Perl: Examples

```
#!/usr/bin/perl -w
# Storing DNA in a variable, and printing it out

# First we store the DNA in a variable called $DNA
$DNA = 'ACGGGAGGACGGGAAAATTACTACGGCATTAGC';

# Next, we print the DNA onto the screen
print $DNA;

# Finally, we'll specifically tell the program to
  exit.
exit;  #perl1.pl
```

Perl: Strings

```
#!/usr/bin/perl -w
$DNA1 = 'ACGGGAGGACGGGAAAATTACTACGGCATTAGC';
$DNA2 = 'ATAGTGCCGTGAGAGTGATGTAGTA';
# Concatenate the DNA fragments
$DNA3 = "$DNA1$DNA2";
print "Concatenation 1):\n\n$DNA3\n\n";
# An alternative way using the "dot operator":
$DNA3 = $DNA1 . $DNA2;
print "Concatenation 2):\n\n$DNA3\n\n";
# transcribe from DNA to RNA; make rev comp; print;
$RNA = $DNA3; $RNA =~ s/T/U/g;
$rev = reverse $DNA3; $rev =~ tr/AGCTacgt/TCGAtgca/;
print "$RNA\n$rev\n";
exit;  #perl2.pl
```

Perl: arrays

```
#!/usr/bin/perl -w
# Read filename & remove newline from string
$protFile = <STDIN>; chomp $protFile;
# First we have to "open" the file
unless (open(PROTEINFILE, $protFile) {
   print "File $protFile does not exist"; exit;}
# Each line becomes an element of array @protein
@protein = <PROTEINFILE>;
print @protein;
# Print line #3 and number of lines
print $protein[2], "File contained ", scalar @protein, "
   lines\n";
# Close the file.
close PROTEINFILE;
exit; #perl3.pl
```

Perl: subroutines

```
#!/usr/bin/perl –w
# using command line argument
$dna1 = $ARGV[0]; $dna2 = $ARGV[1];
# Call subroutine with arguments; result in $dna
$dna = addACGT($dna1, $dna2);
print "Add ACGT to $dna1 & $dna2 to get $dna\n\n";
exit;
##### addACGT: concat $dna1, $dna2, & "ACGT". #####
sub addGACAGT {
    my($dnaA, $dnaB) = @_; my($dnaC) = $dnaA.$dnaB;
    $dnaC .= ‘GACAGT';
    return $dnaC;
} #perl4.pl
```

Sequence Formats in BioPerl

```
#! /local/bin/perl -w

use strict;
use Bio::SeqIO;
my $in  = Bio::SeqIO->newFh ( -file   => '<seqs.html',
                              -format => 'swiss' );
my $out = Bio::SeqIO->newFh ( -file   => '>seqs.fasta',
                              -format => 'fasta' );

print $out $_ while <$in>;

exit; #bioperl1.pl
```

Sequence Formats in BioPerl

```
#! /local/bin/perl -w
use strict;
use Bio::SeqIO;
my $in  = Bio::SeqIO->new ( -file   => 'seqs.html', -format => 'swiss' );
my $out = Bio::SeqIO->new ( -file   => 'seqs.fas', -format => 'fasta' );

while ($seq = $in->next_seq()) {
    $accNum = $seq->accession_number();
    print "Accession# = $accNum\n";
    $out->write_seq($seq);
}

exit; #bioperl2.pl
```

BioPerl

```
#!/usr/bin/perl –w
# define a DNA sequence object with given sequence
$seq = Bio::Seq->new('-seq'=>'actgtggcgtcaact',
   '-desc'=>'Sample Bio::Seq object',
   '-display_id' => 'somethingxxx',
   '-accession_number' => 'accnumxxx',
   '-alphabet' => 'dna' );
$gb = new Bio::DB::GenBank();

$seq = $gb->get_Seq_by_id('MUSIGHBA1'); #returns Seq object
$seq = $gb->get_Seq_by_acc('AF303112'); #returns Seq object
# this returns a SeqIO object :
$seqio = $gb->get_Stream_by_batch([ qw(J00522 AF303112)]));
exit; #bioperl3.pl
```

Sequence Manipulations

```
#!/local/bin/perl -w
use Bio::DB::GenBank;
$gb = new Bio::DB::GenBank();
$seq1 = $gb->get_Seq_by_acc('AF303112');
$seq2=$seq1->trunc(1,90);
$seq2 = $seq2->revcom();
print $seq2->seq(), "\n";
$seq3=$seq2->translate;
print $seq3->seq(), "\n";
exit; #bioperl4.pl
```

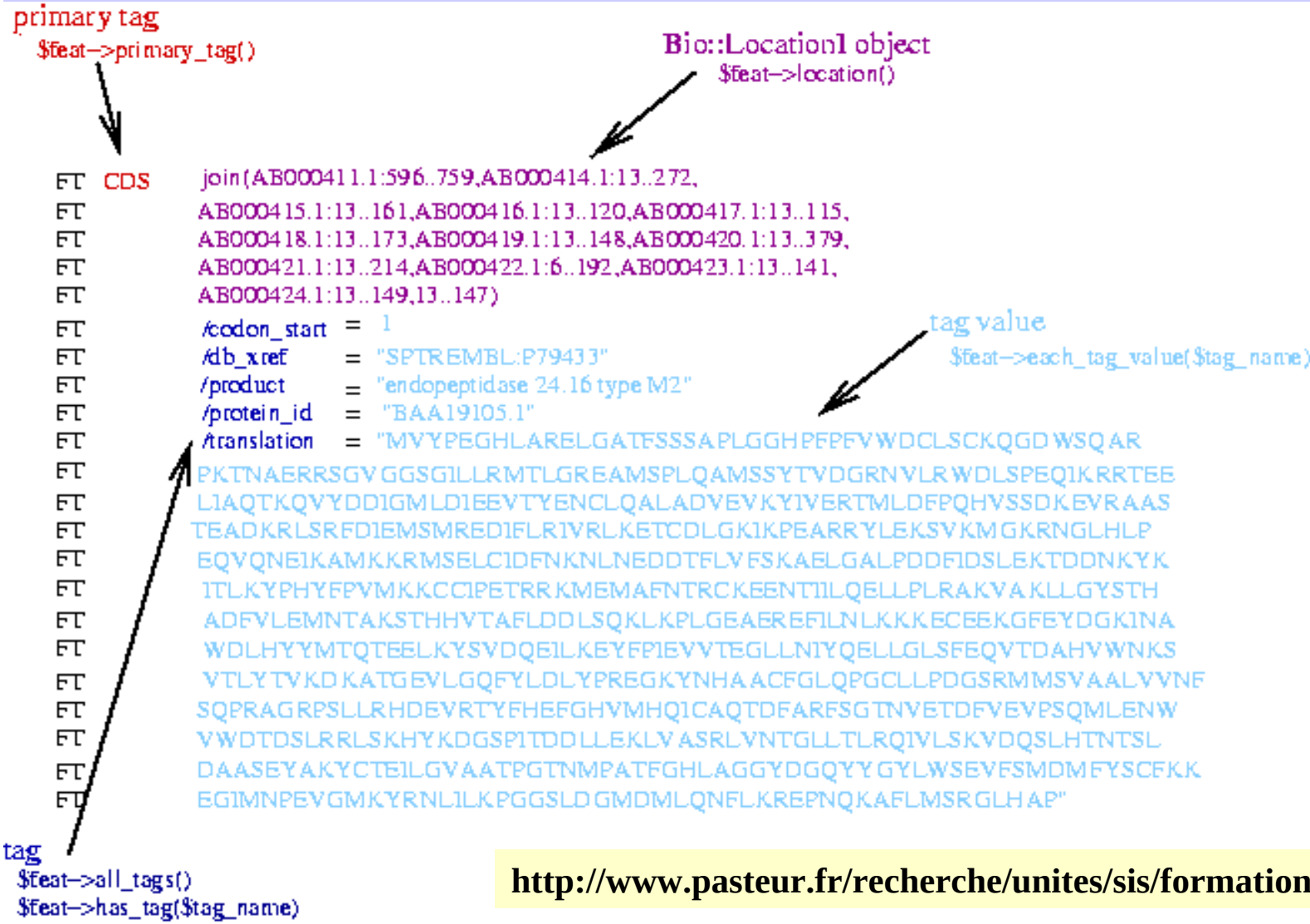
BioPerl:Download GenBank Sequences

```
#!/local/bin/perl -w

use Bio::DB::GenBank;

my $gb = new Bio::DB::GenBank(
    -retrievaltype=>'tempfile', -format=>'Fasta');

my ($seq) = $seq = $gb->get_Seq_by_id("5802612");
print $seq->id, "\n";
print $seq->desc(), "Sequence: \n";
print $seq->seq(), "\n";
exit; #bioperl5.pl
```

BioPerl: Structure

```
use Bio::Structure::IO;
$in = Bio::Structure::IO->new(-file => "inputfilename" , '-
   format' => 'pdb');
$out = Bio::Structure::IO->new(-file =>
   ">outputfilename" , '-format' => 'pdb');
# note: we quote -format to keep older perl's from
   complaining.
while ( my $struc = $in->next_structure() ) {
   $out->write_structure($struc);
   print "Structure ",$struc->id," number of models: ",
        scalar $struc->model,"\n";
}
exit; #bioperl7.pl
```

EMBL Sequence Features, BioPerl Tags



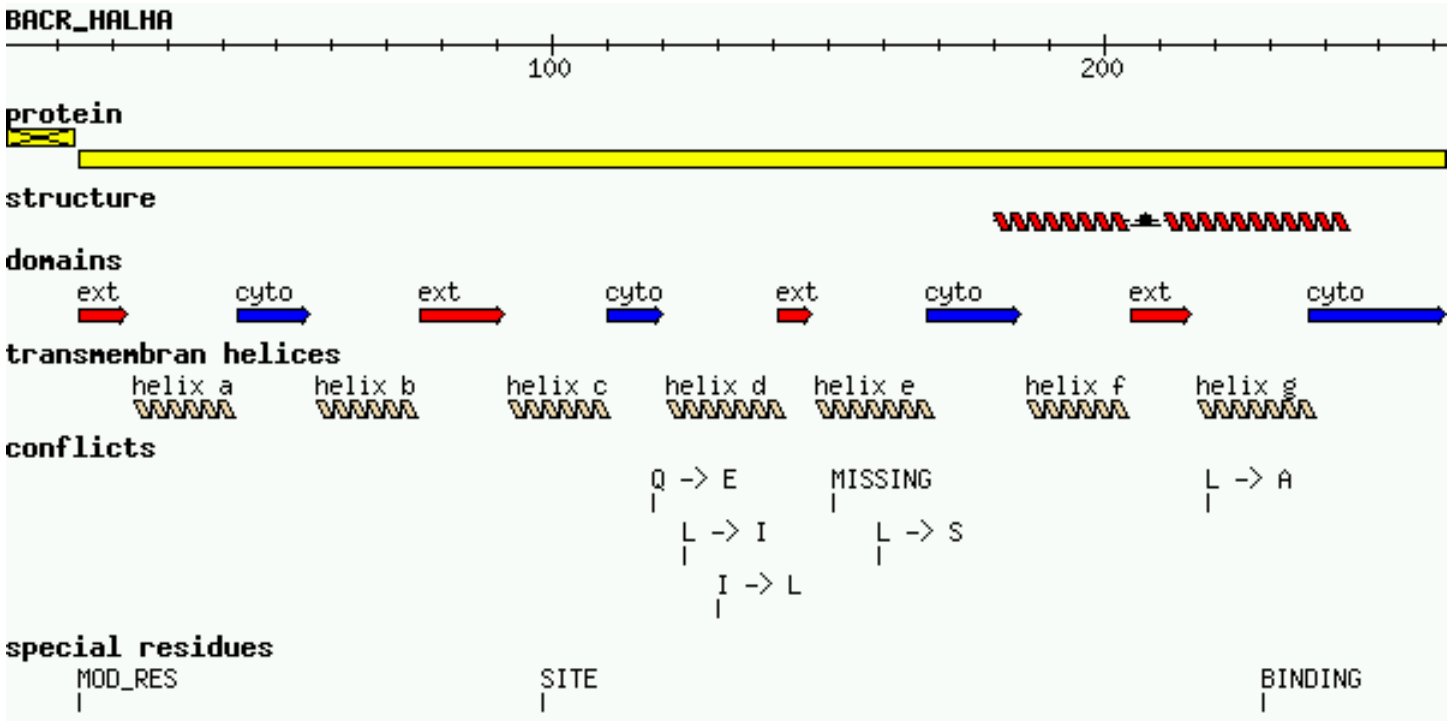
http://www.pasteur.fr/recherche/unites/sis/formation/bioperl/

BioPerl: Seq and SeqIO

```
use Bio::SeqIO;
$seqin = Bio::SeqIO->new(-format =>'EMBL', -file=>‘f1');
$seqout= Bio::SeqIO->new(-format =>'Fasta',-file=>'>f1.fa');
while((my $seqobj = $seqin->next_seq())) {
    print "Seq: ", $seqobj->display_id, ", Start of seq ",
          substr($seqobj->seq,1,10),"\n";
    if( $seqobj->moltype eq 'dna') {
          $rev = $seqobj->revcom;
          $id = $seqobj->display_id();
          $id = "$id.rev";
          $rev->display_id($id);
          $seqout->write_seq($rev); } #end if
    foreach $feat ( $seqobj->top_SeqFeatures() ) {
          if( $feat->primary_tag eq 'exon' ) {
             print STDOUT "Location ",$feat->start,":",
             $feat->end," GFF[",$feat->gff_string,"]\n";}
    } # end foreach
} # end while
exit; #bioperl6.pl
```

BioPerl Graphics Objects

Graphical view of some features of the SwissProt entry BACR_HALHA



textx2.pl can create such a graphics object from a SWISS-PROT file.

BioPerl Sequence Analysis Tools

```
$seq_stats = Bio::Tools::SeqStats->new(-seq=>$seqobj);
$seq_stats->count_monomers();
$seq_stats->count_codons();
$weight = $seq_stats->get_mol_wt($seqobj);


$pat = 'T[GA]AA...TAAT';
$pattern = new Bio::Tools::SeqPattern(-SEQ =>$pat, -TYPE
    =>'Dna');
$pattern->expand;
$pattern->revcom;
$pattern->alphabet_ok;
```

BioPerl Restriction Enzymes

- Locating restriction enzyme cutting sites:
  - RestrictionEnzyme object ;
  - data for over 150 restriction enzymes built in.
  - Access list of available enzymes using available_list()
- Restriction sites can be obtained by cut_seq().
- Adding an enzyme not in the default list is easy.

Restriction Enzymes example

```
#!/local/bin/perl -w

$re=new Bio::Tools::RestrictionEnzyme('-name'=>'EcoRI');
@sixcutters = $re->available_list(6);

$re1 = new Bio::Tools::RestrictionEnzyme(-name=>'EcoRI');
# $seqobj is the Seq object for the dna to be cut
@fragments = $re1->cut_seq($seqobj);

$re2 = new Bio::Tools::RestrictionEnzyme('-NAME' =>'EcoRV--
   GAT^ATC', '-MAKE' =>'custom');

exit; #bioperl8.pl
```

Alignment Object

```
#! /local/bin/perl -w
use strict;
use Bio::AlignIO;
my $inform = shift @ARGV || 'clustalw';
my $outform = shift @ARGV || 'fasta';
my $in = Bio::AlignIO->newFh ( -fh => \*STDIN,
   -format => $inform );
my $out = Bio::AlignIO->newFh ( -fh => \*STDOUT, -
   format => $outform );

print $out $_ while <$in>;
exit; #bioperl9.pl
```

Alignment Object

```
#! /local/bin/perl -w
use strict;
use Bio::AlignIO;
my $in = new Bio::AlignIO ( -file =>, $ARGV[0], -format => 'clustalw'
    );
my $aln = $in->next_aln();
print " all seqs same length: ",($aln->is_flush()) ? "yes" : "no", "\n";
print "alignment length: ", $aln->length(), "\n";
printf "identity: %.2f %%\n", $aln->percentage_identity();
printf "identity of conserved columns: %.2f %%\n",
    $aln->overall_percentage_identity();

Exit; #bioperlx.pl
```

BioPerl: Pairwise Sequence Alignment

```
use Bio::Tools::pSW;

$factory = new Bio::Tools::pSW( '-matrix'
  => 'blosum62.bla', '-gap' => 12, '-ext' =>
  2, );

$factory->align_and_show($seq1, $seq2,
  STDOUT);
```

BioPerl: Running BLAST

```
# This program only shows how to invoke BLAST and store the result
use Bio::SeqIO;
use Bio::Tools::Run::RemoteBlast;
my $Seq_in = Bio::SeqIO->new (-file => $ARGV[0], -format => 'fasta');
my $query = $Seq_in->next_seq();
my $factory = Bio::Tools::Run::RemoteBlast->new( '-prog' => 'blastp',
    '-data' => 'swissprot', _READMETHOD => "Blast" );
my $blast_report = $factory->submit_blast($query);
my $result = $blast_report->next_result;
while( my $hit = $result->next_hit()) {
    print "\thit name: ",
    $hit->name(), " significance: ", $hit->significance(), "\n";
}
Exit; #bioperlx1.pl
# There are programs on the bioperl website that can help you automatically
    parse the information returned by BLAST.
```

BioPerl: Multiple Sequence Alignment

```
@params = ('ktuple' => 2, 'matrix' => 'BLOSUM');
$factory =
Bio::Tools::Run::Alignment::Clustalw->new(@params);
$aln = $factory->align(\@seq_array);
foreach $seq ( $aln->eachSeq() )
   {
        print $seq->seq(), "\n";
}
Exit; #bioperlx2.pl
```

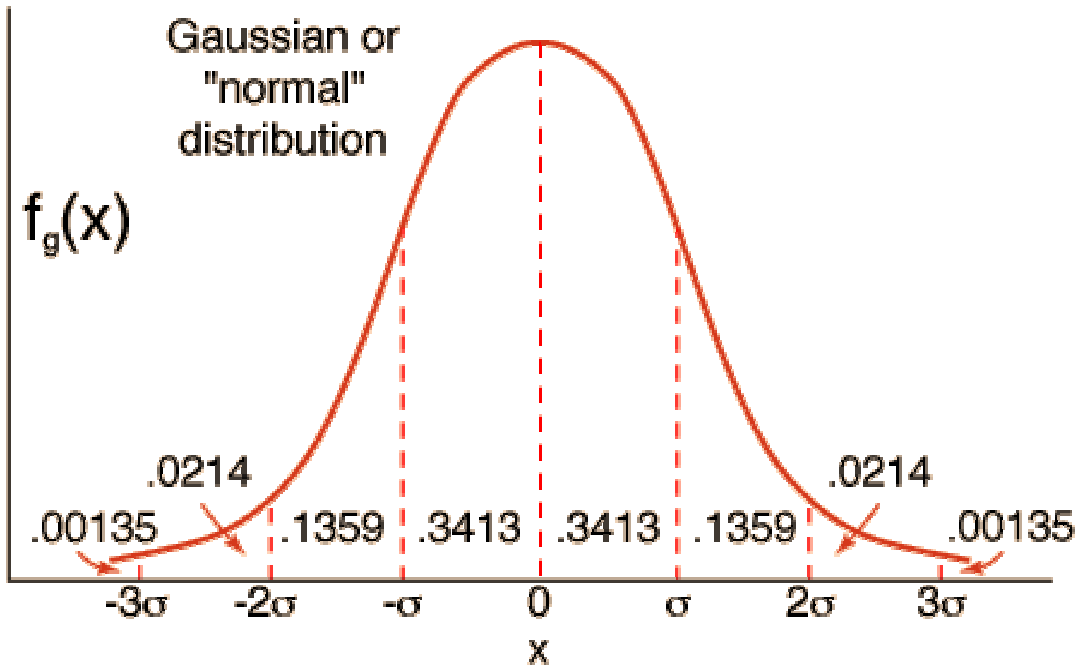
BioPerl: Structure

- Ability to store and manipulate structures.
- Modules: Atom, Chain, Residue, Model, Entry, IO
- Atom
  - new, x, y, z, xyz, residue, element,
- Chain, Residue
- Entry
  - Add_model, chain, add_chain, residue, add_residue, get_residue, add_atom, get_atoms, conect, get_atom_by_serial, seqres, …
- Model

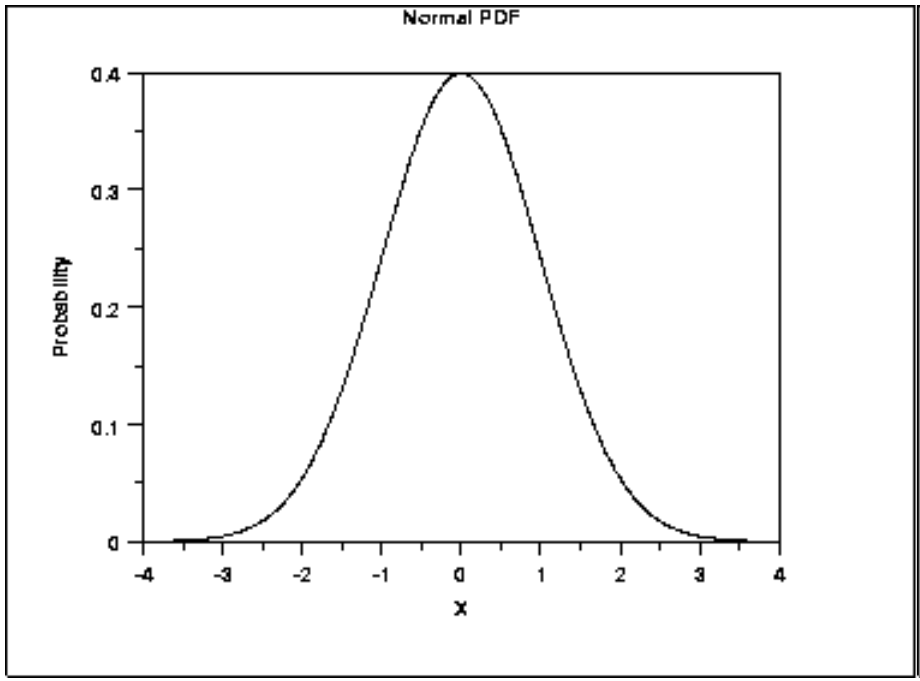
Why is Statistics important in Bioinformatics?

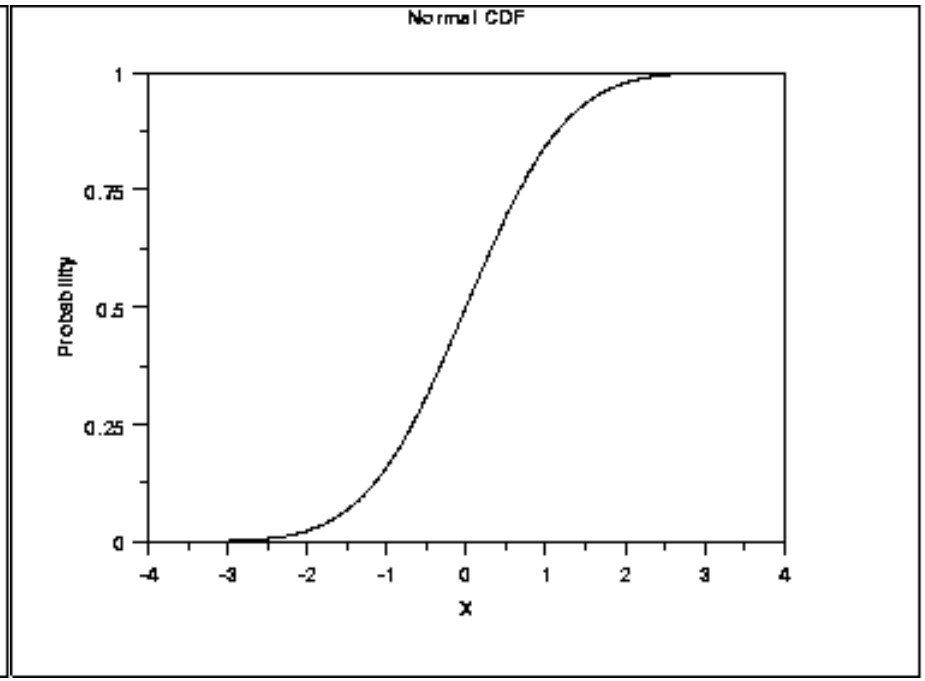
- Random processes are inherent in biological processes (e.g., evolution) & in sampling (data collection).
- Errors are often unavoidable in the data collection process.
- Statistics helps in studying *trends, interpolations, extrapolations, categorizations, classifications, inferences, models, predictions, assigning confidence to predictions, …*

Normal/Gaussian Distribution

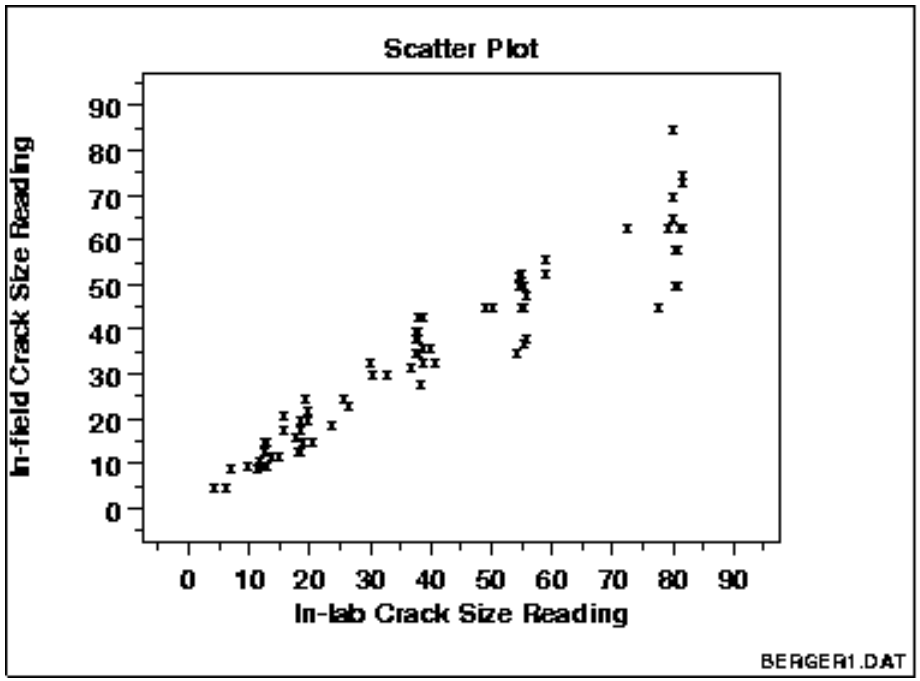


Density & Cumulative Distribution Functions

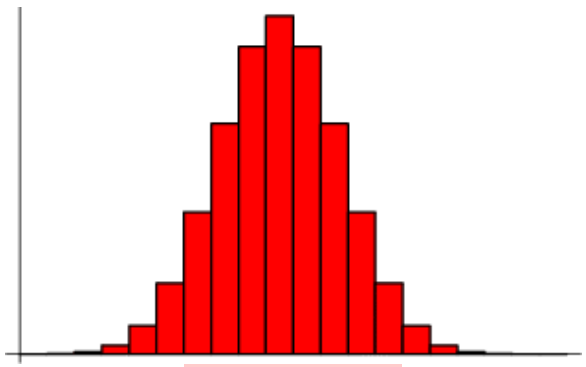




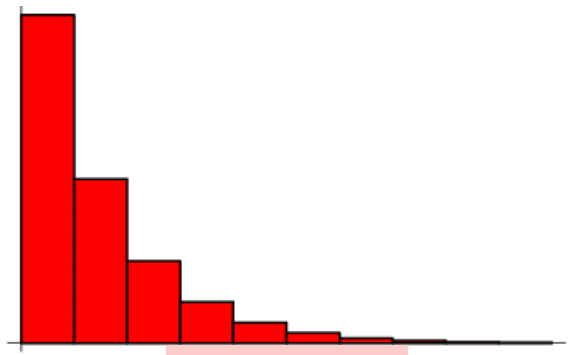
Graphical Techniques: Scatter Plot



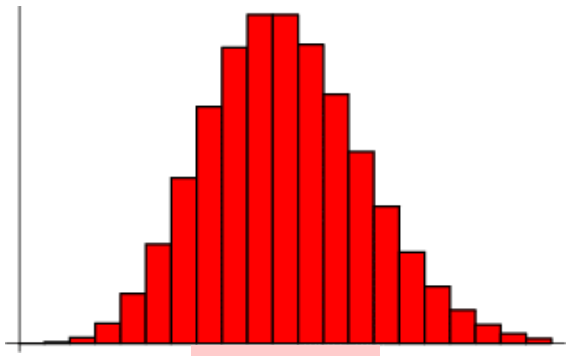
Common Discrete Distributions



Binomial



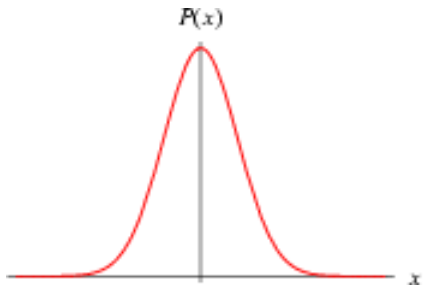
Geometric

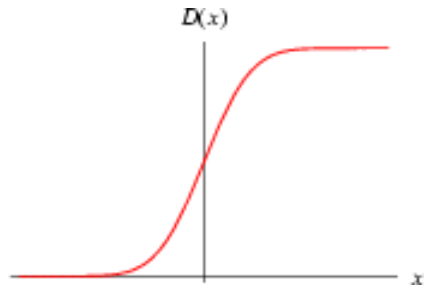


Poisson

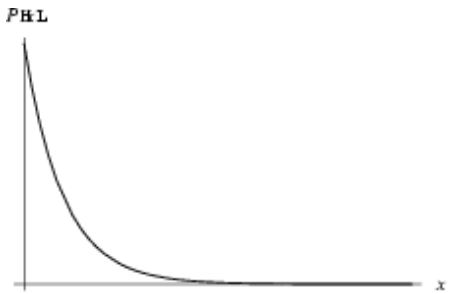
Common Continuous Distributions

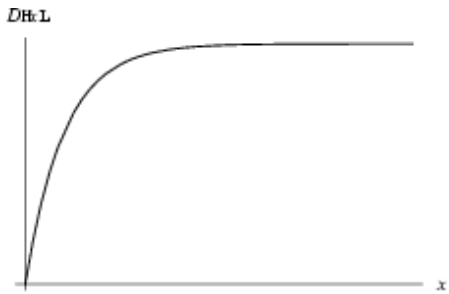
Normal



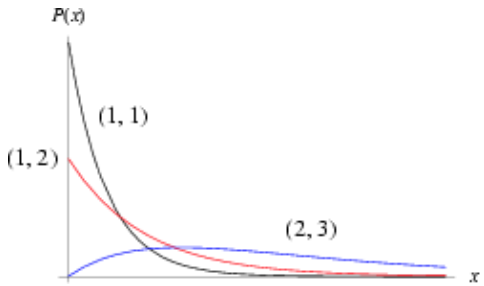


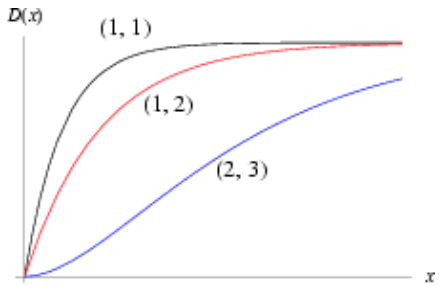
Exponential





Gamma





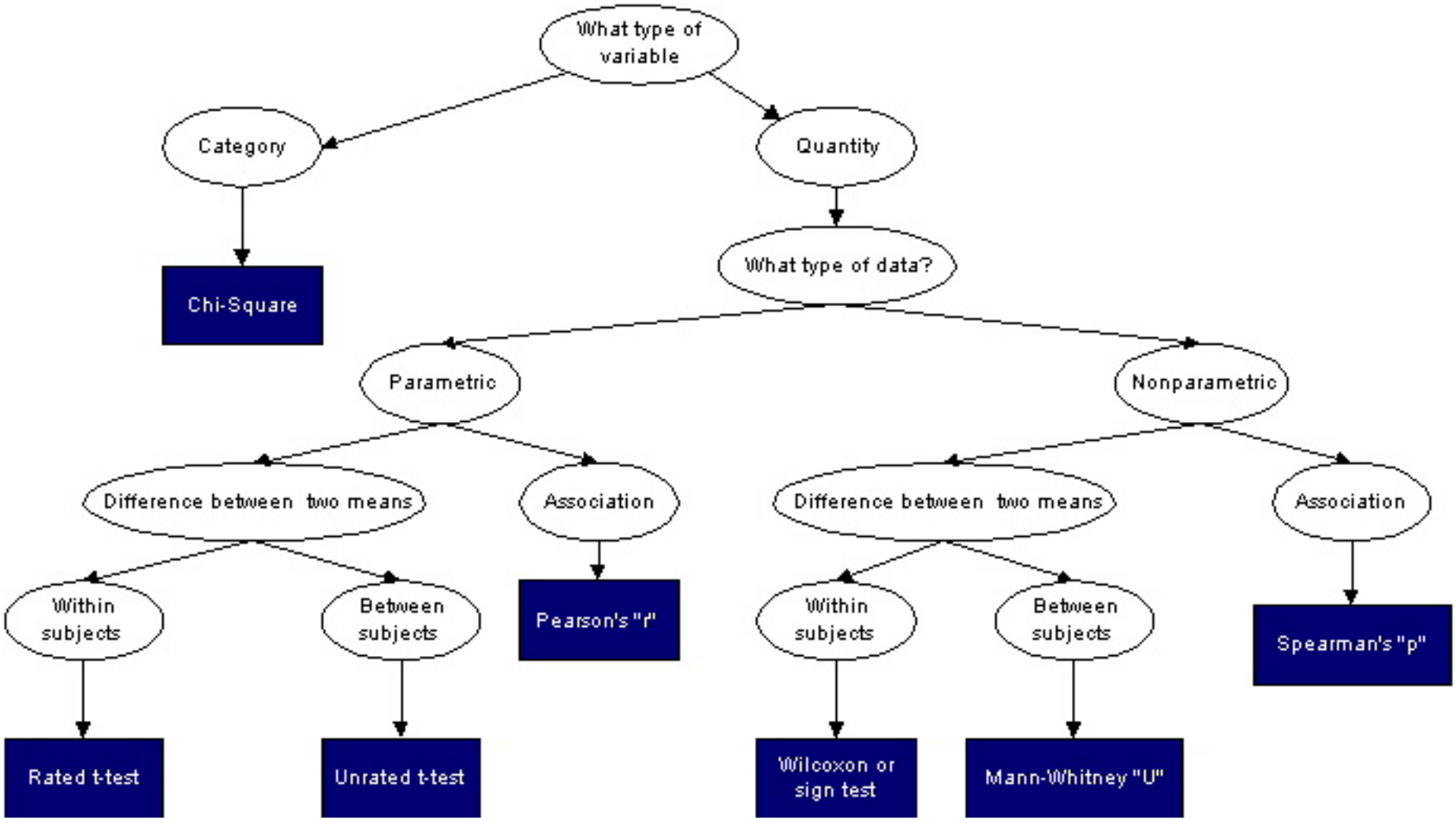
Monte Carlo methods

- Numerical statistical simulation methods that utilize sequences of random numbers to perform the simulation.
- The primary components of a Monte Carlo simulation method include the following:
  - *Probability distribution functions (pdf's)* --- the system described by a set of pdf's.
  - *Random number generator* -- uniformly distributed on unit interval.
  - *Sampling rule* --- a prescription for sampling from the specified pdf's.
  - *Scoring (or tallying)* --- the outcomes must be accumulated into overall tallies or scores for the quantities of interest.
  - *Error estimation* --- an estimate of the statistical error (variance) as a function of the number of trials and other quantities must be determined.
  - *Variance reduction techniques* --- methods for reducing the variance in the estimated solution to reduce the computational time for Monte Carlo simulation.

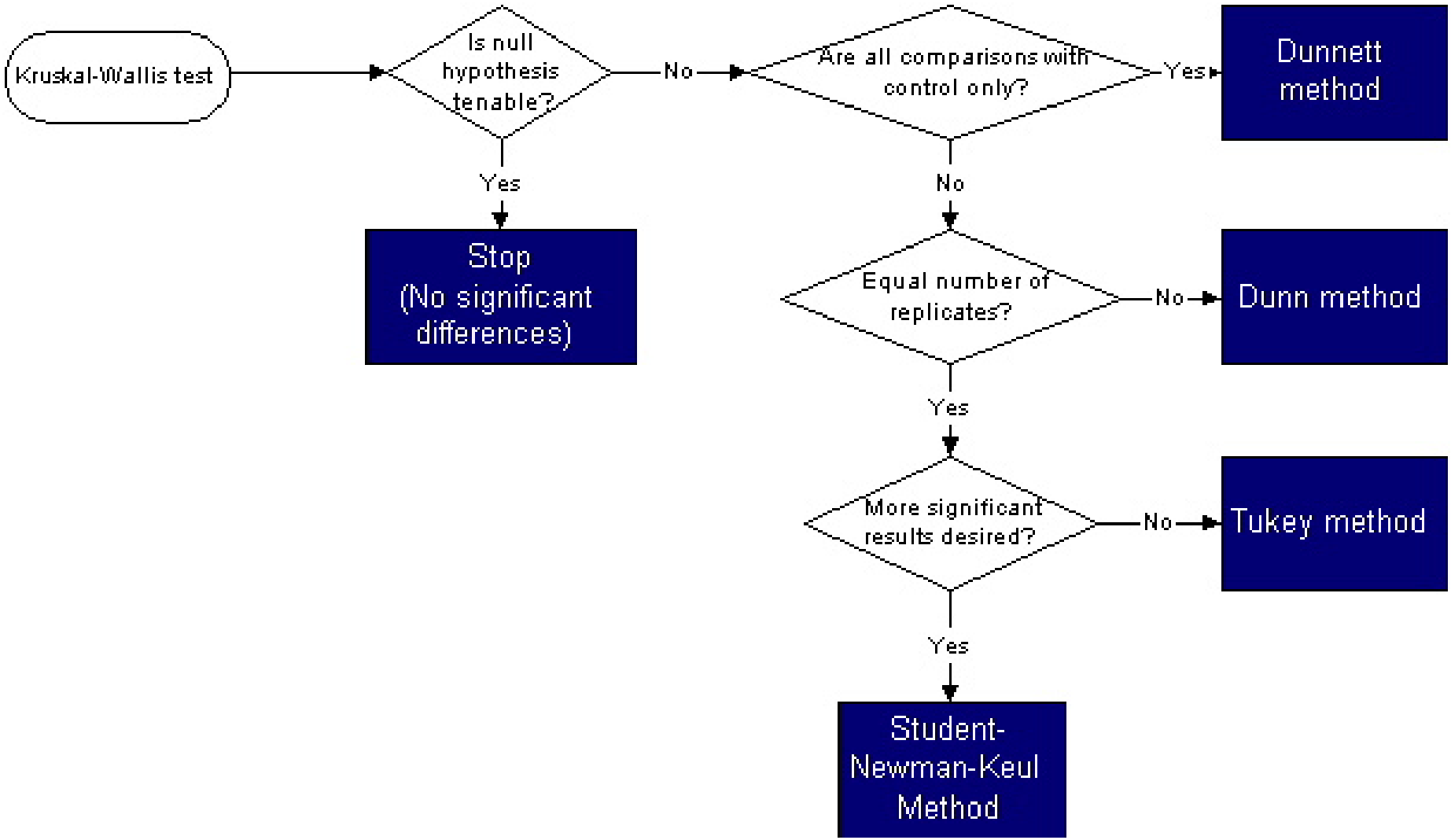
Parametric & Non-parametric equivalents

Type of test	Parametric test	Nonparametric test
2-sample	t-test	Mann-Whitney U-test
Paired sample	Paired t-test	Wilcoxon
Distribution	Chi-square	Kolmogorov-Smirnov
>2 samples	1-way ANOVA	Kruskal-Wallis
Correlation	Pearson's correlation	Spearman's correlation
Crossed comparisons	Factorial ANOVA	Friedman's Quade
Multiple comparisons	Tukey, SNK, Dunnett's, Scheffe's	Nonparametric version of its parametric equivalents

Selection of Statistical Tests



Selection of Multiple Comparison Tests



Multiple Alignments: CLUSTALW

* identical

: conserved substitutions

. semi-conserved substitutions

```
gi|2213819  CDN-ELKSEAIIEHLCASEFALR------------MKIKEVKKENGDKK 223
gi|12656123 ----ELKSEAIIEHLCASEFALR------------MKIKEVKKENGD-- 31
gi|7512442  CKNKNDDDNDIMETLCKNDFALK------------IKVKEITYINRDTK 211
gi|1344282  QDECKFDYVEVYETSSSGAFSLLGRFCGAEPPPHLVSSHHELAVLFRTDH 400
                 : .   : *  . . *:*               . :*:
```

Red: AVFPMLW (Small & hydrophobic)

Blue: DE (Acidic)

Magenta: RHK (Basic)

Green: STYHCNGQ (Hydroxyl, Amine, Basic)

Gray: Others

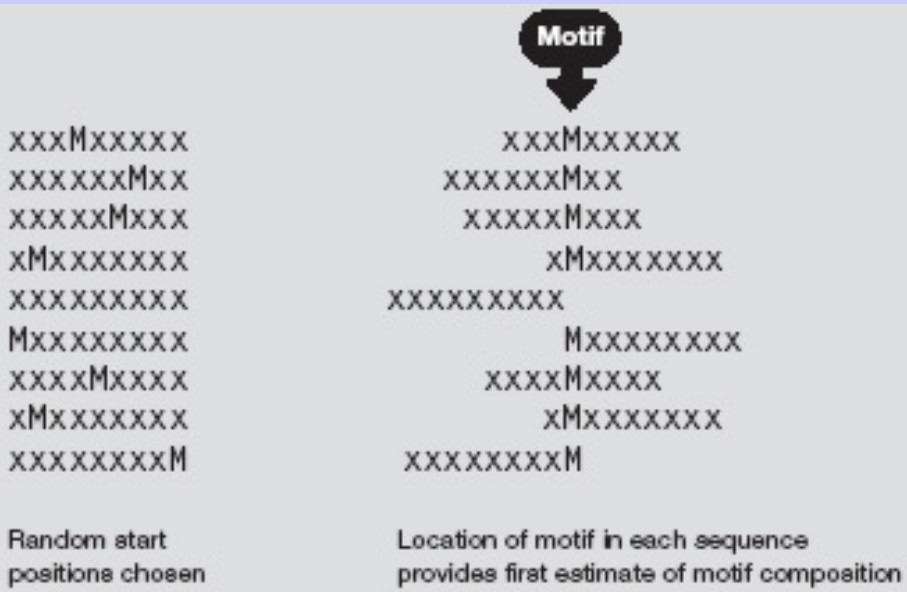
Multiple Alignments

- **Family alignment for the ITAM domain (Immunoreceptor tyrosine-based activation motif)**

```
CD3D_MOUSE/1-2   EQLYQPLRDR EDTQ-YSRLG GN
Q90768/1-21      DQLYQPLGER NDGQ-YSQLA TA
CD3G_SHEEP/1-2   DQLYQPLKER EDDQ-YSHLR KK
P79951/1-21      NDLYQPLGQR SEDT-YSHLN SR
FCEG_CAVPO/1-2   DGIYTGLSTR NQET-YETLK HE
CD3Z_HUMAN/3-0   DGLYQGLSTA TKDT-YDALH MQ
C79A_BOVIN/1-2   ENLYEGLNLD DCSM-YEDIS RG
C79B_MOUSE/1-2   DHTYEGLNID QTAT-YEDIV TL
CD3H_MOUSE/1-2   NQLYNELNLG RREE-YDVLE KK
CD3Z_SHEEP/1-2   NPVYNELNVG RREE-YAVLD RR
CD3E_HUMAN/1-2   NPDYEPIRKG QRDL-YSGLN QR
CD3H_MOUSE/2-0   EGVYNALQKD KMAEAYSEIG TK
Consensus/60%    -.lYpsLspc pcsp.YspLs pp
```

Simple Modular Architecture Research Tool

Multiple Alignment



How to Score Multiple Alignments?

- Sum of Pairs Score (SP)
  - Optimal alignment: $O(d^N)$ [Dynamic Prog]
  - Approximate Algorithm: Approx Ratio 2
    - Locate Center: $O(d^2N^2)$
    - Locate Consensus: $O(d^2N^2)$

Consensus char: char with min distance sum

Consensus string: string of consensus char

Center: input string with min distance sum

Multiple Alignment Methods

- Phylogenetic Tree Alignment (NP-Complete)
  - Given tree, task is to label leaves with strings
- Iterative Method(s)
  - Build a MST using the distance function
- Clustering Methods
  - Hierarchical Clustering
  - K-Means Clustering

Multiple Alignment Methods (Cont'd)

- Gibbs Sampling Method
  - Lawrence, Altschul, Boguski, Liu, Neuwald, Winton, *Science,* 1993
- Hidden Markov Model
  - Krogh, Brown, Mian, Sjolander, Haussler, *JMB,* 1994

Profile Method

PROFILE METHOD, [M. Gribskov et al., '90]

Location in Seq.	Sequence 1	2	3	4	5	6	7	Protein Name
14	G	V	S	A	S	A	V	Ka RbtR
32	G	V	S	E	M	T	I	Ec DeoR
33	G	V	S	P	G	T	I	Ec RpoD
76	G	A	G	I	A	T	I	Ec TrpR
178	G	C	S	R	E	T	V	Ec CAP
205	C	L	S	P	S	R	L	Ec AraC
210	C	L	S	P	S	R	L	St AraC
13	G	V	N	K	E	T	I	Br MerR

FREQUENCY TABLE

	A	C	D	E	F	G	H	I	K	L	M	N	P	Q	R	S	T	V	W	Y
1	0	2	0	0	0	6	0	0	0	0	0	0	0	0	0	0	0	0	0	0
2	1	1	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	4	0	0
3	0	0	0	0	0	1	0	0	0	0	0	1	0	0	0	6	0	0	0	0
4	1	0	0	1	0	0	0	1	1	0	0	0	3	0	1	0	0	0	0	0
5	1	0	0	2	0	1	0	0	0	0	1	0	0	0	0	3	0	0	0	0
6	1	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	5	0	0	0
7	0	0	0	0	0	0	0	4	0	2	0	0	0	0	0	0	0	2	0	0

7

Profile Method

FREQUENCY TABLE

	A	C	D	E	F	G	H	I	K	L	M	N	P	Q	R	S	T	V	W	Y
1	0	2	0	0	0	6	0	0	0	0	0	0	0	0	0	0	0	0	0	0
2	1	1	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	4	0	0
3	0	0	0	0	0	1	0	0	0	0	0	1	0	0	0	6	0	0	0	0
4	1	0	0	1	0	0	0	1	1	0	0	0	3	0	1	0	0	0	0	0
5	1	0	0	2	0	1	0	0	0	0	1	0	0	0	0	3	0	0	0	0
6	1	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	5	0	0	0
7	0	0	0	0	0	0	0	4	0	2	0	0	0	0	0	0	0	2	0	0

WEIGHT MATRIX

	A	C	E	G	I	K	L	M	N	P	R	S
1	0	108	0	101	0	0	0	0	0	0	0	0
2	21	78	0	0	0	0	44	0	0	0	0	0
3	0	0	0	23	0	0	0	0	46	0	0	102
4	21	0	32	0	38	32	0	0	0	86	39	0
5	21	0	62	23	0	0	0	74	0	0	0	72
6	21	0	0	0	0	0	0	0	0	0	69	0
7	0	0	0	0	98	0	44	0	0	0	0	0

$$Weight[i, AA] = \log\left(\frac{Freq[i,AA]}{p[AA] \cdot N}\right) \cdot 100$$

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Profile Method

WEIGHT MATRIX

	A	C	E	G	I	K	L	M	N	P	R	S
1	0	108	0	101	0	0	0	0	0	0	0	0
2	21	78	0	0	0	0	44	0	0	0	0	0
3	0	0	0	23	0	0	0	0	46	0	0	102
4	21	0	32	0	38	32	0	0	0	86	39	0
5	21	0	62	23	0	0	0	74	0	0	0	72
6	21	0	0	0	0	0	0	0	0	0	69	0
7	0	0	0	0	98	0	44	0	0	0	0	0

Given the following protein sequence:

M T E D L F G D L Q D D T I L A H L D N
P A E D T S R F P A L L A E L N D L L R
G E L S R L G V D P A H S L E I V V A I
C K H L G G G Q V Y I P R G Q A L D S L
I R D L R I W N D F N G R N V S E L T T
R Y G V T F N T V Y K A I R R M R R L K

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CpG Islands

- Regions in DNA sequences with increased occurrences of substring "*CG*"
- Rare: typically *C* gets methylated and then mutated into a T.
- Often around promoter or "start" regions of genes
- Few hundred to a few thousand bases long

Problem 1:

- Input: Small sequence S
- Output: Is S from a CpG island?
  - Build Markov models: M+ and M —
  - Then compare

Markov Models

+	A	C	G	T
A	0.180	0.274	0.426	0.120
C	0.171	0.368	0.274	0.188
G	0.161	0.339	0.375	0.125
T	0.079	0.355	0.384	0.182

–	A	C	G	T
A	0.300	0.205	0.285	0.210
C	0.322	0.298	0.078	0.302
G	0.248	0.246	0.298	0.208
T	0.177	0.239	0.292	0.292

How to distinguish?

- Compute

$$S(x) = \log\left(\frac{P(x \mid M+)}{P(x \mid M-)}\right) = \sum_{i=1}^{L} \log\left(\frac{p_{x(i-1)xi}}{m_{x(i-1)xi}}\right) = \sum_{i=1}^{L} r_{x(i-1)xi}$$

r	A	C	G	T
A	-0.740	0.419	0.580	-0.803
C	-0.913	0.302	1.812	-0.685
G	-0.624	0.461	0.331	-0.730
T	-1.169	0.573	0.393	-0.679

Problem 1:

- Input: Small sequence S
- Output: Is S from a CpG island?
  - Build Markov Models: M+ & M-
  - Then compare

Problem 2:

- Input: Long sequence S
- Output: Identify the CpG islands in S.
  - Markov models are inadequate.
  - Need Hidden Markov Models.

Problem 1:

- Input: Small sequence S
- Output: Is S from a CpG island?
  - Build Markov models: M+ and M —
  - Then compare

Markov Models

+	A	C	G	T
A	0.180	0.274	0.426	0.120
C	0.171	0.368	0.274	0.188
G	0.161	0.339	0.375	0.125
T	0.079	0.355	0.384	0.182

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How to distinguish?

- Compute

$$S(x) = \log\left(\frac{P(x \mid M+)}{P(x \mid M-)}\right) = \sum_{i=1}^{L} \log\left(\frac{p_{x(i-1)xi}}{m_{x(i-1)xi}}\right) = \sum_{i=1}^{L} r_{x(i-1)xi}$$

r=p/m	A	C	G	T
A	-0.740	0.419	0.580	-0.803
C	-0.913	0.302	1.812	-0.685
G	-0.624	0.461	0.331	-0.730
T	-1.169	0.573	0.393	-0.679

Score(GCAC)
= .461-.913+.419
< 0.
GCAC not from CpG island.

Score(GCTC)
= .461-.685+.573
> 0.
GCTC from CpG island.

Problem 1:

- Input: Small sequence S
- Output: Is S from a CpG island?
  - Build Markov Models: M+ & M-
  - Then compare

Problem 2:

- Input: Long sequence S
- Output: Identify the CpG islands in S.
  - Markov models are inadequate.
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Markov Models

+	A	C	G	T
A	0.180	0.274	0.426	0.120
C	0.171	0.368	0.274	0.188
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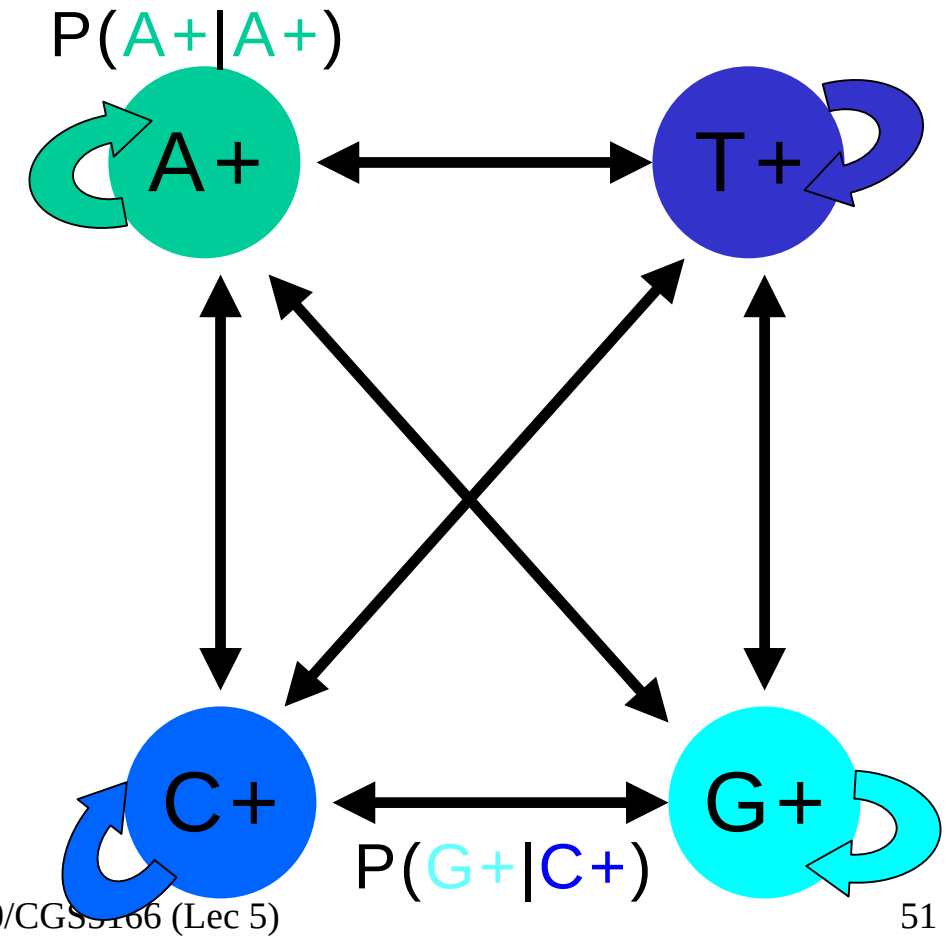
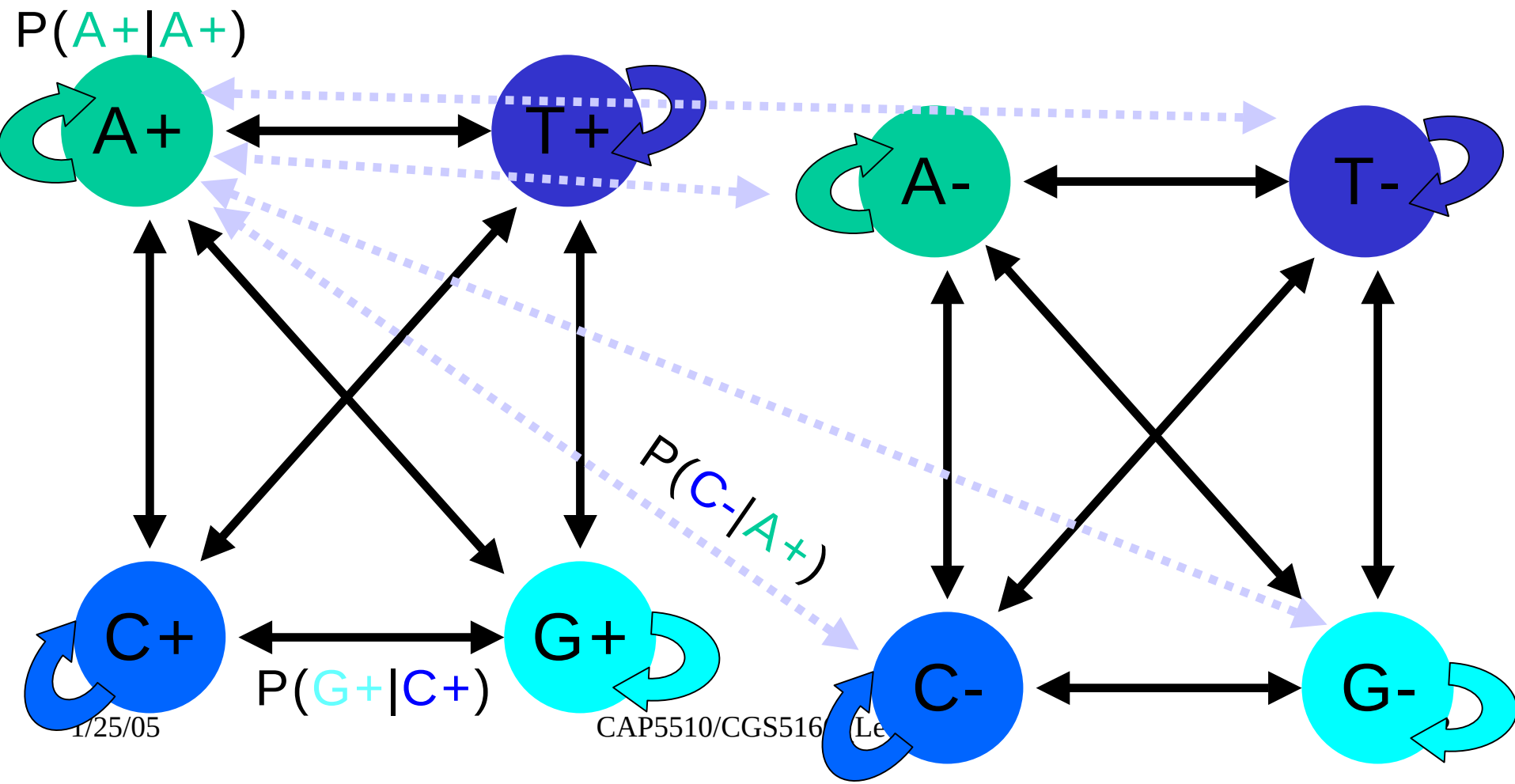


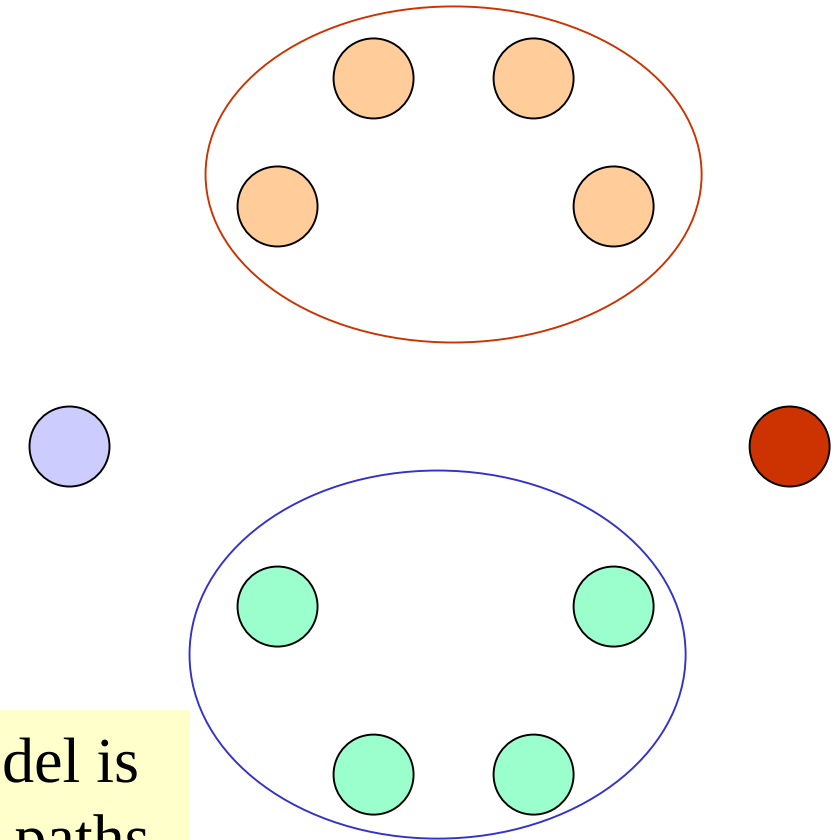
CpG Island + in an ocean of –
First order Hidden Markov Model

MM=16, HMM= 64 transition probabilities (adjacent bp)



Hidden Markov Model (HMM)

- States
- Transitions
- Transition Probabilities
- Emissions
- Emission Probabilities



- What is hidden about HMMs?

Answer: The path through the model is hidden since there are many valid paths.

How to Solve Problem 2?

- Solve the following problem:

Input: Hidden Markov Model M,

parameters Θ, emitted sequence S

Output: Most Probable Path Π

How: Viterbi's Algorithm (Dynamic Programming)

Define $\Pi[i,j]$ = MPP for first j characters of S ending in state i

Define P[i,j] = Probability of $\Pi[i,j]$

- Compute state i with largest P[i,j].