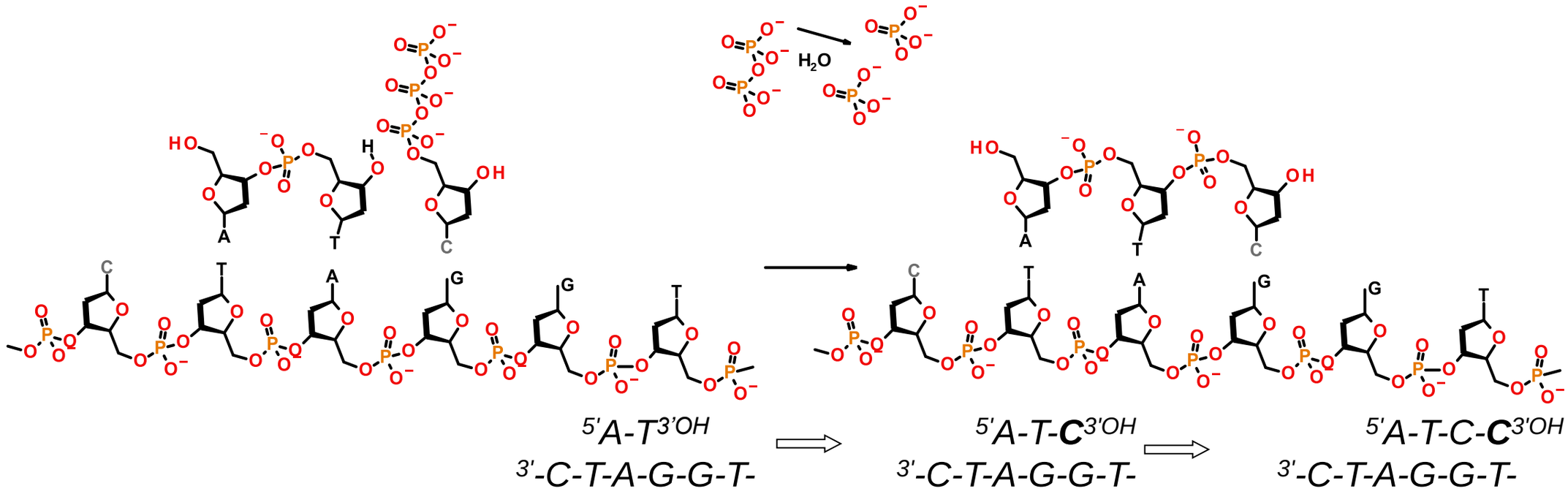
Lecture 4
DNA Technologies, Immunology

- Review of DNA polymerases
- DNA Sequencing
- Polymerase chain reaction (PCR) & Applications

Please view the posted video on Enzyme Kinetics before our next class.

DNA Polymerases – Used in DNA Sequencing and PCR

- **DNA polymerases** utilize a **template** to direct the order of added bases,
- The enzyme will continue to the end of the template.
- Require a basepaired **primer** with a 3'OH. Primer can be DNA or RNA, DNA is used for laboratory work, RNA is used by the cell during replication
- New dNTP added to the 3' hydroxyl of the existing polymer, elongation in the **5' to 3' direction.**
- Pyrophosphate (PP) is released and hydrolyzed to two inorganic phosphates.



Expectations: Know the features of this reaction.

DNA Polymerase – Fundamental Activity.

```
                    5′ A-T-C-A

3′A-T-G-C-C-G-T-A-G-T-C-G-T-A-C-A-G-T-A-C-G-T-G-C-A
  1 2 3 4 5 6 7 8 9 0 1 2 3 4 5 6 7 8 9 0 1 2 3 4 5
                    1                   2
```

A short 4 base primer (ATCA) is added to a template, and the temperature is lowered to allow annealing (basepairing) of the primer to the template.

1. *Where (what position) will this primer anneal?*
2. *What is the first base added by the polymerase? A G C T*
3. *What is the last base added by the polymerase? A G C T*

Repeat Expansion Diseases – Errors in DNA Replication

Handbook of Clinical Neurology
Volume 147, 2018, Pages 105-123

Chapter 9 - Repeat expansion diseases

Henry Paulson

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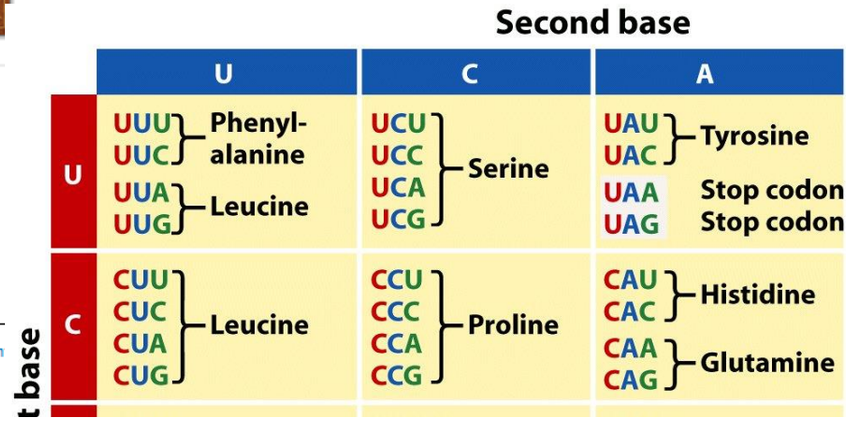
https://doi.org/10.1016/B978-0-444-63233-3.00009-9



- CAG – at least 10 diseases (Huntington disease, spinal and bulbar muscular atrophy, dentatorubral-pallidoluysian atrophy and seven SCAs)
- CGG – fragile X, fragile X tremor ataxia syndrome, other fragile sites (GCC, CCG)
- CTG – myotonic dystrophy type 1, Huntington disease-like 2, spinocerebellar ataxia type 8, Fuchs corneal dystrophy
- GAA – Friedreich ataxia
- GCC – FRAXE mental retardation
- GCG – oculopharyngeal muscular dystrophy
- CCTG – myotonic dystrophy type 1
- ATTCT – spinocerebellar ataxia type 10
- TGGAA – spinocerebellar ataxia type 31
- GGCCTG – spinocerebellar ataxia type 36
- GGGGCC – C9ORF72 frontotemporal dementia/amyotrophic lateral sclerosis
- CCCCGCCCCGCG – EPM1 (myoclonic epilepsy)

- Repeats in coding regions of genes will generate long stretches of the same amino acid.

  CAGCAGCAG = GluGluGlu
- Repeats outside of coding regions can affect gene expression by changing binding of transcription factors.
- These repeats can grow due to slippage of primer during replication
- More repeats = more chance of developing disease.

- The number of repeats can be detected by:
  - DNA sequencing
  - PCR

Repeat Expansions – How Do They Grow?

Original Sequence - 3 repeats

–TATATCCAGCAGCAGAGTATA–
–ATATAGGTCGTCGTCTCATAT–

During Replication in the cell

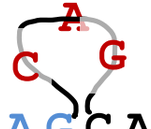
–TA
–ATATAGGTCGTCGTCTCATAT–

–TATATCCAGCAGCAG
–ATATAGGTCGTCGTCTCATAT–

Primer slippage 3’ end comes loose

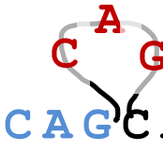
–TATATCCAGCAGCAG
–ATATAGGTCGTCGTCTCATAT–

Looped out DNA



–TATATCCAGCAG
–ATATAGGTCGTCGTCTCATAT–

Replication continues



–TATATCCAGCAGCAGAGTATA–
–ATATAGGTCGTCGTCTCATAT–

Next replication (upper strand as the template)

–TATATCCAGCAGCAGCAGAGTATA–
TCATAT–

4 repeats

–TATATCCAGCAGCAGCAGAGTATA–
–ATATAGCTCGTCGTCGTCTCATAT–

DNA Sequencing – Sanger (dideoxy) Sequencing

DNA Sequencing - Determining the Order of Bases in the DNA.

Sanger Sequencing:

- Second method to generate long (~1000 base) sequence information (an earlier chemical method developed by Gilbert proved to be impractical for most laboratories (hydrazine = rocket fuel was required)
- Sanger was awarded his 2nd Nobel prize for this work in 1980, shared with Gilbert.

Determine the position of all four bases in a DNA strand = Sequence (video)

Sanger Sequencing:

Primer 5′**C-A-T-A-T-G**^{OH}

Template 3′G-A-A-G-T-C-G-A-A-G-G-T-A-T-A-C-**C**-A-T-T-A-G-G-C-C-A-T-G-C-A-C-G-T----

Known Seq (plasmid) Unknown sequence (insert)

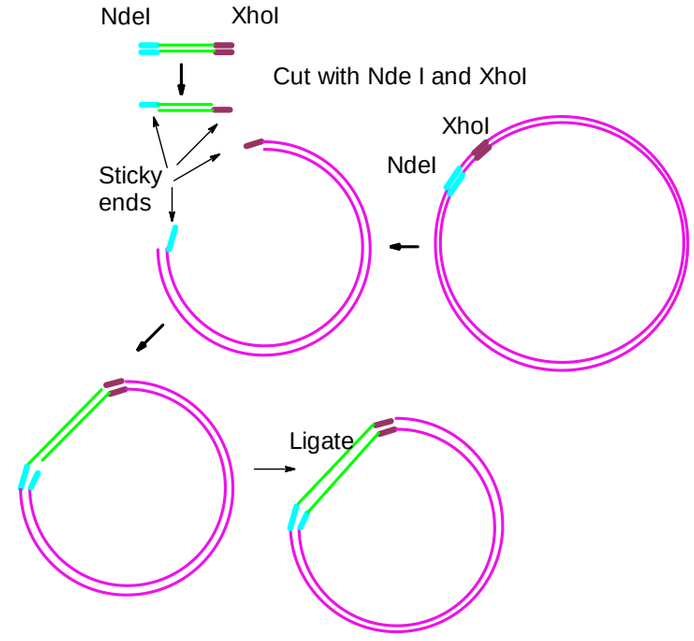
Sequenced region (~1000 bases)

5′C-A-T-A-T-G-G-T-A-A-T-C-C-G-G-T-A-C-G-T-G-C-A----

Template 3′G-A-A-G-T-C-G-A-A-G-G-T-A-T-A-C-**C**-A-T-T-A-G-G-C-C-A-T-G-C-A-C-G-T----

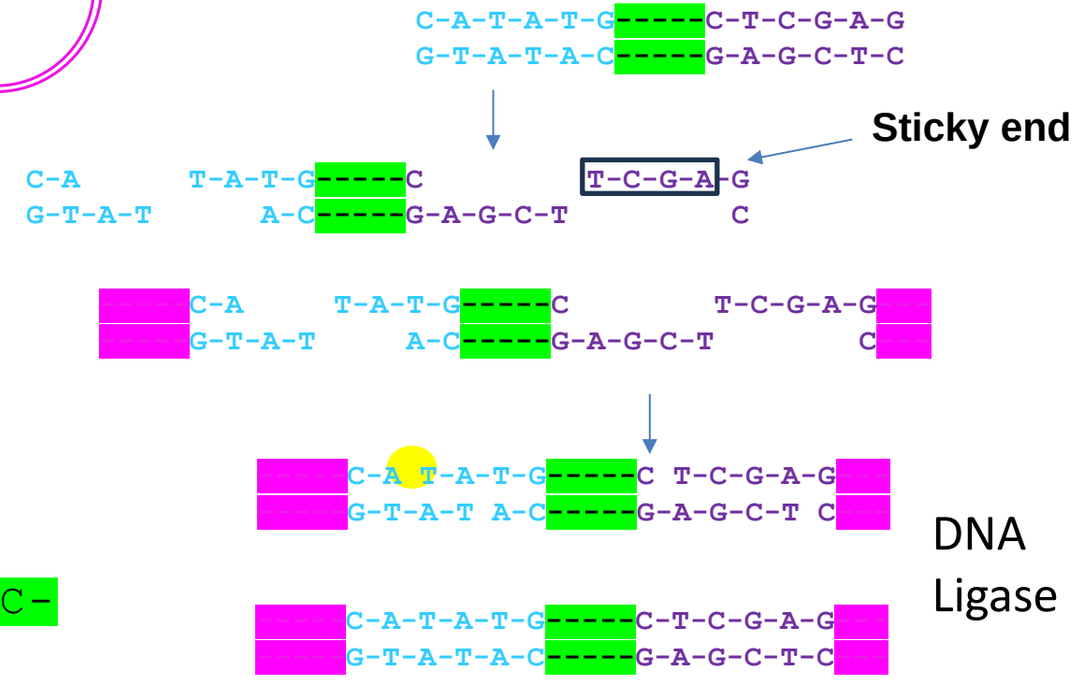
DNA Sequencing - Determining the Order of Bases Added by DNA Polymerase

- The DNA to be sequenced is inserted into a circular piece of double stranded DNA called a plasmid. The DNA sequence of the plasmid is known.
- The insertion is often accomplished using restriction enzymes that generate single stranded overhangs that allow DNA molecules to be efficiently joined.
- Restriction sites can be added to any DNA fragment using a number of techniques:
  - Addition of a short linker (same site on both ends)
  - PCR (different sites on each end)



Restriction Enzymes

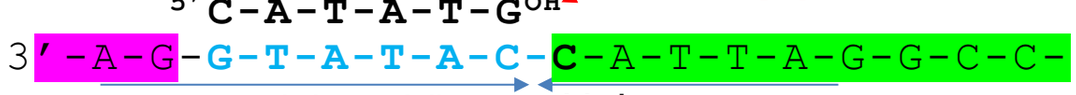
- Recognize a specific sequence in the DNA
- Sequence has 2-fold symmetry – same on the top and bottom strand
- Cuts both strands, most generate single-stranded DNA (sticky ends).
- Complementary sticky ends can bind to each other.



1. Start sequencing at known location with primer that anneals at a ***unique*** location on the plasmid, "upstream" from the region to be sequenced.

All DNA molecules begin here.

First base is added here.

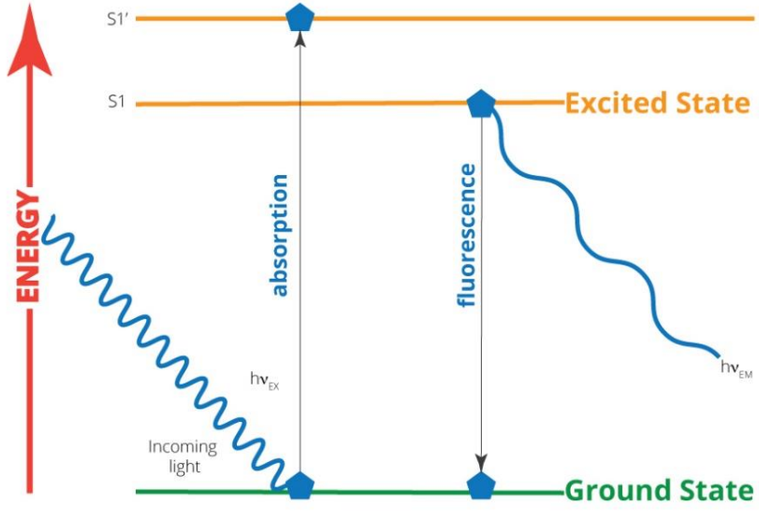


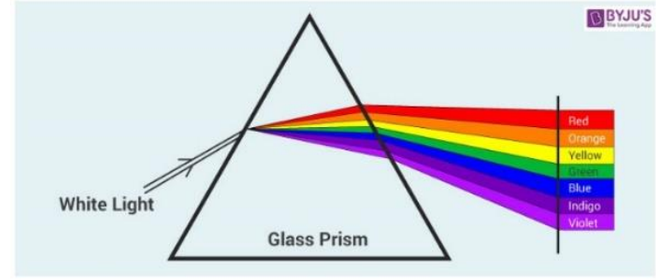
Known Seq (plasmid)

Unknown sequence (insert)

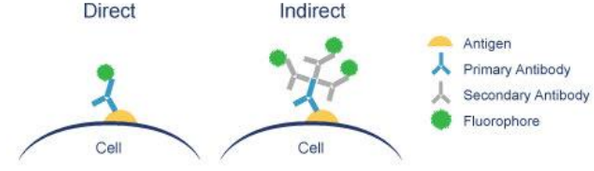
DNA Sequencing Methods Use Fluorescent Bases - What is Fluorescence?

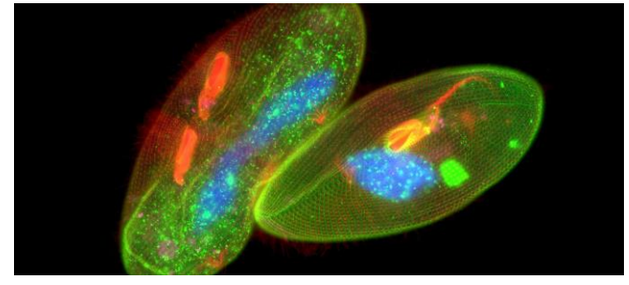
- When molecules absorb light an electron goes from a lower shell to a higher shell. This is where the energy from the light goes.
- In most molecules the electron goes back down to its original shell with the release of heat.
- Fluorescent molecules emit the energy as light of a longer wavelength (different color).
- The color that is emitted depends on the molecule.





Fluorescently tagged antibodies can be used to stain components of cell with fluorophores.

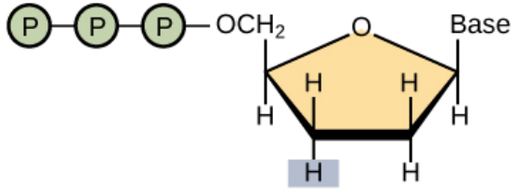




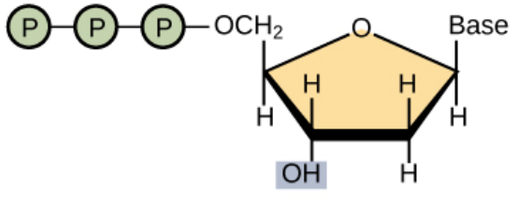
DNA Sequencing - Determining the Order of Bases Added by DNA Polymerase

2. Use a mixture of normal bases (dNTPs) and dideoxy bases (ddNTP) for polymerization. Ratio of dNTP to ddNTP is (100:1), ***most of the time elongation occurs.***

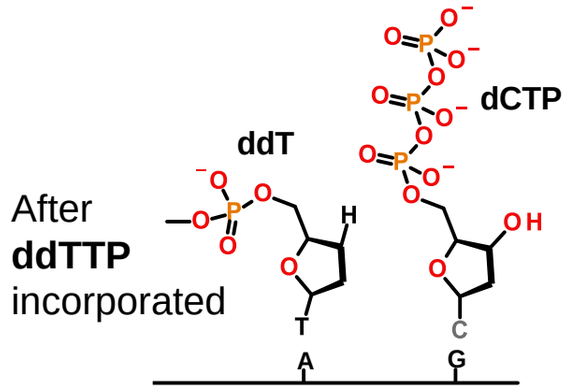
- ddNTPs can be added to the DNA since they have a 5'-triphosphate but ***terminate*** the chain due to the lack of a 3'-OH. ~ 1 in 100 chains terminate at each base addition

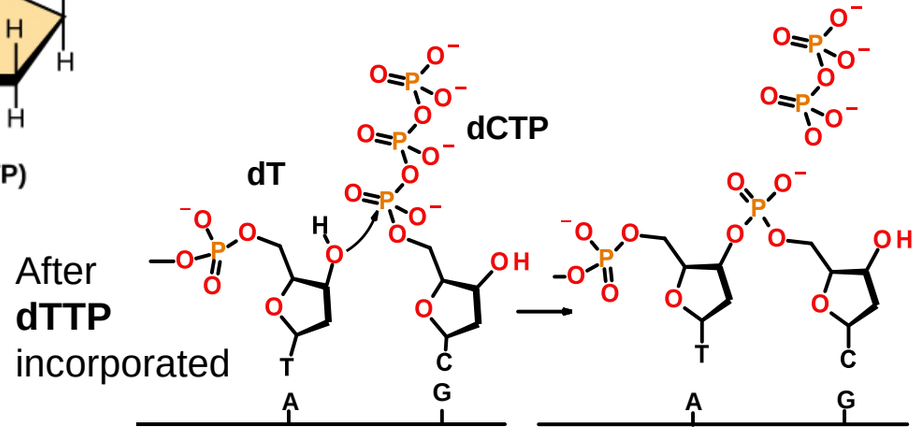


Dideoxynucleotide (ddNTP)



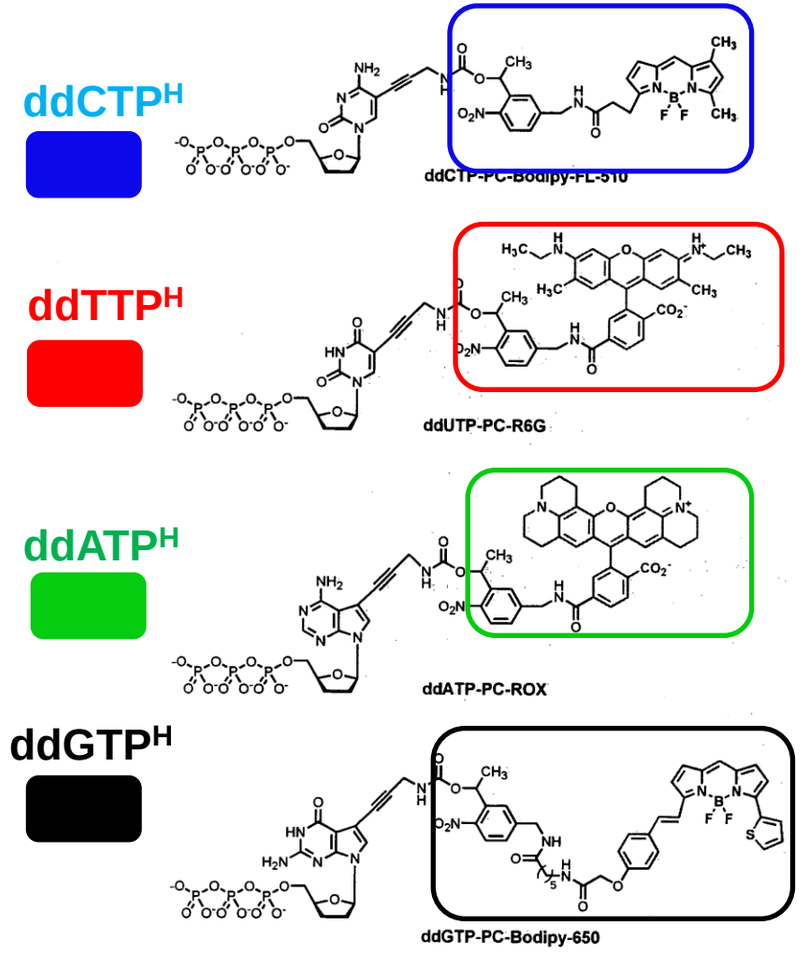
Deoxynucleotide (dNTP)



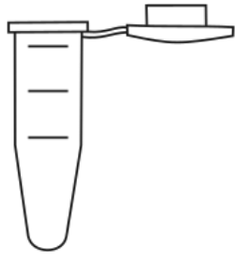


3. The ddNTPs are color coded by different fluorescent emission wavelengths.

The ddNTP that terminated the chain is known from its fluorescent color.



DNA Sequencing – Generation of Fluorescent Fragments



Template
Primer
DNA Pol
dTNP, ddNTP

5′-C-A-T-A-T-G
3′-A-G-G-T-A-T-A-C-C-A-T-T-A-G-G-C

(1000 molecules)

Length=7, Black fluor.

(10) 5′-C-A-T-A-T-G-G^{H}
3′-A-G-G-T-A-T-A-C-C-A-T-T-A-G-G-C

(990) 5′-C-A-T-A-T-G-G^{OH}
3′-A-G-G-T-A-T-A-C-C-A-T-T-A-G-G-C

5′-C-A-T-A-T-G-G^{OH}
3′-A-G-G-T-A-T-A-C-C-A-T-T-A-G-G-C

(990 molecules)

Length=8, Red fluor.

(10) 5′-C-A-T-A-T-G-G-T^{H}
3′-A-G-G-T-A-T-A-C-C-A-T-T-A-G-G-C

(980) 5′-C-A-T-A-T-G-G-T^{OH}
3′-A-G-G-T-A-T-A-C-C-A-T-T-A-G-G-C

5′-C-A-T-A-T-G-G-T^{OH}
3′-A-G-G-T-A-T-A-C-C-A-T-T-A-G-G-C

(980 molecules)

Length=9, Green fluor

(10) 5′-C-A-T-A-T-G-G-T-A^{H}
3′-A-G-G-T-A-T-A-C-C-A-T-T-A-G-G-C

(970) 5′-C-A-T-A-T-G-G-T-A^{OH}
3′-A-G-G-T-A-T-A-C-C-A-T-T-A-G-G-C

5′-C-A-T-A-T-G-G-T-A^{OH}
3′-A-G-G-T-A-T-A-C-C-A-T-T-A-G-G-C

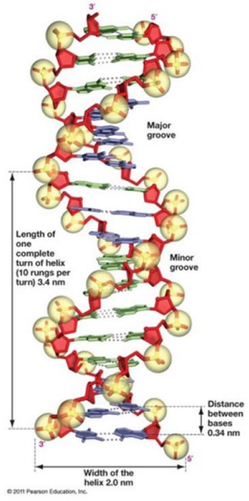
***All* Possible Fragments are Made:**

1. Each begins with the primer
2. Each ends with a *known* ddNTP, based on the color of the fluorescence.
3. Each is one longer than the previous.

C-A-T-A-T-G-**G**
C-A-T-A-T-G-G-**T**
C-A-T-A-T-G-G-T-**A**
C-A-T-A-T-G-G-T-A-**A**
C-A-T-A-T-G-G-T-A-A-**T**
C-A-T-A-T-G-G-T-A-A-T-**C**
C-A-T-A-T-G-G-T-A-A-T-C-**C**
C-A-T-A-T-G-G-T-A-A-T-C-C-**G**

Primer — Added by Pol.

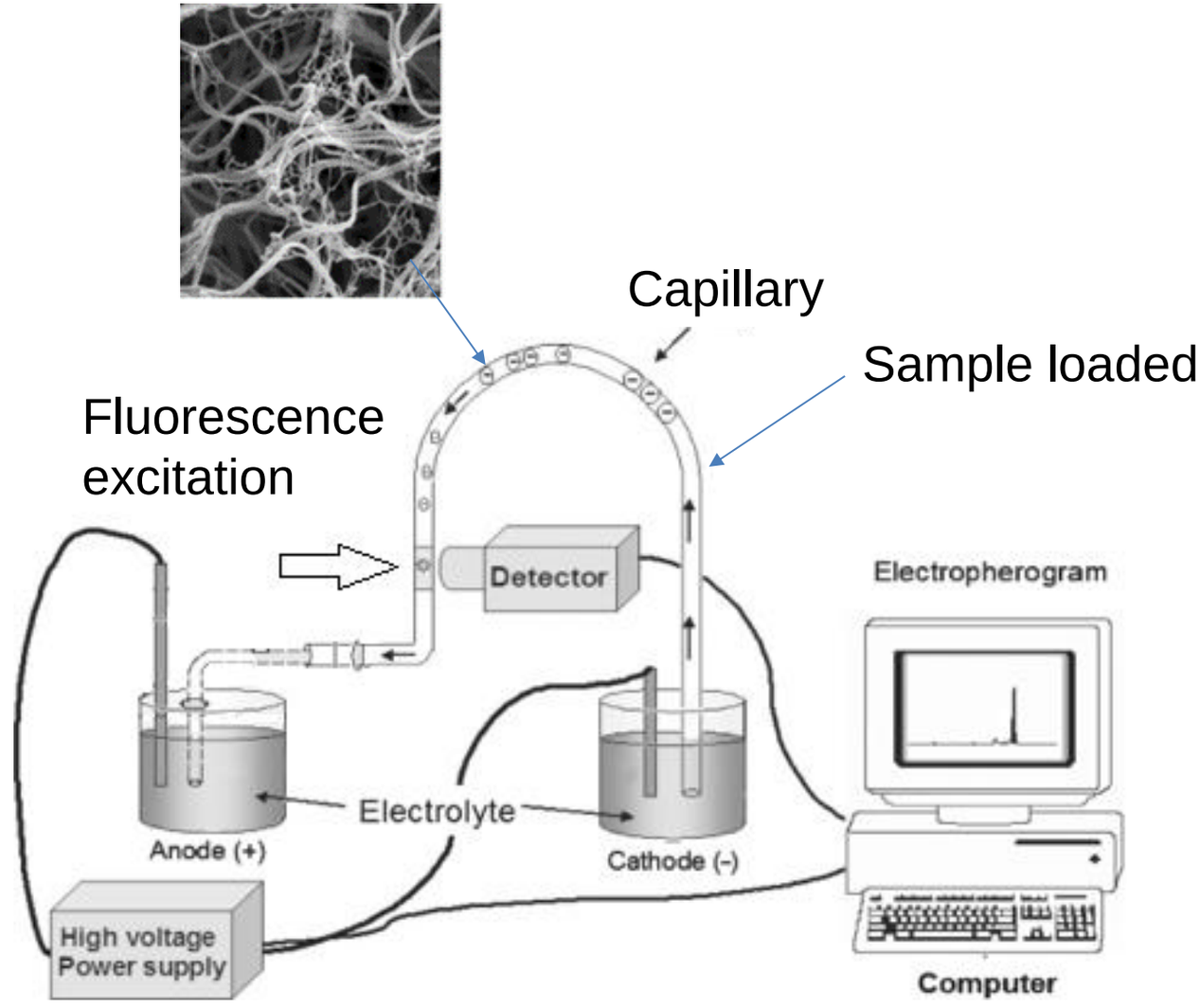
Size Determination of Fragments from DNA Sequencing Capillary Electrophoresis



DNA has a negative charge.
It will migrate towards the anode.

Capillary is filled with a gel that causes separation by size.

DNA molecules that are smaller migrate _____.

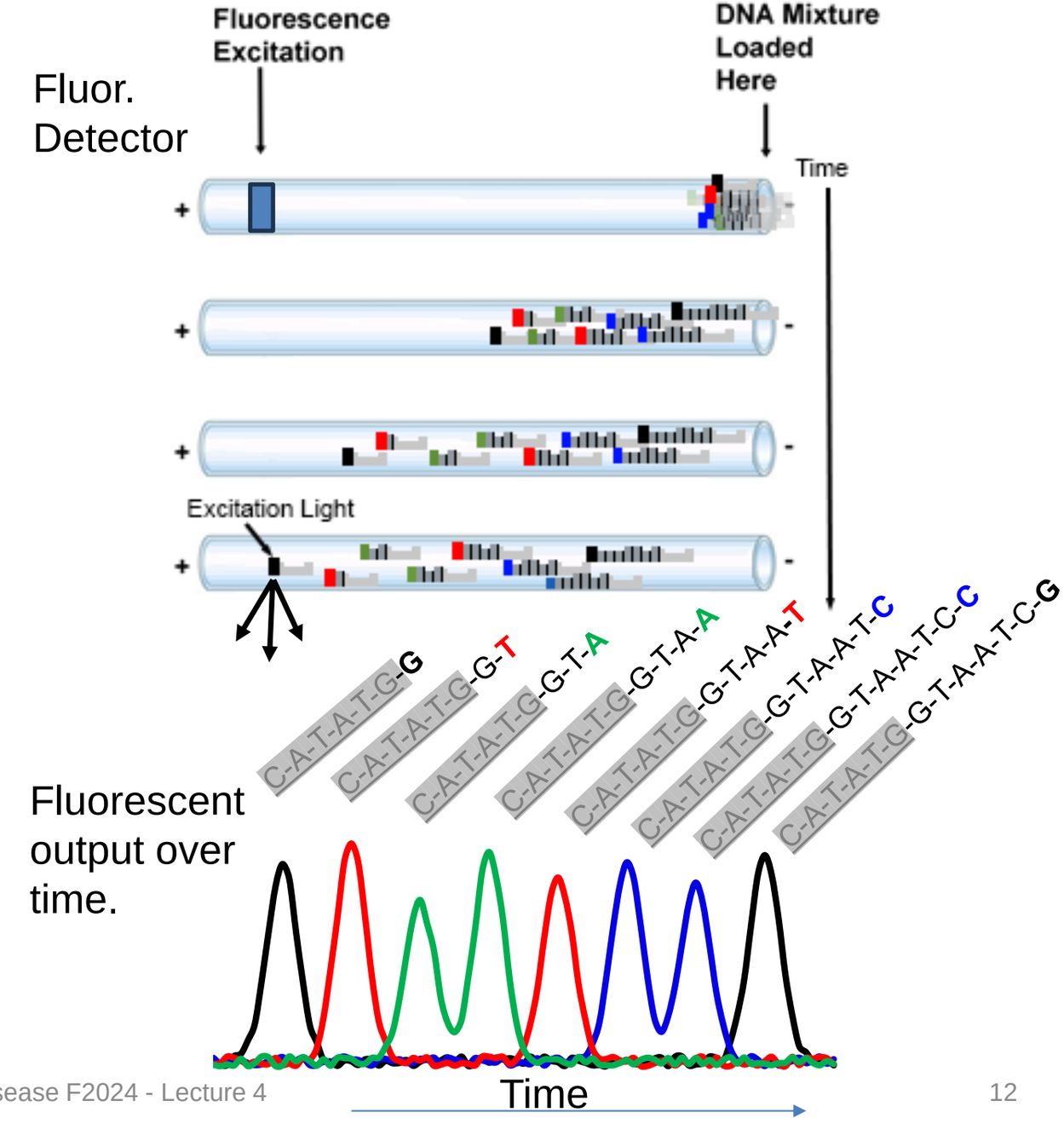


DNA Sequencing – Analysis of Fragments to Determine Order of Addition

4. Capillary Gel Electrophoresis

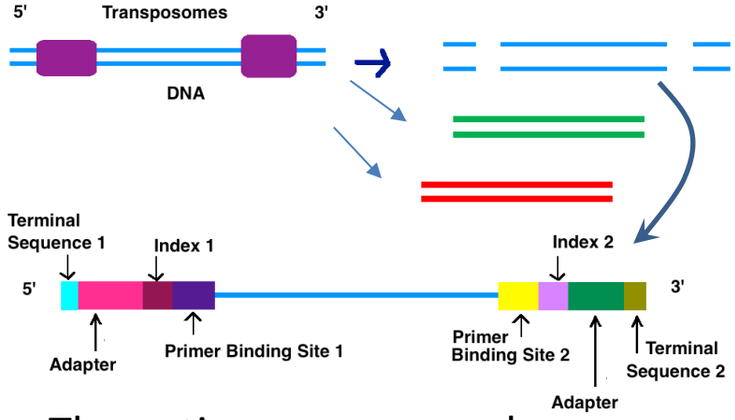
- Migration due to the voltage because of the neg. charge on DNA phosphates
- Separation of DNA molecules by size, smaller travel through gel faster.
- Fragments reach the detector in the order of their length: primer+1 first, primer+2 second, etc.
- At the detector, a laser excites the fluorescence.
- Only fluorescent DNA molecules (terminated with ddNTP) give a signal.
- The color of the emitted fluorescence gives the dideoxy base at the 3' end of the DNA fragment.
- The order of peaks gives the sequence that is complementary to the template (= strand with primer).

5'-C-A-T-A-T-G G-T-A-A-T-C-C-G
3'-A-G-G-C-T-A-T-A-C-C-A-T-T-A-G-G-C

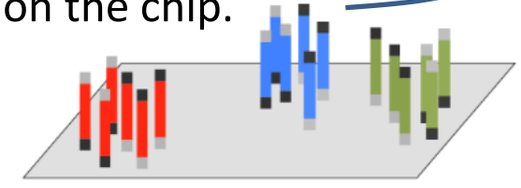


Newer Sequencing Methods-Illumina Dye Sequencing – Next Generation High Throughput

A. Obtaining the DNA

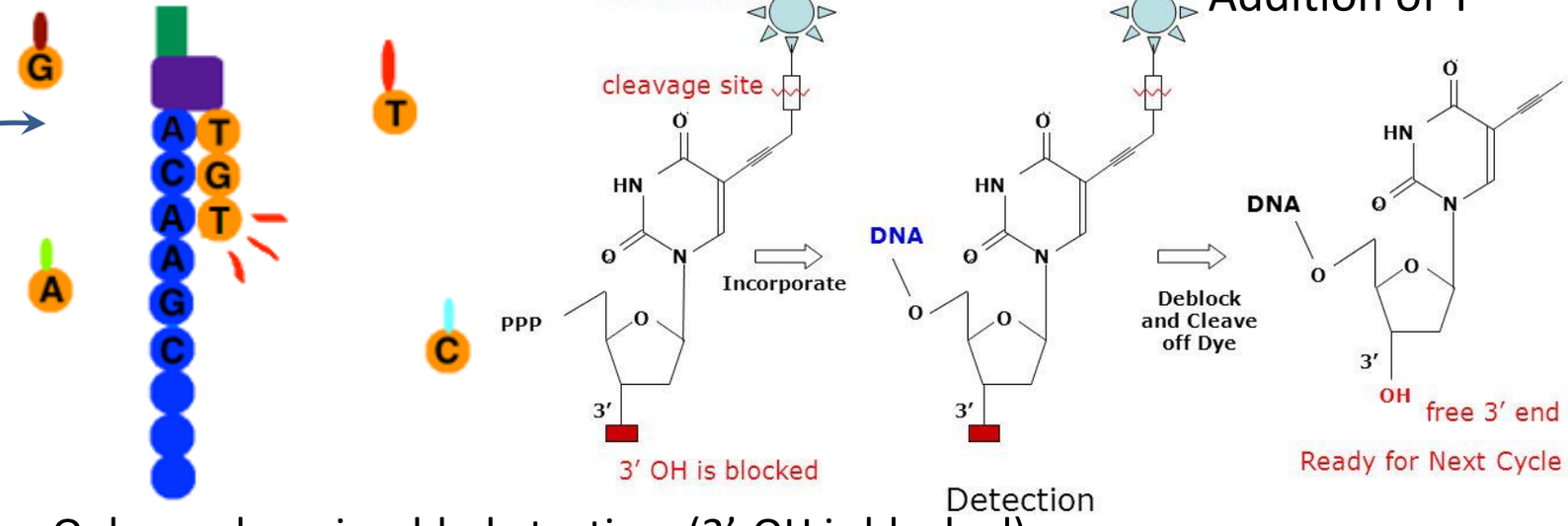


- The entire genome can be sequenced.
- The DNA is fragmented into small 100 base pieces.
- Synthetic DNA is added to the ends (sites for primers for sequencing)
- Different fragments are bound to different location on a solid surface (chip).
- All fragments are sequenced at the same time on the chip.



Cluster formation

B. Sequencing by synthesis – Fluorescent labeling & *reversible* 3'-OH blocking



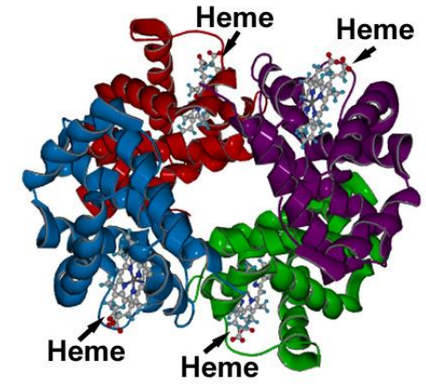
1. Only one base is added at a time (3'-OH is blocked)
2. The base that is added is determined by the color of the fluorescent base.
3. 3'-OH blocking group and the fluorescent group are removed prior to the next addition. ~100 cycles can be performed.

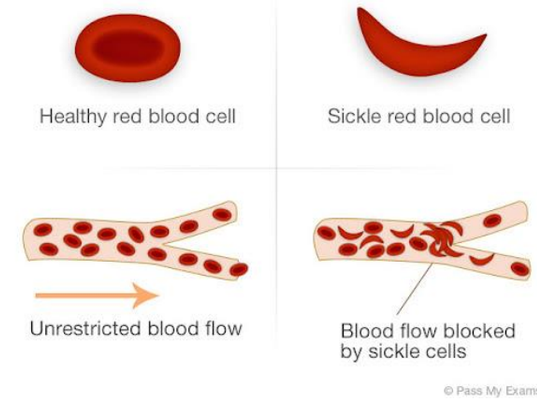
By DMLapato - Own work, CC BY-SA 4.0, https://commons.wikimedia.org/w/index.php?curid=43777596

Method	Read Length	Samples Processed
Sanger	~1000	1
Illumina	~100	~10,000s

Genotyping at the Molecular Level with DNA Sequencing.

- Sickle cell anemia is caused by a single mutation in the beta chain of hemoglobin
- This mutation causes the hemoglobin to form long polymers that distort the shape of the red blood cell.
- Determining whether someone has the mutation can be useful for treatment.



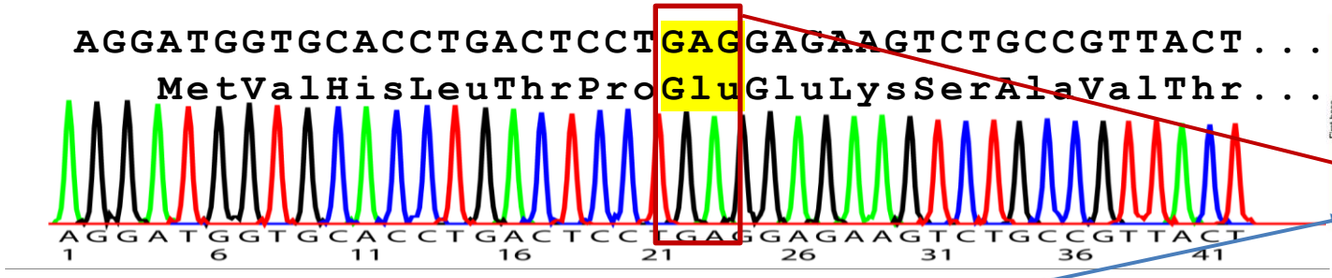


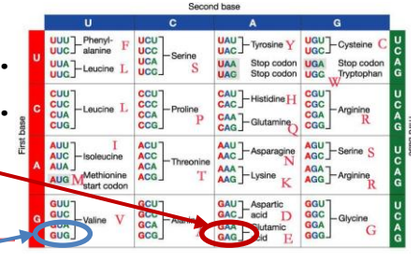
The 5' end of the Hb gene is shown on the right (ATG=start). Using GGTGCCAG as a sequencing primer gives the following sequences for the normal and mutant genes:

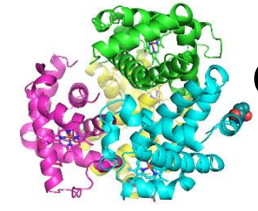
First dd-base added by polymerase

GGTGCCAGAGGATGGTGCACCTGACTCCTGAGGAGAAGTC..
CCACGGTCTCCTACCACGTGGACTGAGGACTCCTCTTCAG..

Sequencing data for the normal beta chain is:

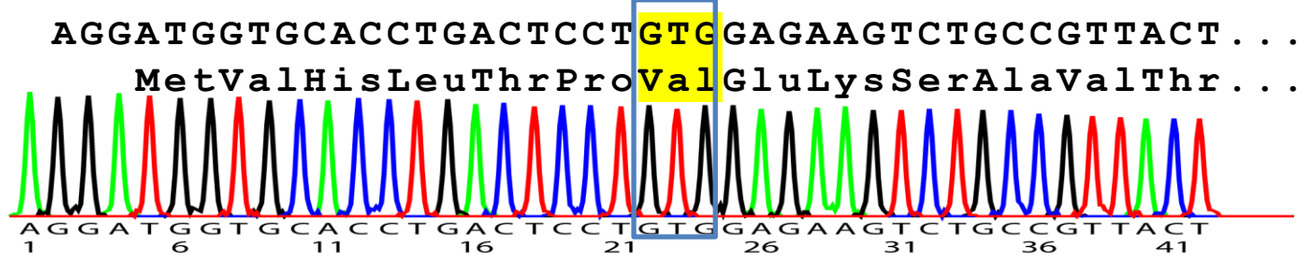






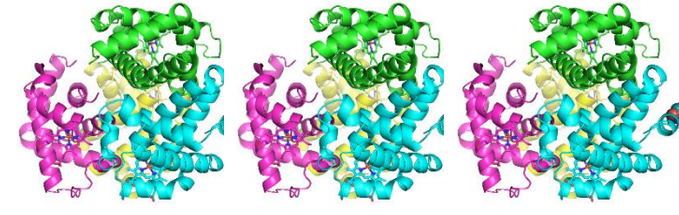
Glu6

Sequencing data for the mutation:



False color code:

A=Green
G=Black
T=Red
C=Blue



Val6

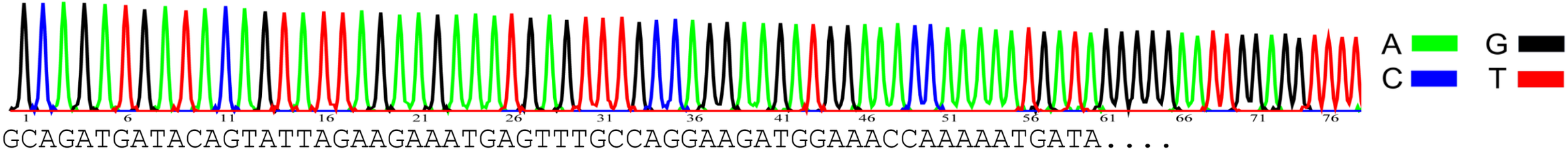
Sequencing Summary & Expectations

Sanger Sequencing:

- Gives the sequence that is complementary to the template strand = “top” strand, same strand at the primer.
- The start of the sequencing information is defined by a primer that anneals to the template (therefore some of the sequence has to be known, how this is done will be described later)
- Dideoxy sequencing is carried out by adding both dideoxynucleotide triphosphates (ddNTPs) and deoxyribonucleotide triphosphates (dNTPs) to the synthesis reactions, at a ratio of 1:100. Most growing chains do not terminate.
- ddNTPs are identical to dNTPs except that they lack the 3' hydroxyl group. Because of the missing 3’-OH, DNA polymerization stops once one ddNTP is added to a growing strand.
- The type of the added base is determined by “color coding” each base.
- The location of added bases is determined by measuring the size of the DNA fragment that was terminated by the ddNTP.
- It is possible to sequence approximately 1000 bases by this method.

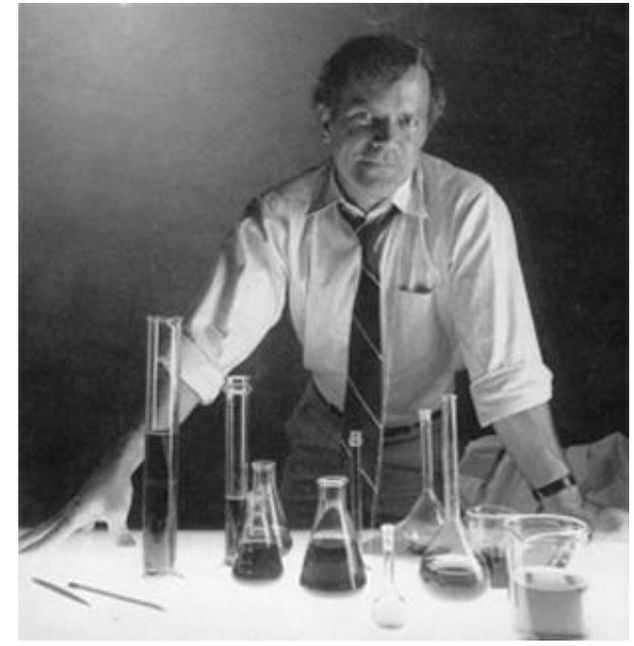
Next Gen-Sequencing:

- Simultaneous sequencing of a large number of fragments
- Shorter “reads” 100 versus 1000 bases/template



Polymerase Chain Reaction - PCR

- In 1983, Kary Mullis developed the molecular biology technique that has since revolutionized genetic research, earning him the Nobel Prize in 1993.
- PCR had an impact on four main areas of biotechnology: gene mapping, cloning, DNA sequencing, and gene detection (e.g. coronavirus).
- PCR is now used as a medical diagnostic tool to detect specific mutations that may cause genetic disease, in criminal investigations and courts of law to identify suspects on a molecular level, and in the sequencing of the human genome.

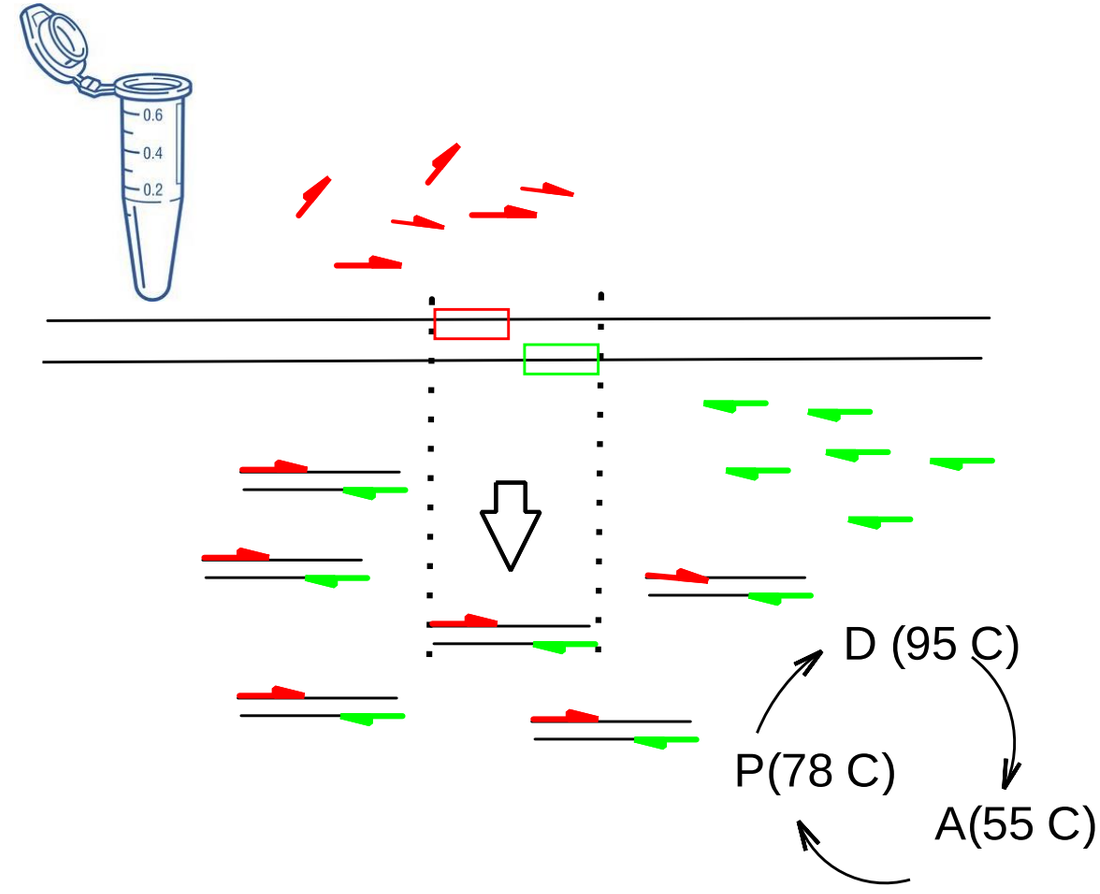


Expectations:

1. Be able to explain how PCR works to amplify a segment of DNA.
2. Be able to give the left and right primers.
3. Apply PCR approaches to determine genotype and detection of viruses.

Polymerase Chain Reaction

- PCR is an *in vitro* DNA synthesis reaction in which a specific section of DNA is replicated over and over generating exponentially large amounts of a specific piece of DNA from trace amounts of starting material (template).
- Template can be trace amounts of DNA from a drop of blood, a single hair follicle, or a cheek cell.
- The region of DNA that is copied is specified by the sequence of two primers, which are short ssDNA that initiate polymerase activity. The primers are in vast excess over the DNA.
- The location of the amplified segment is *defined* by two primers (left = upstream, right = downstream):
  - they anneal to their templates according to Watson-Crick pairing rules (A-T, G-C),
  - initiate polymerization from those sites,
  - they are incorporated into the final PCR product.
  - Left primer = sequence of top strand at left boundary
  - Right primer = sequence of bottom strand at right boundary
- The primers are DNA and are synthesized chemically, they can be any desired sequence.
- If there is no homology between the primers and the input DNA, then no PCR product will be formed.

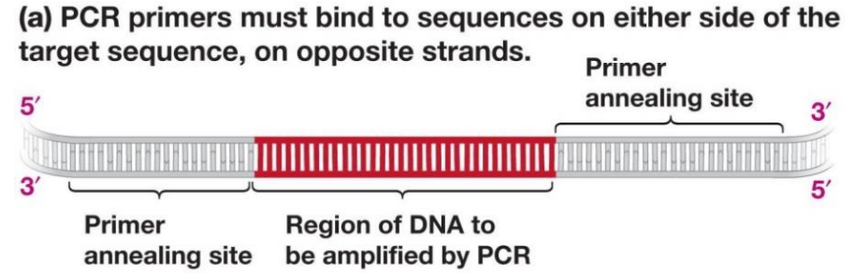


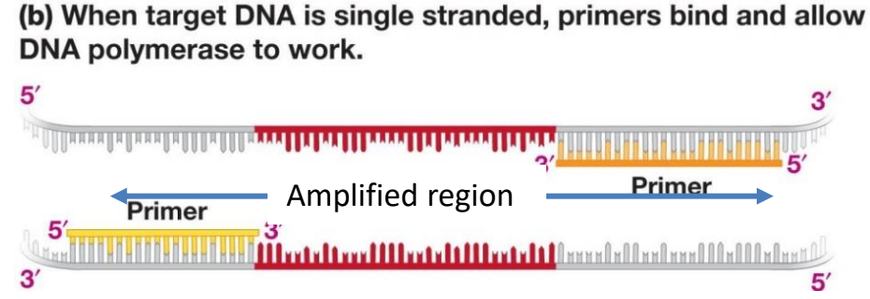
Each PCR cycle consists of three steps:

1. Denaturation of the DNA to make it single stranded (2 min at 98 C)
2. Lowering of temperature to let the primers form double-stranded DNA (1 min at 55 C)
3. Elongation by DNA polymerase (1 min/kb at 78 C)

PCR – Primer Design

- Before a region of DNA can be amplified, one must identify and determine the sequence of a piece of DNA upstream and downstream of the region of interest.
- These areas are then used to determine the sequence of oligonucleotide primers that will be synthesized and used as starting points for DNA replication.
- Primers are complimentary to the up- and down-stream regions of the sequence to be amplified, so they stick, or anneal, to those regions.
  - Left primer = sequence of top strand on the left. This primer will anneal to the bottom strand.
  - Right primer = sequence of bottom strand on the right. This primer will anneal to the top strand.
- Primers are in large excess over the template DNA, they are never used up.
- The primers are incorporated into the final PCR product.





Amplified region

5′--AAG**CTGAC**TAGTCGATGCGAATGTGCGGTGC—3′
3′--TTCGACTGATCAGCTACGCTT**ACACG**CCACG—5′

5′CTGAC

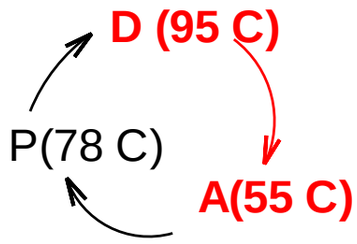
ACACG5′ = 5′GCACA

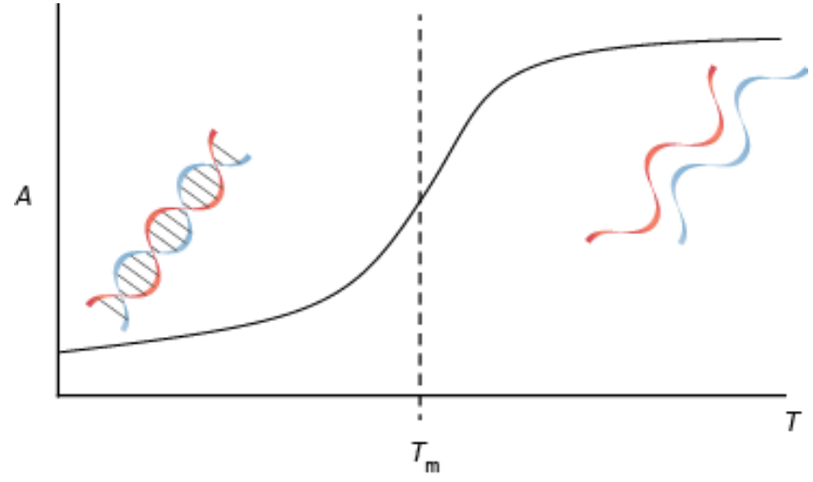
Know these rules!

CTGACTAGTCGATGCGAATGTGC
GACTGATCAGCTACGCTT**ACACG**

Note: Actual primer lengths are 20-30 bases, in the illustrations here and on problem sets, much shorter primers are used.

PCR Step 1 - Thermal Stability of Double Stranded DNA (dsDNA)





5′ A-T-C-A
3′A-T-G-C-C-G-T-A-G-T-C-G-T-A-C-A-G-T-A-C-G-T-G-C-A

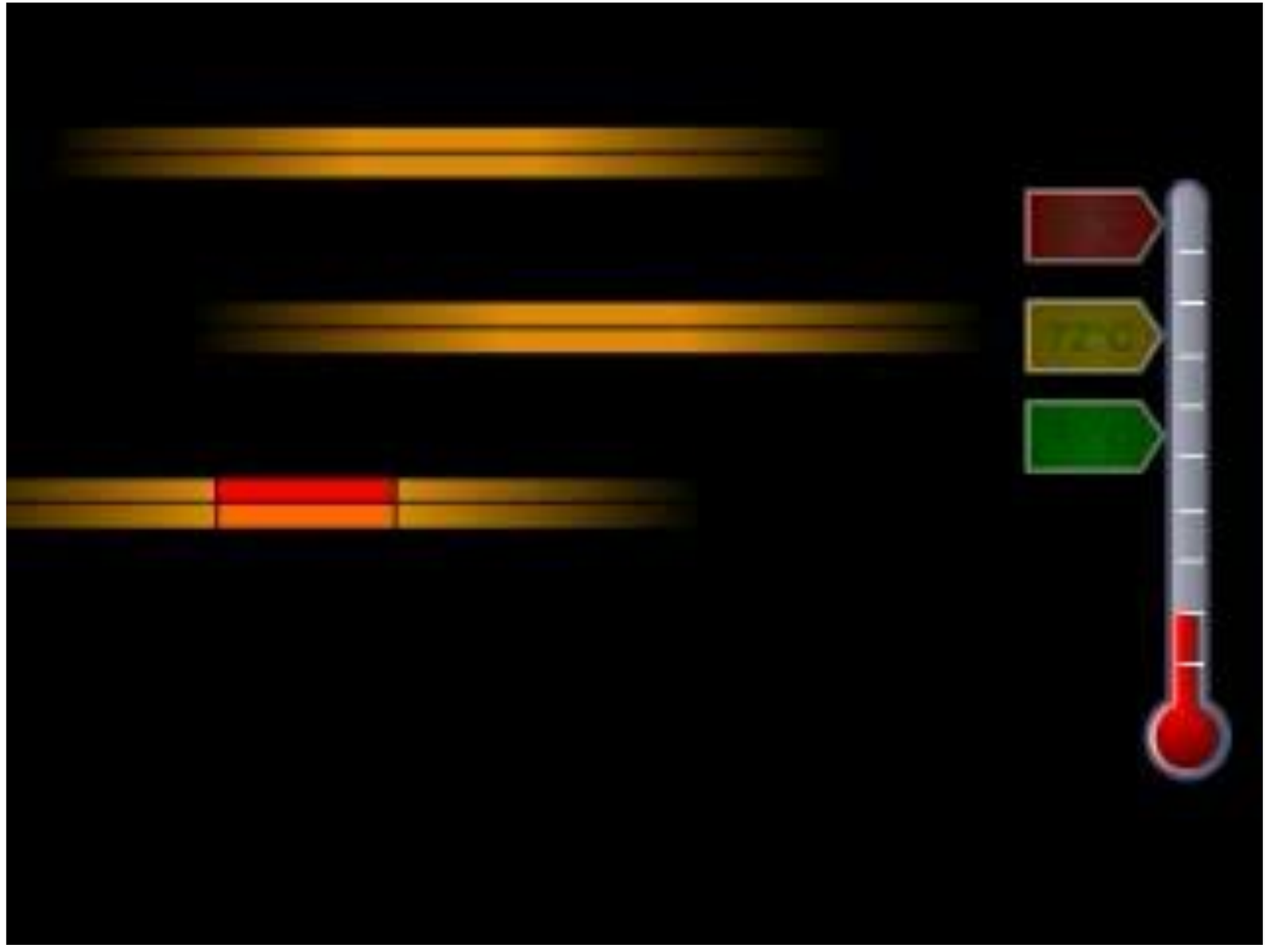
5′ A-T-C-A
3′A-T-G-C-C-G-T-A-G-T-C-G-T-A-C-A-G-T-A-C-G-T-G-C-A

Polymerase Characteristics

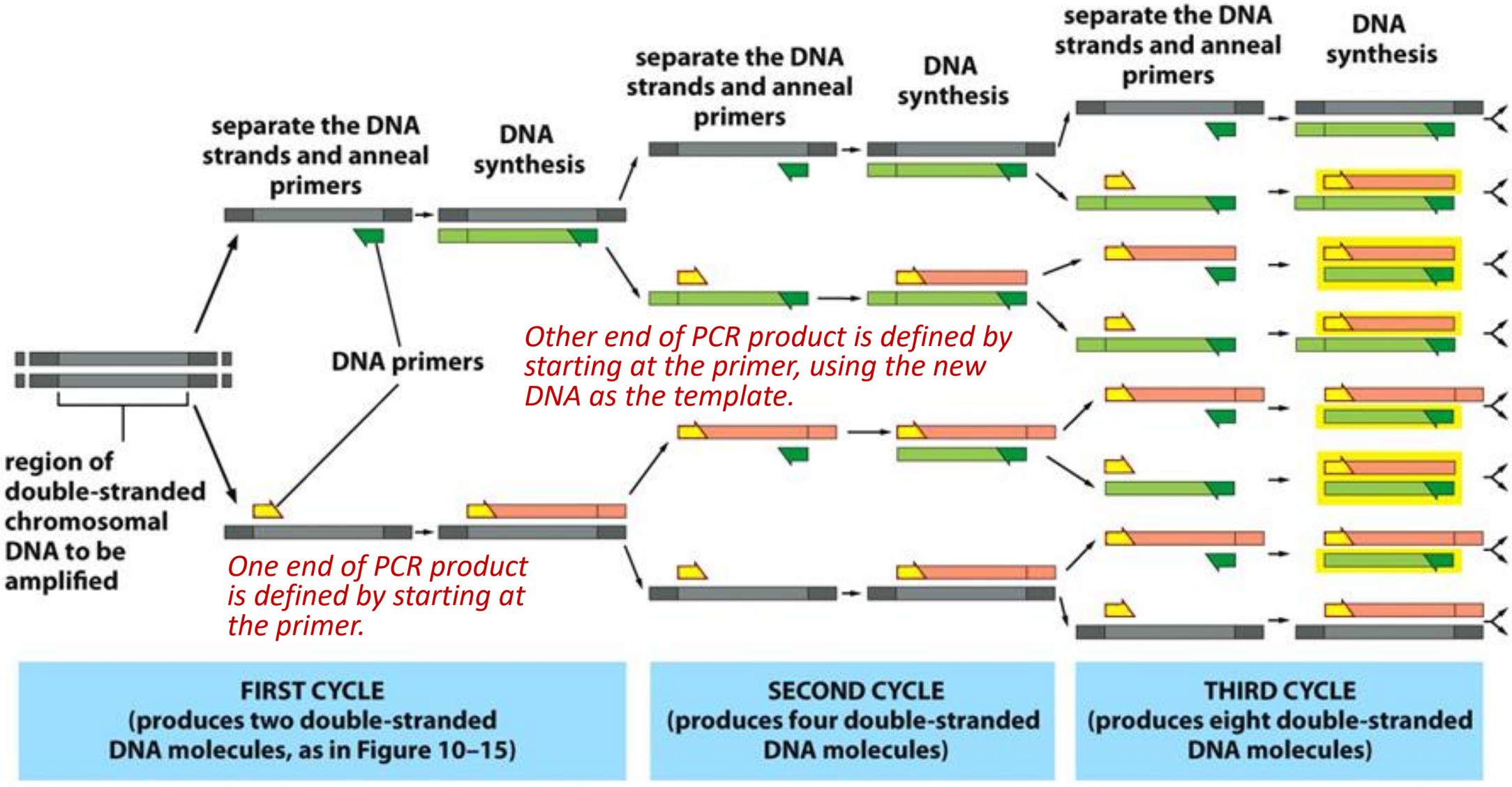
- Since the first step of each cycle (D) requires heating to high temperature, a thermostable polymerase is required.
- The first polymerase, Taq, was isolated from *Thermus Aquaticus,* a bacterial living in hot springs (Yellowstone National Park)
- A number of different polymerases with improved properties have been developed.

PCR Animation

Watch Me!



Three PCR Cycles



After 30 cycles there will be 2^{30}, or over 1 billion times more copies than at the beginning!!!

Detailed Events During First Three PCR Cycles

Note:

- Polymerization starts at the primer (add to 3'-OH)
- Polymerase always goes to the end of the template.

Cycle I

--AAG**CTGAC**TAGTCGATGCGAATGTGCGGTGC--U
--TTCGACTGATCAGCTACGCTT**ACACG**CCACG--L

Denature & Anneal

--AAG**CTGAC**TAGTCGATGCGAATGTGCGGTGC-U
ACACG5'

5'CTGAC
--TTCGACTGATCAGCTACGCTT**ACACG**CCACG-L

Polymerase

--AAG**CTGAC**TAGTCGATGCGAATGTGCGGTGC-U
<-**TTCGACTGATCAGCTACGCTTACACG5'**

5'**CTGACTAGTCGATGCGAATGTGCGGTGC->**
--TTCGACTGATCAGCTACGCTTACACGCCACG-L

So far - defined one end of the product

Final Product

CTGACTAGTCGATGCGAATGTGC
GACTGATCAGCTACGCTT**ACACG**

Cycle II

--AAG**CTGAC**TAGTCGATGCGAATGTGCGGTGC-U
--TTCGACTGATCAGCTACGCTTACACG5'

5'CTGACTAGTCGATGCGAATGTGCGGTGC--
--TTCGACTGATCAGCTACGCTT**ACACG**CCACG-L

Denature & Anneal

--AAG**CTGAC**TAGTCGATGCGAATGTGCGGTGC--

5'CTGAC
--TTCGACTGATCAGCTACGCTTACACG5' **L strand**

5'CTGAC**TAGTCGATGCGAATGTGCGGTGC- U Strand**

Polymerase **ACACG5'**

--TTCGACTGATCAGCTACGCTT**ACACG**CCACG--

--AAG**CTGAC**TAGTCGATGCGAATGTGCGGTGC--

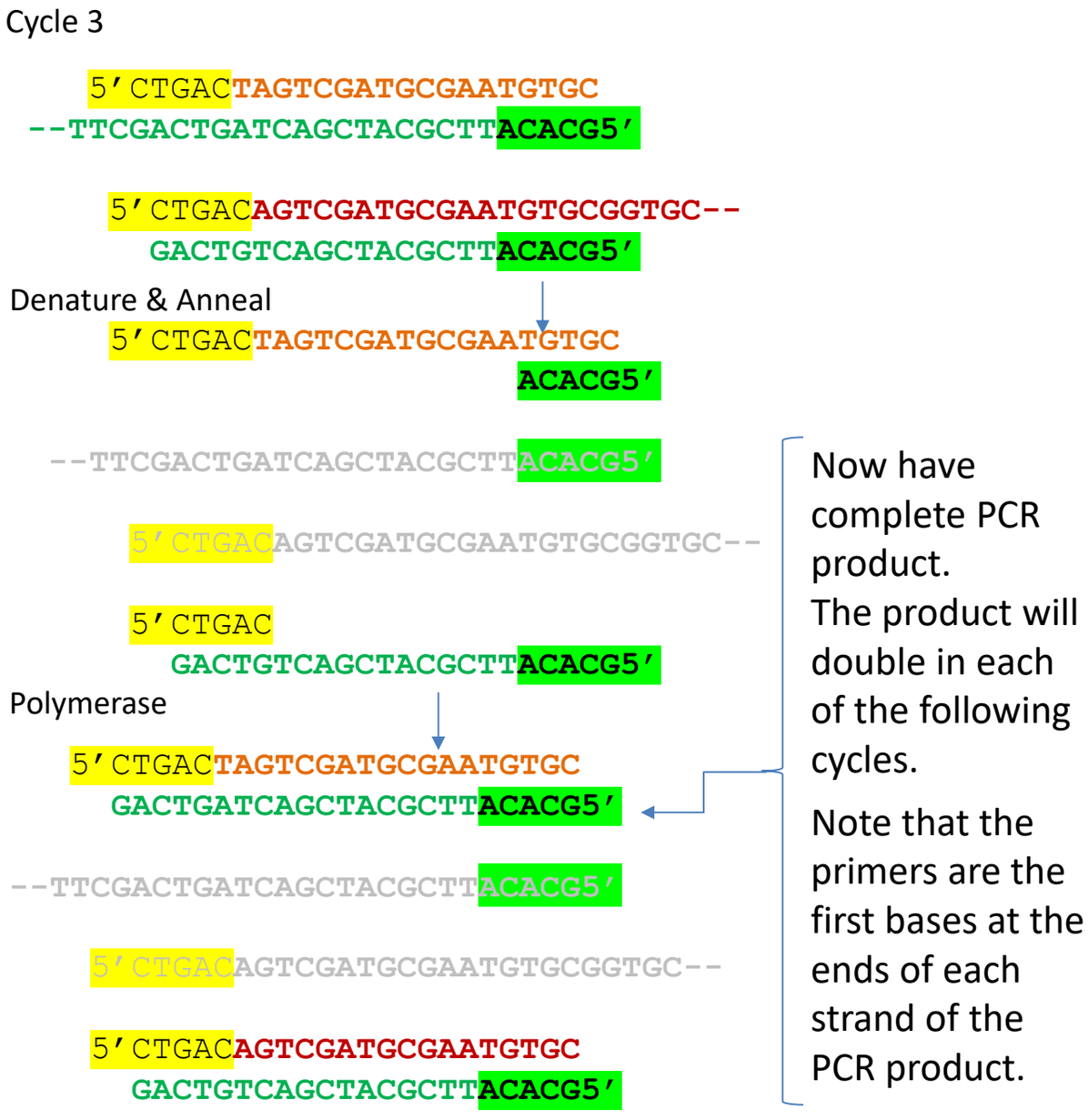
5'CTGAC**TAGTCGATGCGAATGTGC**
--TTCGACTGATCAGCTACGCTTACACG5'

5'CTGAC**TAGTCGATGCGAATGTGCGGTGC--**
GACTGATCAGCTACGCTTACACG5'

--TTCGACTGATCAGCTACGCTT**ACACG**CCACG--

Now have one strand of the product

Detailed Events during first Three PCR Cycles



Now have complete PCR product.
The product will double in each of the following cycles.

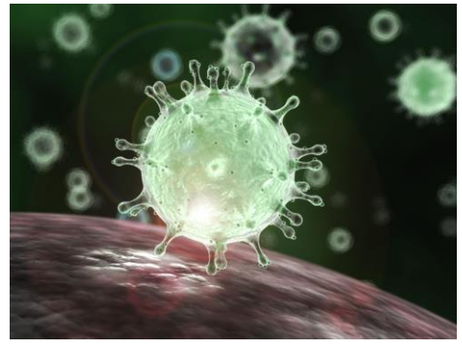
Note that the primers are the first bases at the ends of each strand of the PCR product.

Example – follow the PCR cycles for the following template with primers 5’ AATT (left) and 5’ GGCC (right)

```
----AATT-------------------------GGCC----
----TTAA-------------------------CCGG----
```

PCR Applications - Detection of Viruses

Coronavirus



Sequence of Covid-19 (top strand only)

```
1     attaaaggtt tataccttcc caggtaacaa accaaccaac tttcgatctc ttgtagatct
61    gttctctaaa cgaactttaa aatctgtgtg gctgtcactc ggctgcatgc ttagtgcact
121   cacgcagtat aattaataac taattactgt cgttgacagg acacgagtaa ctcgtctatc
181   ttctgcaggc tgcttacggt ttcgtccgtg ttgcagccga tcatcagcac atctaggttt

28261 cgaacaaact aaaatgtctg ataatggacc ccaaaatcag cgaaatgcac cccgcattac
28321 gtttggtgga ccctcagatt caactggcag taaccagaat ggagaacgca gtggggcgcg
28381 atcaaaacaa cgtcggcccc aaggtttacc caataatact gcgtcttggt tcaccgctct
28441 cactcaacat ggcaaggaag accttaaatt ccctcgagga caaggcgttc caattaacac

29701 gggaggactt gaaagagcca ccacattttc accgaggcca cgcggagtac gatcgagtgt
29761 acagtgaaca atgctaggga gagctgccta tatggaagag ccctaatgtg taaaattaat
29821 tttagtagtg ctatccccat gtgattttaa tagcttctta ggagaatgac aaaaaaaaaa
29881 aaaaaaaaaa aaaaaaaaaa aaa
```

CDC Recommended PCR Primers

2019-Novel Coronavirus (2019-nCoV) Real-time rRT-PCR Panel Primers and Probes

Name	Description	Oligonucleotide Sequence (5'>3')	Label[1]	Working Conc.
2019-nCoV_N1-F	2019-nCoV_N1 Forward Primer	5'-GAC CCC AAA ATC AGC GAA AT-3'	None	20 µM
2019-nCoV_N1-R	2019-nCoV_N1 Reverse Primer	5'-TCT GGT TAC TGC CAG TTG AAT CTG-3'	None	20 µM

dsSeq of above bold & circled region

```
28271 aaaatgtctgataatgGACCCCAAAATCAGCGAAATgcaccccgcattacgtttggtggaccctcagattcaactggcagtaaccagaatggagaacgca
      ttttacagactattacctggggttttagtcgctttacgtggggcgtaatgcaaaccacctgggaGTCTAAGTTGACCGTCATTGGTCTtacctcttgcgt
```

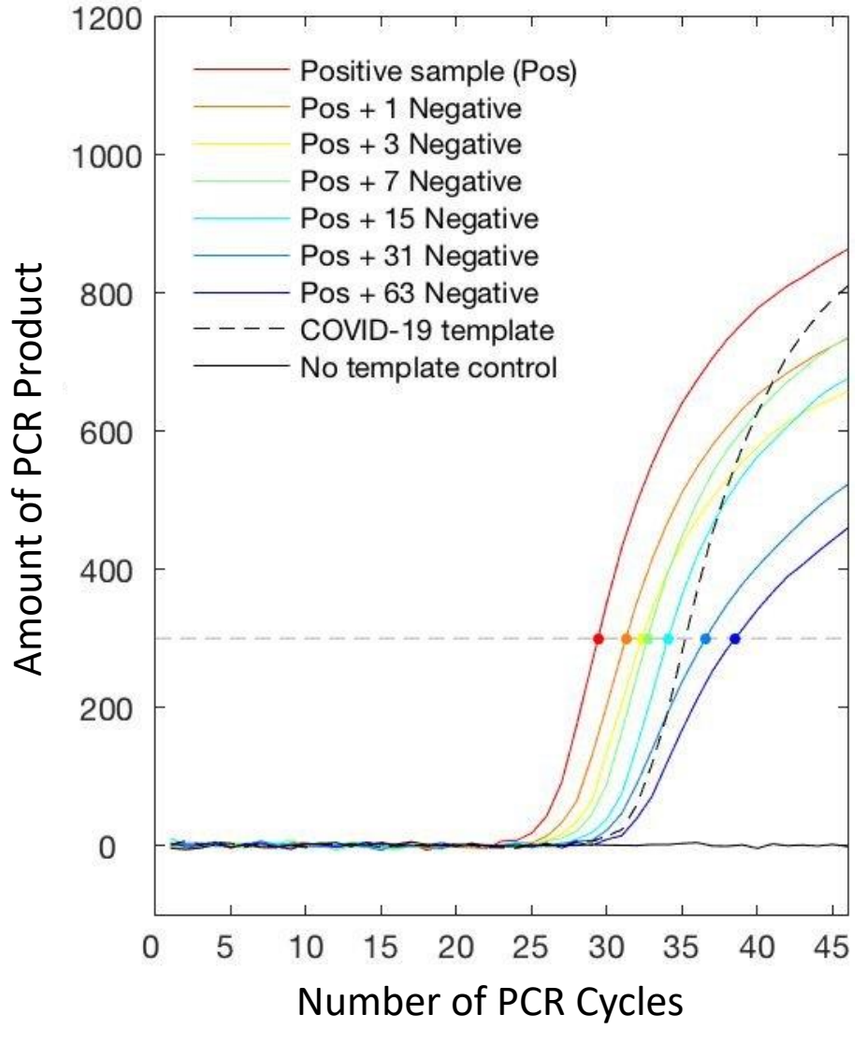
PCR Product

```
GACCCCAAAATCAGCGAAATGCACCCCGCATTACGTTTGGTGGACCCTCAGATTCAACTGGCAGTAACCAGA
CTGGGGTTTTAGTCGCTTTACGTGGGGCGTAATGCAAACCACCTGGGAGTCTAAGTTGACCGTCATTGGTCT
```

Will PCR generate products if the viral DNA is not present?

Covid 19 PCR Test: Detection of the PCR Product.

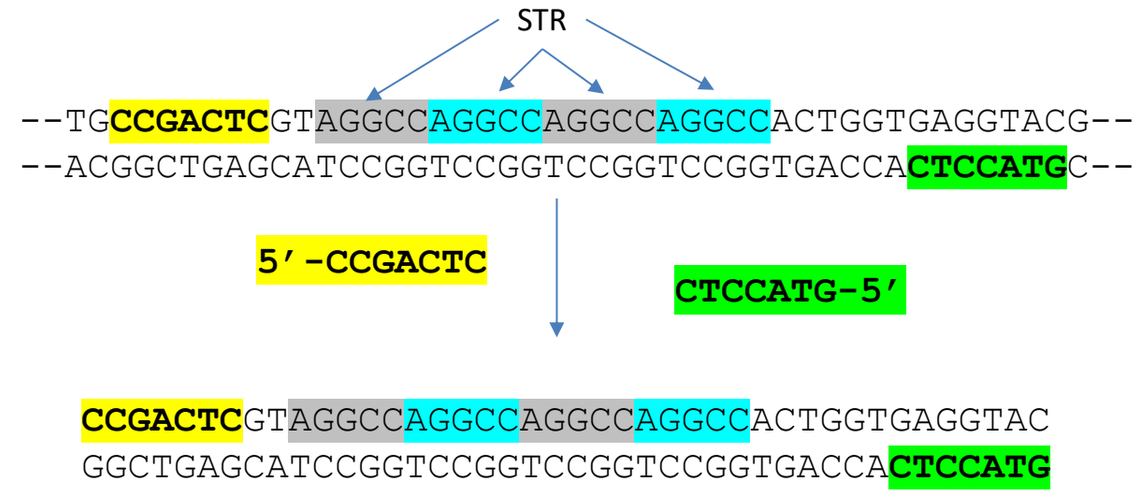
https://www.medrxiv.org/content/10.1101/2020.03.26.20039438v1



- Production of PCR products (double stranded DNA) causes an increase in signal (fluorescence)
- Signal above 300 considered to be positive (dashed gray line)
- Dots represent when a sample crosses the fluorescence threshold.
- Red curve (Positive sample) shows a threshold level of PCR product after 27 cycles.
- Next 6 samples are the positive sample mixed with up to 63 negative samples, showing that it is possible to test pooled samples.
- - - - is a ***positive control*** amount of Covid template. It shows that you can detect a PCR product if the covid genome is present.
- Solid black line is a ***negative control***, no Covid DNA. It shows that addition of covid template will lead to a signal.

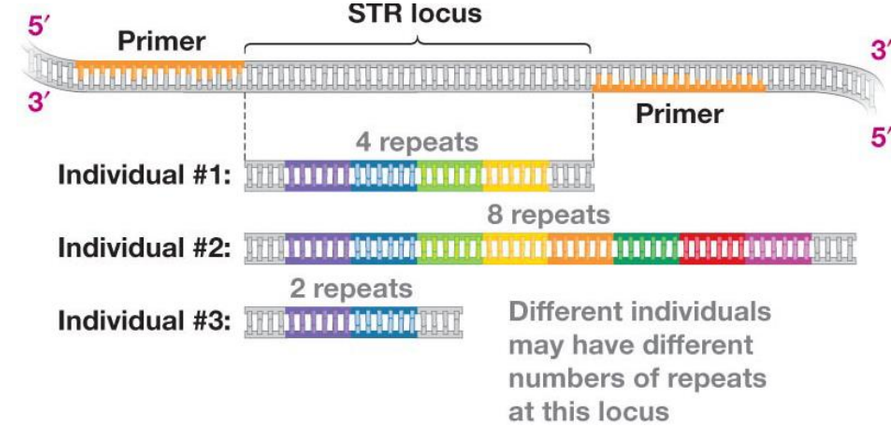
PCR Applications – Identification of Individuals

- Regions of DNA have variable numbers of repeated DNA sequences (Short tandem repeats, STR). The number of STR can differ from one person to the next.
- Individuals will inherit one copy of the repeat from each parent. The length of the inherited DNA can be the same or different.
- PCR Primers are designed to be outside the repeated region, so that they will anneal to a single location on the chromosome and then amplify the region containing the STR
- PCR Product length = primer lengths + number of tandem repeats (+ any DNA between the primers and the repeats). *Individuals can be differentiated by the length of the PCR product if they have different numbers of STR*



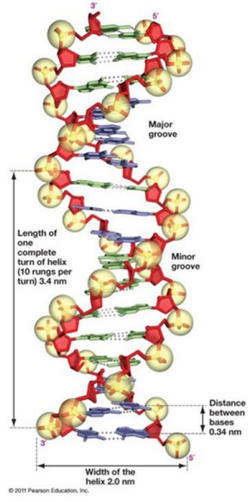
Which individual has the shortest PCR product?

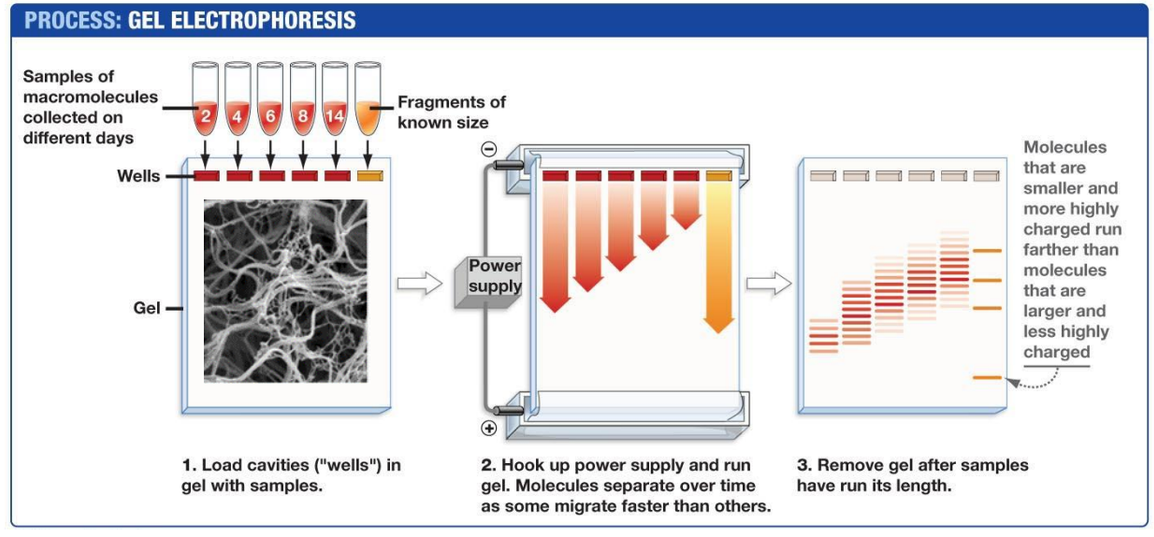
Which has the longest?

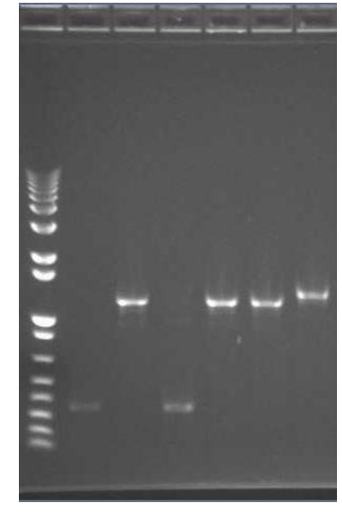


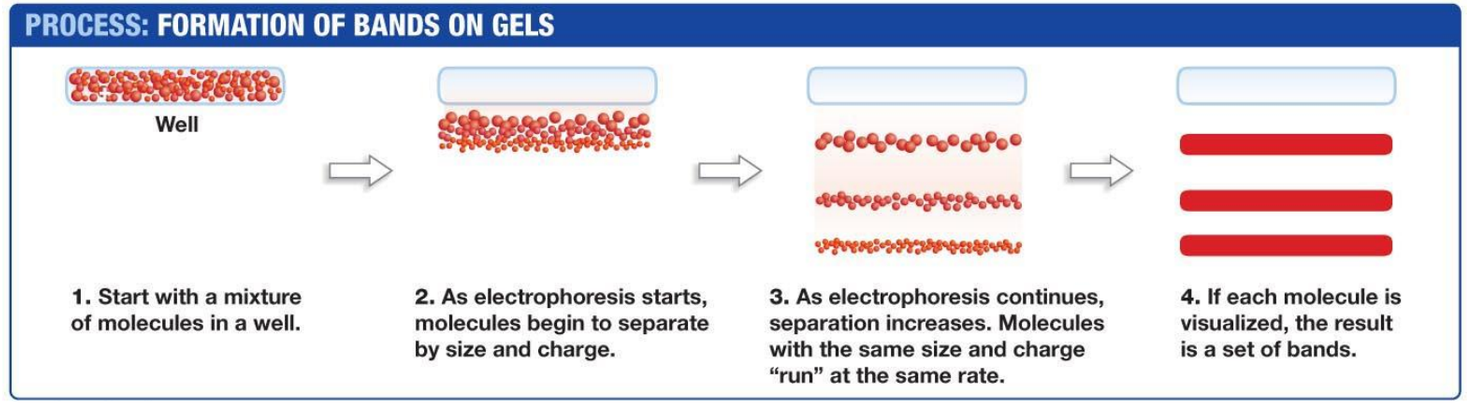
Size Determination of PCR products - Agarose Gel Electrophoresis.

https://dnalc.cshl.edu/resources/animations/gelelectrophoresis.html





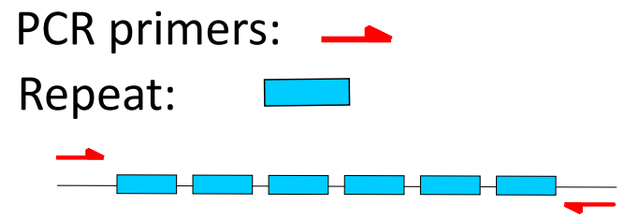




Which are the smallest PCR fragments?

Short Tandem Repeats to Test Paternity

1. DNA samples (blood, cheek cells) would be obtained from:
   - Mother
   - Child
   - Candidate fathers.
2. PCR would be preformed using primers that amplify a segment of the chromosome containing repeats.
3. Each individual would show 2 bands on the gel, corresponding to the PCR product from each chromosome (we have two copies of each chromosome).
4. The child would inherit one copy from the mother and the other from the father:
   - One of the child's PCR product would match one of the mothers.
   - The other PCR product from the child would match one of the PCR products from the father.

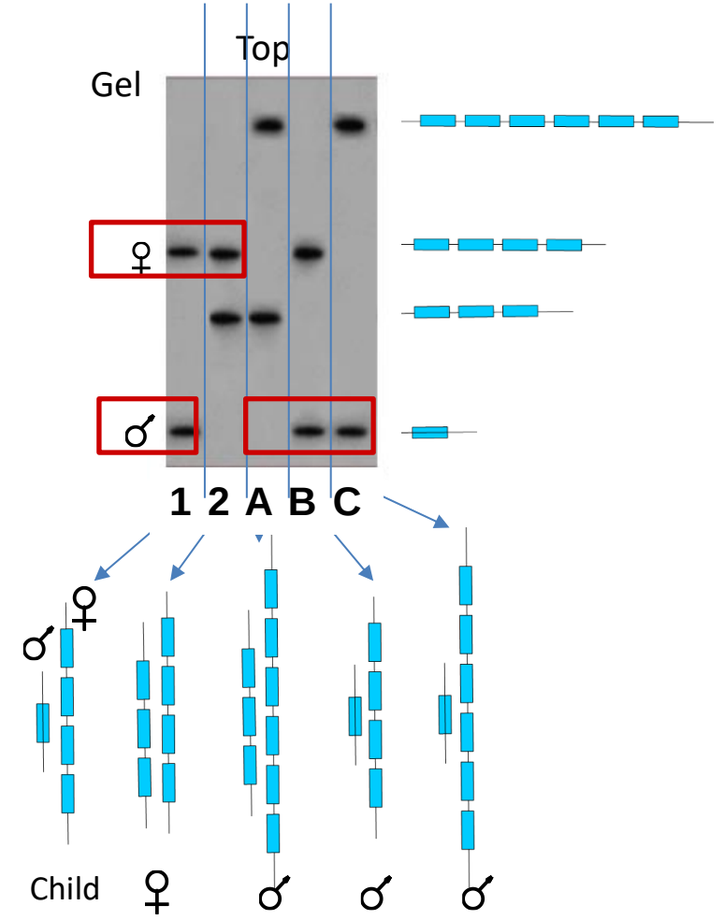


Lane 1: Child
Lane 2: Mother

Lanes A, B, C: Possible Fathers

*2. Who is **not** the father?*

*3. Who **may** be the father?*



Introduction to Immunology

1. Branches of the immune system (Innate and acquired)
2. Properties of antibodies (Quaternary structure, antigen recognition)
3. How antibodies are produced:
   - Genome DNA changes
   - mRNA splicing

4. How antibodies eliminate pathogens

Key Questions:

1. Why is the innate system important?
2. What is the origin of diversity in acquired immunity?
3. How are antibodies made.

The Nobel Prize in Physiology or Medicine 2018

Ill. Niklas Elmehed. © Nobel Media

James P. Allison

Prize share: 1/2

Ill. Niklas Elmehed. © Nobel Media

Tasuku Honjo

Prize share: 1/2

The Nobel Prize in Physiology or Medicine 2018 was awarded jointly to James P. Allison and Tasuku Honjo "for their discovery of cancer therapy by inhibition of negative immune regulation."

Some Important Definitions:

Antigen = something that is recognized by the immune system, e.g. bacteria, virus, pollen.

Epitope = the part of the antigen that is contacted by the antibody.

Antibody (Ab) = Y-shaped protein that recognizes antigens, found on the surface of B-cells or secreted by plasma cells. When bound to antigen, it can initiate a process that results in the destruction of the antigen. *Specificity is high due to AA sequence in the variable segments.*

Immunoglobulin (Ig) = antibody.

B-cell = involved in antibody production and recognition of pathogen. Has antibody molecule on its surface (as part of the B-cell receptor). Develops into plasma cells after activation by T_H cells. Called B-cells because they are generated in the organ called the Bursa in birds.

Plasma cell = derived from B-cell after activation of the B-cell, produces secreted antibodies with the *same specificity as the original B-cell.*

T_H cell = T-helper: *Required* to activate both B and T_C cells, as well as other cells in the immune system. Called T-cells because they mature in the thymus.

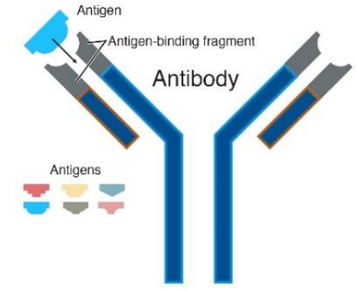
T_c cell = T-cellular: Involved in defense against viruses and cancer.

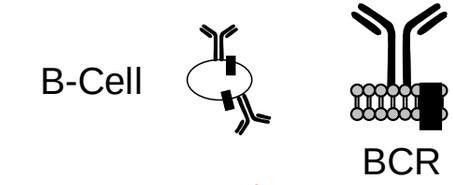
TCR = T-cell receptor – found on the surface of T-cells, recognizes MHC proteins + bound peptide, RTK.

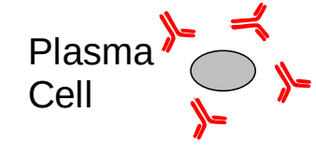
- **T_c cell** = recognizes MHC I + peptide
- **T_H cell** = recognizes MHC II + peptide

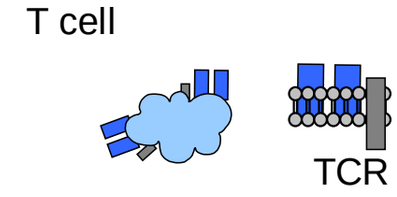
MHC = major histocompatibility complex – required for acquired immunity (basis of transplant rejection)

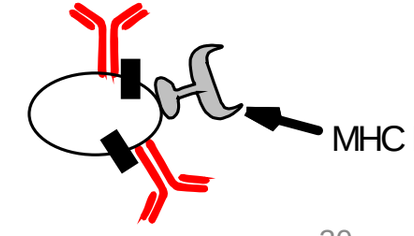
- MHC I = protein found on the surface of ***all*** cells, "presents" peptides derived from the proteins that were made by the cell. The MHC-peptide complex is recognized by T_c cells. ***Only foreign peptides produce a response***.
- MHC II = on the surface of B-cells, macrophages, and dendritic cells. Presents external peptides to T_H cells, leading to activation of the cell by T_H cells. ***Only foreign peptides produce a response***.





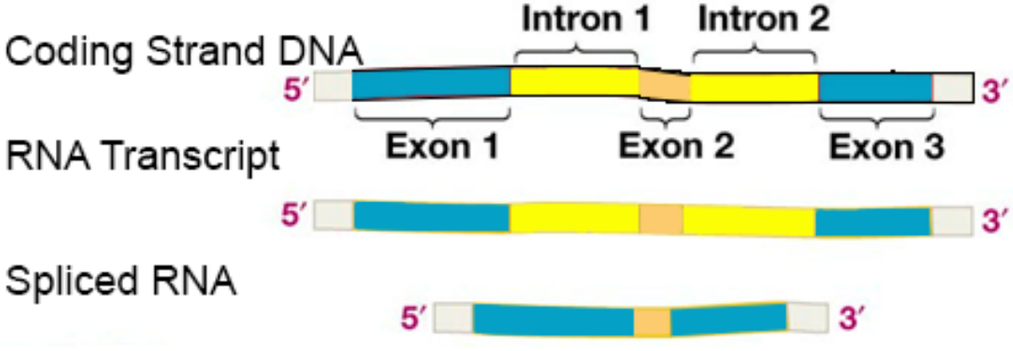




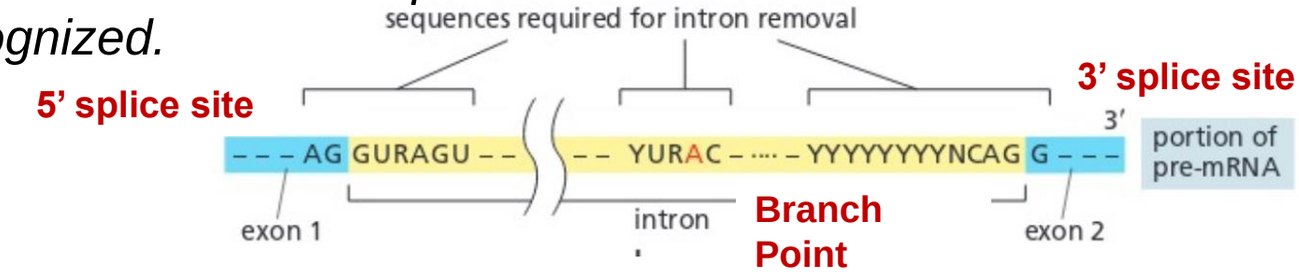


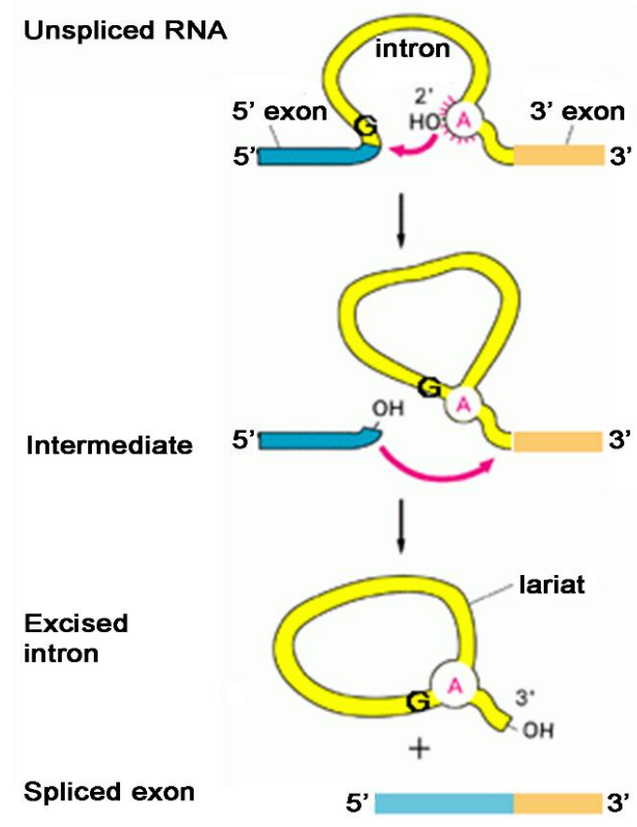
mRNA Splicing Required to Produce Functional mRNA

- When DNA sequences were aligned to RNA sequences, it was found that segments were deleted in the final RNA.
- This suggested that the gene encoding a protein was coded by segments of the DNA:
  - Those to be in the final mRNA were called **exons**.
  - Those sections not in the mRNA were called introns (intervening sequences).



Splice sites are recognized due to specific sequences at the exon-intron boundaries. *Sequences in both the exon and intron are recognized.*





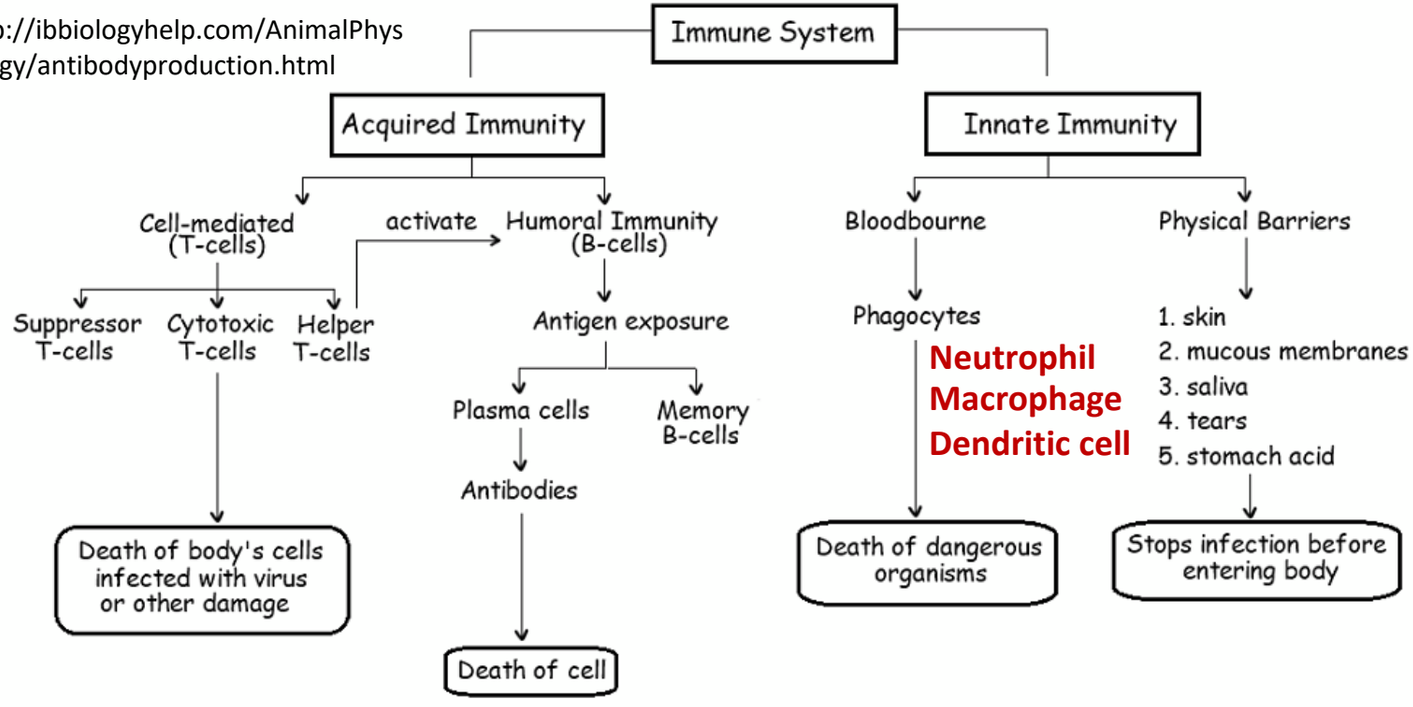
1. There is a 5’ splice site with a conserved sequence: (A/C)AG|GU(A/G)AGU
2. There is a 3’ splice site with a conserved sequence: CAG|G
3. There is an A in the intron (branch point) required for splicing.

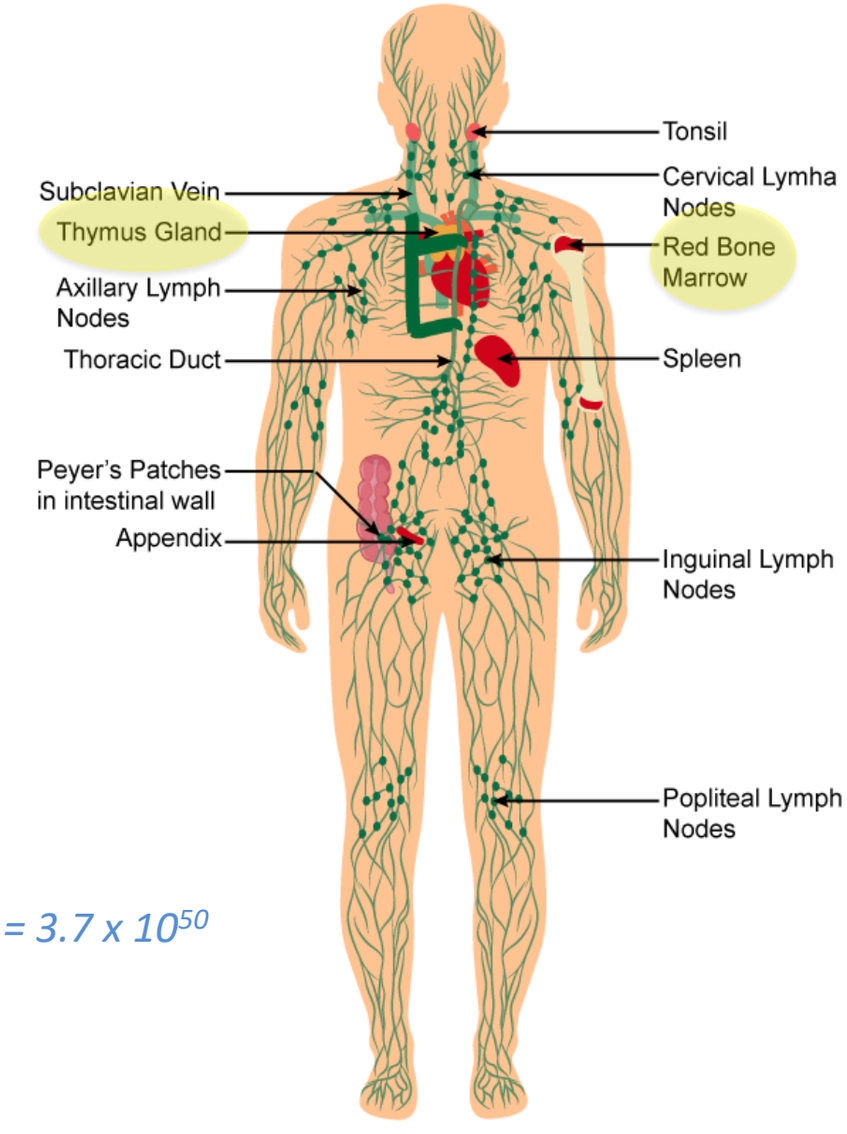
Steps:

1. The branch A breaks at the 5’ splice site, forming a lariat.
2. The 5’-OH is joined to the 5’ end of the downstream 3’ exon.

Branches of the Immune System:

http://ibbiologyhelp.com/AnimalPhysiology/antibodyproduction.html





https://www.topperlearning.com/

Why is the innate system essential?

- *A pathogen doubles every hour.*
- *It takes 7 days to produce antibody (after 1st exposure)*
- *How many bacteria would be present if they grew uncontrolled for 7 days: $2^{24 \times 7} = 3.7 \times 10^{50}$*

(there are approximately 10^{13} cells in the human body)

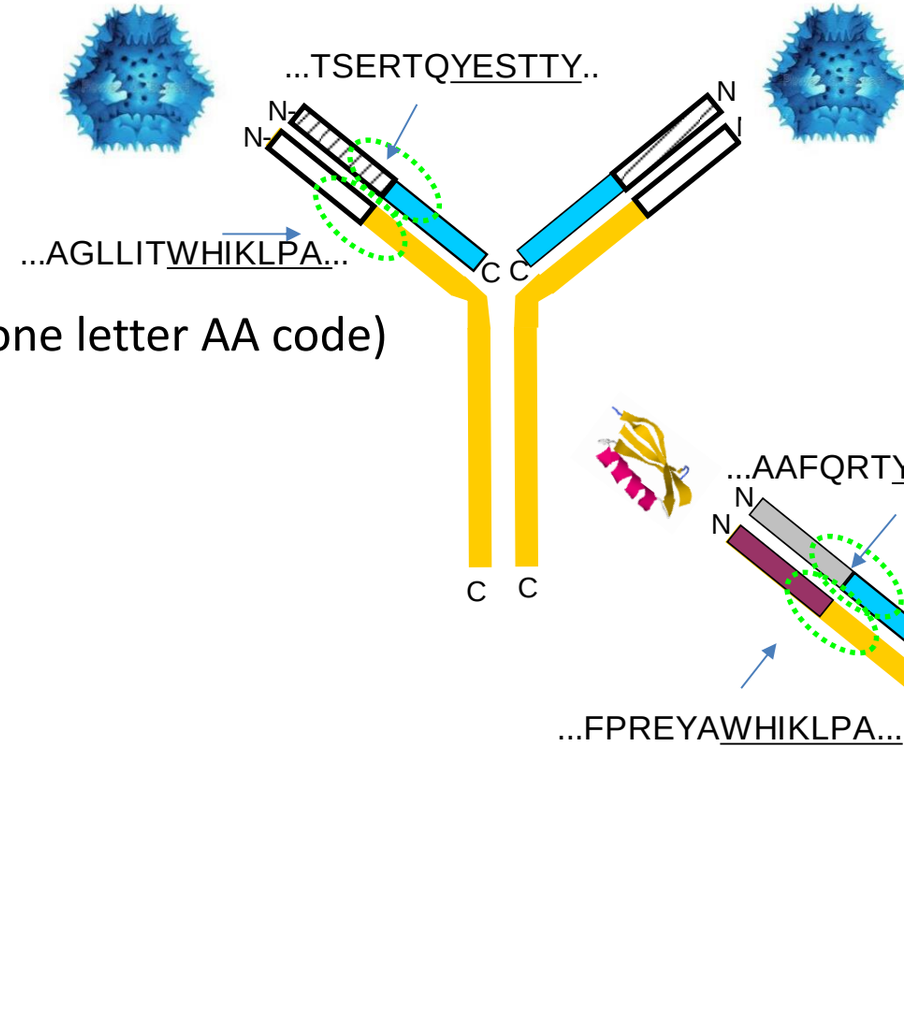
Important **primary** lymphatic organs: bone marrow (B), thymus (T)- Generate all immune cell.

Important **secondary** lymphatic organs: lymph nodes, spleen, Peyer's patches – Activation of immune cells.

Antibody Structure and Diversity

Each Antibody:

- Two identical light chains
- Two identical heavy chains
- First ~100 Amino acids on each chain are called the variable region and differ from antibody to antibody.
- Unique sequence for variable region of both heavy and light chains ***– defines specificity – different antibodies bind different antigens.***
- Constant regions - same protein sequence for all.



Light chain sequences:

```
M..TSERTQYESTTY..RGEC
M..AAFQRTYESTTY..RGEC
1.....110.........210
```

Coded by different exons

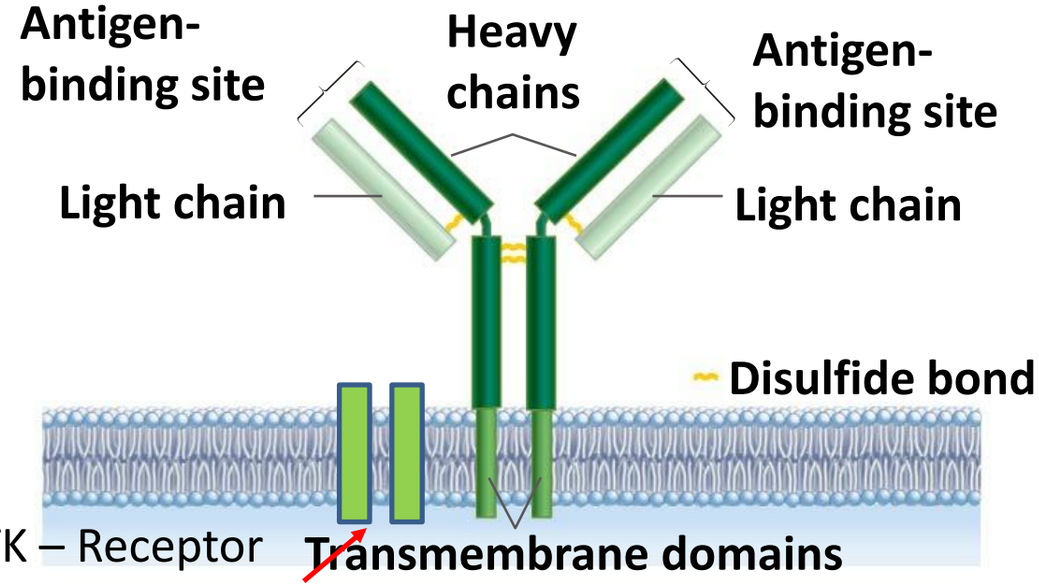
Heavy chain sequences:

```
M...AGLLITWHIKLPA...SPGK
M...FPREYAWHIKLPA...SPGK
1......120............449
```

Production of Antibodies by B-cells & Plasma Cells

B- Cells & B-cell Receptor (BCR)

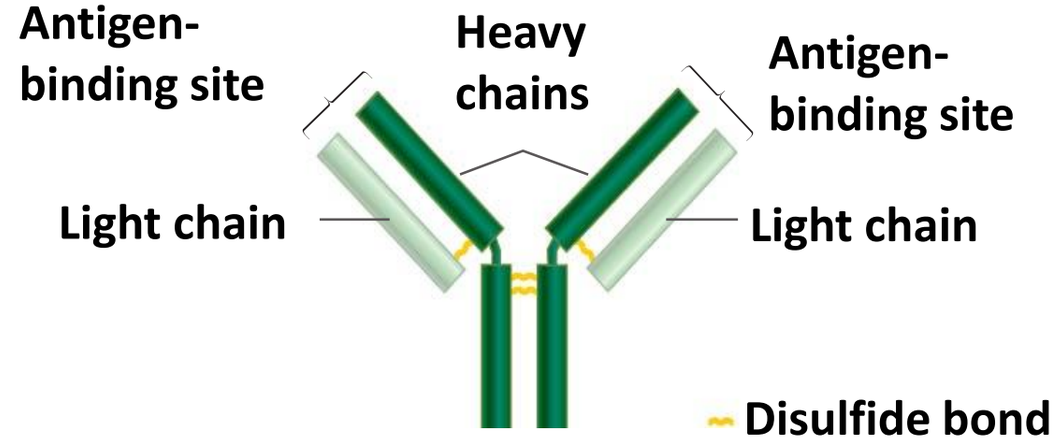
- Each B-cell has only one type of antibody as part of its BCR (B-cell receptor), i.e. the 10^5 BCRs are *homogeneous* on the same cell.
- Approximately 10^8 different specificities at any one time. i.e. *10^8 different B-cells!*



RTK – Receptor tyrosine kinase – signaling domain.

Plasma Cells:

- After activation, a B-cell develops into a plasma cell.
- The antibody is secreted.
- The same light chains are produced.
- The heavy chains differ only in the absence of the transmembrane domains.



mRNA that codes for antibodies contains two types of sequences:

- Exons – contain codons for the amino acids
- Introns – removed before translation

Different exons are used to produce membrane bound or soluble antibodies.

B-Cell Biology - From Stem Cells to Pathogen Killing.

1. Generation of high diversity of chains during development of stem cells to B-cells in bone marrow.

- DNA rearrangements to generate functional exons for variable segments of both light and heavy chain.

2. Molecular & cellular biology of membrane bound antibodies on cell surface = B-cell receptor (BCR)

- Transcriptional enhancers, mRNA splicing
- Light chain and heavy chain exported to surface of B-cells.

3. Self tolerance test to prevent autoimmune diseases, autoreactive B-cells eliminated.

4. Encounter and capture of antigen in lymph nodes

5. Activation of B-cells by T_H cells

- Peptides from pathogen presented on major histocompatibility proteins (MHC II).
- T-cell activation by tyrosine kinase receptors (T-cell Receptor, TCR), secretion of signaling molecules.

6. Development of

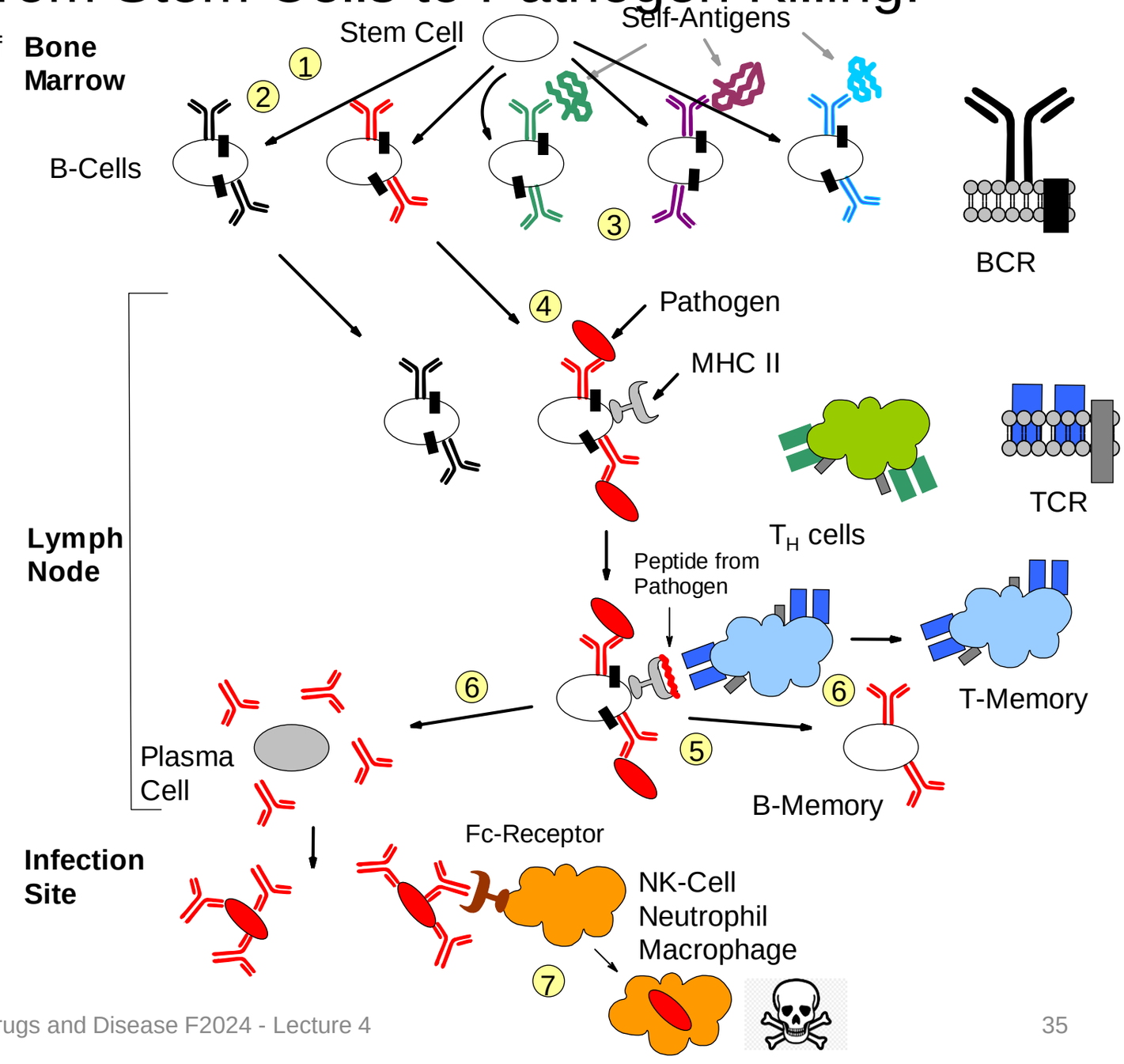
- Plasma cells - Production of soluble antibodies of the same specificity as the parent B-cell.
- B-memory cells (basis of immunity)
- T-memory cells (basis of immunity)

7. Destruction of Pathogens

- Fc region of antibody binds to Fc Receptor on NK cells, neutrophiles, macrophages
- Pathogen internalized and destroyed.

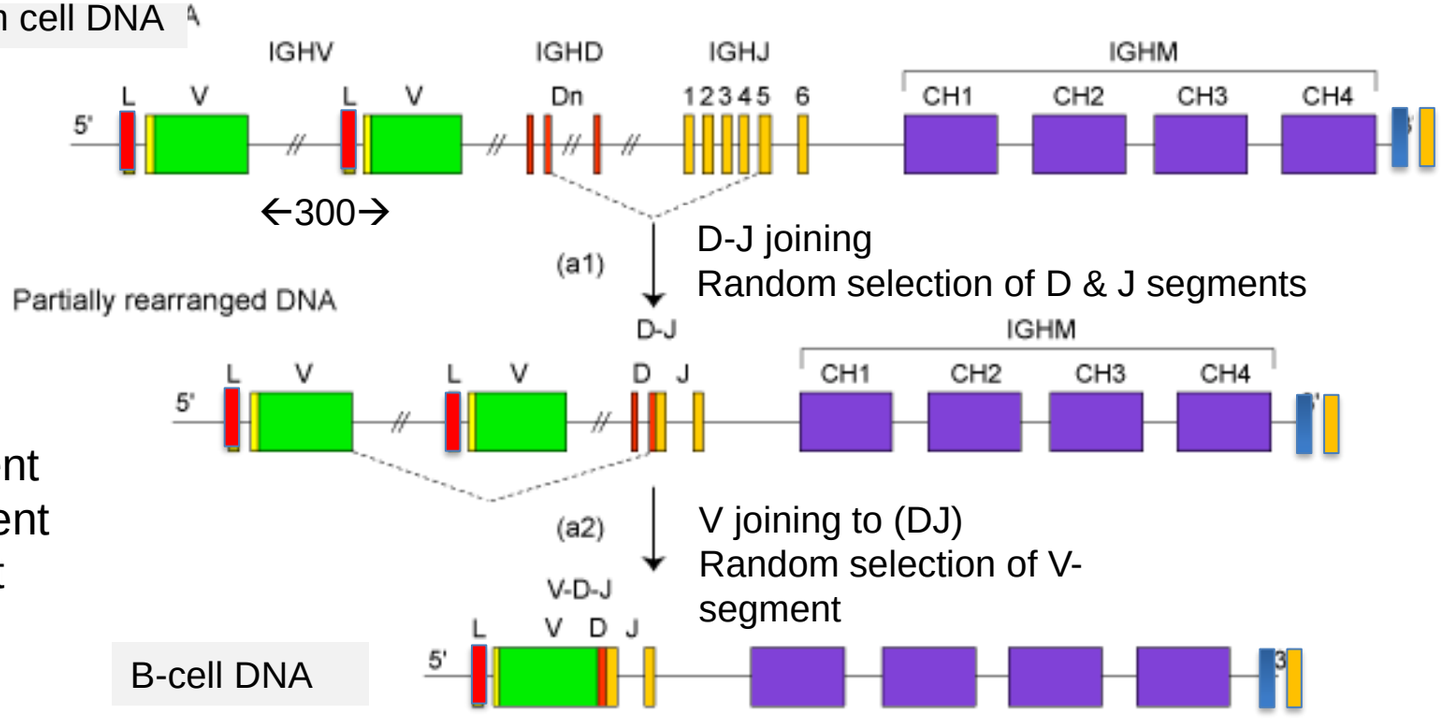
BCR – B-cell receptor = antibody + signaling chains.

TCR – T cell receptor = MHC-peptide recognition + signaling.



Antibody Genes are Assembled From DNA Segments: Giving many different sequences.

Production of Heavy Chain Gene:
V_H exon = V+D+J segment (selected at random)

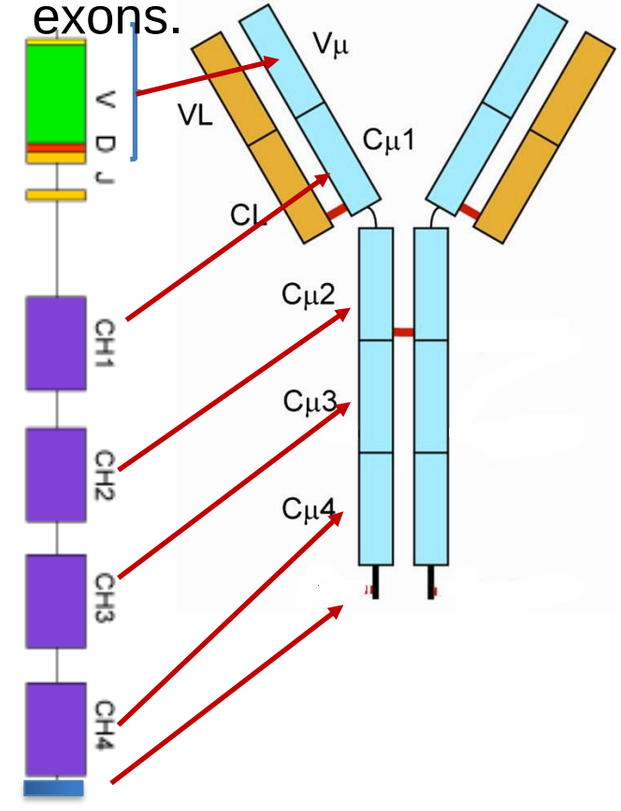


L = leader (signal)
V = variable segment
D = diversity segment
J = joining segment

V_H exon differs from one cell to the next.

Heavy chains have the same constant sequences in all B-cells.

The mRNA coding for antibodies contains 5 exons.



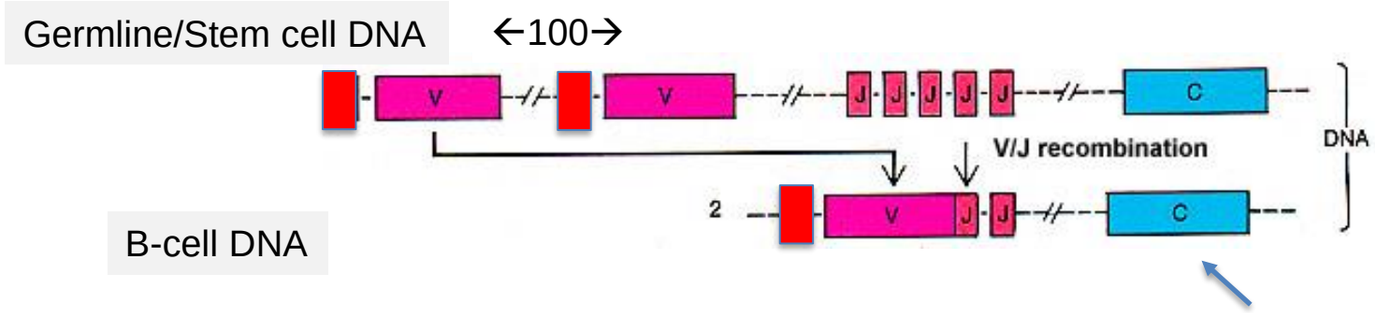
Stem cells -> B-cells

- The exon that codes for the variable region of the heavy chain is generated by the random joining of a V, D, and J DNA segments.
- Each B-cell will generate a unique sequence for its heavy and light chain DNA.
- This is a permanent change to the DNA (***genome)*** of the B-cell.

1. If there are 300 possible V-heavy segments, 10 possible D segments, and 6 possible J segments, how many different heavy chains can be made?

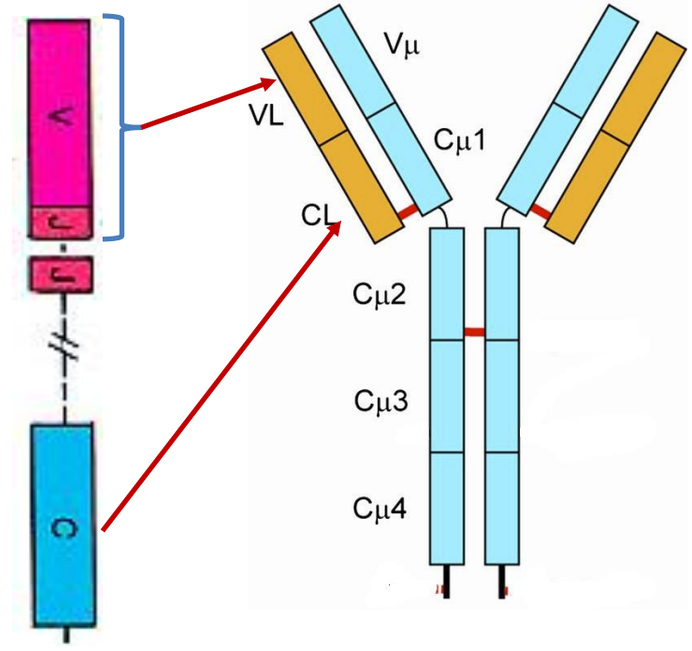
Light-chain Genes are Assembled From DNA Segments: Giving many different sequences.

Production of Light Chain Gene



Light chains have different V-sequences in different B-cells.

Light chains have the same constant sequences in all B-cells.



Antibody Diversity

1. If there are 100 possible V-heavy segments and 5 possible J segments, how many different light chains can be made?

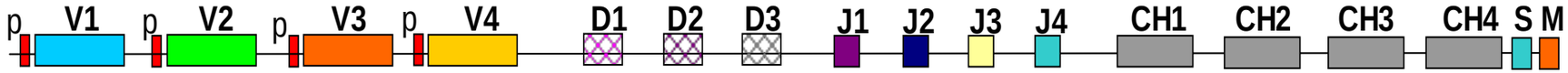
2. If any possible heavy chain can pair with any possible light chain, how many different antibodies can be generated, assuming there are 10,000 possible heavy chains and 500 different light chains?

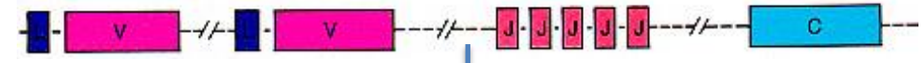
Stem cells -> B-cells

- In the case of the light chain, the variable region is generated by VJ joining.
- Each B-cell will generate a unique sequence for its heavy and light chain DNA.
- This is a permanent change to the DNA (***genome)*** of the B-cell.

Antibody Production – From Stem Cells to B-Cells

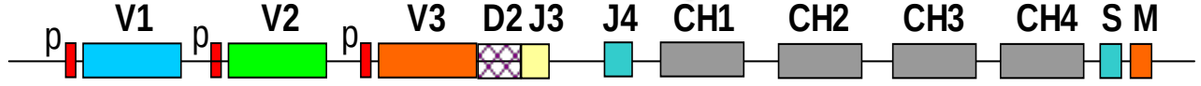
Stem Cell





B-Cell

Rearranged heavy chain gene

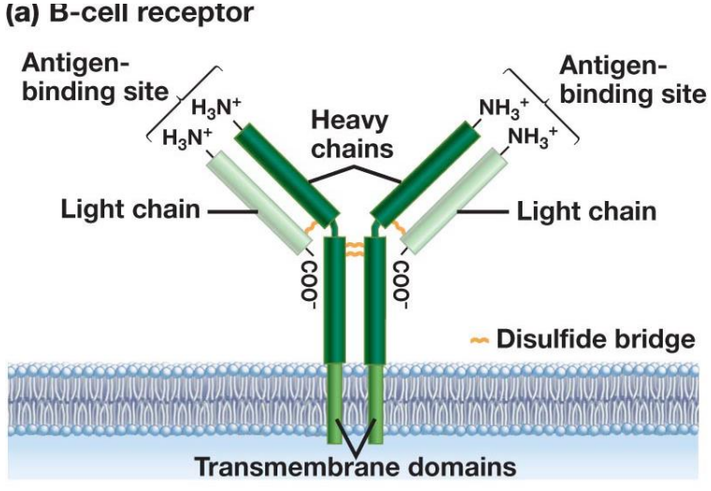


Rearranged light chain gene

Transcription

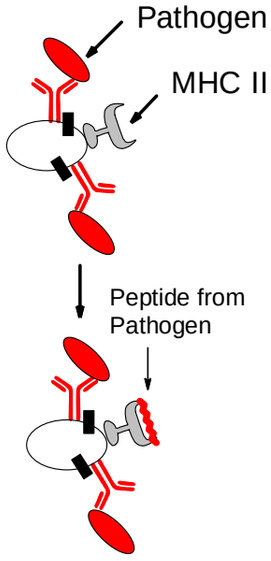
mRNA Splicing

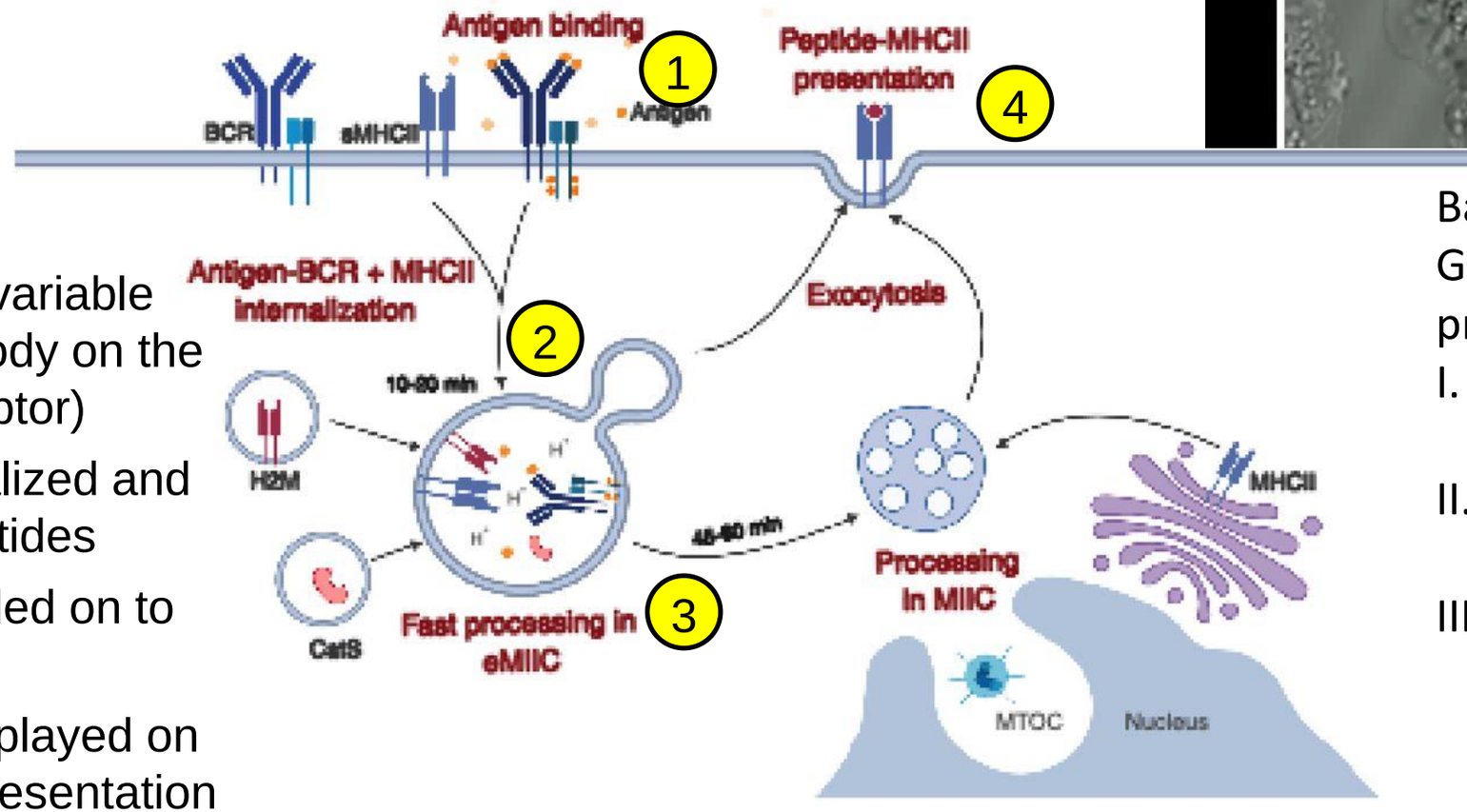
Translation

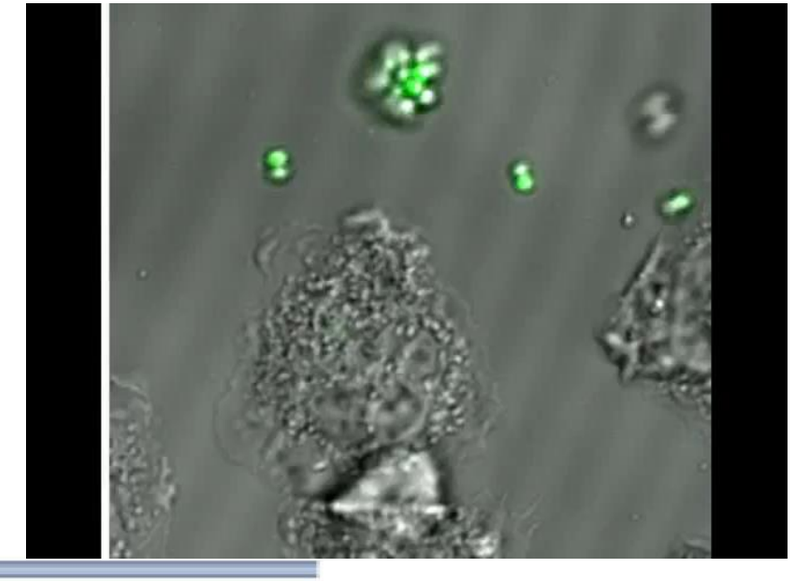


Export to cell surface (ER -> golgi -> Membrane)

Antigen Capture by B-Cells - Endocytic Pathways







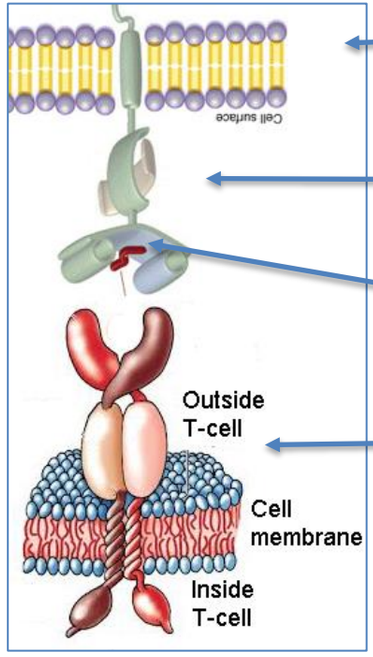
Bacteria labeled with Green fluorescent protein.

I. Capture of the bacteria
II. Internalization (endocytosis)
III. Degradation of the bacterial proteins, producing peptides.

1. Antigen binds to variable domains of antibody on the BCR (B-cell receptor)
2. Antigen is internalized and digested into peptides
3. Peptides are loaded on to class II MHC
4. Peptide-MHC displayed on membrane for presentation to T-cells

Journal of Cell Science doi: [10.1242/jcs.235192](https://doi.org/10.1242/jcs.235192)

Activation of B cells by Antigen - Lymph Node



B-cell

MHC II - Major histocompatibility complex

Peptide from pathogen (foreign peptide)

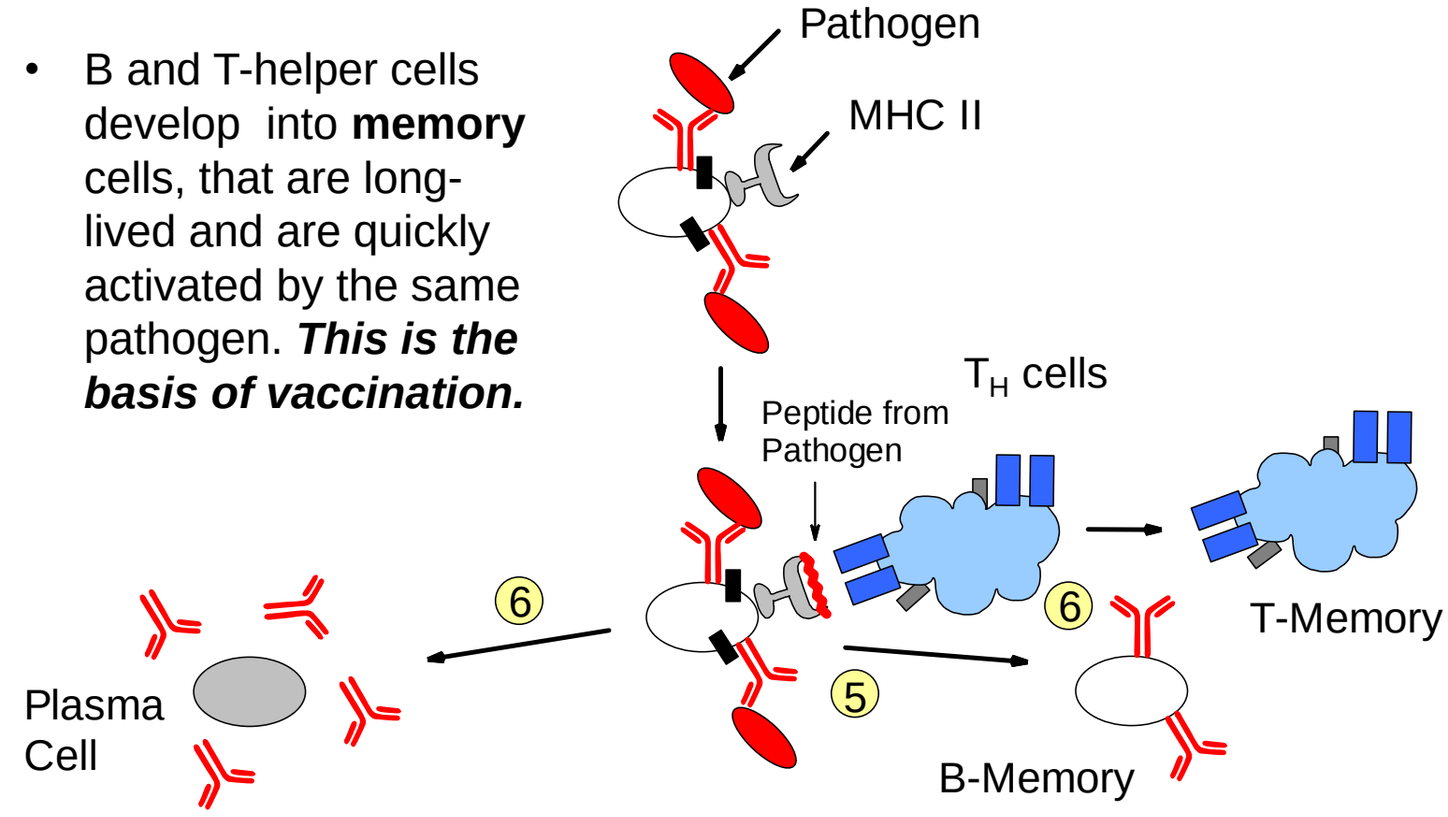
T-cell Receptor (TCR)

T_H cell

Events:

1. Recognition of MHC II-peptide by TCR
2. Tyrosine kinase signaling in T_H cell
3. Cytokines (protein messengers) produced.
4. Cytokines activate B-cells.

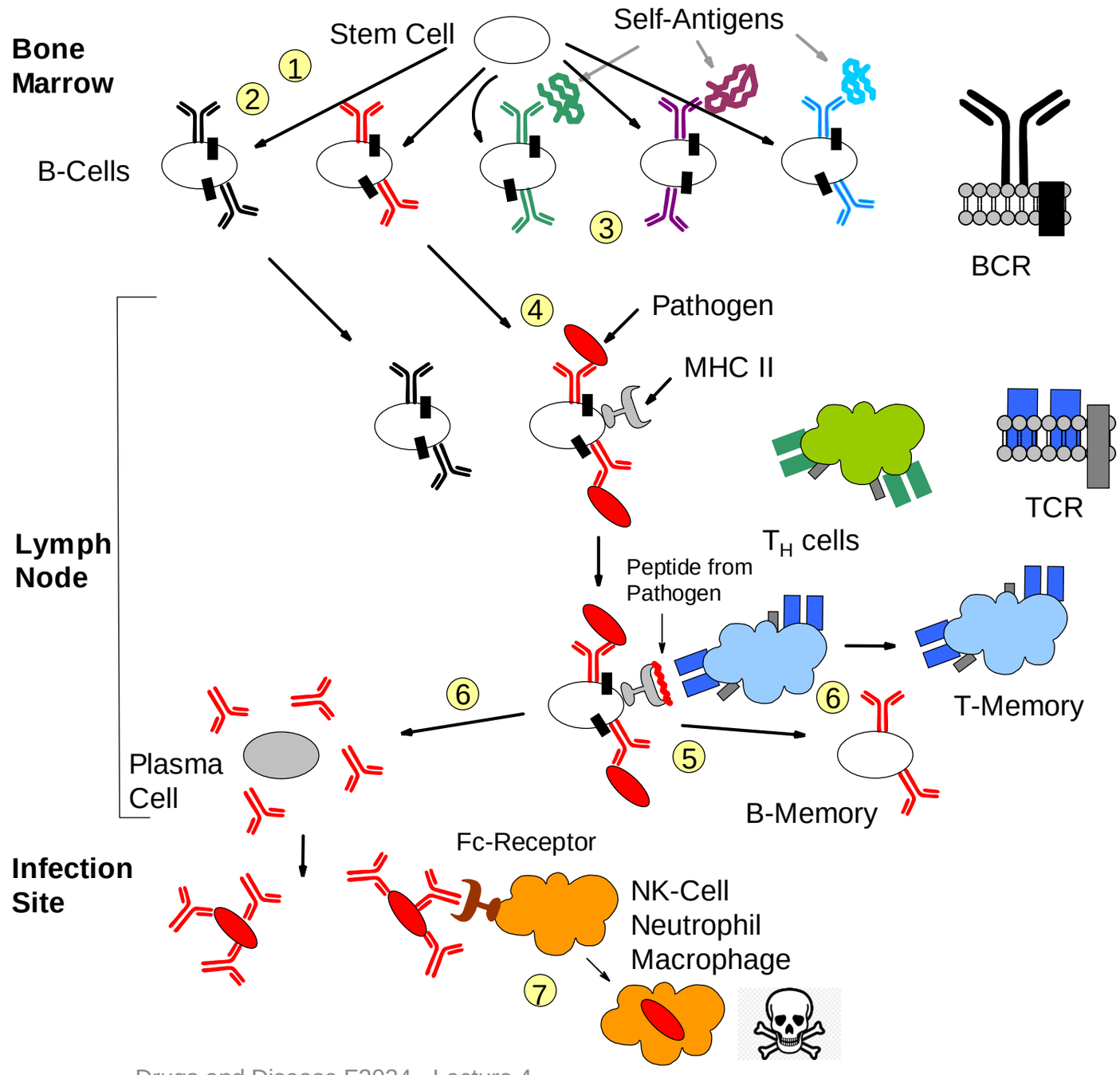
- B-cells develop into antibody secreting *plasma cells*.
- B and T-helper cells develop into **memory** cells, that are long-lived and are quickly activated by the same pathogen. ***This is the basis of vaccination.***



- Soluble antibody from plasma cells has the same light and heavy chains as the original B-cell.
- Membrane anchors are missing, so antibody is secreted outside the cell.

Can you:

- Describe how the genes for the heavy and light chain are generated, and how this give rise to many different antibodies?
- Do you understand the process of B-cell activation, including presentation of foreign peptides on MHC II and the role of the T-helper cell.
- Describe how antibodies inactivate pathogens?



Cell Based Immunology

Key Questions:

1. How does your immune system fight viruses?
2. How does your immune system detect and destroy cancer cells?
3. How can the immune response be engineered to fight cancer?

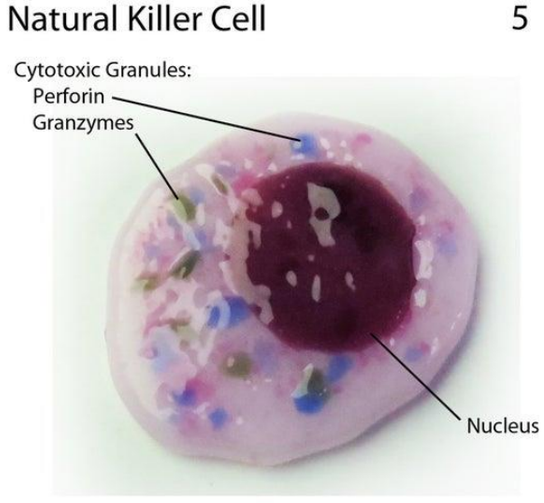
Cell Types:

Innate

- Natural Killer (NK) cell

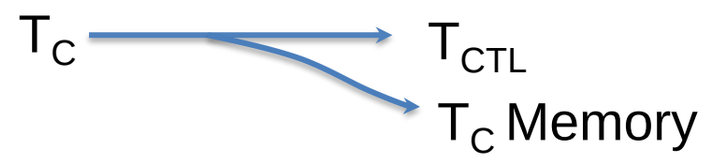
Acquired

- T_H
- T_C, T_{CTL}



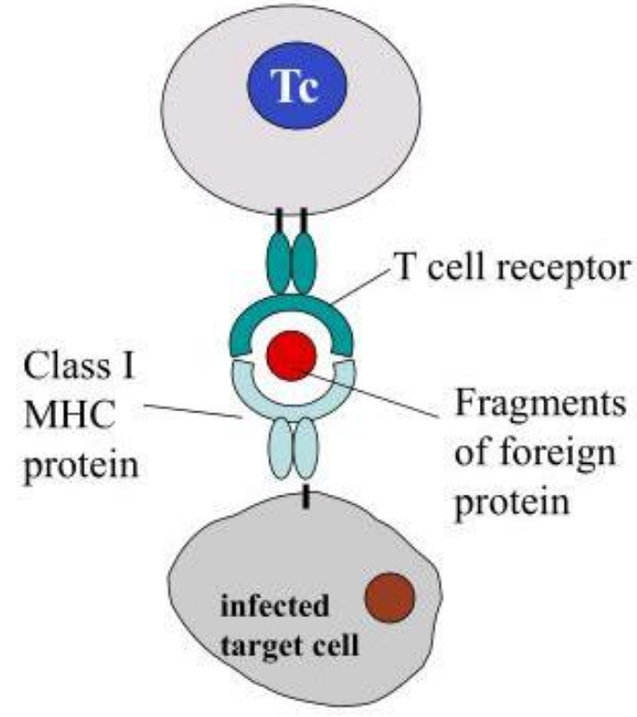
NK: Innate

- Kill virally infected cells
- Kill cancer cells



Activation of Tc cells requires:

1. Recognition of ***foreign*** peptide on MHC I.
2. Assistance from T-helper cells.



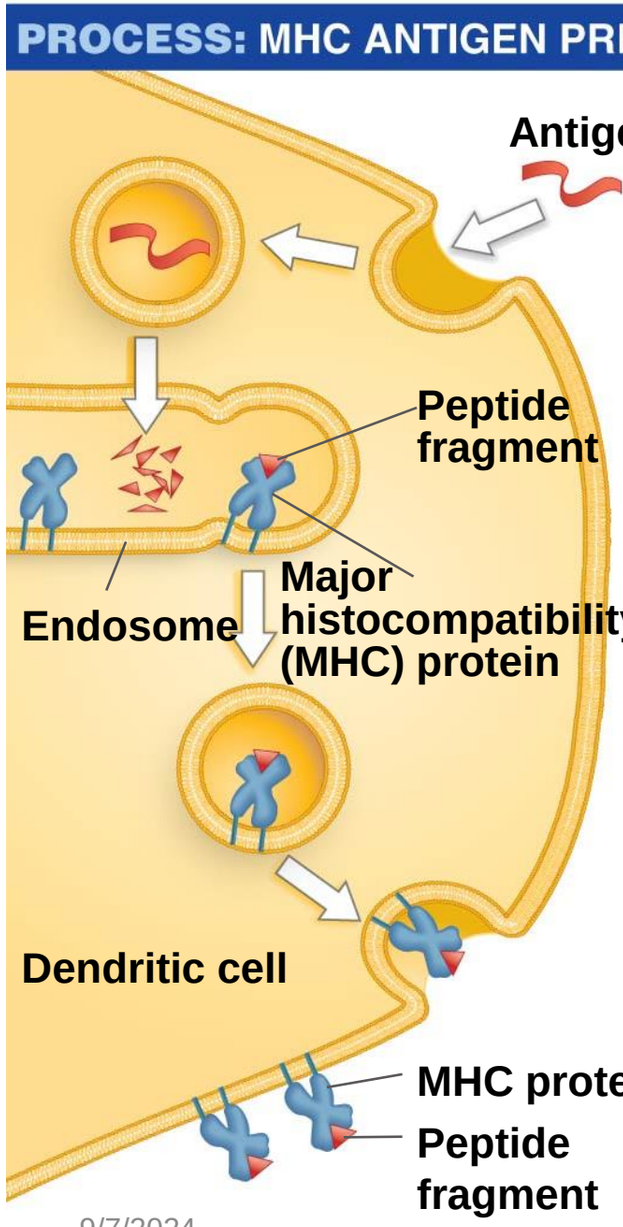
Activated Tc cell becomes a cytotoxic T-lymphocyte T_{CTL}

T_{CTL}

- Kill virally infected cells
- Kill cancer cells

Activation of Tc-Cells

A. Dendritic Cells Acquire Antigen from Viruses and Cancerous Cells



1. Dendritic cell ingests antigen via ***phagocytosis*** (intact virus, cell debris from cancer cell).

2. Enzymes break antigen proteins into peptide fragments.

3. Peptide fragments are loaded onto ***both*** class I and class II MHC proteins in endosomes.

4. MHC I & II –peptide complex is transported to cell surface.

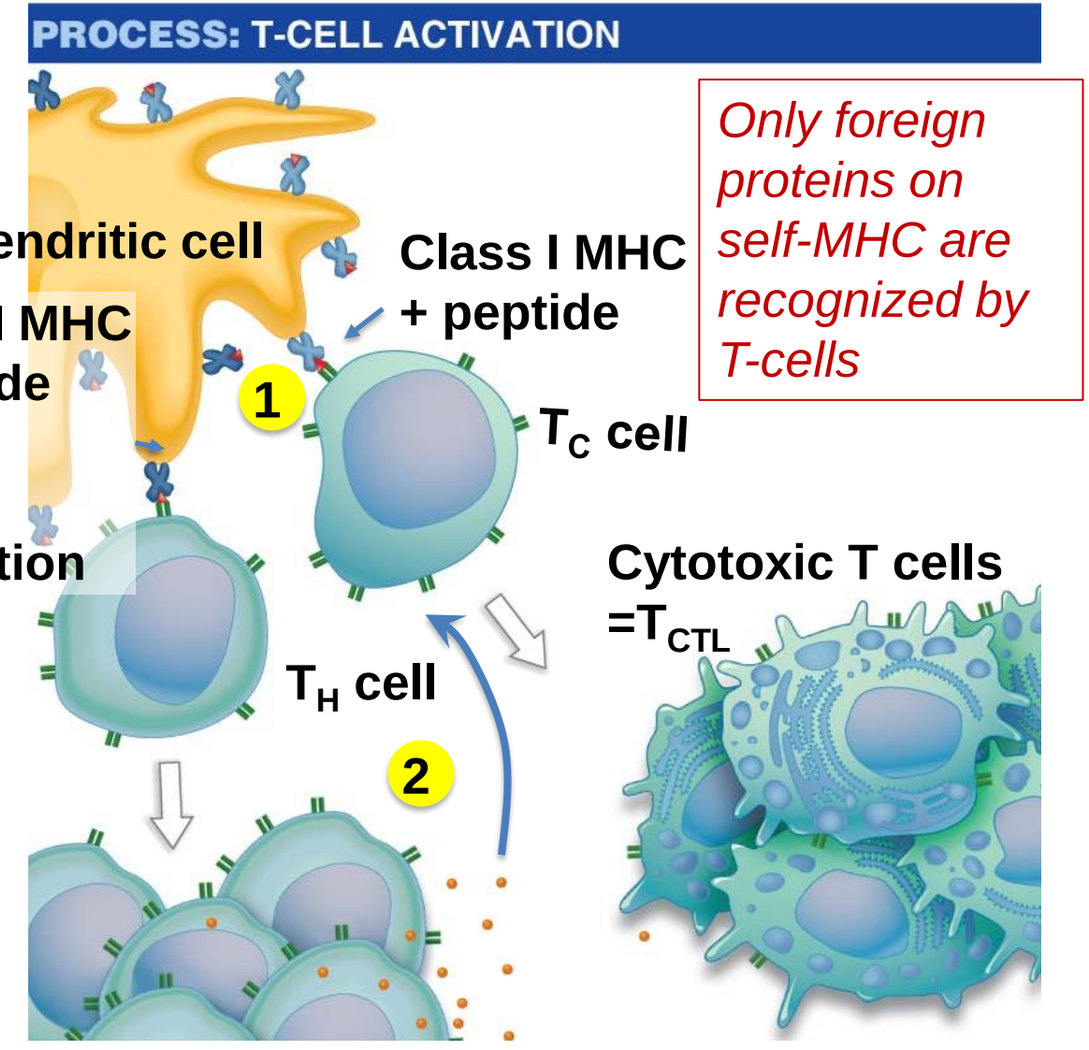
5. MHC protein presents peptide fragment on cell Surface to T-H and T-C cells.

Activation of Tc-Cells
B. Dendritic Cells Activate T_H and T_C cells.

Activation of Tc cells requires:

1. Recognition of foreign peptide on MHC I by TCR on Tc cell

2. Assistance from T-helper cells via secreted messengers (small proteins called cytokines)

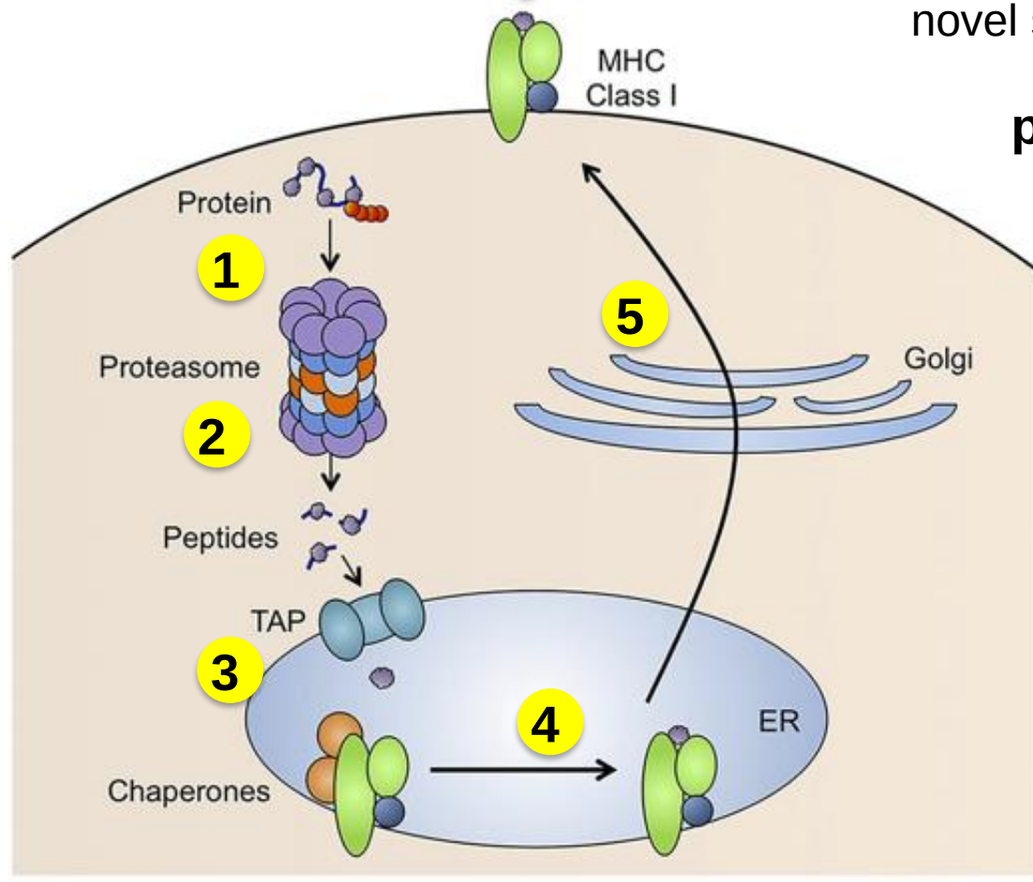


Only foreign proteins on self-MHC are recognized by T-cells

T_c Detection of Diseased/Cancer Cells - Role of MHC I

- MHC I present peptides
- Peptides are generated from of ***all*** of the proteins that are made in the cell.
- Steps:
  1. protein targeted for degradation by ubiquitin
  2. Protein digested by proteasome
  3. Peptides transported into ER
  4. Peptides loaded on to MHC I
  5. Peptide/MHC complex transported to cell membrane.

Only foreign peptides activate T-cells



Foreign Peptide Source:

1. From replication of viruses in the cell
2. From replication of intracellular bacteria (e.g. TB)
3. New coding sequences in cancer cells due to genetic changes (e.g. mutations in p53 lead to novel sequences).

p53 Protein Sequence

Zn Fingers (DNA binding)

```
        10         20         30         40         50
MEEPQSDPSV EPPLSQETFS DLWKLLPENN VLSPLPSQAM DDLMLSPDDI
        60         70         80         90        100
EQWFTEDPGP DEAPRMPEAA PPVAPAPAAP TPAAPAPAPS WPLSSSVPSQ
       110        120        130        140        150
KTYQGSYGFR LGFLHSGTAK SVTCTYSPAL NKMFCQLAKT CPVQLWVDST
       160        170        180        190        200
PPPGTRVRAM AIYKQSQHMT EVVRRCPHHE RCSDSDGLAP PQHLIRVEGN
       210        220        230        240        250
LRVEYLDDRN TFRHSVVVPY EPPEVGSDCT TIHYNYMCNS SCMGGMNRRP
       260        270        280        290        300
ILTIITLEDS SGNLLGRNSF EVRVCACPGR DRRTEEENLR KKGEPHHELP
       310        320        330        340        350
PGSTKRALPN NTSSSPQPKK KPLDGEYFTL QIRGRERFEM FRELNEALEL
       360        370        380        390
KDAQAGKEPG GSRAHSSHLK SKKGQSTSRH KKLMFKTEGP DSD
```

EVVRRCPHHE Normal seq., ***ignored*** by TCR

EVVGGCPHHE Mutant seq. in cancer, ***detected*** by TCR

T_c Detection of Diseased/Cancer Cells - Role of MHC I

- MHC I present peptides
- Peptides are generated from of ***all*** of the proteins that are made in the cell.
- Steps:
  1. protein targeted for degradation by ubiquitin
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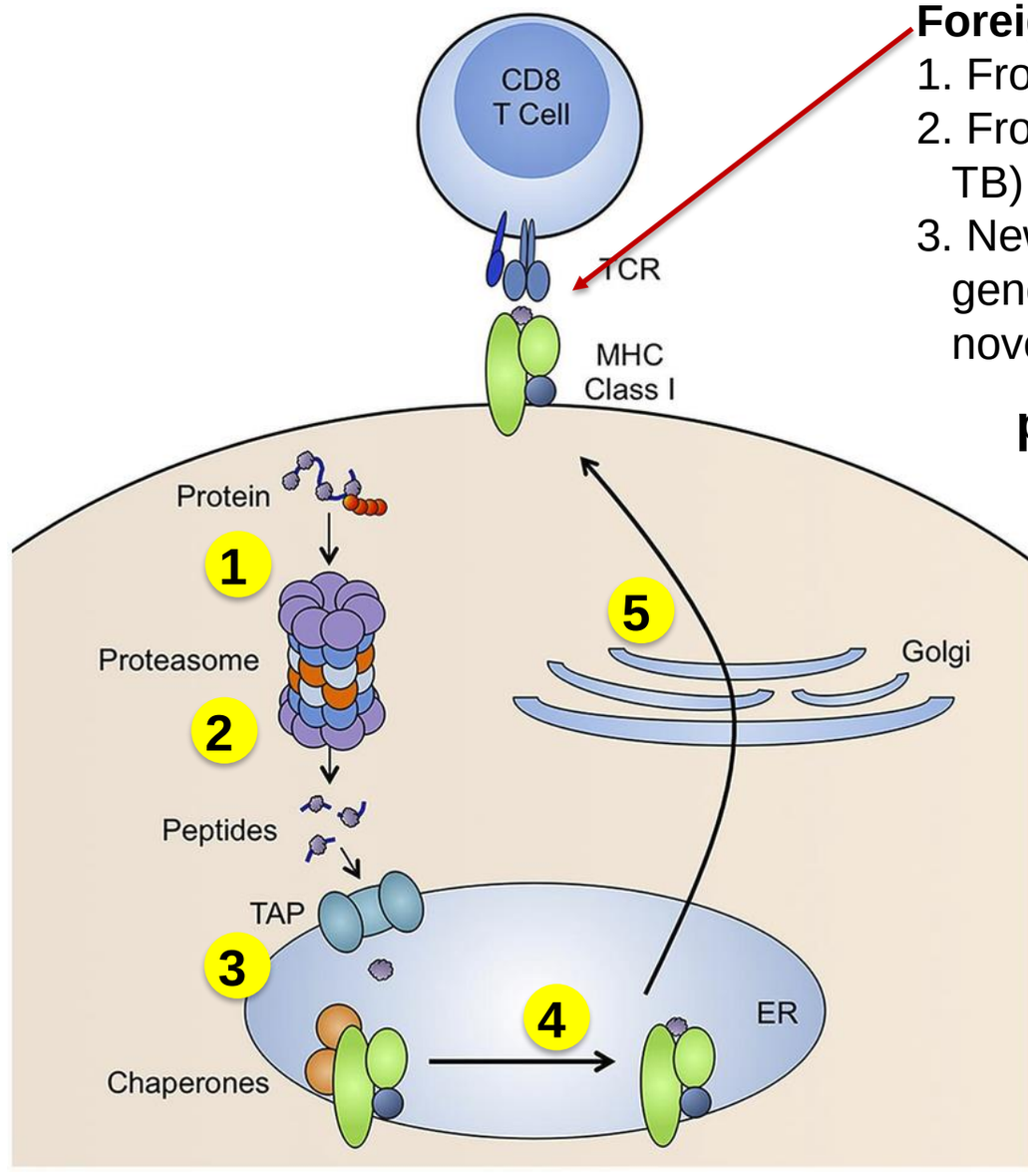

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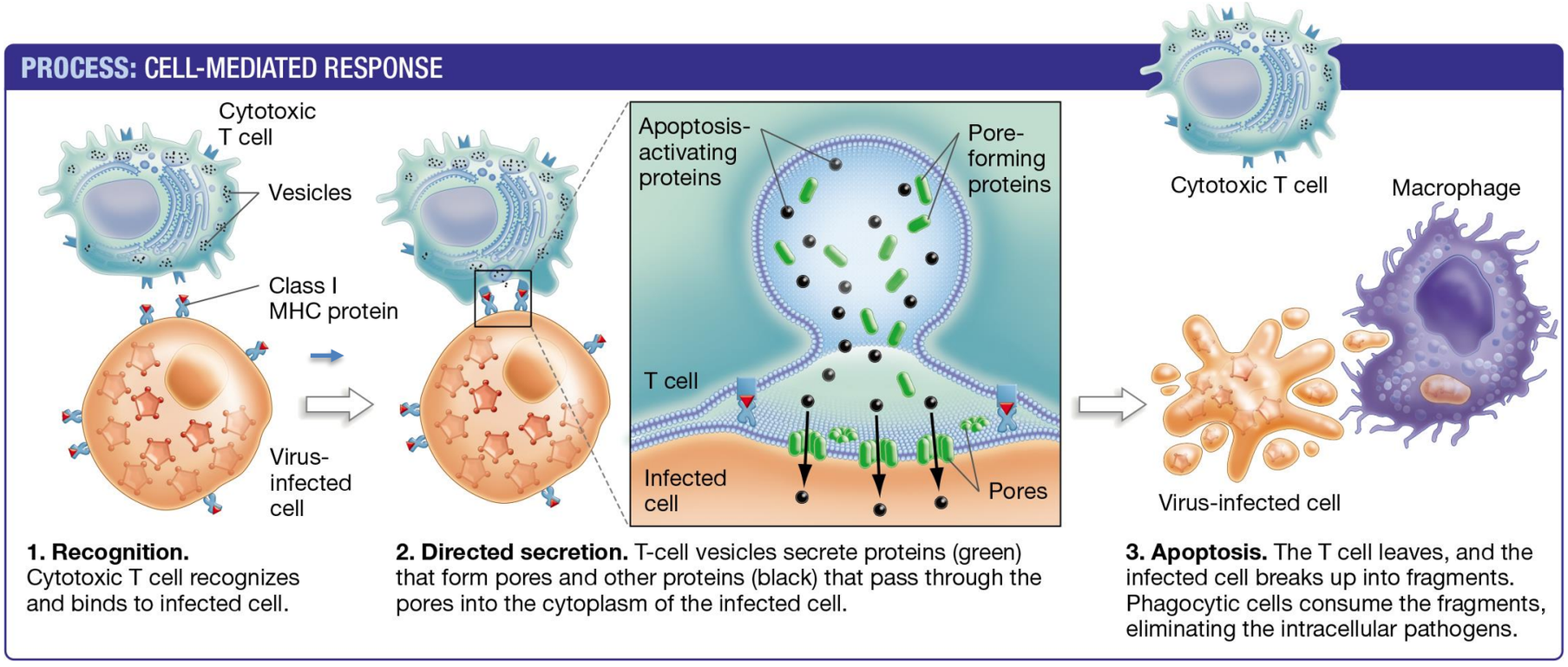
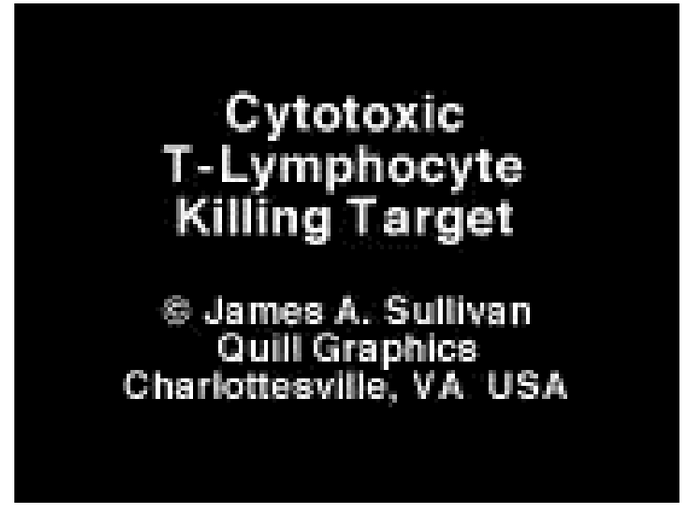
       110        120        130        140        150
KTYQGSYGFR LGFLHSGTAK SVTCTYSPAL NKMFCQLAKT CPVQLWVDST
       160        170        180        190        200
PPPGTRVRAM AIYKQSQHMT EVVRRCPHHE RCSDSDGLAP PQHLIRVEGN
       210        220        230        240        250
LRVEYLDDRN TFRHSVVVPY EPPEVGSDCT TIHYNYMCNS SCMGGMNRRP
       260        270        280        290        300
ILTIITLEDS SGNLLGRNSF EVRVCACPGR DRRTEEENLR KKGEPHHELP
       310        320        330        340        350
PGSTKRALPN NTSSSPQPKK KPLDGEYFTL QIRGRERFEM FRELNEALEL
       360        370        380        390
KDAQAGKEPG GSRAHSSHLK SKKGQSTSRH KKLMFKTEGP DSD
```

EVVRRCPHHE Normal seq., ***ignored*** by TCR

EVV**GG**CPHHE Mutant seq. in cancer, ***detected*** by TCR

T_C Cells: Detection and Killing of Virally Infected or Cancer Cells





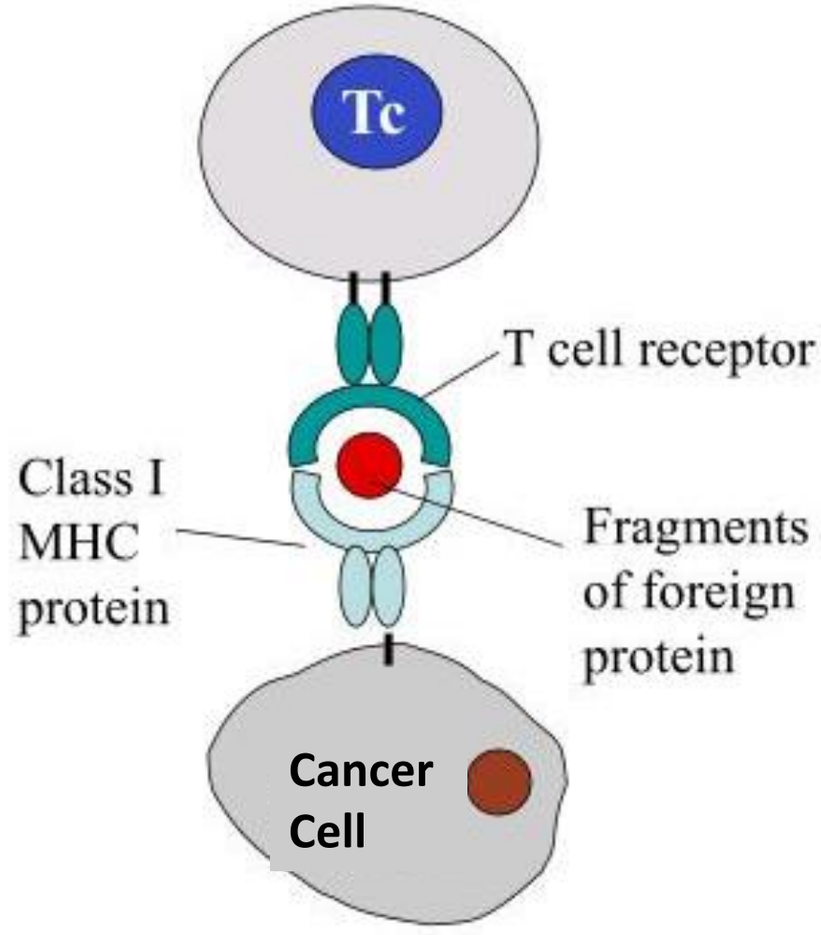
Cancer cell or Infected cell

- Granzymes enter through perforin pore and cause cell undergo programmed cell death (apoptosis)

Cancer Evasion Mechanism I - Loss of MHC I on Tumor Cell

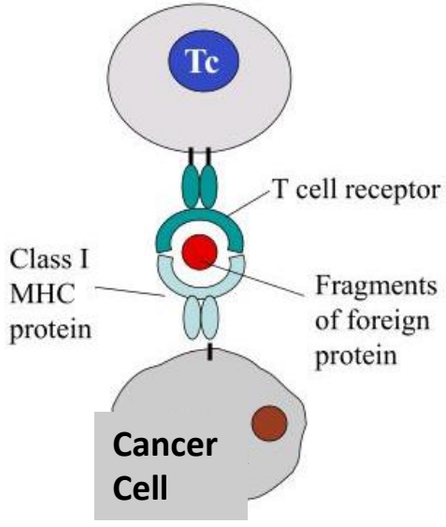
Loss of MHC I expression means that T_{CTL} cells can no longer recognize and kill cancer cells because T-cell activation requires recognition of the MHC-peptide complex.

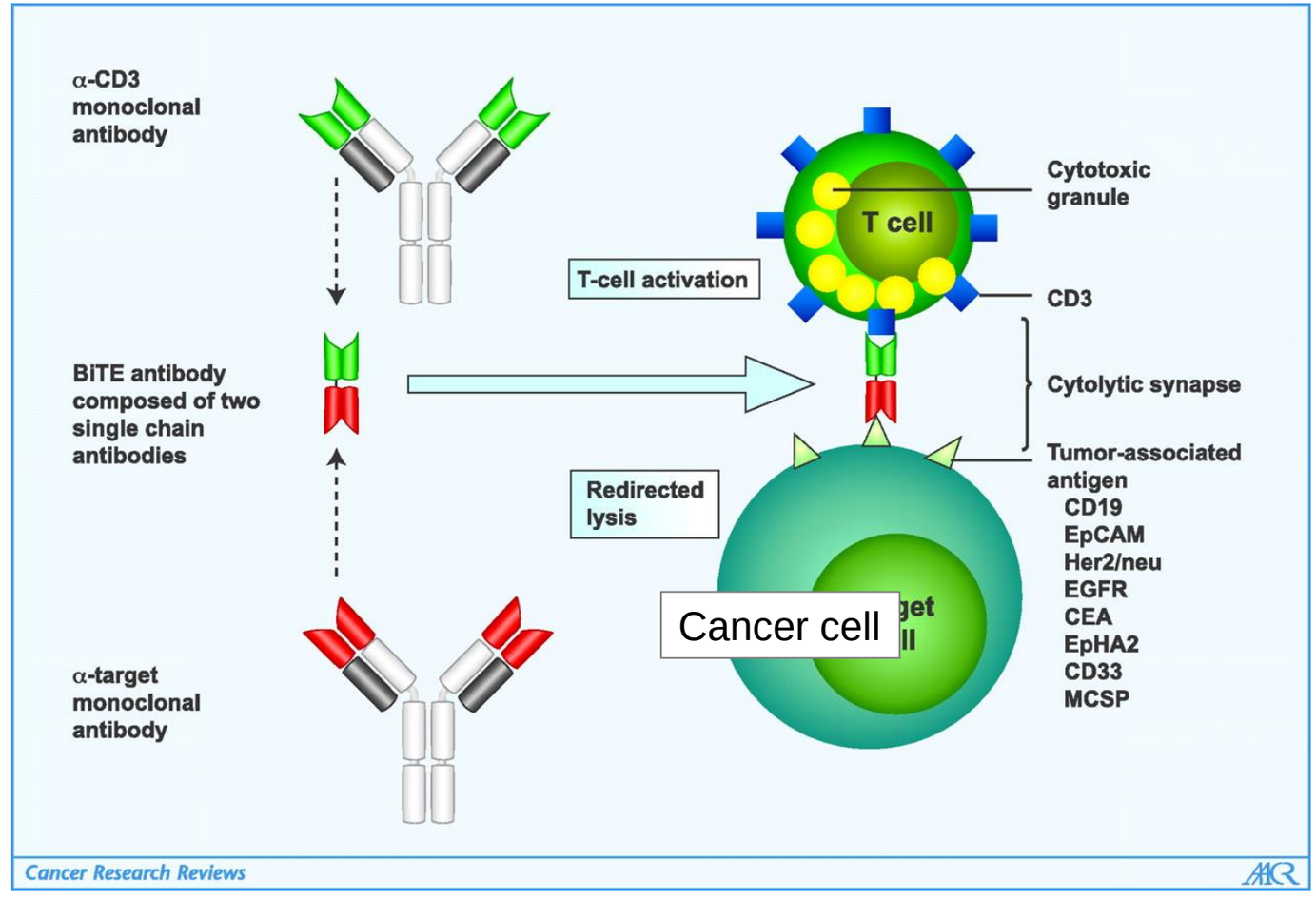
How to re-establish T_C contact with tumor cell and activation of the T-cell so that the cancer cell is killed?



Cancer Treatment with Antibodies - 1. Cancer Evasion - Loss of MHC I on Tumor Cell

Loss of MHC I expression means that T_{CTL} cells can no longer recognize and kill cancer cells because T-cell activation requires recognition of the MHC-peptide complex.



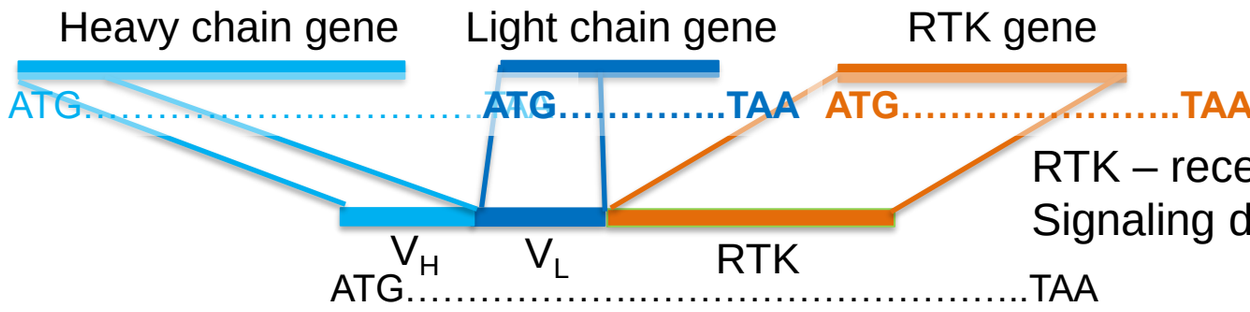


How to re-establish T_C contact with tumor cell and activation of the T-cell so that the cancer cell is killed?

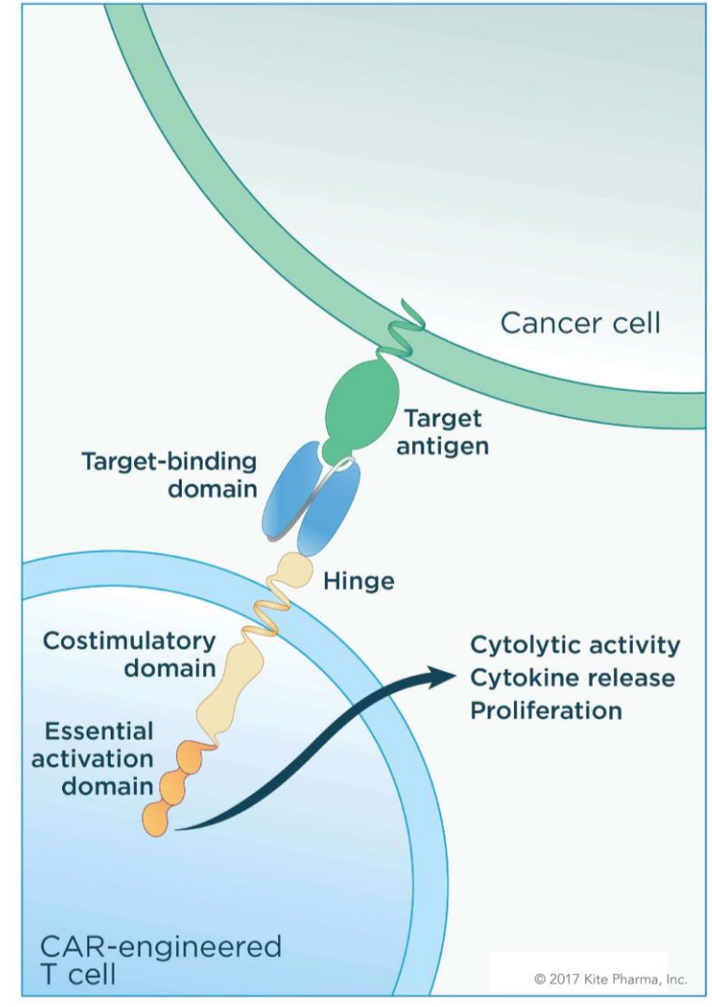
- Bispecific antibodies are generated from two separate antibodies:
  - One recognizes CD3, which is part of the T-cell receptor (TCR)
  - Other recognizes a tumor antigen.
- The two variable regions are linked into a single polypeptide chain.
- The dual binding event mimics the original MHC-I TCR interaction.,

Chimeric Antigen Receptor T-cells = CAR T-Cells

A. Obtain antibodies against cancer antigen, isolate genes that code for light and heavy chains for those antibodies.
B. Fuse coding region for variable light and heavy domains to coding region for RTK on T-cells = single CAR-T gene.

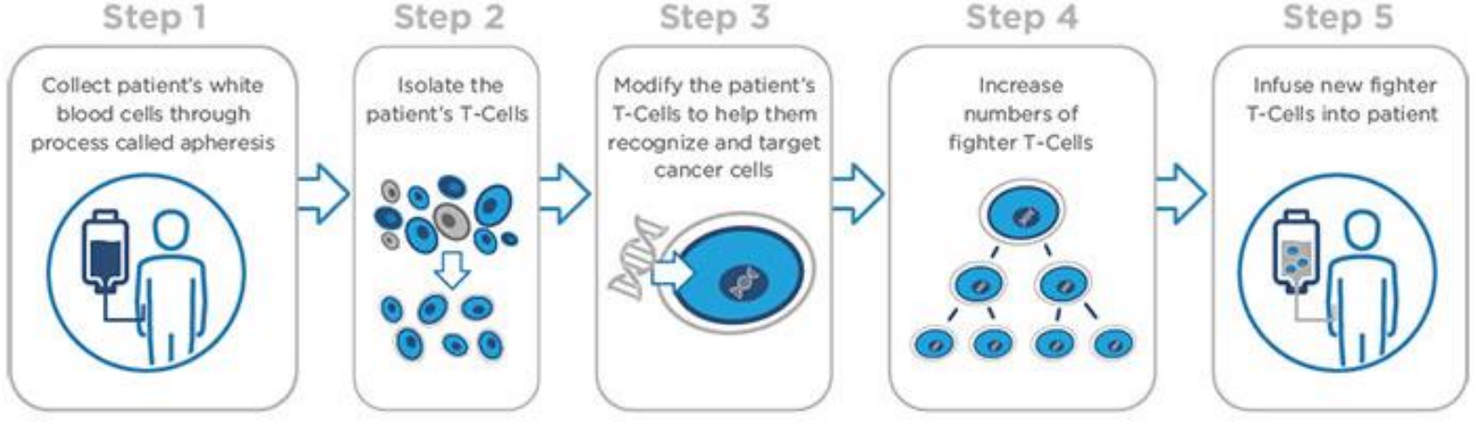


RTK – receptor tyrosine kinase
Signaling domain on T-cells



C. Introduce gene for CAR-T cell into Patient
1. Obtain white blood cells from patient
2. Isolate T-cells
3. Introduce DNA into T-cells
4. Obtain large amounts of T-cells by cell culture
5. Inject CAR-T cells into cancer patient.

D. What happens when cancer cell is encountered by CarT cell?



Cancer Evasion Mechanism II – Downregulation/killing of Tc cells.

The Nobel Prize in Physiology or Medicine 2018

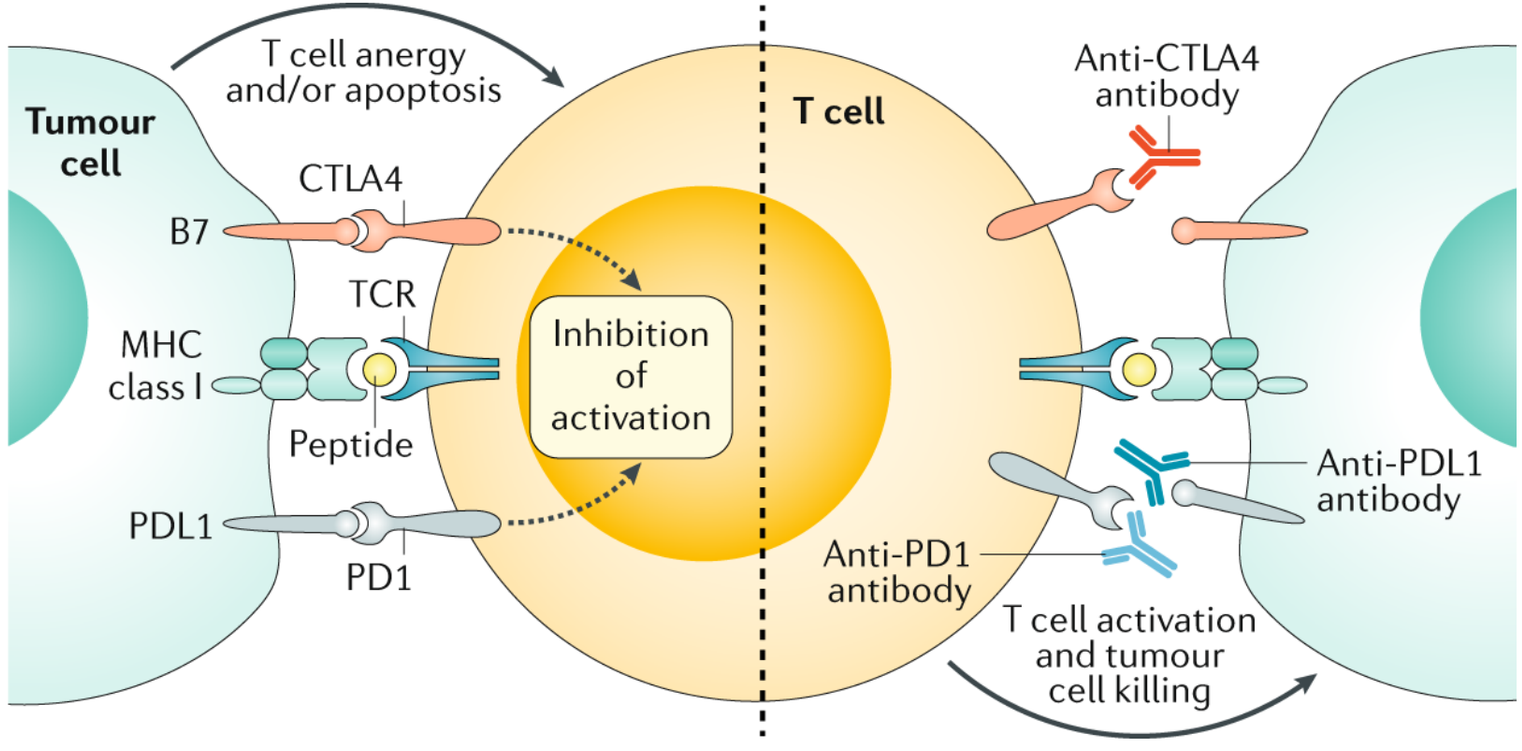

James P. Allison
Prize share: 1/2


Tasuku Honjo
Prize share: 1/2

The Nobel Prize in Physiology or Medicine 2018 was awarded jointly to James P. Allison and Tasuku Honjo "for their discovery of cancer therapy by inhibition of negative immune regulation."

1. **Cancer cells overproduce b7.** Binding of b7 to CTLA4 receptor on the surface of the Tc cell deactivates the Tc cell - immunosuppressive reaction called anergy.

2. **Cancer cells overproduce PDL1.** PDL1 binds to PD1 on T-cells. Activation of signaling causes Tc cell to enter apoptosis (programmed cell death).

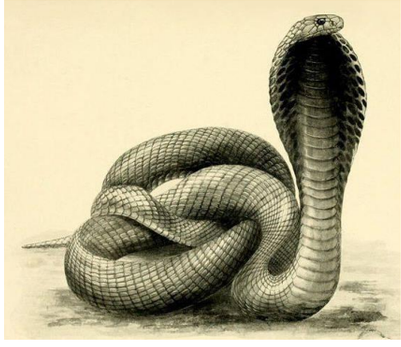


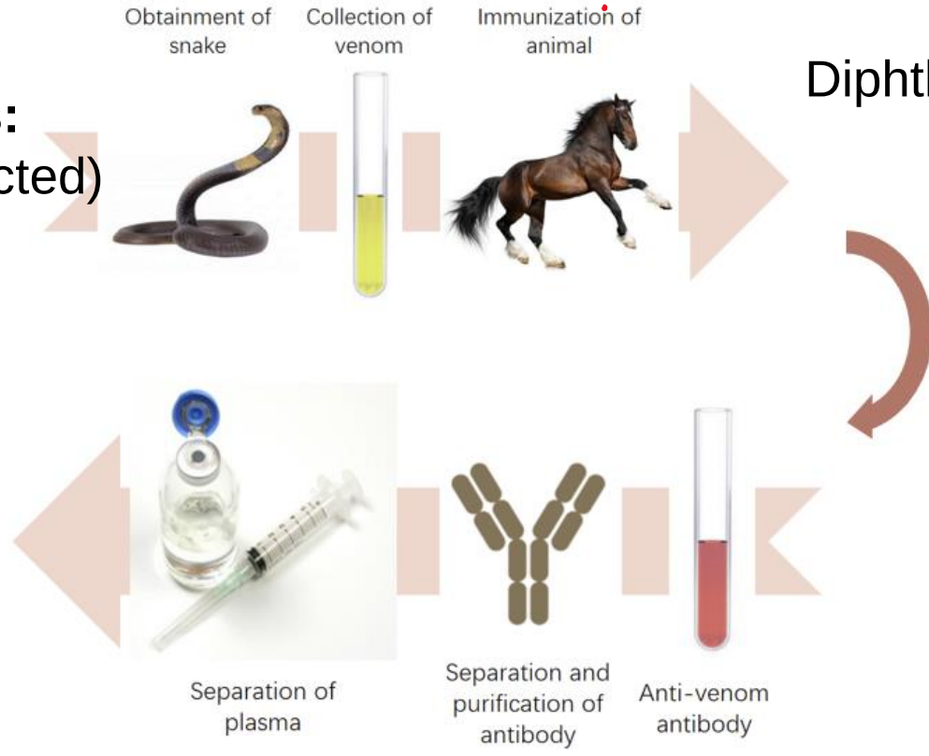
How to block this signaling?

Vaccination

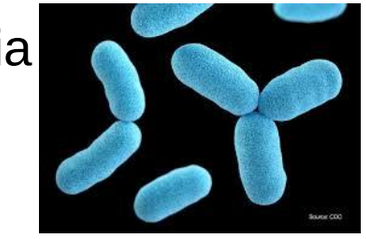
Types of vaccines:

1. Passive (Ab injected)





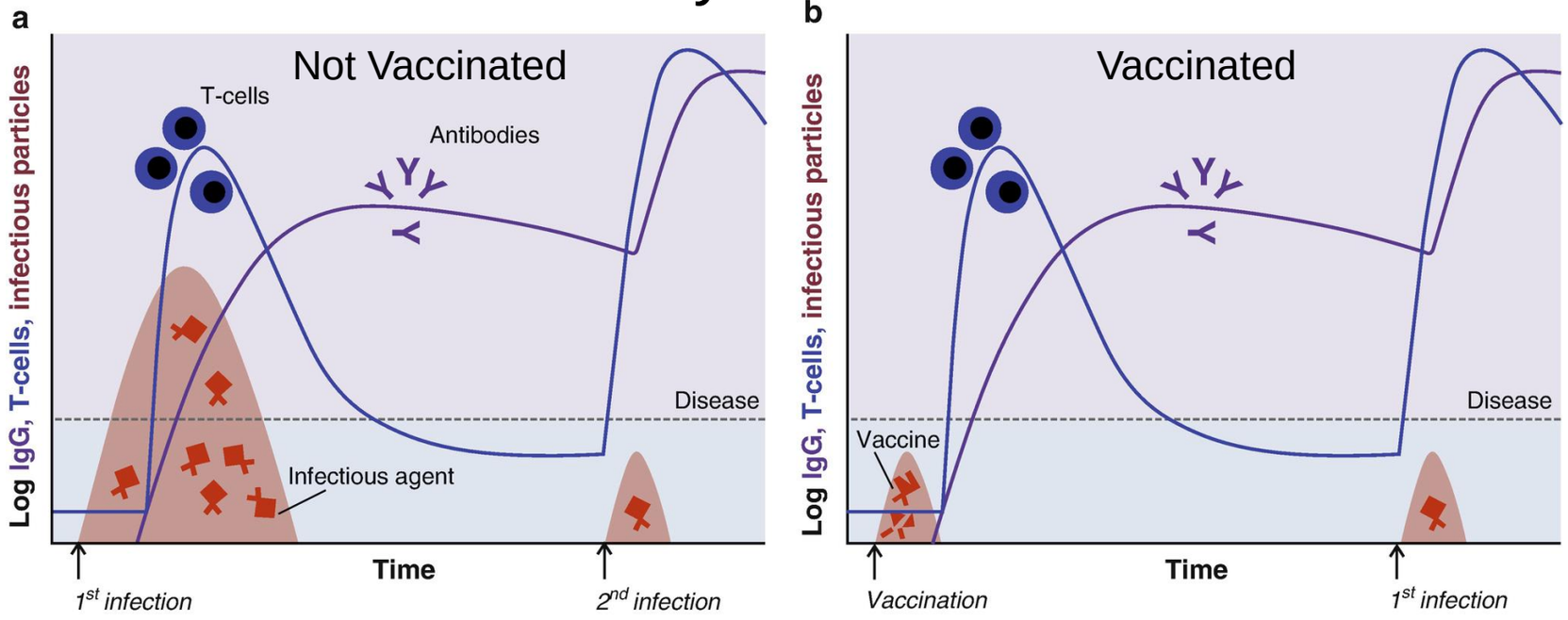
Diphtheria

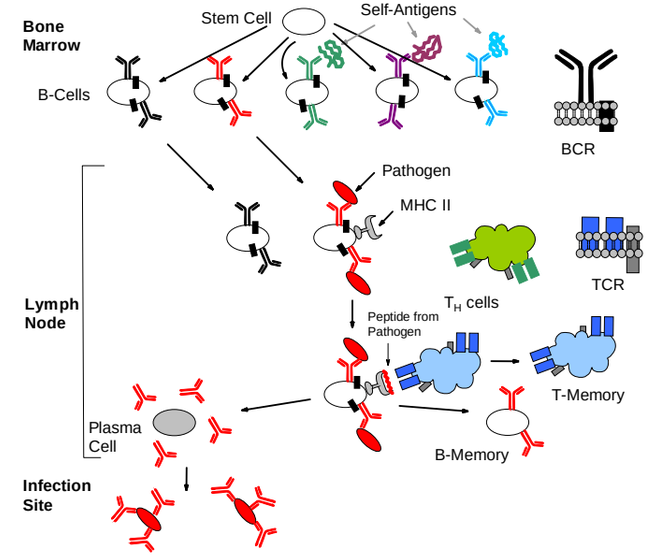


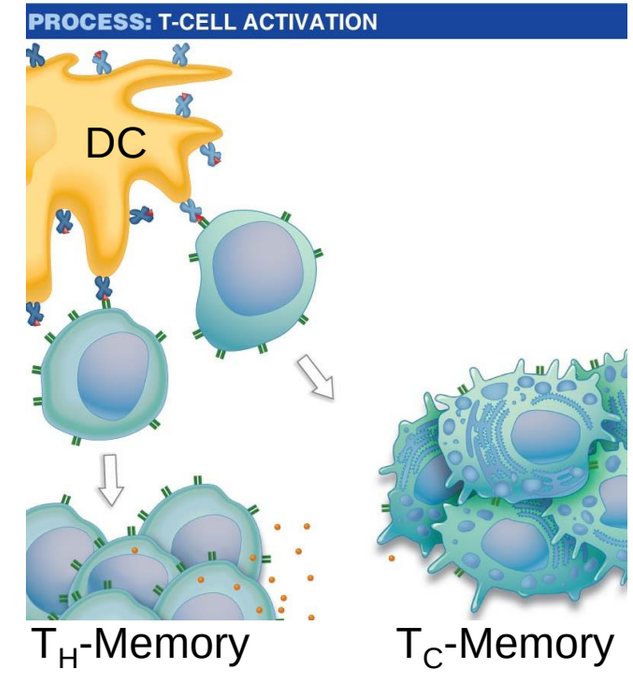
Breast feeding provides IgA to the newborn.

2. Active (Antigen Provided)

Primary and Secondary Response & Protection by Vaccines







Large number of pathogens during first (primary) infection causes disease symptoms

More rapid secondary response prevents extensive pathogen growth – no symptoms.

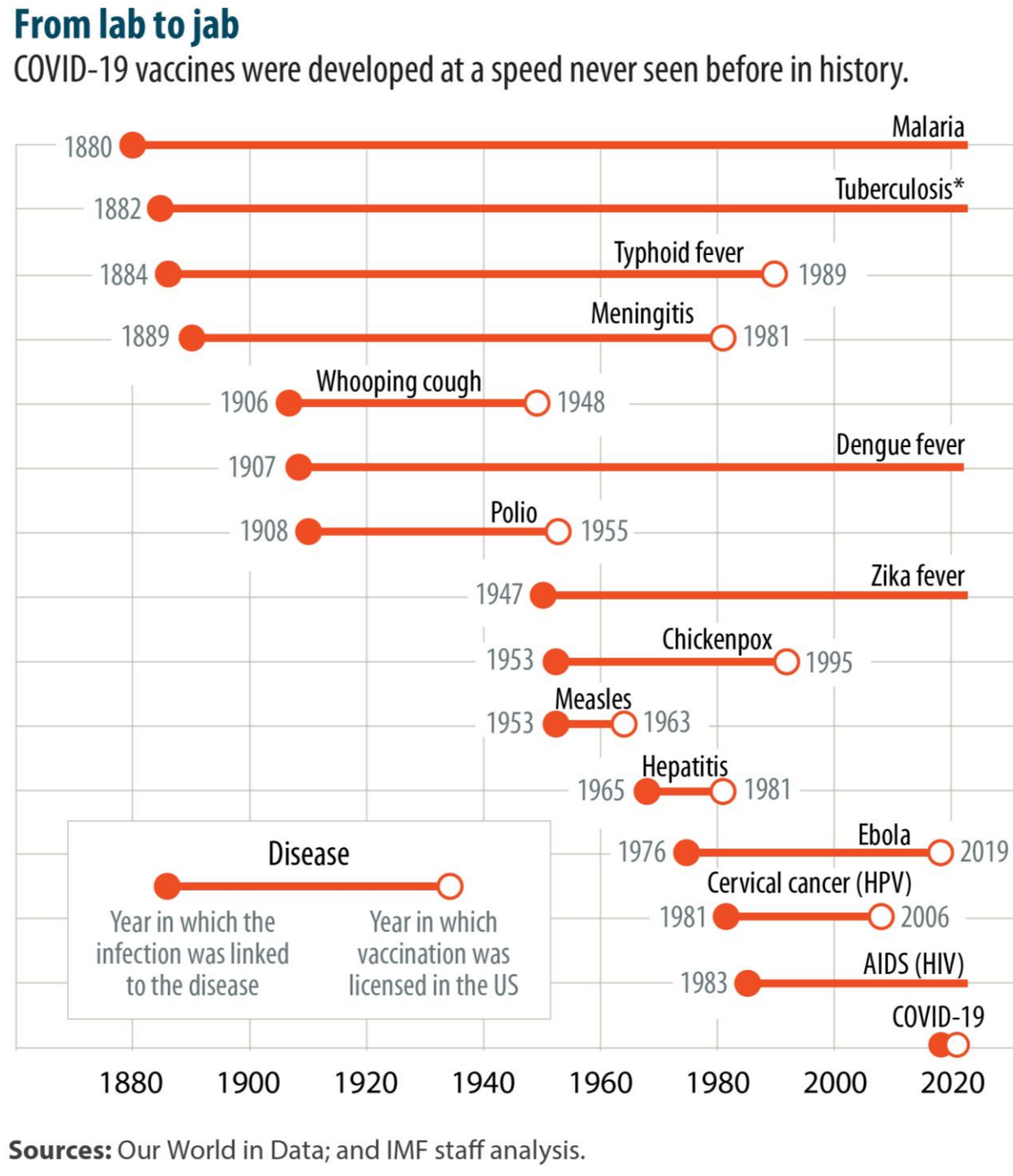
Vaccine: **antigen** induces primary response = memory B and T cells specific for that antigen.

Jiskoot W., Kersten G.F.A., Mastrobattista E., Slütter B. (2019) Vaccines. In: Crommelin D., Sindelar R., Meibohm B. (eds) Pharmaceutical Biotechnology. Springer, Cham. https://doi.org/10.1007/978-3-030-00710-2_14

Vaccine History

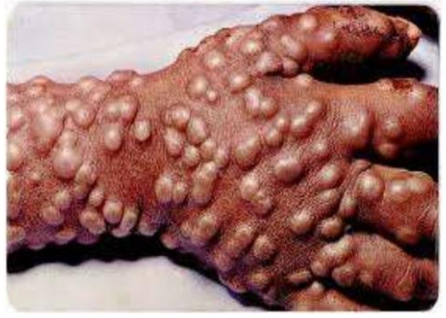
- Some diseases still do not have vaccines (Malaria)
- Most diseases are controlled by vaccination.
- A few diseases have been completely eliminated by vaccination (Smallpox)

https://www.imf.org/en/Publications/fandd/issues/2021/12/Journey-covid-19-vaccine-Stanley



Smallpox - A Success Story for Vaccination

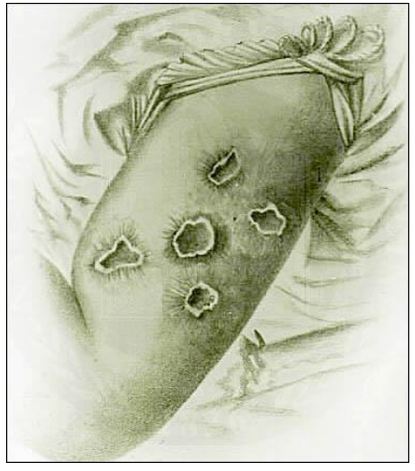
Vaccination – to introduce immunity prior to infection by pathogen

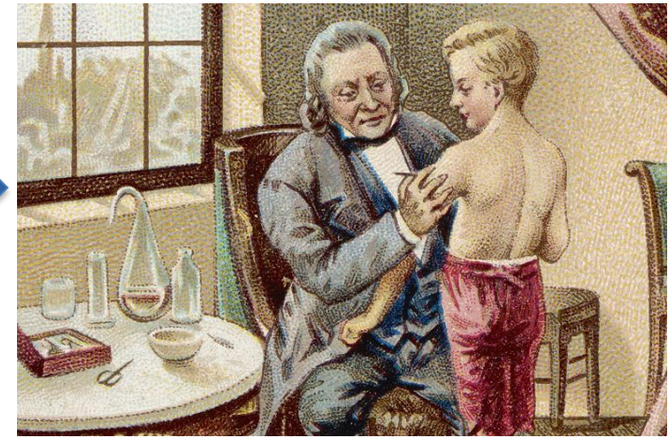


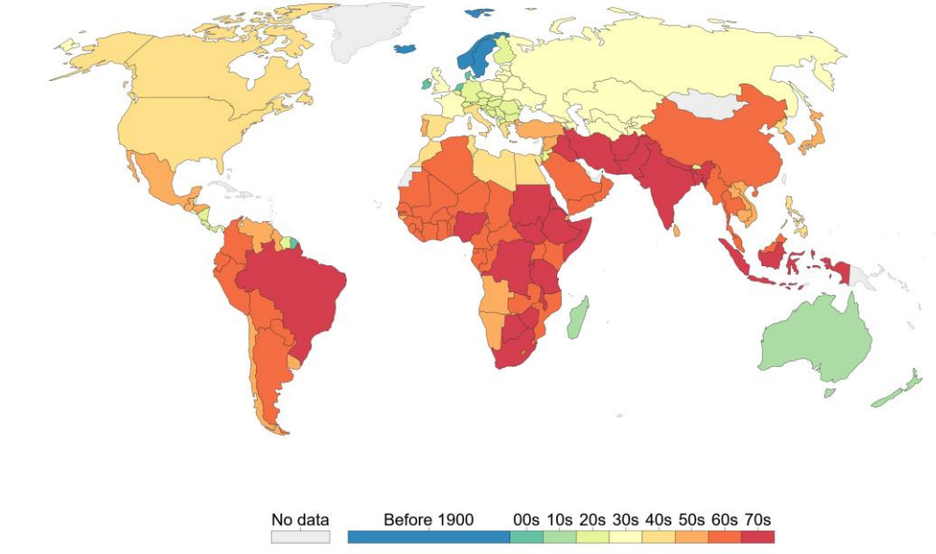
Variolation (1800) provided protection by exposing people to small amounts of smallpox virus (obtained from blisters on infected people).

Risky because smallpox was used to vaccinate

Smallpox - 30% lethality







Cowpox virus causes production of antibodies against smallpox
Jenner was the first to use cowpox to vaccinate against smallpox

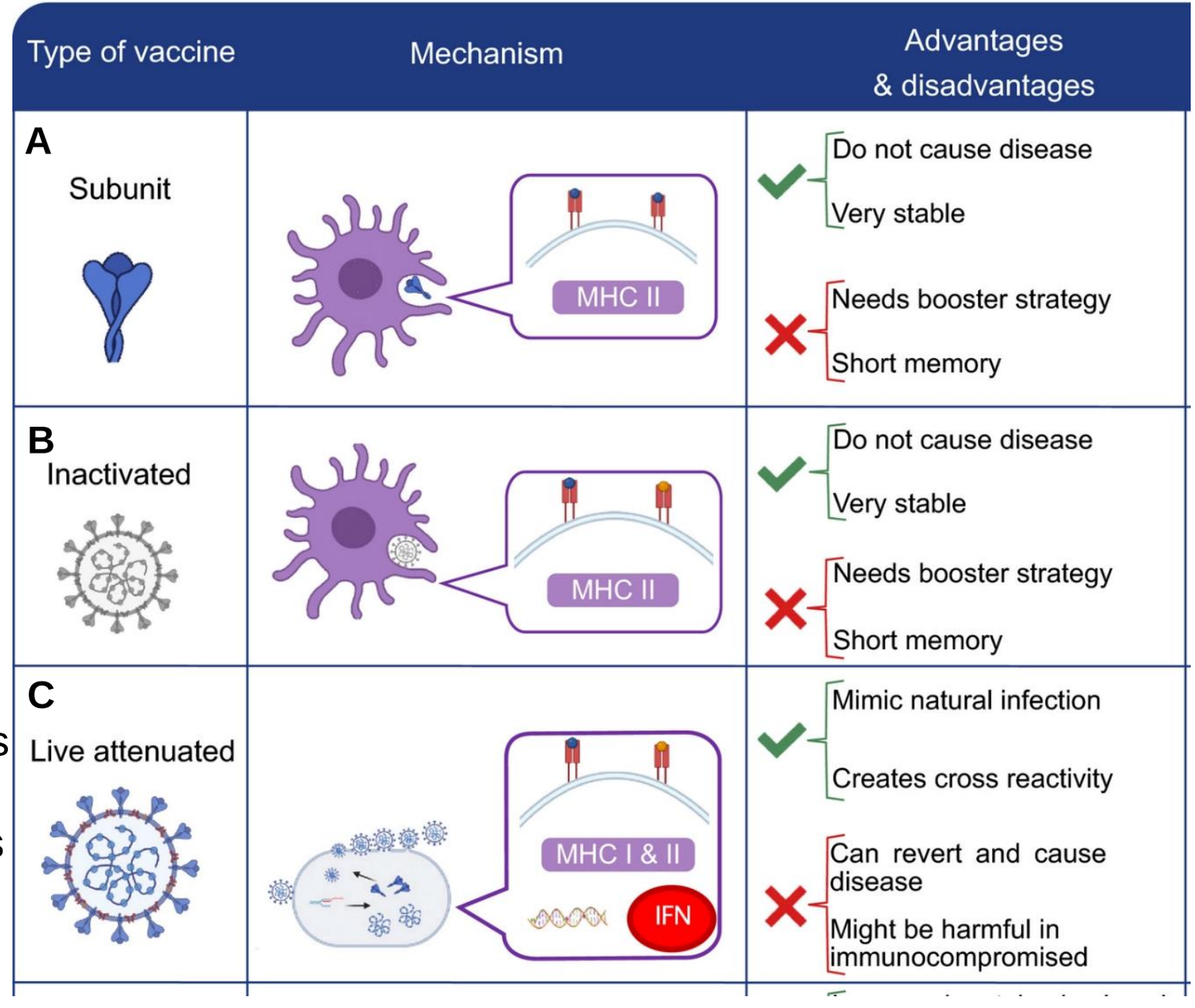
Vaccinia virus (similar to smallpox) is one form of the current vaccine.

Types of Vaccines

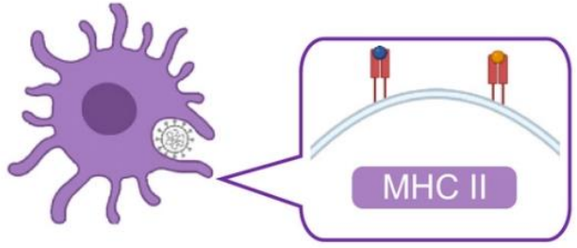
A. Subunit Vaccine:
A protein from the pathogen is used to induce memory cells, e.g. spike protein from the virus. The protein can be produced by recombinant DNA technology.

B. Inactivated Virus
The virus is chemically inactivated before administration.

C. Live Attenuated
The virus is grown under conditions that select for mutant viruses that:
i) Induce memory cells in humans
ii) Do not cause disease symptoms



B. Inactivated – Salk Polio Vaccine

Inactivated 		✓ Do not cause disease Very stable ✗ Needs booster strategy Short memory

THE TAMPA DAILY TIMES NIGHT FINAL

SIXTY-THIRD YEAR—No. 56 TAMPA, FLORIDA, TUESDAY, APRIL 12, 1955 PRICE FIVE CENTS

POLIO VACCINE 'SUCCESSFUL'

End of Quick Divorce Law Gains Favor

Senate Committee Clears Bill for 6 Months' Residence

Top Prize Story In Today's News

Oakland, Cal., April 12 (AP). John Cox's headache turned out to be one for his whole fire station here last night.

Cox, a tillerman on a hook and ladder rig, obtained permission from his captain to step across the street to get some aspirin.

While he was in the drug store, an alarm rang.

And away went the hook and ladder truck—without a tillerman.

At the first corner, the wild-

Auditorium, Bridge Bonds Get Okay

Consultants Give Go Ahead on Tampa Improvement Projects

By BOB TURNER Times Staff Writer

...e Hixon said today the bond consultants in ...ork have given the go-for floating a $5,000,-...id issue to finance con-...n of a new auditorium, ...idges and several build-...provements here.

Paralysis Prevention Proved in Mass Tests

'Effective, Safe' Verdict Reported After Long Study of Innoculations

By ALTON L. BLAKESLEE Associated Press Science Reporter

Ann Arbor, Mich., April 12 (AP). The Salk polio vaccine works—safely, potently—and can virtually end the icy fear that long has gripped the hearts of parents.

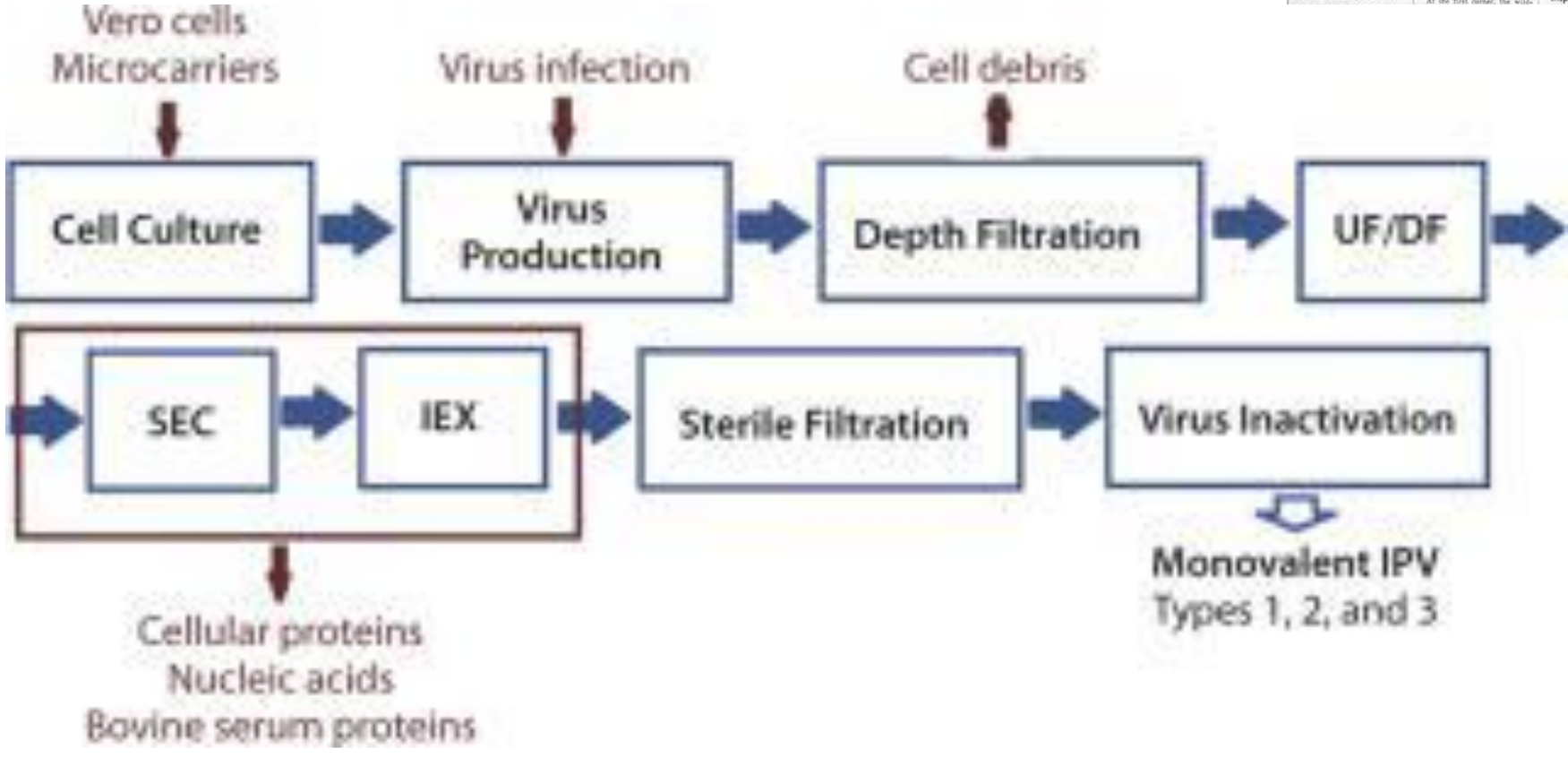
The vaccine was officially declared today to have proved to be 80 to 90 percent effective in preventing paralytic polio.

And young Dr. Jonas E. Salk, who developed it, declared he is certain it can be potentially made almost 100 percent potent to smash polio's terror and tragedies.

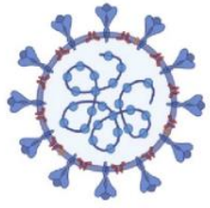
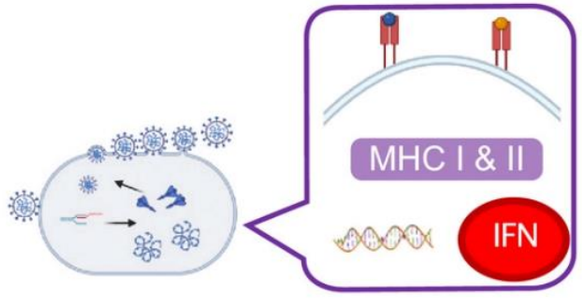
Supercharged Atmosphere

The triumph of the vaccine was announced in an atmosphere of

Dr. Salk Says 'Too Busy Now For Comment'



C. Attenuated – Sabin Polio Vaccine Viruses

Live attenuated 		✓ Mimic natural infection Creates cross reactivity ✗ Can revert and cause disease Might be harmful in immunocompromised

THE CINCINNATI ENQUIRER

122nd YEAR NO. 76—DAILY SUNDAY MORNING, JUNE 24, 1962 FINAL EDITION—PRICE 25 CENTS

'Sabin Sundaes' Ready For Area Polio Drive Beginning At 11 A. M.

You can start getting rid of polio in Metropolitan Cincinnati today.

Take a few minutes and go to a vaccine station in a school near you and get your cube of sugar enriched with Sabin vaccine.

This campaign is for everyone over six weeks of age — everyone, babies to grandmothers, who has never had Sabin vaccine.

There are a million doses waiting—there's plenty for everybody. Most stations in this five-county area will be open from 11 a. m. to 5 p. m.

Crushing polio is a big job. Today you can take a giant first step against Type III polio. The other two types will be dealt with later, using Sabin vaccine, a swallow at a time.

On page 3-A is a list of places, most of them schools, where the vaccine is being given. Near the list is a coupon for the head of the household to sign to give consent for the giving of the vaccine.

Donations, of 25 cents a dose, will be accepted at the distribution stations to help pay for the vaccine. You may give more if you

suburbs were doing the same.

Business and industry have risen to the challenge giving time and materials and men.

Firemen will help keep order, giving up their off days. So will the doctors, nurses, druggists and Boy Scouts you see today when you get your vaccine.

Dr. Kenneth Macleod, Cincinnati Health Commissioner, with Dr. J. Edwin Reed, Hamilton County Health Commissioner, have thrown their weight behind the campaign. With Dr. Sabin they make up the top planners of the operation.

Dr. Macleod said if half the population got vaccine it would be a "good" effort considering the short time of preparation. Sixty per cent would be "superlative." Over 70% would be a miracle, he said.

Any bets on a miracle?

(Related Photo, Page 6A)

Where To Go

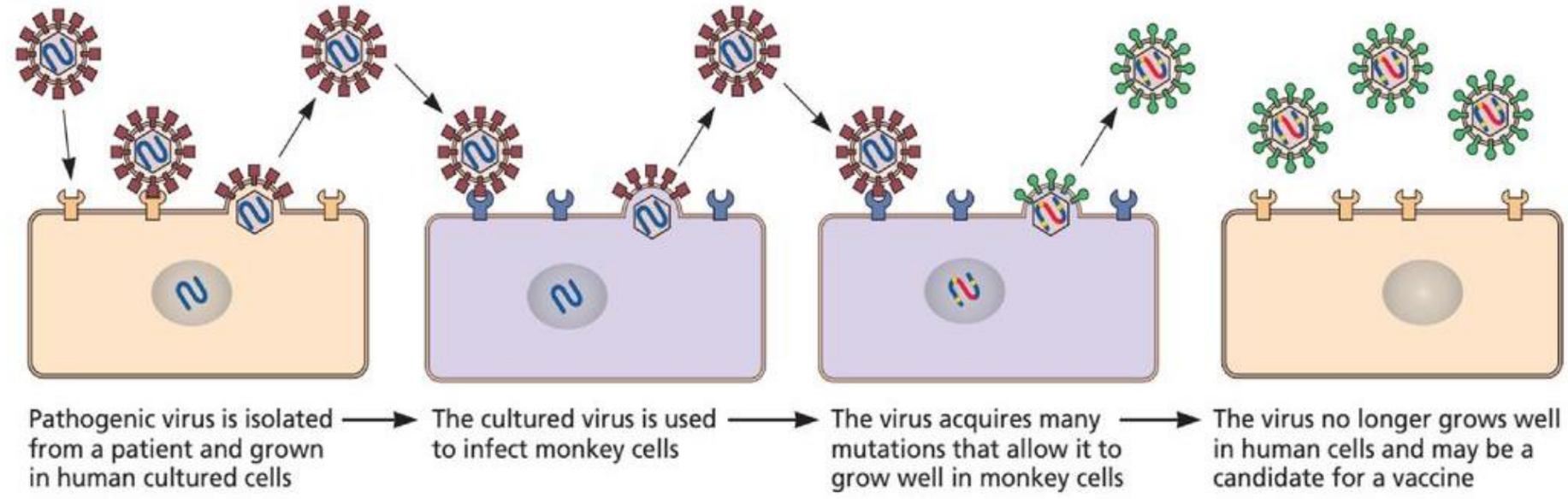
A list of places where you can get the polio vaccine and a form for you to sign to give your consent for minors are on page 3A.

On Page 6-A is a picture showing volunteers at work getting the vaccine ready to dispense today.

Don't forget—this is the vaccine you eat. There are not shots.

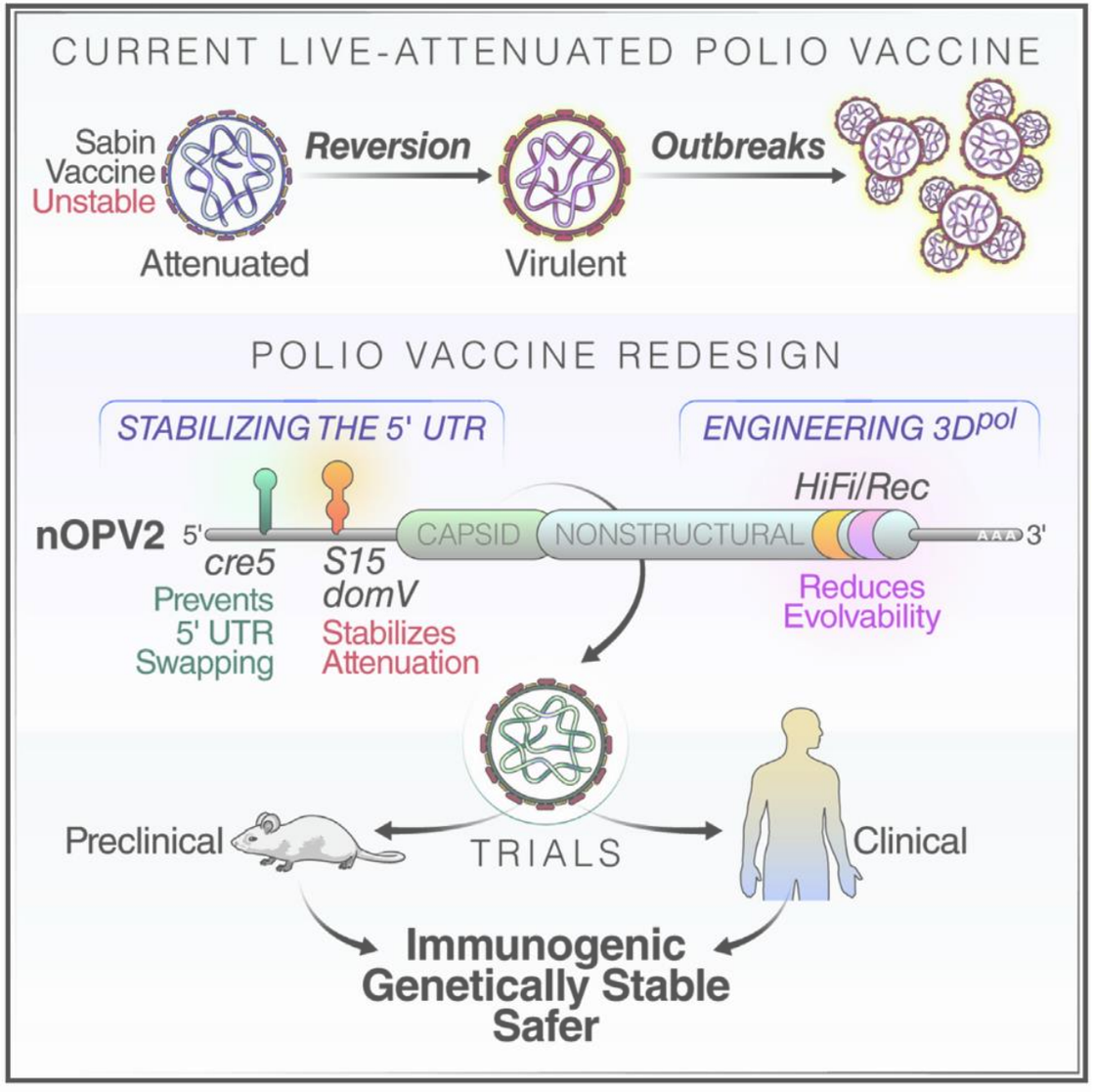
'First' Family Gets Sabin Vaccine On Sugar

... Dr Sabin oversees feeding to Tom and Marilyn Bross and grandmother, Mrs. Ruby Johnson



Pathogenic virus is isolated from a patient and grown in human cultured cells → The cultured virus is used to infect monkey cells → The virus acquires many mutations that allow it to grow well in monkey cells → The virus no longer grows well in human cells and may be a candidate for a vaccine

C. Attenuated Viruses – Can Return to Virulence by Reversion



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—Enquirer (Bob Free) Photo

Cell Host & Microbe

CellPress

Volume 27, Issue 5, 13 May 2020, Pages 736-751.e8

Article

Engineering the Live-Attenuated Polio Vaccine to Prevent Reversion to Virulence

Ming Te Yeh [1], Erika Bujaki [2], Patrick T. Dolan [1], Matthew Smith [2], Rahnuma Wahid [3], John Konz [3], Amy J. Weiner [4], Ananda S. Bandyopadhyay [4], Pierre Van Damme [5], Ilse De Coster [5], Hilde Revets [5], Andrew Macadam [2], Raul Andino [1 6]

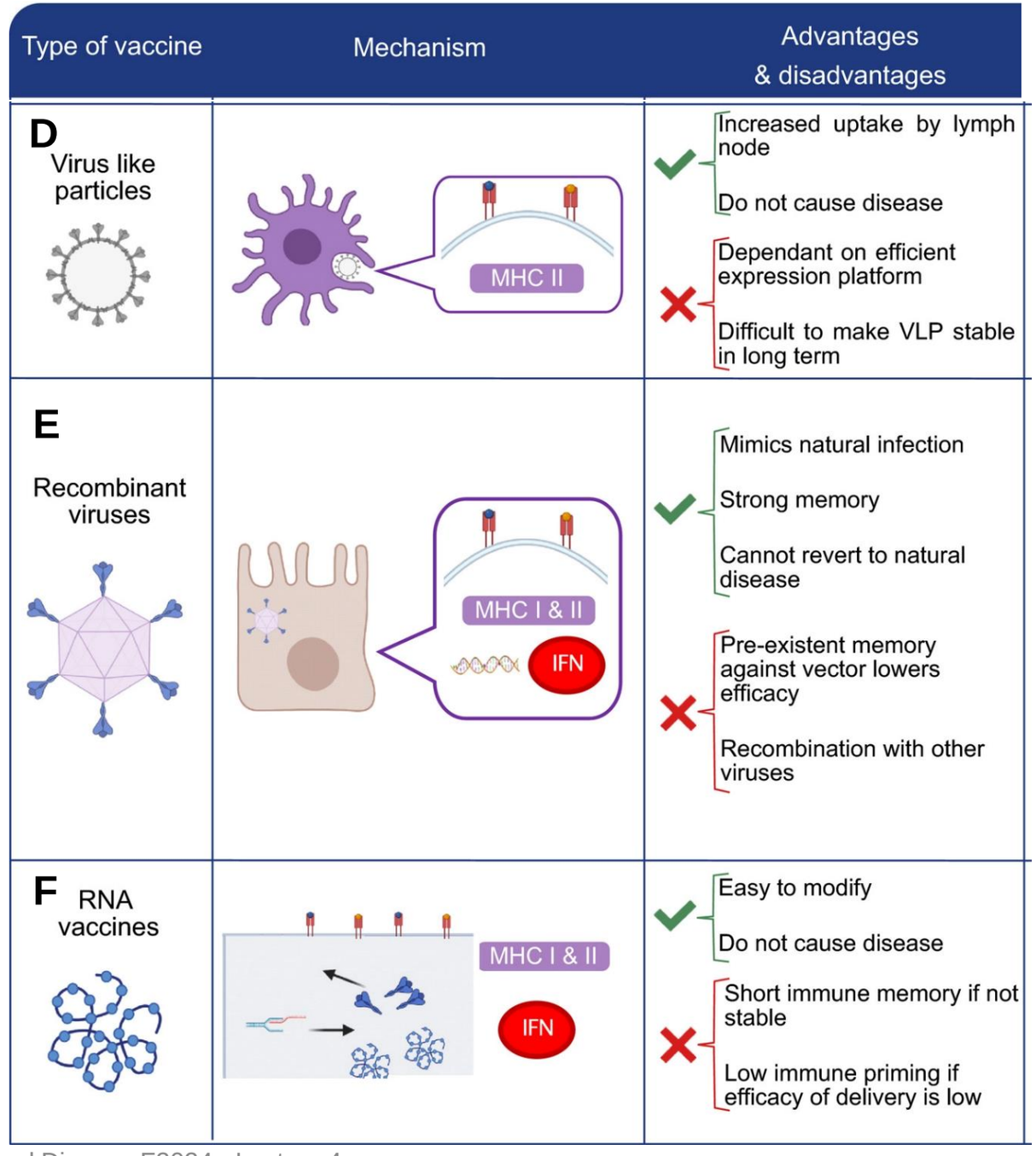
D – Virus Like Particles:
Proteins isolated from the virus form virus-like-particles, *without* the genetic material of the virus

E. Recombinant Virus:
A “safe virus” is used (e.g. cold virus)
Gene for a protein from a pathogen is inserted into the DNA of the virus.

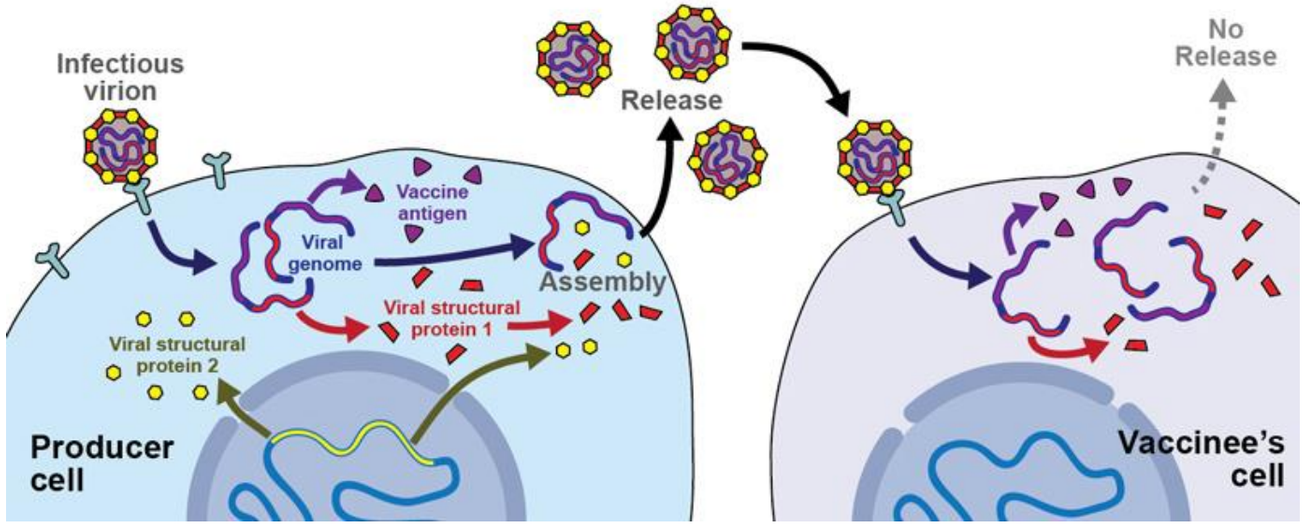
- When virus grows it produces the protein from the pathogen generating immunity.

Also includes vaccines that are a mixture of genetic material from human and animal viruses (reassortment viruses)

F. RNA Vaccines (Pfizer Covid Vaccines)
RNA coding for a viral protein is introduced into cells. The RNA is used by the cell to make viral proteins, inducing an immune response.



E. Viral Vectors for Antigen Delivery AstraZeneca Covid

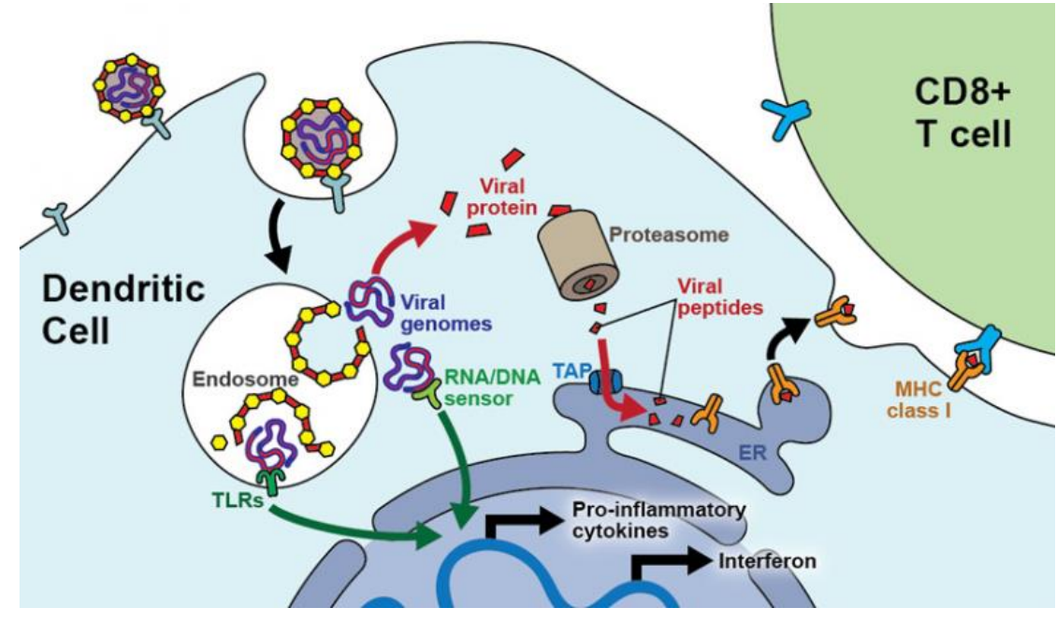


2. Action of vaccine

a) Virus infects host cell in vaccinated person.
b) Viral genome is used to make viral proteins, including proteins from the pathogen.
c) Activate T_C cells to become T_C – memory cells (can be re-activated by MHC I + Peptide).
d) B-cell response can occur due to antigens that are sent to the surface of the cell, generating B- and T_H-memory cells.

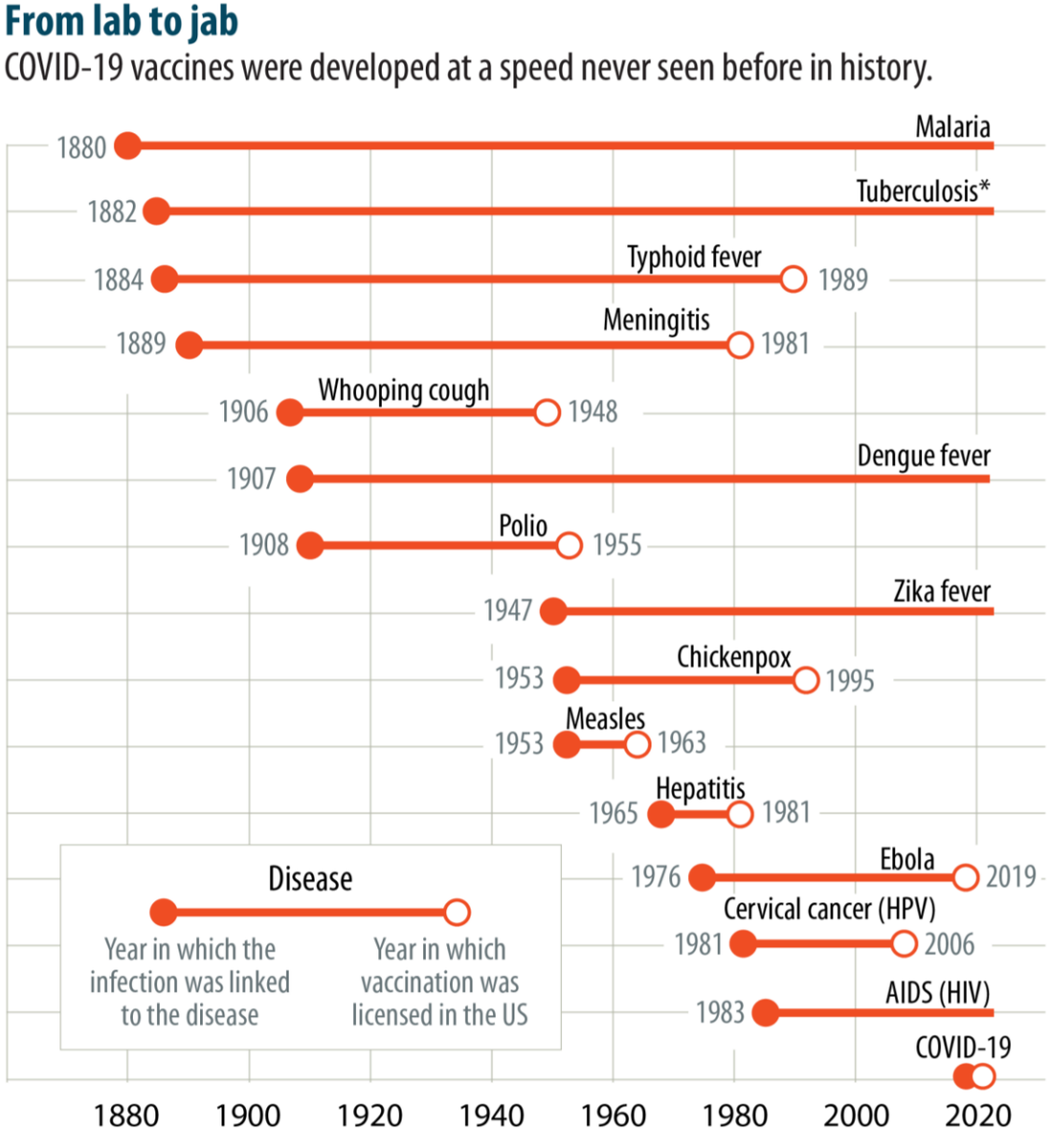
1. Production of vaccine

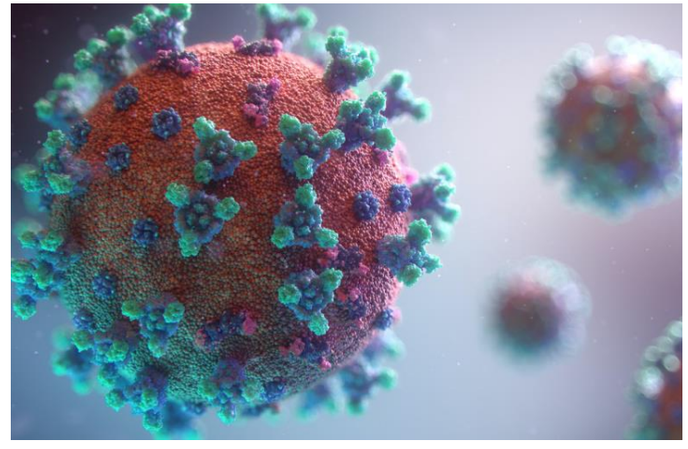
I. Genes from the pathogenic virus are added to an adenovirus (common cold)
II. Adenovirus is defective and cannot replicate without key structural proteins that are provided in the producer cell.
III. No viral particles are released in the vaccinee's cells because they lack the key structural proteins.

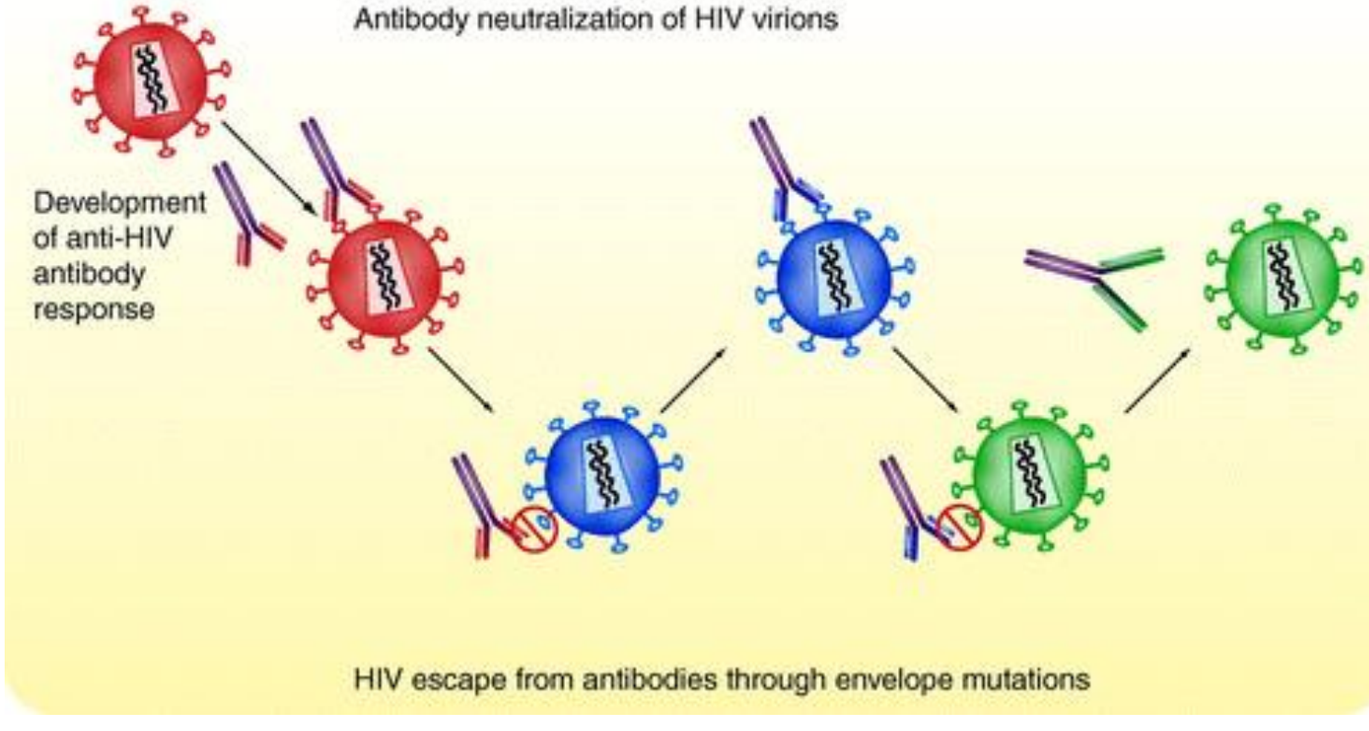


DOI:
https://doi.org/10.4414/smw.2017.14465
Publication Date: 08.08.2017
Swiss Med Wkly. 2017;147:w14465

Why Are There No Vaccines for HIV?







Summary Questions for Immunology:

1. What are the two major branches of the immune system? Why are both important?
2. What are the roles of different cell types in each system, e.g. what would happen if T_H-cells disappeared?
3. What is the quaternary structure of an antibody? Can you sketch an antibody and indicate where the antigen binds?
4. What defines the specificity of antibodies?
5. What are the steps in the production of antibody genes, at the molecular level:
   a) How do DNA rearrangements produce functional heavy and light chain genes
   b) How are is the mature mRNA generated in B-cells and Plasma cells.
   c) What is the difference between the heavy chain export process for B-cells and plasma cells.
6. Can you describe how antibodies kill/inactivate pathogens

7. How are virally infected cells and tumor cells recognized by Tc cells?
8. How does the Tc cell kill those cells?
9. What evasion mechanisms are used by cancer cells and how have these been addressed by antibody therapy?

10. What was the origin of the idea for vaccination?
11. What was one of the first “safe” vaccines? What disease has now been eradicated due to this vaccine?
12. Can you describe one way to generate a vaccine for a pathogen? Do you know the pros and cons for that method?