Genetic engineering Part 2:

Designing Basic DNA

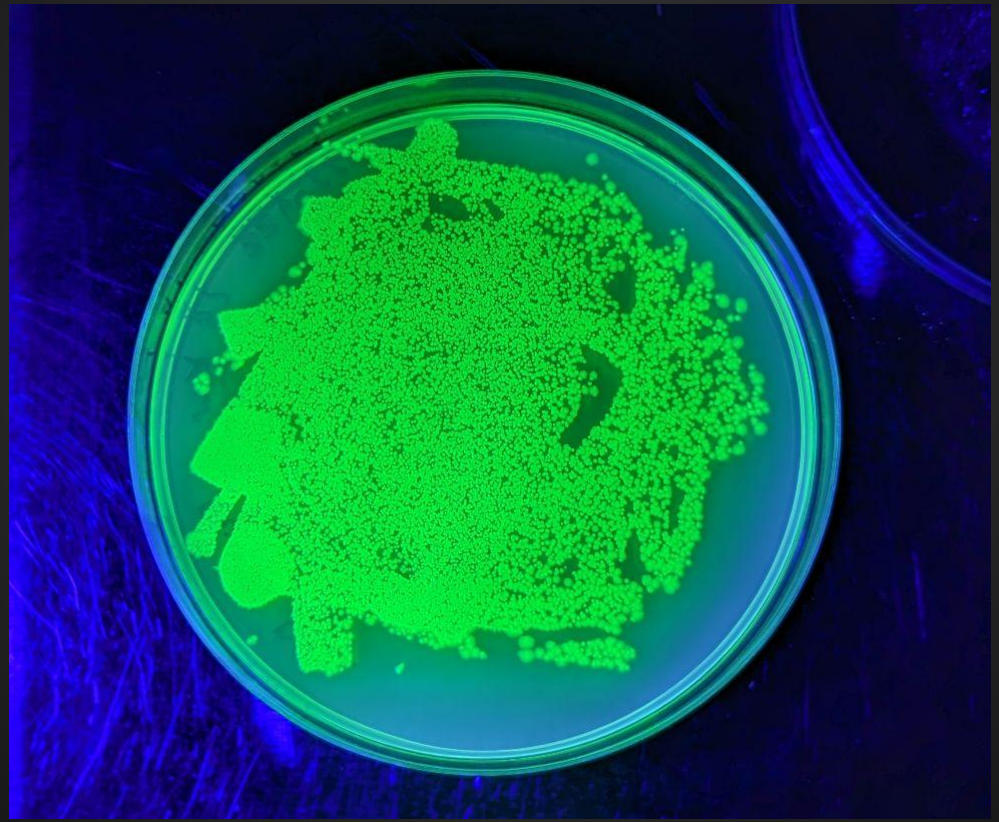
What We'll Cover Today:

- What is a gene? What are it's 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

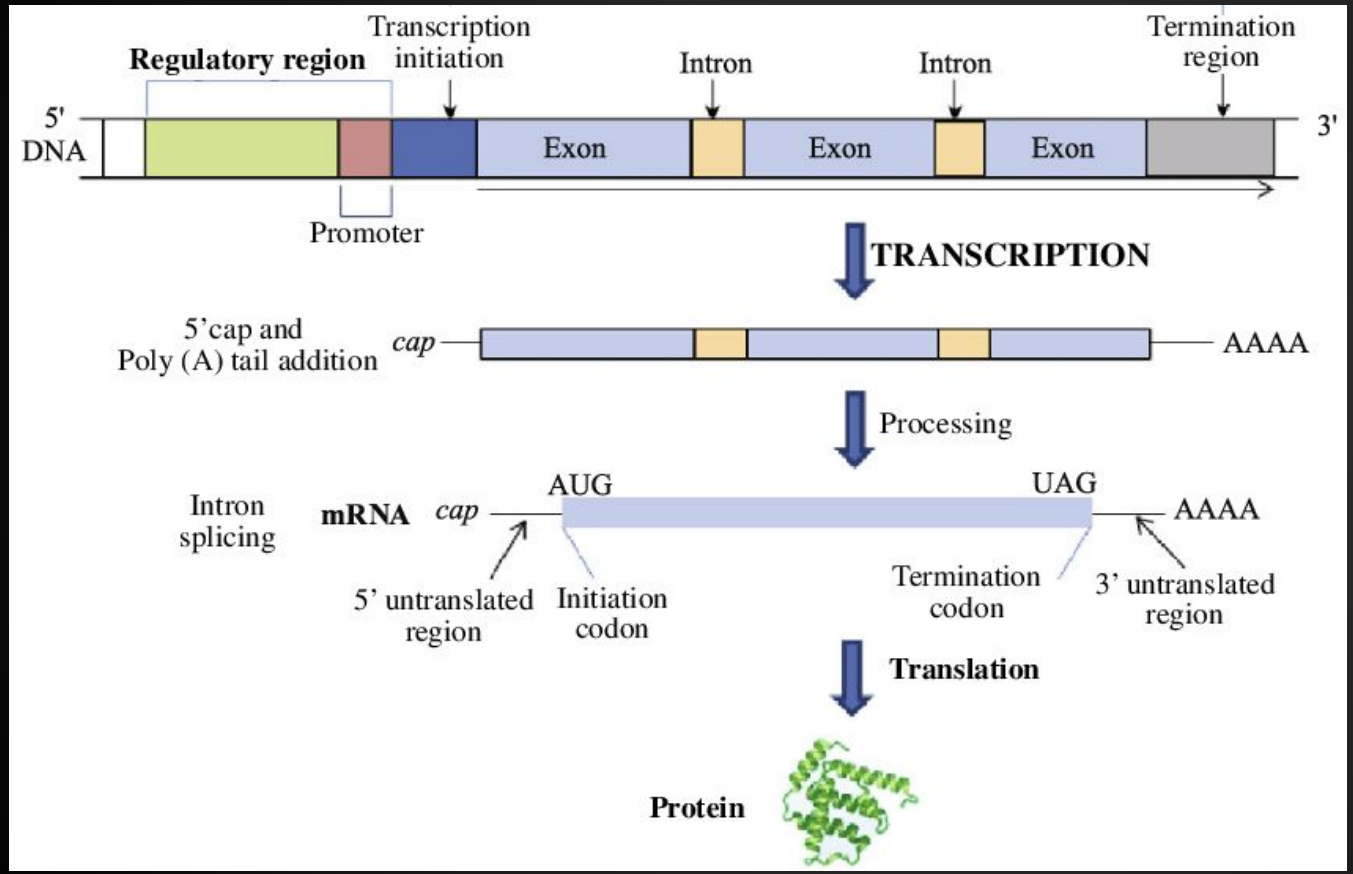
```
>>> print("Hello World!")
Hello World!
>>>
```

The “Hello World” Equivalent: GFP



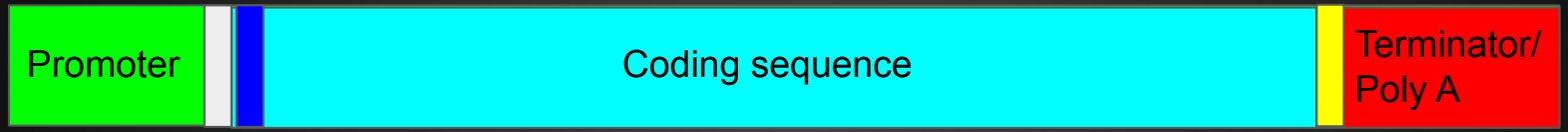
Part 1: The basics

Starting simple: The central dogma



A Basic Gene

Kozac/RBS (White)

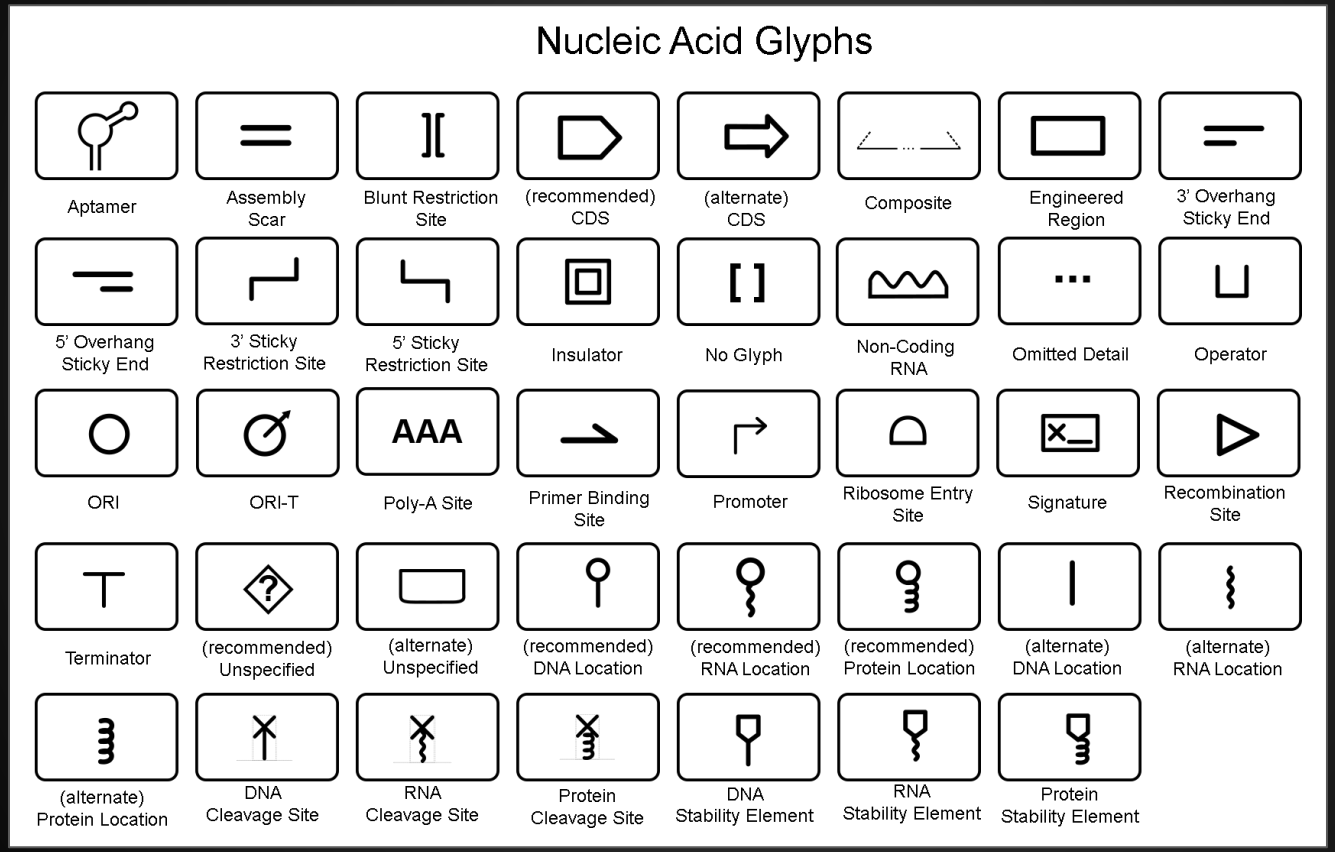


Start codon (blue)

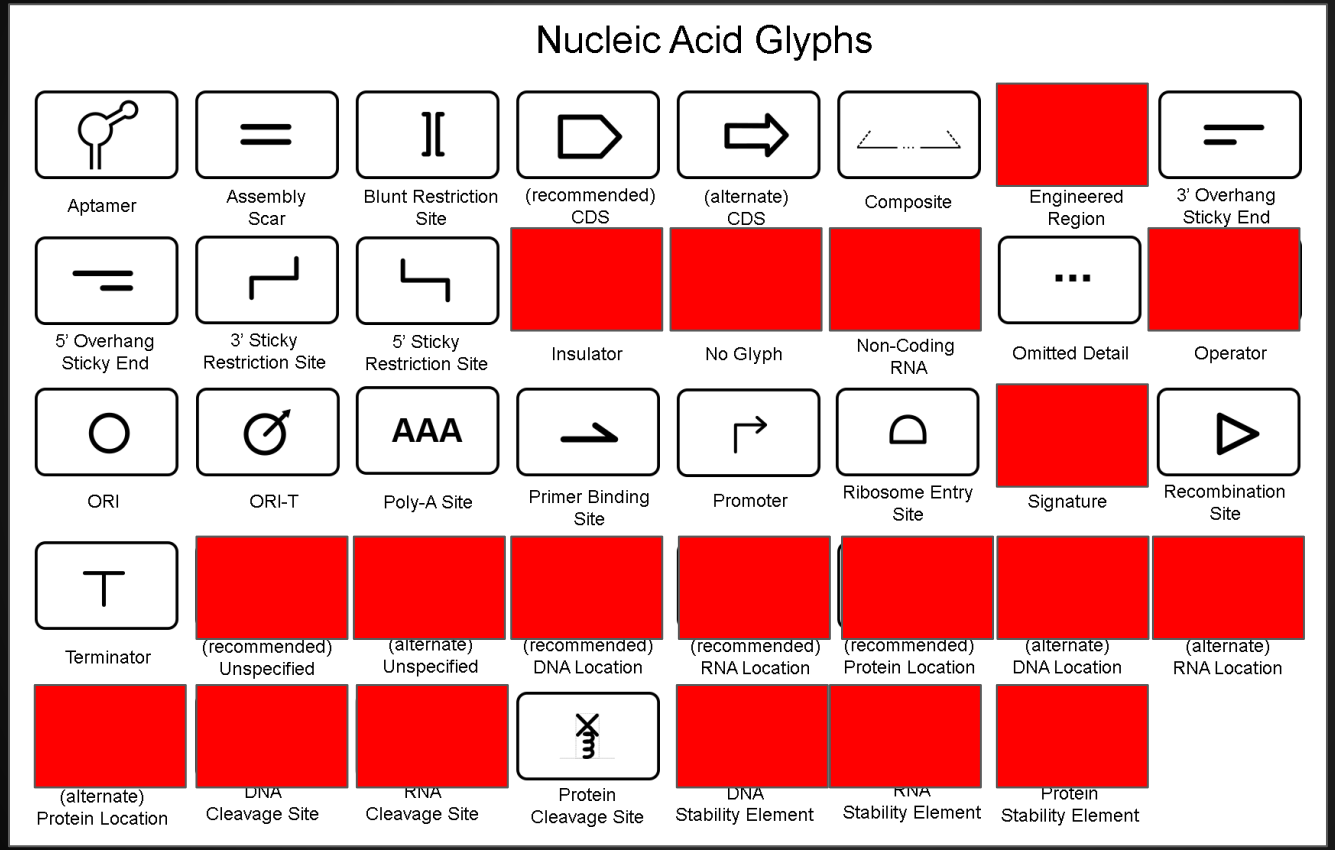
Stop codon

*The exact details vary slightly between bacteria/animals/plants
**This example uses no introns or regulatory elements

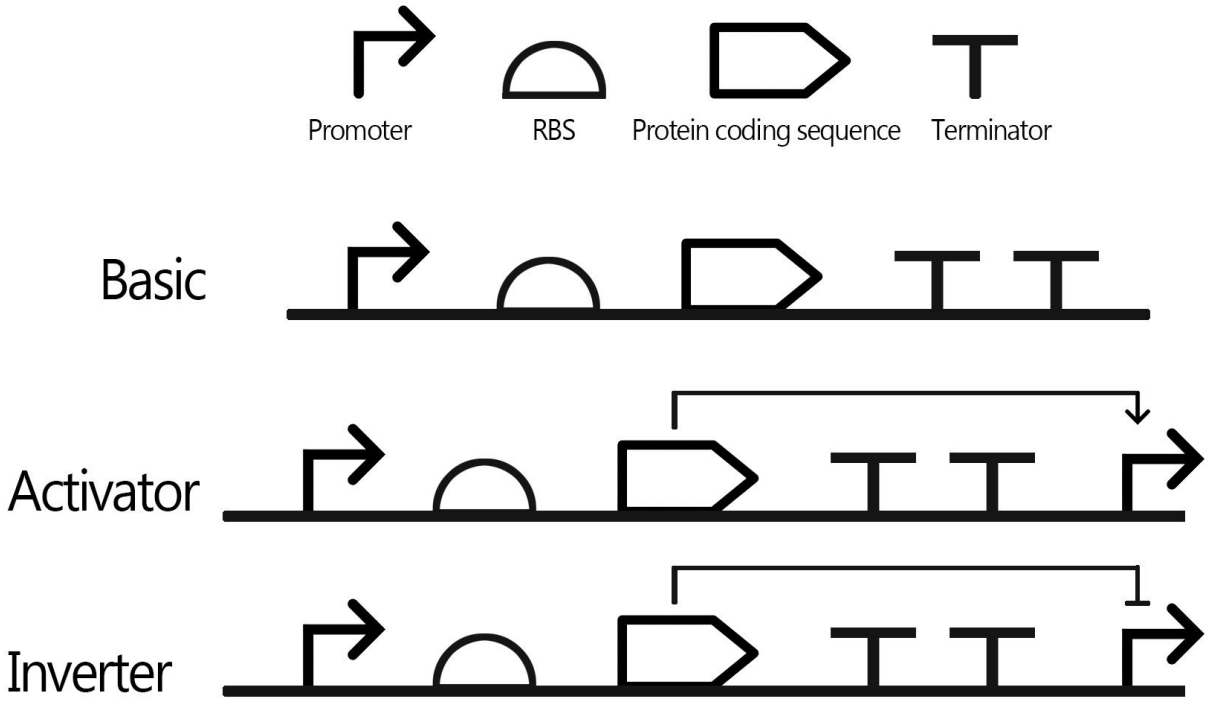
Gene notation



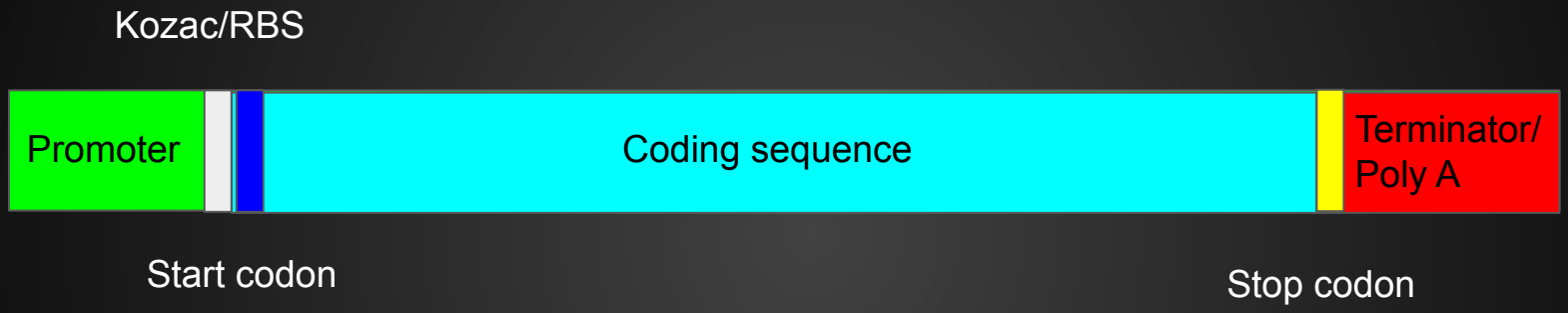
Gene notation: actually useful ones I've seen used



Basic Gene Notation Example

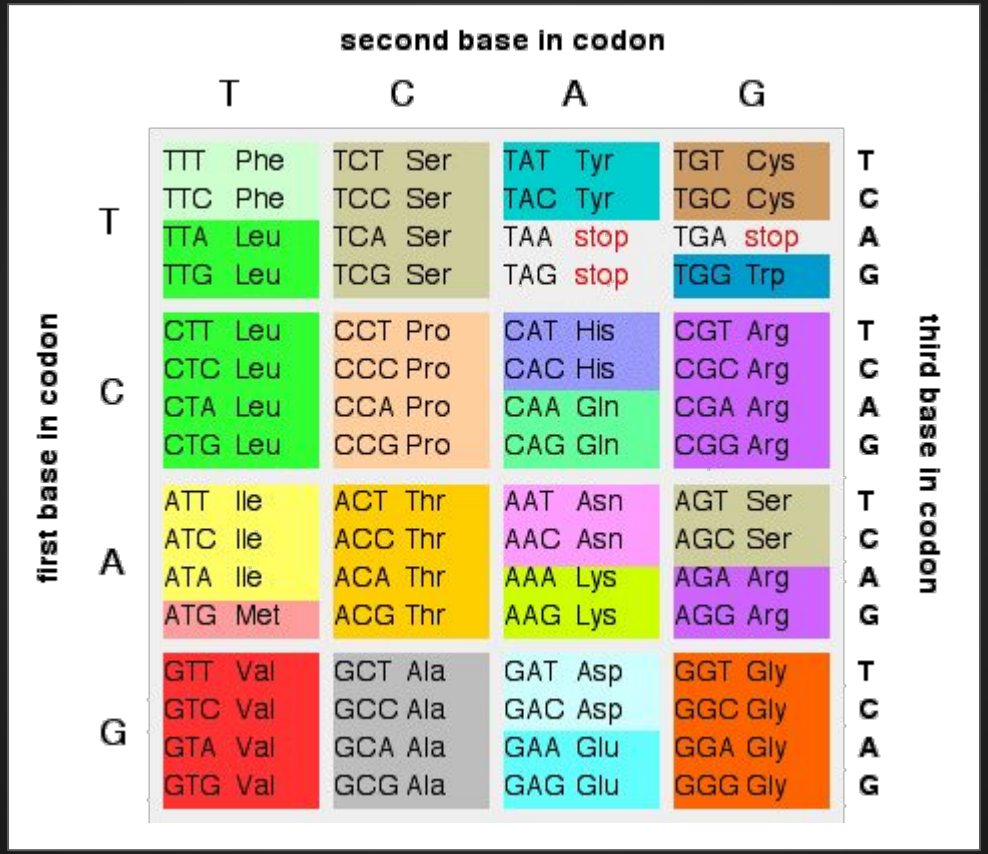


A Basic Gene

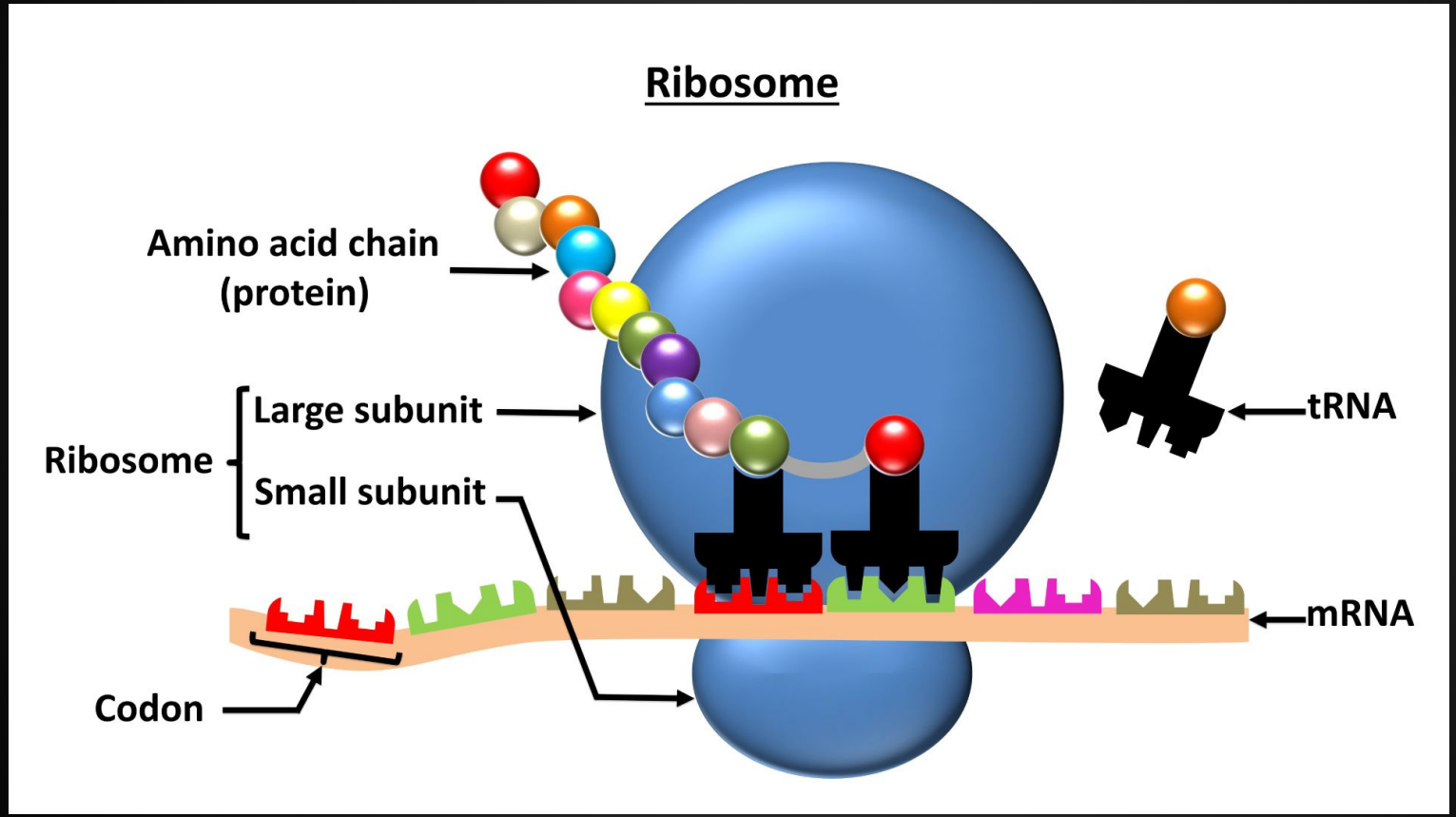


*The exact details vary slightly between bacteria/animals/plants

Genetic code

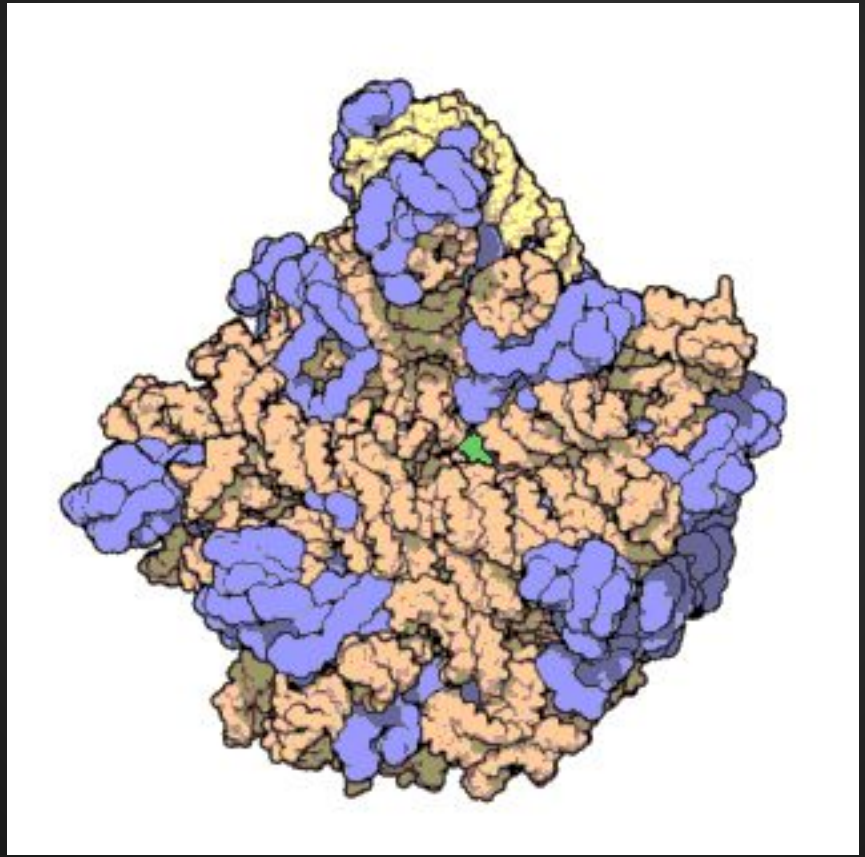


The ribosome

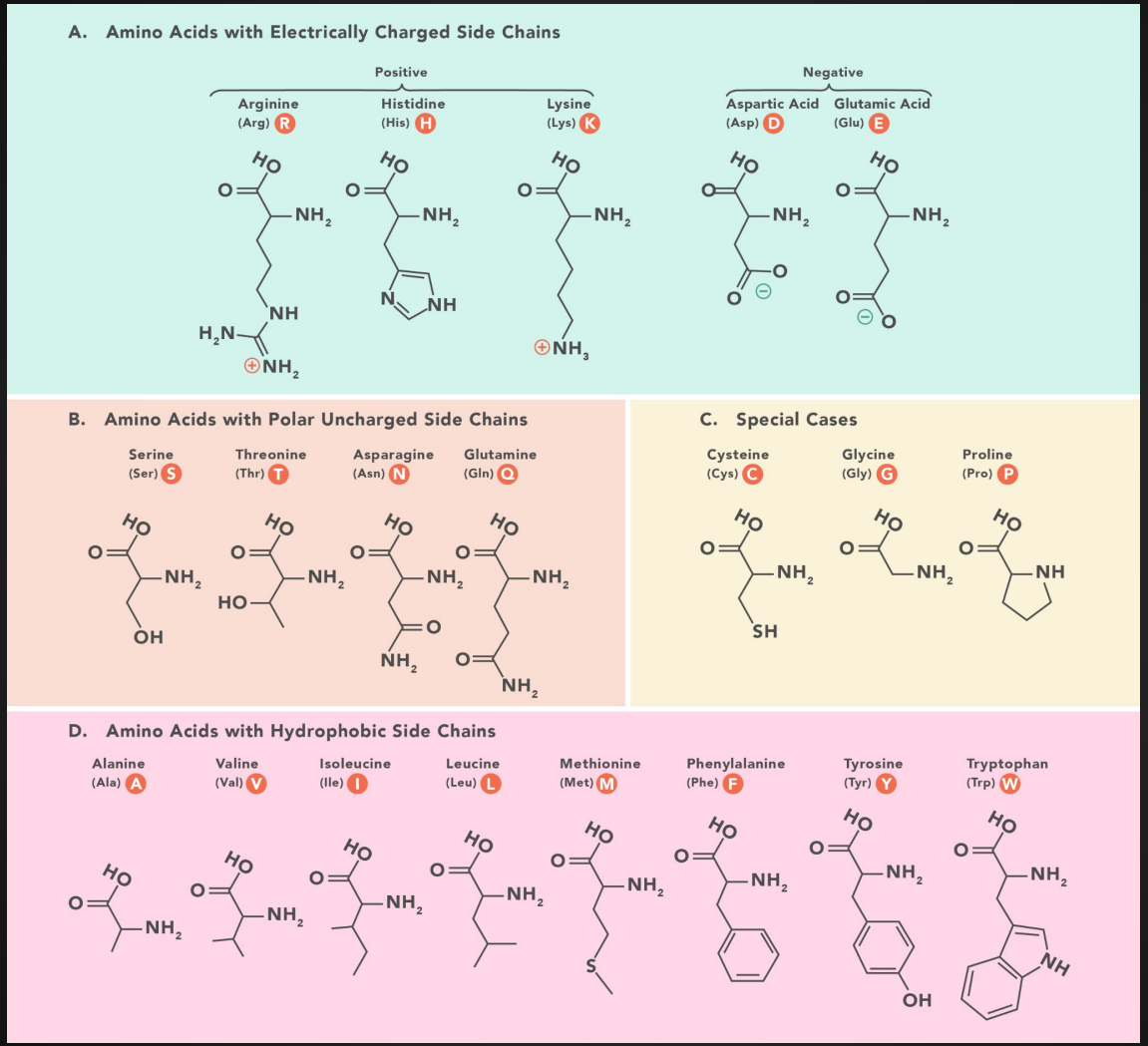


A quick reminder: Cartoons =/= reality

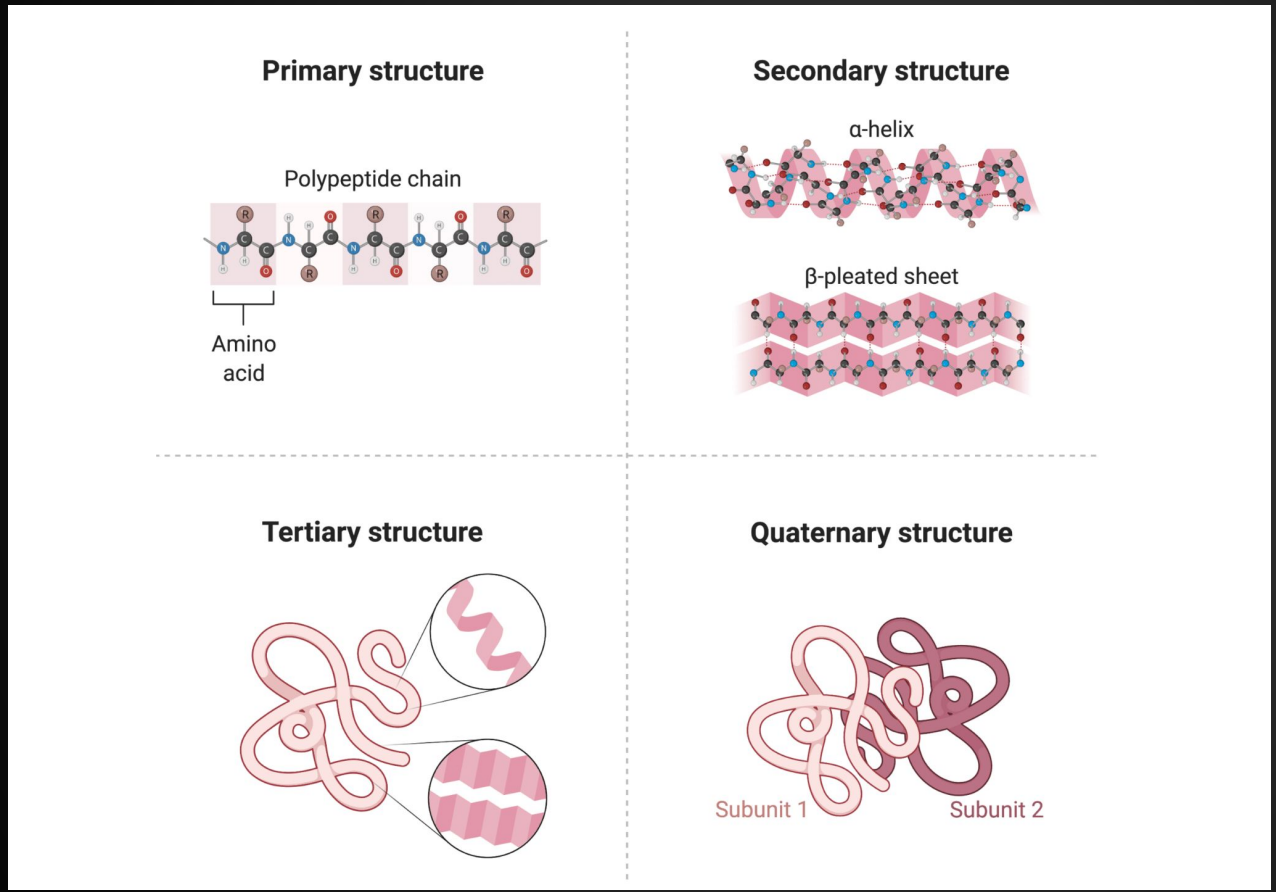
What a ribosome actually looks like



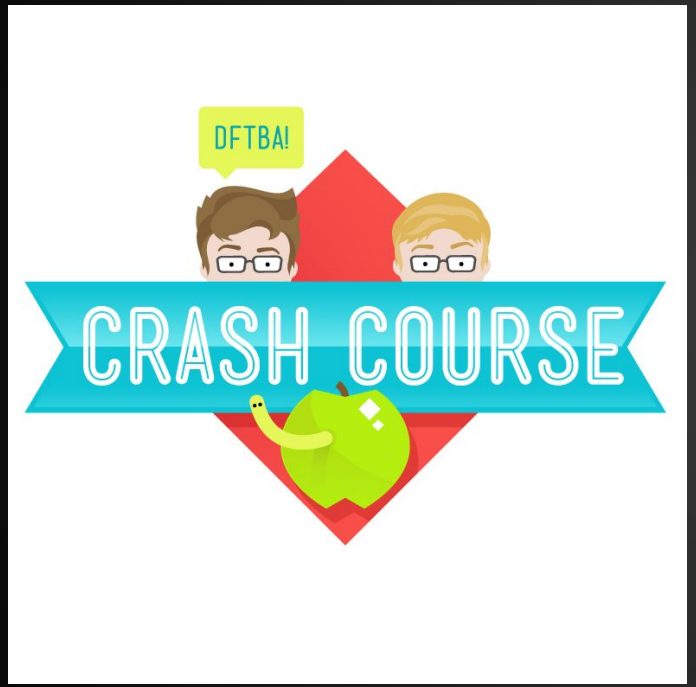
Aminos

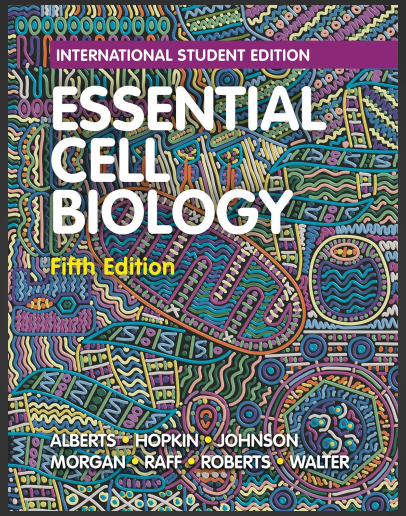

A. Amino Acids with Electrically Charged Side Chains
Positive
Arginine (Arg) R
Histidine (His) H
Lysine (Lys) K
Negative
Aspartic Acid (Asp) D
Glutamic Acid (Glu) E
B. Amino Acids with Polar Uncharged Side Chains
Serine (Ser) S
Threonine (Thr) T
Asparagine (Asn) N
Glutamine (Gln) Q
C. Special Cases
Cysteine (Cys) C
Glycine (Gly) G
Proline (Pro) P
D. Amino Acids with Hydrophobic Side Chains
Alanine (Ala) A
Valine (Val) V
Isoleucine (Ile) I
Leucine (Leu) L
Methionine (Met) M
Phenylalanine (Phe) F
Tyrosine (Tyr) Y
Tryptophan (Trp) W

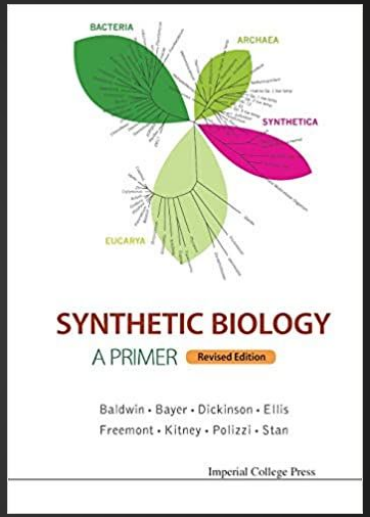
Proteins

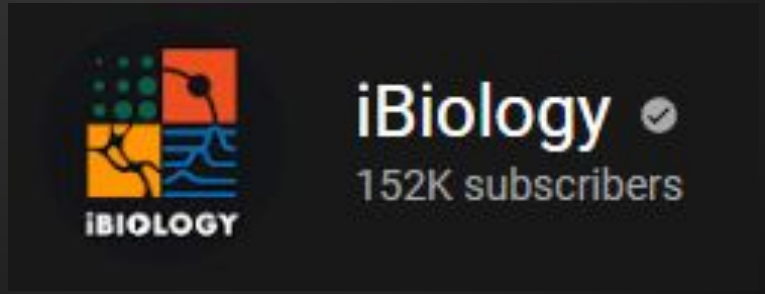

Primary structure
Polypeptide chain
Amino acid
Secondary structure
α-helix
β-pleated sheet
Tertiary structure
Quaternary structure
Subunit 1
Subunit 2

For more detail on the basics, see these resources:









Part 2: Plasmids

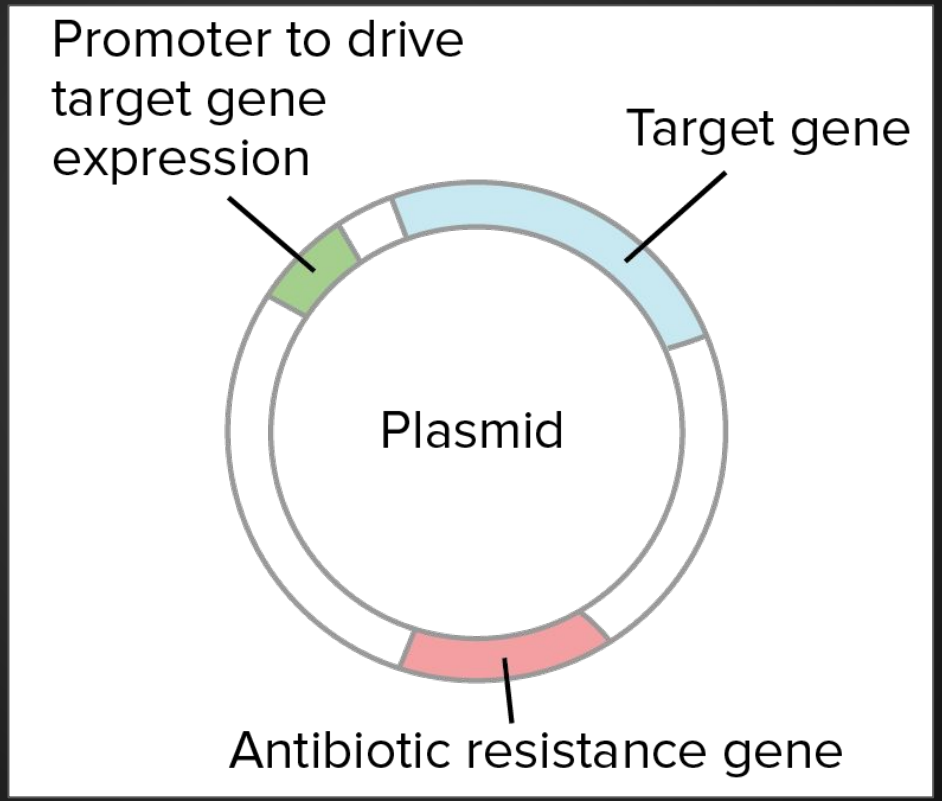
Plasmids: the most basic genetic tool

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 goinng 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

addgene

iGEM

Part 3: Enough Talk

It’s time to cook.