H3ABioNet

Pan African Bioinformatics Network for H3Africa

Introduction to Bioinformatics online course: IBT

Bioinformatics resources and databases: Lecture 3: DNA sequence analysis

Nicola Mulder

Learning Objectives

- Objective: Basic DNA sequence analysis – finding sequence features
- Sub objectives:
  - Understand how to extract a DNA sequence from the database
  - Use online or local tools for simple DNA sequence analysis -finding features on the sequence and their applications

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Learning Outcomes

- Understand how to find a DNA sequence and save it in the correct format
- Identify features on the sequence such as coding regions, restriction enzyme sites, etc.
- Design primers for amplification of a DNA sequence
- Interpret sequence analysis results and understand the biological impact of functional regions

Fundamental biology you need to know

- DNA is made up of 4 nucleotides/bases: ACGT
- DNA is usually double stranded through base pairing: A-T and C-G
- G-C bond is a bit stronger than A-T bond
- Double strand has top strand (sense strand) running 5' to 3'
- Bottom strand is complementary

5' ACCTTGCGTTAAGCAATTGGCT 3'
3' TGGAACGCAATTCGTTAACCGA 5'

Reverse complement: bottom strand read 5' to 3'

Genes can be encoded by either strand, + or – and are transcribed into RNA

How do you get the sequence?

- Sequencing technologies to determine the sequence of bases
- Not in the scope of this module

Two major components to sequence analysis

- Storing and retrieving data:
  - Biological databases
  - Querying these to retrieve data
- Manipulating the data –tools e.g:
  - Finding features on sequences
  - Sequence similarity searches
  - Protein families and function prediction
  - Comparing sequences –phylogenetics
  - Etc.

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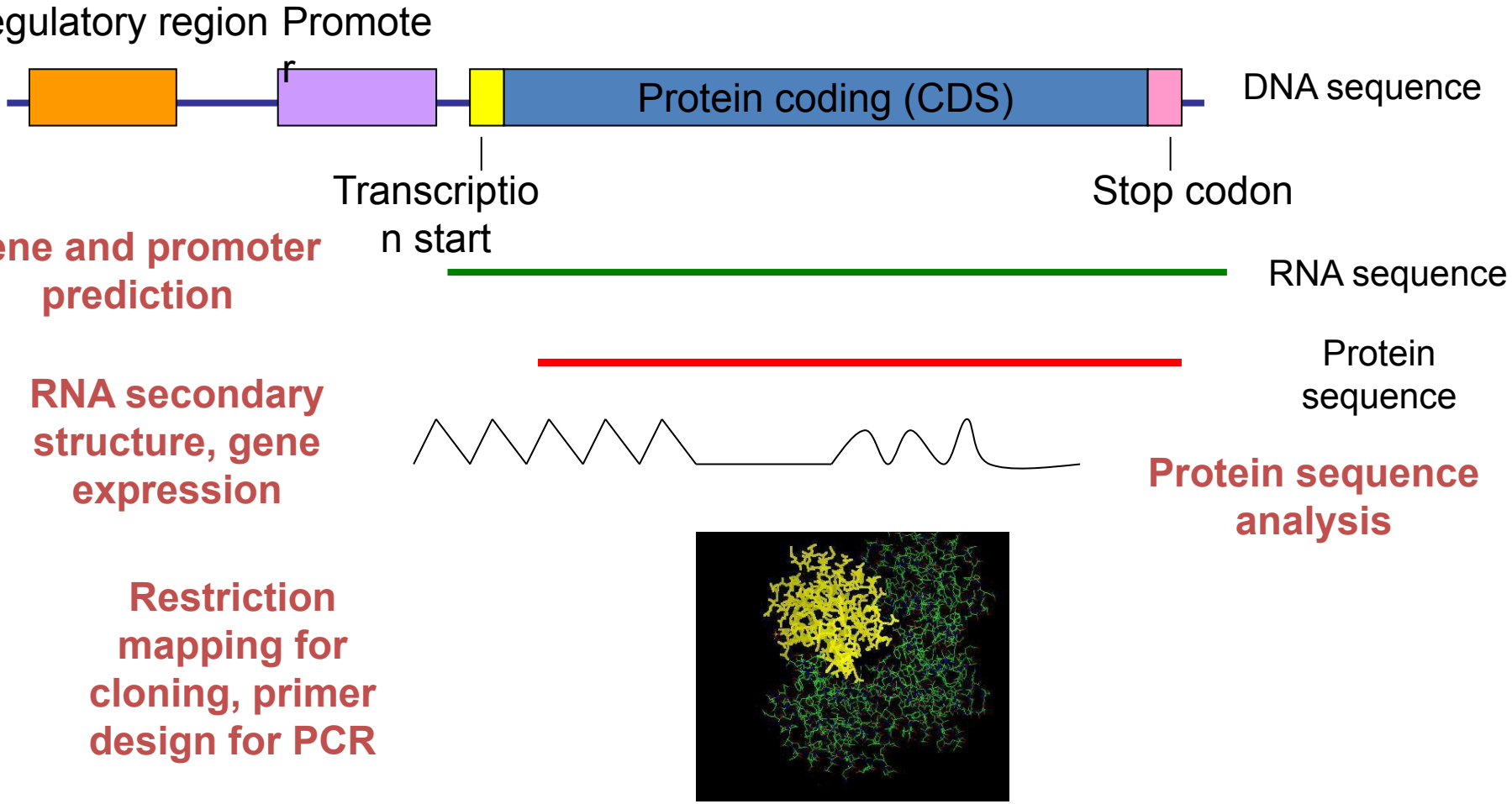
Aspects of sequence analysis



Sequence formats: Fasta

> [title]
[sequence]

>seq1
GGAAAATTAGATGCATGGGAAAAAATTA
GGAAAATTAGACAAATGGGAAAAAATTA
>seq2
AAGTCCCTGGATTTACCCAATGCAGTCG
A
CATCGCATTT

Sequence formats: GenBank

```
LOCUS       525-42       1588 bp
DEFINITION  525-42       1588 bp
TITLE       525-42
FEATURES             Location/Qualifiers
     exon            39..70
                     /note="exon1 is believed to have an alternative splice donor site"
ORIGIN

1     ATGTT AAGAG GGGGA AAATT AGATG CATGG GAAAA AATTA GGTTA AGGCC
51    AGGGG GAAAG AAATG CTATA NGATA AAACA CCTAG TATGG GCAAG CAGGG
101   AGCTG GAAAG ATTTG CACTT AACCC TGGCC TTTTA GAGAC ATCAG ANGGC
151   TGTAA ACAAA TAATG NAACA GATAC AACCA GCTCT TCAGA CAGGA ACAGA
```

Converting between sequence formats (save options)

DNA sequence composition

- Nucleotide composition (% GC vs AT content)
- GC bonds are stronger than AT bonds
- Applications:
  - Horizontal gene transfer analysis
  - Gene prediction
  - Primer design

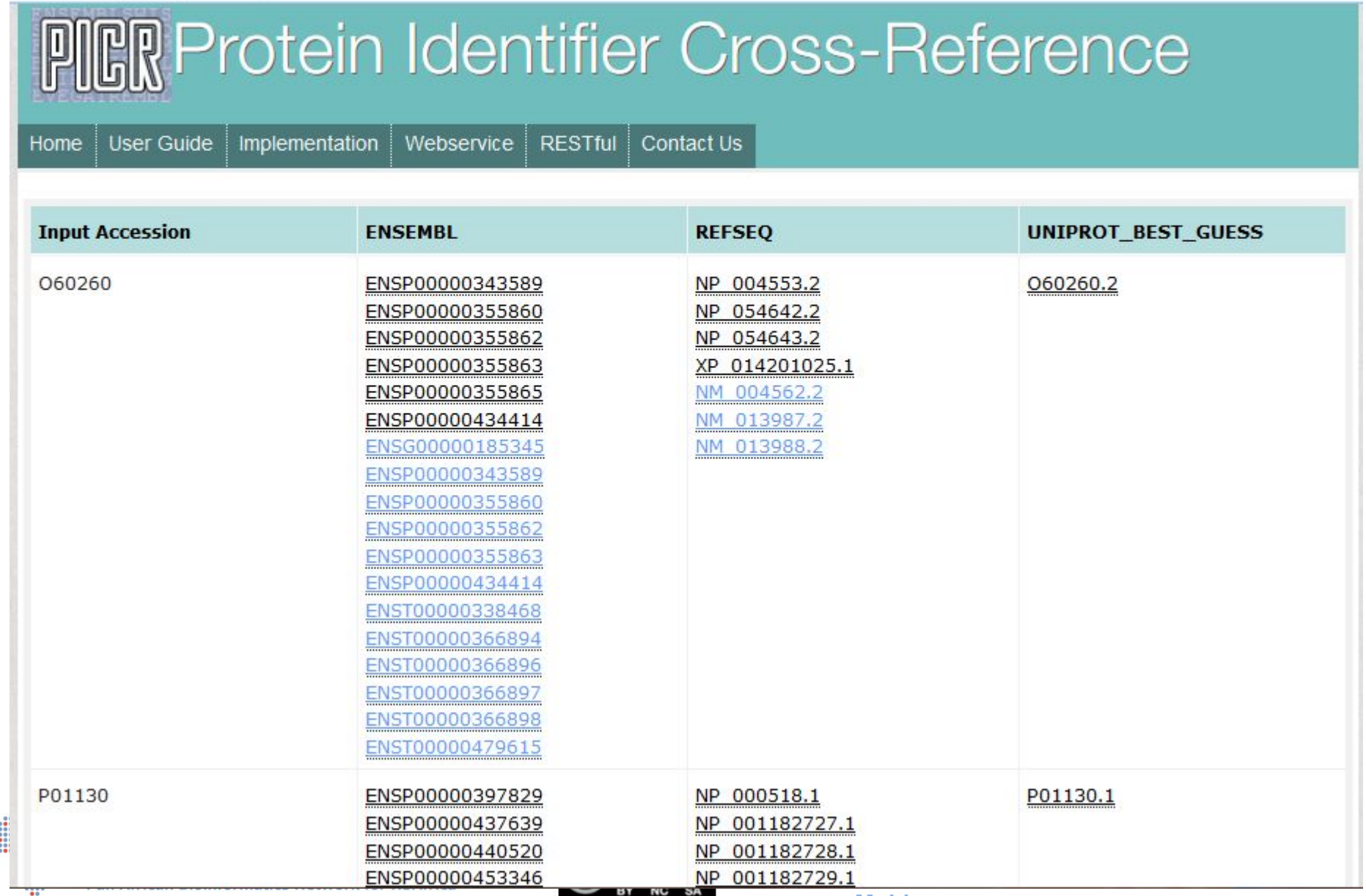
Accession numbers

- **GenBank/EMBL/DDBJ**: 1 letter & digits, e.g.: U12345 or 2 letters & 6 digits, e.g.: AY123456
- **GenPept** Sequence Records -3 letters & 5 digits, e.g.: AAA12345
- **UniProt** -All 6 characters: [A,B,O,P,Q] [0-9] [A-Z,0-9] [A-Z,0-9] [A-Z,0-9] [0-9], e.g.: P12345 and Q9JJS7

Cross-referencing identifiers

- So many different IDs for same thing, e.g. Ensembl, EMBL, HGNC, UniGene, UniProt, Affy ID, etc.
- Need mapping files to move between them to avoid having to parse every entry
- UniProt website mapper (www.uniprot.org)
- PICR (http://www.ebi.ac.uk/Tools/picr/) enables mapping between IDs

Example conversion



PICR Protein Identifier Cross-Reference

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Input Accession	ENSEMBL	REFSEQ	UNIPROT_BEST_GUESS
O60260	ENSP00000343589 ENSP00000355860 ENSP00000355862 ENSP00000355863 ENSP00000355865 ENSP00000434414 ENSG00000185345 ENSP00000343589 ENSP00000355860 ENSP00000355862 ENSP00000355863 ENSP00000434414 ENST00000338468 ENST00000366894 ENST00000366896 ENST00000366897 ENST00000366898 ENST00000479615	NP_004553.2 NP_054642.2 NP_054643.2 XP_014201025.1 NM_004562.2 NM_013987.2 NM_013988.2	O60260.2
P01130	ENSP00000397829 ENSP00000437639 ENSP00000440520 ENSP00000453346	NP_000518.1 NP_001182727.1 NP_001182728.1 NP_001182729.1	P01130.1

DNA sequence analysis

- Restriction analysis e.g. for cloning –looks for recognition sites
- Primer design
- Finding features on a sequence
- Gene prediction:
  - Translation
  - Promoter prediction

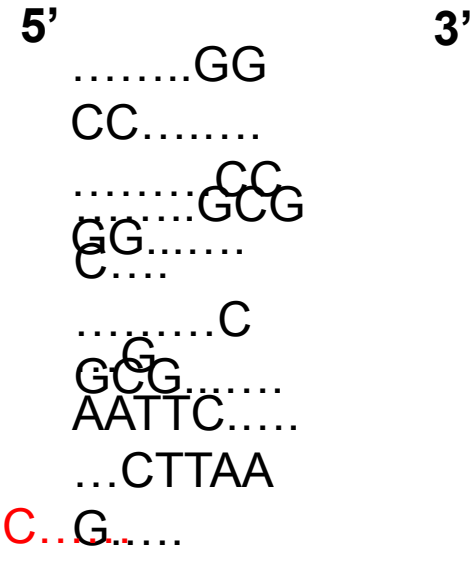
Bioinformatics and cloning

- Retrieving sequence of interest
- Identifying restriction enzyme sites
- Matching these to RE sites in cloning vector

Restriction enzyme analysis

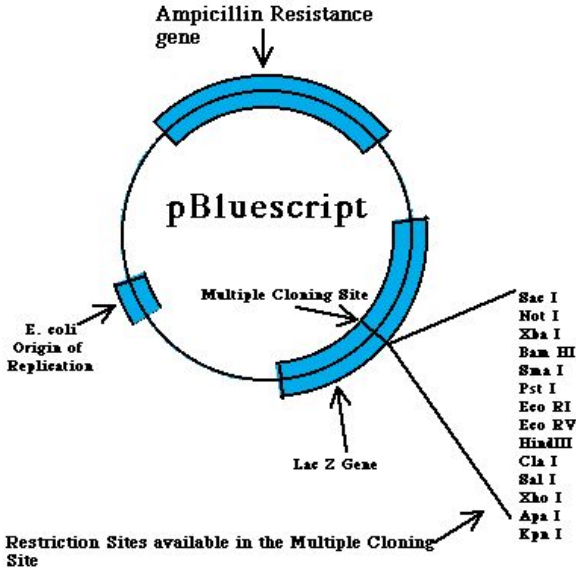
- Restriction enzymes recognize specific or defined 4 to 8 base pair sequences on DNA and cut

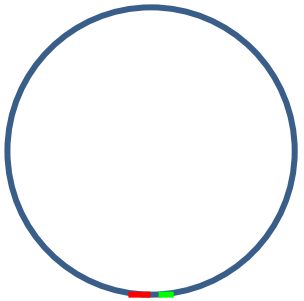
Microorganism	Enzyme	Sequences	Notes
Haemophilus aegitius	*Hae*III	5'…GG|CC..3' 3'…CC|GG..5'	Blunt end
Haemophilus haemolytica	*Hha*I	5'…GC G|C..3' 3'…C|G C G..5'	3' single strand
Escherichia coli	*Eco*RI	5'…G|AATT C..3' 3'…C TTAA|G..5'	5' single strand



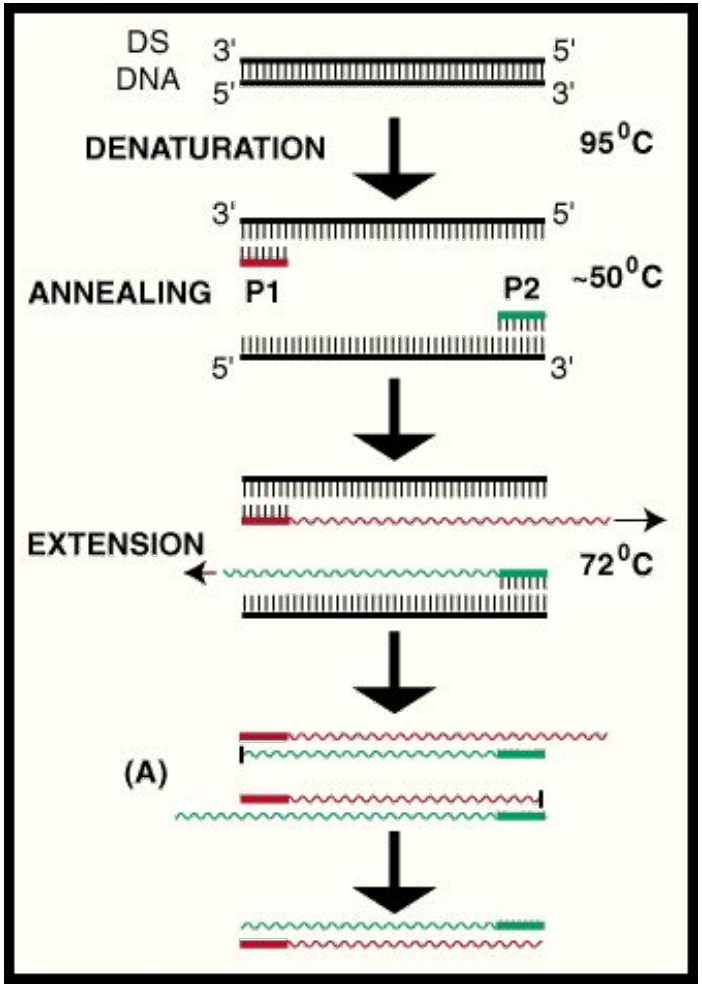
Restriction map

```
Restriction Enzyme Map:

1       TACATGCATGTTCATGGTAGCATTATTCACAAAGCCAAAAAGATGCAAACAGCCCCAATGTCCATAGATGAATAAACTGTG   80
1       ATGTACGTACAAGTACCATCGTAATAAGTGTTTCGGTTTTCTACGTTTGTCGGGGTTACAGGTATCTACTTATTTGACAC   80
              NspI                        SfaNI
               BfrBI
                 NsiI
                   NspI
                    MslI



81      GCATACATGATACACACACACACACGCACACACATATACATATACACACACAAACACTATTCAGTCATAAAAAGGAATAA   160
81      CGTATGTACTATGTGTGTGTGTGTGCGTGTGTGTATATGTATATGTGTGTGTTTGTGATAAGTCAGTATTTTTCCTTATT   160
          TspDTI



161     AGTCTGTTACATGCTACCTGAGGATGAACCTCGAAAACATGCTAAGTGAAAGACACAAAAGTCCACACACTGTGATTCCG   240
161     TCAGACAATGTACGATGGACTCCTACTTGGAGCTTTTGTACGATTCACTTTCTGTGTTTTCAGGTGTGTGACACTAAGGC   240
                BseMII    Bsu36I     BstF5I      TspDTI             DrdI      TspGWI
                  BspCNI                      FokI   NspI                 Hpy8I    DraIII
                     NspI                       MnlI                              TspRI
                     MnlI



241     TTTATATGAAGTATCTAAAGTAAGTAAATATAGAGACAGAAGTAGACTGGTAATTGCCAGGGGCTGGGGGGGAAGAGGGC   320
241     AAATATACTTCATAGATTTCATTCATTTATATCTCTGTCTTCATCTGACCATTAACGGTCCCCGACCCCCCCTTCTCCCG   320
                        TspDTI                 AccI     BsrI   BsaJI      EarI
                              BsmAI              Hpy8I              BslI
                                                                  PflMI
                                                                  AlwNI
                                                                  BseYI
                                                                       MnlI
```



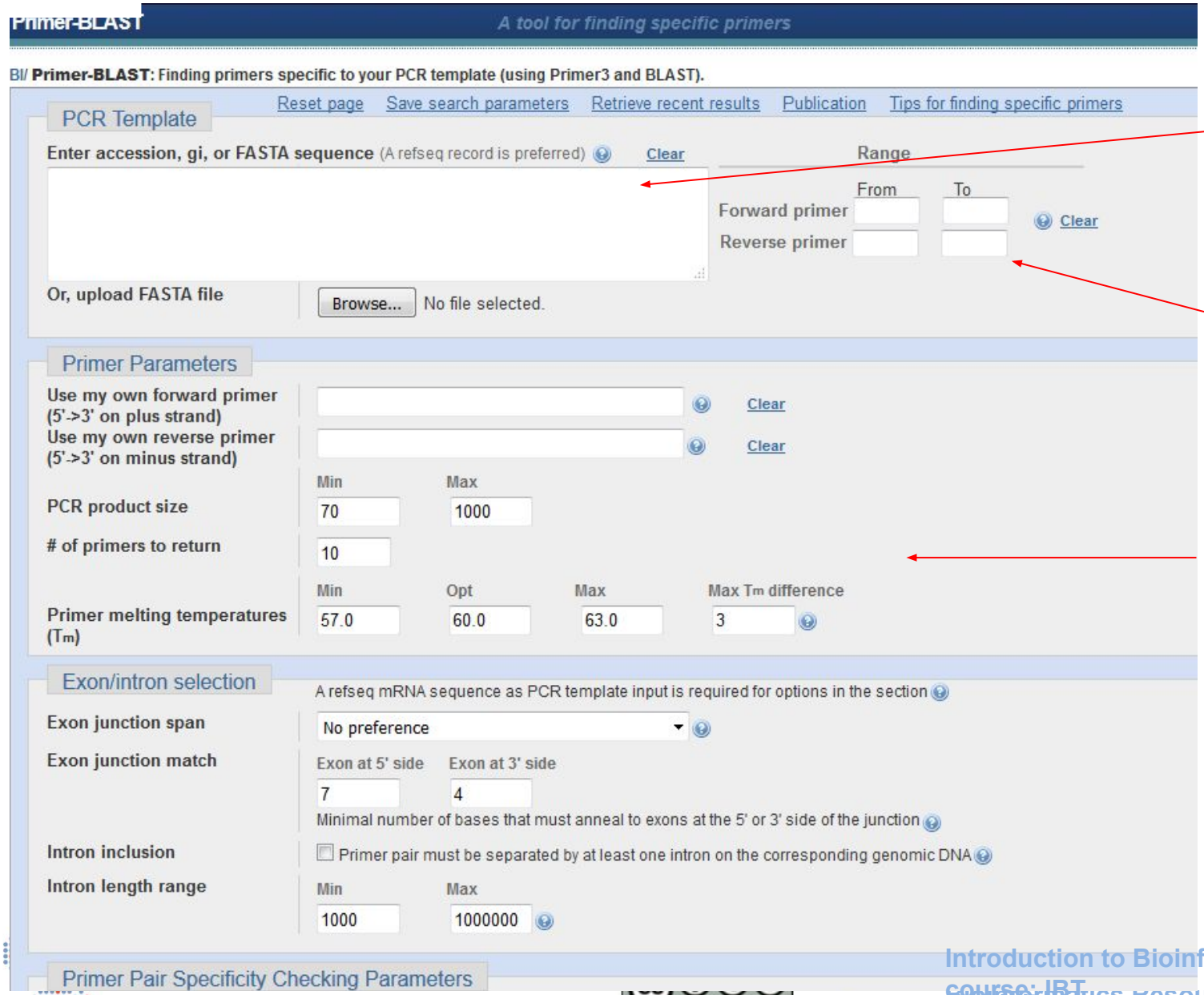


PCR and primer design



- Primers should be similar length and Tm
- Should amplify only required piece from genome

Example with Primer BLAST



Enter accession number of DNA entry or paste in sequence

Select where on sequence primers should be

Select size range of product and Tm

Primer BLAST output

Detailed primer reports

Primer pair 1

	Sequence (5'->3')	Length	Tm	GC%	Self complementarity
Forward primer	ATGAGGCCAAGGACCCAAGAC	21	62.08	57.14	4.00
Reverse primer	GATGAGGGGCTGACAGGAGTGG	22	64.35	63.64	5.00

Products on target templates

>NC_000020.11 Homo sapiens chromosome 20, GRCh38.p7 Primary Assembly

```
product length = 690
Features associated with this product:
   glutathione synthetase

   glutathione synthetase

Forward primer  1         ATGAGGCCAAGGACCCAAGAC  21
Template        34929242  ......................  34929222

Reverse primer  1         GATGAGGGGCTGACAGGAGTGG  22
Template        34928553  ......................  34928574
```

```
product length = 1995
Features flanking this product:
   62286 bp at 5' side: zinc finger protein 217
   297816 bp at 3' side: breast carcinoma-amplified sequence 1 isoform 1

Forward primer  1         ATGAGGCCAAGGACCCAAGAC  21
Template        53645111  C..........A.TT......  53645131

Forward primer  1         ATGAGGCCAAGGACCCAAGAC  21
Template        53647105  C......A.T.A........G  53647085
```

Other places in the genome primers may bind

>NC_018931.2 Homo sapiens chromosome 20, alternate assembly CHM1_1.1, whole genome shotgun sequence

```
product length = 690
Features associated with this product:
   glutathione synthetase

   glutathione synthetase
```

Gene Prediction

Wikipedia: A **gene** is a locatable region of genomic sequence, corresponding to a unit of inheritance, which is associated with regulatory regions, transcribed regions
and/or other functional sequence regions

- Look for gene structures
- Move along sequence looking for coding regions and intergenic regions
- Check reading frame -translate
- Look for promoters and poly-adenylation signals
- In eukaryotes look for introns and exons
- Use EST or BLAST support (reduce pseudogenes)

Translation

- Can choose frame if you know it
- Otherwise 6-frame translation:
  - Choose start codon ATG
  - Otherwise lists all codons between stop codons
- Results –for bacteria, usually the longest ORF starting with Met and ending in stop, & no stop codons inside
- Can confirm this with promoter prediction
- Should use appropriate **codon usage table**

Open reading frame

- String of in-frame combinations/triplets of bases that specify an amino acid
- Starts with ATG (Meth) or Val
- Ends with stop codon
- One base insertion or deletion –out of frame/frameshift

Genetic code

- Each amino acid is specified by a triplet of 3 bases
- 4 bases: A,C,G,T = 64 possible codons. Actually 61 codons + 3 stop codons

	T		C		A		G		
T	TTT	phe	TCT	ser	TAT	tyr	TGT	cys	T
	TTC		TCC		TAC		TGC		C
	TTA	leu	TCA		TAA	stop	TGA	stop	A
	TTG		TCG		TAG		TGG	try	G
C	CTT	leu	CCT	pro	CAT	his	CGT	arg	T
	CTC		CCC		CAC		CGC		C
	CTA		CCA		CAA	gln	CGA		A
	CTG		CCG		CAG		CGG		G
A	ATT	ile	ACT	thr	AAT	asp	AGT	ser	T
	ATC		ACC		AAC		AGC		C
	ATA	ile	ACA		AAA	lys	AGA	arg	A
	ATG	met	ACG		AAG		AGG		G
G	GTT	val	GCT	ala	GAT	asp	GGT	gly	T
	GTC		GCC		GAC		GGC		C
	GTA		GCA		GAA	glu	GGA		A
	GTG		GCG		GAG		GGG		G

Translating sequences

- 6 possible reading frames, 3 in each direction

5’-AGTCGGCTGACTGCGTTTACGAATGCGATTACTCCCTT-3’ +strand
3-’TCAGCCGACTGACGCAAATGCTTACGCTAATGAGGGAA-5’ -strand

Reverse complement

5’-AAGGGAGTAATCGCATTCGTAAACGCAGTCAGCCGACT-3’

Translating sequences

- 6 possible reading frames, 3 in each direction

AGTCGGCTGACTGCGTTTACGAATGCGATTACT

+1

Reverse complement

AAGGGAGTAATCGCATTCGTAAACGCAGTCAG

-1

	T		C		A		G		
T	TTT	phe	TCT	ser	TAT	tyr	TGT	cys	T
	TTC		TCC		TAC		TGC		C
	TTA	leu	TCA		TAA	stop	TGA	stop	A
	TTG		TCG		TAG		TGG	try	G
C	CTT	leu	CCT	pro	CAT	his	CGT	arg	T
	CTC		CCC		CAC		CGC		C
	CTA		CCA		CAA	gln	CGA		A
	CTG		CCG		CAG		CGG		G
A	ATT	ile	ACT	thr	AAT	asp	AGT	ser	T
	ATC		ACC		AAC		AGC		C
	ATA	ile	ACA		AAA	lys	AGA	arg	A
	ATG	met	ACG		AAG		AGG		G
G	GTT	val	GCT	ala	GAT	asp	GGT	gly	T
	GTC		GCC		GAC		GGC		C
	GTA		GCA		GAA	glu	GGA		A
	GTG		GCG		GAG		GGG		G

Translating sequences

- 6 possible reading frames, 3 in each direction

Ser Arg Leu

AGTCGGCTGACTGCGTTTACGAATGCGATTACTCCCTT

+1

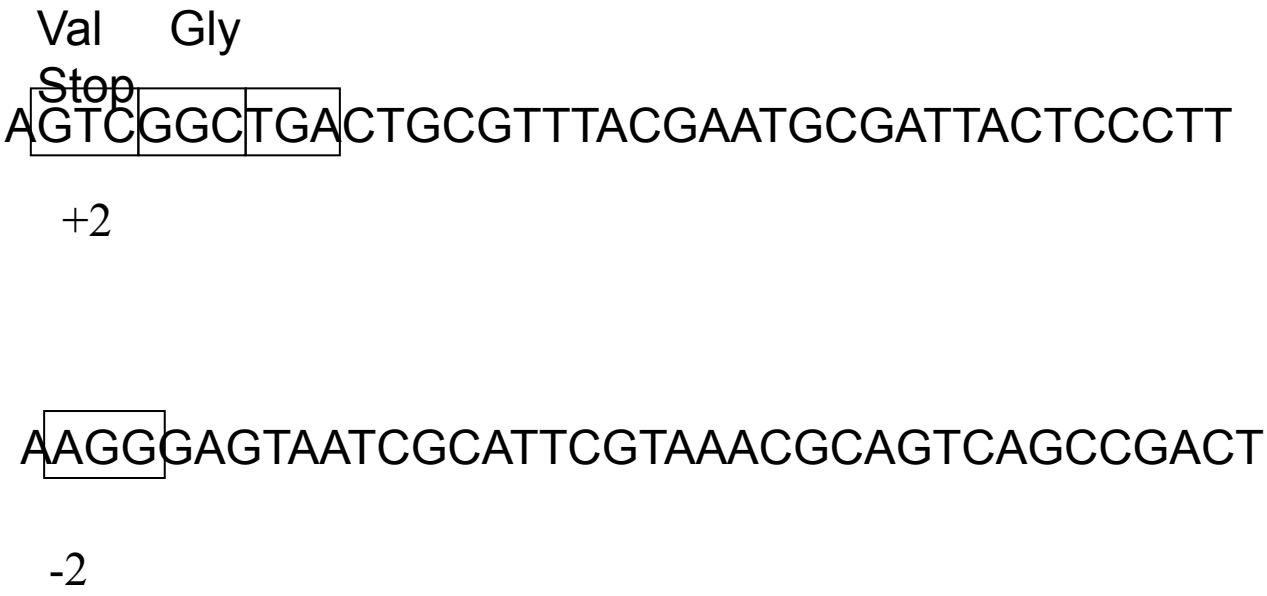
Reverse complement

AAGGGAGTAATCGCATTCGTAAACGCAGTCAGCCGACT

-1

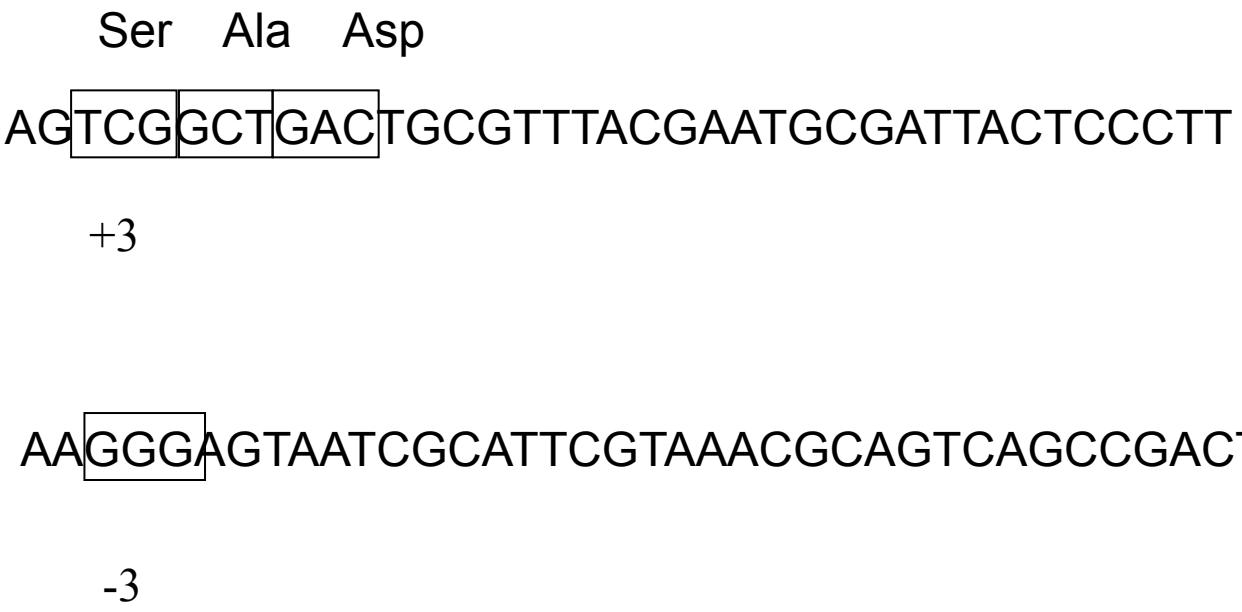
Translating sequences

- 6 possible reading frames, 3 in each direction



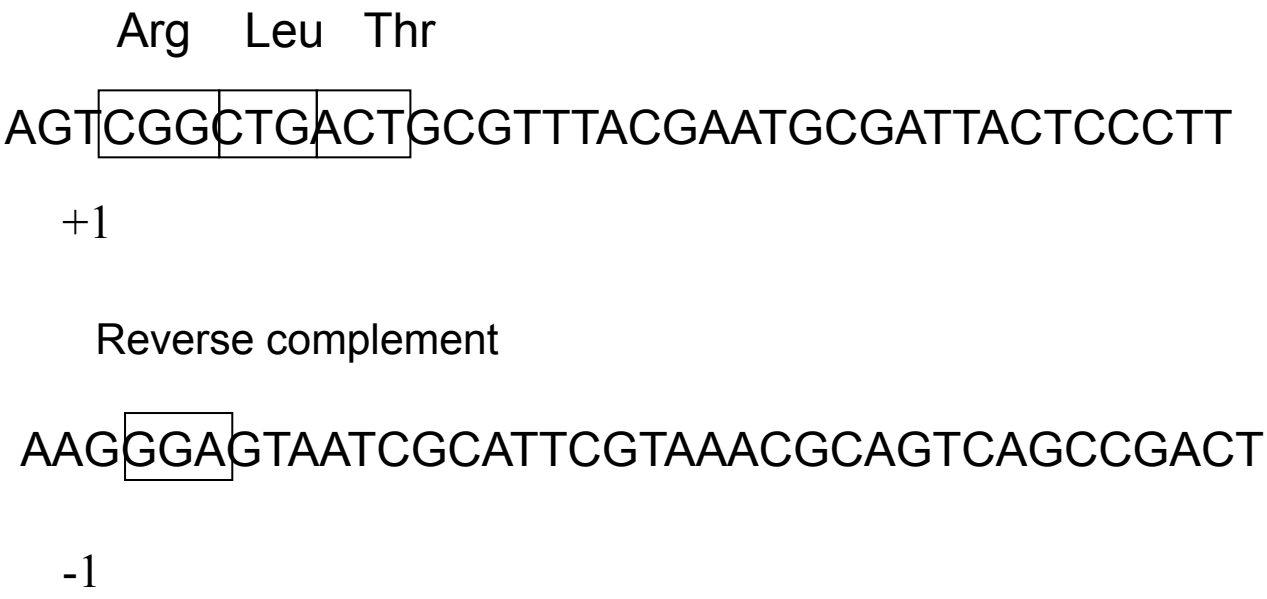
Translating sequences

- 6 possible reading frames, 3 in each direction



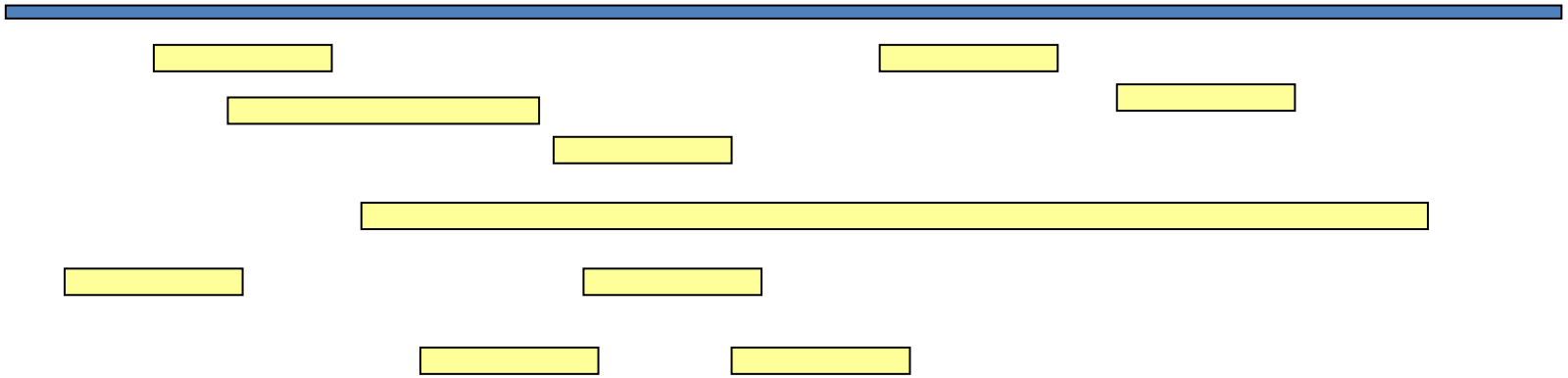
Translating sequences

- 6 possible reading frames, 3 in each direction



Getting the final protein

- Six-frame translation
- Find longest ORF with initiation site, start codon and ending with stop codon



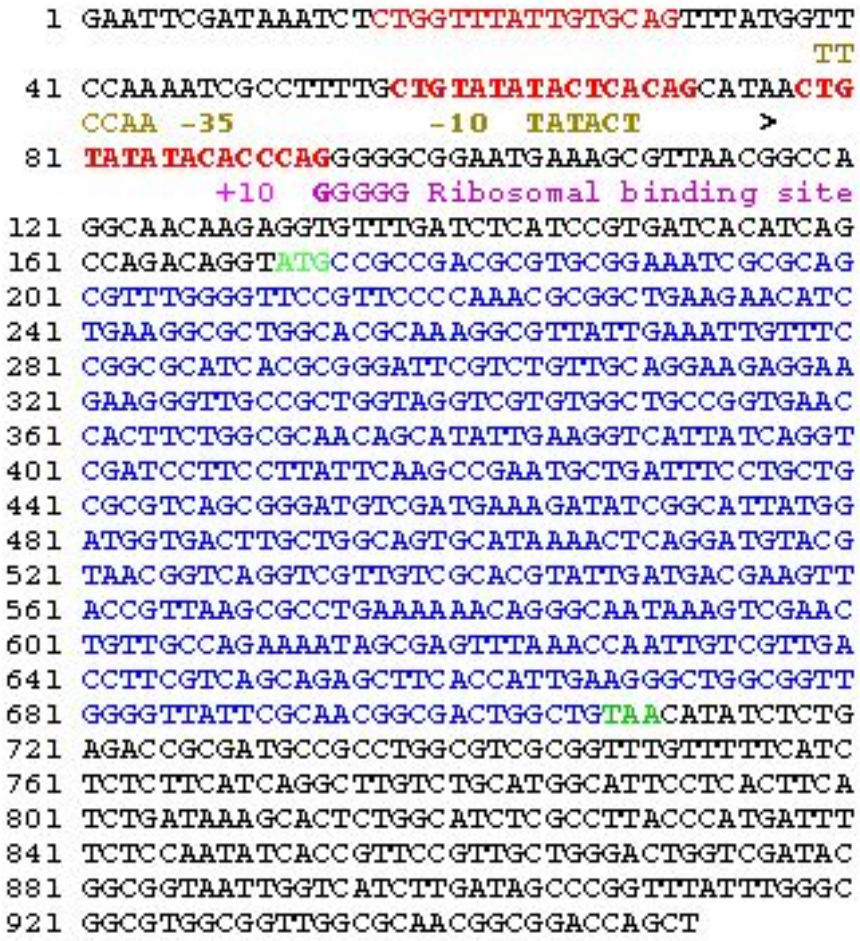
Gene Prediction -bacteria

Promoter

Start codon

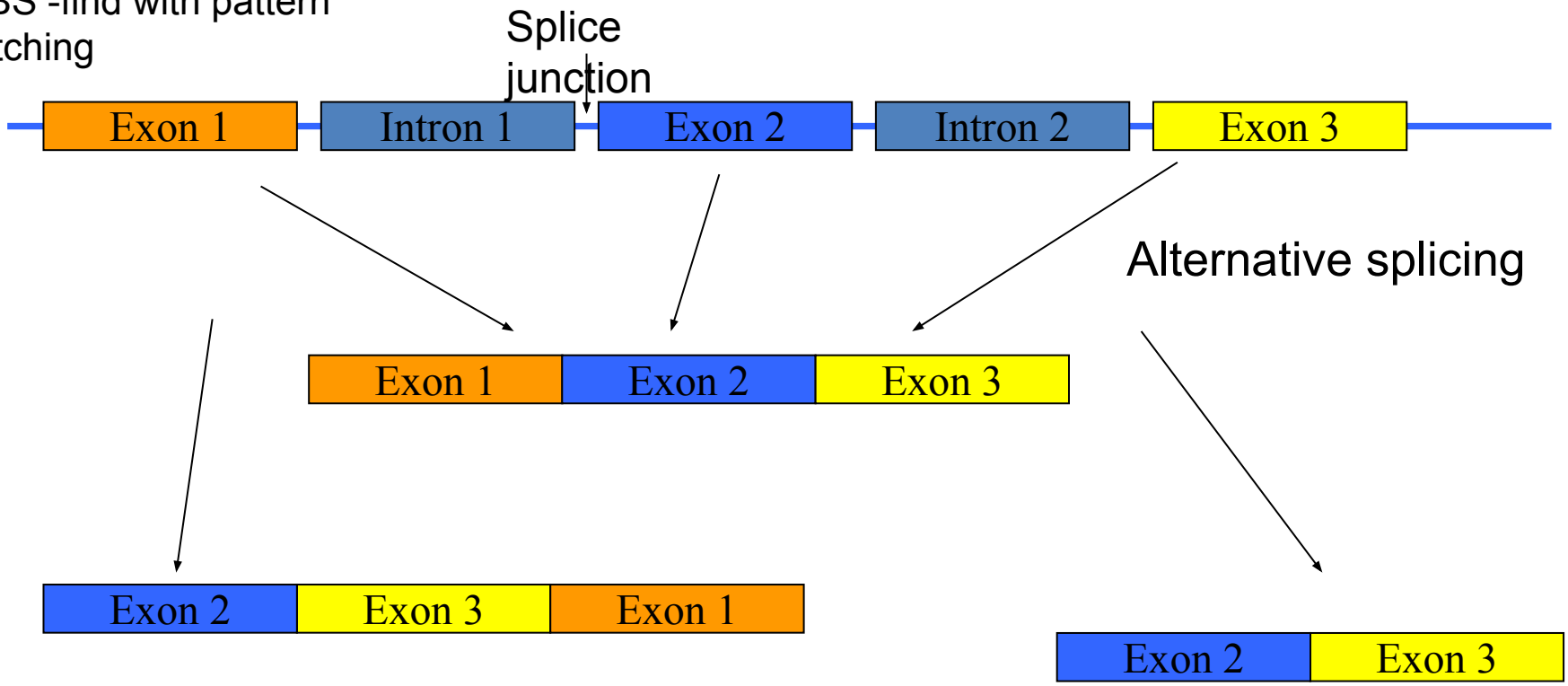
CDS

Stop codon



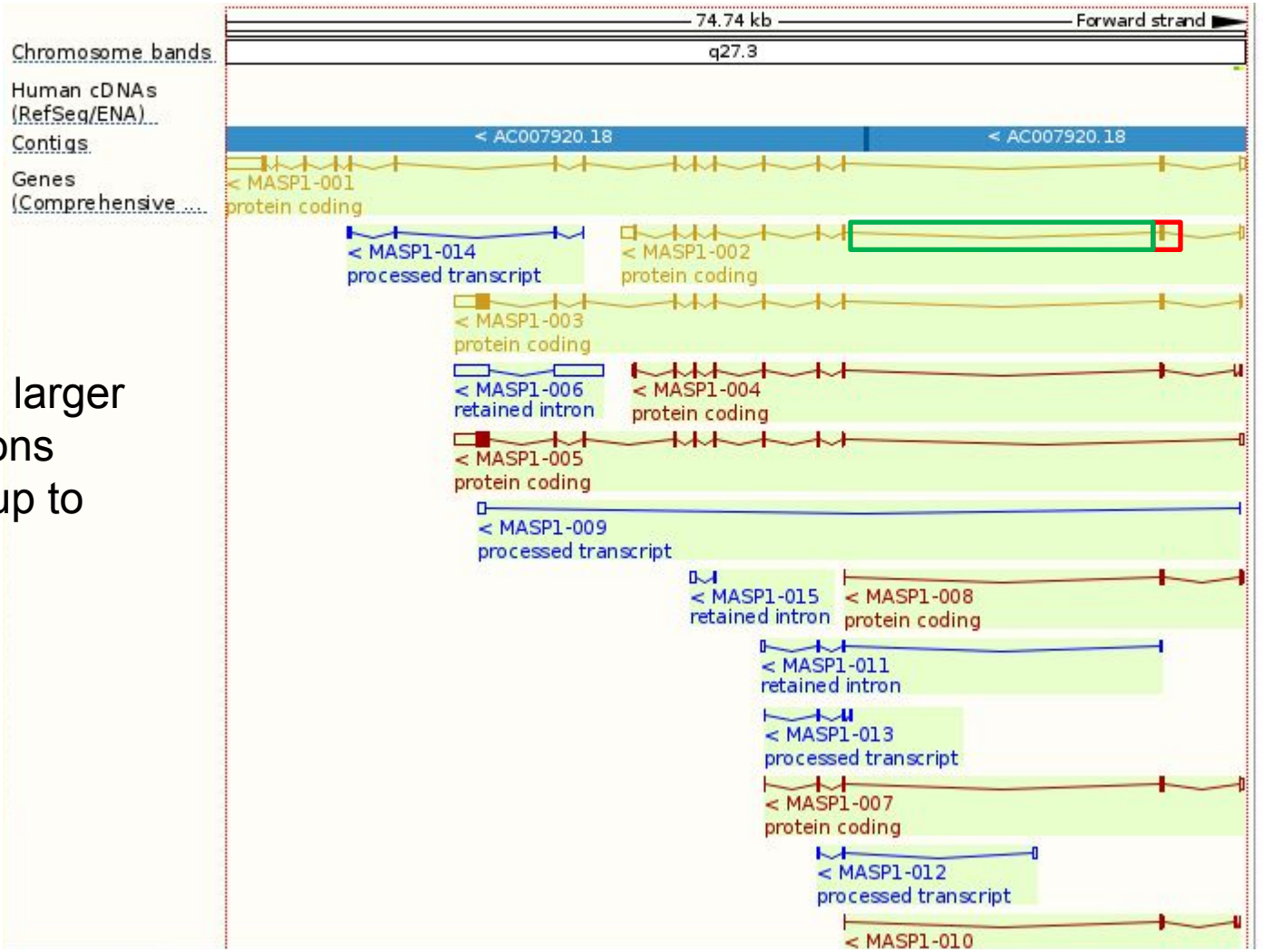
Complex Eukaryotic systems

Promoter region –many TFBS -find with pattern matching



Human introns and exons

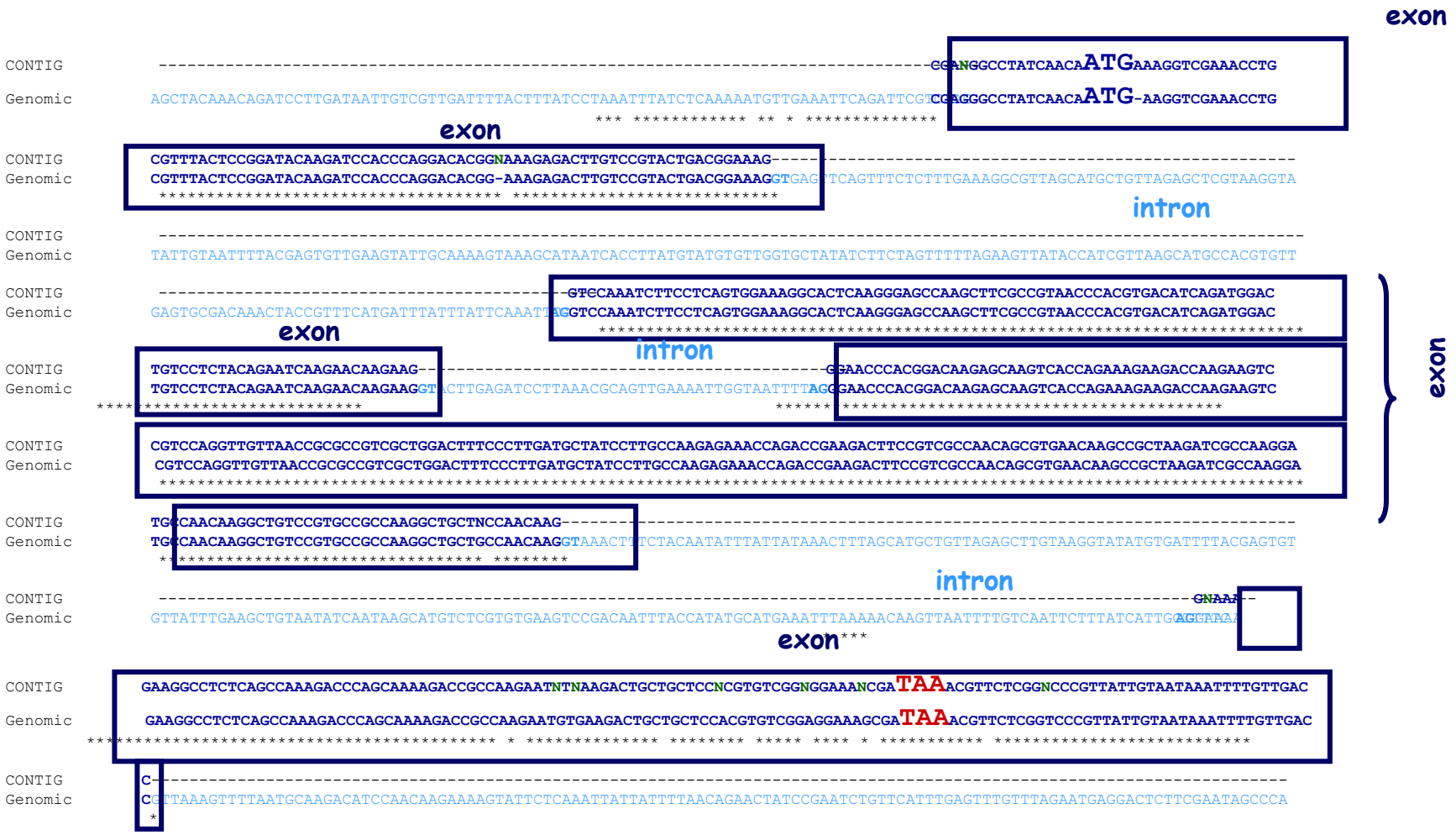
Introns are much larger than exons, introns could represent up to 95% of gene



Gene prediction in eukaryotes

- Identifying features (sometimes by PSSMs):
  - splice sites
  - start and stop sites
- Predict exons based on these signals
- Score exons based on signals and exon characteristics (coding sequences may have compositional biases)
- Use composition and homology information
- Assemble components into predicted gene structure
- Some methods use HMMs -features are states
- Use EST (expressed sequence tag –sequenced RNA) info

Using EST data: mRNA against genomic sequence



Gene Prediction software

- GeneMark –gene prediction for prokaryotes, eukaryotes and viruses: http://opal.biology.gatech.edu/GeneMark/
- GENSCAN –for vertebrate, maize and Arabidopsis sequences: http://genes.mit.edu/GENSCAN.html
- Microbial Gene Prediction System http://compbio.ornl.gov/generation/
- Glimmer –bacteria, archae and viruses http://www.tigr.org/software/glimmer/
- GRAIL –for eukaryotes, includes splice info, homology, etc. http://compbio.ornl.gov/grailexp/

Other translators and promoter prediction

- NCBI ORF Finder: (http://www.ncbi.nlm.nih.gov/gorf/gorf.htm)
- Promoter 2.0 Prediction Server (http://www.cbs.dtu.dk/services/Promoter/)
- MCPromoter MM:II (http://genes.mit.edu/McPromoter.html)
- BPROM -prediction of bacterial promoters, etc.

RNA sequence analysis

- Many different types of RNA e.g. tRNA, rRNA, mRNA etc.
- Some have activities e.g. ribozymes
- Many new programs for identification of non-coding RNA, miRNAs etc and their targets
- Secondary structure of RNA is NB for stability and often function
- RNA levels are NB for final protein levels, they measure gene expression –ESTs, microarrays

Summary and conclusions

- Basic sequence analysis is finding features on a sequence
- This could be small features
  - Restriction sites -> cloning
  - Primer sites -> PCR
- Or combinations of features:
  - Gene signals -> gene prediction
- Features found by nature of their “conservation” or pattern matching
- ***Practical assignment*** *–retrieve a DNA sequence, run some basic analysis programs*