# Bioperl II

Jason Stajich

## Getting Sequences from GenBank

- Through Web Interface Bio::DB::GenBank (don’t abuse!!)
- Alternative is to download all of genbank, index with Bio::DB::Flat (will be **much** faster in long run)

# Sequence Retrieval Script

```
#!/usr/bin/perl -w
use strict;

use Bio::DB::GenPept;
use Bio::DB::GenBank;
use Bio::SeqIO;

my $db = new Bio::DB::GenPept();
# my $db = new Bio::DB::GenBank(); # if you want NT seqs
# use STDOUT to write sequences
my $out = new Bio::SeqIO(-format => 'fasta');

 my $acc = ‘AB077698’;
 my $seq = $db->get_Seq_by_acc($acc);
 if( $seq ) {
   $out->write_seq($seq);
 } else {
   print STDERR "cannot find seq for acc $acc\n";
}
$out->close();
```

## Sequence Retrieval from Local Database

```
use Bio::DB::Fasta;

my $db = Bio::DB::Fasta->new($dir_with_fa_files);

my $seqstr = $db->seq('SEQUENCE1');
my $seq_part = $db->seq('SEQUENCE2', 100 => 300);
my $seqobj = $db->get_Seq_by_acc('SEQUENCE1');
```

## Use Entrez Queries

```
use Bio::DB::GenBank;
use Bio::DB::Query::GenBank;
my $db = Bio::DB::GenBank->new;
my $query = Bio::DB::Query::GenBank->new
             (-db => ‘nucleotide’,
              -query => “Oryza[Organism] and EST[keyword]”);
my $count = $query->count;
my @ids   = $query->ids;

my $stream = $db->get_Stream_by_query($query);
while (my $seq = $stream->next_seq) {
   # do something with the sequence object
}

# set the IDs from a list
my $query = Bio::DB::Query::GenBank
             (-db => ‘nucleotide’,
              -ids => \@myids);
```

# Bio::DB::Flat

For EMBL, Swissprot, Genbank formats

```
$db = Bio::DB::Flat->new(-directory  => '/usr/share/embl',
                           -dbname     => 'mydb',
                           -format     => 'embl',
                           -index      => 'bdb',
                           -write_flag => 1);
  $db->build_index('/usr/share/embl/primate.embl',
                   '/usr/share/embl/protists.embl');
  $seq       = $db->get_Seq_by_id('BUM');
  @sequences = $db->get_Seq_by_acc('DIV' => 'primate');
  $raw       = $db->fetch_raw('BUM');
```

## Sequence databases

- Represent Sequences, Features, Annotations
- Denormalize relationships
- Allow efficient queries for
    - “All exons in species X”
    - “All genes with description Y”
    - “Longest mRNA from each gene”

## Feature databases

- BioPerl databases
  - Bio::DB::GFF
  - Bio::DB::SeqFeature
- SQL databases
  - EnsEMBL
  - Biosql
  - Chado
  - UCSC

## Bio::DB::GFF

- Version 1 of a GFF database in BioPerl (LD Stein)
- Gbrowse backend
- Some limitations due to ambiguity in GFF spec
- Mysql, Postgres, flatfile implementations

## Bio::DB::GFF usage

```
use Bio::DB::GFF;
# Open the sequence database
my $db      = Bio::DB::GFF->new( -adaptor => 'dbi::mysqlopt',
                                 -dsn     => 'dbi:mysql:elegans');

# fetch a 1 megabase segment of sequence starting at landmark "ZK909
my $segment = $db->segment('ZK909', 1 => 1000000);
# pull out all transcript features
my @transcripts = $segment->features('transcript');

# for each transcript, total the length of the introns
my %totals;
for my $t (@transcripts) {
  my @introns = $t->Intron;
  $totals{$t->name} += $_->length foreach @introns;
}
```

## GFF3 for two loci

```
ylip_A genbank gene 2659 5277  . + . ID=YALI0A00110g.gene;Dbxref=GeneID:2906259
ylip_A genbank mRNA 2659 5277  . + . ID=YALI0A00110g; Parent=YALI0A00110g.gene;
ylip_A genbank cds  2659 5277  . + . ID=50542874.cds1;Parent=YALI0A00110g

ylip_A genbank gene 529296 531064 . - . ID=YALI0A05027g.gene;Dbxref=GeneID:2905838
ylip_A genbank mRNA 529296 531064 . - . ID=YALI0A05027g;Parent=YALI0A05027g.gene
ylip_A genbank cds  530918 531064 . - . ID=50543212.cds1;Parent=YALI0A05027g
ylip_A genbank cds  529296 529928 . - . ID=50543212.cds2;Parent=YALI0A05027g
```

## Bio::DB::SeqFeature

- Fully handle GFF3 parent->child relationships
- Flatfile and mysql implementation
- Simpler interface than Bio::DB::GFF
- Only in Bioperl 1.5.2+

# Write CDS seqs for GFF3 DB

```
use Bio::DB::SeqFeature;
use Bio::Seq;
use Bio::SeqIO;
my $seqio = Bio::SeqIO->new(-format => ‘fasta’);
my $db = Bio::DB::SeqFeature::Store->new(-adaptor => 'berkeleydb',
                                         -dir     => "$dir/$genome");
for my $feature ( $db->get_features_by_type(’gene’) ) {
 for my $mRNA ( $feature->get_SeqFeatures(’mRNA’) ) {
  my $cds;
  for my $exon ( $mRNA->get_SeqFeatures(’cds’) ) {
     $cds .= $exon->dna;
   }
   $seqio->write_seq(Bio::Seq->new(-id => $mRNA->load_id,
                                   -seq => $cds));
 }
}
```

## Multiple Sequence Alignments

- Bio::AlignIO to read alignment files
- Produces Bio::SimpleAlign objects
- Interface and objects designed for round-tripping and some functional work
- Could really use an overhaul or a parallel MSA representation

## Using AlignIO

```
use Bio::AlignIO;
my $in = Bio::AlignIO->new(-format => ‘clustalw’,
                           -file   => ‘filename.aln’);
my $out = Bio::AlignIO->new(-format => ‘phylip’,
                            -file   => ‘>slice.phy’);


while( my $aln = $in->next_aln ) {
  print $aln->no_sequences,” sequence in alignment\n”;
  for my $sequence( $aln->each_seq ) {
    print $sequence->display_id, “\n”;
  }
  my $slice = $aln->slice(10,30); # slice of alignment
  $out->write_aln($slice);
}
```

## Graphics

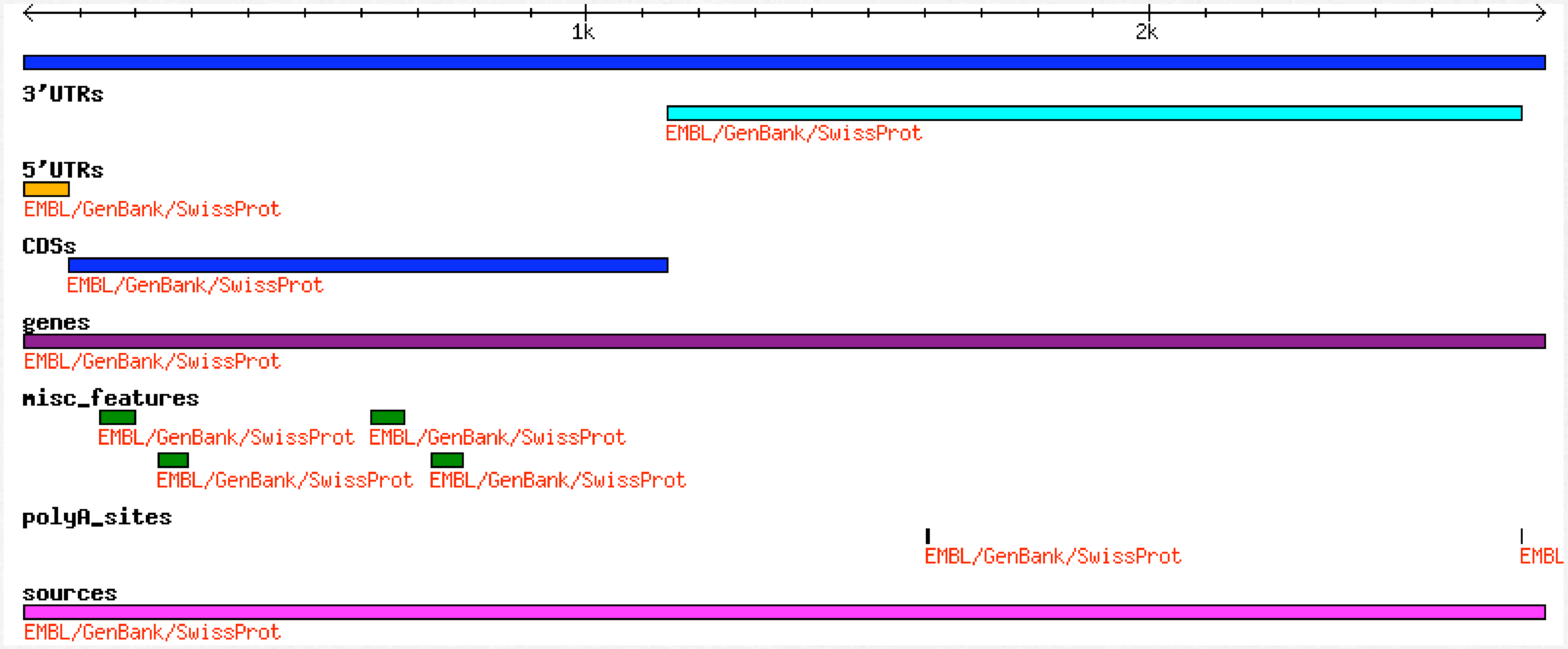
- Simple code to render Sequence with ‘tracks’
- Basic component of Generic Genome Browser (GBrowse)
- Highly customizable, extensible

# Generate a Graphics Panel

```perl
 use Bio::Graphics;
 use Bio::SeqIO;
 use Bio::SeqFeature::Generic;

 my $file = shift
  or die "provide a sequence file as the argument";
 my $io = Bio::SeqIO->new(-file=>$file)
         or die "couldn't create Bio::SeqIO";
 my $seq = $io->next_seq
         or die "couldn't find a sequence in the file";

 my @features = $seq->all_SeqFeatures;

 # sort features by their primary tags
 my %sorted_features;
 for my $f (@features) {
   my $tag = $f->primary_tag;
   push @{$sorted_features{$tag}},$f;
 }
 my $panel = Bio::Graphics::Panel->new(
    -length => $seq->length,
    -key_style => 'between',
    -width     => 800,
    -pad_left  => 10,
    -pad_right => 10);

$panel->add_track(arrow =>
Bio::SeqFeature::Generic->new(-start => 1,
                             -end => $seq->length),
    -bump => 0,
    -double=>1,
    -tick => 2);

$panel->add_track(generic =>
   Bio::SeqFeature::Generic->new(-start => 1,
                                -end => $seq->length,
  -bgcolor => 'blue',
  -label  => 1,);

 # general case
 my @colors = qw(cyan orange blue purple green
                chartreuse magenta yellow aqua);
 my $idx    = 0;

for my $tag (sort keys %sorted_features) {
   my $features = $sorted_features{$tag};
   $panel->add_track($features,
        -glyph    =>  'generic',
        -bgcolor  =>  $colors[$idx++ % @colors],
        -fgcolor  => 'black',
        -font2color => 'red',
        -key      => "${tag}s",
        -bump     => +1,
        -height   => 8,
        -label    => 1,
        -description => 1,
       );
 }

 print $panel->png;
```

# Graphics output

```
[babelfish]$ perl graphics.pl AB077698.gbk > AB077698.png
```



## Tools for Evolutionary and Population analyses

- Population Genetics Modules
- Taxonomy
- Molecular Evolution
- Phylogenetic Tree Building and Manipulation

## Taxonomy data

- Local indexed files or access NCBI (HTTP)
- Also access to BioSQL
- scripts/taxa: local_taxonomydb_query, taxid4species

## Querying Local Taxonomy DB

Download nodesfile and namesfile from NCBI /pub/taxonomy

```
use Bio::DB::Taxonomy;
my $db = Bio::DB::Taxonomy->new(
         -source  => ‘flatfile’,
         -nodesfile=> $nodefile,
         -namesfile=> $namesfile);
$node = $db->get_Taxonomy_Node(
-taxonid => ‘9606’);
$node = $db->get_Taxonomy_Node(
-name => ‘Homo sapiens’);
```

## Querying Remote Taxonomy DB

```
use Bio::DB::Taxonomy;
my $db = new Bio::DB::Taxonomy(
         -source  => 'entrez');

$node = $db->get_Taxonomy_Node(
-taxonid => '9606');
$node = $db->get_Taxonomy_Node(
-gi => $gi); # lookup a taxonid for seq GI
```

Careful! Like RemoteBlast and DB::GenBank you can get your site cut off from NCBI!!

## Put it together

```
use Bio::DB::Taxonomy;
use Bio::SearchIO;
my $db = Bio::DB::Taxonomy->new(
         -source  => ‘flatfile’,
         -nodesfile=> $nodefile,
         -namesfile=> $namesfile);
my $in = Bio::SearchIO->new(-format => ‘fasta’,
                            -file =>’blastfile.FASTX’);

while( my $r = $in->next_result ) {
  while( my $h = $r->next_hit ) {
    my ($gi) = ( $h->name =~ /gi\|(\d+)/ );
    my $kingdom = &gi_to_kingdom($gi);
    if( $kingdom ) {
     $classify{$r->query_name}->{$kingdom}++;
    }
  }
}
```

```
sub gi_to_kingdom {
 my $gi = shift;
 my $taxid = $GI2TaxId{$gi}; # build a local index from NCBI files
 my $node = $TaxDB->get_Taxonomy_Node($taxid);
 if( ! $node ) {
  warn("cannot find node for gi=$gi ($hname) (taxid=$taxid)\n");
  next;
 }
 my $kingdom;
 my $nm = $taxon->scientific_name;
 while( my $n = $taxon->ancestor ) {
        my $rank = lc $n->rank;
        my $name = $n->scientific_name;

        if( $rank eq 'kingdom' ||
            $rank eq 'superkingdom' || $name eq 'Viruses') {
            $kingdom = $name;
            last;
        }
        $taxon = $n;
    }
}
```

## A replacement for Bio::Species?

- DB aware Taxonomy Nodes
- $pnode = $node->get_Parent_Node();
  - $parentid = $node->parent_id;
- my @class = $node->classification;
- my $division = $node->division();
- $node->name; $node->scientific_name;

## Molecular Evolution Tools

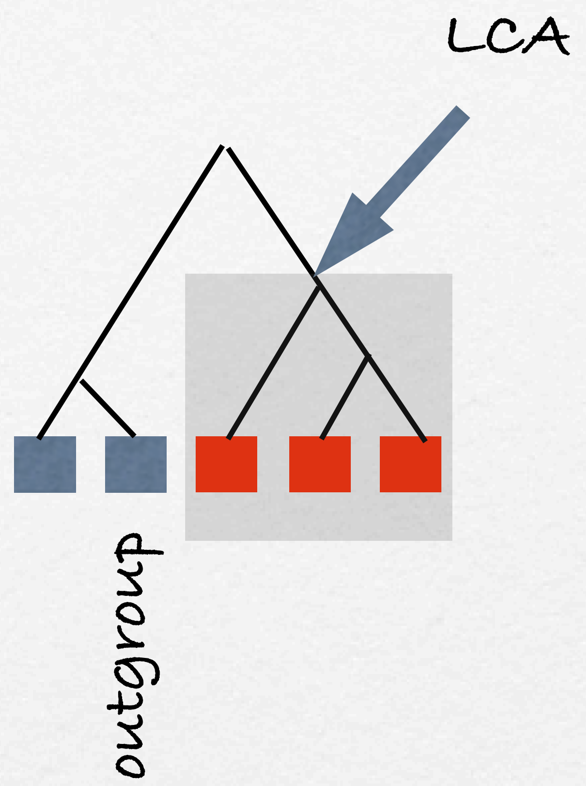
- Bio::Align::DNAStatistics - native calculation of distances, Ks,Ka
- Bio::Align::Utilities - translate aa to nt alignments
- Bio::Tools::Phylo::PAML - parse Codeml output
- Bio::Tools::Run::Phylo::PAML::Codeml - run Codeml (or YN00)

## Phylogenetic Trees

- Support reading of Tree data
  - newick, nexus, nhx formats
- Manipulation of Trees
- Trees are connections of Nodes which have ancestor and children pointers

# Simple Tree Routines

- Lowest Common Ancestor
- Tests of monophyly, paraphyly
- Reroot tree
- Distances between two nodes
- Find a node by name



## Constructing Trees

- Bio::Tree::DistanceFactory has UPGMA, Neighbor-Joining implemented
- Build a matrix with Align::DNAStatistics or Align::ProteinStatistics  OR read in one from Phylip with Bio::Matrix::IO
- Create NJ tree

## Interfacing with Phylip

- Bioperl 1.5.1 supports Phylip 3.6
- Can run tools with bioperl-run package
- Bio::Tools::Run::Phylo::Phylip
    - ProtDist (also parser Bio::Matrix::IO)
    - Neighbor, ProtPars, SeqBoot, Consense
    - DrawGram, DrawTree

## Reading/Writing a Tree

```
#!/usr/bin/perl -w
use Bio::TreeIO;
use strict;
my $in = Bio::TreeIO->new(-format => ‘nexus’,
                          -file   => ‘trees.nex’);
my $out = Bio::TreeIO->new(-format => ‘newick’,
                           -file   => ‘>trees.nh’);
while( my $tree = $in->next_tree ) {
 $out->write_tree($tree);
}
```

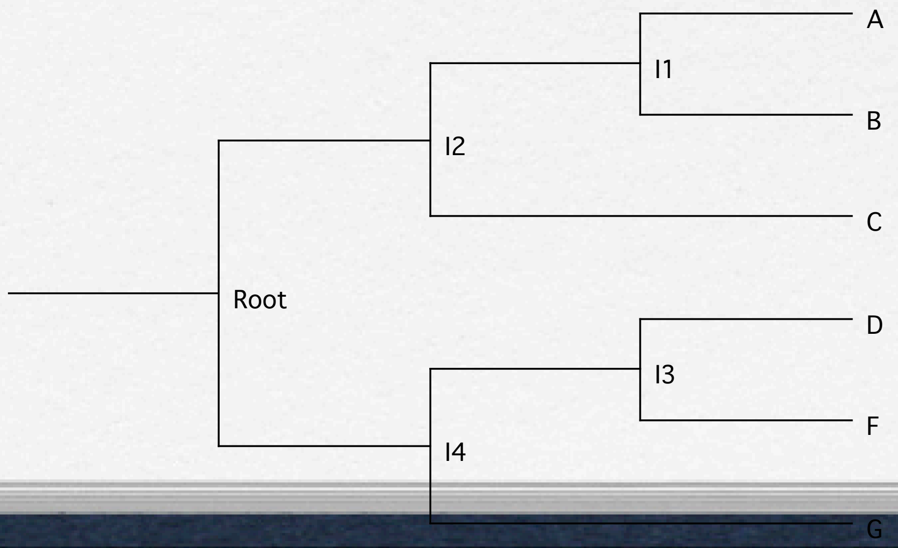
# Fetching subset of nodes

```
#!/usr/bin/perl -w
use strict;
use Bio::TreeIO;
my $in = Bio::TreeIO->new(-format => ‘newick’,
                          -fh     => \*DATA);
if( my $tree = $in->next_tree ) {
   my @nodes = $tree->get_nodes;
   my @tips = grep { $_->is_Leaf } @nodes;
   print "there are ",scalar @tips, " tips\n";
   my @internal = grep { ! $_->is_Leaf } @nodes;
   print "there are ",scalar @internal, " internal
nodes\n";
   my ($A_node) = $tree->find_node(-id => 'A');
   print "branch length of Ancestor of ",$A_node->id,
   " is ", $A_node->ancestor->branch_length, "\n";
}
__DATA__
(((A:10,B:11):2,C:5),((D:7,F:6):17,G:8));
```

# Walking up the tree (tips to root)

```
if( my $tree = $in->next_tree ) {
   my @tips = grep { $_->is_Leaf } $tree->get_nodes;
   for my $node ( @tips ) {
      my @path;
      while( defined $node) {
        push @path, $node->id;
        $node = $node->ancestor;
      }
      print join(“,“, @path), “\n”;
   }
}
__DATA__
(((A:10,B:11)I1:2,C:5)I2,((D:7,F:6)I3:17,G:8)I4)Root;
```

```
C,I2,Root
A,I1,I2,Root
B,I1,I2,Root
G,I4,Root
D,I3,I4,Root
F,I3,I4,Root
```



## From Alignments to Trees

- Bio::AlignIO to parse the alignment
- Bio::Align::ProteinStatistics to compute pairwise distances
- Bio::Tree::DistanceFactory to build a tree based on a matrix of distances using NJ or UPGMA
- More sophisticated tree building should be done with tools like phyml, PAUP, MOLPHY, PHYLIP, MrBayes, or PUZZLE

## Testing phylogenetic hypotheses

- No sophisticated ML methods are currently built in Bioperl for testing for phylogenetic correlations, etc
- Can export trees and use tools like Mesquite
- Work to be finished in Dec 2006 to fully integrate more phylogenetic tools into BioPerl

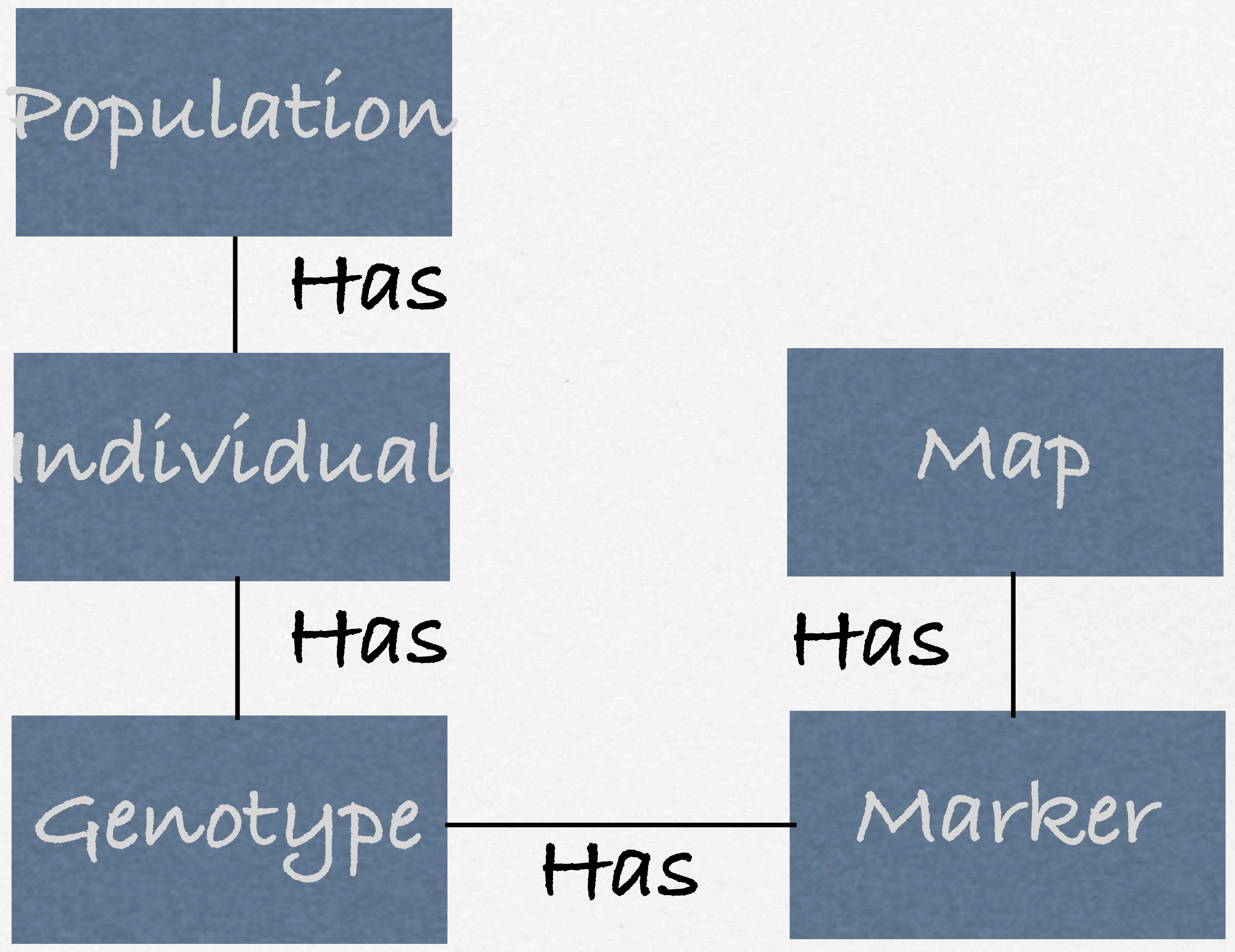
## Bioperl & Population Genetics

- Basic data
  - Marker - polymorphic region of genome
  - Individual - individual sampled
  - Genotype - observed allele(s) for a marker in an individual
  - Population - collection of individuals
  - See http://bioperl.org/wiki/PopGen_modules

## The Players

- Bio::PopGen::Population - container for Individuals, calculate summary stats
- Bio::PopGen::Individual - container for Genotypes
- Bio::PopGen::Marker - summary info about a Marker (primers, genome loc, allele freq)
- Bio::PopGen::Genotype - pairing of Individual & Marker; allele container

# Population Genetics Data Objects



## Some Data Formats

- PrettyBase format (Seattle SNP data)

```
MARKER      SAMPLEID   ALLELE1 ALLELE2
ProcR2973EA      01      C        T
ProcR2973EA      02      N        N
ProcR2973EA      03      C        C
ProcR2973EA      04      C        T
ProcR2973EA      05      C        C
```

- Comma Delimited (CSV)

```
SAMPLE,MARKERNAME1,MARKERNAME2,...
SAMPLE,ProcR2973EA,Marker2
sample-01,C T,G T
sample-02,C C,G G
```

- Phase format

```
3
5
P 300 1313 1500 2023 5635
MSSSM
#1
12 1 0 1 3
11 0 1 0 3
```

## Reading in Data

```
use Bio::PopGen::IO;
my $in = Bio::PopGen::IO->new(-format => ‘csv’
                              -file   => ‘dat.csv’);
my @pop;
while( my $ind = $in->next_individual ) {
  push @pop, $ind;
}
# OR
my $pop = $in->next_population;
```

## Ready Built Stuff

- Bio::PopGen::PopStats - population level statistics (only $F_{ST}$ currently)
- Bio::PopGen::Statistics - suite of Population Genetics statistical tests and summary stats.
- Bio::PopGen::Simulation::Coalescent - primitive Coalescent simulation
  - Basic tree topology and branch length assignment.

## Using the Modules

```
use Bio::PopGen::Statistics;
my $stats = Bio::PopGen::Statistics->new();
my $pi = $stats->pi($population);
# or use an array reference of Individuals
my $pi = $stats->pi(\@individuals);
# Tajima’s D
my $TajimaD = $stats->tajima_D($population);
# Fu and Li’s D
my $FuLiD = $stats->fu_and_li_D($ingroup_pop,
                                $outgroup_ind);
# Fu and Li’s D*
my $FLDstar = $stats->fu_and_li_D_star($population);

# pairwise composite LD
my %LDstats = $stats->composite_LD($population);
my $LDarray = $LDstats{’marker1’}->{’marker2’};
my ($ldval,$chisq) = @$LDarray;
```

## Getting Data from Alignments

- use Bio::AlignIO to read in Multiple Sequence Alignment data
- Bio::PopGen::Utilities aln_to_population will build Population from MSA
  - Will make a “Marker” for every polymorphic site (or if asked every site)
  - Eventually will have ability to only get silent/non-silent coding sites

## Automating PAML

- PAML - phylogenetic analysis with maximum likelihood
- Estimate synonymous and non-synonymous substitution rates
- Along branches of a tree or in a pairwise fashion

## Preparing Data

- Multiple sequence alignments of protein coding sequence
- No stop codons!
- Must be aligned on codon boundaries
- Easiest way is to align at protein level, then project back into CDS alignment

## Doing Protein Alignments

- Bio::Tools::Run::Alignment::Clustalw or Bio::Tools::Run::Alignment::MUSCLE or just prepare the sequence files and run the alignment programs via scripts
- Bio::AlignIO to parse the alignment data
- Bio::Align::Utilities to project back into CDS space

## Build tree or assume a tree

- If doing analysis of genomes which have a know species tree - use that tree
- Branch lengths are not part of PAML.  Multiple topologies can be provided to test alternative hypotheses (by comparing maximum likelihood values)

## Running PAML

```
#!/usr/bin/perl -w
use strict;
use Bio::Tools::Run::Phylo::PAML::Codeml;
use Bio::AlignIO;
my $factory = Bio::Tools::Run::Phylo::PAML::Codeml->new(
              -params => { ‘runmode’ => -2,
                           ‘seqtype’ => 1});
my $alnio = Bio::AlignIO->new(-format => ‘clustalw’,
                              -file   => ‘cds.aln’);
my $aln = $alnio->next_aln; # get the alignment from file
$factory->alignment($aln);  # set the alignment
my ($returncode,$parser) = $factory->run();
my $result = $parser->next_result;
my $MLmatrix = $result->get_MLMatrix;

print "Ka = ", $MLmatrix->[0]->[1]->{’dN’},"\n";
print "Ks = ", $MLmatrix->[0]->[1]->{’dS’},"\n";
print "Ka/Ks = ", $MLmatrix->[0]->[1]->{’omega’},"\n";
```

## Parsing PAML

```
#!/usr/bin/perl -w
use strict;
use Bio::Tools::Phylo::PAML;
my $parser = Bio::Tools::Phylo::PAML->new
  (-file => ‘results/mlc’, -dir => ‘results’);
if( my $result = $parser->next_result ) {
  my @otus = $result->get_seqs;
  # get Nei & Gojobori dN/dS matrix
  my $NGmatrix = $result->get_NGmatrix;
  printf “%s and %s dS=%.4f dN=%.4f Omega=%.4f\n”,
    $otus[0]->display_id, $otus[1]->display_id,
    $NGMatrix->[0]->[1]->{dS}, $NGMatrix->[0]->[1]->{dN},
    $NGMatrix->[0]->[1]->{omega};
}
```

## Getting the Trees out

```
my @trees = $result->get_trees;
for my $tree ( @trees ) {
  print “likelihood is “, $tree->score, “\n”;
  # do something else with the trees,
  # for non runmode -2 results
# inspect the tree, branch specific rates
# the "t" (time) parameter is available via
# ("omega", "dN", etc.) are available via
# ($omega) = $node->get_tag_values('omega');
for my $node ( $tree->get_nodes ) {
  print $node->id, “ t=”,$node->branch_length,
   “ omega “, $node->get_tag_values(‘omega’);
 }
}
```

## Running BLAST Remotely

- Allow submission of BLAST queries to NCBI via scripts
- Need to be careful - infinite loops, over submitting jobs can get your access shutdown!

```
use Bio::Tools::Run::RemoteBlast;
my $prog = ‘blastp’;
my $db   = ‘ecoli’;
my $e_val= ‘1e-10’;
my $remote_blast = Bio::Tools::Run::RemoteBlast->new(
   -prog   => $prog,
   -data   => $db,
   -expect => $e_val);
my $r = $remote_blast->submit_blast($inputfilename);
while( my @rids = $remote_blast->each_rid ) {
  for my $rid ( @rids ) {
  my $rc = $remote_blast->retrieve_blast($rid);
  if( ! ref($rc) ) {
    if( $rc < 0 ) { $remote_blast->remove_rid($rid); }
    print STDERR “.”; sleep(10);
  } else {
    $remote_blast->remove_rid($rid);
    my $result = $rc->next_result;
    while( my $hit = $result->next_hit ) {
     print $hit->name, “ “, $hit->significance, “\n”;
    }
  }
```

Fin