Sequence Analysis

BBSI 2006: Lecture #(χ+1)

Takis Benos (2006)

Molecular Genetics 101

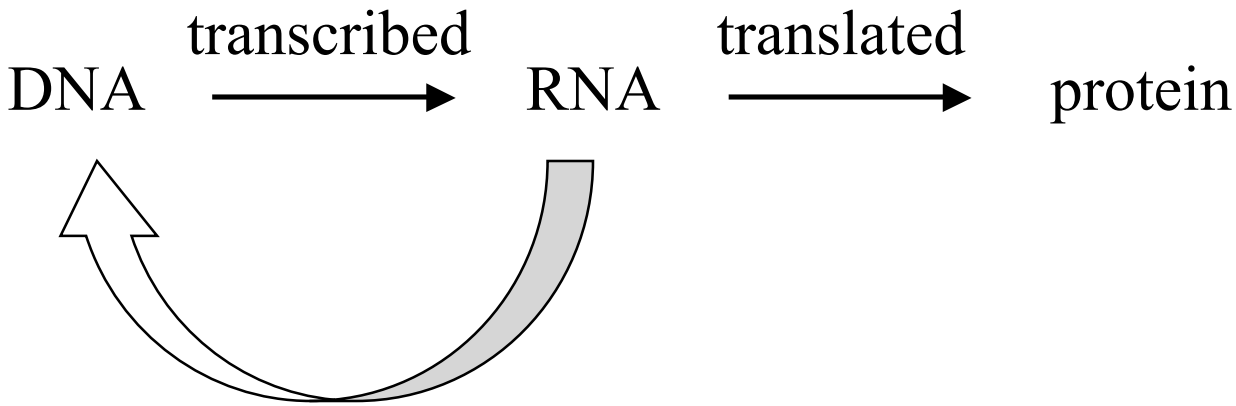
What is a "gene"?

- We cannot define it (but we know it when we see it…)
- A loose definition:

> "Gene" is a *DNA/RNA* ***information unit*** that is able to perform a function in a cellular environment

Protein coding genes

Central Dogma:

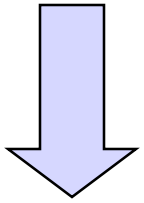


Open Reading Frames (ORFs)

```
aatagcgaat tttccaacga caaaagctaa atatcgcaaa aacctcagta aaaatcttgc  60
tggagctatt attgctaagt aacatttacc ccctgaagtt aatggatcaa tcaagagaga 120
tgtgggctgt aATGaatcgt cttattgaat taacaggttg gatcgttctt gtcgtttcag 180
tcattcttct tggcgtggcg agtcacattg acaactatca gccacctgaa cagagtgctt 240
cggtacaaca caagTAAgct ctgcacttgt ggagcgacat gctgcccgtc cgggtgcatg 300
ttttcacttg tcggatatta aaccaggaat ttattatctt gttcgatgtt gtaataaa   358
```

Open Reading Frames (ORFs)

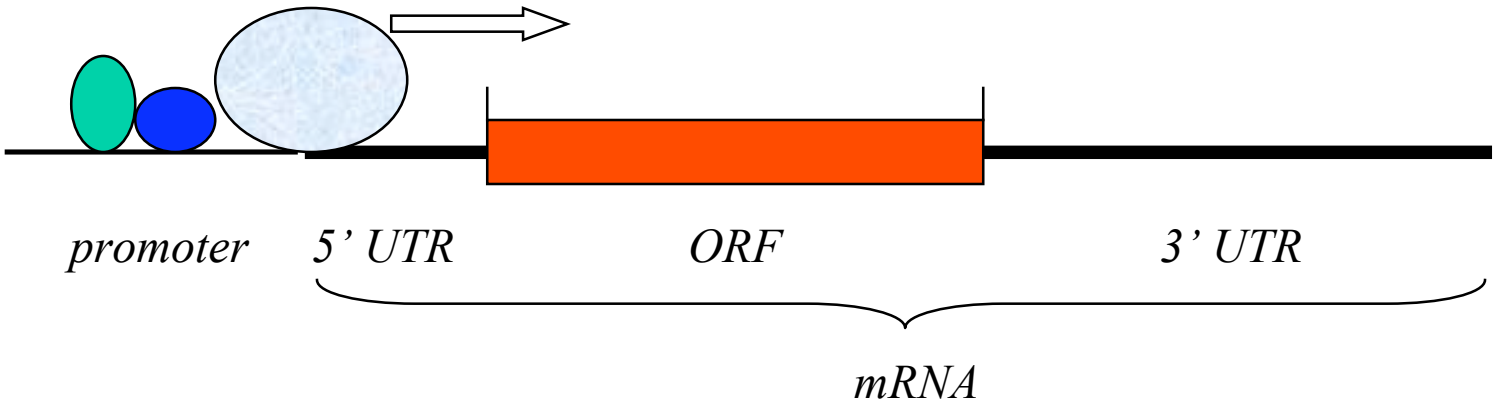
```
aatagcgaat tttccaacga caaaagctaa atatcgcaaa aacctcagta aaaatcttgc  60
tggagctatt attgctaagt aacatttacc ccctgaagtt aatggatcaa tcaagagaga 120
tgtgggctgt aATGaatcgt cttattgaat taacaggttg gatcgttctt gtcgtttcag 180
          M   N   R   L
tcattcttct tggcgtggcg agtcacattg acaactatca gccacctgaa cagagtgctt 240
cggtacaaca caagTAAgct ctgcacttgt ggagcgacat gctgcccgtc cgggtgcatg 300
          K   stop
ttttcacttg tcggatatta aaccaggaat ttattatctt gttcgatgtt gtaataaa   358
```



MNRLIELTGWIVLVVSVILLGVASHIDNYQPPEQSASVQHK

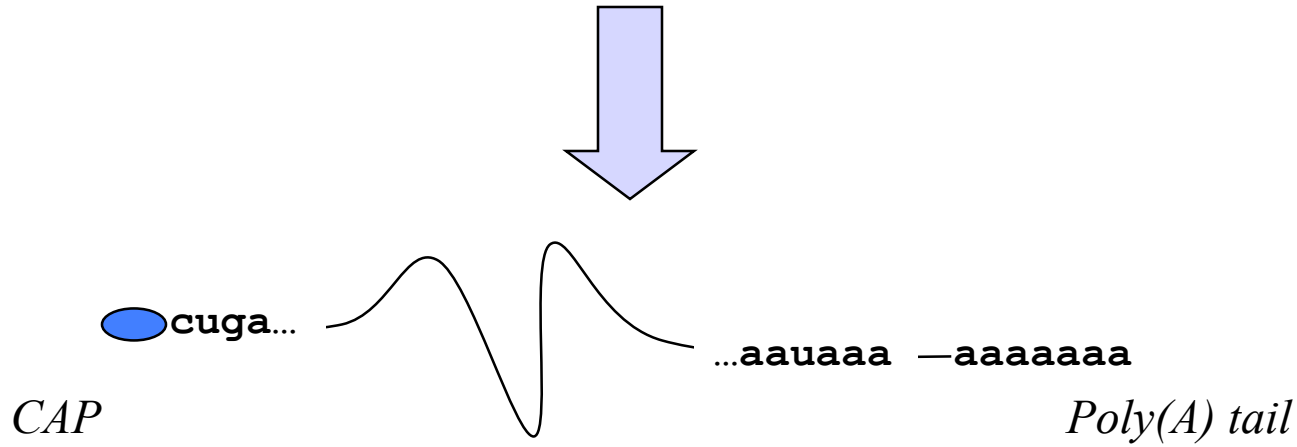
Gene's characteristics

```
aatagcgaat tttccaacga caaaagctaa atatcgcaaa aacctcagta aaaatcttgc  60
tggagctatt attgctaagt aacatttacc ccctgaagtt aatggatcaa tcaagagaga 120
tgtgggctgt aATGaatcgt cttattgaat taacaggttg gatcgttctt gtcgtttcag 180
tcattcttct tggcgtggcg agtcacattg acaactatca gccacctgaa cagagtgctt 240
cggtacaaca caagTAAgct ctgcacttgt ggagcgacat gctgcccgtc cgggtgcatg 300
ttttcacttg tcggatatta aaccaggaat ttattatctt gttcgatgtt gtaataaa   358
```

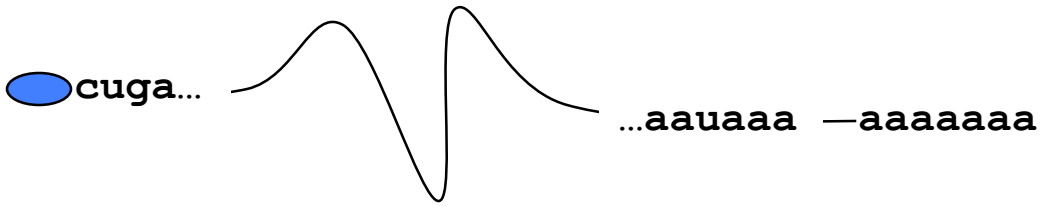


Transcription

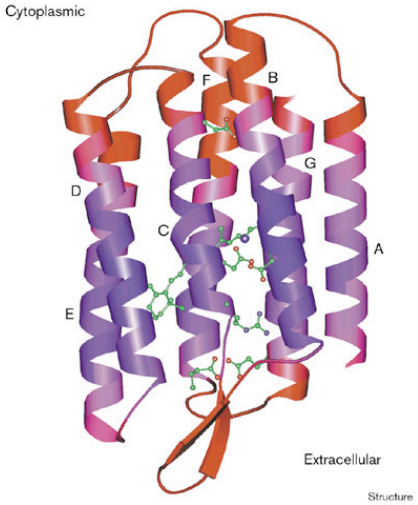
```
                                        cugaaguu aauggaucaa ucaagagaga 120
ugugggcugu aAUGaaucgu cuuauugaau uaacagguug gaucguucuu gucguuucag 180
ucauucuucu uggcguggcg agucacauug acaacuauca gccaccugaa cagagugcuu 240
cgguacaaca caagUAAgcu cugcacuugu ggagcgacau gcugcccguc cgggugcaug 300
uuuucacuug ucggauauua aaccaggaau uuauuaucuu guucgauguu guaauaaa   358
```



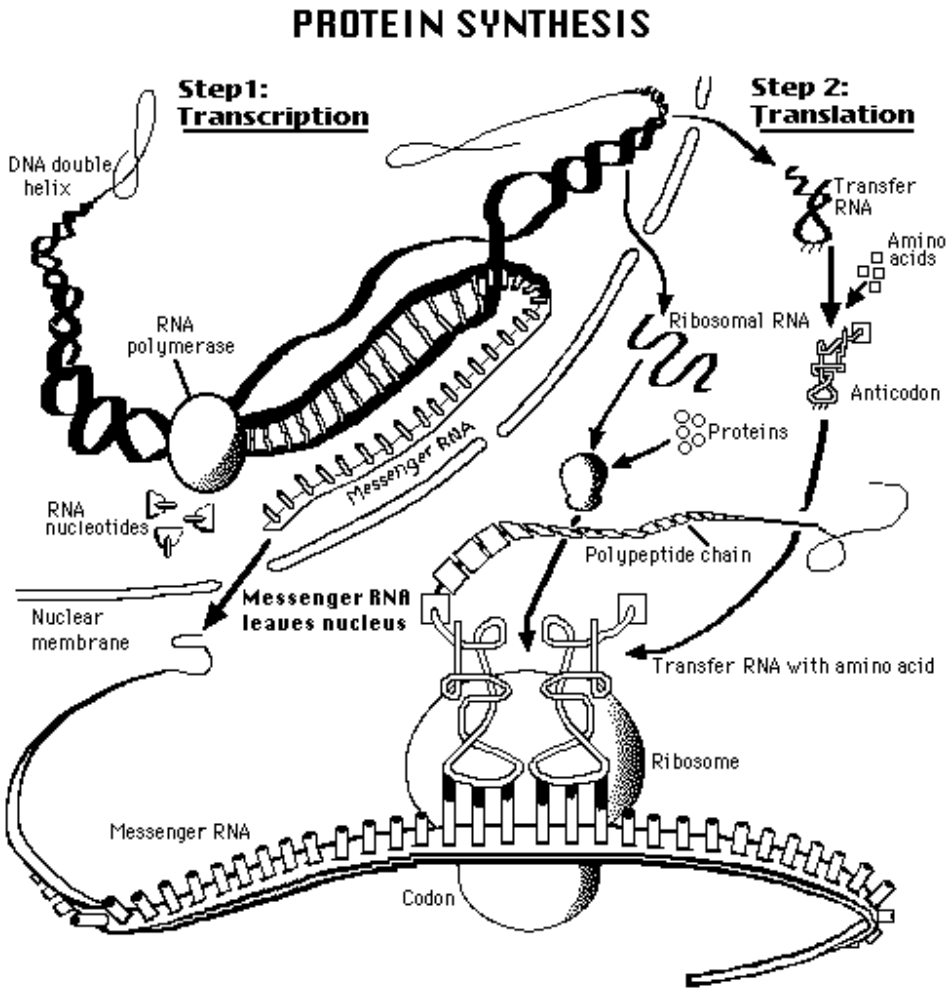
Translation



MNRLIELTGWIVLVVSVILLGVASHIDNYQPPEQSASVQHK



Protein coding genes (cntd)



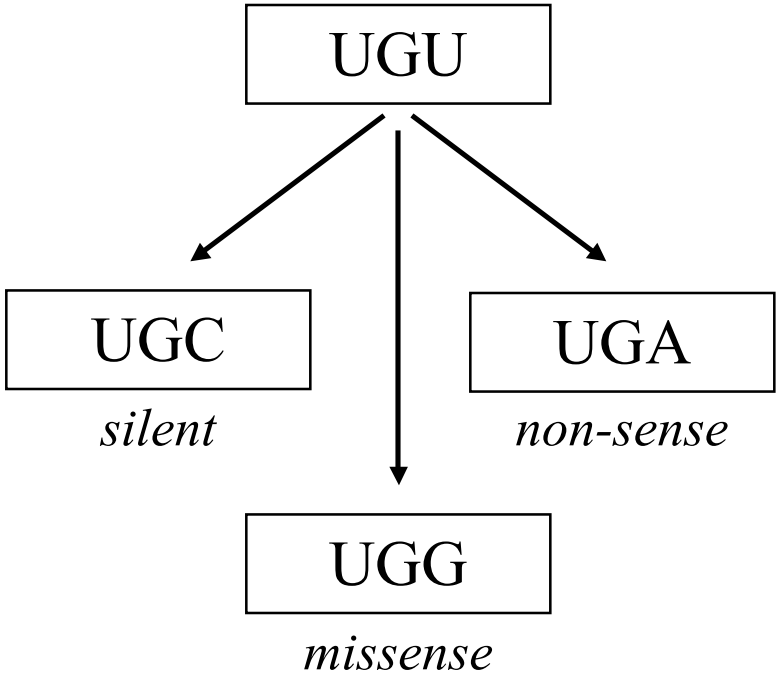
Source:
http://www.emc.maricopa.edu/faculty/farabee/BIOBK/BioBookPROTSYn.html

Alterations of the DNA

Base substitutions:

- silent: no a.a. replacement
- missense: a.a. replacement
- non-sense: a.a. → stop codon replacement

Alterations of the DNA (cntd)



Second letter

First letter	U		C		A		G		
U	UUU, UUC	Phenyl-alanine	UCU, UCC, UCA, UCG	Serine	UAU, UAC	Tyrosine	UGU, UGC	Cysteine	U C A G
	UUA, UUG	Leucine			UAA	Stop codon	UGA	Stop codon	
					UAG	Stop codon	UGG	Tryptophan	
C	CUU, CUC, CUA, CUG	Leucine	CCU, CCC, CCA, CCG	Proline	CAU, CAC	Histidine	CGU, CGC, CGA, CGG	Arginine	U C A G
					CAA, CAG	Glutamine			
A	AUU, AUC, AUA	Isoleucine	ACU, ACC, ACA, ACG	Threonine	AAU, AAC	Asparagine	AGU, AGC	Serine	U C A G
	AUG	Methionine; initiation codon			AAA, AAG	Lysine	AGA, AGG	Arginine	
G	GUU, GUC, GUA, GUG	Valine	GCU, GCC, GCA, GCG	Alanine	GAU, GAC	Aspartic acid	GGU, GGC, GGA, GGG	Glycine	U C A G
					GAA, GAG	Glutamic acid			

Source: http://www.emc.maricopa.edu/faculty/farabee/BIOBK/BioBookPROTSYn.html

Molecular evolution

Two species will acquire mutations proportionally to their divergence time. However:

- all proteins do not change in the same pace
- a given protein does not necessarily change in the same pace throughout time
- different parts of the same protein change at different paces

Molecular evolution (cntd)

Human (C11A_HUMAN; P05108) *vs.* Pig (C11A_PIG; P10612)

```
Query:   1   MLAKGLPPRSVLVKGYQTFLSAPREGLGRLRVPTGEGAGISTRSPRPFNEIPSPGDNGWL  60
             MLA+GL  RSVLVKG Q FLSAPRE  G  RV TGEGA IST++PRPF+EIPSPGDNGW+
Sbjct:   1   MLARGLALRSVLVKGCQPFLSAPRECPGHPRVGTGEGACISTKTPRPFSEIPSPGDNGWI  60

Query:  61   NLYHFWRETGTHKVHLHHVQNFQKYGPIYREKLGNVESVYVIDPEDVALLFKSEGPNPER 120
             NLY FW+E GT K+H HHVQNFQKYGPIYREKLGN+ESVY+IDPEDVALLFK EGPNPER
Sbjct:  61   NLYRFWKEKGTQKIHYHHVQNFQKYGPIYREKLGNLESVYIIDPEDVALLFKFEGPNPER 120

Query: 121   FLIPPWVAYHQYYQRPIGVLLKKSAAWKKDRVALNQEVMAPEATKNFLPLLDAVSRDFVS 180
             + IPPWVAYHQ+YQ+P+GVLLKKS AWKKDR+ LN EVMAPEA KNF+PLLD VS+DFV
Sbjct: 121   YNIPPWVAYHQHYQKPVGVLLKKSGAWKKDRLVLNTEVMAPEAIKNFIPLLDTVSQDFVG 180

Query: 181   VLHRRIKKAGSGNYSGDISDDLFRFAFESITNVIFGERQGMLEEVVNPEAQRFIDAIYQM 240
             VLHRRIK+ GSG +SGDI +DLFRFAFESITNVIFGER GMLEE+V+PEAQ+FIDA+YQM
Sbjct: 181   VLHRRIKQQGSGKFSGDIREDLFRFAFESITNVIFGERLGMLEEIVDPEAQKFIDAVYQM 240

Query: 241   FHTSVPMLNLPPDLFRLFRTKTWKDHVAAWDVIFSKADIYTQNFYWELRQKGSVHHDYRG 300
             FHTSVPMLNLPPDLFRLFRTKTW+DHVAAWD IF+KA+ YTQNFYW+LR+K    ++Y G
Sbjct: 241   FHTSVPMLNLPPDLFRLFRTKTWRDHVAAWDTIFNKAEKYTQNFYWDLRRKRE-FNNYPG 299

Query: 301   MLYRLLGDSKMSFEDIKANVTEMLAGGVDTTSMTLQWHLYEMARNLKVQDMLRAEVLAAR 360
             +LYRLLG+ K+  ED+KANVTEMLAGGVDTTSMTLQWHLYEMAR+L VQ+MLR EVL AR
Sbjct: 300   ILYRLLGNDKLLSEDVKANVTEMLAGGVDTTSMTLQWHLYEMARSLNVQEMLREEVLNAR 359

Query: 361   HQAQGDMATMLQLVPLLKASIKETLRLHPISVTLQRYLVNDLVLRDYMIPAKTLVQVAIY 420
              QAQGD + MLQLVPLLKASIKETLRLHPISVTLQRYLVNDLVLRDYMIPAKTLVQVA+Y
Sbjct: 360   RQAQGDTSKMLQLVPLLKASIKETLRLHPISVTLQRYLVNDLVLRDYMIPAKTLVQVAVY 419

Query: 421   ALGREPTFFFDPENFDPTRWLSKDKNITYFRNLGFGWGVRQCLGRRIAELEMTIFLINML 480
             A+GR+P FF +P  FDPTRWL K++++ +FRNLGFGWGVRQC+GRRIAELEMT+FLI++L
Sbjct: 420   AMGRDPAFFSNPGQFDPTRWLGKERDLIHFRNLGFGWGVRQCVGRRIAELEMTLFLIHIL 479

Query: 481   ENFRVEIQHLSDVGTTFNLILMPEKPISFTFWPFNQEATQ 520
             ENF+VE+QH SDV T FNLILMP+KPI   F PFNQ+  Q
Sbjct: 480   ENFKVELQHFSDVDTIFNLILMPDKPIFLVFRPFNQDPLQ 519
```

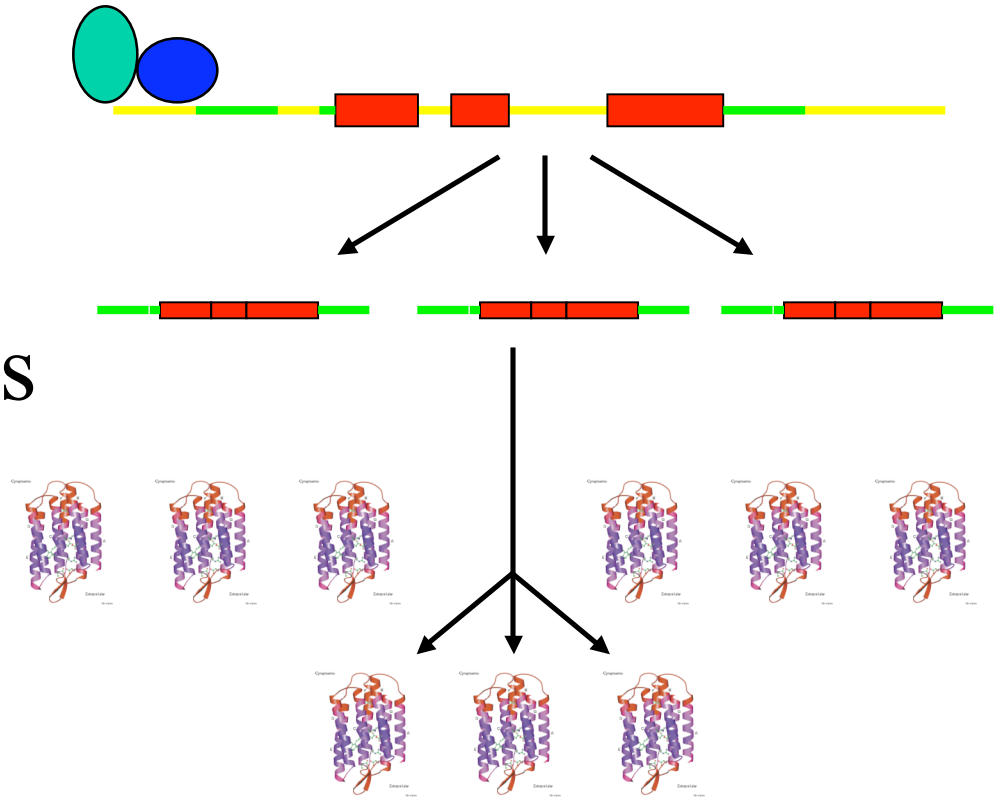
Molecular evolution (cntd)

Human (C11A_HUMAN; P05108) *vs.* zebrafish (Cyp11a1; Q8JH93)

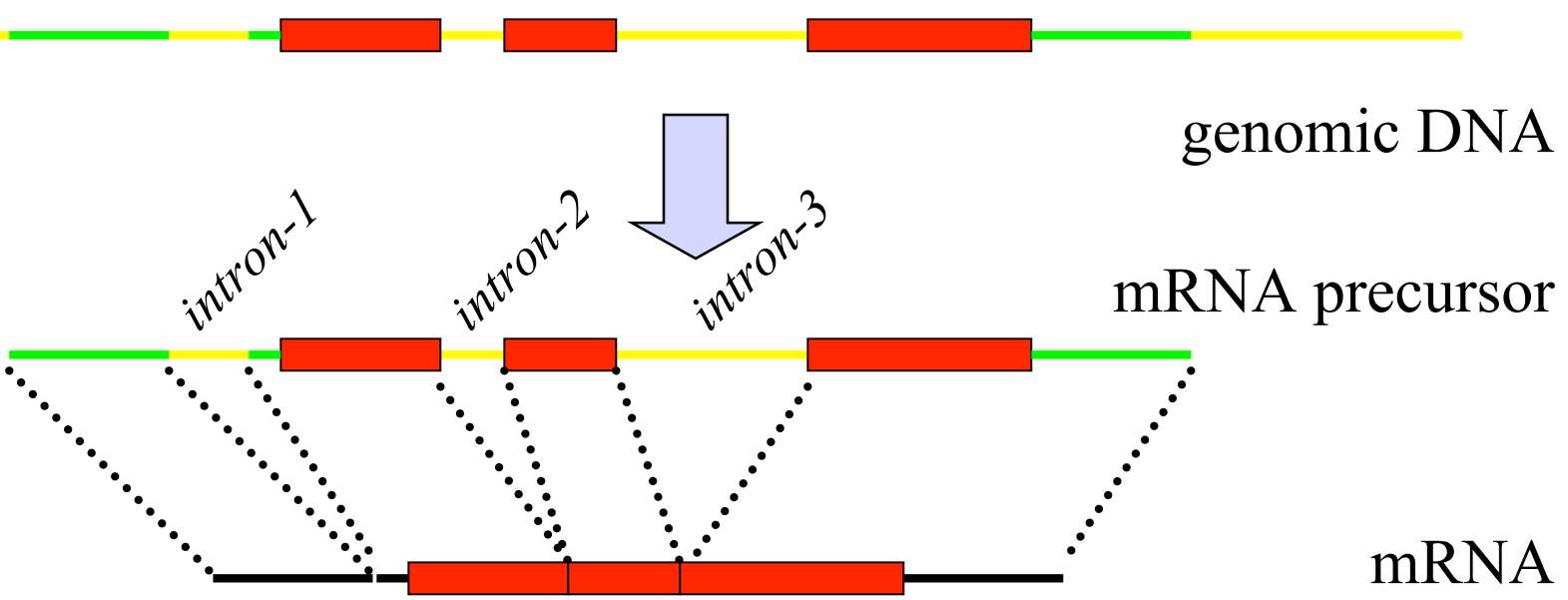
```
Query:  34  TGEGAGISTRSPRPFNEIPSPGDNGWLNLYHFWRETGTHKVHLHHVQNFQKYGPIYREKL  93
            T  G      + +PFN+IP    N  L++  F +  G   VH   V NF+ +GPIYREK+
Sbjct:  27  TRSGRAPQNSTVQPFNKIPGRWRNSLLSVLAFTKMGGLRNVHRIMVHNFKTFGPIYREKV  86

Query:  94  GNVESVYVIDPEDVALLFKSEGPNPERFLIPPWVAYHQYYQRPIGVLLKKSAAWKKDRVA 153
            G  +SVY+I PED A+LFK+EG +P R  +  W AY  Y  +  GVLLK+  AWK DR+
Sbjct:  87  GIYDSVYIIKPEDGAILFKAEGHHPNRINVDAWTAYRDYRNQKYGVLLKEGKAWKTDRMI 146

Query: 154  LNQEVMAPEATKNFLPLLDAVSRDFVSVLHRRIKKAGSGNYSGDISDDLFRFAFESITNV 213
            LN+E++ P+    F+PLLD V +DFV+ ++++I+++G   ++ D++ DLFRF+ ES++ V
Sbjct: 147  LNKELLLPKLQGTFVPLLDEVGQDFVARVNKQIERSGQKQWTTDLTHDLFRFSLESVSAV 206

Query: 214  IFGERQGMLEEVVNPEAQRFIDAIYQMFHTSVPMLNLPPDLFRLFRTKTWKDHVAAWDVI 273
            ++GER G+L + ++PE Q FID +  MF T+ PML LPP L R   +  WK+HV AWD I
Sbjct: 207  LYGERLGLLLDNIDPEFQHFIDCVSVMFKTTSPMLYLPPGLLRSIGSNIWKNHVEAWDGI 266

Query: 274  FSKADIYTQNFYWELRQKGSVHHDYRGMLYRLLGDSKMSFEDIKANVTEMLAGGVDTTSM 333
            F++AD   QN + + ++    +  Y G+L  LL   K+S EDIKA+VTE++AGGVD+ +
Sbjct: 267  FNQADRCIQNIFKQWKENPEGNGKYPGVLAILLMQDKLSIEDIKASVTELMAGGVDSVTF 326

Query: 334  TLQWHLYEMARNLKVQDMLRAEVLAARHQAQGDMATMLQLVPLLKASIKETLRLHPISVT 393
            TL W LYE+AR   +QD LRAE+ AAR  +GDM  M+++++PLLKA++KETLRLHP++++
Sbjct: 327  TLLWTLYELARQPDLQDELRAEISAARIAFKGDMVQMVKMIPLLKAALKETLRLHPVAMS 386

Query: 394  LQRYLVNDLVLRDYMIPAKTLVQVAIYALGREPTFFFDPENFDPTRWLSKDKNITYFRNL 453
            L RY+  D V+++Y IPA TLVQ+ +YA+GR+  FF  PE + P+RW+S ++   YF++L
Sbjct: 387  LPRYITEDTVIQNYHIPAGTLVQLGVYAMGRDHQFFPKPEQYCPSRWISSNRQ--YFKSL 444

Query: 454  GFGWGVRQCLGRRIAELEMTIFLINMLENFRVEIQHLSDVGTTFNLILMPEKPISFTFWP 513
            GFG+G RQCLGRRIAE EM IFLI+MLENFR+E Q   +V + F L+LMPEKPI  T  P
Sbjct: 445  GFGFGPRQCLGRRIAETEMQIFLIHMLENFRIEKQKQIEVRSKFELLLMPEKPIILTIKP 504

Query:  514 FN 515
            N
Sbjct:  505 LN 506
```

Gene expression regulation

- promoter region
- expression rates
- degradation
- post modifications

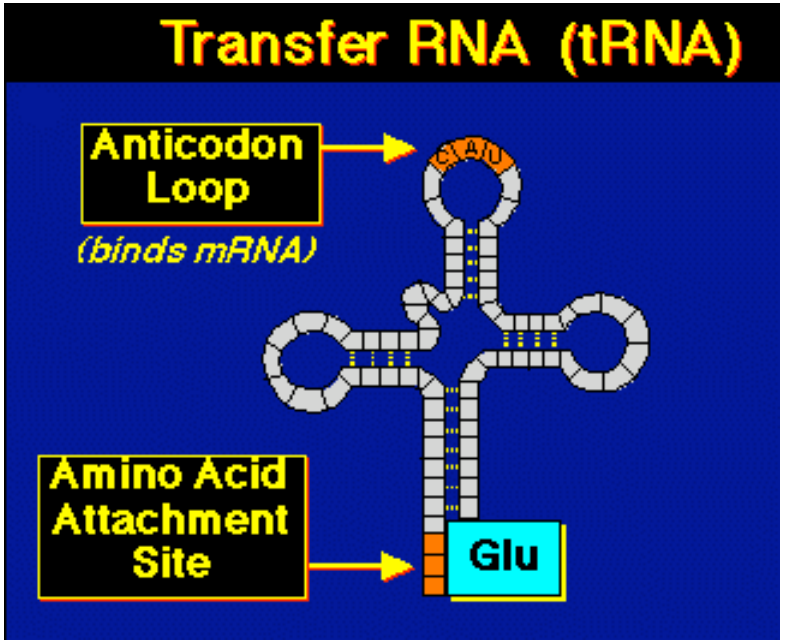


Splicing



Non-coding genes

- tRNA
- ribosomal RNA
- snoRNA
- microRNA
- etc



Source: http://www.emc.maricopa.edu/faculty/farabee/BIOBK/BioBookPROTSYn.html

Elements of Probability Theory (with examples)

Outline

- Conditional Probabilities
- Markov Chains
- Hidden Markov Models
- Information measures

Probabilities

Definition:

$$P(x) = \frac{\text{\# favourable outcomes } (x)}{\text{total \# possible outcomes}}$$

Conditional probabilities:

$$P(x|A) = \frac{\text{\# favourable outcomes (x) given A}}{\text{total \# possible outcomes given A}}$$

Conditional Probabilities

- *Joint probability*: $P(X,Y) = P(X|Y)\,P(Y)$
- If $P(X|Y) = P(X)$ then X,Y *independent*

$$P(X,Y) = P(X)\,P(Y)$$

- *Marginal probability:*

$$P(X) = \sum_{Y} P(X,Y) = \sum_{Y} P(X \mid Y)P(Y)$$

Conditional Probabilities (cntd)

- *Bayes' theorem*

$$P(X \mid Y) = \frac{P(Y \mid X)P(X)}{P(Y)} = \frac{P(Y \mid X)P(X)}{\sum_{x} P(Y \mid X)P(X)}$$

- *Posterior probabilities are the compromise between data and prior information.*

Bayes: Application-1

- Problem (from Durbin *et al.*, *1998*):

> A rare genetic disease is discovered with population frequency one in 1 million. An extremely good genetic test is 100% sensitive (always correct if you have the disease) and 99.99% specific (false positive rate 0.01%). Will you be willing to take such a test?

- *Hint:* What is the probability that you have the disease, if the test is positive?

Bayes: Application-1 (cntd)

- Answer:

$$P(D \mid +) = P(+ \mid D)\, P(D) / P(+) =$$

$$= 1.0 * 10^{-6} / [1.0 * 10^{-6} + 10^{-4} * (1 - 10^{-6})] =$$

$$= 0.0099$$

Application of Bayes-2

- Problem:

Given a set of transmembrane proteins with specified membrane domains of length *L (training set*), can you develop a probabilistic model that predicts which parts of a new transmembrane protein are likely to be membrane domains?

Application of Bayes-2 (cntd)

- Solution:
  - Suppose that we suspect that the amino acid frequencies differ between membrane and non-membrane regions.
  - Using the *training* set, calculate the probabilities, P(a_i|D), that each amino acid a_i is part of a membrane domain (D). Also, using the non-membrane parts, calculate the corresponding probabilities, P(a_i|not D).

Application of Bayes-2 (cntd)

- Solution *(cntd)*:
  - Divide the new protein into segments.
  - Using Bayes theorem, calculate the posterior probability of each segment being a membrane domain using the P(a_i|D).

$$P(X \mid M); M := \arg\max_M \frac{P(M \mid D)P(D)}{\sum_d P(M \mid d)P(d)}$$

Markov chains

- What is a Markov chain?

➢ Markov chain of order *n* is a stochastic process of a series of outcomes, in which the probability of outcome *x* depends on the state of the previous *n* outcomes.

Markov chains (cntd)

- Markov chain (of *first* order):

$$P(x) = P(X_L, X_{L-1}, \ldots, X_1) =$$
$$= P(X_L \mid X_{L-1}, \ldots, X_1) P(X_{L-1} \mid X_{L-2}, \ldots, X_1) \ldots P(X_1) =$$
$$= P(X_L \mid X_{L-1}) P(X_{L-1} \mid X_{L-2}) \ldots P(X_2 \mid X_1) P(X_1) =$$
$$= P(X_1) \prod_{i=2}^{L} P(X_i \mid X_{i-1})$$

- *Transition probabilities*: $P(X_i \mid X_{i-1})$

Application of Markov chains

- Problem (from Durbin *et al.*): CpG islands

Given two sets of sequences from the human genome, one with CpG islands and one without, can you calculate a model that can predict the CpG islands?

Application of Markov chains (cntd)

- Solution:

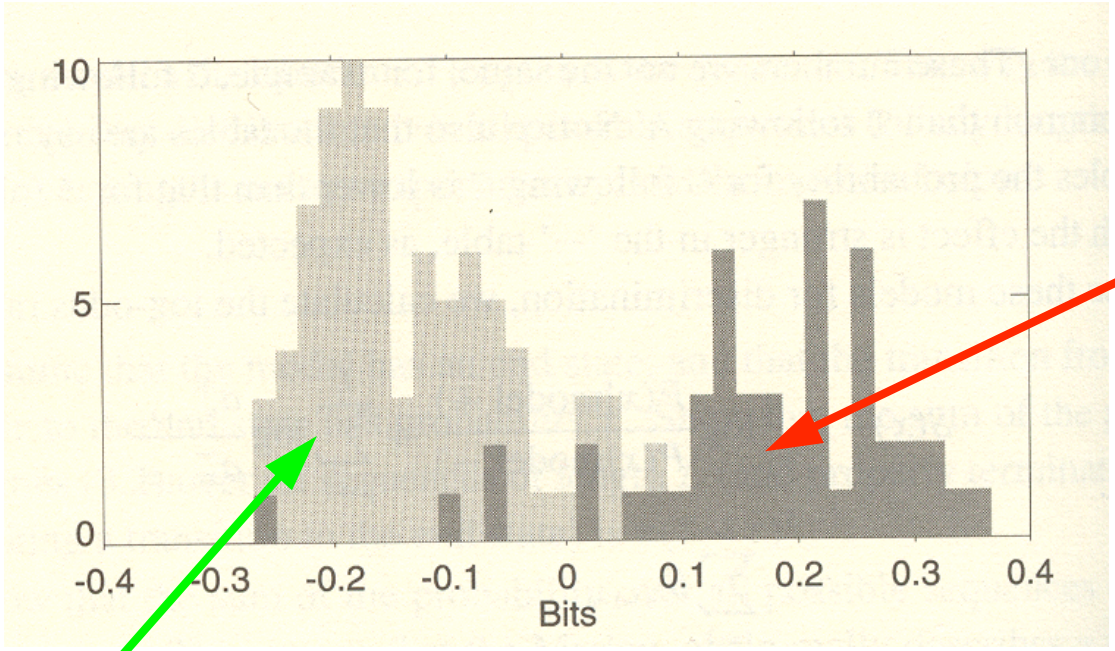
+	A	C	G	T
A	0.180	0.274	0.426	0.120
C	0.171	0.368	0.274	0.188
G	0.161	0.339	0.375	0.125
T	0.079	0.355	0.384	0.182

-	A	C	G	T
A	0.300	0.205	0.285	0.210
C	0.322	0.298	0.078	0.302
G	0.248	0.246	0.298	0.208
T	0.177	0.239	0.292	0.292

	A	C	G	T
A	-0.740	0.419	0.580	-0.803
C	-0.913	0.302	1.812	-0.685
G	-0.624	0.461	0.331	-0.730
T	-1.169	0.573	0.393	-0.679

Application of Markov chains (cntd)

- Histogram of scores (CpG islands):



Hidden Markov Models

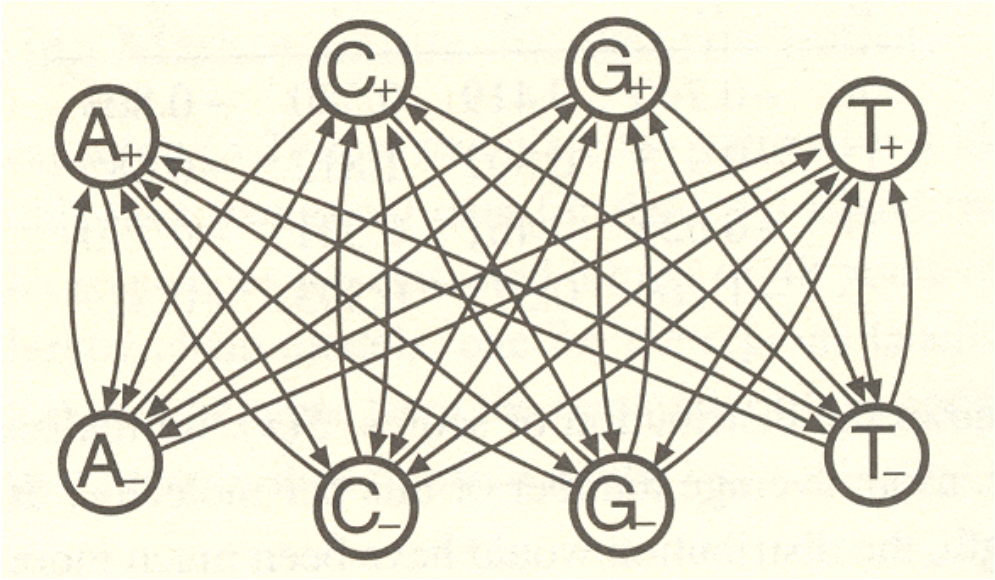
- What is a Hidden Markov Model?
- A Markov process in which the probability of an outcome depends also in a (hidden) random variable (state).
- *Transition* probability: the probability of reaching a state given the previous state.
- E*mission* probability: the probability of an outcome given the state.

Hidden Markov Models (cntd)

- Graphical representation of the HMM:

<u>CpG islands</u>

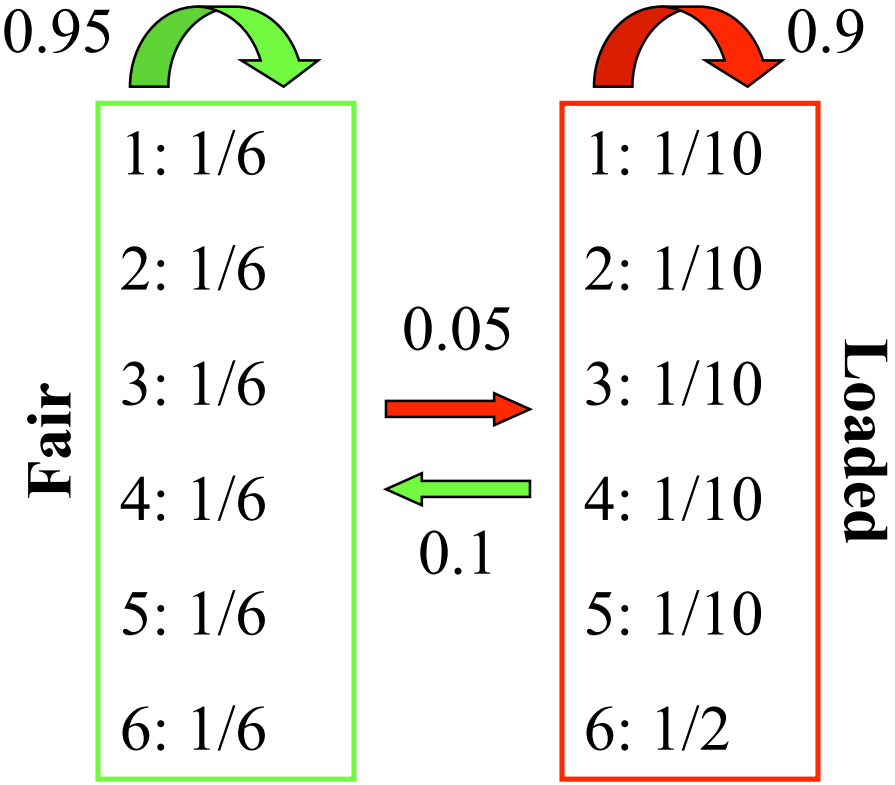
(transition probabilities)



- *<u>Question</u>:* Where is the Markov process here?

Application of HMMs

- Problem (from Durbin *et al.*): dishonest casino



Application of HMMs (cntd)

- Problem (from Durbin *et al.*): dishonest casino

Given (1) the previous model and (2) a series of die rolls (x_i, i=1,…,L), can we predict which of the rolls are coming from the fair and which from the loaded die?

- *Question:* What is "hidden" here?

Application of HMMs (cntd)

- Answer: YES
  - Viterbi algorithm (best path)
  - Forward-backward algorithm (probability of state k in outcome x_i)

HMMs: Viterbi algorithm

- Viterbi predictions: 300 rolls of die

```
Rolls   315116246446644245311321631164152133625144543631656626566666
Die     FFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFLLLLLLLLLLLLLLL
Viterbi FFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFLLLLLLLLLLLLL

Rolls   651166453132651245636664631636663162326455236266666625151631
Die     LLLLLLFFFFFFFFFFFLLLLLLLLLLLLLLLLLFFFLLLLLLLLLLLLLLLFFFFFFFFF
Viterbi LLLLLLFFFFFFFFFFFLLLLLLLLLLLLLLLLLLLLLLLLLLLLLLLLLLFFFFFFFF

Rolls   222555441666566563564324364131513465146353411126414626253356
Die     FFFFFFFLLLLLLLLLLLLLFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFLL
Viterbi FFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFL

Rolls   366163666466232534413661661163252562462255265252266435353336
Die     LLLLLLLLFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFF
Viterbi LLLLLLLLLLLLFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFF

Rolls   233121625364414432335163243633665562466662632666612355245242
Die     FFFFFFFFFFFFFFFFFFFFFFFFFLLLLLLLLLLLLLLLLLLLLLLFFFFFFFFFFF
Viterbi FFFFFFFFFFFFFFFFFFFFFFFFFFFFFLLLLLLLLLLLLLLLLLLLFFFFFFFFFF
```

HMMs in biology

- General comments:

- Usually the structure of the model is unknown
- The transition and emission probabilities are calculated based on trusted training set(s) and the postulated model
- Predictions are based on the Viterbi or the forward-backward algorithm, depending on the question asked

Information measures

- Definitions:
  - Entropy: $$\mathrm{H}(P) = \mathbf{E}(-\log P) = -\sum_{i=1}^{n} p_i \log p_i$$
  - Relative Entropy: $$\mathrm{H}(P,Q) = \sum_{i=1}^{n} p_i \log \frac{p_i}{q_i}$$
  - Mutual Information: $$M(X,Y) = \sum_{i,j} P(x_i, y_j) \log \frac{P(x_i, y_j)}{P(x_i)P(y_j)}$$