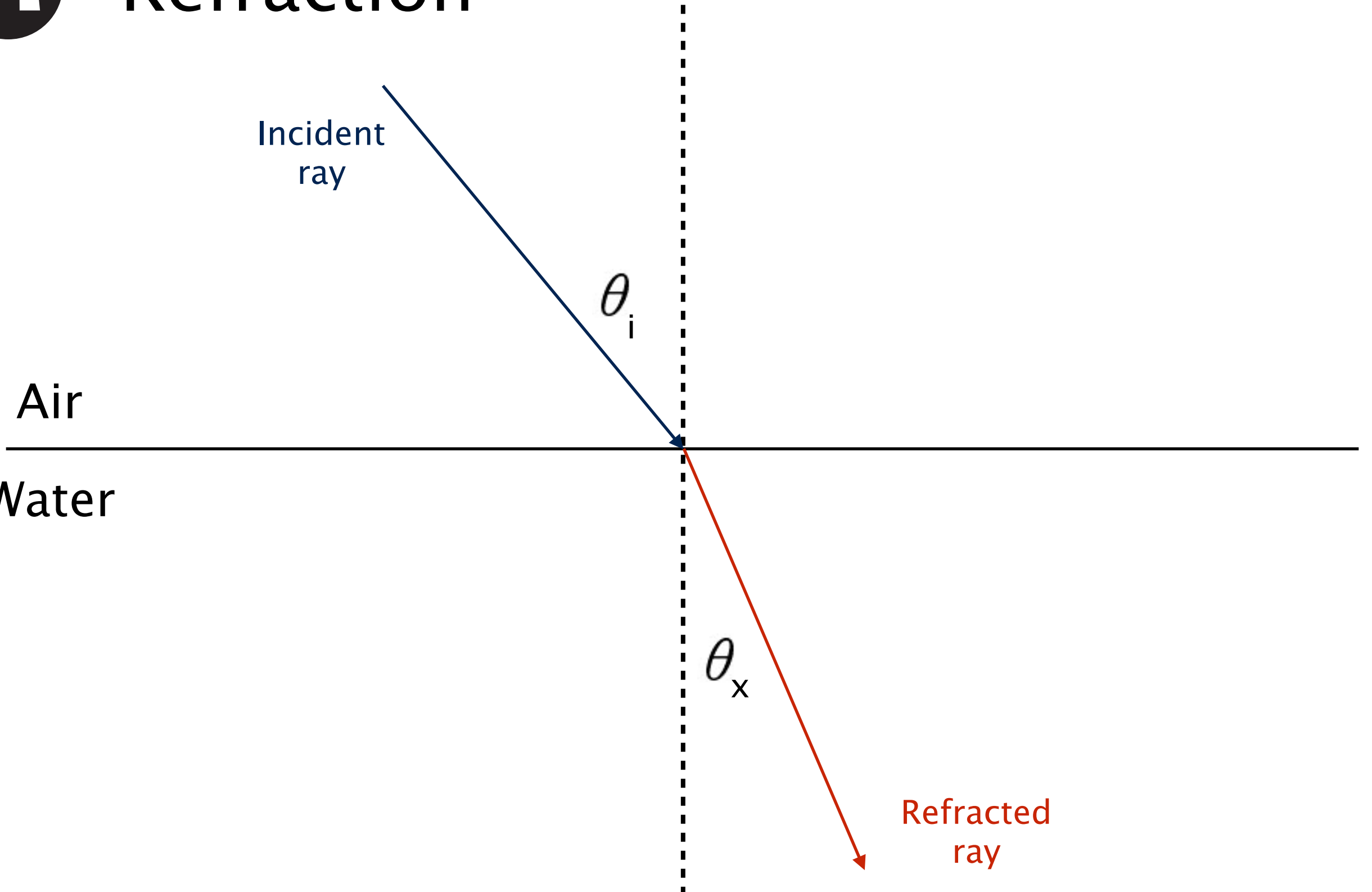
Water

θ_x

Refracted
ray

θ_i Angle of incidence

θ_x Angle of refraction



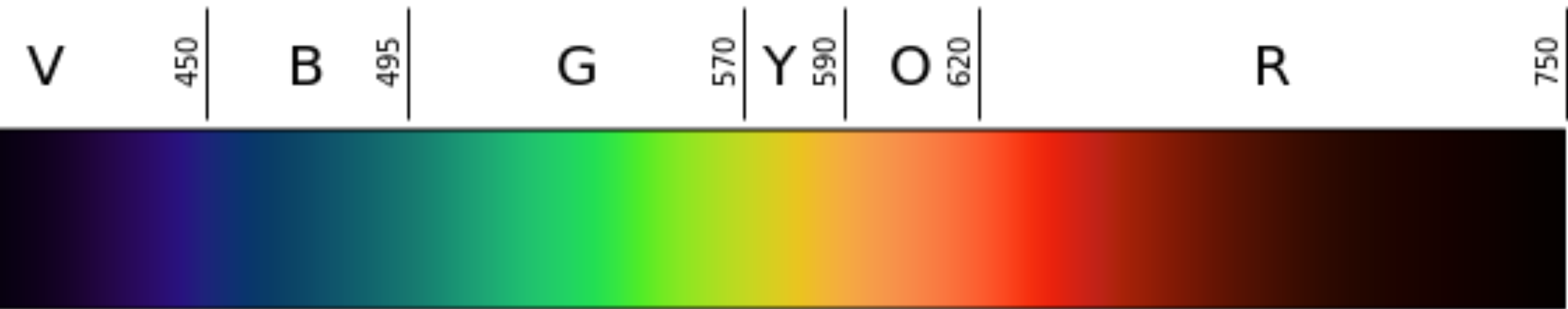


Snell's Law

$$\sin(\theta_i) = n_{\text{material}} \times \sin(\theta_x)$$

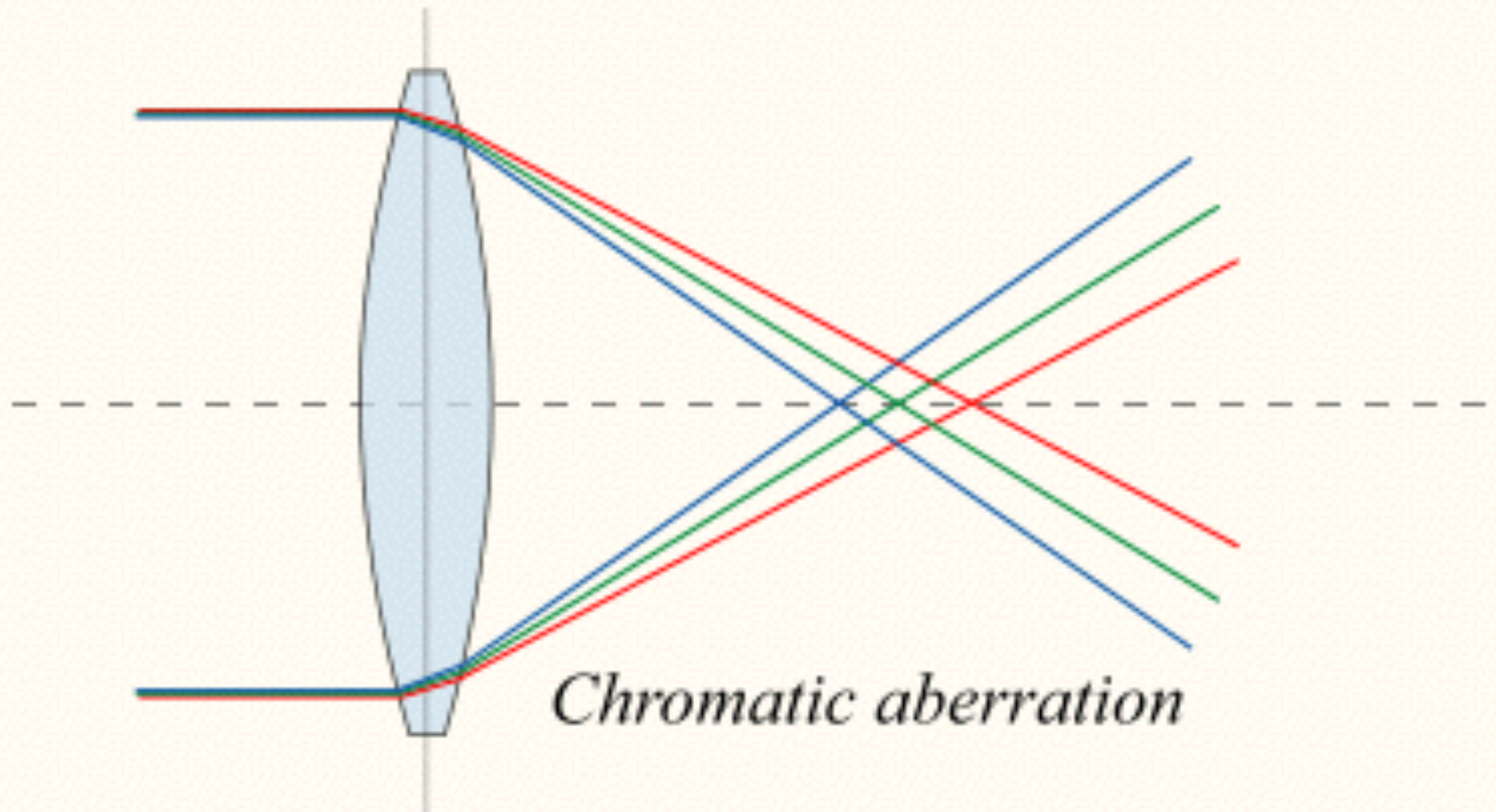


Visible light spectrum





Chromatic aberration



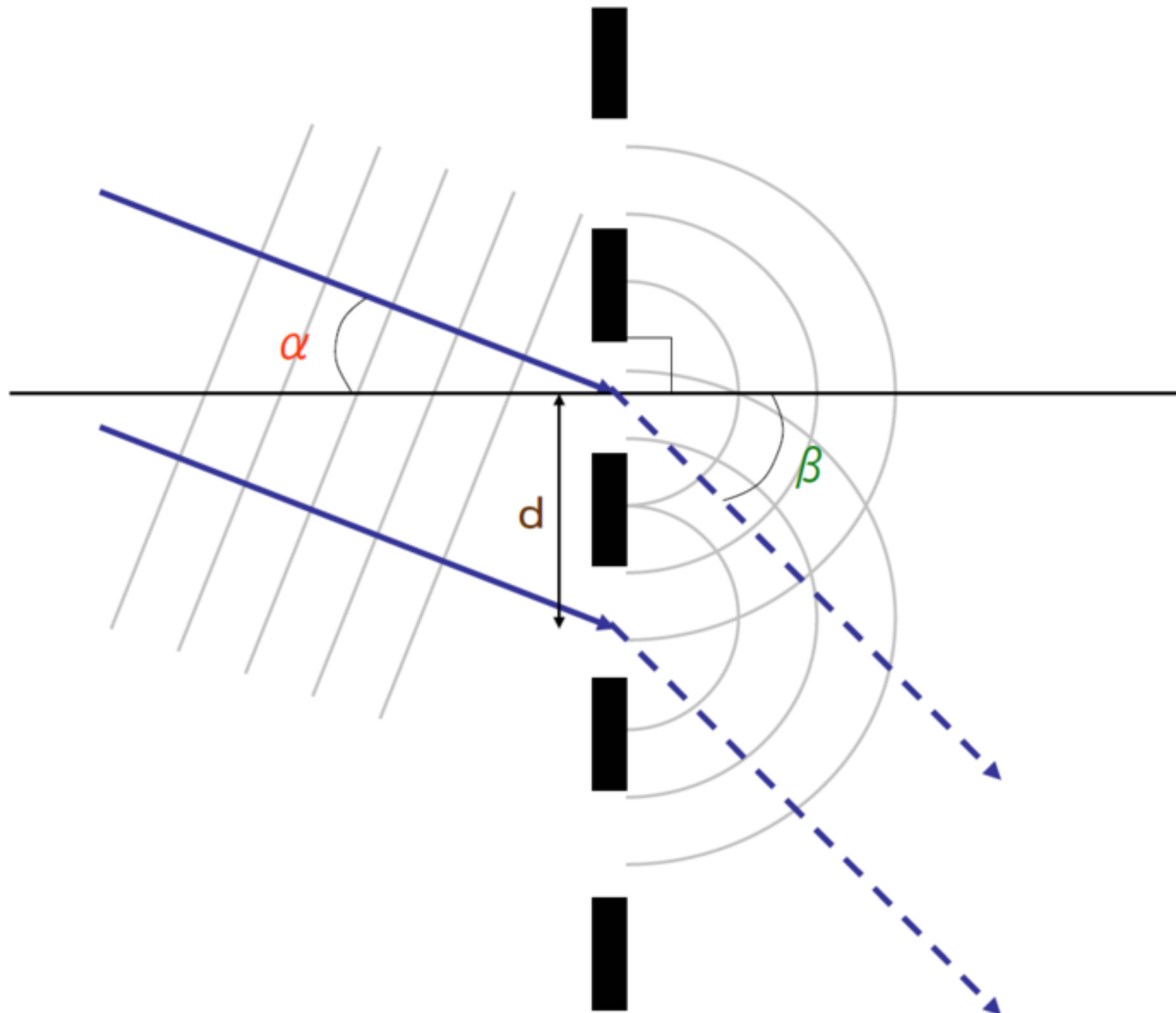


Grating diffraction



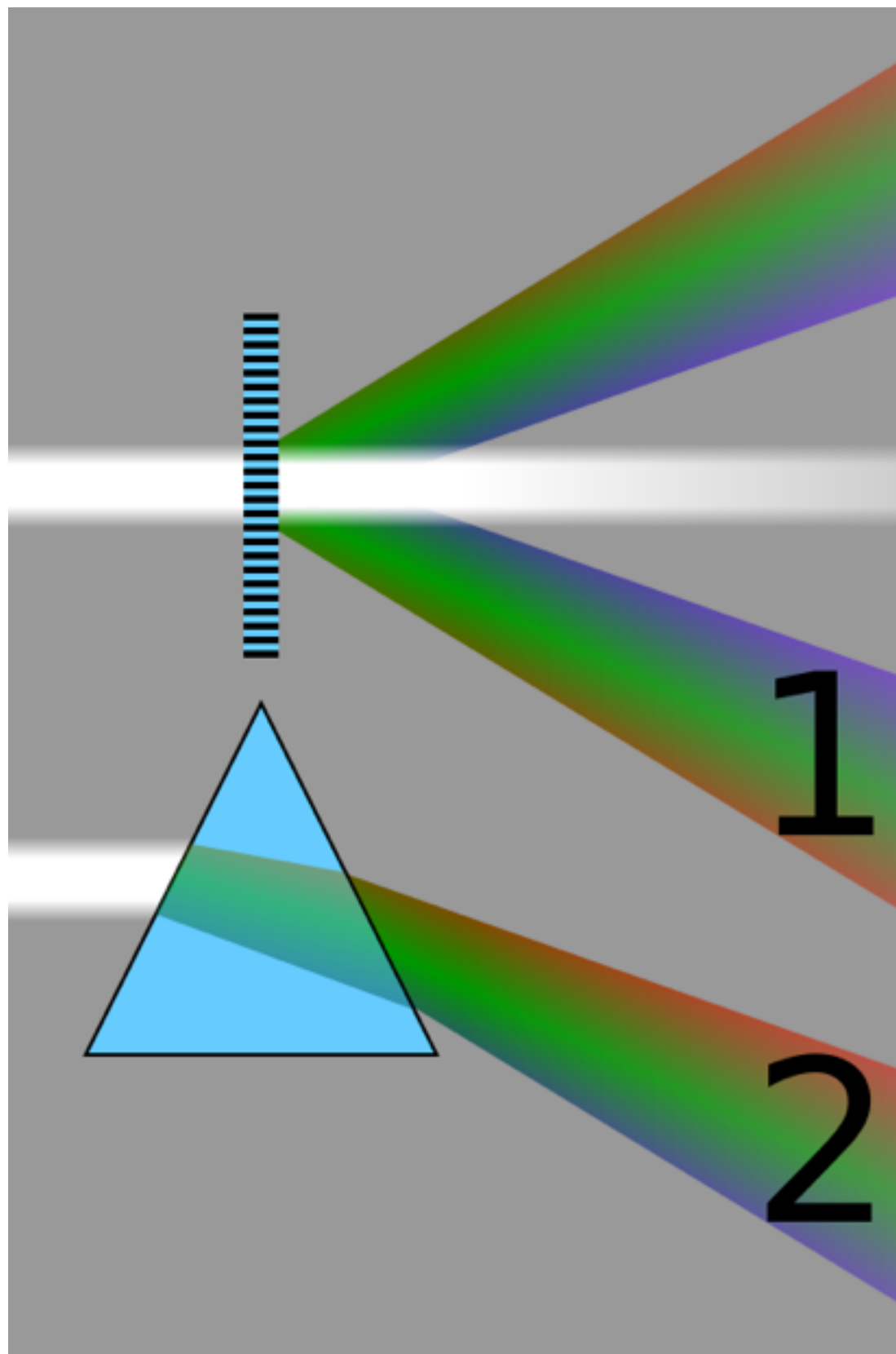


Diffraction grating principle





Just like a prism



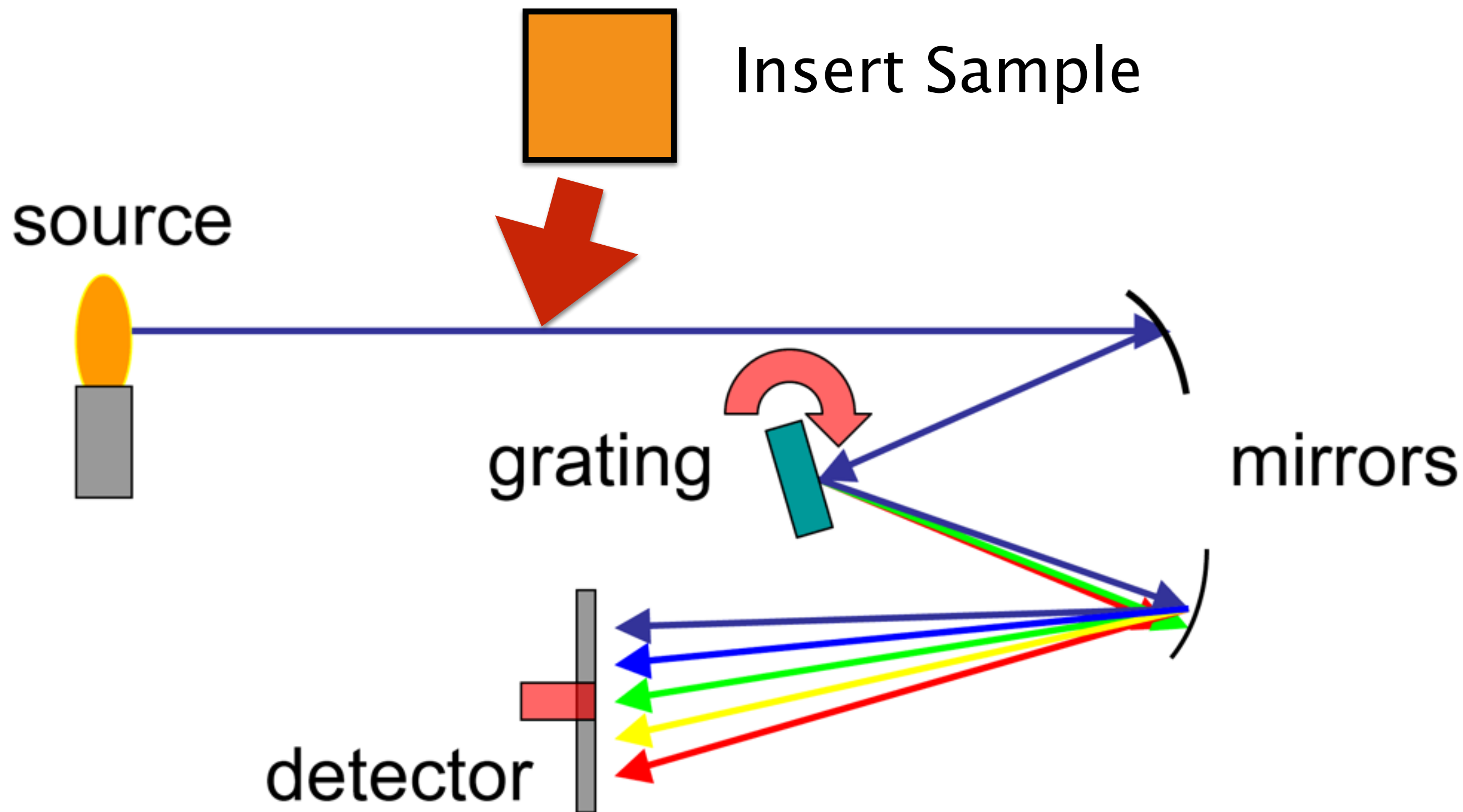


Industry standard



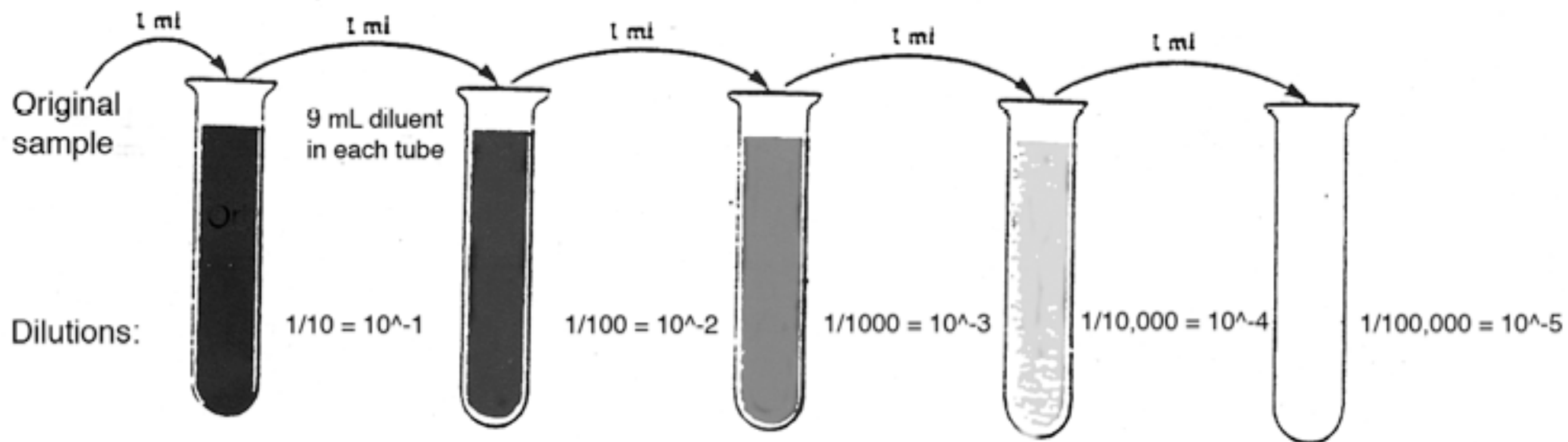
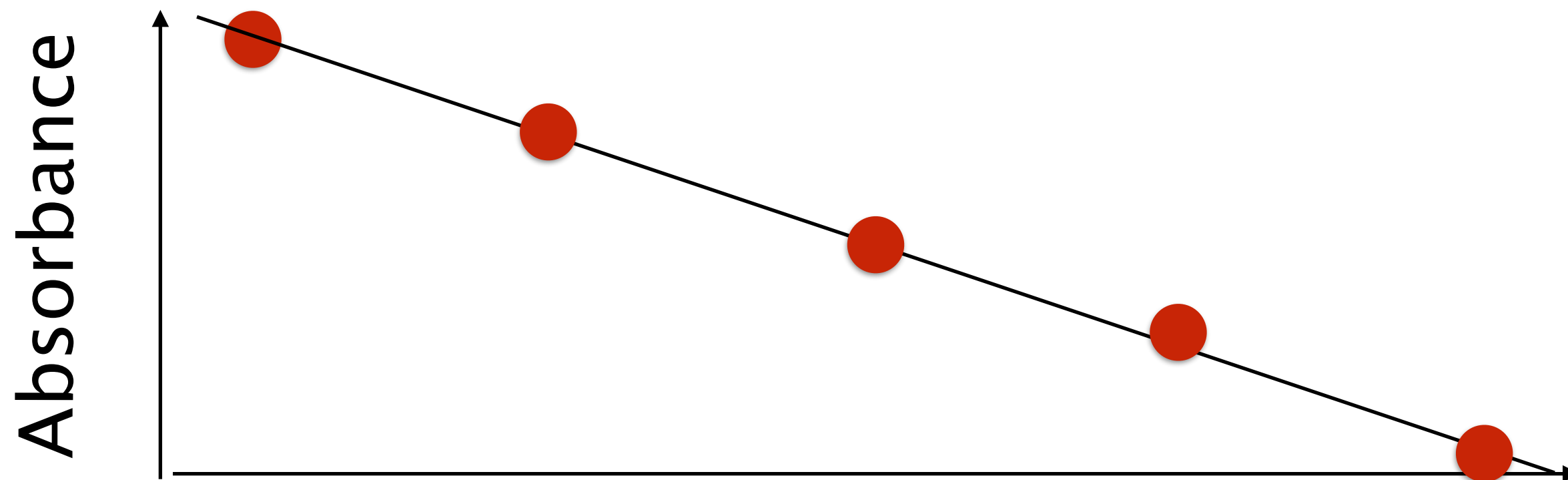


Inside



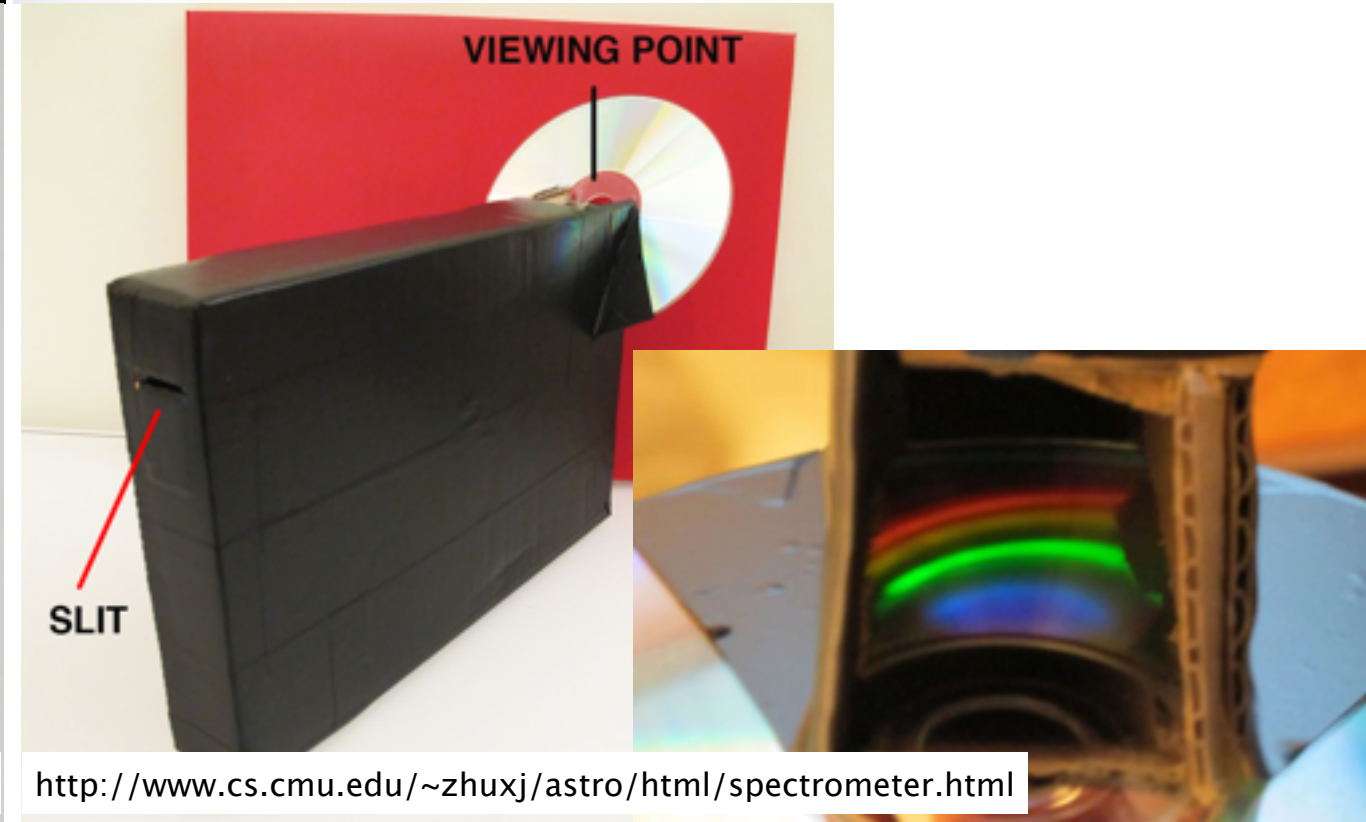
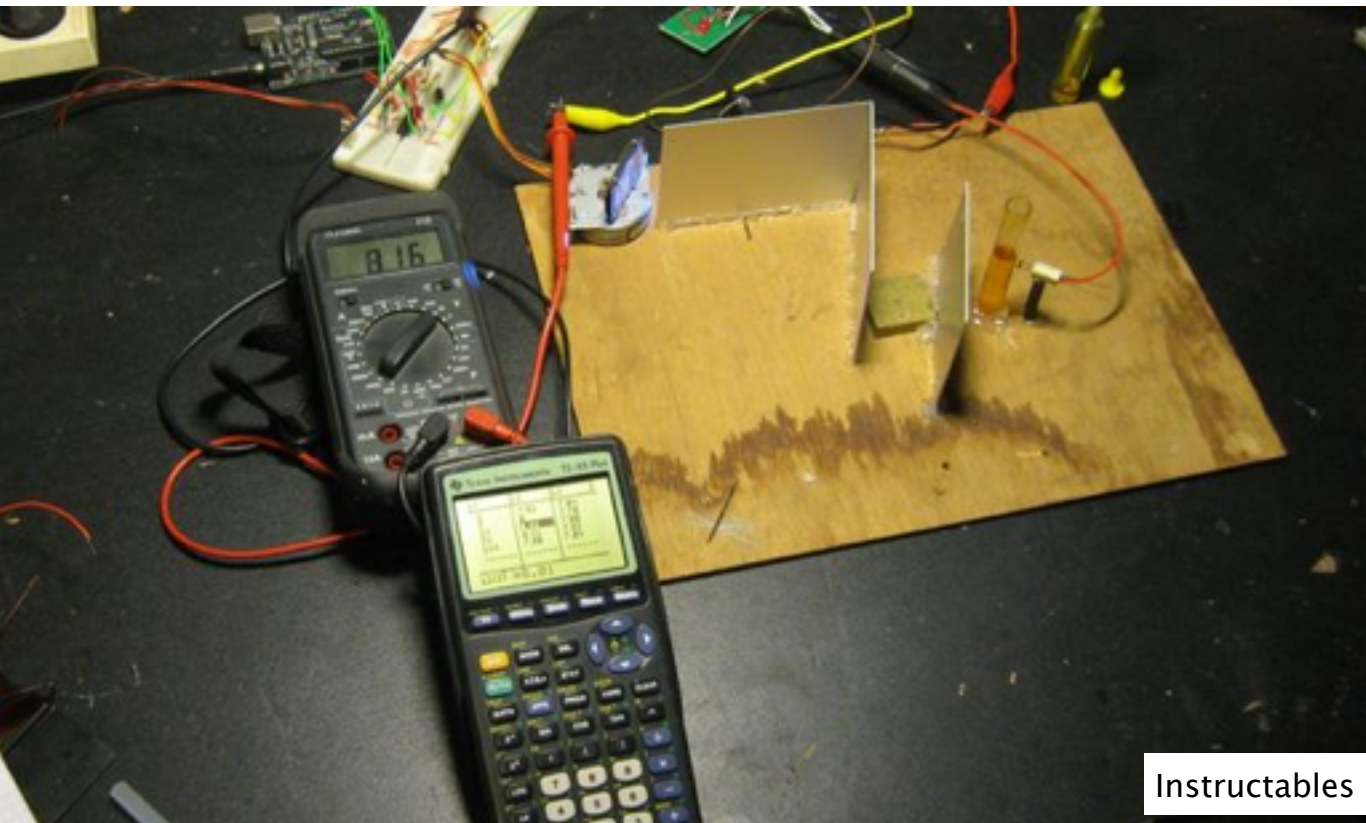


Dilution series





Previous hacks





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Raman Spectrometry



Border security





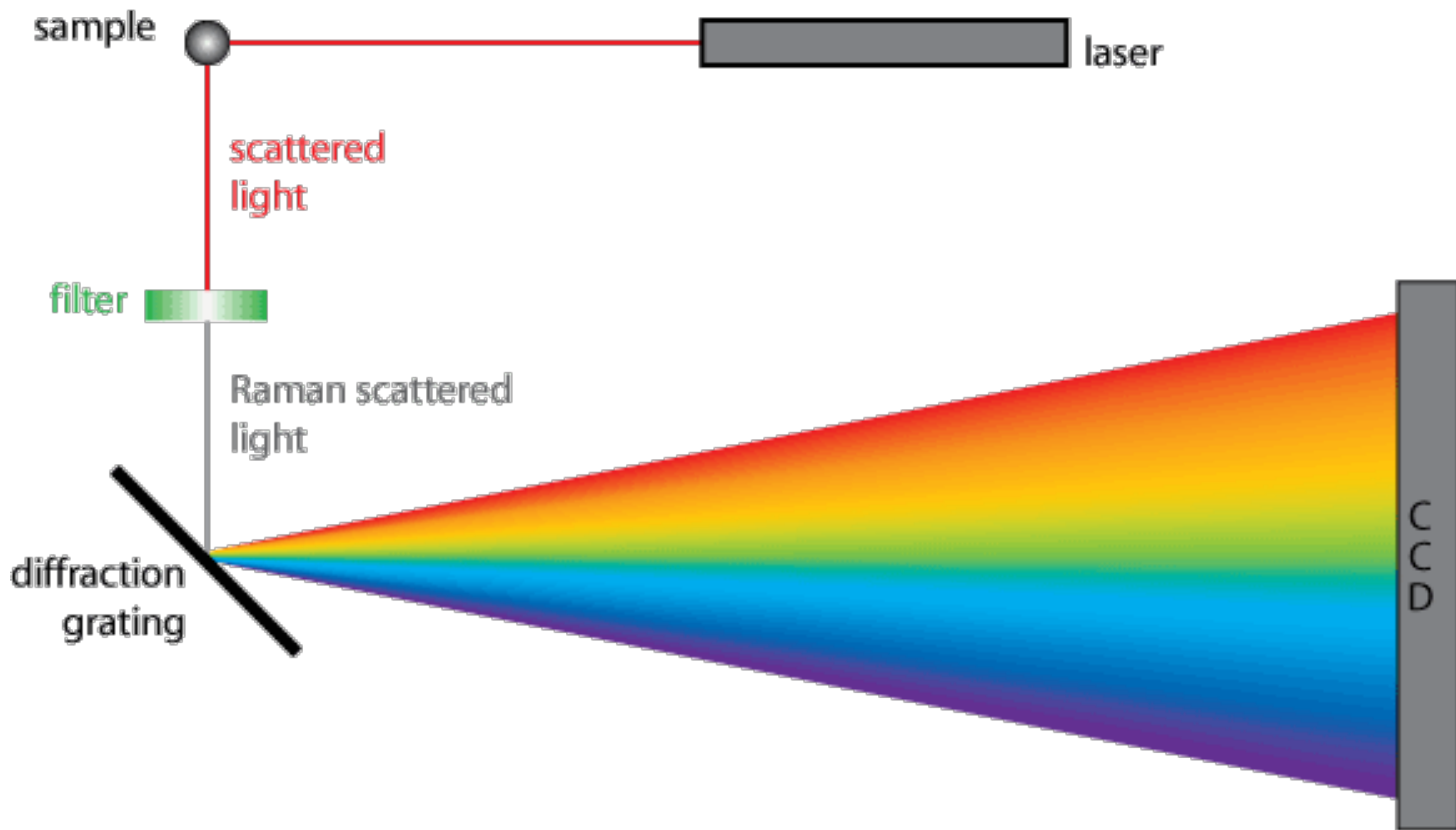
C.V. Raman

- Nobel Prize 1930
- „Raman Scattering”





Principle





DIY versions

KICKSTARTER

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Start



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12,958 backers pledged \$2,762,571 to help bring this project to life.



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Chromatography

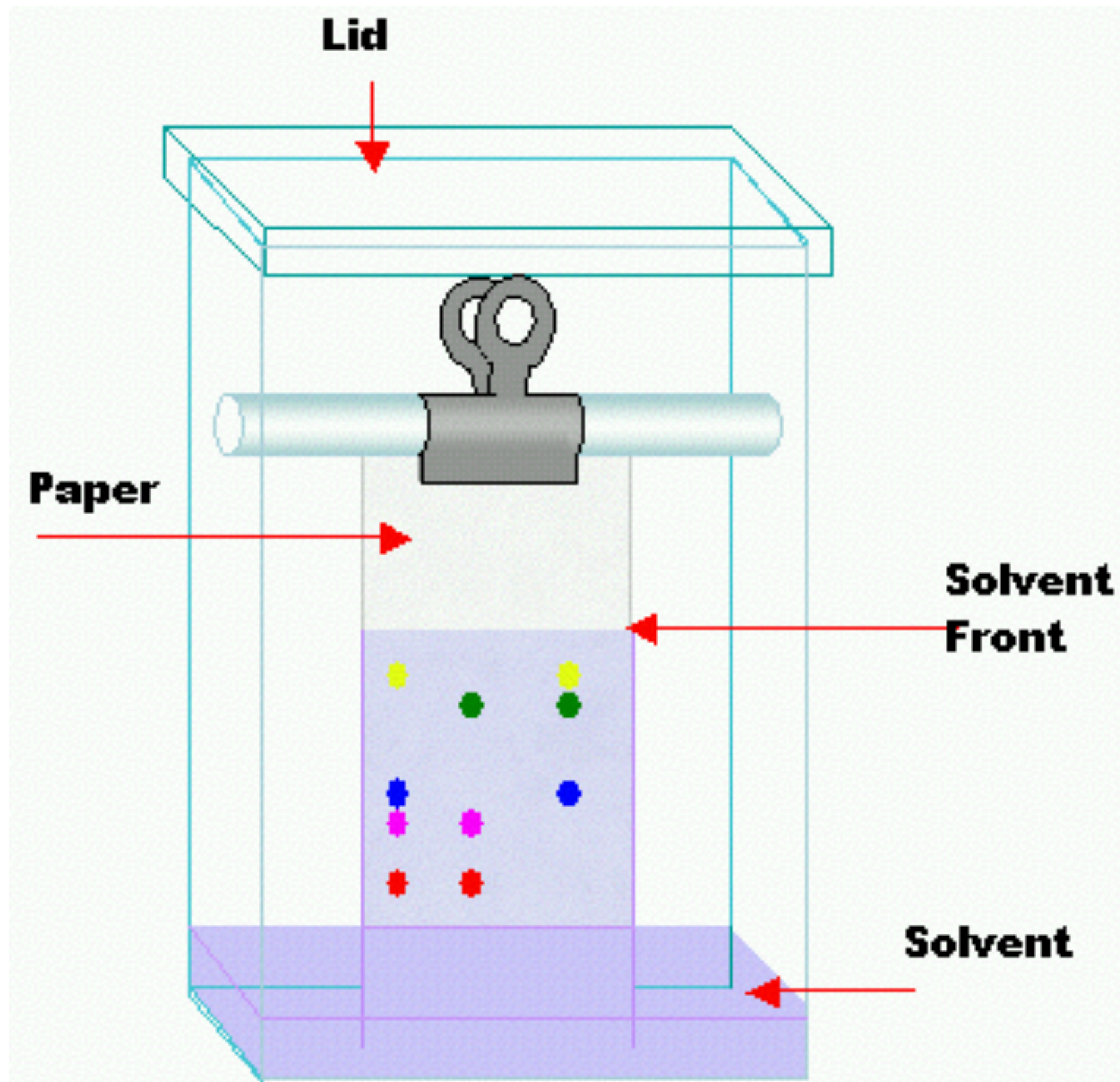


What's in here?



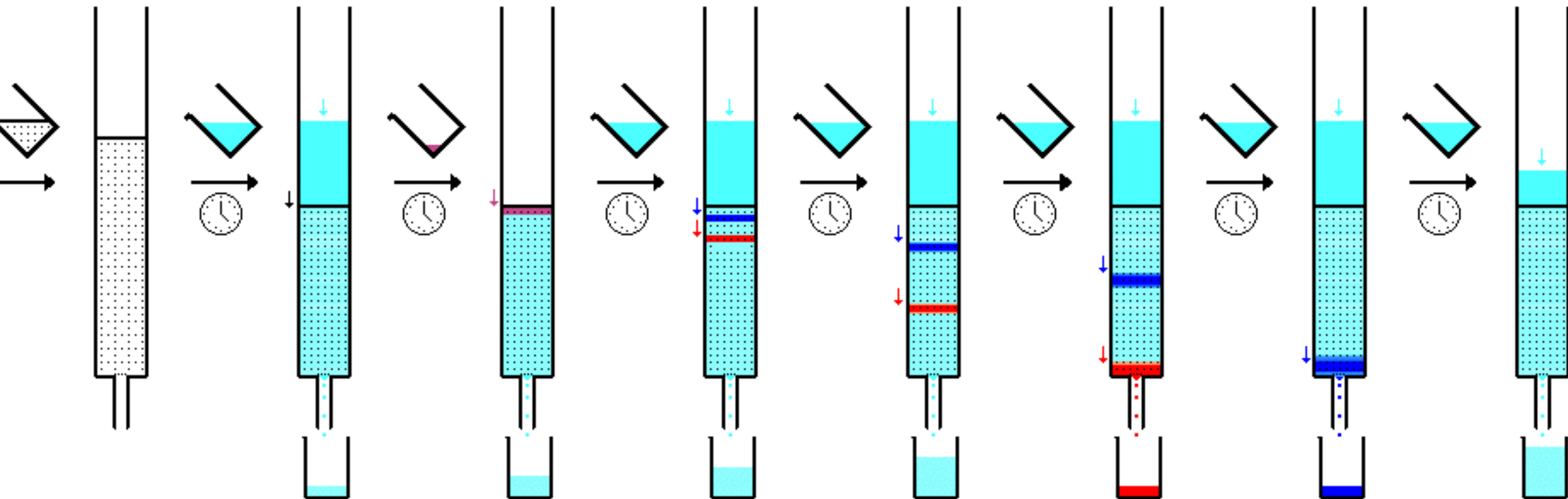


Paper



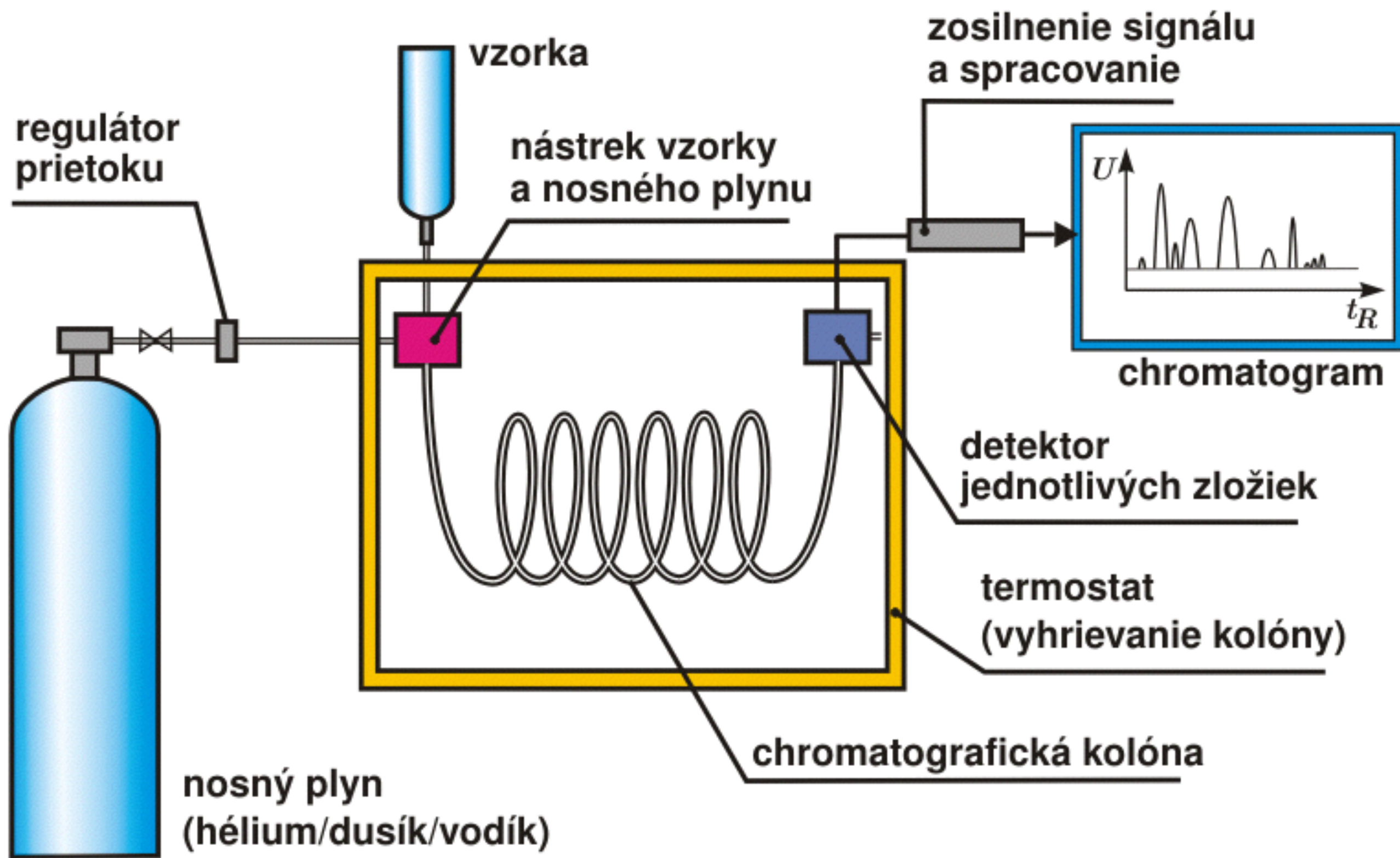


Liquid





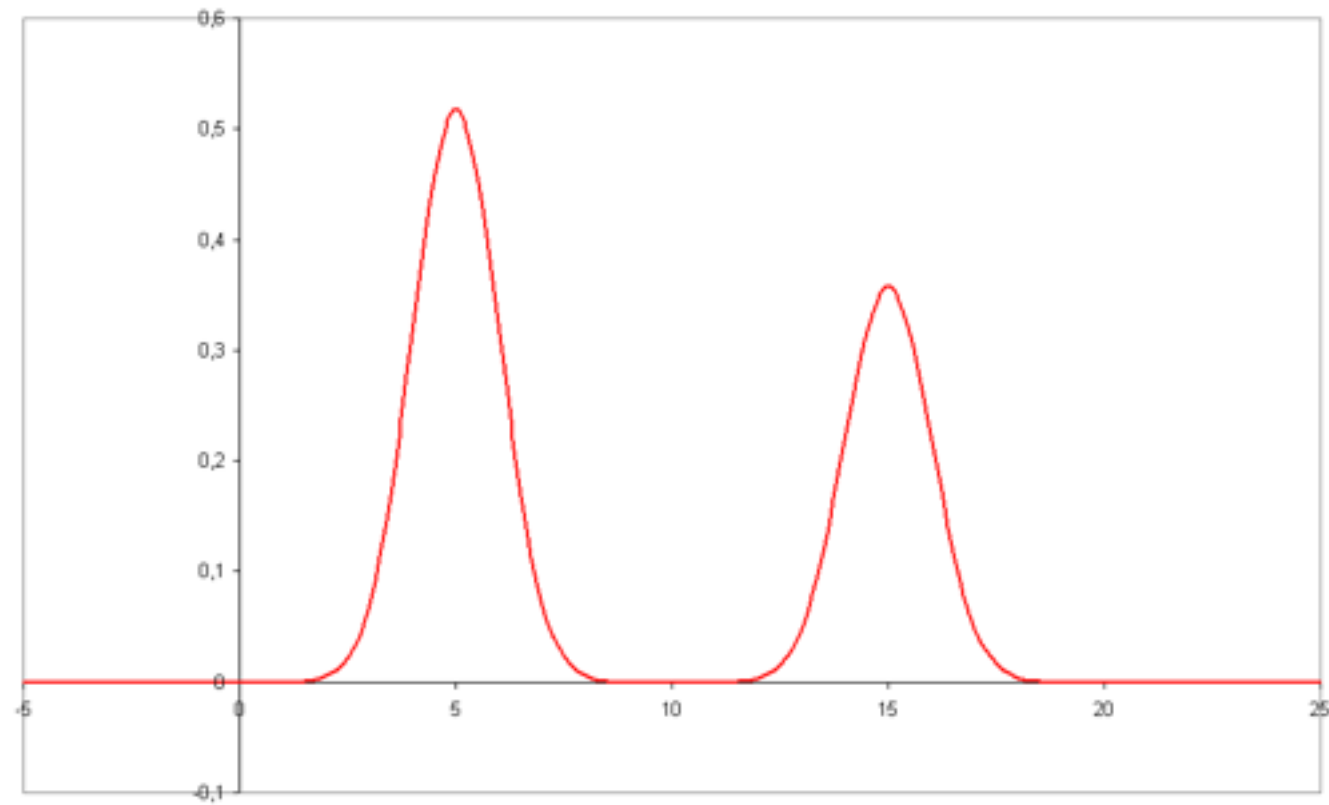
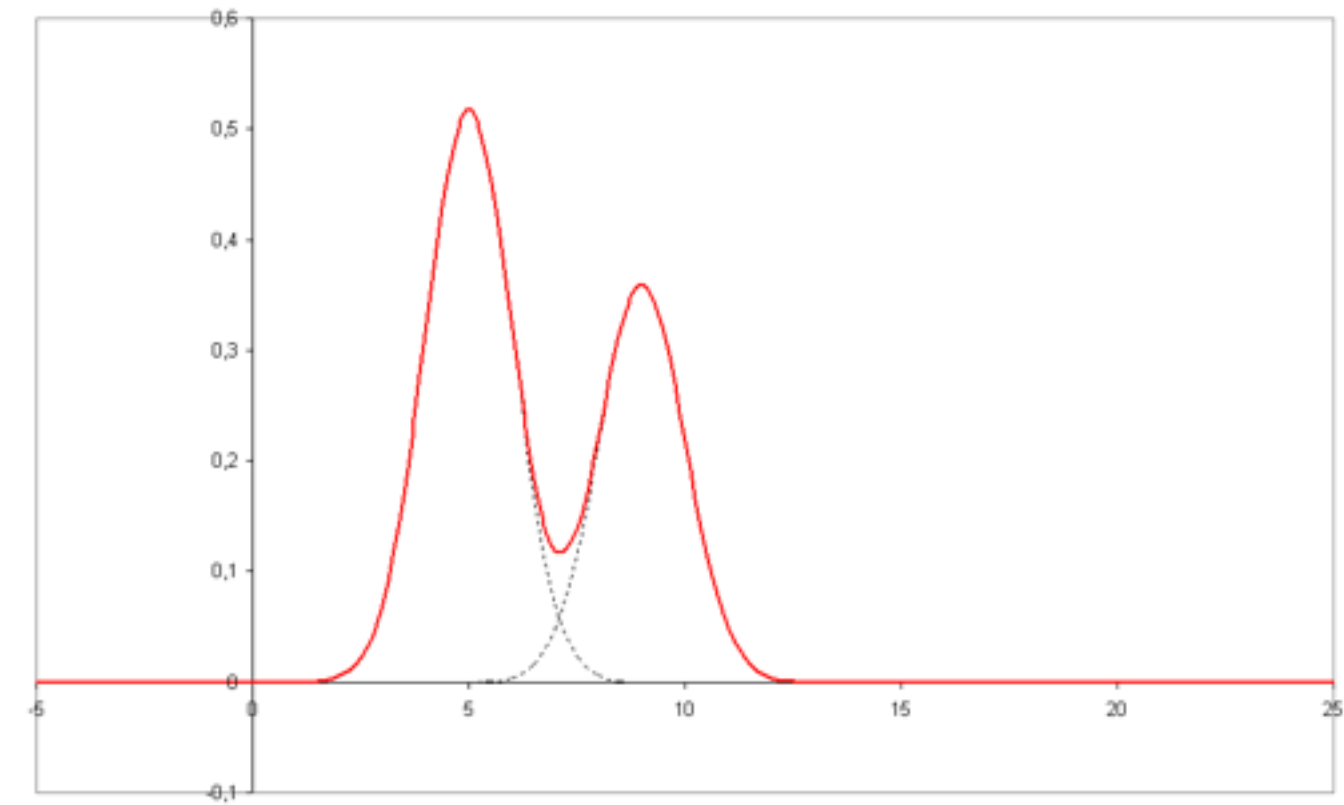
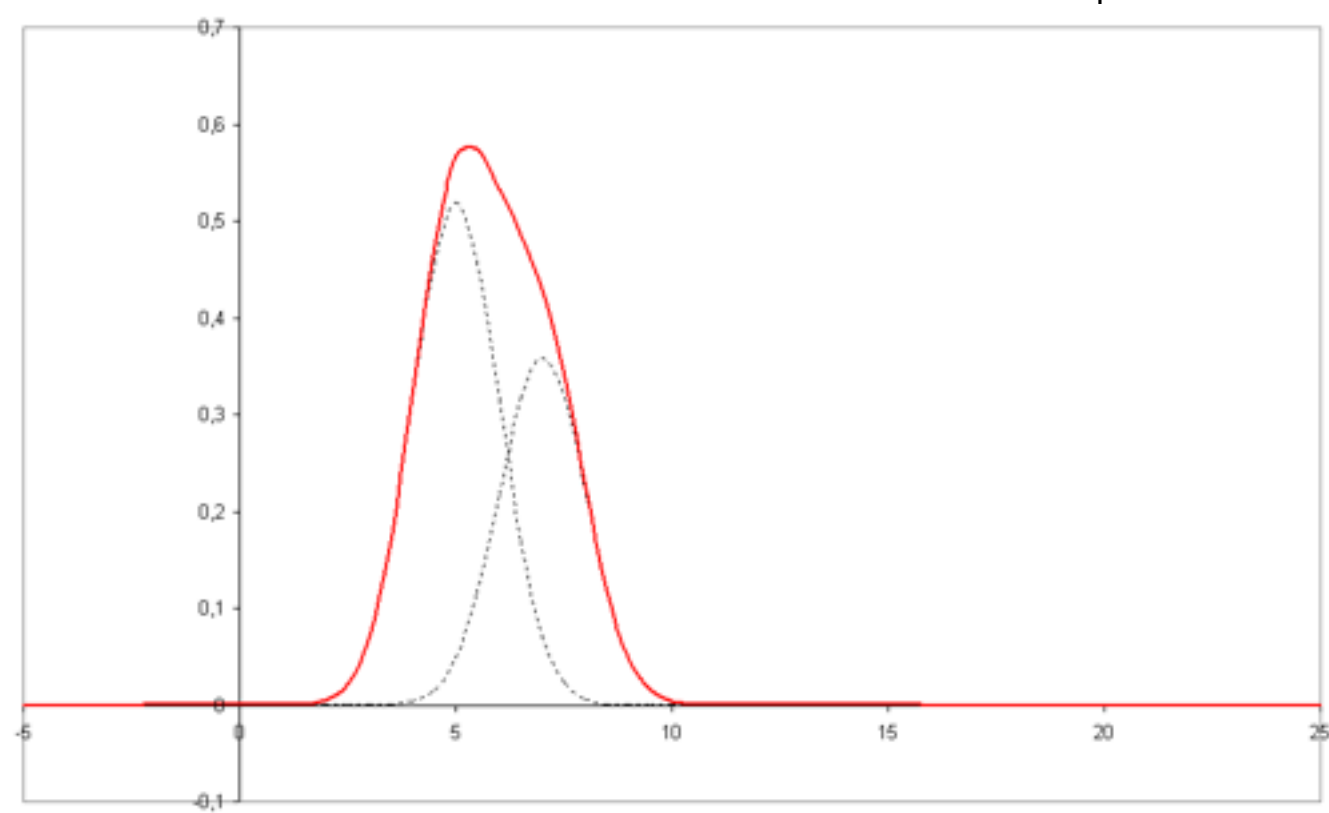
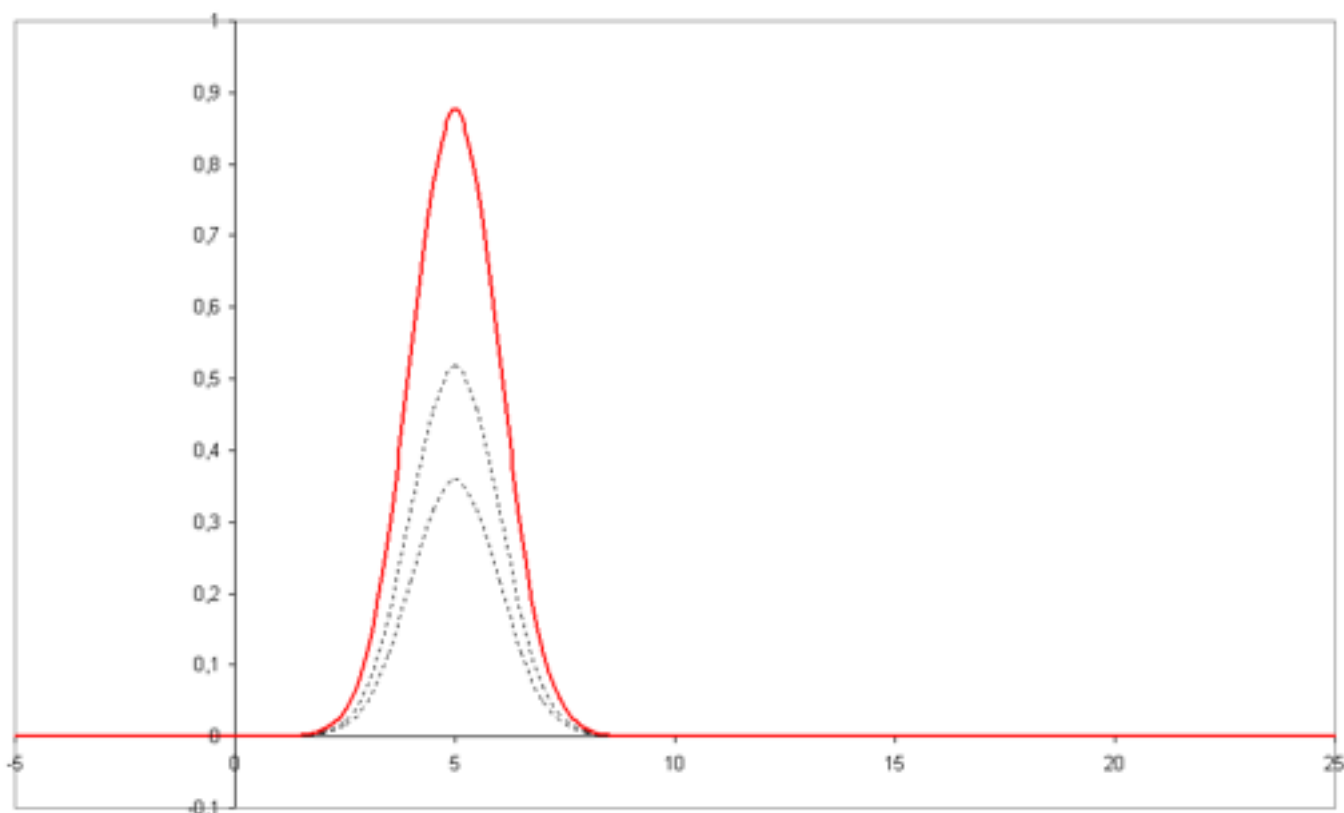
Gas





Chromatogram

public domain





HPLC

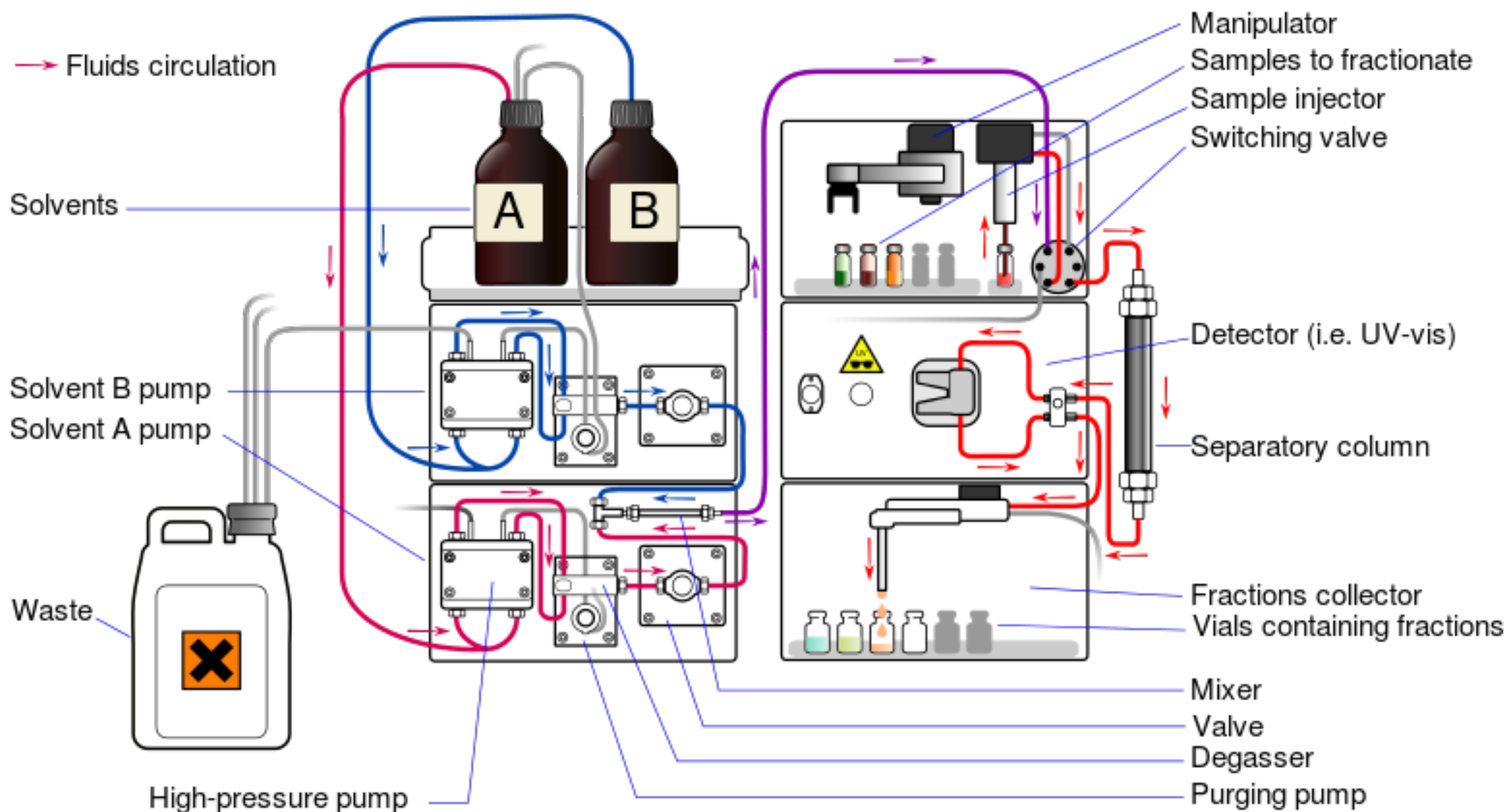
Superchilem – CC-BY-SA 3.0





HPLC

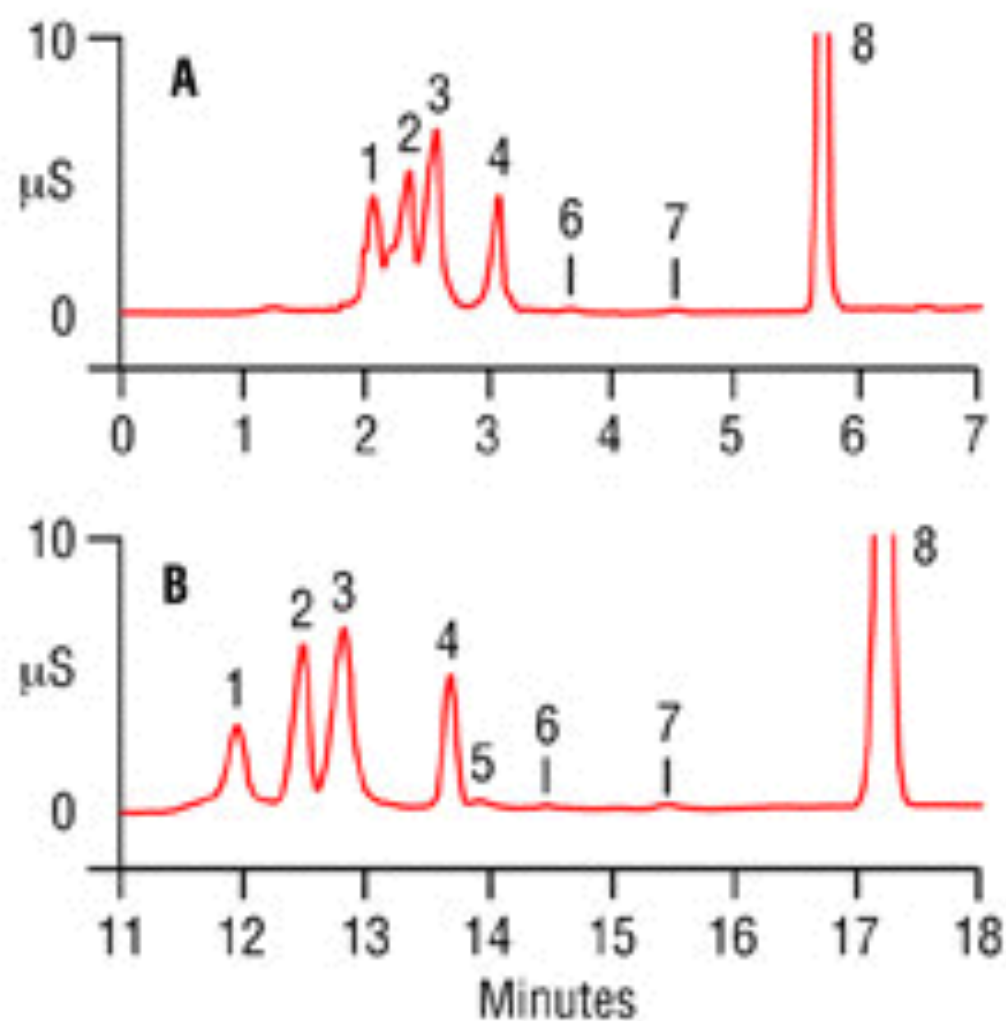
YassinMbarat – CC-BY-SA 3.0





Typical HPLC graph

Yeast Fermentation Broth Culture (10-fold Dilution) Using the IonPac[®] AS11 and AS11-HC



Column: A. IonPac AS11, AG11 Guard
B. IonPac AS11-HC, AG11-HC Guard

Eluent: A. 0.5 – 38 mM Sodium hydroxide
B. 0.5 – 90 mM Sodium hydroxide

Flow Rate: A. 2.0 mL/min
B. 1.5 mL/min

Inj. Volume: 10 μ L

Temperature: A. Ambient
B. 30 °C

Detection: Suppressed conductivity, ASRS[®],
AutoSuppression[®] recycle mode

Peaks: 1. Unknown
2. Lactate
3. Acetate/Glycolate
4. Formate
5. Butyrate
6. Pyruvate/Isovalerate
7. Valerate
8. Chloride



DIY HPLC?



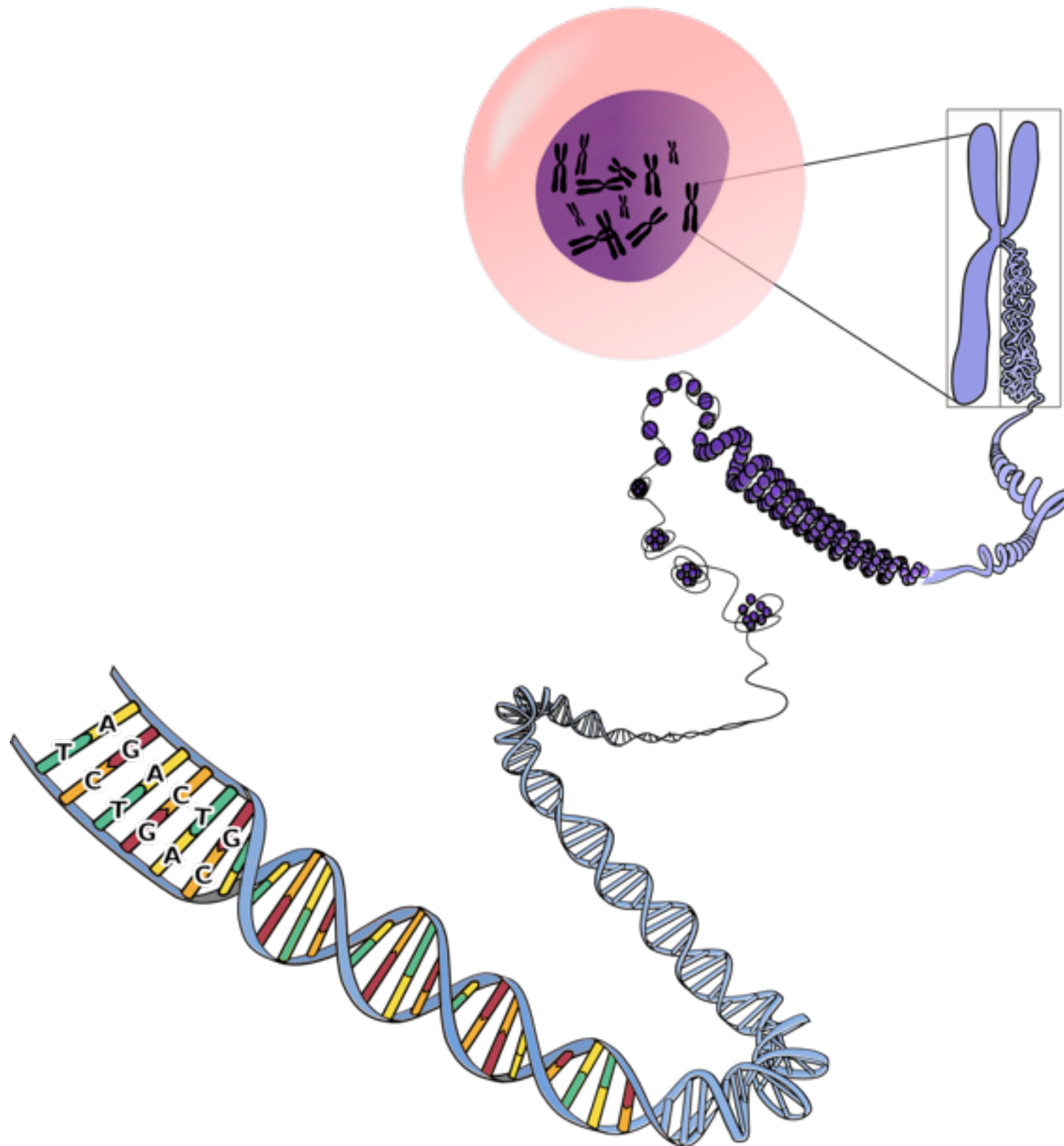
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Electrophoresis



How to analyse DNA





Sushi test





PooPrints



Customer Information Card

**Required Information*

Account Information

*Country: _____
*Email: _____
*Your Name: _____
*Address: _____
*City, State, Zip: _____
Phone: _____

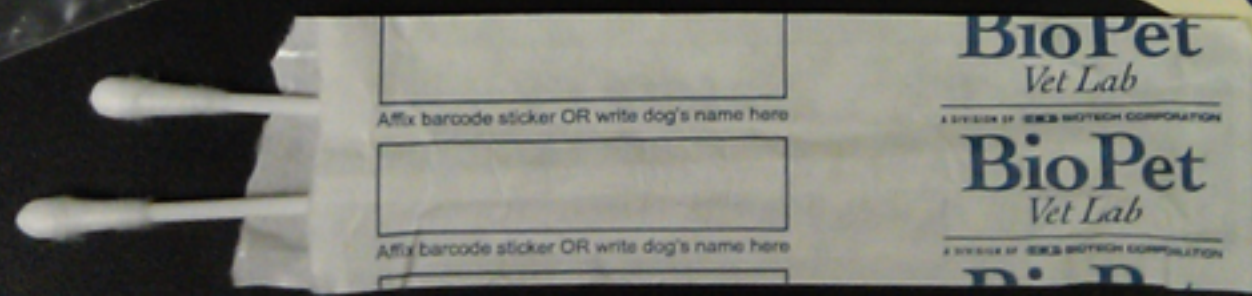
Pet Information

Apply Barcode Sticker Here

Pet's Name: _____
*Pet's Species: _____ Dog _____ Cat
Where did you purchase your DNA Pet ID Kit?
Company: _____

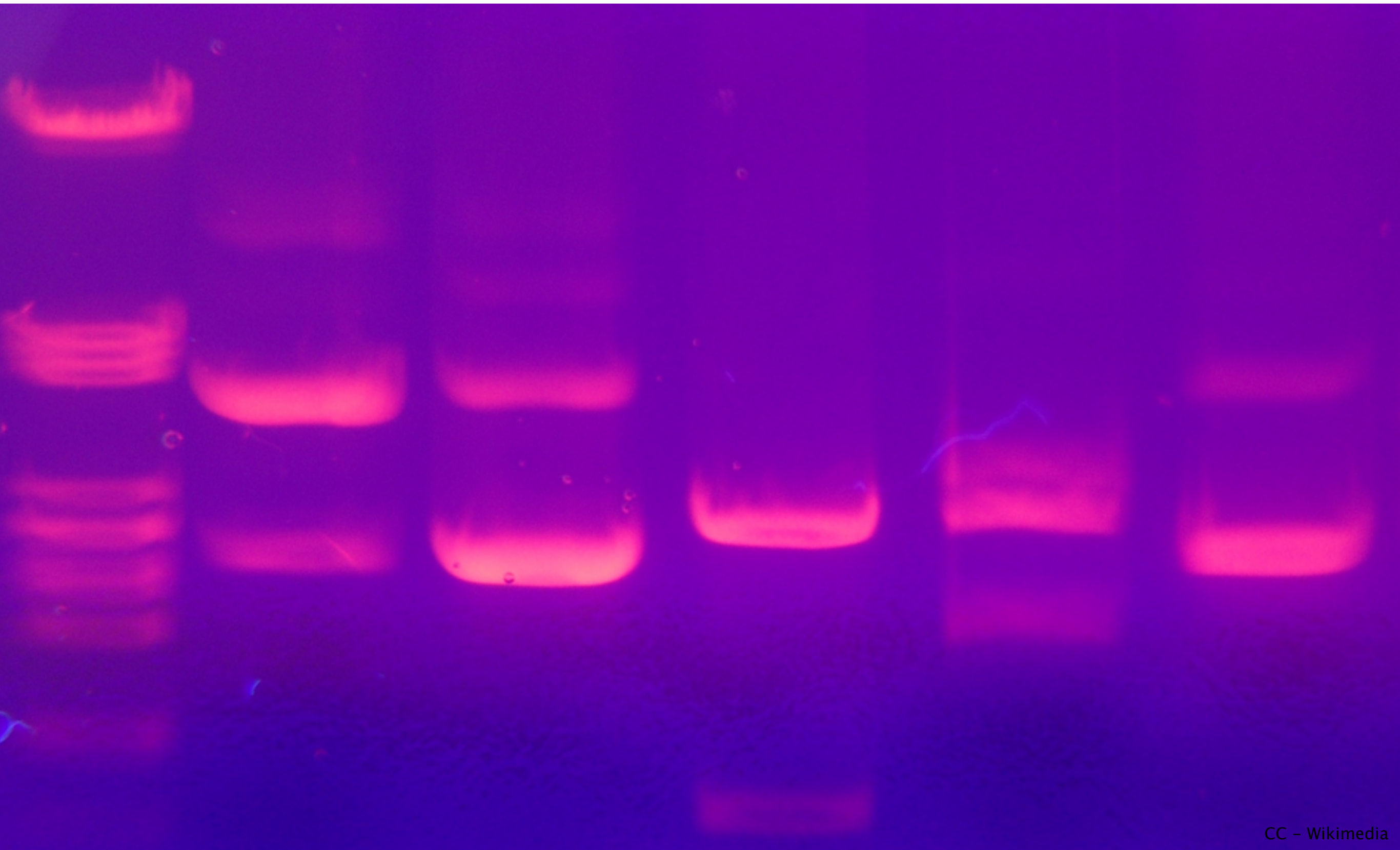
BioPet Vet Lab
A DIVISION OF GENES BIOTECH CORPORATION

DNA World Pet Registry





DNA fingerprint



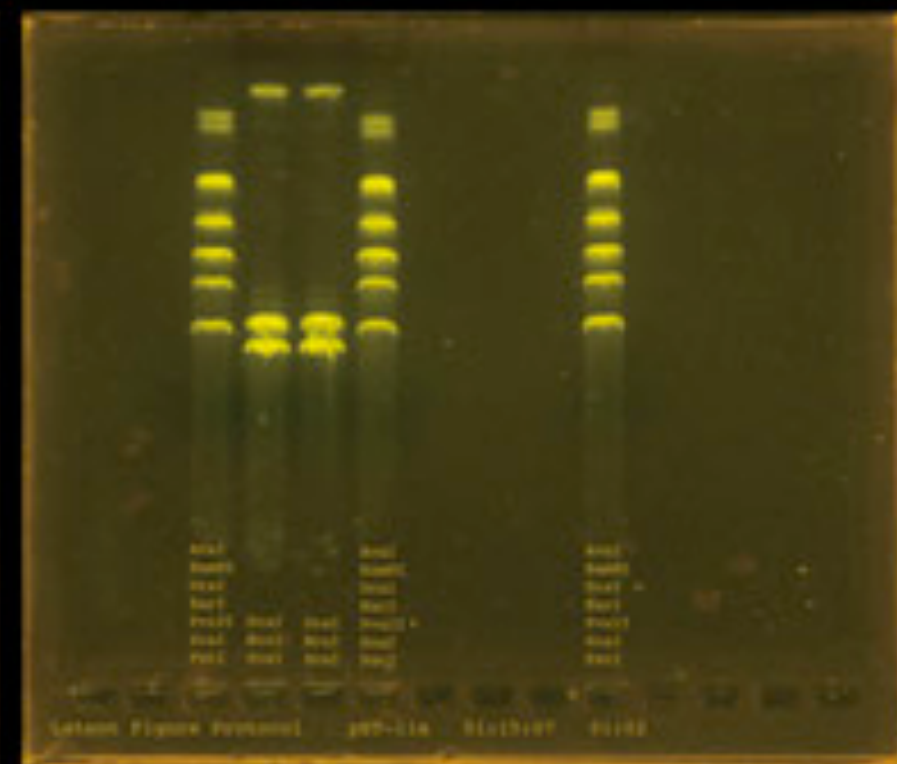
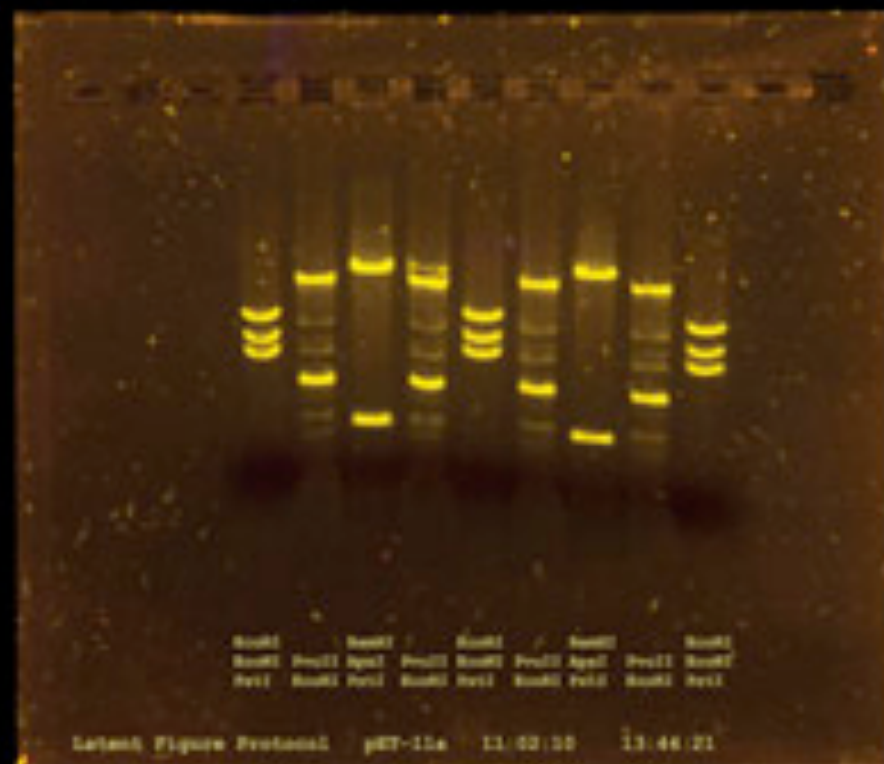
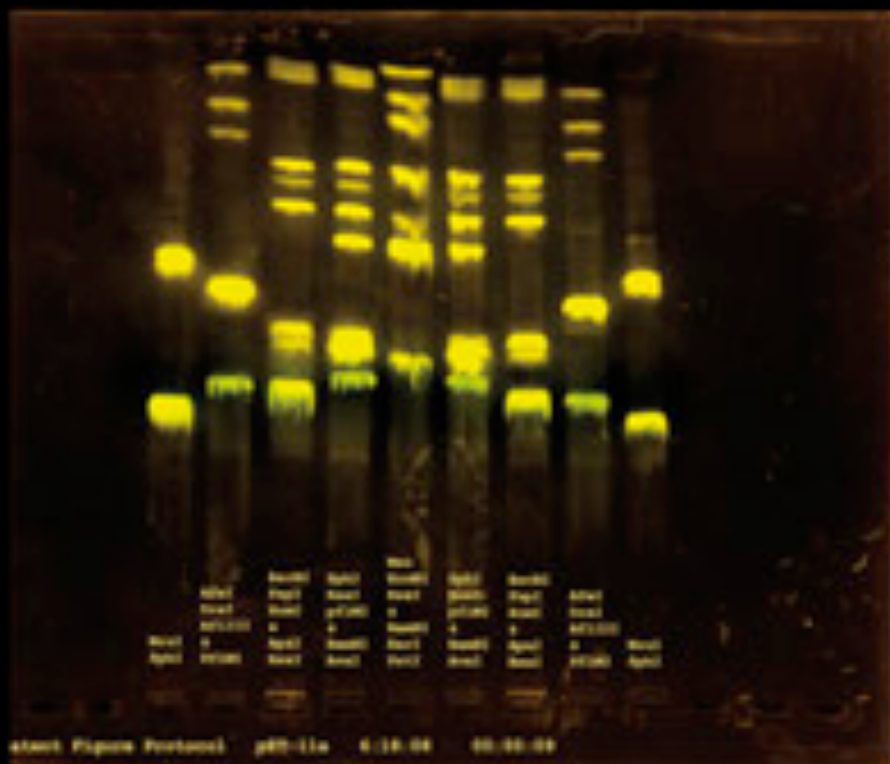
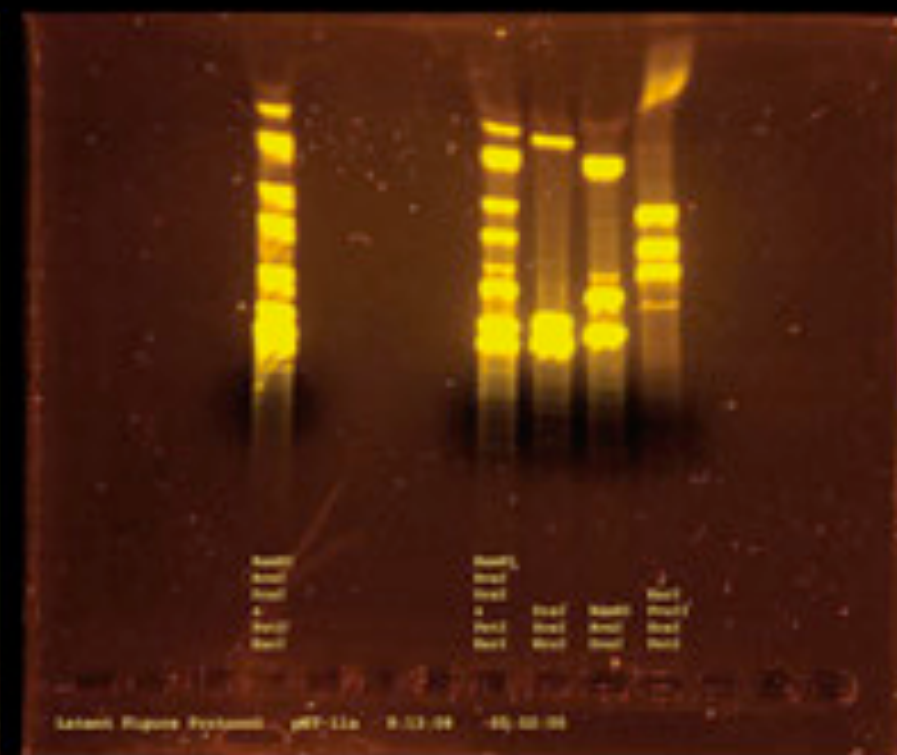
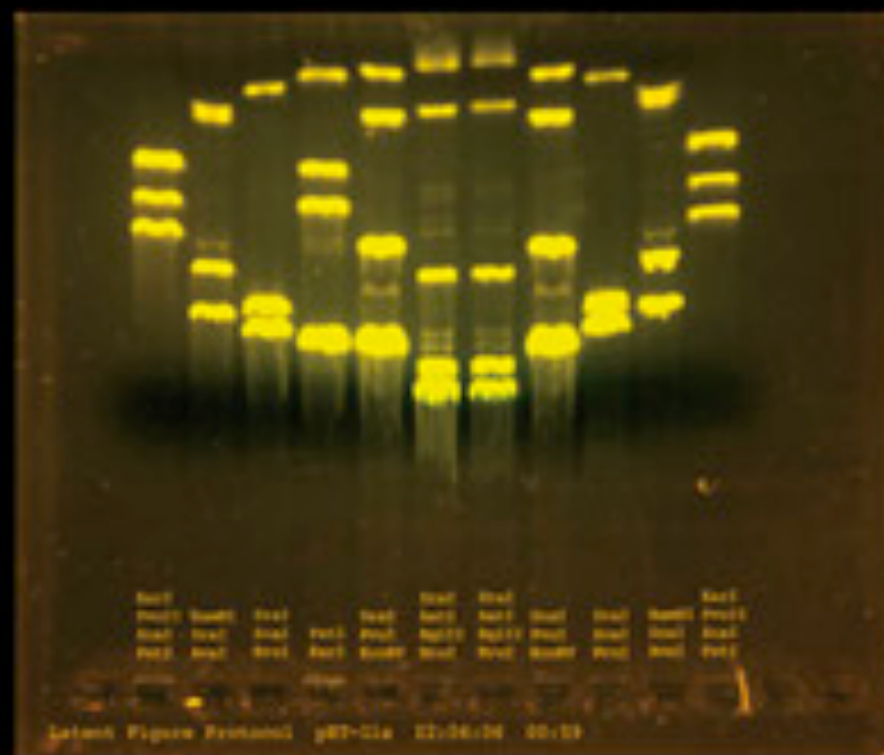
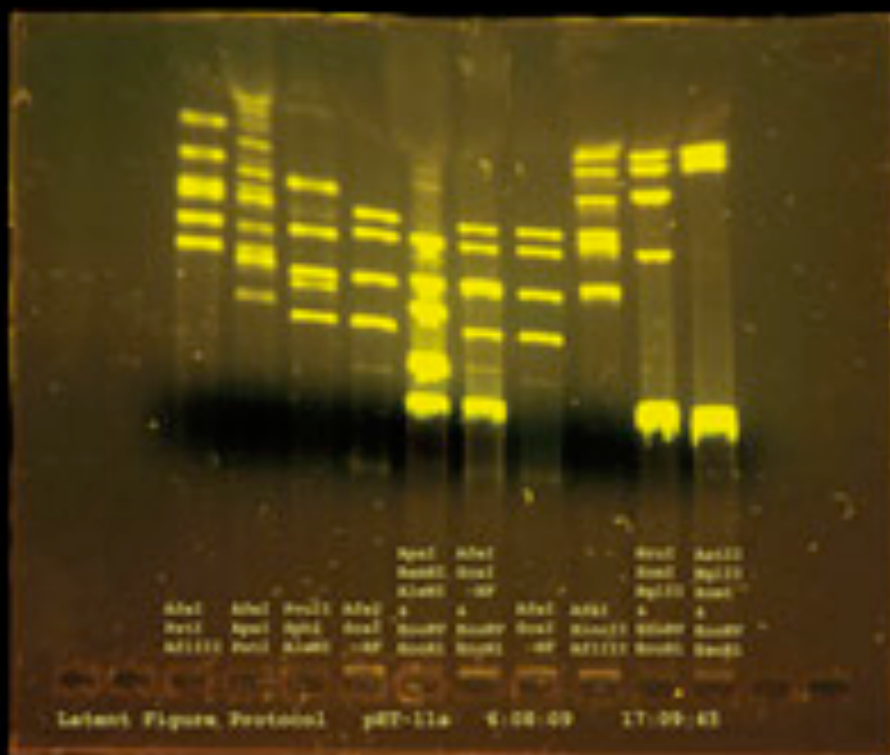


Barcode





Paul Vanouse





Paul Vanouse





Sequence specific cuts

Restrictie site

Palindrome



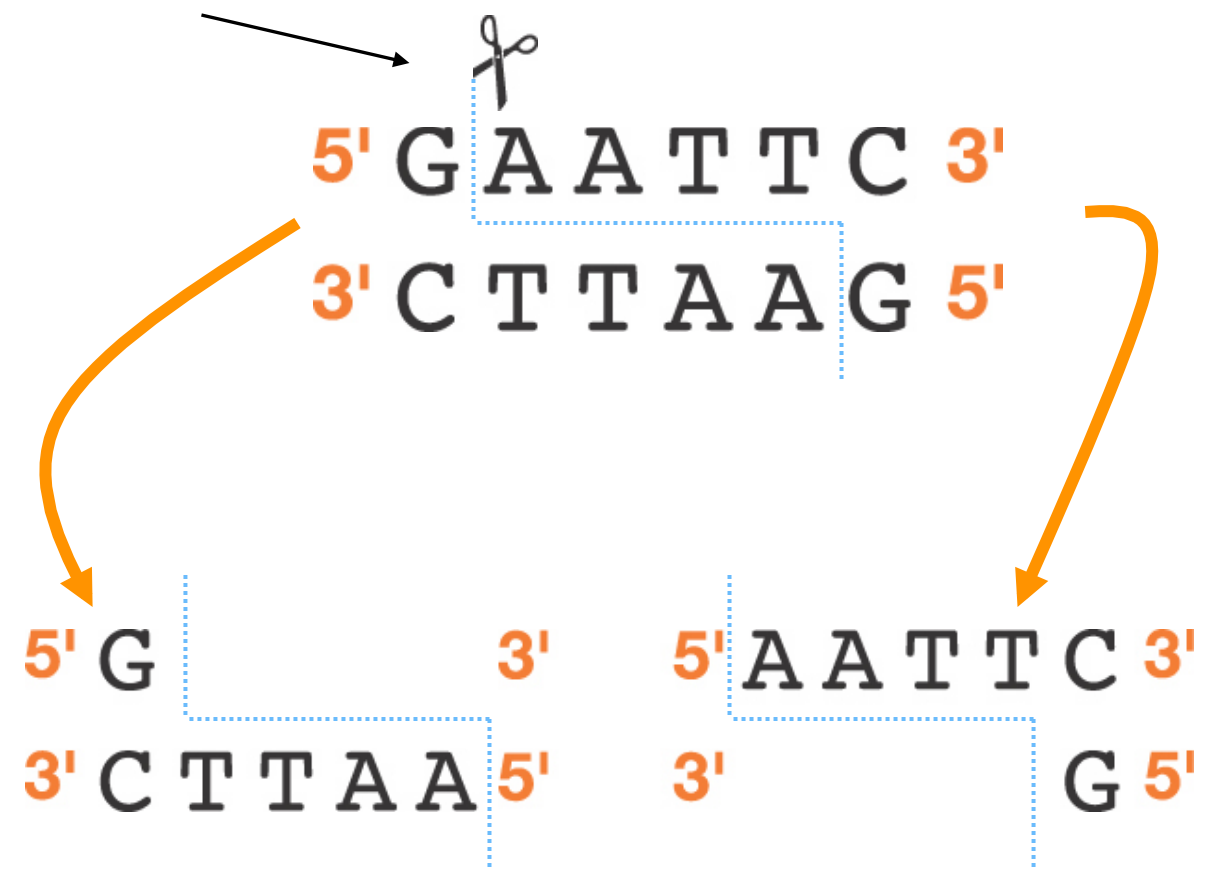
Fragment 1

Fragment 2



5 vs 3 accent overlap

cut site





EcoRI en PstI



EcoRI

- Escherichia coli
- 5 prime overlap



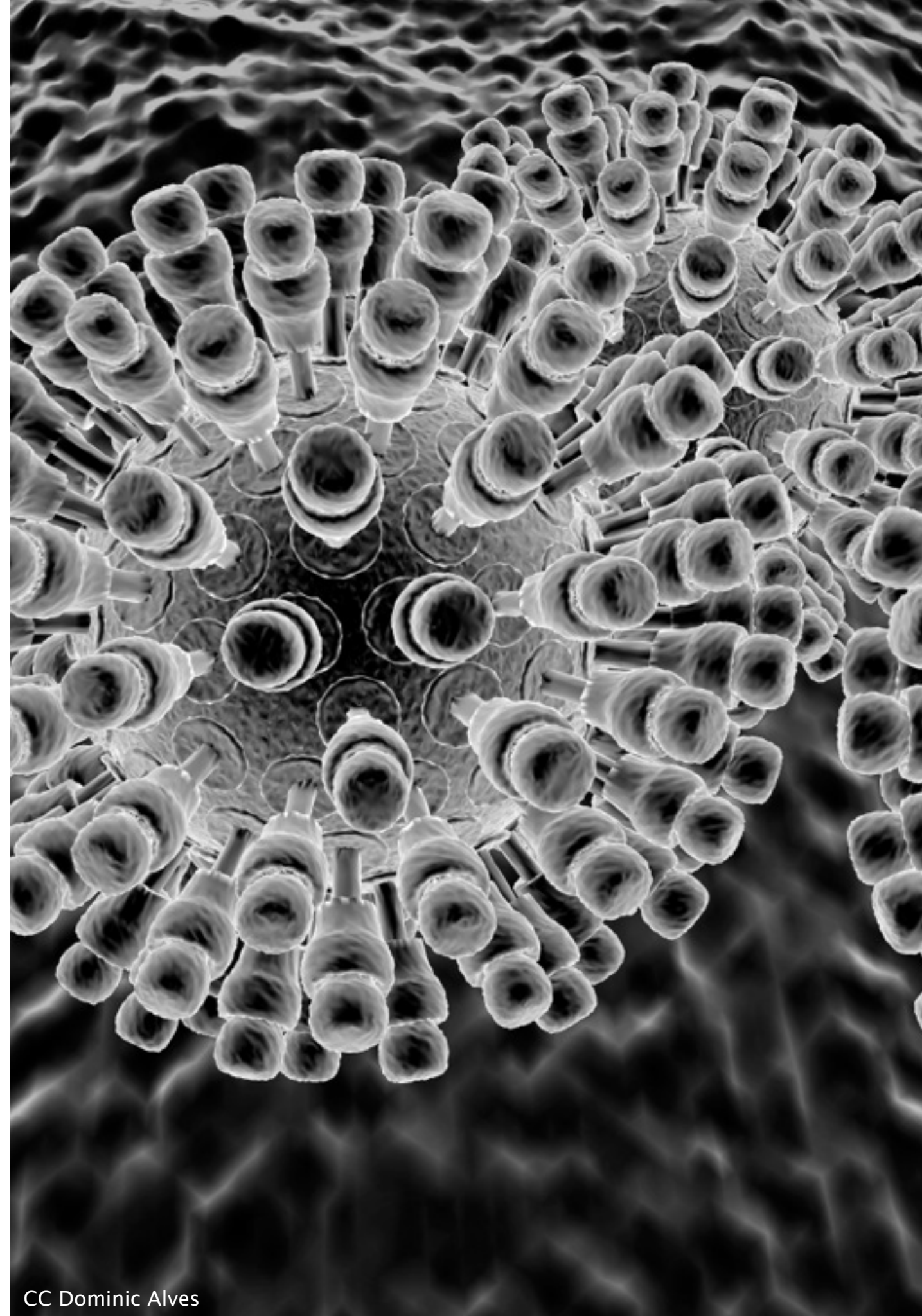
PstI

- Providencia stuartii
- 3 prime overlap



DNA restriction enzymes

- Protect against viral infections
- Over 3000 types known





Step 1: samples and enzymes

Get DNA and enzymes

Crime Scene Suspects DNA reference

1 2 3 4 5



Take the 5 samples



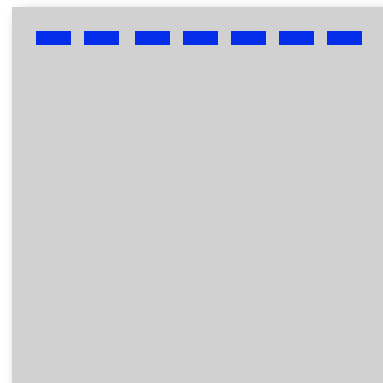
Cut it using a EcoRI/
PstI restriction-
enzymmix

Incubate 45 minutes at 37 degrees

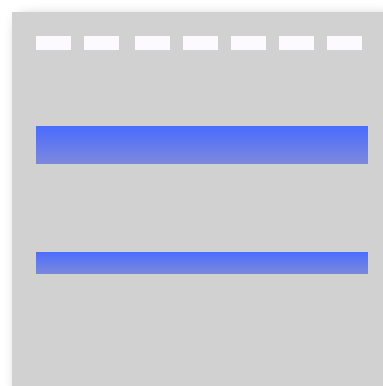


Step 2: Gel electrophoreses

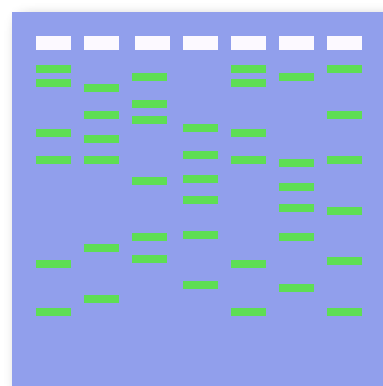
Mix the samples with loading dye



Load the samples in a gel



Apply current

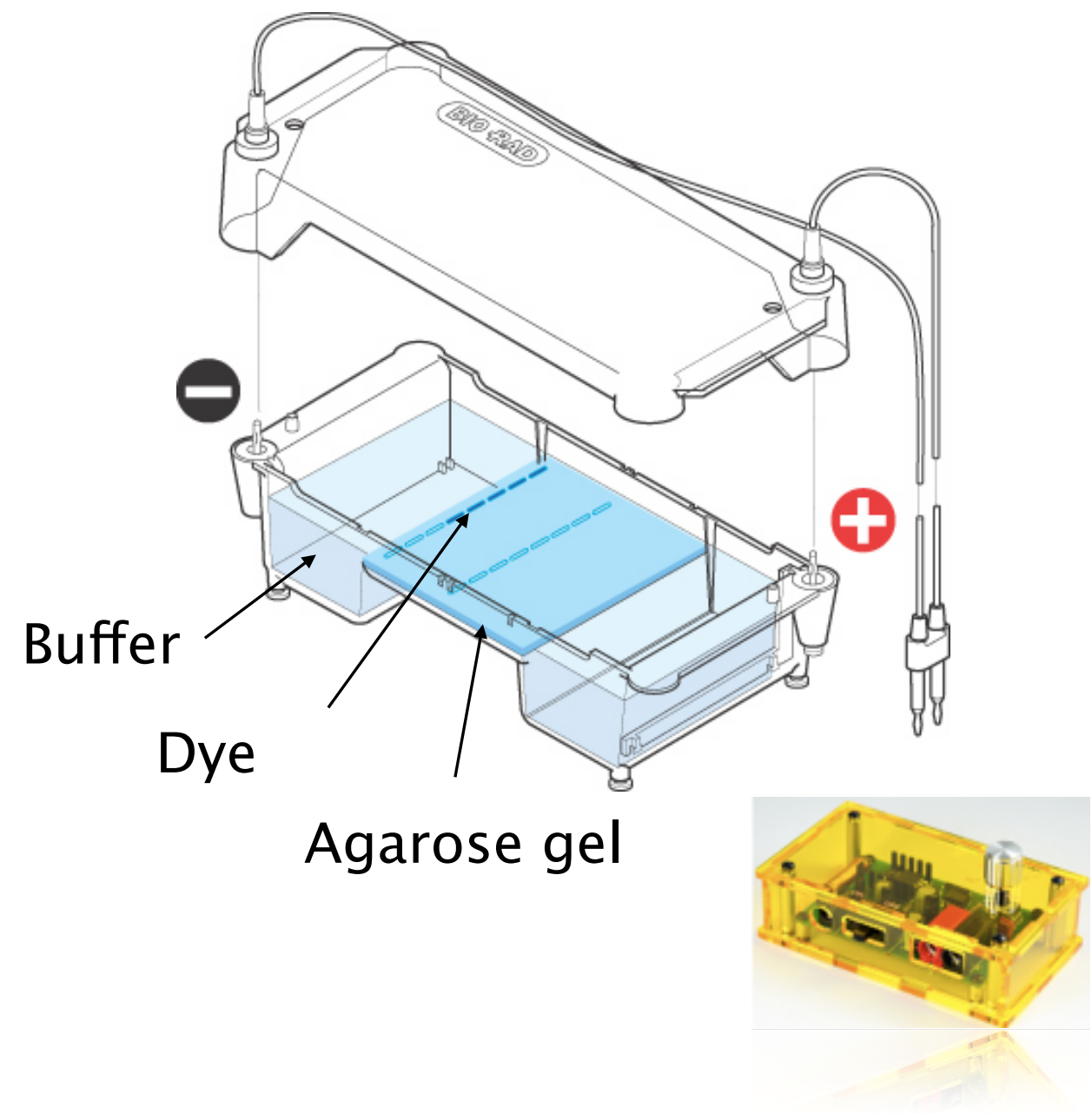


Read the pattern

Identify the killer

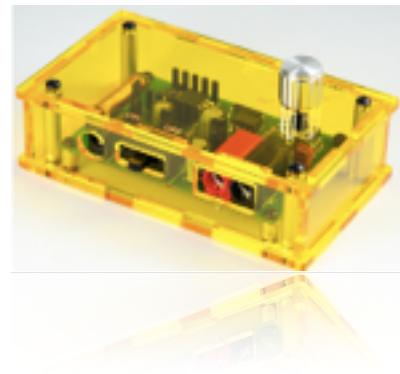
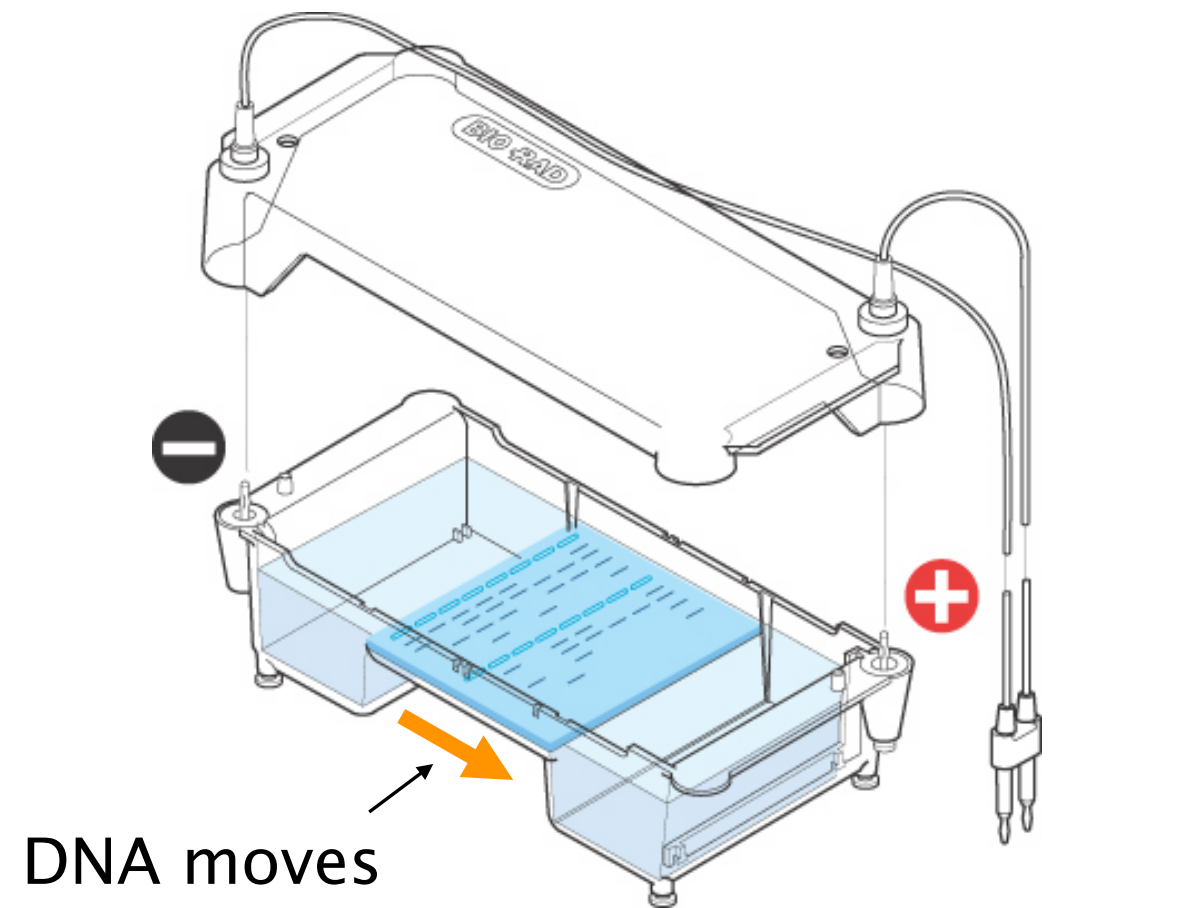
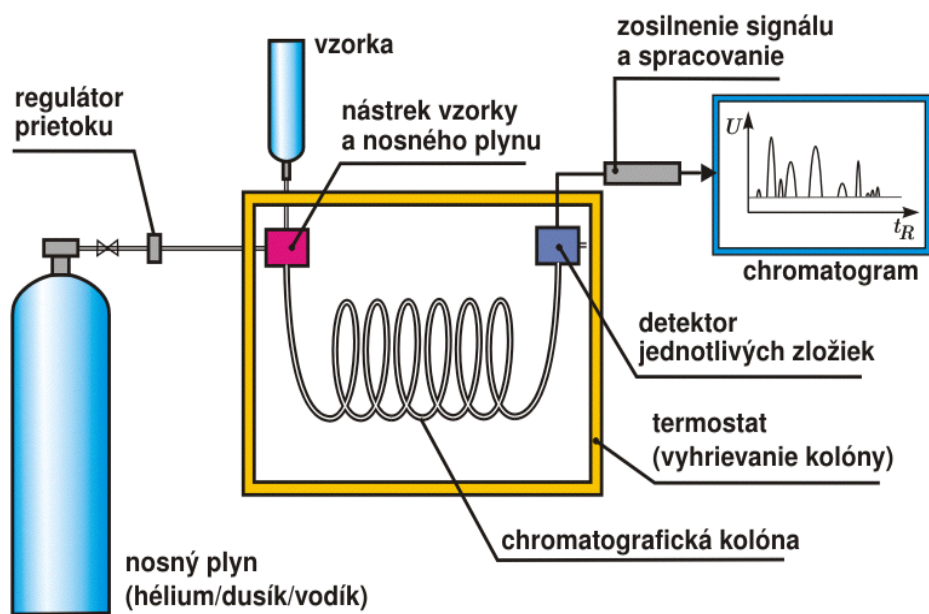


DNA is attracted by the anode





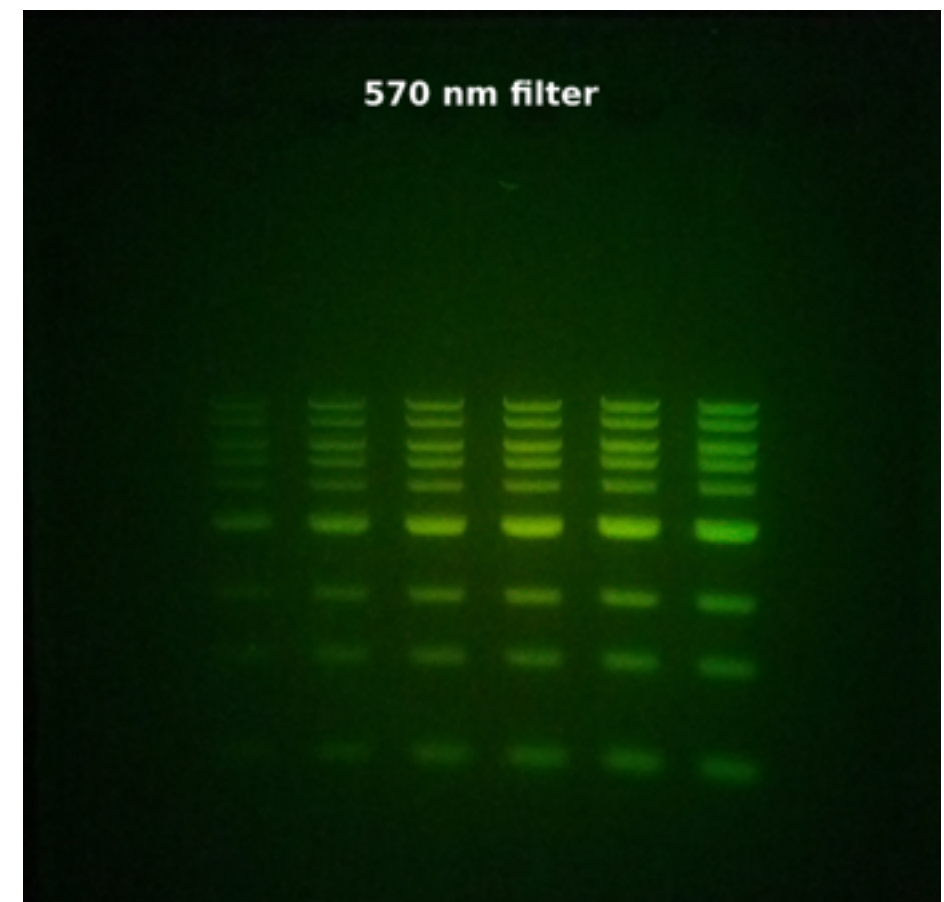
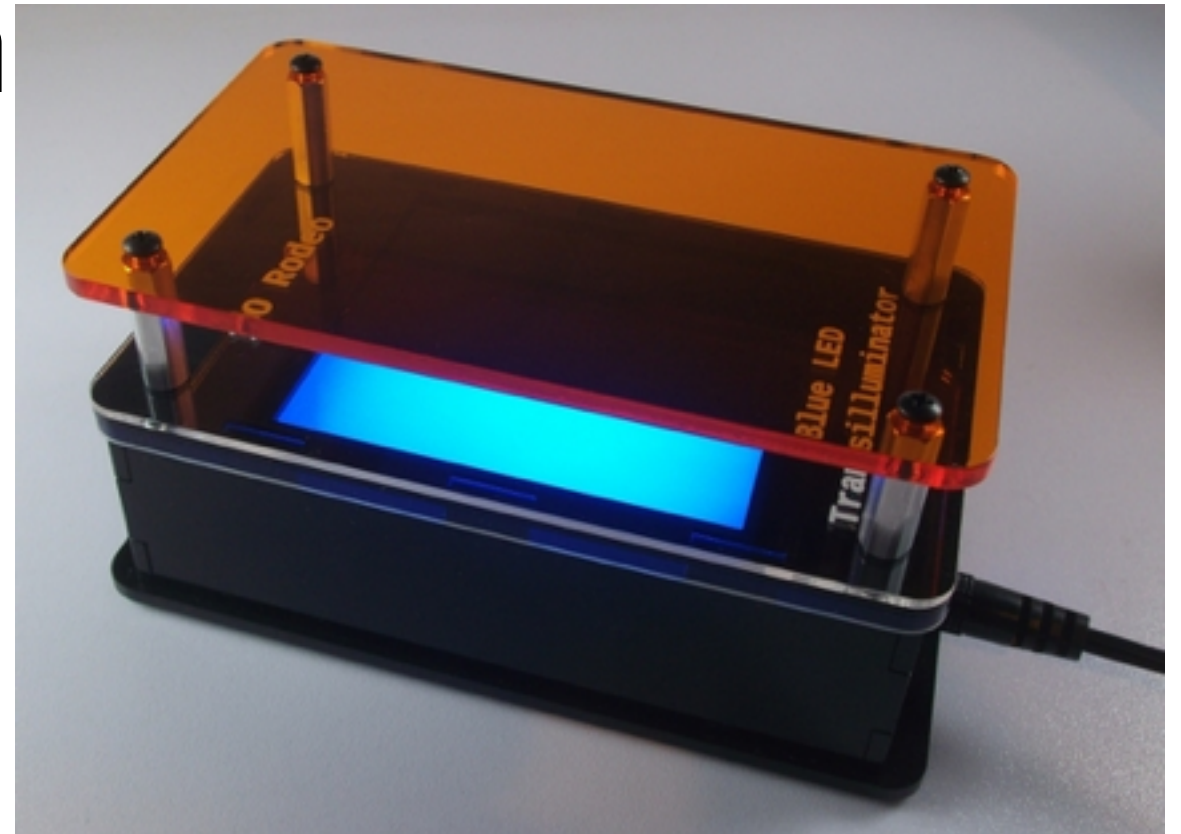
Short pieces move faster than long pieces





Transillumination

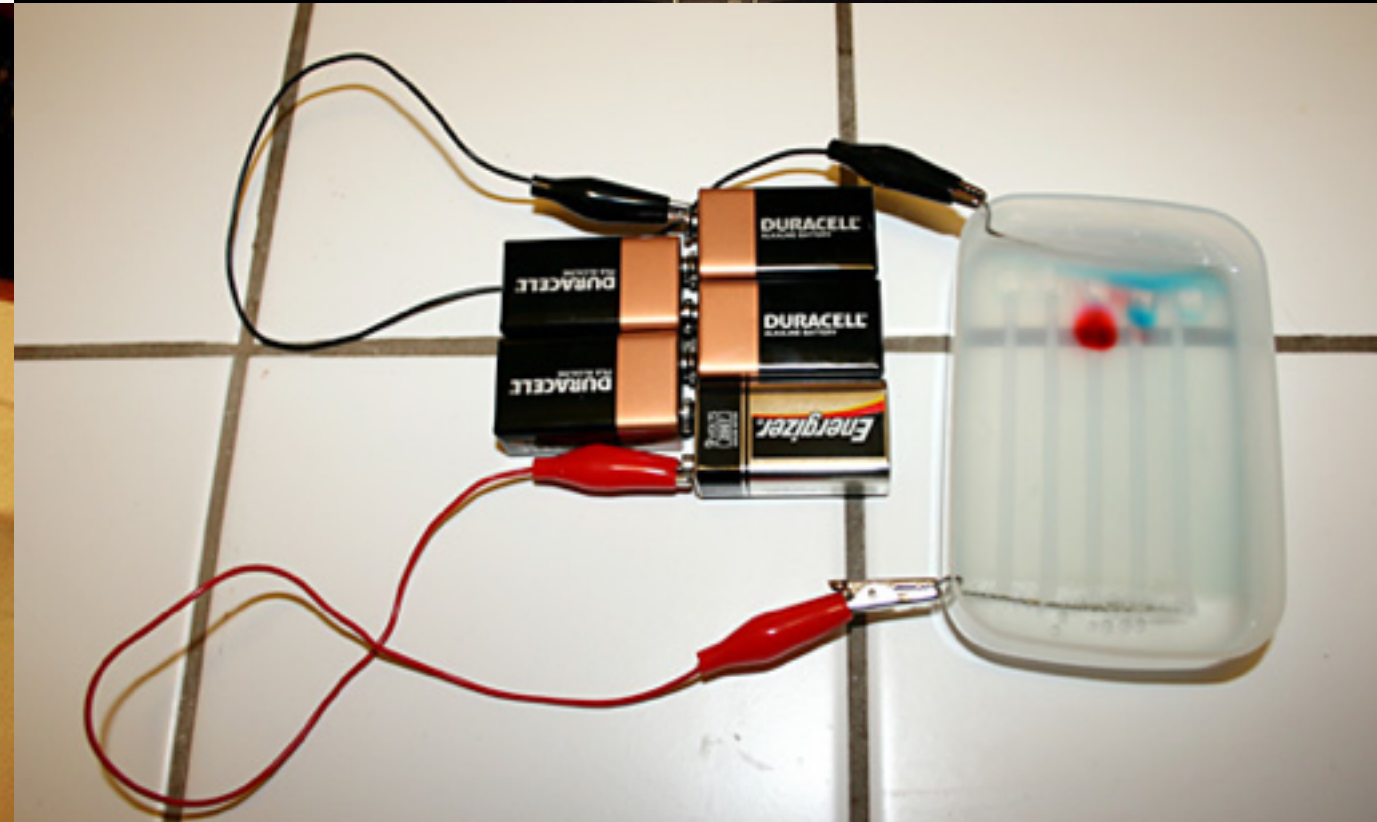
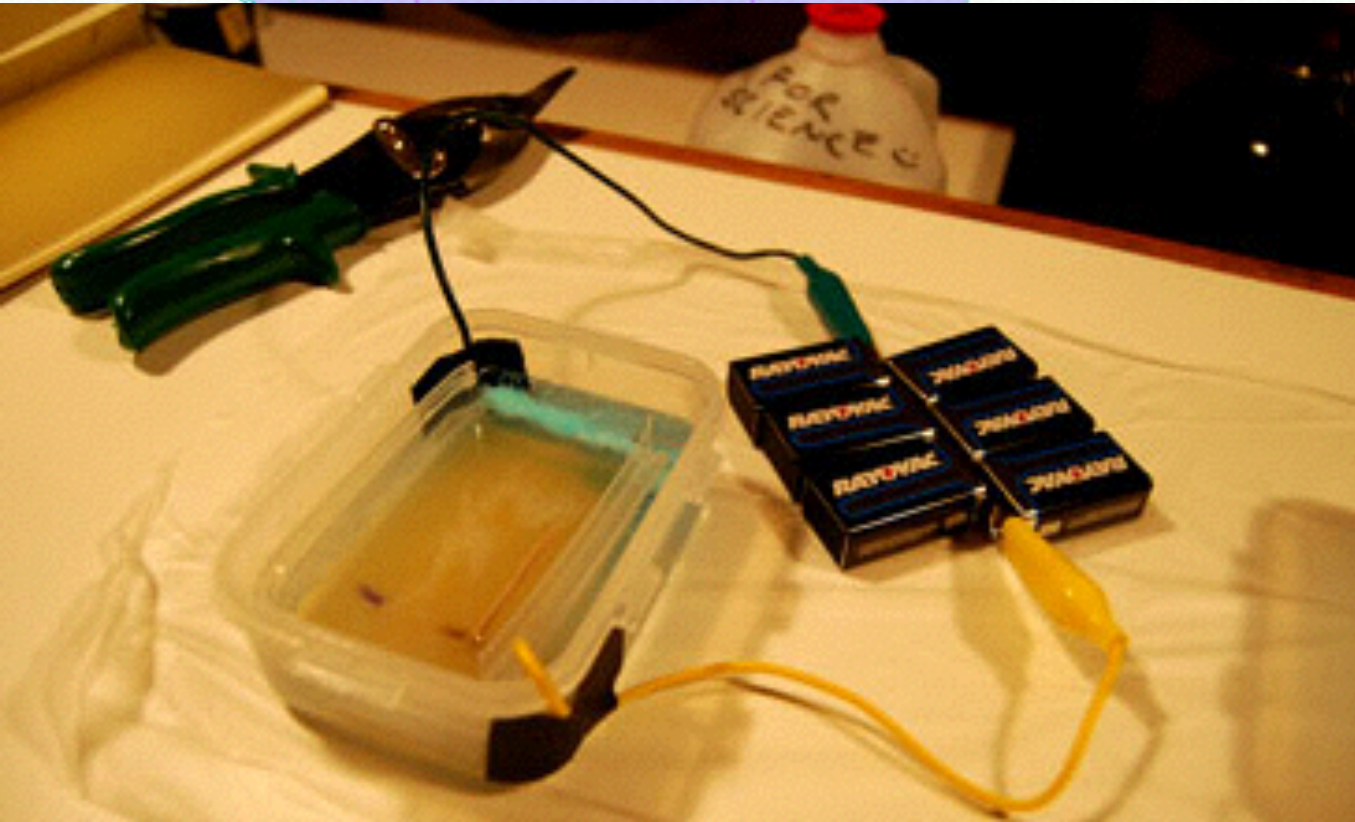
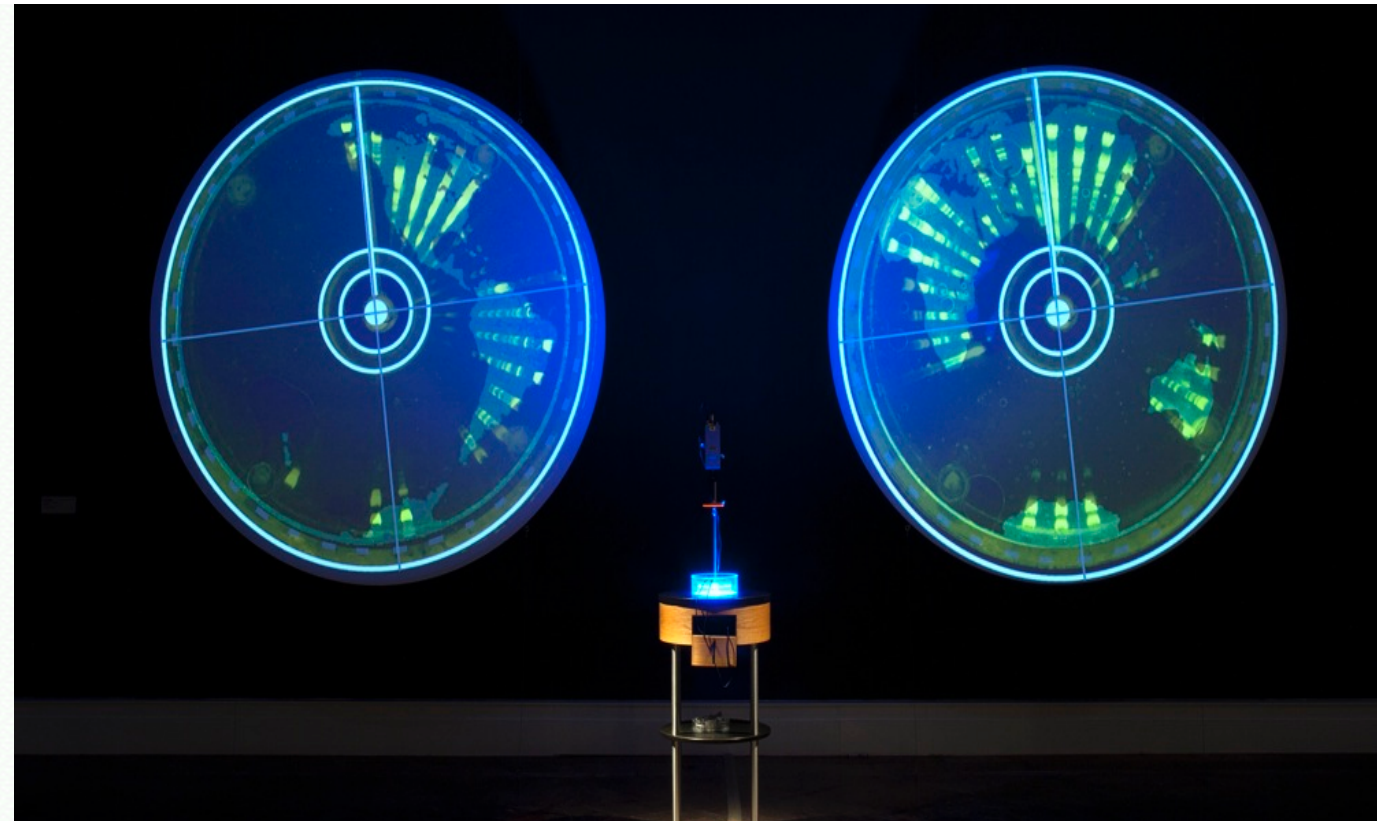
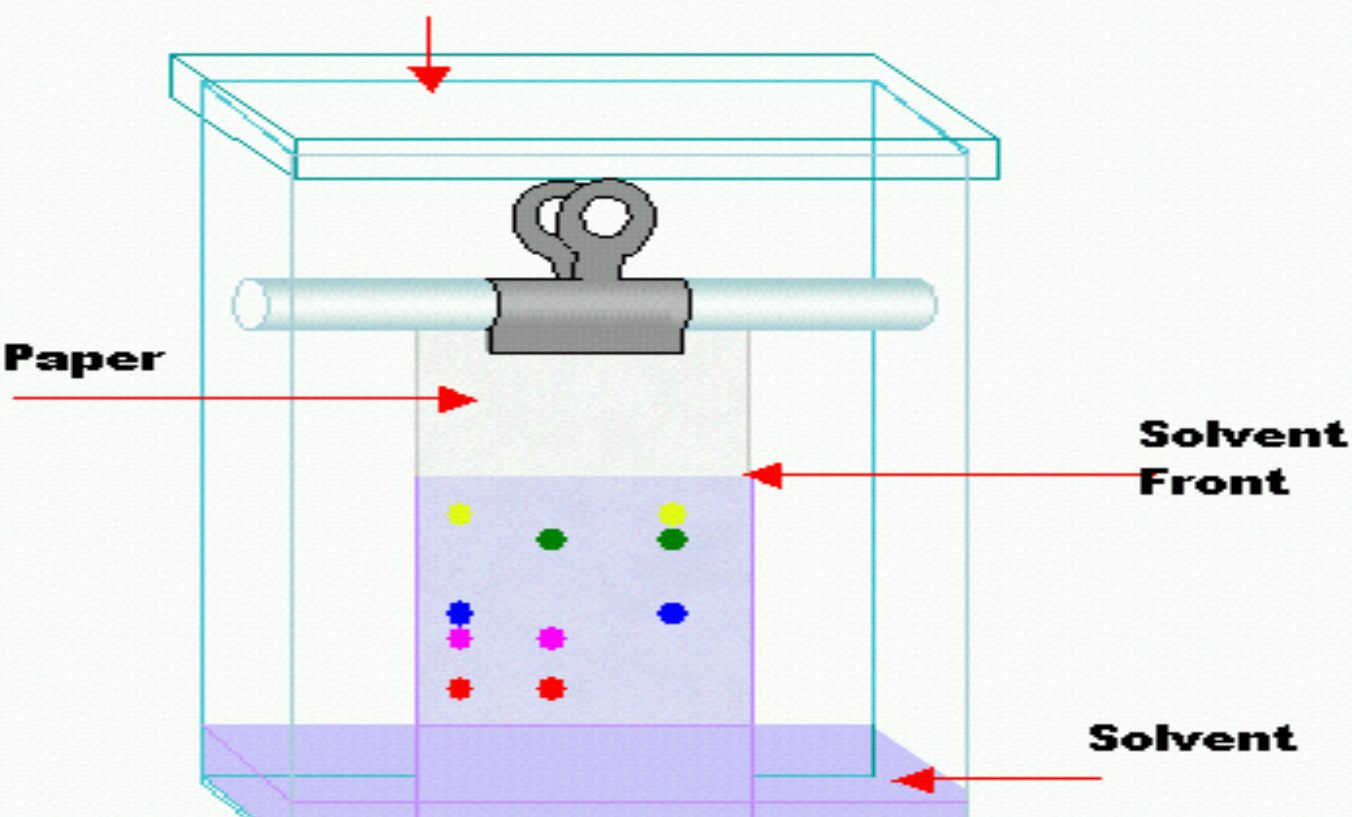
- Fluorescent DNA dye
- Sensitive to blue light
- Emits green light
- Orange filter blocks blue light





DIY Electrophoresis

<http://fablab.waag.org/project/ow-dna-gel-electrophoresis-box>





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Nuclear Magnetic Resonance



What is this?



Simon Eugstar - CC-BY-SA 3.0



Debstart - CC-BY-SA 3.0



NMR Machines

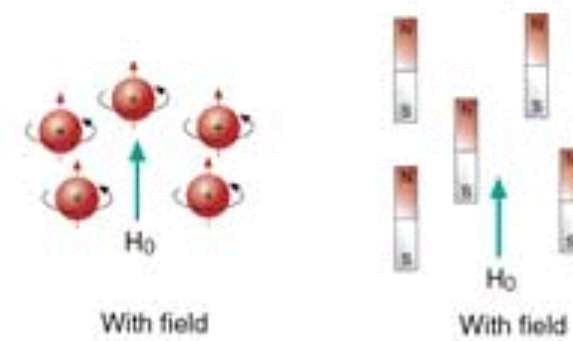
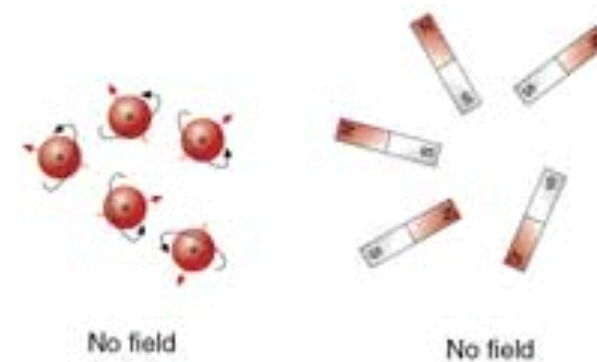
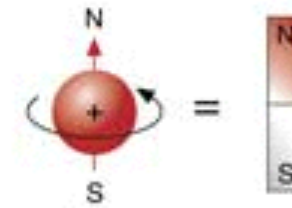
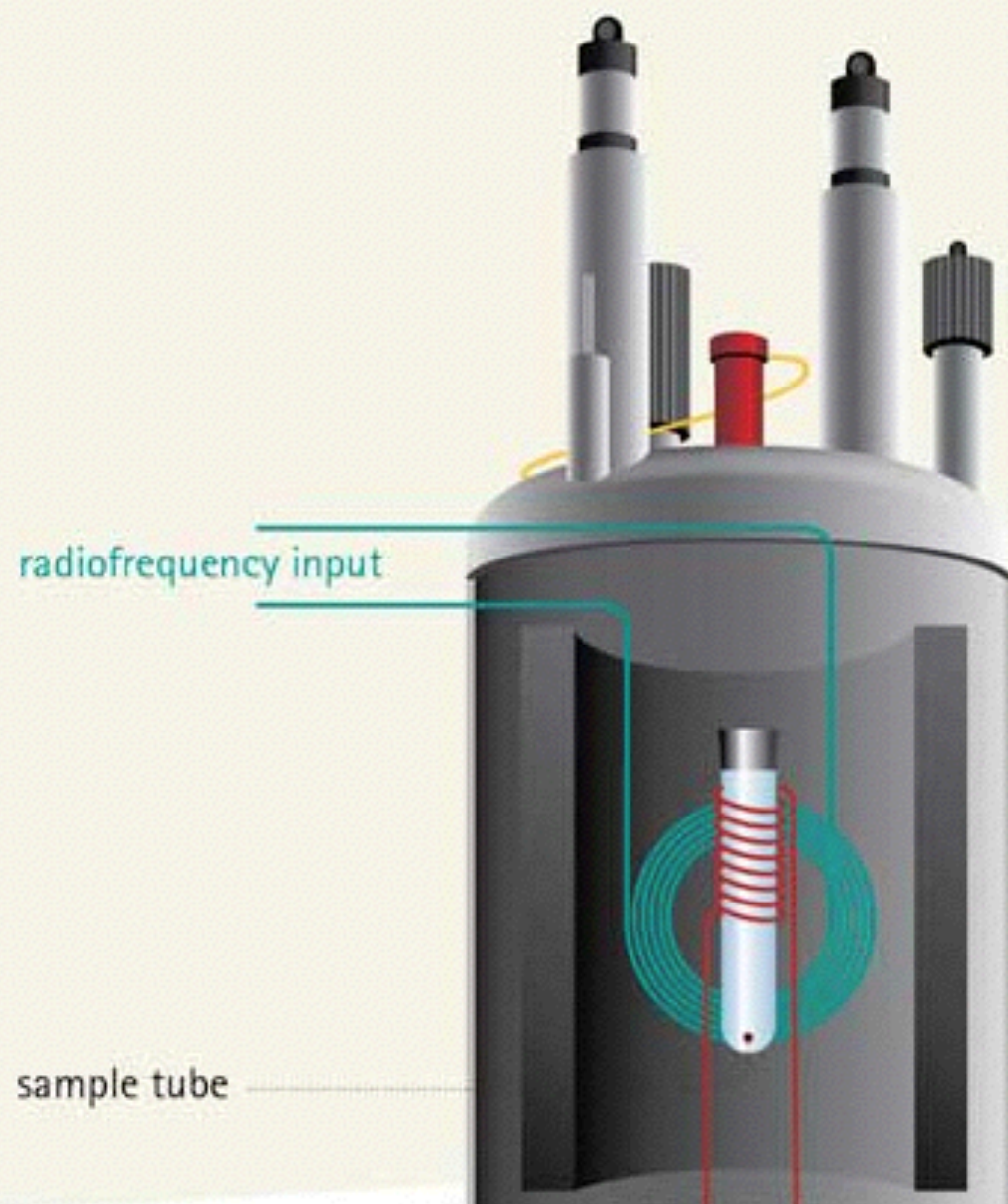
MartinSaunders

Public Domain





NMR principles

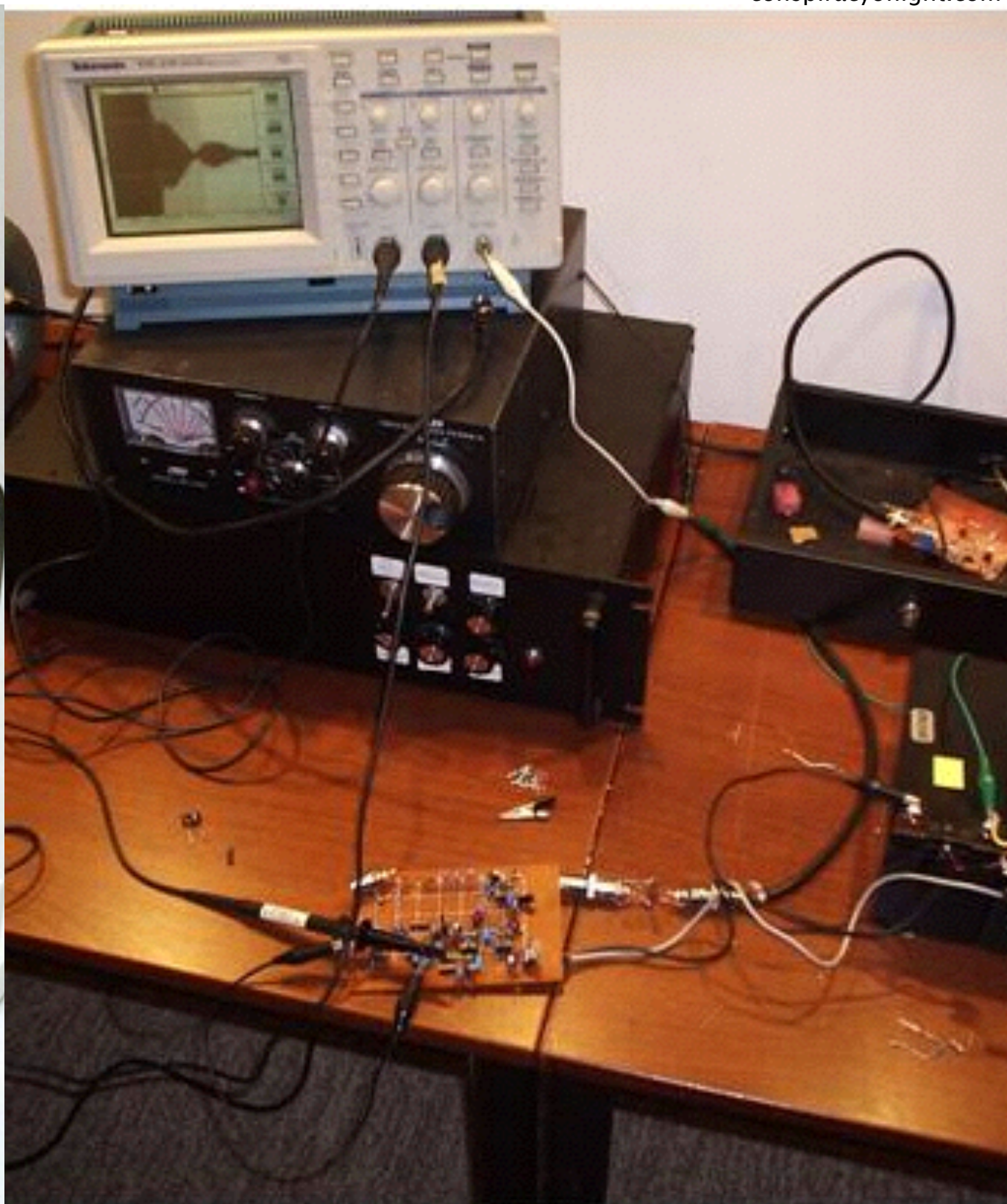
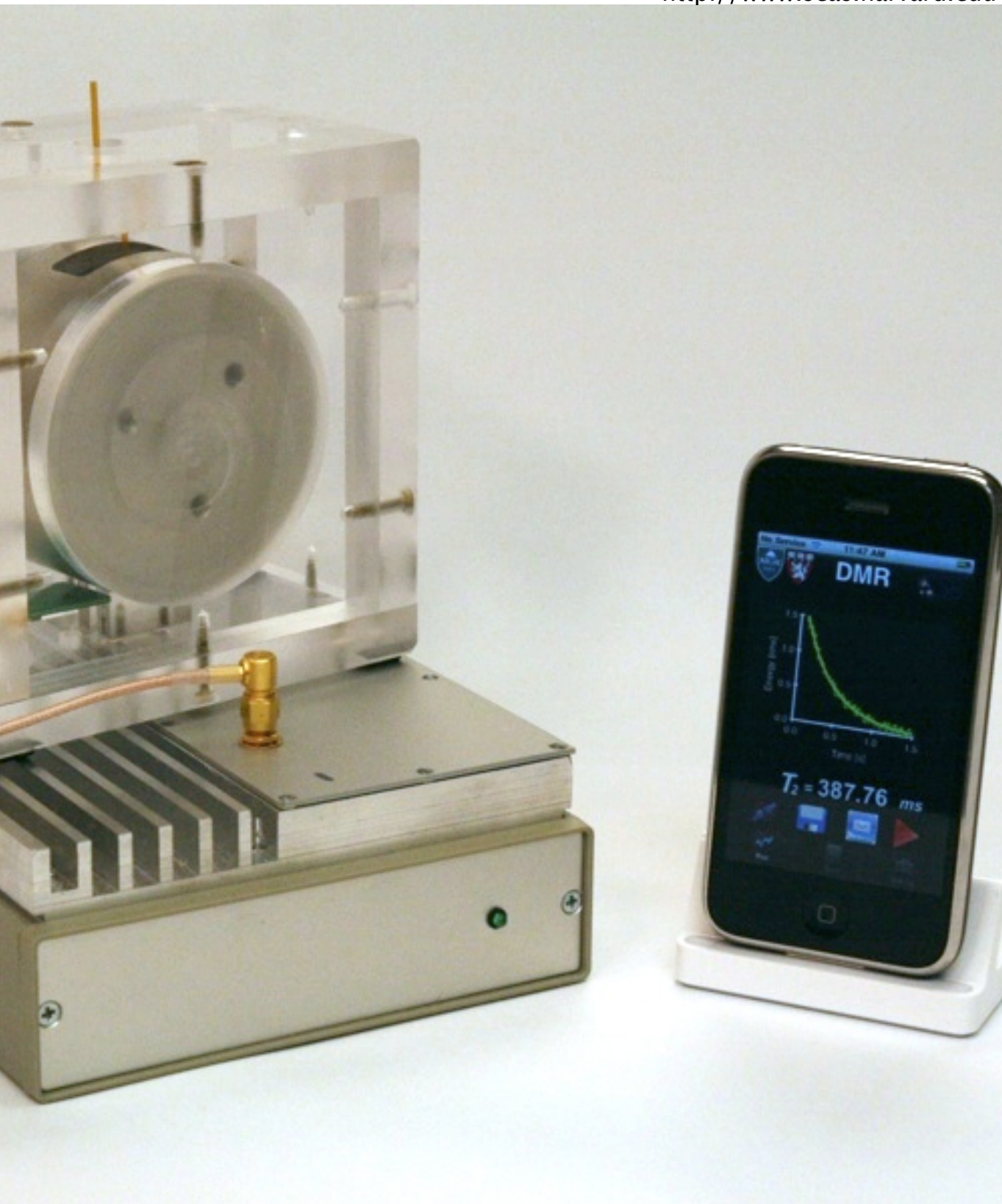




DIY NMR?

<http://www.seas.harvard.edu>

conspiracyoflight.com





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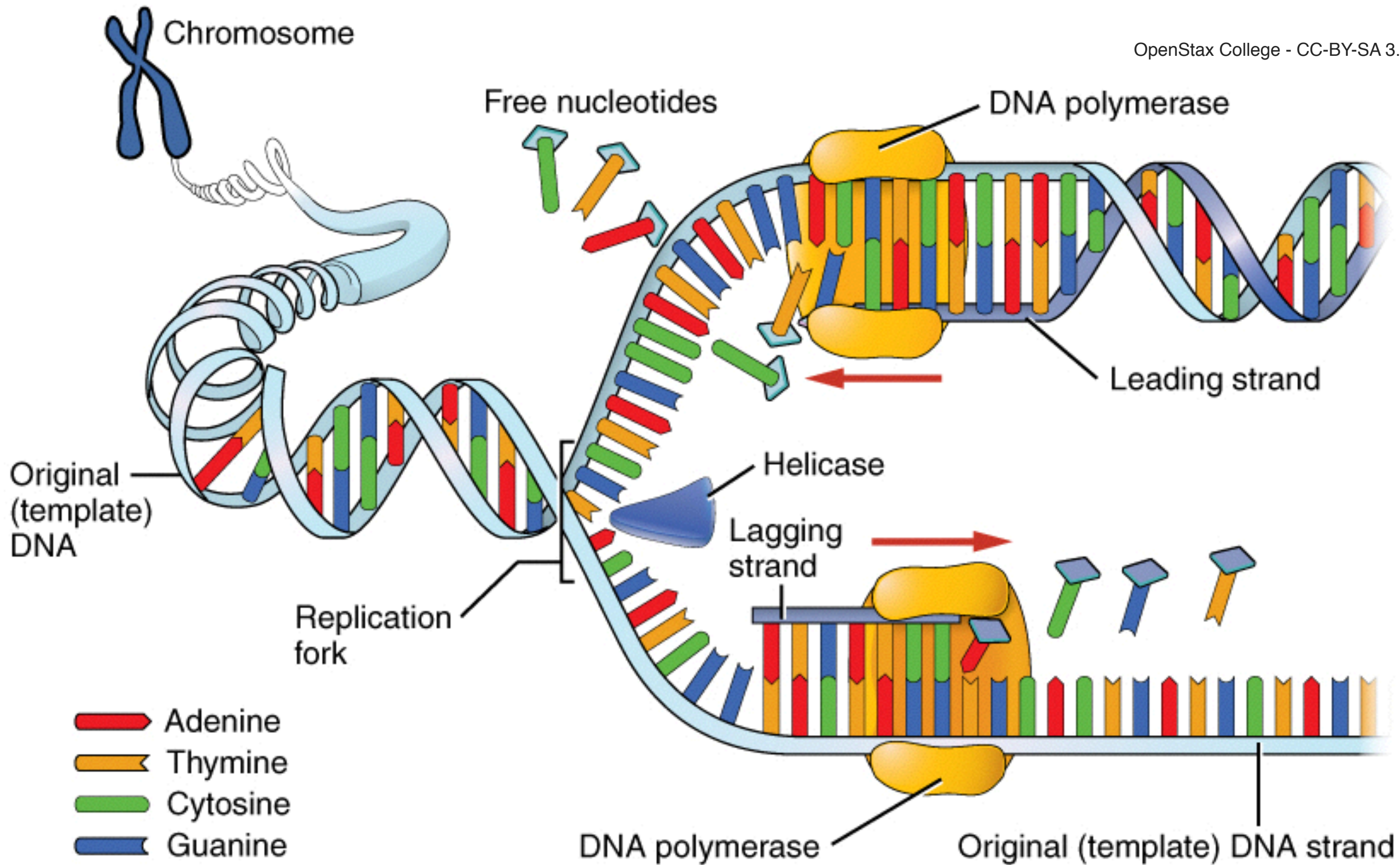
institute for art, science and technology

DNA analytics



DNA Replication

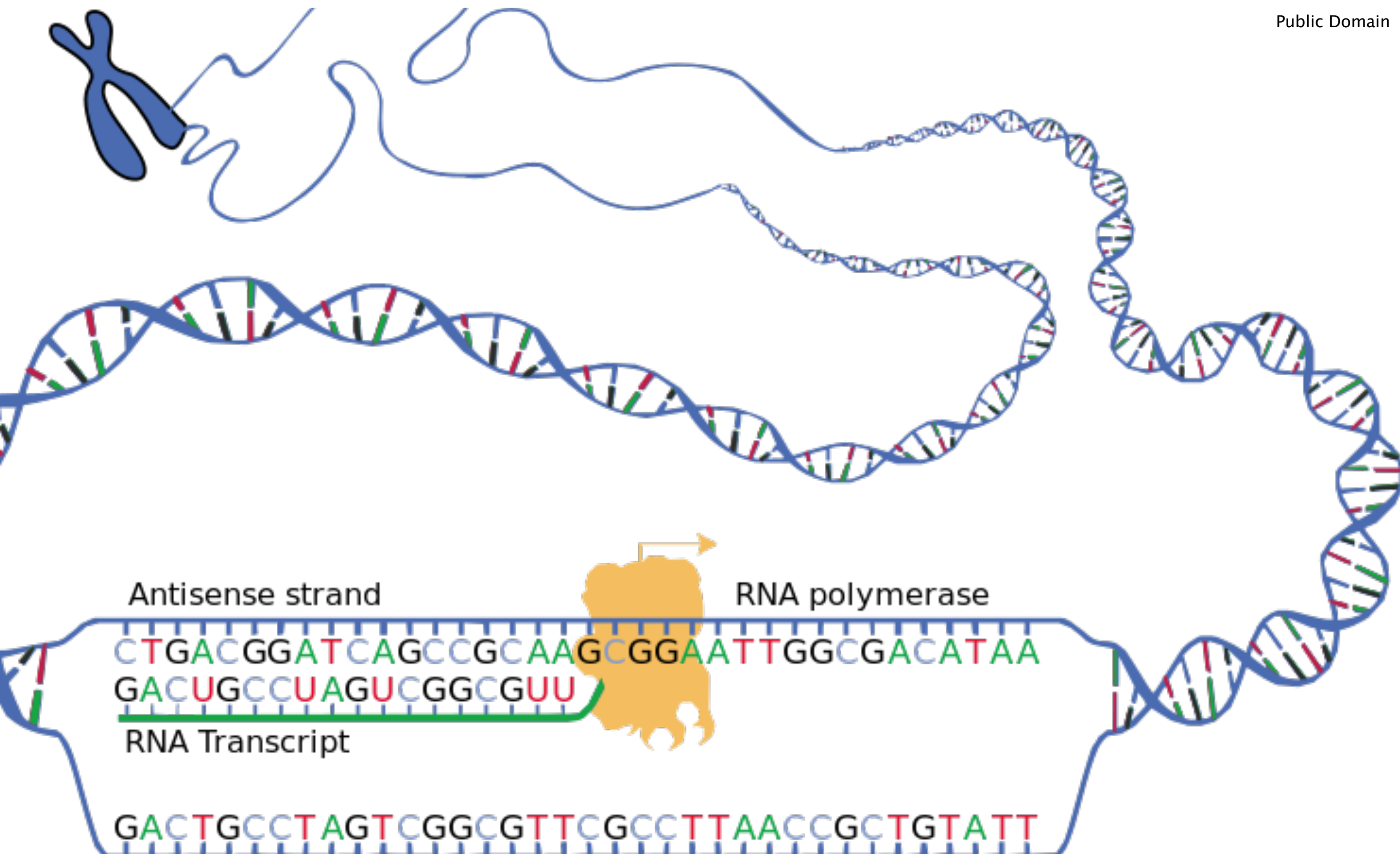
OpenStax College - CC-BY-SA 3.0





DNA Transcription

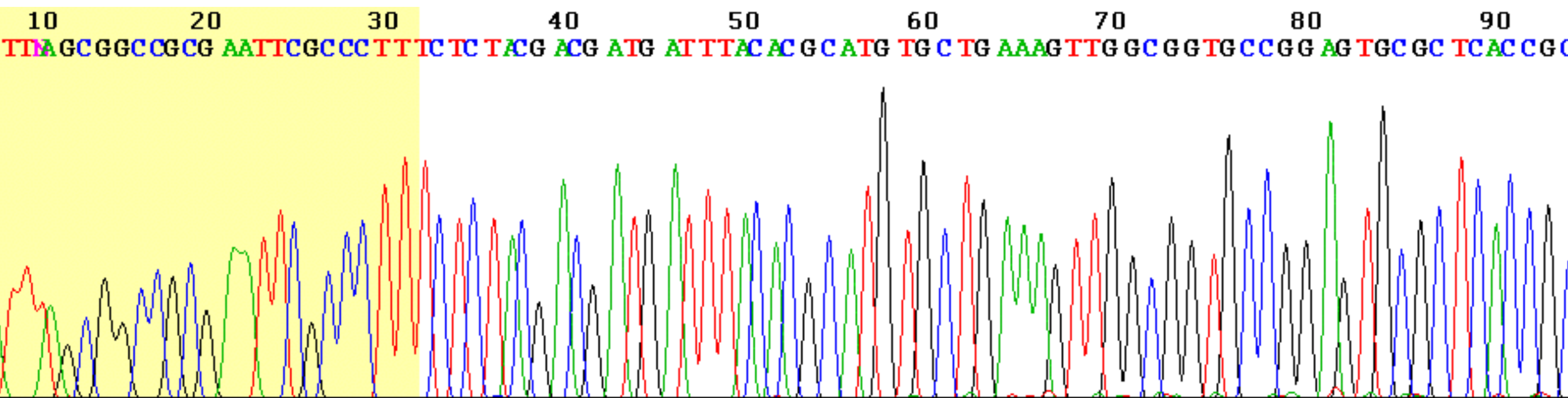
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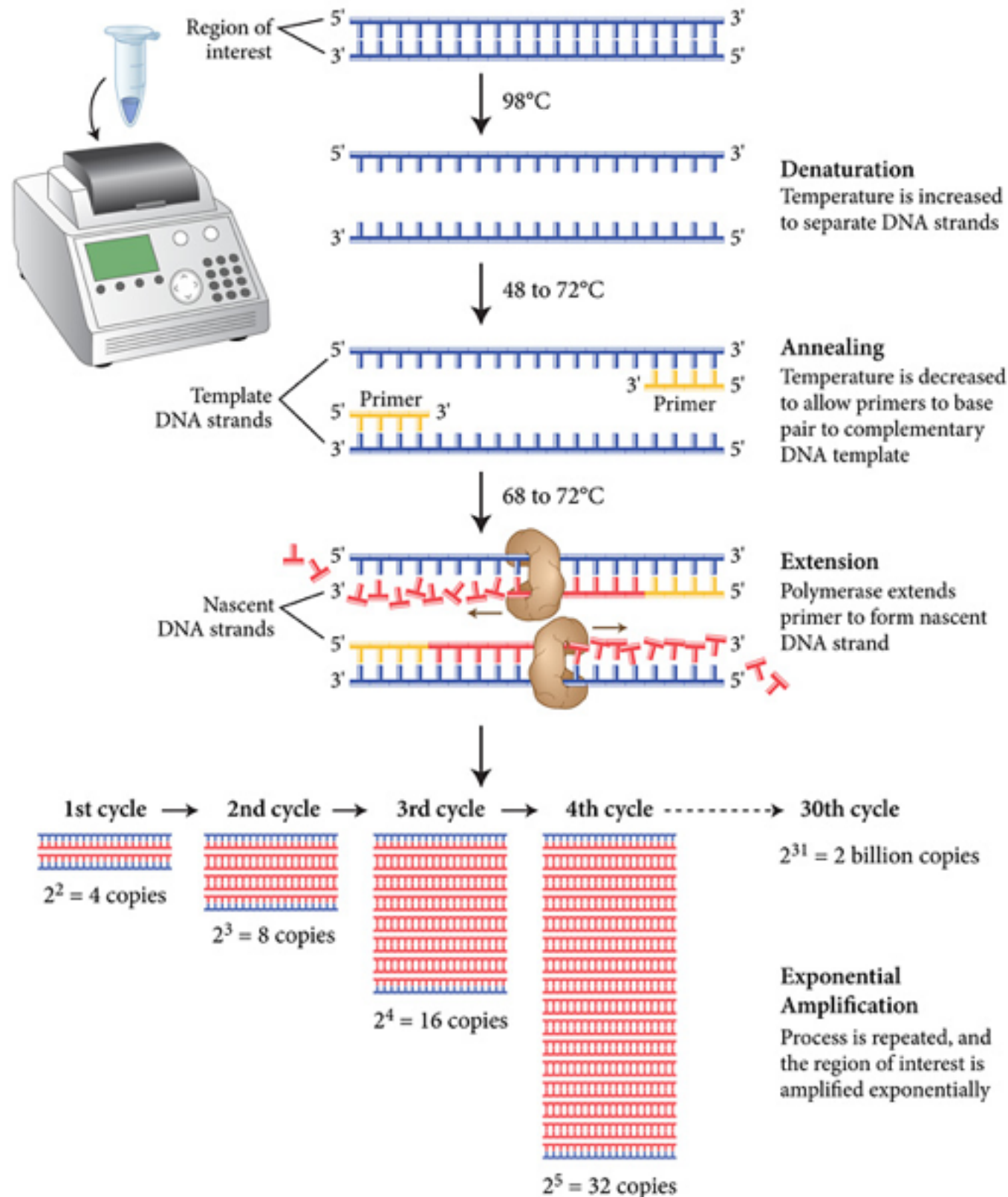
Sequencing

Public Domain



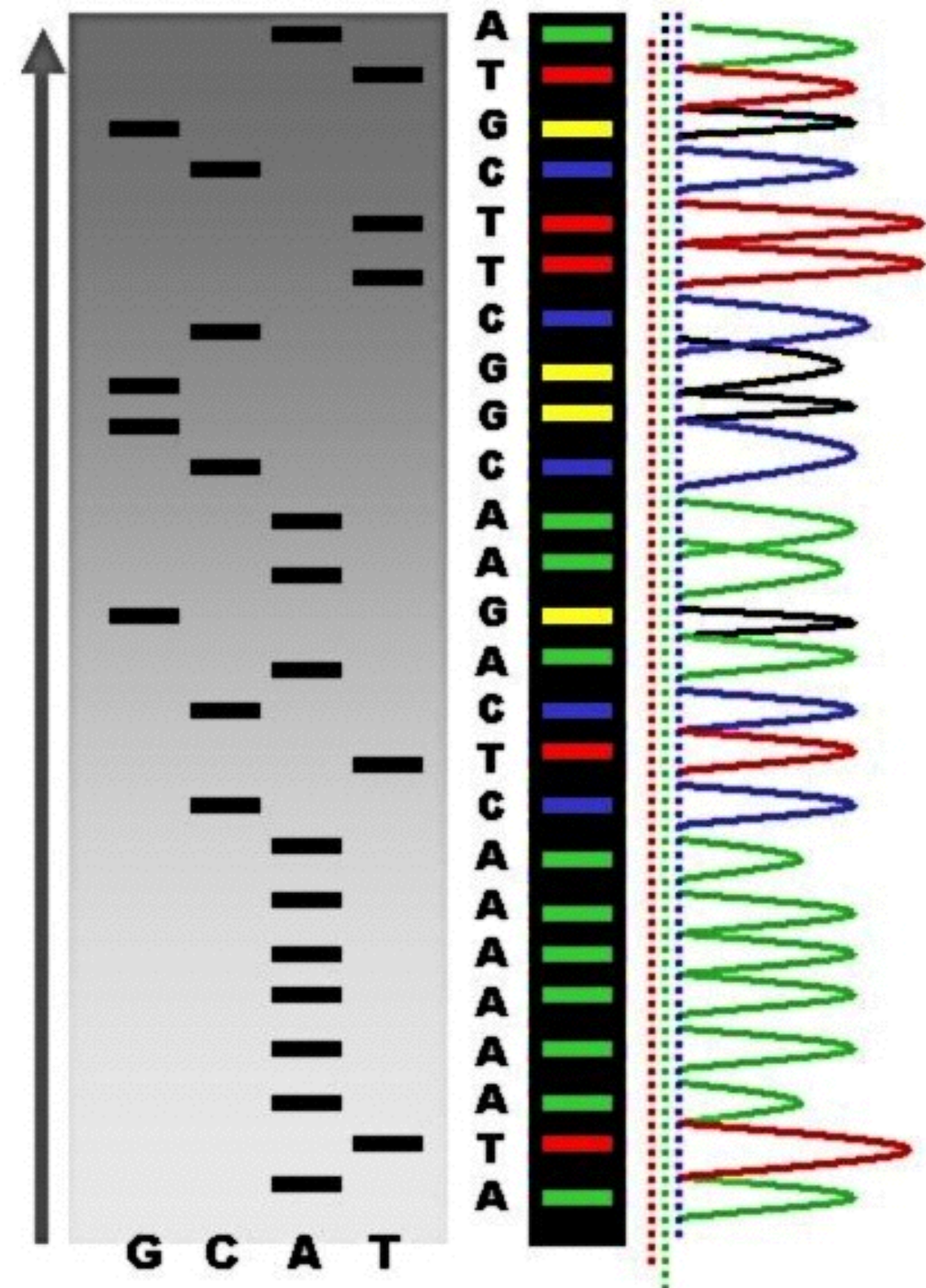


Polymerase Chain Reaction





Sanger Sequencing – chain termination





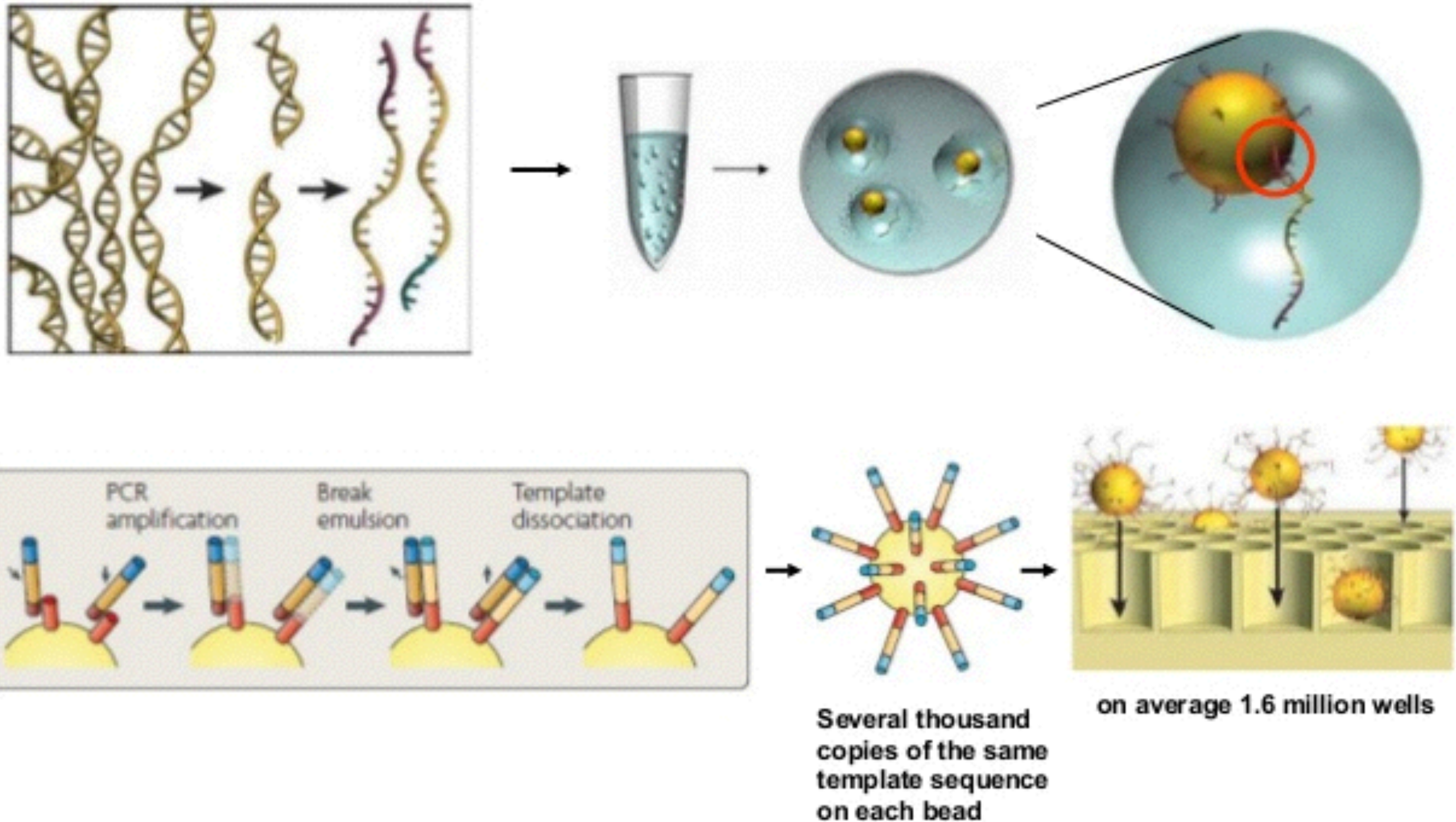
454 sequencer





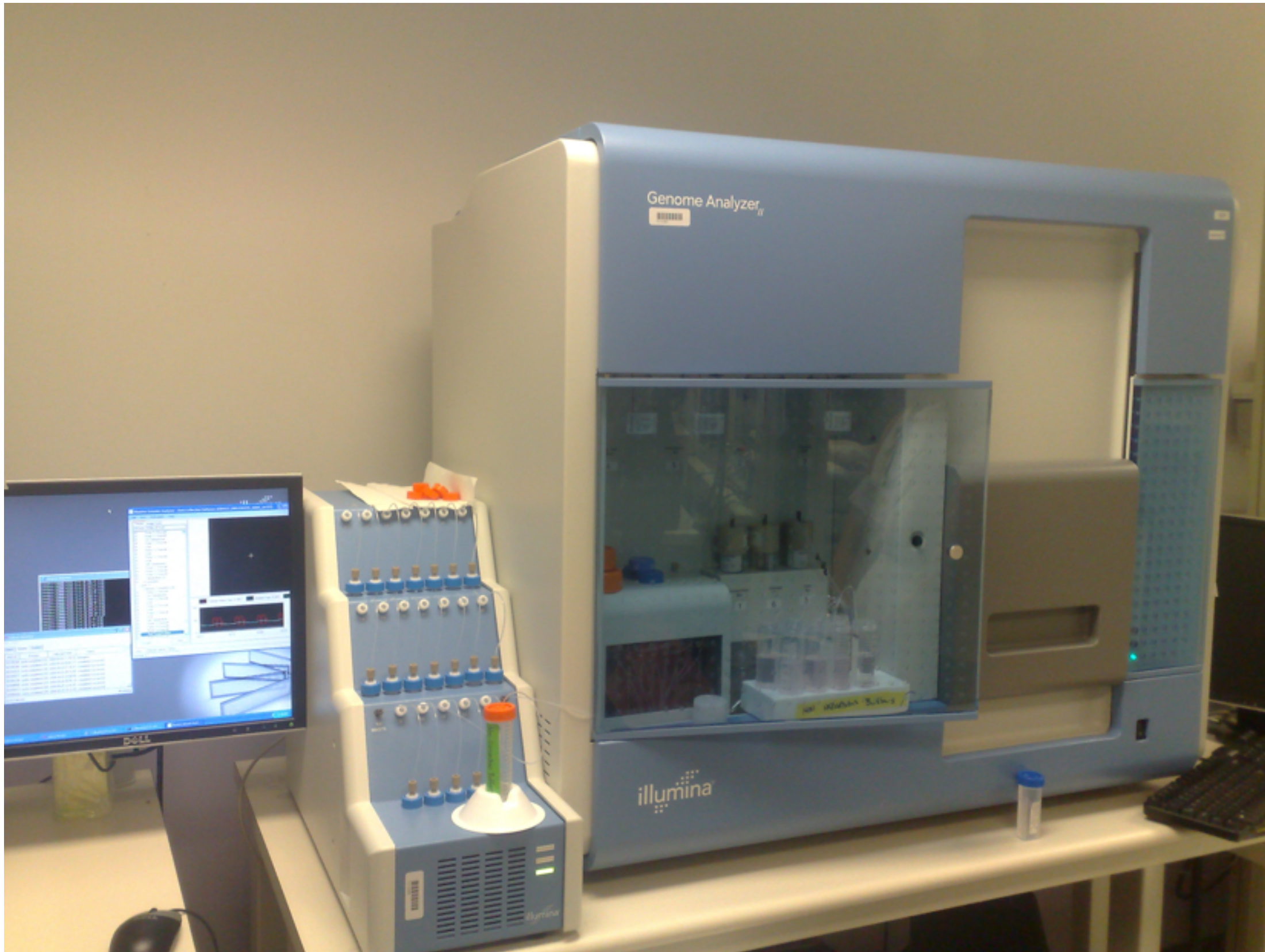
454 Pyrosequencing

1. Emulsion-based sample preparation (emPCR)





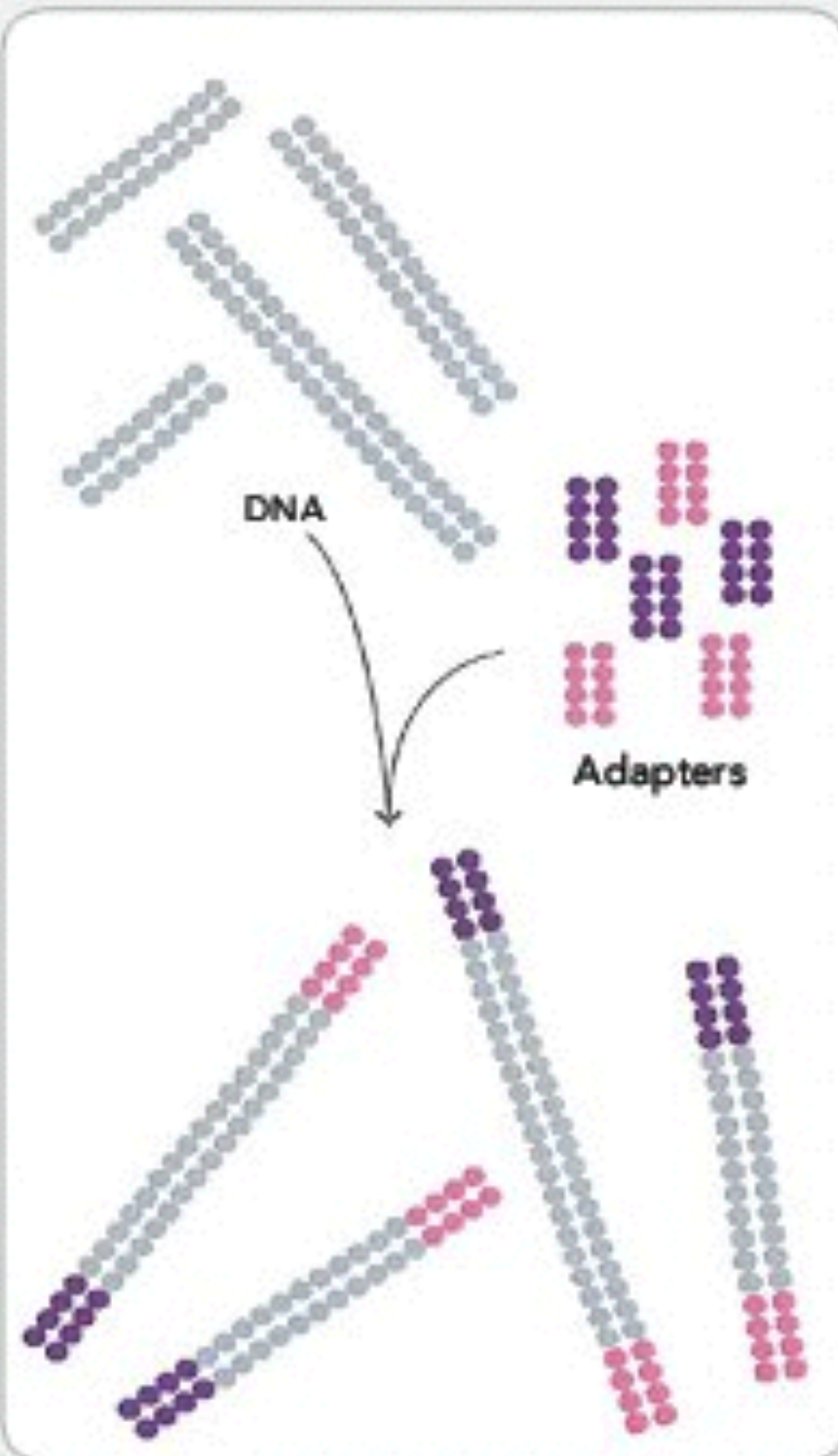
Illumina – Solexa



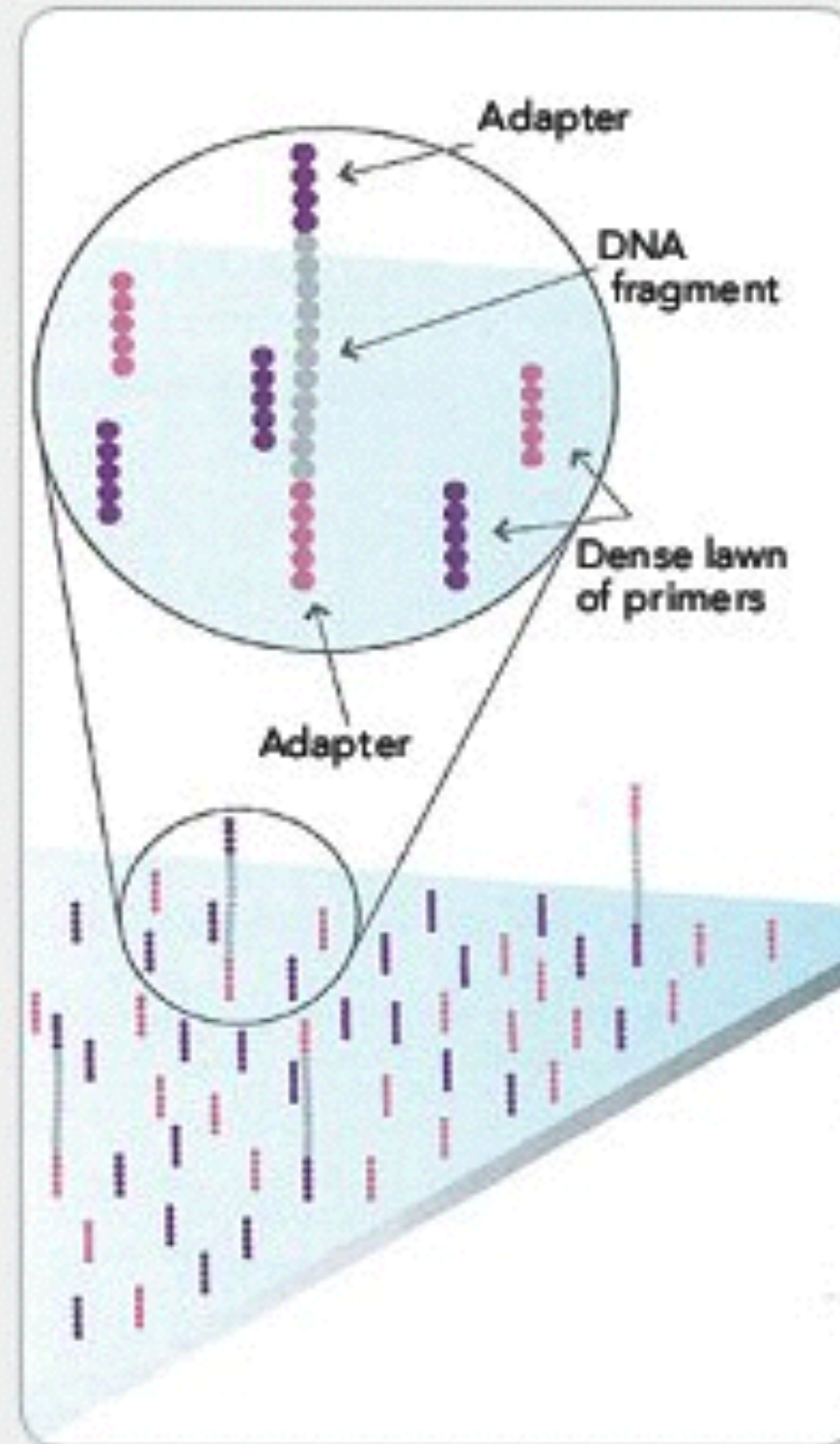


Solexa – Illumina sequencing

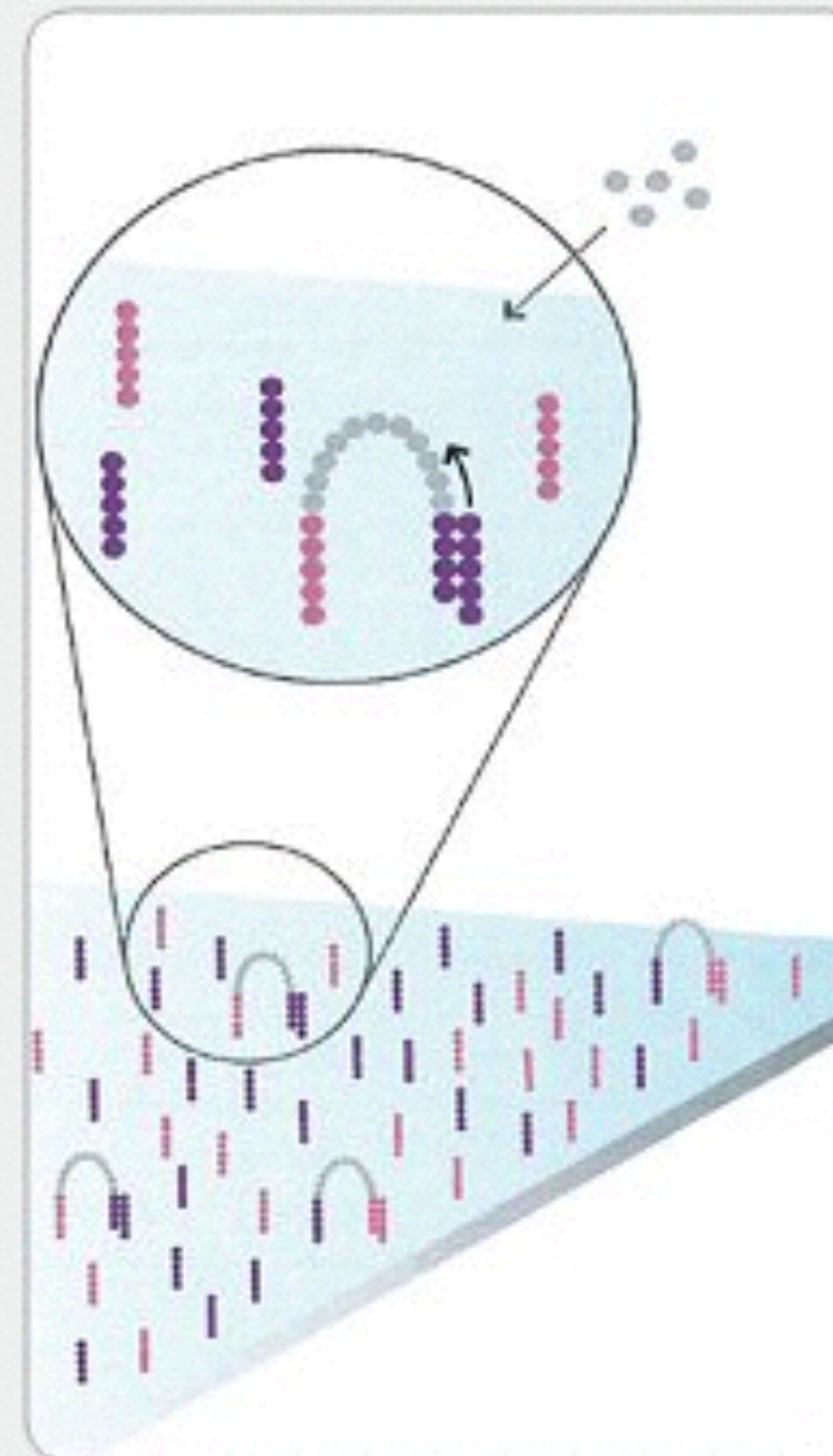
1. PREPARE GENOMIC DNA SAMPLE



2. ATTACH DNA TO SURFACE

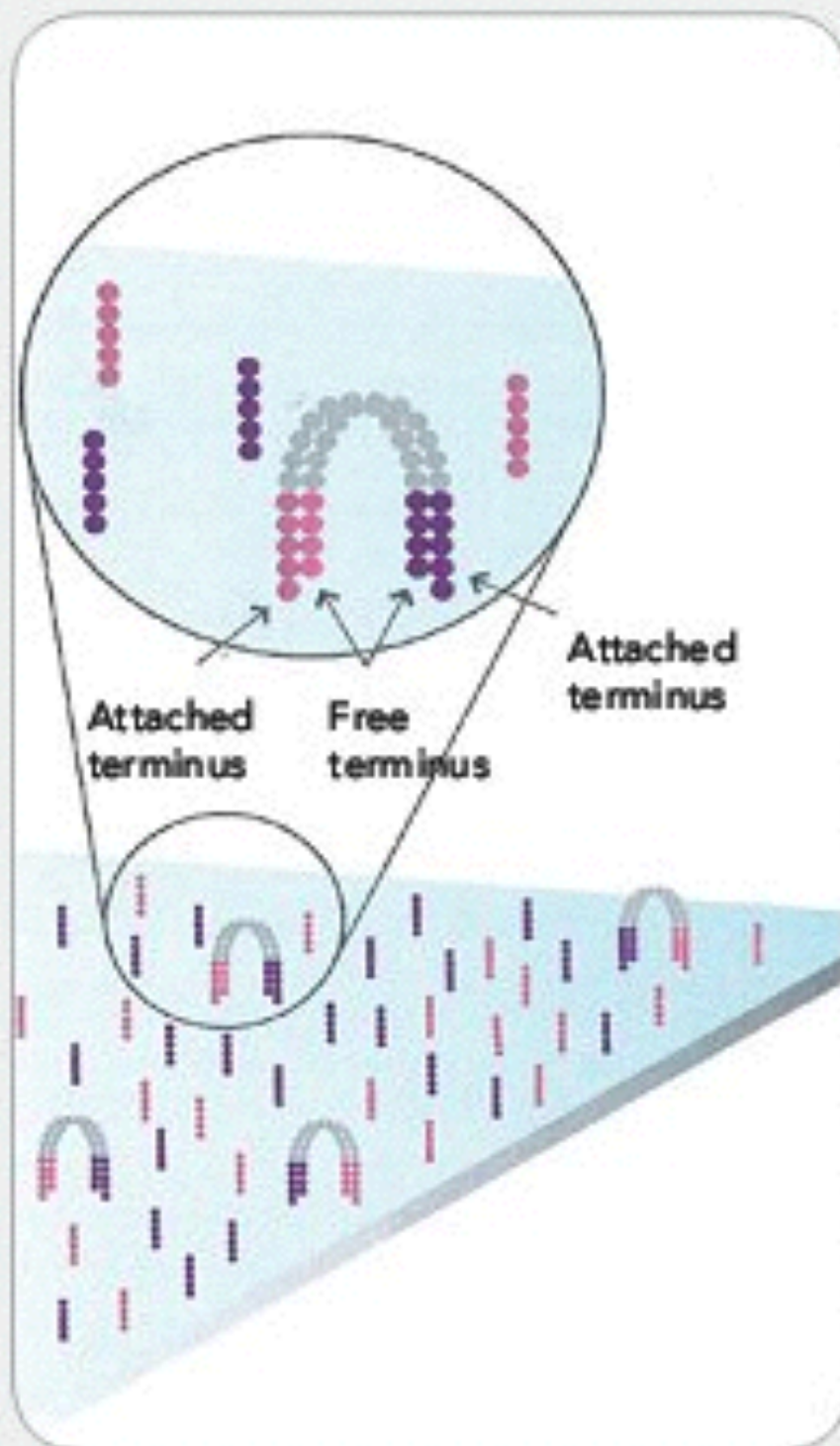


3. BRIDGE AMPLIFICATION

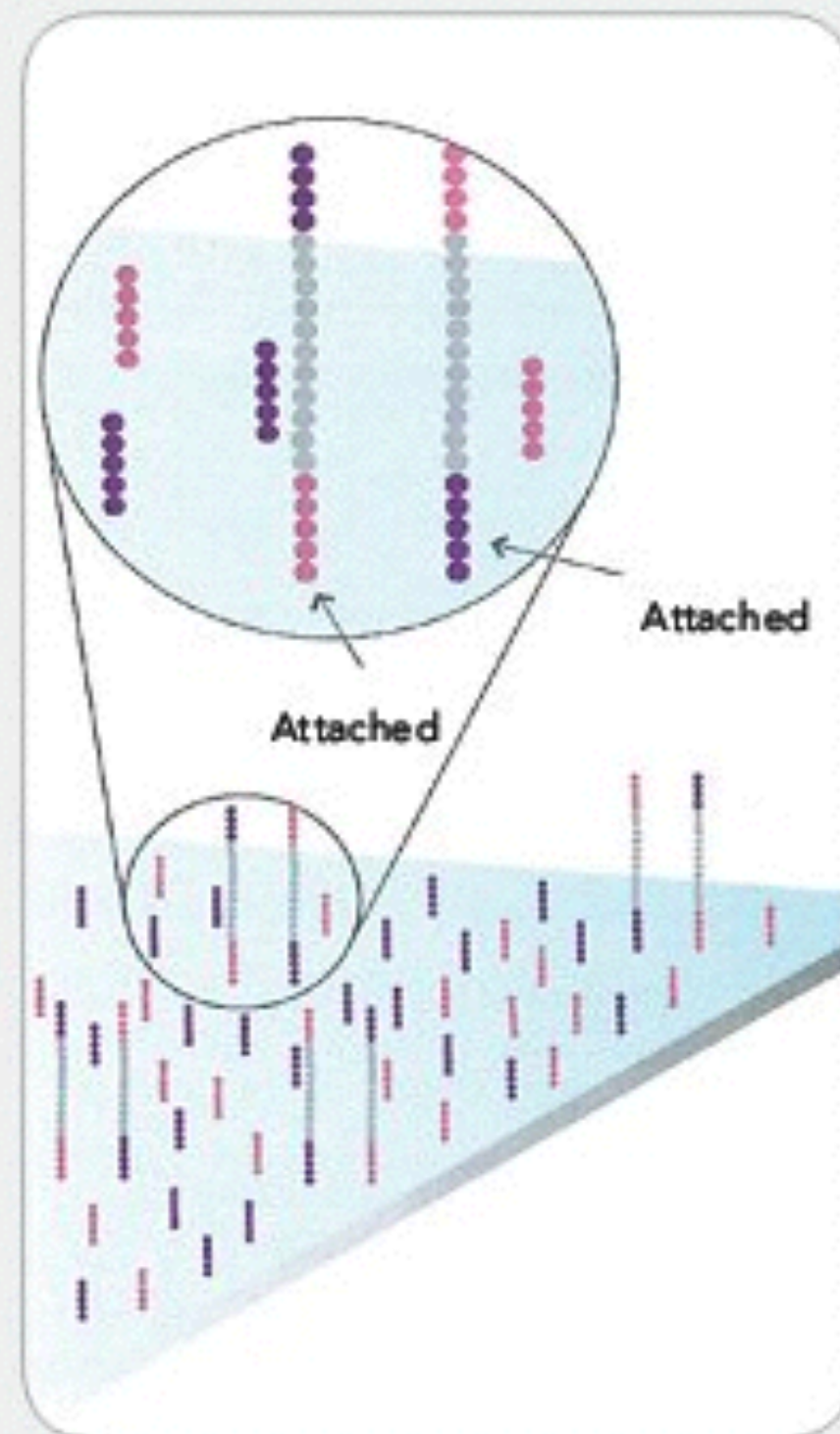




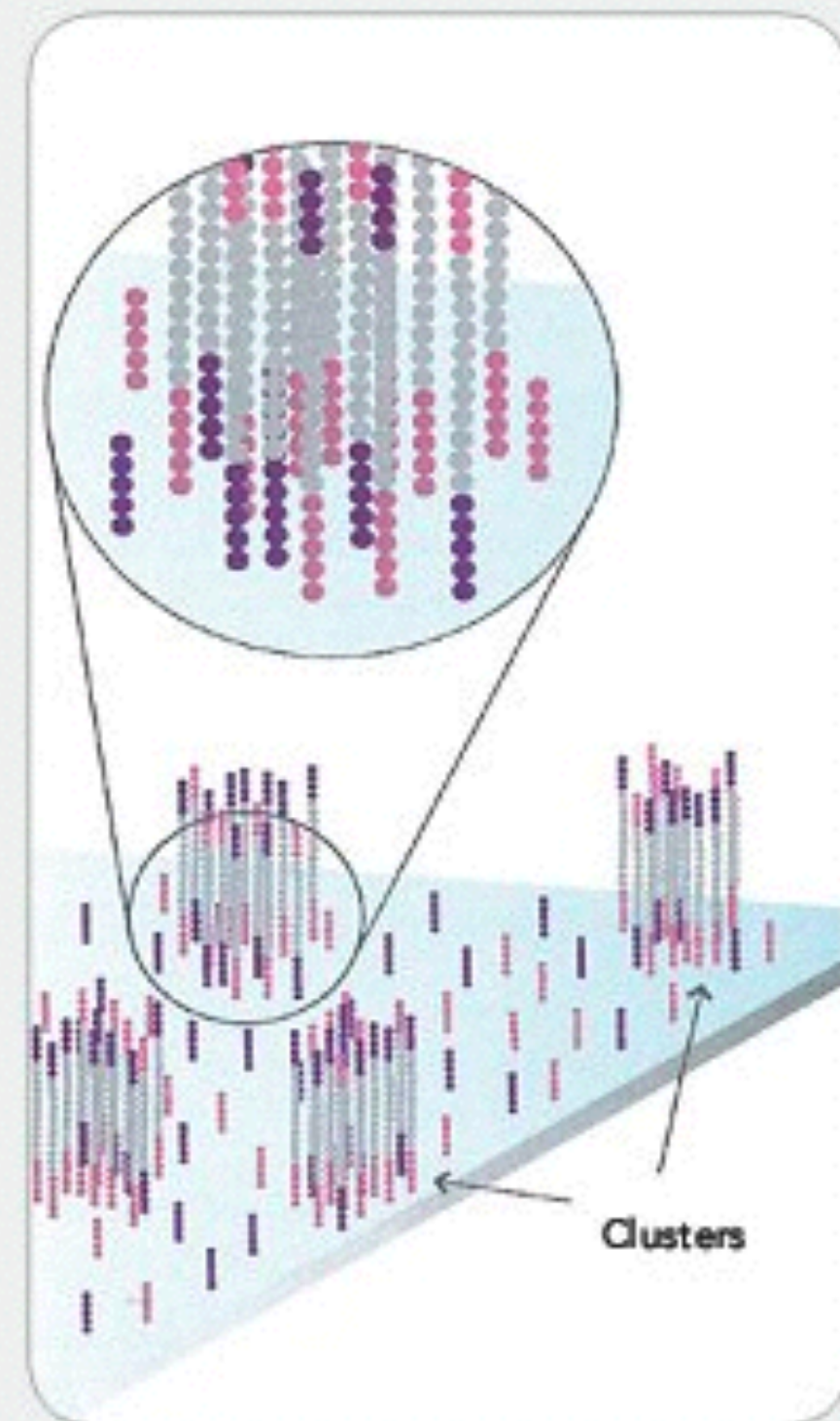
4. FRAGMENTS BECOME DOUBLE STRANDED



5. DENATURE THE DOUBLE-STRANDED MOLECULES

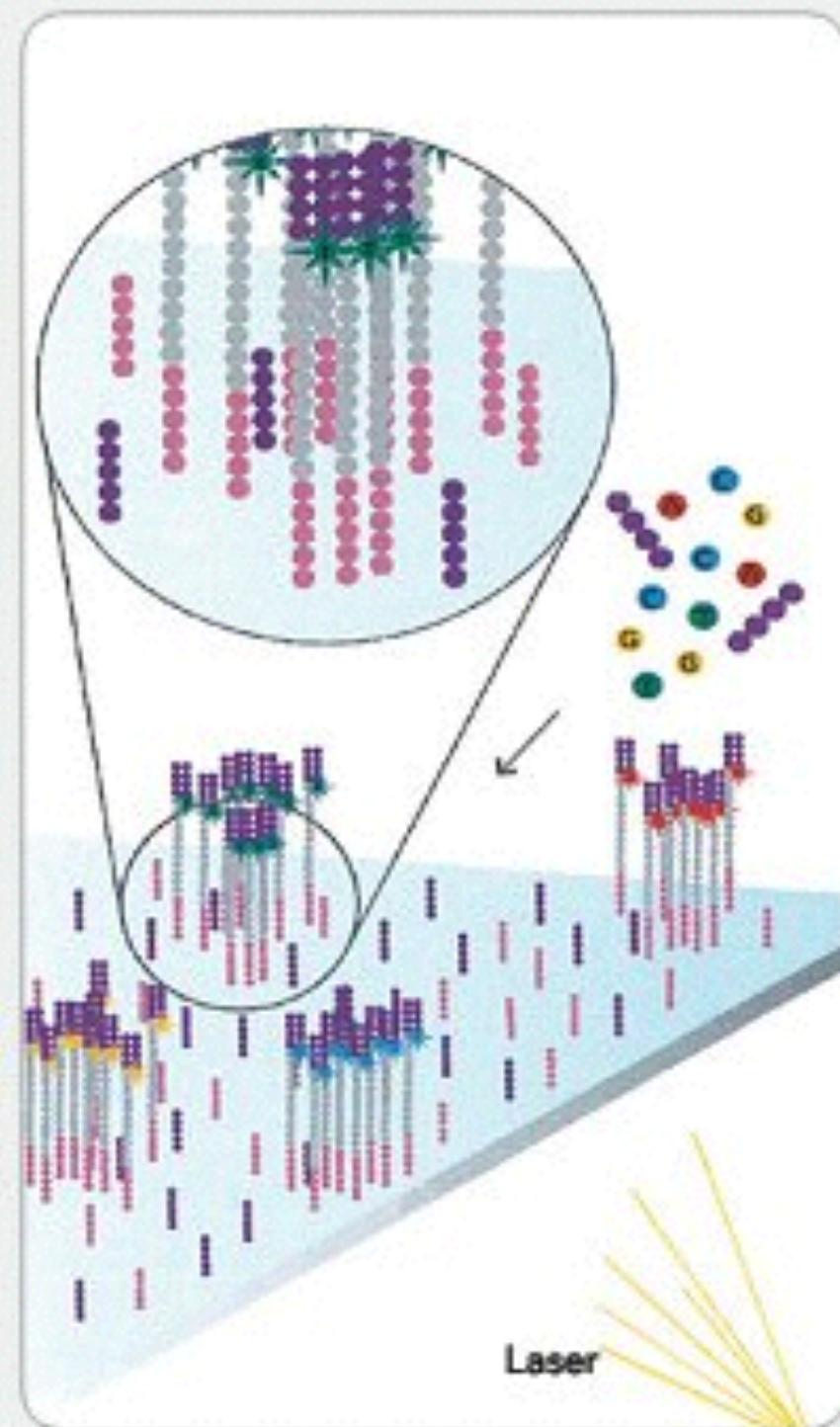


6. COMPLETE AMPLIFICATION





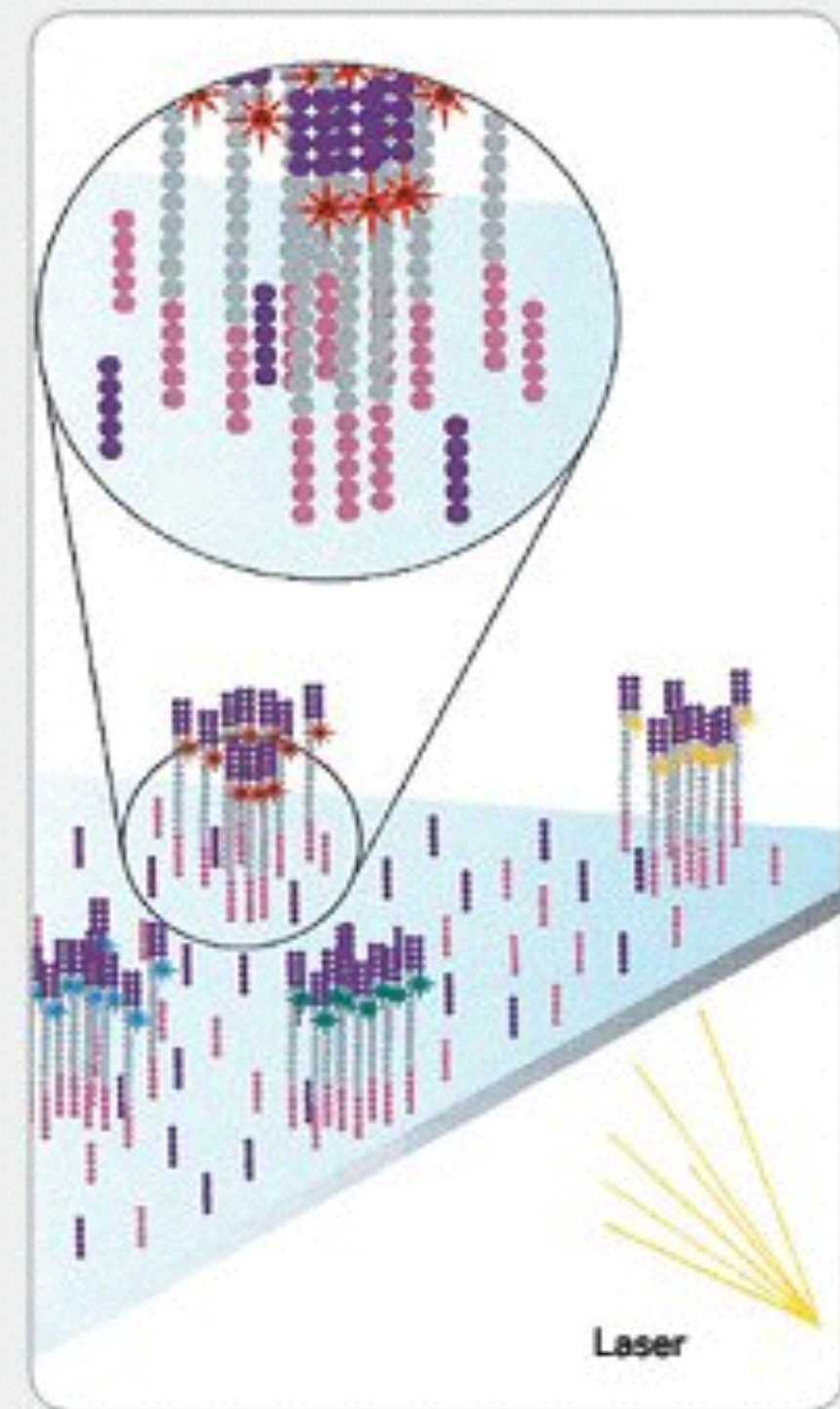
7. DETERMINE FIRST BASE



8. IMAGE FIRST BASE

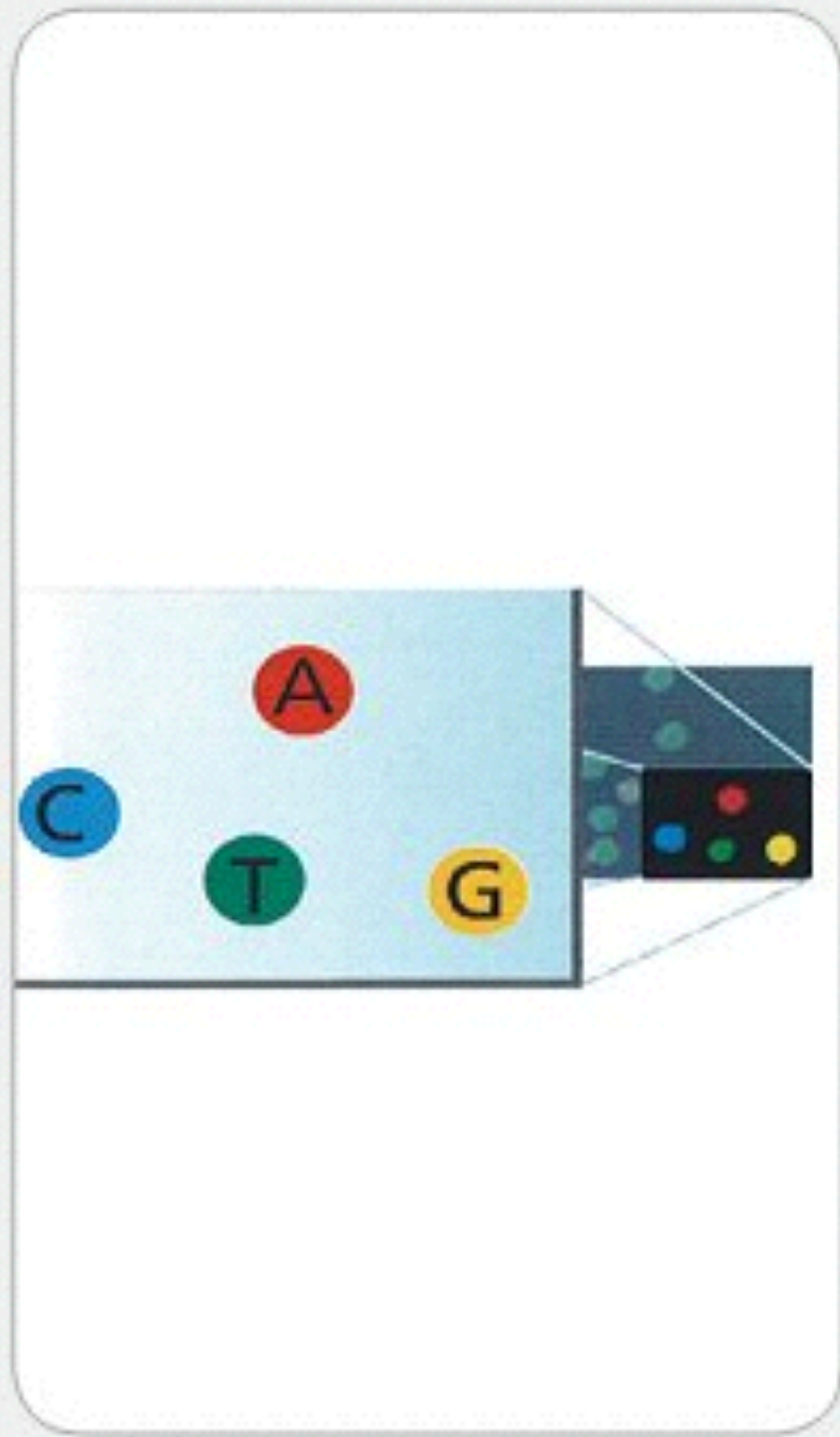


9. DETERMINE SECOND BASE

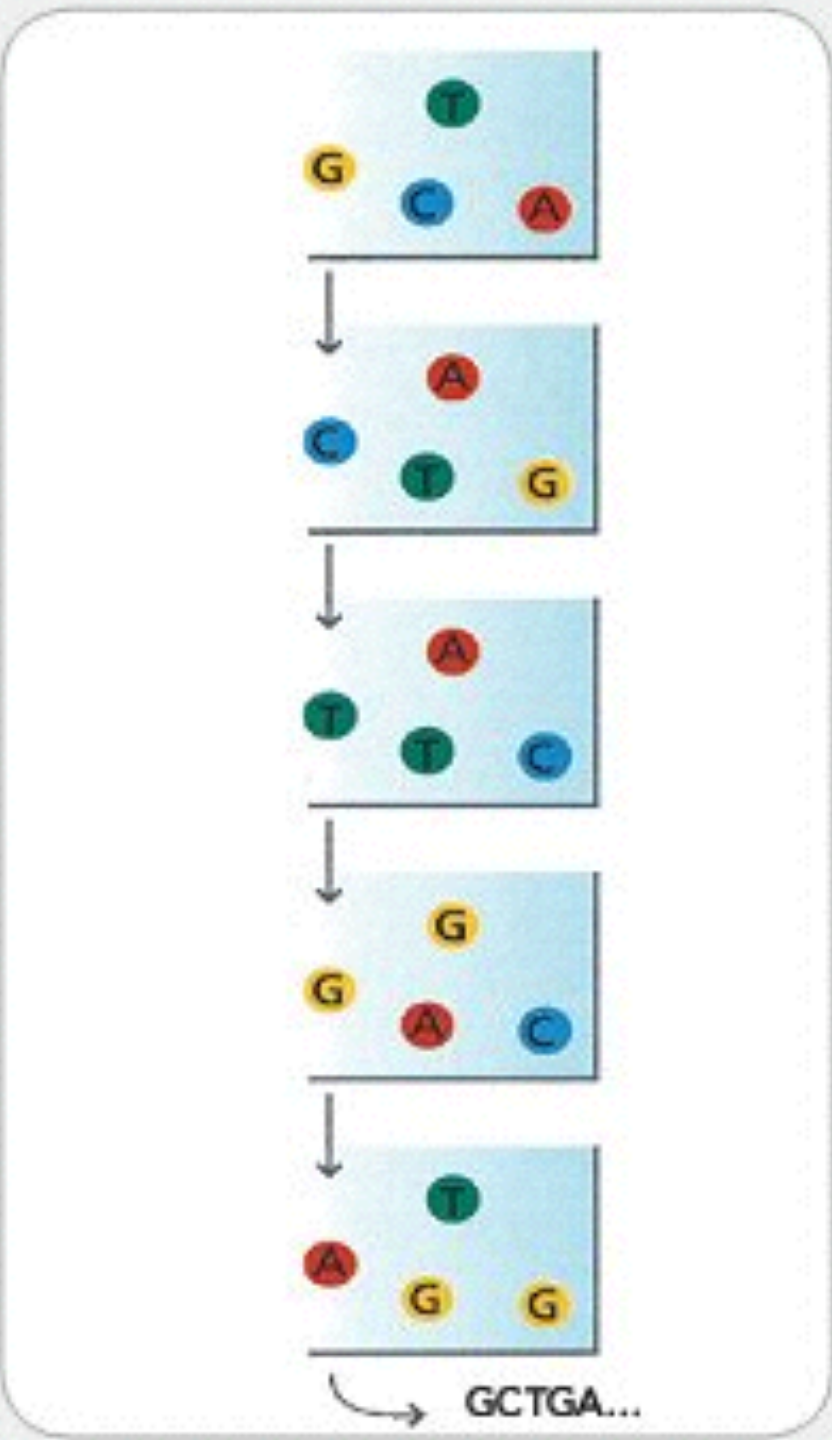




10. IMAGE SECOND CHEMISTRY CYCLE



11. SEQUENCE READS OVER MULTIPLE CHEMISTRY CYCLES

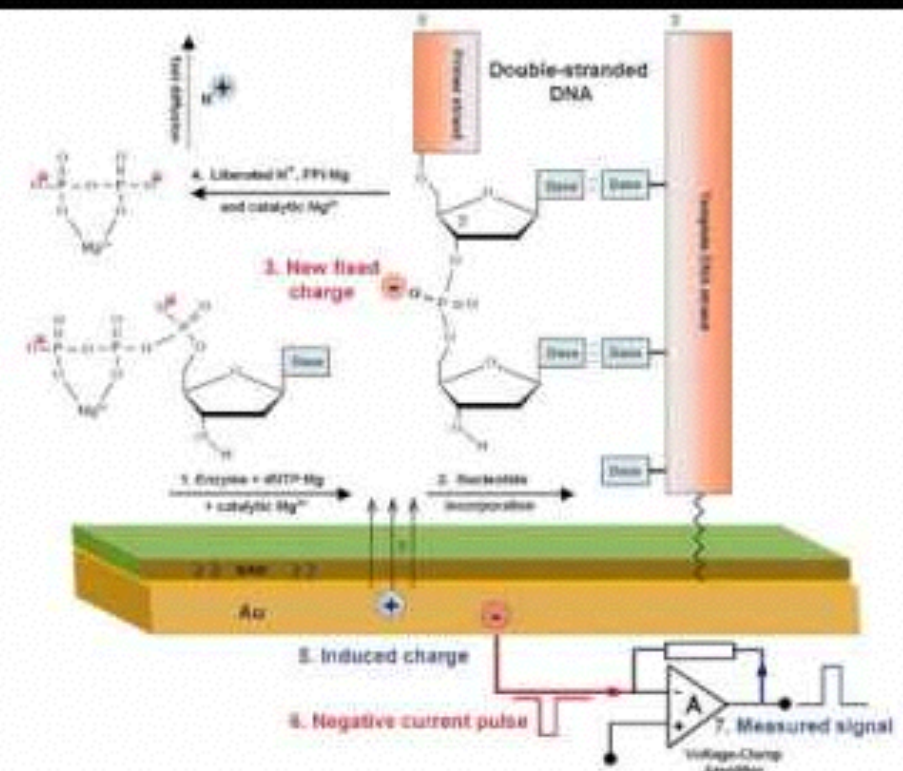
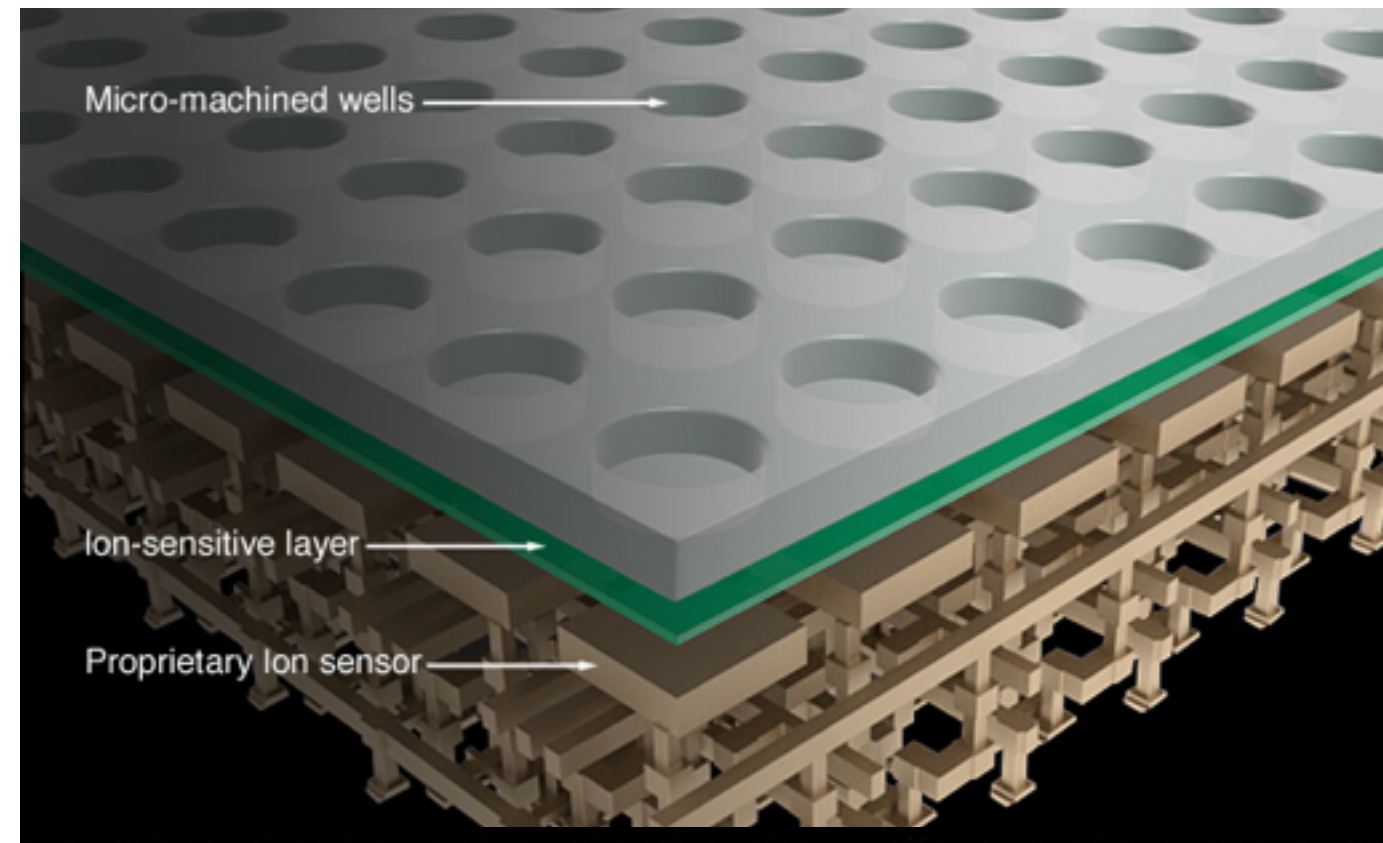


12. ALIGN DATA



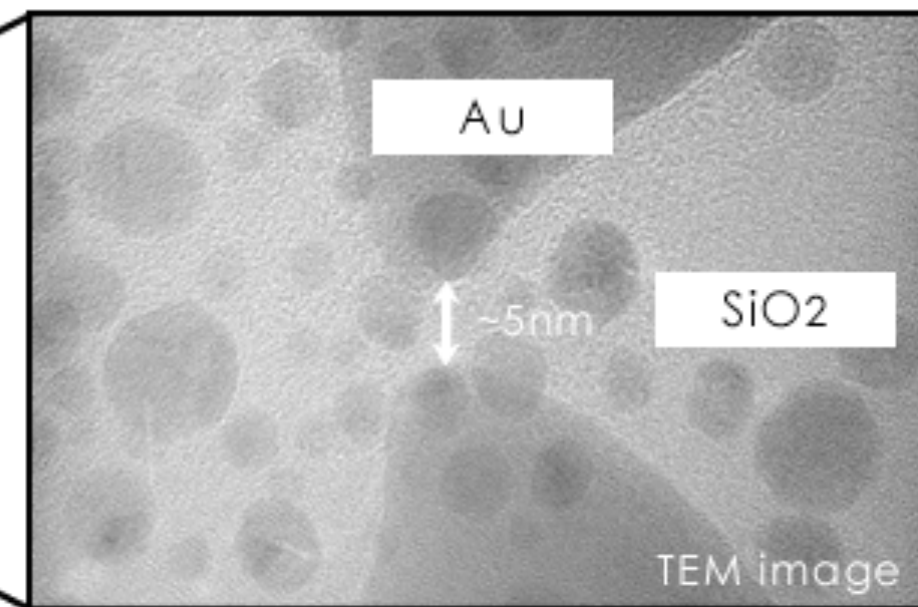
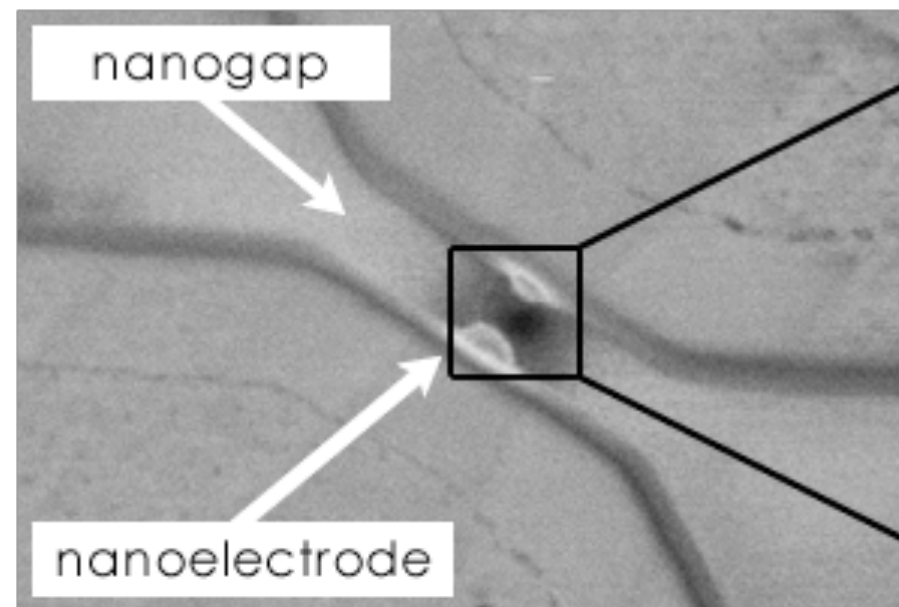
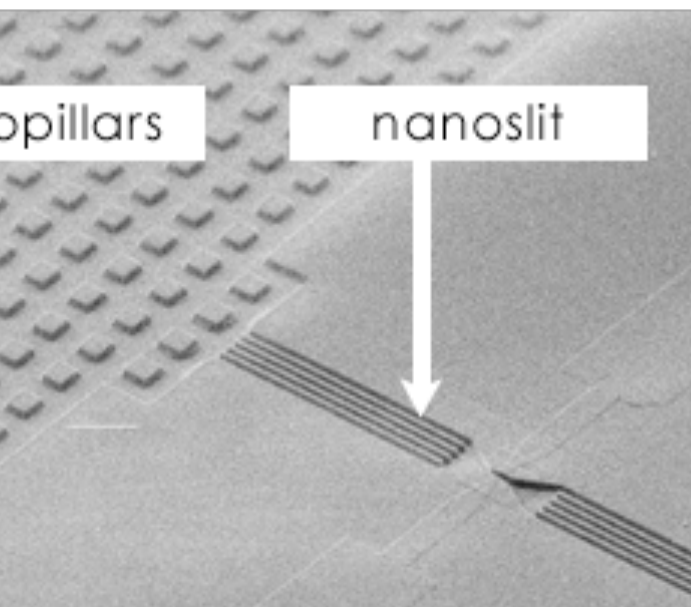
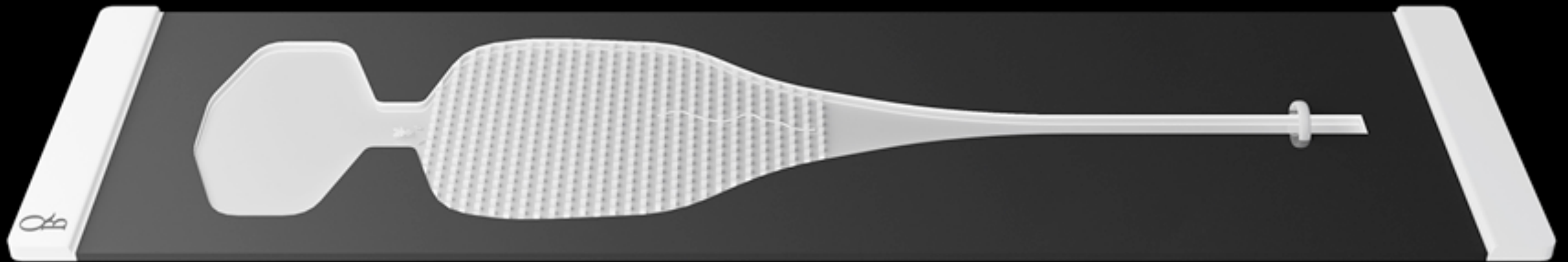


IonTorrent sequencing



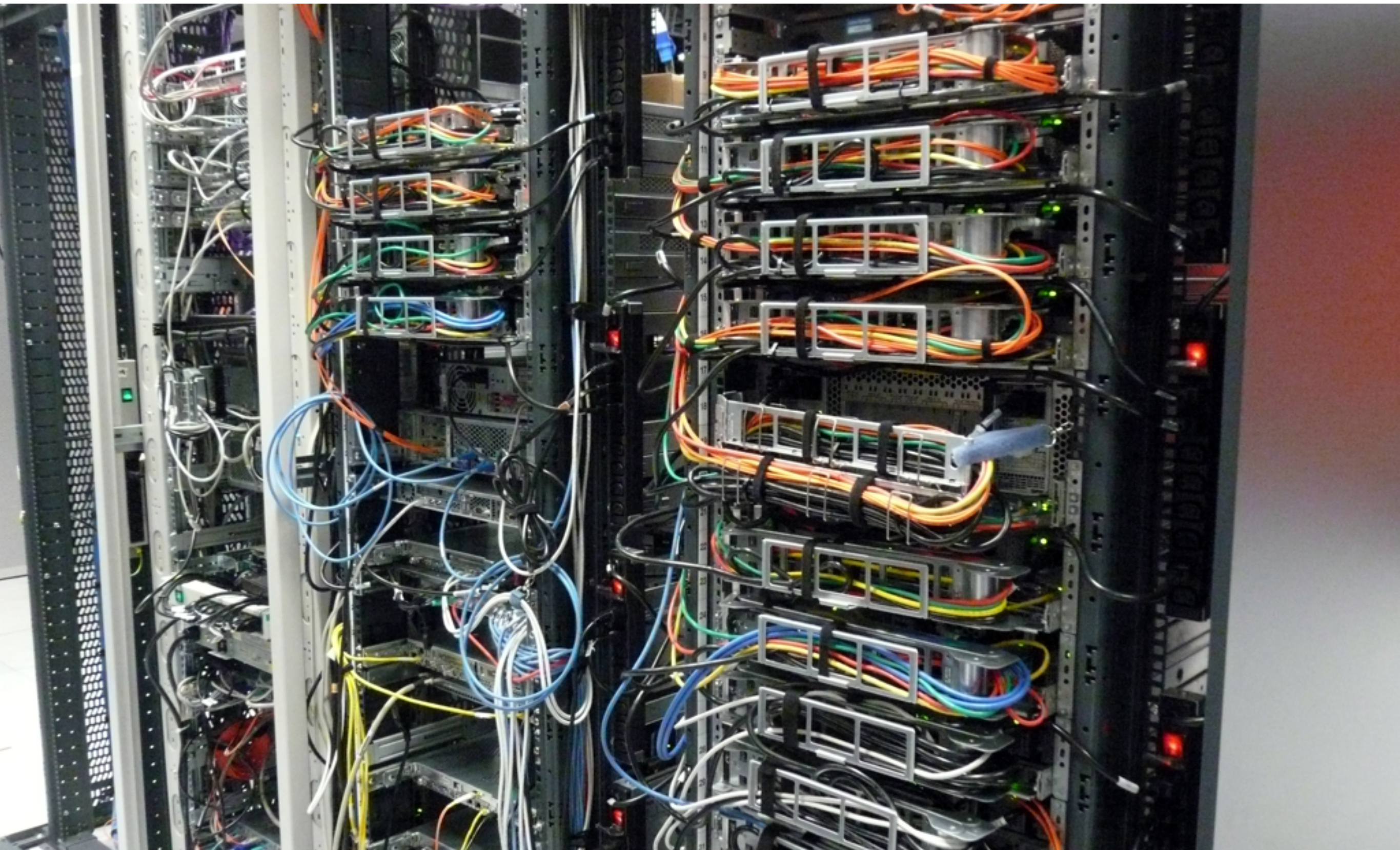


Nanopore sequencing





Bioinformatics





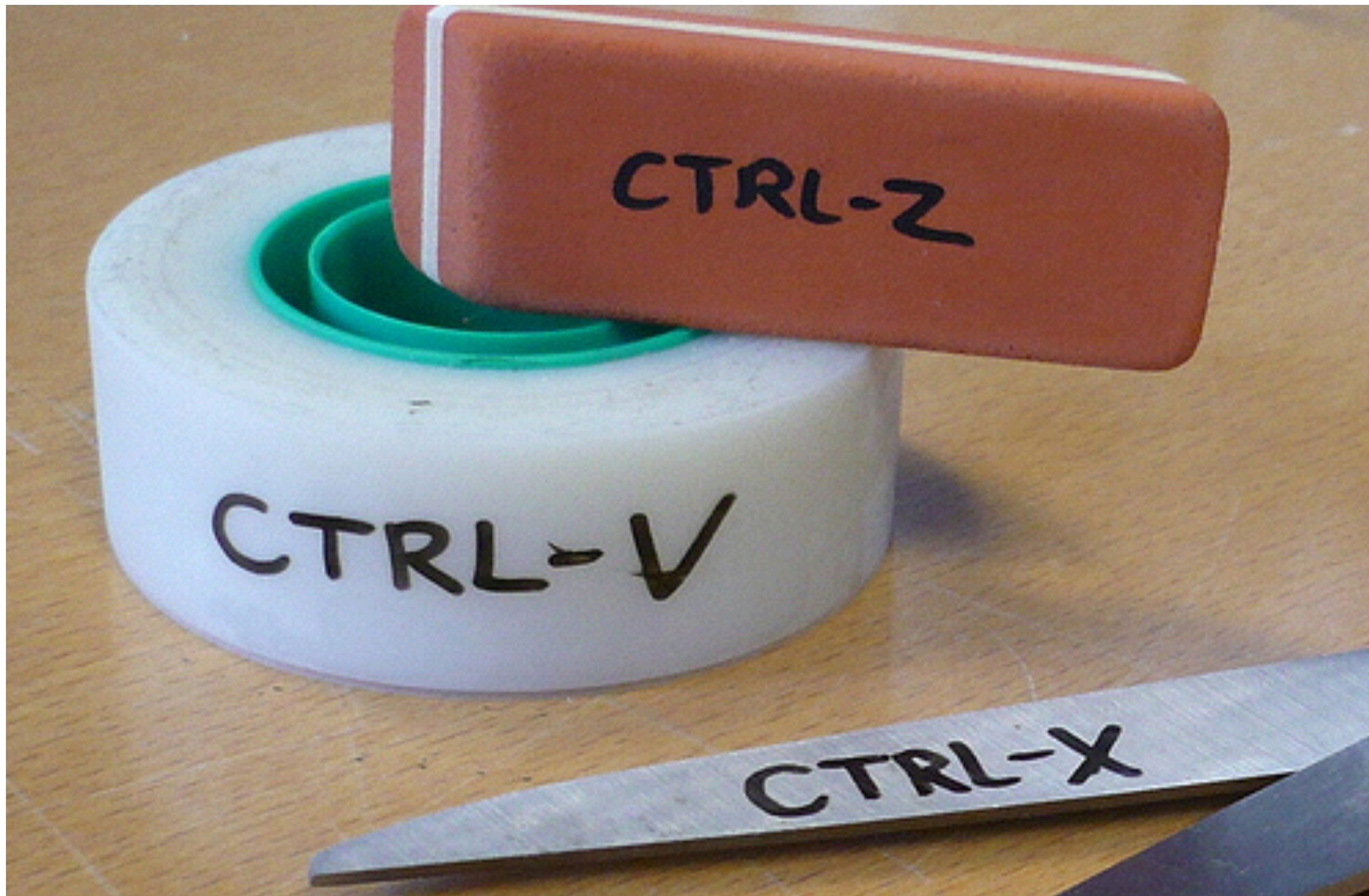
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DNA editing

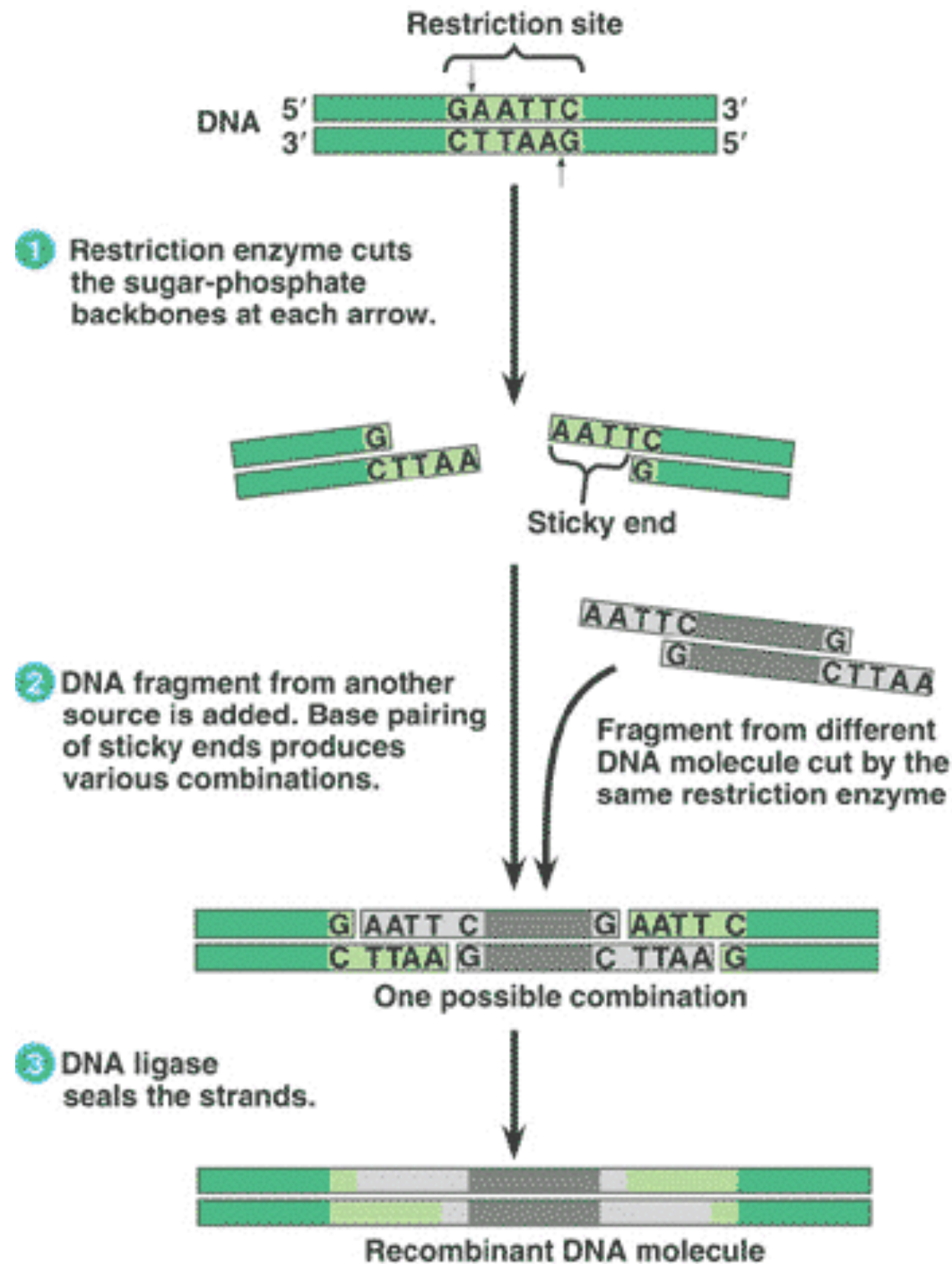


Cutting & Pasting



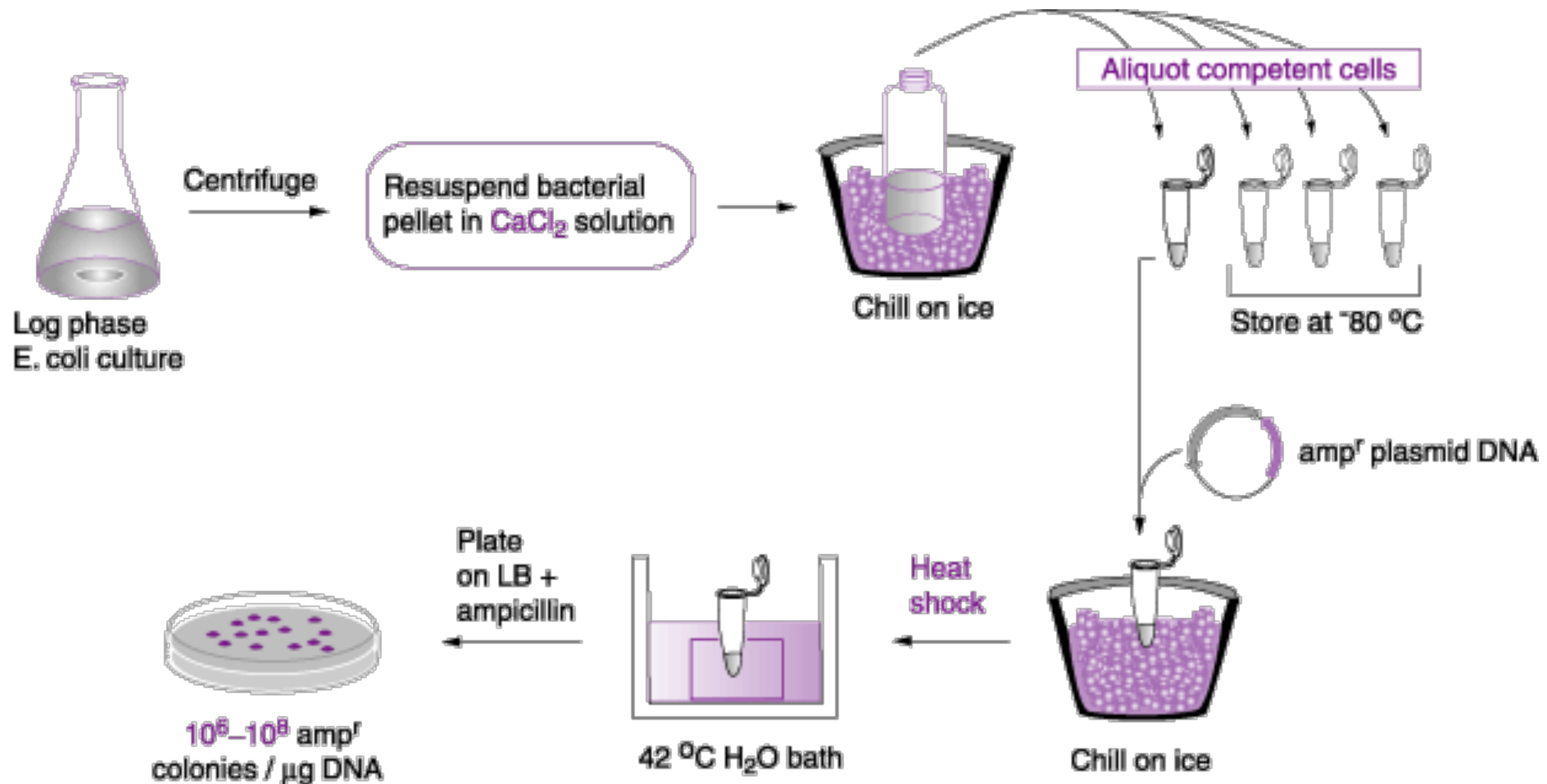


DNA Restriction Ligation



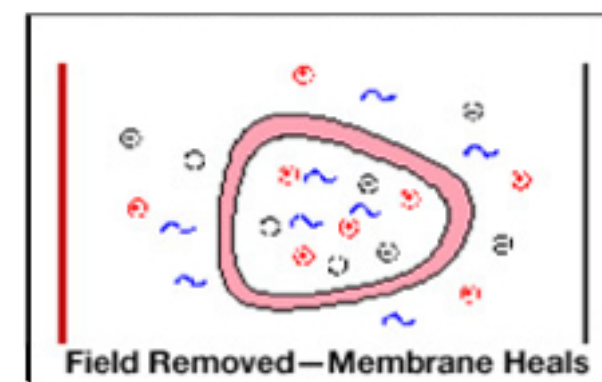
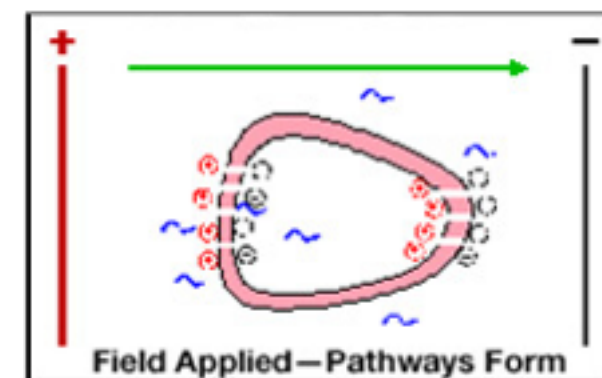
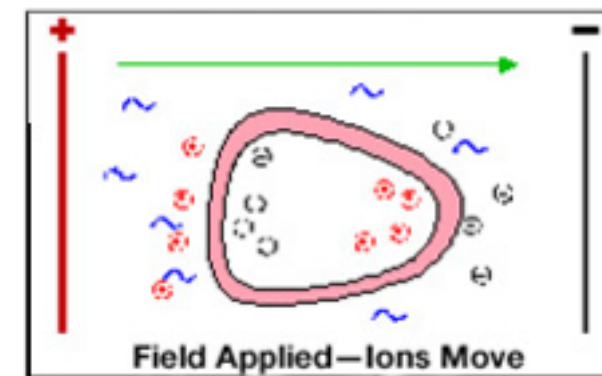
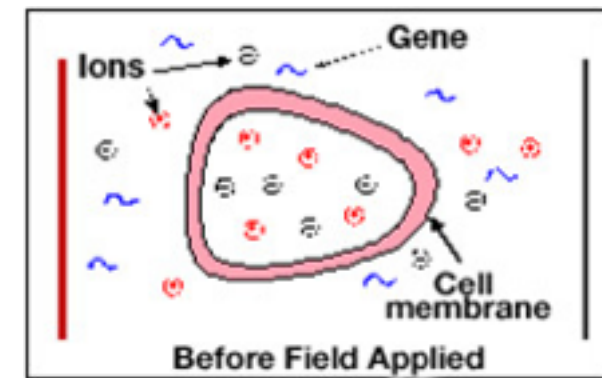


Heat Shock Transformation





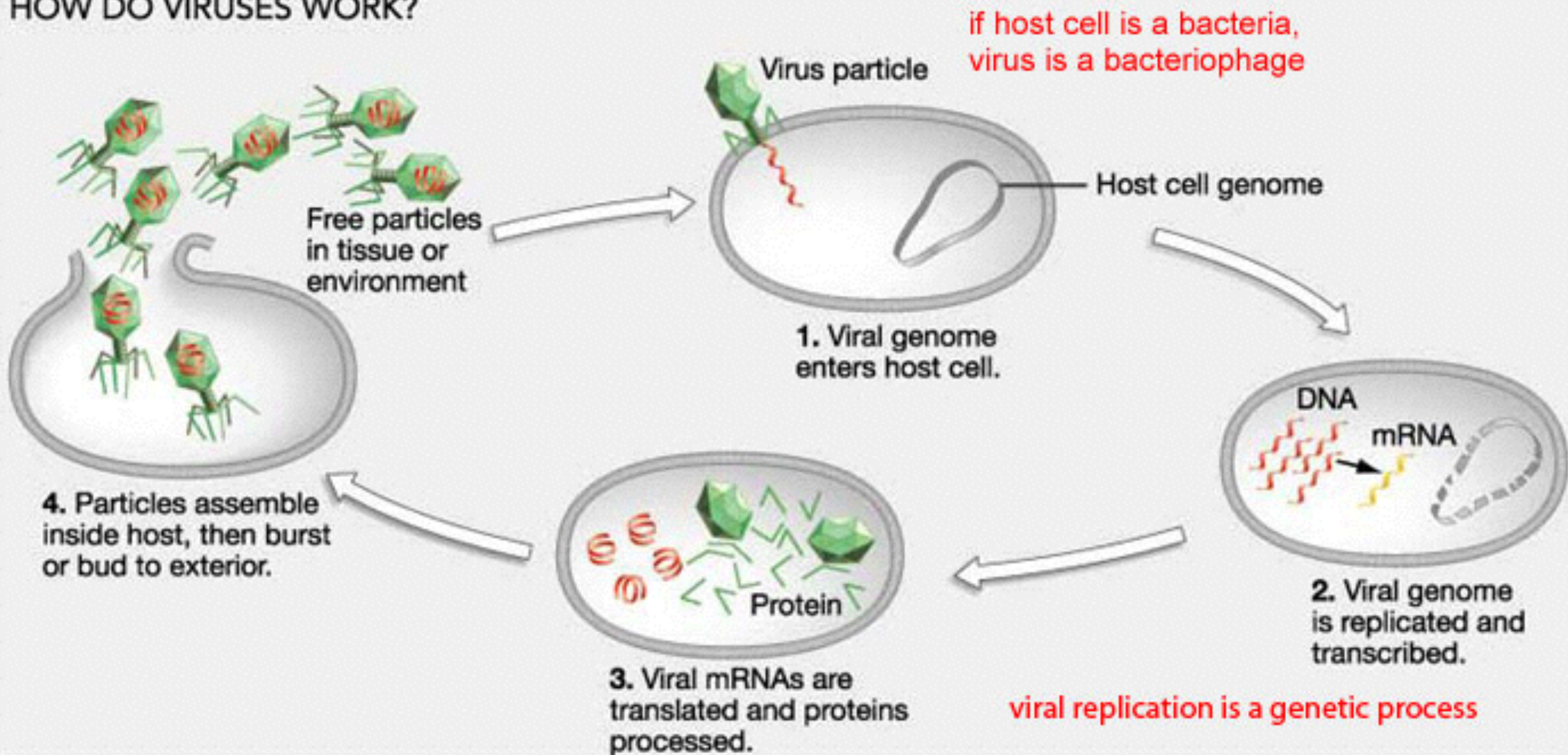
GeneGun – Electroporation





Viral Transformation

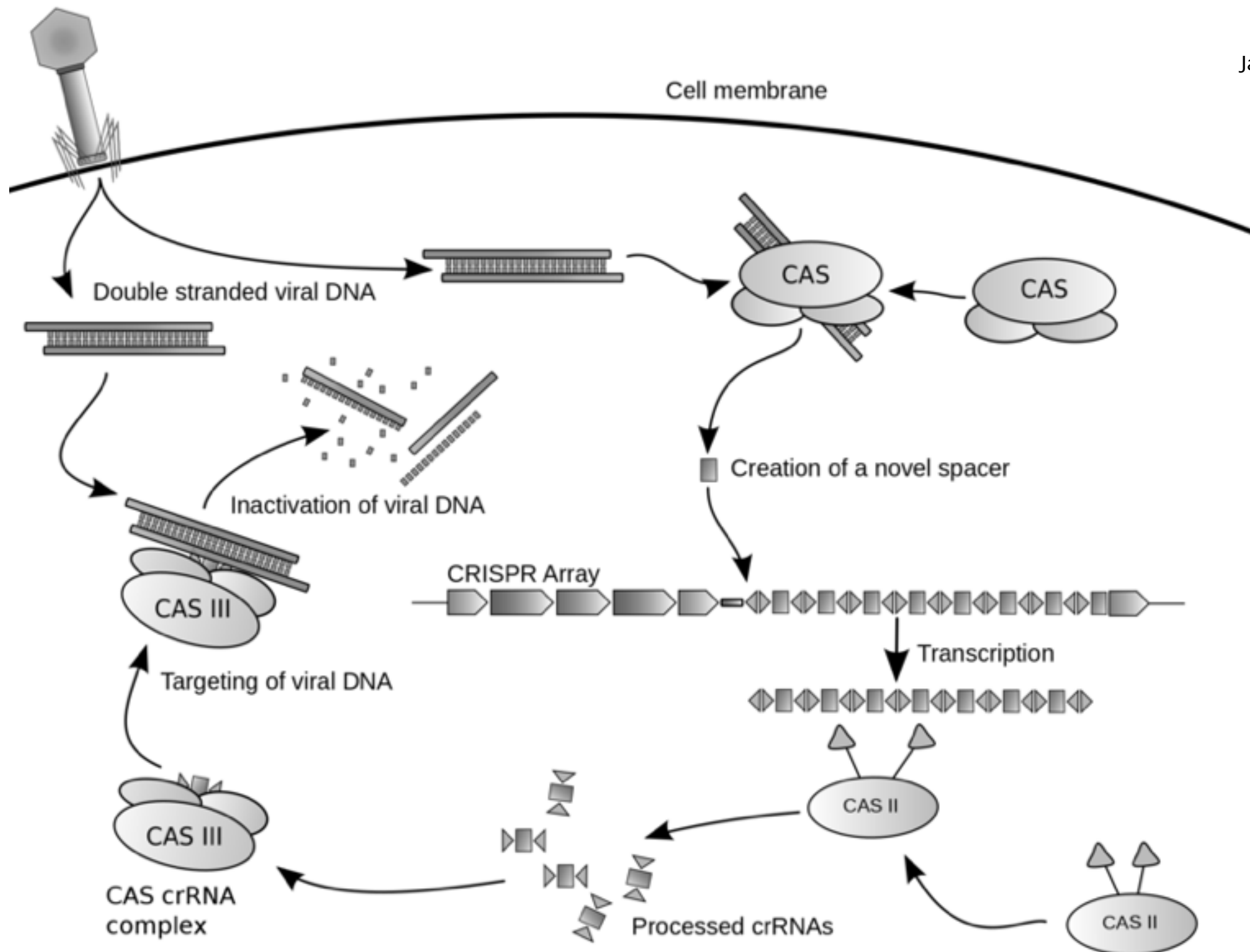
HOW DO VIRUSES WORK?





CRISPR – Cas9

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