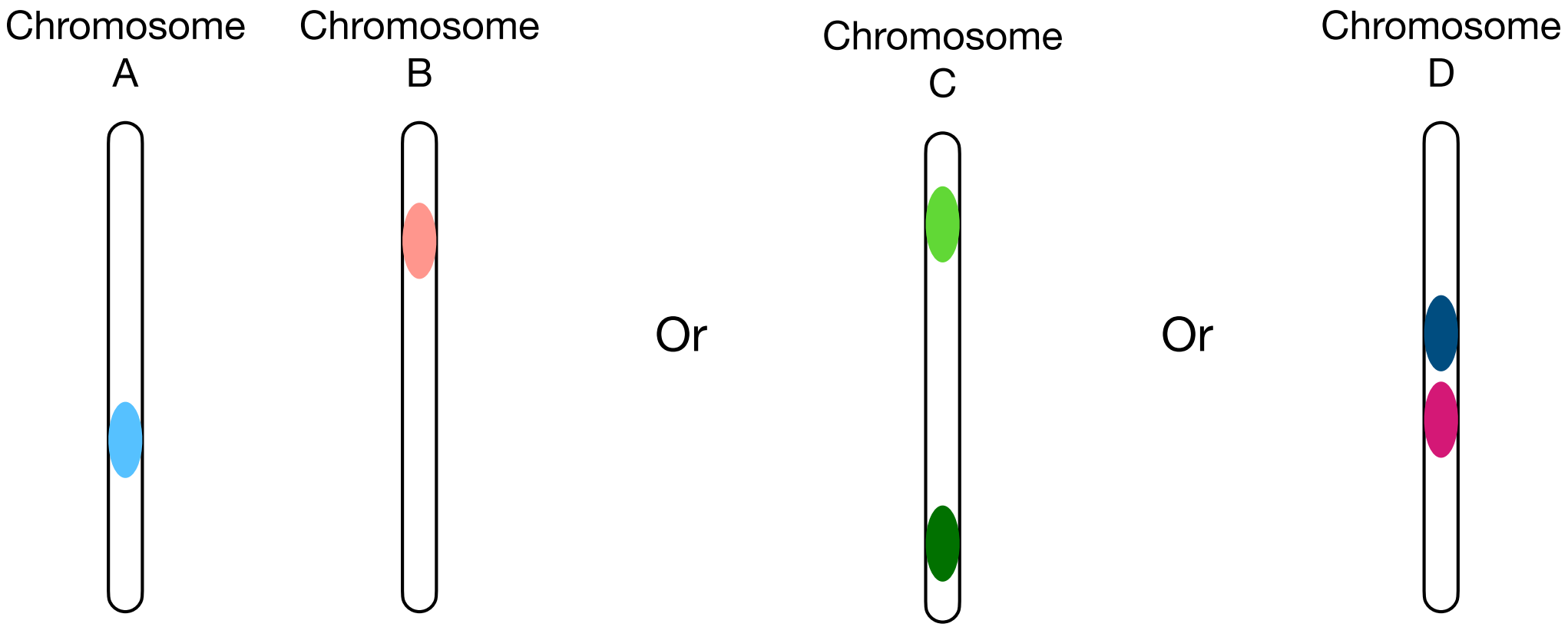
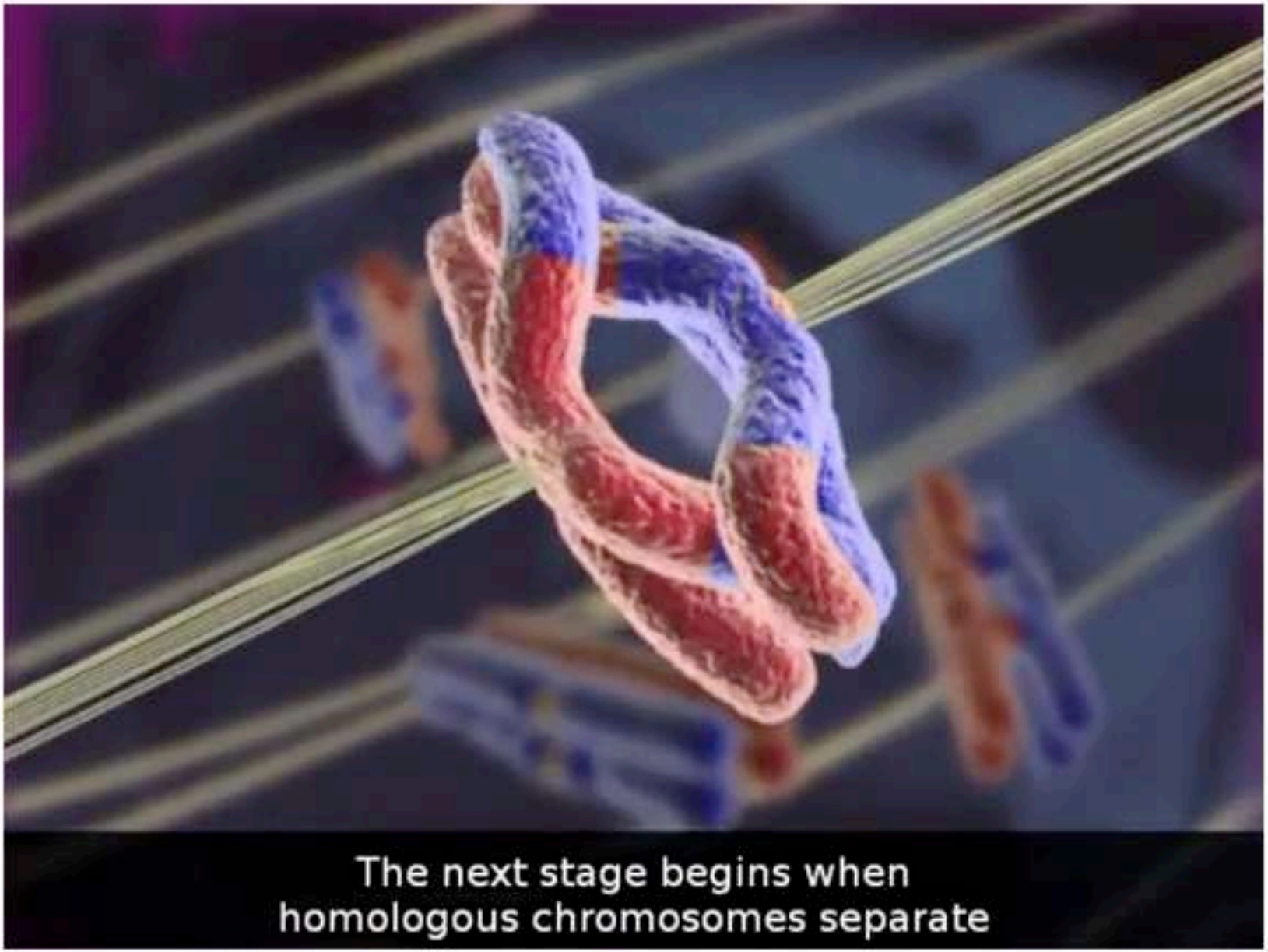
Genetic Linkage

- Genes on different chromatids are not physically contiguous on the same DNA molecule and can be inherited independently
- Genes on same chromatid are physically contiguous on the same DNA molecule and can be inherited independently, if they are far from each other
- Genes on same chromatid are physically contiguous on the same DNA molecule and are likely be inherited independently extremely rarely, if they are near to each other.
- Closer they are to each other, the less likely it is for them to be inherited independently



Only one chromatid shown in diagrams above. Ovals represent genes

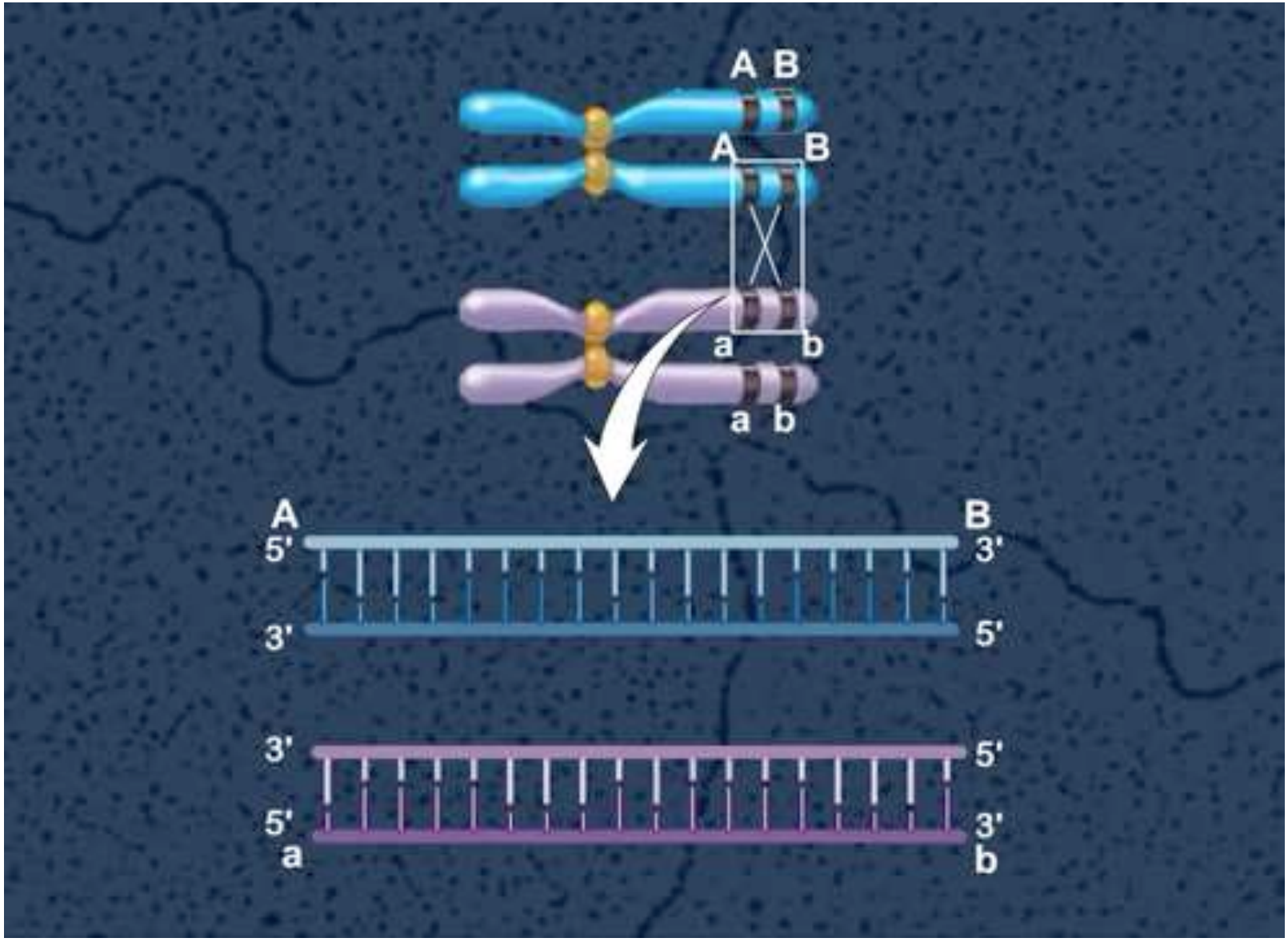
BioFlix: Meiosis



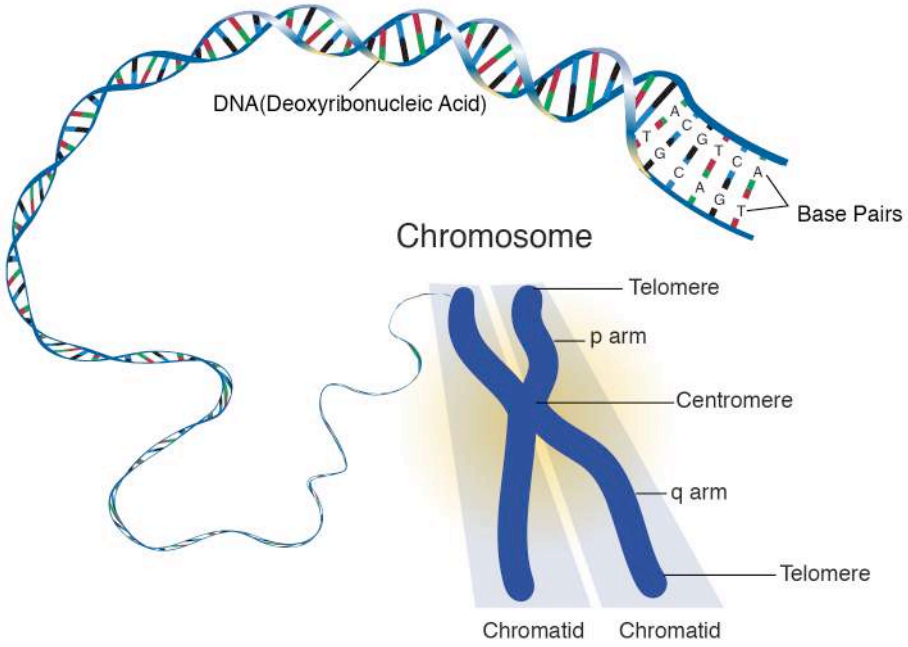
https://www.youtube.com/watch?v=EvRJ7yeF23k

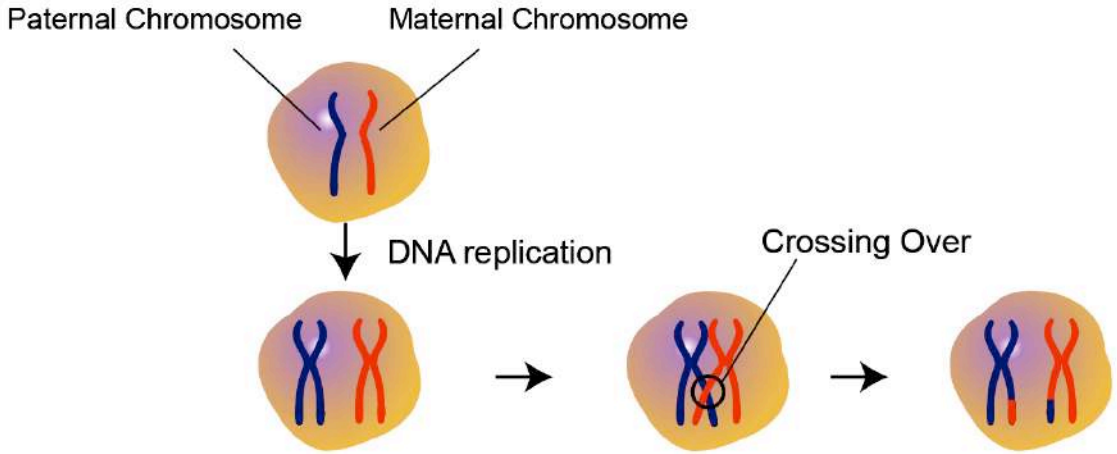
Recombination between non-sister chromatids during meiosis

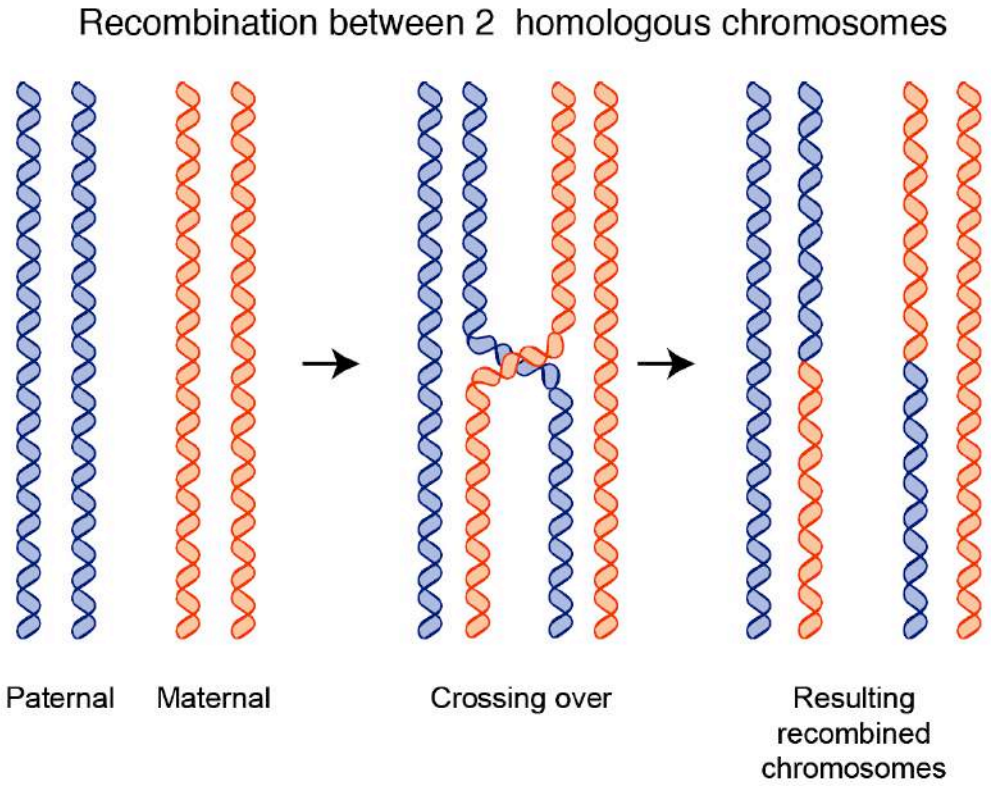
- Occurs in all species when sperm and eggs are produced
- Basis of genetic diversity amongst individuals of a species



Fundamental importance of recombination in the context of gene positions on a chromosome

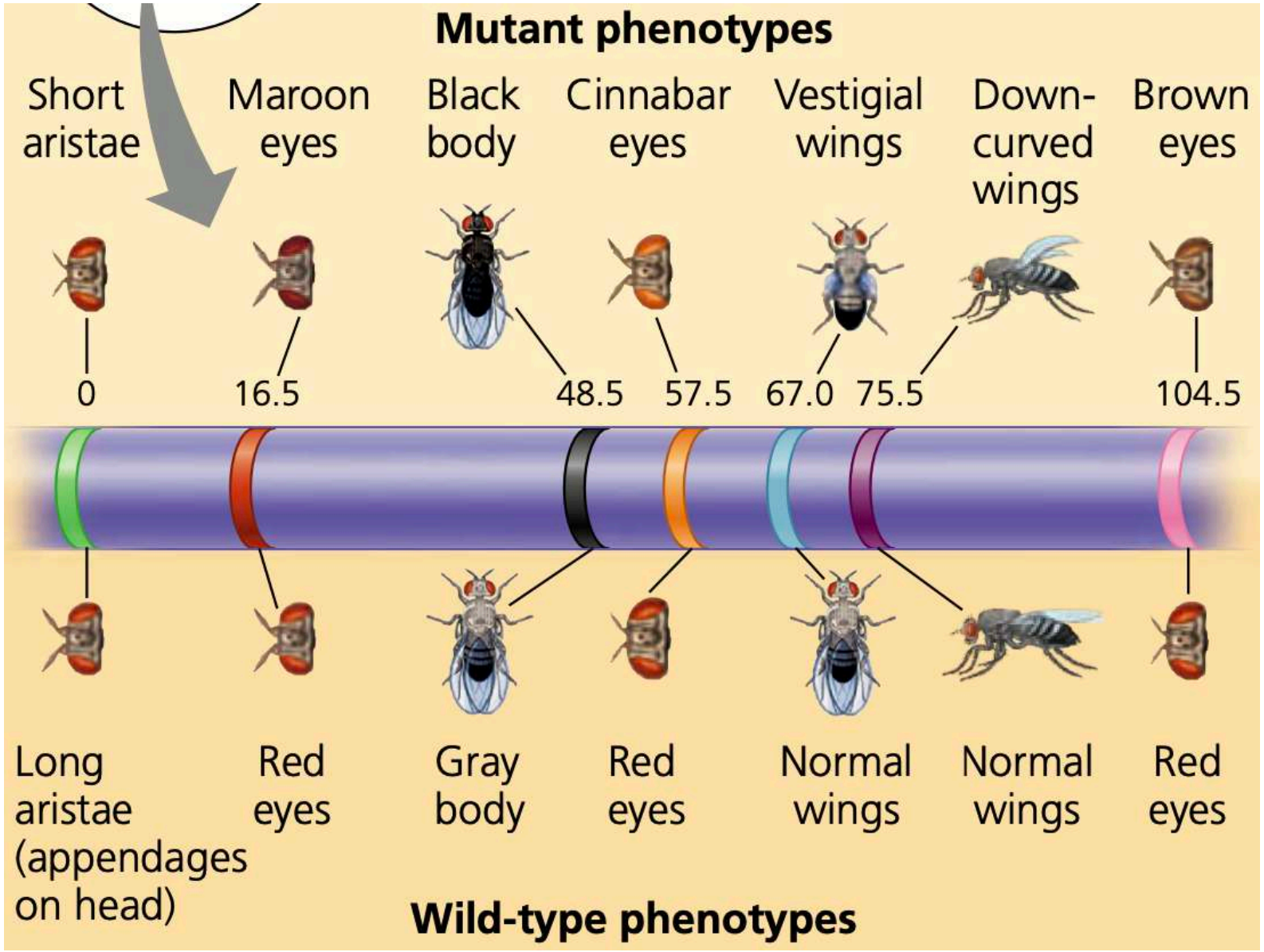






https://www.nature.com/scitable/topicpage/mitosis-14046258/

Recombination between non-sister chromatids during meiosis



If a cross between two individuals produced 100 progeny, in how many of these progeny do two traits manifest together?

Depends on how close 2 genes are on the same chromosome

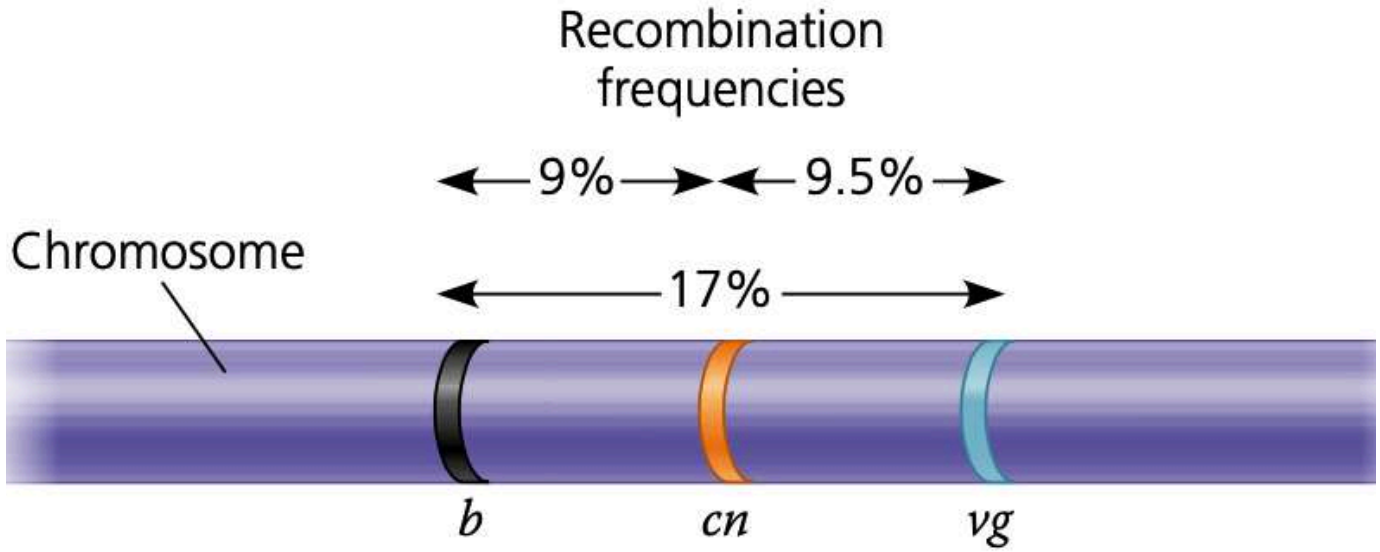
The closer the genes are, more likely they will be inherited together or not recombined away

Chapter 15 and Figure 15.12 of Campbell's Biology: a global approach

Genetic Linkage

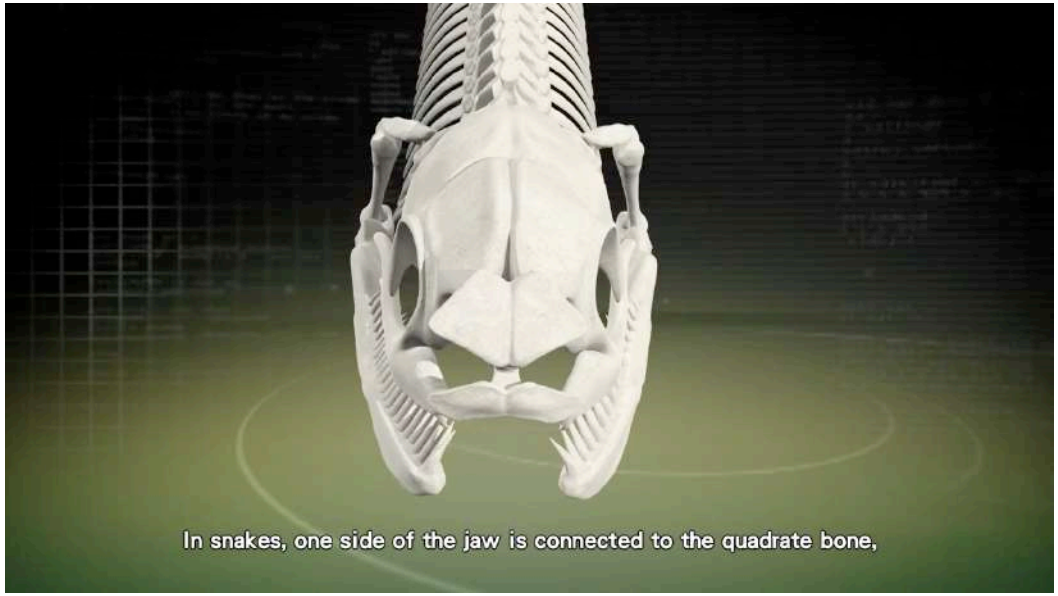
How does one estimate the distances between genes on chromosomes?

Typically if traits are separated (recombined) in 1 out of 100 progeny (1%), then the genes controlling those traits are 1centimorgan (cM) apart



Ignore X-inactivation sections in Chapter 15

Maternal genes and maternal inheritance of traits



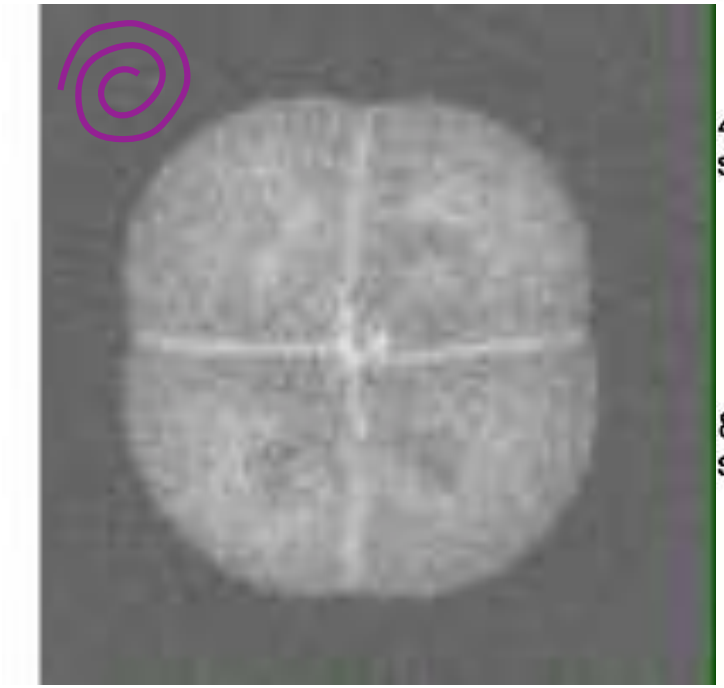
PMID: 21139578

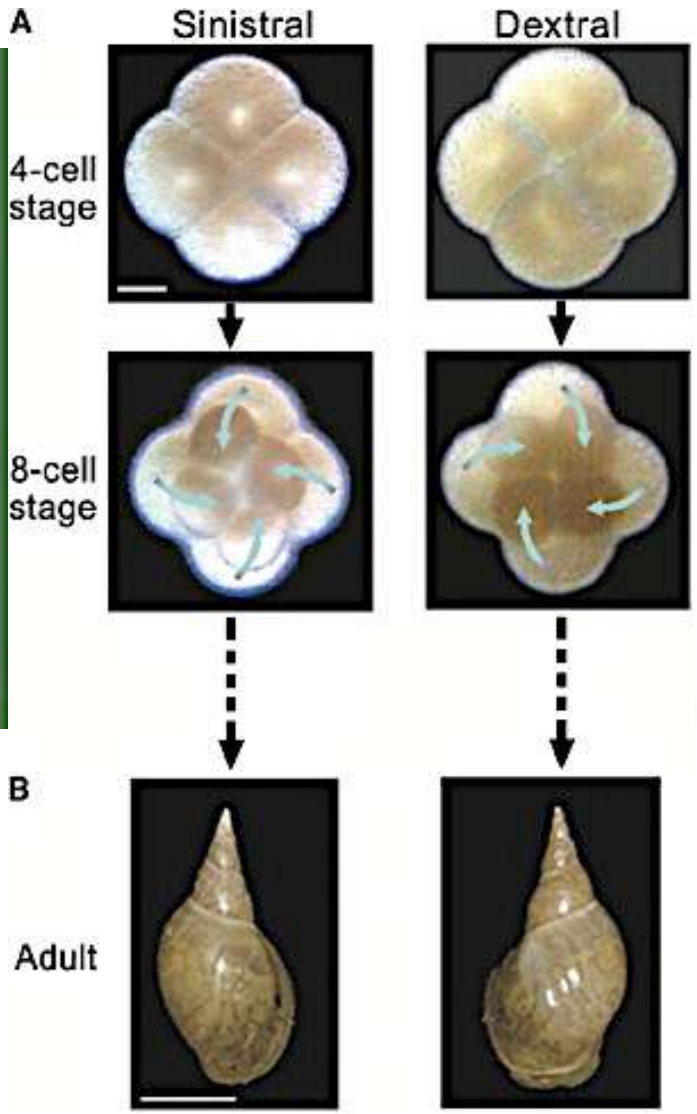
Coiling of shell - predator vs prey

- The snake extracts the snail by alternately protracting and retracting the asymmetric right and left mandibles, more teeth on the right side
- This approach works for a clockwise coil but not an anticlockwise coil snail

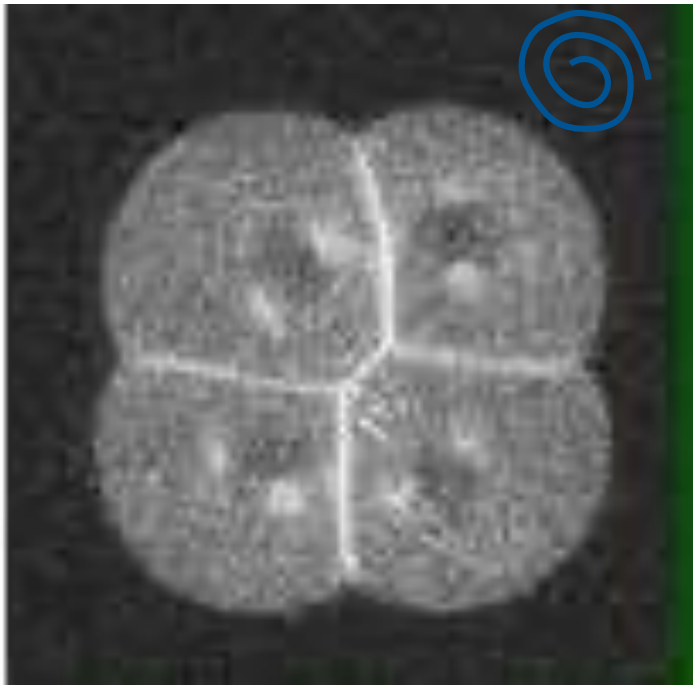
At the third cell division after fertilisation, snail cells undergo a chiral twist

4 new cells twist anticlockwise in an anticlockwise snail





4 new cells twist clockwise in a clockwise snail

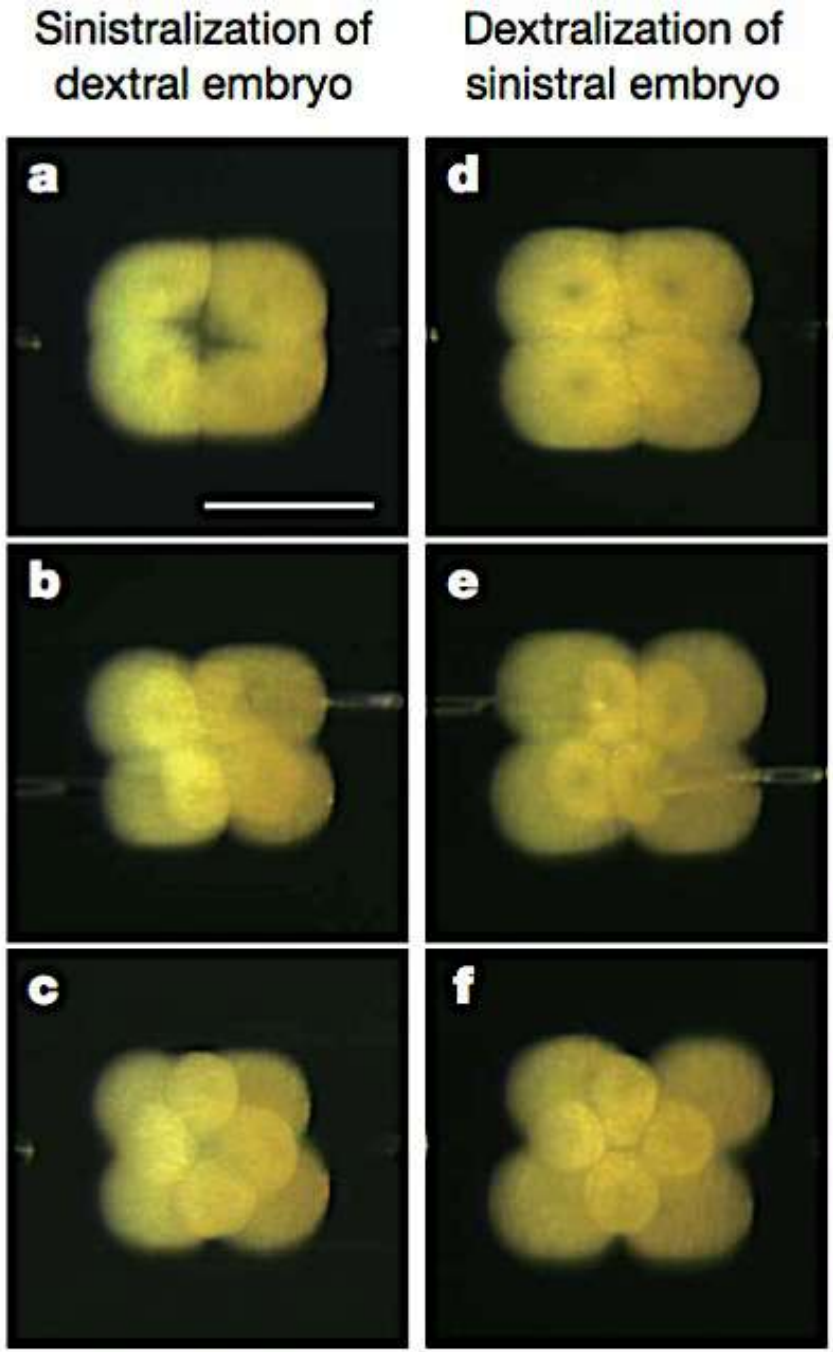


First observed in *Physa* a snail species which is normally an anticlockwise coil, by Henry Crampton in 1893

Lymnaea stagnalis
pond snail

PMID: 15324662

Can the clockwise and anticlockwise twist of cells be over-ridden?



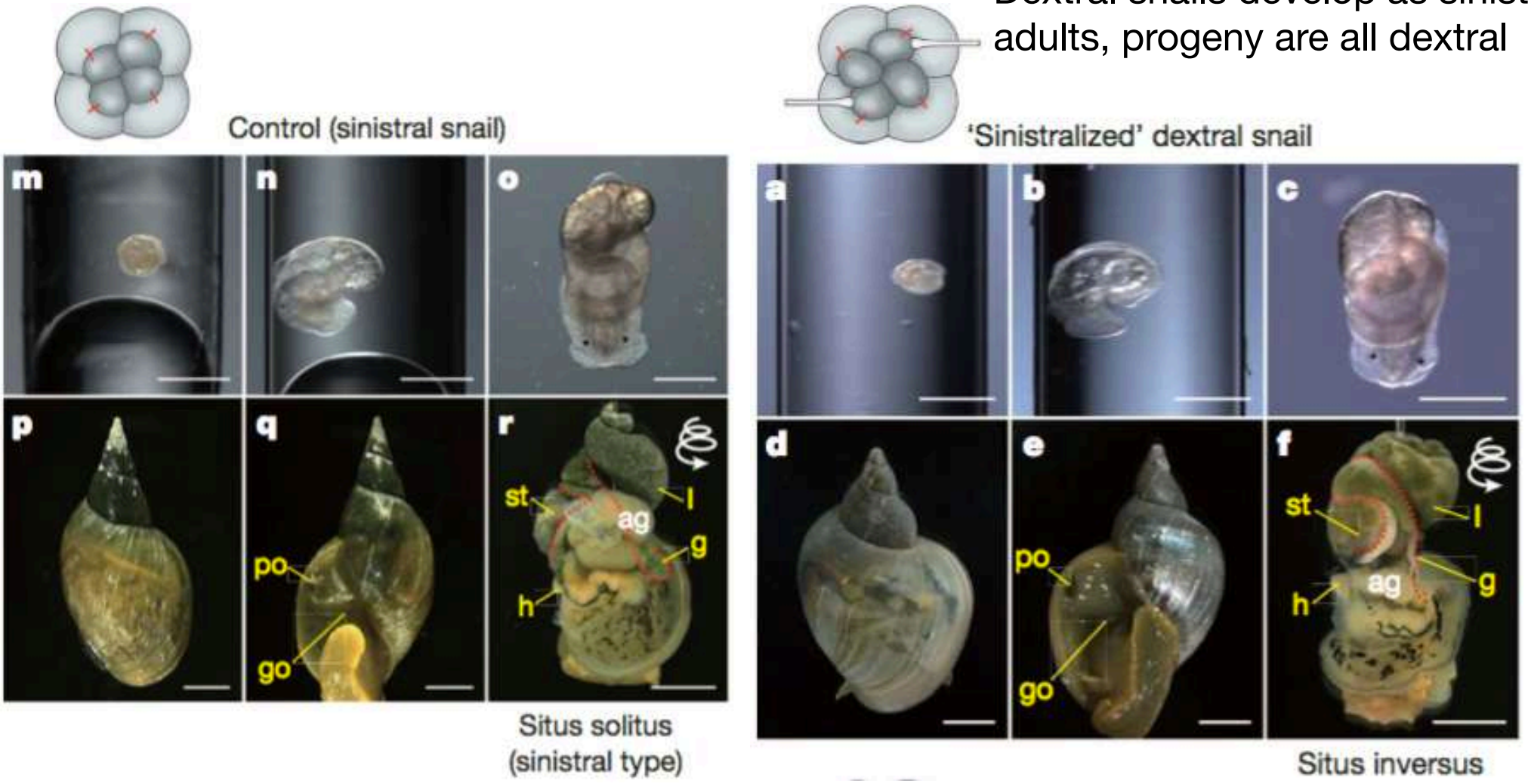
The 4 new cells (micromeres) were continuously pushed towards the direction opposite to normal by glass rods

Now the 4 new cells are placed opposite of how they would be normally placed in embryos

PMID: 19940849

Can the clockwise and anticlockwise twist of cells be over-ridden? - yes

Dextral snails develop as sinistral adults, progeny are all dextral

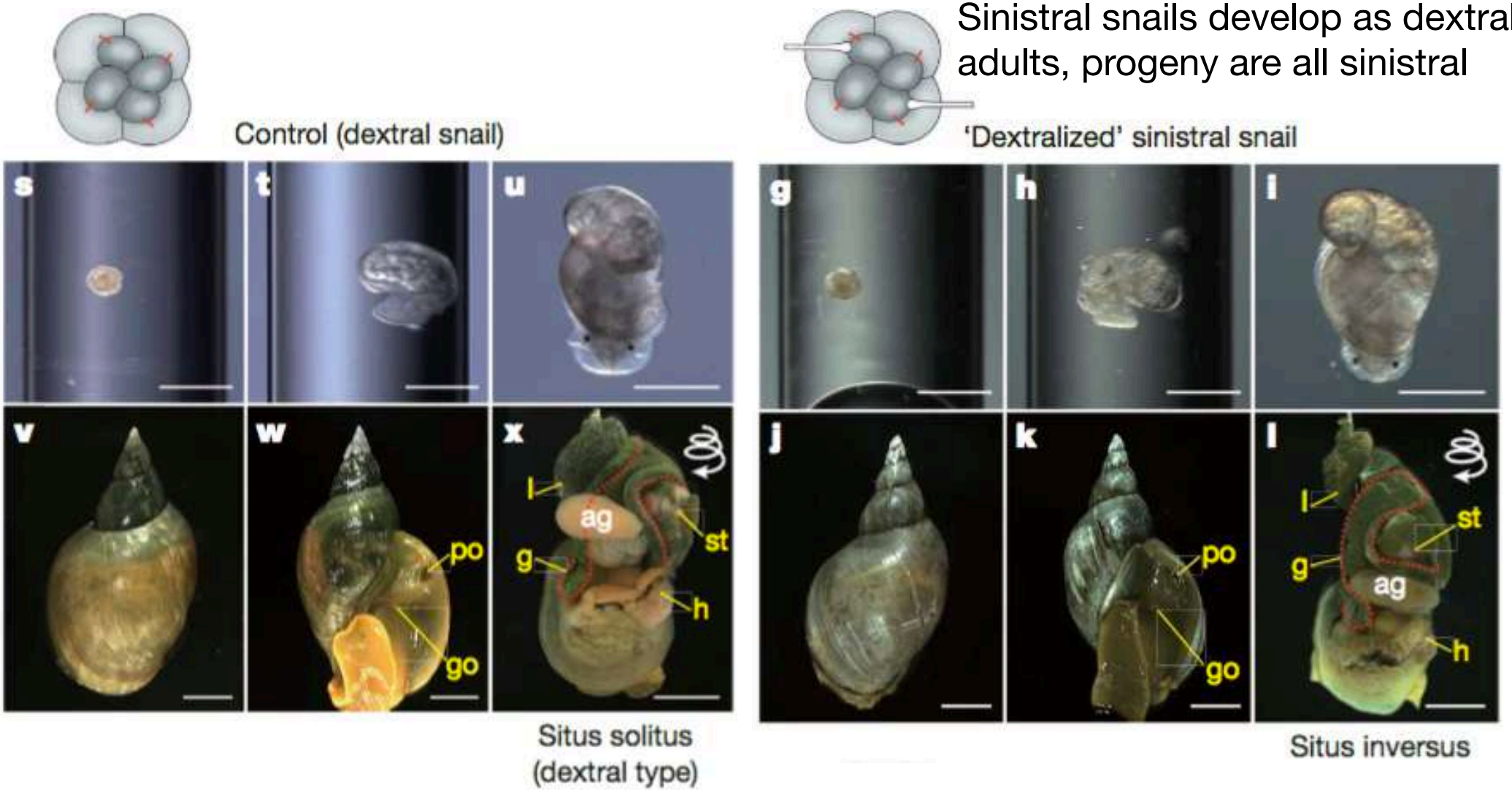


ag, albumen gland; g with dotted red line, gut; go, female genital opening; h, heart; l with white coil, liver; st, stomach; po, pulmonary sac opening

PMID: 19940849

Can the clockwise and anticlockwise twist of cells be over-ridden? - yes

Sinistral snails develop as dextral adults, progeny are all sinistral

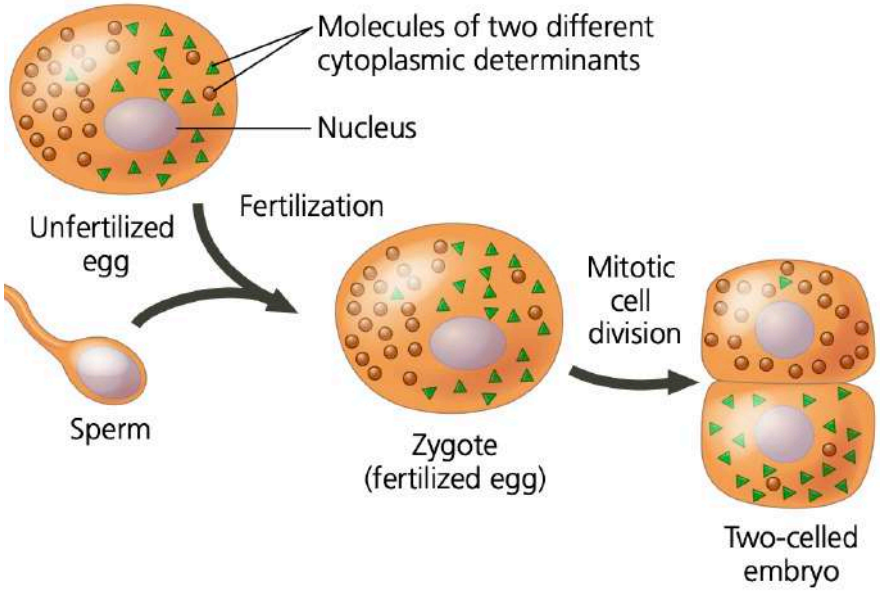


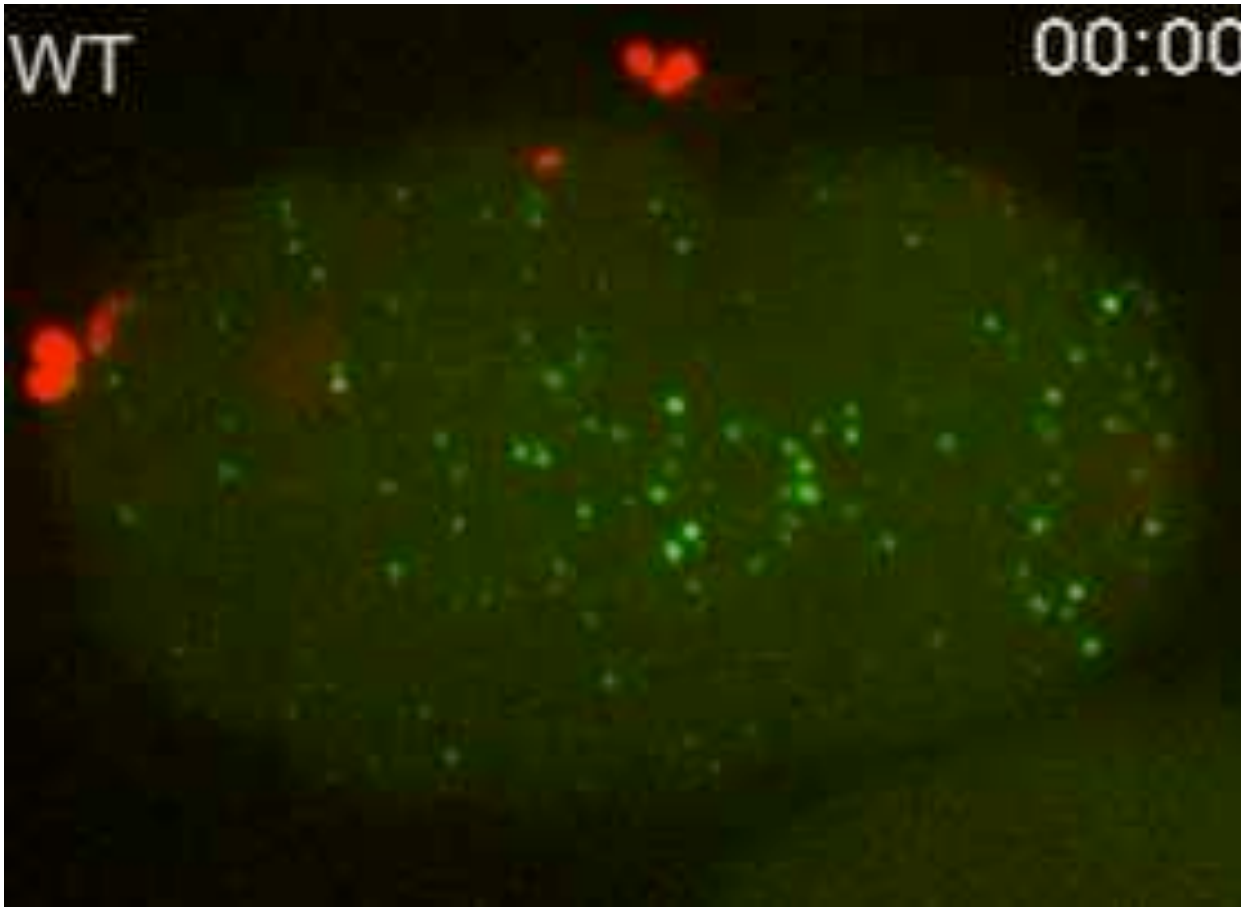
ag, albumen gland; g with dotted red line, gut; go, female genital opening; h, heart; l with white coil, liver; st, stomach; po, pulmonary sac opening

PMID: 19940849

Cytoplasmic Determinants - inheritance of a different kind

- RNAs and proteins deposited into the egg or sperm by the adult individual
- Controls early phases of embryonic development in all species
- Essential for cell fate determination in some species





PMID: 25535836

Figure 18.17 of Campbell's Biology: a global approach

PMID: 30103856

Cell Communication

Do cells “talk” and “listen” to their environment and each other?

Yes

- *E. coli* expressing Proteorhodopsin
- Protein is close to cell surface, acts like a solar panel and captures energy from light
- Intensity of light determines speed of swimming
- Applications in bioprinting, diagnostics etc

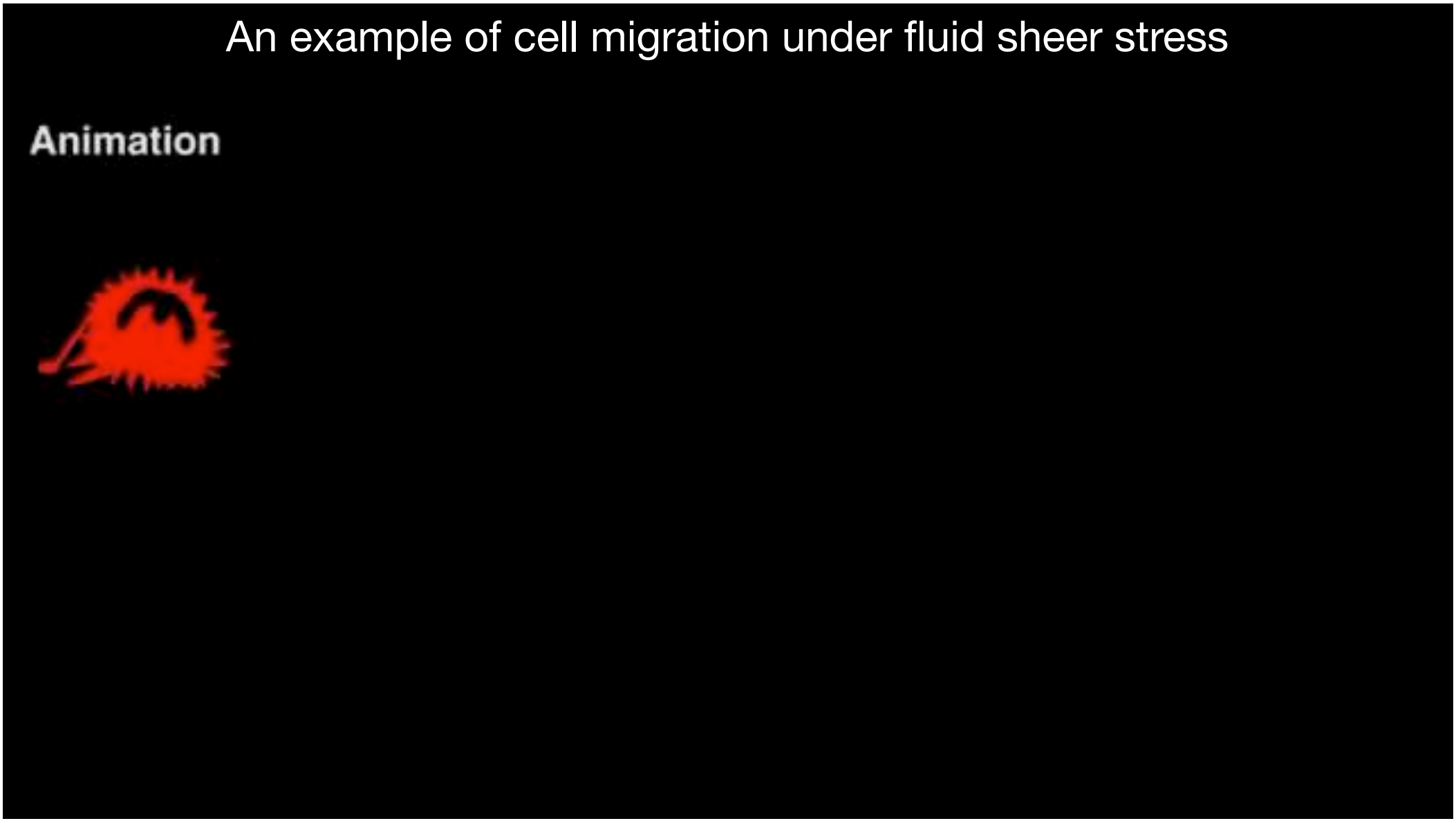
How do cells “sense” and “respond”?

PMID: 30103856

Cell Communication

Cells “talk” to each other and their environment

An example of cell migration under fluid sheer stress

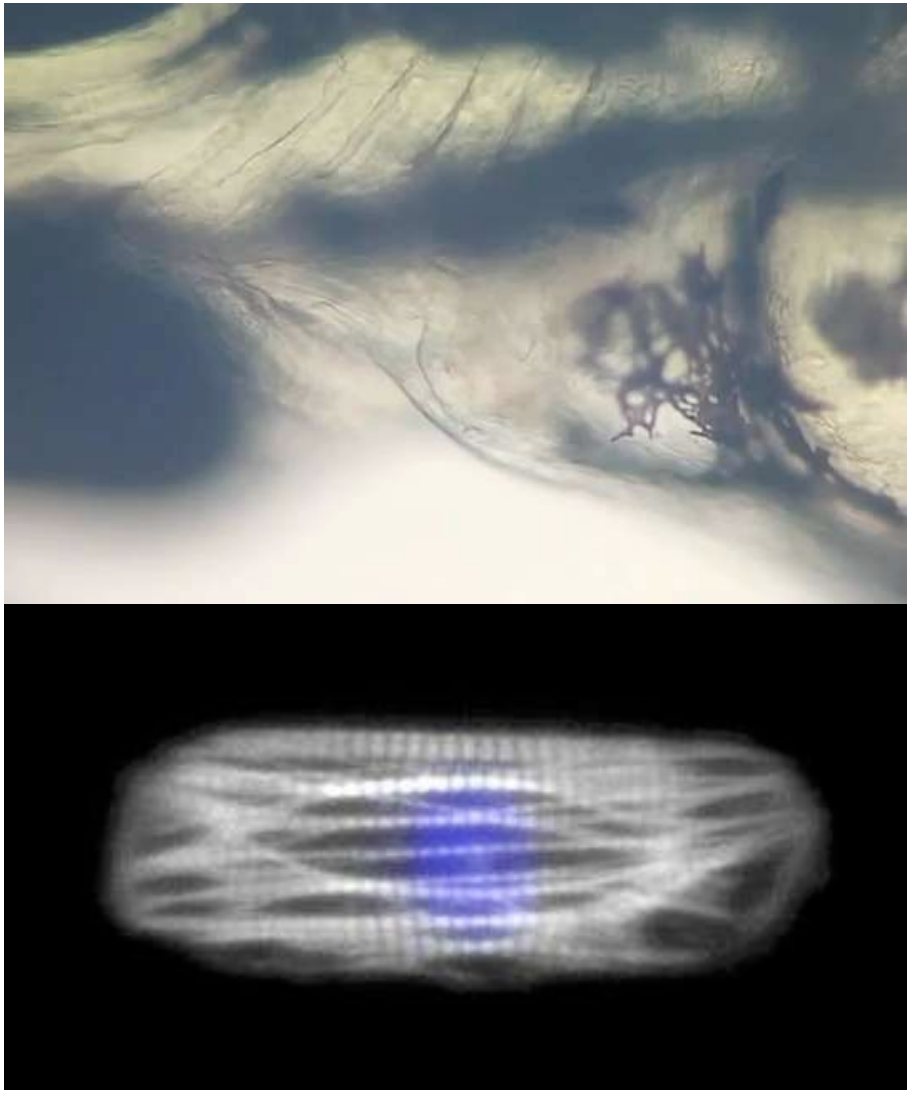


PMID: 22763437

Cell Communication

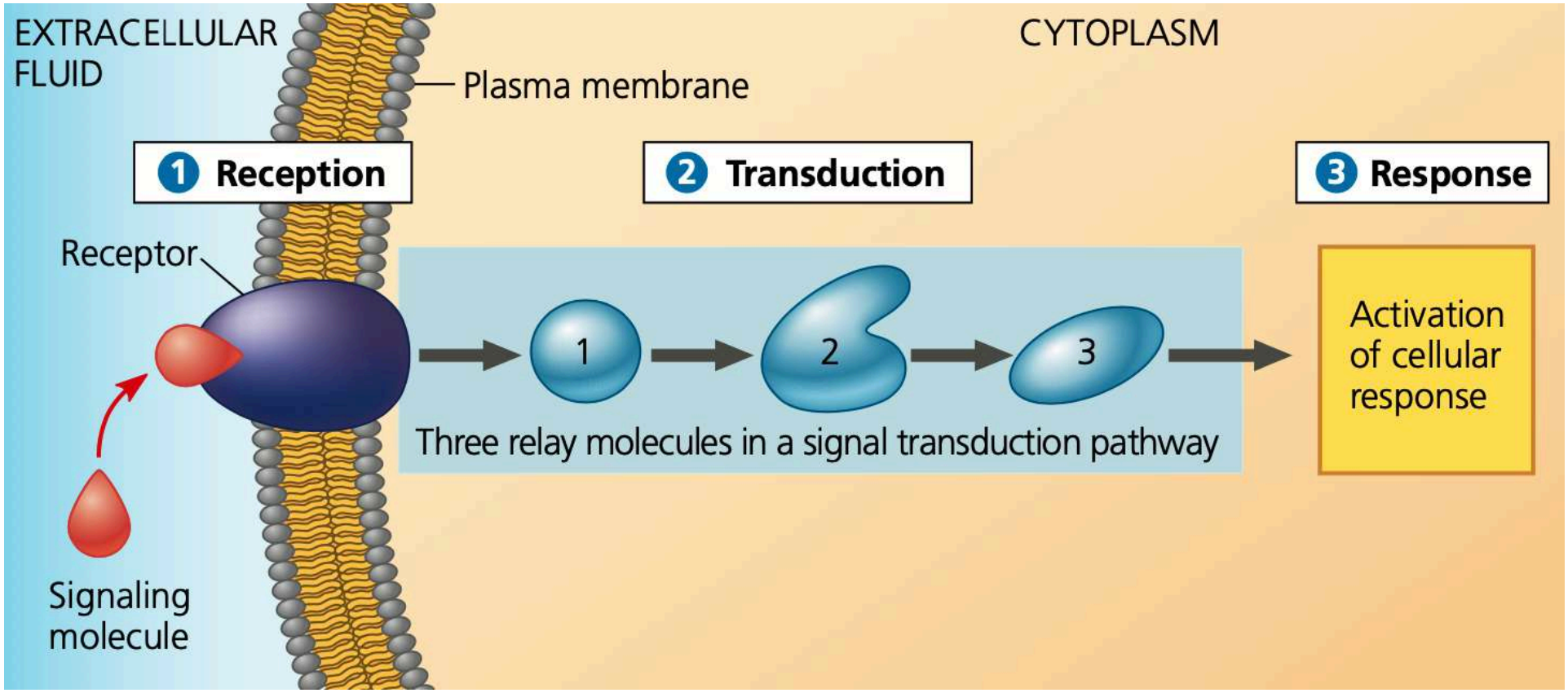
Cells “talk” to each other and their environment

- zebrafish embryo undergoing gastrulation



- Heart function in zebrafish larvae
- Single cardiomyocyte (from human iPSCs) exhibiting actomyosin driven contractility

Cell Communication



Sensing that someone is talking

Listening and reacting to what was heard

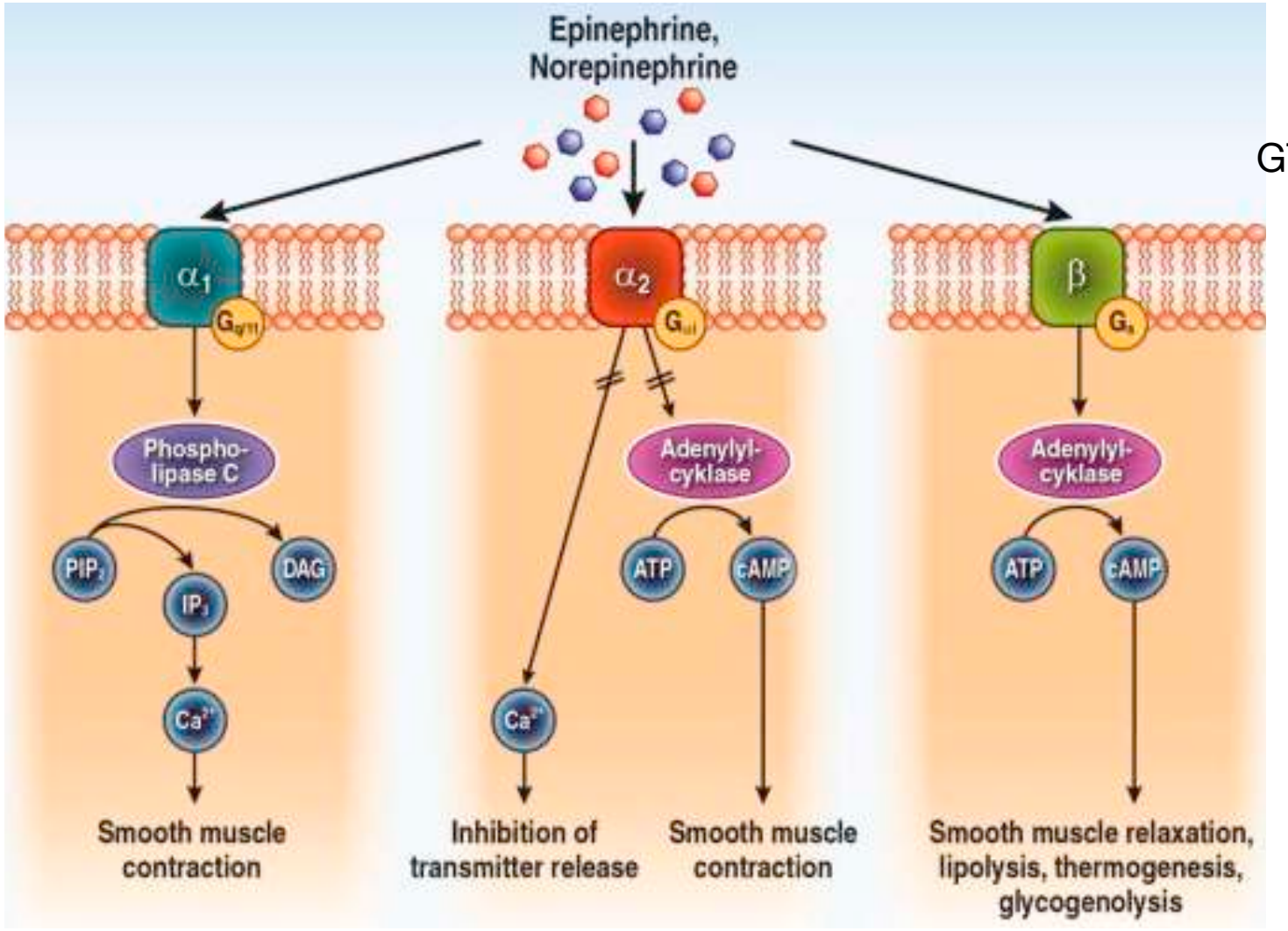
Deciding what to do based on what was heard

A general module cells use to talk and listen to each other and their environment

Figure 11.6 of Campbell's Biology: a global approach

Cell Communication

- Epinephrine - hormone, made and released by adrenal glands (located on top kidneys)
- Does many things - including production of glucose from glycogen when blood sugar is low (gluconeogenesis)
- Triggered by the sympathetic nervous system



First message

language “translator”

GTP, GDP, ATP, ADP, AMP, iP etc

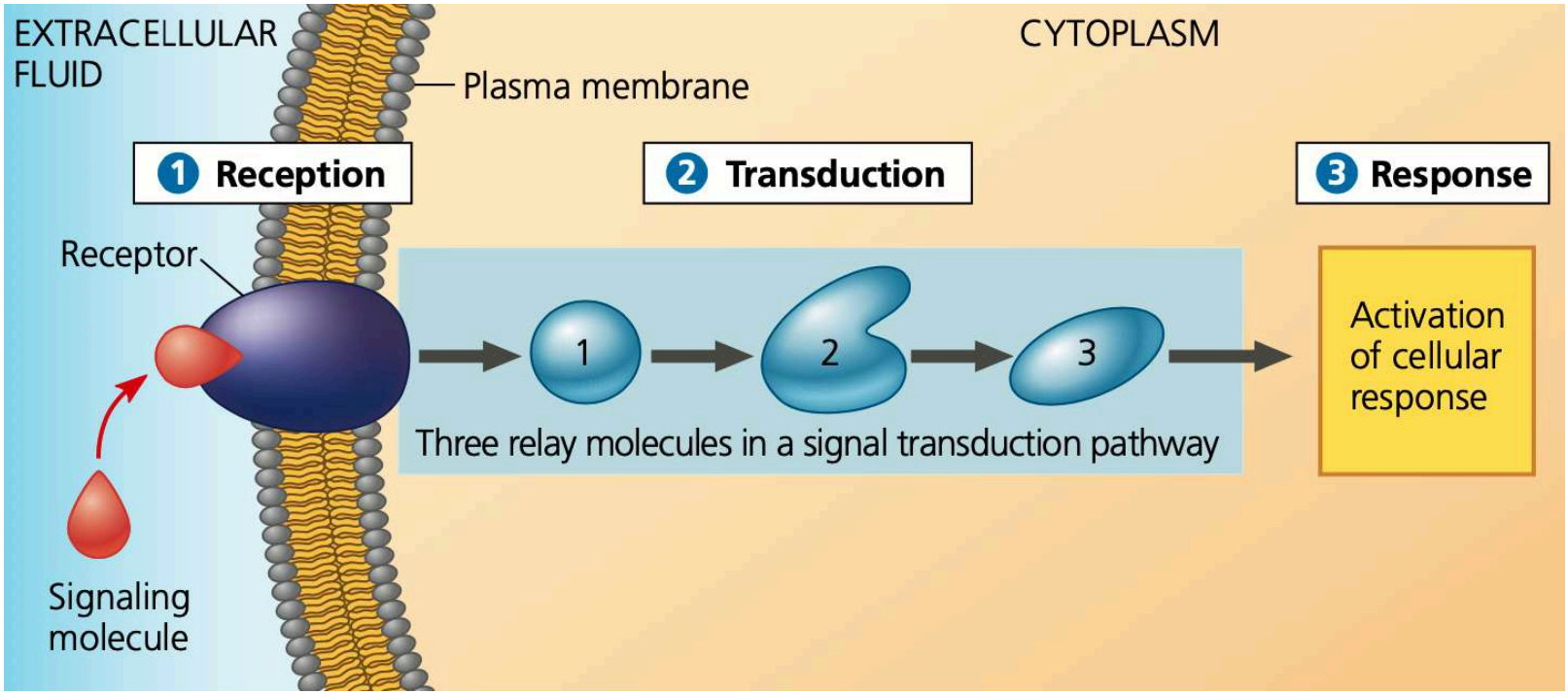
GPCRs located in various tissues: heart, muscles, liver, lungs, brain etc

Second message
Amplifies the signal

Allows cells to tune signal and responses

Figure 11.8, 11.10, 11.12, 11.13, 11.14 of Campbell’s Biology: a global approach

The concept that cells "sense" talking at the cell membrane won the 1971 Nobel Prize (work done by Earl W. Sutherland)



Sensing that someone is talking

Listening and reacting to what was heard

Deciding what to do based on what was heard

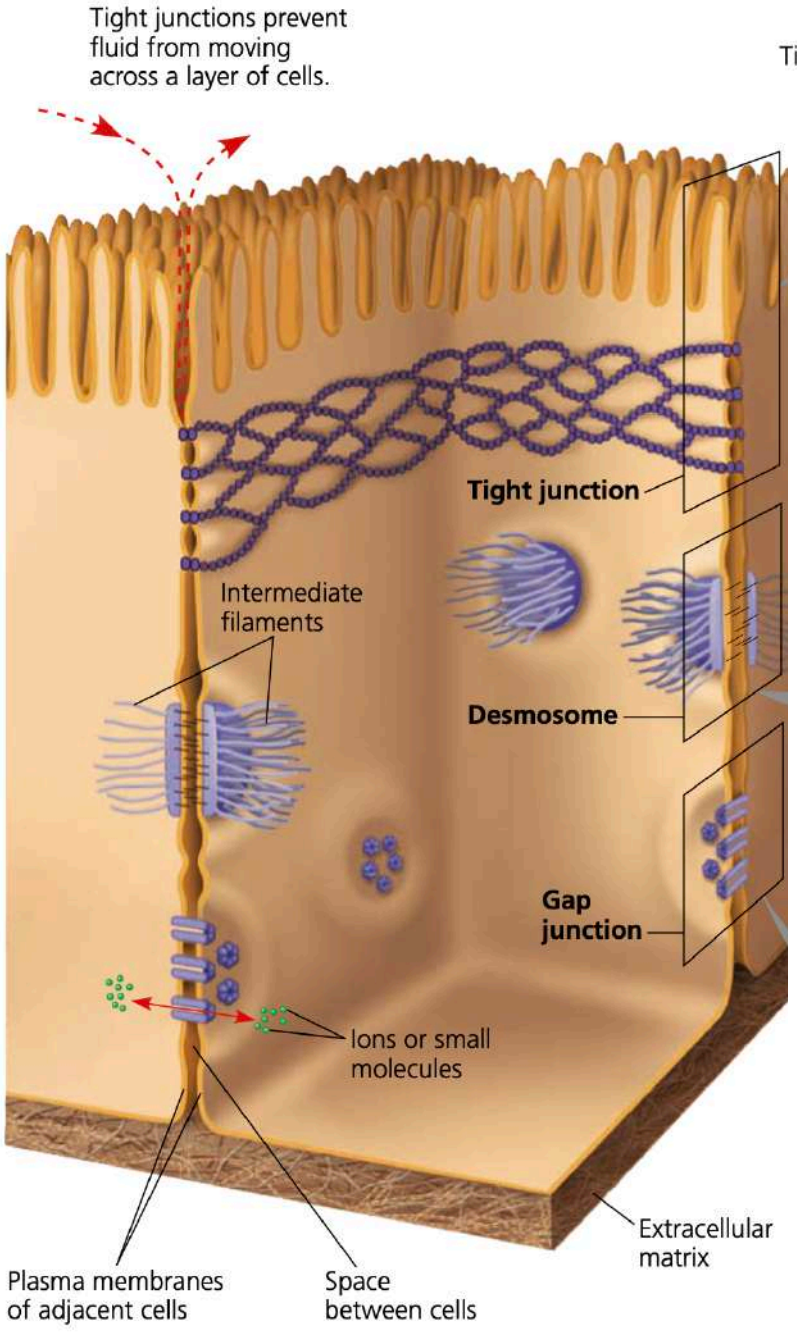
In a tube **A** **+B** did not make **C**

A needed intact cells **+B** to make **C**

The "ear" on a cell's membrane is known as the "receptor"

Cell Communication

Example: Gap junctions connect the cytoplasm of two cells to each other



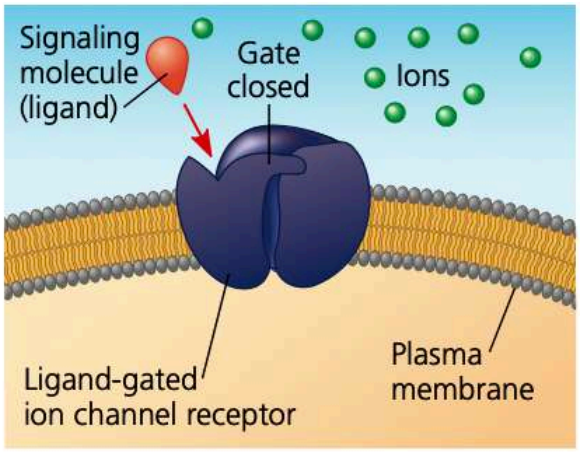
- Gap junction signaling allows cells to pass small molecules to each other via gap junction
- intercellular channel that allows the passage of small (s) molecules (up to 1.5 kDa), such as Ca ++ , IP 3 , and cAMP etc
- Gap-junctional channels are composed of hexamers of integral proteins: connexins in chordates and innexins in precordates
- Found in all cell and organ types

Gap junctions are like holes in two walls. If the holes match up to each other, cells can hear what is happening in their neighbour cell

Figure 6.3 of Campbell's Biology: a global approach

Sometimes cells use a “gate pass” to communicate

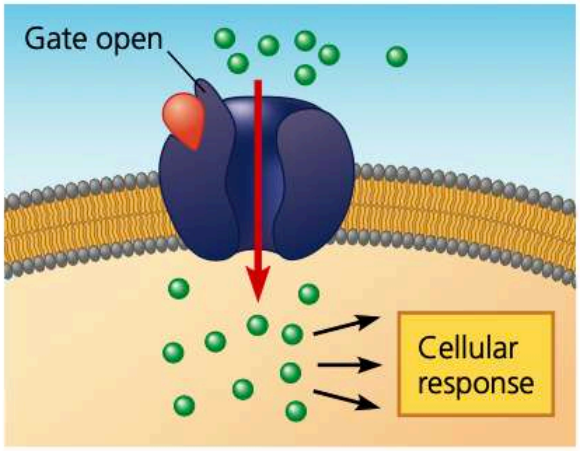
1. Here we show a ligand-gated ion channel receptor in which the gate remains closed until a ligand binds to the receptor.



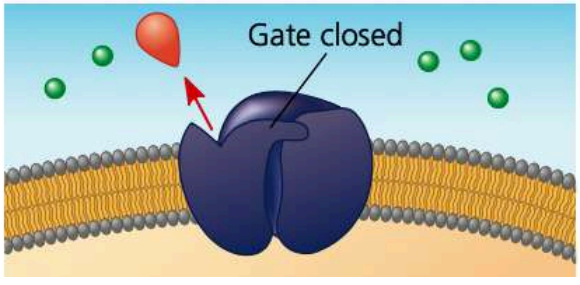
with the correct gate pass cells don’t need a translator

Ligand gated ion-channels

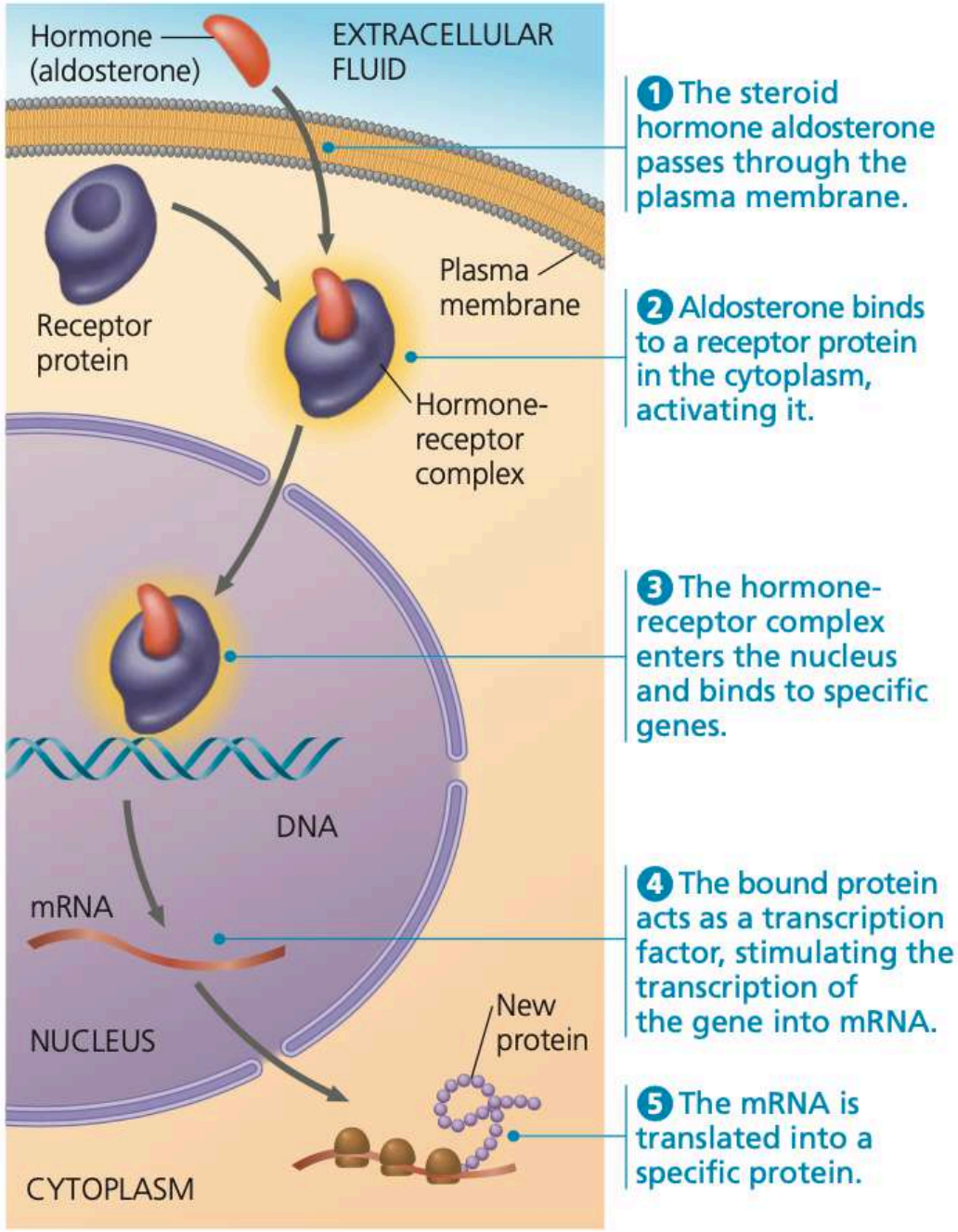
2. When the ligand binds to the receptor and the gate opens, specific ions can flow through the channel and rapidly change the concentration of that particular ion inside the cell. This change may directly affect the activity of the cell in some way.



3. When the ligand dissociates from this receptor, the gate closes and ions no longer enter the cell.

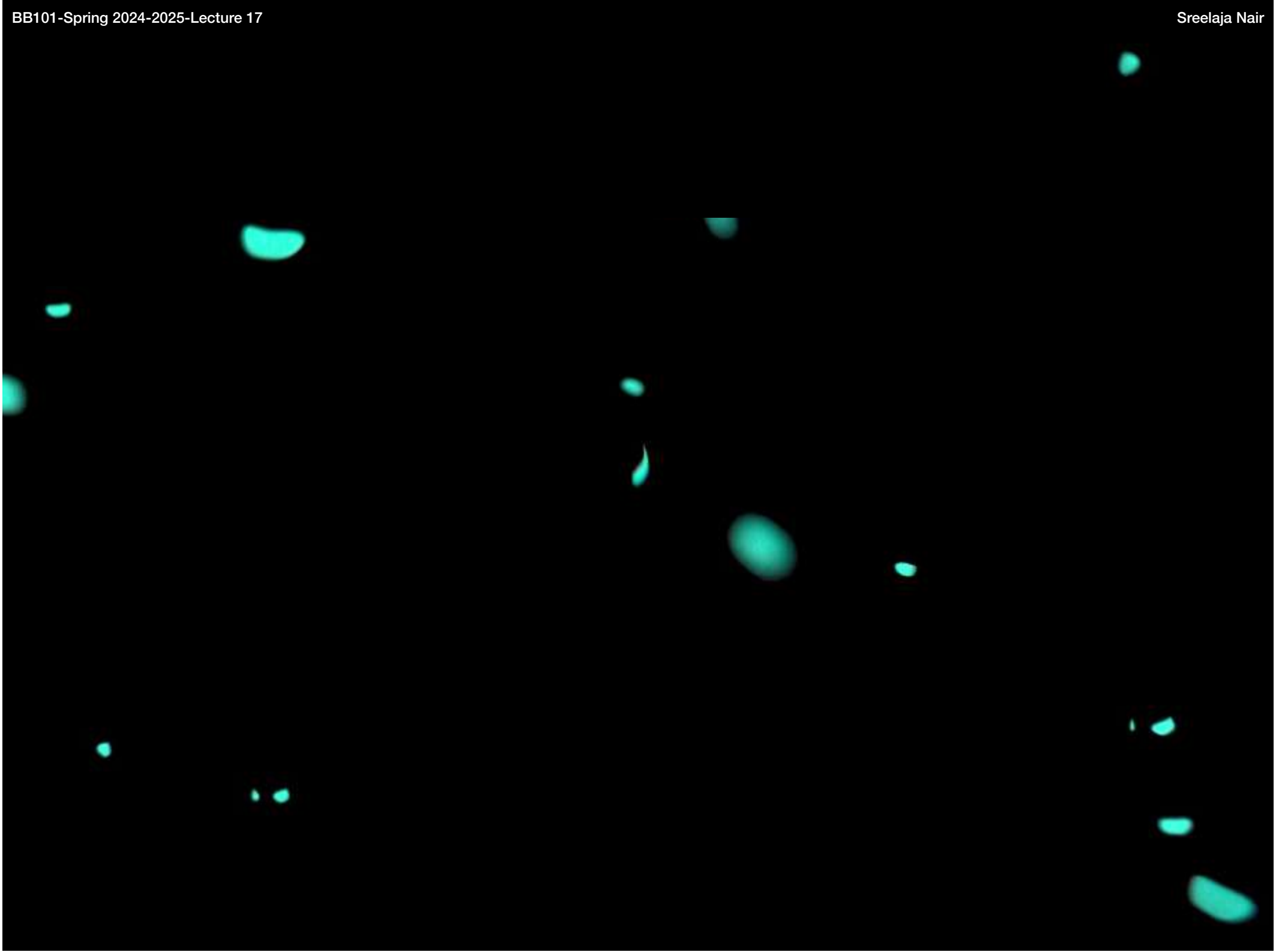


Can cells “hear” even if their receptors are floating inside them and are not on the membrane



Intracellular receptor based signaling

Figure 11.9 of Campbell’s Biology: a global approach

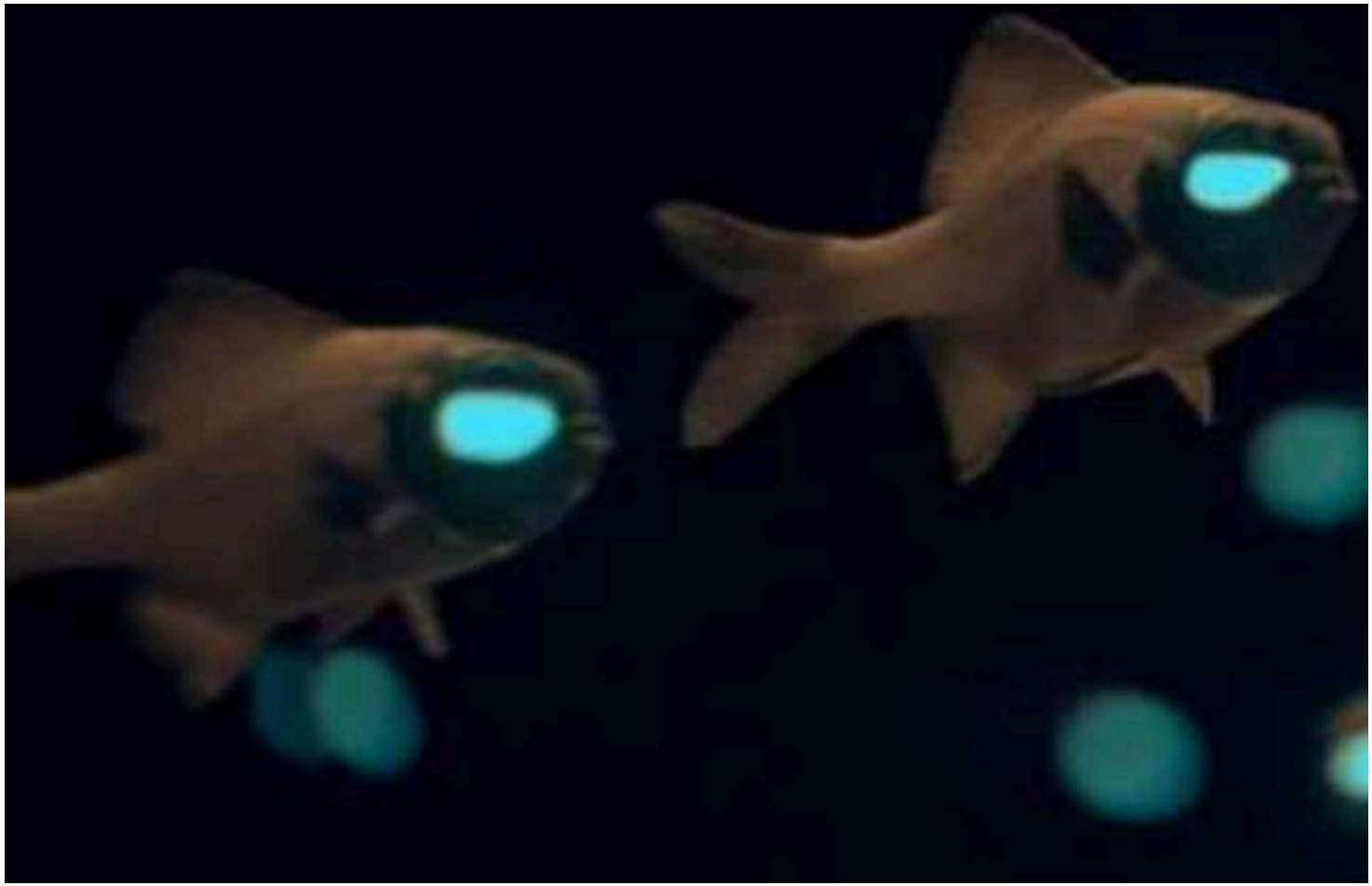


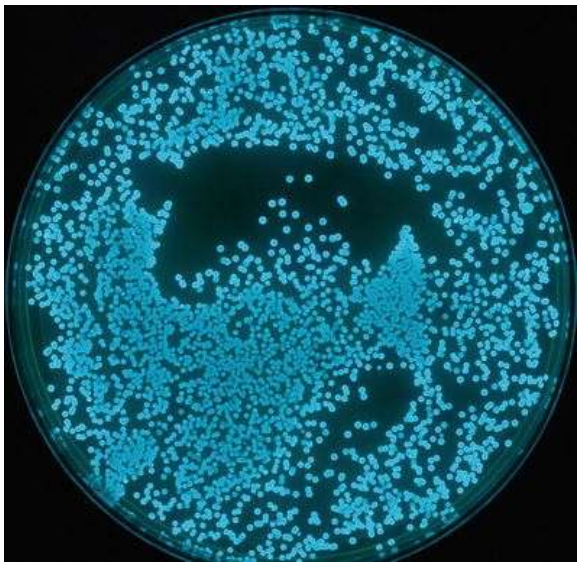
Cell Communication

Cells “talk” to each other and their environment

Allows emergence of unique interactions between species
Example: flashlight fish and their bioluminescent organ

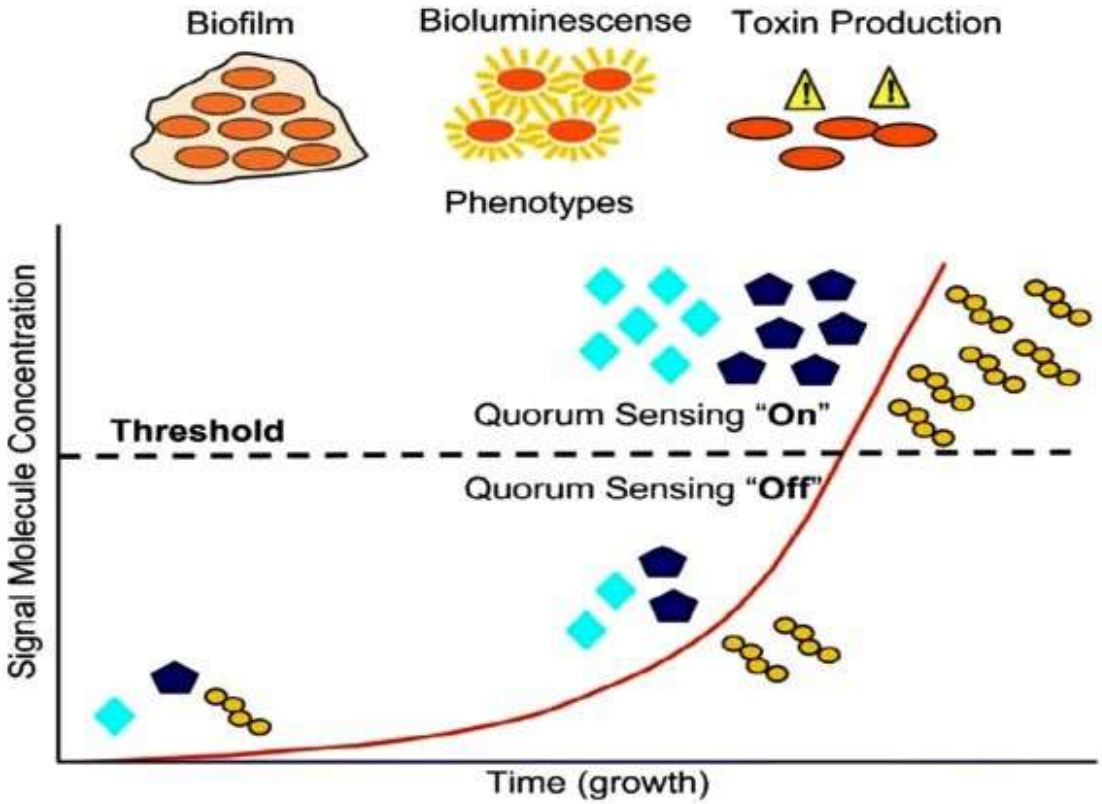
Occurs because the organ is colonized by bioluminescent bacteria

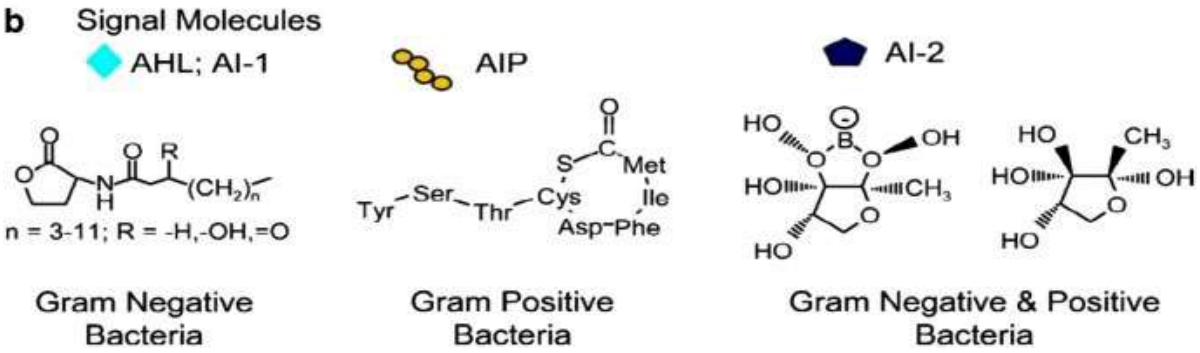




Cell Communication

Quorum sensing



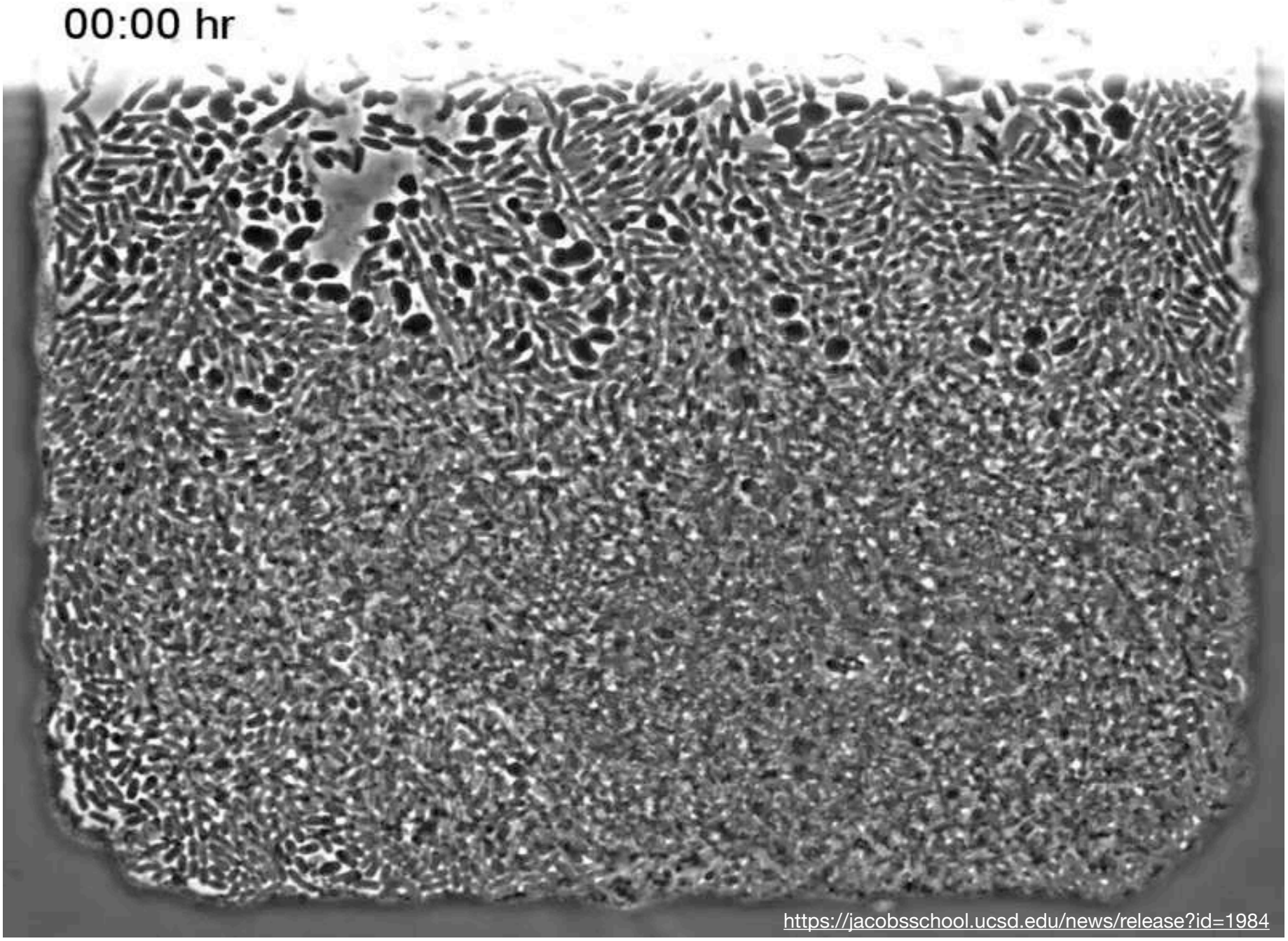


- Individual bacteria produce and secrete "autoinducers"
- Various types of autoinducers are made by bacteria
- When the concentration of autoinducers reach a threshold, a group of bacteria engage in a phenomenon collectively
- This is known as quorum sensing

https://doi.org/10.1007/s12602-019-09555-4

Cell Communication

Quorum sensing - triggering bacterial lysis for auto-timed release of drugs



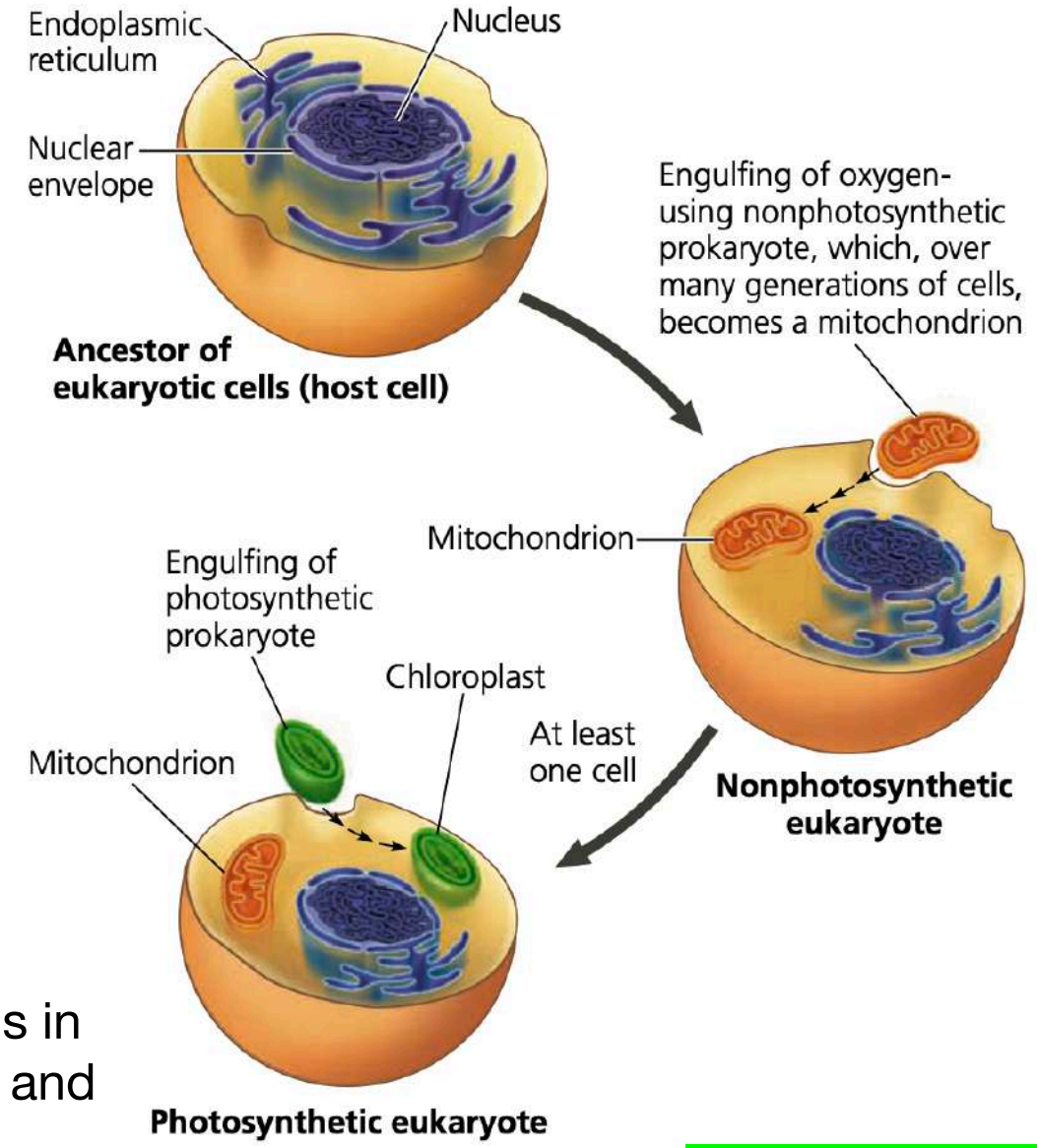
https://jacobsschool.ucsd.edu/news/release?id=1984

Cell Communication

- Cells communicate when in direct contact with each other
- Cells communicate at a distance
- Cells communicate using small molecule chemicals and gases
- Cells communicate using proteins
- Cells communicate using electrical signals

Evolution

Endosymbiont theory of the origins of mitochondria and chloroplasts in eukaryotic cells



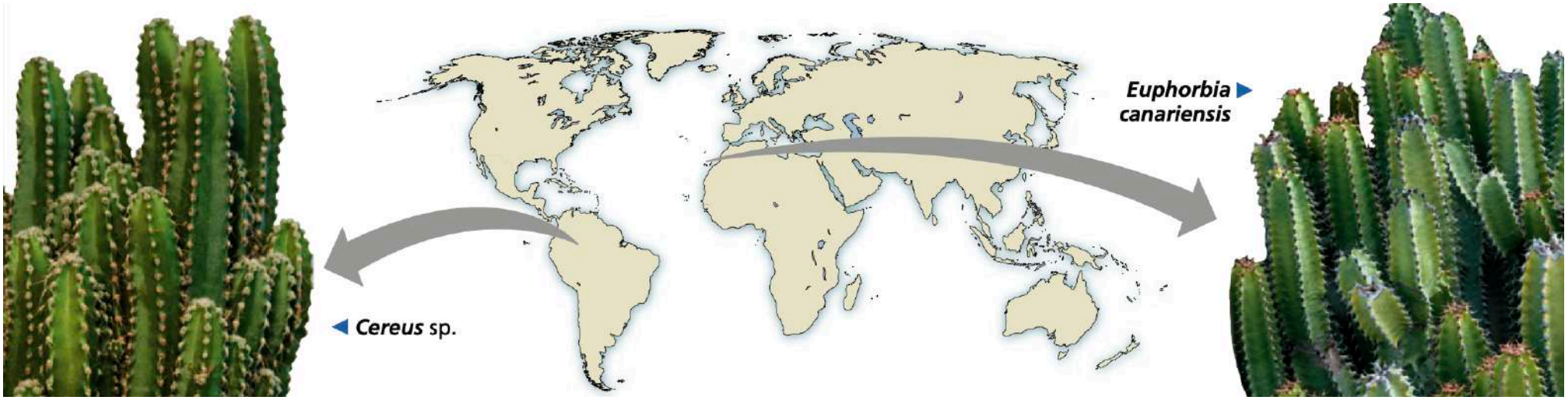
Advantageous in some context and survived

Advantageous in some context and survived

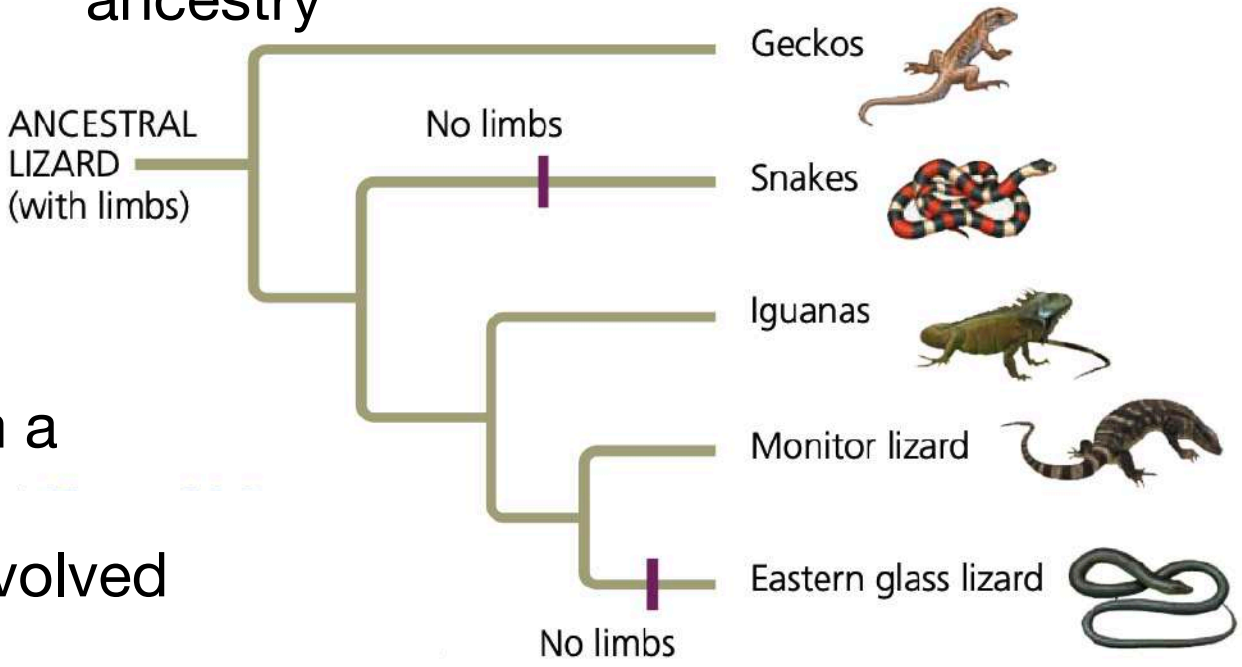
Figure 25.10 and 6.16 of Campbell's Biology: a global approach

Evolution

Convergent vs Divergent evolution



Convergent: same traits evolve independently multiple times - different ancestry



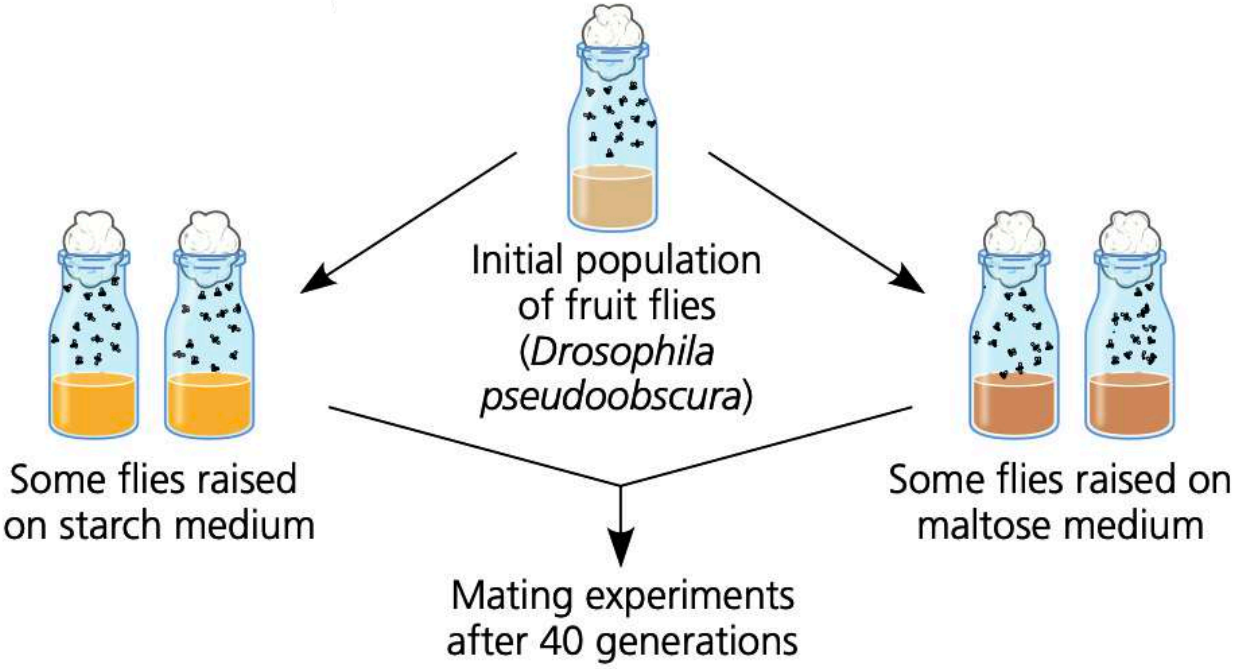
Divergent examples:

- All dog species evolved from a common wolf ancestor
- Elephants and Mammoths evolved from a common ancestor

Campbell's Biology: a global approach

Evolution

Reproductive barrier - defines a species



- Flies grown only on maltose or starch prefer to mate with like-partners
- Over the course of time, the two types of flies can evolve into two different species

		Female	
		Starch	Maltose
Male	Starch	22	9
	Maltose	8	20

Number of matings in experimental group

		Female	
		Starch population 1	Starch population 2
Male	Starch population 1	18	15
	Starch population 2	12	15

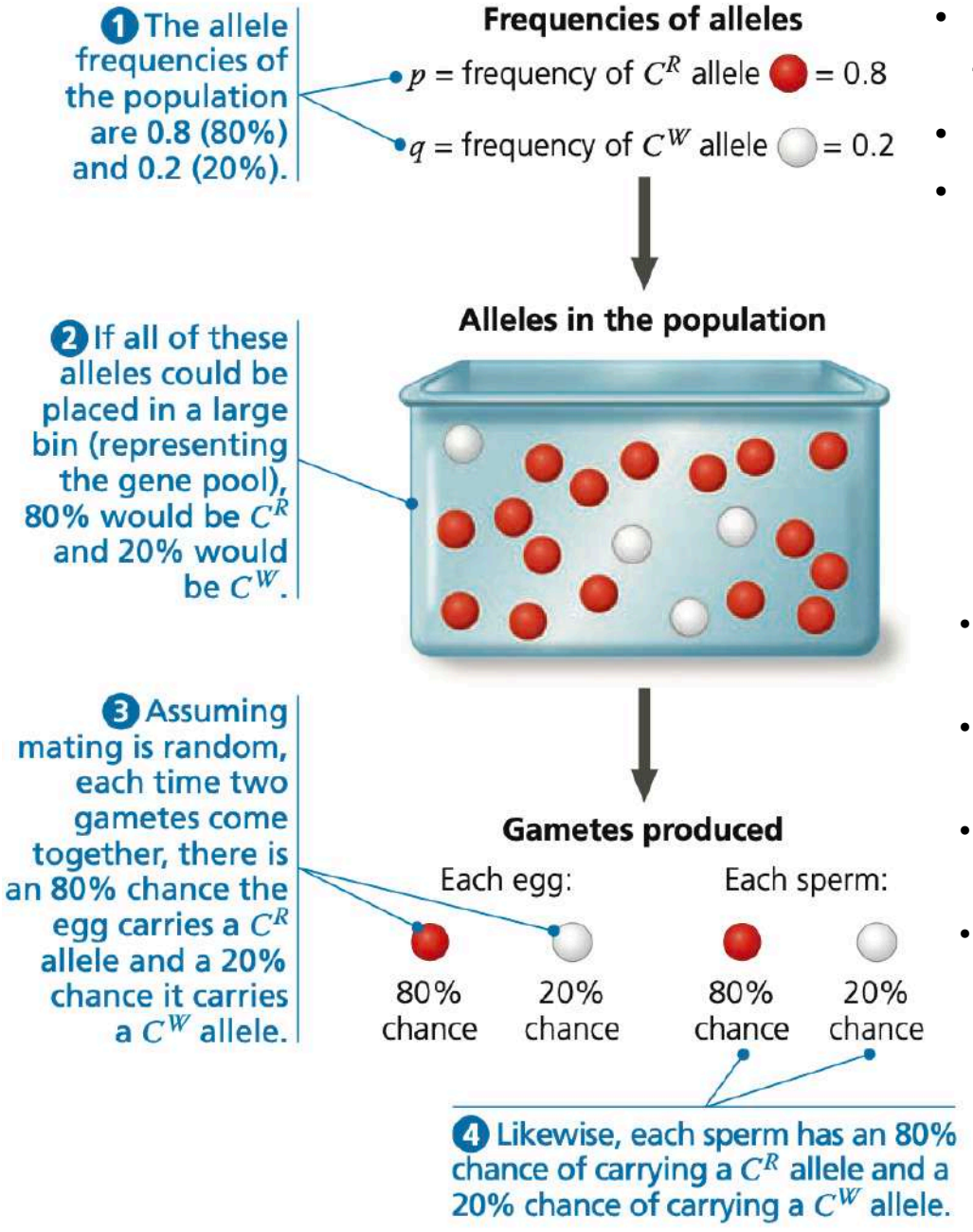
Number of matings in control group

- Groups experience different selection pressures - this may change physiology, behavior, genes etc leading to reproductive isolation
- Groups may also be separated geographically, leading to reproductive isolation

Figure 24.3 and 24.7 of Campbell's Biology: a global approach

Evolution

Hardy Weinberg equilibrium - How to tell if a species is evolving?



- 500 plants with two alleles, C^R and C^W, for a locus that codes for flower pigment, incomplete dominance
- C^RC^R = Red, C^RC^W = Pink, C^WC^W = White
- Each allele has a frequency (proportion) in the population

  p = frequency of one allele (C^R)

  q = frequency of the other allele (C^w)

  Example data: in the 500 plants

  320 = red flowers

  160 = pink flowers

  20 = white flowers

- Because these are diploid, the 500 individual plants have a total of 1,000 copies of the gene for flower color
- The C^R allele accounts for 800 of these copies (320x2 = 640 for C^RC^R plants + 160x1 = 160 for C^RC^W plants).
- The C^W allele accounts for 200 of these copies (20x2 = 4 for C^WC^W plants + 160x1 = 160 for C^RC^W plants).
- Frequency of the C^R allele in the population is 800/1000 = 0.8 (80%) and of the C^W allele in the population is 200/1000 = 0.2 (20%)

sum of all allele frequencies, irrespective of number of alleles = 1 i.e. (100%)

Figure 23.7 of Campbell's Biology: a global approach

Evolution

Hardy Weinberg equilibrium - How to tell if a species is evolving?

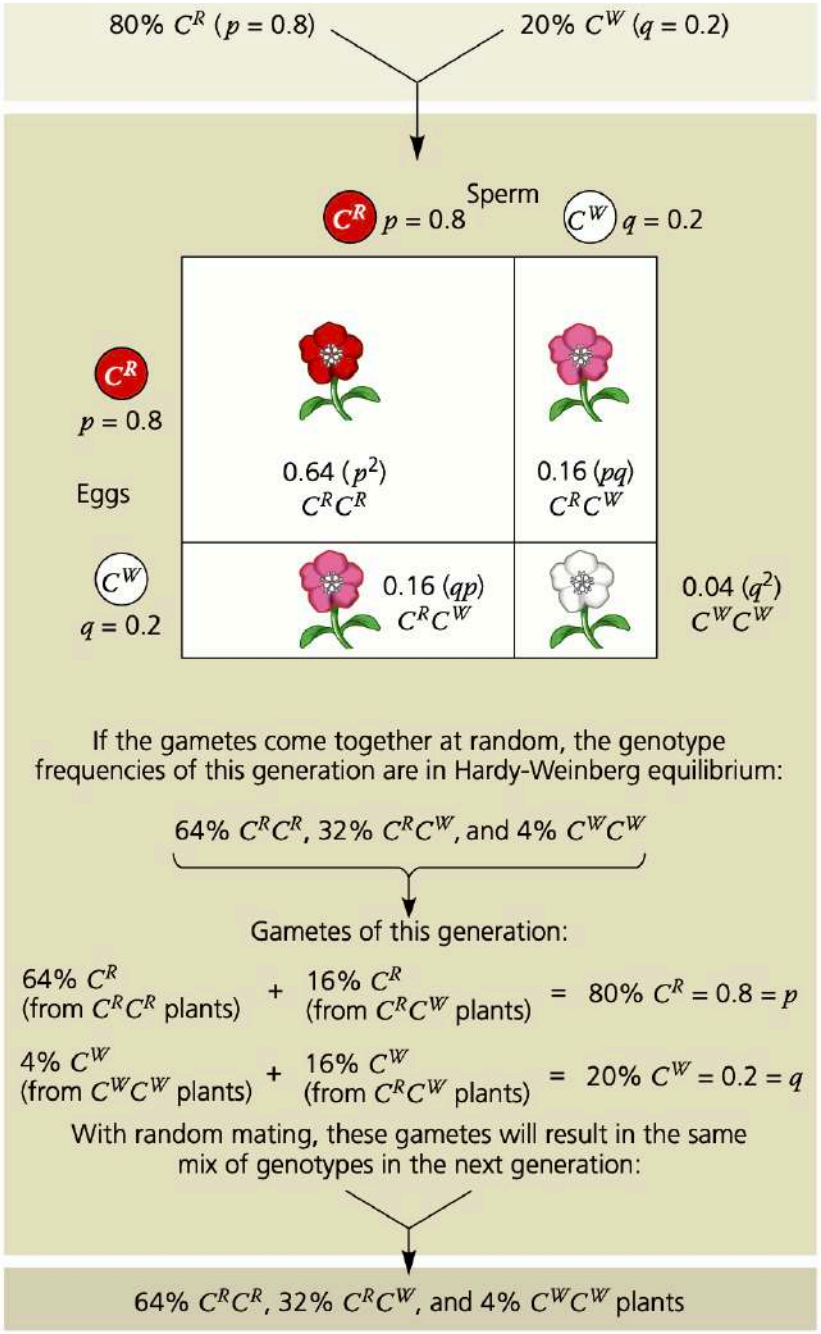
- A population's genetic makeup = its gene pool
- Gene pool = all copies of every type of allele at every locus in all members of the population
- To assess whether natural selection or other factors are causing evolution at a particular locus, determine what the genetic makeup of a population would be if it were not evolving at that locus.
- Compare that scenario with data actually observed for the population
- If there are no differences between theoretical number and actual data = population is not evolving
- If there are differences = population may be evolving, can figure out reason

- In a population that is not evolving, allele and genotype frequencies will remain constant from generation to generation, provided that only Mendelian segregation and recombination of alleles are at work
- Such a population is said to be in Hardy-Weinberg equilibrium

- Named for the British mathematician and German physician, respectively, who independently developed this idea in 1908

Evolution

Hardy Weinberg equilibrium - How to tell if a species is evolving?



Gametes for each generation are drawn at random from the gene pool of the previous generation

- The probability that two C^R alleles will come together from 2 gametes is p x p = p^2 = 0.8 x 0.8 = 0.64. Thus, about 64% of the plants in the next generation will have the genotype C^RC^R.
- The probability that two C^W alleles will come together from 2 gametes q x q = q^2 = 0.2 x 0.2 = 0.04. Thus, about 4% of the plants in the next generation will have the genotype C^WC^W.

  C^RC^W heterozygotes can arise in two different ways
- If the sperm provides the C^R allele and the egg provides the C^W allele, the resulting heterozygotes = p x q = 0.8 x 0.2 = 0.16, or 16% of the total.
- If the sperm provides the C^W allele and the egg the C^R allele, the heterozygous offspring will make up q x p = 0.2 x 0.8 = 0.16, or 16%.
- Frequency of C^RC^W heterozygotes is thus the sum of these possibilities: pq + qp = 2pq = 0.16 + 0.16 = 0.32, or 32%

Figure 23.8 of Campbell's Biology: a global approach

Evolution

Hardy Weinberg equilibrium - How to tell if a species is evolving?

Sum of all allele frequencies, irrespective of number of alleles and regardless of whether the population is in H-W equilibrium = 1 i.e. (100%)

Hardy-Weinberg equilibrium states that at a locus with two alleles, the three genotypes will appear in the following proportions:

$$p^2 + 2pq + q^2 = 1$$

p^2	+	$2pq$	+	$q^2 = 1$
Expected frequency of genotype C^RC^R		Expected frequency of genotype C^RC^W		Expected frequency of genotype C^RC^W

A population is in Hardy-Weinberg equilibrium only if the genotype frequencies are such that the actual frequency seen in the population is as given above

For the flower color example discussed, unless something happens, generation after generation the expected genotype frequencies will match the actual occurrence of allele combinations in that population of 500 plants

Evolution

Hardy Weinberg equilibrium - How to tell if a species is evolving?

The Hardy-Weinberg approach describes a hypothetical population that is not evolving

But in real populations, the allele and genotype frequencies often do change over time. Such changes can occur when at least one of the following five conditions of Hardy-Weinberg equilibrium is not met:

1. No mutations - The gene pool is modified if mutations alter alleles or if entire genes are deleted or duplicated
2. Random mating - If individuals tend to mate within a subset of the population, such as their near neighbors or close relatives (inbreeding), random mixing of gametes does not occur, and genotype frequencies change
3. No natural selection - Differences in the survival and reproductive success of individuals carrying different genotypes can alter allele frequencies
4. Extremely large population size - The smaller the population, the more likely it is that allele frequencies will fluctuate by chance from one generation to the next (a process called genetic drift)
5. No gene flow - By moving alleles into or out of populations, gene flow can alter allele frequencies

Evolution

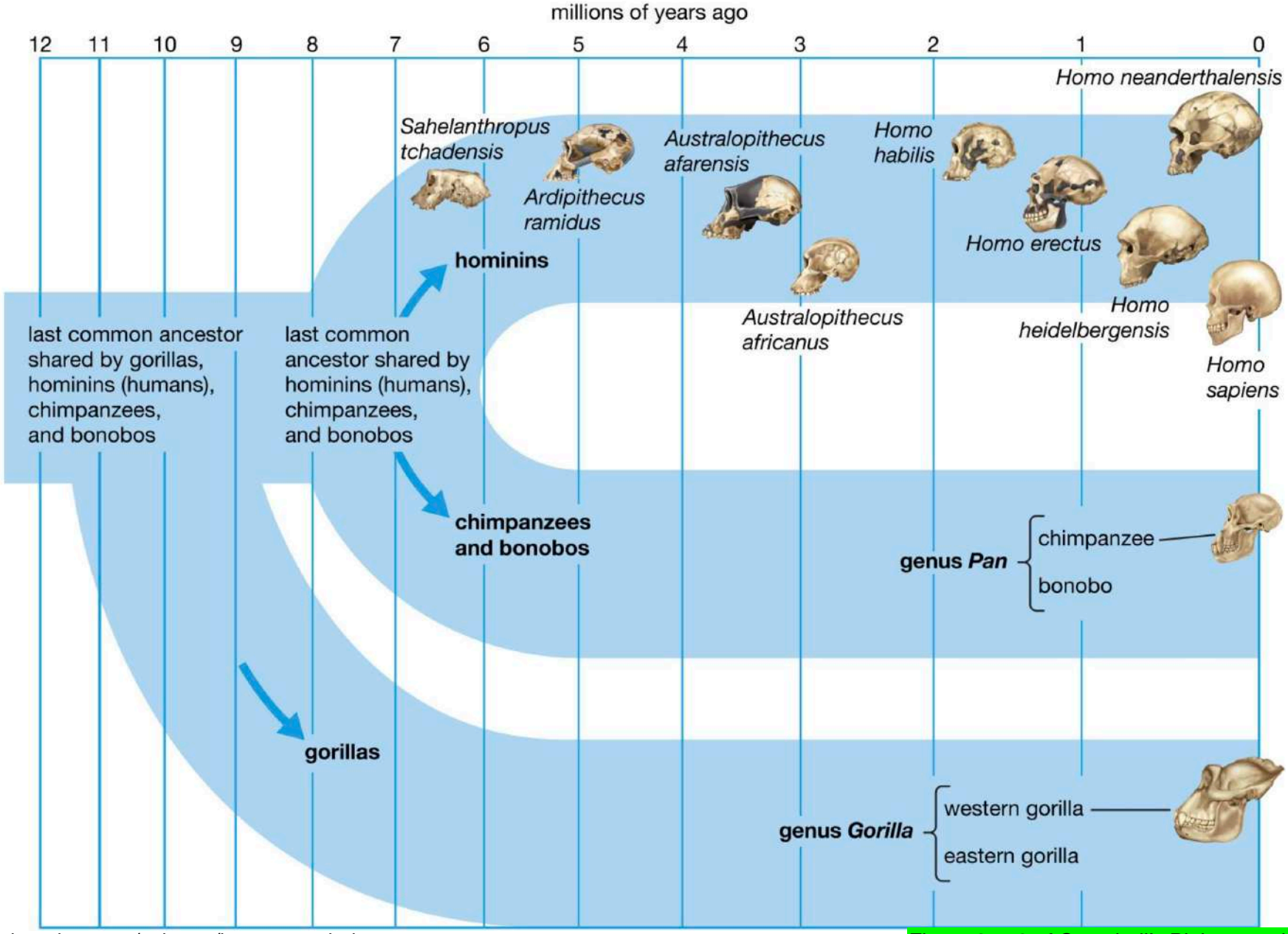
What criteria to use to check for evolution or relatedness?

Physical traits or comparative morphology
Organization of organs and cellular structures etc

Molecular signatures
Similarity or dissimilarity of DNA, RNA and protein sequences

Evolution

H. sapiens and *H. neanderthalensis* did interbreed - some populations of *H. sapiens* have 1-4 % of neanderthal genes

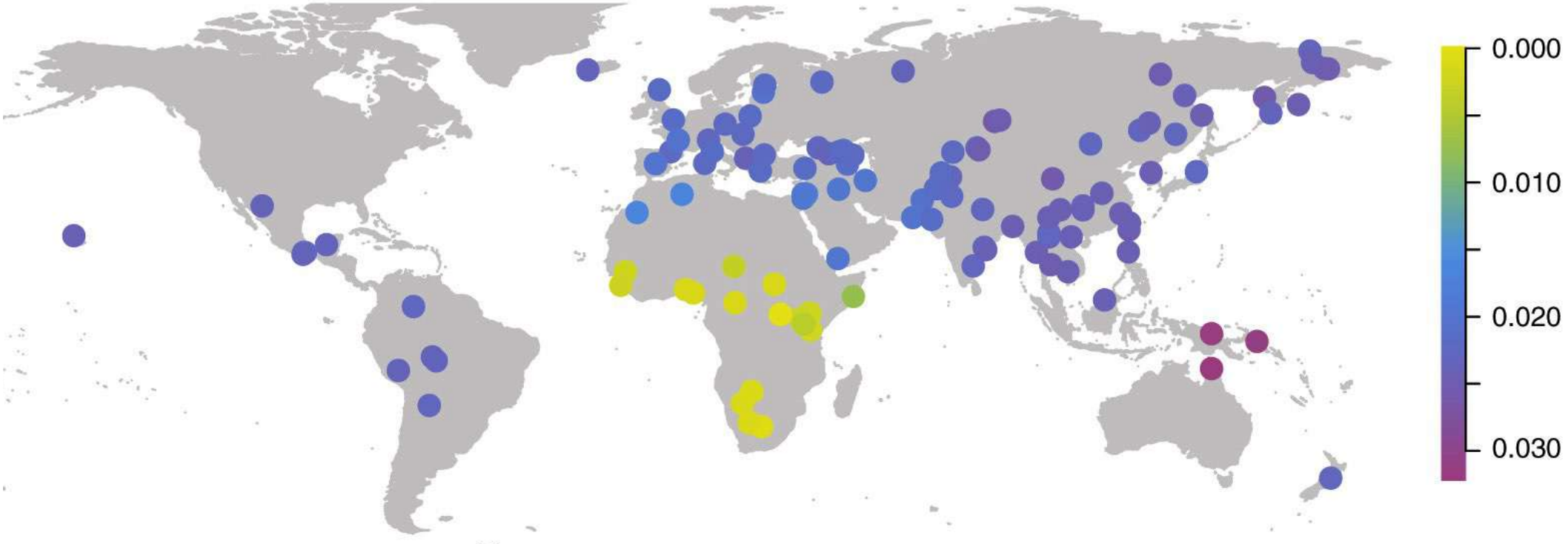


https://www.britannica.com/science/human-evolution

Figure 34.49 of Campbell's Biology: a global approach

Evolution

Neanderthals are extinct - pieces of their DNA lives on in us



Out of Africa theory: *H. sapiens* originated in Africa and migrated out

In doing so, *H. sapiens* encountered *H. neanderthalensis (and other Homininis* and interbred

PMID: 36167050

Figure 34.49 of Campbell's Biology: a global approach

Evolution

Neanderthals and Denisovans are extinct - pieces of their DNA lives on in us

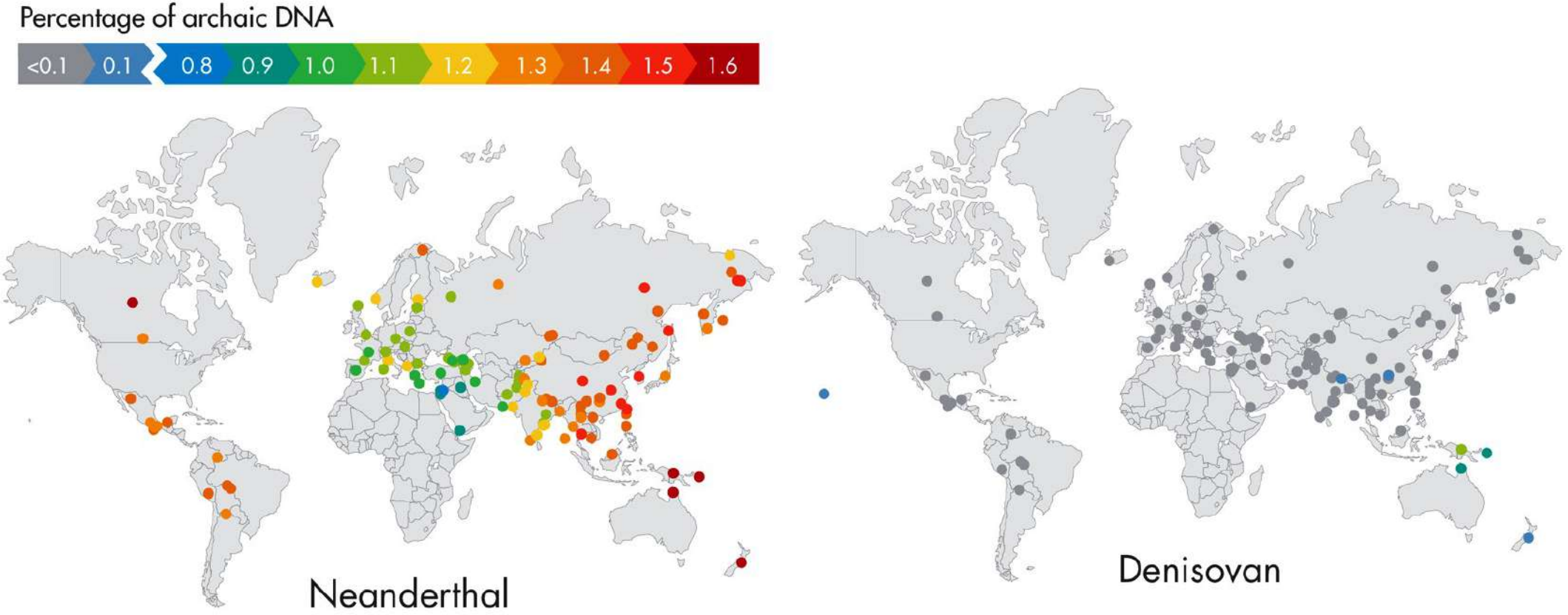
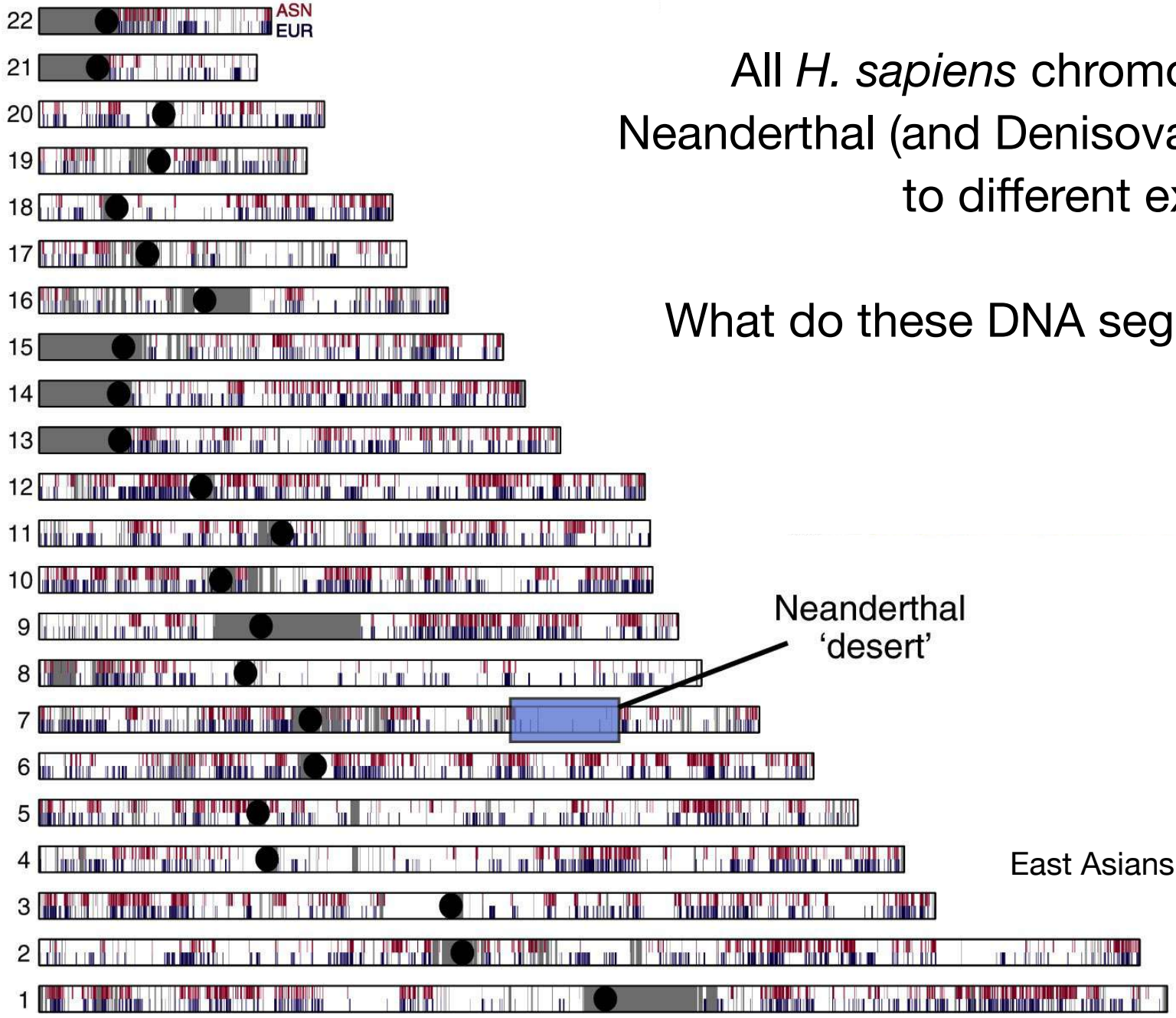


Figure 34.49 of Campbell's Biology: a global approach

Evolution

Neanderthals and Denisovans are extinct - pieces of their DNA lives on in us



All *H. sapiens* chromosomes have Neanderthal (and Denisovan) DNA segments to different extent

What do these DNA segments do in us?

East Asians (red) and Europeans (blue)

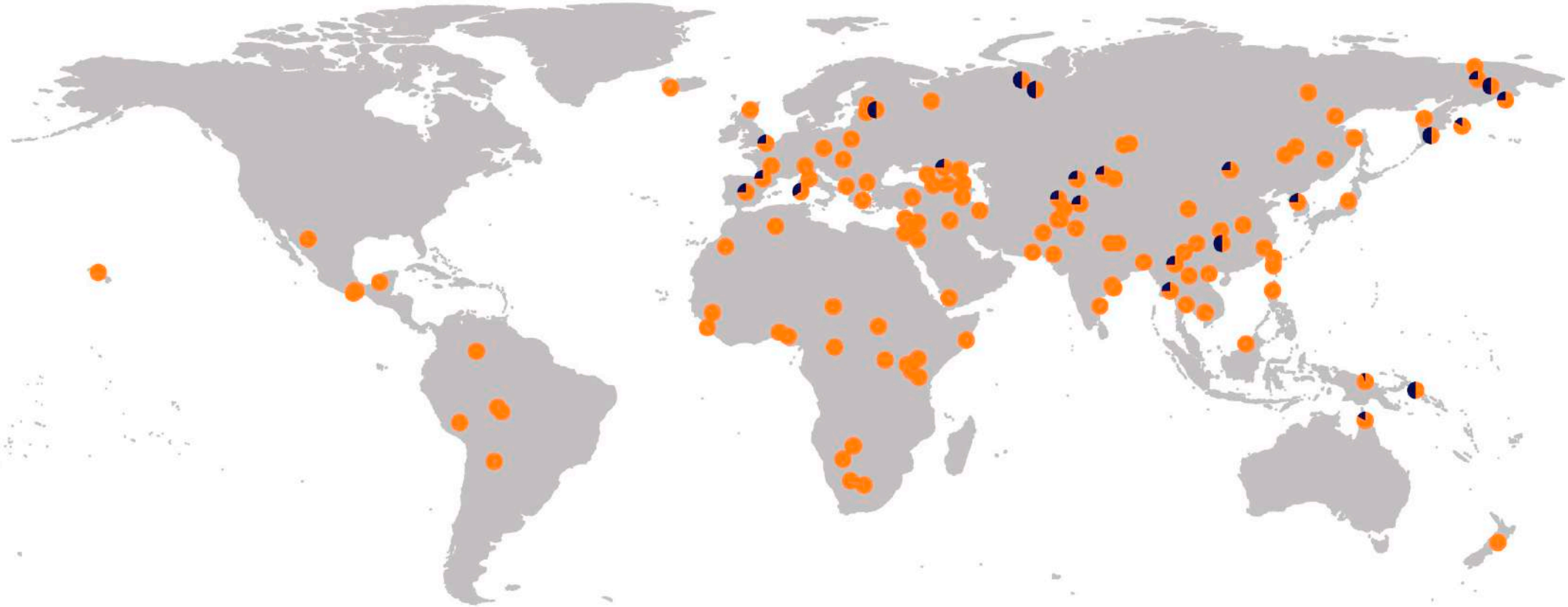
PMID: 36167050

Evolution

Neanderthals and Denisovans are extinct - pieces of their DNA lives on in us

Chronotypes and ancient DNA

One of the Neanderthal introgressed SNPs modifies the coding sequence of ASB1. Archaic alleles near ASB1 and EXOC6 are associated with a preference for being an “evening person” and an increased tendency for daytime napping and narcolepsy, respectively.

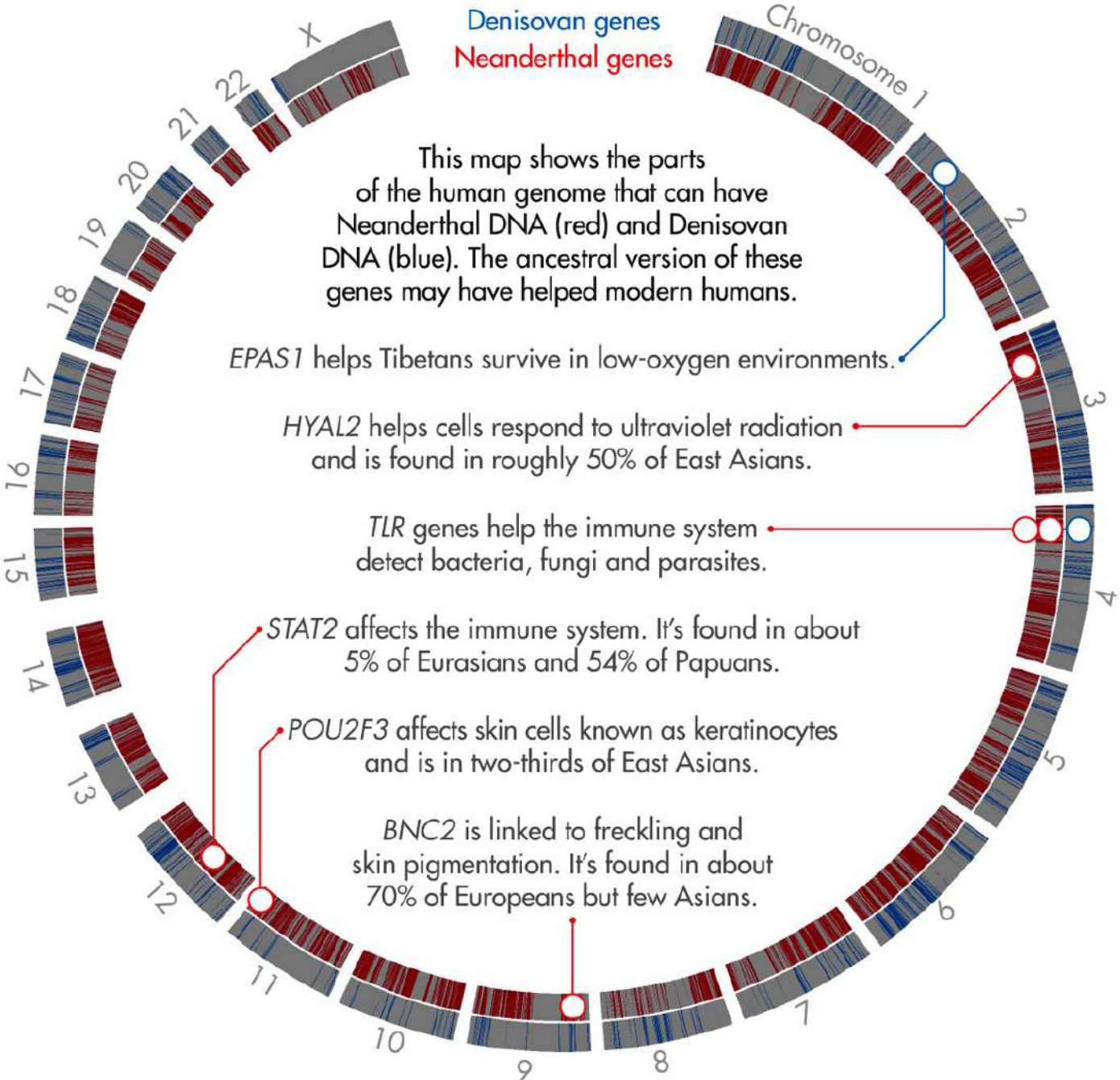


Worldwide frequency of the archaic allele (C, blue) and the modern human allele (T, orange) for ASB1 in the Simons Genome Diversity Panel populations

PMID: 28985494

Evolution

Neanderthals and Denisovans are extinct - pieces of their DNA lives on in us



- 143,000 DNA base-pairs long Neanderthal DNA spans 3 genes which encode toll-like receptors (innate immunity)
- People carrying one of the Neanderthal variants are less likely to be infected with *H. pylori*, a microbe that causes ulcers, but more likely to suffer from common allergies such as hay fever
- These variants also made cytokine storms after SARS-CoV2 infection worse in these individuals

PMID: 26748514

Evolution

Neanderthals and Denisovans are extinct - pieces of their DNA lives on in us

Advantages as well as disadvantages - must be understood in terms of context

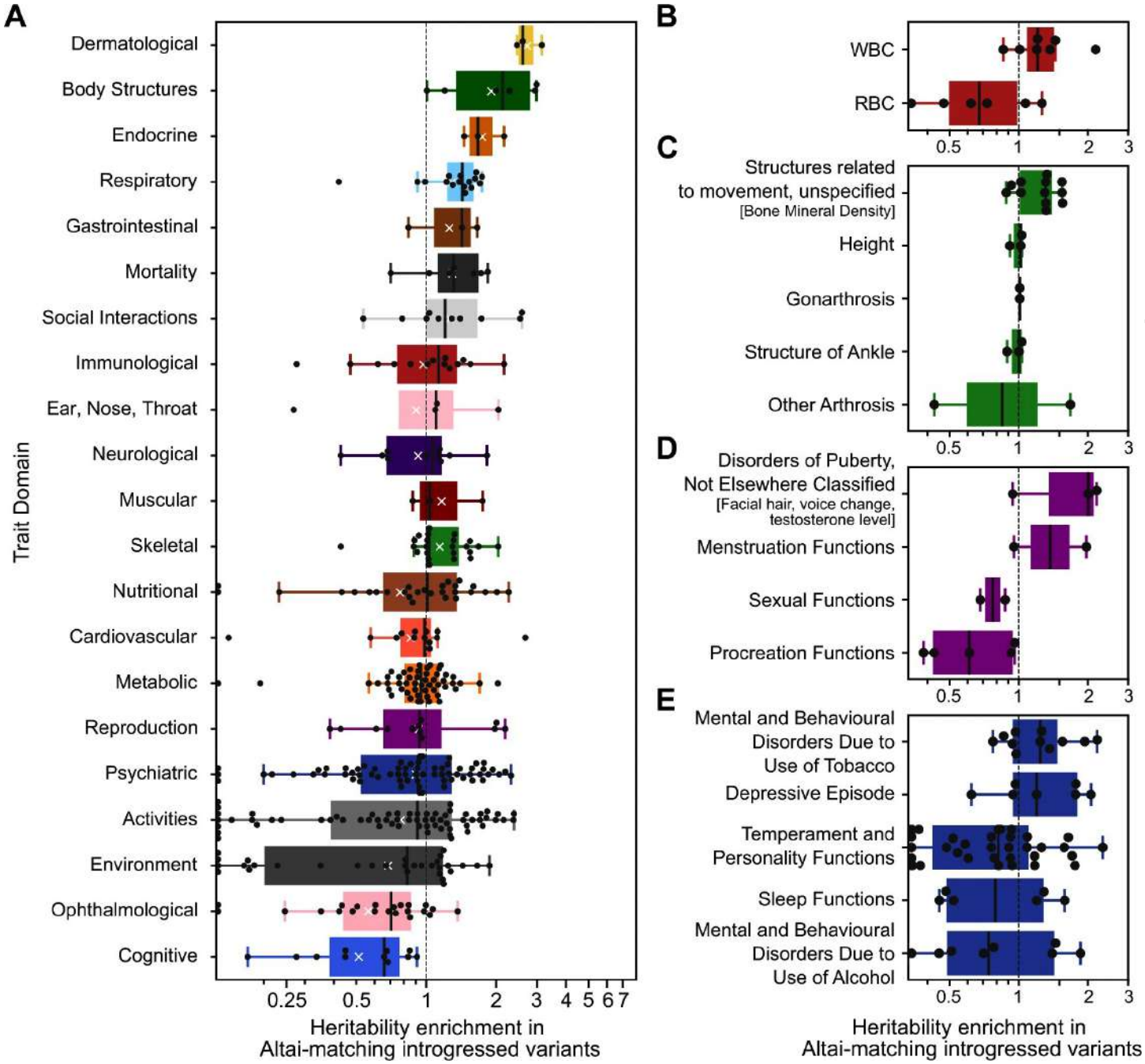
Some examples

- Metabolism: fatty acid and lipid metabolism associated with increased risk of type 2 diabetes, metabolism of some drugs
- Pain sensation: Neanderthal DNA (3 amino acid change) affects the function of a sodium channel. Result is individuals with the neanderthal variant are sensitive to pain. In *H. sapiens*, biallelic loss-of-function mutations in this sodium channel causes congenital insensitivity to pain but also reduces life expectancy
- Gestation: Neanderthal version is associated with an increased risk of premature births
- Virus infection: Neanderthal version is associated with decreased risk of HIV

PMID: 38367615

Evolution

Neanderthals and Denisovans are extinct - pieces of their DNA lives on in us



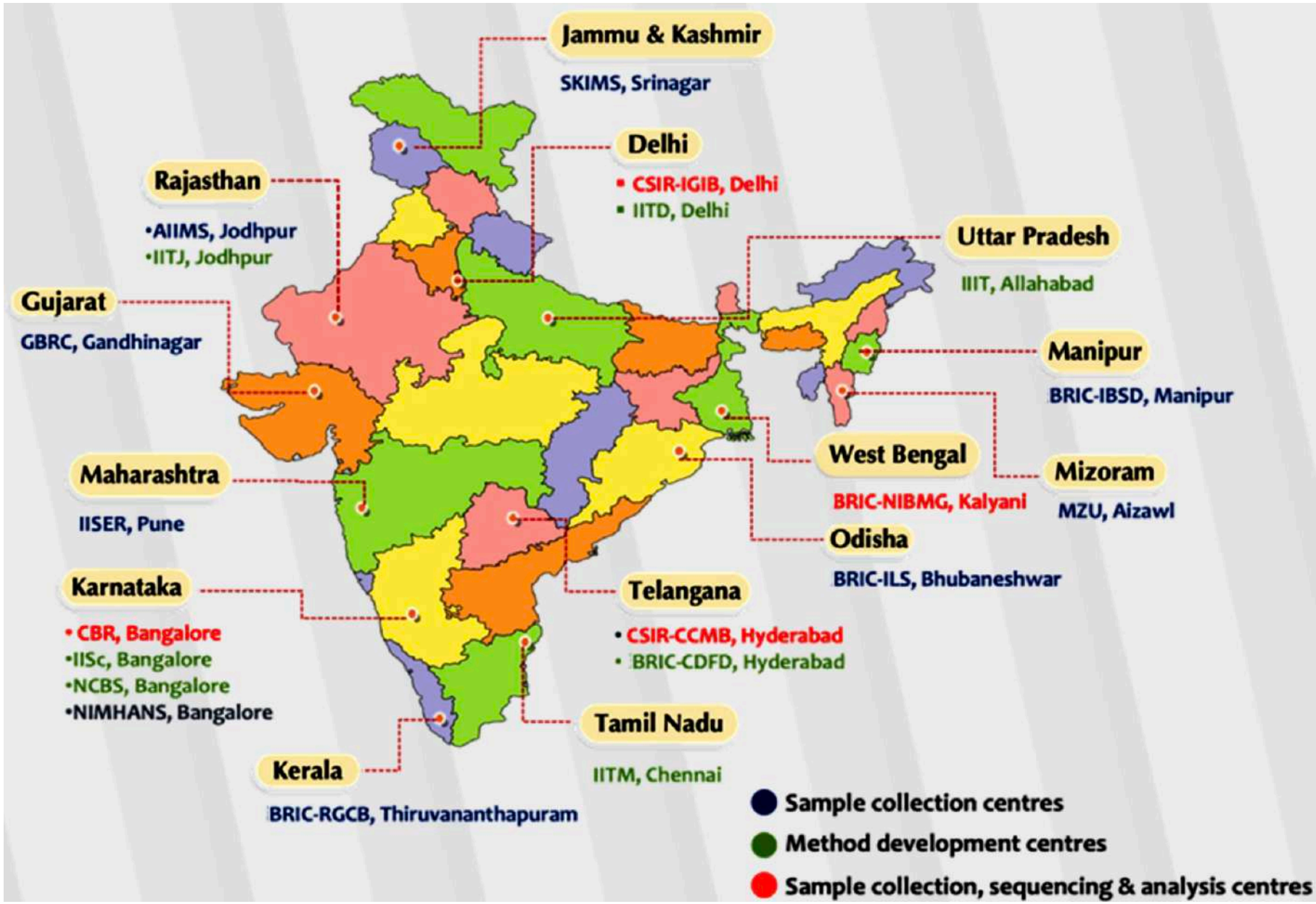
Introgressed variants for cognitive traits were most likely selected against

PMID: 34294692

The Genome India Project

Database for understanding genome diversity in Indian population

10000 genomes - data available



The Genome India Project

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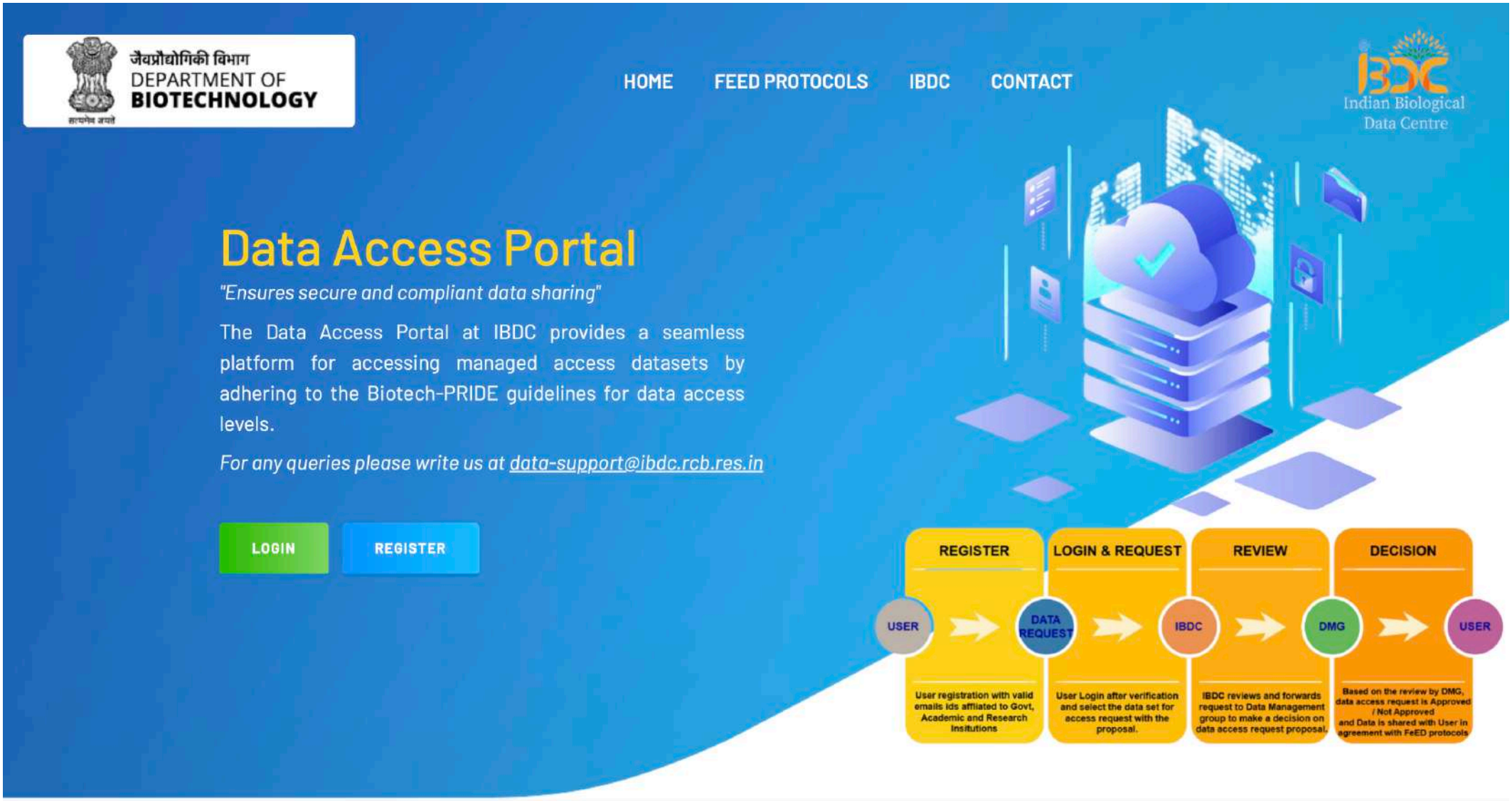
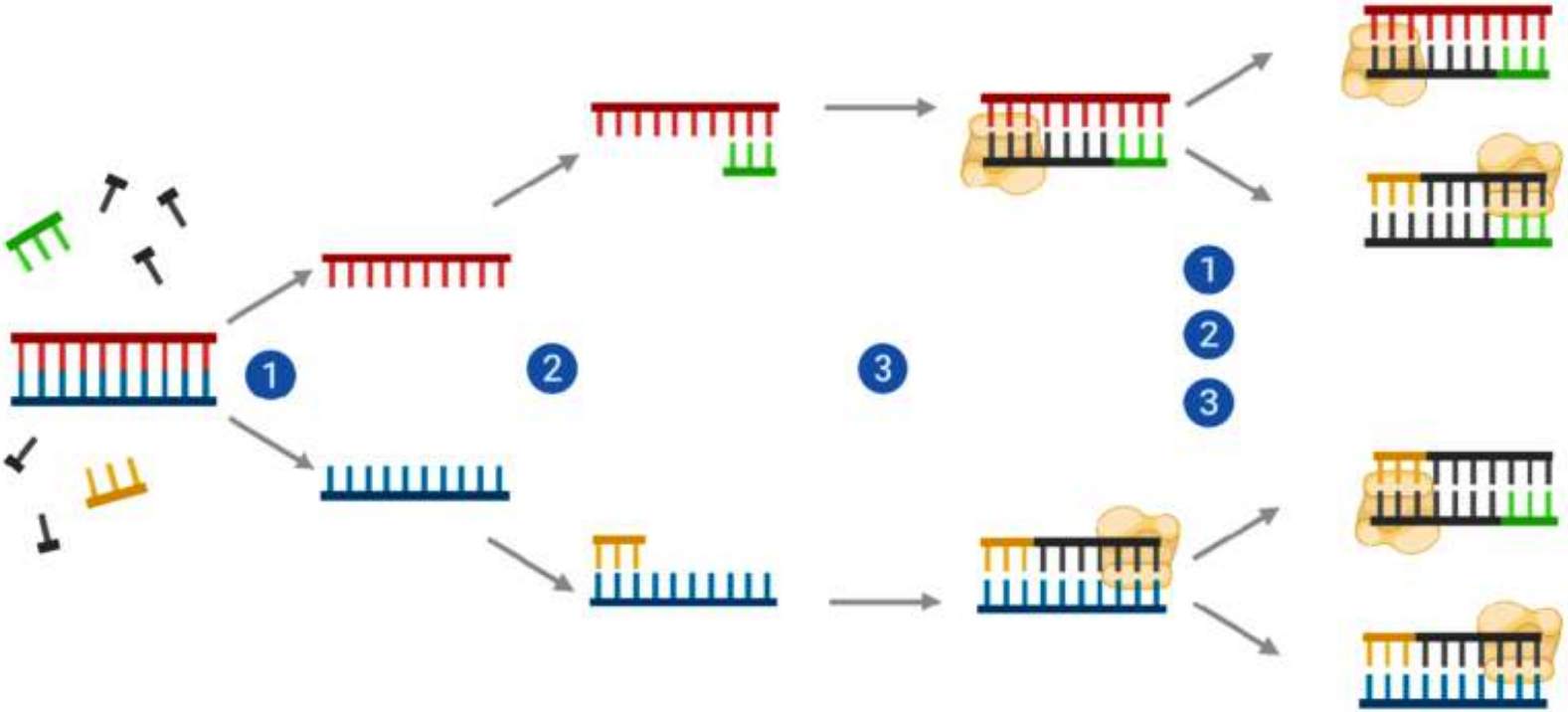
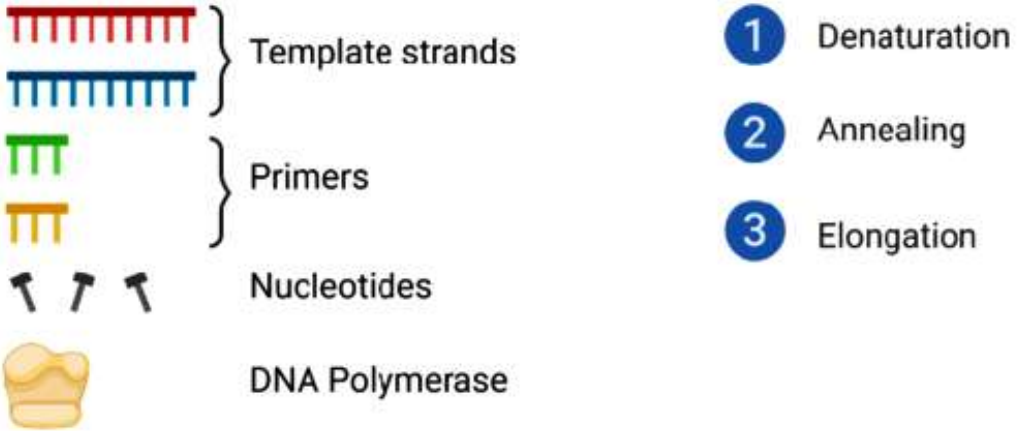


Polymerase Chain Reaction

Method used to amplify DNA fragments by synthesizing new strands complementary to the original ones

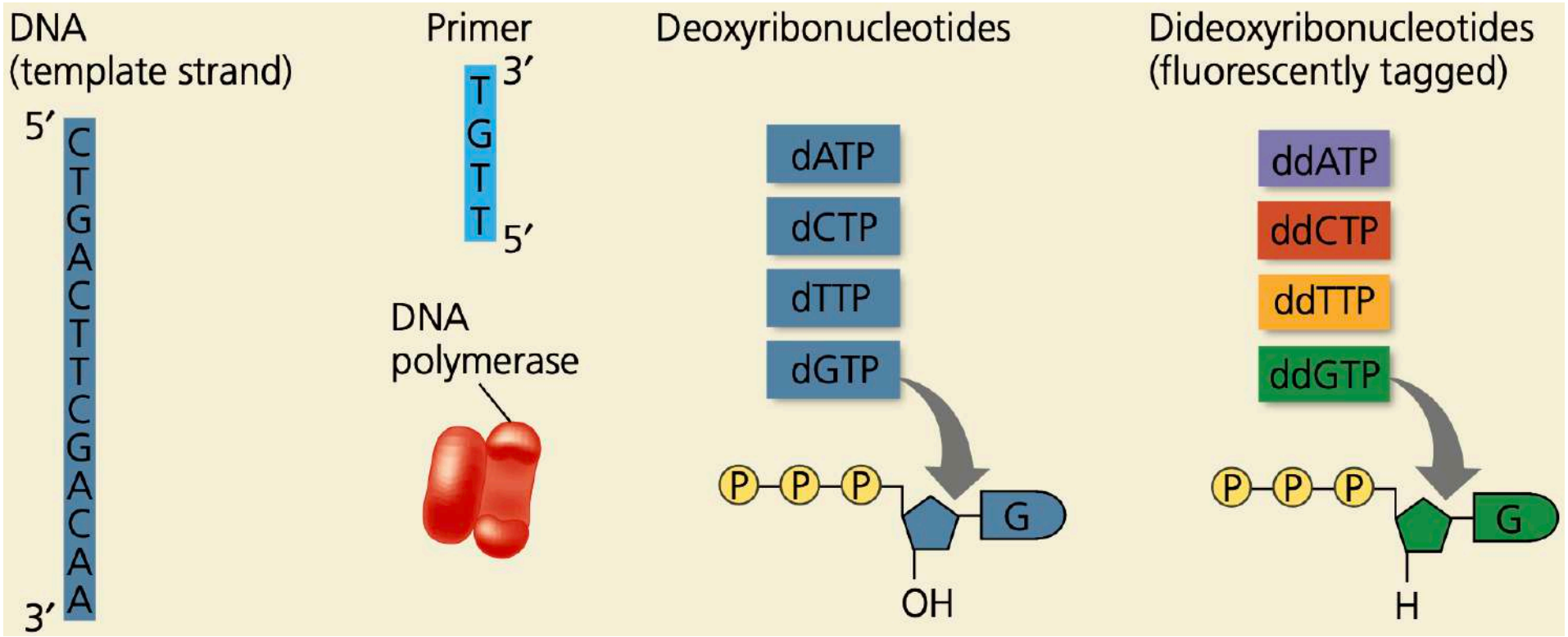




- Exponential amplification
- Can use this for “sequence by synthesis” method to figure out sequence of a DNA fragment

Figure 20.8 of Campbell’s Biology: a global approach

Sanger Sequencing



- Synthesis of new strand terminates when ddNTP is incorporated
- ddNTPs are labelled fluorescently
- Fluorescence is read to figure out which ddNTP got added resulting in termination of DNA synthesis

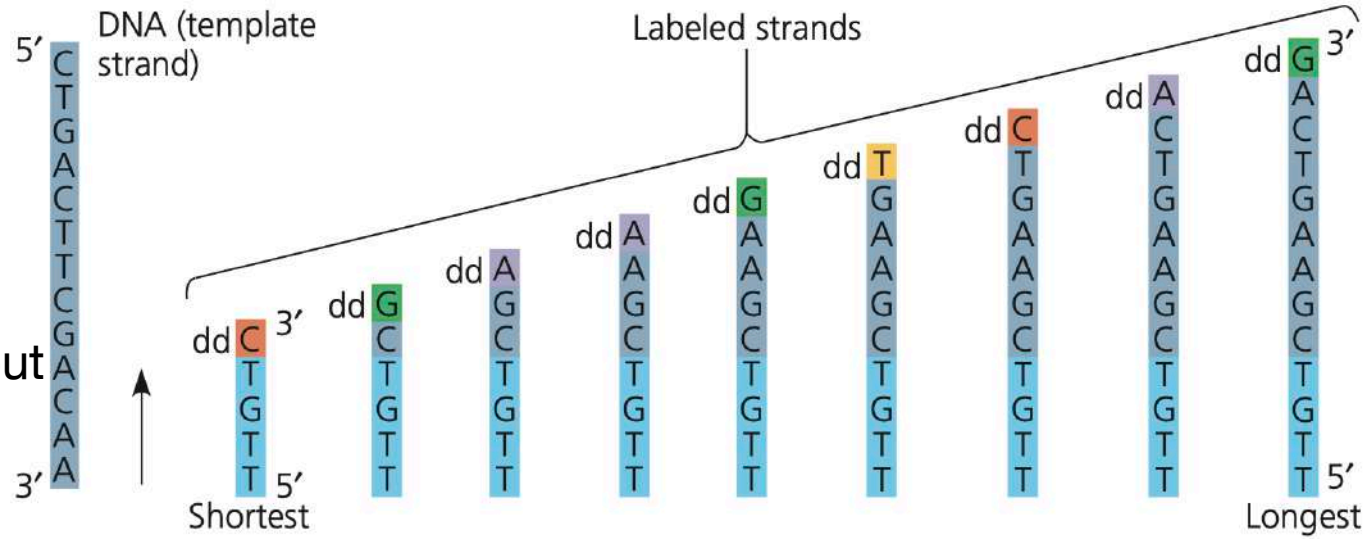


Figure 20.3 of Campbell's Biology: a global approach

Sanger Sequencing

- Fluorescence is read to figure out which ddNTP got added resulting in termination of DNA synthesis
- Results are in the form of a chromatogram - peaks indicate fluorescence above a threshold
- Fluorescence is specific to one of four nucleotides
- Typically used to read fragments of ~1000 - 1500 base pairs

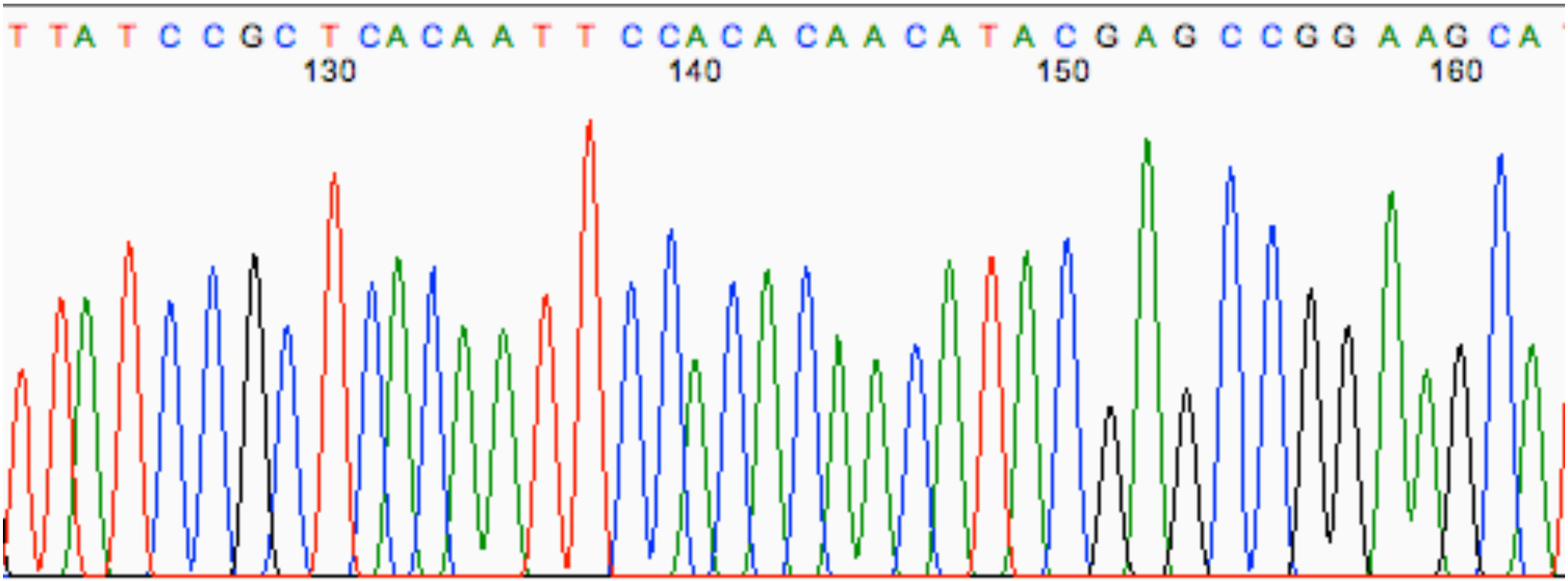


Figure 20.3 of Campbell's Biology: a global approach

Pyrosequencing of DNA

- DNA polymerase incorporates dNTP complementary to the template strand
- Pyrophosphate (PPi) is released in the reaction
- PPi is used to elicit luminescence using luciferase enzyme
- The luminescence is detected and displayed in a pyrogram
- Luminescence occurs each time a dNTP is incorporated successfully

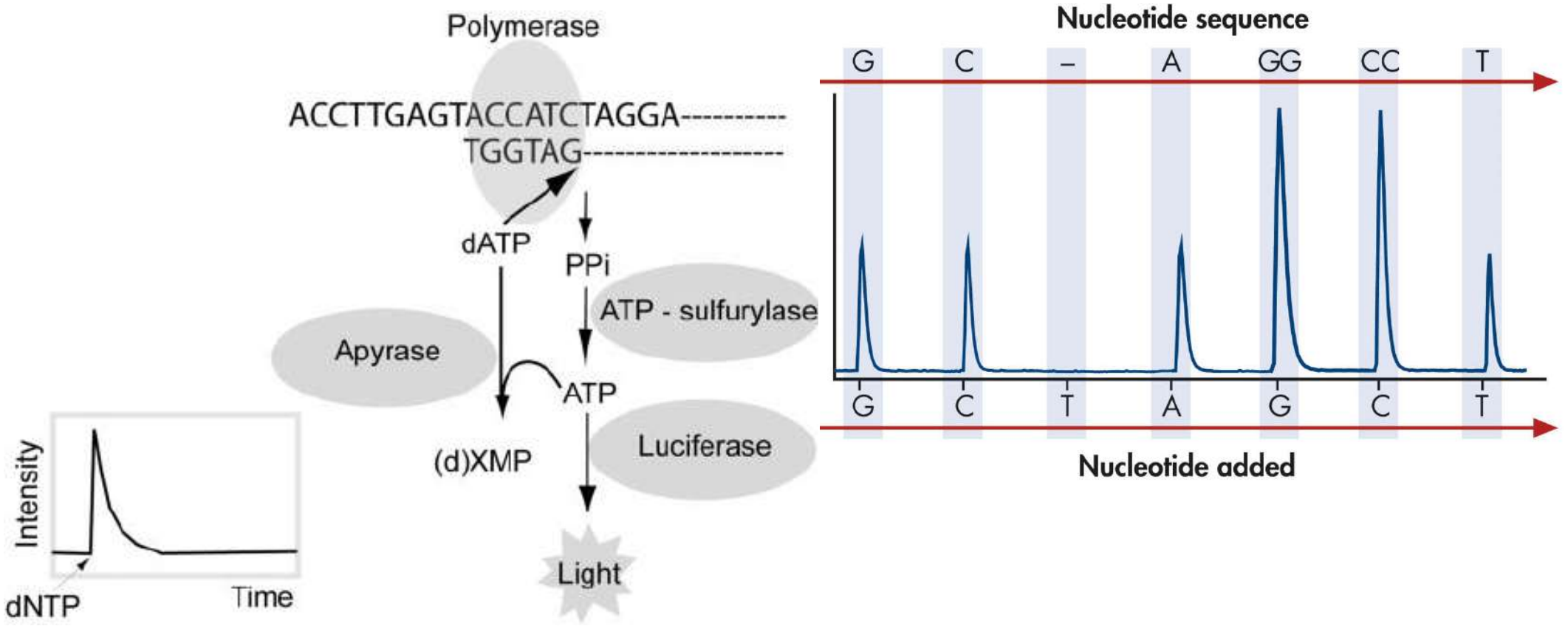
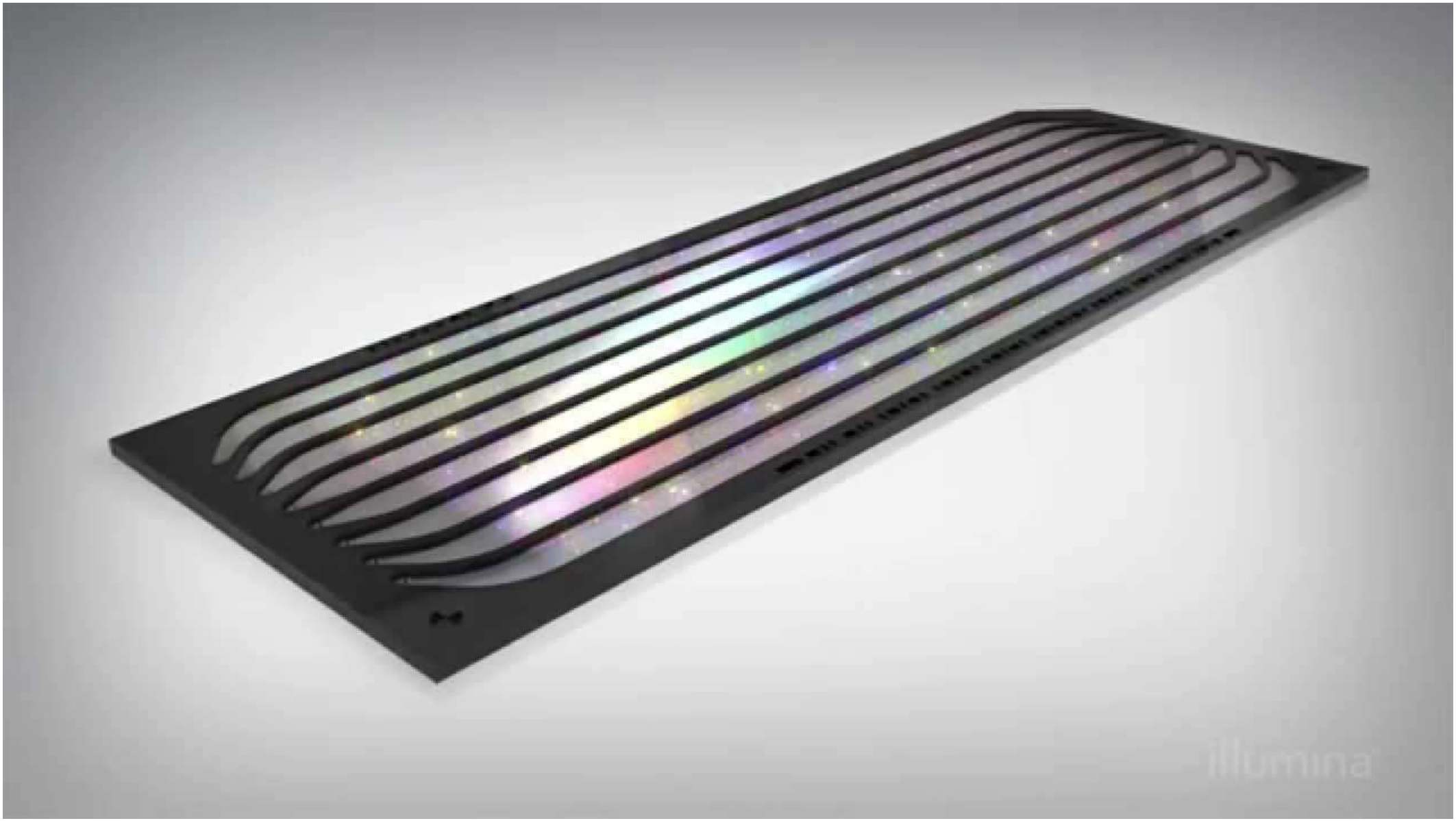


Figure 20.4 of Campbell's Biology: a global approach

Sequencing by synthesis

Next Gen Sequencing (NGS)

Large number of wells in a flow cell - each well is for one strand of DNA and its amplification



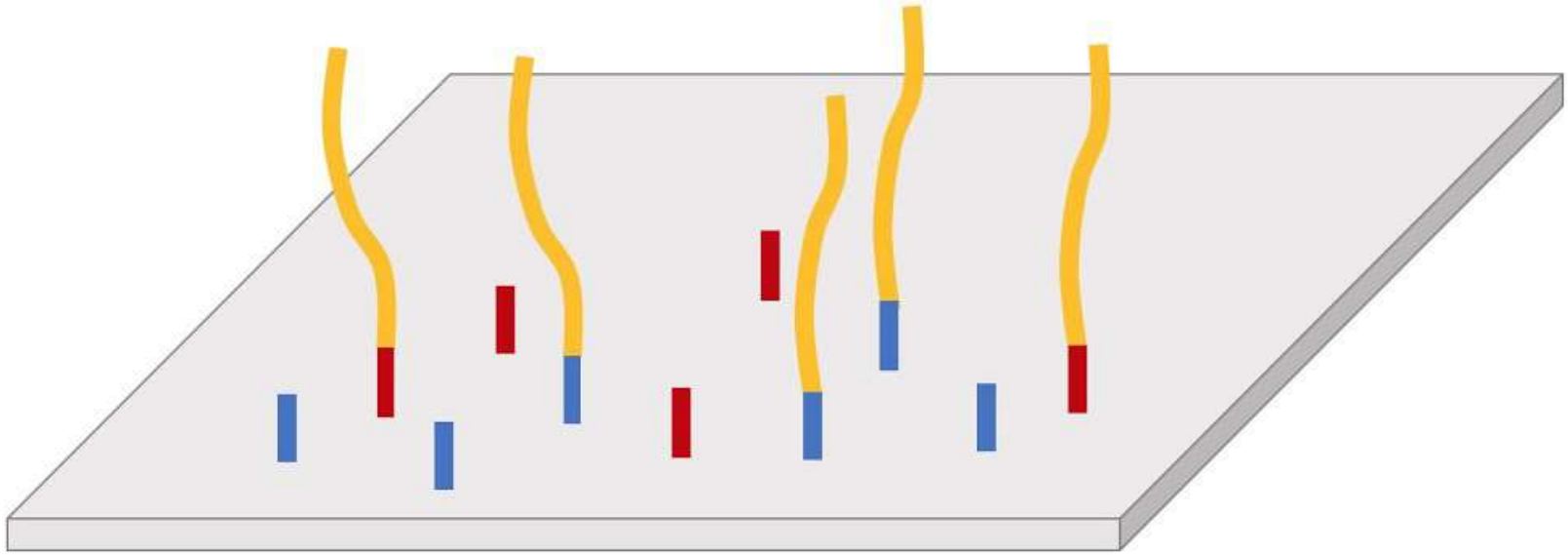
https://www.youtube.com/watch?v=pfZp5Vgsbw0

Sequencing by synthesis

Next Gen Sequencing (NGS)

Large number of wells in a flow cell - each well is for one strand of DNA and its amplification

Next Gen Sequencing

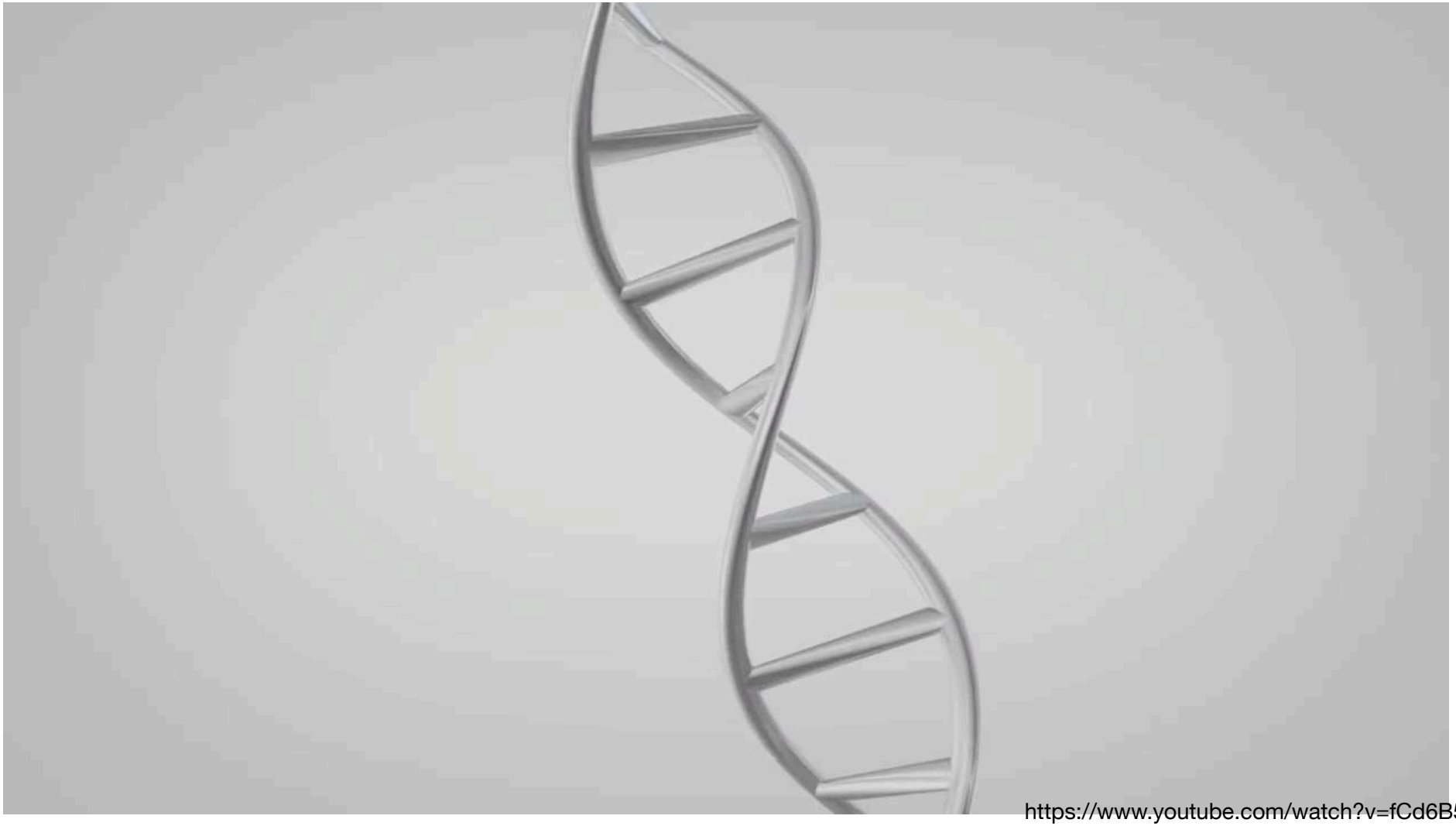


https://www.youtube.com/watch?v=CZeN-IgjYCo

Sequencing by synthesis

Next Gen Sequencing (NGS)

- Developed by Solexa - bought by Illumina (also known as Illumina sequencing)
- Next-Generation Sequencing (NGS) technology allows massively parallel sequencing of DNA fragments of 150–400 bases
- Can sequence hundreds of samples - limitation is number of wells in the flow cell



https://www.youtube.com/watch?v=fCd6B5HRaZ8

Phred scores

P = probability of an erroneous base call at a position, ranges from 0 to 1

P = 0.1 = 10 % error = 90% correct
P = 0.01 = 1% error = 99% correct
P = 0.001 = 0.1% error = 99.9% correct
P = 0.0001 = 0.01% error = 99.99% correct

$\log_{10} 0.1 = -1$
$\log_{10} 0.01 = -2$
$\log_{10} 0.001 = -3$
$\log_{10} 0.0001 = -4$

$$Q = -10 \log10(P)$$

-10 x (-1) = 10. Q = 10 - probability that 1 in 10 base call is incorrect
-10 x (-2) = 20. Q = 20 - probability that 1 in 100 base call is incorrect
-10 x (-3) = 30. Q = 30 - probability that 1 in 1000 base call is incorrect
-10 x (-4) = 40. Q = 40 - probability that 1 in 10000 base call is incorrect

Quality scores (Q) are a way to assign confidence to a particular base within a read
Illumina reads are usually ~100 - 150 bases long, Q of >25 is considered excellent

Symbol	ASCII Code	Q-Score
!	33	0
"	34	1
#	35	2
$	36	3
%	37	4
&	38	5
'	39	6
(	40	7
)	41	8
*	42	9
+	43	10
,	44	11
-	45	12
.	46	13
/	47	14
0	48	15
1	49	16
2	50	17
3	51	18
4	52	19
5	53	20
6	54	21
7	55	22
8	56	23
9	57	24
:	58	25
;	59	26
<	60	27
=	61	28
>	62	29
?	63	30
@	64	31
A	65	32
B	66	33
C	67	34
D	68	35
E	69	36
F	70	37
G	71	38
H	72	39
I	73	40

Phred scores and ASCII encoding

- Quality scores started as numbers (0-40)
- Each base has a Q score and for a sequence of 100 - 150 bases, there will be that many Q scores
- Changed to ASCII encoding to reduce file size
- Two formats of encoding - Phred 33 and Phred 64 (adds 33 or 64 to the Q score for ASCII encoding)
- Currently Phred 33 is widely used

- Raw sequence file is in FASTQ format
- First line is identifiers: instrument, run number, lane number etc
- Second line is text version of the sequence
- Third line is +
- Fourth line is ASCII encoded quality score

```
@SIM:1:FCX:1:15:6329:1045 1:N:0:2
TCGCACTCAACGCCCTGCATATGACAAGACAGAATC
+
<>;##=><9=AAAAAAAAAA9#:<#<;<<<????#=
```

https://help.basespace.illumina.com/files-used-by-basespace/fastq-files
https://help.basespace.illumina.com/files-used-by-basespace/quality-scores

Phred scores and FASTQC

- Raw sequence file is in FASTQ format
- A program FASTQC is used to assess quality of the sequence reads
- FASTQC reports on various parameters of the sequencing read is generated
- Each position will have an average Q score - as many many DNA fragments of identical sequence was sequenced together
- Average Q score at a position should be high (>25) for the base call made at that position to be considered correct

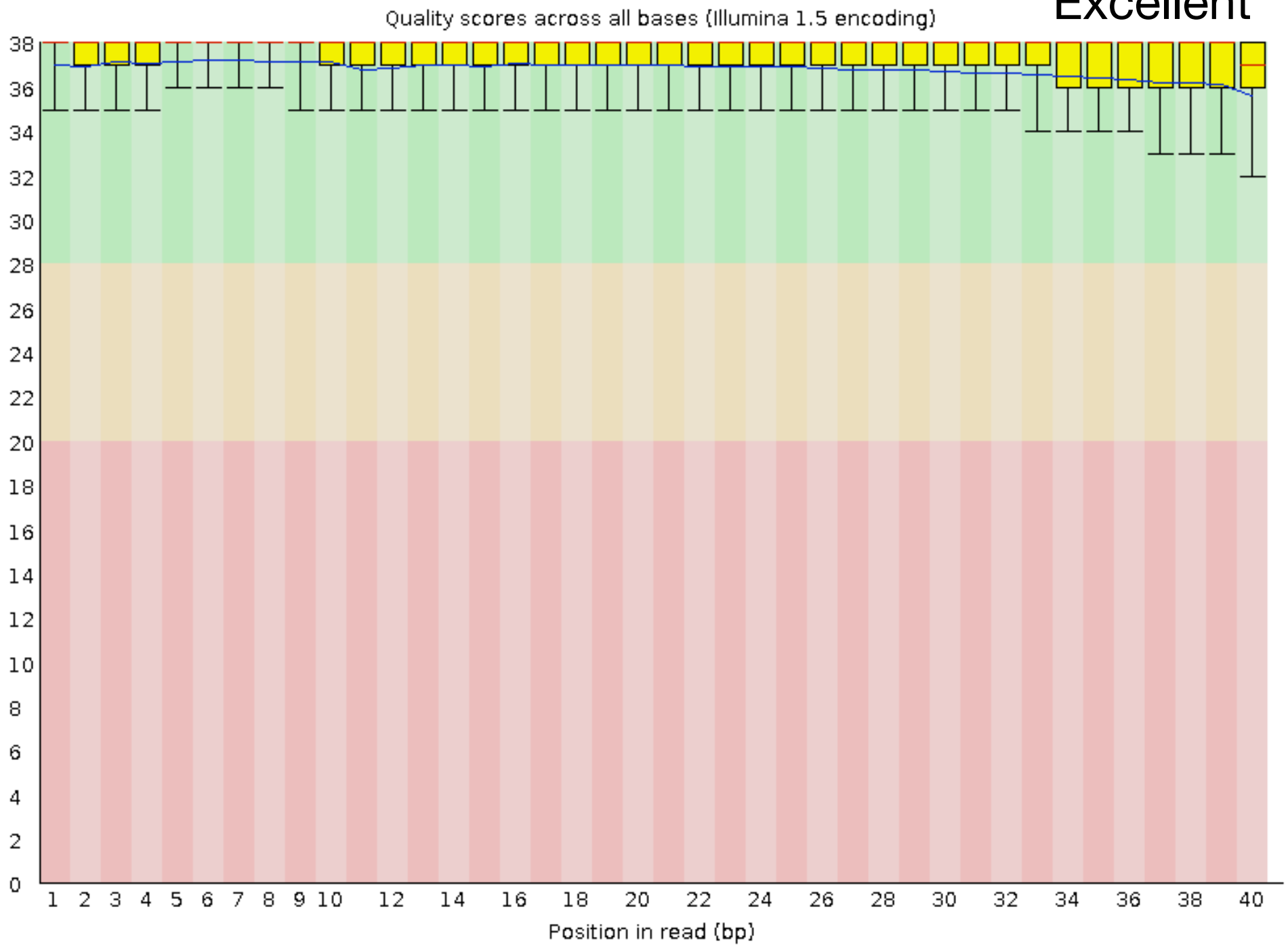
https://www.bioinformatics.babraham.ac.uk/projects/

https://usegalaxy.org/

http://www.ensembl.org/index.html

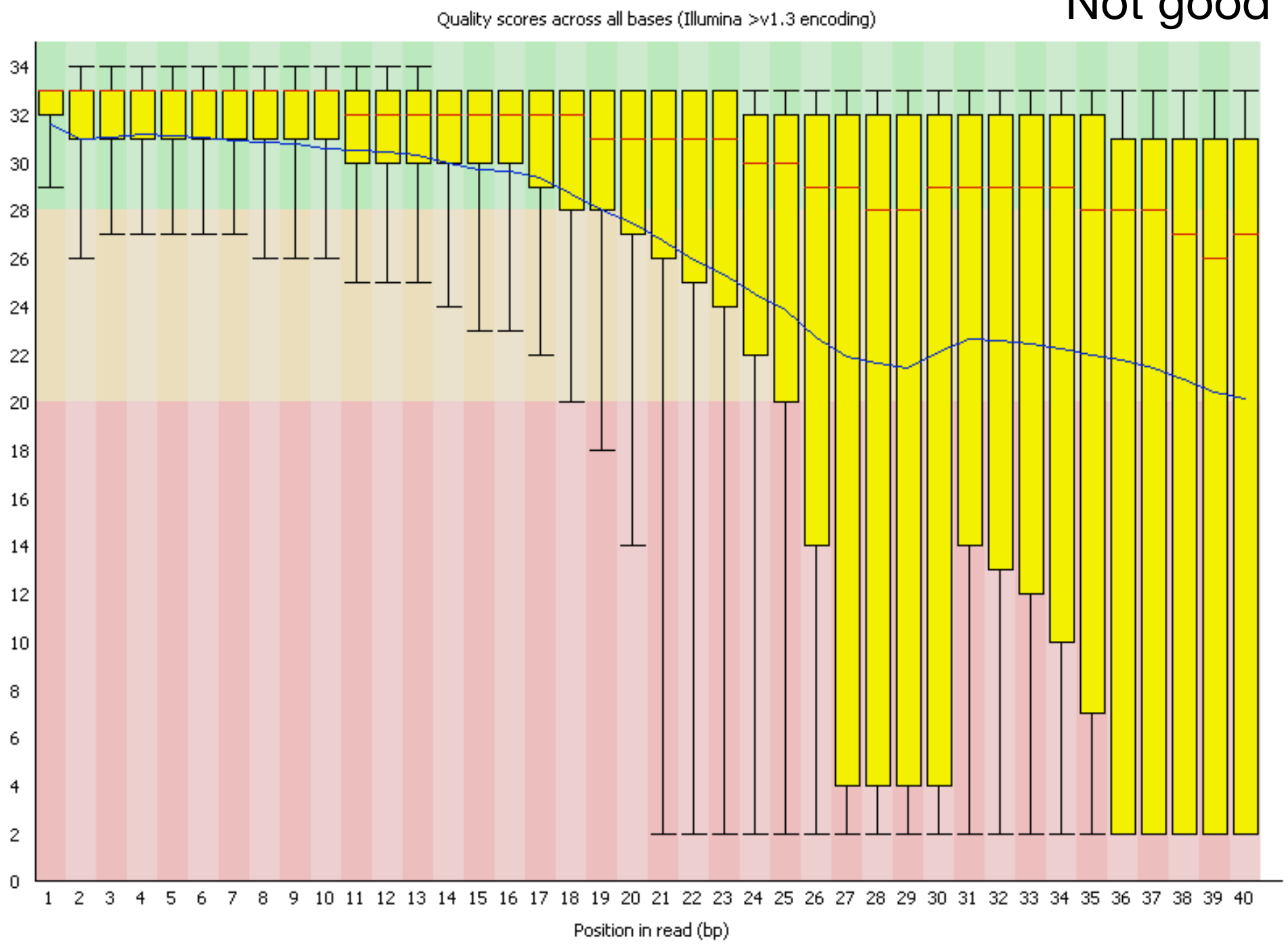
Example of average Q scores at a position on a sequence is shown below

Excellent



Example of average Q scores at a position on a sequence is shown below

Not good



Example of average Q scores at a position on a sequence is shown below

Uh oh.

Direct sequencing
Nanopore Sequencing Technology - Oxford Nanopore

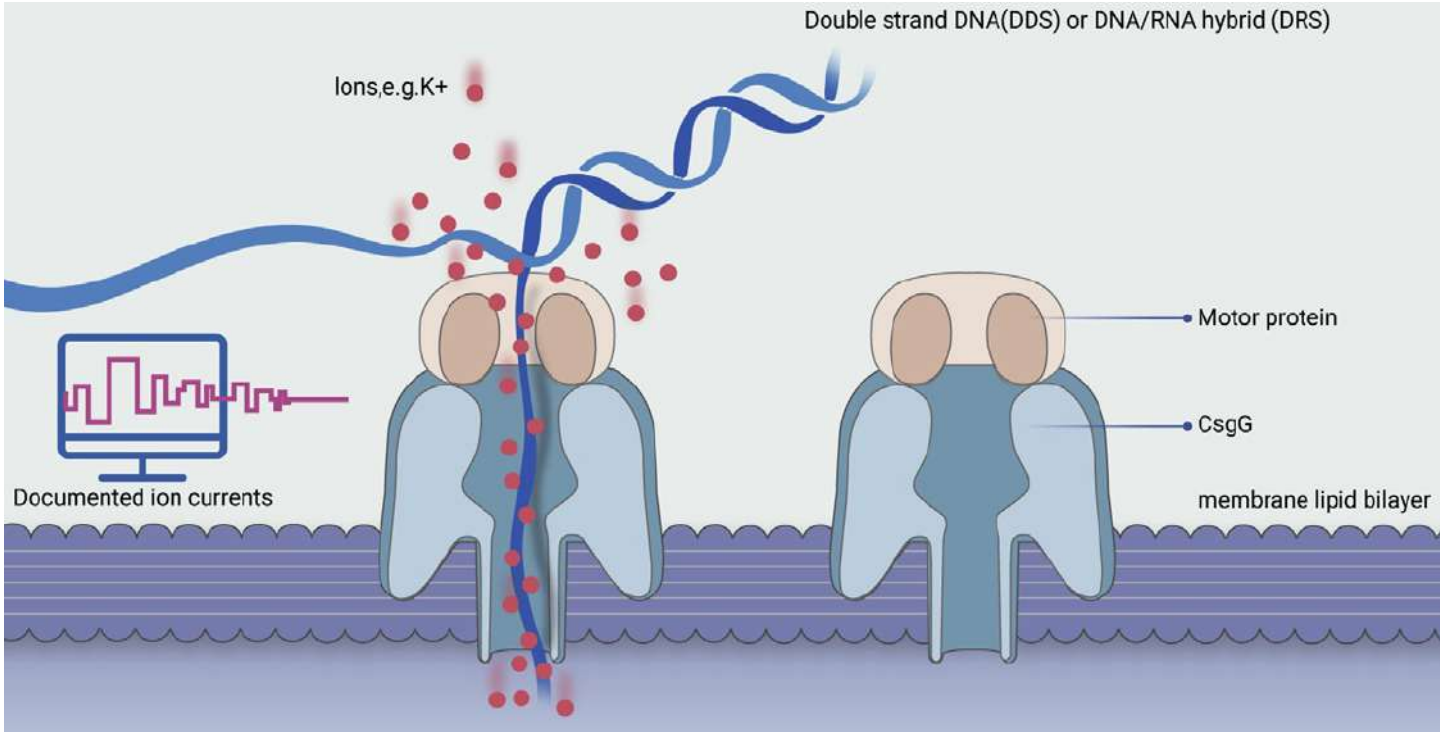
- Nanopores are biological pores embedded in a lipid bilayer
- Pore-forming toxins from bacteria can form nanopores in cell membranes - results in cell death

- Nanopores can be used as a biosensor for DNA sequencing
- A nanopore is embedded in an electrically resistant polymer membrane
- In an electrolytic solution, a constant voltage is applied to produce an ionic current through the nanopore
- DNA or RNA molecules are driven through the nanopore from negatively charged ‘cis’ side to positively charged ‘trans’ side

PMID: 26639780 PMID: 34750572

Direct sequencing

https://nanoporetech.com/platform/technology



- A motor protein ratchets the nucleic acid molecule through the nanopore in a step-wise manner
- Changes in the ionic current within the pore during translocation correspond to the nucleotide passing through the pore - unique for each nucleotide
- Changes in ionic current as the nucleotide passed through is decoded using computational algorithms
- Real-time sequencing of single molecules, no size limitation

PMID: 26639780 PMID: 34750572