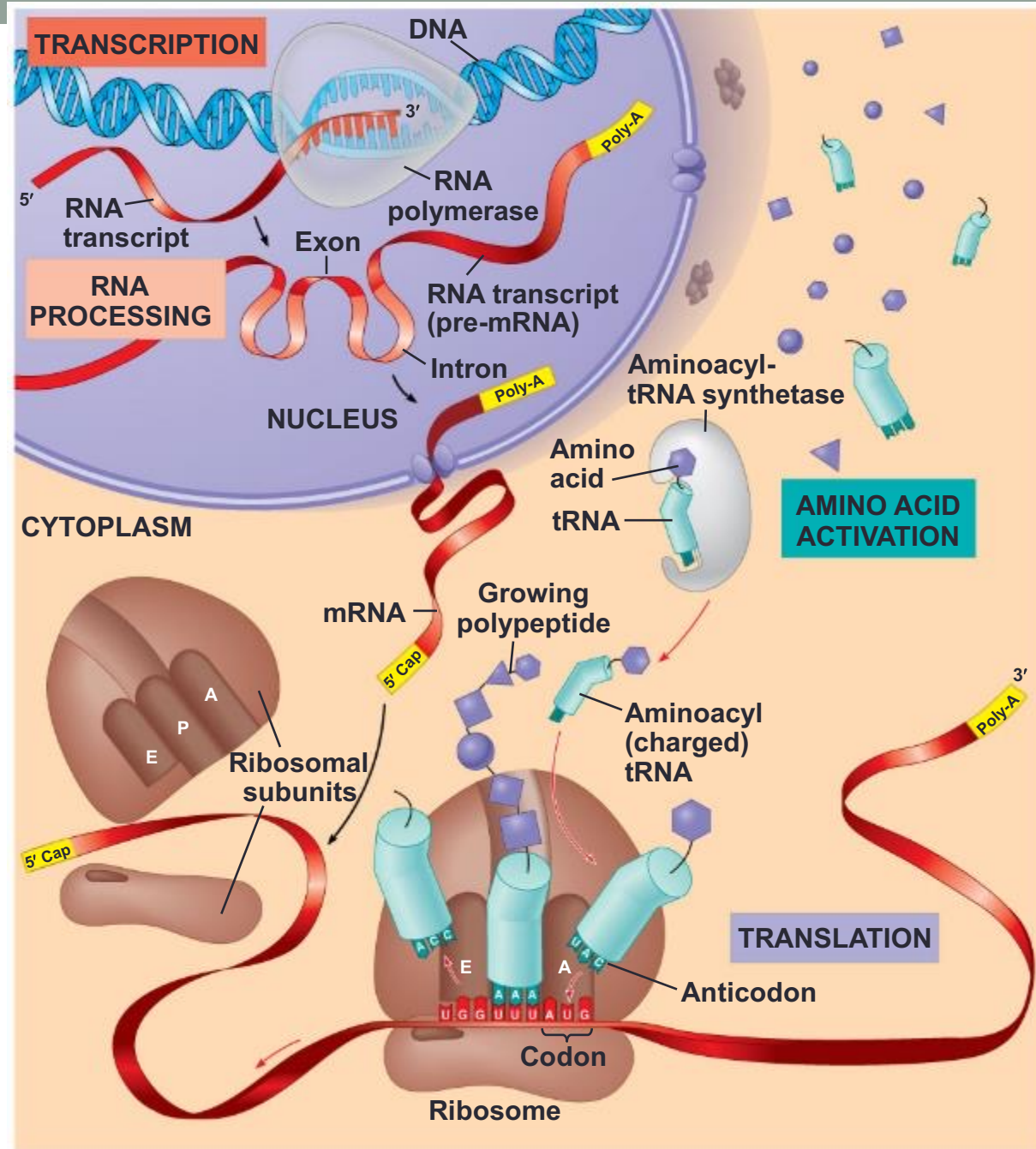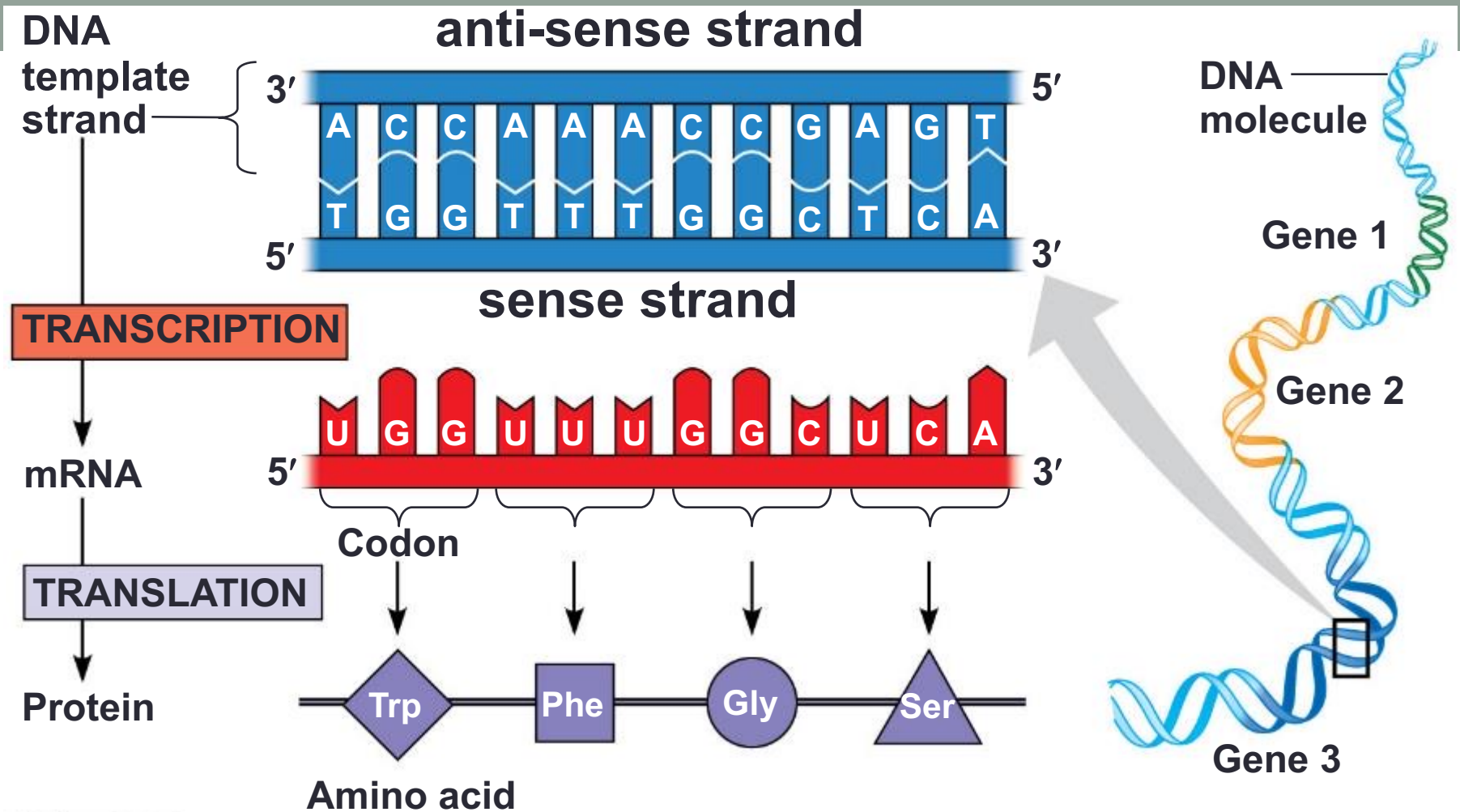# CHAPTER 9: GENE PREDICTION

Dr. Garrett Dancik

What is a gene?

- a region of DNA that can be expressed to produce a final functional product, either
  - a polypeptide or
  - an RNA molecule

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- The genetic code is a triplet code where a 3-nucleotide DNA word codes for a 3-nucleotide mRNA word (a **codon**) which codes for an amino acid

Gene Prediction by Homology

- New DNA sequences can be searched (e.g., BLASTED) against various databases
  - blastx – search a <u>protein</u> database using a <u>translated nucleotide query</u>
  - tblastx – search a <u>translated nucleotide</u> database using a <u>translated nucleotide</u> query
- Generally, >50% of prokaryotic genes can be identified by homology
- Gene prediction in this manner is more difficult for eukaryotic organisms
  - Why?

Sequence Translation Revisited

- Suppose you have a sequence of DNA that includes a gene (you don't know exactly where the gene is). What are the possible polypeptide sequences that could (theoretically) be produced?

  - 5' – GATGGATGACGCGATGATCC – 3'

- Let's look at the Expasy Translate tool:
  - http://web.expasy.org/translate/

Sequence Translation Revisited

- We don't know where the first codon (in the sequence) begins.
 - 5' – GATGGATGACGCGATGA – 3'

Reading frame 1	GAT	GGA	TGA	CGC	GAT	GA
Reading frame 2	ATG	GAT	GAC	GCG	ATG	A
Reading frame 2	TGG	ATG	ACG	CGA	TGA	

- We don't know which *strand* is the *sense* strand (need to consider the *reverse complement*)
  - 5' – TCATCGCGTCATCCATC – 3'

Reading frame 4	TCA	TCG	CGT	CAT	CCA	TC
Reading frame 5	CAT	CGC	GTC	ATC	CAT	C
Reading frame 6	ATC	GCG	TCA	TCC	ATC	

# Annotation of a segment of the *E. coli* genome
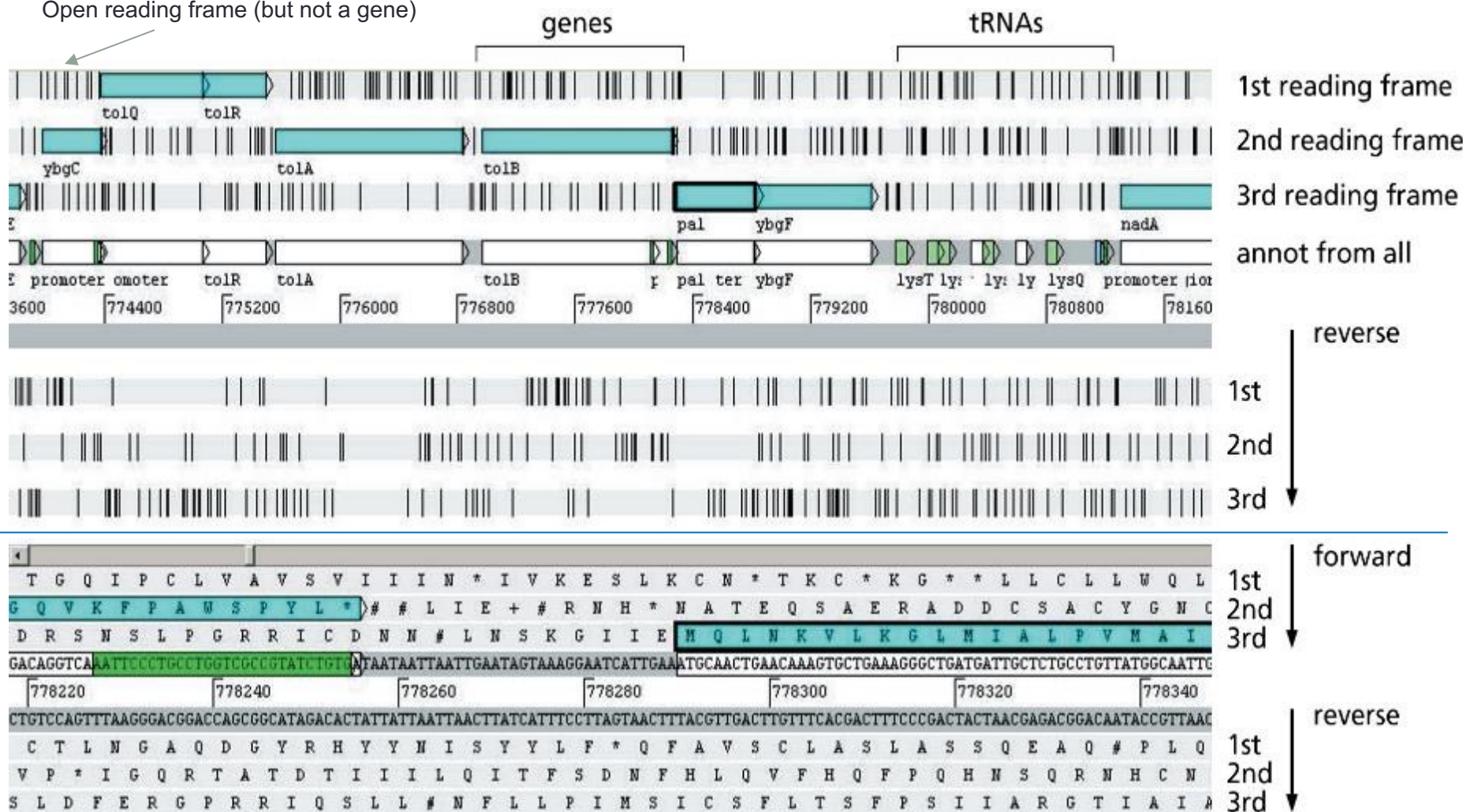
Observations

- Non-coding genes such as tRNAs do not have corresponding proteins
  - These have conserved structures that aid in their identification
- Definition: an *open reading frame* is a DNA sequence that contains no stop codons
- Actual protein-coding genes correspond to regions of DNA with large open reading frames, that begin with a start codon and end with a stop codon.
- Simple algorithm (for prokaryotes):
  - Search for a start codon. If not found, then there are no protein coding genes in this sequence
  - Search for a stop codon in the same reading frame as the start codon. Discard the ORF if its length is less than a threshold (e.g., 100 amino acids)
 - Repeat until all candidate genes are found

Promoter identification
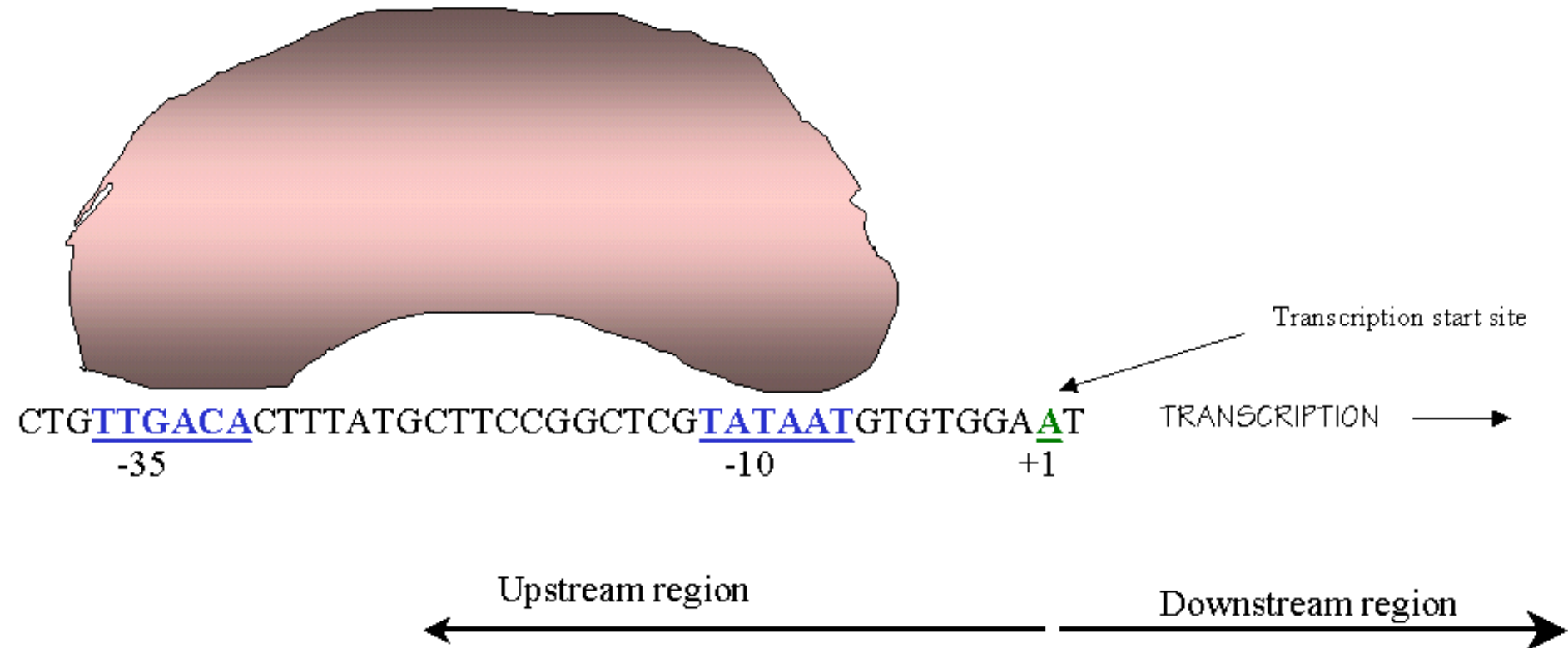
- A promoter is a region of DNA where RNA polymerase binds.
- Prokaryotic gene promoters have two conserved sequences
  - -10 sequence: TATAAT approximately 10 bp upstream of the transcription start site
  - -35 sequence TTGACA approximately 35 bp upstream of transcription start site
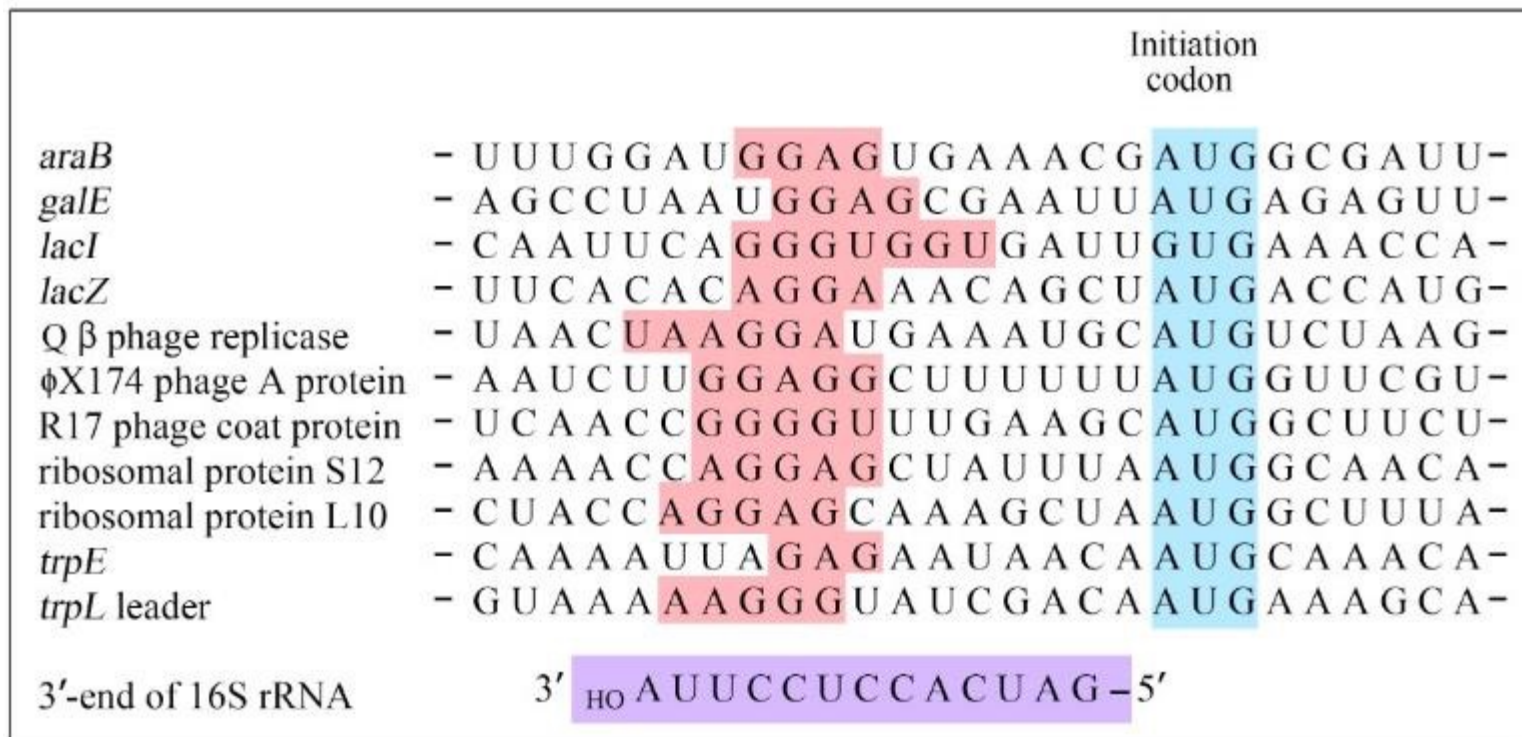 - The two above sequences may not be exact

Sequence logo of -10 sequence
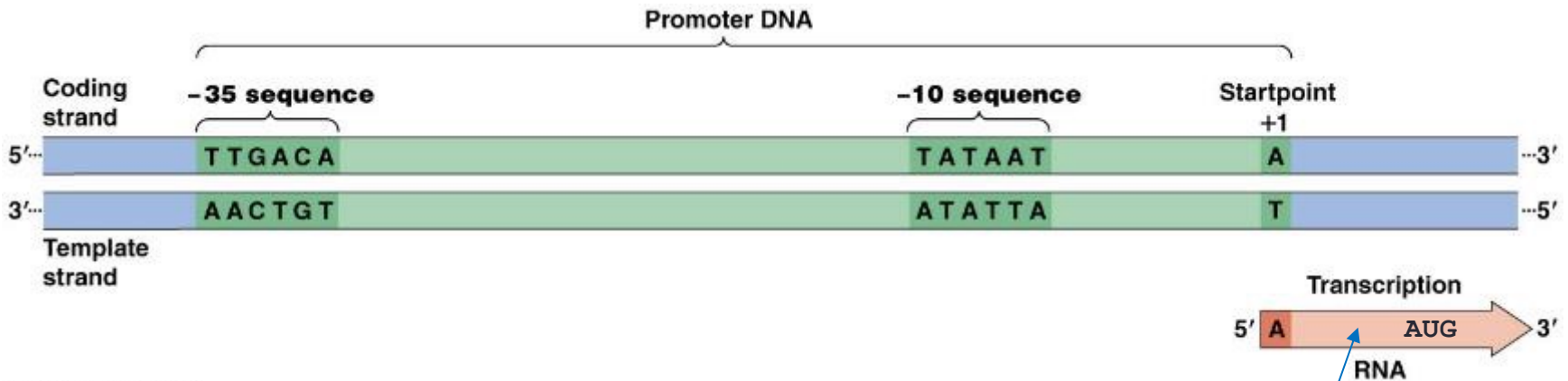

The -10 region of 350 E. coli promoters
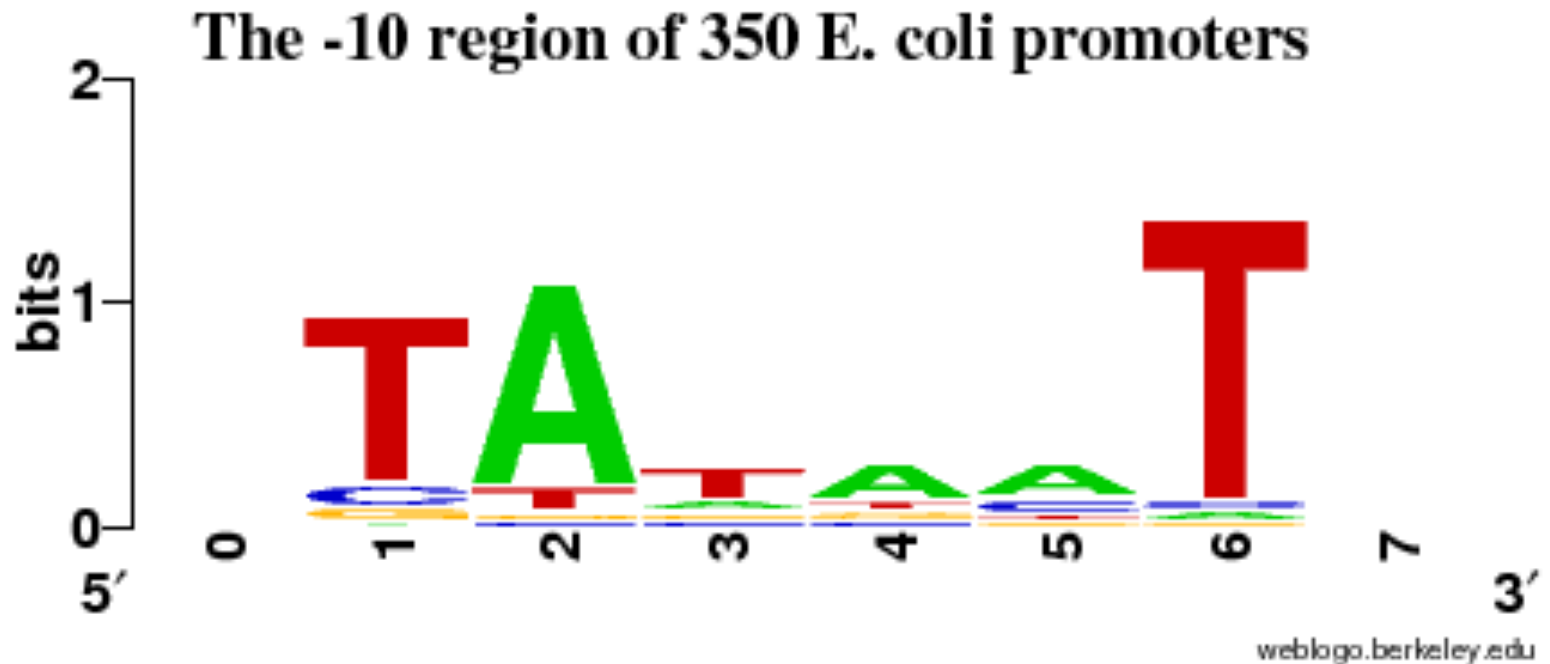
- The height of a *position* corresponds to how conserved the position is
- At each position, the height of each character is proportional to its frequency

Shine-Delgarno sequence (prokaryotes)

- The Shine-Dalgarno sequence (or ribosome binding site) precedes the start codon by a few bases and is where the ribosome binds to the corresponding mRNA.
- Consensus sequence is AGGAGG

Putting it together…

Promoter DNA

Coding strand
−35 sequence
−10 sequence
Startpoint +1

5′··· TTGACA ··· TATAAT ··· A ···3′
3′··· AACTGT ··· ATATTA ··· T ···5′

Template strand

Transcription

5′ A ······ AUG 3′
RNA

Shine-Dalgarno Sequence, AGGAGG, ~ 5bp upstream of start codon

Prokaryotic Gene Prediction Algorithm

Sequences that include an ORF of a minimum length, a Shine-Dalgarno sequence, and conserved promoter elements are candidate genes:

1. Search for the next start codon. If no start codon is found, end.
2. Search for a stop codon in the same reading frame as the start codon. Continue only if the ORF length is greater than a threshold (e.g., 100 amino acids). Otherwise start over.
3. Search for a Shine-Dalgarno sequence 3-7 bases upstream of the start codon. The sequence should pass a matching threshold (e.g., 5/6 identity). If not found, start over.
4. Search 500 nucleotides upstream of the Shine-Dalgarno sequence for a promoter. The TTGACA promoter should be located 15-19 nucleotides upstream of TATAAT. Allow for one mismatch in each sequence (use of other consensus sequences is possible)

Gene expression in eukaryotes
## (introns are spliced out)
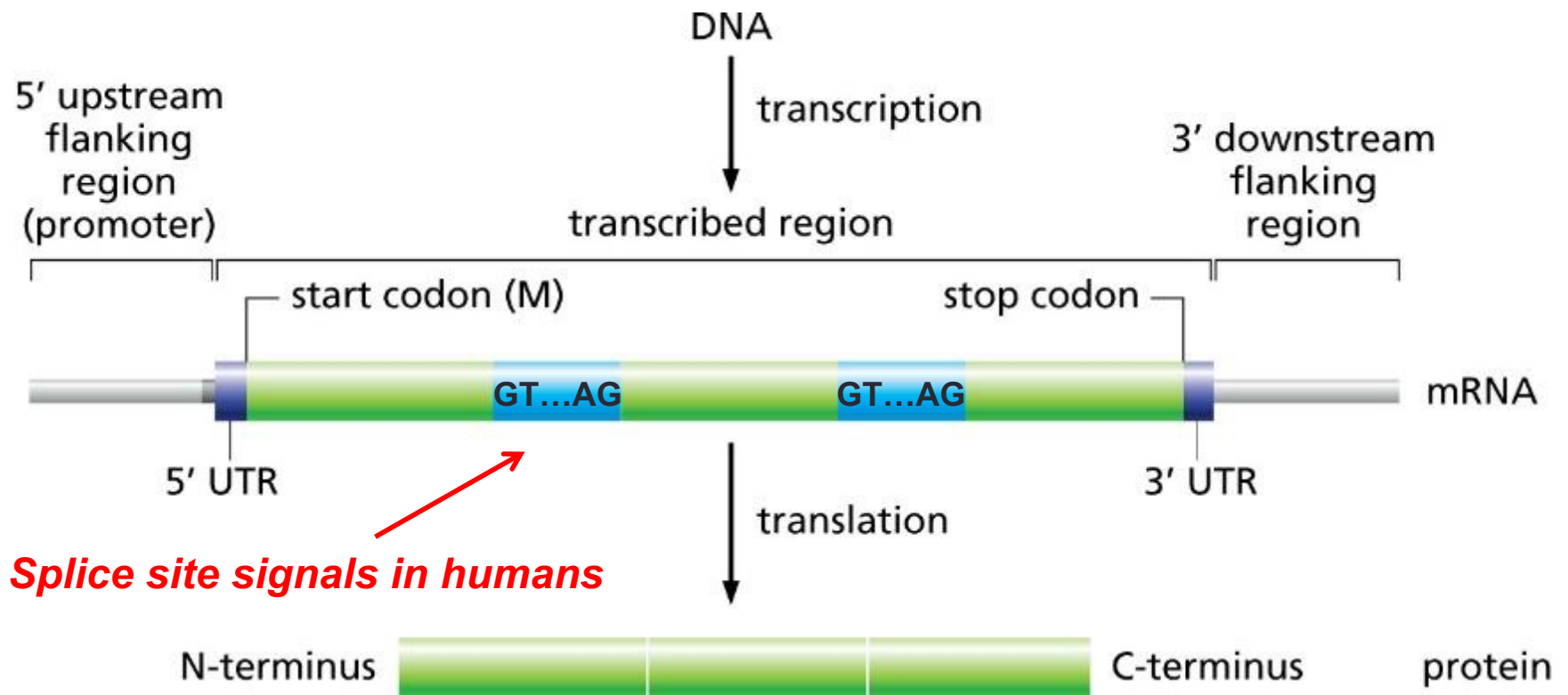
Sequence conservation of splice sites in humans
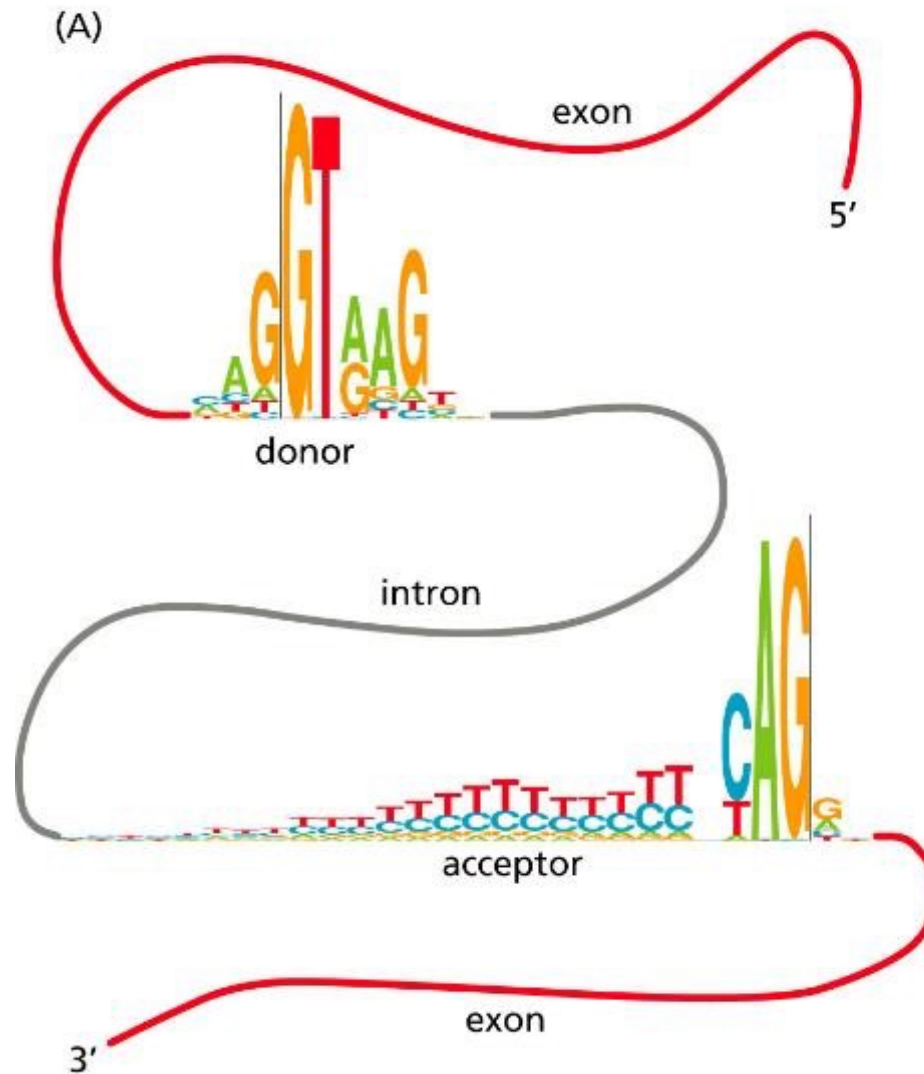
Gene Prediction in Eukaryotes

- Involves prediction of exons and introns
  - Based on statistical gene models and query sequence
  - Based on statistical gene models, sequence similarity, and a query sequence
- Must preserve the correct reading frame
- Involves prediction of the promoter

Eukaryotic Gene Prediction and Gene Annotation

Augustus
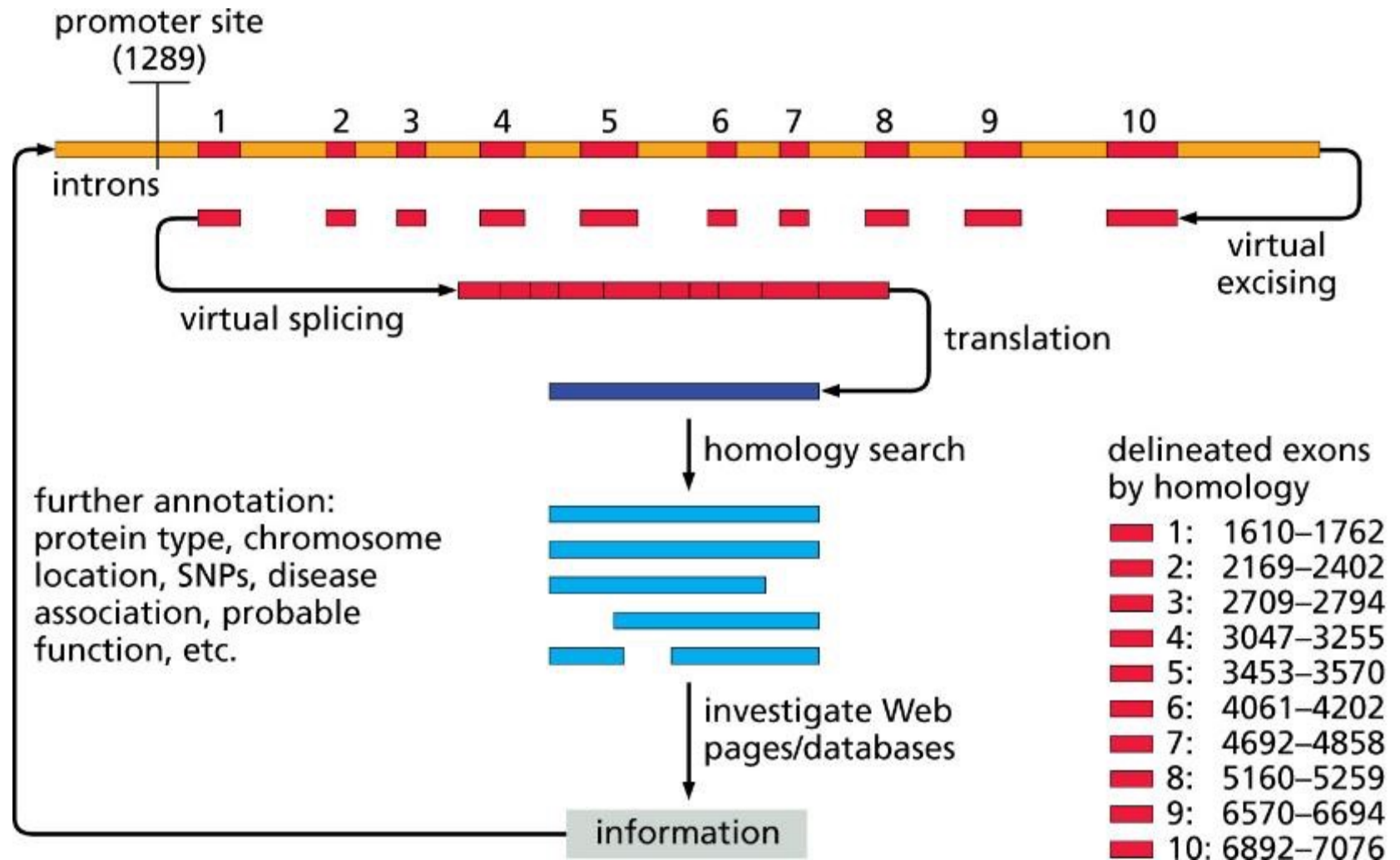
- http://bioinf.uni-greifswald.de/augustus/
- Uses a Hidden Markov Model (HMM)
- Probabilistic intron length model
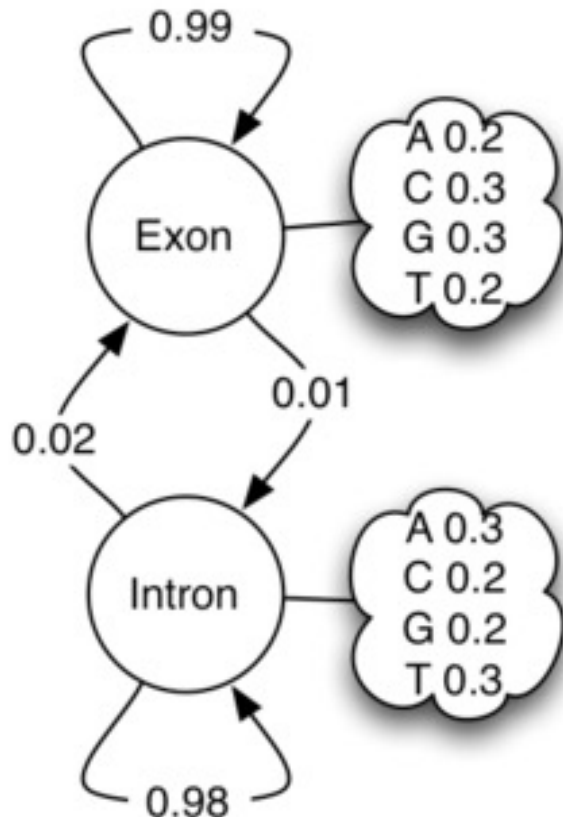
A very simple HMM for gene structure

- Hidden states: exon and intron
- Transition probabilities

  exon → exon: 0.99   intron → intron: 0.98

  exon → intron: 0.01   intron → exon: 0.02

- Emission probabilities for observed values
  - Exon: A,C,G,T (0.2, 0.3, 0.3, 0.2)
  - Intron: A,C,G,T (0.3, 0.2, 0.2, 0.3)
- Objective: identify the most likely states (gene structure) given the observed values (the sequence)?