


National Institute of Allergy and Infectious Diseases

**Phylogenetics and Sequence Analysis**

**Lecture 2**  
**BLAST and Sequence Alignment**

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Bioinformatics and Computational Biosciences Branch  
Office of Cyber Infrastructure and Computational Biology

Fall 2015



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**We Are BCBB!**



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- Group of 37
  - Bioinformatics Software Developers
  - Computational Biologists
  - Project Management & Analysis Professionals



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**Course Organization**

- Building a clean sequence
- **Collecting homologs**
- **Aligning your sequences**
- Building trees
- Further analyses



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## Previously

- Hierarchical and genealogical data
- Comparative sequence analysis
- Generating clean sequence
  - Trim vector contamination
  - Trim low-quality ends
  - Align fragment overlap to build contig
  - Export contig (consensus)

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## Today...

**Pairwise sequence alignment**

- How does it work?

**BLAST**

- How does it work?
- The many flavors of BLAST
- Demo

**Multiple Sequence Alignment**

- How does it work?
- Demo
- Inspect and correct your MSA

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## PAIRWISE ALIGNMENT

and **BLAST**: Basic Local Alignment Search Tool

- Sequence Alignment: Assigning homology to sites among a group of known sequences
- BLAST: Alignment of one sequence with many unknown sequences

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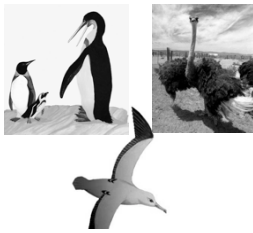
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### HOMOLOGY vs. ANALOGY

common ancestry



convergence



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### PAIRWISE ALIGNMENT

Pairing of sites based on an assessment of homology

Homology assessed using Substitution Matrices

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### PAIRWISE ALIGNMENT

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HBA_HUMAN  GSAQVKGHGKKVADALTNAVAHVDDMPNALSALSDLHAHKL
              G+ +VK+HGKKV  A++++AH+D++ +++++LS+LH  KL
HBB_HUMAN  GNPVKAHGKKVLGAFSDGLAHLDLKGTFATLSELHCDKL

HBA_HUMAN  GSAQVKGHGKKVADALTNAVAHV---D---DMPNALSALSDLHAHKL
              ++ +++++H+ KV  + +A  ++                +L+ L+++H+ K
LGB2_LUPLU  NNPELQAHAGKVFKLVEAAIQLVQTGVVVTDATLKNLGSVHVSKG

HBA_HUMAN  GSAQVKGHGKKVADALTNAVAHVDDMPNALSALS---LHAHKL
              GS+ + G +   +D L  ++ H+ D+  A +AL D  ++AH+
F11G11.2   GSGYLVGDSLTFVDLL---VAQHTADLLAANAALLDEFPQPKAQHE
  
```

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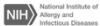
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## PAIRWISE ALIGNMENT

Substitution Matrices

- ➔ Derived mathematically
- ➔ Derived from data

“A substitution matrix (even one derived by arbitrarily assigning probabilities to pairs) is a statement of the probability of observing these pairs in real alignment.”



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## PAIRWISE ALIGNMENT

DNA Substitution Matrices

- Single parameter - Jukes-Cantor
  - Equal base frequencies
  - Uniform rates of change
- Two parameter - Kimura
  - Equal base probabilities
  - Two rates of change



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## PAIRWISE ALIGNMENT

DNA Substitution Matrices

- More parameters - HKY
  - Unequal base frequencies
  - Two rates of change
- Fully parameterized - GTR
  - Unequal base probabilities
  - Six rates of change



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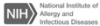
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## PAIRWISE ALIGNMENT

Jukes-Cantor Substitution Probabilities

$$P_{ij}(t) = \begin{cases} \frac{1}{4} + \frac{3}{4}e^{-4\mu t} & i = j \\ \frac{1}{4} - \frac{1}{4}e^{-4\mu t} & i \neq j \end{cases}$$


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## PAIRWISE ALIGNMENT

Jukes-Cantor Substitution Probabilities

$\mu t = 0.25$

	A	C	G	T
A	0.5259	0.1580	0.1580	0.1580
C	0.1580	0.5259	0.1580	0.1580
G	0.1580	0.1580	0.5259	0.1580
T	0.1580	0.1580	0.1580	0.5259



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## PAIRWISE ALIGNMENT

Kimura Two-Parameter Substitution Model

If the probability of transitions ( $A \leftrightarrow G, C \leftrightarrow T$ ) is different from the probability of transversions ( $A \leftrightarrow T, G \leftrightarrow T, A \leftrightarrow C, G \leftrightarrow C$ ), then there are two relative rate parameters expressed as the transition/transversion rate ratio  $\kappa$



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### PAIRWISE ALIGNMENT

Kimura Two-Parameter Substitution Probabilities

$$P_{ij}(t) = \begin{cases} \frac{1}{4} - \frac{1}{4}e^{-4\mu t} & i \neq j, \text{transversion} \\ \frac{1}{4} + \frac{1}{4}e^{-4\mu t} - \frac{1}{2}e^{-2(\kappa+1)\mu t} & i \neq j, \text{transition} \\ \frac{1}{4} + \frac{1}{4}e^{-4\mu t} + \frac{1}{2}e^{-2(\kappa+1)\mu t} & i = j \end{cases}$$


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### PAIRWISE ALIGNMENT

Kimura Two-Parameter Substitution Probabilities

$\mu t = 0.25 \quad \kappa = 2.0$

	A	C	G	T
A	0.4535	0.1580	0.2304	0.1580
C	0.1580	0.4535	0.1580	0.2304
G	0.2304	0.1580	0.4535	0.1580
T	0.1580	0.2304	0.1580	0.4535



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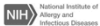
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### PAIRWISE ALIGNMENT

HKY Substitution Probabilities

$$P_{ij}(t) = \begin{cases} \pi_j + \pi_j \left( \frac{1}{\Pi_j} - 1 \right) e^{-\mu t} + \left( \frac{\Pi_j - \pi_j}{\Pi_j} \right) e^{-\mu A} & (i = j) \\ \pi_j + \pi_j \left( \frac{1}{\Pi_j} - 1 \right) e^{-\mu t} + \left( \frac{\pi_j}{\Pi_j} \right) e^{-\mu A} & (i \neq j, \text{transition}) \\ \pi_j (1 - e^{-\mu t}) & (i \neq j, \text{transversion}) \end{cases}$$


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## PAIRWISE ALIGNMENT

HKY Substitution Probabilities

$\Pi_j = \pi_A + \pi_G$  if  $j$  is a purine  
 $\Pi_j = \pi_C + \pi_T$  if  $j$  is a pyrimidine  
 $A = 1 + \Pi_j(\kappa - 1)$



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## Substitution Models



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## PAIRWISE ALIGNMENT

### Protein Score Matrices Similarity of Amino Acids

- A** alanine (ala)
- R** arginine (arg)
- N** asparagine (asn)
- D** aspartic acid (asp)
- C** cysteine (cys)
- Q** glutamine (gln)
- E** glutamic acid (glu)
- G** glycine (gly)
- H** histidine (his)
- I** isoleucine (ile)
- L** leucine (leu)
- K** lysine (lys)
- M** methionine (met)
- F** phenylalanine (phe)
- P** proline (pro)
- S** serine (ser)
- T** threonine (thr)
- W** tryptophan (trp)
- Y** tyrosine (tyr)



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From Esquivel RO, et al., 2013. Advances in Quantum Mechanics, Chapter 27 InTech. 22

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
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**PAIRWISE ALIGNMENT**

Protein Score Matrices

- Derived from empirical data
- Account for depth of relationship among the data
- Expressed as log-odds ratio:
  - Logarithm of the ratio of the probabilities of two residues being aligned due to homology versus random chance

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
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**PAIRWISE ALIGNMENT**

Protein Score (Substitution) Matrices

The log-odds ratio:  
 $s(a,b) = \log(p_{ab}/q_a q_b)$

$q_a$  = frequency of residue a in the data  
 $p_{ab}$  = probability that residues a and b have been derived from a common ancestor

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
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**PAIRWISE ALIGNMENT**

Protein Substitution Matrices

- PAM250: Based on phylogenies where all sequences differ by no more than 15%.
- BLOSUM62: Based on clusters of sequences with greater than 62% identical residues.

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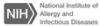




## BLAST and Sequence Alignment

How do two sequences get “aligned”?

- Global alignment (Needleman-Wunsch)
  - Assign homology across the entire sequence
  - Clustal
- Local alignment (Smith-Waterman)
  - Assign homology for subsequences
  - MUSCLE and BLAST
  - Good for aligning very divergent sequences


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## SEQUENCE ALIGNMENT

**HEAGAWGHEE ↔ PAWHEAE**


Build a matrix of score values for all site pairs

PAM250

H	E	A	G	A	W	G	H	E	E	
P	0	-1	1	0	1	-5	0	0	-1	-1
A	-1	0	2	1	2	-6	1	-1	0	0
W	-3	-7	-6	-7	-6	17	-7	-3	-7	-7
H	6	1	-1	-2	-1	-3	-2	6	1	1
E	1	4	0	0	0	-7	0	1	4	4
A	-1	0	2	1	2	-6	1	-1	0	0
E	1	4	0	0	0	-7	0	1	4	4

BLOSUM62

H	E	A	G	A	W	G	H	E	E	
P	-2	-1	-1	-2	-1	-4	-2	-2	-1	-1
A	-2	-1	4	0	4	-3	0	-2	-1	-1
W	-2	-3	-3	-2	-3	11	-2	-2	-3	-3
H	8	0	-2	-2	-2	-2	8	0	0	0
E	0	5	-1	-2	-1	-3	-2	0	5	5
A	-2	-1	4	0	4	-3	0	-2	-1	-1
E	0	5	-1	-2	-1	-3	-2	0	5	5


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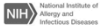
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## SEQUENCE ALIGNMENT

What about gaps?

- Score penalty for opening
- Score penalty for extending

Penalties are log probabilities of a gap of a specific length


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## SEQUENCE ALIGNMENT

Standard gap costs

Substitution Matrix	Gap Costs (Open, Extend)
PAM30	(9,1)
PAM70	(10,1)
BLOSUM80	(10,1)
BLOSUM62	(10,1)
BLOSUM45	(15,2)

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## SEQUENCE ALIGNMENT

Dynamic Programming:  
Calculate a matrix of alignment scores

BLOSUM62			
	H	E	A
P	-2	-1	-1
A	-2	-1	4
W	-2	-3	-3

		H	E	A
	0	-8	-16	-24
P	-8	-2	-9	-17
A	-16	-10	-3	-5
W	-24	-18	-11	-6

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## SEQUENCE ALIGNMENT

Dynamic Programming

- 1) Calculate a full matrix
- 2) Traceback to get the Global Alignment

	H	E	A	G	A	W	G	H	E	E	
P	-8	-2	-9	-24	-32	-40	-48	-56	-64	-72	-80
A	-16	-10	-3	-5	-13	-29	-37	-45	-53	-61	-69
W	-24	-18	-11	-6	-7	-15	-18	-26	-34	-41	-49
H	-32	-16	-18	-13	-8	-9	-17	-10	-18	-26	-34
E	-40	-24	-11	-19	-15	-9	-12	-19	-5	-13	-21
A	-48	-32	-19	-7	-15	-11	-12	-12	-20	-6	-14
E	-58	-40	-27	-15	-9	-16	-14	-14	-12	-15	-23

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## SEQUENCE ALIGNMENT

### Local Alignment

- Alignment of subsequences
- Good for aligning very divergent sequences

### Score Calculation

- Minimum score is zero
- Traceback begins at the highest score
- Score = 0 → End of subsequence


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## SEQUENCE ALIGNMENT

### Local Alignment

	H	E	A	G	A	W	G	H	E	E
O	0	0	0	0	0	0	0	0	0	0
P	0	0	0	0	0	0	0	0	0	0
A	0	0	0	4	0	0	0	0	0	0
W	0	0	0	0	0	0	0	0	0	0
H	0	8	0	0	0	0	7	13	0	7
E	0	0	13	5	0	0	0	5	13	12
A	0	0	5	17	9	4	0	0	5	12
E	0	0	5	9	15	8	0	0	0	10


Repeat Match

HEAGAWGHEE  
pawHEEae

Overlap Match

AWGHE  
AW-HEE

HEAGAWGHEE  
pAW-HEEae


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## SEQUENCE ALIGNMENT


### Scoring alignments and expect values

**Score** := Value in the dynamic programming matrix where the traceback began.

**Expect (E) value** := Number of matches expected due to chance, with a score greater than **S**, based on a stochastic sequence model.

**P value** := Probability of finding at least one match with score ≥ **S**

$$P = 1 - e^{-E(S)}$$


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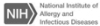
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**BLAST**  
**(Basic Local Alignment Search Tool)**

How does BLAST work?

- Create a list of query sequence "words"
  - Word lengths: 11 nucleotides, 3 amino acids
- Create a list of neighborhood words
  - Similar to query words and above a score threshold
- Search for matches in the database
- Extend matches
  - Below threshold? Discard!
  - Above threshold? Keep it!
- Format and output maximally extended matches

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**BLAST**  
**(Basic Local Alignment Search Tool)**

How does BLAST work?

How does BLAST evaluate matches?

It uses (local) alignment scores

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**BLAST**

The Many Flavors of BLAST

- BLASTn and BLASTp
- short, nearly-exact match BLAST
- Translated BLAST
  - BLASTx nt → aa ⇔ protein db
  - tBLASTn aa ⇔ protein db ← DNA db
  - tBLASTx nt → aa ⇔ protein db ← DNA db
- PSI-BLAST (Position-Specific Iterated BLAST)
- PHI-BLAST (Pattern Hit Initiated BLAST)
- bi2seq

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**BLAST**

short, nearly-exact match BLAST

- Increase Expect threshold
- Reduce word size (7 for nt, 2 for aa)
- Turn off low complexity filter
- Protein: Use a more stringent substitution matrix

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**BLAST**

**PSI-BLAST**  
(Position-Specific Iterated BLAST)

- Perform initial BLASTp search
- Generate a sequence profile from results
- BLASTp using the profile
- Iterate until no new sequences are found
- Convergence

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**BLAST**

**PHI-BLAST**  
(Pattern Hit Initiated BLAST)

Sequence Profile

[ LIVMF ] -G-E-x- [ GAS ] - [ LIVM ] -x(5,11) -R- [ STAQ ] -A-x- [ LIVMA ] -x- [ STACV ]

[ ] = Any of the residues within the brackets  
 - = spacer separating sites in the profile  
 x = Any residue  
 x(a,b) = Any residues a to b in length

VGERGLEEDKRRKRSAWMQC  
 MGETALRRRKKEDEERTANVYT  
 FGEAAMPGGPHQSRSAFAWV

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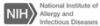
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## BLAST

Access to BLAST

- NCBI
- Your own computer
- NIAID HPC cluster



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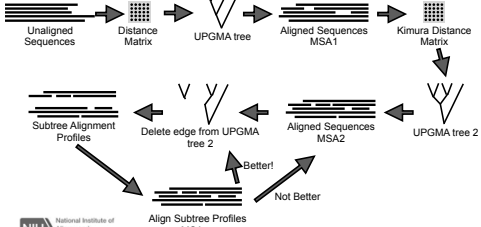
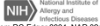
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## Multiple Sequence Alignment

### The Progressive Alignment Algorithm

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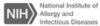
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## Multiple Sequence Alignment

### Programs

- Clustal
  - Your own computer
  - Web Server
  - NIAID HPC cluster
- MUSCLE
  - Your own computer
  - Web Server
  - NIAID HPC cluster
- MAFFT
  - Web Server



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### Multiple Sequence Alignment

**NEVER**  
directly input the output of a MSA program into  
an analysis program!

**ALWAYS**  
inspect the alignment to improve it.



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### Multiple Sequence Alignment

#### Multiple Sequence Alignment Editors

- MacVector
  - Commercial software
- MegAlign (Lasergene)
  - Commercial software
- AliView
  - Public domain
- GeneDoc
  - Public domain
- BioEdit
  - Public domain



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### Web Resources

**ClustalW**  
<http://www.clustal.org/>



**Muscle**  
<http://www.drive5.com/muscle/download3.6.html>

**MAFFT**  
<http://mafft.cbrc.jp/alignment/server/>

**AliView**  
<http://www.orbunkar.se/aliview/>

**GeneDoc**  
<http://www.nrbsc.org/downloads/>

**BioEdit**  
<http://www.mbio.ncsu.edu/BioEdit/bioedit.html>



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## Recapitulation

- BLAST search for contig0001 homologs
- Download selected sequence records
- Align sequence records with Clustal2

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## Seminar Follow-Up Site

For access to past recordings, handouts, slides visit this site from the NIH network: <http://collab.niaid.nih.gov/sites/research/SIG/Bioinformatics/>

**1. Select a Subject Matter**

**Recommended Browsers:**

- IE for Windows,
- Safari for Mac (Firefox on a Mac is incompatible with NIH Authentication technology)

**Login**

- If prompted to log in use "NIH" in front of your username

**View:**

- Seminar Details
- Handout and Reference Docs
- Relevant Links
- Seminar Recording Links

**2. Select a Topic**

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## Retrieving Slides/Handouts

**This lecture series**

**1. Select a Subject Matter**

- 2013 Bioinformatics Festival
- Bioinformatics Development
- Bioinformatics Festival
- Biostatistics
- Data Presentation
- General Bioinformatics
- Genomics
- Library Management
- Homology Analysis
- Next-Gen Sequencing
- NIAD SIGs
- Networks Analysis
- Phylogenetics**
- RNA, Boot Camp
- Sequence Analysis
- Structural Biology

**2. Select a Seminar Title**

- Annotating Our Sequences
- Building Trees - Phylogenetics I
- Building Trees - Phylogenetics II
- Homology Searching and Sequence Alignment
- Introduction to Phylogenetics and Sequence Assembly
- Making Presentation Quality Phylogenetic Trees
- Making Publication Quality Tree Figures
- Flow Tools for Adding Genomic and Functional Context to Your Data
- Phylogenetic Analysis Using BEAST
- Phylogenetic Molecular Evolution I
- Phylogenetic Molecular Evolution II
- Selection Analysis Using HyPhy
- Selection Analysis Using TRAC

**Seminar Details**

**Seminar Handouts and Reference Documents**

There are no items to show in this view.

**Links Relevant to this Seminar**

There are no items to show in this view.

**Seminar Recording Links**

There are no items to show in this view.

NIAD

NIH National Institute of Allergy and Infectious Diseases 52

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