Introduction to Genome Annotation

What is Annotation?

- **dictionary definition of "to annotate":**
  - "to make or furnish critical or explanatory notes or comment"
- **some of what this includes for genomics**
  - gene product names
  - functional characteristics of gene products
  - physical characteristics of gene/protein/genome
  - overall metabolic profile of the organism
- **elements of the annotation process**
  - gene finding
  - homology searches
  - functional assignment
  - ORF management
  - data availability
- **manual vs. automatic**
  - automatic = computer makes the decisions
    - good on easy ones
    - bad on hard ones
  - manual = human makes the decisions
    - highest quality

**Due to the VOLUMES of genome data today, most genome projects are annotated primarily using automated methods with limited manual annotation

Annotation pipeline

Generation of Open Reading Frames

↓

Homology Searches

↓

Putative ID
Frameshift Detection
Ambiguity Report

↓

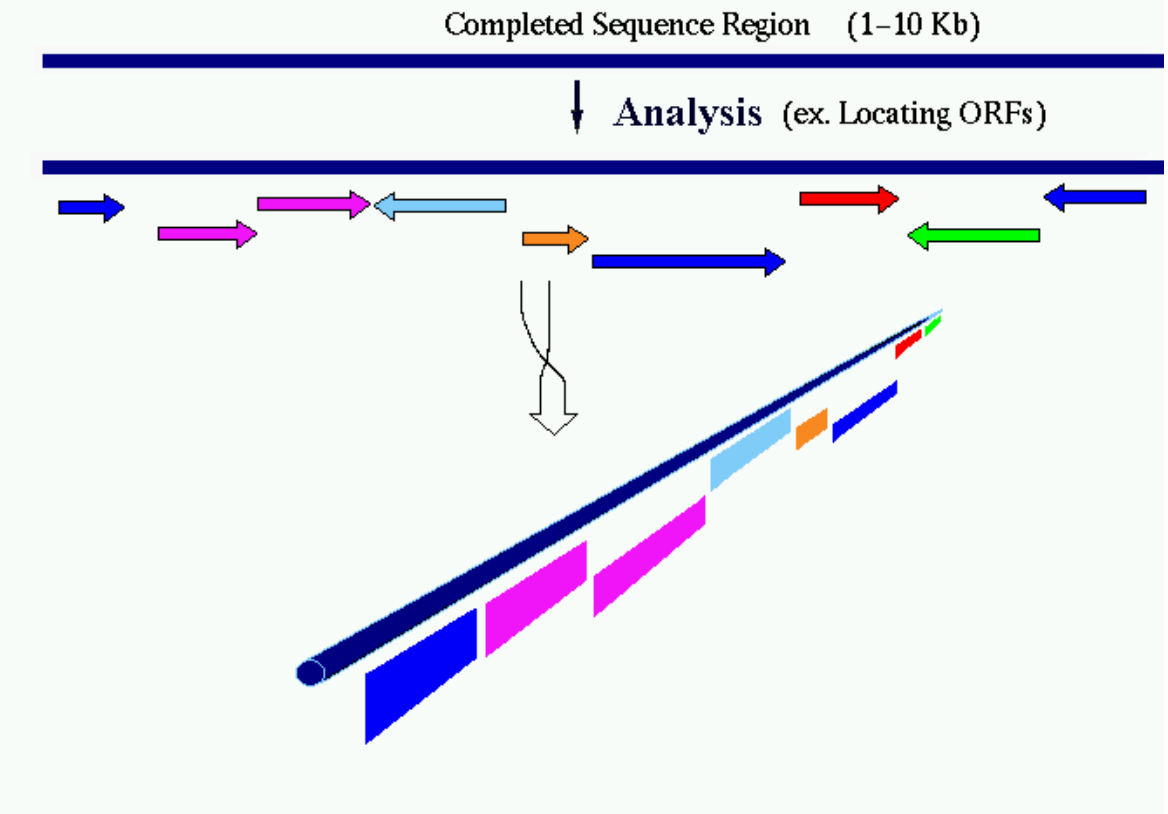
Role Assignment
Metabolic Pathways
Gene Families

↓

DNA Motifs
Regulatory Elements
Repetitive Sequences

↓

Comparative Genomics



Genome Structure

Prokaryote

Intergenic Region

Monocistronic Gene

Intergenic Region

Polycistronic Genes in an Operon

Eukaryote

Gene

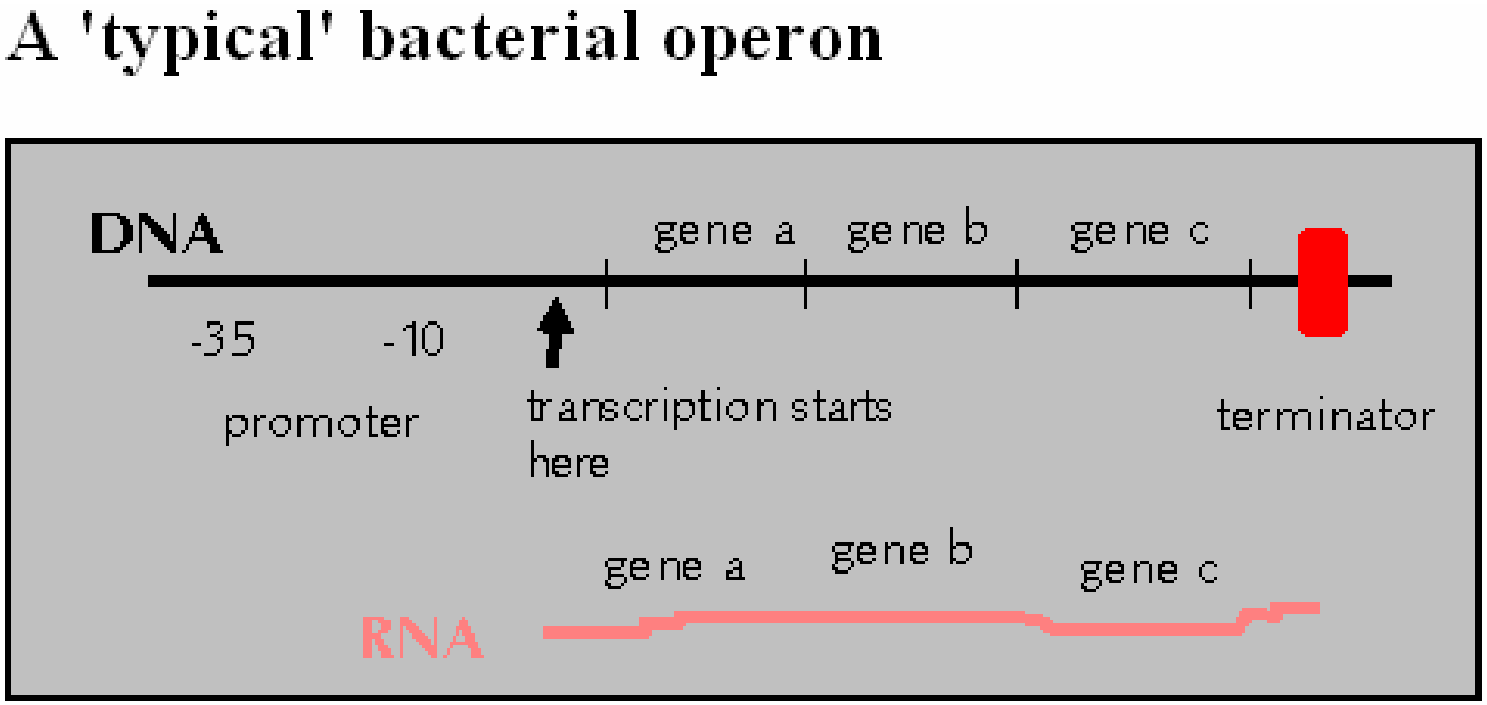
Intergenic Region

Gene

Intergenic Region

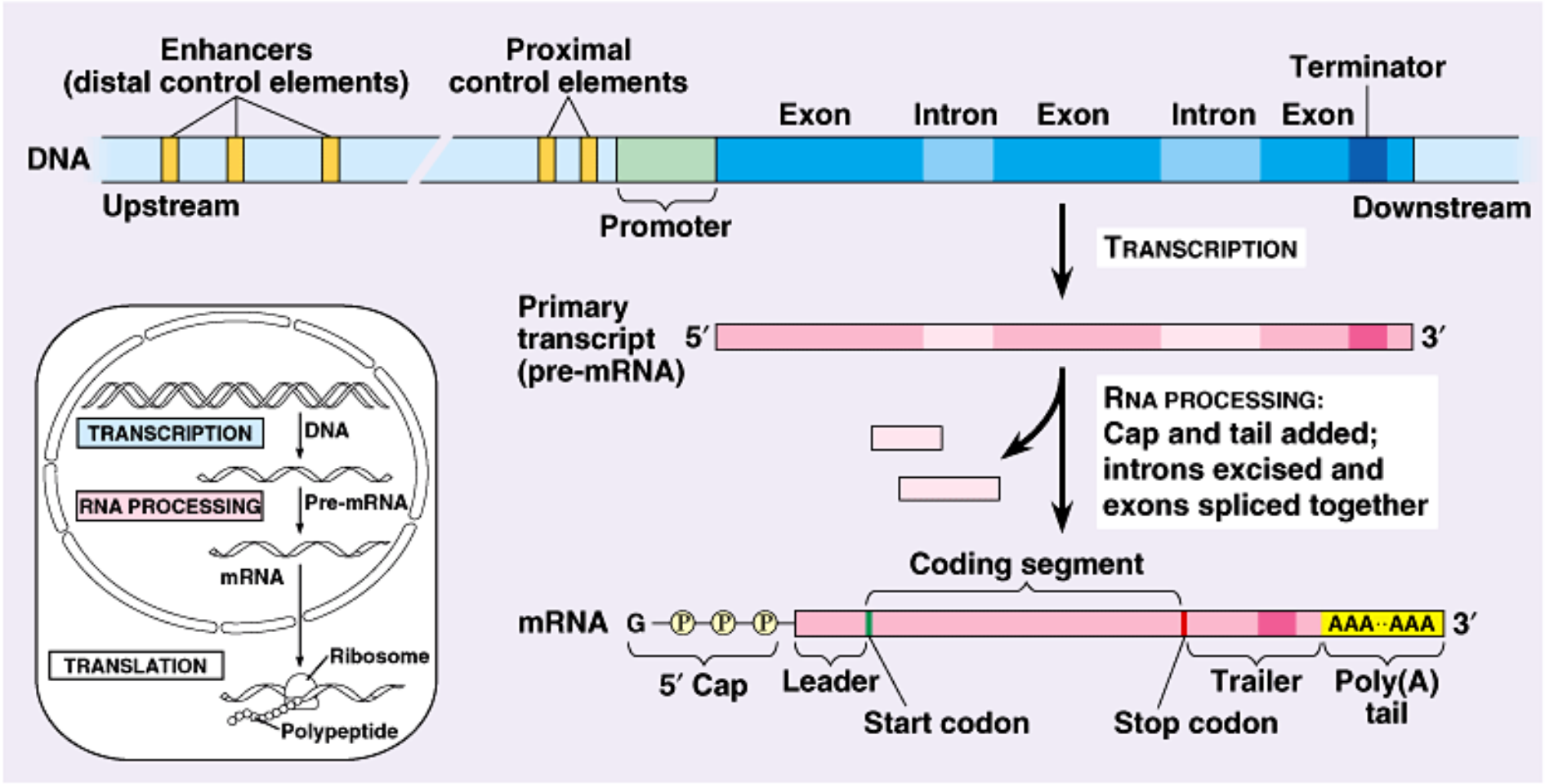
Gene

Prokaryotic Gene Structure and Transcript Processing



http://pps00.cryst.bbk.ac.uk/course/section6/henryb/genestrp.htm

Eukaryotic Gene Structure and Transcript Processing



Structural Annotation: Finding the Genes in Genomic DNA

Two main types of data used in defining gene structure:

Prediction based: algorithms designed to find genes/gene structures based on nucleotide sequence and composition

Sequence similarity (DNA and protein): alignment to mRNA sequences (ESTs) and proteins from the same species or related species; identification of domains and motifs

Finding Genes (ORFs)

Gene finders a programs that can identify genes computationally

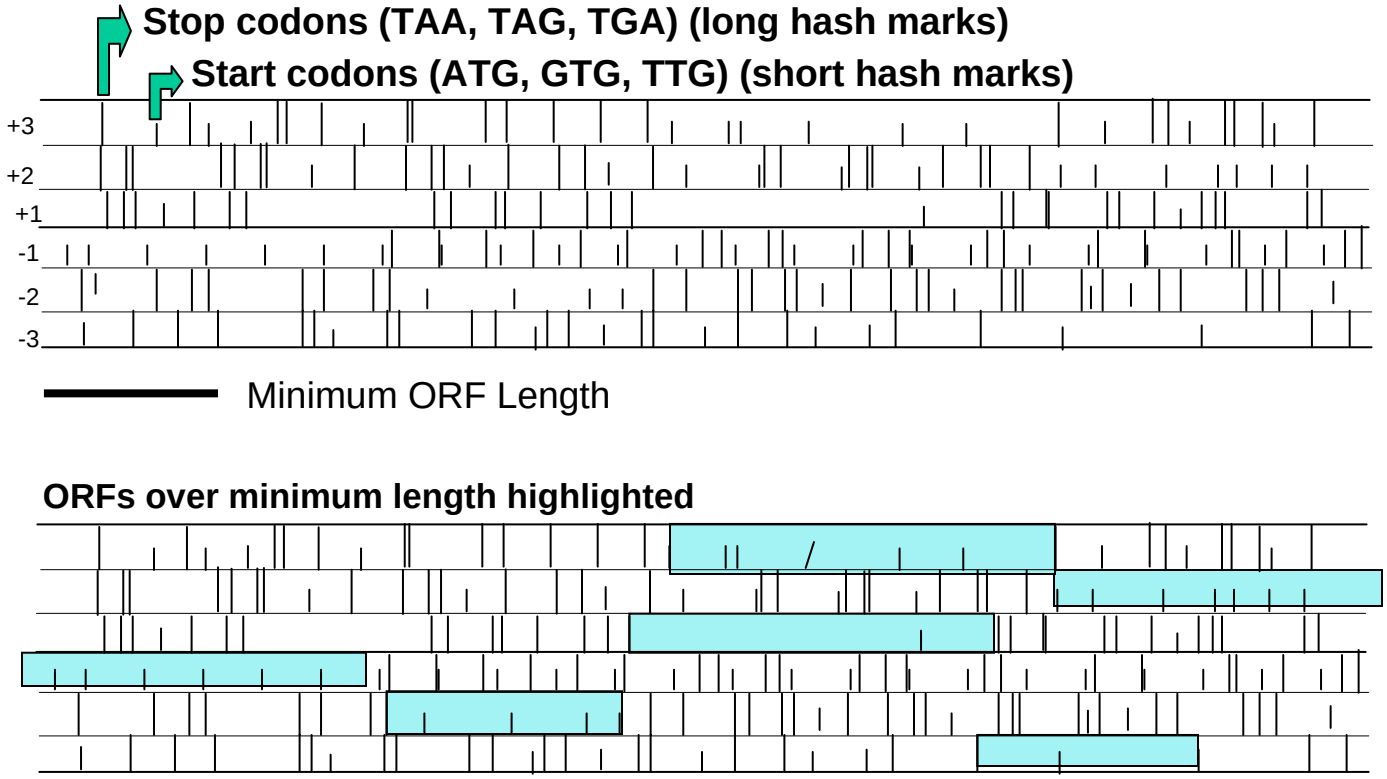
Running a Gene-finder
is a two-part process

1) Train Gene finder for the organism you have sequenced.

2) Run the trained Gene finder on the completed sequence.

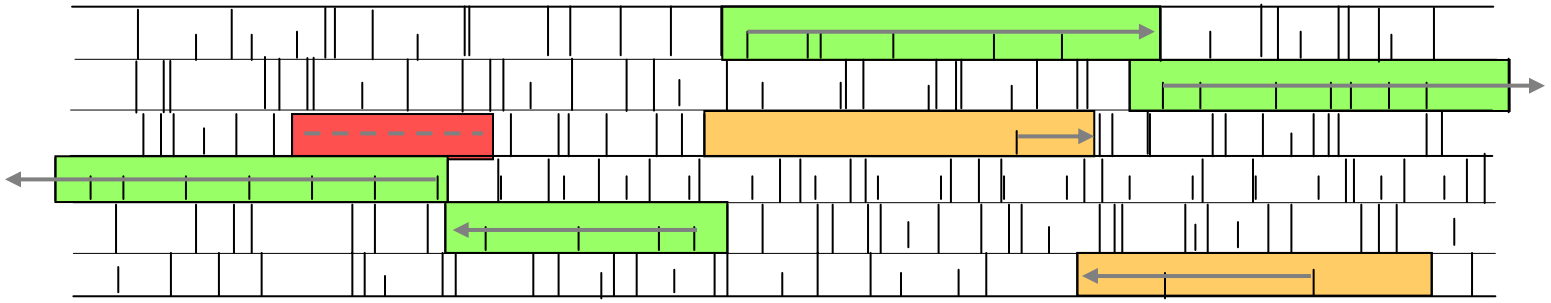
Candidate Genes

6-frame ORF map

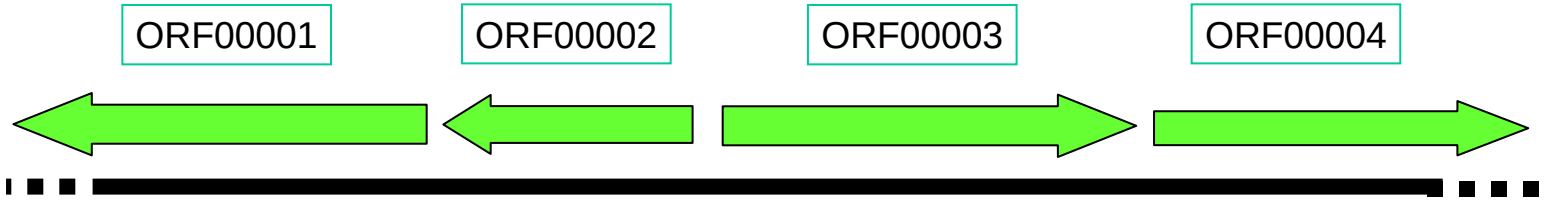


Annotating ORFs

Possible translations represented by arrows, moving from start to stop, the dotted line represents an ORF with no start site.



Glimmer chooses the set of likely genes.



Eukaryotic Gene Finding

Identifying the protein coding region of genes

AAAGCATGCATTTAACGAGTGCATCAGGACTCCATACGTAATGCCG

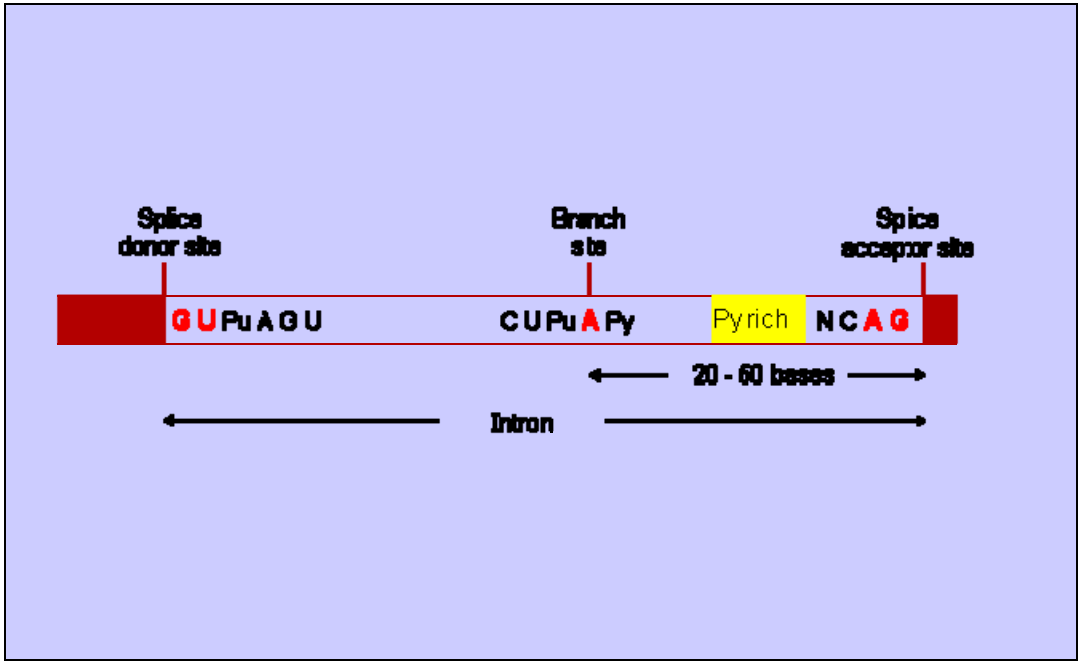
Gene finder (many different programs)

AAAGC ATG CAT TTA ACG A GT GCATC AG **GA CTC CAT ACG TAA TGCCG**

*This is a eukaryotic gene as evidenced by the intron

Signals Within DNA

- Splice sites to identify intron/exon junctions
- Transcription start and stop codons
- Promoter regions
- PolyA signals



Experimental Evidence

DNA sequence evidence: Transcript sequence (EST, full length cDNA, other expression types); more restrictive in evolutionary terms

Protein Evidence: alignment to protein that suggests structural similarity at the amino acid level; can be more distant evolutionarily

Experimental Evidence

Transcript evidence:

-Demonstrates gene is transcribed

-Delineates exon boundaries

-Defines splice sites and alternative transcripts

-If EST based, indicates expression patterns

Functional Assignments

Name

Descriptive common name for the protein, with as much specificity as the evidence supports; gene symbol.

Role

Describe what the protein is doing in the cell and why.

Associated information:

Supporting evidence:Domain and motifs
EC number if protein is an enzyme.
Paralogous family membership.

Evidence for Gene Function

- PROSITE Motifs
  - collection of protein motifs associated with active sites, binding sites, etc.
  - help in classifying genes into functional families when HMMs for that family have not been built

- InterPro
  - Brings together HMMs (both TIGR and Pfam) Prosite motifs and other forms of motif/domain clustering
  - Results in motif “signatures” for families or functions
  - GO terms have been assigned to many of these

Sequence Alignments

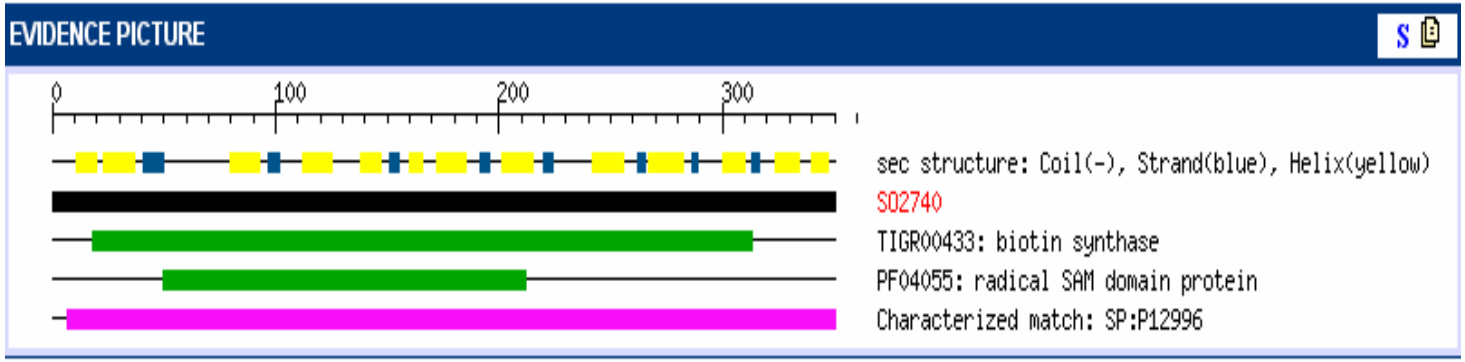
Compare sequence against other databases

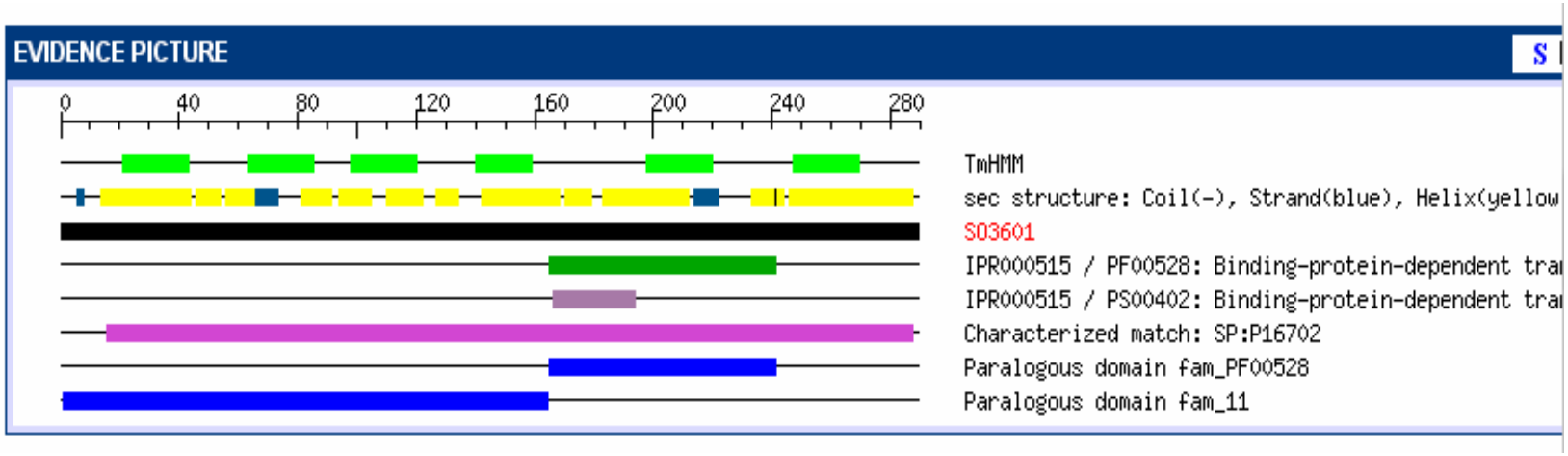
66.0/79.7% over 342aa | *Escherichia coli*

- EGAD|12981| biotin synthase Insert characterized
- OMNI|NTL01EC00751 biotin synthesis, sulfur insertion? Insert characterized
- SP|P12996|BIOB_ECOLI BIOTIN SYNTHASE (EC 2.8.1.6) (BIOTIN SYNTHETASE). Edit chara
- GP|1786992|gb|AAC73862.1||AE000180 biotin synthesis, sulfur insertion? {Escherichia coli K12} Inse
- GP|490219|emb|CAA00965.1||A11530 BIOB Insert characterized
- GP|2294845|emb|CAA02325.1||A38246 BIOTIN SYNTHASE Insert characterized
- GP|6741863|emb|CAB69590.1||A93674 BIOTIN SYNTHASE Insert characterized
- PIR|JC2517|SYECBB biotin Insert characterized

```
EGAD|12981|EC0775(4 - 346 of 346) biotin synthase (Escherichia coli)OMNI|NTL01EC0
%Match = 42.3
%Identity = 66.0  %Similarity = 79.7
Matches = 227  Mismatches = 69  Conservative Sub.s = 47
Gaps = 1  InDels = 3  Frame Shifts = 0
Primary Frame = 1 [343, 0, 0]

tcctgtgcccacgcacgctgccacggcgttataggatgctcacacgatgtacagtagattggactcttgtagtgcatttc
gaaatagagctccggtgagtcgaaataacggaagcaaggaagaagtagcaatccttaaacttataagtctctagtgtcac
ctaatcctaatatcctgctacaagagcgaggtgtcatgctatataatcaagcaggtataagacgtcgggatagggacga
      -81       -71       -61       -51       -41       -31       -21       -11
CHQ*VYGHRPIPARSLGHCVPRKQEVHESWRYKGAKYGRYQSQNRVNCA*KLTALIEN*SVVNLYHWLALTV*GLRLS*P



ataaaaagttatctcgccgtacggaggttgccaagtttagcaaccggtgcaggcaactttaaaggtcggtattccagctg
gaacataggctcatatgaagagaatacttctctaatttacagtagaaaacaatatggttctacgcgcaagaagcagcgaa
gataaaagtaggggattttgggaacacatggggtcaatacctcctagcttcaggcccaggcatgtttgttattggtgtcc
      -1        10        20        30        40        50        60        70
R*NTKIKGCSMSQLQVRHDWKREEIEALFALPMNDLLFKAHSIHREEYDPNEVQISRLLSIKTGACPEDCKYCPQSARYD
              :   |   |    ::   ||   |: ||||:|: :|||: :|| :|||| ||||||||||||||||||:||
             MAHRPRWTLSQVTELFEKPLLDLLFEAQQVHRQHFDPRQVQVSTLLSIKTGACPEDCKYCPQSSRYK
                      10        20        30        40        50        60

agcgagcctgagagcaggcagaggggtcttagggtcacagagactcacagcggagcgagataatgataggcgagtggggc
cgtaaagttctacttcacggcaccgccgtgtgccggacaaaatcataattaatactgtacgtctgttgcaacaatcacgt
tctaagtcaggacgccagtcgaggctgtctgcctgtcgatatgaccgaggaggacccgactgcaggatcgactggcaact
      80        90        100       110       120       130       140       150
TGLEKERLLAMETVLTEARSAKAAGASRFCMGAAWRNPKDKDMPYLKQMVQEVKALGMETCMTLGMLSAEQANELAEAGL
|||| |||: :| ||  || ||||||::|||||||||:|| ::|||||:|||| ||||:|:| ||||| ||  ||  || |||
TGLEAERLMEVEQVLESARKAKAAGSTRFCMGAAWKNPHERDMPYLEQMVQGVKAMGLEACMTLGTLSESQAQRLANAGL
            80        90        100       110       120       130       140

gttacatgatcgttgggaaacatcactgatacgcgtgaagttggaggaggagagaggttcccgatccccgtgcaaatgag
aaaaaatacccaaagattccgcaaagtactgatgccgtatgcggttgtgaaccagcgttaatcatcaacactctatttat
ctccctatcgtaccctgccctctaccatcactgcagcgatctcctccgcggttcactaaaagttacgtgttggctgacaa
      160       170       180       190       200       210       220       230
DYYNHNLDTSPEYYGDVITTRTYQNRLDTLSHVRASGMKVCSGGIVGMGEKATDRAGLLQQLANLPQHPDSVPINMLVKV
||||||||||||:||::|||||||| |||||  || :|:|||||||||:||  |||||| |||||| |:||||||||||
DYYNHNLDTSPEFYGNIITTRTYQERLDTLEKVRDAGIKVCSGGIVGLGETVKDRAGLLLQLANLPTPPESVPINMLVKV
          160       170       180       190       200       210       220

ggactgacggtgccgtgcaagggcatacctcgcttggcgaaaggccgatttgggatattgtatcaacacggaggagttcc
cgcctaataatactattgctctcgtttctcgtgtccggaatgaatactgttcgcacttaggattcccacaagaatgttgg
gtcctaatttatacgtcacccggttagagggggtacactatgctaggcgtctgcgcgttcctgagcgcccaatttgggctc
      240       250       260       270       280       290       300       310
AGTPFEKLDDLDPLEFVRTIAVARILMPLSRVRLSAGRENMSDELQAMCFFAGANSIFYGCKLLTTPNPEESDDMGLFRR
|||:   ||:| ::|:|||||||:|| | |||||||| |::: ||||| |||||||||||||||||||||  |: |||:
KGTPLADNDDVDAFDFIRTIAVARIMMPTSYVRLSAGREQMNEQTQAMCFMAGANSIFYGCKLLTTPNPEEDKDLQLFRK
          240       250       260       270       280       290       300

cgtccgcggggtagggcggtgagggtcgagtgcttggggctcacagagacgttgtttgactaaacagttgttgtgaatga
tgtgcaagcccctaaaacttcacccaaaacccataaccctacattcctatcctatgttatggcacatgggaggccgtgct
gtactggcacctttgagaatatgttatatatgtttggaaaaattcatacatgcgcctggacatcaaacggaccgataacc
      320       330       340       350       360       370       380       390
LGLRPEQGAAASIDDEQAVLAKAAAYQDKASAQFYDAAAL*PKLIATVKLASLDLCFVKL*STNPKV*CG**GSARI*AI
||| |:| |  : |:||    : |      : ::|:||||
LGLNPQQTAVLAGDNEQQQRLEQA-LMTPDTDEYYNAAAL
          320       330       340
```

Gene function evidence





Functional Annotation: Gene Product Names

Gene Name Assignment: Based on similarity to known proteins in nraa database

Categories:

Known or Putative: Identical or strong similarity to documented gene(s) in Genbank or has high similarity to a Pfam domain; e.g. kinase, Rubisco

Expressed Protein: Only match is to an EST with an unknown function; thus have confirmation that the gene is expressed but still do not know what the gene does

Hypothetical Protein: Predicted solely by gene prediction programs and matches another hypothetical or expressed protein

Hypothetical Protein: Predicted solely by gene prediction programs; no database match

Annotation example

- A good example of this is seen with transporters, what you'll see:
  - Multiple hits to a specific type of transporter
  - -The substrate identified for the proteins your protein matches are not all the same, but fall into a group, for example they are all sugars.

- Give the protein a name with specific function but a more general substrate specificity:
  - "sugar ABC transporter, permease protein"

- Sometimes it will not be possible to identify particular substrate group, in that case:
  - "ABC transporter, permease protein"

Automated Annotation is Not a Solved Problem

What you are getting is output from a series of prediction tools or alignment programs

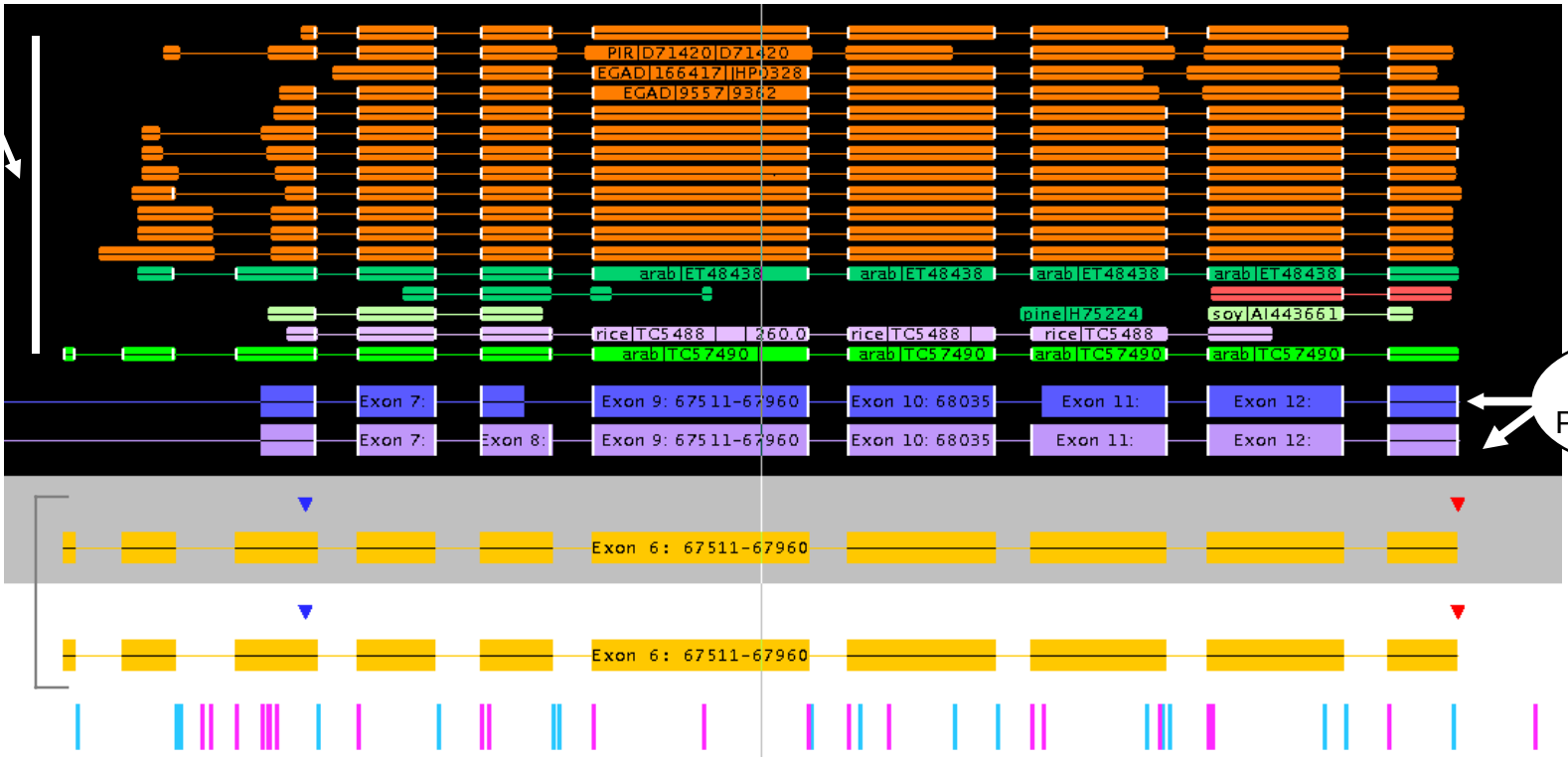
- Manual curation is often used to assess various types of evidence and improve upon automated gene calls and alignment output

- Ultimately, experimental verification is the only way to be sure that a gene structure is correct

Structural Annotation: Graphic Viewer Annotation Station

Sequence Database Hits
Top: Protein matches
Bottom: EST matches

Not shown graphically: gene name, nucleotide and protein sequence, MW, pI, organellar targeting sequence, membrane spanning regions, other domains.



Gene Predictions

Annotated Gene
Top: editing panel
Bottom: final curation

Splice site predictions:
red: acceptor sites
blue: donor sites

Screenshot of a component within Neomorphic's annotation station: www.neomorphic.com

Features Typically Resolved During Manual Annotation

- incorrect exon boundaries
- merged, split, missing genes
- missing untranslated regions (UTRs)
- missing alternative splicing isoform annotations
- degenerate transposons annotated as protein-coding genes

Increasing Complexity of Genome Annotation

	# Genes	bp
Mycoplasma pulmonis	780	964,000
Escherichia coli K-12	4,300	4,641,000
Saccharomyces cerevisiae	6,300	12,100,000
Plasmodium falciparum	5,400	22,850,000
Caenorhabditis elegans	19,000	97,000,000
Drosophila melanogaster	16,000	120,000,000
Arabidopsis thaliana	25,000	115,400,000
Fugu rubripes	35,000	365,000,000
Homo sapiens	30,000	2,910,000,000

Decrease in gene density and the presence of more, larger introns

Caveats of Genome Annotation

-Greatly impacted by the quality of the sequence; the impact of draft sequencing on whole genome annotation has yet to be seen by Joe/Jane Scientist. There will be disappointment when the research communities realize that they don't have the "gold" standard of sequence as present in Arabidopsis and rice.

-Annotation is challenging, highly UNDER-estimated in difficulty, highly UNDER-valued until a community goes to use its genome sequence

-Annotation can be done to high accuracy on a single gene level by single investigators with expertise in gene families. The challenge is how to extrapolate this to the whole genome

-Blends of automated, semi-automated, and manual annotation is perhaps the best way to approach genomes in which there are not large communities

-Iterative, never perfect, can always be improved with new evidence and improved algorithms