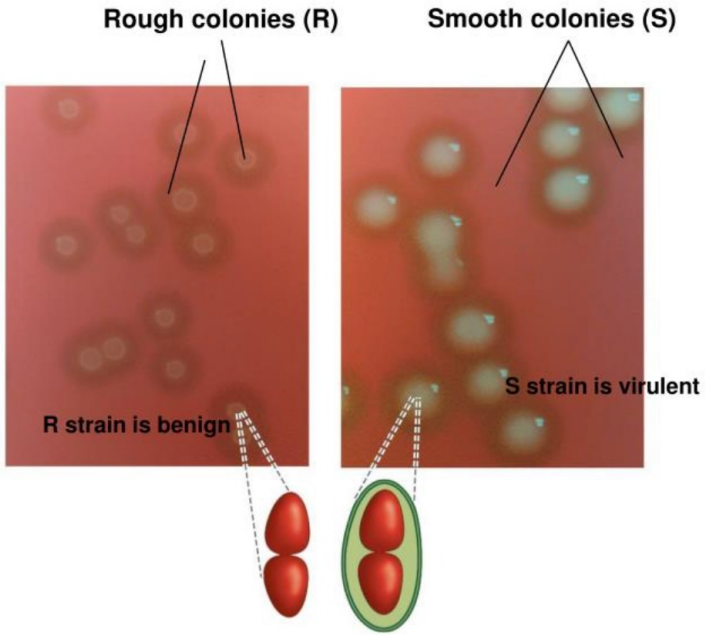
BB 101
Molecular Basis of Inheritance & DNA Tools

Tutorial 4

01.02.2024

Experimental proofs-Frederick Griffith



R- non-virulent strain
S- Virulent strain

S strain ⟶ Inject into mice ⟶ Mice die

R strain ⟶ Inject into mice ⟶ Mice live

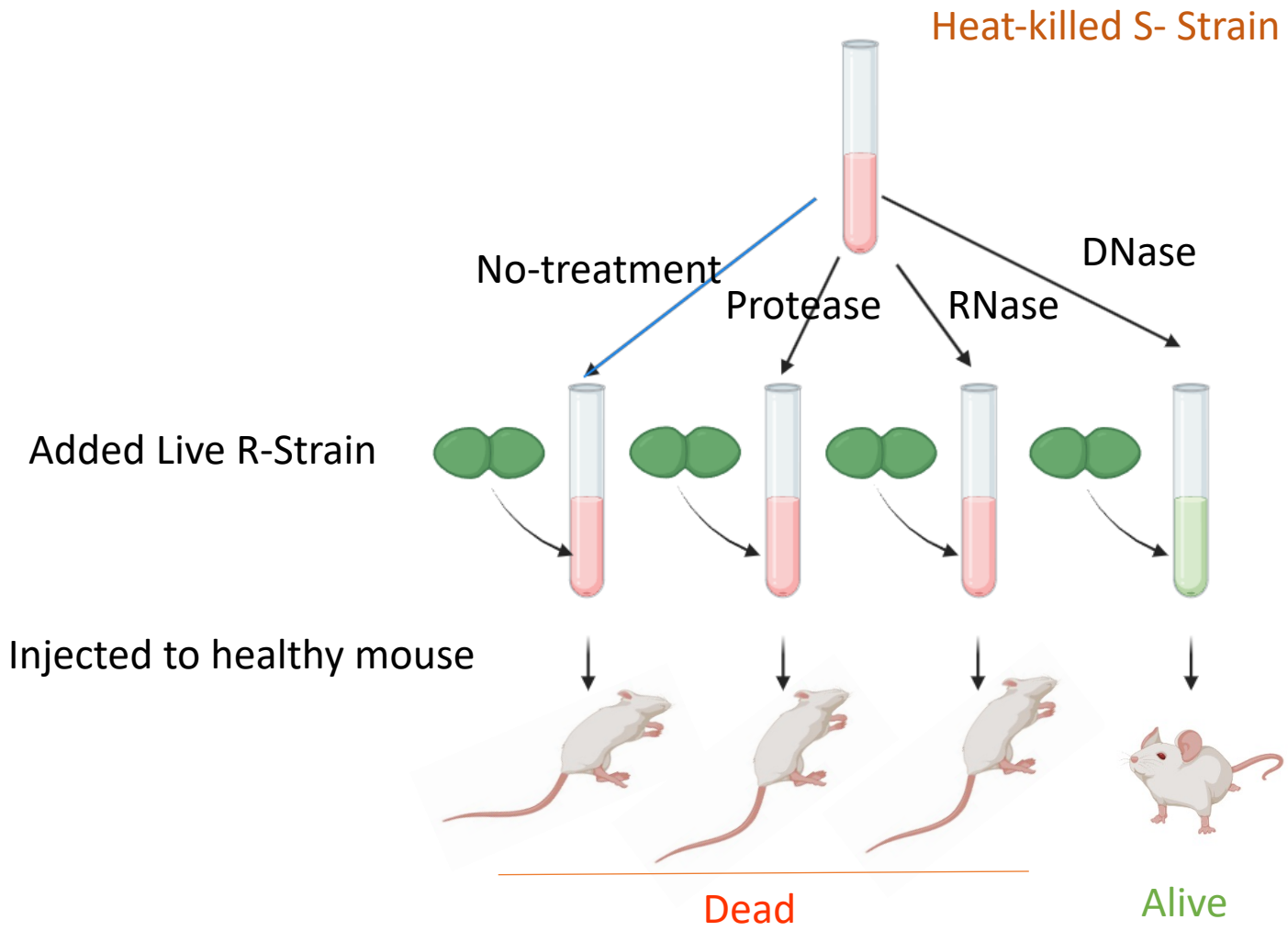
S strain (heat-killed) ⟶ Inject into mice ⟶ Mice live

S strain (heat-killed) + R strain (live) ⟶ Inject into mice ⟶ Mice die

Conclusion-Some 'heritable substance ', transferred from the heat-killed S strain and enabled the R strain to synthesise a smooth polysaccharide coat and become virulent.

How to confirm the nature of the heritable substance out of known biomolecules, protein, RNA and DNA?

Experimental proofs-Frederick Griffith (2)

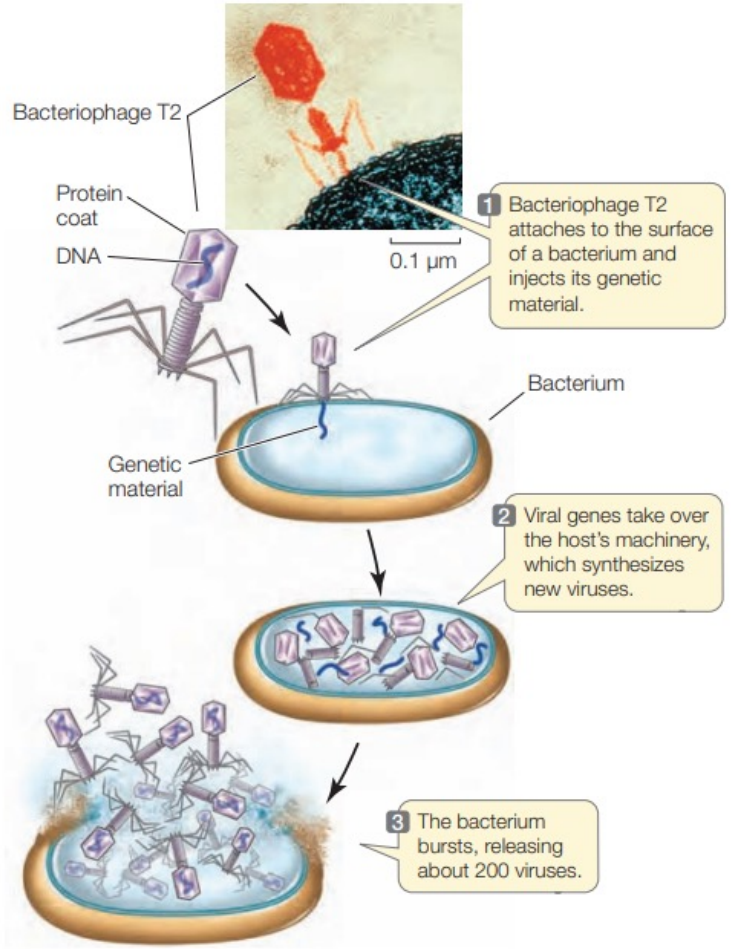


If Mouse Dies:
Transformation occurred

If Mouse Lives:
Transformation Disrupted

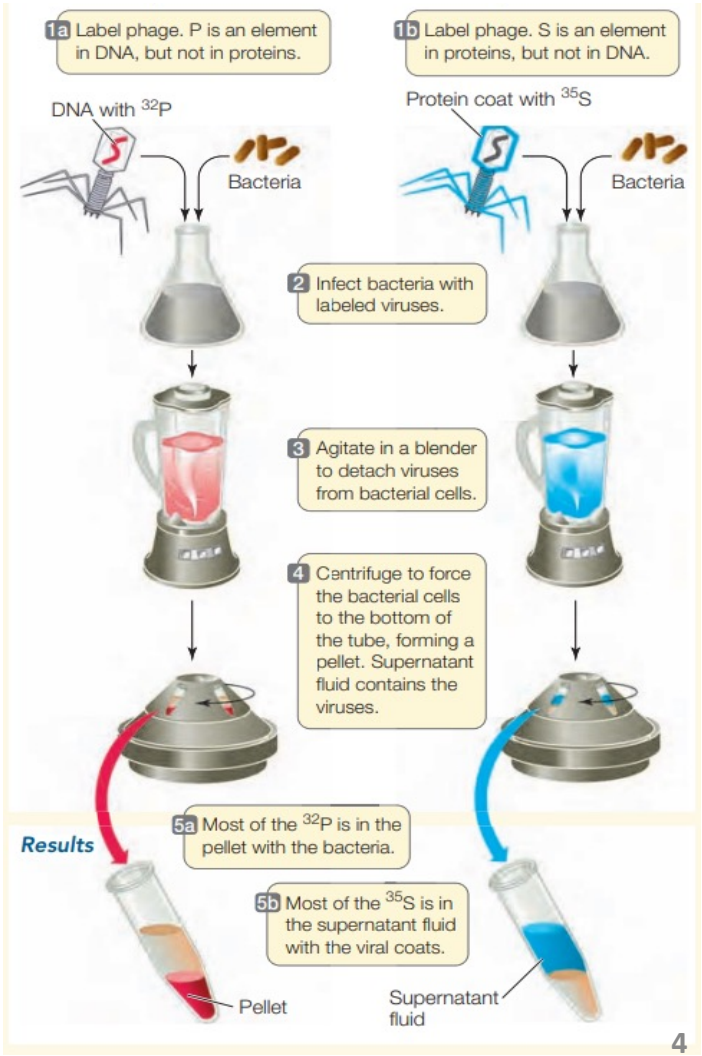
Conclusion: DNA is the transforming principle

Final proof- Hershey and Chase experiment



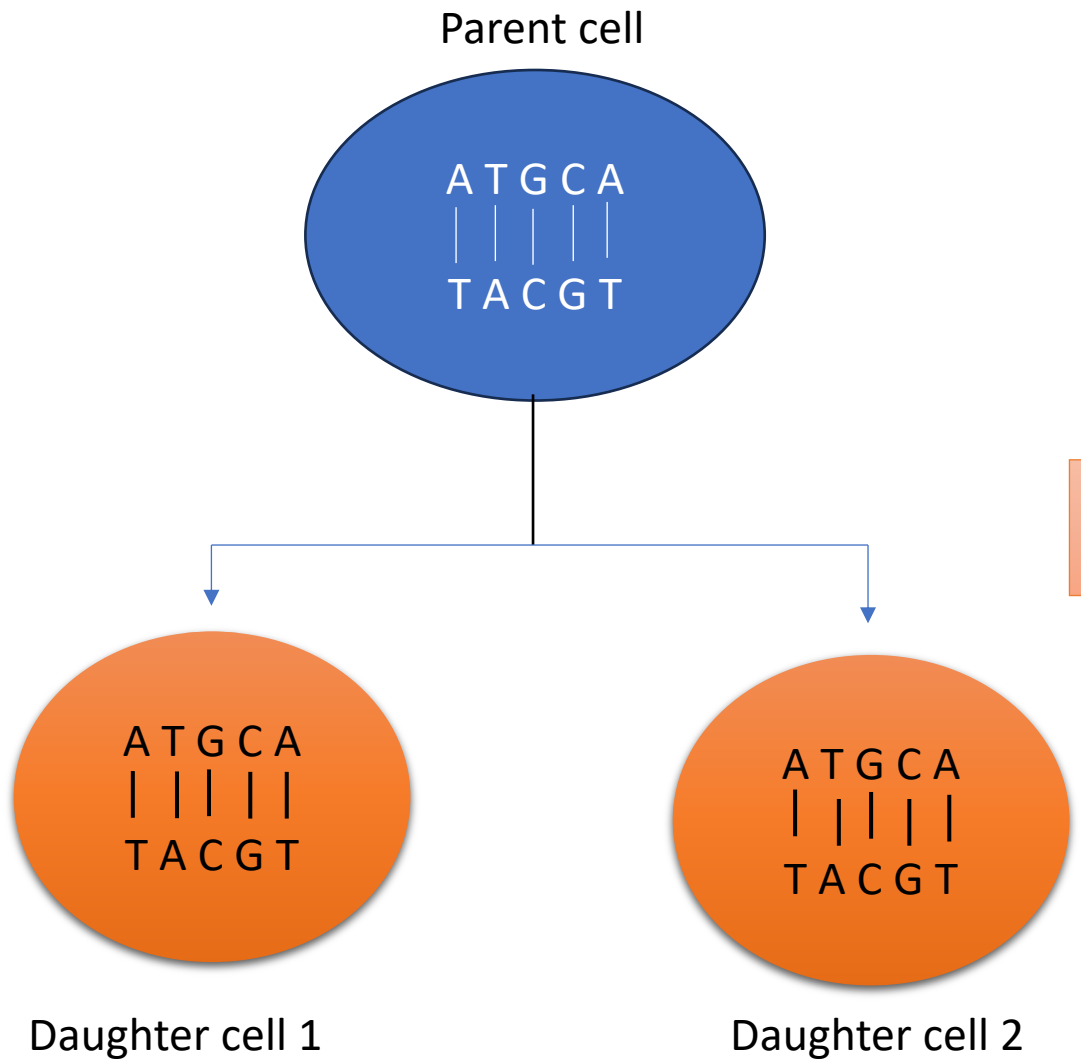
Bacteriophage T2 Reproduction Cycle

HYPOTHESIS: Either component of a bacteriophage—DNA or protein—might be the hereditary material that enters a bacterial cell to direct the assembly of new viruses.



CONCLUSION: DNA, not protein, enters bacterial cells and directs the assembly of new viruses.

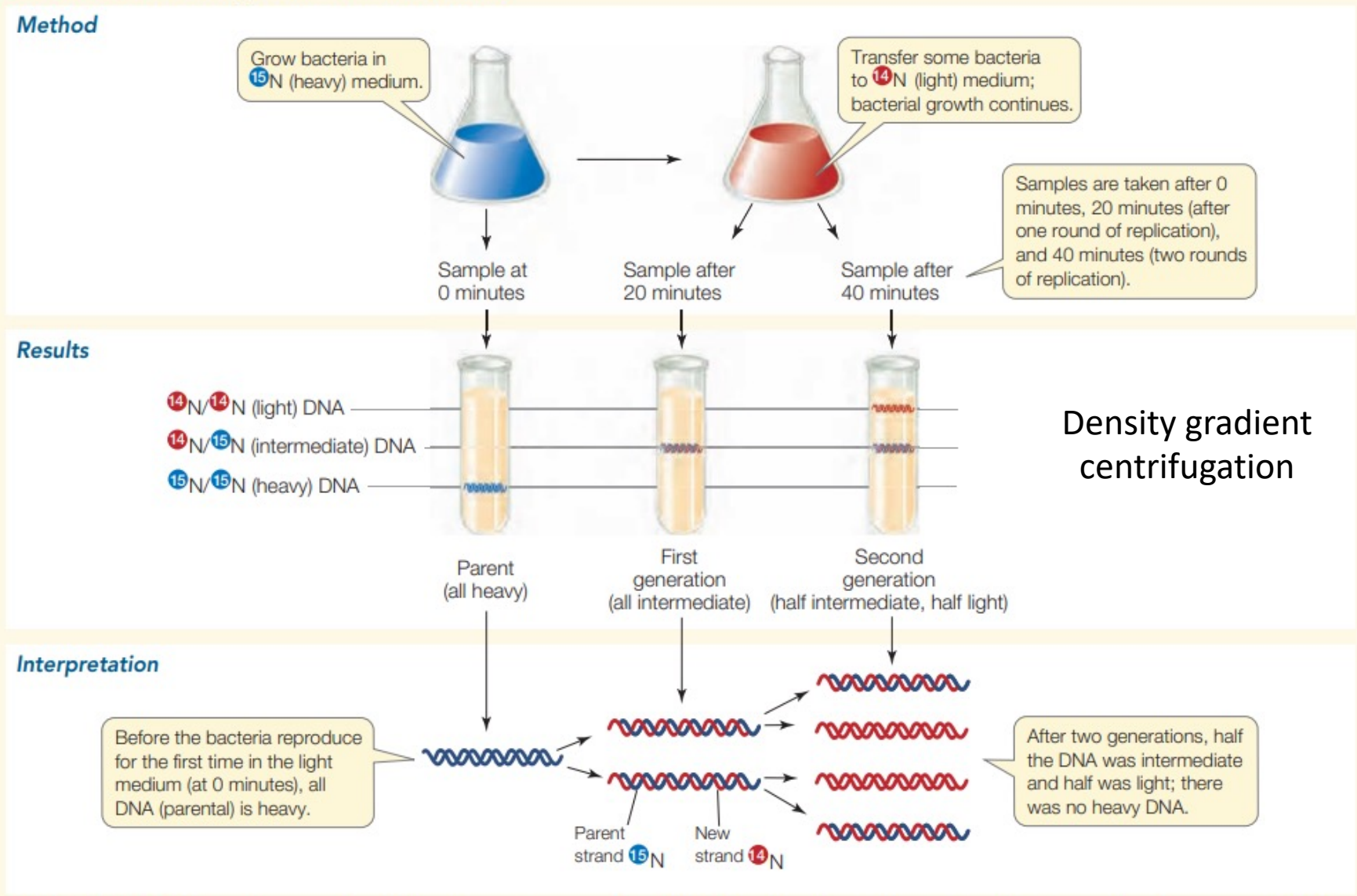
Transfer of genetic material form parent to daughter cell



The two new daughter cells have same genetic information!

Then how do you think DNA replication happens?

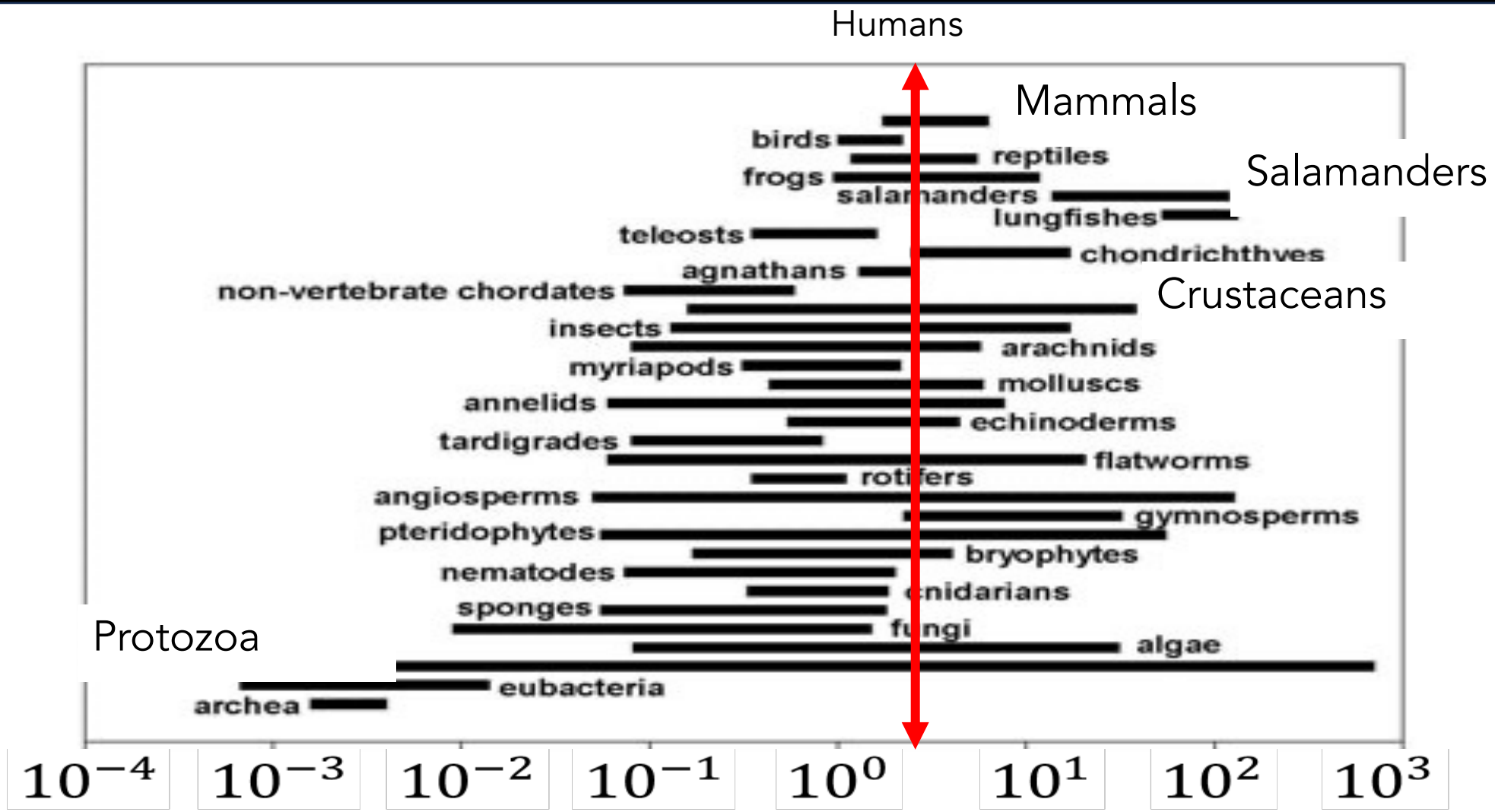
Meselson and Stahl experiment



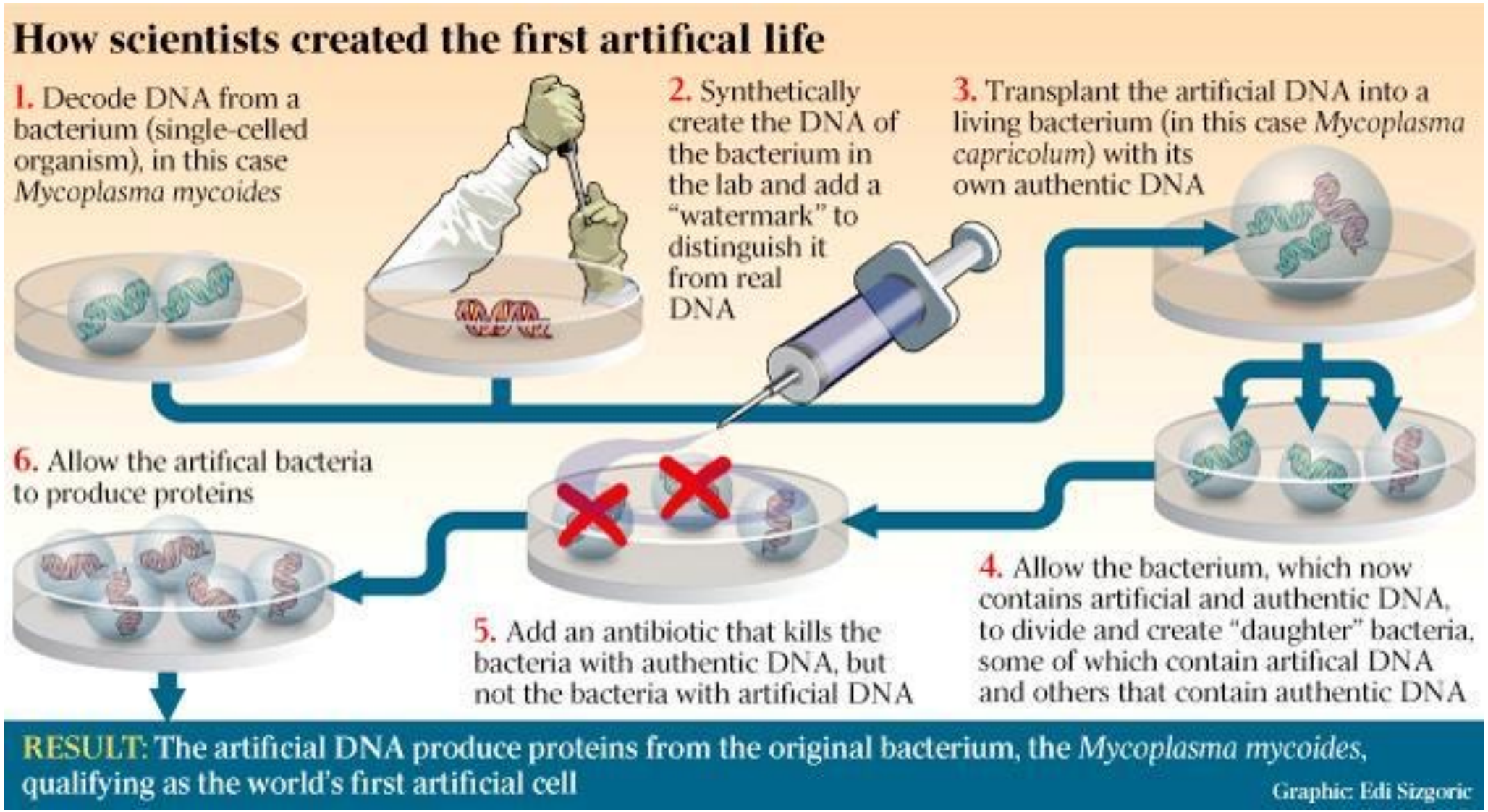
Density gradient centrifugation

CONCLUSION: This pattern could only have been observed if each DNA molecule contains a template strand from the parental DNA; thus DNA replication is semiconservative.

Do humans have the largest genome?



Can we make an organism with a synthetic instruction manual (genome)?



The synthetic genome in each cell contained only 473 key genes thought to be essential for life (half the size of the original genome, approx. 531 kbp, which is smaller than any other autonomously replicating organism found in nature.

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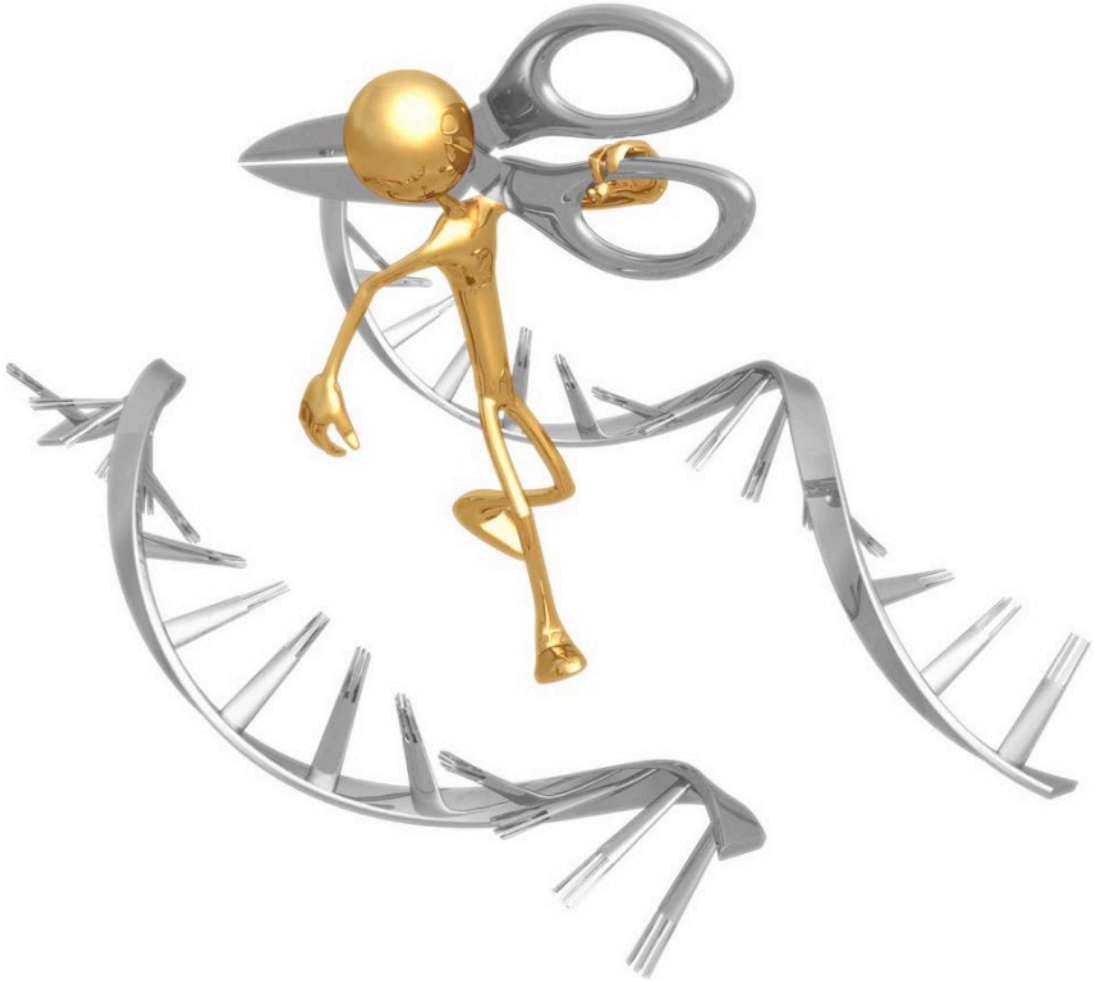
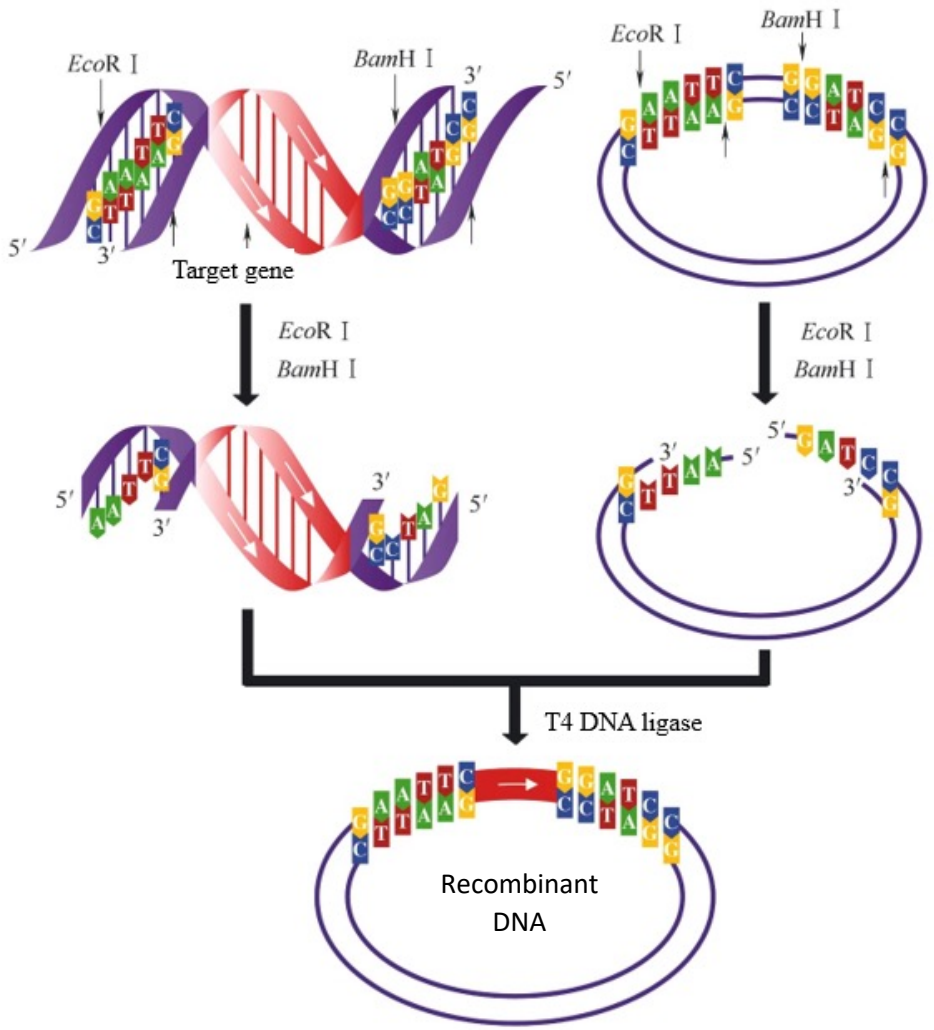
How do People manipulate DNA in Lab?



DNA Tools

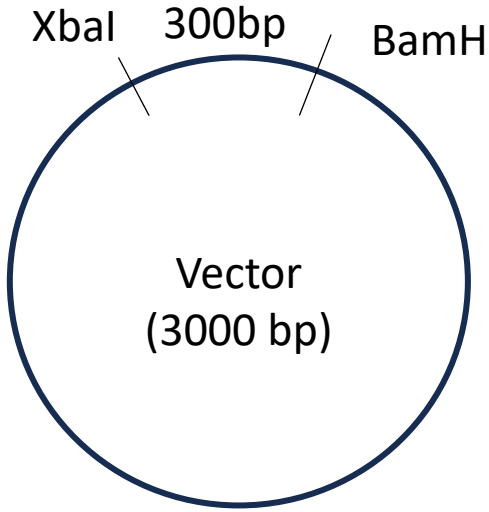
Overview of Genetic Engineering

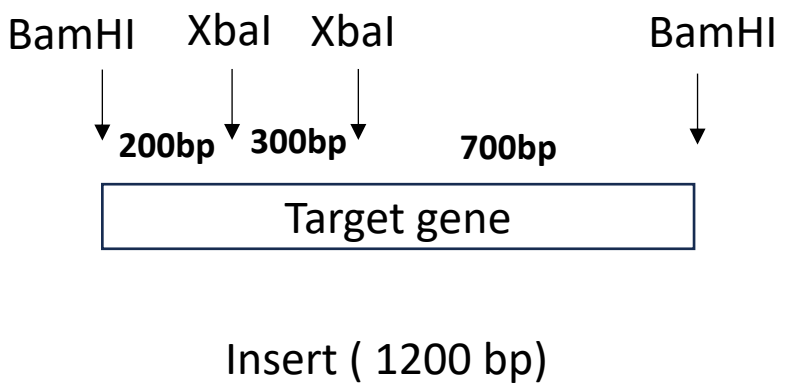
- Both the target gene and the vector are digested with same set of restriction enzyme to produce compatible ends.
- These cut ends are then joined by DNA ligases to form the recombinant DNA molecule.



Problem

The maps of a 1200 base pairs (bp) long target gene and a 3000 bp vector is shown below. The gene is cloned at the BamHI site of the vector using only this restriction enzyme producing a recombinant DNA. The lengths of the DNA in between the restriction sites are indicated in base pairs (bp). What will be the sizes of the fragments produced following complete digestion with XbaI if the insert is cloned in **(i)** correct orientation (right to left) and **(ii)** wrong orientation (left to right)?

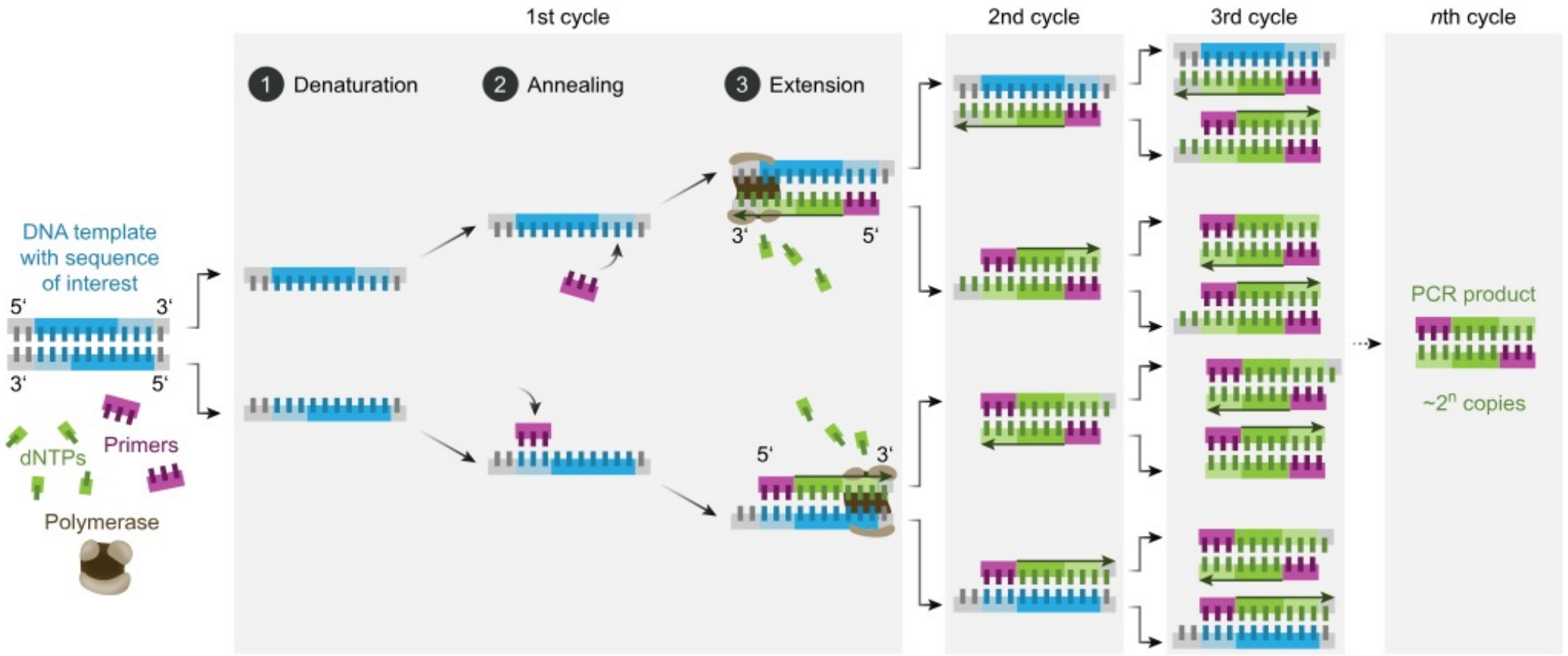




Insert (1200 bp)

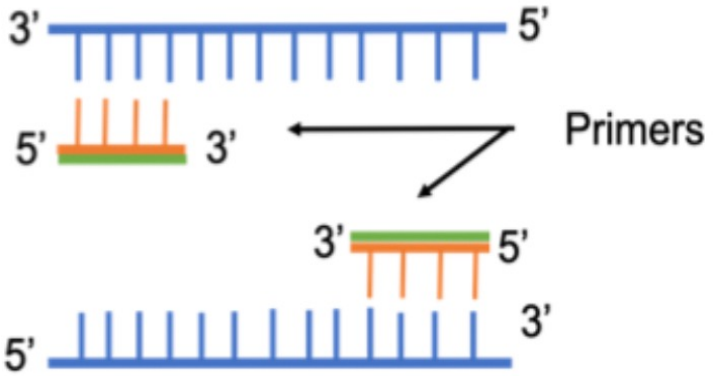
Ans. i) 500 bp, 300bp & 3400 bp
ii) 1000 bp, 300 bp & 2900 bp.

PCR (Polymerase Chain Reaction)



Primers

- Short strand of nucleotides (about 18-28 nucleotides in length) that serves as a starting point for DNA synthesis
- 50-60% GC composition
- Have a balanced distribution of G/C and A/T domains
- No long strings of a single base (<4)
- Tm= (A+T) X 2 + (G+C) X 4
- Primers should not be self complimentary



Q. For the primer 5' GATCCGATTGGACACTGTACTA 3' calculate the Tm.

Ans: 64 °C

Primer designing

Design primers for the following sequence. Location of 2 primers is indicated by >>'s. (Remember, that when both strands of DNA are shown the top strand runs 5'-3')

CTGTCCACACAATCTGCCCTTTCGAAACCATGGGATCCCAACGAAAAGAATTCCCACATGGTCCTT -upper strand
GACAGGTGTGTTAGACGGGAAAGCTTTGGTACCCTAGGGTTGCTTTTCTTAAGGGTGTACCAGGAA –lower strand
>>>>>>>>>>>>>>>>>>

CTTGAATTCGTAACAGCTGCTGGGATTACACATGGCATGGATGAACTATACAAATAA

GAACTTAAGCATTGTCGACG**ACCCTAATGTGTACCGTAC**CTACTTGATATGTTTATT
<<<<<<<<<<<<<<<<<<

The forward primer (>>>>>) will be complementary to the lower strand and must run 5'-3'
5'-CTGTCCACACAATCTGCC -3'

For the reverse primer, you will need to write the sequence of the other DNA strand. The reverse primer (<<<<<) which will be complementary to the upper strand and must run 3'-5'. However, we always write DNA sequences in the 5'-3' direction so the reverse primer would be written: 5'-CATGCCATGTGTAATCCCAG-3'

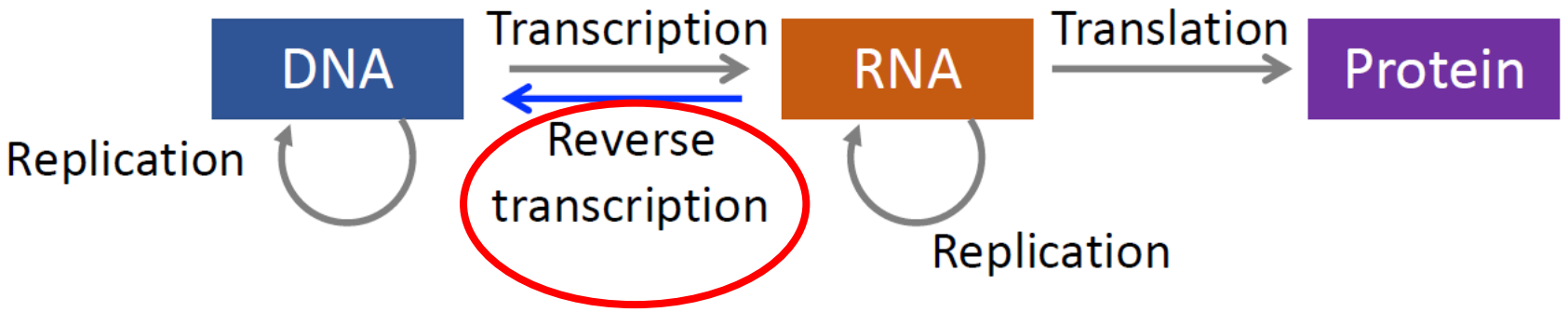
Primer 3 : https://bioinfo.ut.ee/primer3-0.4.0/

Primer- BLAST : https://www.ncbi.nlm.nih.gov/tools/primer-blast/

Central Dogma

A new disease causing organism is spreading quickly in your city. To determine whether a person is infected or not, his or her nasal swabs are being tested through a technique that involves a process called "**Reverse Transcription**". Given this information, can you comment about the type of genetic material that the organism have?

Recall...



Genetic Code

A popular brand yogurt has been shown to provide several health benefits including alleviation of negative emotions like depression. After a lot of research it was found that a bacteria used in the yogurt secretes a small protein molecule (20 amino acids) which have anti-depressant qualities. What would be the size of the gene encoding this protein?

Recall…

- Each codon consists of 3 nucleotide bases coding for an amino acid.
- A stop codon in a gene signals for end of the synthesis of that protein.

- So, the size of the gene would be (20*3)+3 = 63 base pairs.

Genetic Code

Now, you want to find out which part of the bacterial genome is responsible for secretion of this protein. What are the basic criteria that you would include in your search string?

Things to remember…

- START Codon: AUG (ATG)
- STOP Codon: UAA (TAA) or, UAG (TAG) or, UGA (TGA).

So, We need to look for presence of “ATG” and any one of “TAA”, “TAG” or “TGA”; and these two separated by 57 other nucleotides (19 codons, none of them being a STOP codon) in the sequence of the bacterial genome.

Genetic Code

Further research showed that, another gene with the following sequence is also important in promoting anti-depressant behaviour.
5’-ATGGACAGCCCAGCCGACTACTAA-3’
3’-TACCTGTCGGGTCGGCTGATGATT-5’

- What would be the mRNA sequence encoded by this gene?

Answer..
“T” would replaced by “U” in the coding sequence of the gene for mRNA.

So, it would be: 5’-AUGGACAGCCCAGCCGACUACUAA-3’

Genetic Code

Now as you have the mRNA sequence (5’-AUGGACAGCCCAGCCGACUACUAA-3’), find out the amino acid sequence of the protein that the gene encodes by referring to the given Genetic code table:

Answer…
Met-Asp-Ser-Pro-Ala-Asp-Tyr.

No need to mug the table.

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