

'Glass Half Full or Empty': Illuminating the Human Transcriptome

Lecture 2

Any questions from last class?

Worksheet Answers!

(from last class)

Let's review some big themes!

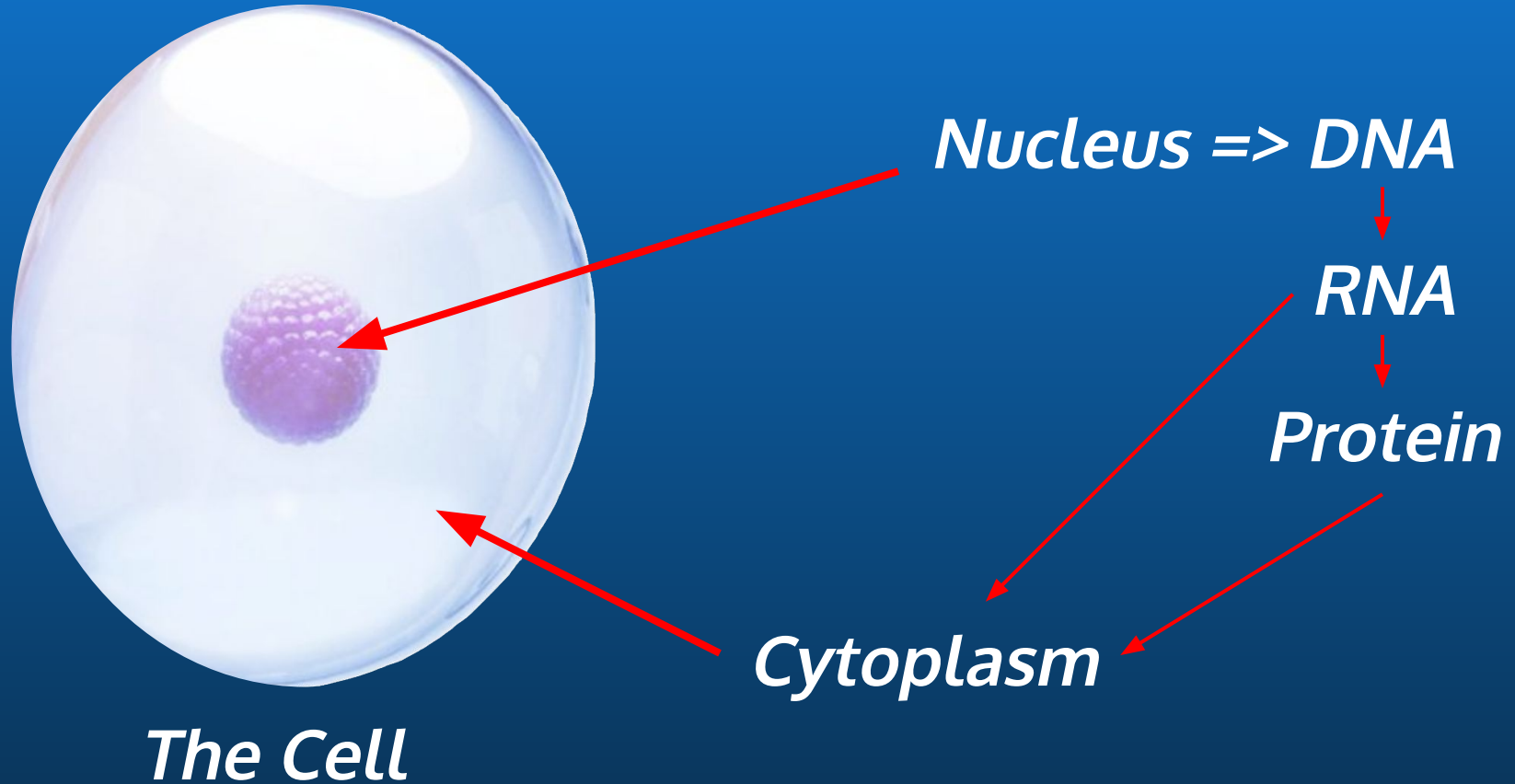
- 1) Central dogma of biology
- 2) DNA has two strands that pair with each other
- 3) Alternative splicing

The World of Biology

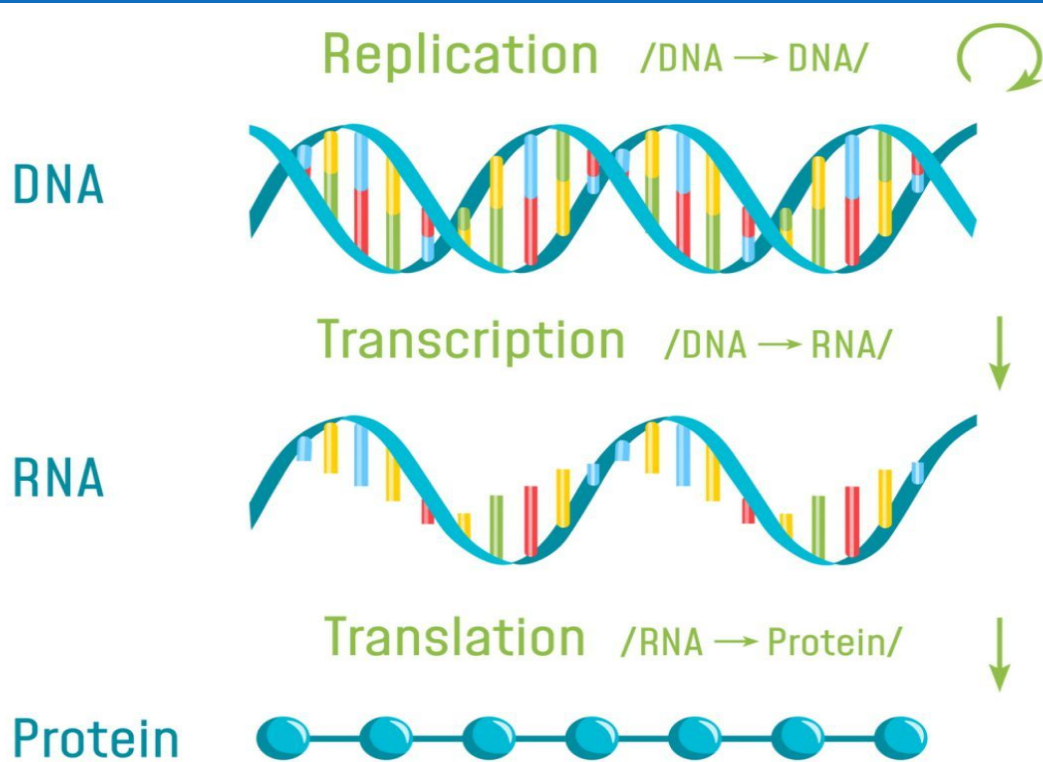


The Cell

The Central Dogma

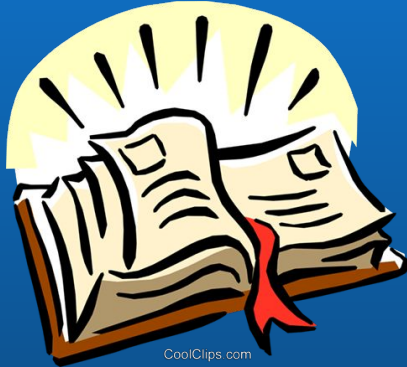


The Central Dogma



The Central Dogma

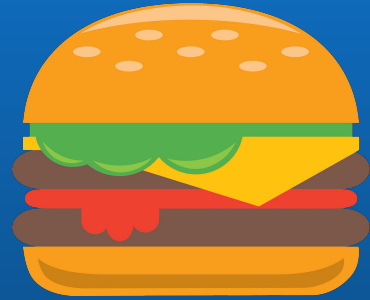
DNA



RNA

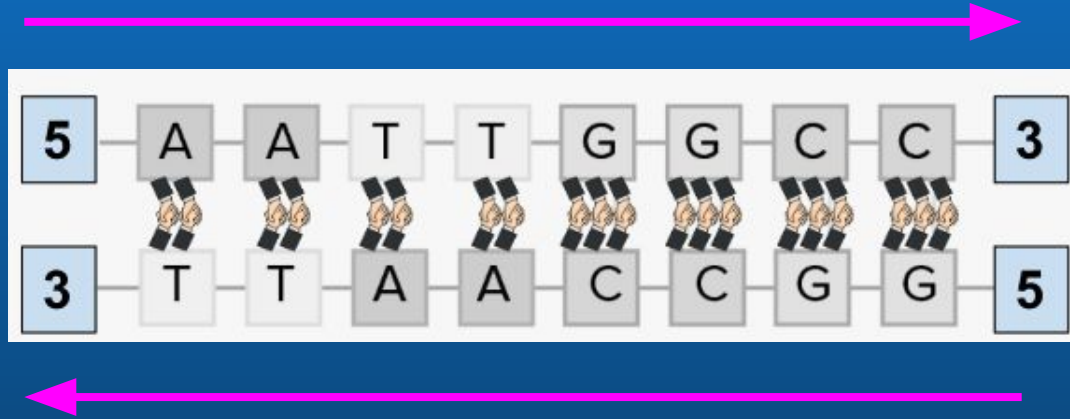


Protein



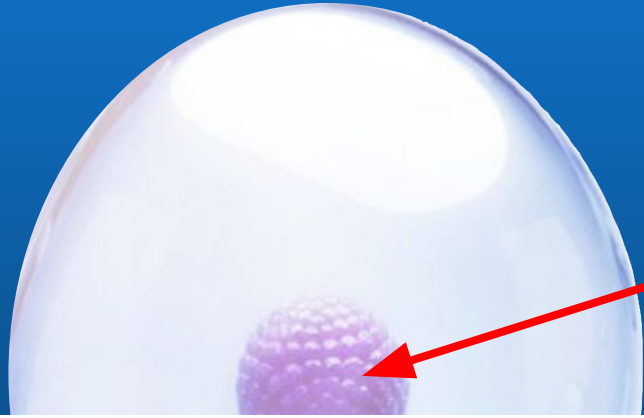
Eggs
Beef
Cheese
Bacon
Lettuce
Pickles
Tomato
Bread
Mayo

DNA has two strands that base pair with each other



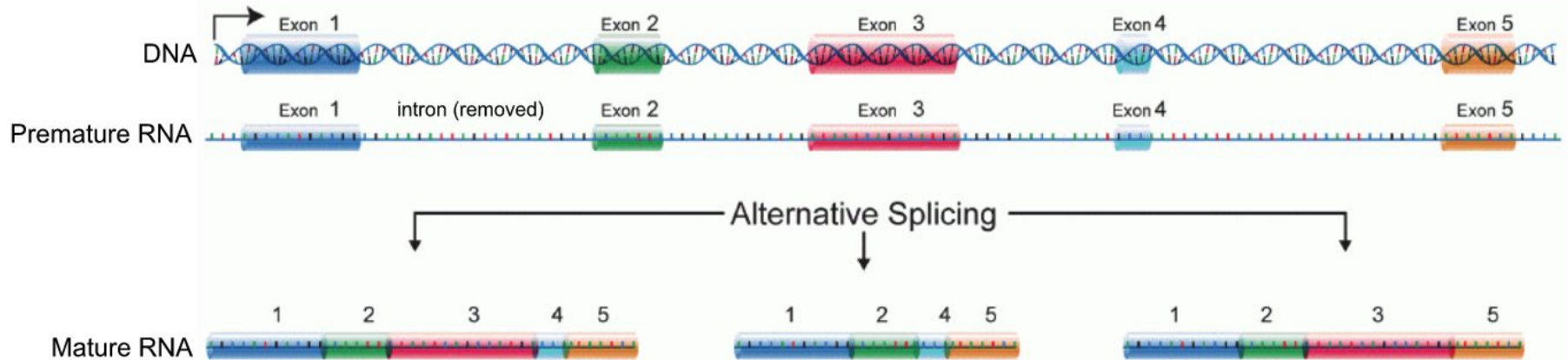
NOTICE: The 5' end of one strand pairs with the 3' end of the other!

Alternative splicing



Nucleus => DNA

RNA



Alternative splicing

DNA

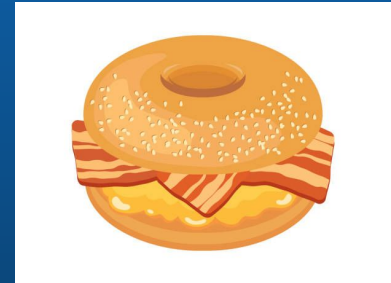
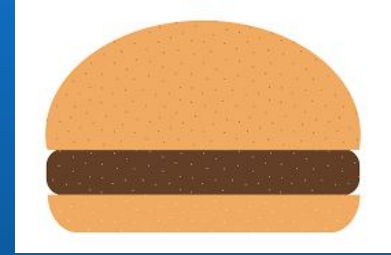


CoolClips.com

RNA



Protein



Eggs
Beef
Cheese
Bacon
Lettuce
Pickles
Tomato
Bread
Mayo

Eggs
Beef
Cheese
Bacon
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Pickles
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Bread
Mayo

So... what's next?

You are working on a NOVEL research project!

So.... what's the research question?



Can we predict NEW transcripts (mature RNA) and prove that they exist?

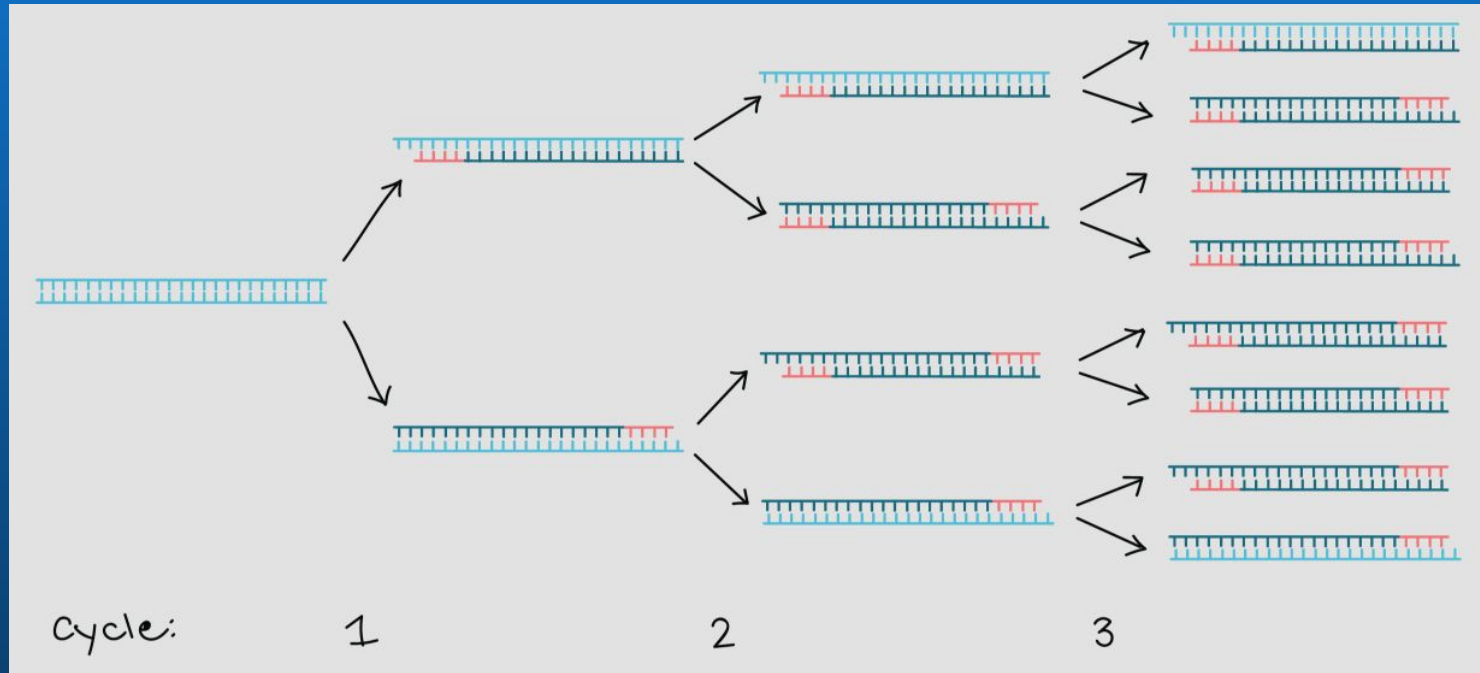
Yes!!!

- 1) Predict new transcripts (RNA)
- 2) Harvest transcripts (as stable DNA) from cells
- 3) Amplify your transcript
- 4) Isolate your transcript
- 5) Sequence the transcript

Yes!!!

- 1) Predict new transcripts (RNA)
- 2) Harvest transcripts (as stable DNA) from cells
- 3) Amplify your transcript
- 4) Isolate your transcript
- 5) Sequence the transcript

Polymerase chain reaction (PCR)



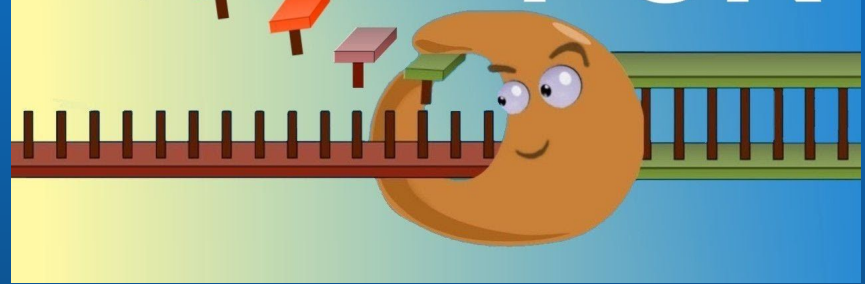
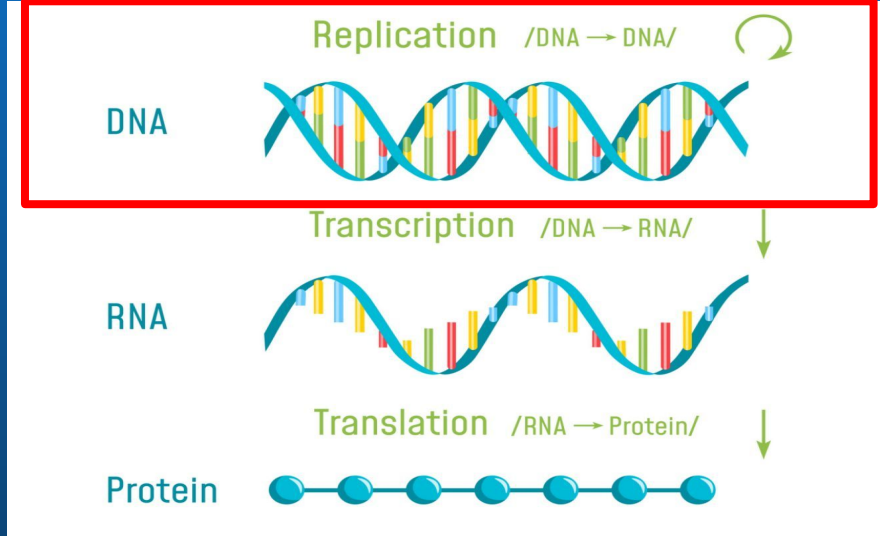
Polymerase chain reaction (PCR)

Where have you heard this term...



Polymerase chain reaction (PCR)

... is inspired by DNA replication!

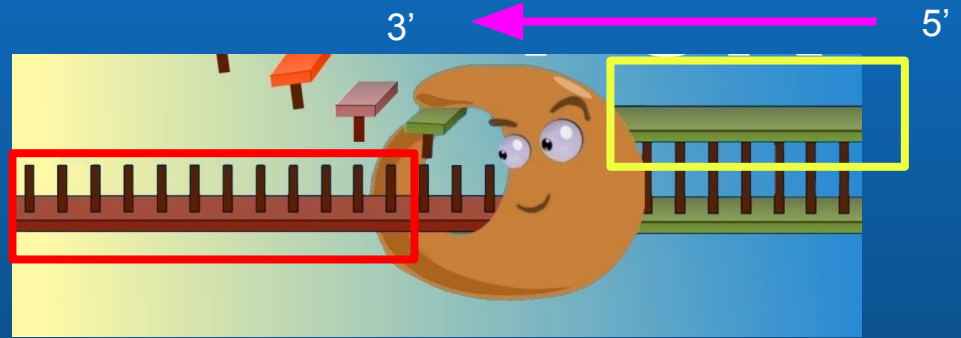


**DNA polymerase
'the DNA builder'**

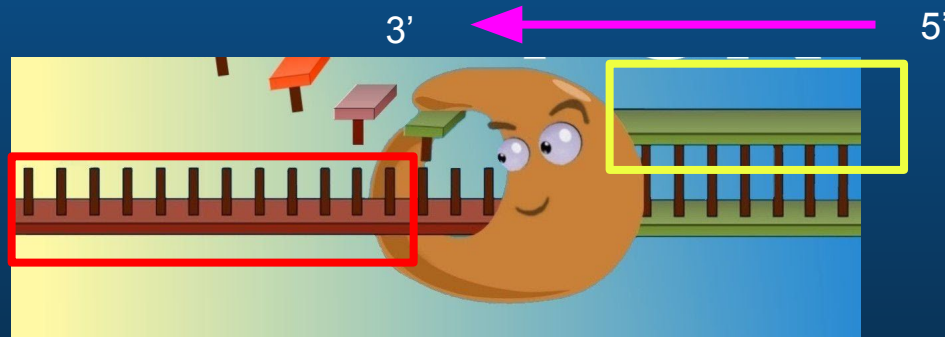
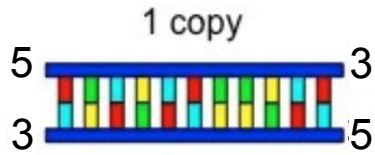
Polymerase chain reaction (PCR)

DNA polymerase needs 3 things

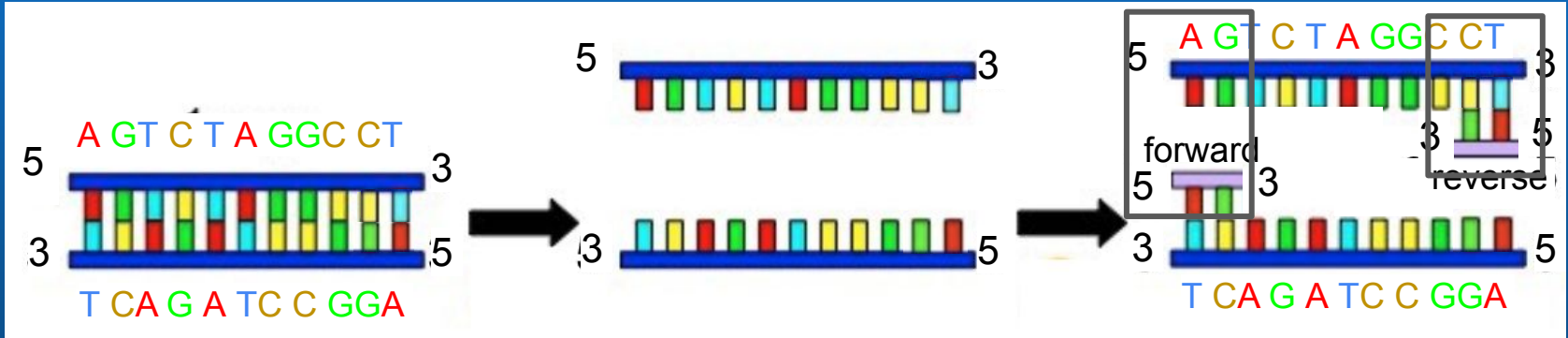
1. **Template DNA** (red)
2. **Primer** (yellow) = 'an anchor'
3. **New bases**



Polymerase chain reaction (PCR)



Polymerase chain reaction (PCR)

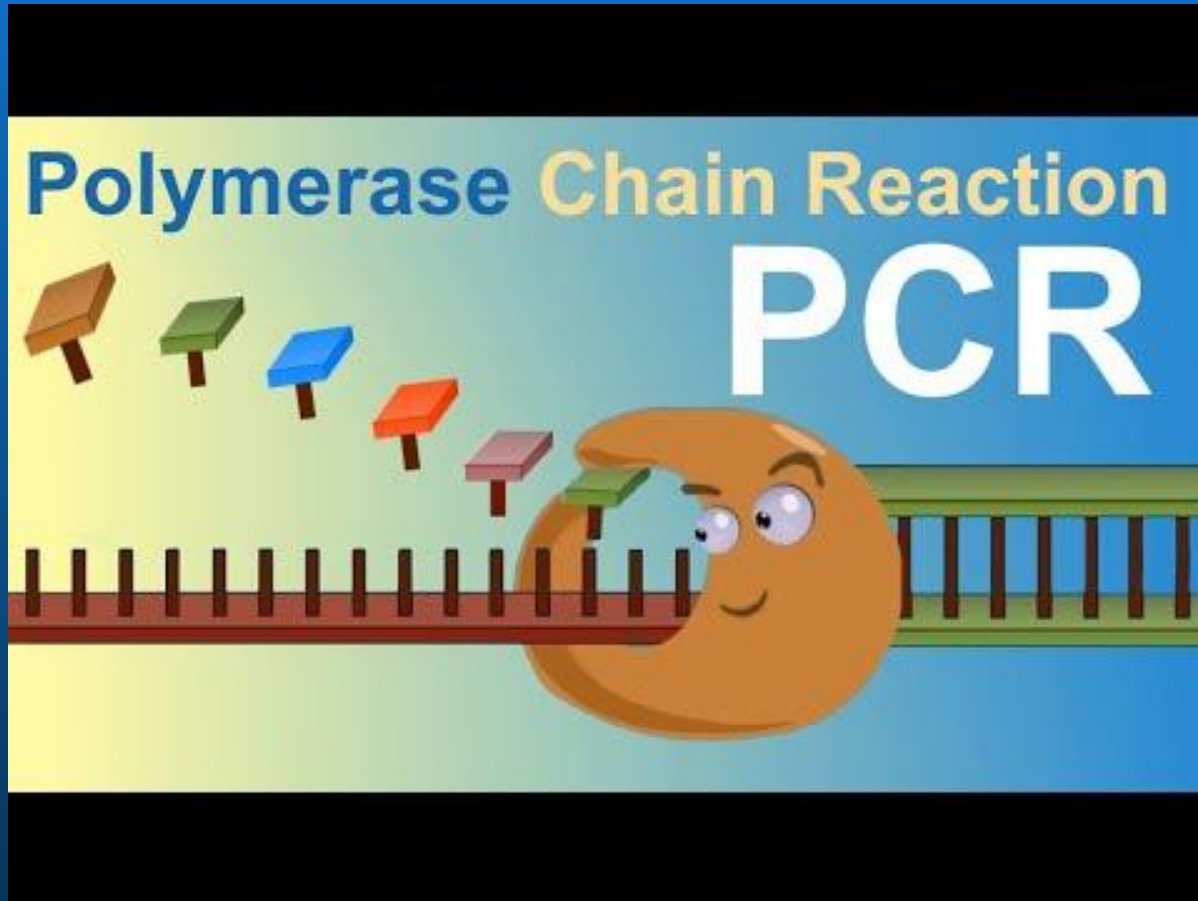


What's the sequence of the 2-base forward primer? **5'-AG-3'**
same as top strand

What's the sequence of the 2-base reverse primer? **5'-AG-3'**
'reverse complement' of
top strand 5'-CT-3'

Answer by writing it in the 5' -> 3' orientation.

Polymerase chain reaction (PCR)



Worksheet Time!

**Time to select some transcripts
& design some primers!**

After designing primers go to...

https://www.bioinformatics.org/sms2/pcr_primer_stats.html

gatatattatagacatatattatatat
gaaacacagatattacacaccagg
cagaccggaatcagagatagg
cacaaagacaaagacagcagcatalat

SMS

Format Conversion

- Combine FASTA
- EMBL to FASTA
- EMBL Feature Extractor
- EMBL Trans Extractor
- Filter DNA
- Filter Protein
- GenBank to FASTA
- GenBank Feature Extractor
- GenBank Trans Extractor
- One to Three
- Range Extractor DNA
- Range Extractor Protein
- Reverse Complement
- Split Codons
- Split FASTA
- Three to One
- Window Extractor DNA
- Window Extractor Protein

Sequence Manipulation Suite: PCR Primer Stats

PCR Primer Stats accepts a list of PCR primer sequences and returns a report describing the properties of each primer, including melting temperature, percent GC content, and PCR suitability. Use PCR Primer Stats to evaluate potential PCR primers.

Paste the raw sequence or one or more FASTA sequences into the text area below. Input limit is 5,000,000 characters. The maximum accepted primer length is 50 bases.

```
>reverse  
aacagctatgacatg  
>forward  
agtcgatcgatgagaa
```