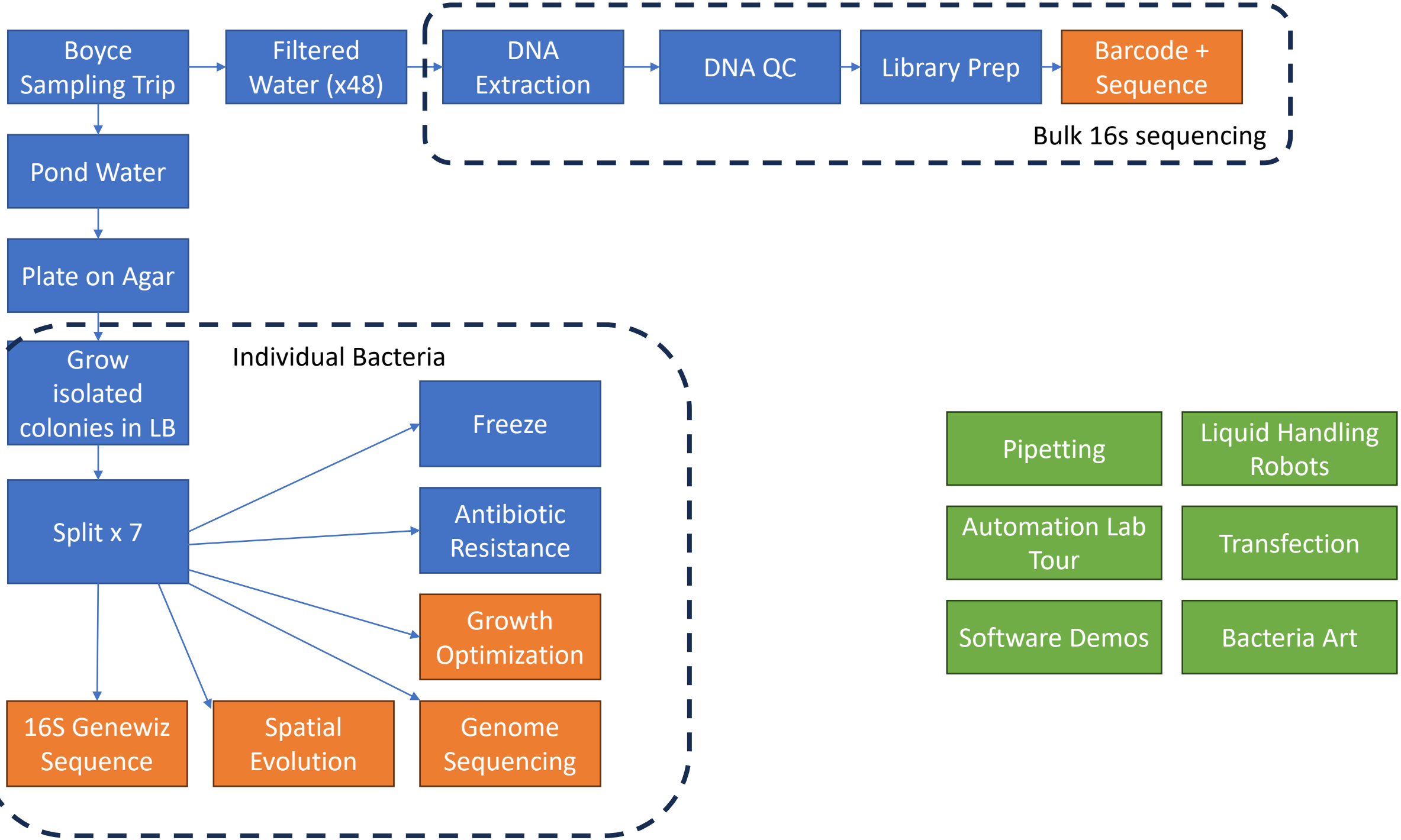
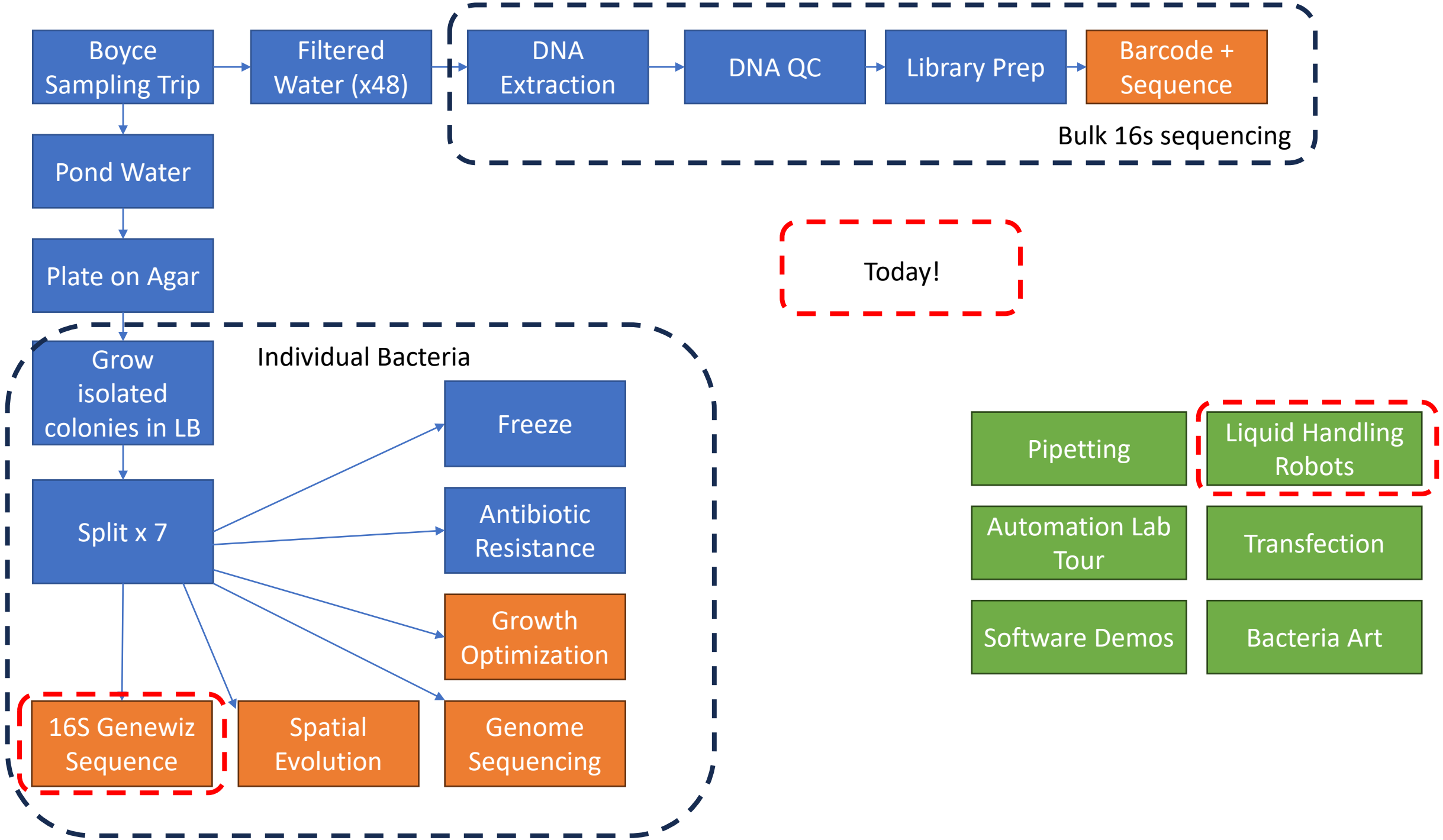
CompBio Pre-College Lab


Boyce Sampling Trip
Filtered Water (x48)
DNA Extraction
DNA QC
Library Prep
Barcode + Sequence
Bulk 16s sequencing
Pond Water
Plate on Agar
Individual Bacteria
Grow isolated colonies in LB
Split x 7
Freeze
Antibiotic Resistance
Growth Optimization
Genome Sequencing
Spatial Evolution
16S Genewiz Sequence
Pipetting
Liquid Handling Robots
Automation Lab Tour
Transfection
Software Demos
Bacteria Art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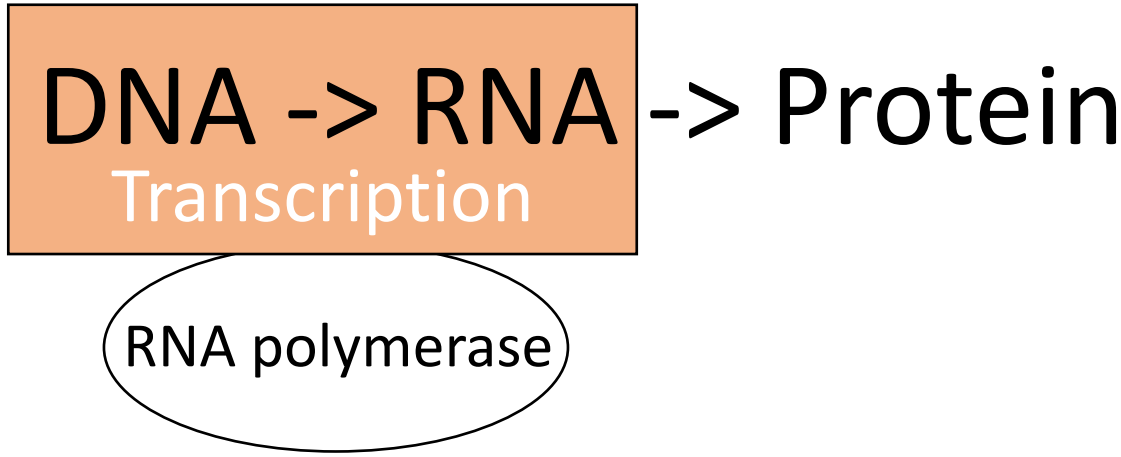
Molecular Biology - Central Dogma



article

Nature **227**, 561 - 563 (08 August 1970); doi:10.1038/227561a0

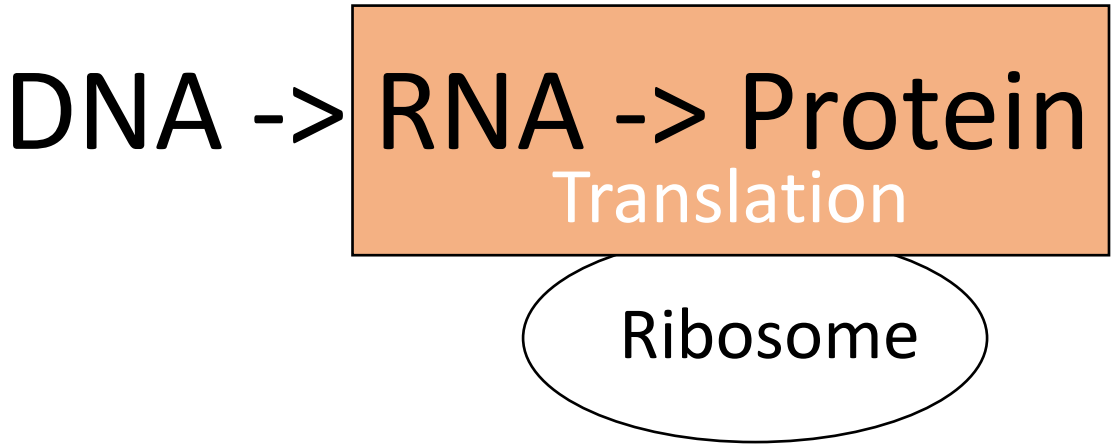
Central Dogma of Molecular Biology

FRANCIS CRICK

MRC Laboratory of Molecular Biology, Hills Road, Cambridge CB2 2QH

The central dogma of molecular biology deals with the detailed residue-by-residue transfer of sequential information. It states that such information cannot be transferred from protein to either protein or nucleic acid.

Molecular Biology - Central Dogma



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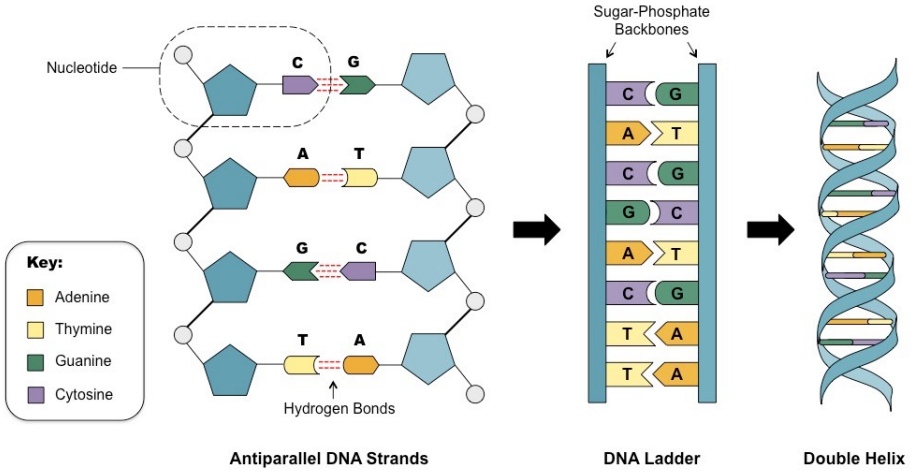
DNA Structure

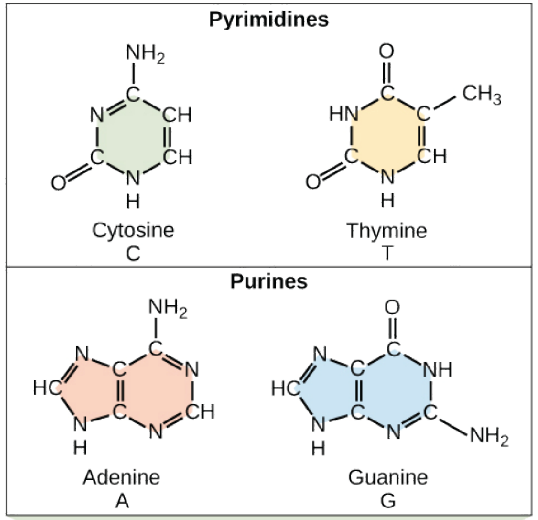
- Molecule carrying genetic information to allow a cell to generate proteins and function.
- Each strand is composed of nucleotides
- Nucleotides:

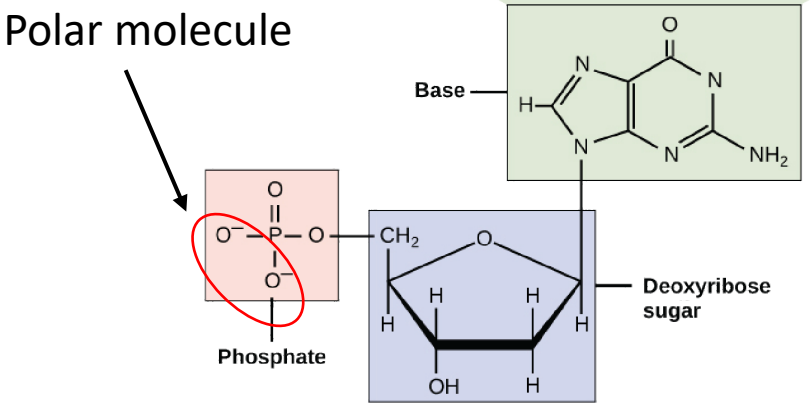
  nitrogen containing base (adenine, guanine, cytosine, thymine)
  (A,G,C,T)
  Deoxyribose
  Phosphate group
- Double Helix Structure
  - A <-> T
  - G <-> C

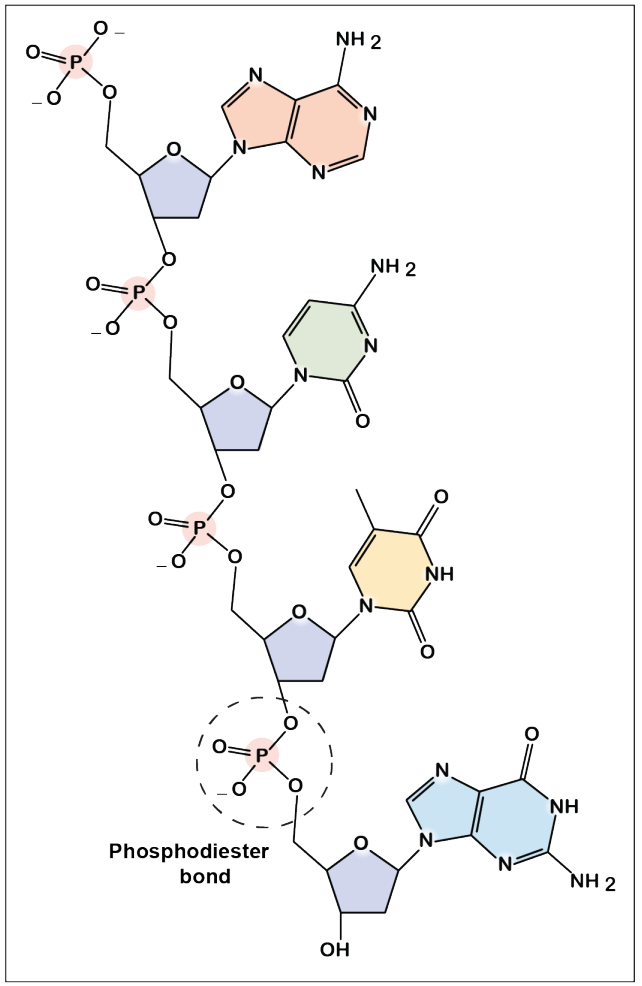


https://ib.bioninja.com.au/standard-level/topic-2-molecular-biology/26-structure-of-dna-and-rna/dna-structure.html

DNA structure





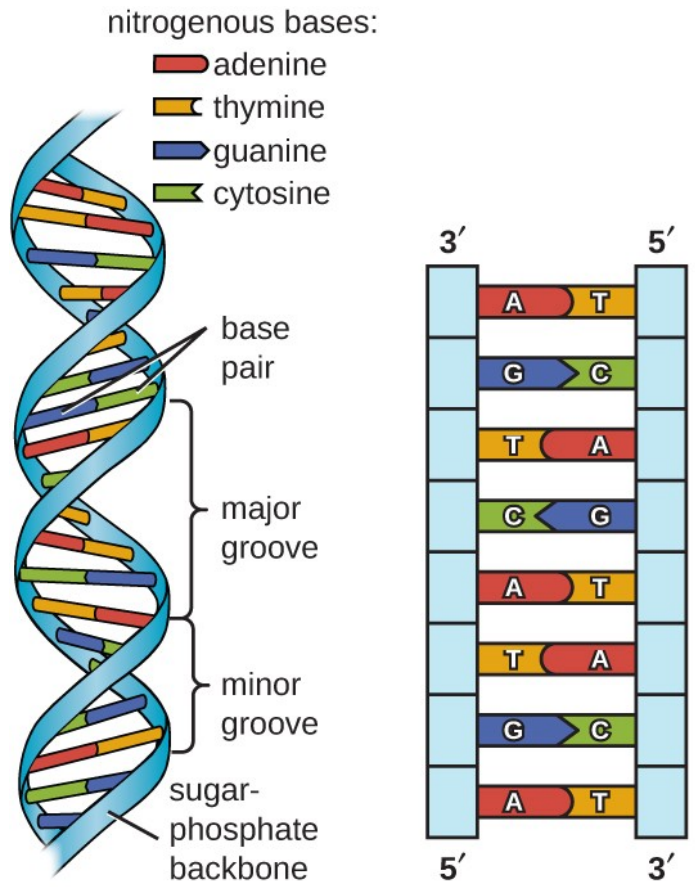


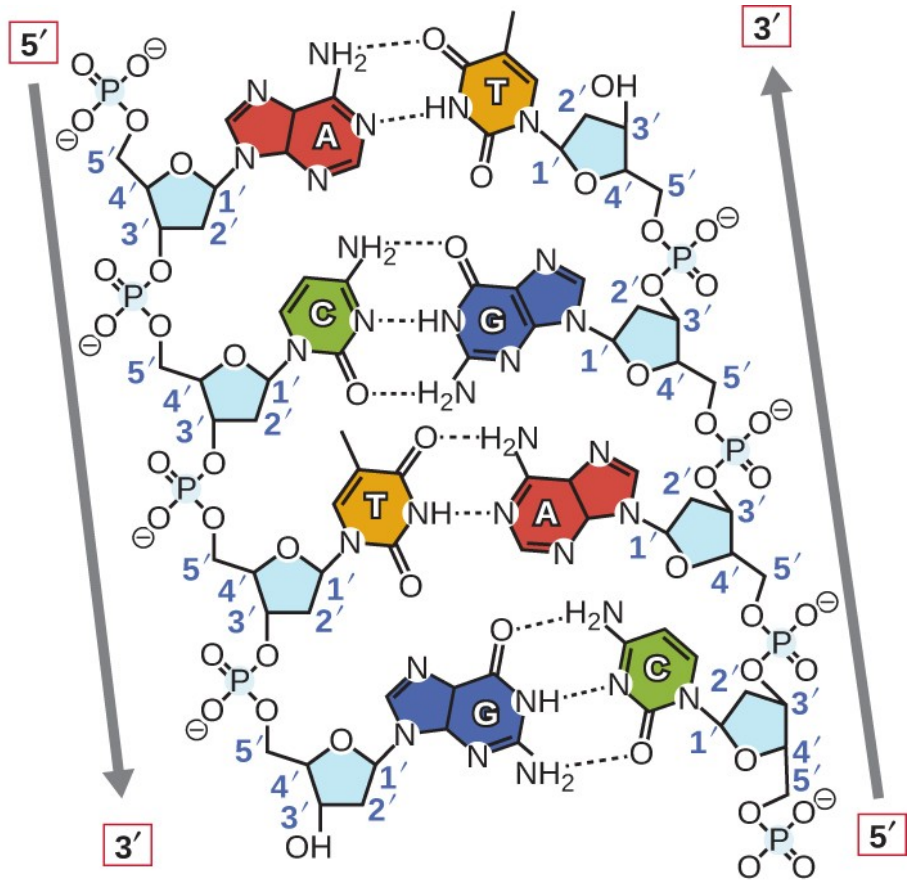
Dissolves in water (polar)

Precipitate in Ethanol (non-polar)

https://www.khanacademy.org/science/high-school-biology/hs-molecular-genetics/hs-discovery-and-structure-of-dna/a/discovery-of-the-structure-of-dna

DNA primary and secondary structures

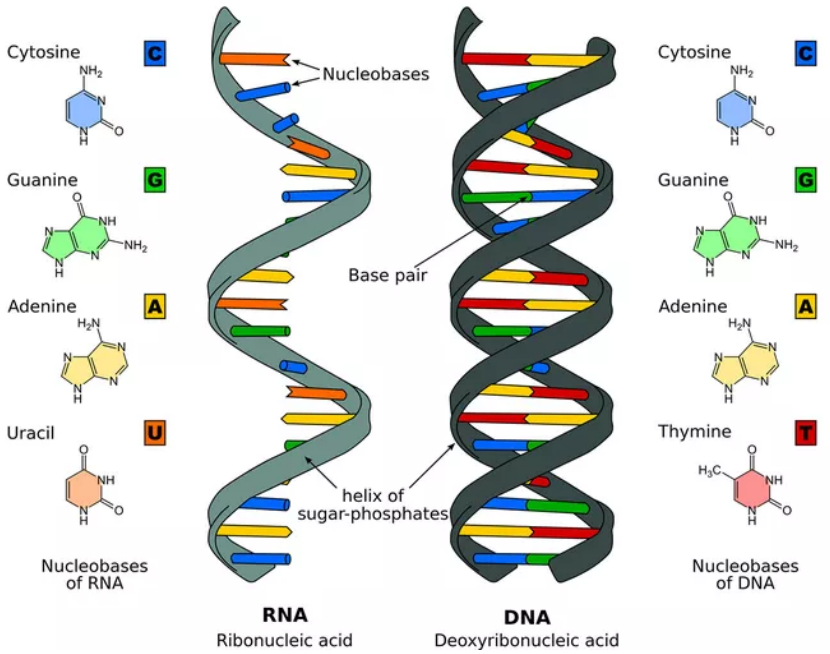




https://courses.lumenlearning.com/suny-microbiology/chapter/structure-and-function-of-dna/

RNA structure

Comparison	DNA	RNA
Name	DeoxyriboNucleic Acid	RiboNucleic Acid
Function	Long-term storage of genetic information; transmission of genetic information to make other cells and new organisms.	Used to transfer the genetic code from the nucleus to the ribosomes to make proteins. RNA is used to transmit genetic information in some organisms and may have been the molecule used to store genetic blueprints in primitive organisms.
Structural Features	B-form double helix.	A-form helix. shorter chains of nucleotides.
Composition of Bases and Sugars	deoxyribose sugar phosphate backbone adenine, guanine, cytosine, thymine bases	ribose sugar phosphate backbone adenine, guanine, cytosine, uracil bases
Propagation	self-replicating.	synthesized from DNA on an as-needed basis.
Base Pairing	A <->T G<->C	A <-> U G <-> C
Reactivity	The C-H bonds make it fairly stable, The small grooves in the helix also serve as protection.	The O-H bond in the ribose of RNA makes the molecule more reactive. The large grooves in the molecule make it susceptible to enzyme attack. Constantly produced, used, degraded, and recycled.
Ultraviolet Damage	susceptible to UV damage.	relatively resistant to UV damage.

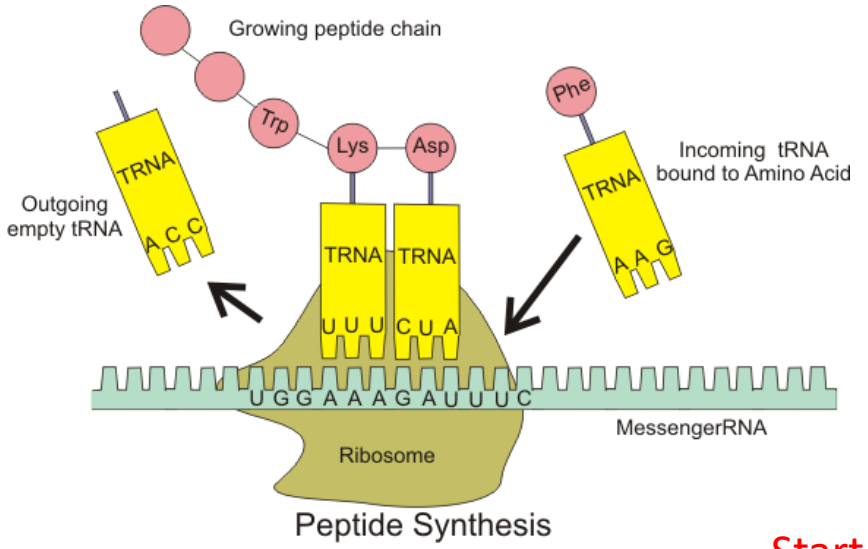


https://www.thoughtco.com/dna-versus-rna-608191

Types of RNA

- Messenger RNA (mRNA) – conveys genetic information that directs the synthesis of specific proteins.
- Transfer RNA (tRNA) – delivers amino acids to the ribosome.
- Ribosomal RNA (rRNA) – links amino acids together to create coded proteins.
- Micro RNA (miRNA) – small non-coding RNA that post transcriptionally regulate gene expression.

Protein synthesis - 64 different codons produce 20 different amino acids



Second Letter

1st letter	U	C	A	G	3rd letter
U	UUU Phe, UUC Phe, UUA Leu, UUG Leu	UCU Ser, UCC Ser, UCA Ser, UCG Ser	UAU Tyr, UAC Tyr, UAA Stop, UAG Stop	UGU Cys, UGC Cys, UGA Stop, UGG Trp	U C A G
C	CUU Leu, CUC Leu, CUA Leu, CUG Leu	CCU Pro, CCC Pro, CCA Pro, CCG Pro	CAU His, CAC His, CAA Gln, CAG Gln	CGU Arg, CGC Arg, CGA Arg, CGG Arg	U C A G
A	AUU Ile, AUC Ile, AUA Ile, AUG Met	ACU Thr, ACC Thr, ACA Thr, ACG Thr	AAU Asn, AAC Asn, AAA Lys, AAG Lys	AGU Ser, AGC Ser, AGA Arg, AGG Arg	U C A G
G	GUU Val, GUC Val, GUA Val, GUG Val	GCU Ala, GCC Ala, GCA Ala, GCG Ala	GAU Asp, GAC Asp, GAA Glu, GAG Glu	GGU Gly, GGC Gly, GGA Gly, GGG Gly	U C A G

Start codon

No amino acids

Stretch between AUG and stop codon called Open reading frame (ORF).

https://en.wikipedia.org/wiki/Transfer_RNA

http://biology.kenyon.edu/courses/biol114/Chap05/Chapter05.html

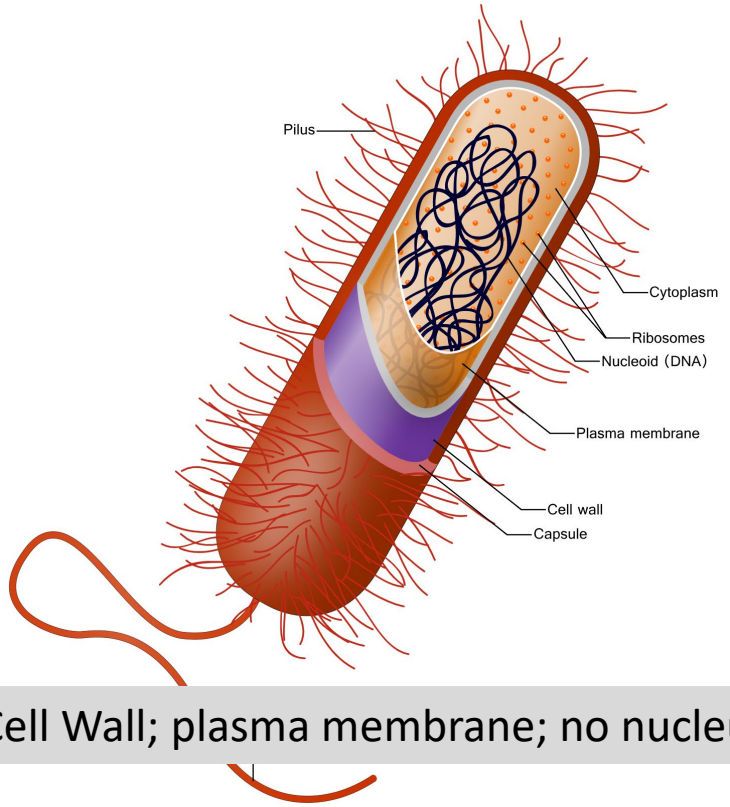
https://youtu.be/gG7uCskUOrA

Common Operations on DNA

1. Collect it (**DNA Extraction**)
2. Quantify total (**fluorescent tagging, light absorbance**)
3. Estimate size distribution (**gel electrophoresis**)
4. Make copies
5. Read it
6. Cut it
7. Connect it
8. Move it
9. Change it

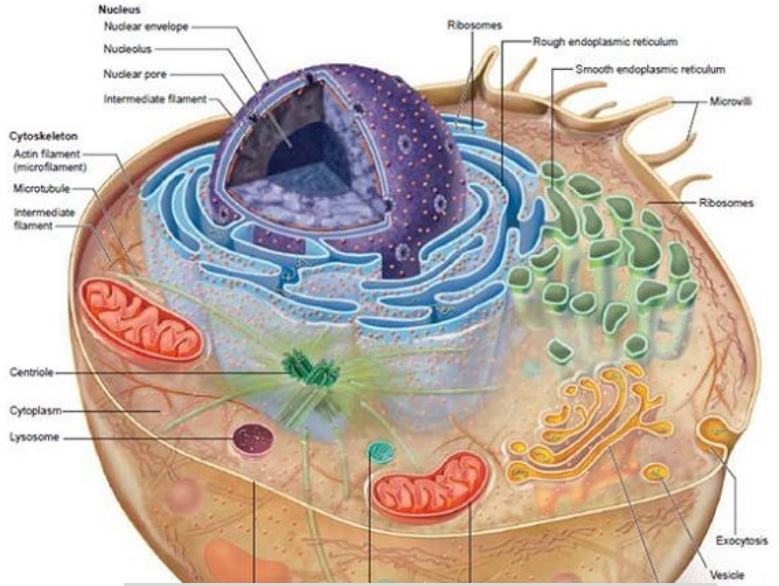
Cellular Organization affects how we extract DNA

Prokaryotes (bacteria)



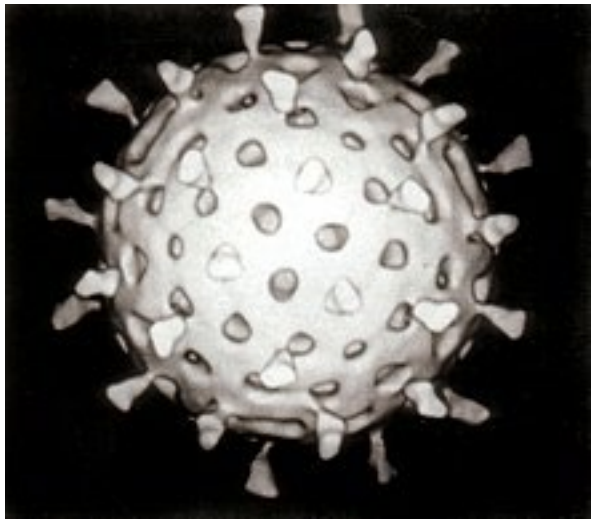
Cell Wall; plasma membrane; no nucleus

Eukaryotes (algae, plants, animals, etc.)



Cell Wall (in plants, algae only); plasma membrane; DNA in nucleus

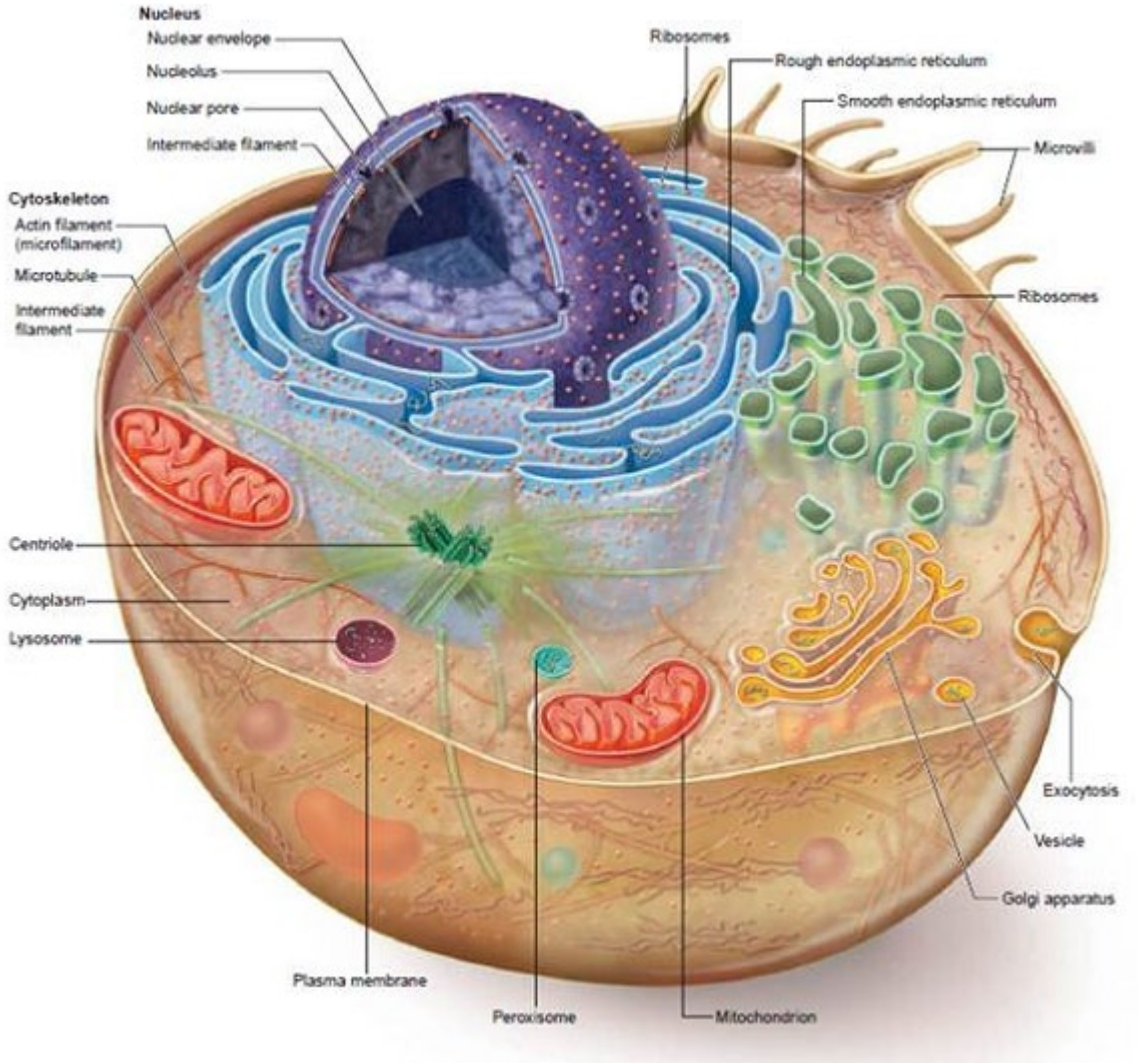
Viruses



Capsid; plasma membrane (sometimes); RNA or DNA

DNA Extraction

- Collect cells/tissue sample
- Lyse the cells
- Break down lipids
- Break down proteins
- Remove debris
- Assess quality of extraction



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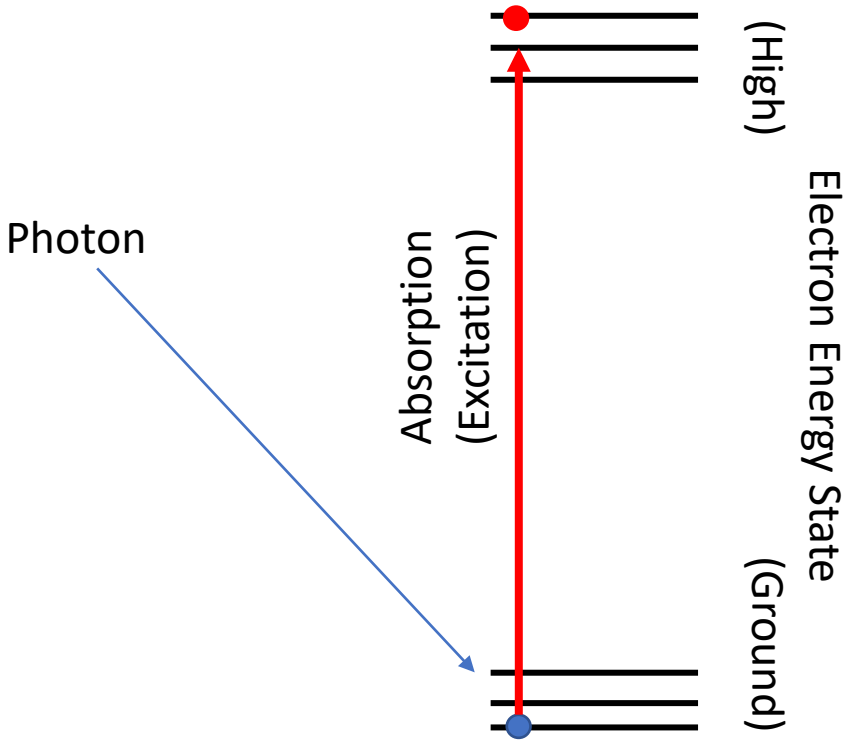
Fluorescence Spectroscopy

Generate signal by modifying and controlling wavelength of light reaching the sensor.

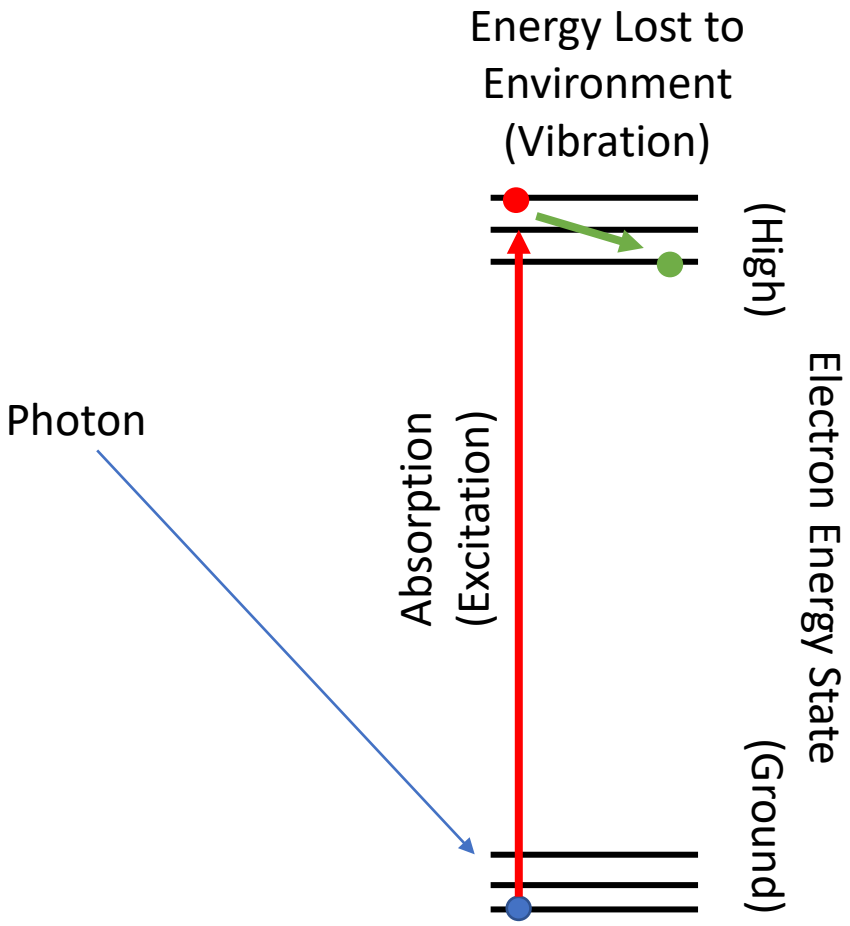
1. Attach fluorescent tag to protein/target
2. Excite tag using light
3. Measure emission from excited tag

Excitation and Emission (very important for modern biological research!)

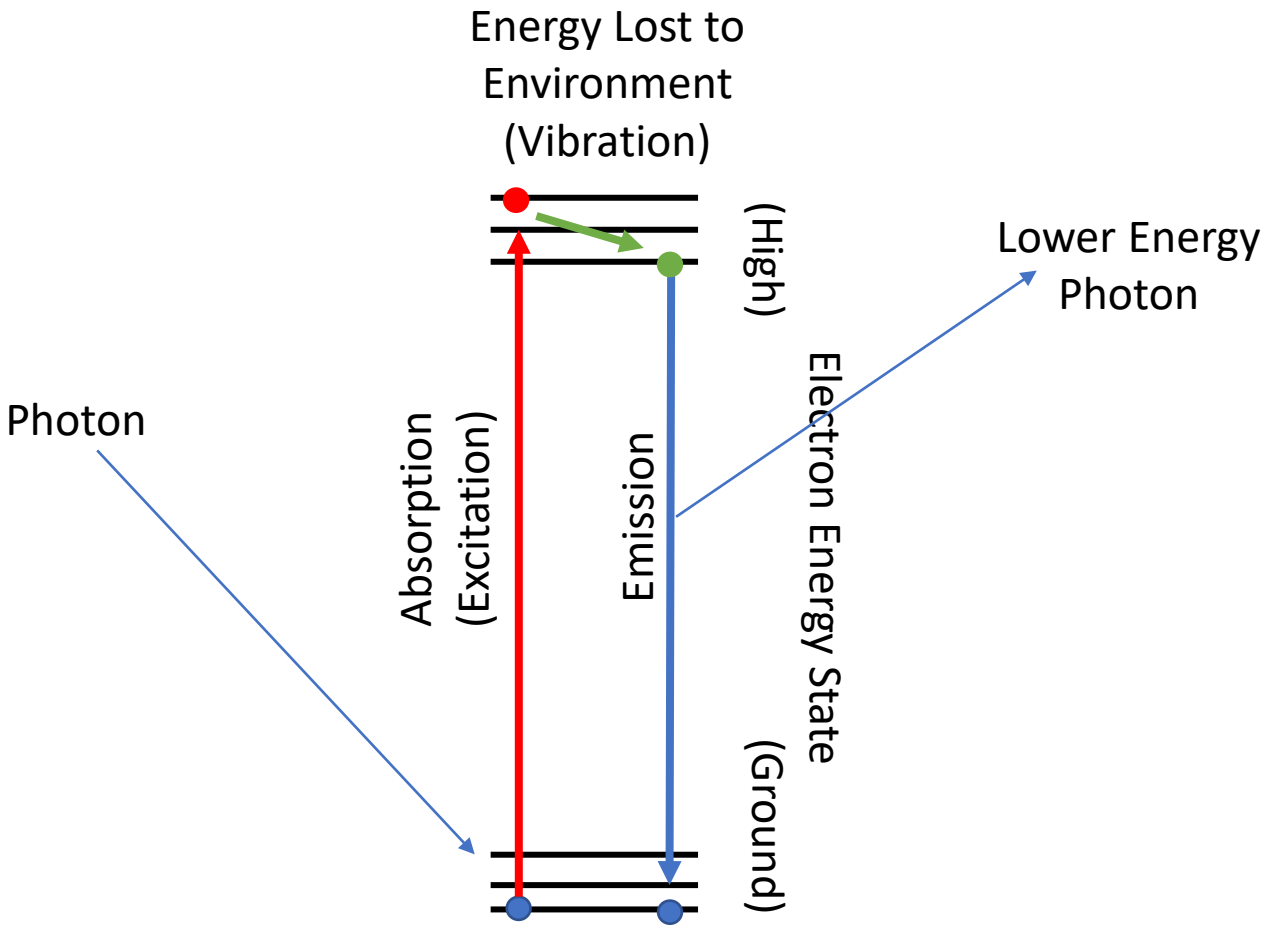
Excitation and Emission



Excitation and Emission



Excitation and Emission



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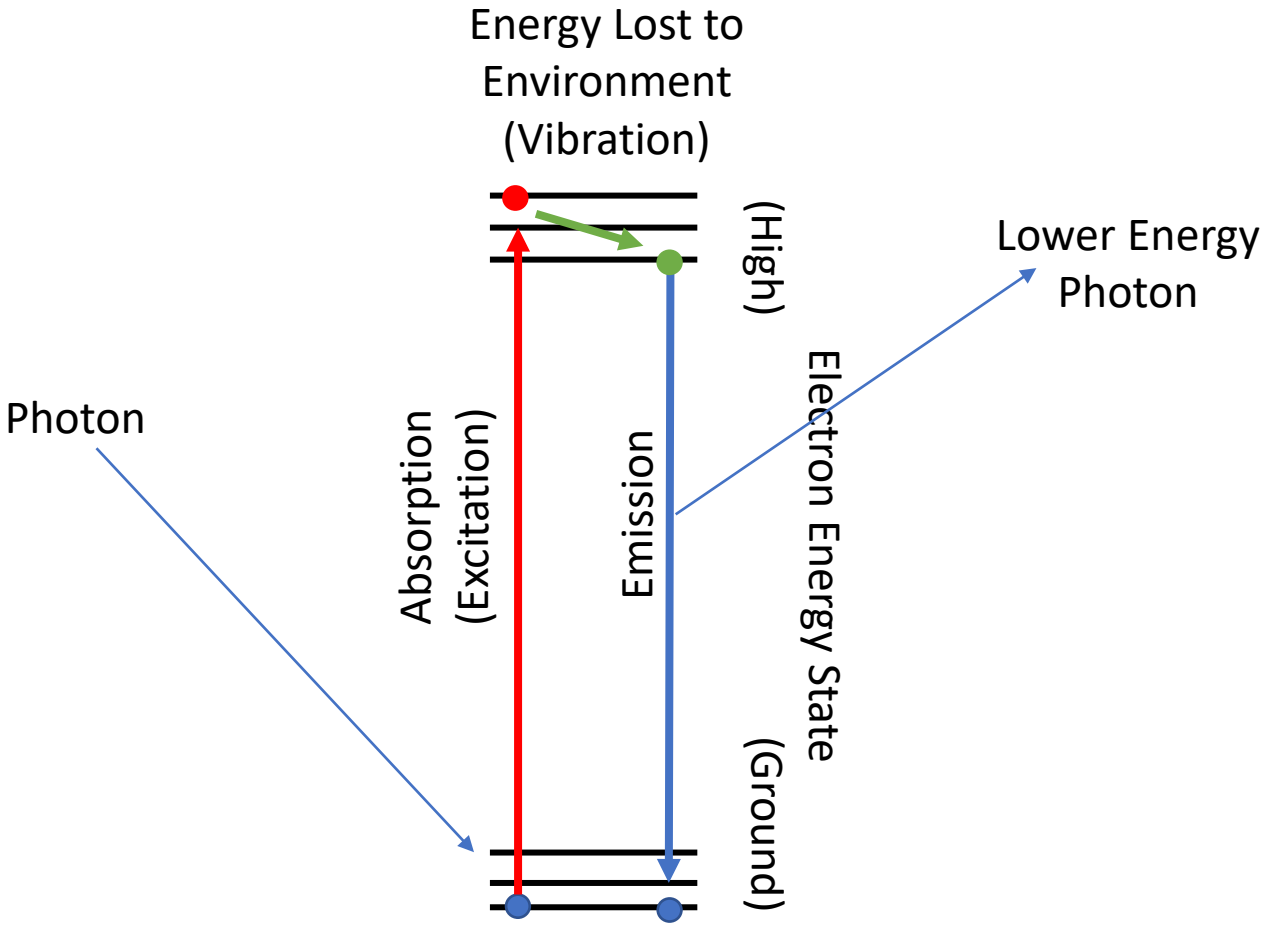

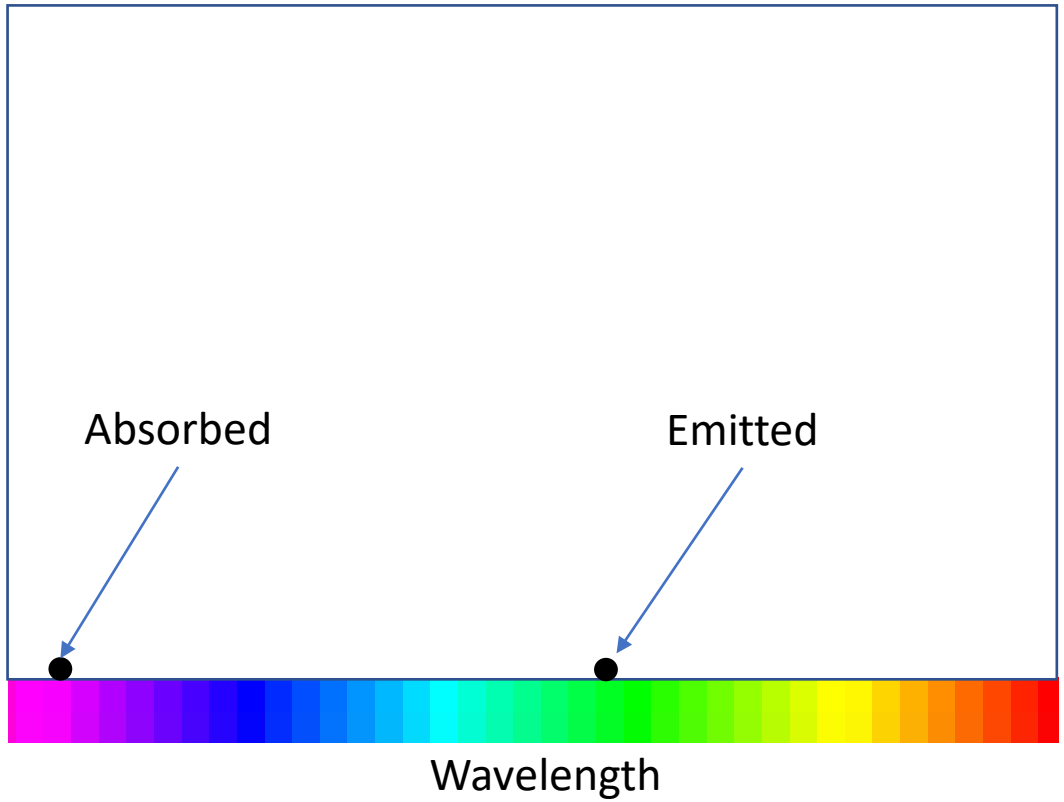

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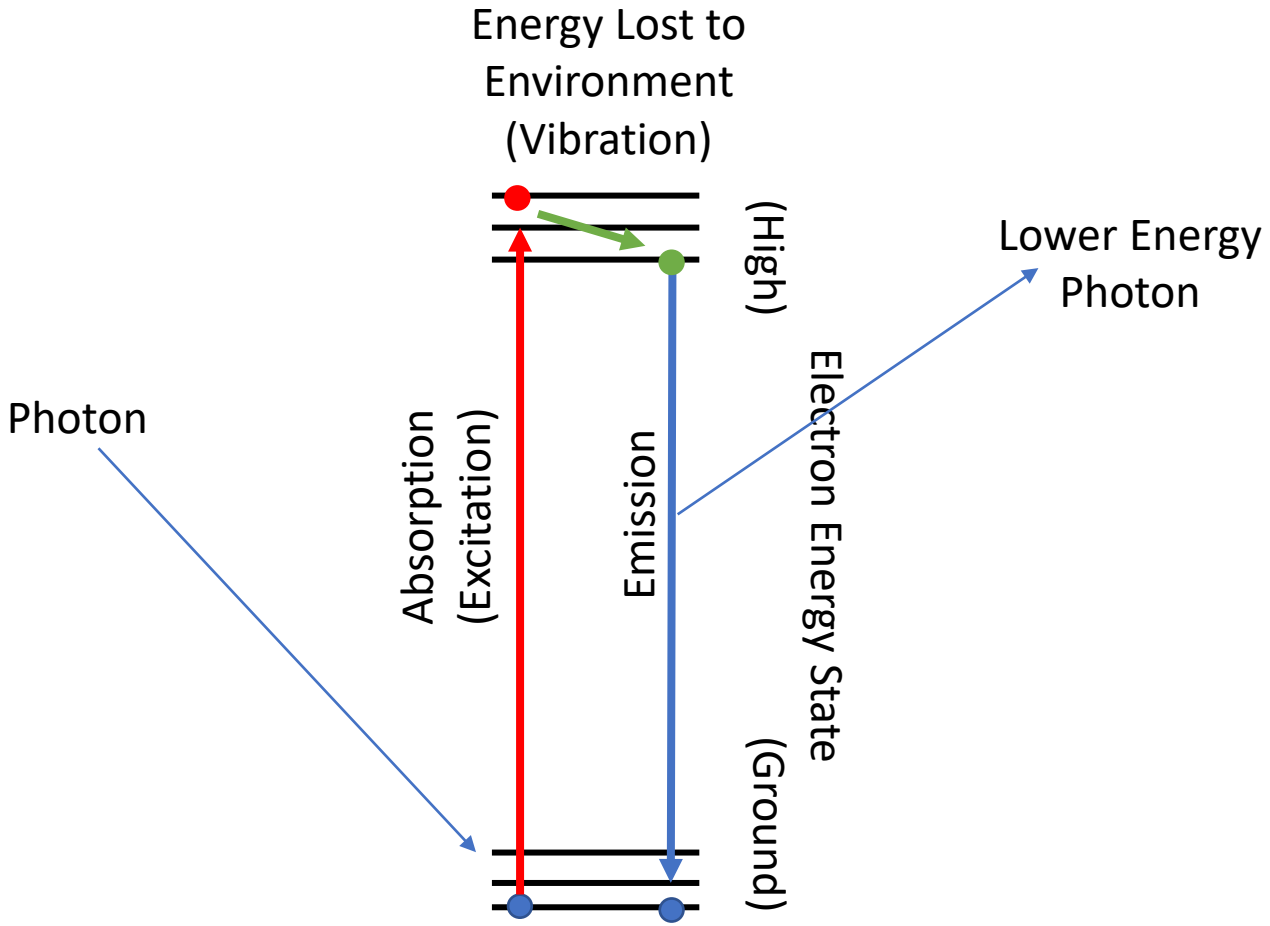
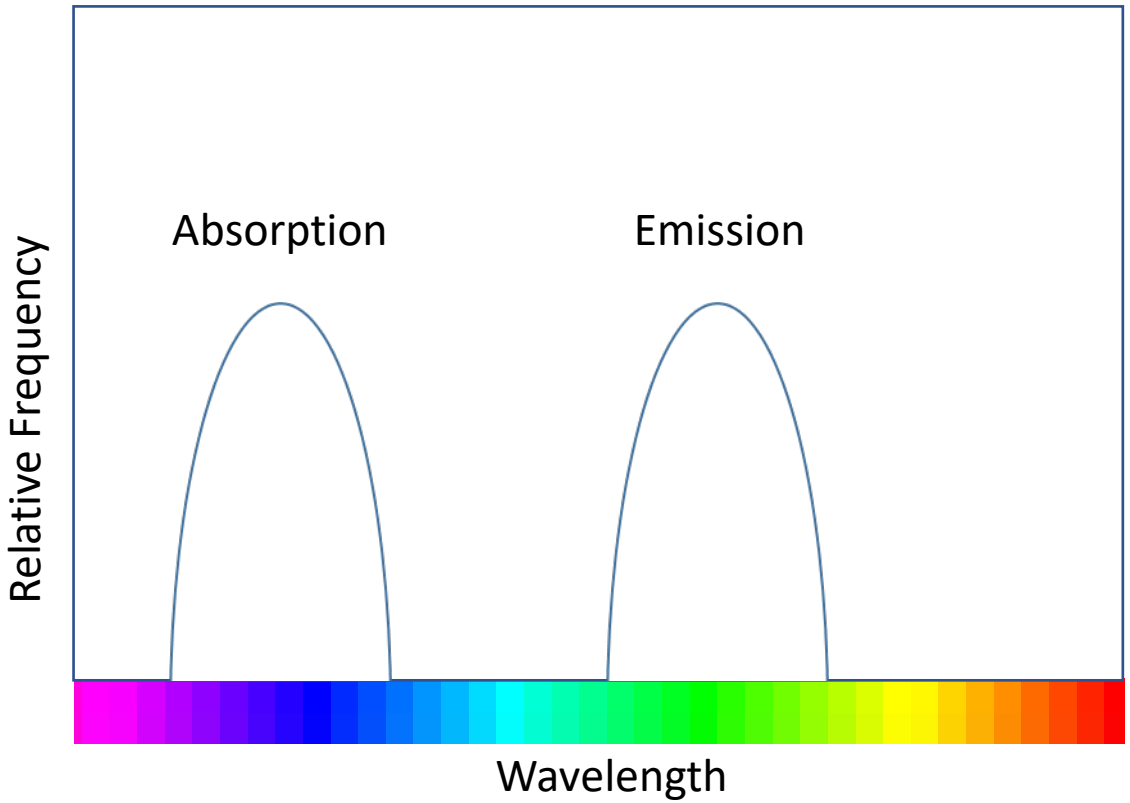
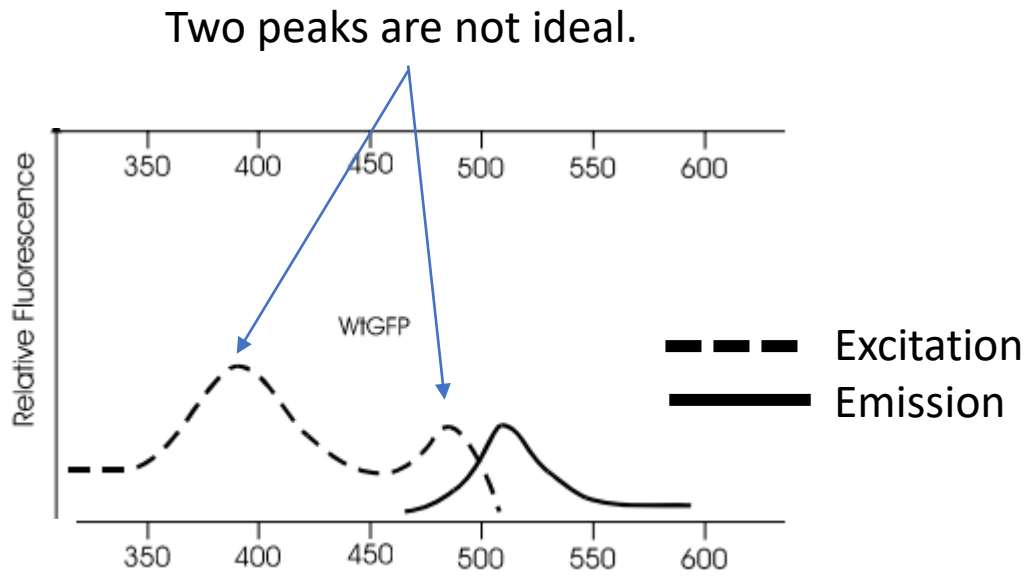
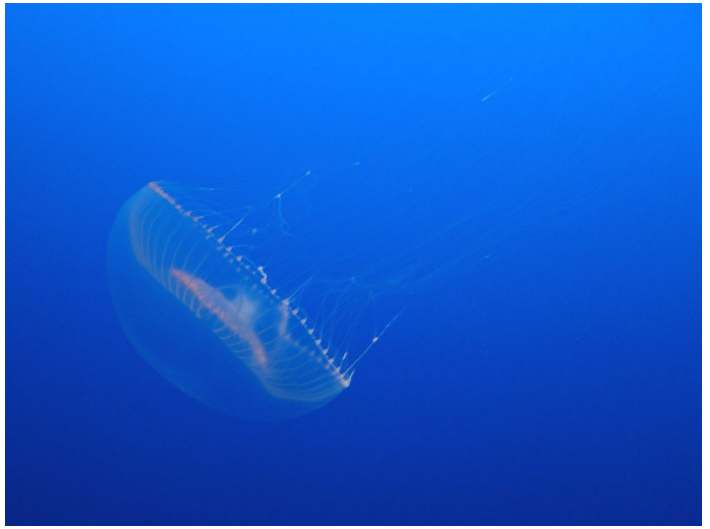




Green Fluorescent Protein

- Derived from *Aequorea Victoria*
- Required Ca^{2+} to fluoresce





By Mnolf (Photo taken in the Monterey Bay Aquarium, CA, USA) [GFDL (http://www.gnu.org/copyleft/fdl.html), CC-BY-SA-3.0 (http://creativecommons.org/licenses/by-sa/3.0/) or CC BY-SA 2.0 (http://creativecommons.org/licenses/by-sa/2.0)], via Wikimedia Commons

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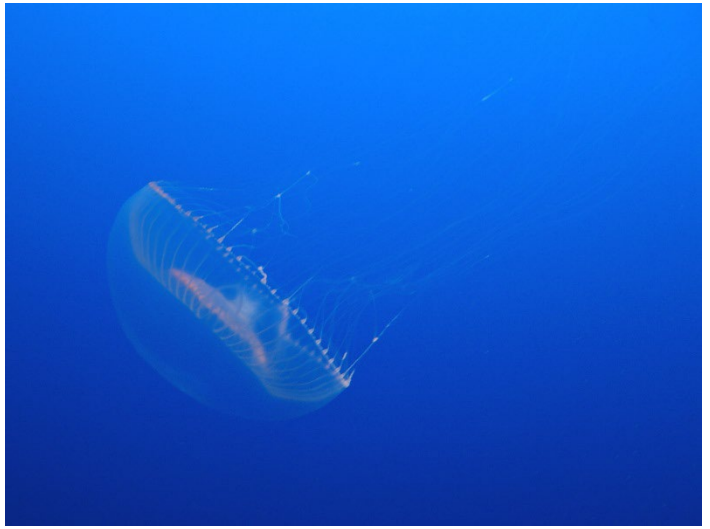
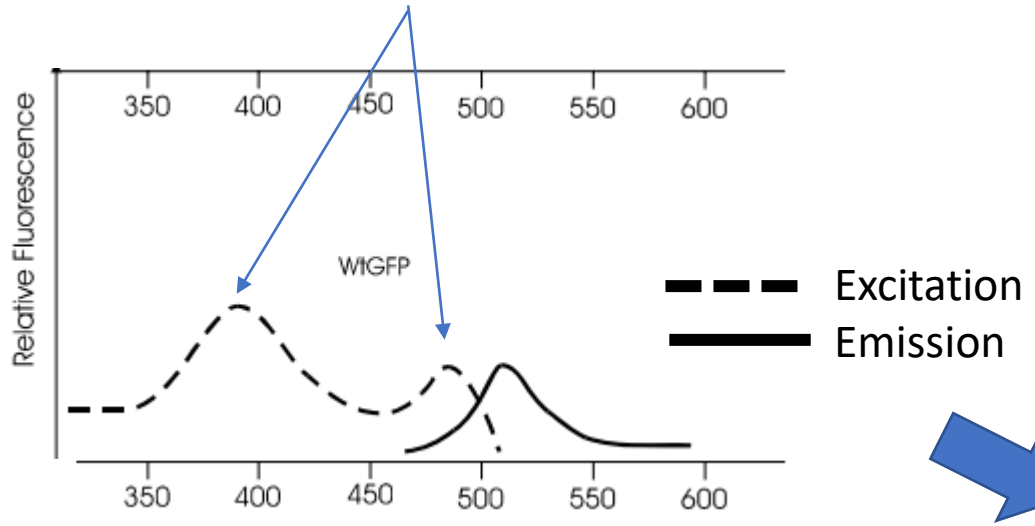
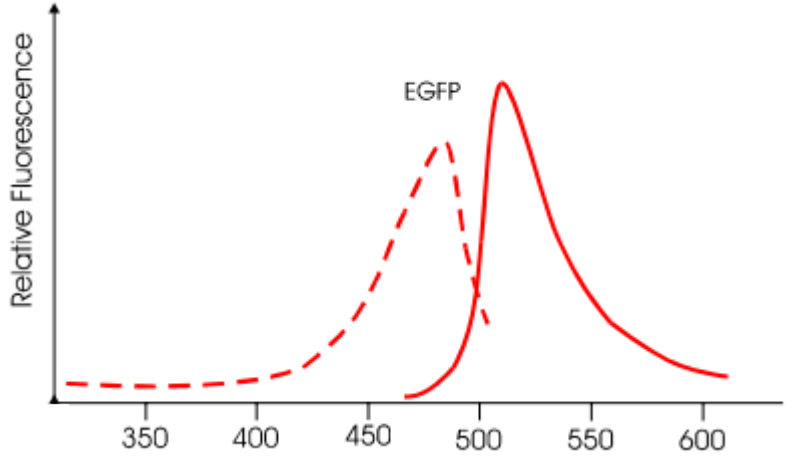


By Mnolf (Photo taken in the Monterey Bay Aquarium, CA, USA) [GFDL (http://www.gnu.org/copyleft/fdl.html), CC-BY-SA-3.0 (http://creativecommons.org/licenses/by-sa/3.0/) or CC BY-SA 2.0 (http://creativecommons.org/licenses/by-sa/2.0)], via Wikimedia Commons

Two peaks are not ideal.



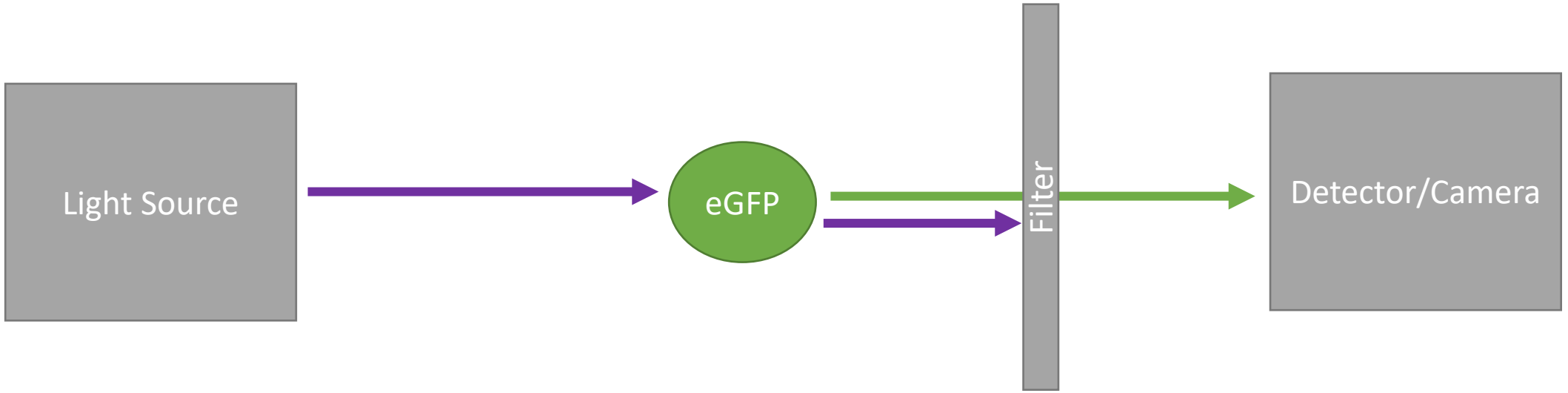
Nobel Prize in Chemistry – 2008



http://www.biotek.com/resources/docs/FL600_Excitation_and_Emission_of_Green_Fluorescent_Proteins_Tech_Note.pdf

Hardware Approaches

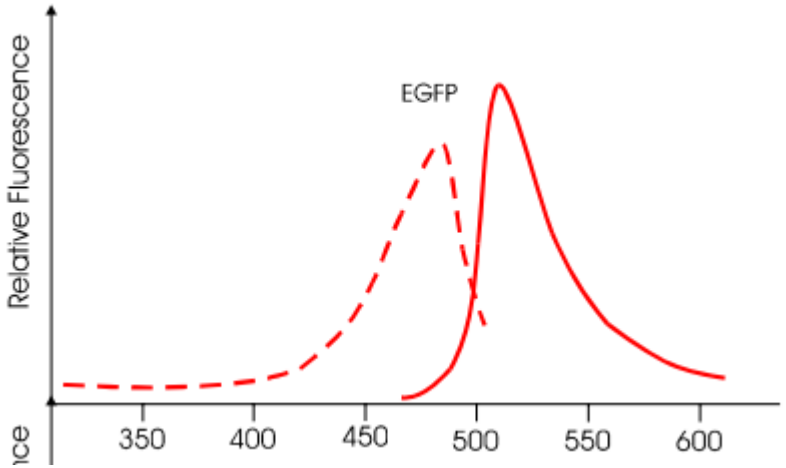
- Excite EGFP with UV light and detect emitted green light



Why is fluorescence a big deal?

Specificity!

- Location within cells
  - Modify genes to include fluorescent marker
  - Chemical control
- Fluorescent activation



This gives researchers a lot of flexibility to control fluorescence in their experiments.

Common Operations on DNA

1. Collect it (**DNA Extraction**)
2. Quantify total (**fluorescent tagging, light absorbance**)
3. Estimate size distribution (**gel electrophoresis**)
4. Make copies
5. Read it
6. Cut it
7. Connect it
8. Move it
9. Change it

Gel Electrophoresis

Overall Goal: sort substances (nucleic acid strands or proteins) based on size/charge

- Construct Gel
- Prepare samples
- Add experimental samples (as well as ladder and control) to gel
- Run current through gel
- Observe change in gel

Gel Electrophoresis – Step 1 – The Gel

- Goal: generate a mesh through which sample will flow slowly in an organized fashion
- Lanes – lanes are added using a comb to generate wells in the gel
- DNA/RNA:
  - Gel - Agarose gel 0.2-5% weight/ buffer volume – thermal set
  - Buffer – Tris/(Borate or Acetic acid)/EDTA – TBE or TAE
    - Prevent/reduces enzymatic activities that might degrade the DNA
  - Stain – Ethidium bromide – fluorescent DNA intercalator

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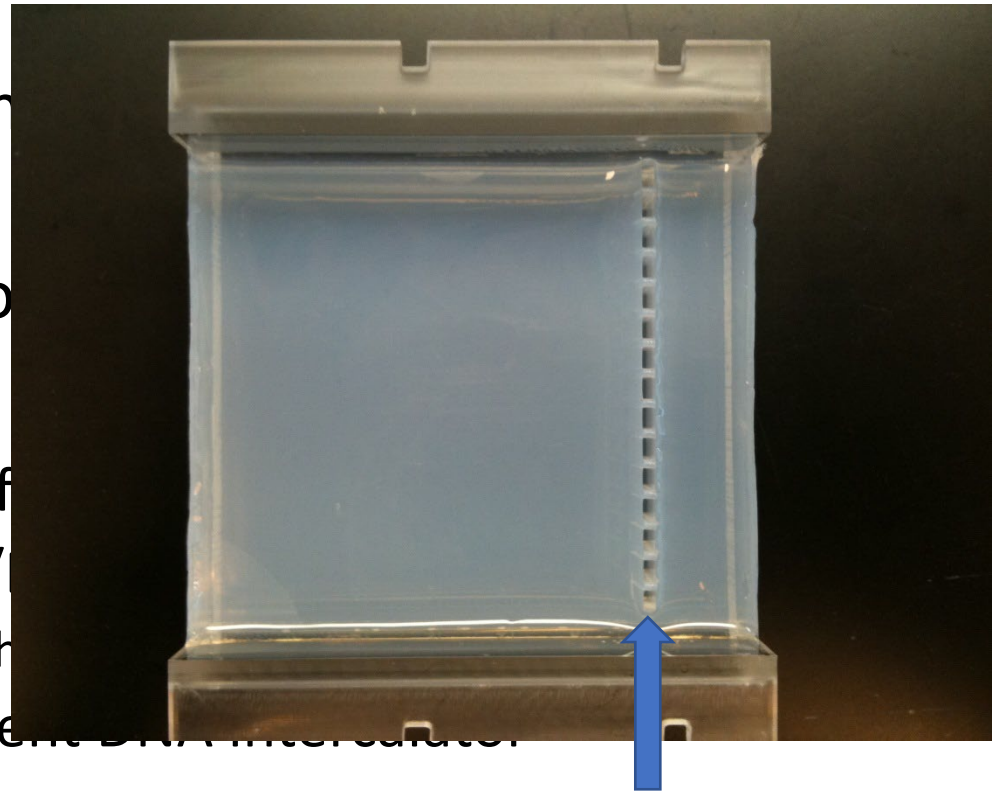


Gel Electrophoresis – Step 2 – Sample Prep

1. Denature DNA/RNA
2. Add loading dye, stain

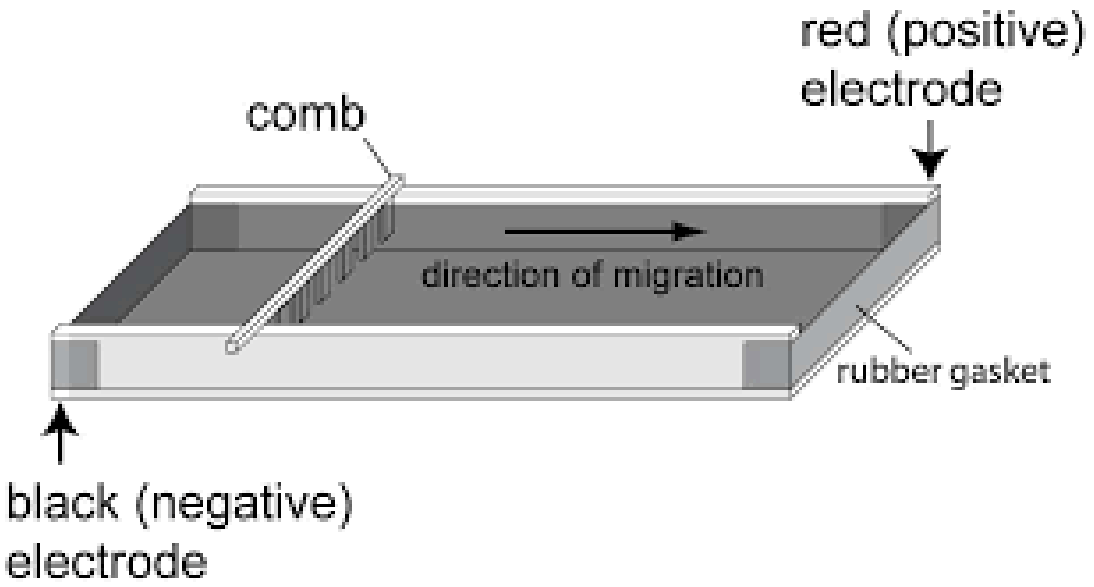
Gel Electrophoresis – Step 3 – Load Sample

5-20 uL total volume per sample

1. Ladder with known sizes (base pairs for DNA/RNA)
2. Controls
3. Experimental samples

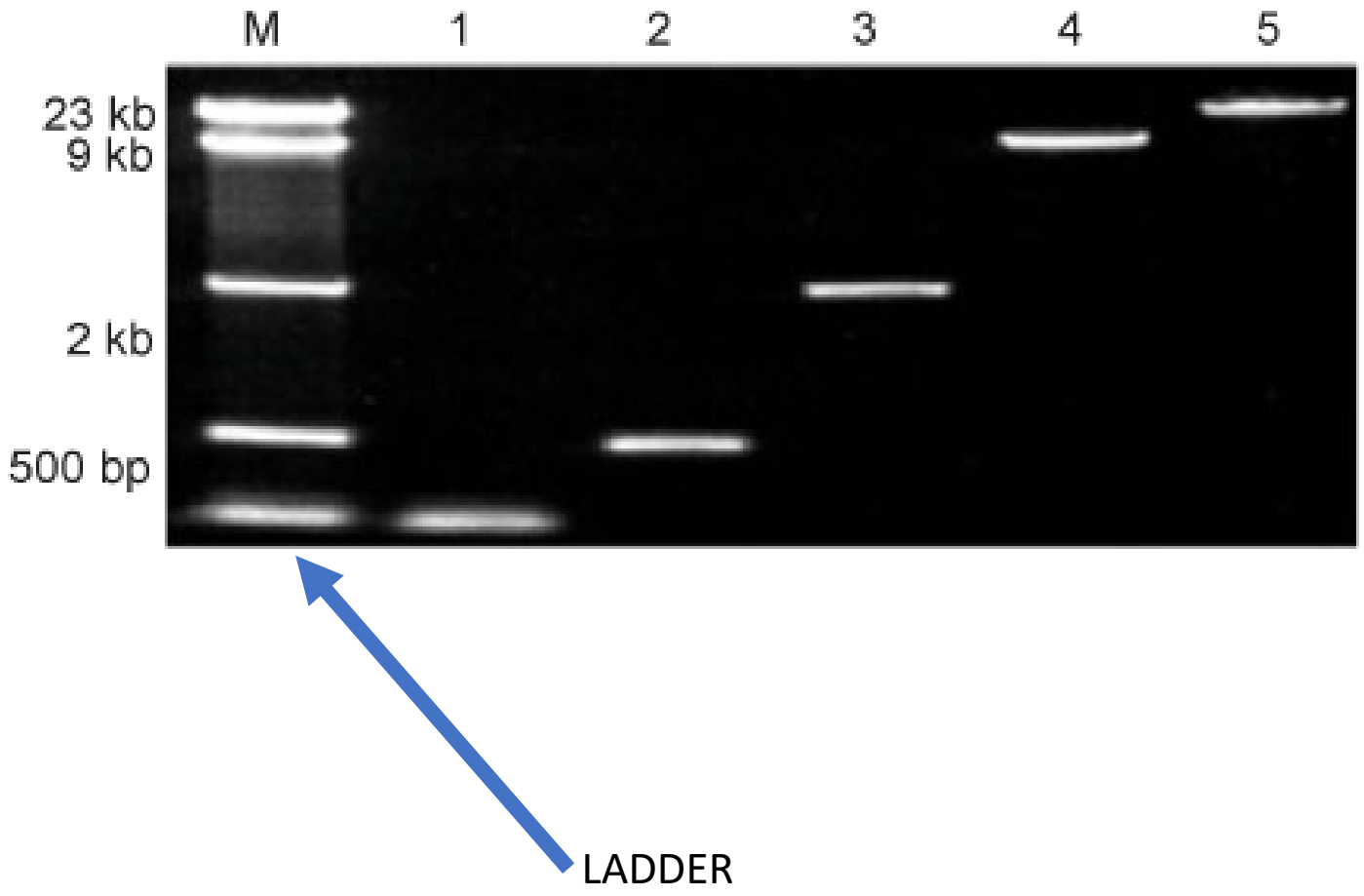
Gel Electrophoresis – Step 4 – Run the Gel

- Run current through the gel
- Negatively charged molecules flow from negative to positive



Agarose (DNA/RNA)

Gel Electrophoresis – Step 5 - Observe



Gel Electrophoresis - Uses

- Measure size of molecules
- Isolate molecules of specific size
- Quantify samples

Common Operations on DNA

1. Collect it (**DNA Extraction**)
2. Quantify total (**fluorescent tagging, light absorbance**)
3. Estimate size distribution (**gel electrophoresis**)
4. Make copies (**polymerase chain reaction**)
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DNA Notation (primary and secondary structure)

ssDNA = 5’-ACTGCGATAGACGATGTCCGGATGACA-3’ ← Shows sequence

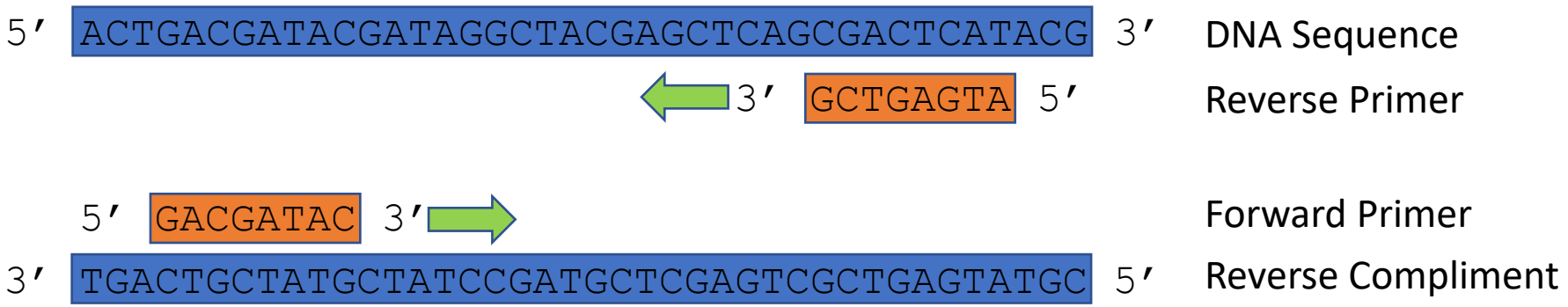
dsDNA = 5’-ACTGCGATAGACGATGTCCGGATGACA-3’
3’-TGACGCTATCTGCTACAGGCCTACTGT-5’ ← Shows sequence and pairing

dsDNA = 5’-  -3’
3’--5’ ← Shows pairing

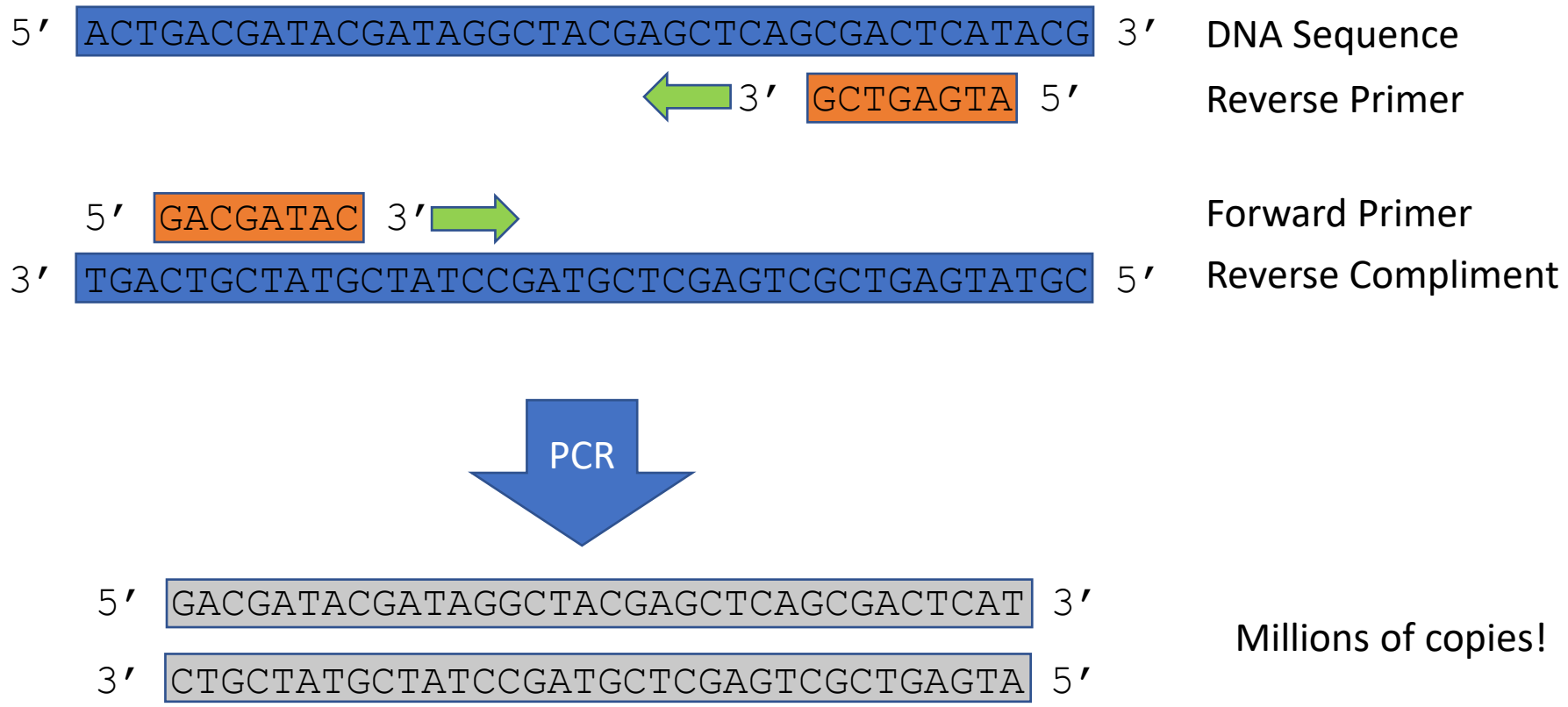
Polymerase Chain Reaction

- Purpose : generate many copies of small segments of DNA amounts of DNA
- Requirements:
  - Polymerase
  - Free nucleotides (dNTP)
  - DNA to be copied
  - Primers – very short segments of beginning/end of DNA to be copied
  - Thermocycler
- Polymerase – DNA repair/extension enzyme

Polymerase Chain Reaction



Polymerase Chain Reaction




95
72
53
°C
Cycle 1
www.dnalc.org

Common Operations on DNA

1. Collect it (**DNA Extraction**)
2. Quantify total (**fluorescent tagging, light absorbance**)
3. Estimate size distribution (**gel electrophoresis**)
4. Make copies (**polymerase chain reaction**)
5. Read it (**sequencing**)
6. Cut it (**restriction endonuclease enzymes**)
7. Connect it
8. Move it
9. Change it

Standard Polymerase Chain Reaction

- Purpose : generate many copies of small segments of DNA amounts of DNA
- Requirements:
  - Polymerase
  - Free nucleotides (dNTP)
  - DNA to be copied
  - Primers – very short segments of beginning/end of DNA to be copied
  - Thermocycler
- Polymerase – DNA repair/extension enzyme

Multiple Cycles Product: Millions+ copies of desired DNA

Polymerase Chain Reaction (for Sanger Sequencing)

- Purpose : generate many copies of small segments of DNA amounts of DNA
- Requirements:
  - Polymerase
  - Free nucleotides (dNTP) **+ fluorescent terminating nucleotides**
  - DNA to be copied
  - ~~Primers – very short segments of beginning/end of DNA to be copied~~
  - Single primer at beginning of region to be sequenced
  - Thermocycler
- Polymerase – DNA repair/extension enzyme

One cycle product: Millions+ copies of **truncated** desired DNA

Sanger Sequencing

1. Amplify region of interest using standard PCR
2. Purify product (remove primers and undesired PCR product)
3. Add one primer for side of strand you wish to sequence
4. Replace ***small*** fraction of dNTPs with terminating dNTPs

   Terminating dNTPs: dNTPs which stop the polymerase reaction

   Each terminating nucleotide (G,A,T,C) may be uniquely fluorescently tagged
5. Run one cycle of PCR

   Note: If fluorescent, only one reaction is needed. If not, four reactions are required (one each for G,A,T,C)
6. Run reaction(s) in gel

Sanger Sequencing

Terminating nucleotides terminate polymerase strand extension

1. Amplify region of interest using standard PCR
2. Purify product (remove primers and undesired PCR product)
3. Add one primer for side of strand you wish to sequence
4. Replace ***small*** fraction of dNTPs with terminating dNTPs

   Terminating dNTPs: dNTPs which stop the polymerase reaction

   Each terminating nucleotide (G,A,T,C) may be uniquely fluorescently tagged
5. Run one cycle of PCR

   Note: If fluorescent, only one reaction is needed. If not, four reactions are required (one each for G,A,T,C)
6. Run reaction(s) in gel

Terminating Nucleotides Examples

- Nucleotides which terminate polymerase strand extension

ACTGTCTGACATGTGCGCGT

ACTG 

TGACAGACTGTACACGCGCA

	A	C	T	G
Normal	100%	100%	100%	100%
Terminating	0%	0%	0%	0%

Product(s)?

Terminating Nucleotides

- Nucleotides which terminate polymerase strand extension

ACTGTCTGACATGTGCGCGT

ACTG 

TGACAGACTGTACACGCGCA

	A	C	T	G
Normal	100%	100%	100%	100%
Terminating	0%	0%	0%	0%

Product(s)? ACTGTCTGACATGTGCGCGT

Terminating Nucleotides

- Nucleotides which terminate polymerase strand extension

ACTGTCTGACATGTGCGCGT

ACTG 

TGACAGACTGTACACGCGCA

	A	C	T	G
Normal	0%	100%	100%	100%
Terminating	100%	0%	0%	0%

Product(s)?

Terminating Nucleotides

- Nucleotides which terminate polymerase strand extension

ACTGTCTGACATGTGCGCGT

ACTG 

TGACAGACTGTACACGCGCA

	A	C	T	G
Normal	0%	100%	100%	100%
Terminating	100%	0%	0%	0%

Product(s)? ACTGTCTGA

Terminating Nucleotides

- Nucleotides which terminate polymerase strand extension

ACTGTCTGACATGTGCGCGT

ACTG ➡

TGACAGACTGTACACGCGCA

	A	C	T	G
Normal	50%	100%	100%	100%
Terminating	50%	0%	0%	0%

Product(s)?

Terminating Nucleotides

- Nucleotides which terminate polymerase strand extension

ACTGTCTGACATGTGCGCGT

ACTG 

TGACAGACTGTACACGCGCA

	A	C	T	G
Normal	50%	100%	100%	100%
Terminating	50%	0%	0%	0%

Product(s)? ACTGTCTGA; ACTGTCTGACA; ACTGTCTGACATGTGCGCGT

Multiple Reactions

ACTGTCTGACATGTGCGCGT

	A	C	T	G
Normal	50%	100%	100%	100%
Terminating	50%	0%	0%	0%
Normal	100%	50%	100%	100%
Terminating	0%	50%	0%	0%
Normal	100%	100%	50%	100%
Terminating	0%	0%	50%	0%
Normal	100%	100%	100%	50%
Terminating	0%	0%	0%	50%

Products

ACTGTCTGA
ACTGTCTGACA

ACTGTC
ACTGTCTGAC
ACTGTCTGACATGTGC
ACTGTCTGACATGTGCGC

ACTGT
ACTGTCT
ACTGTCTGACAT
ACTGTCTGACATGT

ACTGTCTG
ACTGTCTGACATG
ACTGTCTGACATGTG
ACTGTCTGACATGTGCG
ACTGTCTGACATGTGCGCG

Multiple Reactions

ACTGTCTGACATGTGCGCGT

	A	C	T	G
Normal	50%	100%	100%	100%
Terminating	50%	0%	0%	0%
Normal	100%	50%	100%	100%
Terminating	0%	50%	0%	0%
Normal	100%	100%	50%	100%
Terminating	0%	0%	50%	0%
Normal	100%	100%	100%	50%
Terminating	0%	0%	0%	50%

Products

ACTGTCTGA
ACTGTCTGACA

ACTGTC
ACTGTCTGAC
ACTGTCTGACATGTGC
ACTGTCTGACATGTGCGC

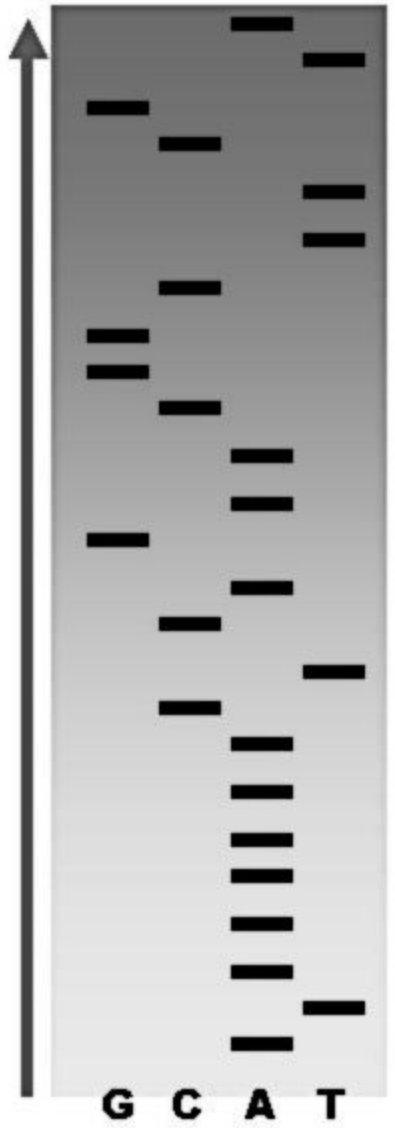
ACTGT
ACTGTCT
ACTGTCTGAC
ACTGTCTGAC

ACTGTCTG
ACTGTCTGACATG
ACTGTCTGACATGTG
ACTGTCTGACATGTGCG
ACTGTCTGACATGTGCGCG

What is the relative frequency of products ending in G?

Sanger Sequencing Gel

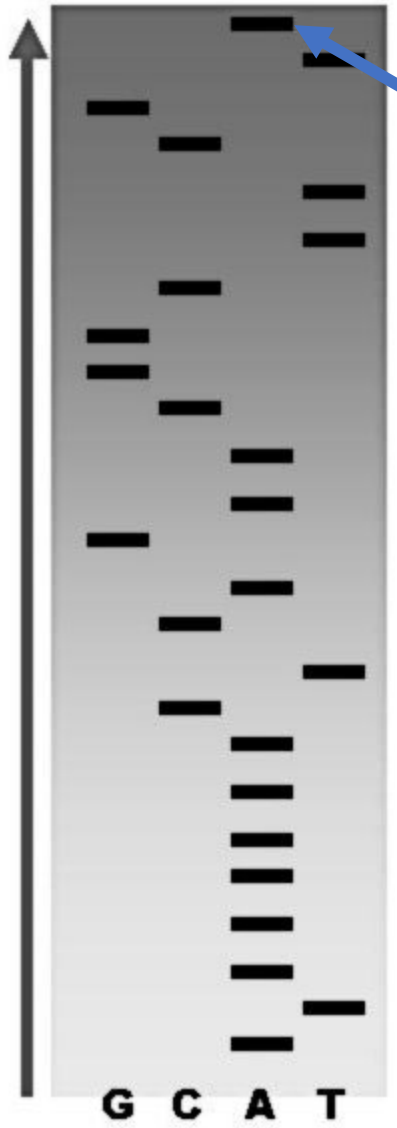
https://commons.wikimedia.org/wiki/File:Radioactive_Fluorescent_Seq.jpg



Where are the smaller PCR products?

Sanger Sequencing Gel

https://commons.wikimedia.org/wiki/File:Radioactive_Fluorescent_Seq.jpg

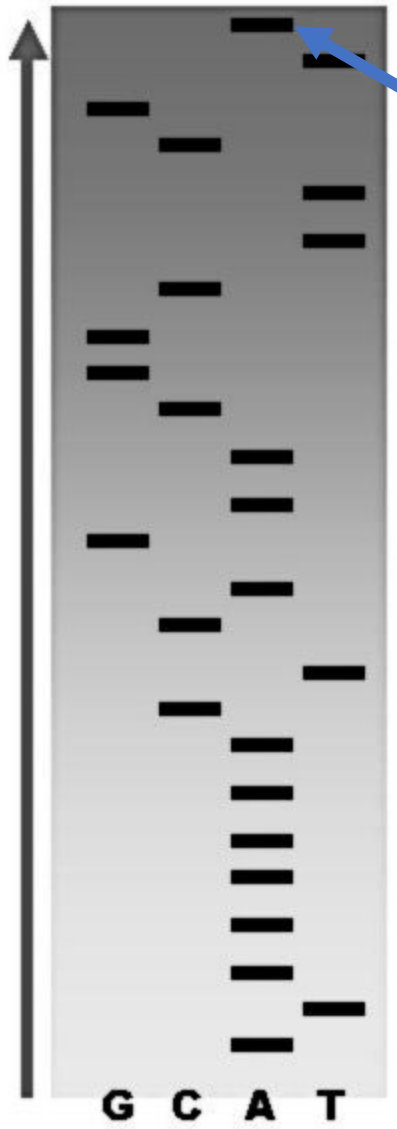


Where are the smaller PCR products?

What is the sequence of product here?

Sanger Sequencing Gel

https://commons.wikimedia.org/wiki/File:Radioactive_Fluorescent_Seq.jpg

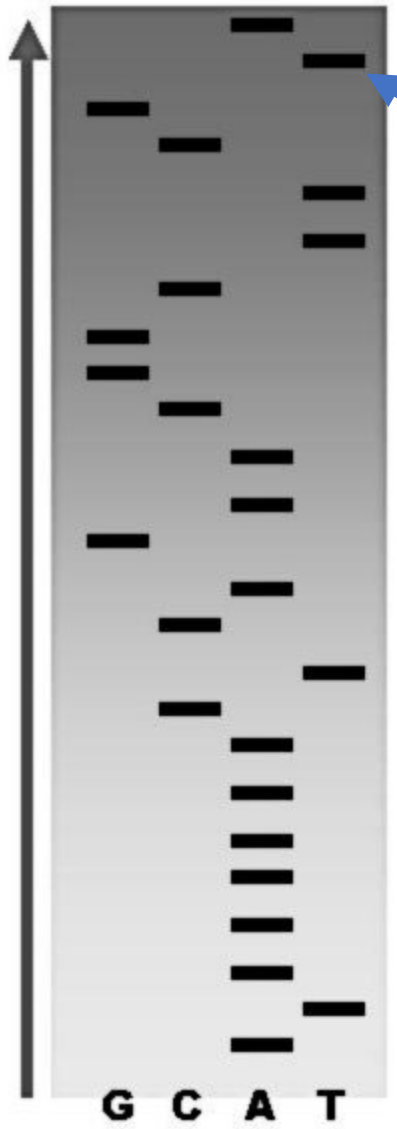


Where are the smaller PCR products?

What is the sequence of product here? [Primer] + A

Sanger Sequencing Gel

https://commons.wikimedia.org/wiki/File:Radioactive_Fluorescent_Seq.jpg

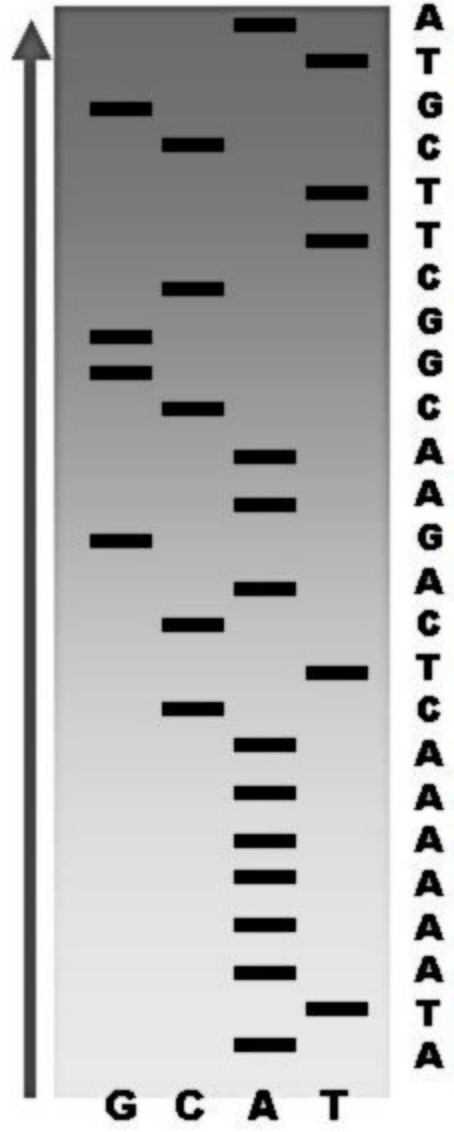


Where are the smaller PCR products?

What is the sequence of product here? [Primer] + AT

Sanger Sequencing Gel

https://commons.wikimedia.org/wiki/File:Radioactive_Fluorescent_Seq.jpg



Single Reaction with Fluorescent Terminating Nucleotides

ACTGTCTGACATGTGCGCGT

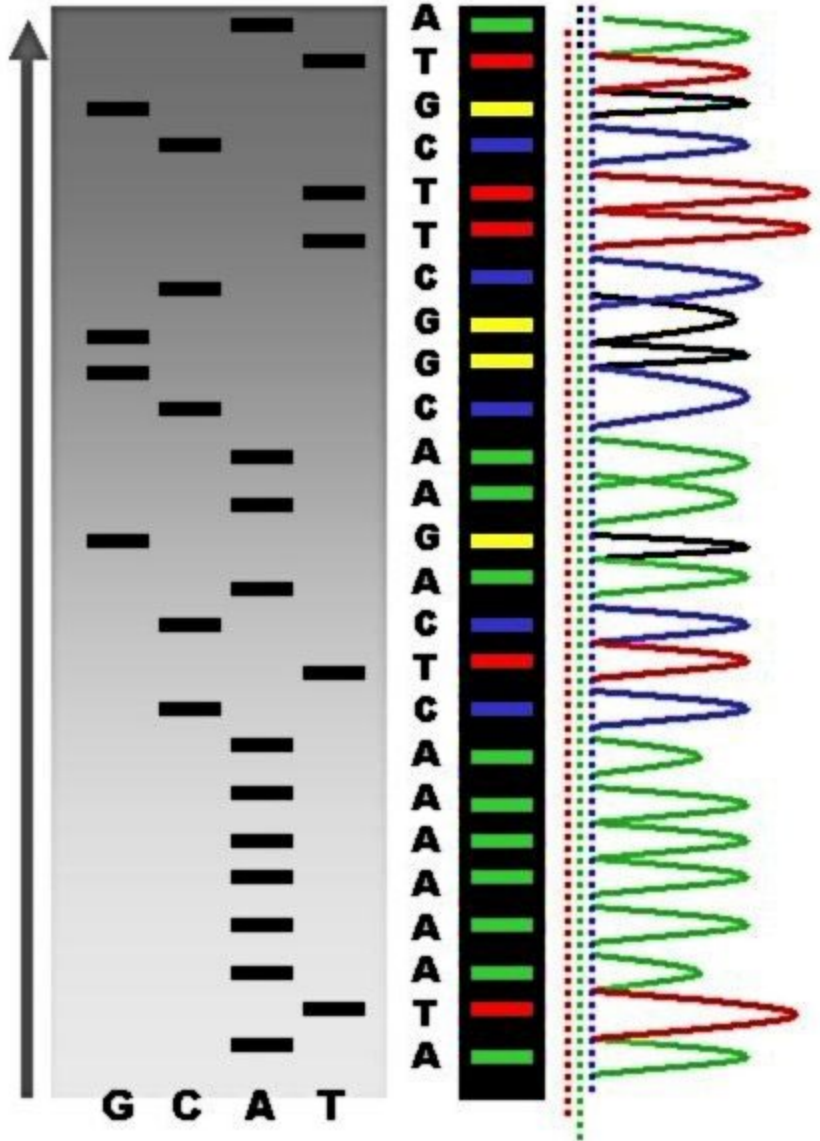
	A	C	T	G
Normal	50%	50%	50%	50%
Terminating	50%	50%	50%	50%

Products

ACTG**T**
ACTGT**C**
ACTGTC**T**
ACTGTCT**G**
ACTGTCTG**A**
ACTGTCTGA**C**
ACTGTCTGAC**A**
ACTGTCTGACA**T**
ACTGTCTGACAT**G**
ACTGTCTGACATG**T**
ACTGTCTGACATGT**G**
ACTGTCTGACATGTG**C**
ACTGTCTGACATGTGC**G**
ACTGTCTGACATGTGCG**C**
ACTGTCTGACATGTGCGC**G**
ACTGTCTGACATGTGCGCG**T**

Sanger Sequencing Gel

https://commons.wikimedia.org/wiki/File:Radioactive_Fluorescent_Seq.jpg



Sequencing Traces

- Snapgene Viewer (free)
- Sequence file on Canvas

Today

- Protocol 7 for remaining bacteria
  - Skip WGS prep
  - Only make 2 extra tubes per sample
  - Blank labels provided…