معهد بحوث ودراسات البيولوجيا الجزيئية
Molecular Biology Researches & Studies Institute

جامعة أسيوط

INTERPRETATION OF SEQUENCE RESULTS & DIFFERENT APPLICATIONS OF DNA SEQUENCING

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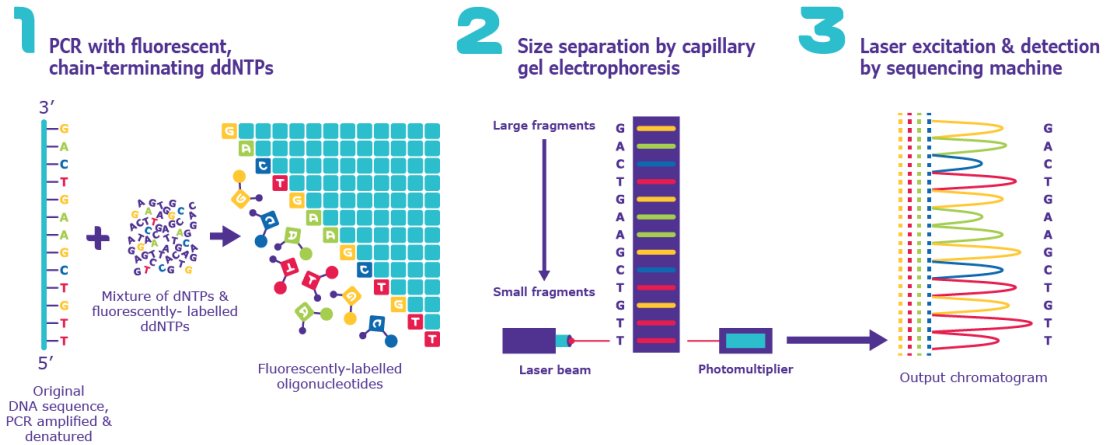
An overview on DNA sequencing:

- DNA sequencing involves the determination of the sequence of nucleotides in a sample of DNA.
- It use a modified PCR reaction where both normal and labeled dideoxy-nucleotides are included in the reaction mix.
- Each dideoxy-nucleotides were labeled with different fluorescent dyes (Each nucleotide has a different color).

An overview on DNA sequencing:

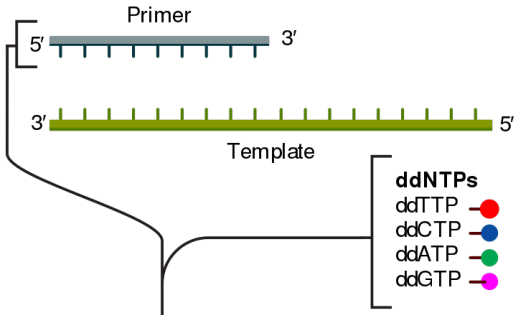
These fluorescent-colored nucleotides will be scanned with a laser detection device, the laser excites the dye, and the color of fluorescence is read by a photocell and recorded on a computer (inside the sequencer).

Sanger sequencing, also known as the “chain termination

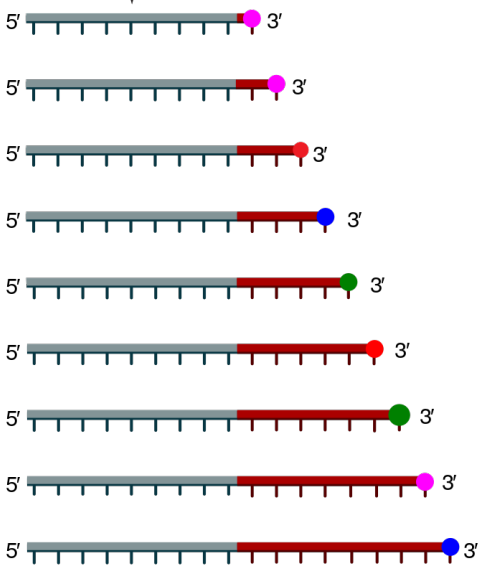


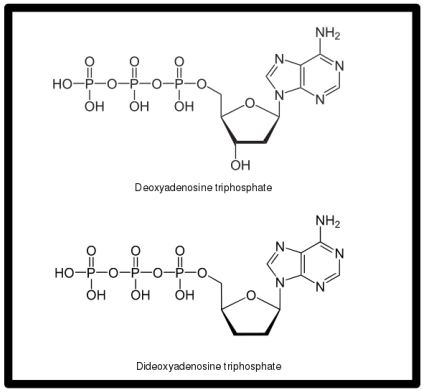
① Reaction mixture

- Primer and DNA template
- DNA polymerase
- ddNTPs with flourochromes
- dNTPs (dATP, dCTP, dGTP, and dTTP)

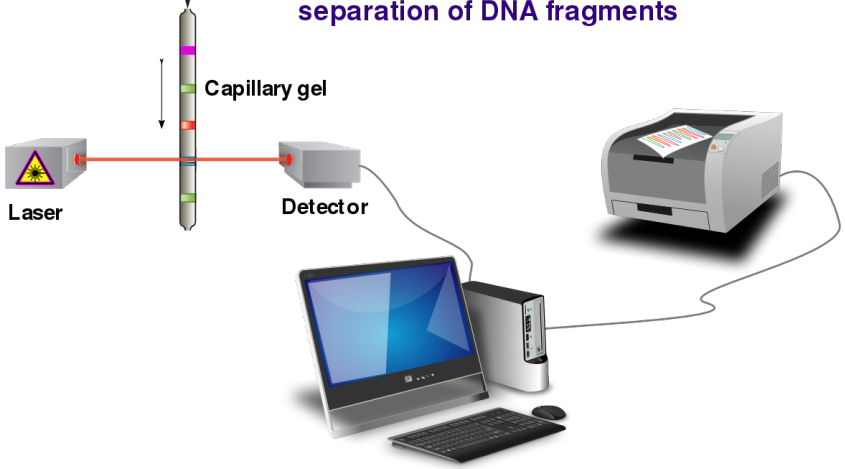


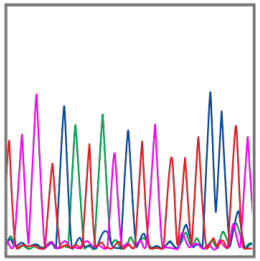
② Primer elongation and chain termination





③ Capillary gel electrophoresis separation of DNA fragments





Chromatograph

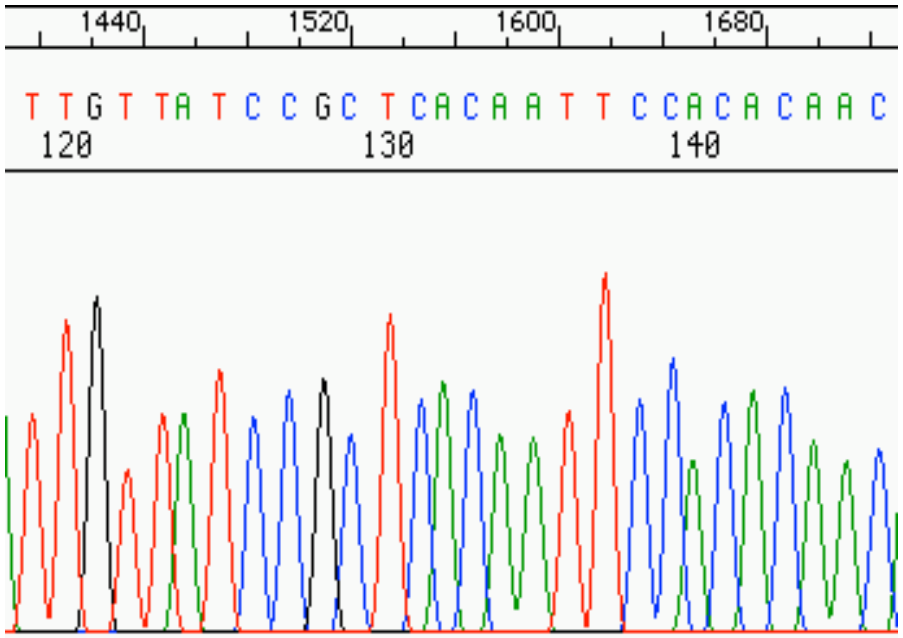
④ Laser detection of flourochromes and computational sequence analysis

Interpreting Sequencing Results

DNA Sequencers generate

1- A four-color chromatogram showing the results of the sequencing run.

2- In addition to a text file of sequence data.



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CGCTGGGTTGGAAACCCGGCCCGGCAGGGAGCGGGGAAGGCGCGCTTTCCCGGAGGTCGGCGCGGGGCCGGGGCCGGGGC
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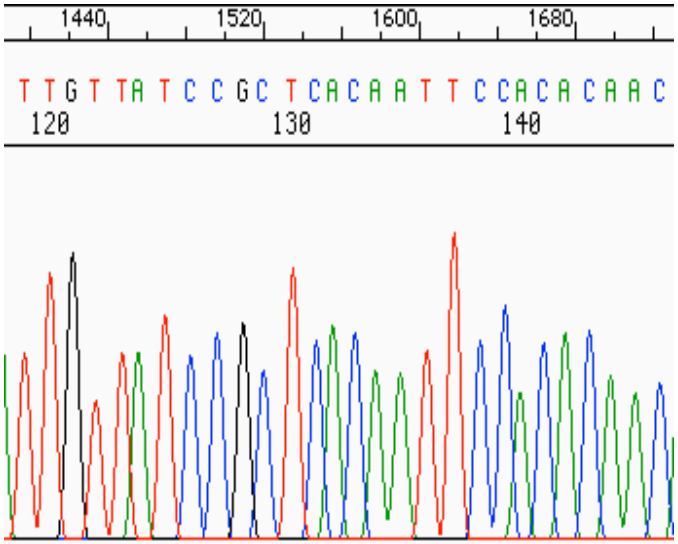
GCGGCGCCGGCGCGGGCGGGGCGGAGGATCTGGAGAGGGAAGGGGCGTGCGAGCCCCGCGGACCCCGGGCGCGCCCGGGC
CGCCTGAGCTGGGCCAGCCGCGCGGCGGGCGCGGGCGCGGGCGCGGGCGCGGGCGCGGGCGGGTGGGGAGCCCCAGCCCC
GGGGCCGCGGGGGCGCGTGACCGGCTGTCTGCGTGGGGCCCGCGCGC

Interpreting Sequencing Results

- When you obtain a sequence you should proofread it to ensure that all ambiguous sites are correctly called and determine the overall quality of your data.

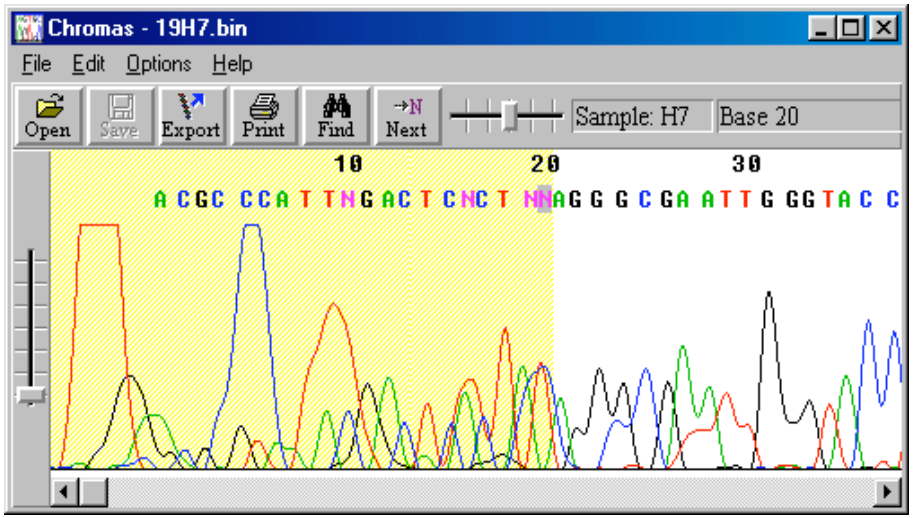
Base Designations

- *"A" designation*—green peaks
- *"G" designation*—black peaks
- *"T" designation*—red peaks
- *"C" designation*—blue peaks
- *"N" designation*—peaks that,

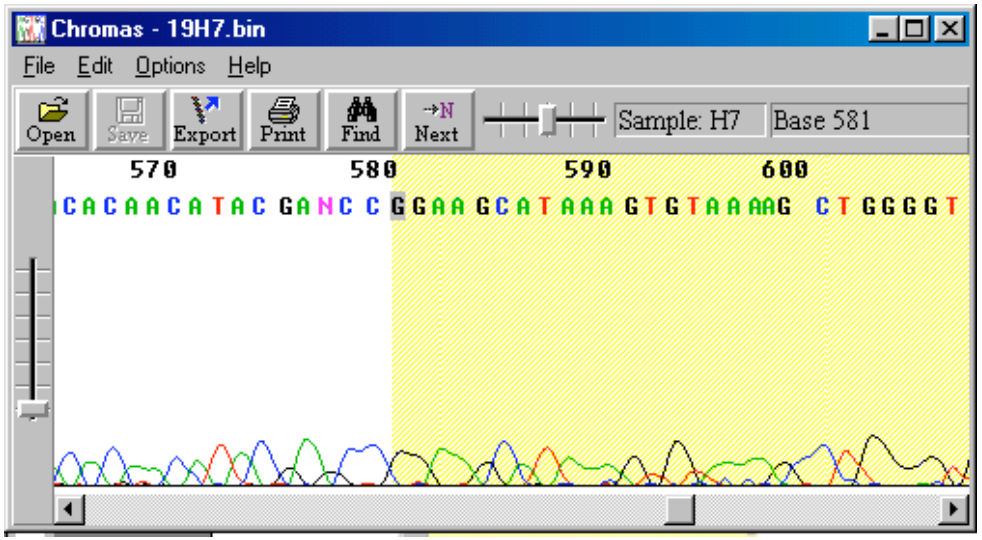


Interpreting Sequencing Chromatograms

Good sequence generally begins roughly around base 20.



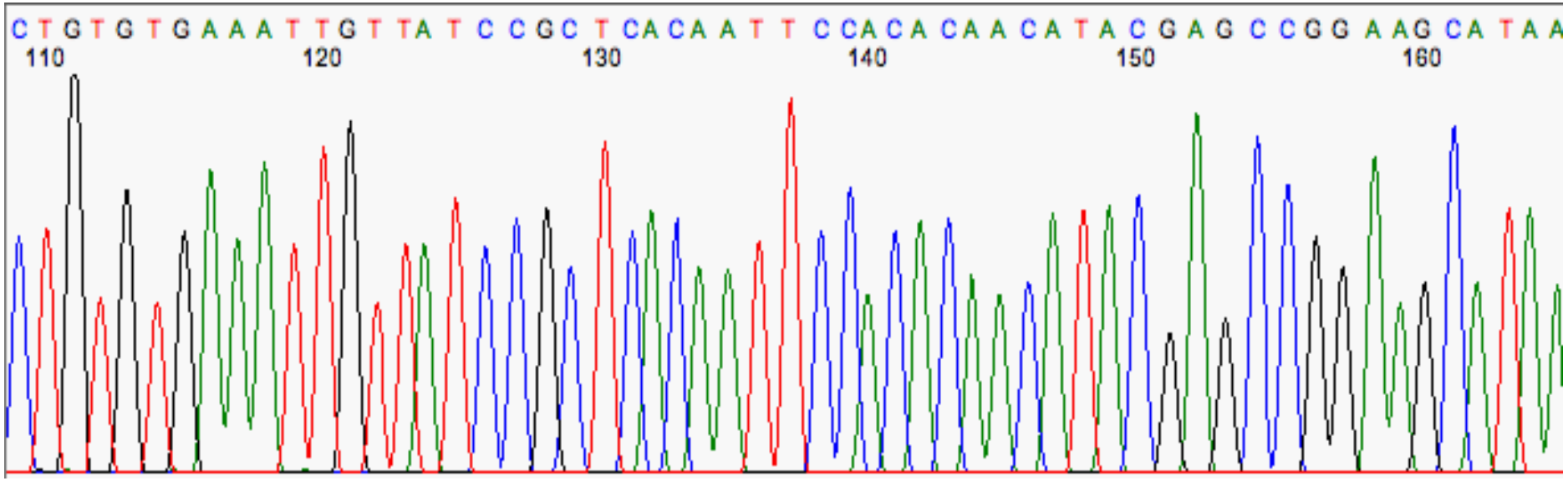
Beginning of Sequence



End of sequence

Interpreting Sequencing Chromatograms

With a little practice, you can scan a chromatogram in less than a minute and spot problems.

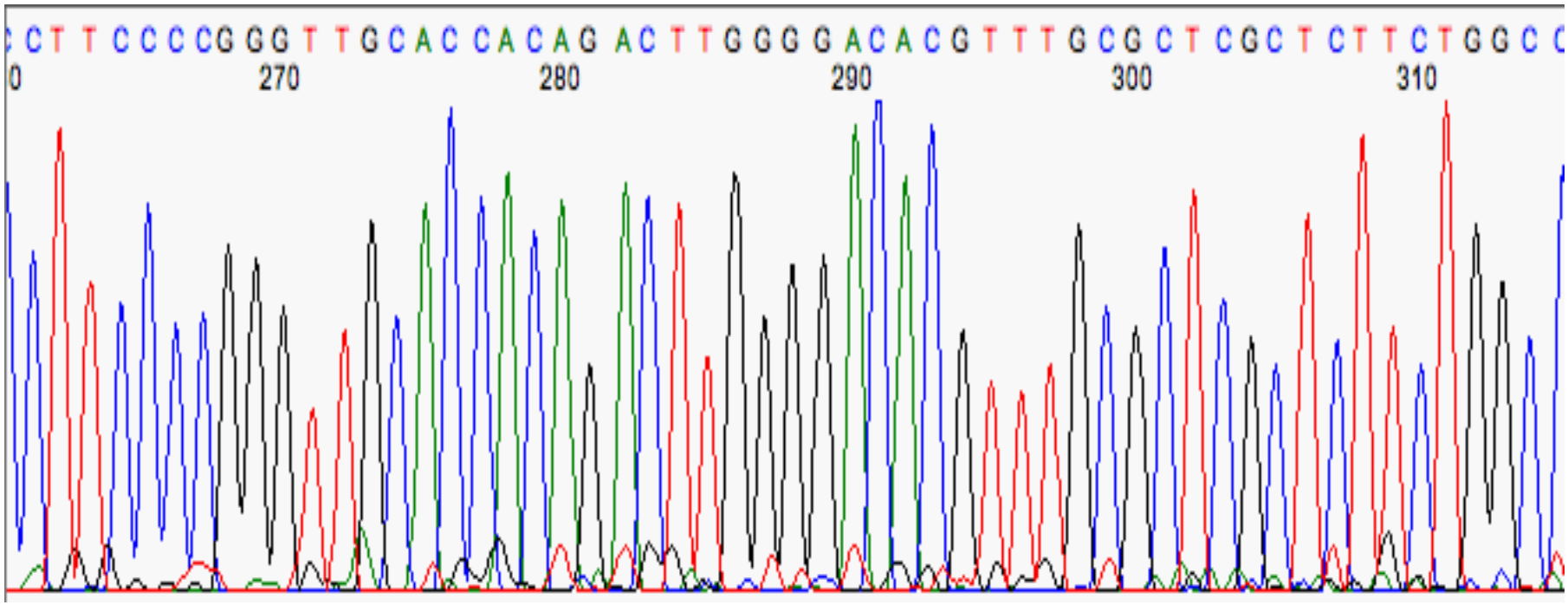


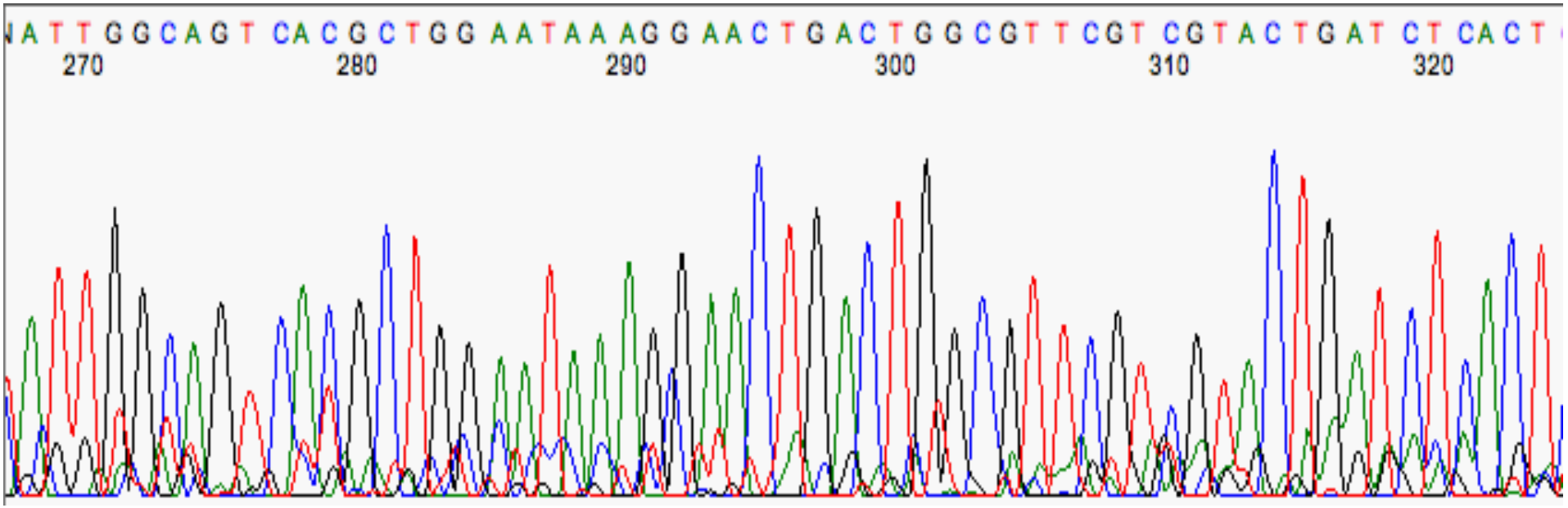
An example of excellent sequence. Note the evenly-spaced peaks and the lack of baseline 'noise'

Possible Defects Could be Detected during Interpreting of Chromatograms

1. Background noise

This example has a little baseline noise, but the 'real' peaks are still easy to call, so there's no problem with this sample





Noise like the above most commonly arises when the sample itself is too dim.

2. Types of Polymorphisms

a. Transitions: A ←→ G or C ←→ T

(purines to purines OR pyrimidines to pyrimidines)

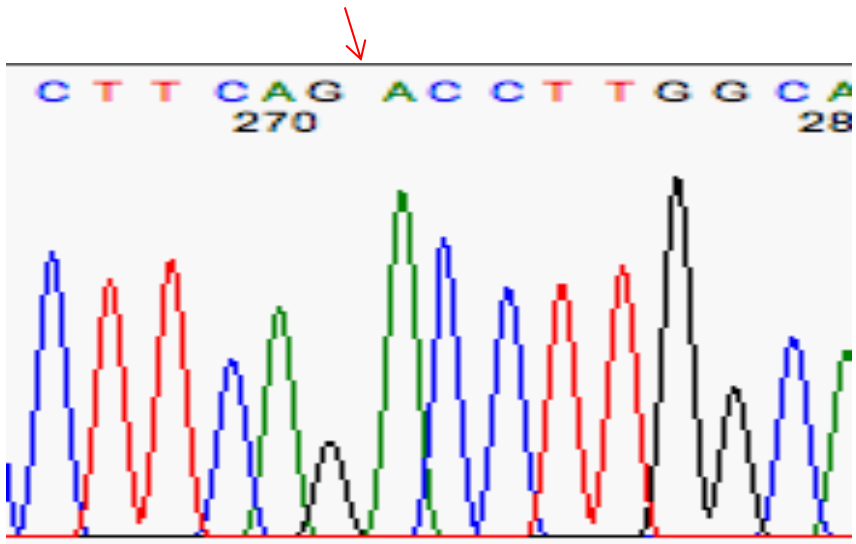
b. Insertions: an extra base is present.

c. Deletions: a base may be missing.

d. Mis-Called

(I) Irregular spacing:

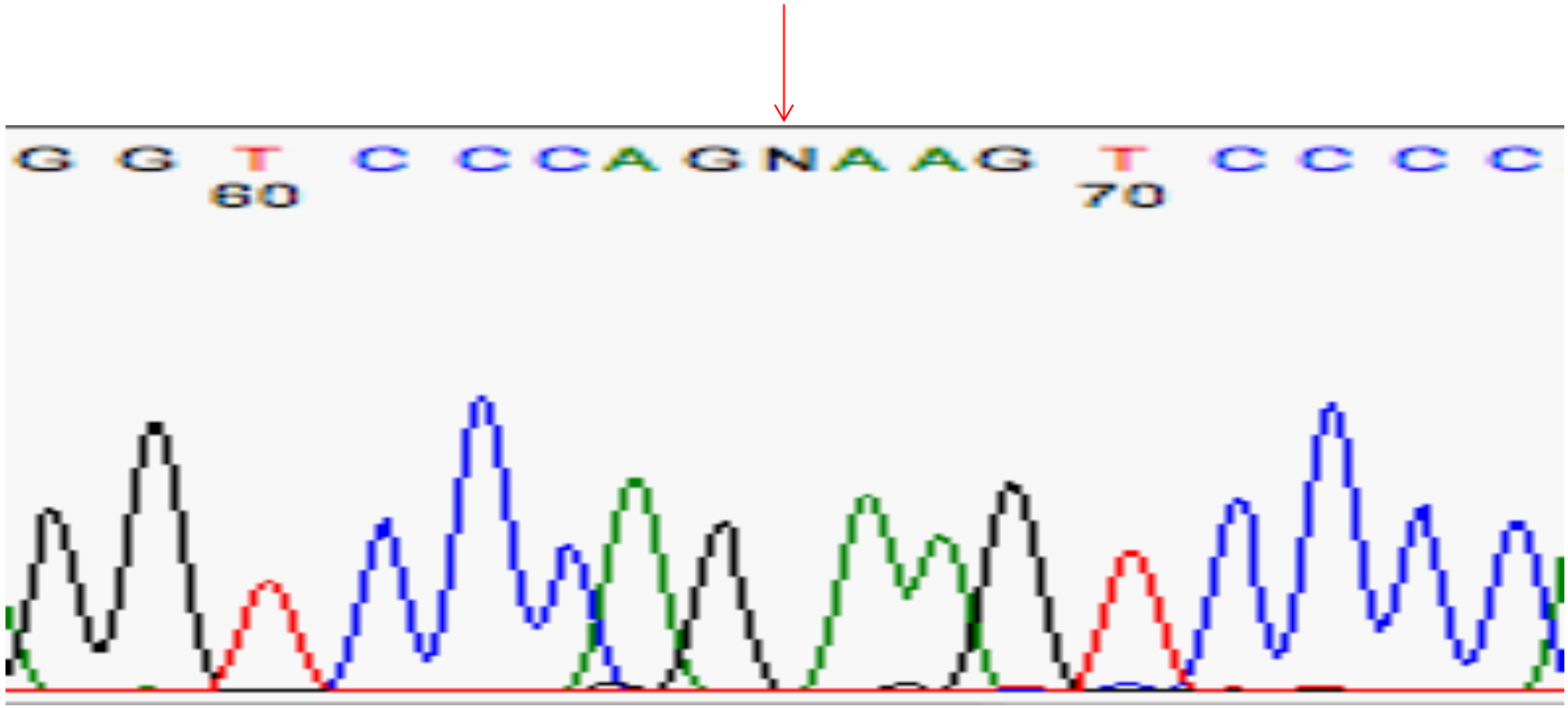
Common one for us is a G-A dinucleotide, which leaves a little extra space between them.

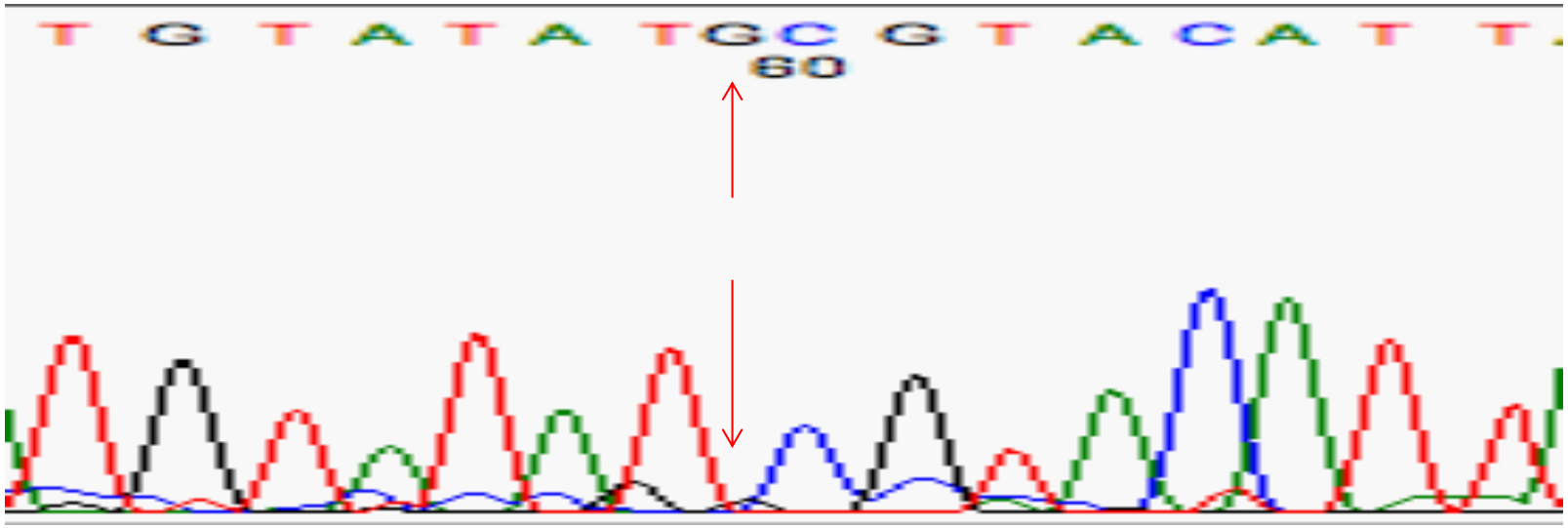


d. Mis-Called

(II) Mis-call a nucleotide:

Sometimes the computer will mis-call a nucleotide when a human could do better. Most often, this occurs when the base caller calls a specific nucleotide, when the peak really was ambiguous and should have been called as 'N'.





d. Mis-Called

(III) The real problem comes when the base caller attempts to interpret a gap as a real nucleotide.

Note the real T peak (nt 58) and the real C peak (nt 60), with the G barely visible between them. Despite its size, the baseline-noise G peak was picked as if it were real. The clues to spot are (i) the oddly-spaced letters, with the G squeezed in, and (ii) the gap in the 'real' peaks, containing a low noise peak.

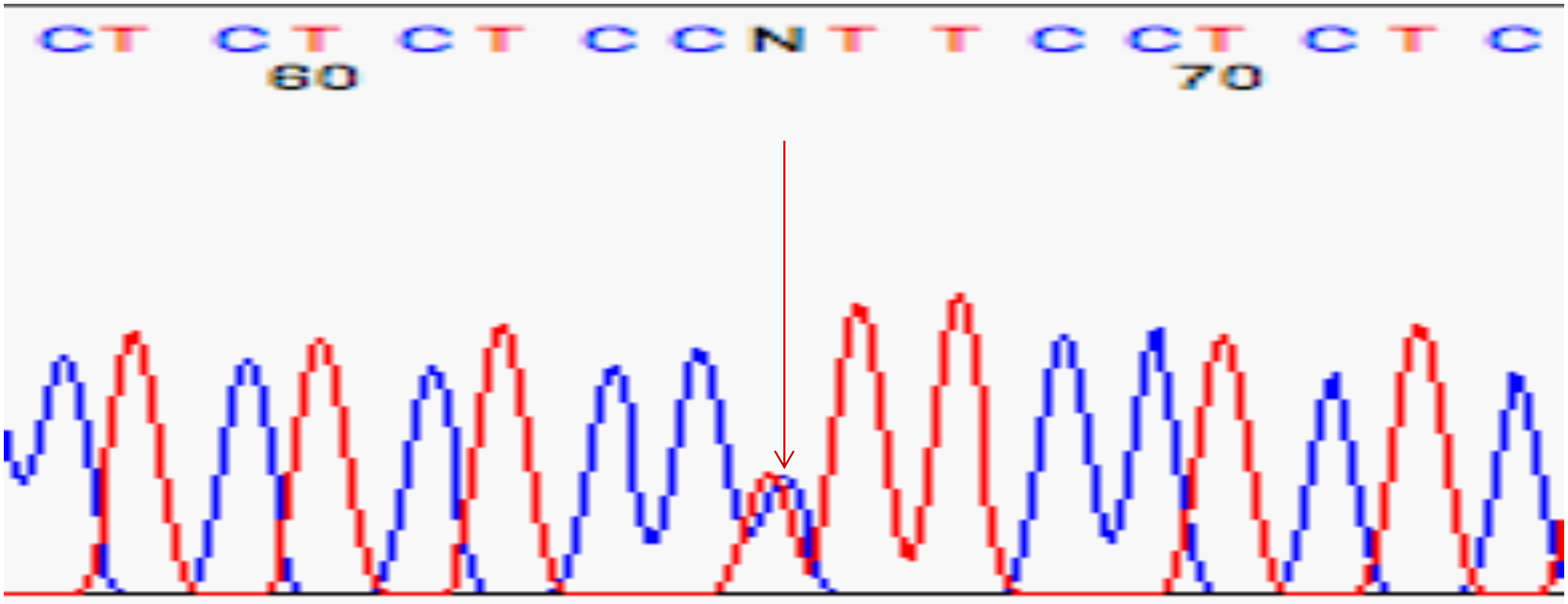
This is a great example of why a weak sample, with its consequent noisy chromatogram, is untrustworthy.

3- Heterozygous (double) peaks:

A single peak position within a trace may have but two peaks of different colors instead of just one.

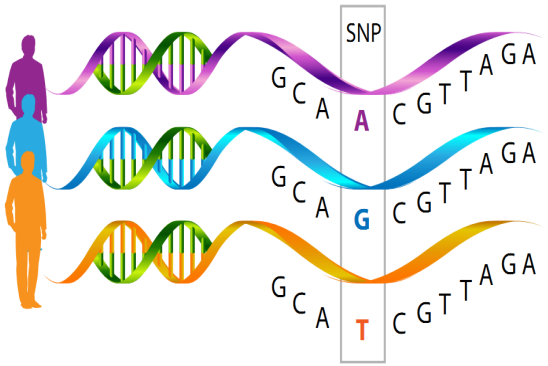
Note that the base caller may list that base position as an 'N', or it may simply call the larger of the two peaks.

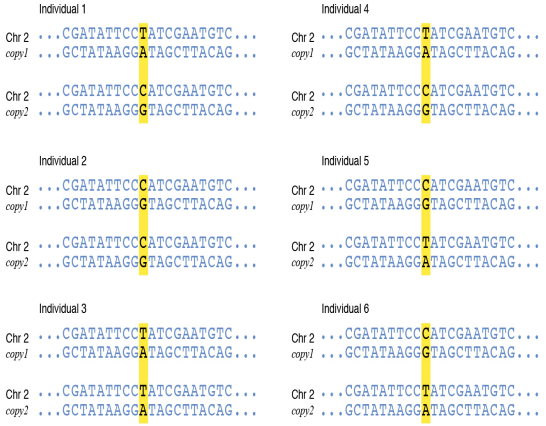
Here's a great example of a PCR

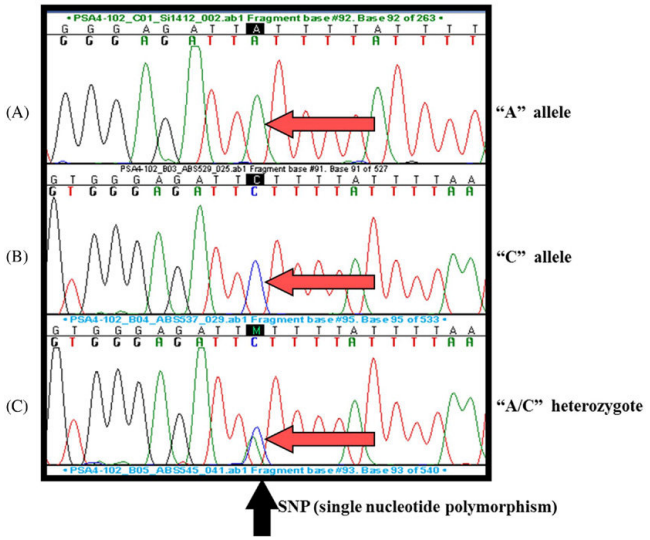


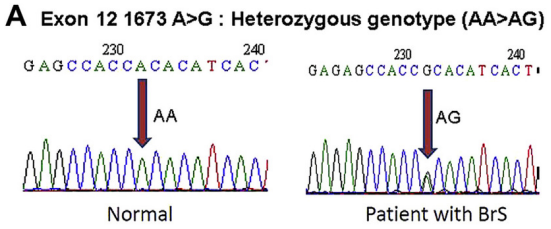
4. Single-nucleotide polymorphism (SNP).

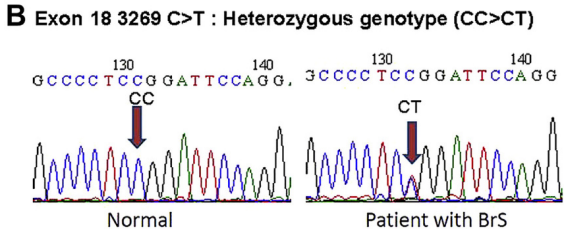
A single peak position within a trace may have two peaks different colors instead of just one.

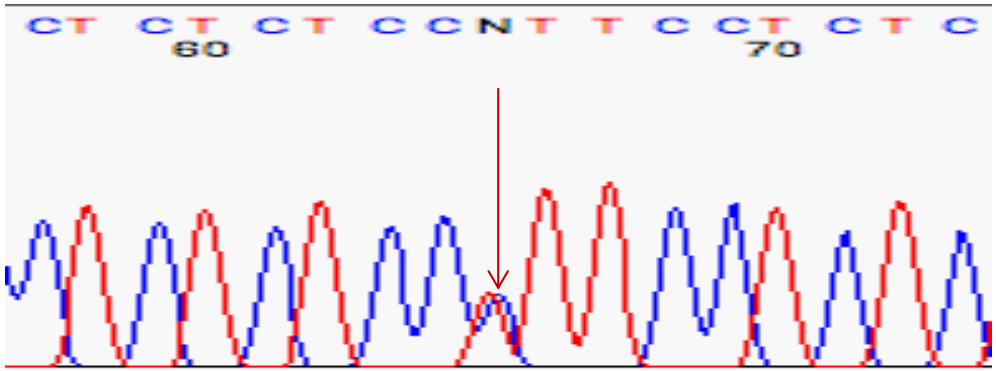




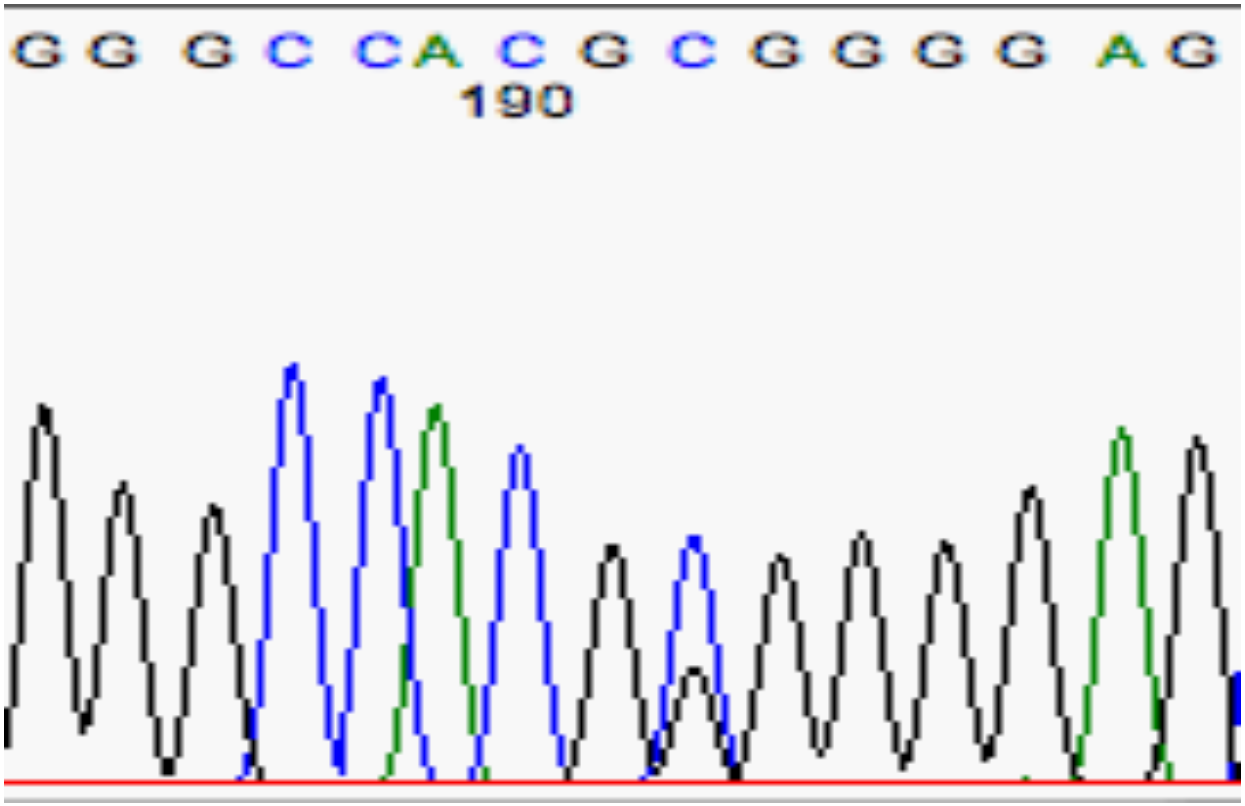






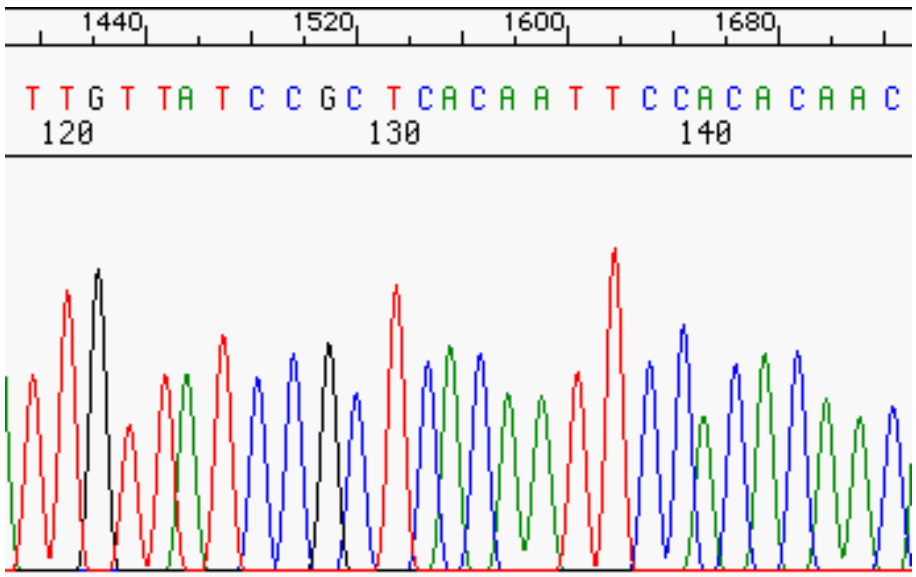


5- *Negative samples / No DNA*—chromatograms displaying peaks from which no useable sequence can be obtained may be due to an absence of DNA. These chromatograms generally have one or two predominant colors.

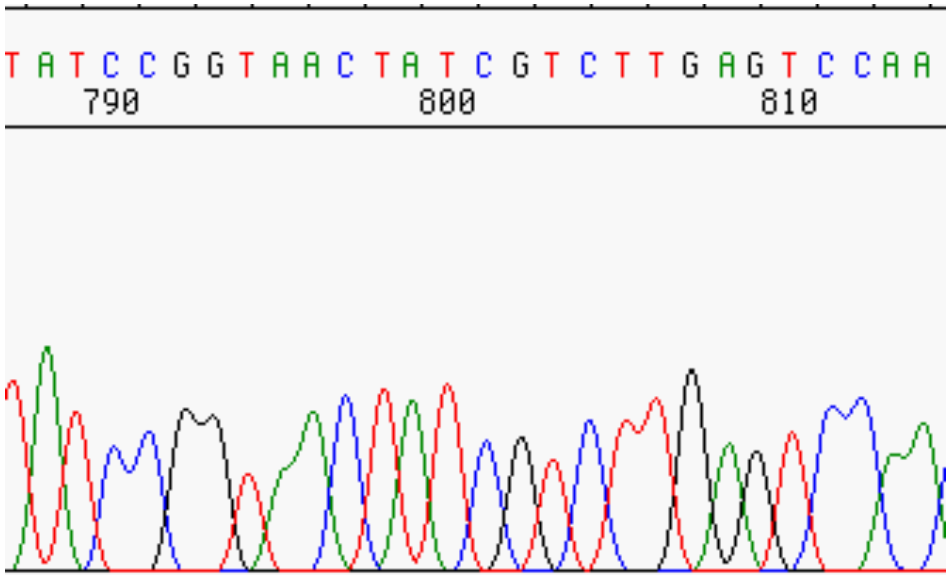


6- *Loss of resolution later in the gel:*

As the gel progresses, it loses resolution. This is normal; peaks broaden and shift, making it harder to make them out and call the bases accurately.



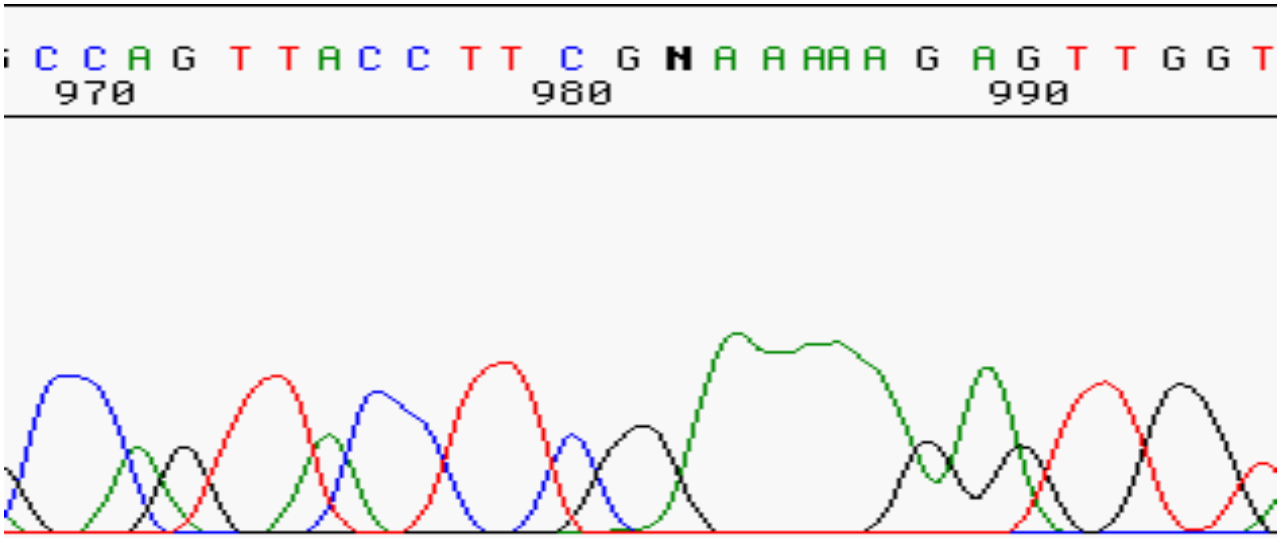
This is a typical example of data from a very good sample



the spacing between the basecall letters at top is regular, which is often a good indication of the reliability of the data.

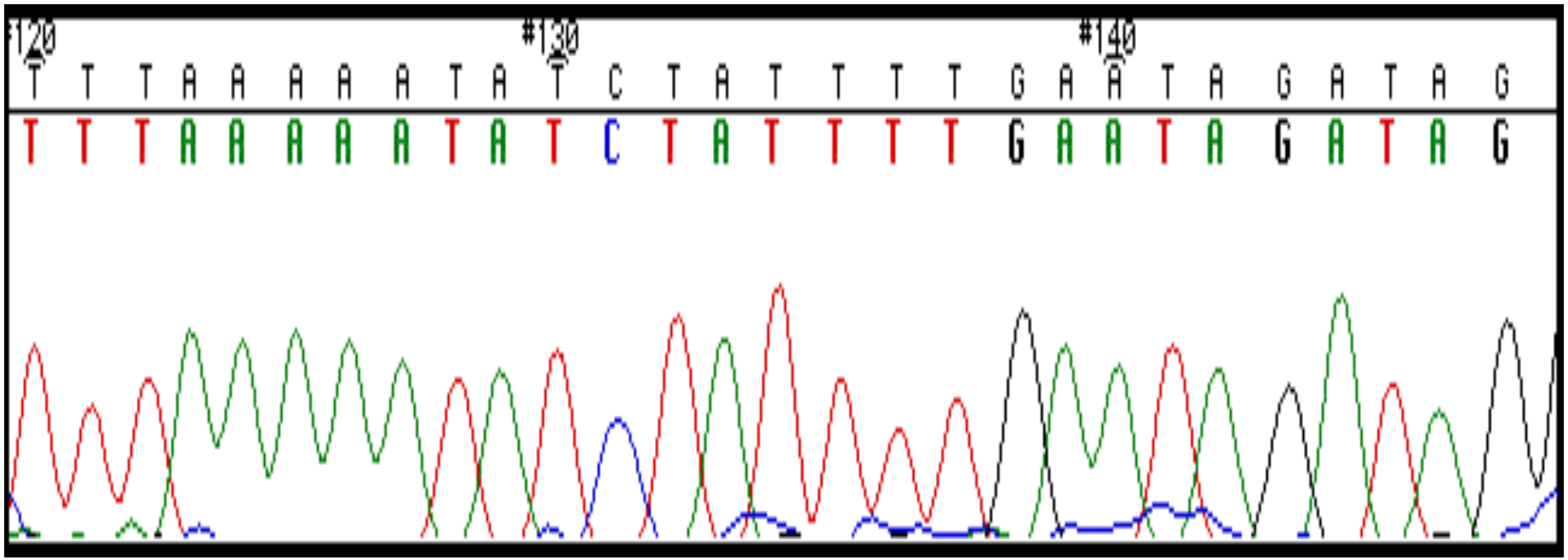
6- *Loss of resolution later in the gel:*

The sequencer will continue attempting to "read" this data, but errors become more and more frequent.



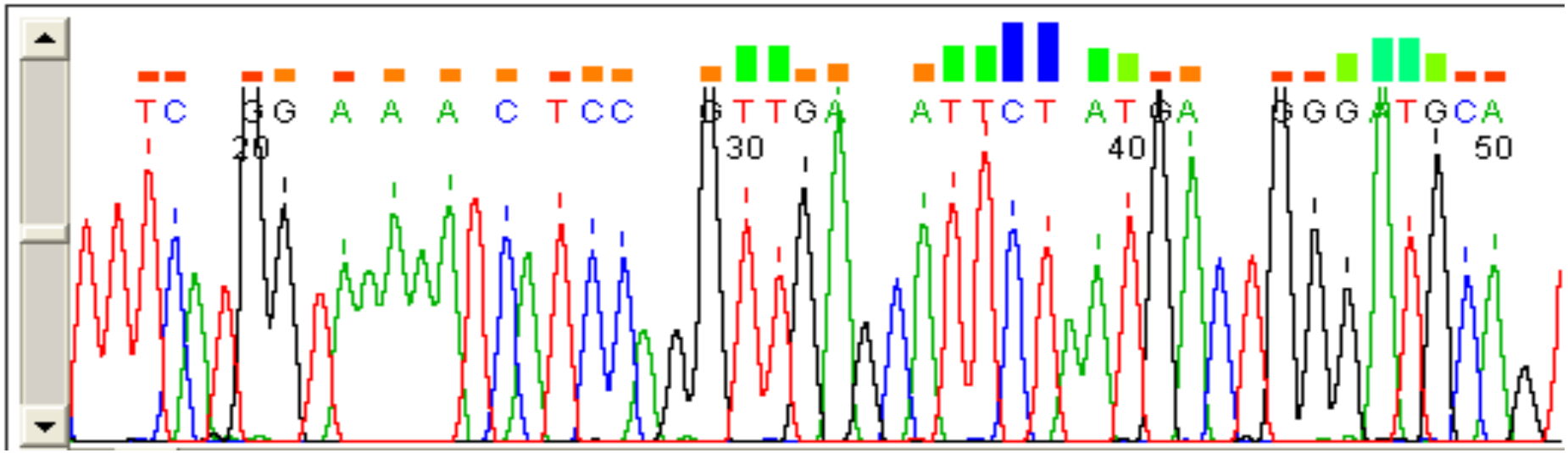
There are only a few base calls that can be considered reliable. The G at 981 may in fact be two G's, the N could be a G or an A, and who knows how many A's there are afterwards.

7- Non-discrete peaks: these may occur when several of the same nucleotide appears in a row. For example, if the sequence includes the region TAAAAAT, it may be represented by one wavy peak as opposed to 5 distinct peaks.



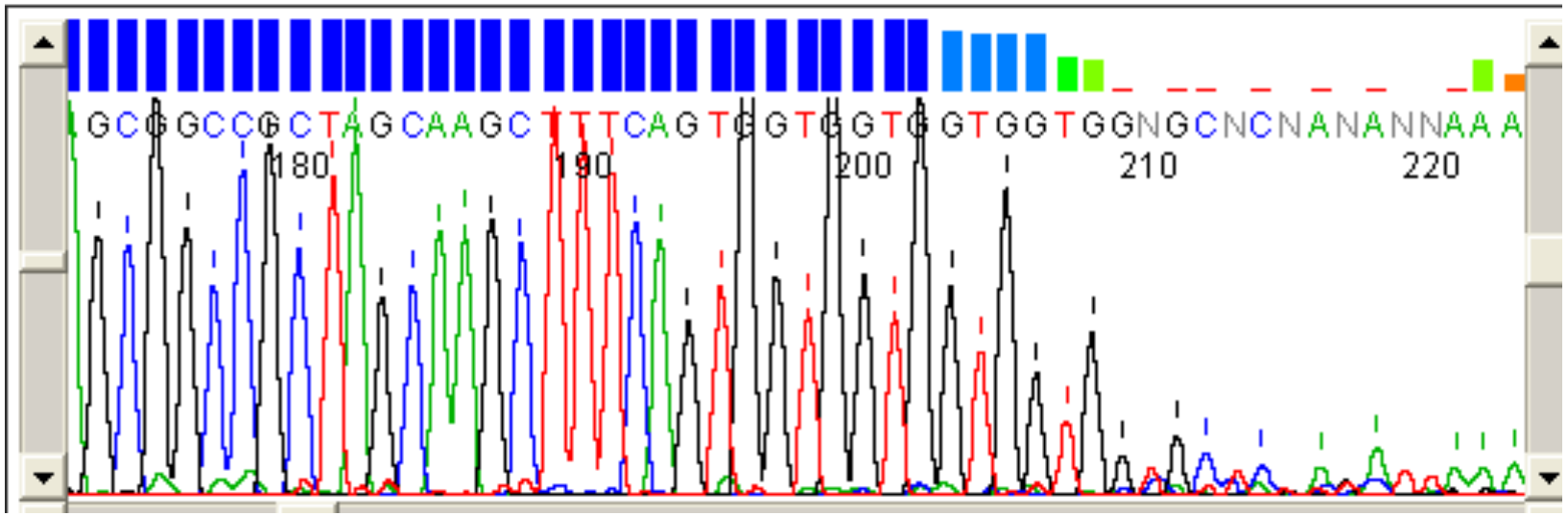
8- Good sequence with bad base calling:

Failed analysis
Ask the Sequencing Service to reanalyze the sequence.



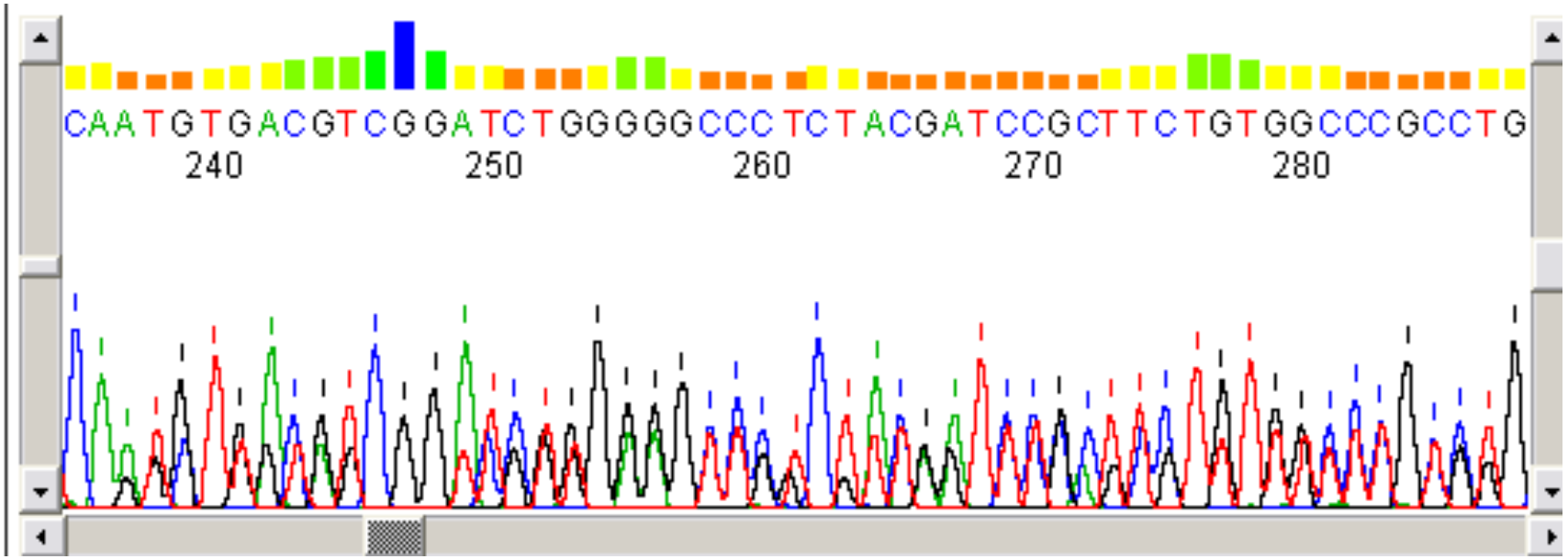
9- DNA template has a secondary structure:

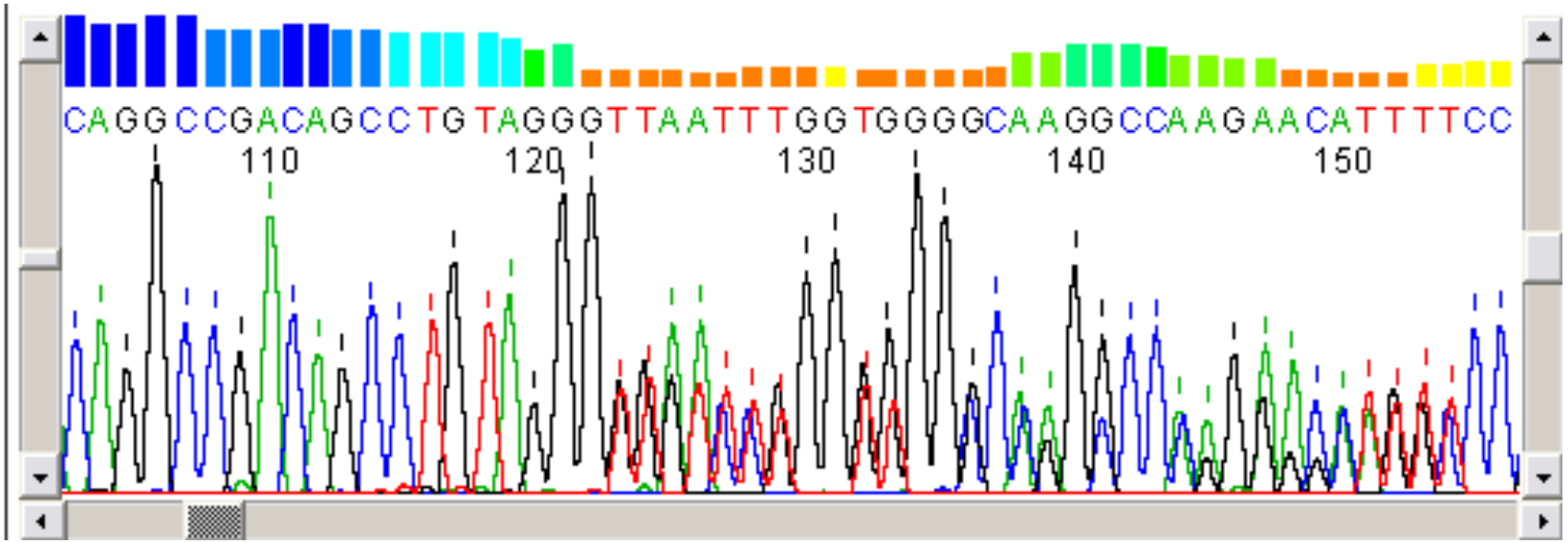
Secondary structures create a distortion that makes it impossible for elongation to continue and so the sequence ends abruptly.



The sequence ends after approximately 200 bp

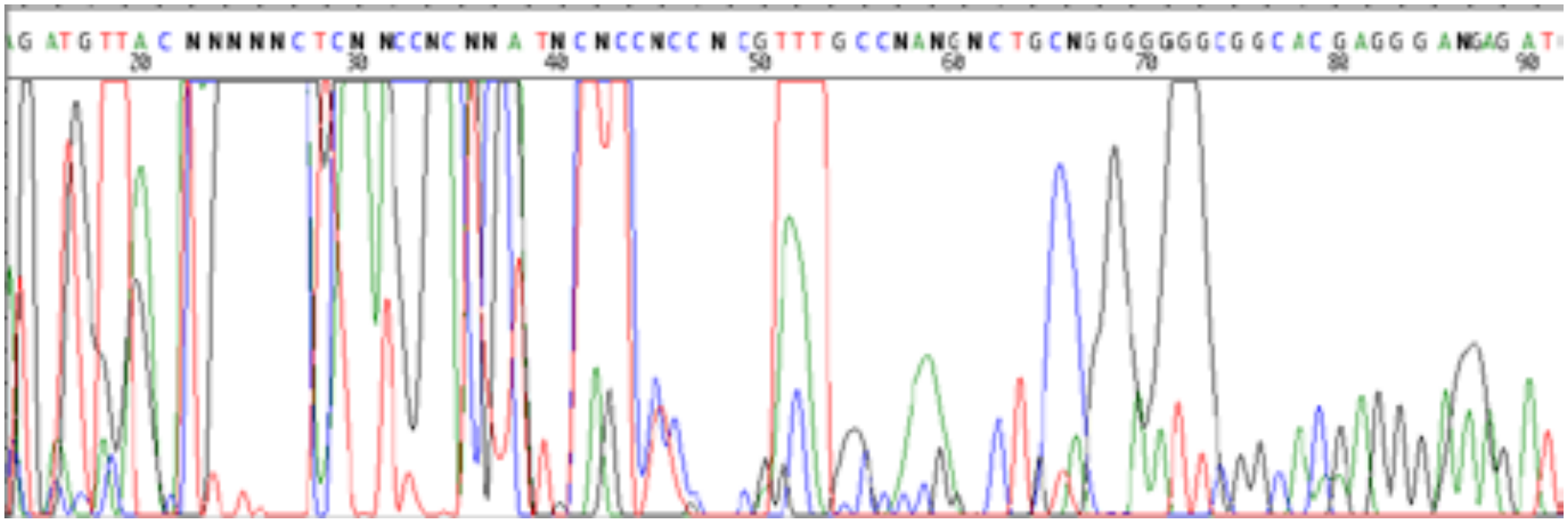
10- DNA contamination:



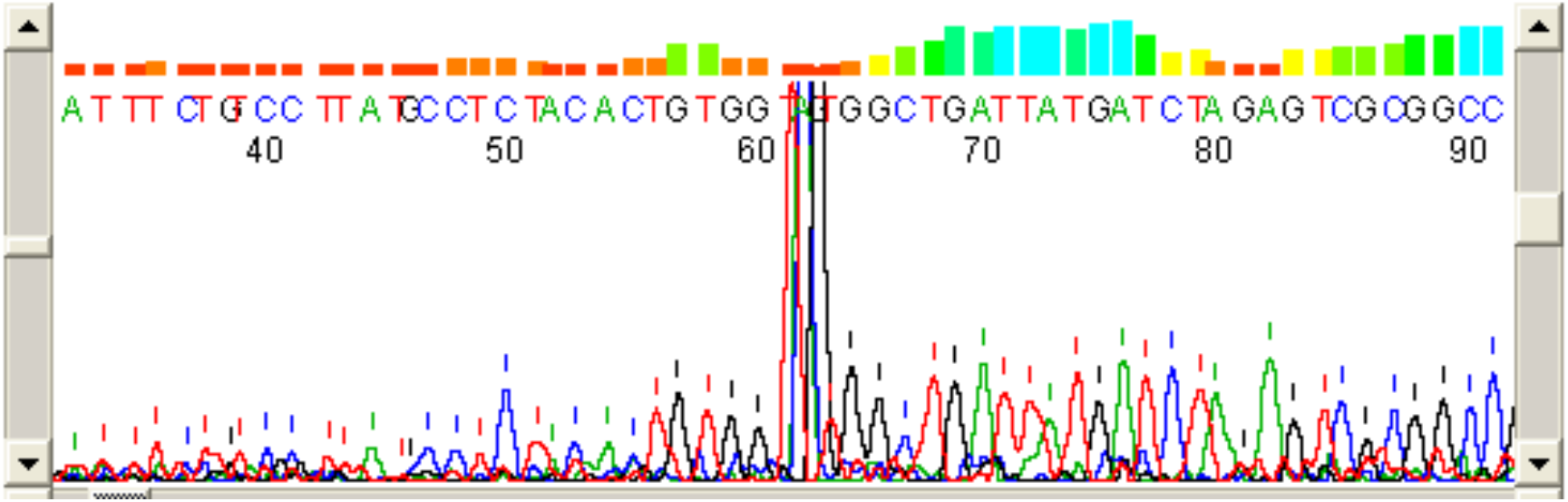


11- Excess dye peaks at the beginning of the sequence

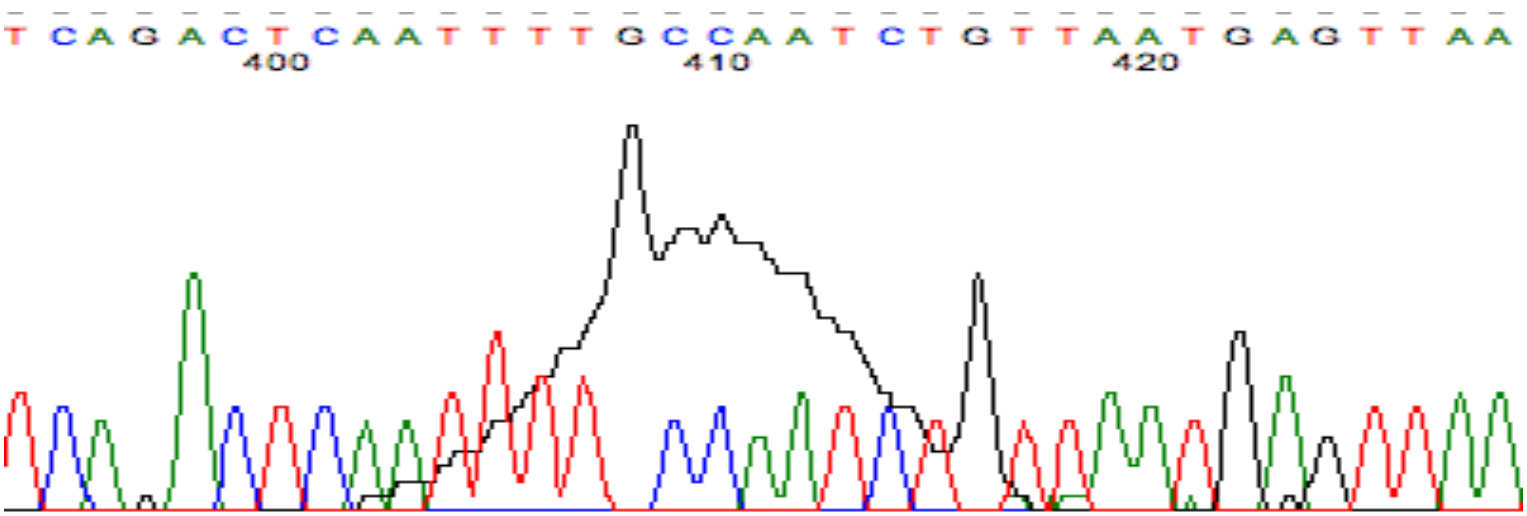
Cause related to sequencing: Poor removal of unincorporated dye terminators during the post-sequencing clean up



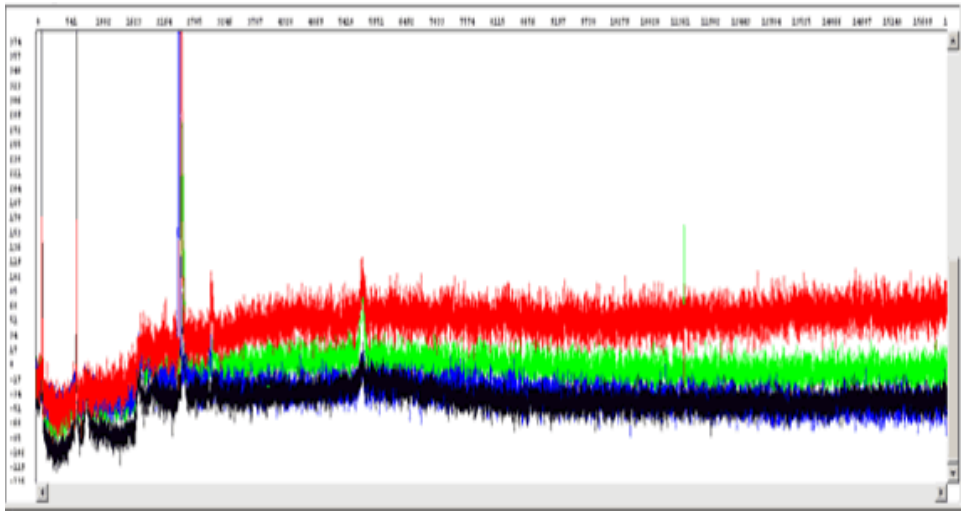
12- Sharp peaks in the sequence:

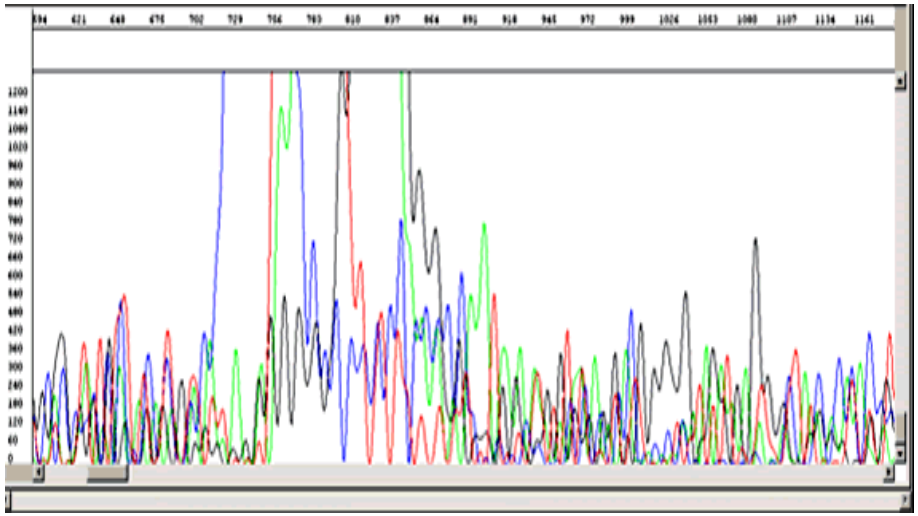


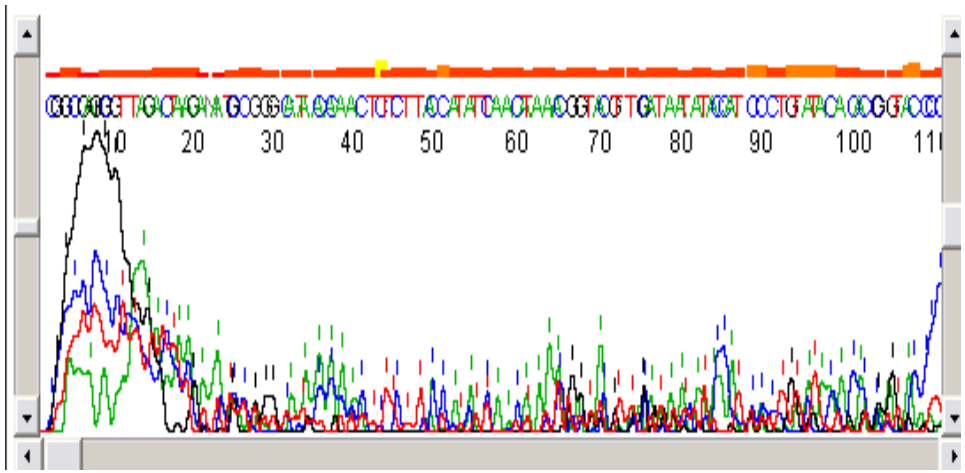
13- Sequence with "G" dye blob

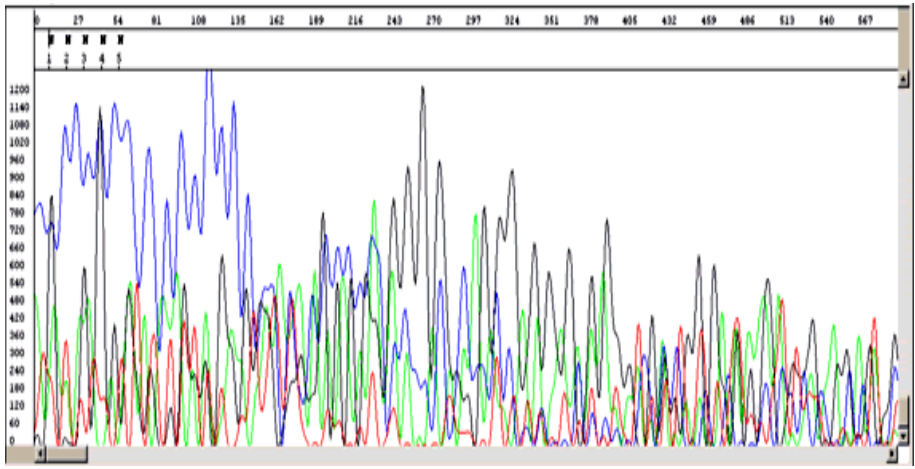


14- Reaction failed, No sequencing data









Realize, too, that it's easy for a human to miss these. If you want to be sure you've detected all the polymorphic positions, you should be using a computer program to scan your chromatograms!

Interpreting of FASTA format of the Sequencing Results

>GXP_210035 loc=GXL_175098|sym=FAM149A|taxid=9606|spec=Homo sapiens|chr=4|ctg=NC_000004|str=(+)|start=187065495|end=187066181|len=687|comm=Promoter Region

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CGCCTGAGCTGGGCCAGCCGCGCGGCGGGCGCGGGCGCGGGCGCGGGCGCGGGCGCGGGCGGGTGGGGAGCCCCAGCCCC
GGGGCCGCGGGGGCGCGTGACCGGCTGTCTGCGTGGGGCCCGCGCGC

1. Determining homology:

In other words, is your sequence like any other published sequences and if so, to what degree?

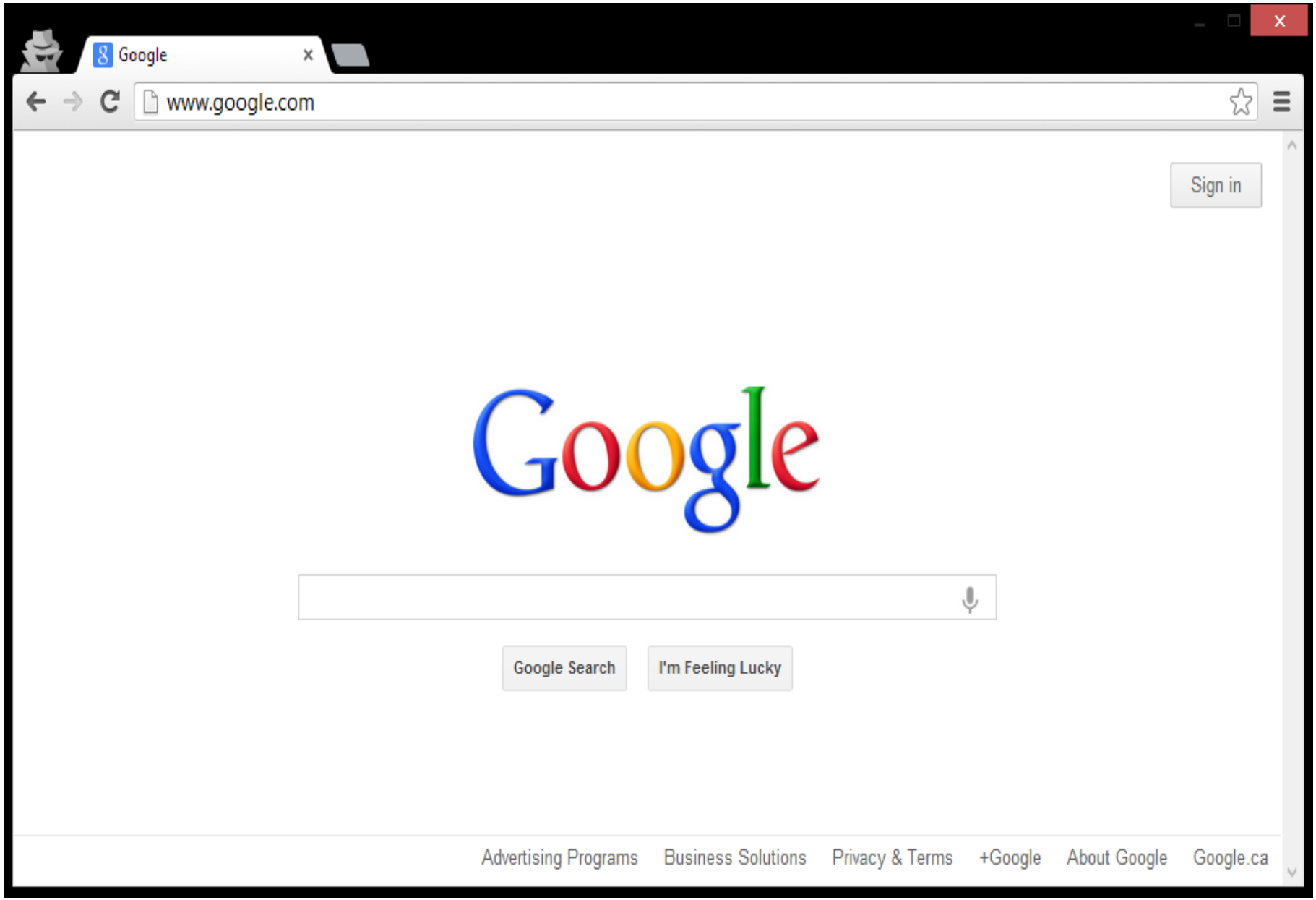
This can be accomplished using:

BLAST, (Basic Local Alignment Search Tool)

It is a program supported by the National Center for Biotechnology Information (NCBI).

The program compares nucleotide or protein sequences to sequence databases and calculates the statistical significance of matches.

This program is accessible at: http://www.ncbi.nlm.nih.gov/BLAST/ (GenBank database; National Center for Biotechnology Information, National Institutes of health).


Google
www.google.com
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Advertising Programs
Business Solutions
Privacy & Terms
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About Google
Google.ca

BLAST: Basic Local Alignment Search Tool

blast.ncbi.nlm.nih.gov/

The **Basic Local Alignment Search Tool** (**BLAST**) finds regions of local similarity between sequences. The program compares nucleotide or protein sequences to **...**

Align two or more - Protein BLAST: ***search ... - Nucleotide BLAST Rat - sequences

Nucleotide **BLAST**: Search nucleotide databases using a nucleotide **...**

blast.ncbi.nlm.nih.gov/Blast.cgi?...blastn...BlastSearch...

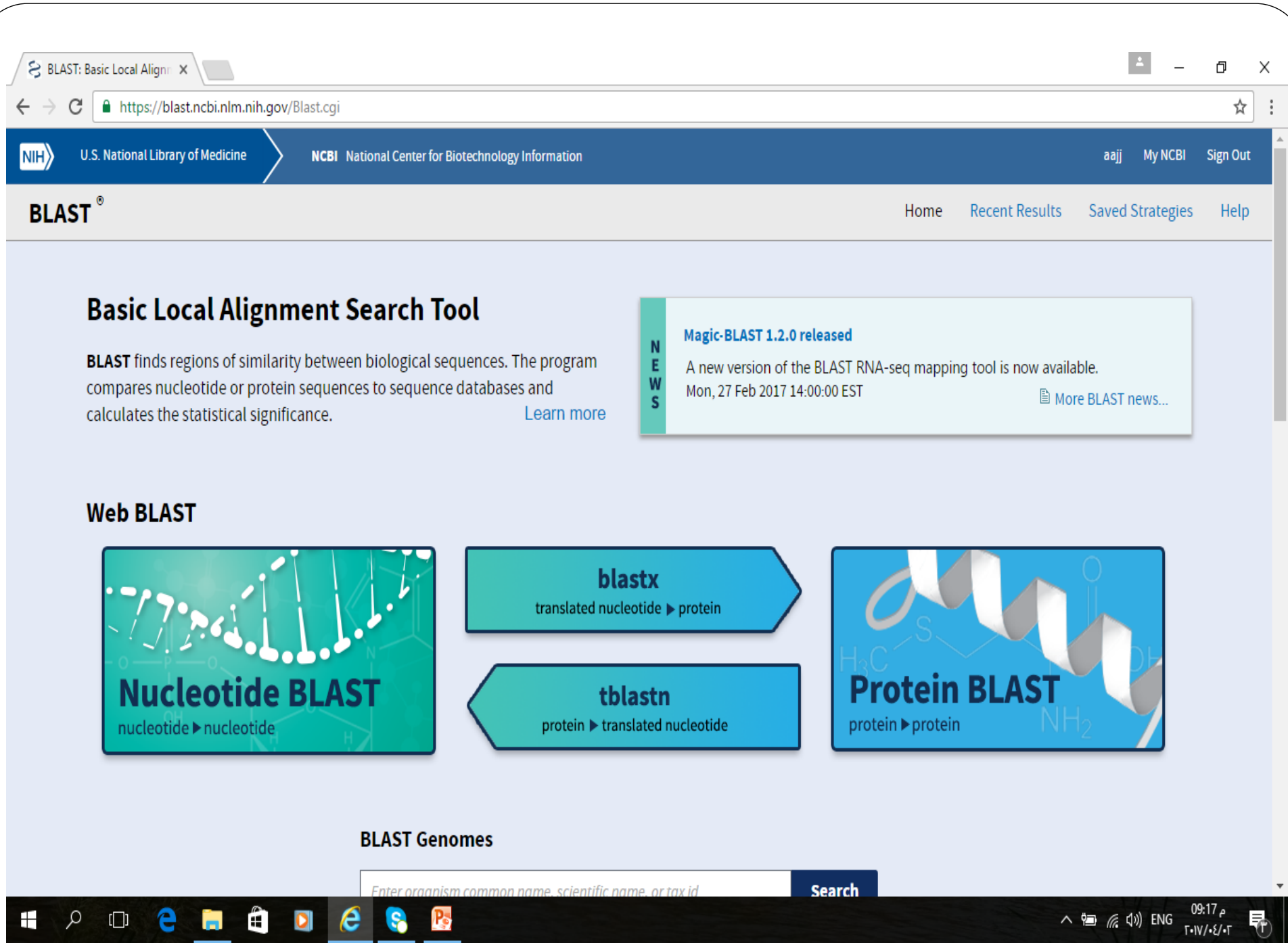
No BLAST database contains all the sequences at NCBI. BLAST databases **...**

BLAST - Wikipedia, the free encyclopedia

en.wikipedia.org/wiki/BLAST

In bioinformatics, **Basic Local Alignment Search Tool**, or **BLAST**, is an algorithm for comparing primary biological sequence information, such as the amino-acid **...**

Process - Output - Input - Background


BLAST: Basic Local Align
https://blast.ncbi.nlm.nih.gov/Blast.cgi
U.S. National Library of Medicine
NCBI National Center for Biotechnology Information
aajj
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Sign Out
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Home
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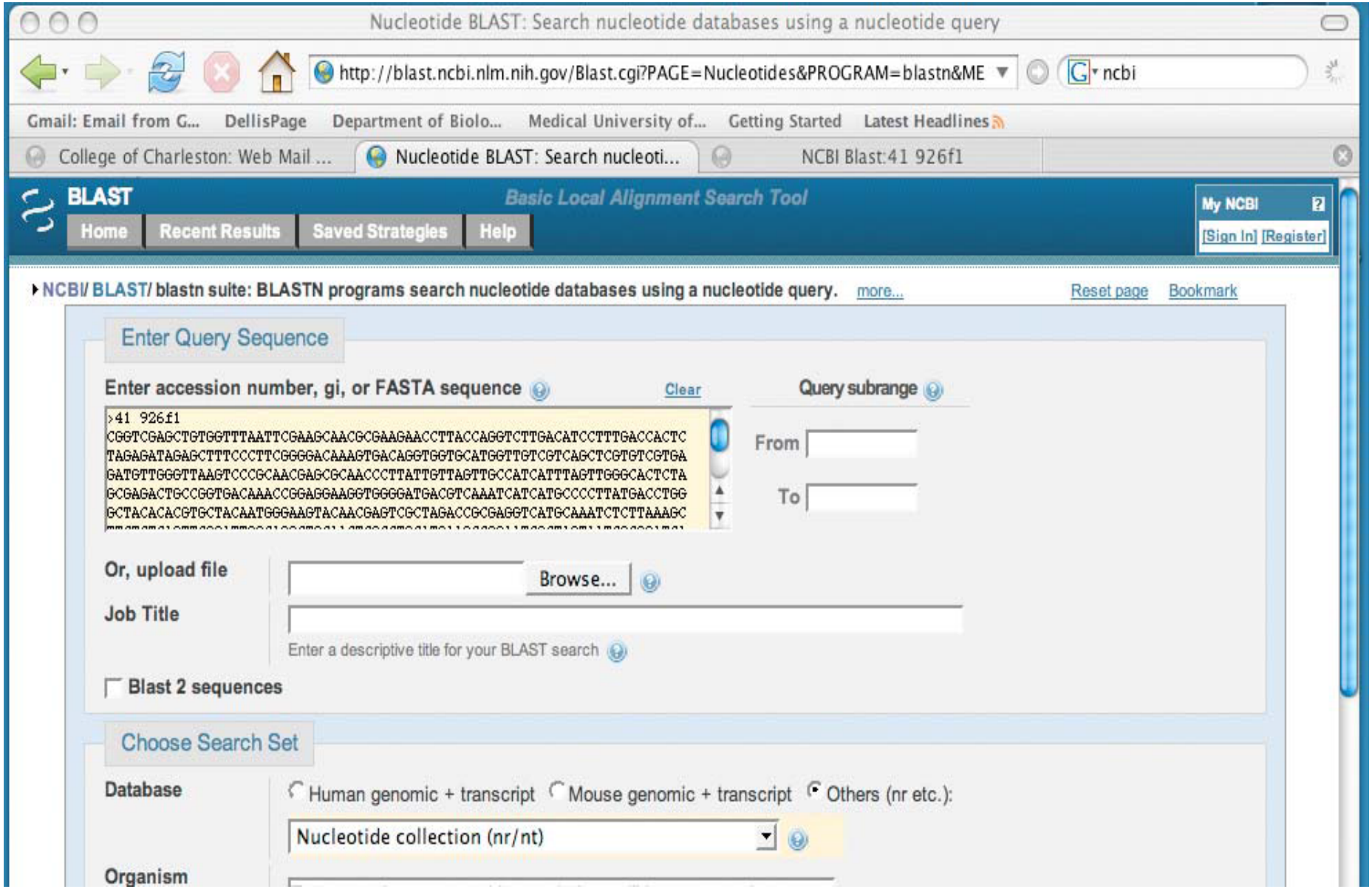
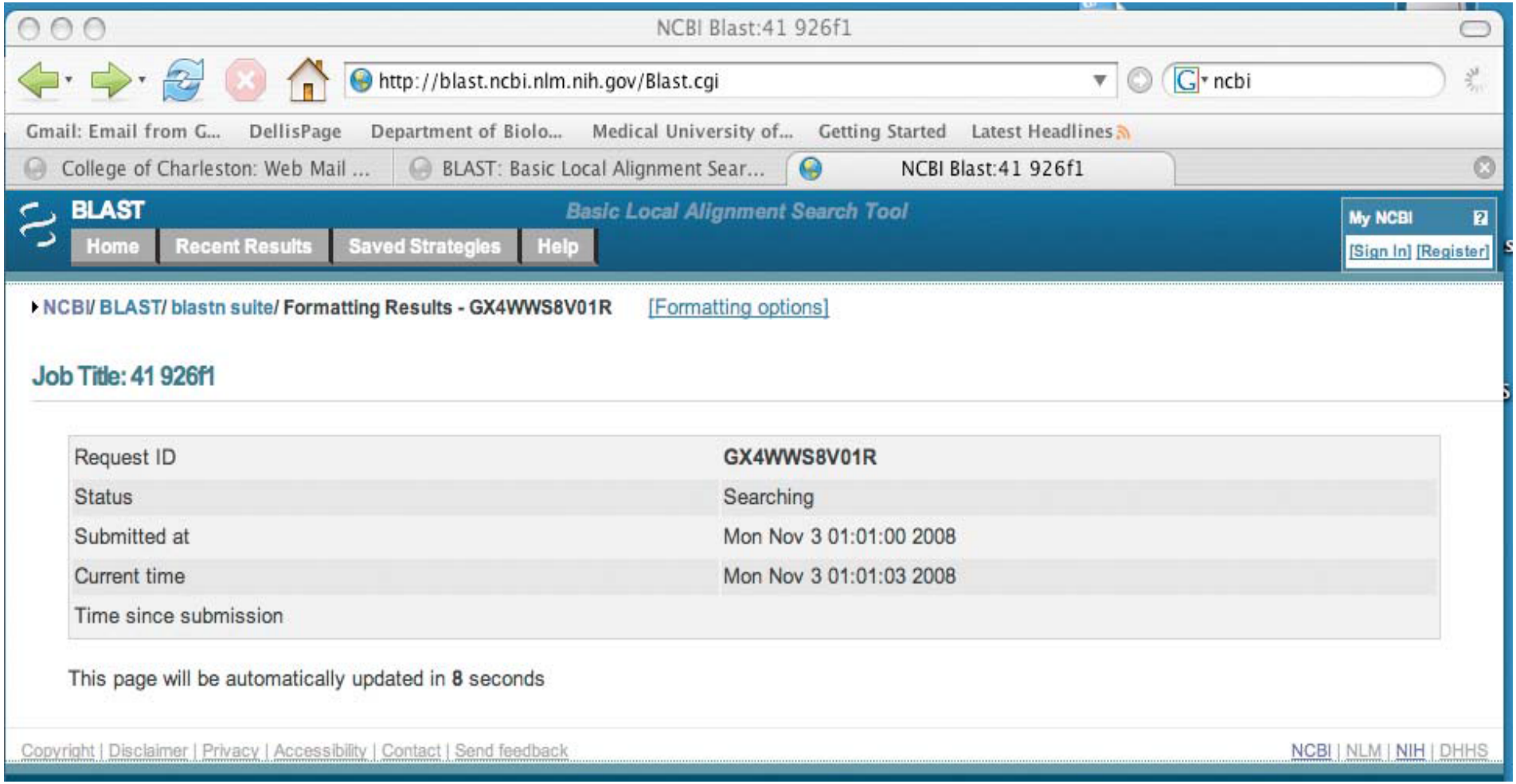
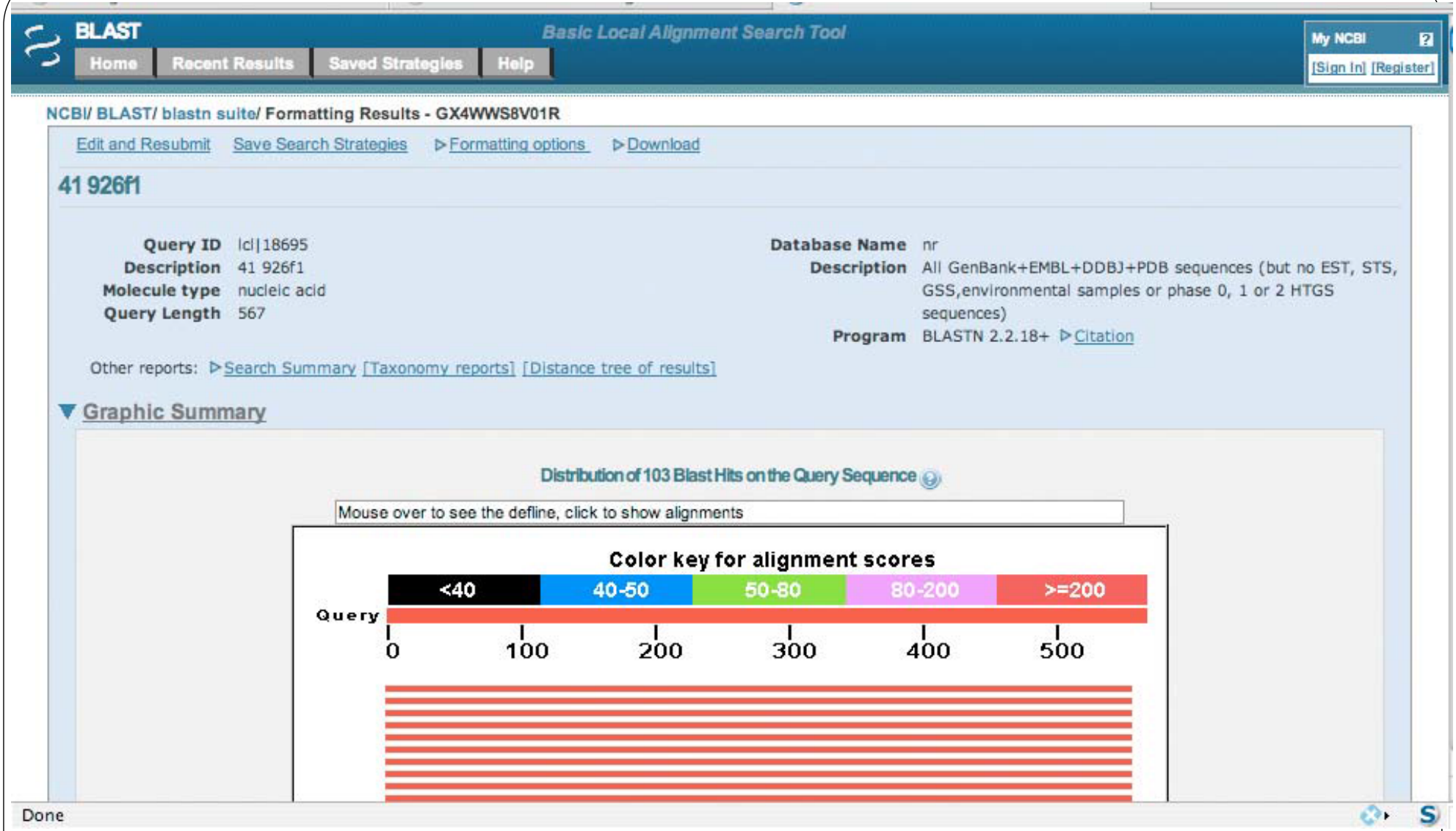
Saved Strategies
Help
Basic Local Alignment Search Tool
BLAST finds regions of similarity between biological sequences. The program compares nucleotide or protein sequences to sequence databases and calculates the statistical significance.
Learn more
NEWS
Magic-BLAST 1.2.0 released
A new version of the BLAST RNA-seq mapping tool is now available.
Mon, 27 Feb 2017 14:00:00 EST
More BLAST news...
Web BLAST
Nucleotide BLAST
nucleotide ▶ nucleotide
blastx
translated nucleotide ▶ protein
tblastn
protein ▶ translated nucleotide
Protein BLAST
protein ▶ protein
BLAST Genomes
Search
ENG
09:17 م



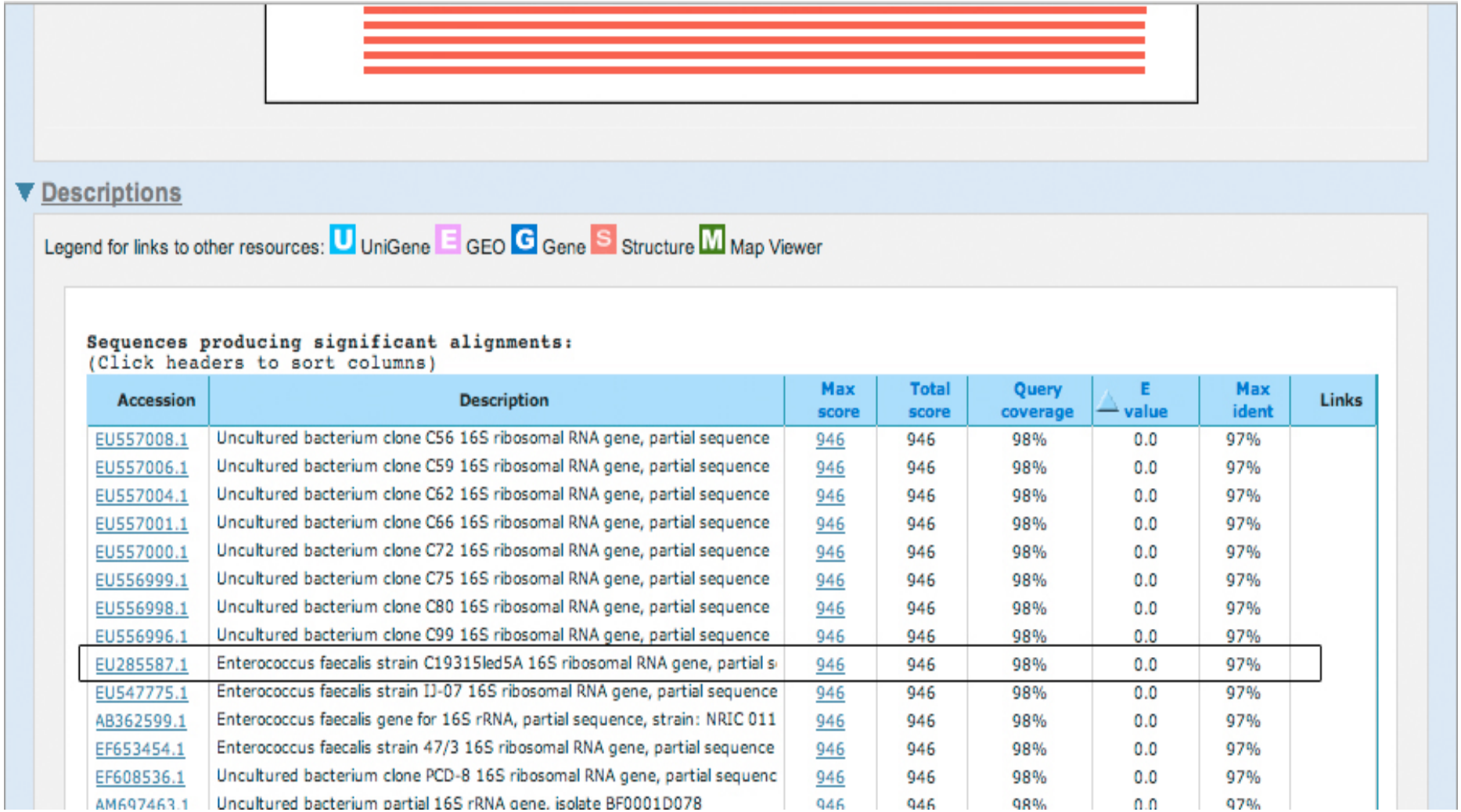
Click the "Blast!" button at the bottom to submit your sequence data.



This screen will come up next. Finally (sometimes after a lengthy wait), a new window will appear showing any "hits" your sequence made. The results will be color coded and annotated



The bars show what places along your sequence are similar to other published sequences; the colors indicate how many bases were involved in homology determination.



Descriptions

Legend for links to other resources: U UniGene E GEO G Gene S Structure M Map Viewer

Sequences producing significant alignments:
(Click headers to sort columns)

Accession	Description	Max score	Total score	Query coverage	E value	Max ident	Links
EU557008.1	Uncultured bacterium clone C56 16S ribosomal RNA gene, partial sequence	946	946	98%	0.0	97%	
EU557006.1	Uncultured bacterium clone C59 16S ribosomal RNA gene, partial sequence	946	946	98%	0.0	97%	
EU557004.1	Uncultured bacterium clone C62 16S ribosomal RNA gene, partial sequence	946	946	98%	0.0	97%	
EU557001.1	Uncultured bacterium clone C66 16S ribosomal RNA gene, partial sequence	946	946	98%	0.0	97%	
EU557000.1	Uncultured bacterium clone C72 16S ribosomal RNA gene, partial sequence	946	946	98%	0.0	97%	
EU556999.1	Uncultured bacterium clone C75 16S ribosomal RNA gene, partial sequence	946	946	98%	0.0	97%	
EU556998.1	Uncultured bacterium clone C80 16S ribosomal RNA gene, partial sequence	946	946	98%	0.0	97%	
EU556996.1	Uncultured bacterium clone C99 16S ribosomal RNA gene, partial sequence	946	946	98%	0.0	97%	
EU285587.1	Enterococcus faecalis strain C19315led5A 16S ribosomal RNA gene, partial s	946	946	98%	0.0	97%	
EU547775.1	Enterococcus faecalis strain IJ-07 16S ribosomal RNA gene, partial sequence	946	946	98%	0.0	97%	
AB362599.1	Enterococcus faecalis gene for 16S rRNA, partial sequence, strain: NRIC 011	946	946	98%	0.0	97%	
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EF608536.1	Uncultured bacterium clone PCD-8 16S ribosomal RNA gene, partial sequenc	946	946	98%	0.0	97%	
AM697463.1	Uncultured bacterium partial 16S rRNA gene, isolate BF0001D078	946	946	98%	0.0	97%	

Clicking on a "gi" link at the beginning of any line will take you to the GenBank accession page for a sequence showing similarity to yours. There you can find a wealth of information about the published sequence to which yours showed some homology.

>gb|EU285587.1| Enterococcus faecalis strain C19315led5A 16S ribosomal RNA gene, partial sequence
Length=1456

Score = 946 bits (512), Expect = 0.0
Identities = 550/566 (97%), Gaps = 12/566 (2%)
Strand=Plus/Plus

```
Query  1     CGGTCGAGC-TGTGGTTTAATTCGAAGCAACGCGAAGAACCTTACCAGGTCTTGACATCC  59
             |||| |||| ||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  893   CGGTGGAGCATGTGGTTTAATTCGAAGCAACGCGAAGAACCTTACCAGGTCTTGACATCC  952

Query  60    TTTGACCACTCTAGAGATAGAGCTTTCCCTTCGGGGACAAAGTGACAGGTGGTGCATGGT  119
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  953   TTTGACCACTCTAGAGATAGAGCTTTCCCTTCGGGGACAAAGTGACAGGTGGTGCATGGT  1012

Query  120   TGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTATT  179
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1013  TGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTATT  1072

Query  180   GTTAGTTGCCATCATTTAGTTGGGCACTCTAGCGAGACTGCCGGTGACAAACCGGAGGAA  239
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1073  GTTAGTTGCCATCATTTAGTTGGGCACTCTAGCGAGACTGCCGGTGACAAACCGGAGGAA  1132

Query  240   GGTGGGGATGACGTCAAATCATCATGCCCCTTATGACCTGGGCTACACACGTGCTACAAT  299
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1133  GGTGGGGATGACGTCAAATCATCATGCCCCTTATGACCTGGGCTACACACGTGCTACAAT  1192

Query  300   GGGAAGTACAACGAGTCGCTAGACCGCGAGGTCATGCAAATCTCTTAAAGCTTCTCTCAG  359
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1193  GGGAAGTACAACGAGTCGCTAGACCGCGAGGTCATGCAAATCTCTTAAAGCTTCTCTCAG  1252

Query  360   TTCGGATTGGCAGGCTGCAACTCGCCTGCATGAAGCCGGAATCGCTAGTAATCGCGGATC  419
             ||||||||| ||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1253  TTCGGATTG-CAGGCTGCAACTCGCCTGCATGAAGCCGGAATCGCTAGTAATCGCGGATC  1311

Query  420   AGCACGCCGCGGTGAATACGTTGCCGGGGCCTTGTACACACCGCCCGTCACACCACGAGA  479
             |||||||||||||||||||| |||||| ||||||||||||||||||||||||||||||||
Sbjct  1312  AGCACGCCGCGGTGAATACGTTCCCGGG-CCTTGTACACACCGCCCGTCACACCACGAGA  1370

Query  480   GTTTGTAACACCCGAAGTCGG-GAGGTACCCTTTT-GGAGC-A-CCGCCTTAGGTGG-AT  534
             |||||||||||||||||||| ||||||| |||||| ||||| | |||||| |||||| ||
Sbjct  1371  GTTTGTAACACCCGAAGTCGGTGAGGTAACCTTTTTGGAGCCAGCCGCCTAAGGTGGGAT  1430

Query  535   AGATGAT-GGGGTGA-GTTC-TAACA  557
             ||||||| ||||||| || | |||||
Sbjct  1431  AGATGATTGGGGTGAAGT-CGTAACA  1455
```

Interpretation of sequences which coding for protein

Translation and Open Reading Frame Search

Regions of DNA that encode proteins are first transcribed into messenger RNA and then translated into protein.

By examining the DNA sequence alone we can determine the sequence of amino acids that will appear in the final protein.

In translation codons of three nucleotides determine which amino acid will be added next in the growing protein chain.

It is important then to decide which nucleotide to start translation, and when to stop, this is called an **open reading frame**.

Once a gene has been sequenced it is important to determine the correct **open reading frame (ORF).**

Every region of DNA has three/six possible **reading frames**, three in each direction.

The reading frame that is used determines which amino acids will be encoded by a gene.

Typically only one reading frame is used in translating a gene and this is often the longest open reading frame.

Once the open reading frame is known the DNA sequence can be translated into its corresponding amino acid sequence. An open reading frame starts with an **ATG (Met)** in most species and ends with a **stop codon (TAA, TAG or TGA).**

For example,

the following sequence of DNA can be read in six reading frames.

Three in the forward and three in the reverse direction.

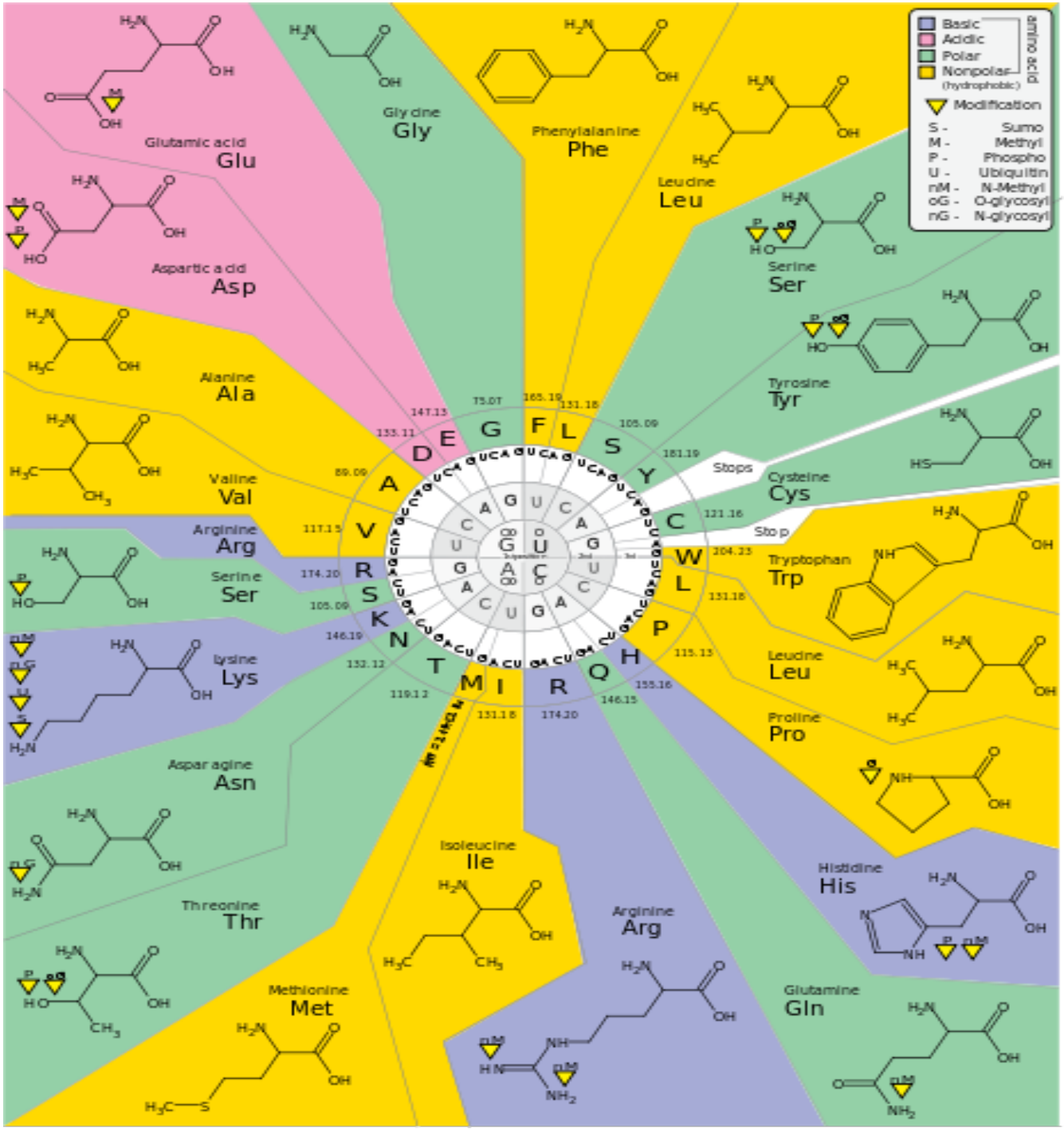
The three reading frames in the forward direction are shown with the translated amino acids below each DNA sequence.

Frame 1 starts with the "a", Frame 2 with the "t" and Frame 3 with the "g". Stop codons are indicated by an "*" in the protein sequence.

5' 3'

atgcccaagctgaatagcgtagaggggttttcatcatttgaggacgatgtataa

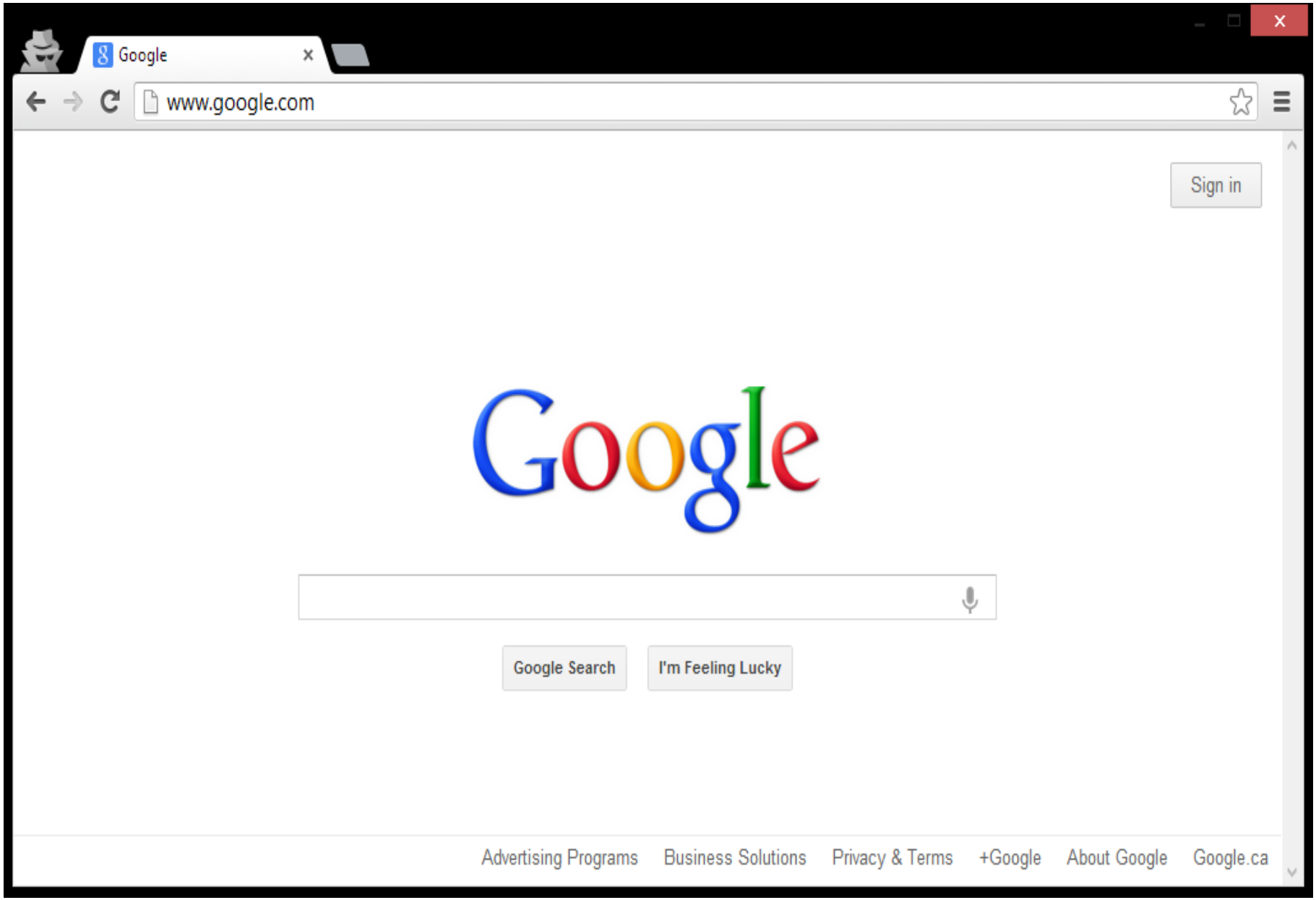
1	atg	ccc	aag	ctg	aat	agc	gta	gag	ggg	ttt	tca	tca	ttt	gag	gac	gat	gta	taa
	M	P	K	L	N	S	V	E	G	F	S	S	F	E	D	D	V	*
2	tgc	cca	agc	tga	ata	gcg	tag	agg	ggt	ttt	cat	cat	ttg	agg	acg	atg	tat	
	C	P	S	*	I	A	*	R	G	F	H	H	L	R	T	M	Y	
3	gcc	caa	gct	gaa	tag	cgt	aga	ggg	gtt	ttc	atc	att	tga	gga	cga	tgt	ata	
	A	Q	A	E	*	R	R	G	V	F	I	I	*	G	R	C	I	


amino acid
Basic
Acidic
Polar
Nonpolar
(hydrophobic)
Modification
S - Sumo
M - Methyl
P - Phospho
U - Ubiquitin
nM - N-Methyl
oG - O-glycosyl
nG - N-glycosyl
Glycine
Gly
Phenylalanine
Phe
Leucine
Leu
Glutamic acid
Glu
Aspartic acid
Asp
Serine
Ser
Tyrosine
Tyr
Alanine
Ala
Valine
Val
Stops
Cysteine
Cys
Stop
Arginine
Arg
Serine
Ser
Tryptophan
Trp
Leucine
Leu
lysine
Lys
Proline
Pro
Asparagine
Asn
Isoleucine
Ile
Histidine
His
Threonine
Thr
Arginine
Arg
Methionine
Met
Glutamine
Gln

Translation:

Each sequence must be translate to its amino acids (aa) by using

Expasy.translatesoftware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Translate tool

Translate is a tool which allows the translation of a nucleotide (DNA/RNA) sequence to a protein sequence.

Please enter a DNA or RNA sequence in the box below (numbers and blanks are ignored).

```
3601 AAGATACTAG TTTTGCTGAA AATGACATTA AGGAAAGTTC TGCTGTTTTT AGCAAAAGCG
3661 TCCAGAAAGG AGAGCTTAGC AGGAGTCCTA GCCCTTTCAC CCATACACAT TTGGCTCAGG
3721 GTTACCGAAG AGGGGCCAAG AAATTAGAGT CCTCAGAAGA GAACTTATCT AGTGAGGATG
3781 AAGAGCTTCC CTGCTTCCAA CACTTGTTAT TTGGTAAAGT AAACAATATA CCTTCTCAGT
3841 CTACTAGGCA TAGCACCGTT GCTACCGAGT GTCTGTCTAA GAACACAGAG GAGAATTTAT
3901 TATCATTGAA GAATAGCTTA AATGACTGCA GTAACCAGGT AATATTGGCA AAGGCATCTC
3961 AGGAACATCA CCTTAGTGAG GAAACAAAAT GTTCTGCTAG CTTGTTTTCT TCACAGTGCA
4021 GTGAATTGGA AGACTTGACT GCAAATACAA ACACCCAGGA TCCTTTCTTG ATTGGTTCTT
4081 CCAAACAAAT GAGGCATCAG TCTGAAAGCC AGGGAGTTGG TCTGAGTGAC AAGGAATTGG
4141 TTTCAGATGA TGAAGAAAGA GGAACGGGCT TGGAAGAAAA TAATCAAGAA GAGCAAAGCA
4201 TGGATTCAAA CTTAGGTGAA GCAGCATCTG GGTGTGAGAG TGAAACAAGC GTCTCTGAAG
4261 ACTGCTCAGG GCTATCCTCT CAGAGTGACA TTTTAACCAC TCAGCAGAGG GATACCATGC
4321 AACATAACCT GATAAAGCTC CAGCAGGAAA TGGCTGAACT AGAAGCTGTG TTAGAACAGC
4381 ATGGGAGCCA GCCTTCTAAC AGCTACCCTT CCATCATAAG TGACTCTTCT GCCCTTGAGG
4441 ACCTGCGAAA TCCAGAACAA AGCACATCAG AAAAAGCAGT ATTAACTTCA CAGAAAAGTA
```

Output format: Verbose ("Met", "Stop", spaces between residues)

Reset or TRANSLATE SEQUENCE

Strand 1:

1st ORF: 2 stop codons

CGA-GAT-GCC-TAA-ATG-AGT-TGG-CCA-GCA-GAG-CGA-GCA-TGG-ATG-TAA-TCA-G

R D A * M S W P A E R A W M * S

2nd ORF: 1 stop codons

GAG-ATG-CCT-AAA-TGA-GTT-GGC-CAG-CAG-AGC-GAG-CAT-GGA-TGT-AAT-CAG

E M P K * V G Q Q S E H G C N Q

3rd ORF: 0 stop codons

AGA-TGC-CTA-AAT-GAG-TTG-GCC-AGC-AGA-GCG-AGC-ATG-GAT-GTA-ATC-AG

R C L N E L A S R A S M D V I

Reverse complementary strand:

4th ORF: 0 stop codons

CTG-ATT-ACA-TCC-ATG-CTC-GCT-CTG-CTG-GCC-AAC-TCA-TTT-AGG-CAT-CTC-G

L I T S M L A L L A N S F R H L

5th ORF: 1 stop codons

TGA-TTA-CAT-CCA-TGC-TCG-CTC-TGC-TGG-CCA-ACT-CAT-TTA-GGC-ATC-TCG

* L H P C S L C W P T H L G I S

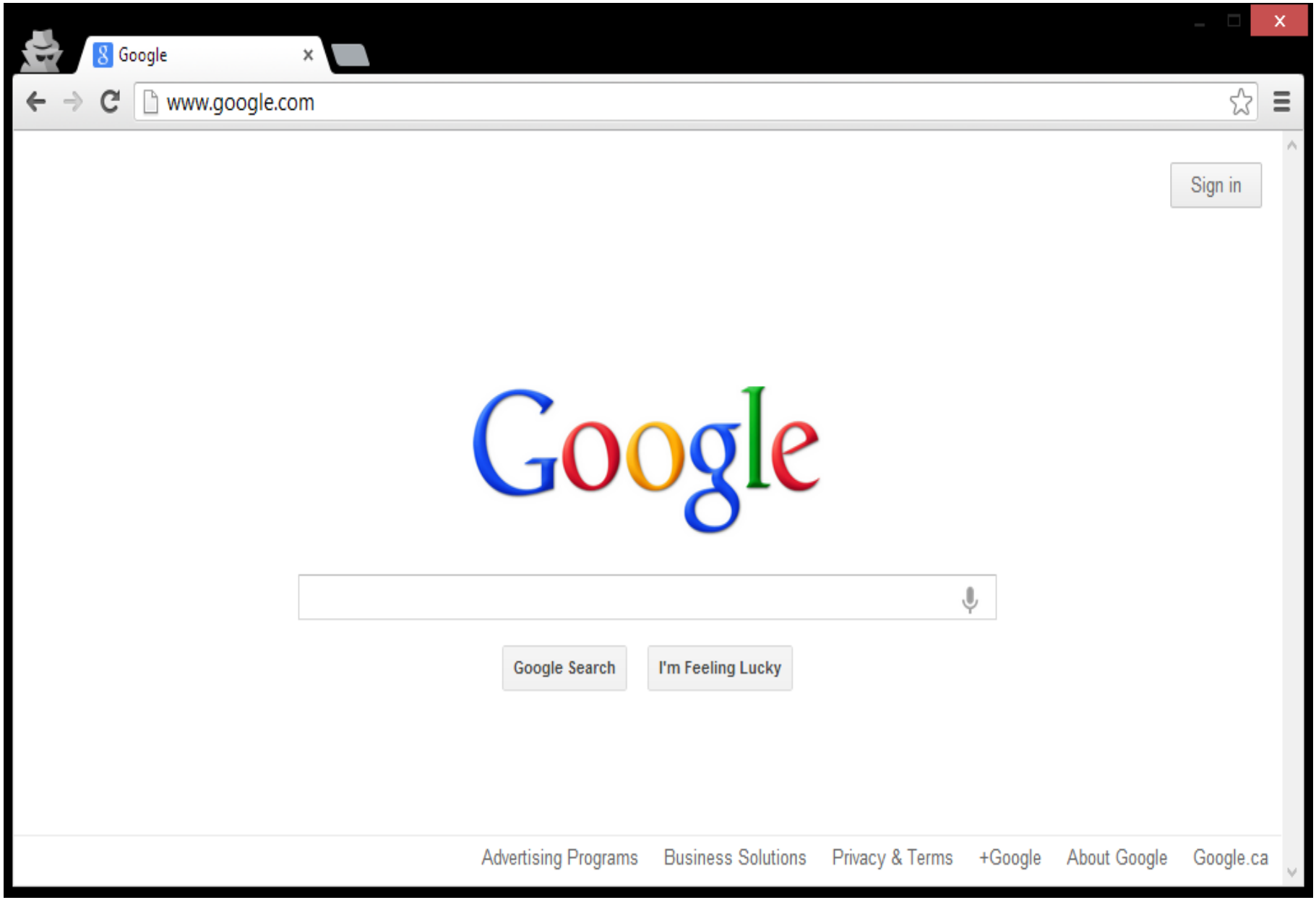
6th ORF: 1 stop codons

GAT-TAC-ATC-CAT-GCT-CGC-TCT-GCT-GGC-CAA-CTC-ATT-TAG-GCA-TCT-CG

D Y I H A R S A G Q L I * A S

>Seq3,

MLQMRMKRKR RKKKDVVLDV TLTSCENVTF DTRDPNSVVL TVKDGFRFKT LKVGDKTLFN
VDTGKHTPVK AFKLKHDSEE WFRLDLHAAQ PKMFKKKGDK EYSESKFETY YDEVLFKGKS
AKELDVSKFE DPALFTSANF GTGKKYTFKK DFKPSKVLFE KKEVGKPNNA KYLEVVVFVG
SDSKKLVKLY YFYTGDSRLK ETYFELKDDK WVQMTQADAN KALNAMNSSW STDYKPVVDK
FSPLAVFASV LIVFSSV


Google
www.google.com
Sign in
Google
Google Search
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BLAST: Basic Local Alignment Search Tool

blast.ncbi.nlm.nih.gov/

The **Basic Local Alignment Search Tool** (**BLAST**) finds regions of local similarity between sequences. The program compares nucleotide or protein sequences to **...**

Align two or more - Protein BLAST: ***search ... - Nucleotide BLAST Rat - sequences

Nucleotide **BLAST**: Search nucleotide databases using a nucleotide **...**

blast.ncbi.nlm.nih.gov/Blast.cgi?...blastn...BlastSearch...

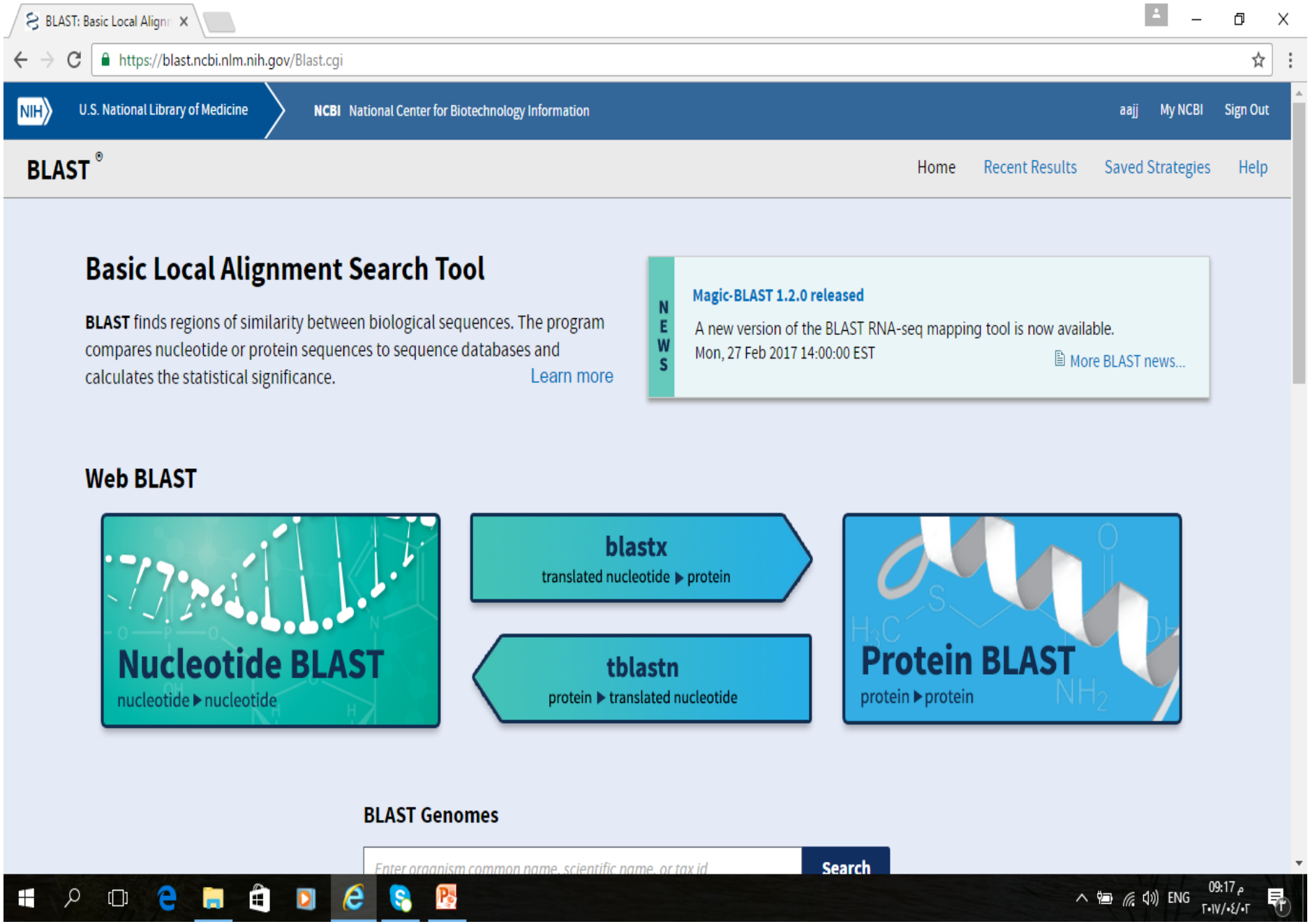
No BLAST database contains all the sequences at NCBI. BLAST databases **...**

BLAST - Wikipedia, the free encyclopedia

en.wikipedia.org/wiki/BLAST

In bioinformatics, **Basic Local Alignment Search Tool**, or **BLAST**, is an algorithm for comparing primary biological sequence information, such as the amino-acid **...**

Process - Output - Input - Background


BLAST: Basic Local Align
https://blast.ncbi.nlm.nih.gov/Blast.cgi
U.S. National Library of Medicine
NCBI National Center for Biotechnology Information
aajj
My NCBI
Sign Out
BLAST
Home
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Basic Local Alignment Search Tool
BLAST finds regions of similarity between biological sequences. The program compares nucleotide or protein sequences to sequence databases and calculates the statistical significance.
Learn more
NEWS
Magic-BLAST 1.2.0 released
A new version of the BLAST RNA-seq mapping tool is now available.
Mon, 27 Feb 2017 14:00:00 EST
More BLAST news...
Web BLAST
Nucleotide BLAST
nucleotide ▶ nucleotide
blastx
translated nucleotide ▶ protein
tblastn
protein ▶ translated nucleotide
Protein BLAST
protein ▶ protein
BLAST Genomes
Search
ENG
09:17 م
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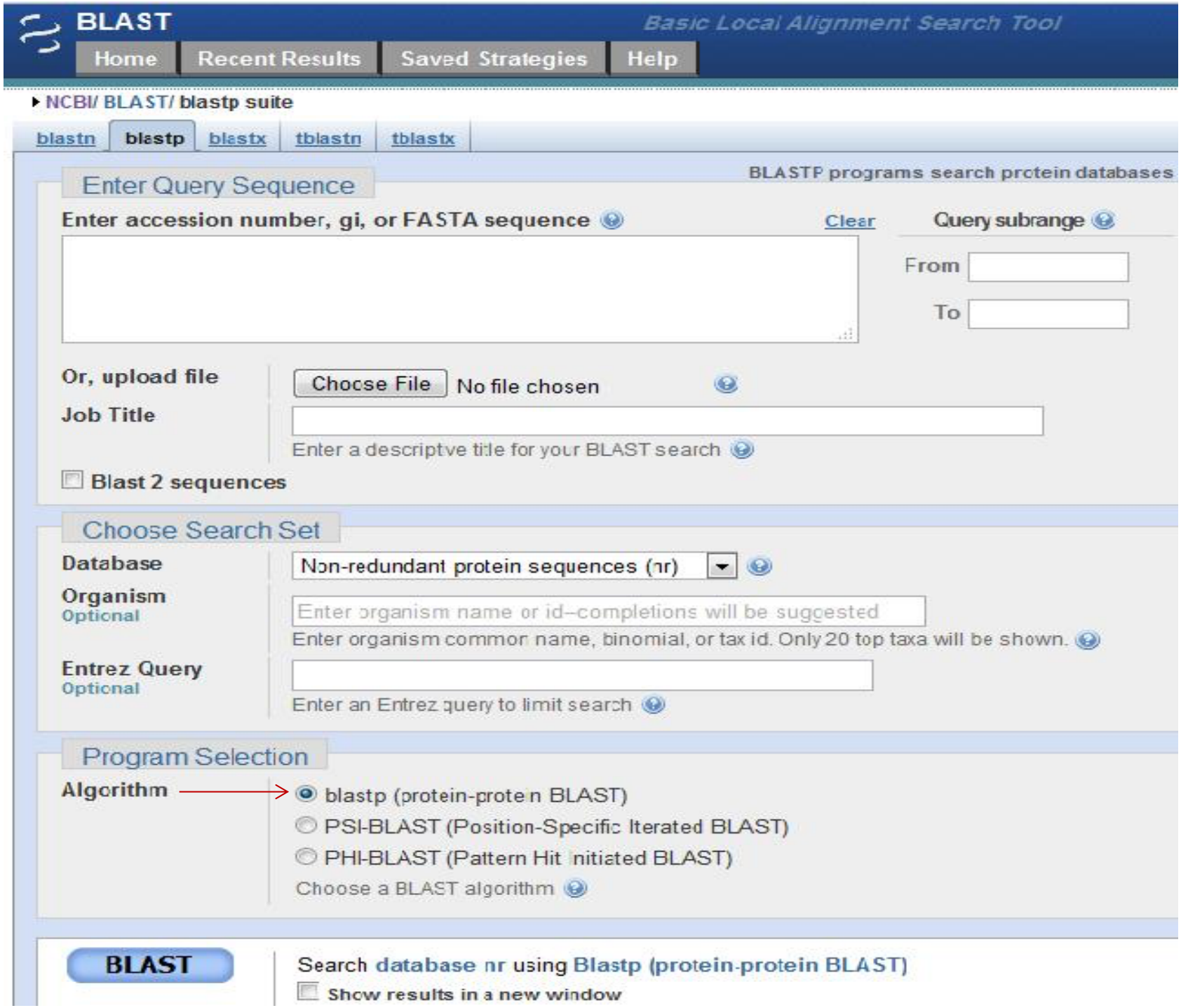
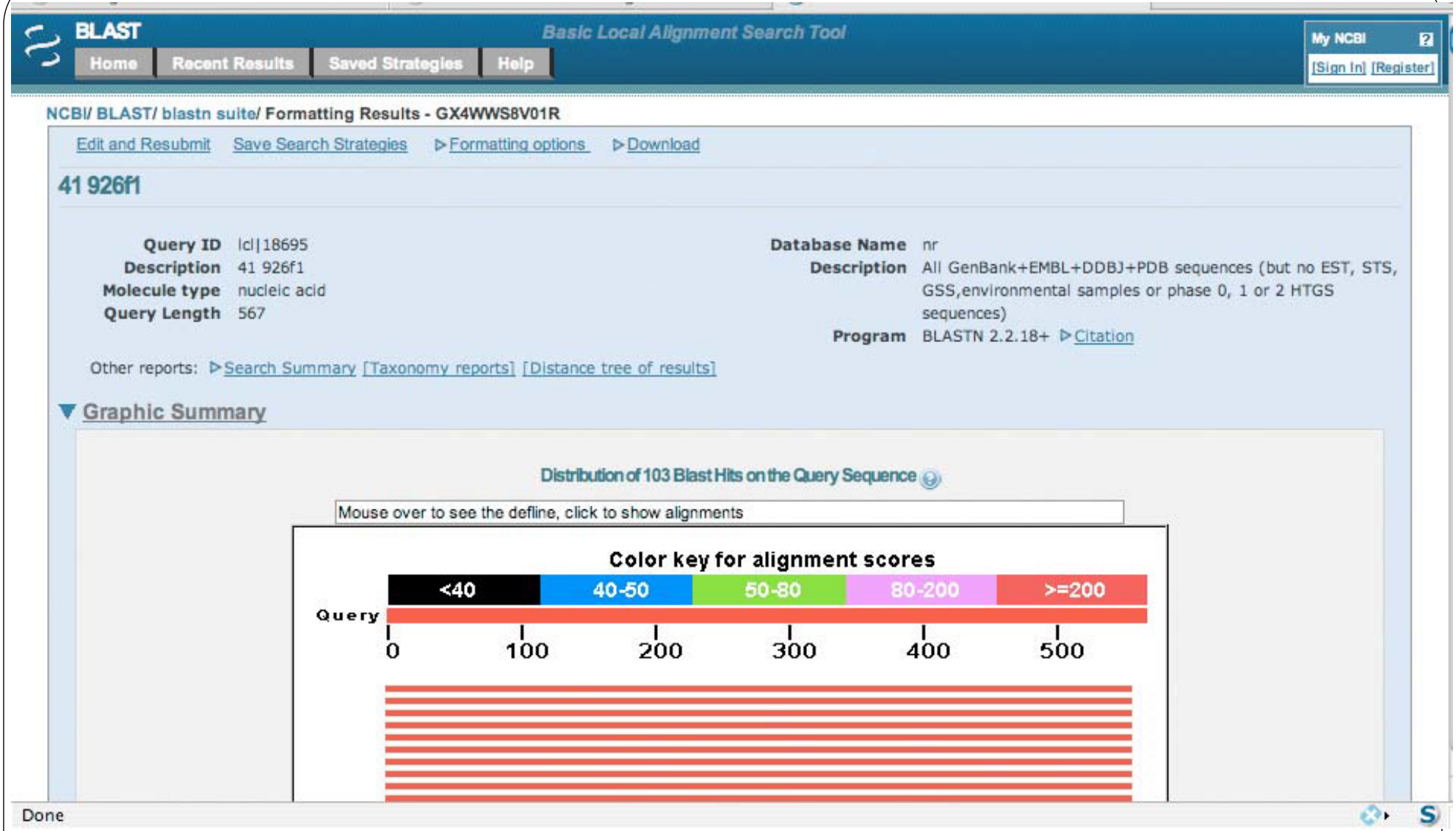

BLAST
Basic Local Alignment Search Tool
Home
Recent Results
Saved Strategies
Help
NCBI/ BLAST/ blastp suite
blastn
blastp
blastx
tblastn
tblastx
BLASTP programs search protein databases
Enter Query Sequence
Enter accession number, gi, or FASTA sequence
Clear
Query subrange
From
To
Or, upload file
Choose File
No file chosen
Job Title
Enter a descriptve title for your BLAST search
Blast 2 sequences
Choose Search Set
Database
Non-redundant protein sequences (nr)
Organism
Optional
Enter organism name or id--completions will be suggested
Enter organism common name, binomial, or tax id. Only 20 top taxa will be shown.
Entrez Query
Optional
Enter an Entrez query to limit search
Program Selection
Algorithm
blastp (protein-protein BLAST)
PSI-BLAST (Position-Specific Iterated BLAST)
PHI-BLAST (Pattern Hit Initiated BLAST)
Choose a BLAST algorithm
BLAST
Search database nr using Blastp (protein-protein BLAST)
Show results in a new window



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Descriptions

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AM697463.1	Uncultured bacterium partial 16S rRNA gene, isolate BF0001D078	946	946	98%	0.0	97%	

>Seq3,

ATGCTGCAAATGAGGATGAAAAGAAAAAGGAGGAAAAAAAAAAGATGTTGTTCTCGATGTTACTCTCACTTCATGTGAGA
ATGTAACCTTTGATACTCGCGACCCTAACTCCGTCGTGTTAACTGTCAAGGATGGCTTCCGTTTCAAGACCCTTAAGGT
CGGGGACAAGACCTTATTCAATGTTGACACAGGAAAACATACCCCAGTAAAGGCATTCAAACTTAAGCATGATTCCGAG
GAGTGGTTCAGGCTTGATCTTCATGCTGCCCAACCAAAGATGTTCAAGAAGAAGGGAGACAAGGAATATTCTGAGTCCA
AATTCGAGACCTACTACGATGAAGTCTTGTTCAAGGGAAAATCCGCCAAGGAACTAGATGTTTCCAAGTTCGAAGATCC
AGCTTTGTTCACCTCCGCTAACTTCGGCACTGGAAAGAAGTACATTCTTTAAAAAGGATTTCAAACCTTCCAAAGTTCT
CTTCGAAAAGAAAGAAGTCGGAAAACCAAACAATGCCAAGTATCTTGAAGTTGTTGTCTTTGTCAGTTCTGATTCCAAG
AAGGTCGTCAGACTCGACTACTTCTATACCGGTGACTCAAGGTTGAAGGAGACCTACTTCGAGCTTAAGGACGATAAGT
GGGTGCAAATGTCACAGGCAGATGCAAACAAGGCCTTGAATGCCATGGACCCAGCCTGGTCCTCAGATTACAAACCAGT
TGTCGACAAGTTCTCCCCCCTTGCAGTCTTCGCCTCAGTACTCATCGTCTTCTCATCAGTCCAATCAC

>Seq3,

MLQMRMKRKR RKKKDVVLDV TLTSCENVTF DTRDPNSVVL TVKDGFRFKT LKVGDKTLFN
VDTGKHTPVK AFKLKHDSEE WFRLDLHAAQ PKMFKKKGDK EYSESKFETY YDEVLFKGKS
AKELDVSKFE DPALFTSANF GTGKKYTFKK DFKPSKVLFE KKEVGKPNNA KYLEVVVFVG
SDSKKLVKLY YFYTGDSRLK ETYFELKDDK WVQMTQADAN KALNAMNSSW STDYKPVVDK
FSPLAVFASV LIVFSSV

Second Generation sequencing

Its Idea:

There are number of different NGS platforms using different sequencing technologies.

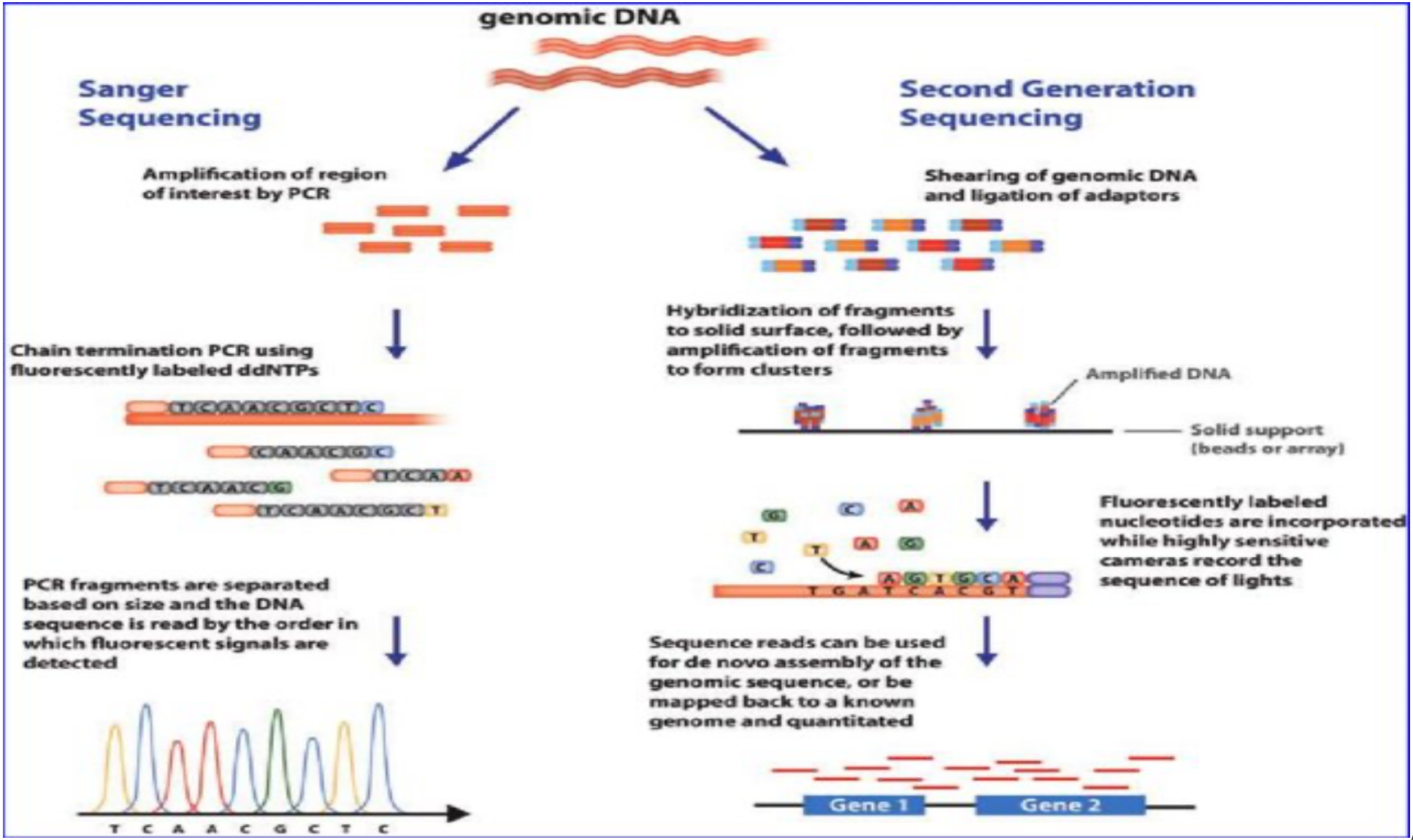
But all NGS platforms perform sequencing of millions of small fragments of DNA in parallel.

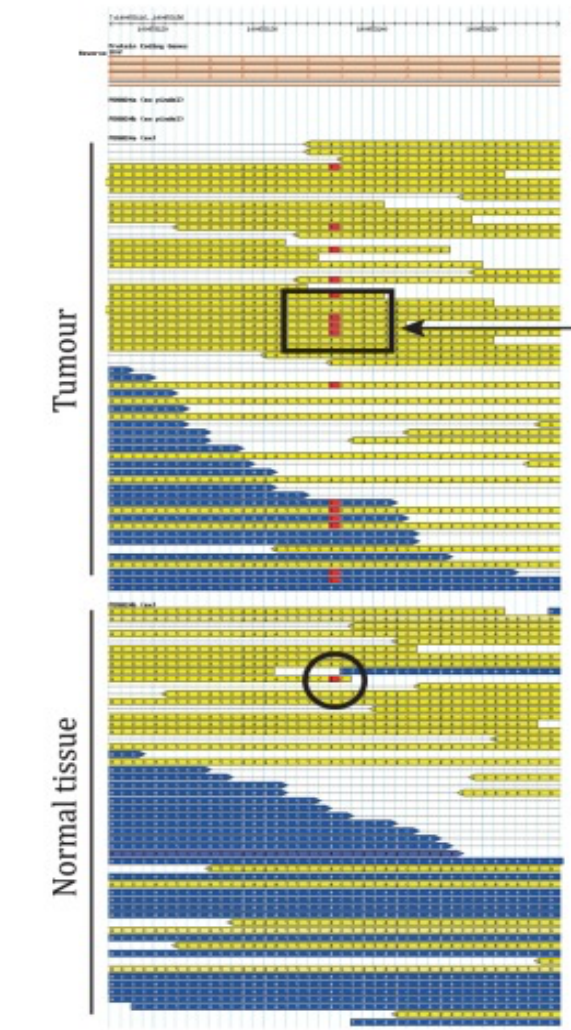
Then Bioinformatics analyses are used to piece together these fragments by mapping the individual reads to reference genome.

Its Power:

Next generation sequencing (NGS) can be sequenced the human genome in a single day while sanger sequencing mat require over a decade to deliver the final draft.

The diffrences between Sanger sequencing and 2nd/Next generation sequencing





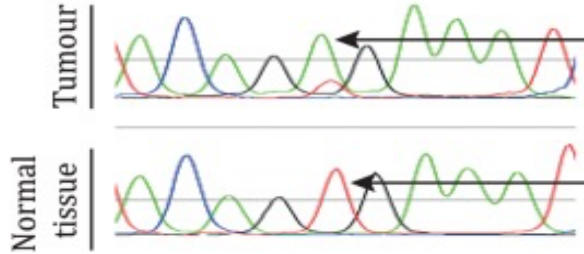
Genomic coordinate. We are looking at a 40 bp window on chromosome 7 within the BRAF gene

Amino acids encoded by DNA codons

Each bar reprents a sequencing read, in the forward (blue) or reverse (yellow) direction. The DNA sequence on the read is the DNA sequence of a single fragment of DNA. The sum of reads covering a particular base is the sequencing depth in that position.

Bases that are yellow or blue are "normal", i.e. the same base in that particular position is found in the human reference genome. Red indicates deviation from the reference genome which can either be due to a mutation or due to a sequencing artefact. Here, amongst tumour reads at base 140,453,136 on chromosome 7, the thymine of the human referene genome has been substituted with an adenine. The codon that the base belongs to normally encodes the 600th amino acids of BRAF, a valine (V). The mutation changes this to glutamic acid (E). The resulting BRAF V600E mutation is one of the first somatic mutations discovered in human cancer for which a clinically effective specific drug was developed. Note that the mutation is present in roughly half of the reads of the tumour, indicating that it affects one allele, i.e. that it is heterozygous.

Note absence of mutation in normal tissue from the same individual, demonstrating that the mutation is somatic. The one mutant base (circle) seen amongst reads of normal tissue represents a sequencing artefact (a thymine to guanine change, in this case).



Sanger sequencing trace of the tumour showing the mutation (green peak = adenine) on top of the normal base (red peak = thymine)

In normal tissue only the normal base, thymine, is seen.

DIFFERENT APPLICATIONS OF DNA SEQUENCING

1. In Medicine and pharmacy:

a. The spread of disease/organism through populations

Especially the appearance of new virulent sub-types.

The sub-types of an organism that were responsible for earlier epidemics can also be determined by PCR analysis.

b. Detection of Mutation: In case of genetic diseases, there is a mutation resulting in a detectable change in the length of the restriction fragment.

c. Detection of gene defects and their related diseases.

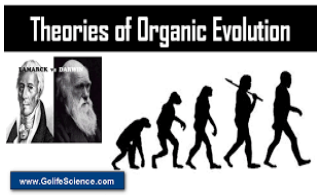
d. Diagnosis of retroviral infection and cancers. Many forms of cancer involve alterations to oncogenes

e. Prenatal testing.

f. Gene Therapy: helps to monitor the gene in gene therapy

g. Genomic studies: helps to compare the genomes of two organisms and identify the difference between them.

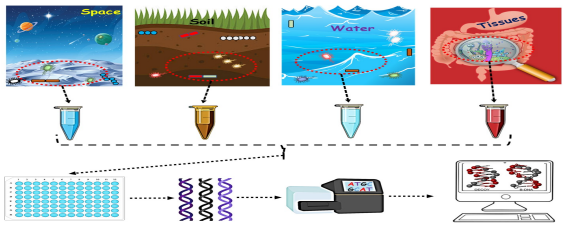
2. Evolutionary studies: The differences in the genomes of two organisms can be detected.



It plays an important role in phylogenetic analysis.

Minute quantities of DNA from any source such a fossilized material, hair, bones, mummified tissues can be amplified using PCR technique.

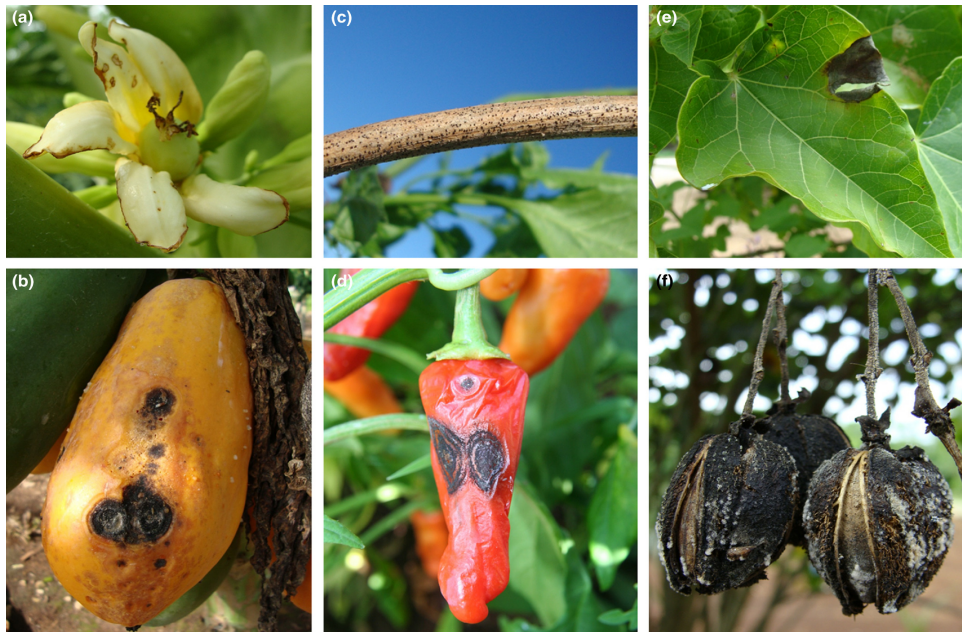
3. Metagenomics: study of genetic material obtained from environmental samples (Ecological studies).



4. In Agriculture:

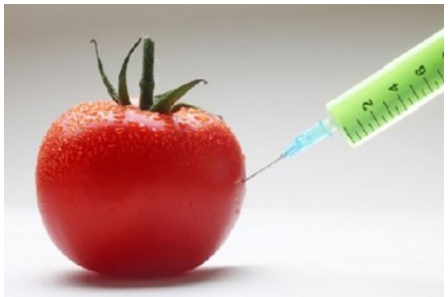
a. Detection of phytopathogens:

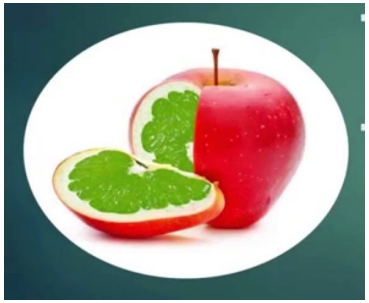
The agricultural industry is constantly striving to produce plant propagules or seedlings that are free of pathogens in order to prevent economic losses and safeguard health.



b. Genetically modified organisms (GMO)

- **Genetically modified organism (GMO) is a living organism whose genetic composition has been altered by means of gene technology.**
- **The genetic modification usually involves insertion of a piece of DNA (the insert).**
- **These smaller pieces of DNA are usually taken from other naturally occurring organisms.**





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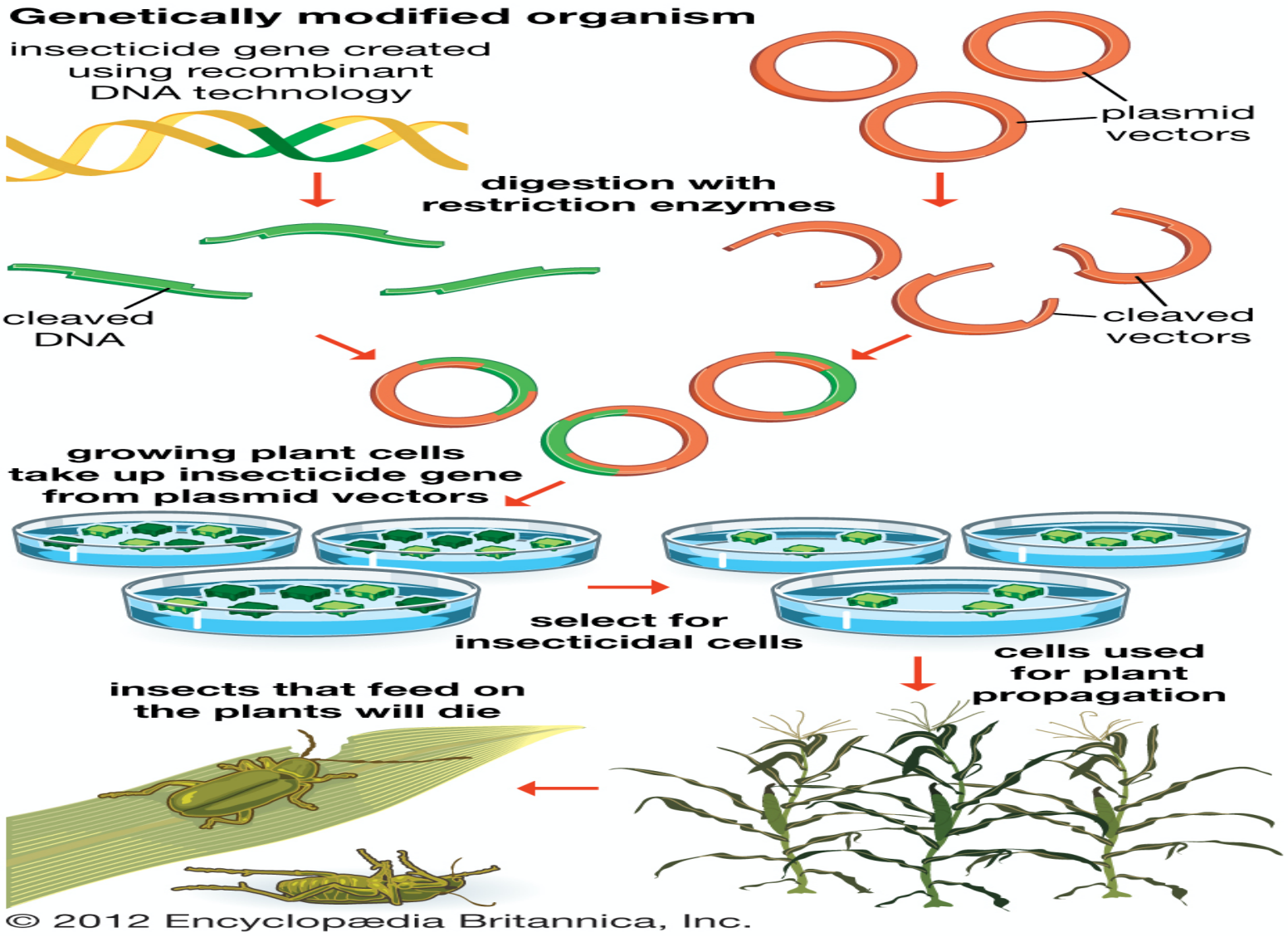

c. Detection of genetically modified organisms (GMOs):

Thanks a lot

with my Best Regards and My Best wishes

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