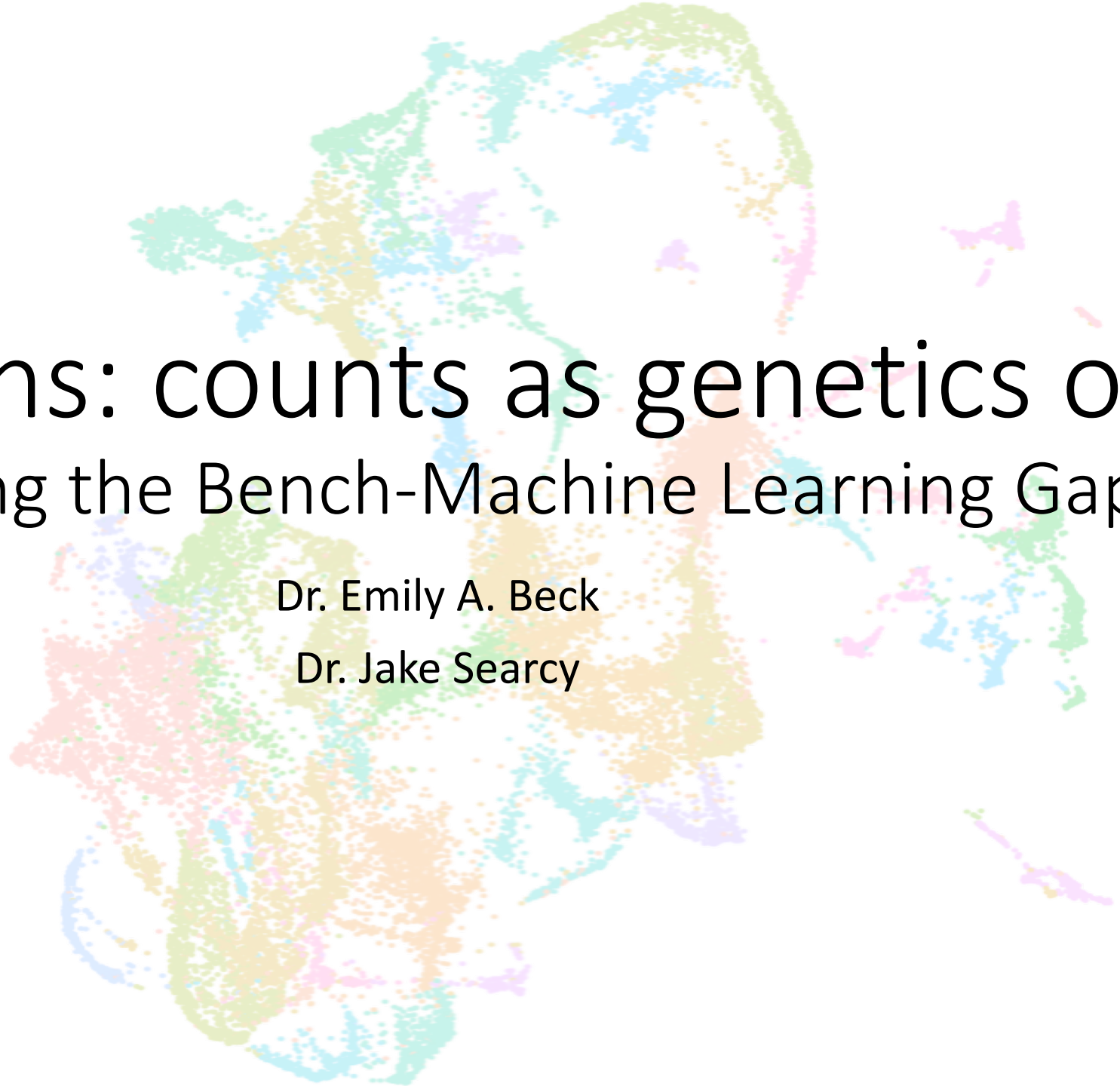


Proteins: counts as genetics ok?

Bridging the Bench-Machine Learning Gap

Dr. Emily A. Beck

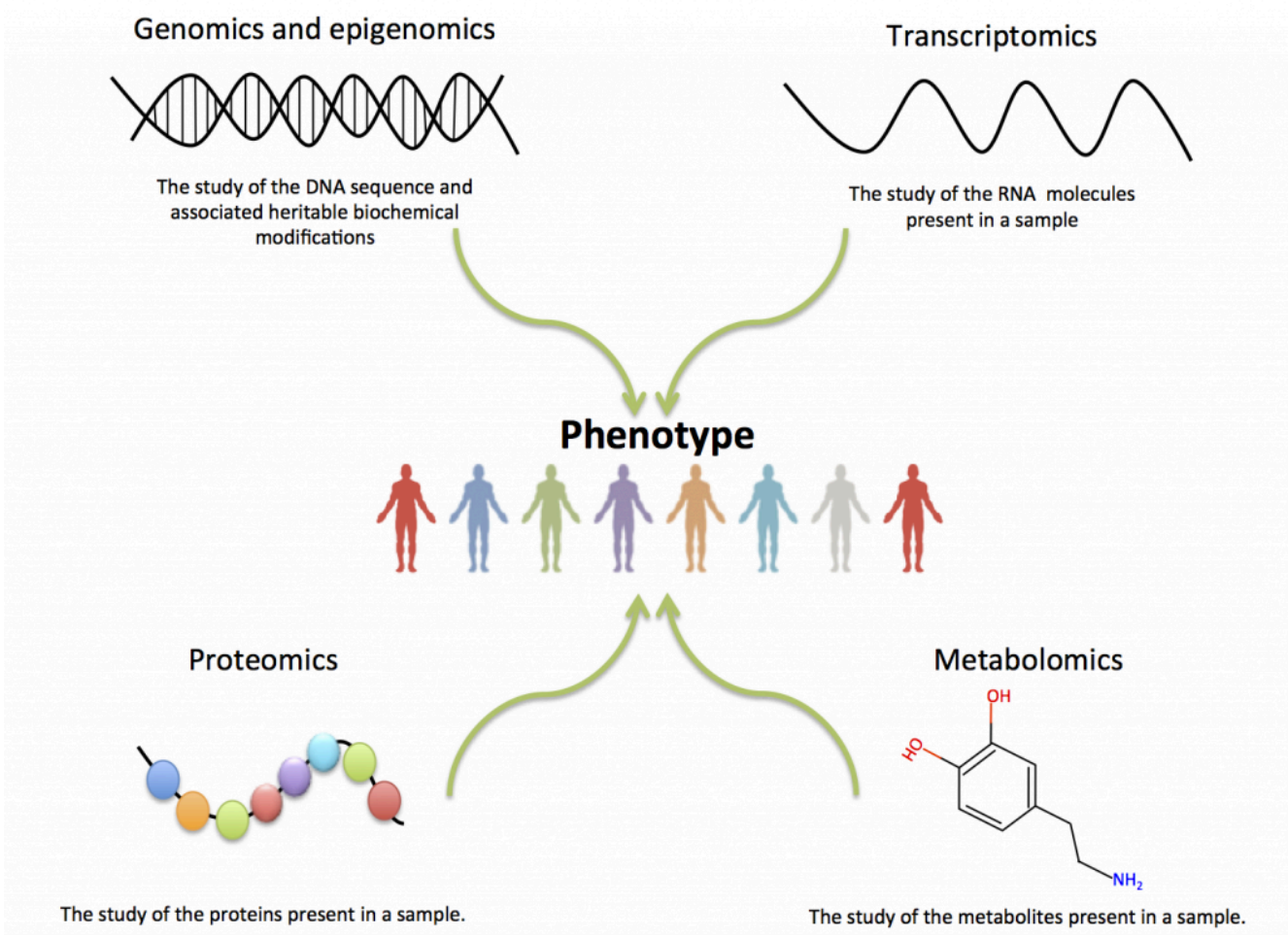
Dr. Jake Searcy

Learning Objectives

Learn the basics of protein structures and why ML is needed
Understand basic structures of protein sequencing files
Learn about the cool things you can do with single amino acid sequences
Learn about cool things you can do with multiple alignments of amino acid seq

After the break we will learn to make a multiple alignment file via command line, run software assessing amino acid changes for deleterious effects on function, predict structure changes in our proteins

Many questions in genomics are trying to get at biological significance of mutations



We have spent a lot of time focusing on SNPs in DNA/RNA.

How those impact amino acids and protein structure is a fundamental question in biology.

There is a suite of questions biologists and biochemists have struggled to answer

Does this mutation cause a change in the amino acid sequence of a protein?

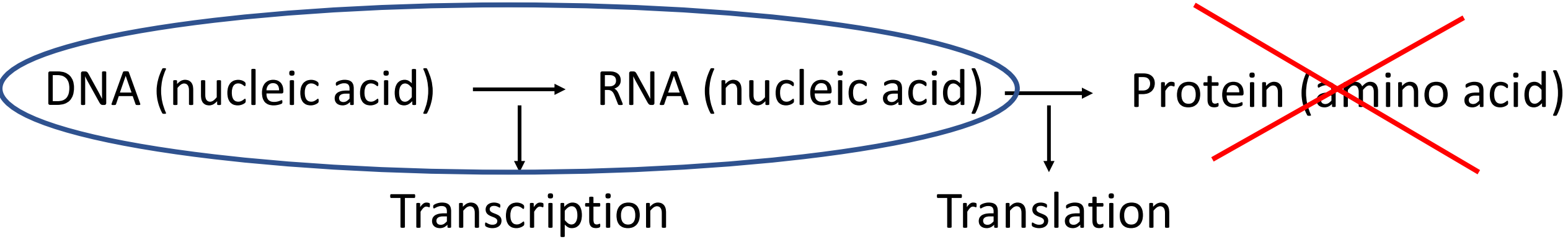
How different are the amino acid changes?

Do the amino acid changes impact the function of my protein?

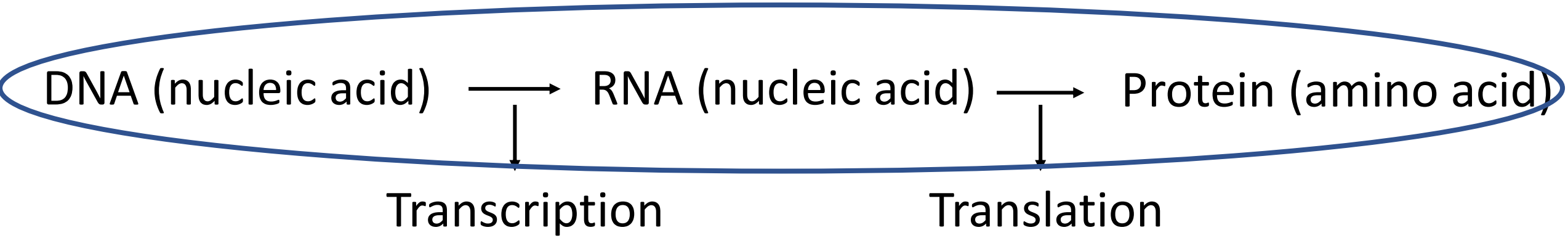
Do the amino acid changes impact the structure of my protein?

Synonymous or Silent mutations can occur in noncoding regions and have no impact on protein sequence

Synonymous changes can also occur within protein coding regions but do not result in an amino acid change

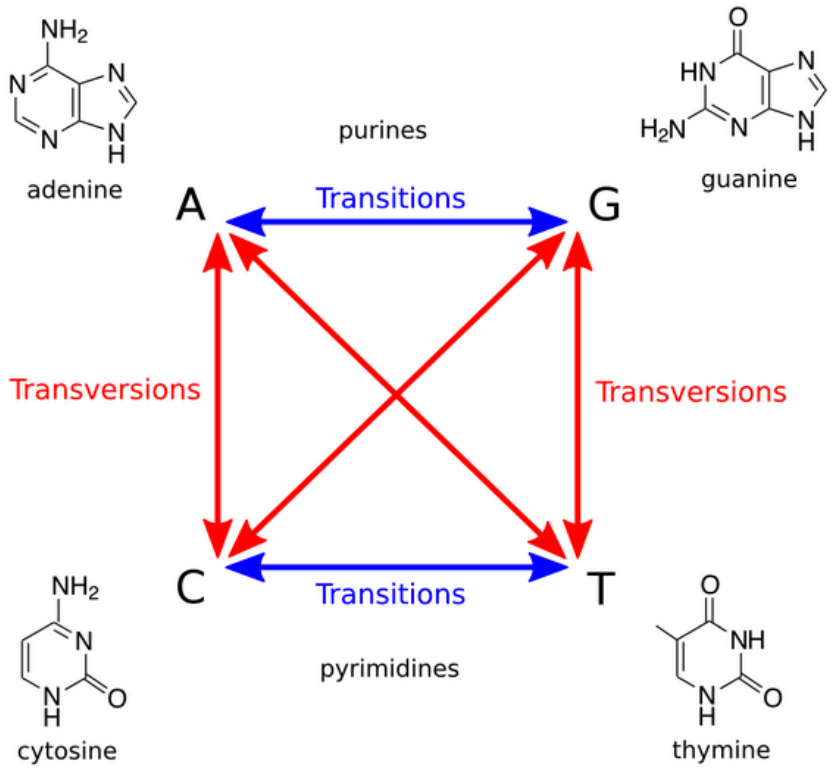


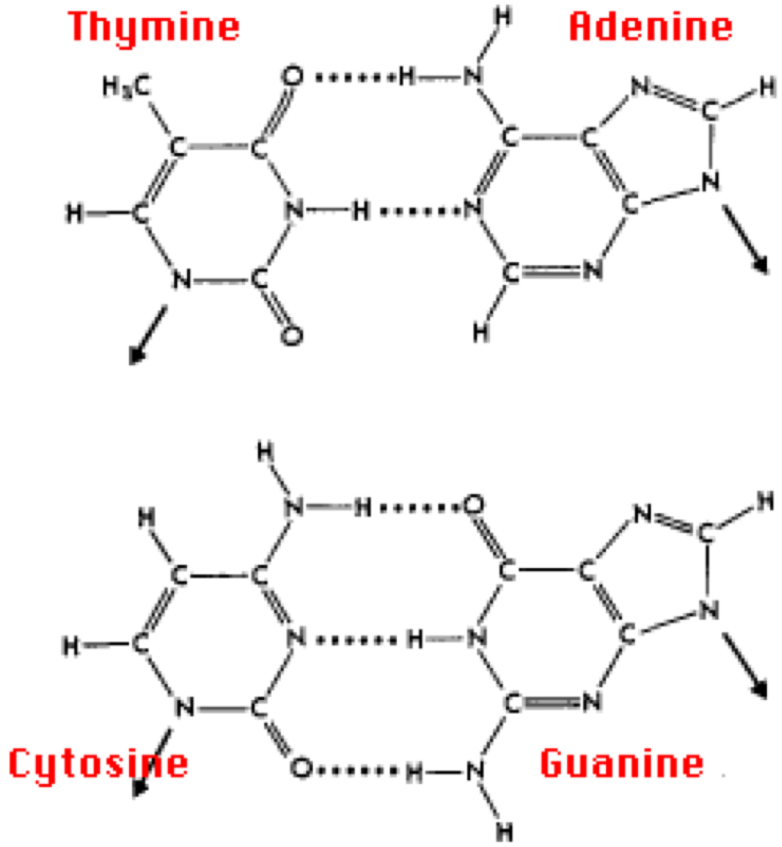
Nonsynonymous changes (replacement changes) persist through translation and a different amino acid is used



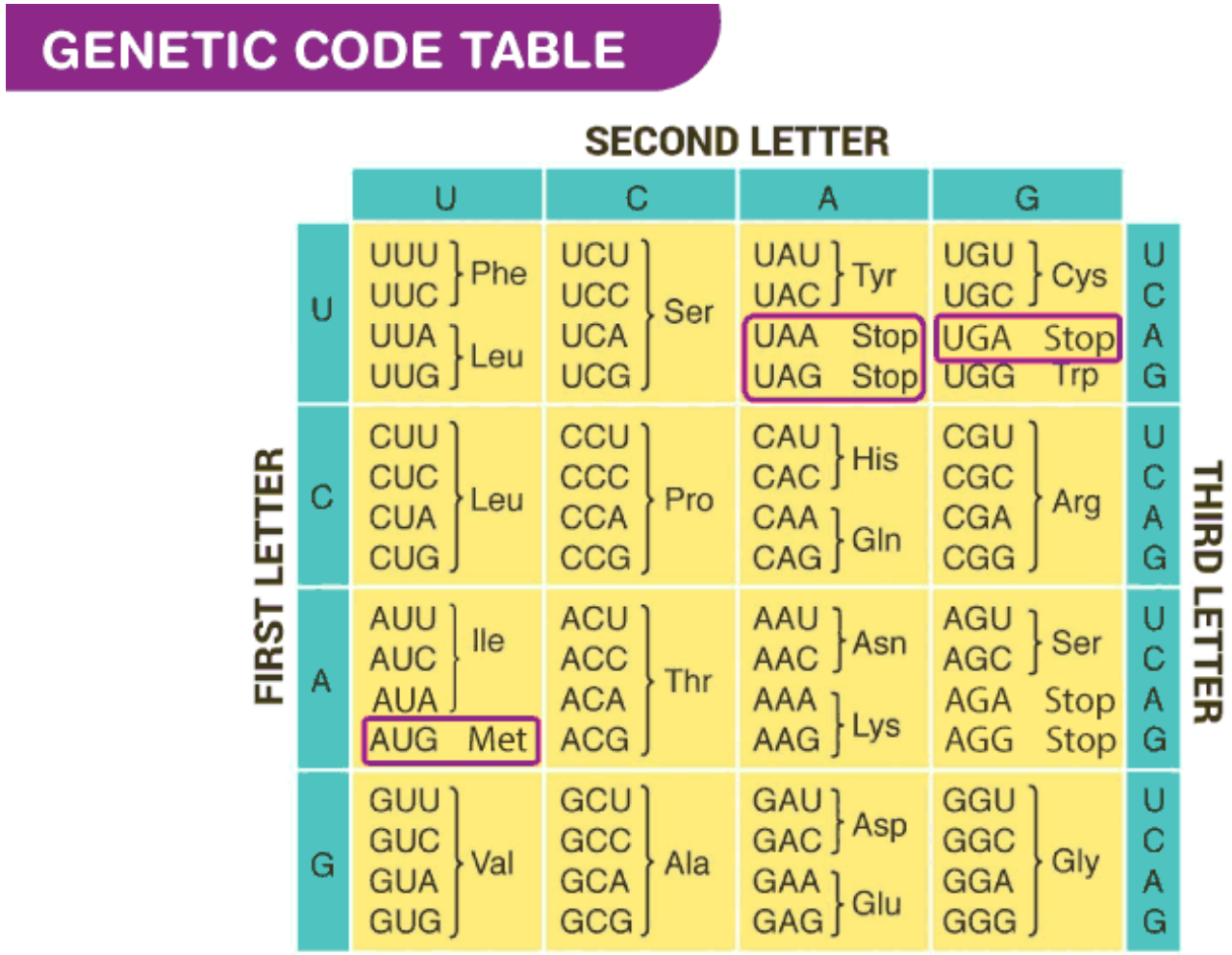
Take a quick step back to DNA

There are two classes of DNA mutations **Transitions** and **Transversions**

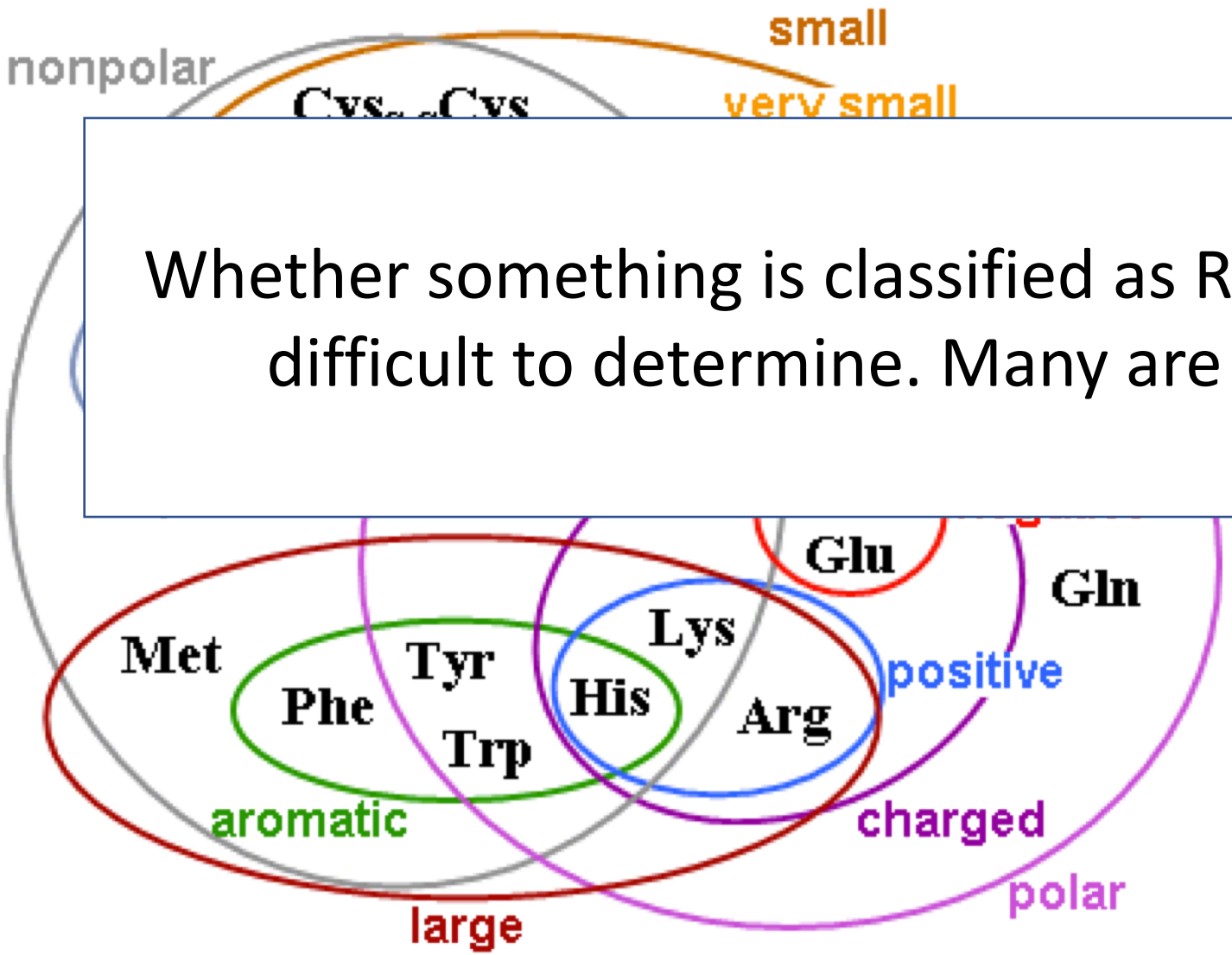




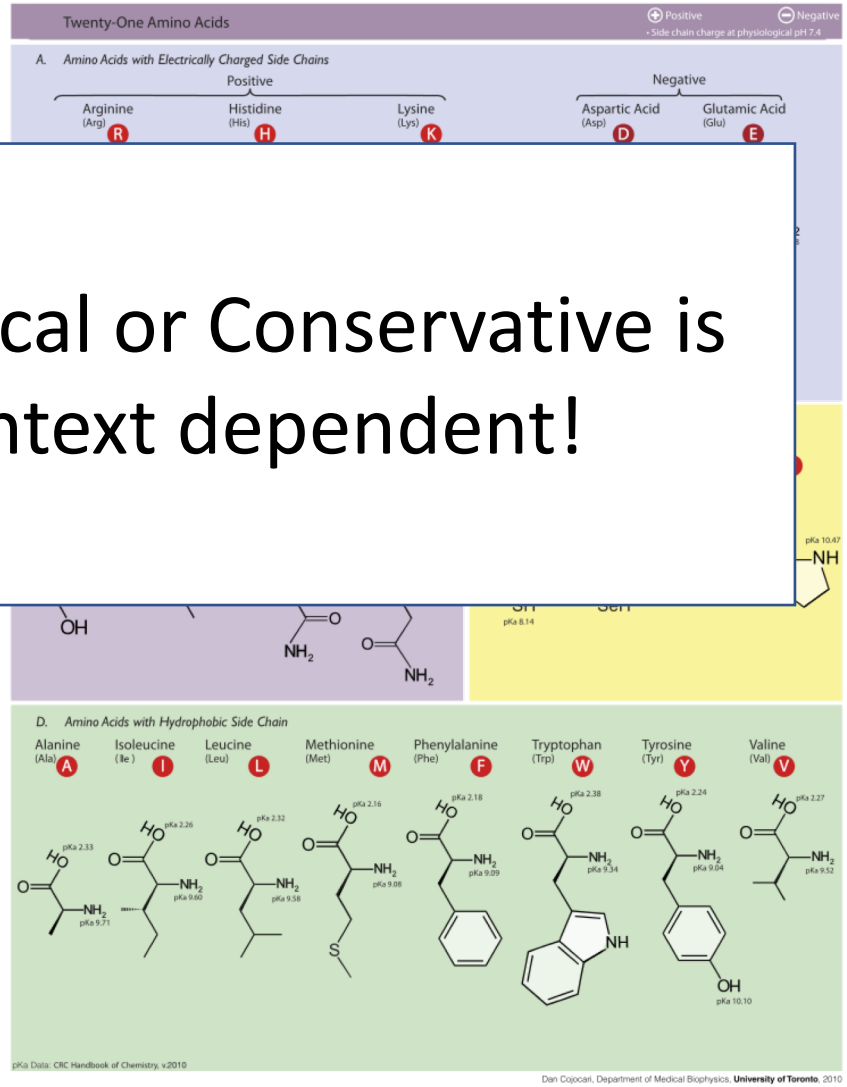
Transversions are more likely to impact amino acid sequences



Within Nonsynonymous changes there are two groups **Radical** and **Conservative** changes.



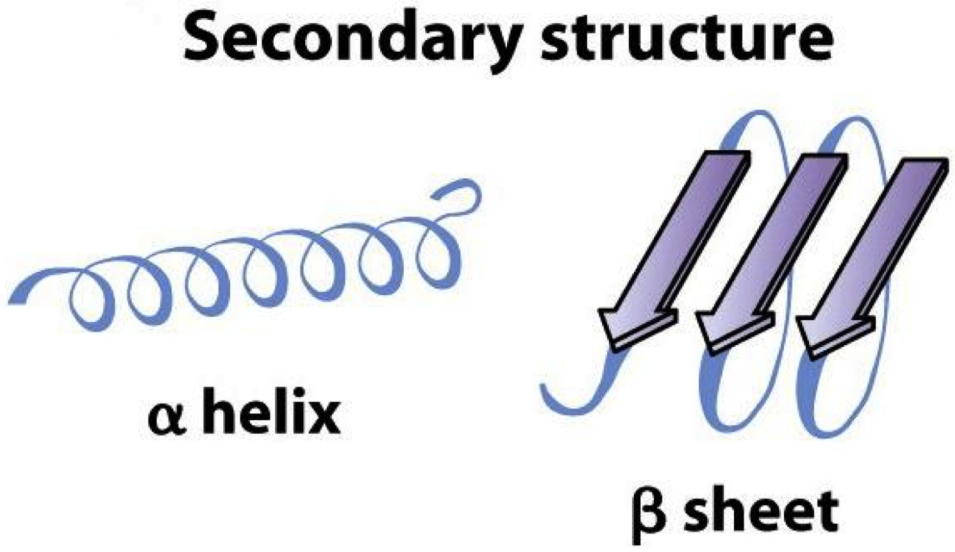
Whether something is classified as Radical or Conservative is difficult to determine. Many are context dependent!

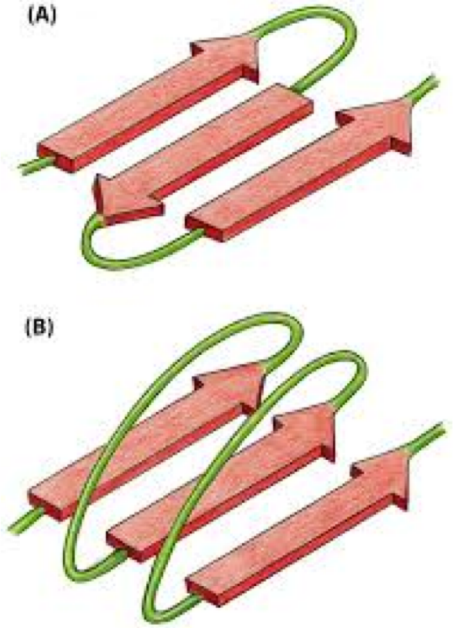


There are a lot of software packages to help you assess the impacts of nonsynonymous changes.

Primary structure: Changes in amino acid sequences

Secondary structure: Changes in amino acid sequences

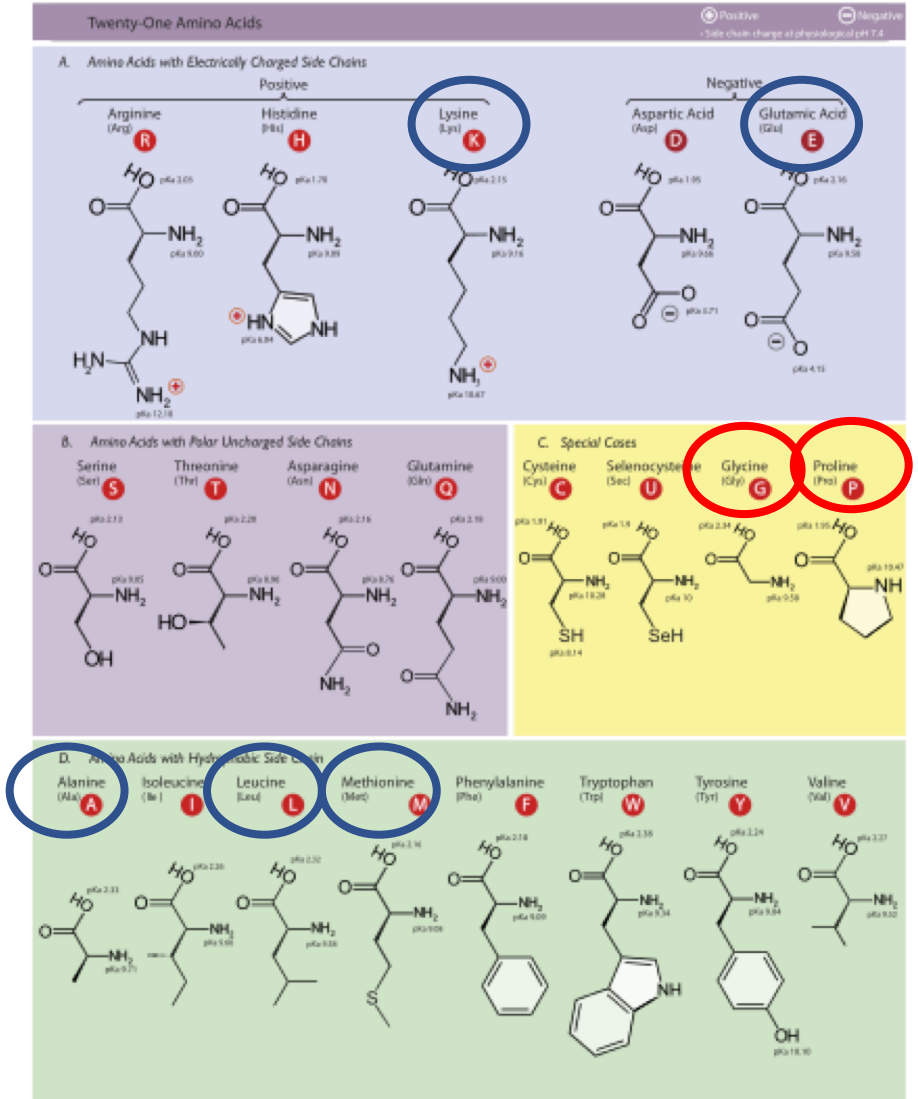




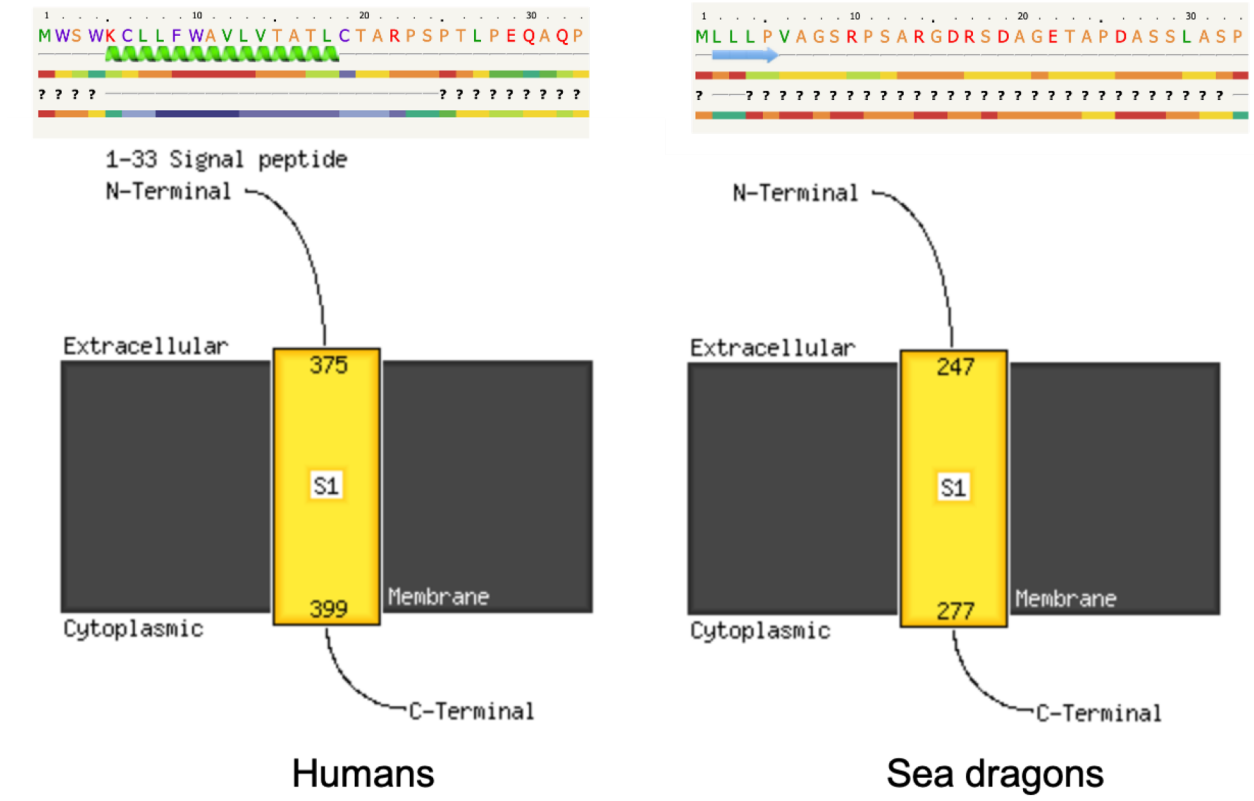
Some amino acids have properties that favor alpha helices or Beta pleated sheets

"MALEK" Amino acids readily form Alpha helices

Glycine and Proline DO NOT work well in alpha helices



Using Secondary structure predictions you can also assess impacts on alpha helices, transmembrane domains, beta sheets etc.



You can also compare sequences to assess impact of mutations.

Example: A SNP that determines sex

HHTRANSMEMBRANEDOMAINSEQUENCE**RR**
HHTRANSMEMBRANEDOMAINSEQUENCE**RR**
HHTRANSMEMBRANEDOMAINSEQUENCE**RR**

YHTRANSMEMBRANEDOMAINSEQUENCE**LR**
YHTRANSMEMBRANEDOMAINSEQUENCE**LR**
YHTRANSMEMBRANEDOMAINSEQUENCE**LR**

Two Nonsynonymous changes fixed between the sexes flanking a transmembrane domain

-In Both cases you have repeated amino acids HH & RR

-in males there is a change: HH -> YH and RR -> LR

Both are changes from a positively charged aa to a hydrophobic aa

- PROVEAN Tools
  - PROVEAN Protein
  - PROVEAN Protein Batch
    - Human
    - Mouse
  - PROVEAN Genome Variants
    - Human
    - Mouse
- About
- FAQ
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- Related Links

PROVEAN (**Pro**tein **V**ariation **E**ffect **An**alyzer) is a software tool which predicts whether an amino acid substitution or indel has an impact on the biological function of a protein.

PROVEAN is useful for filtering sequence variants to identify nonsynonymous or indel variants that are predicted to be functionally important.

The performance of PROVEAN is comparable to popular tools such as SIFT or PolyPhen-2 [1]. Read more.

A fast computation approach to obtain pairwise sequence alignment scores enabled the generation of precomputed PROVEAN predictions for 20 single AA substitutions and a single AA deletion at every amino acid position of all protein sequences in human and mouse [2].

This work is funded by the National Institutes of Health [grant number 5R01HG004701-04].

References:

1. Choi Y, Sims GE, Murphy S, Miller JR, Chan AP (2012) Predicting the Functional Effect of Amino Acid Substitutions and Indels. *PLoS ONE* 7(10): e46688.
2. Choi Y (2012) A Fast Computation of Pairwise Sequence Alignment Scores Between a Protein and a Set of Single-Locus Variants of Another Protein. *In Proceedings of the ACM Conference on Bioinformatics, Computational Biology and Biomedicine* (BCB '12). ACM, New York, NY, USA, 414-417.
(* This is the author's version of the work. It is posted here by permission of ACM for your personal use. Not for redistribution. The definitive version was published in ACM BCB '12. http://doi.acm.org/10.1145/2382936.2382989)
3. Choi Y, Chan AP (2015) PROVEAN web server: a tool to predict the functional effect of amino acid substitutions and indels. *Bioinformatics* 31(16): 2745-2747.

PROVEAN web server functions are currently using PROVEAN v1.1.3.

There are also online tools specifically designed to assess impacts on TM domains

DeepTMHMM

A Deep Learning Model for Transmembrane Topology Prediction and Classification

Protein structure prediction using deep learning methods have seen several advancements within the last years. In this project, we investigate deep learning for prediction of the membrane topology of transmembrane proteins. Transmembrane proteins are relevant for drug development since they make up more than 50% of all human drug targets .

DeepTMHMM is currently the most complete and best-performing method for the prediction of the topology of both alpha-helical and beta-barrel transmembrane proteins. The model encodes the primary amino acid sequence by a pre-trained language model and decodes the topology by a state space model to produce topology and type predictions at unprecedented accuracy. DeepTMHMM makes it possible to scan full proteomes in order to detect both classes of transmembrane proteins, and we anticipate our method to be very valuable for the research community.

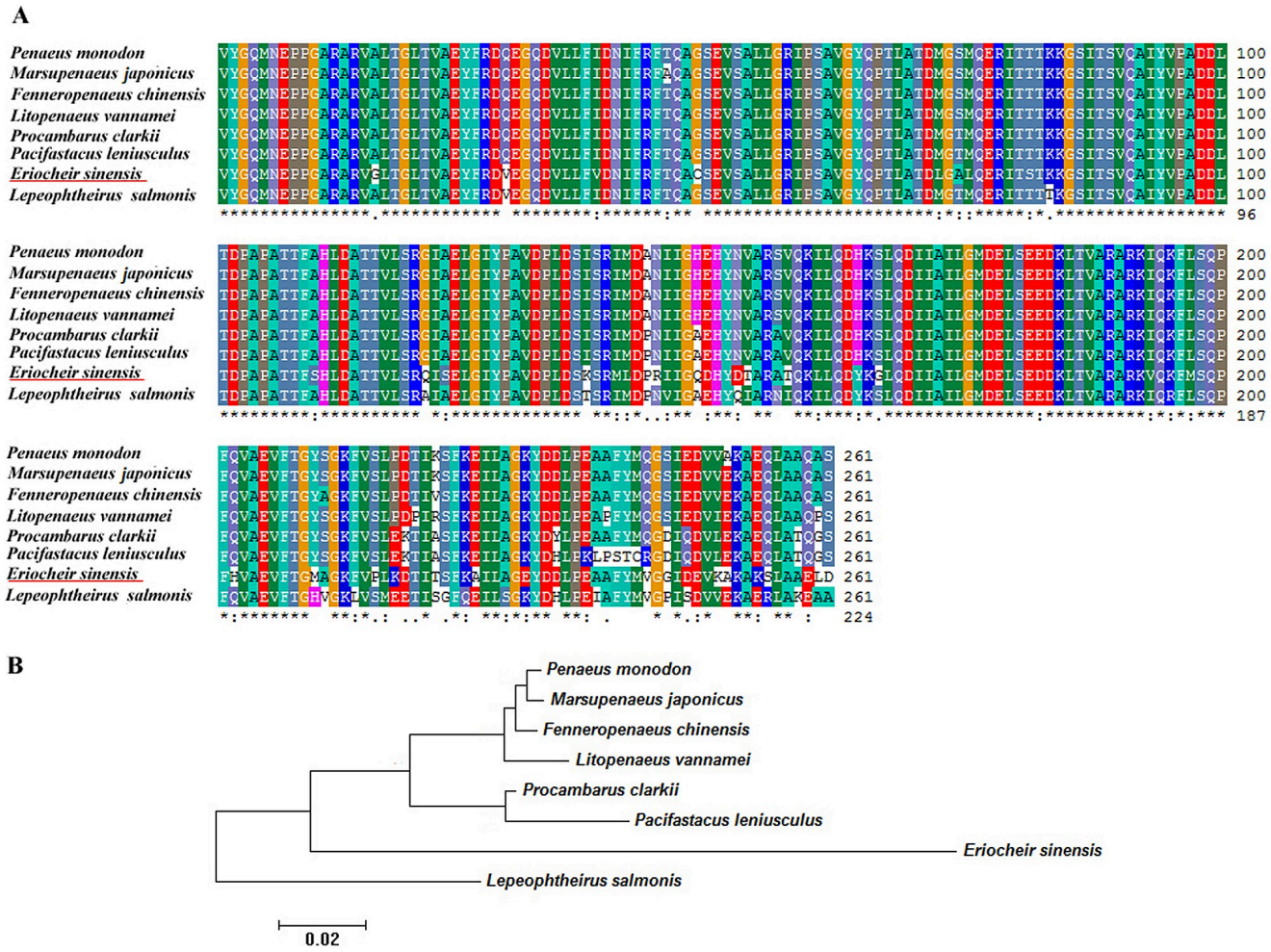
Note: If you want to run DeepTMHMM on more than 10000 sequences per run, then you can run it locally by following the instructions below.

Running DeepTHMMM From the Terminal

You can run DeepTMHMM directly from the terminal through the `pybiolib` package:

To perform tasks comparing between sequences you need a high quality multiple alignment

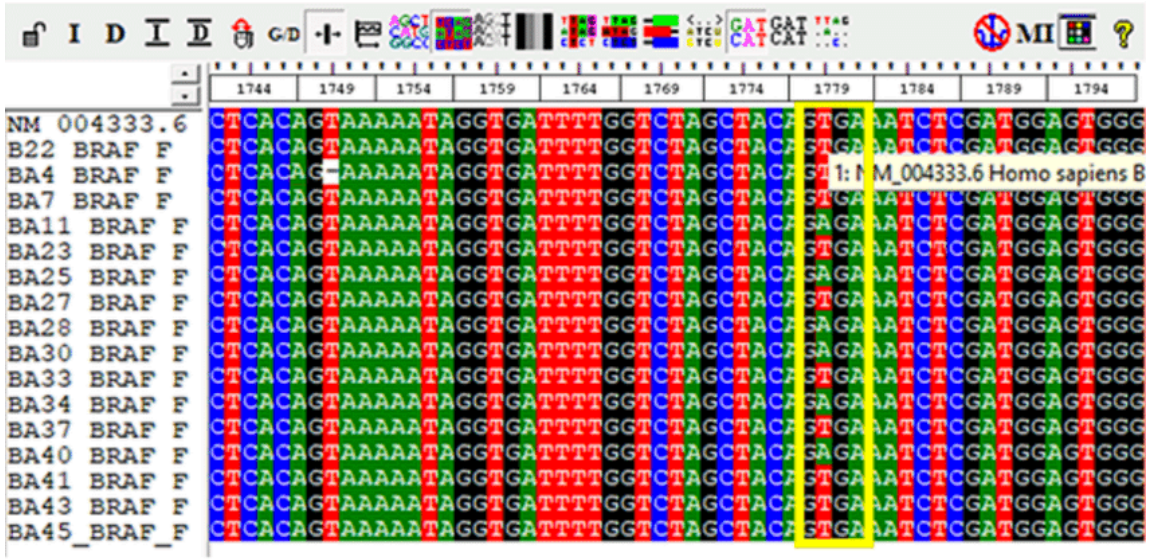
Up until now when we have talked about “alignments” we have been referring to reads aligned to a reference



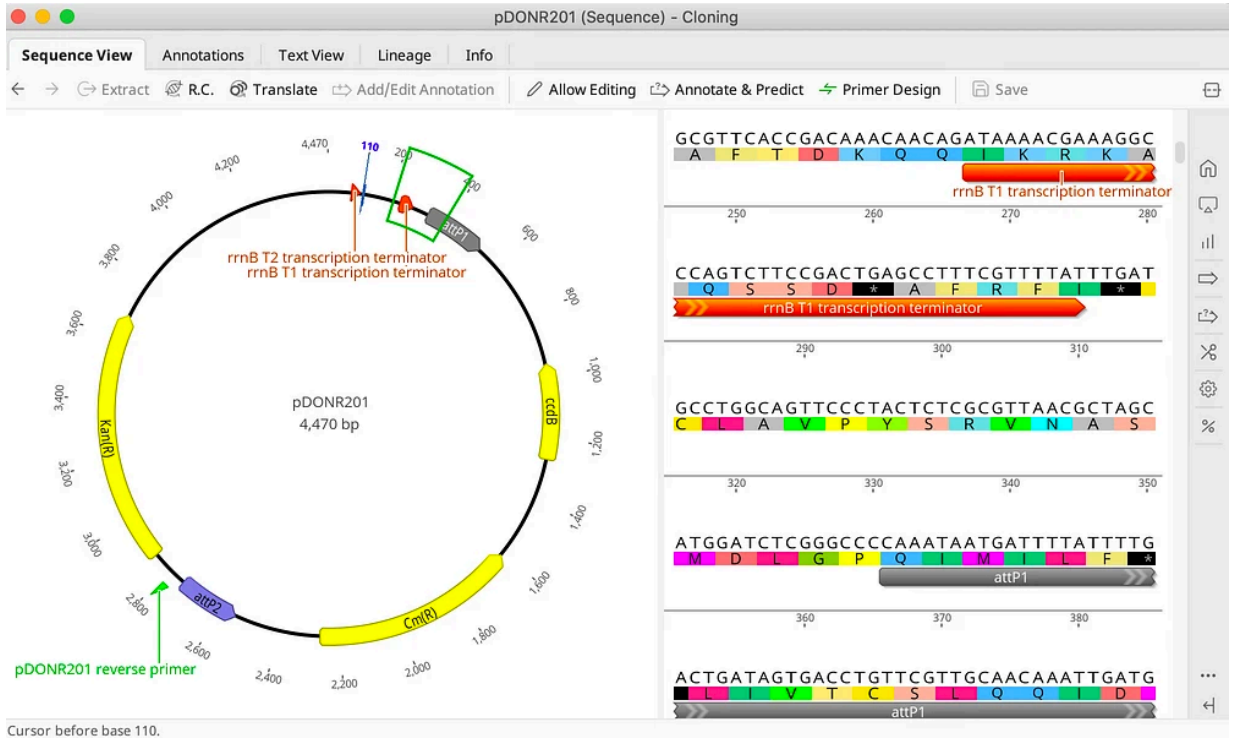
We are now focusing on **multiple alignments** from Fasta formats which can be used for lots of biological inferences

(Hui et al. 2014)

Multiple alignments can be in nucleotide or amino acid formats.



Bioedit



Geneious

I use Geneious for most things, and even find the free version (with most features locked) helpful for visualization

-visualize bam files

-call consensus

-annotate sequences

-design primers

-translate/reverse/complement/align/edit

Multiple alignments can also be done on the command line for example with MAFFT

MAFFT

Section: Mafft Manual (1)
Updated: 2007-06-09
Index Return to Main Contents

NAME

mafft - Multiple alignment program for amino acid or nucleotide sequences

SYNOPSIS

mafft [**options**] *input* [> *output*]
linsi *input* [> *output*]
ginsi *input* [> *output*]
einsi *input* [> *output*]
fftnsi *input* [> *output*]
fftns *input* [> *output*]
nwns *input* [> *output*]
nwnsi *input* [> *output*]
mafft-profile *group1 group2* [> *output*]

input, *group1* and *group2* must be in FASTA format.

DESCRIPTION

MAFFT is a multiple sequence alignment program for unix-like operating systems. It offers a range of multiple alignment methods.

Accuracy-oriented methods:

*L-INS-i (probably most accurate; recommended for <200 sequences; iterative refinement method incorporating local pairwise alignment information):

mafft --localpair --maxiterate *1000 input* [> *output*]
linsi *input* [> *output*]

*G-INS-i (suitable for sequences of similar lengths; recommended for <200 sequences; iterative refinement method incorporating global pairwise alignment information):

mafft --globalpair --maxiterate *1000 input* [> *output*]
ginsi *input* [> *output*]

*E-INS-i (suitable for sequences containing large unalignable regions; recommended for <200 sequences):

mafft --ep *0* **--genafpair --maxiterate** *1000 input* [> *output*]
einsi *input* [> *output*]

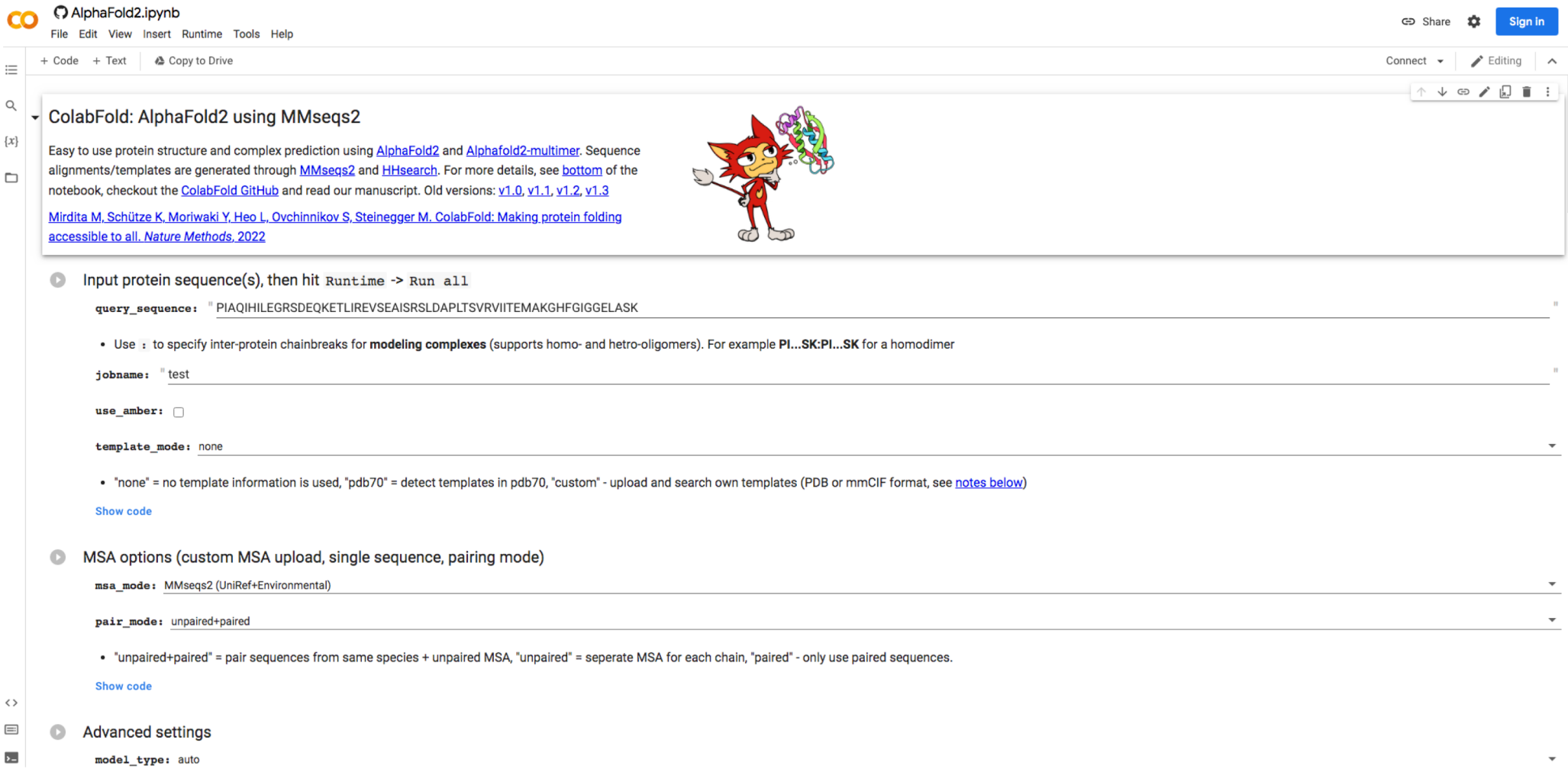
For E-INS-i, the **--ep** *0* option is recommended to allow large gaps.

The protein folding problem:

We have come a long way in predicting impacts on secondary structures but how changes impact folding and tertiary structure is still a massive problem

“Maybe more than any other area of biology, Machine Learning is needed to solve the protein folding problem”

Enter AlphaFold2



Keep in mind that proteins are dynamic and changes in the environment can impact structure

This adds a layer of complexity to the protein folding problem

After our break we will work through using prediction software for assessing impacts of nonsynonymous changes on protein structure and function

(1) Phyre2
(2) PROVEAN
(3) AlphaFold2