HC70AL Spring 2011

An Introduction to Bioinformatics By

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April 7, 2011

Outline

- 1. Review of Dideoxy Sequencing
- 2. Obtaining and Processing DNA Sequences
 - 3. What is a Gene?
 - 4. Sequence Analysis Using BLAST
 - 5. Identifying Knockout Lines from SALK





DNA Polymerase reads the template strand and synthesizes a new second strand to match:

5' - TACGCGGTAACGGTATGTTCGACCGTTTAGCTACCGA

IF 5% of the T nucleotides are actually <u>dideoxy</u> T, then each strand will terminate when it gets a ddT on its growing end:

- 5' TAC6C66TAAC66TAT6TTC6ACC6TTTA6CTACC6AT•
- 5' TACGCGGTAACGGTATGTTCGACCGTTTAGCT•
- 5' TACGCGGTAACGGTATGTTCGACCGTTT•
- 5' TACGCGGTAACGGTATGTTCGACCGTT•
- 5' TACGCGGTAACGGTATGTTCGACCGT•
- 5' TACGCGGTAACGGTATGTT•
- 5' TACGCGGTAACGGTATGT•
- 5' TACGCGGTAACGGTAT•
- 5' TACGCGGTAACGGT•
- 5' TACGCGGT•





GCGAATGCGTCCACAACGCTACAGGTG GCGAATGCGTCCACAACGCTACAGGT GCGAATGCGTCCACAACGCTACAGG GCGAATGCGTCCACAACGCTACAG GCGAATGCGTCCACAACGCTACA GCGAATGCGTCCACAACGCTAC GCGAATGCGTCCACAACGCTA GCGAATGCGTCCACAACGCT GCGAATGCGTCCACAACGC GCGAATGCGTCCACAACG GCGAATGCGTCCACAAC GCGAATGCGTCCACAA GCGAATGCGTCCACA GCGAATGCGTCCAC GCGAATGCGTCCA GCGAATGCGTCC GCGAATGCGTC GCGAATGCGT GCGAATGCG GCGAATGC GCGAATG



Each Lane is One DNA Sequence



FINCH TV

MAC - http://mac.softpedia.com/get/Math-Scientific/FinchTV.shtml

PC - http://www.softpedia.com/get/Science-CAD/FinchTV.shtml

4Peaks

MAC - http://www.mekentosj.com/science/4peaks

Goldberg EST-DB

Current disk usage: 15G free (43% used) / 0.18,0.21,0.09 / Jobs

Process

Process trace files, either existing remotely via an FTP server or provided by an outside source and uploaded to this server. Or, input a new sequence manually, without processing any trace files.

<u>Summary,</u> <u>Search &</u> <u>Select</u>

Obtain summaries, search the database, select specific sets of sequences on which to perform actions.

Browse/manipulate datasets

Browse the cap3 analysis data or the databases available for BLAST. Manually update these datasets, or delete custom blast databases. To create a BLAST database, use the <u>Search form</u> to first select the desired sequences, then use the "Make Blast DB" action.

Preferences

Update database entries such as projects, function groups, etc.

<u>Help</u>

Online help manual

Links to some useful software.

http://estdb.biology.ucla.edu/~goldberg

Processing Sequence Files - PART I





Current disk usage: 15G free (43% used) / 0.06,0.16,0.08 / Jobs

Process files from UCLA sequencing facility

In order to process files from the UCLA sequencing facility, you must first download the files from <u>WebSeq</u>. The file, containing all of the sequences you wish to process should be called "webseq.zip". Please use the following to uplead your webseq.zip to estdb for processing by clicking the "Browse" button, then navigating to the location of webseq.zip on your computer. Please note that the upload and unzipping process make take a bit of time. Please be patient and do not cancel the process prematurely.

You may also use this form to upload a single (non-zipped) sequence file.

(Go)

Choose File no file selected

Process local files

Select this item if you wish to process files not located at UCLA's sequencing facility. These files should have been uploaded to estdb.biology.ucla.edu via ftp prior to selecting this option.

Process local files

Obtaining Sequences From UCLA Sequencing Facility

WWWWWWWWWWWWWWWWWWWWWWWWWWWWWWWWWWWWWW	Web	Seq	
<u>Help</u>	Welcome to We	ebSeq	
Sequencing & Genotyping Core Home Page	Username: Password:	goldberg_r •••••• Login	
	Copyright (C) 2002-2 Last modified 20 Janu	2004 UCLA Human Genetics uary 2004 14:44:07.	

http://www.genetics.ucla.edu/webseq/

Selecting Sequence to Download



Choose File to Upload to ESTDB



Select Individual Files to Process in ESTDB



:: process sequence trace files into the database

File selection

Directory: /home/goldberg/tmp/12755102524090db5f13178

Which of the following files should be processed?

⊟ 11012GoldR_F-JW_083.ab1
⊟ 11012GoldR_R-JW_091.ab1
Select All | Deselect All

Continue with selected

Renaming and Initial Annotation of Files



Renaming and initial annotation

Below is a list of the sequences selected from the **8810816084090df4038ca0** directory. Please review it before providing initial annotation and inputing new names for these files. Once complete, click the continue button at the bottom.

Project: Manual Sequencing and Others 🛟 Pri	mer: n.d. + Vector: n.d. + Sequenced from 3' + end
Original Name New Name 8966GoldR_EM- K16JL_008.ab1 8966GoldR_EM- K16RV_007.ab1 Gene_Superpool_Rv	Base name : Starting number : Starting number : Fill In Clear Values Use the above two fields ("Base name" and "Starting number") to automatically fill a basename plus a starting number in the rename fields to the left. This does not finalize this form- you have to click the continue button at the bottom- but eases repetitive entry. For example, if you want the first sequence on the left (8966GoldR_EM-K16JL_008.ab1) to be named PCEP00100, you would type in "PCEP00" for the base name, and "100" for the starting number, then click the "Fill In" button.
	Continue

Interpreting Sequence Chromatogram Information



Rename sequences

The files will now be renamed. Do not interrupt this process.

Please wait till transfer is complete before reviewing and selecting an option at the bottom.

Trying to make /home/goldberg/tmp/2da08cbf25b0588d96fe66cd57176513...OK Moving /home/goldberg/tmp/12755102524090db5f13178/11012GoldR_F-JW_083.abl to /home/goldberg/tmp/2da08cbf25 Moving /home/goldberg/tmp/12755102524090db5f13178/11012GoldR R-JW 091.abl to /home/goldberg/tmp/2da08cbf25

Next step: base call with phred

CATTAT	GGCCGGG	: Tell phred to trim
12		: Hilite poly-A of length
		: Other sequences to highlight (separate multiple with commas)
		: Omit sequences less than or equal to this length
(Start p	hred	

Successful Interpretation of Chromatogram

Base Calling with Phred

The files are now being base called.

Do not interrupt this process or click on a link until the reads are done- wait until this is complete before reviewing and selecting an option at the bottom.

```
LEN : length of sequence read, after trimming
PRET : length of sequence read, pre-trimming
Ns : number of Ns in sequence read
STAT : status
OMIT : sequence was omitted due to short read; sequence not inputted into database
ERR! : an error occurred during base calling by phred; sequence not inputted into database
A : when an A occurs, a poly A sequence greater than or equal to 12 in length was found
M : when an M occurs, a match to one of given sequences was found (none given)
```

Trying to make /home/goldberg/tmp/9fb2c99b2e9f5edc660880ec4a283858-phred...OK

					10	20	30	40	50	
NAME	LEN	PRET	Ns	STAT	++.	+.	+	+ .	+	•••
Gene_Superpool_JL202	420	909	0		TTCTTTTTCTCC	TATTGACCAT	CATACTCATTC	SCTGATCCAT	GTAGATTTCCCGGAC.	AT
Gene_Superpool_Rv	568	883	0		AAGACTCGAATC	GAAATAACAAA	AAGTTCCAAG	ICCAACAATG	ACAAGGAAACCAAAG	AC

Deleting temp directory for sequence reads... OK

SUMMARY : Sequences added to database : 2 (avg length 494.0000000000000) Sequences omitted/too short : 0 Sequences generating errors : 0

The sequences above, unless marked as OMIT or ERR!, were read and entered into the database.

You may edit or delete a sequence at this point by clicking on it. Sequence editing will occur in a separate window. Trace download is not available until the next step.

Discard reads	Sele para	ct this option if you wish to discard all of the reads performed above. If you wish to change the meters passed to phred on the previous page, select this option before using the Back button to
	go t	p the previous page.
Continue	Sele	ct this option if you wish to continue with the sequences above, as edited, and configure the blast
	runs	with them. Do not use the back button after you have used this option.

Sequence Record Information

Edit Sequence Record for Gene_Superpool_JL202							
Show BLAST/PFAM data							
Perform action: Submit changes 🛟 Go							
TTCTTTTCTCCTATTGACCATCATACTCATTGCTGATCCATGTAGA	Project	Manual Sequencing and Others 🛟					
ATTAGGTCAGGTGTGTAATTAATGACCTGTATTTGCAAAATCTGTG GTCAACAATGTTTGCCGGAATTGTTGGTAGAGCCAGGACTCATGAA	Vector	n.d. 🛟					
CAGATAATGGCTGATGCTGCTGGAAACTTCAATGGAAATCTCCAAA TAGTAAGTCTTCTACACTAATATAGAGTTATACAAAGAAAAAAAC	Primer	n.d. 🗘					
TTCATAGATGAGTGCTGAGTACCAAGTGCTTTCCCCGCTAGTCACA ACCCGCGAAAGCTACTTCGTCCGCTACTGAAAGCAACAAGGAGAG GGTTT	Sequenced from	3' + end					
	cDNA size						
	Function group	No Significant Hits					
Length : 420 # Ns : 0	Most						
Date entered : 04/29/2004 Date modified : 04/29/2004	homologous	1					
Session : 9102c9902e915edc660880ec4a283858	Origin	n.a. 🗘					
	Arabidopsis hit?	⊖ Yes ⊙ No					
	poly-A tail?	⊖ Yes ⊙ No					
	Accession #	(none)					
	DBESTID	(none)					

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

	S NCBI		translatir	BLAST
	Nucleotide	Protein	Translations	Retrieve results for ar RID
1				
	<u>Search</u>			
	Choose a translation	TRANSLATED query - PRO	TEIN database (blastx)	•
	Set subsequence	From: To:		
	Choose database	nr 🛟		
	Genetic codes	Standard (1)	\$	
	Now:	BLAST! or Reset qu	Reset all	

What are the different BLAST Programs?



Anatomy of a BLAST Result -- Part I

Distribution of 339 Blast Hits on the Query Sequence



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 📕
gi 13559241 emb CAB65792.2 dJ842G6.2 (novel protein imilar	282	2e-74
gi 19923669 ref NP_005056.3 sel-1 suppressor of lin-12-lik	268	4e-70 👢
gi 6851089 gb AAF29413.1 SEL1L [Homo sapiens] >gi 17646138	268	4e-70 👢
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 📘
gi 20857527 ref XP_127076.1 Sell (suppressor of lin-12) 1	261	4e-68
gi 4159995 gb AAD05210.1 SELLL [Mus musculus] >gi 20073079	259	1e-67 📕
gi 29336095 ref NP_808794.1 Sell (suppressor of lin-12) 1	259	2e-67 👢
gi 29612522 gb AAH49959.1 Sellh protein [Mus musculus]	258	4e-67 📕
gi 17563256 ref NP_506144.1 Suppressor/Enhancer of Lin-12	247	9e-64 👢
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
          MRILSYGIVILSLLVFSFIEFGVHARPVVLV
                                                     v
Sbjct: 1
          MRILSYGIVILSLLVFSFIEFGVHARPVVLVLSNDDLNSGGDDNGVGESSDFDEFGESEP 60
Query: 61 XXXXXLDPGSWRSIFEPDDSTVQAASPQYYSGLKKILSAASEGNFRLMEEAVDEIEAASS 120
               LDPGSWRSIFEPDDSTVQAASPQYYSGLKKILSAASEGNFRLMEEAVDEIEAASS
Sbjct: 61 KSEEELDPGSWRSIFEPDDSTVQAASPQYYSGLKKILSAASEGNFRLMEEAVDEIEAASS 120
Query: 121 AGDPHAQSIMGFVYGIGMMREKSKSKSFLHHNFAAAGGNMQSKMALAFTYLRODMHDKAV 180
          AGDPHAQSIMGFVYGIGMMREKSKSKSFLHHNFAAAGGNMQSKMALAFTYLRQDMHDKAV
Sbjct: 121 AGDPHAQSIMGFVYGIGMMREKSKSKSFLHHNFAAAGGNMQSKMALAFTYLRODMHDKAV 180
Query: 181 OLYAELAETAVNSFLISKDSPVVEPTRIHSGTEENKGALRKSRGEEDEDFOILEYOAOKG 240
          OLYAELAETAVNSFLISKDSPVVEPTRIHSGTEENKGALRKSRGEEDEDFOILEYOAOKG
Sbjct: 181 QLYAELAETAVNSFLISKDSPVVEPTRIHSGTEENKGALRKSRGEEDEDFQILEYQAQKG 240
Query: 241 NANAMYKIGLFYYFGLRGLRRDHTKALHWFLKAVDKGEPRSMELLGEIYARGAGVERNYT 300
          NANAMYK GLFYYFGLRGLRRDHTKALHWFLKAVDKGEPRSMELLGEIYARGAGVERNYT
bjct: 241 NANAMYKNGLFYYFGLRGLRRDHTKALHWFLKAVDKGEPRSMELLGEIYARGAGVERNYT 300
```

What is a Gene?

An <u>ordered</u> 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 <u>ordered</u> sequence of nucleotides
- A unique position/location in the genome
- Polarity (5' to 3')
- Exons and/or 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

Nonsense Strand

What Gene Are You Working With?

Task: Use the DNA sequence you've obtained from the sequencing facility to identify your gene

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

Procedure:

- 1. Go to the TAIR BLAST page (http://arabidopsis.org/Blast/index.jsp)
- 2. Select BLASTN
- 3. For Dataset, Select "TAIR10 Genes"
- 4. Run BLAST

Results/Question:

1. What is the gene that you're working with?

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)

Procedure:

- 1. Select Seqviewer
- 2. Enter gene number (ex. AT2G26320)
- 3. Submit

- 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

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

Genes Have Exons and/or Introns!

Task: How many exons and/or 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

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

A Gene Encodes a Protein

Task: Determine the protein encoded by your gene

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

- 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: TAIR (http://arabidopsis.org)

> NCBI Pubmed (http://ncbi.nlm.nih.gov/pubmed)

PubMed - Endless Resources

(http://www.ncbi.nlm.nih.gov/pubmed)



Ex. Author search - Goldberg RB, Bui AB Keyword - T-DNA Mutagenesis, etc...



http://signal.salk.edu/cgi-bin/tdnaexpress

SALK T-DNA Lines How Were T-DNA Lines Created?



SALK T-DNA Lines How Were T-DNA Lines Created?





000	O T-DNAexpress : The SIGnAL Arabidopsis Gene Mapping Tool							
A A C http://sign	nal.salk.edu/cgi-		 Q→ Google 					
□ Lab ▼ HC70A ▼ Bioinformatics ▼	Arabidopsis 🔻	Phytome	Scholar	Softpedia				
GSLT cDNA PYRt1901010 RIKEN EST	RATM13-3326-1_G €					ŕ		
Please note: We are using A	GI V5 pseudo-n	nolecules (Feb-19-2	004)/annotation (Feb-23	-2005, revised Mar-17-2005).	L		
1. Edit Preference:			4	. Blast:		l		
Click to edit your preference for T-DNA Expres	5.		P	rogram: blastn, DNA 🛟 E	-value: 1e-04 🛟 Hits: 1 🛟	L		
2. Search:			C	ut and paste your sequence into	here.	L		
Gene name:	for examp	ole: <u>At1q0101</u>	0			n		
or Function:	for examp (Case se	ole: <u>auxin</u> nsitive)						
or cDNA/T-DNA:	for exan <u>SALK_0</u>	nple: 03854, <u>R1004</u>		ile :	Submit			
			(Choose File no file selected	Clear	Ш		
	Sub	mit Clear	5	. Multiple Search Search by	Gene/Affy/GO/Sequence and plain text return only.			
3. Locate: Position:	;) (Submi	t) Clear)	6 re	• SIGnAL iSect Tools to de trieve sequences and to compare to	≥sign primers for T-DNA or your sequences, to wo lists.			
ANNOUNCEMENT : ** • We requested that users include this r	eference (below) in	publications	describing t	he use of SSP gold standard cDN	A ORF clones obtained from ABRC. Thanks!			
Kayoko Yamada, Jun Lim, Joseph M. Dale, Huaming Chen, Paul Shinn, Curtis J. Palm, Audrey M. Southwick, Hank C. Wu, Christopher Kim, Michelle Nguyen, Paul Pham, Rosa Cheuk, George Karlin-Newmann, Shirley X. Liu, Bao Lam, Hitomi Sakano, Troy Wu, Guixia Yu, Molly Miranda, Hong L. Quach, Matthew Tripp, Charlie H. Chang, Jeong M. Lee, Mitsue Toriumi, Marie M. H. Chan, Carolyn C. Tang, Courtney S. Onodera, Justine M. Deng, Kenji Akiyama, Yasser Ansari, Takahiro Arakawa, Jenny Banh, Fumika Banno, Leah Bowser, Shelise Brooks, Piero Carninci, Qimin Chao, Nathan Choy, Akiko Enju, Andrew D. Goldsmith, Mani Gurjal, Nancy F. Hansen, Yoshihide Hayashizaki, Chanda Johnson-Hopson, Vickie W. Hsuan, Kei Iida, Meagan Karnes, Shehnaz Khan, Eric Koesema, Junko Ishida, Paul X. Jiang, Ted Jones, Jun Kawai, Asako Kamiya, Cristina Meyers, Maiko Nakajima, Mari Narusaka, Motoaki Seki, Tetsuya Sakurai, Masakazu Satou, Racquel Tamse, Maria Vaysberg, Erika K. Wallender, Cecilia Wong, Yuki Yamamura, Shiaulou Yuan, Kazuo Shinozaki, Ronald W. Davis, Athanasios Theologis, and Joaseph R. Ecker (2003) Empirical Analysis of Transcriptional Activity in the Arabidopsis Genome Science 302: 842-846. [Abstract] [Full Text] [Supporting data] [Supplementary GEO Analysis Files]								
 We requested that users include this r 	eference (below) in	publications	describing t	he use of Salk lines obtained fro	m ABRC or NASC. Thanks!			
José M. Alonso, Anna N. Stepanova, Thom Carmelita Gadrinab, Collen Heller, Albert J Hazari, Emily Hom, Meagan Karnes, Celen	as J. Leisse, Christopl eske, Eric Koesema, C e Mulholland, Ral Ndu	ner J. Kim, Hua ristina C. Meye baku, Ian Schi	aming Chen, ers, Holly Parl midt, Plinio G	Paul Shinn, Denise K. Stevenson, Ju ker, Lance Prednis, Yasser Ansari, N Juzman, Laura Aguilar-Henonin, Mar	istin Zimmerman, Pascual Barajas, Rosa Cheuk, athan Choy, Hashim Deen, Michael Geralt, Nisha kus Schmid, Detlef Weigel, David E. Carter, Trudy	4 +		
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