

Biochemistry

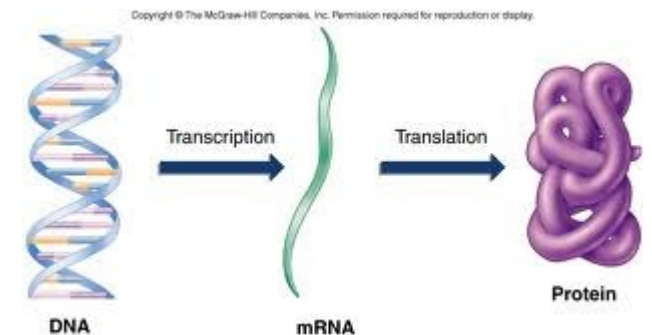
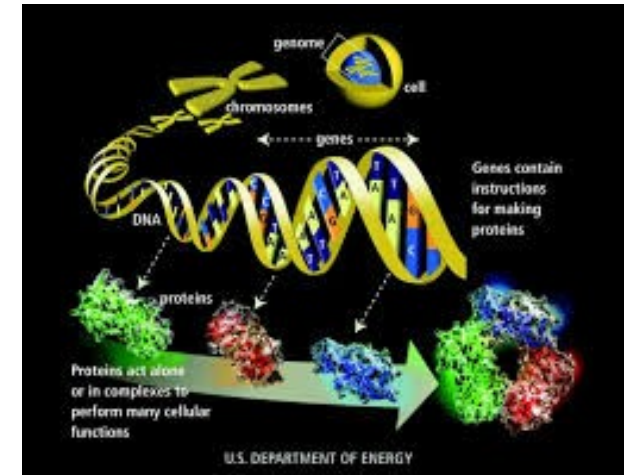
Dr. Shariq Syed

What is DNA Sequence ??

- Our Genome is made up of DNA
- Biological instructions are written in our DNA in chemical form
- The order (sequence) in which nucleotides are placed in gene determines biological information stored
- DNA in other words in chemical memory of life

Why do we Sequence DNA ??

- To understand the instructions “encoded” in a DNA molecule, one must start by determining the sequence of its bases (A,T,G,C)
- “Reading” of the sequence is called sequencing
- Gene Annotation:
 - The next step after sequencing is finding locations of genes & then
 - Determining what those genes do



Human Genome Project

- Primary aim of project was to find the sequence of entire human genome
- Results from project:
 - April 2003 sequencing of the full human genome was completed and published
 - Project told us the order, or sequence, of the more than 3 billion bases in human [DNA](#)
 - In addition location of genes on our chromosomes - no easy task, given the 20,500 genes in a human genome

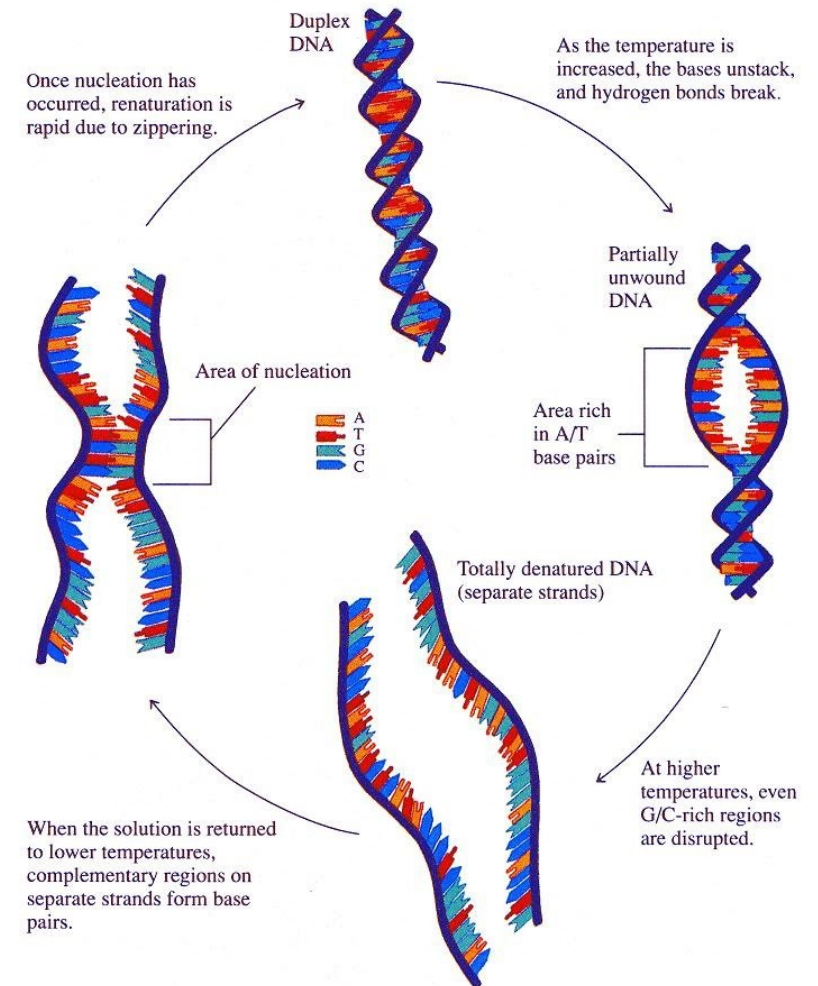


How do we Sequence DNA ??

- **This method is also called “Sanger dideoxy Method”**

- **Step 1:**

- We need to separate the two DNA strands
- This process is called DNA denaturation



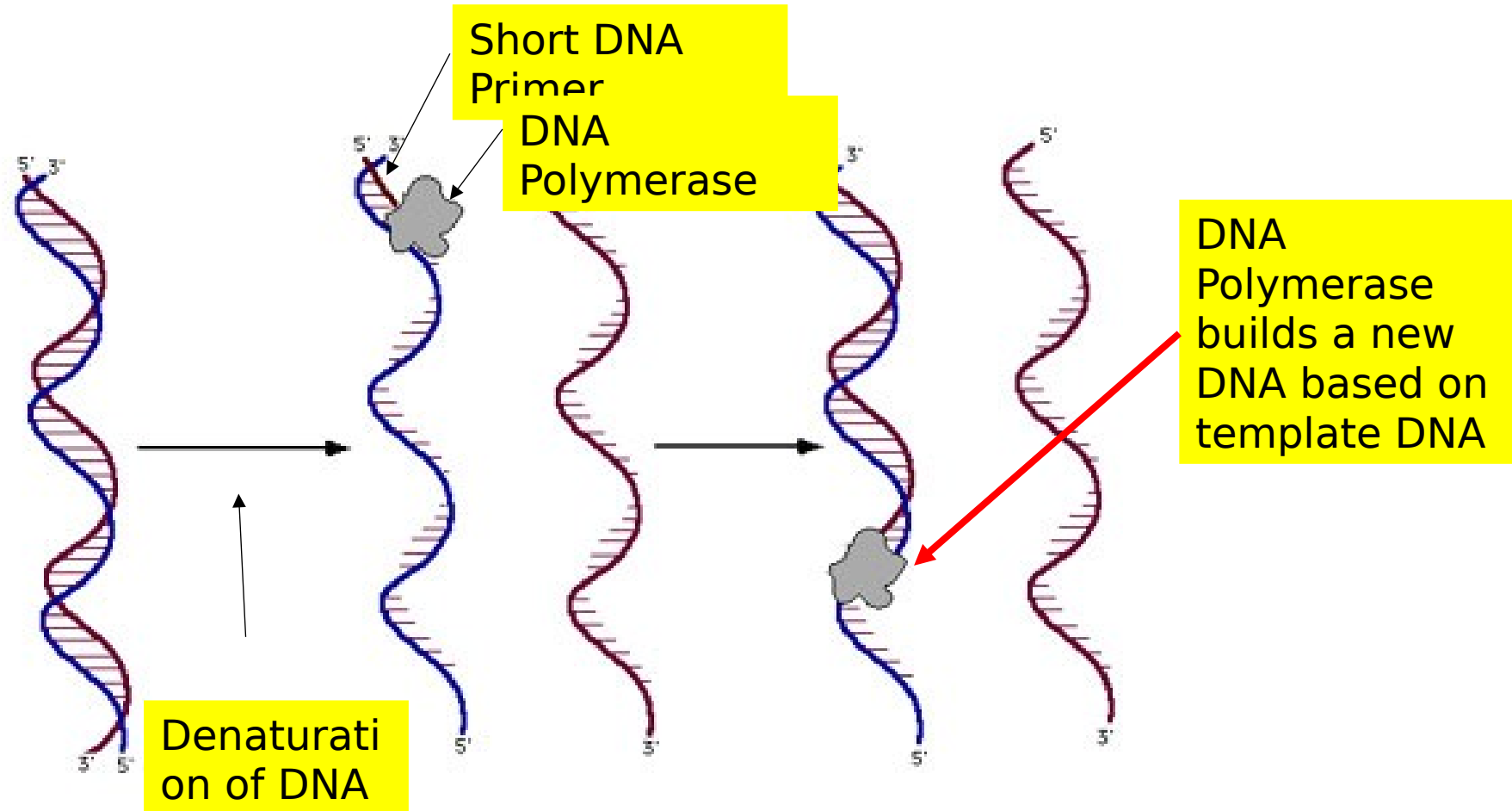
How do we Sequence DNA ??

- **Step 2:**

- Single strands of DNA now act as a template
- To this reaction the following are added
 1. Free nucleotides
 2. DNA Primer (Short DNA sequence, 20-30 nts)
 3. DNA polymerase
- DNA polymerase starts to build DNA chain based on template DNA

How do we Sequence DNA ??

- **Step 2:**



How do we Sequence DNA ??

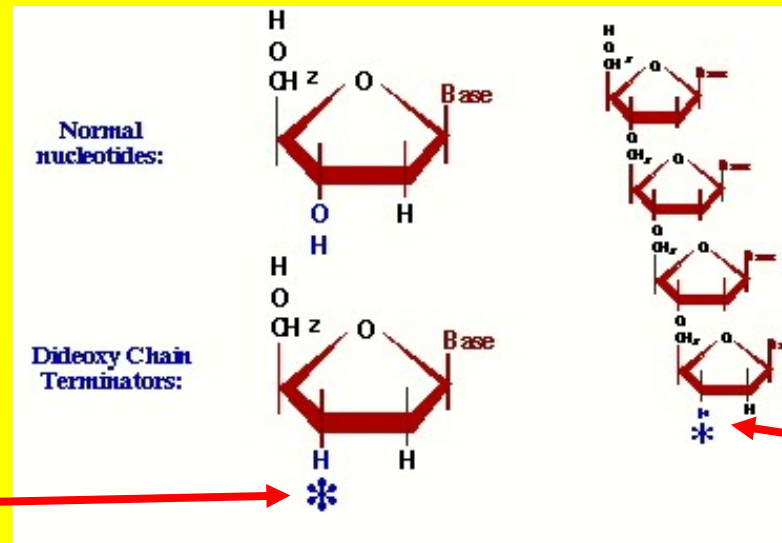
- **Step 2:**

- In addition to regular nucleotides, special nucleotides are added
- Role of these nucleotide is to stop chain reaction
- Also called “chain terminators”

How do we Sequence DNA ??

• Step 2:

- How do “Chain Terminators” stop reaction ??



These special nucleotides DO NOT have 3-OH group

When these nucleotides get added in chain, they STOP further DNA elongation

There is NO 3-OH grp to add another nucleotide

How do we Sequence DNA ??

• Step 2:

- Let's take an example
- Suppose we added small amount (5%) of Special "Thymine" nucleotide to our reaction
- DNA polymerase will add regular "T" most of time when needed
- But 5 % of time it will add Special "T"
- This will stop reaction, this DNA strand will break away from enzyme
- Sooner or later ALL of the copies will get terminated by a "T"

How do we Sequence DNA ??

• Step 2:

- Each time the enzyme makes a new strand, the place it gets stopped will be random
- ALL of the strands we make started at one exact position
- ALL of them end with a “T”

- To find out where all the T's are in our newly synthesized strand, all we have to do is find out the sizes of all the terminated products!

How do we Sequence DNA ??

• Step 2:

DNA Polymerase reads the template strand and synthesizes a new second strand to match:



IF 5% of the T nucleotides are actually dideoxy T, then each strand will terminate when it gets a ddT on its growing end:

5' - TACGCGGTACGGTATGTTTCGACCGTTTAGCTACCGAT•
5' - TACGCGGTACGGTATGTTTCGACCGTTTAGCT•
5' - TACGCGGTACGGTATGTTTCGACCGTT•
5' - TACGCGGTACGGTATGTTTCGACCGTT•
5' - TACGCGGTACGGTATGTTTCGACCGT•
5' - TACGCGGTACGGTATGTT•
5' - TACGCGGTACGGTATGT•
5' - TACGCGGTACGGTAT•
5' - TACGCGGTACGGT•
5' - TACGCGGT•

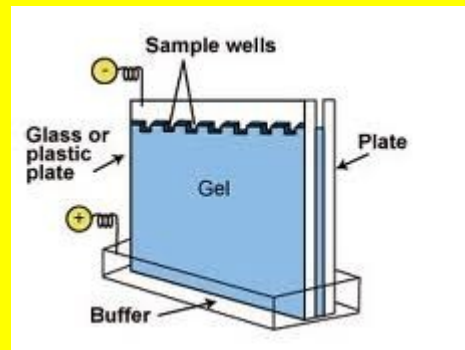
How do we Sequence DNA ??

• Step 3:

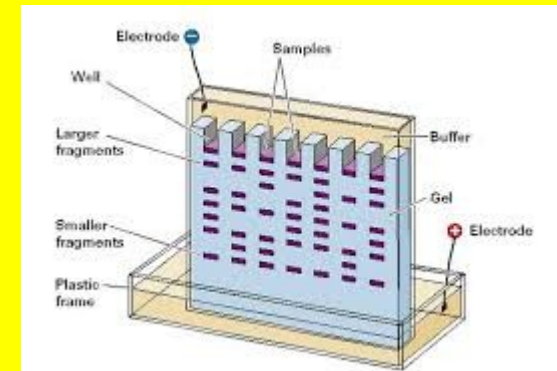
• *How do we separate these DNA fragments ?*

- **Gel electrophoresis can be used to separate the fragments by size and measure them**

**Add
sample**



Separation of DNA fragments based on **size** under influence of current

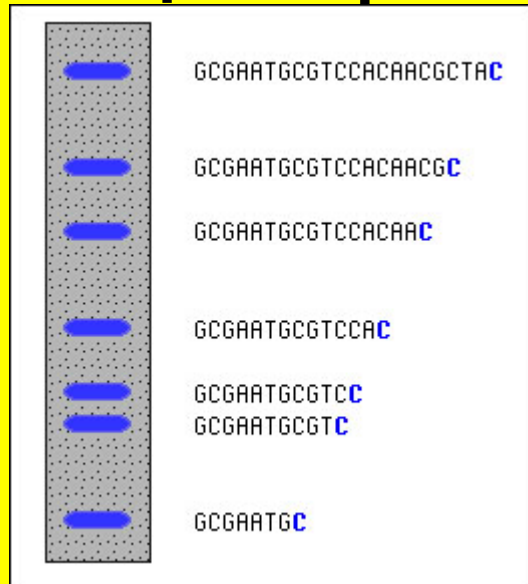


How do we Sequence DNA ??

• Step 3:

• *How do we separate these DNA fragments ?*

- **Gel electrophoresis can be used to separate the fragments by size and measure them**


























- **Special Cytidine (Dideoxy-cytidine) are chemically modified to fluoresce under light, glows blue**
- **Smallest fragments are at the bottom, largest at the top**
- **The positions and spacing shows the relative sizes**
- **We can tell where are all the “C”**

How do we Sequence DNA ??

• Step 3:

- If we had added all four special nucleotides along with all regular nucleotides
- Each nucleotide has a separate color under UV

Gel:

	G	GCGAATGCGTCCACACGCTACAGGTG
	T	GCGAATGCGTCCACACGCTACAGGT
	G	GCGAATGCGTCCACACGCTACAGG
	G	GCGAATGCGTCCACACGCTACAG
	A	GCGAATGCGTCCACACGCTACA
	C	GCGAATGCGTCCACACGCTAC
	A	GCGAATGCGTCCACACGCTA
	T	GCGAATGCGTCCACACGCT
	C	GCGAATGCGTCCACACGCT
	G	GCGAATGCGTCCACACGCT
	C	GCGAATGCGTCCACACG
	A	GCGAATGCGTCCACAC
	A	GCGAATGCGTCCACA
	C	GCGAATGCGTCCAC
	A	GCGAATGCGTCCAC
	C	GCGAATGCGTCC
	C	GCGAATGCGTCC
	C	GCGAATGCGTCC
	T	GCGAATGCGTCC
	G	GCGAATGCGTCC
	C	GCGAATGCGTCC
	G	GCGAATGCGTCC
	T	GCGAATGCGTCC

How do we Sequence DNA ??

**Run DNA
replication
reaction in
presence of
terminator
nucleotides**



**Run
electrophoresis
to separate the
fragments by
size**

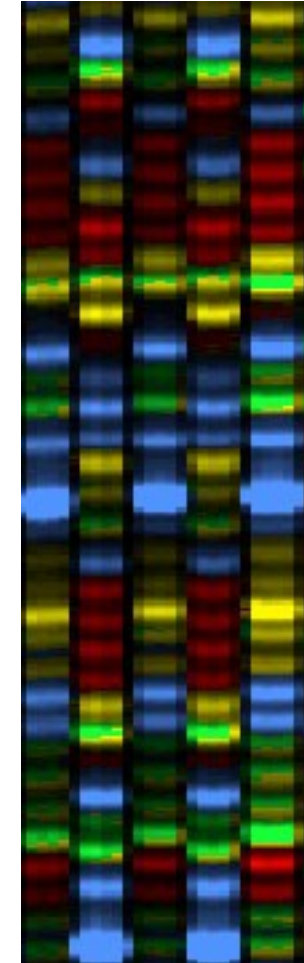


**Read the
sequence
based on color**

How can we automate Sequence DNA ??

- **Automated DNA sequencers**

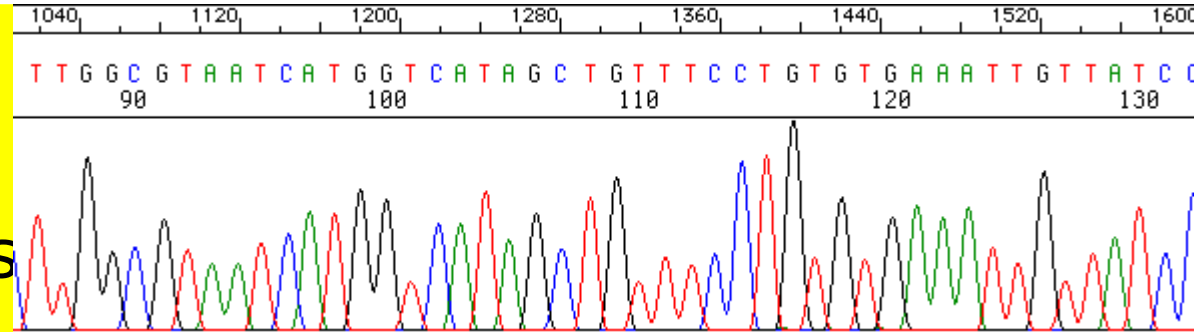
- These use capillary electrophoresis
- Fragments are piped through a tiny glass-fiber capillary during the electrophoresis step
- Fragments come out in size-order
- ultraviolet laser built checks for which Special nucleotide is coming out
- Computer reads the color & tells us the nucleotide



How can we automate Sequence DNA ??

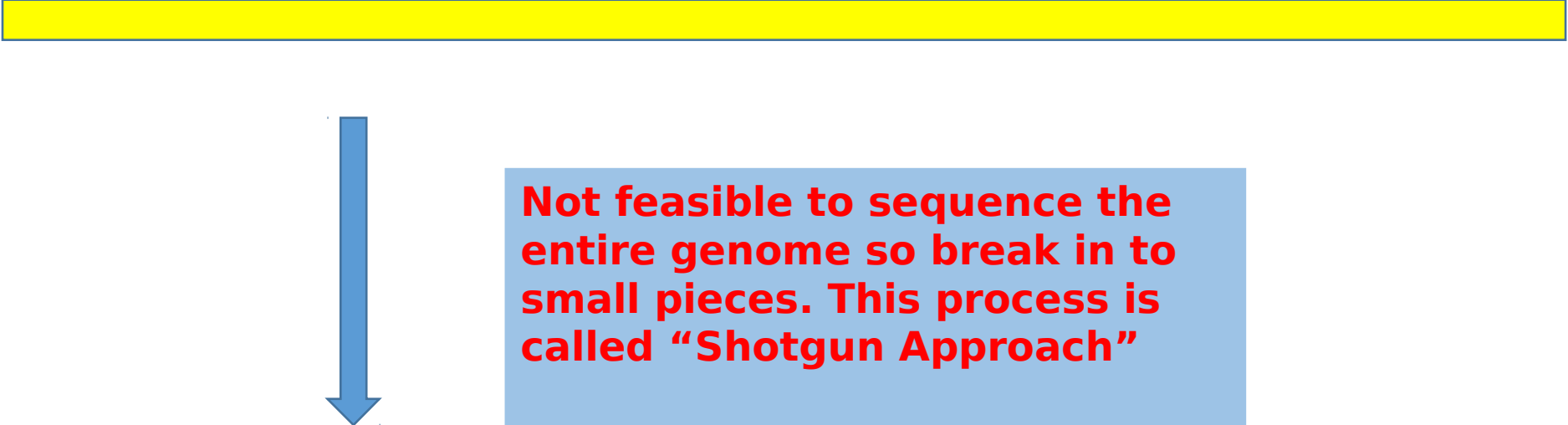
- **Automated DNA sequencers**

- These use capillary electrophoresis
- Fragments are piped through a tiny glass-fiber capillary during the electrophoresis step
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How can we automate Sequence DNA ??

**Human
Genome:
3 billion
nucleotide
long**



Not feasible to sequence the entire genome so break in to small pieces. This process is called "Shotgun Approach"

We can sequence 900 base pairs. Computer can then all these to get final sequence



Internet Resources

- [http://
seqcore.brcf.med.umich.edu/doc/educ/dnapr/sequencing.html](http://seqcore.brcf.med.umich.edu/doc/educ/dnapr/sequencing.html)
- [http://
www.wellcome.ac.uk/Education-resources/Education-and-learning/Resources/Animation/WTDV026689.htm](http://www.wellcome.ac.uk/Education-resources/Education-and-learning/Resources/Animation/WTDV026689.htm)
- <http://www.dnalc.org/resources/animations/sangerseq.html>
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