

EnsEMBL COMPARA PERL API TUTORIAL

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Revisions: CW Jun 04, AUV Aug 04.

WARNING: this is still a first draft. A polished version of it in sync with ensembl release 24 (in September 2004) should be available at the time of release. By now this tutorial should work with the cvs main trunk of the code (so don't specify -r branch-ensembl-24), and with ensembl databases release 23 (at least for whole genome alignment part, as some changes have been done to the tables that hold the orthologues data).

INTRODUCTION

This tutorial is an introduction to the ensembl compara API. A knowledge of the ensembl core API is presumed, it is assumed that concepts and conventions presented in the ensembl core API tutorial have been assimilated by the user. The ensembl core API tutorial can be found at <http://www.ensembl.org/?????> (in cvs, ensembl/docs/tutorial/ensembl_tutorial.pdf) and should be read first as it provides a comprehensive guide to the ensembl environment.

A documentation about the compara database schema is available <http://www.ensembl.org/????> (in cvs ensembl-compara/docs/docs/schema_doc.html) and while not necessary for this tutorial, an understanding of the database tables may help, as many of the Adaptor modules are table specific.

Obtaining the code

To use the ensembl compara API, you have the same requirement that when using the ensembl core API i.e. perl 5.6 or later, bioperl 1.2 or later, DBI, DBD::mysql and ensembl core code. Please refer to the ensembl core API tutorial that will tell you everything about these modules, how and where to get them.

In addition, you will need the ensembl compara code that is available by cvs from the ensembl cvs repository using the following cvs commands:

```
cvs -d :pserver:cvsuser@cvsro.sanger.ac.uk:/cvsroot/CVSmaster login
```

When prompted the password is 'CVS'.

```
cvs -d :pserver:cvsuser@cvsro.sanger.ac.uk:/cvsroot/CVSmaster co -r branch-ensembl-24
ensembl-compara
```

This will check out ensembl-compara code for stable branch 24. Make sure the ensembl core code you have already checked out is on the same branch. Note that the branch that is checked out should correspond to the database version being used. Thus `ensembl_compara_24_1` and e.g. `homo-sapiens_core_24_34e` and `mus_musculus_core_24_33` should be used with the above ensembl branch 24 code.

Environment Variables

The following PERL5LIB environment variables should be set up:

under tcsh/csh shell with

```
setenv PERL5LIB ${PERL5LIB}:${HOME}/src/bioperl-live: \
${HOME}/src/ensembl/modules:${HOME}/src/ensembl-compara/modules
```

under bash shell with

```
export PERL5LIB=${PERL5LIB}:${HOME}/src/bioperl-live: \
${HOME}/src/ensembl/modules:${HOME}/src/ensembl-compara/modules
```

These presume that bioperl and ensembl are in a directory called src set up in your home directory.

Code Conventions (and unconventions)

Refer to the `ensembl` core tutorial for a good description of the coding conventions normally used in `ensembl`. Due to historical accidents, there may be exceptions to these rules in `compara`, especially with respect to variable names.

Connecting a ensembl compara database

Explicitly, using the Bio::EnsEMBL::Compara::DBSQL::DBAdaptor

Ensembl compara data as ensembl core data, is stored in a MySQL relational database. If you want to access a compara database, you will need to connect to it. This is done in exactly the same way as when connecting an ensembl core database, but using a Compara specific DBAdaptor.

[illegible]

```
-dbname => $dbname);
```

As for a ensembl core connection, in addition to the parameters provided above, the optional port, driver and pass parameters can also be used to specify the TCP connection port, the type of database driver and the password respectively. These values have sensible defaults and can often be omitted.

Implicitely, using the Bio::EnsEMBL::Registry (recommended)

To use the registry you will need to have a registry configuration file set up. An example of such file can be found in ensembl/modules/Bio/EnsEMBL/Utils/ensembl_init.example, and below you have a slightly modified copy of it.

```
# Example of configuration file used by Bio::EnsEMBL::Registry::load_all method
# to store/register all kind of Adaptors.

use strict;
use Bio::EnsEMBL::Utils::ConfigRegistry;
use Bio::EnsEMBL::DBSQL::DBAdaptor;
use Bio::EnsEMBL::Compara::DBSQL::DBAdaptor;

my @aliases;

new Bio::EnsEMBL::DBSQL::DBAdaptor(-host => 'ensembl.db.ensembl.org',
                                   -user => 'anonymous',
                                   -port => 3306,
                                   -disconnect_when_inactive => 1,
                                   -species => 'Homo sapiens',
                                   -group => 'core',
                                   -dbname => 'homo_sapiens_core_23_34e');

@aliases = ('H_Sapiens', 'homo sapiens', 'Homo_Sapiens', 'Homo_sapiens', 'Homo',
            'homo', 'human');

Bio::EnsEMBL::Utils::ConfigRegistry->add_alias(-species => "Homo sapiens",
                                                -alias => \@aliases);

new Bio::EnsEMBL::DBSQL::DBAdaptor(-host => 'ensembl.db.ensembl.org',
                                   -user => 'anonymous',
                                   -port => 3306,
                                   -disconnect_when_inactive => 1,
                                   -species => 'Mus musculus',
                                   -group => 'core',
                                   -dbname => 'mus_musculus_core_23_32c');

@aliases = ('M_Musculus', 'mus musculus', 'Mus_Musculus', 'Mus_musculus', 'Mus', 'mus',
            'mouse');

Bio::EnsEMBL::Utils::ConfigRegistry->add_alias(-species => "Mus musculus",
                                                -alias => \@aliases);

new Bio::EnsEMBL::DBSQL::DBAdaptor(-host => 'ensembl.db.ensembl.org',
                                   -user => 'anonymous',
                                   -port => 3306,
                                   -disconnect_when_inactive => 1,
                                   -species => 'Fugu rubripes',
                                   -group => 'core',
                                   -dbname => 'fugu_rubripes_core_23_2c');

@aliases = ('F_Rubripes', 'fugu rubripes', 'Fugu_Rubripes', 'Fugu_rubripes', 'Fugu',
            'fugu');

Bio::EnsEMBL::Utils::ConfigRegistry->add_alias(-species => "Fugu rubripes",
                                                -alias => \@aliases);

new Bio::EnsEMBL::Compara::DBSQL::DBAdaptor(-host => 'ensembl.db.ensembl.org',
```

```

        -user => 'anonymous',
        -port => 3306,
        -disconnect_when_inactive => 0,
        -species => 'Compara23',
        -dbname => 'ensembl_compara_23_1');

@aliases = ('ensembl_compara_23_1', 'compara23');

Bio::EnsEMBL::Utils::ConfigRegistry->add_alias(-species => "Compara23",
        -alias => \@aliases);

1;

```

In this configuration file, you can list all the parameters needed to connect a compara database. The compara database is a multi-species database that contains comparative genomic information on all ensembl species. One should then be able not only to connect to a compara database but also to every species ensembl core database. The use of the registry configuration file lets you the freedom to list connection parameters for all ensembl core databases you might need to access in relation to ensembl compara data (in our example, only 3 are mentioned, human, mouse and rat). All this information is then in a single central place, easy to maintain (modify or update).

The access to database adaptor is done using either the species alias (specified by the `-species` parameter) or one of the aliases specified (in the `@aliases` array). No need to remember the complete database name, an alias is enough.

The parameter `disconnect_when_active` set to 1 for all ensembl core databases. The main purpose of this is that many time when accessing compara data, you won't need to access any of the core databases. There is no need then to keep open connections to unused databases. The `disconnect_when_active` set to 1, automatically disconnect from databases when no use of the connection is done, and will reconnect automatically when needed. This helps to reduce connection load to the MySQL server used.

Alias.

Ensembl compara Adaptors available

GenomeDBAdaptor	to fetch Bio::Ensembl::Compara::GenomeDB objects
DnaFragAdaptor	to fetch Bio::Ensembl::Compara::DnaFrag objects
GenomicAlignAdaptor	to fetch Bio::Ensembl::Compara::GenomicAlign objects
DnaAlignFeatureAdaptor	to fetch Bio::Ensembl::DnaDnaAlignFeature objects (note that this adaptor return a ensembl core object)
SyntenAdaptor	to fetch Bio::Ensembl::Compara::SyntenRegion objects
MemberAdaptor	to fetch Bio::Ensembl::Compara::Member objects
TaxonAdaptor	to fetch Bio::Ensembl::Compara::Taxon objects
HomologyAdaptor	to fetch Bio::Ensembl::Compara::Homology objects
FamilyAdaptor	to fetch Bio::Ensembl::Compara::Family objects
PeptideAlignFeatureAdaptor	to fetch Bio::Ensembl::Compara::PeptideAlignFeature objects
MetaContainer	to query the meta table

Only some of these adaptors will be used for illustration as part of this tutorial through commented perl scripts code.

Whole Genome Alignments

The compara database contains a number of different types of whole genome alignments. A much detailed information about what are these different types can be found [here](http://www.ensembl.org/Homo_sapiens/helpview?se=1&kw=contigview#mus_musculus_match)

http://www.ensembl.org/Homo_sapiens/helpview?se=1&kw=contigview#mus_musculus_match

The full range of comparisons available may be found in the method_link table as shown below.

```
mysql> select * from method_link;
```

method_link_id	type
1	BLASTZ_NET
2	BLASTZ_NET_TIGHT
3	BLASTZ_RECIP_NET
4	PHUSION_BLASTN
5	PHUSION_BLASTN_TIGHT
6	TRANSLATED_BLAT
101	SYNTENY
201	HOMOLOGOUS_GENE
301	FAMILY

The designation 'TIGHT' denotes that the alignments have been rescored using the 'TIGHT' matrix (in cvs, ensembl-compara/scripts/hcr/tight.mat) so only the most highly conserved alignments are reported.

The whole genome comparisons are accessed through the API by way of the adaptors mentioned above. Specifically, the DnaAlignFeatureAdaptor, which returns DnaAlignFeatures (illustrated in this tutorial) and the GenomicAlignAdaptor, which allows direct access to all of the data in the genomic_align_block table (not illustrated in this tutorial for now).

DnaDnaAlignFeature objects (for pairwise alignments only)

Below it is a commented perl script which a simplified version of the ensembl-compara/scripts/dumps/DumpAlignments.pl script that you can get from the ensembl-compara cvs repository.

```
use strict;
use Bio::Ensembl::Registry;
use Bio::Ensembl::Compara::DBSQL::DBAdaptor;
use Bio::AlignIO;
use Bio::LocatableSeq;
use Getopt::Long;

my $usage = "
$0
  [--help]                this menu
  --dbname string          (e.g. compara23) one of the compara database
                           Bio::Ensembl::Registry aliases
  --seq_region string      (e.g. 22)
  --seq_region_start integer (e.g. 50000000)
```

```

--seq_region_end integer    (e.g. 50500000)
--qy string                 (e.g. human) the query species (i.e. a
                             Bio::Ensembl::Registry alias) from which alignments
                             are queried and seq_region refer to
--tg string                 (e.g. mouse) the target species (i.e. a
                             Bio::Ensembl::Registry alias) to which alignments are
                             queried
[--alignment_type string]  (e.g. TRANSLATED_BLAT) type of alignment stored
                             (default: BLASTZ_NET)
[--tsl]                   print out a translated alignment
[--oo]                   Original orientation output from the alignment
                             program. Mostly useful in association with --tsl
                             option, when a full translated alignment program has
                             been used e.g TRANSLATED_BLAT (helps to obtain the
                             right translation phase used during the alignment
                             process)
[--ft string]            alignment format, available in bioperl Bio::AlignIO
                             (default: clustalw)
[--uc]                   print out sequence in upper cases (default is lower
                             cases)
[--limit integer]        (e.g. 2) limit the output to the number of alignments
                             specified
[--reg_conf filepath]    the Bio::Ensembl::Registry configuration file. If none
                             given, the one set in ENSEMBL_REGISTRY will be used if
                             defined, if not ~/.ensembl_init will be used.

\n";

my $dbname;
my ($seq_region,$seq_region_start,$seq_region_end);
my ($qy_species,$tg_species);
my $help = 0;
my $alignment_type = "BLASTZ_NET";
my $limit;
my $reg_conf;
my $format = "clustalw";
my $translated = 0;
my $uc = 0;
my $original_orientation = 0;

unless (scalar @ARGV) {
    print $usage;
    exit 0;
}

GetOptions('help' => \$help,
           'dbname=s' => \$dbname,
           'seq_region=s' => \$seq_region,
           'seq_region_start=i' => \$seq_region_start,
           'seq_region_end=i' => \$seq_region_end,
           'qy=s' => \$qy_species,
           'tg=s' => \$tg_species,
           'alignment_type=s' => \$alignment_type,
           'tsl' => \$translated,
           'ft=s' => \$format,
           'uc' => \$uc,
           'oo' => \$original_orientation,
           'limit=i' => \$limit,
           'reg_conf=s' => \$reg_conf);

$|=1;

if ($help) {
    print $usage;
    exit 0;
}

# Setting up Bio::Ensembl::Registry

Bio::Ensembl::Registry->load_all($reg_conf);

$format = lc $format;

# Getting the core SliceAdaptor for the query species

my $qy_sa = Bio::Ensembl::Registry->get_adaptor($qy_species,'core','Slice');

# Fetching a Slice. In compara, all slices are 'toplevel' coordinate system.

```

```

my $qy_slice = $qy_sa->fetch_by_region('toplevel',$seq_region,
                                       $seq_region_start,$seq_region_end);

# Getting the core MetaContainer adaptor for the target species

my $tg_mc = Bio::EnsEMBL::Registry->get_adaptor($tg_species,'core','MetaContainer');

# Getting a Bio::Species object and from it the Species genus (e.g. Mus
# musculus) of the target species, using the binomial call

my $tg_binomial = $tg_mc->get_Species->binomial;

# Getting the compara DnaAlignFeatureAdaptor to query the compara database

my $dafad = Bio::EnsEMBL::Registry->get_adaptor($dbname,'compara','DnaAlignFeature');

# Fetching DnaDnaAlignFeatures object (these are core objects) using the
# fetch_all_by_Slice. The 3rd argument that can specify the assembly version
# can be undef. The compara API will find for you the default assembly for
# the target species.

my @DnaDnaAlignFeatures = sort {$a->start <=> $b->start
                               || $a->end <=> $b->end}
    @{$dafad->fetch_all_by_Slice($qy_slice,$tg_binomial,undef,$alignment_type,$limit)};

# Go through each alignment to print out in the requested format

foreach my $ddaf (@DnaDnaAlignFeatures) {

    # if the original alignment strand orientation is requested
    # ($original_orientation is true) and effectively the alignment obtained
    # is reverse complement from the originally obtained by the alignment
    # program used (if $ddaf->strands_reversed is true), then reverse
    # complement the alignment.

    if ($original_orientation && $ddaf->strands_reversed) {
        $ddaf->reverse_complement;
    }

    # Create a list of flags to be used in the get_SimpleAlign method call

    my @flags;
    push @flags, 'translated' if ($translated);
    push @flags, 'uc' if ($uc);

    # Get a Bio::SimpleAlign from the DnaDnaAlignFeature object

    my $sa = $ddaf->get_SimpleAlign(@flags);

    # Create a Bio::AlignIO with the requested output format

    my $alignIO = Bio::AlignIO->newFh(-interleaved => 0,
                                       -fh => \*STDOUT,
                                       -format => $format,
                                       -idlength => 20);

    # print out the alignment (Bio::SimpleAlign object) in the requested
    # output format through the Bio::AlignIO handler

    print $alignIO $sa;
}

exit 0;

```

So to pull out BLASTZ_NET_TIGHT alignments, let's say on part of ENCODE region ENm004 on human chromosome 22, between position 30184430 and position 30184485, against the mouse genome in clustalw format, we can use know the following command line,

```
% perl DumpAlignmentsLight.pl --dbname Compara23 --seq_region 22
--seq_region_start 30184430 --seq_region_end 30184485 --qy human --tg mouse
--alignment_type BLASTZ_NET_TIGHT

CLUSTAL W(1.81) multiple sequence alignment

22/30184223-30184547      tgaaacgcttgctccttgaagtcctctctcgggtctctgtctctcaagtcgccgaggtcct
11/3118113-3118437      tgaaacgtttgtcctttagtccctctctctgtctcgggtctctcaagtcgcgaggtcct
*****

22/30184223-30184547      tatcgctaagacgggtgatccttctcaaaggtccgggcagagattatcctcccactgccaa
11/3118113-3118437      tatcactgagacgggtgatccttttcaaaggccgggcagaaattatccttccactgccaa
**** *

22/30184223-30184547      tcctacgtccaccaagcagacgaagcccatcactatctttctctaattggacttcctgagc
11/3118113-3118437      ttcttcgtccaccaagcaggcgaagtccatcactgtcttttccaatggactgccagatc
* **

22/30184223-30184547      gccgggagctaacacgaggctgtcacgtggcagccccctccaaagctccgtctctgagggc
11/3118113-3118437      gtcgggagctaacacgagctgtcacatggcagccacctccaaagcttcgtctctgtgggc
* *****

22/30184223-30184547      tgagaacaacatctaagtcacatcttctttcacacgctctcgtggatctggaaggacgtggg
11/3118113-3118437      tgagaacaacatctaagtcacatcttctttcactcgtctcgtggatctgaaagatgccag
*****

22/30184223-30184547      aaagacaaagttaaacaaccaaca
11/3118113-3118437      aaagagaaaggtaagcaaaccaaca
*****
```

Now on the same region, TRANSLATED_BLAT alignments against fugu in clustalw format, but at translation level now (-tsl) not nucleotide level, we can run the following command line,

```
% perl DumpAlignmentsLight.pl --dbname Compara23 --seq_region 22 \
--seq_region_start 30184430 --seq_region_end 30184485 --qy human --tg fugu \
--alignment_type TRANSLATED_BLAT --tsl

CLUSTAL W(1.81) multiple sequence alignment

22/30184431-30184484      aapskapslraenni*vi
scaffold_2267/1347-1400  tspskaaplwa*yyi*ii
                        :*****.* * **:*
```

To make sure that the alignment, we got is on the strand on which it was originally generated using the -oo option will check that and restore the right strandness. See below the difference in the translation level alignment obtained.

```
% perl DumpAlignmentsLight.pl --dbname Compara23 --seq_region 22 \
--seq_region_start 30184430 --seq_region_end 30184485 --qy human --tg fugu \
--alignment_type TRANSLATED_BLAT --tsl --oo

CLUSTAL W(1.81) multiple sequence alignment

22/30184431-30184484      ddldvvlspqrrsfgggc
scaffold_2267/1347-1400  ddldvilspqrrsfgggc
*****
```


SyntenyRegion objects

NB: This part needs an update. Some part of it may be out of date soon....

The DNA/DNA whole genome comparisons are also used to create long regions of synteny between chromosomes of different species. For example, the ENCODE region on chromosome 22 used previously is syntenous to regions on chromosomes 10, 5 and 11 of mouse. This information is available through the SyntenyAdaptor. The following script returns the mouse regions that are syntenous with this ENCODE region.

```
use strict;
use Bio::Ensembl::Compara::DBSQL::DBAdaptor;
use Bio::Ensembl::Transcript;

my $host = 'ensembl.ensembl.org';
my $user = 'anonymous';
my $dbname = 'ensembl_compara_22_1';
my $conf_file = '/nfs/acari/cara/src/ensembl_main/ensembl-compara/modules/Bio/Ensembl/Compara/Compara.conf';

my $qy_species = "Mus musculus";
my $cs_species = "Homo sapiens";

my $cs_chr = "22";
my $cs_start = 30128508;
my $cs_end = 31828507;

my $comparadb = new Bio::Ensembl::Compara::DBSQL::DBAdaptor (-host => $host,
                                                             -user => $user,
                                                             -dbname => $dbname,
                                                             -conf_file => $conf_file);

my $synteny_adaptor = $comparadb->get_SyntenyAdaptor();

$synteny_adaptor->setSpecies($comparadb, $cs_species, $qy_species);
my $synteny_regions = $synteny_adaptor->get_synteny_for_chromosome($cs_chr, $cs_start,
                                                                    $cs_end);

foreach my $synt (@$synteny_regions){#this object uses a hash to access the info rather than having true methods
    print $synt->{'synteny_id'}.", ";
    print $synt->{'seq_type'}.", ";
    print $synt->{'chr_name'}.", ";
    print $synt->{'start'}.", ";
    print $synt->{'end'}.", ";
    print $synt->{'chr_start'}.", ";
    print $synt->{'chr_end'}.", ";
    print $synt->{'start'}.", ";
    print $synt->{'end'}.", ";
    print $synt->{'hit_seq_type'}.", ";
    print $synt->{'hit_chr_name'}.", ";
    print $synt->{'hit_chr_start'}.", ";
    print $synt->{'hit_chr_end'}.", ";
    print $synt->{'rel_ori'}.", "\n";
}
```

Orthologues and Protein clusters

NB : This following is very much a draft at this stage with some piece of code to give examples, but not much comments. Will be added by September 2004.

Member objects (for pairwise alignments only)

```
my $ma = Bio::Ensembl::Registry->get_adaptor($dbname, 'compara', 'Member');
my $member = $ma->fetch_by_source_stable_id("ENSEMBLGENE", ENSP00000000233);

print join " ", map { $member->$_ } qw(chr_name chr_start chr_end description
source_name taxon_id taxon), "\n";
```

chr_name, chr_start, chr_end and description are self-explanatory.

source_name tells about the origin of the Member entry, and can be either

ENSEMBLPEP, derived from ensembl translation,

or ENSEMBLGENE, derived from an ensembl gene,

or SWISSPROT, derived from a swissprot entry,

or SPTREMBL, derived from a sptrembl entry.

taxon_id e.g. 9606 correspond to the NCBI taxonomy identifier (see www.ncbi.nih.gov for more details).

taxon returns a Bio::Ensembl::Compara::Taxon object that inherits itself from Bio::Species, so from this object you can get

```
my $taxon = $member->taxon;
print join "; ", map { $taxon->$_ } qw(common_name genus species binomial
classification), "\n";
```

respectively for these method call and in the case of human species, you will obtain

```
human; Homo; sapiens; Homo sapiens; sapiens Homo Hominidae Catarrhini Primates
Eutheria Mammalia Euteleostomi Vertebrata Craniata Chordata Metazoa Eukaryota
```

Homology objects

first you have to get a Member object. In case of homology is a gene, in case of family it can be a gene or a protein

```
my $ma = Bio::Ensembl::Registry->get_adaptor($dbname, 'compara', 'Member');
my $member = $ma->fetch_by_source_stable_id("ENSEMBLGENE", ENSP00000000233);
```

then you get the homologies where the member is implicated

```
my $ha = Bio::Ensembl::Registry->get_adaptor($dbname, 'compara', 'Homology');
my $homologies = $ha->fetch_by_Member($member);
fetch_by_Member_Homology_source (fetch_by_Member_MethodLink)
```

Then for each homology, you get all the Members implicated

```
foreach my $homology (@{$homologies}) {
    print $homology->description
```

each homology relation have only 2 members, you should find there the initial member used in the first fetching

```
for each my $member_attribute (@{$homology->get_all_Member_Attribute})
```

for each Member, you get information on the Member specifically or in relation to the homology relation via Attribute object

```
my ($member, $attribute) = @{$member_attribute};
print join " ", map { $member->$_ } qw(stable_id taxon_id), "\n";
print join " ", map { $attribute->$_ } qw(perc_id perc_pos perc_cov), "\n";
}
```

```

# You can even retrieve the HSP alignment between the 2 proteins,
# HSP used to build the homology relationship at the peptide level

my $sa = $homology->get_SimpleAlign();
my $alignIO = Bio::AlignIO->newFh(-interleaved => 0,
                                   -fh => \*STDOUT,
                                   -format => "clustalw",
                                   -idlength => 20);

print $alignIO $sa;

# or at the nucleotide level. You will need to make you have a connection to
# the corresponding core databases through the Bio::Ensembl::Registry

$sa = $homology->get_SimpleAlign("cdna");
my $alignIO = Bio::AlignIO->newFh(-interleaved => 0,
                                   -fh => \*STDOUT,
                                   -format => "phylip",
                                   -idlength => 20);

print $alignIO $sa;
}

```

Family objects

```

my $ma = Bio::Ensembl::Registry->get_adaptor($dbname, 'compara', 'Member');
my $member = $ma->fetch_by_source_stable_id("ENSEMBLGENE", ENSP00000000233);

my $fa = Bio::Ensembl::Registry->get_adaptor($dbname, 'compara', 'Family');
my $families = $fa->fetch_by_Member($member);

foreach my $family (@{$families}) {
    print $family->description;

    for each my $member_attribute (@{$family->get_all_Member_Attribute})
        my ($member, $attribute) = @{$member_attribute};
        print $member->stable_id, " ", $member->taxon_id, "\n";
        print $attribute->cigar_line, "\n";
    }

    my $sa = $family->get_SimpleAlign();
    my $alignIO = Bio::AlignIO->newFh(-interleaved => 0,
                                       -fh => \*STDOUT,
                                       -format => "phylip",
                                       -idlength => 20);

    print $alignIO $sa;

    $sa = $family->get_SimpleAlign("cdna");
    my $alignIO = Bio::AlignIO->newFh(-interleaved => 0,
                                       -fh => \*STDOUT,
                                       -format => "phylip",
                                       -idlength => 20);

    print $alignIO $sa;
}

```