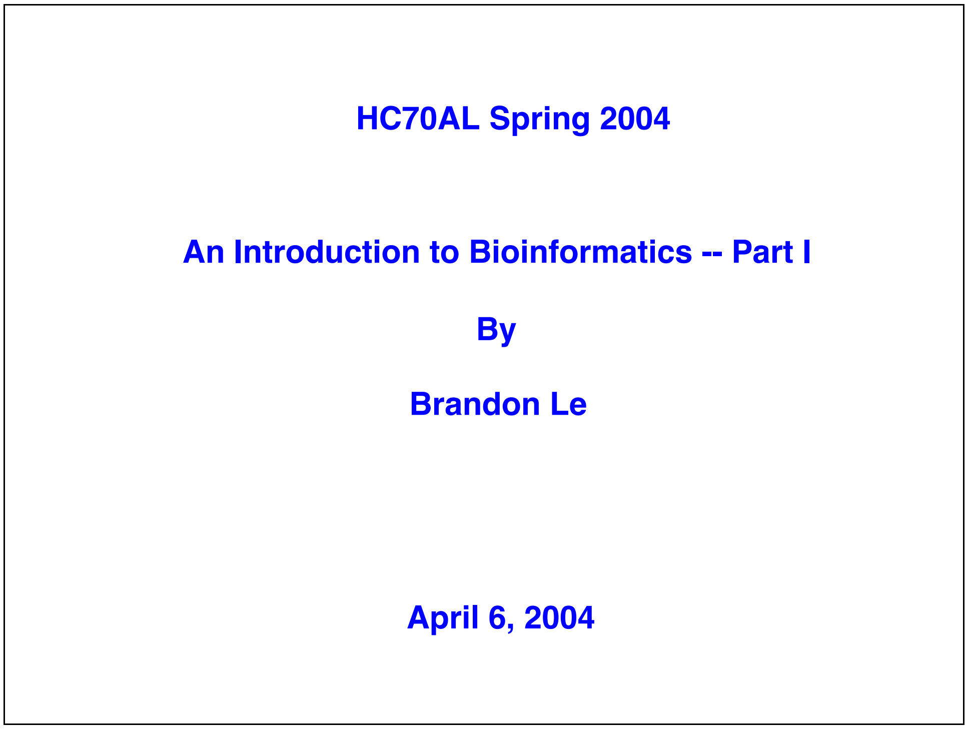
APPENDIX 2 - BIOINFORMATICS (PARTS I AND II)



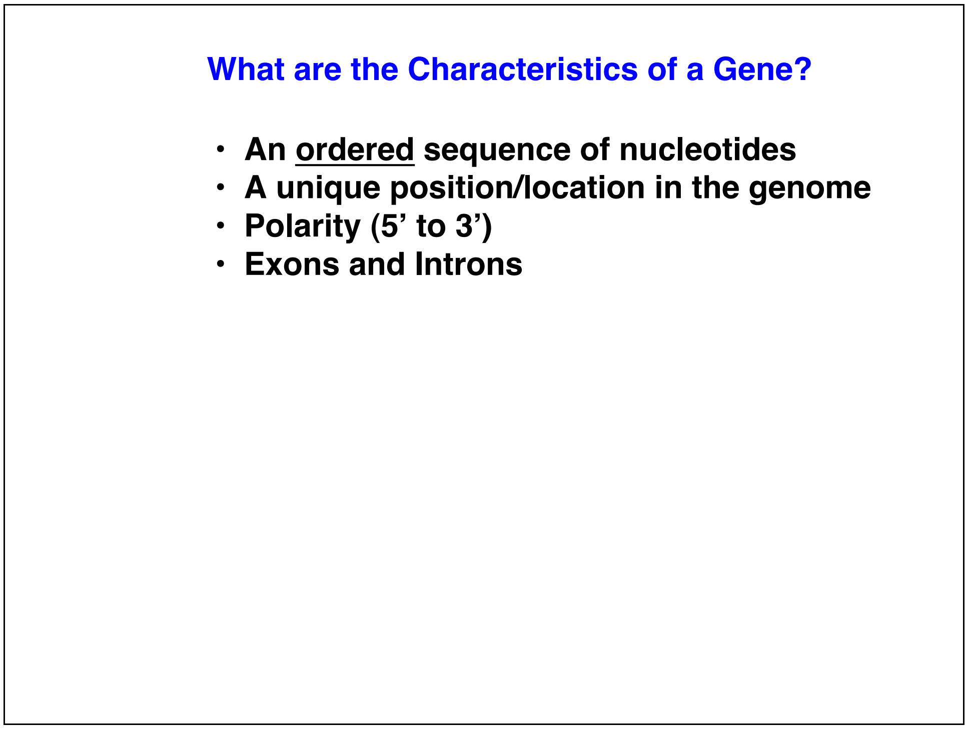
HC70AL Spring 2004

An Introduction to Bioinformatics -- Part I

By

Brandon Le

April 6, 2004



What are the Characteristics of a Gene?

- An ordered sequence of nucleotides
- A unique position/location in the genome
- Polarity (5’ to 3’)
- Exons and Introns

What are the Anatomical Features of Genes?

- Discrete beginning and discrete end
- Two strands of DNA
- Double helical
- Strand one (5’ to 3’)
- Strand two (3’ to 5’)
- Sense strand (5’ to 3’)
  - specifies the trait
- Nonsense strand (3’ to 5’)
  - template for transcription

Sense Strand

```
5′ – ACGTCAGTCGATGCATGCTAGCTAGC – 3′
3′ – TGCAGTCAGCTACGTACGATCGATCG – 5′
```

Nonsense Strand

Genes Have a Unique Position in the Genome!

Task: Where is your gene located in the genome?

Tools: The Arabidopsis Information Resources (TAIR) (http://www.arabidopsis.org)

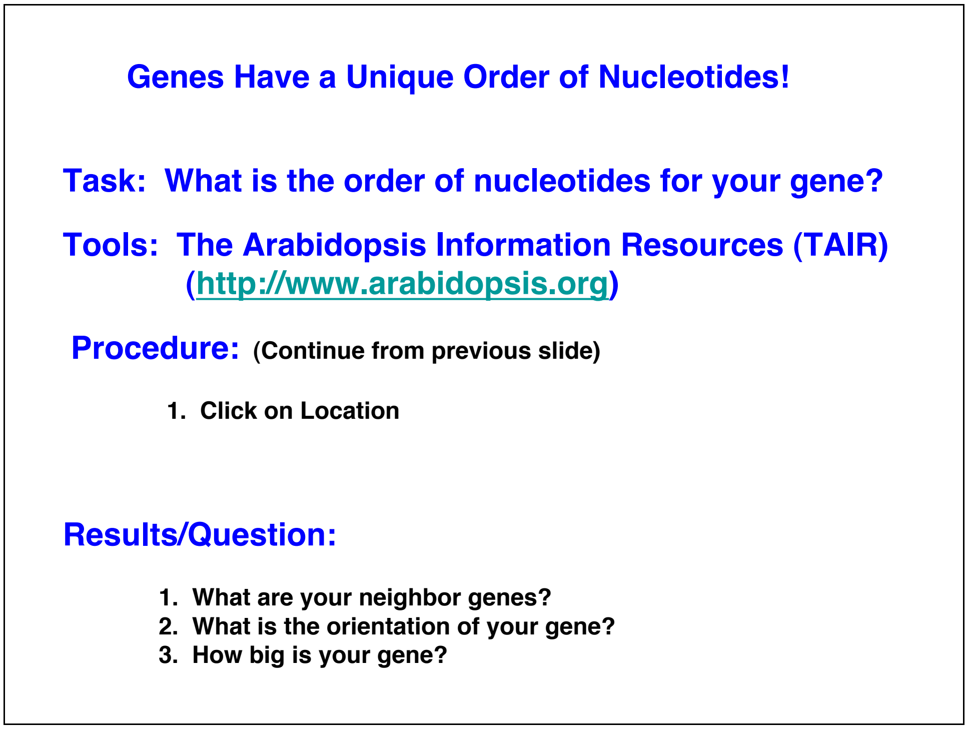
01	AT2G22800
02	AT2G23290
03	AT2G37120
04	AT3G09735
05	AT3G12840
06	AT3G50060
07	AT3G53370
08	AT4G37260
09	AT4G37790
10	AT5G03220
11	AT5G03500
12	AT5G19490
13	AT5G67300

Procedure:

1. Select Seqviewer
2. Enter gene number (ex. AT1G18260)
3. Submit

Results/Question:

1. What chromosome is your gene in?
2. What other genes/markers are next to your gene?
3. What is the exact position of your gene in the genome?



Genes Have a Unique Order of Nucleotides!

Task: What is the order of nucleotides for your gene?

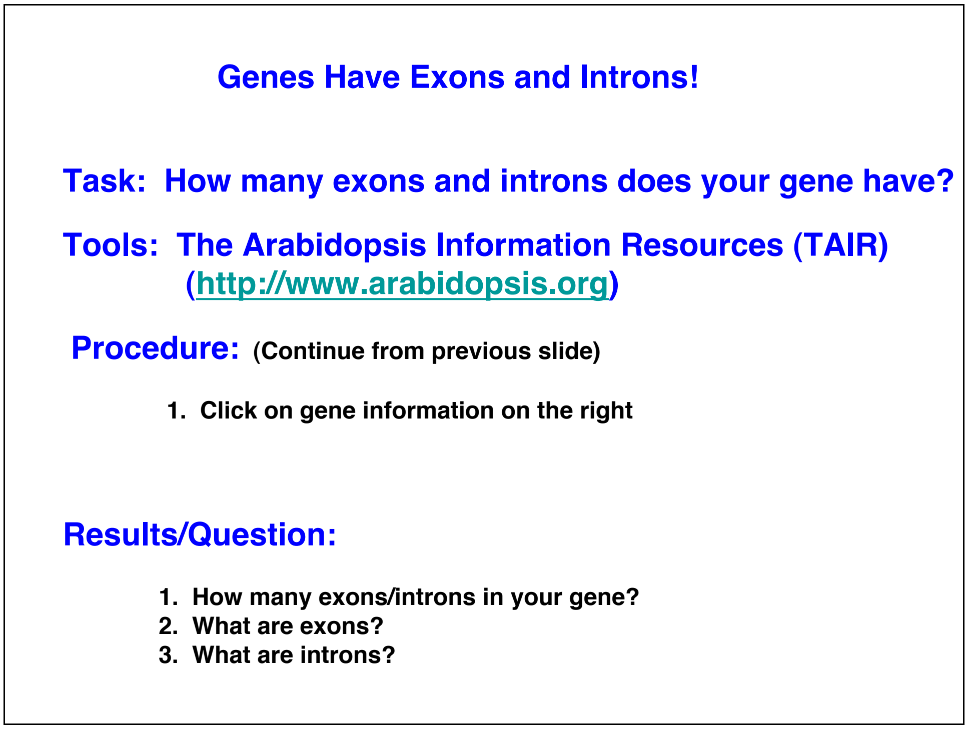
Tools: The Arabidopsis Information Resources (TAIR)
(http://www.arabidopsis.org)

Procedure: **(Continue from previous slide)**

1. **Click on Location**

Results/Question:

1. **What are your neighbor genes?**
2. **What is the orientation of your gene?**
3. **How big is your gene?**



Genes Have Exons and Introns!

Task: How many exons and introns does your gene have?

Tools: The Arabidopsis Information Resources (TAIR)
(http://www.arabidopsis.org)

Procedure: **(Continue from previous slide)**

1. **Click on gene information on the right**

Results/Question:

1. **How many exons/introns in your gene?**
2. **What are exons?**
3. **What are introns?**

Gene Encodes a Protein

Task: Determine the protein encoded by gene?

Tools: The Arabidopsis Information Resources (TAIR)
(http://www.arabidopsis.org)

Results/Question:

1. How large is your protein?
2. What are the anatomy of a protein?

N-terminal
C-terminal

What is the identity of your gene?

Task: What does your gene code for?

Tools: NCBI BLAST Tools
(http://www.ncbi.nlm.nih.gov/BLAST)

What is BLAST?

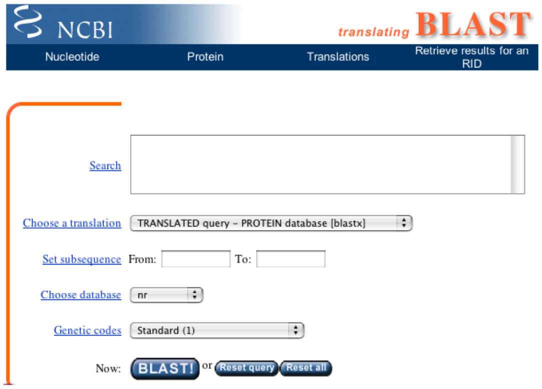
Basic Local Alignment Search Tool (BLAST)

What does BLAST do?

A family of programs that allows you to input a query sequence and compare it to DNA or protein sequences in db.

What are the steps to performing BLAST search?

Paste sequence of interest into BLAST input box
Select BLAST program
Select db
Select Optional Parameters



Choose a translation: TRANSLATED query – PROTEIN database [blastx]

Set subsequence From: To:

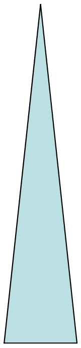
Choose database: nr

Genetic codes: Standard (1)

Now: BLAST! or Reset query Reset all

What are the different BLAST Programs?

Fastest



Slowest

blastp - protein query vs protein db

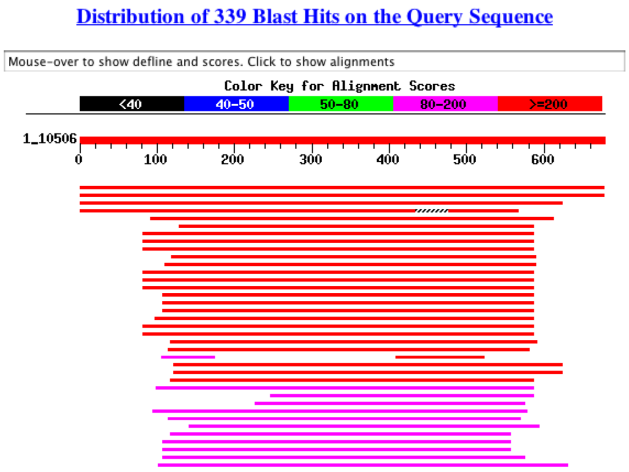
blastn - DNA query vs DNA db

blastx - translated DNA query vs protein db

tblastx - protein query vs translated DNA db

tblastn - translated DNA query vs translated DNA db

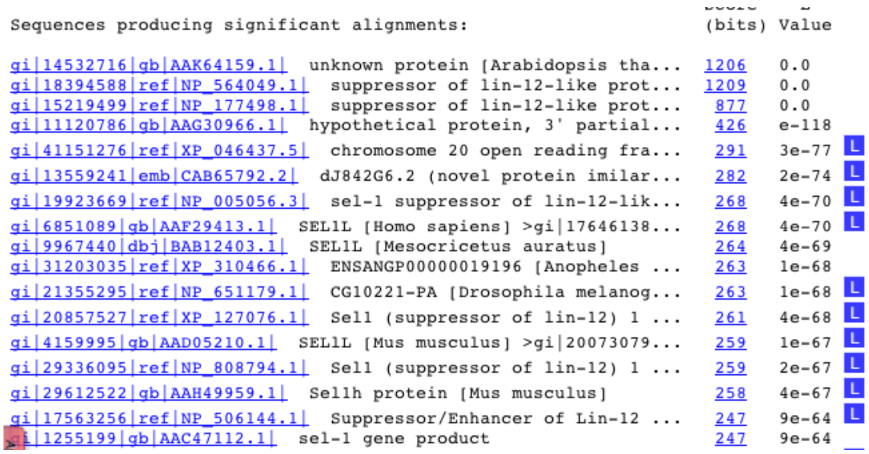
Anatomy of a BLAST Result -- Part I



Distribution of 339 Blast Hits on the Query Sequence

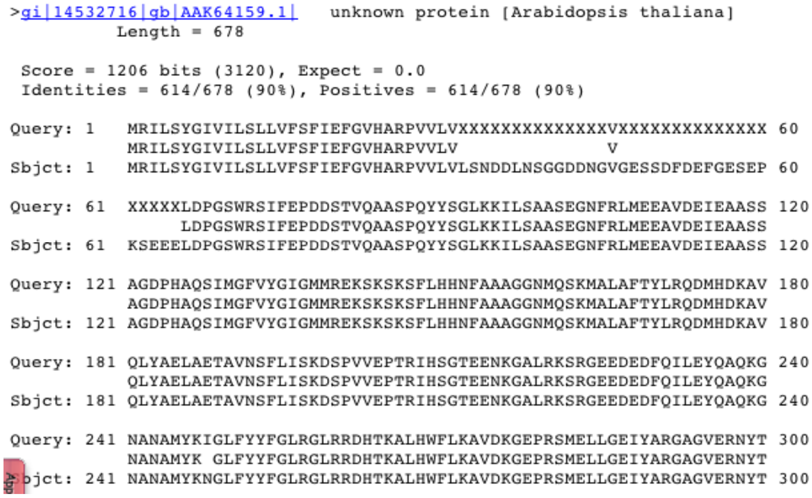
Mouse-over to show defline and scores. Click to show alignments

Anatomy of a BLAST Result -- Part II



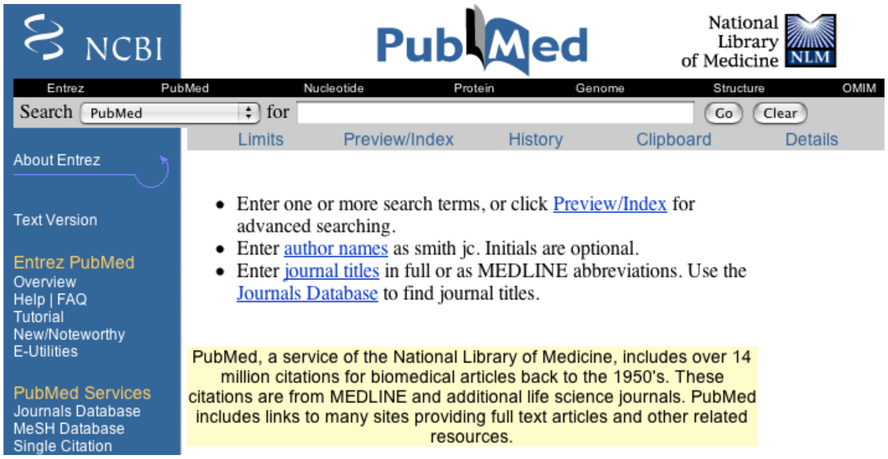
```
                                                                 Score    E
Sequences producing significant alignments:                      (bits) Value

gi|14532716|gb|AAK64159.1|  unknown protein [Arabidopsis tha...  1206   0.0
gi|18394588|ref|NP_564049.1|  suppressor of lin-12-like prot...  1209   0.0
gi|15219499|ref|NP_177498.1|  suppressor of lin-12-like prot...   877   0.0
gi|11120786|gb|AAG30966.1|  hypothetical protein, 3' partial...   426   e-118
gi|41151276|ref|XP_046437.5|  chromosome 20 open reading fra...   291   3e-77 L
gi|13559241|emb|CAB65792.2|  dJ842G6.2 (novel protein imilar...   282   2e-74 L
gi|19923669|ref|NP_005056.3|  sel-1 suppressor of lin-12-lik...   268   4e-70 L
gi|6851089|gb|AAF29413.1|  SEL1L [Homo sapiens] >gi|17646138...   268   4e-70 L
gi|9967440|dbj|BAB12403.1|  SEL1L [Mesocricetus auratus]          264   4e-69
gi|31203035|ref|XP_310466.1|  ENSANGP00000019196 [Anopheles ...   263   1e-68
gi|21355295|ref|NP_651179.1|  CG10221-PA [Drosophila melanog...   263   1e-68 L
gi|20857527|ref|XP_127076.1|  Sel1 (suppressor of lin-12) 1 ...   261   4e-68 L
gi|4159995|gb|AAD05210.1|  SEL1L [Mus musculus] >gi|20073079...   259   1e-67 L
gi|29336095|ref|NP_808794.1|  Sel1 (suppressor of lin-12) 1 ...   259   2e-67 L
gi|29612522|gb|AAH49959.1|  Sel1h protein [Mus musculus]          258   4e-67 L
gi|17563256|ref|NP_506144.1|  Suppressor/Enhancer of Lin-12 ...   247   9e-64 L
gi|1255199|gb|AAC47112.1|  sel-1 gene product                     247   9e-64
```

Anatomy of a BLAST Result -- Part III



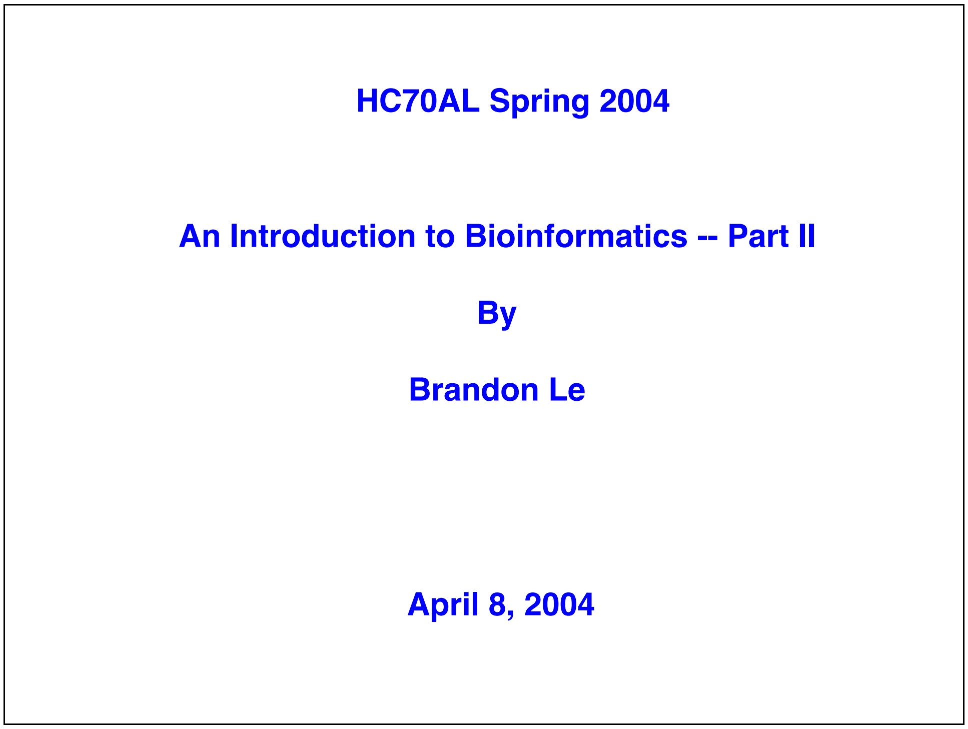
```
>gi|14532716|gb|AAK64159.1|   unknown protein [Arabidopsis thaliana]
          Length = 678

 Score = 1206 bits (3120), Expect = 0.0
 Identities = 614/678 (90%), Positives = 614/678 (90%)

Query: 1   MRILSYGIVILSLLVFSFIEFGVHARPVVLVXXXXXXXXXXXXXXVXXXXXXXXXXXXXX 60
            MRILSYGIVILSLLVFSFIEFGVHARPVVLV              V
Sbjct: 1   MRILSYGIVILSLLVFSFIEFGVHARPVVLVLSNDDLNSGGDDNGVGESSDFDEFGESEP 60

Query: 61  XXXXXLDPGSWRSIFEPDDSTVQAASPQYYSGLKKILSAASEGNFRLMEEAVDEIEAASS 120
                 LDPGSWRSIFEPDDSTVQAASPQYYSGLKKILSAASEGNFRLMEEAVDEIEAASS
Sbjct: 61  KSEEELDPGSWRSIFEPDDSTVQAASPQYYSGLKKILSAASEGNFRLMEEAVDEIEAASS 120

Query: 121 AGDPHAQSIMGFVYGIGMMREKSKSKSFLHHNFAAAGGNMQSKMALAFTYLRQDMHDKAV 180
            AGDPHAQSIMGFVYGIGMMREKSKSKSFLHHNFAAAGGNMQSKMALAFTYLRQDMHDKAV
Sbjct: 121 AGDPHAQSIMGFVYGIGMMREKSKSKSFLHHNFAAAGGNMQSKMALAFTYLRQDMHDKAV 180

Query: 181 QLYAELAETAVNSFLISKDSPVVEPTRIHSGTEENKGALRKSRGEEDEDFQILEYQAQKG 240
            QLYAELAETAVNSFLISKDSPVVEPTRIHSGTEENKGALRKSRGEEDEDFQILEYQAQKG
Sbjct: 181 QLYAELAETAVNSFLISKDSPVVEPTRIHSGTEENKGALRKSRGEEDEDFQILEYQAQKG 240

Query: 241 NANAMYKIGLFYYFGLRGLRRDHTKALHWFLKAVDKGEPRSMELLGEIYARGAGVERNYT 300
            NANAMYK GLFYYFGLRGLRRDHTKALHWFLKAVDKGEPRSMELLGEIYARGAGVERNYT
Sbjct: 241 NANAMYKNGLFYYFGLRGLRRDHTKALHWFLKAVDKGEPRSMELLGEIYARGAGVERNYT 300
```

PubMed - Endless Resources



- Enter one or more search terms, or click Preview/Index for advanced searching.
- Enter author names as smith jc. Initials are optional.
- Enter journal titles in full or as MEDLINE abbreviations. Use the Journals Database to find journal titles.

PubMed, a service of the National Library of Medicine, includes over 14 million citations for biomedical articles back to the 1950's. These citations are from MEDLINE and additional life science journals. PubMed includes links to many sites providing full text articles and other related resources.



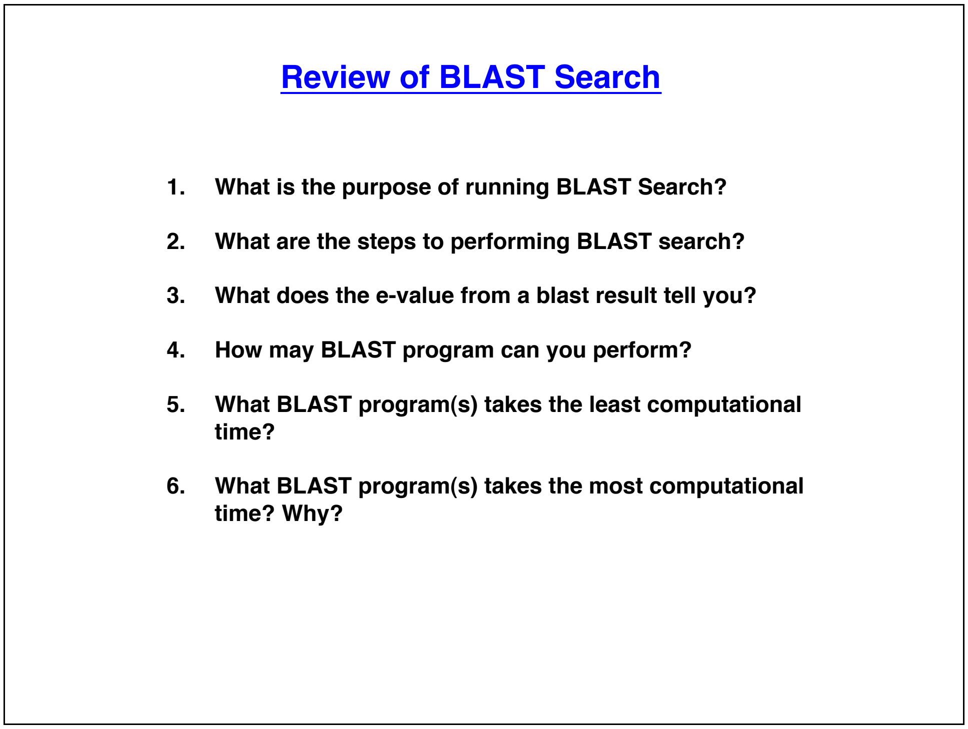
HC70AL Spring 2004

An Introduction to Bioinformatics -- Part II

By

Brandon Le

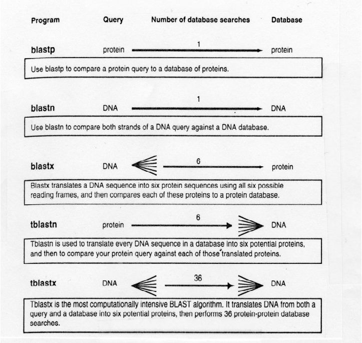
April 8, 2004



Review of BLAST Search

1. What is the purpose of running BLAST Search?
2. What are the steps to performing BLAST search?
3. What does the e-value from a blast result tell you?
4. How may BLAST program can you perform?
5. What BLAST program(s) takes the least computational time?
6. What BLAST program(s) takes the most computational time? Why?

What are the Five BLAST Search Programs?



Program	Query	Number of database searches	Database
blastp	protein	1	protein
blastn	DNA	1	DNA
blastx	DNA	6	protein
tblastn	protein	6	DNA
tblastx	DNA	36	DNA

Use blastp to compare a protein query to a database of proteins.

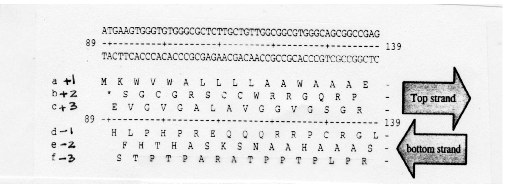
Use blastn to compare both strands of a DNA query against a DNA database.

Blastx translates a DNA sequence into six protein sequences using all six possible reading frames, and then compares each of these proteins to a protein database.

Tblastn is used to translate every DNA sequence in a database into six potential proteins, and then to compare your protein query against each of those translated proteins.

Tblastx is the most computationally intensive BLAST algorithm. It translates DNA from both a query and a database into six potential proteins, then performs 36 protein-protein database searches.

•How many proteins can a short DNA sequence potentially encode?



```
            ATGAAGTGGGTGTGGGCGCTCTTGCTGTTGGCGGCGTGGGCAGCGGCCGAG
         89 -+---------+---------+---------+---------+--------- 139
            TACTTCACCCACACCCGCGAGAACGACAACCGCCGCACCCGTCGCCGGCTC

a +1        M  K  W  V  W  A  L  L  L  L  A  A  W  A  A  A  E   -
b +2         *  S  G  C  G  R  S  C  C  W  R  R  G  Q  R  P     -
c +3          E  V  G  V  G  A  L  A  V  G  G  V  G  S  G  R    -
         89 -+---------+---------+---------+---------+--------- 139
d -1          H  L  P  H  P  R  E  Q  Q  Q  R  R  P  C  R  G  L -
e -2           F  H  T  H  A  S  K  S  N  A  A  H  A  A  A  S   -
f -3            S  T  P  T  P  A  R  A  T  P  P  T  P  L  P  R  -
```

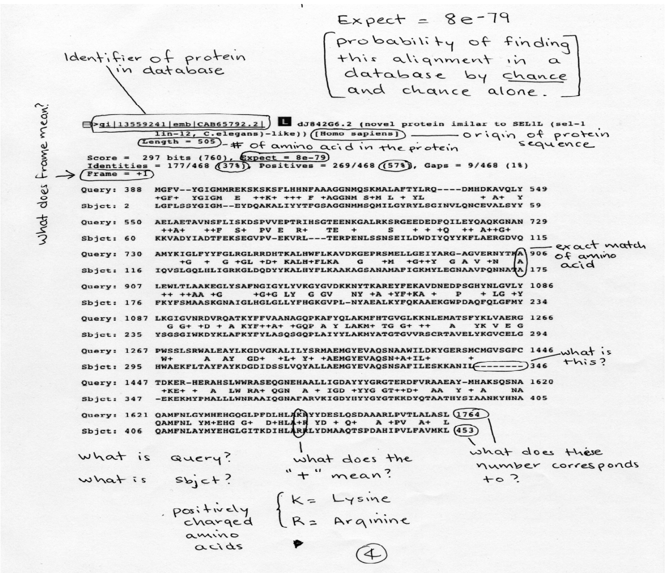
Question:

You have DNA Sequence. You want to know which protein in the main protein database is most similar to some protein encoded by your DNA.

Which BLAST program should you use?

Suppose you have a protein sequence.
Which BLAST program should you use?

HOW to interpret BLAST results?



```
>gi|13559241|emb|CAB65792.2|  L  dJ842G6.2 (novel protein imilar to SEL1L (sel-1
          lin-12, C.elegans)-like)) [Homo sapiens]
        Length = 505

 Score =  297 bits (760), Expect = 8e-79
 Identities = 177/468 (37%), Positives = 269/468 (57%), Gaps = 9/468 (1%)
 Frame = +1

Query: 388  MGFV--YGIGMMREKSKSKSFLHHNFAAAGGNMQSKMALAFTYLRQ----DMHDKAVQLY 549
            +GF+  YGIGM  E  ++K+ +++ F +AGGNM S+M L + YL         + A+  Y
Sbjct: 2    LGFLSSYGIGM--EYDQAKALIYYTFGSAGGNMMSQMILGYRYLSGINVLQNCEVALSYY 59

Query: 550  AELAETAVNSFLISKDSPVVEPTRIHSGTEENKGALRKSRGEEDEDFQILEYQAQKGNAN 729
             ++A+   ++F  S+  PV E  R+   TE  +     S   + + +Q  ++ A++G+
Sbjct: 60   KKVADYIADTFEKSEGVPV-EKVRL---TERPENLSSNSEILDWDIYQYYKFLAERGDVQ 115

Query: 730  AMYKIGLFYYFGLRGLRRDHTKALHWFLKAVDKGEPRSMELLGEIYARG-AGVERNYTKA 906
                +G  +  G +GL +D+ KALH+FLKA   G   +M  +G++Y  G A V +N   A
Sbjct: 116  IQVSLGQLHLIGRKGLDQDYYKALHYFLKAAKAGSANAMAFIGKMYLEGNAAVPQNNATA 175

Query: 907  LEWLTLAAKEGLYSAFNGIGYLYVKGYGVDKKNYTKAREYFEKAVDNEDPSGHYNLGVLY 1086
             ++ ++AA +G     +G+G LY  G GV   NY +A +YF+KA +   P   + LG +Y
Sbjct: 176  FKYFSMAASKGNAIGLHGLGLLYFHGKGVPL-NYAEALKYFQKAAEKGWPDAQFQLGFMY 234

Query: 1087 LKGIGVNRDVRQATKYFFVAANAGQPKAFYQLAKMFHTGVGLKKNLEMATSFYKLVAERG 1266
              G G+ +D + A KYF++A+ +GQP A Y LAKM+ TG G+ ++   A   YK V E G
Sbjct: 235  YSGSGIWKDYKLAFKYFYLASQSGQPLAIYYLAKMYATGTGVVRSCRTAVELYKGVCELG 294

Query: 1267 PWSSLSRWALEAYLKGDVGKALILYSRMAEMGYEVAQSNAAWILDKYGERSMCMGVSGFC 1446
             W+     A  AY  GD+  +L+ Y+ +AEMGYEVAQSN+A+IL+      +
Sbjct: 295  HWAEKFLTAYFAYKDGDIDSSLVQYALLAEMGYEVAQSNSAFILESKKANIL-------- 346

Query: 1447 TDKER-HERAHSLWWRASEQGNEHAALLIGDAYYYGRGTERDFVRAAEAY-MHAKSQSNA 1620
             +KE+ +  A  LW RA+ QGN  A + IGD +YYG GT++D+  AA  Y + A    NA
Sbjct: 347  -EKEKMYPMALLLWNRAAIQGNAFARVKIGDYHYYGYGTKKDYQTAATHYSIAANKYHNA 405

Query: 1621 QAMFNLGYMHEHGQGLPFDLHLAKRYYDESLQSDAAARLPVTLALASL 1764
            QAMFNL YM+EHG G+  D+HLA+R YD + Q+   A +PV  A+  L
Sbjct: 406  QAMFNLAYMYEHGLGITKDIHLARRLYDMAAQTSPDAHIPVLFAVMKL 453
```

Expect = 8e-79

probability of finding this alignment in a database by chance and chance alone.

Identifier of protein in database

origin of protein sequence

# of amino acid in the protein

What does Frame mean?

exact match of amino acid

what is this?

what is Query?

what is Sbjct?

what does the "+" mean?

what does these number corresponds to?

positively charged amino acids

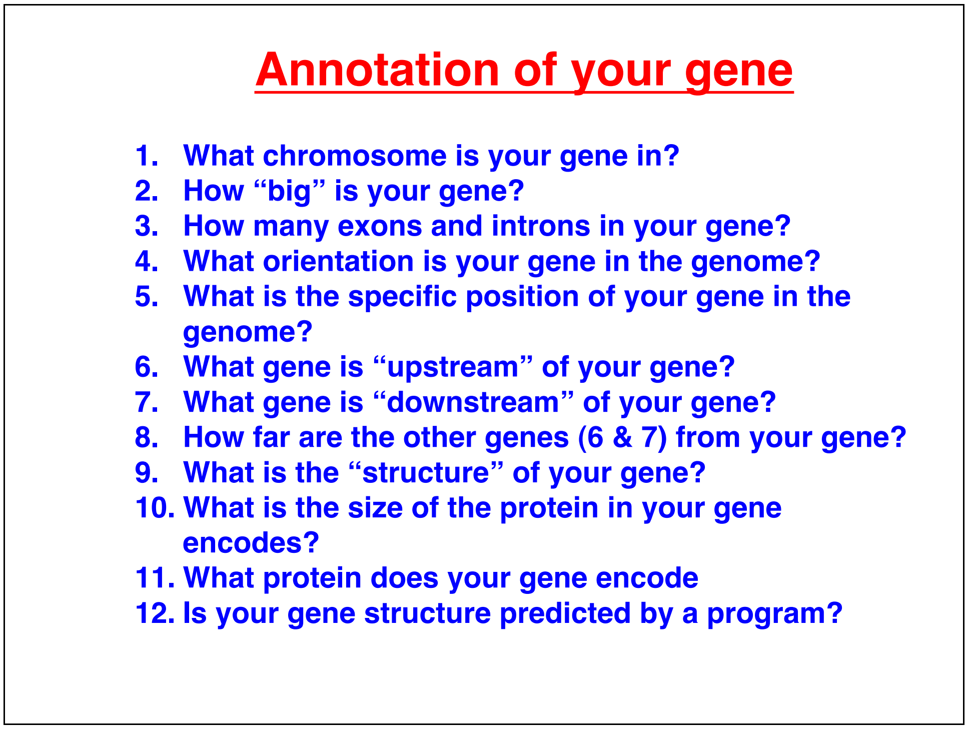
K = Lysine

R = Arginine

4

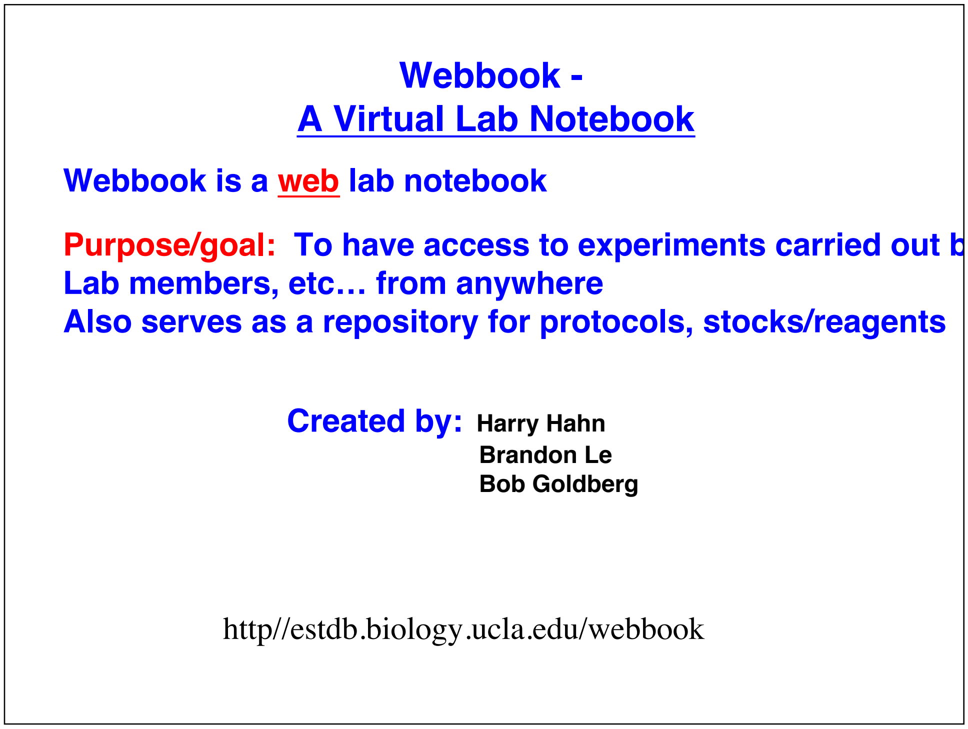
Review of gene transcription

1. What product is made after transcription?
2. How is the product similar/different from the gene?
3. What is cDNA?
4. What important information does a cDNA tell you about a gene?
5. What are ESTs?
6. What important information does ESTs tell you about a gene?



Annotation of your gene

1. What chromosome is your gene in?
2. How "big" is your gene?
3. How many exons and introns in your gene?
4. What orientation is your gene in the genome?
5. What is the specific position of your gene in the genome?
6. What gene is "upstream" of your gene?
7. What gene is "downstream" of your gene?
8. How far are the other genes (6 & 7) from your gene?
9. What is the "structure" of your gene?
10. What is the size of the protein in your gene encodes?
11. What protein does your gene encode
12. Is your gene structure predicted by a program?



Webbook - A Virtual Lab Notebook

Webbook is a web lab notebook

Purpose/goal: To have access to experiments carried out b
Lab members, etc… from anywhere
Also serves as a repository for protocols, stocks/reagents

Created by: Harry Hahn
Brandon Le
Bob Goldberg

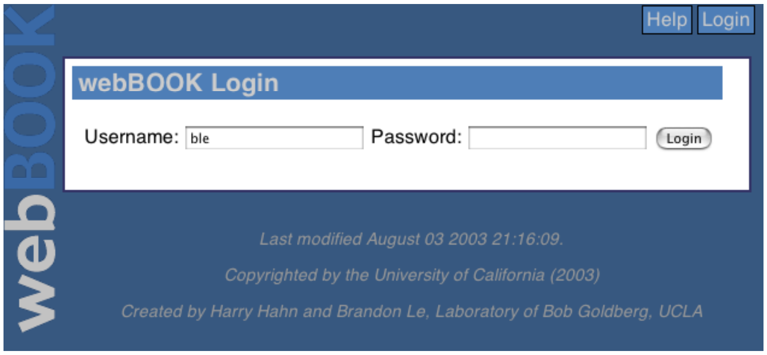
http//estdb.biology.ucla.edu/webbook

Using the Webboook

1. Username: email username
   Password: 9 digit student id
2. Check message board for important news/updates
3. An overview of the different sections

Projects	-	list of experiments
Stocks	-	catalog of stocks/reagent in the lab
Protocols	-	procedures carried out in the lab (pdf format)
Calendar	-	calendar to plant your experiments
Browse	-	search and look at other members experiments
Contact	-	email for help
Logout	-	will logout if idle for 30 min

Webbook Login Page



webBOOK Login

Username: ble Password:

Last modified August 03 2003 21:16:09.

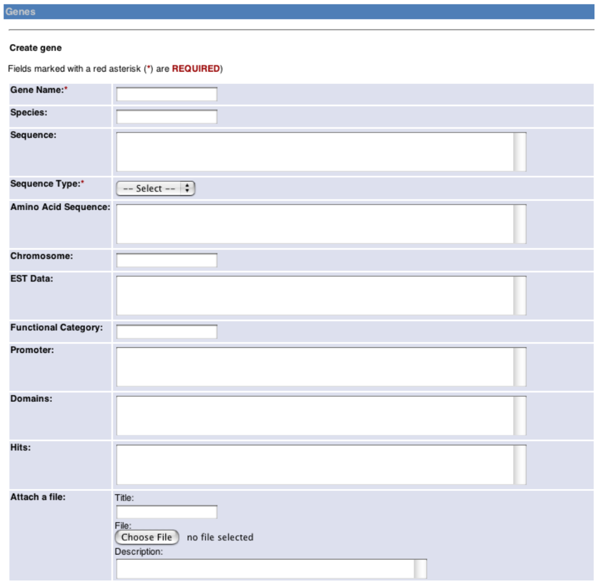
Copyrighted by the University of California (2003)

Created by Harry Hahn and Brandon Le, Laboratory of Bob Goldberg, UCLA

Creating Projects / Experiments

1. Title of project
2. Questions/Purpose of project
3. Summary of project (ideas)

Entering Gene Information



Genes

Create gene

Fields marked with a red asterisk (*) are REQUIRED)

Gene Name:*

Species:

Sequence:

Sequence Type:* -- Select --

Amino Acid Sequence:

Chromosome:

EST Data:

Functional Category:

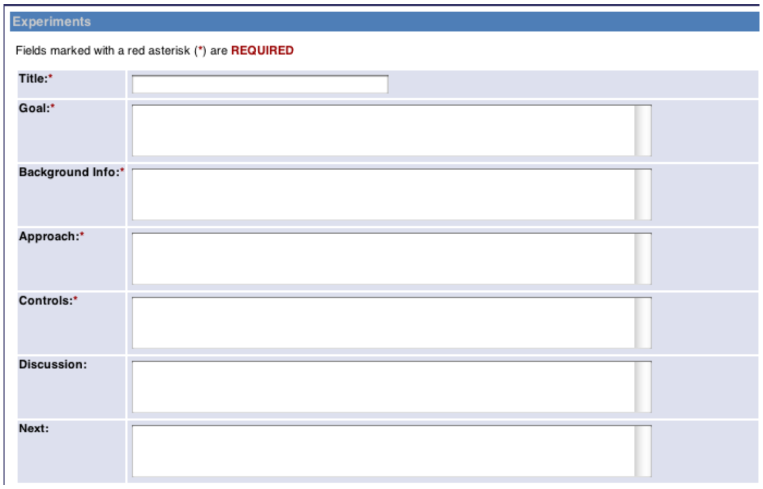
Promoter:

Domains:

Hits:

Attach a file: Title:
File: Choose File no file selected
Description:

Entering Experiments Information
Part 1



Experiments

Fields marked with a red asterisk (*) are REQUIRED

Title:*

Goal:*

Background Info:*

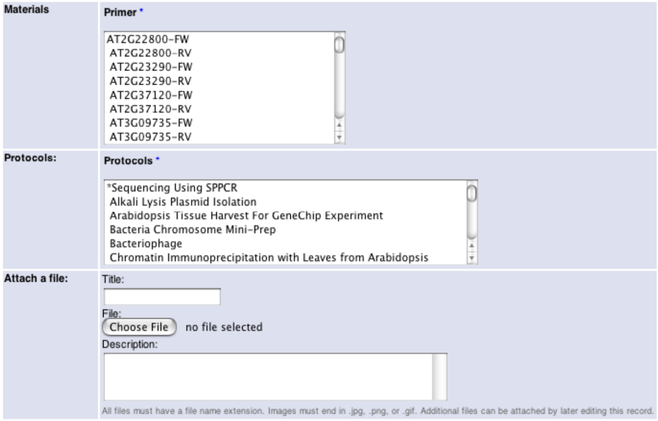
Approach:*

Controls:*

Discussion:

Next:

Entering Experiment Information
Part II



Materials

Primer *

AT2G22800-FW
AT2G22800-RV
AT2G23290-FW
AT2G23290-RV
AT2G37120-FW
AT2G37120-RV
AT3G09735-FW
AT3G09735-RV

Protocols:

Protocols *

*Sequencing Using SPPCR
Alkali Lysis Plasmid Isolation
Arabidopsis Tissue Harvest For GeneChip Experiment
Bacteria Chromosome Mini-Prep
Bacteriophage
Chromatin Immunoprecipitation with Leaves from Arabidopsis

Attach a file:

Title:

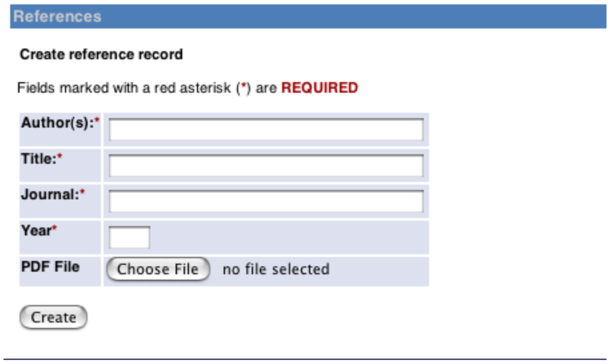
File:

Choose File no file selected

Description:

All files must have a file name extension. Images must end in .jpg, .png, or .gif. Additional files can be attached by later editing this record.

Entering References Relating to your Gene



References

Create reference record

Fields marked with a red asterisk (*) are **REQUIRED**

Author(s):*	
Title:*	
Journal:*	
Year*	
PDF File	Choose File no file selected