1. Given the following segment of DNA, identify the various components and processes involved in the translation from DNA to protein.
   DNA coding sequence: ATGGCCGTAGGCTAAAAGGGTGCCCGATAG
   a) Identify the coding and template strands and write them down.
      The coding strand is provided:
      5' - ATGGCCGTAGGCTAAAAGGGTGCCCGATAG - 3'

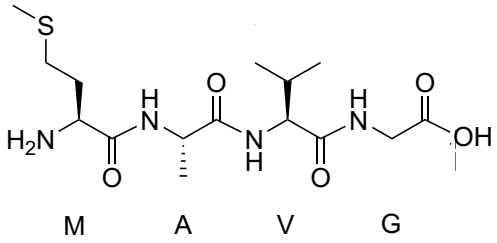
      The template strand (complementary to the coding strand) is:
      3' - TACCGGCATCCGATTTTCCCACGGGCTATC - 5'

   b) Write down the mRNA sequence transcribed from the template strand. Indicate the directionality of the strand.
      The mRNA sequence transcribed from the template strand is:
      5' - AUGGCCGUAGGCUAAAAGGGUGCCCGAUAG - 3'

   c) List the sequence of codons in the mRNA and translate the codons into the corresponding amino acids using the genetic code chart.
      AUG (Methionine), GCC (Alanine), GUA (Valine), GGC (Glycine), UAA (**Stop**), AAG (Lysine), GGU (Glycine), GCC (Alanine), CGA (Arginine), UAG (**Stop**)

   d) Write down the final protein sequence in single letter code in the direction of translation and comment the potential observations.
      The direction of the translation is from N-terminus to C-terminus and thus the protein sequence in that direction would be N-MAVG-C. As UGA yields a stop codon, the translation would end at this point and the rest of the mRNA-sequence would not be translated

   e) Draw the polypeptide chain that would result from this mRNA strand.



2. Given the following list of amino acids, classify them based on their side chain properties:
   - Alanine (Ala)
   - Glutamic Acid (Glu)
   - Serine (Ser)
   - Valine (Val)
   - Lysine (Lys)
   - Tyrosine (Tyr)
   - Leucine (Leu)
   - Arginine (Arg)

   a) Which amino acids in the list have hydrophobic side chains and which amino acids have hydrophilic or charged side chains?

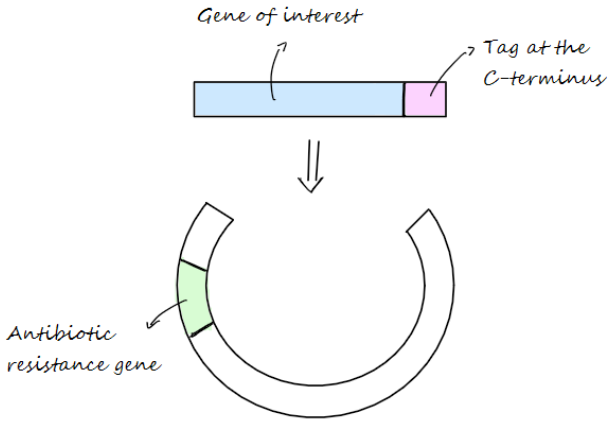
      Hydrophobic side chains: Alanine (Ala), Valine (Val), Leucine (Leu)

      Hydrophilic/Charged side chains: Serine (Ser), Tyrosine (Tyr), Glutamic Acid (Glu) - negative, Lysine (Lys) - positive, Arginine (Arg) – positive

b) Related to the side chain properties of Leucine and Lysine, where would they respectively most likely be found: in the transmembrane region of a cell or on the surface of a cytoplasmic protein?
Leucine, which contains a hydrophobic side chain, interact favorably with the hydrophobic core of the lipid bilayer and will therefore most likely be founds in the transmembrane region of a cell.
Lysine, which contains hydrophilic side chain, are more likely to interact with the aqueous cytosolic environment such as on the surface of a cytoplasmic protein.

3. What does the following DNA-sequence encode:
5'-CACCACCATCACCATCAC-3'
6xHis tag
a) If we now want to couple such a tag to a protein of interest, how would you do this? What is this specific peptide chain used for in a research setting?
Either add the tag at the N-terminus or C-terminus of the DNA fragment that encodes for the protein of interest. Then clone this DNA fragment into a plasmid to infect the cells (E.coli) for protein expression. The His tag is used for the purification of protein using affinity chromatography.

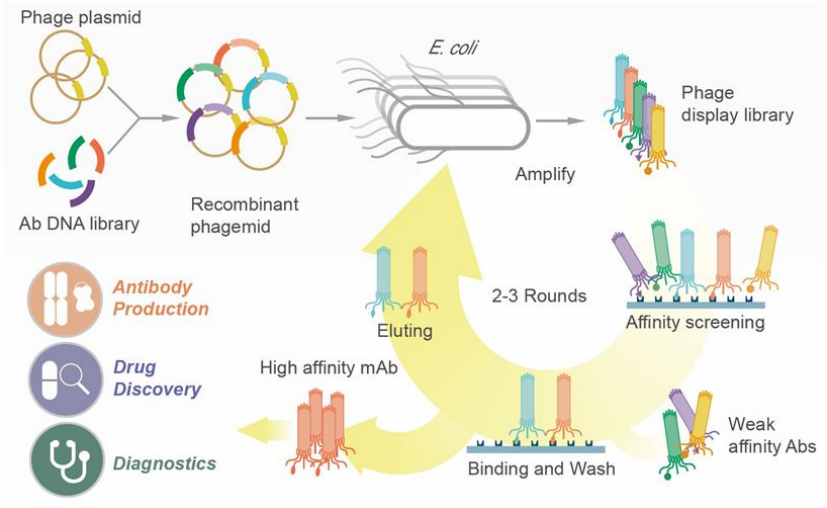
b) Please draw a plasmid and annotate the components in your cartoon.



Blue: Gene of interest
Pink: TAG
Green: Antibiotic-resistant gene
The plasmid needs to be modified to include the gene that encodes the specific protein of interest (BLUE). If we now want to modify the protein to include a tag such as a His-tag, we can easily filter out the protein of interest using a divalent metal ion such as nickel cation (Ni^{2+}) that is a very strong binding partner for the His-tag.

c) When identifying strong binding partners (e.g. tags) for a specific target, which technique is often used? Below is a schematic illustration of this technique, please fill in the gray boxes with the steps or components they refer to.

Phage display.



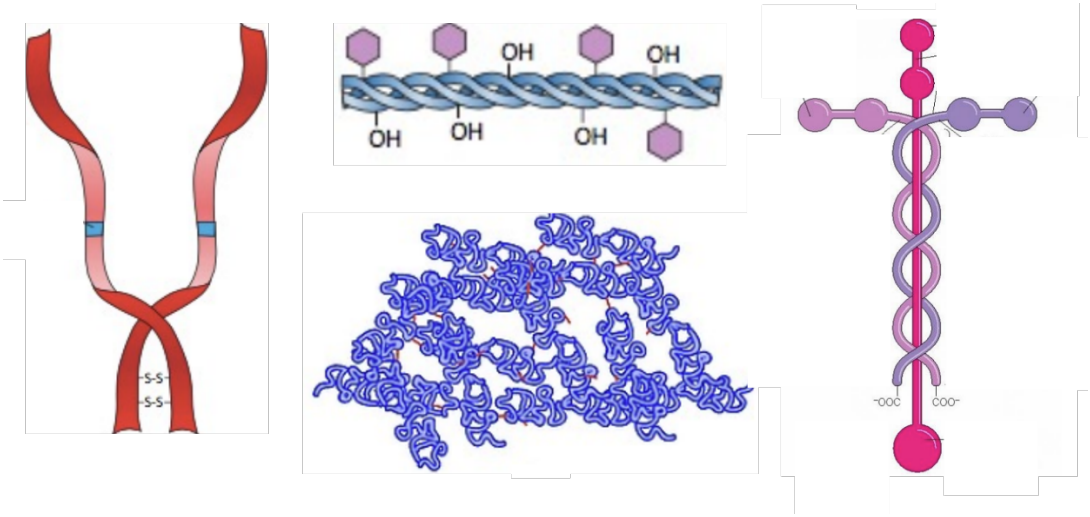
4. Draw a DNA origami scaffold in the shape

a) What is the most important feature that the DNA origami scaffold always has?
A long, single-stranded DNA, and a closed circuit.

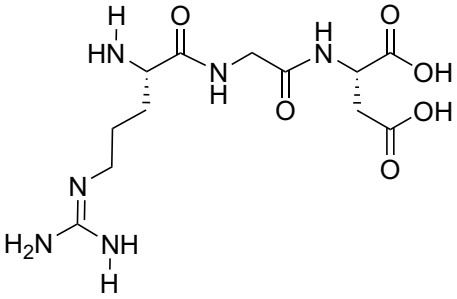
b) If I would like to functionalize a protein onto the surface of my DNA origami nanoparticle, how would I do this? Annotate on the DNA origami scaffold.
Have an extended staple strand (so called handle), that protrudes from the surface of the origami. Couple a strand complementary to this handle overhang (so called anti-handle) to the protein of interest (e.g. via click chemistry) and then let these two strands hybridize to immobilize the protein on the surface of the particle.

c) Assume that the sequence of the strand that you annotate in 4b) is: 5'-GGCTGAAAATCTCCTGACATG-3'. Please give the sequence of the DNA strand that should be conjugated to the protein.
5'-CATGTCAGGAGATTTTCAGCC-3'.

5. Which of the following 4 ECM-proteins are involved in the connection between cells and the extra cellular matrix (ECM)?
Fibronectin on the left, and Laminin on the right.



a) Your cell of interest will connect to a particular 3 amino acid motif within this ECM protein using its integrin receptors. Please annotate the amino acids in the structure below in single letter code.



N-RGD-C

b) Please give a potential mRNA-sequence for this motif in 5’ to 3’ direction.
5’-CGU GGU GAU-3’

c) Please give the coding and template strand of this mRNA both in 5’ to 3’ direction.
Coding strand: 5’-CGT GGT GAT-3’
Template strand: 5’-ATC ACC ACG-3’

6. Ligand and receptors
You are given data from an experiment where a ligand interacts with a receptor.

	Ligand concentration [L] (nM)	Receptor-ligand complex [LR] (nM)
Timepoint 1	10	8
Timepoint 2	20	15
Timepoint 3	50	30
Timepoint 4	80	30

a) Use the provided experimental data to calculate the Kd with the following formula:
[R] = [LR] = 50% of max [LR]
Kd = ([L] * [R]) / [LR]
You use the concentrations of the components at timepoint 2 a there 50% of the receptors on the cells are bound by the ligands. This you know, as the complex of ligand and receptor [LR] saturates at 30 nM.

[LR] = 15 nM → Can directly be taken from above
[L] = 20 nM → Can directly be taken from above
[R] = [LR] = 15 nM

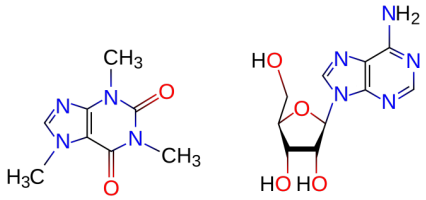
Kd = ([L] * [R]) / [LR]
Kd = (20 nM * 15 nM) / 15 nM = 20 nM

b) Use the provided data to calculate the Ka.
Ka = 1/Kd = 1 / 20 nM = $0.05\ nM^{-1}$

c) Would you consider the ligand and receptor strong binders, based on the Kd that you calculated?
20 nM is a mediocre Kd and thus it would be quite a good binder, but not too strong. There are binders with Kds down into the pM-range, these would be very strong ones.

d) I want to functionalize this ligand on a nanoparticle to target the receptor. Would it be necessary to functionalize many ligands on the nanoparticle, and therefore leverage multivalent binding to ensure a stable interaction between the ligand and receptor? Why?
In that case it would be good to have several binding units to ensure strong association of the nanoparticle with the target. The association strength and thus also the Kd should scale with the number of binding units until a certain point (at some point saturation would be reached, as for example not more receptors would be present on a given surface of the target).

e) During the lecture you heard about adenosine, a natural molecule in our body. Naturally, it binds to adenosine-receptors and makes us feel tired. Often, we drink coffee to feel more awake and alert. Caffeine, a component of coffee, is a ligand for the adenosine-receptor. Based on the effect, is caffeine an agonist or antagonist for the adenosine receptor? Explain why?

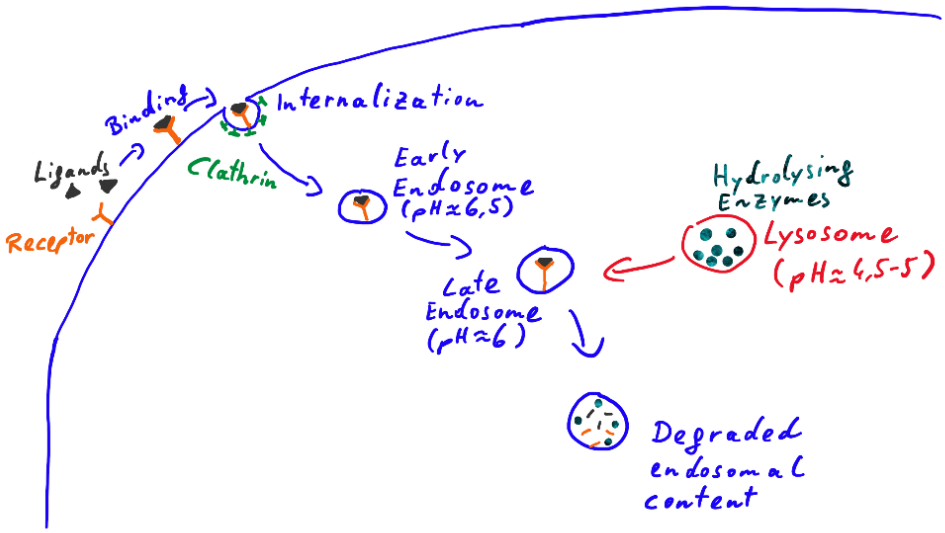


Caféine Adénosine

An Antagonist. Since naturally, when the receptor is active, the result is that we feel tired. With Caffeine bound to the receptor, we do not feel tired, so the natural pathway is blocked. This is the function of an antagonist.

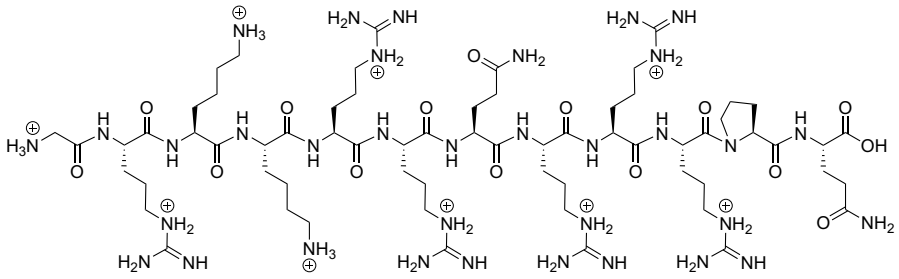
7. The following pathway is very important in the context of many therapeutic applications.

a. Please annotated the differently colored components.



b. During the whole process the pH of the vesicle is changing. Please indicate the trend and why this is important.
Over time the endosomal content is acidifying, and this allows for the activation of the hydrolyzing enzymes, that are contained in the lysosome and added to the endosome in the late stage by fusion of the two vesicles.

c. Nanoparticles of interest can be fused to the following peptide to allow for their escape from the endosome. Please name this peptide and give the amino acid sequence in single letter code.



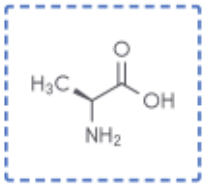
N-GRKKRRQRRRPQ-C

d. Another peptide with similar function has the single letter code N-RRRRRRRR-C. What do these two peptides have in common, that help them carry out the function?
The high positive charge of both sequences, that is helping with the destabilization of the membrane on the outside of the endosomal vesicle.

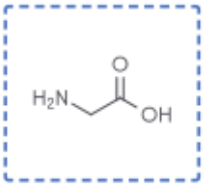
Chart Key

Alkyl | Aromatic | Neutral | Acidic | Basic | Essential | Non-Essential

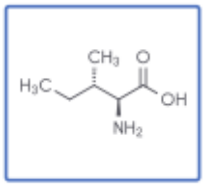
Note: The NH_2 and COOH values listed below are pK_a values.



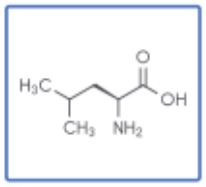
Alanine
Ala A
NH_2: 9.87 COOH: 2.35



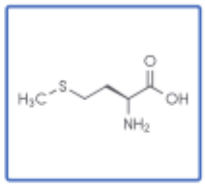
Glycine
Gly G
NH_2: 9.60 COOH: 2.34



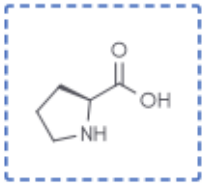
Isoleucine
Ile I
NH_2: 9.76 COOH: 2.32



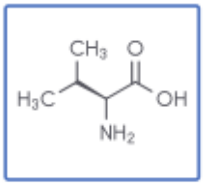
Leucine
Leu L
NH_2: 9.60 COOH: 2.36



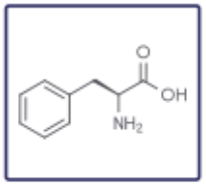
Methionine
Met M
NH_2: 9.21 COOH: 2.28



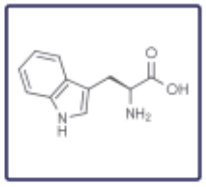
Proline
Pro P
NH_2: 10.60 COOH: 1.99



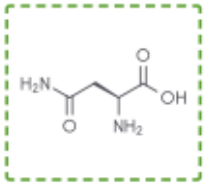
Valine
Val V
NH_2: 9.72 COOH: 2.29



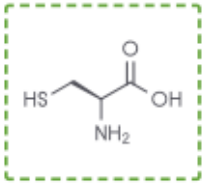
Phenylalanine
Phe F
NH_2: 9.24 COOH: 2.58



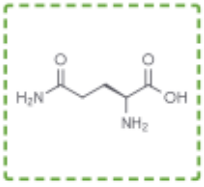
Tryptophan
Trp W
NH_2: 9.39 COOH: 2.38



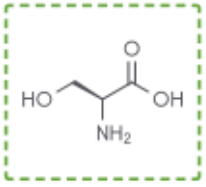
Asparagine
Asn N
NH_2: 8.80 COOH: 2.02



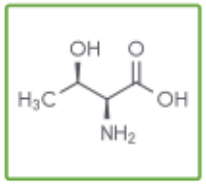
Cysteine
Cys C
NH_2: 10.78 COOH: 1.71



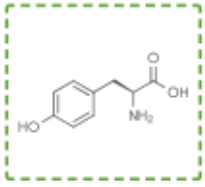
Glutamine
Gln Q
NH_2: 9.13 COOH: 2.17



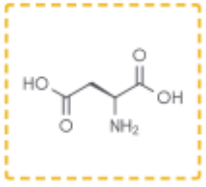
Serine
Ser S
NH_2: 9.15 COOH: 2.21



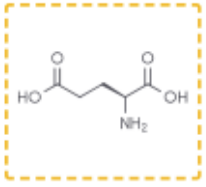
Threonine
Thr T
NH_2: 9.12 COOH: 2.15



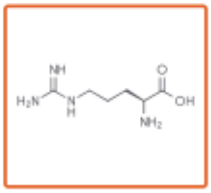
Tyrosine
Tyr Y
NH_2: 9.11 COOH: 2.20



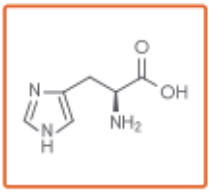
Aspartic Acid
Asp D
NH_2: 9.60 COOH: 1.88



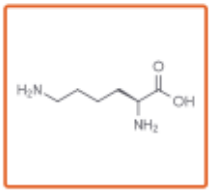
Glutamic Acid
Glu E
NH_2: 9.67 COOH: 2.19



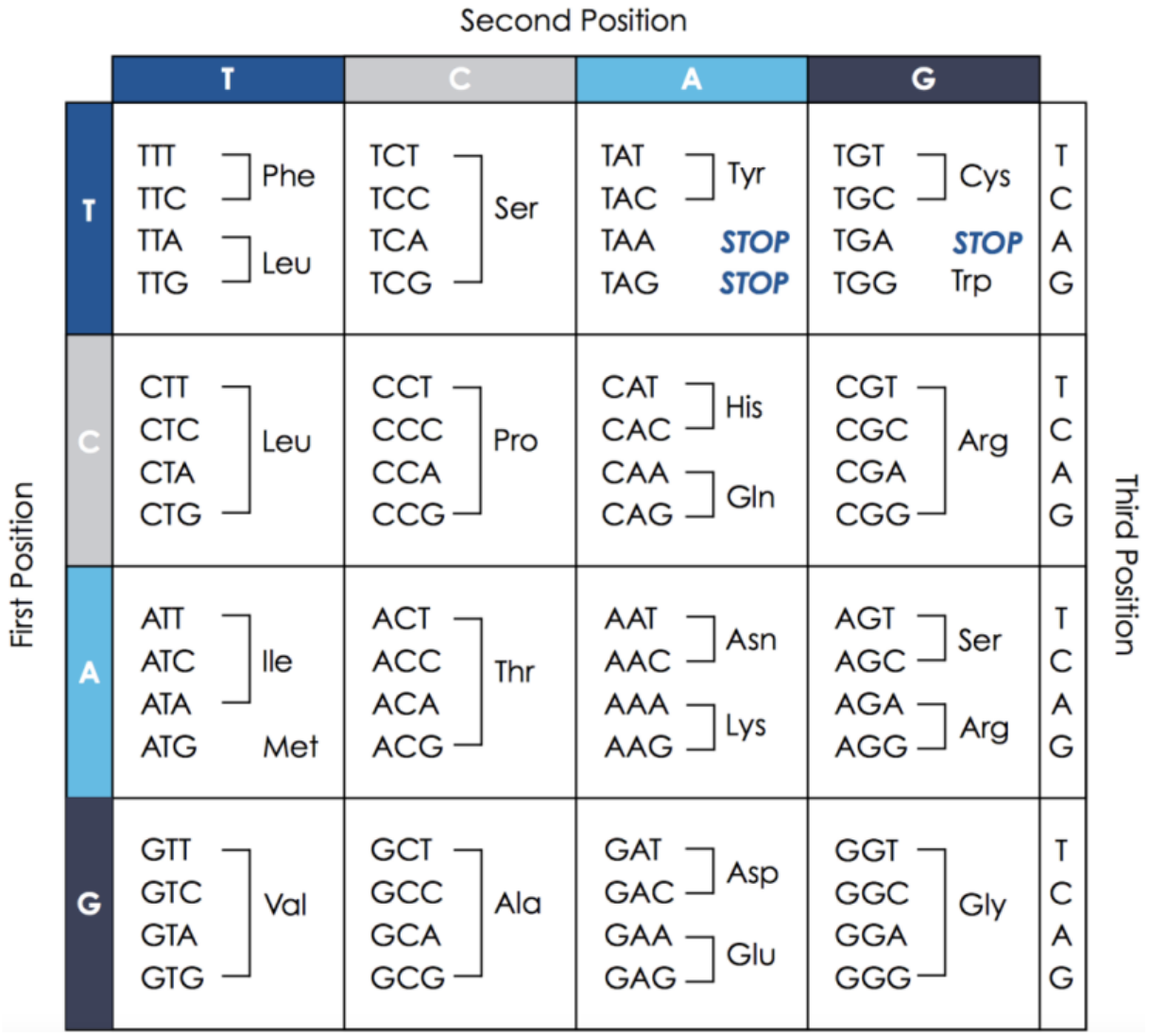
Arginine
Arg R
NH_2: 9.09 COOH: 2.18



Histidine
His H
NH_2: 8.97 COOH: 1.78



Lysine
Lys K
NH_2: 10.28 COOH: 8.90


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