CHAPTER 9: GENE PREDICTION

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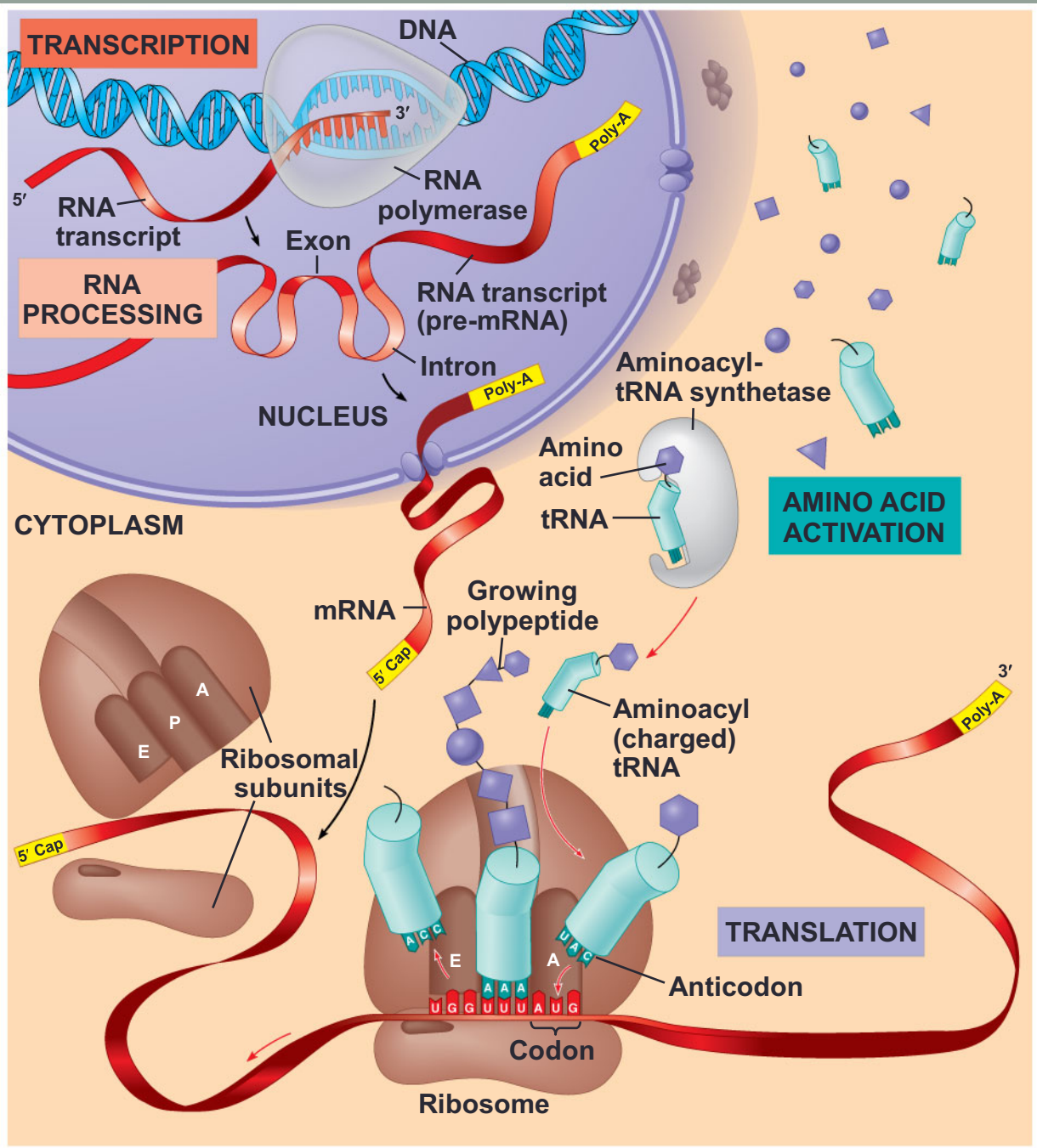
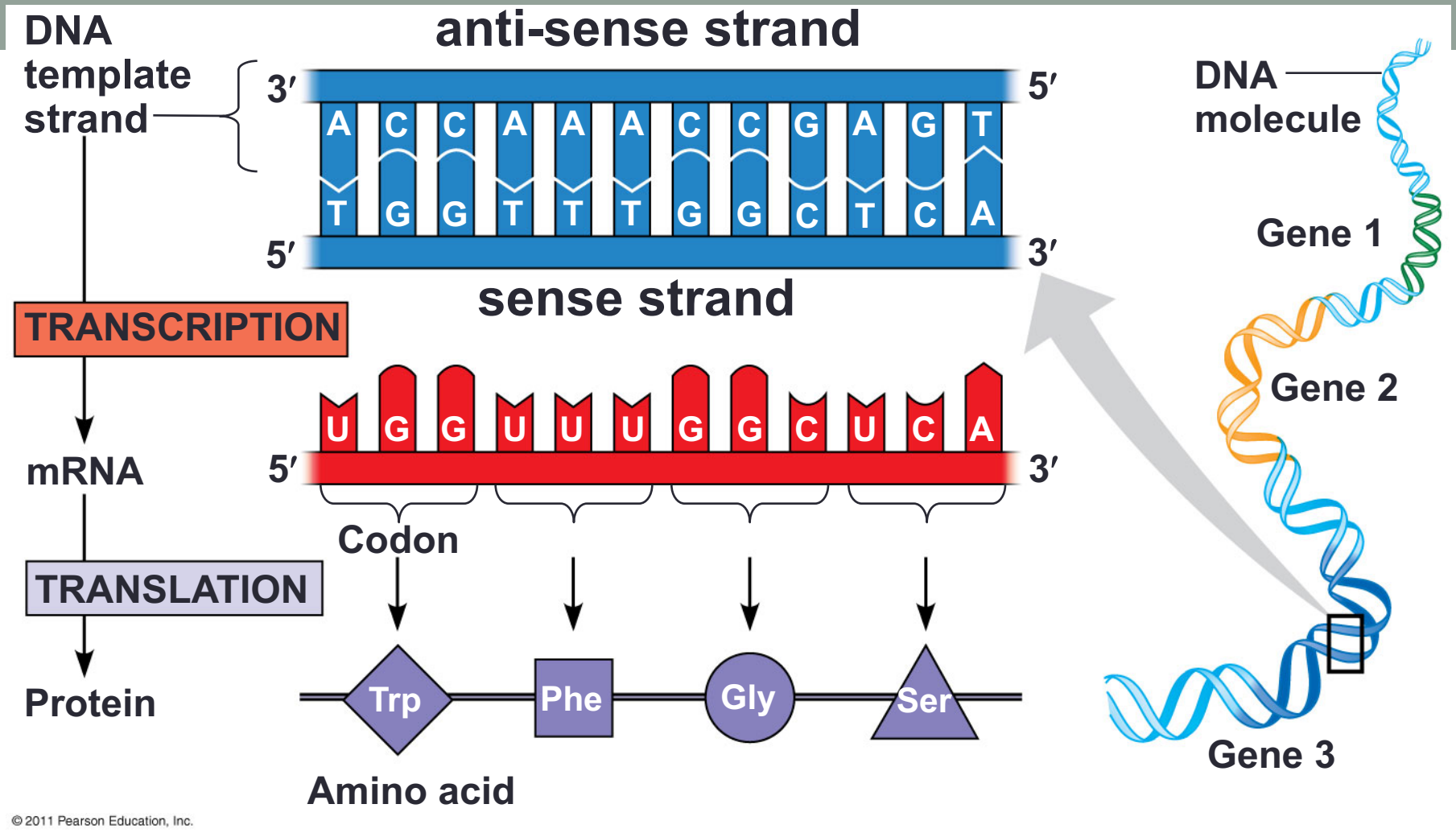
What is a gene?

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





- 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

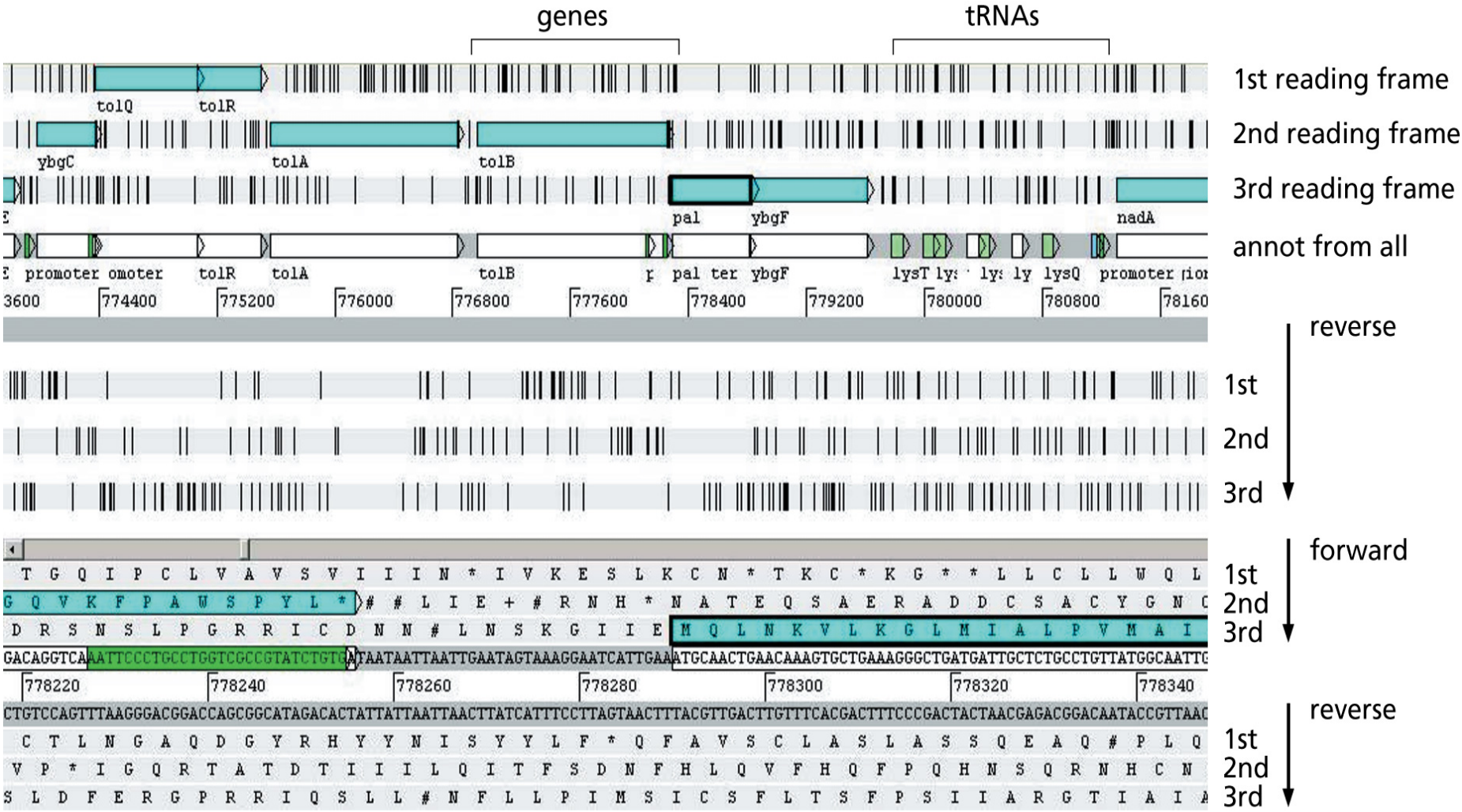
Gene Prediction by Homology

- New DNA sequences can be searched (e.g., BLASTED) against various databases
  - blastx – search a protein database using a translated nucleotide query
  - tblastx – search a translated nucleotide database using a translated nucleotide 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 proteins that are produced?
  - 5' – GATGGATGACGCGATGA – 3'
- Let's look at the Expasy Translate tool:
  - http://web.expasy.org/translate/
- In both prokaryotes and eukaryotes, genes can occur in *any* reading frame and on either strand (always in the 5' to 3' direction)
- Also, not all genes are coding.

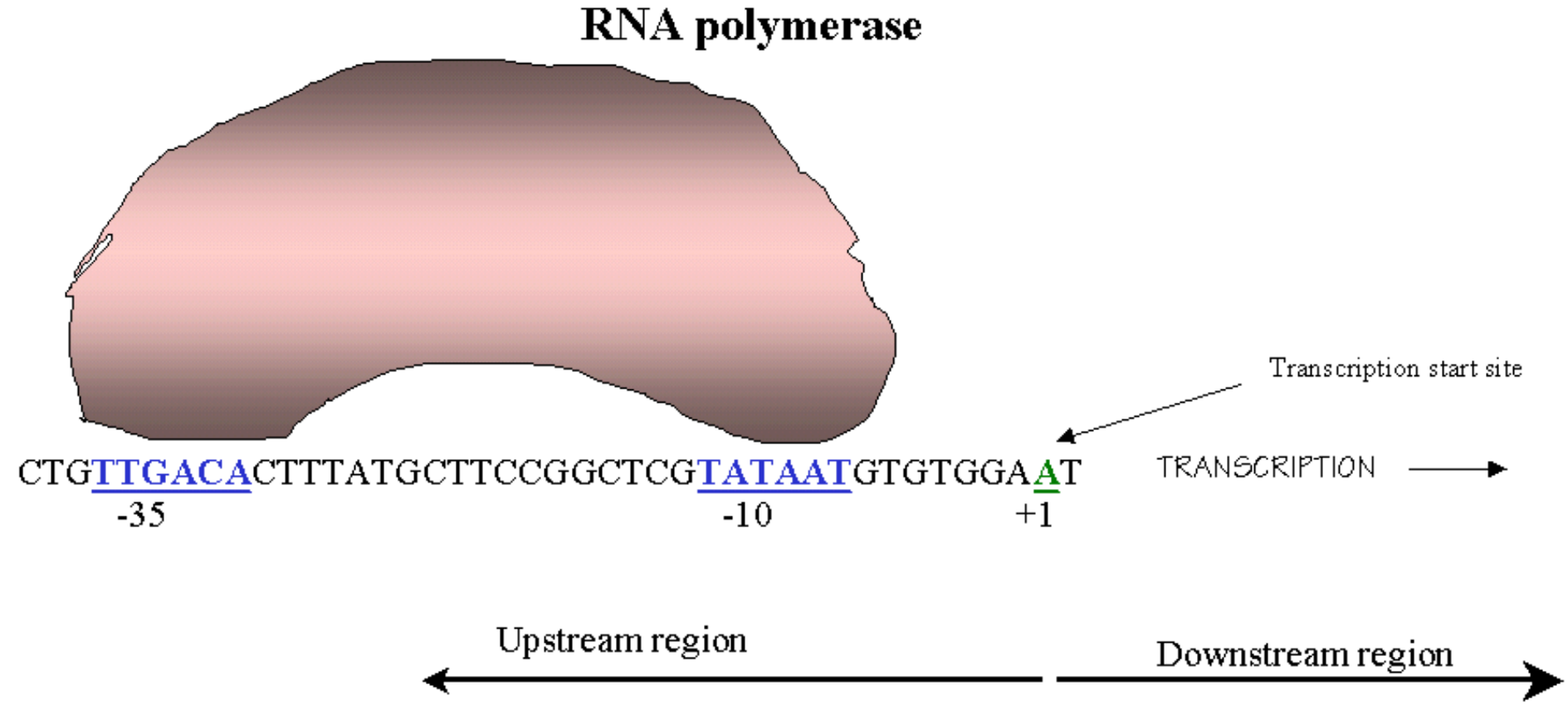
Annotation of a segment of the *E. coli* genome



Observations

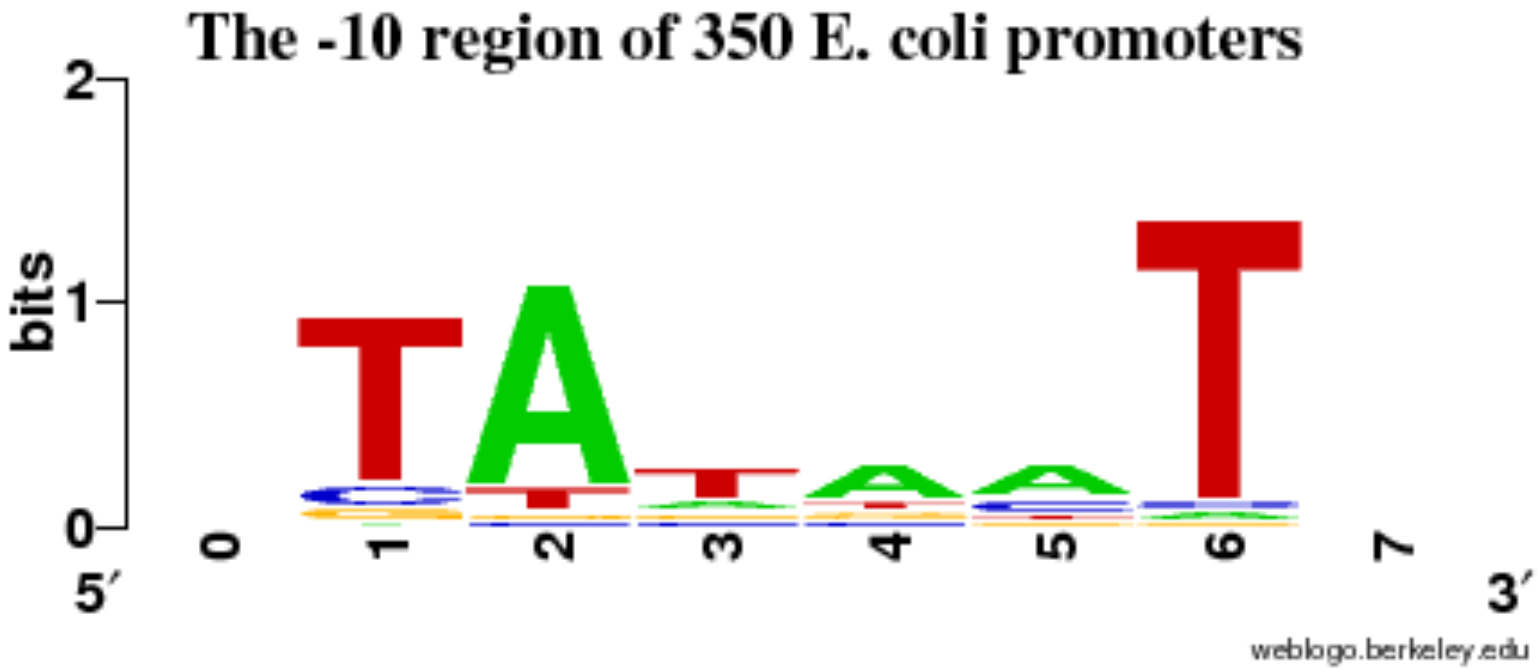
- Non-coding genes such as tRNAs do not have corresponding proteins
  - These have conserved structures that aid in their identification
- Definition: an *open reading frame* is a DNA sequence that begins with the start codon ATG and ends with a stop codon
- The actual genes correspond to regions of DNA with large open reading frames
- Simple algorithm:
  - 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

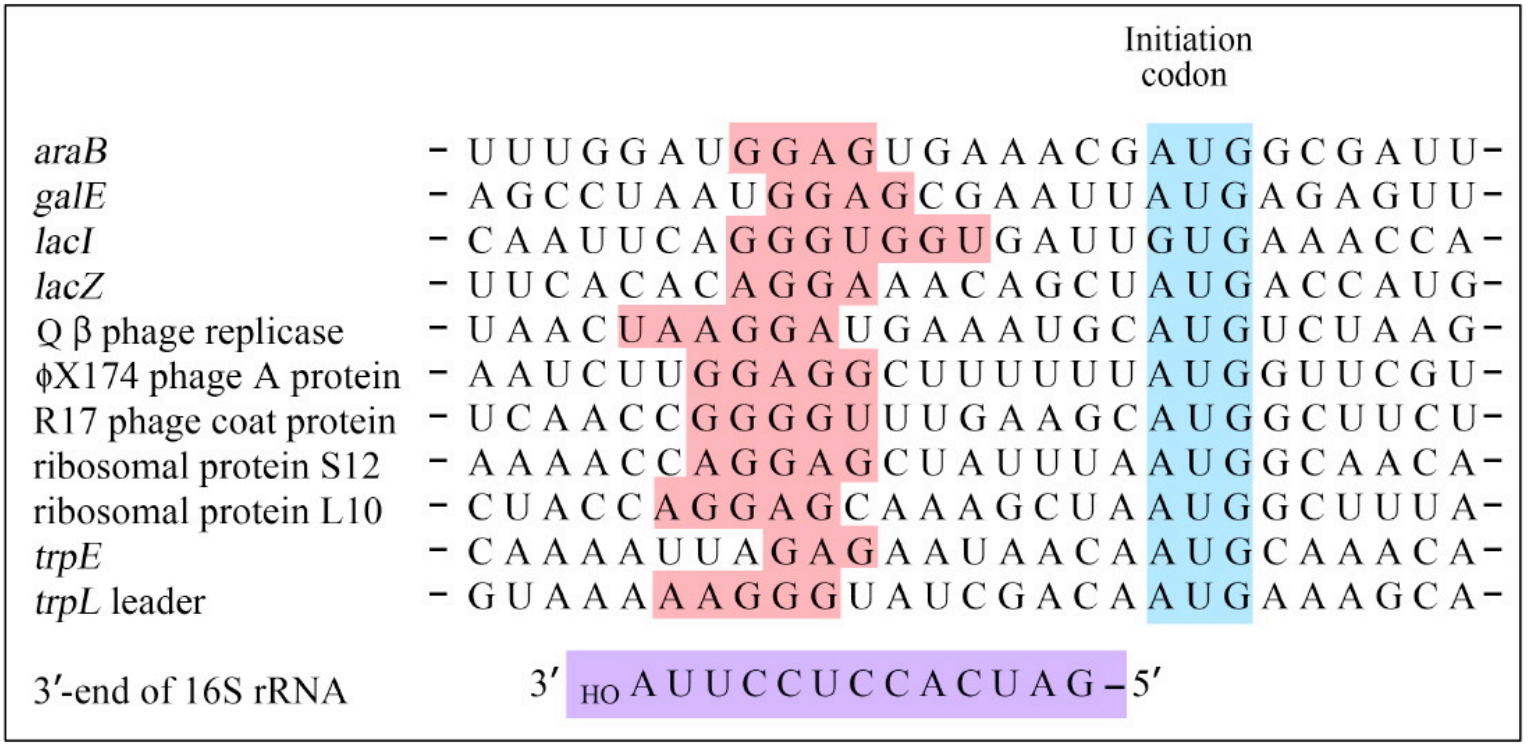
Consensus logo of -10 sequence



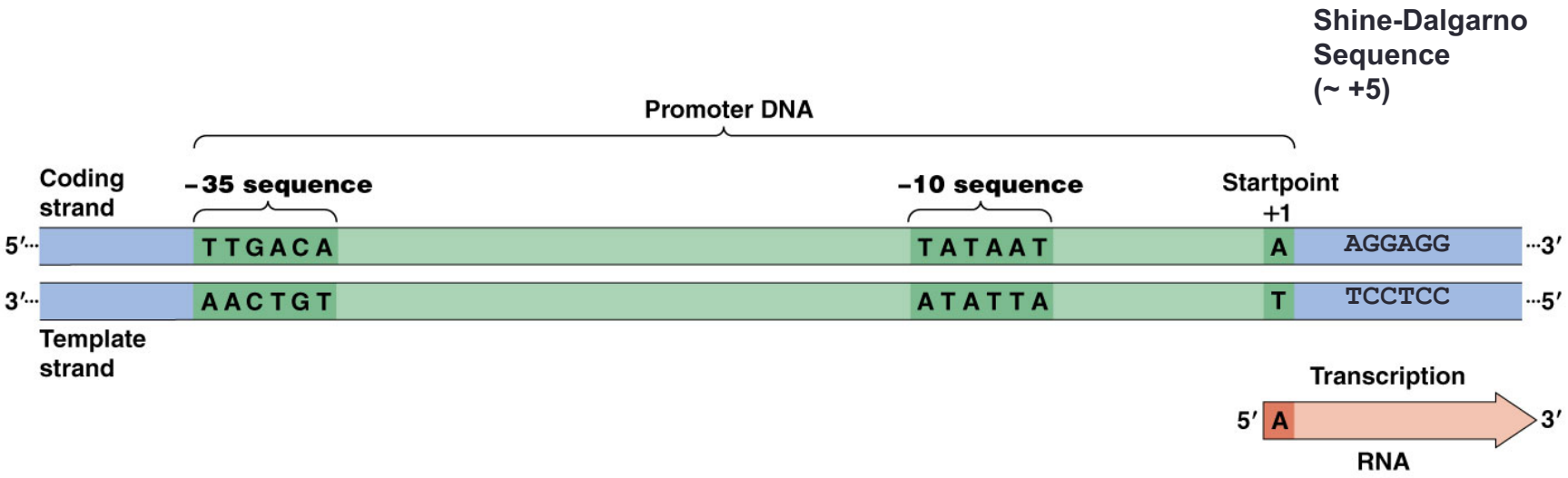
- The height of a *position* corresponds to how conserved the position is
- At each position, the height of each character is proportional to its frequency

Shine-Delgarno sequence

- The Shine-Delgarno 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…

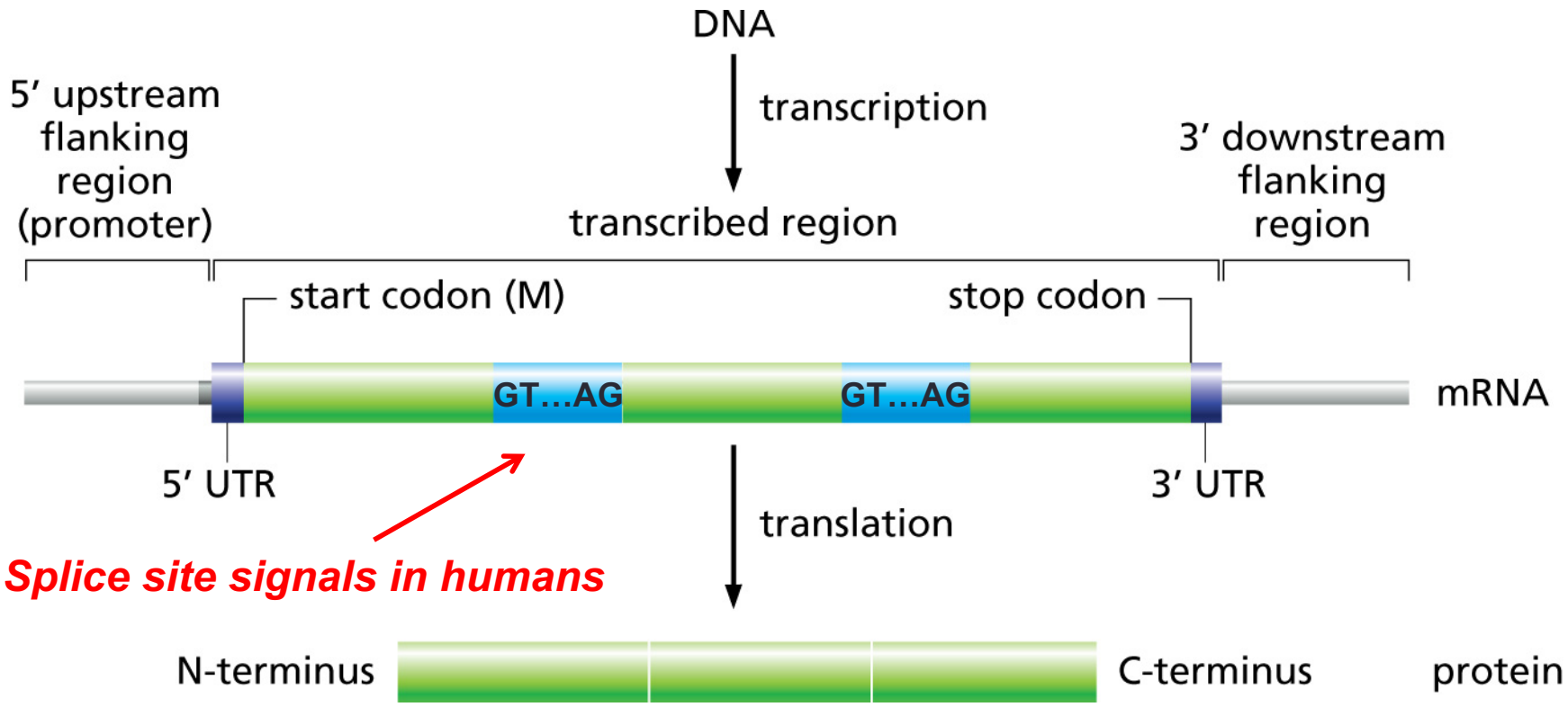


Prokaryotic Gene Prediction Algorithm

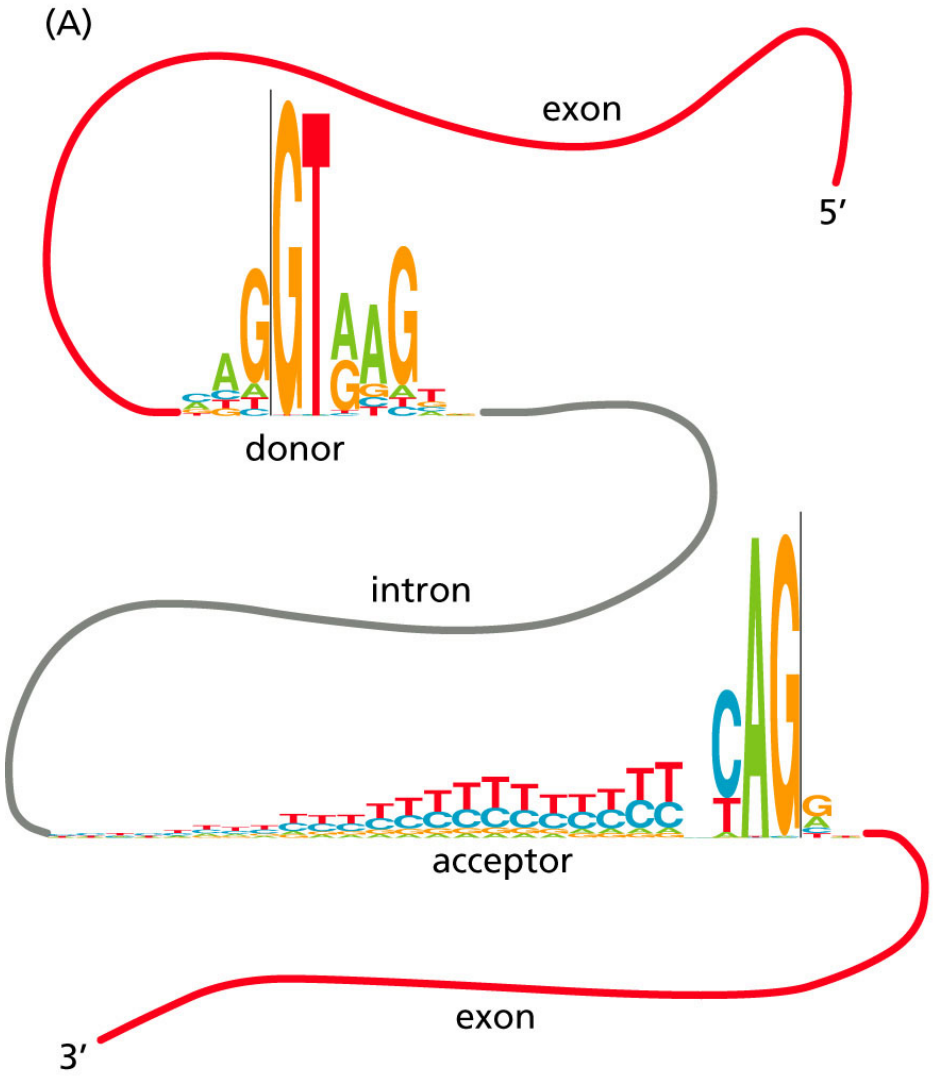
Gene sequences including an ORF of a certain length, a Shine-Dalgarno sequence, and promoter, are candidate genes

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

Gene expression in eukaryotes



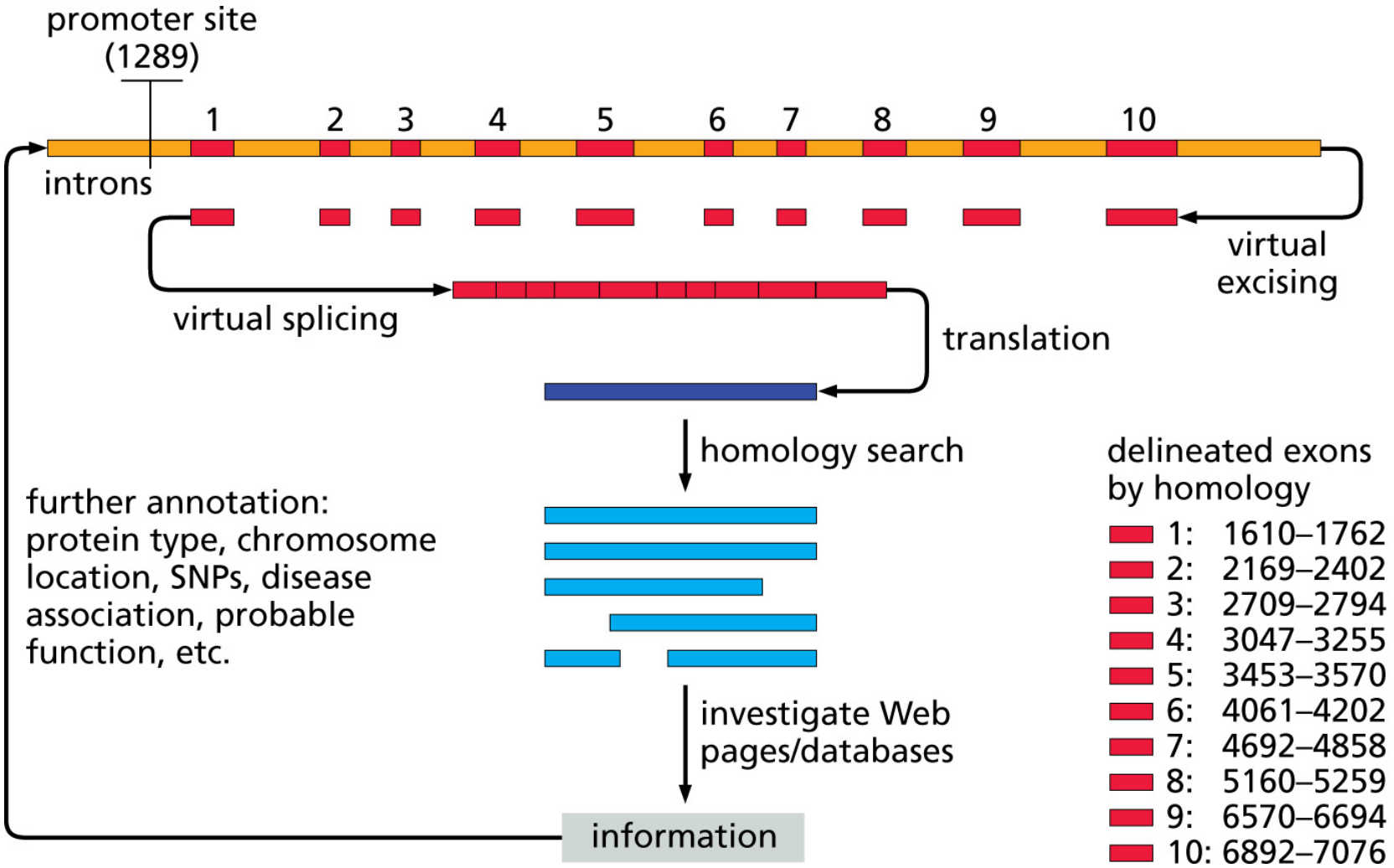
Sequence conservation of splice sites in humans



Gene Prediction in Eukaryotes

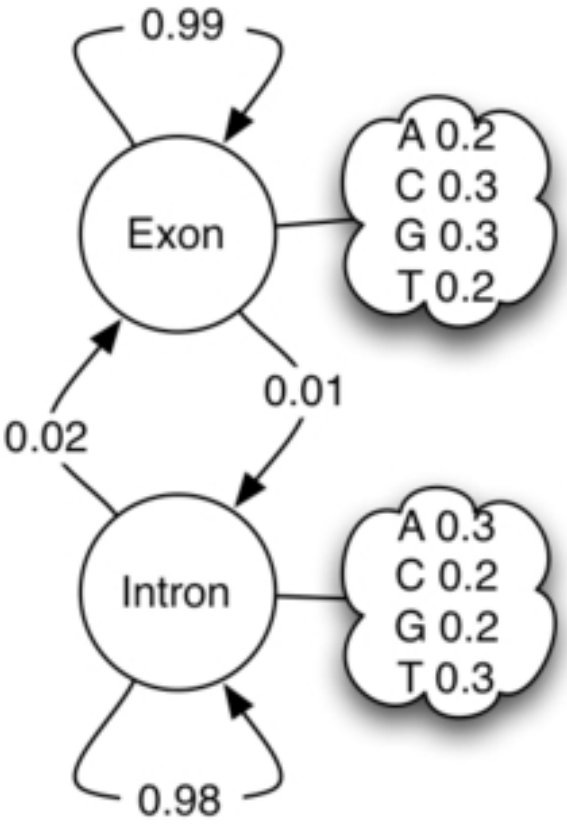
- Involves prediction of exons and introns
  - Based on statistical gene models and query sequence
  - Based on statistical gene models, sequence similarity, and a query sequence
- Must preserve the correct reading frame
- Involves prediction of the promoter

Eukaryotic Gene Prediction and Gene Annotation



Augustus

- http://bioinf.uni-greifswald.de/augustus/
- Uses a Hidden Markov Model (HMM)
- Probabilitistic 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)?