APPENDIXES

Appendix 1A

Preparation of a 1% Agarose Gel for Gel Electrophoresis

2. For a **1% agarose gel**, weigh out **1 gram** of agarose (powder) on a weighing scale.
   *Note: **percentage** of the gel reflects the **amount of agarose** in **gram** in **100 mL** of 1X TAE buffer, depending on the final percentage of agarose in the gel.*
   Example: If you want to make a 0.7% agarose gel (0.7 g/100 mL, w/v), weigh out 0.7 g of agarose for 100 mL of agarose solution
3. Carefully, put the agarose in a 250-mL Erlenmeyer flask.
4. Measure out 100 mL of 1X TAE buffer using a plastic or glass graduated cylinder.
5. Add 100 mL of 1X TAE buffer into the flask in step 2.
6. Cover the flask with a piece of plastic wrap. Poke 3-4 holes on the plastic wrap using a pointed end of a pencil or pen (*Note: the holes allow the steam to escape during microwaving in step 6 below*). Swirl the solution to break up any lumps of agarose granules.
7. Microwave the solution for about 2 minutes or until the agarose granules have completely melted.
   - *Be careful with the flask. The solution gets very hot.*
   - *Constantly watch over the solution because when it starts boiling, it might overflow.*
   - *Swirl gently the solution several times while microwaving to help melt agarose evenly.*
   - Once the agarose has melted completely, the solution is clear.
8. Cool down the agarose solution for at least 30 min in a 55ºC water bath.
9. While the agarose solution is cooling, prepare the gel cast with the appropriate comb.
   - ***The comb depends on the number of samples to be loaded on the gel**. For example, if there are less than 18 samples, then use a 20-tooth comb; but, if there are 21 samples, then use a 30-tooth comb.*

- *Remember to add two more wells to the number of wells needed for the samples. These two wells will be for loading 1kb DNA ladder in the first and the last wells (or left and right sides of loaded samples).*

10. After the agarose solution has been cooled down, add 5 µL of Ethidium Bromide (EtBr) into the solution and swirl the flask GENTLY to mix. <u>*Note:*</u> *Do NOT swirl vigorously to generate many bubbles.*

11. Pour the agarose/EtBr solution into the gel cast. Wait for 30 min for the agarose solution to solidify.

    <u>*Note:*</u> *IMMEDIATELY after pouring the agarose solution, inspect the agarose solution's surface for the present of bubbles. If there are many bubbles floating on the surface of the gel solution, use a pipette tip to pop them before the gel is completely solidified.*

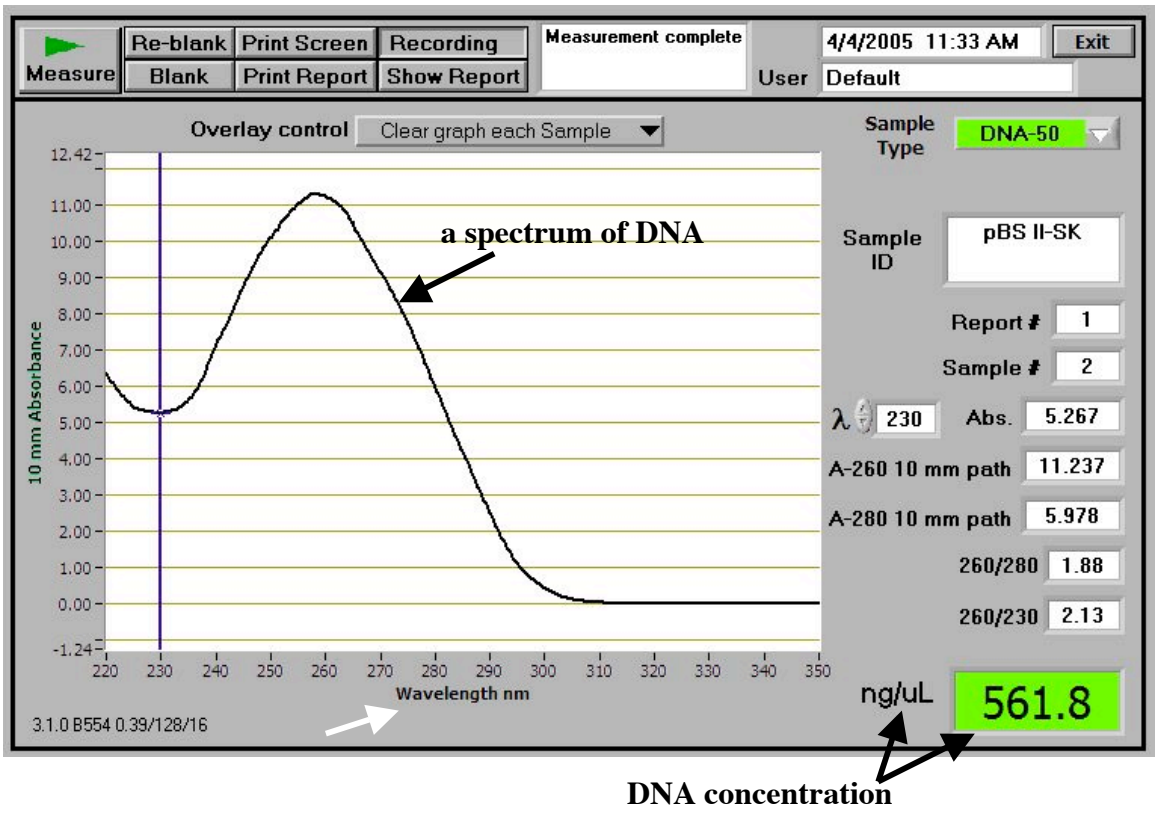
12. Pour ~600 mL of 1X TAE buffer into the gel box.

After the agarose has solidified into a gel, take out the comb gently by pulling it straight up out of the gel and put the gel in the gel box containing the 1XTAE running buffer.

Appendix 1B

What is a **spectrophotometer**?

It is an **instrument** that **measures** the **amount** of **molecules absorbing at a given wavelength of energy**. In this exercise, we measure the amount of DNA molecules in a given volume in the **ultraviolet wavelengths** of **200 – 280 nm** (**nm** stands for **nanometer**, which is **1 billionth** of a **meter**). The bases of DNA have the absorbance at the wavelength of 254 nm. The absorbance of DNA molecules over the wavelength range of 220 - 350 nm is represented as a **spectrum** with a **peak** at **near 260 nm** as illustrated by the **Nanodrop spectrophotometer readout** below:



OPERATION OF NANODROP SPECTROPHOTOMETER ND-1000

Note: The Nanodrop is powered by the computer via the USB port.

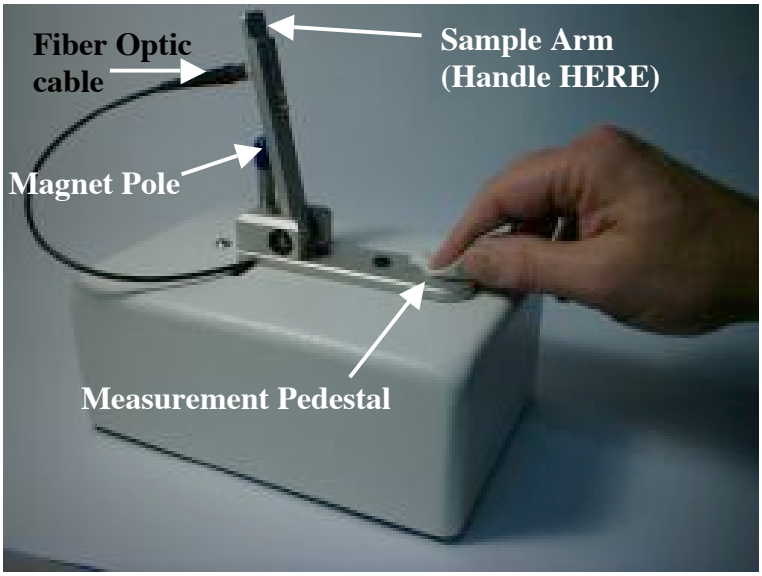
1. **Turn ON** the **Computer (laptop)** that connects to the Nanodrop.
2. **Turn ON** the **Nanodrop** by clicking an "**ND-1000 v3.1.0**" icon on the computer desktop. Wait for a few seconds for the Nanodrop to be up. You see the Nanodrop 3.1.0 Diagnose panel with **User field** as **Default.**
3. **Click** on "**Nucleic Acid**" button on the top left column for reading concentration of DNA and RNA solutions.
   - ❖ You see a following message:

   "Ensure Sample Pedestals are clean and then load a water sample. After loading water sample, click OK to initialize instrument"
4. a. **Clean** the **sample pedestals as followings:**

   i. **Raise** the **Sample Arm up** by holding at its end as shown on the picture below.

   Caution: NEVER hold the Optical Cord when lifting and lowering the Sample Arm because the cord is fragile; and it is very expensive to replace it.

   ii. **Wipe both** the **Measurement Pedestal** and the **Sample Arm** with a piece of Kimwipes slightly wetted with distilled water.



b. **Pipet 1.5 - 2 µL** of water on the **Measurement pedestal**.

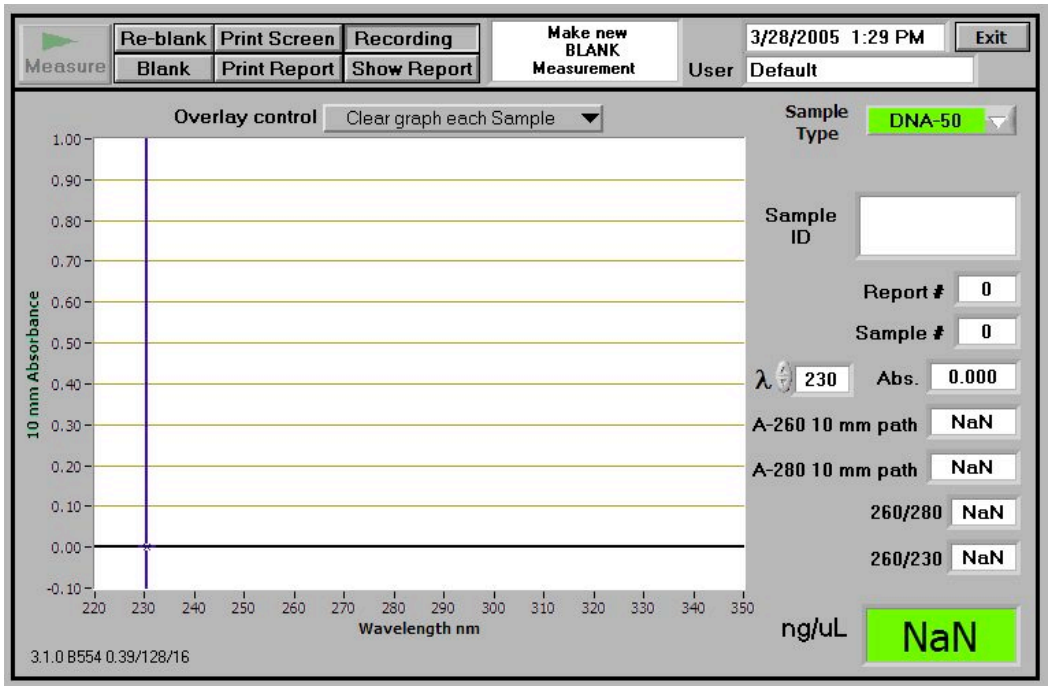
Note: Even though the Nanodrop Inc. claims that the Nanodrop can read as low as **1 µL**, the concentration reading is NOT consistent at this volume. Therefore, the **minimal** volume for the concentration reading is **1.5 µL**

c. **Slowly Lower** the SAMPLE Arm to its horizontal position.

Caution: NEVER let the arm fall freely.

d. **Click** the **OK** button. The Nanodrop is **INITIALIZED.**

You see a **Dialog panel** as shown below



What do you need to do, NEXT?

a. **Change SAMPLE TYPE** (if necessary) from **DNA-50** (by Default) to **RNA-40** or **Other** (for Oligonucleotides), depending on your sample.

b. **Type in** the **SAMPLE ID** field the Information of your sample.

c. **Make** a **NEW BLANK measurement**

d. (Option) **Change** the **OVERLAY CONTROL** field from the Default setting of "CLEAR GRAPH EACH SAMPLE" to "CLEAR GRAPH ON NEW REPORT" or "ACCUMULATE UNTIL CLEAR" or "CLEAR GRAPH NOW"

5. **Wipe off** the **liquid** on **BOTH** the **ARM** and **Measurement pedestals** with a piece of Kimwipes.
6. **Make** a **Blank measurement** by pipetting 1.5 - 2.0 mL of either double-distilled water or TE (depending on whether your sample solution is in double-distilled water or TE) on the Measurement Pedestal. Then lower the Sample Arm to its horizontal position.
7. **Click** the **BLANK** button. The blank was made.
8. After the reading is done, **bring** the Sample Arm **up** to the vertical position and **Wipe off** the **liquid** on **BOTH** the **ARM** and **Measurement pedestals** with a piece of Kimwipes.
9. **Pipet** 1.5 - 2.0 mL of SAMPLE on the Measurement Pedestal and lower the Sample Arm.
10. **Type in** the **SAMPLE ID** field **Information** of a sample solution.
11. **Click** the **MEASURE** button to determine concentration of your sample.
12. After the reading is done, a sample concentration (in **ng/mL**) and a spectrum of the sample along other information are shown. You can either
    a. **Save** the **window** of measured sample by
    clicking on FILE → choose SAVE WINDOW → Select an existing folder or Create a NEW folder (give a name for the NEW folder) → Type in a Name file in the FILE NAME field → Click the SAVE button to save the file **or**
    b. **Print** the **window** by **clicking** the **PRINT SCREEN** button.

Note: To print the current spectrum of the sample, you MUST print it before reading the next sample. Otherwise, you need to repeat reading the sample.

13. **Repeat** steps 8-12 for other samples.
14. After reading the **last sample**, **click** the **PRINT BATCH** button to print concentrations of all read samples.
15. If done with the Nanodrop, **click** the **EXIT** buttons.
16. **Clean** the **Measurement Pedestal** and the **Sample Arm** with a piece of Kimwipes slightly wetted with distilled water.

Appendix 1C

1-kb DNA Ladder (Taken from Invitrogen website)

Invitrogen
life technologies

1 Kb DNA Ladder

Cat. No. 15615-016 **Size: 250 µg**
Conc.: 1.0 µg/µl **Store at -20°C.**

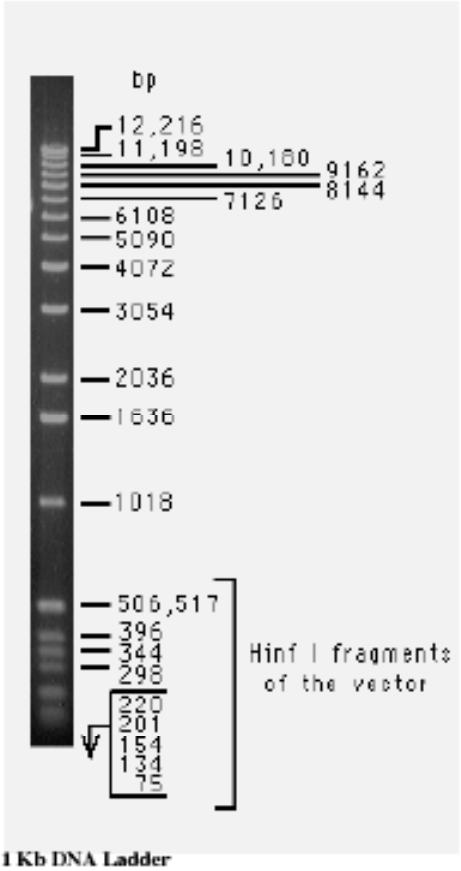
Description:
The 1 Kb DNA Ladder (U.S. Patent No. 4,403,036) is suitable for sizing linear double-stranded DNA fragments from 500 bp to 12 kb. The bands of the ladder each contain from 1 to 12 repeats of a 1018-bp DNA fragment. In addition to these 12 bands, the ladder contains vector DNA fragments that range from 75 to 1636 bp. The 1636-bp band contains 10% of the mass applied to the gel. The ladder may be radioactively labeled by one of the following methods: (i) Partial exonucleolytic degradation and resynthesis with T4 DNA polymerase. This method is preferred because higher specific activity is achieved with less ^{32}P input; (ii) Labeling the 5′ ends with T4 polynucleotide kinase; (iii) Filling in the 3′ recessed ends with *E. coli* DNA polymerase I or the large fragment of DNA polymerase I.

Storage Buffer:
10 mM Tris-HCl (pH 7.5)
50 mM NaCl
0.1 mM EDTA

Recommended Procedure:
Invitrogen recommends the use of 10X BlueJuice® Gel Loading Buffer (10816-015) at a concentration of 2X [for electrophoresis of this ladder on agarose gels]. Alternatively, the DNA ladder can be diluted in a buffer such that the final concentration of NaCl is 20 mM. Apply approximately 0.1 µg of standard per mm lane width. DO NOT HEAT!

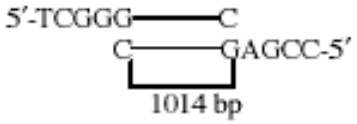
Quality Control:
Agarose gel analysis shows that all bands larger than 500 bp are distinguishable.

Doc. Rev.: 011602



1 Kb DNA Ladder
0.5 µg/lane
0.9% agarose gel
stained with ethidium bromide

Structure of Fragment (1):



Notes:

During 1.2% agarose gel electrophoresis with Tris-acetate (pH 7.6) as the running buffer, bromophenol blue migrates together with the 506/517 bp doublet band.

The 1636 bp band and all bands less than 1000 bp are generated from pBR322.

If the ionic strength of the sample is too low, blurring of the bands can occur.

Cat. No. 15615-016

APPENDIX 2 - BIOINFORMATICS (PARTS I AND II)

HC70AL Spring 2004

An Introduction to Bioinformatics -- Part I

By

Brandon Le

April 6, 2004

What is a Gene?

An ordered sequence of nucleotides

What are the 4 Nucleotides in DNA?

A - Adenine
T - Thymine
C - Cytosine
G - Guanine

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?

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)

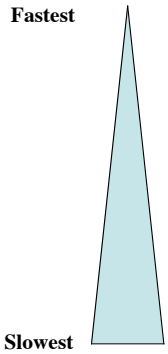
What does BLAST do?

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

What are the different BLAST Programs?



blastp - protein query vs protein db

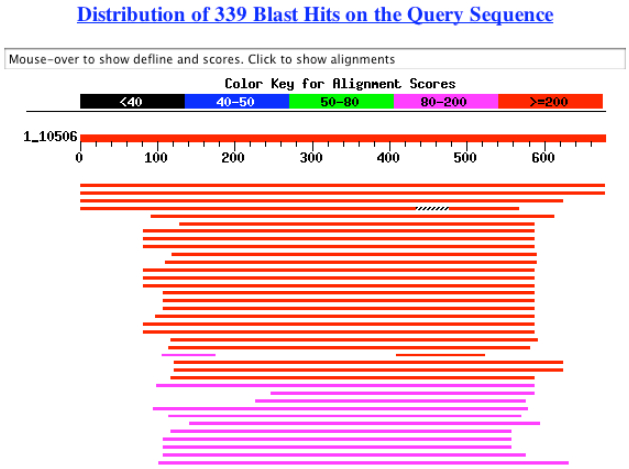
blastn - DNA query vs DNA db

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



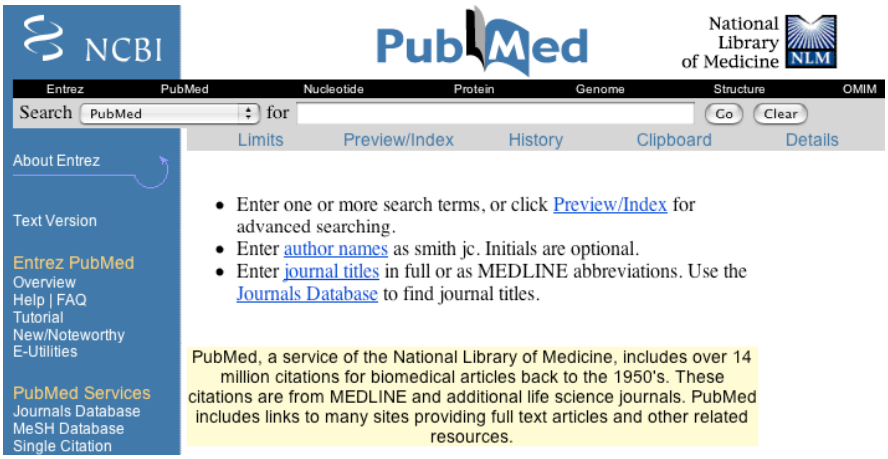
Anatomy of a BLAST Result -- Part II

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



HC70AL Spring 2004

An Introduction to Bioinformatics -- Part II

By

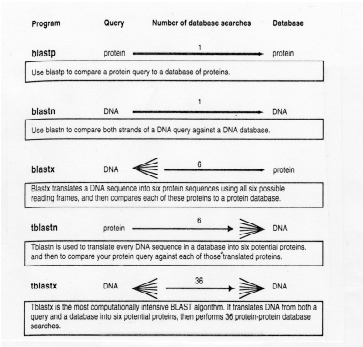
Brandon Le

April 8, 2004

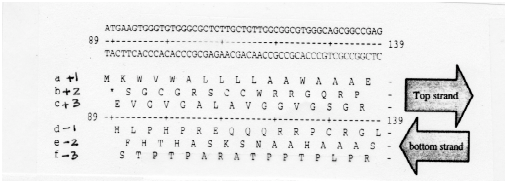
Review of BLAST Search

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?



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



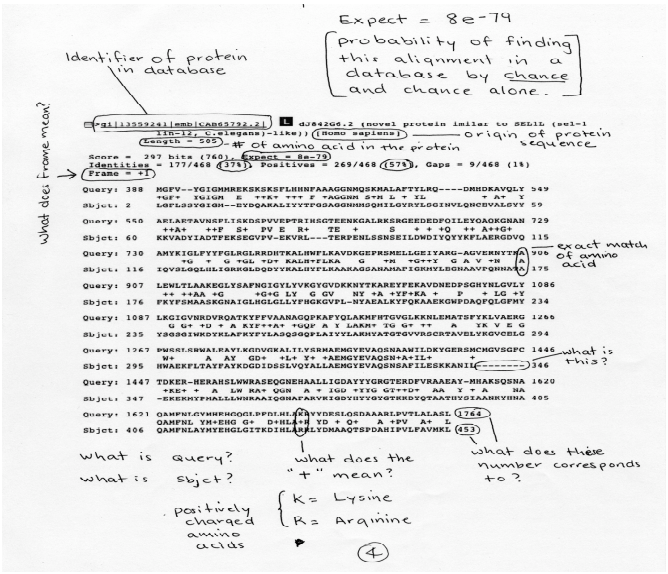
Question:

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?



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**

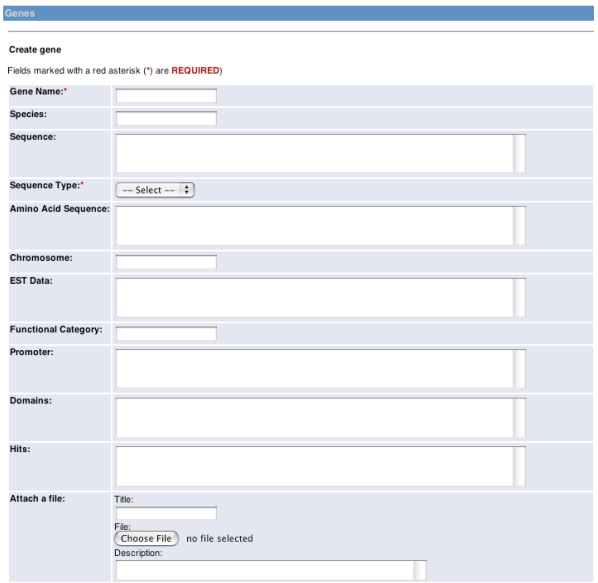
Logout - **will logout if idle for 30 min**

Webbook Login Page

Creating Projects / Experiments

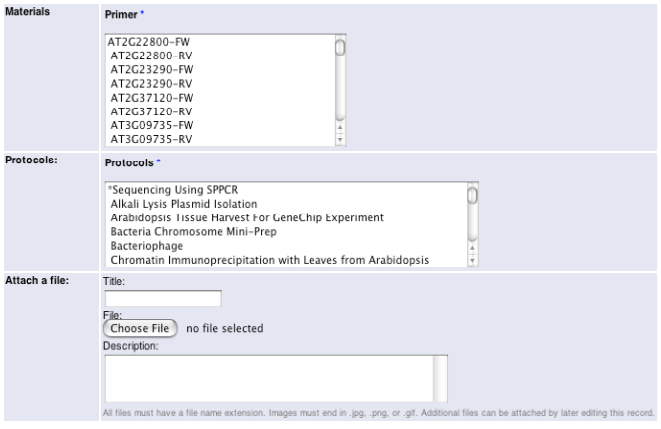
1. Title of project
2. Questions/Purpose of project
3. Summary of project (ideas)

Entering Gene Information



Entering Experiments Information
Part 1

Entering Experiment Information
Part II



Entering References Relating to your Gene