

# Genetic engineering Part 2:

## Designing Basic DNA

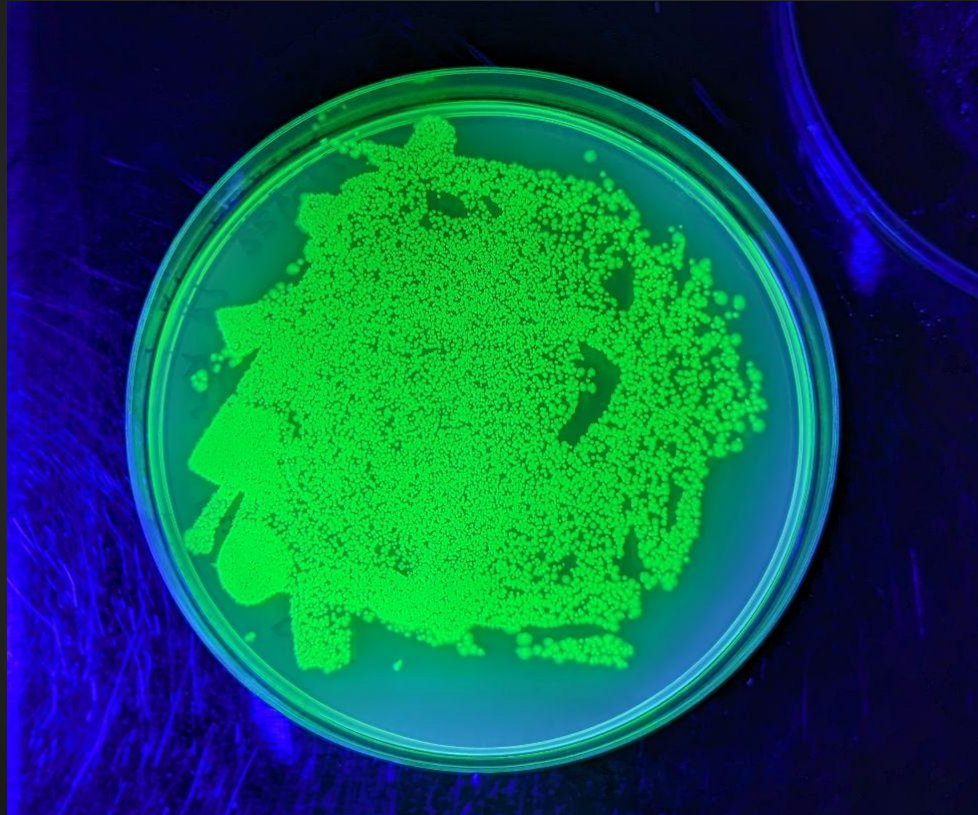
# What We'll Cover Today:

- What is a gene? What are its pieces?
- What is a plasmid? What are the basic requirements?
- High Copy vs Low Copy plasmids (Bacterial)
- Codons, back translation
- Genetic part notation
- Common resources for finding genetic information
- Basic assembly strategies overview
  - Restriction cloning
  - Gibson cloning
- DNA editing program basics
- Designing a few simple examples

## The “Hello World” Equivalent

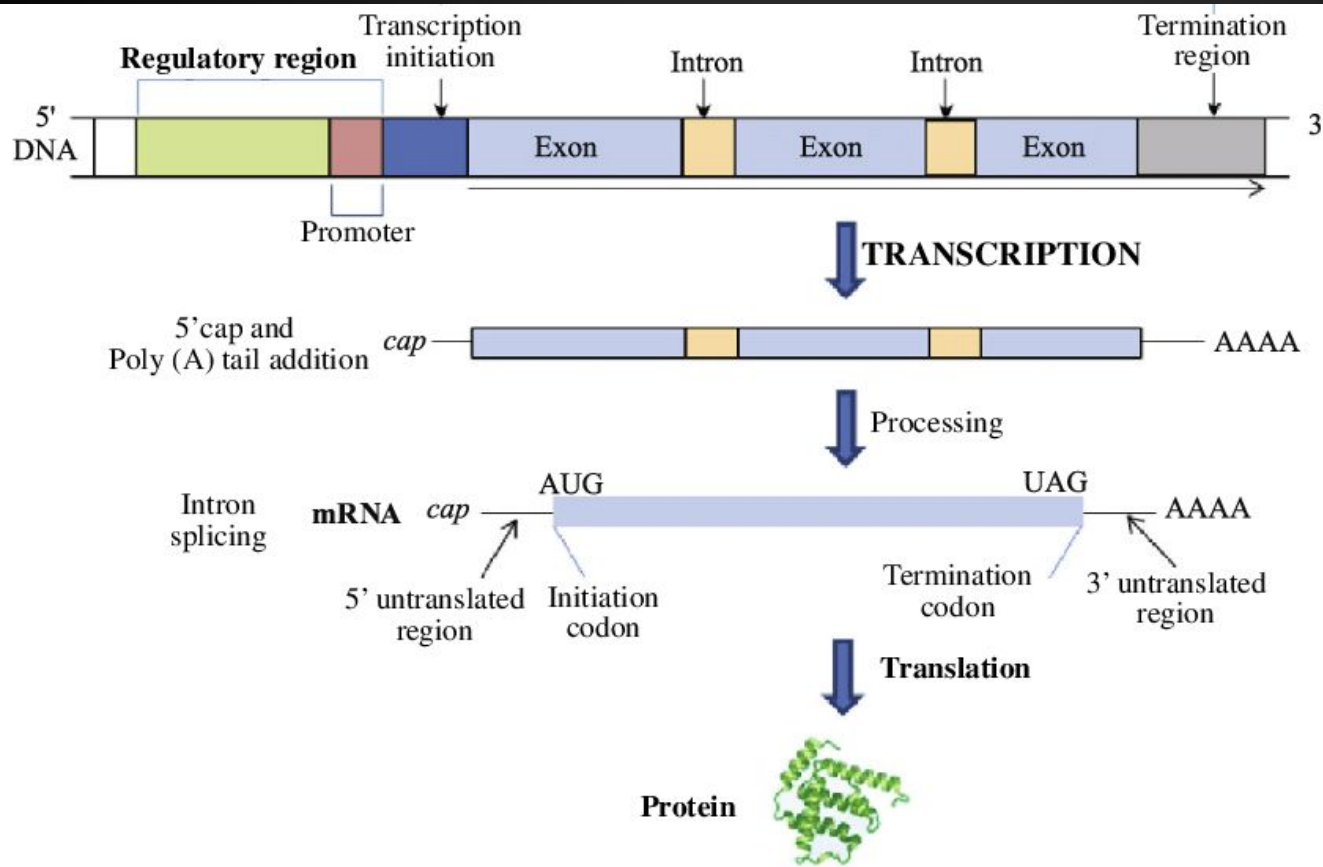
```
>>> print("Hello World!")  
Hello World!  
>>>
```

# The “Hello World” Equivalent: GFP

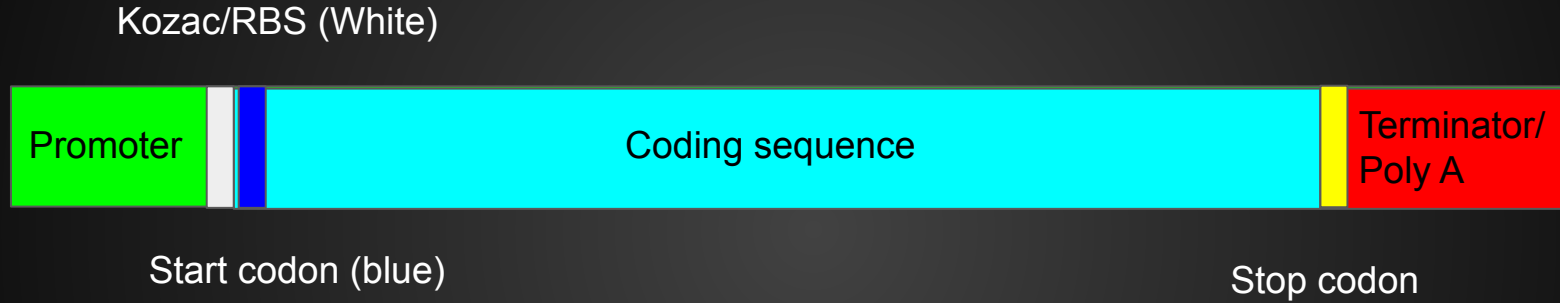


# Part 1: The basics

# Starting simple: The central dogma



# A Basic Gene



\*The exact details vary slightly between  
bacteria/animals/plants

\*\*This example uses no introns or regulatory  
elements

# Gene notation

## Nucleic Acid Glyphs

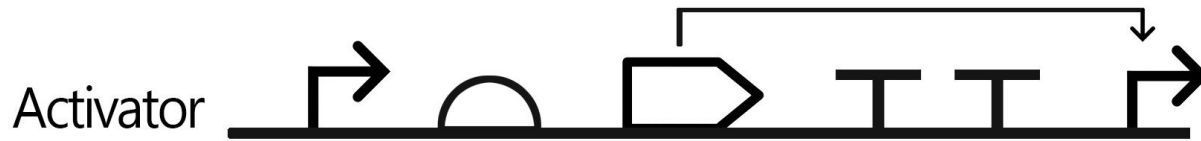
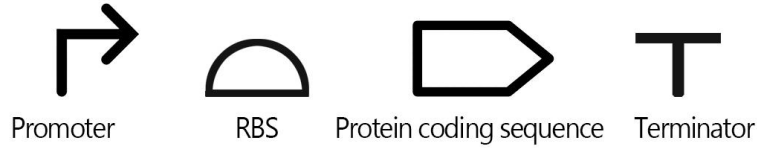
Aptamer	Assembly Scar	Blunt Restriction Site	(recommended) CDS	(alternate) CDS	Composite	Engineered Region	3' Overhang Sticky End
5' Overhang Sticky End	3' Sticky Restriction Site	5' Sticky Restriction Site	Insulator	No Glyph	Non-Coding RNA	Omitted Detail	Operator
ORI	ORI-T	Poly-A Site	Primer Binding Site	Promoter	Ribosome Entry Site	Signature	Recombination Site
Terminator	(recommended) Unspecified	(alternate) Unspecified	(recommended) DNA Location	(recommended) RNA Location	(recommended) Protein Location	(alternate) DNA Location	(alternate) RNA Location
(alternate) Protein Location	DNA Cleavage Site	RNA Cleavage Site	Protein Cleavage Site	DNA Stability Element	RNA Stability Element	Protein Stability Element	



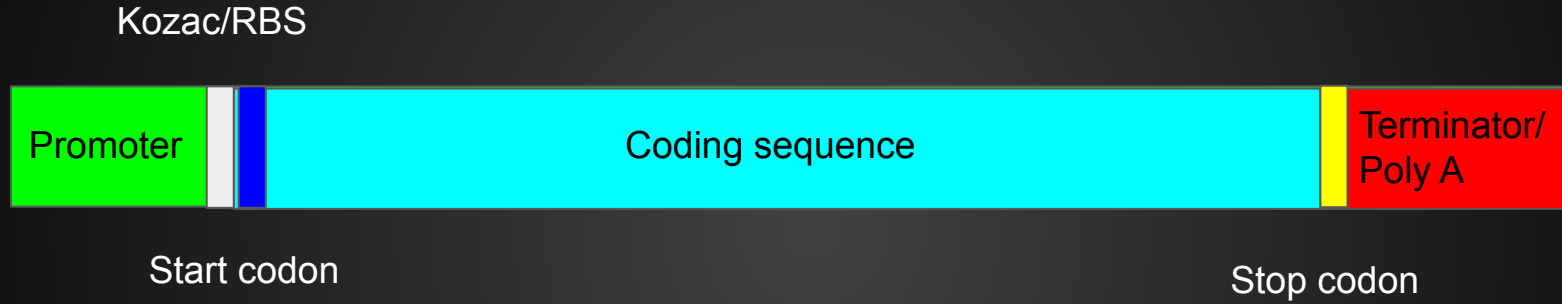
# Gene notation: actually useful ones I've seen used

Nucleic Acid Glyphs							
Aptamer	Assembly Scar	Blunt Restriction Site	(recommended) CDS	(alternate) CDS	Composite	Engineered Region	3' Overhang Sticky End
5' Overhang Sticky End	3' Sticky Restriction Site	5' Sticky Restriction Site	Insulator	No Glyph	Non-Coding RNA	Omitted Detail	Operator
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(alternate) Protein Location	DNA Cleavage Site	RNA Cleavage Site	Protein Cleavage Site	DNA Stability Element	RNA Stability Element	Protein Stability Element	

# Basic Gene Notation Example



# A Basic Gene

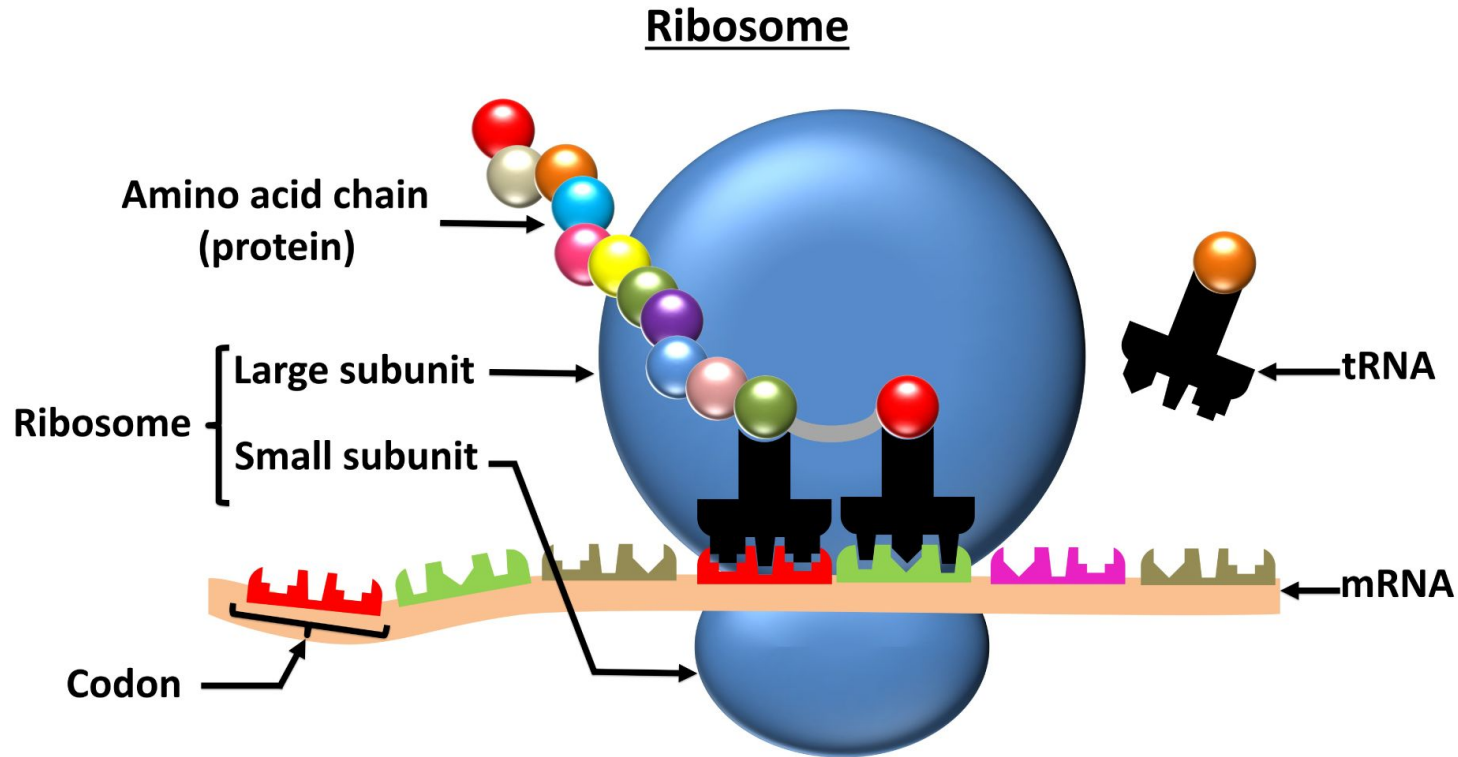


\*The exact details vary slightly between  
bacteria/animals/plants

# Genetic code

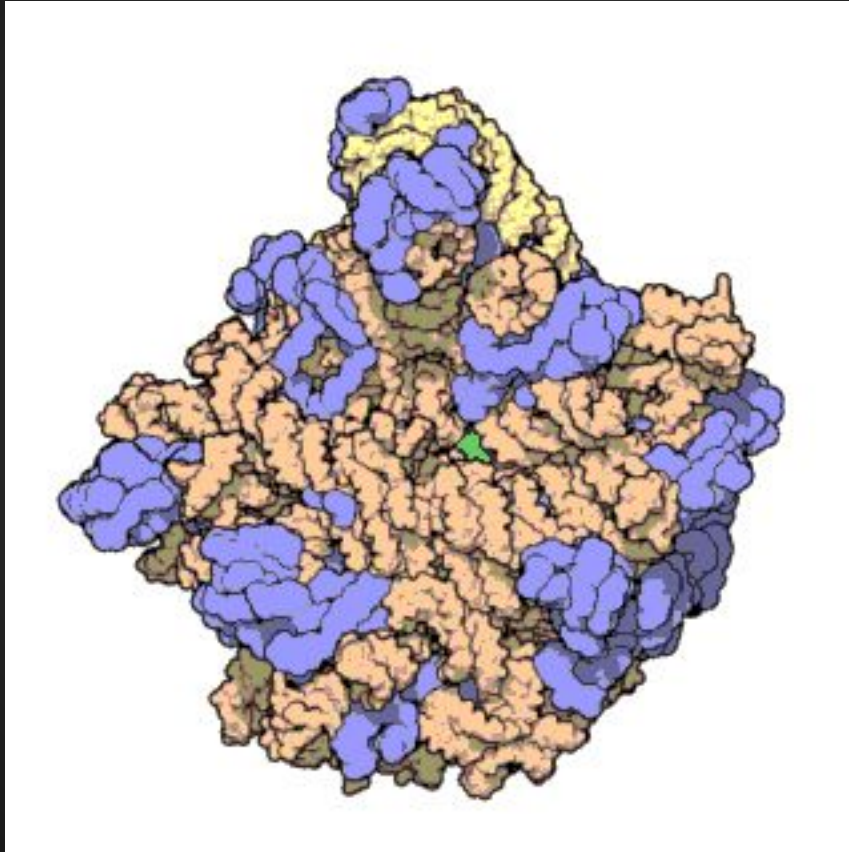
		second base in codon									
		T		C		A		G			
		first base in codon		first base in codon		first base in codon		first base in codon		third base in codon	
T	TTT	Phe	TCT	Ser	TAT	Tyr	TGT	Cys	T		
	TTC	Phe	TCC	Ser	TAC	Tyr	TGC	Cys	C		
	TTA	Leu	TCA	Ser	TAA	stop	TGA	stop	A		
	TTG	Leu	TCG	Ser	TAG	stop	TGG	Trp	G		
C	CTT	Leu	CCT	Pro	CAT	His	CGT	Arg	T		
	CTC	Leu	CCC	Pro	CAC	His	CGC	Arg	C		
	CTA	Leu	CCA	Pro	CAA	Gln	CGA	Arg	A		
	CTG	Leu	CCG	Pro	CAG	Gln	CGG	Arg	G		
A	ATT	Ile	ACT	Thr	AAT	Asn	AGT	Ser	T		
	ATC	Ile	ACC	Thr	AAC	Asn	AGC	Ser	C		
	ATA	Ile	ACA	Thr	AAA	Lys	AGA	Arg	A		
	ATG	Met	ACG	Thr	AAG	Lys	AGG	Arg	G		
G	GTT	Val	GCT	Ala	GAT	Asp	GGT	Gly	T		
	GTC	Val	GCC	Ala	GAC	Asp	GGC	Gly	C		
	GTA	Val	GCA	Ala	GAA	Glu	GGA	Gly	A		
	GTG	Val	GCG	Ala	GAG	Glu	GGG	Gly	G		

# The ribosome



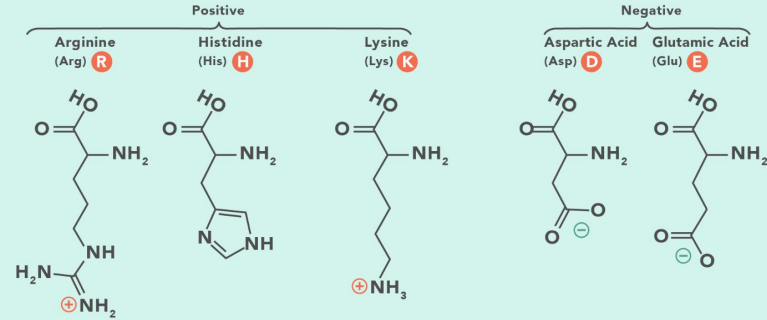
# A quick reminder: Cartoons $\neq$ reality

What a ribosome  
actually looks like

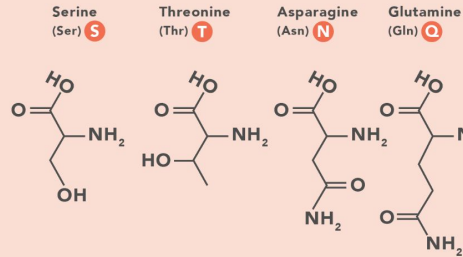


# Aminos

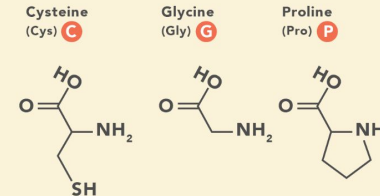
### A. Amino Acids with Electrically Charged Side Chains



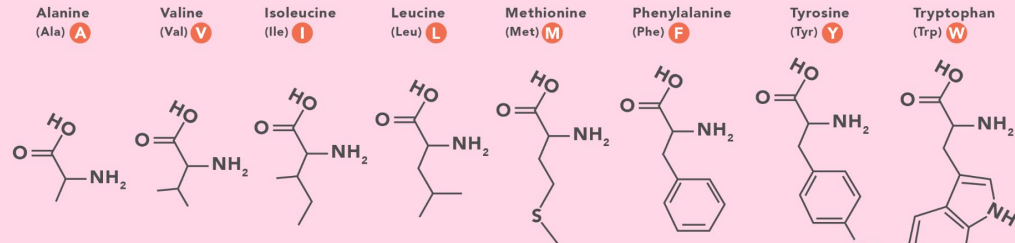
### B. Amino Acids with Polar Uncharged Side Chains



### C. Special Cases

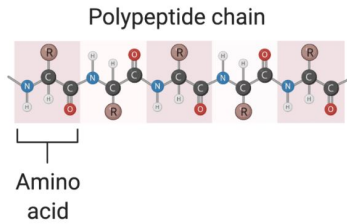


#### D. Amino Acids with Hydrophobic Side Chains

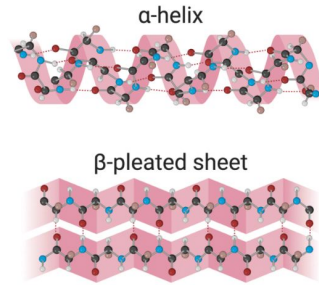


# Proteins

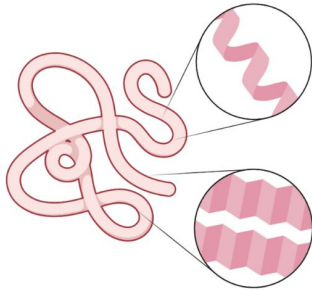
## Primary structure



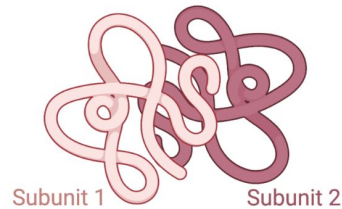
## Secondary structure



## Tertiary structure

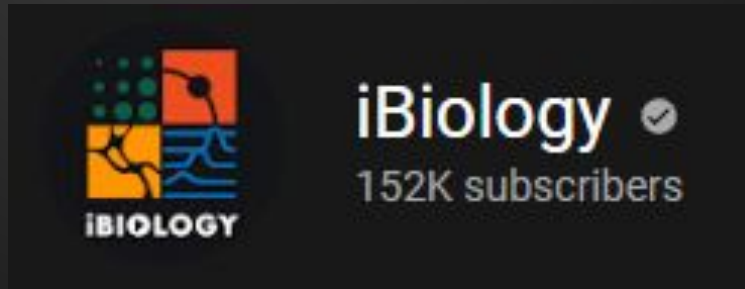
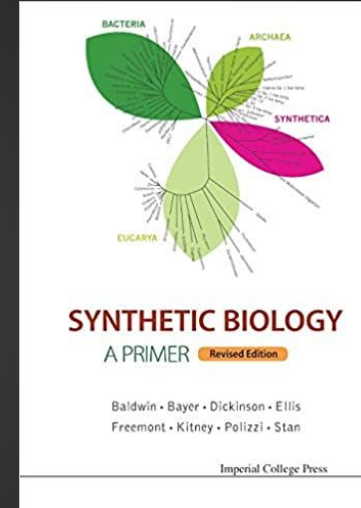
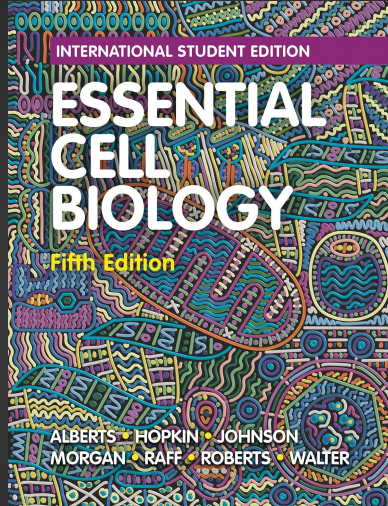


## Quaternary structure





For more detail on the basics, see these resources:



# Part 2: Plasmids

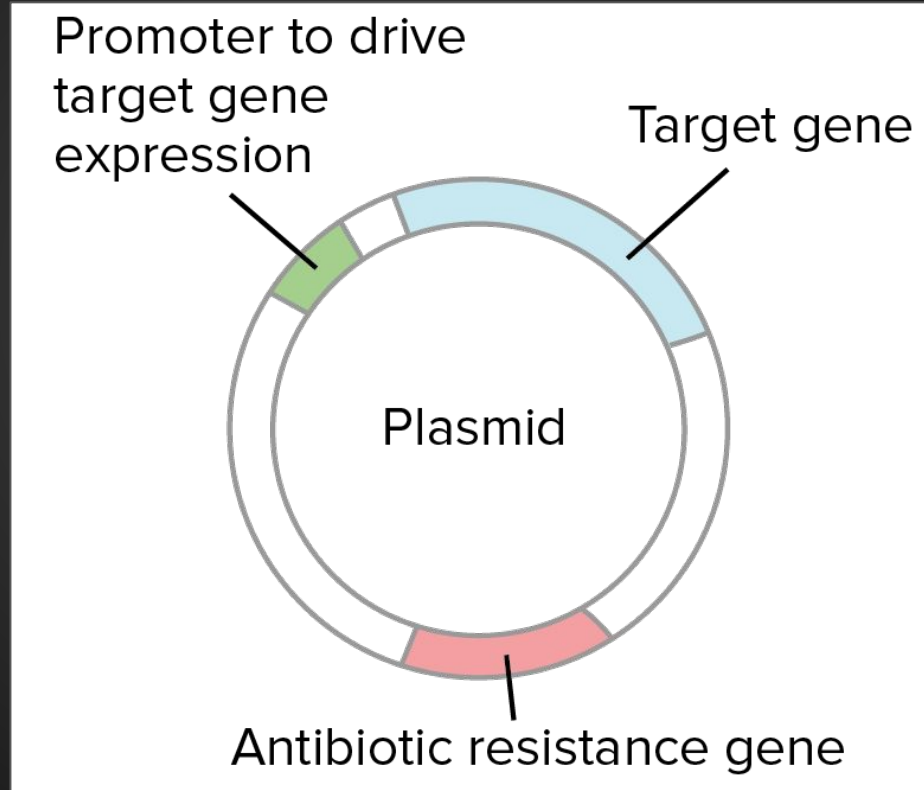
# Plasmids: the most basic genetic tool

3 basic pieces:

Target gene

Resistance gene

ORI (origin of replication)



# Main plasmid characteristics

- Integrative vs Non integrative
  - Do they exist on their own or do they insert themselves into the organism's genome
- High copy vs Low copy
  - If they exist on their own, how many copies exist per cell
- Inducible vs constitutive
  - Does the gene/genes they contain run on their own, or do they only turn on when given a signal
- Single gene vs multi gene
  - How many target genes? Just one? Or more?

# Important terms

- **Backbone**
  - The plasmid you're going to put your gene(s) into
- **Vector**
  - Same as backbone
- **Insert**
  - The gene/construct you're going to put into a backbone/vector
- **MCS**
  - Multiple cloning site. A location with lots of restriction sites for easy gene insertion
- **Restriction Site**
  - A targetable/cuttable bit of code if the DNA is mixed with a specific enzyme
- **Back translation**
  - Converting an amino sequence back into a DNA sequence

Good resources to start your design from



# Part 3: Enough Talk

It's time to cook.