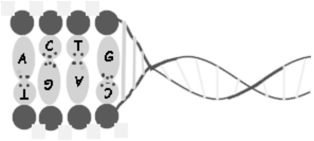
Bio2

Gene and Protein Prediction

Gene prediction

- What is a gene?
  - Simple definition: A stretch of DNA that encodes a protein and includes the regulatory sequences required for temporal and spatial control of gene transcription.
- Characteristics of genes.
  - What genetic features can we use to recognise a gene?

DNA structure



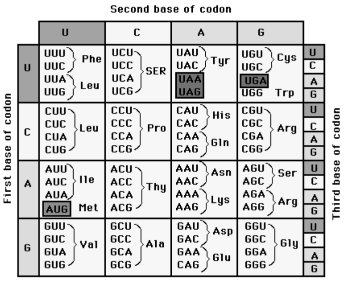
Bases: A,C,G and T

Chemically, A can only pair with T and G with C

Two strands, 5' and 3' Genes are encoded along one side of the DNA molecule. The 5' end being at the left hand side of the gene.

Codons and ORFs

- Three bases that encode an amino acid or stop site.
- A run of valid codons is an Open Reading Frame.
- An ORF usually starts with a Met
- Ends with a nonsense or stop codon.



Second base of codon

First base of codon	U	C	A	G	Third base of codon
U	UUU } Phe UUC UUA } Leu UUG	UCU } SER UCC UCA UCG	UAU } Tyr UAC UAA UAG	UGU } Cys UGC UGA UGG Trp	U C A G
C	CUU } Leu CUC CUA CUG	CCU } Pro CCC CCA CCG	CAU } His CAC CAA } Gln CAG	CGU } Arg CGC CGA CGG	U C A G
A	AUU } Ile AUC AUA AUG Met	ACU } Thy ACC ACA ACG	AAU } Asn AAC AAA } Lys AAG	AGU } Ser AGC AGA } Arg AGG	U C A G
G	GUU } Val GUC GUA GUG	GCU } Ala GCC GCA GCG	GAU } Asp GAC GAA } Glu CAG	GGU } Gly GGC GGA GGG	U C A G

The genetic code, written by convention in the form in which the Codons appear in mRNA. The three terminator codons, UAA, UAG, and UGA, are boxed in red; the AUG initiator codon is shown in green.

Predicting ORFs

- 64 total codons
- 3 stop codons, 61 codons for amino acids
- Random sequence 1:21 ratio for stop:coding.
- = 1 stop codon every 63 base pairs
- Gene lengths average around 1000 base pairs.

Finding ORFs

- One algorithm slides along the sequence looking stop codons.
- Scans back until it finds a start codon.
- Fails to find very short genes since it it looking for long ones
- Also fails to find overlaping ORFs
- There are many more ORFs than genes

Amino Acid Bias

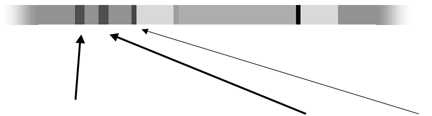
- The amino acids in proteins are not random
  - leucine has 6 codons
  - alanine has 4 codons
  - tryptophan has 1 codon
- The random the ratio would be 6:4:1
- In proteins it is 6.9:6.5:1
  - i.e. it is not random

Gene Prediction

- Take all factors into consideration
- Prokaryotes
  - No Nucleus
  - 70% of the genome encodes protein
  - No introns

Prokaryote gene structure

1. Promoter region



nnnTTGACAnnnnnnnnnnnnnnnnnnTATAATnnnnnnS

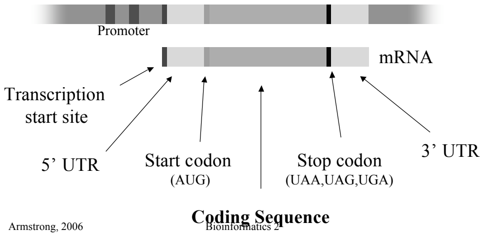
(consensus sequence for *E.coli*.)

Probability matrix for TATA box

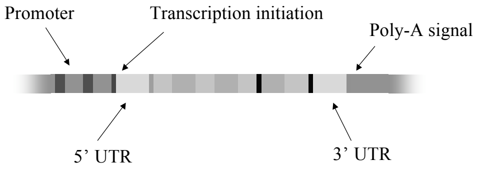
Pos.	1	2	3	4	5	6
A	2	95	26	59	51	1
C	9	2	14	13	20	3
G	10	1	16	15	13	0
T	79	3	44	13	17	96

Prokaryote gene structure

2. Transcribed region (mRNA)



Eukaryote gene structure



Promoter

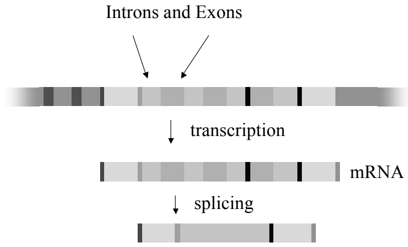
Transcription initiation

Poly-A signal

5’ UTR

3’ UTR

Eukaryote gene structure



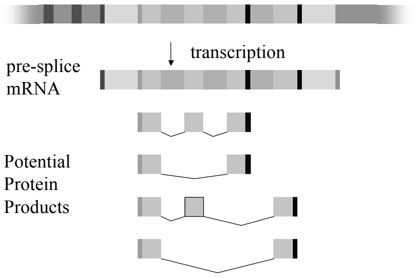
Introns and Exons

transcription

mRNA

splicing

Functional significance of Introns and Exons



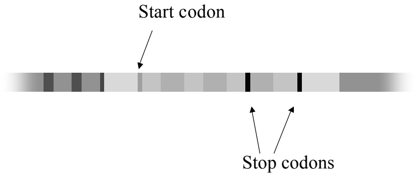
transcription

pre-splice mRNA

Potential Protein Products

1 gene - 4 protein products

Eukaryote gene structure



Start codon

Stop codons

Intron/Exon structure allows multiple start and stop codons

HMMs for codons

- Model based on examining 6 consecutive bases (i.e. all three reading frames).
- Based on statistical differences between coding and non coding regions
- 5th order Markov Model.
- Given 5 preceding bases, what is the probability of the 6th?
- Homogenous model (ignores reading frame)

HMMs for codons

- Homogenous models have two tables, one for coding, one non coding.
- Each table is has 4096 entries for the potential 6 base pair sequences
- Non-homogenous models have three tables for possible reading frames
- Short exons cause these models problems
- Hard to detect splice sites

Glimmer

- Uses non-homogenous HMMs to predict prokaryote gene sequences
- Identifies ORFs
- Trains itself on a prokaryote genome using ORFs over 500 bp
- http://www.cs.jhu.edu/labs/compbio/glimmer.html

Predicting Splice Sites

- There are some DNA features that allow splice sites to be predicted
- These are often species specific
- They are not very accurate.

NetGene2

- Neural network based splice site prediction
- Trained on known genes
- Claims to be 95% accurate
- Human, C. elegans & Arabidopsis thaliana
- http://www.cbs.dtu.dk/services/NetGene2/

HMMgene

- Based on an HMM model of gene structure
- Predicts intron/exon boundries
- Predicts start and stop codons
- Known information can be added (e.g. from ESTS etc)
- Outputs in GFF format

GFF Format

- Exchange format for gene finding packages
- Fields are:
  - <seqname> name, genbank accession number
  - <source> program used
  - <feature> various inc splice sites
  - <start> start of feature

GFF Format

- <end> end of feature
- <score> floating point value
- <strand> +, - (or .. for n/a)
- <frame> 0,1 or 2

GenScan

- Probabilistic model for gene structure based on a general HMM
- Can model intron/exon boundries, UTRs, Promoters, polyA tails etc
- http://genes.mit.edu/GENSCAN.html

Given a new protein sequence…

- What is the function?
- Where is the protein localised?
- What is the structure?
- What might it interact with?

Given a new protein sequence…

- What is the function?
- Have we seen this protein or a very similar one before?
  - If yes then we can infer function, structure, localisation and interactions from homologous sequence.
- Are there features of this protein similar to others?

Protein Families

- Proteins are complex structures built from functional and structural sub-units
  - When studying protein families it is evident that some regions are more heavily conserved than others.
  - These regions are generally important for the structure or function of the protein
  - Multiple alignment can be used to find these regions
  - These regions can form a signature to be used in identifying the protein family or functional domain.

Protein Domains

- Evolution conserves sequence patterns due to functional and structural constraints.
- Different methods have been applied to the analysis of these regions.
- Domains also known by a range of other names:

motifs patterns prints blocks

Profiles

- Given a sequence, we often want to assign the sequence to a family of known sequences
- We often also want to assign a subsequence to a family of subsequences.

Profiles

- Examples include assigning a gene/protein to a known gene/protein family, e.g.
  - G coupled receptors
  - actins
  - globins

Profiles

- Also we may wish to find known protein domains or motifs that give us clues about structure and function
  - Phosphorylation sites (regulated site)
  - Leucine zipper (dna binding)
  - EGF hand (calcium binding)

Creating Profiles

- Aligning a sequence to a single member of the family is not optimal
- Create profiles of the family members and test how similar the sequence is to the profile.
- A profile of a multiply aligned protein family gives us letter frequencies per column.

Matching sequences to profiles

- We can define a distance/similarity cost for a base in each sequence being present at any location based on the probabilities in the profile.
- We define define costs for opening and extending gaps in the sequence or profile.
- Therefore we can essentially treat the alignment of a sequence to a profile as a pairwise alignment and use dynamic programming algorithms to find and score the optimal alignments.

Protein profiles

- Multiple alignments can be used to give a consensus sequence.
- The columns of characters above each entry in the consensus sequence can be used to derive a table of probabilities for any amino acid or base at that position.

Protein profiles

- The table of percentages forms a profile of the protein or protein subsequence.
- With a gap scoring approach - sequence similarity to a profile can be calculated.
- The alignment and similarity of a sequence / profile pair can be calculated using a dynamic programming algorithm.

Protein profiles

- Alternative approaches use statistical techniques to assess the probability that the sequence belongs to a family of related sequences.
- This is calculated by multiplying the probabilities for amino acid *x* occurring at position *y* along the sequence/profile.

Probabilistic models

- Protein sequences are over 300 ave length.
- Random amino acid probability is 0.05
- Multiplying low probabilities together can cause underflow errors.
- Move into log space:
  - Take the log of the probabilities and sum.

HMMs

- A hidden markov model (HMM) is a refinement of this approach:
- HMMs can be visualised as finite state machines with a begin and an end state.
- FSMs move through a series of state emitting some kind of output report either at the end or during a transition from one state to another.

Protein profile HMMs

- In the profile of a protein sequence, there are effectively 3 states the model can be in:
  - 1. Match (exact or substitution)
  - 2. Insertion
  - 3. Deletion

Scoring profile HMMs

- The score of a sequence is the product of the probabilities that describe the path taken through the model used to recreate the sequence.
- Again, a log transformation allows the log of the probabilities to be summed rather than the probabilities multiplied.

Tools for HMM profile searches

- Meme and Mast at UCSD (SDSC)
- http://meme.sdsc.edu/
- MEME
  - input: a group of sequences
  - output: profiles found in those sequences
- MAST
  - input: a profile and sequence database
  - output: locations of the profile in the database

Summary

- Multiple alignment is used to define and find conserved features within DNA and protein sequences
- Profiles of multiply aligned sequences are a better description and can be searched using pairwise sequence alignment.
- Many different programs and databases available.

Secondary Databases

- PDB
- Pfam
- PRINTS
- PROSITE
- ProDom
- SMART
- TIGRFAMs

PDB

RCSB PDB PROTEIN DATA BANK

- Molecular Structure Database
- Contains the 3D structure coordinates of ‘solved’ protein sequences
  - X-ray crystallography
  - NMR spectra
- 29429 protein structures

Superfamily 1.65

HMM library and genome assignments server

SUPERFAMILY is a library of profile hidden Markov models that represent all proteins of known structure, based on SCOP.

The SCOP database aims to provide a detailed and comprehensive description of the structural and evolutionary relationships between all proteins whose structure is known (based on PDB)

Pfam

- Database of protein domains
- Multiple sequence alignments and profile HMMs
- Entries also annotated
- Swiss-Prot DB all pre-searched
- New sequences can be searched as well.
  - 7973 entries in Pfam last update

PRINTS

- Database of ‘protein fingerprints’
- Group of motifs that combined can be used to characterise a protein family
- ~11,000 motifs in PRINTS DB
- Provide more info than motifs alone

‘linear’ motifs

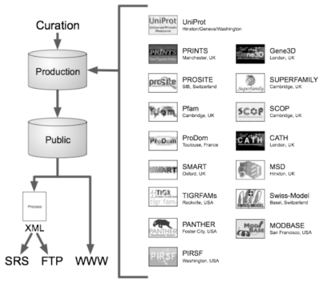
- Not all protein motifs are easy to find
- Linear motifs involved in protein-protein interactions
  - Very degenerate
  - Found in specific regions of proteins
  - Require special treatment
  - Neduva *et al*, PLOS 2005

Linking it all together…

- Database Searches
  - Multiple Alignments
  - Find known motifs and domains
  - Find possible similar folds
- Prediction algorithms
  - Properties of amino acids
  - Predicting folding
  - Finding cysteine bonds

InterPro

- EBI managed DB
- Incorporates most protein structure DBs
- Unified query interface and a single results output.



See http://www.ebi.ac.uk/interpro/

InterPro

DATABASE	VERSION	ENTRIES
SWISS-PROT	48	197228
PRINTS	38	1900
TREMBL	31.1	2342938
PFAM	18	7973
PROSITE	19.10	1882

Currently 15 databases, plans to add 3 new ones this month.

PredictProtein

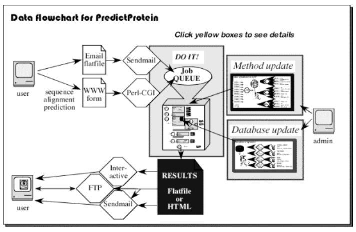
http://www.embl-heidelberg.de/predictprotein/

Database searches:
- generation of multiple sequence alignments (MaxHom)
- detection of functional motifs (PROSITE)
- detection of composition-bias (SEG)
- detection of protein domains (PRODOM)
- fold recognition by prediction-based threading (TOPITS)

PredictProtein

Predictions of:
- secondary structure (PHDsec, and PROFsec)
- residue solvent accessibility (PHDacc, and PROFacc)
- transmembrane helix location and topology (PHDhtm, PHDtopology)
- protein globularity (GLOBE)
- coiled-coil regions (COILS)
- cysteine bonds (CYSPRED)
- structural switching regions (ASP)

Data and methods in PredictProtein



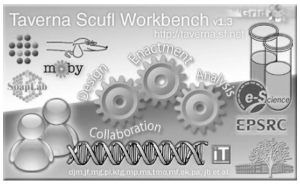
Add data and programs run at central site and updated on a regular basis

Too many programs/databases

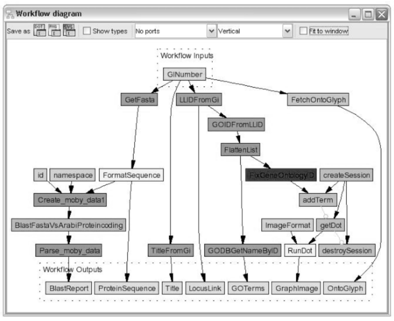
- How do we keep track of our own queries?
  - Repeat an old query
  - Run the same tests on a new sequence
  - Run 100s of sequences..
  - Document the process for a paper or client or for quality assurance

Workflow managers

- Locate and manage connections to software and databases
- Record actions
- Replay a workflow at a later date or against multiple sequences
- Manages redundant external sources (e.g. multiple blast servers)
- Can connect to specialist local sources



- http://taverna.sourceforge.net/
- Open source and free to download
- Runs on PC/linux/mac
- Drag-n-Drop interface to bioinformatics analysis



Example workflow from on-line taverna documentation

Given a new protein sequence…

- *What is the function?*
- *Where is the protein localised?*
- *What is the structure?*
- *What might it interact with?*

These are not fully solved problems. The latest issue of Bioinformatics (today) contains many new studies and tools addressing these problems.

Protein-Ligand interactions

- Most proteins do not act alone
- Most interact with other molecules
  - Other proteins
  - Small molecules
  - Drugs
- The shape and amino acid composition come together to form the site of interaction.
- ‘Grand Challenge’ in Bioinformatics: Can we accurately predict if two molecules will interact with each other based on sequence alone?

Walkinshaw - Feb 8th

- Rational Drug Design
  - Attempt to use shape models that include chemical binding sites to choose new drug candidates.
- Some pointers to protein structure prediction on the lecture notes web site.