





INTERPRETATION OF SEQUENCE RESULTS

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 fluorescent dyes (Each nucleotide has a different color).
- At the end of the sequencing reaction,
- It 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.

Interpreting Sequencing Results

Automated 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.



>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

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

тсасаа

• "N" designation—peaks that,

for whatever reason, are not clear enough to designate as A, G, T, or C.

Good sequence generally begins roughly around base 20.

Beginning of Sequence



End of sequence



With a little practice, you can scan a chromatogram in less than a minute and spot problems. It is not necessary to read each and every base.



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.

Types of Polymorphisms

1- Transitions: $A \leftrightarrow F G \text{ or } C \leftrightarrow T$

(purines to purines OR pyrimidines to pyrimidines)

2-Insertions: an extra base is present.

- **3- Deletions:** a base may be missing.
- 4- Mis-Called

(a) Irregular spacing:

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



4- Mis-Called

(b) Mis-call a nucleotide:

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





4- Mis-Called

(b) 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 it 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.

5- 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 single-nucleotide polymorphism (SNP).



6- *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.



7- 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. 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. **8-** *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.



9- Good sequence with bad base calling:

Failed analysis Ask the Sequencing Service to reanalyze the sequence.



10- 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



12- Excess dye peaks at the beginning of the sequence

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





15- 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 of the polymorphic positions, you should be using a computer program to scan your chromatograms!

Interpreting of 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

Interpreting of Sequencing Results

Determining homology:

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

This can be accomplished using **BLAST**, (**Basic Local Alignment Search Tool**): This 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).

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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



Web BLAST



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

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Status	Searching	
Submitted at	Mon Nov 3 01:01:00 2008	
Current time	Mon Nov 3 01:01:03 2008	
Time since submission		
This page will be automatically updated in 8 seconds		

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 🔲 GEO G Gene 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%	
EF653454.1	Enterococcus faecalis strain 47/3 16S ribosomal RNA gene, partial sequence	946	946	98%	0.0	97%	
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.

> <mark>gb</mark> partia Length	<u>EU2855</u> 1 sequ =1456	87.1 Enterococcus faecalis strain C19315led5A 16S ribosomal ence	RNA gene,
Score Ident Stran	= 94 ities d=Plus	6 bits (512), Expect = 0.0 = 550/566 (97%), Gaps = 12/566 (2%) /Plus	
Query	1	$\tt CGGTCGAGC-TGTGGTTTAATTCGAAGCAACGCGAAGAACCTTACCAGGTCTTGACATCC$	59
Sbjct	893	CGGTGGAGCATGTGGTTTAATTCGAAGCAACGCGAAGAACCTTACCAGGTCTTGACATCC	952
Query	60	TTTGACCACTCTAGAGATAGAGCTTTCCCTTCGGGGGACAAAGTGACAGGTGGTGCATGGT	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	${\tt ttcggattggcaggctgcaactcgcctgcatgaagccggaatcgctagtaatcgccggatc}$	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	GTTTGTAACACCCGAAGTCGGTGAGGTAACCTTTTTGGAGCCAGCC	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 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. atgcccaagctgaatagcgtagaggggttttcatcatttgaggacgatgtataa

5'

ttt ttt gat atq tCa tca] Ctg gta CCCggð aat agc gta gag 999 Qaq QaC taa X M P N S V E G F S S F E V K]] Г 2 ttt Cat Cat ttg tqc tga ata ggt gåð atq agc tag agg acq CCG gcg tat X X S R G P A F H H Y L R T M 3 gtt ttc atc att tgt qct tag Cqt tga QCC Caa Qgg aga ggg QQa CQa ata X E R R G V F X G A A Ι R C Q

3'



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	CACTIGITAT	TIGGTAAAGT	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	CCAARCAAAT	GAGGCATCAG	TCTGAAAGCC	AGGGAGTTGG	TCTGAGTGAC	AAGGAATTGG
4141	TTTCAGATGA	TGAAGAAAGA	GGAACGGGCT	TGGAAGAAAA	TAATCAAGAA	GAGCAAAGCA
4201	TGGATTCAAA	CTTAGGTGAA	GCAGCATCTG	GGTGTGAGAG	TGRAACAAGC	GTCTCTGAAG
4261	ACTGCTCAGG	GCTATCCTCT	CAGAGTGACA	TTTTAACCAC	TCAGCAGAGG	GATACCATGC
4321	AACATAACCT	GATAAAGCTC	CAGCAGGAAA	TEGCTEAACT	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

6th ORF: 1 stop codons GAT-TAC-ATC-CAT-GCT-CGC-TCT-GCT-GGC-CAA-CTC-ATT-TAG-GCA-TCT-CG DYIHARSAGQLI*AS

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

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

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 1

2nd ORF: 1 stop codons GAG-ATG-CCT-AAA-TGA-GTT-GGC-CAG-CAG-AGC-GAG-CAT-GGA-TGT-AAT-CAG F M P K V G Q Q S E H G C N 0

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

>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

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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



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

Descriptions

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

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Thanks a lot

with my Best Regards and My Best wishes

Amira A. T. AL-Hosary