HOWTO:SeqIO

From BioPerl

This HOWTO will teach you about the Bio::SeqIO system for reading and writing sequences of various formats

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The basics

This section assumes you've never seen BioPerl before, perhaps you're a biologist trying to get some information about some sequences or you're some kind of IT expert interested in learning something about this hot topic, "bioinformatics". Your first script may want to get some information from a file containing one or more sequences.

A piece of advice: always use the module Bio::SeqIO! Here's what the first lines of your script might look like:

#!/bin/perl

```
use strict;
use Bio::SeqIO;
my $file = shift; # get the file name, somehow
my $seqio_object = Bio::SeqIO->new(-file => $file);
my $seq_object = $seqio_object->next_seq;
```

Why use Bio::SeqIO? In part because SeqIO understands the many different sequence file formats and creates the proper BioPerl object for each format. Some formats, like FASTA sequence format, are minimal. The fasta format contains a sequence and some kind of identifier, but nothing else is required nor does the format inherently allow for much more detail, like a feature (a sub-sequence, usually with some biological property - see the Feature-Annotation HOWTO for more information). When given fasta SeqIO creates a Bio::Seq object, a more spare object than the Bio::Seq::RichSeq object that's created when Bio::SeqIO is given formats like Genbank or EMBL, which may contain features and annotations.

Now, should you care what kind of BioPerl object is created by SeqIO? For the most part no - let SeqIO take care of those details.

10 second overview

Lots of bioinformatics involves processing sequence information in different formats - indeed, there often seems to be about as many formats as there are programs for processing them. The SeqIO system handles sequences of many different formats and is the way Bioperl pushes sequences in and out of objects. You can think of the SeqIO system as "a smart filehandle for sequences".

Background Information

The SeqIO system handles all of the complexity of parsing sequences of many standard formats that scripters have wrestled with over the years. Given some way of accessing some sequences (flat files, STDIN and STDOUT, variables, etc.), and a format description, it provides access to a stream of Bio::SeqI objects tailored to the information provided by the format. The format description is, technically, optional. SeqIO can try to guess based on known file extensions or content, but if your source doesn't have a standard file extension or comprehensible content, or isn't even a file at all, it throws up its hands and tries fasta. Unless you are always working with FASTA files, it is a good idea to get into the practice of always specifying the format.

Sequences can be fed into the SeqIO system in a variety of ways. The only requirement is that the sequence be contained in some kind of standard Perl 'Handle' (see IO::Handle (http://search.cpan.org /search?query=IO::Handle&mode=all)). Most people will make use of the traditional handles: file handles, and STDIN/STDOUT. However, Perl provides ways of turning the contents of a string into a Handle as well (more on this below), so just about anything can be fed into SeqIO to get at the sequence information contained within it. What SeqIO does is create a Handle, or take a given Handle, and parse it into SeqI compliant objects, one for each entry at a time. It also knows, for each of the supported formats, things like which record-separator (e.g. "//" for the GenBank sequence format, ">header" for the FASTA sequence format, etc.) to use, and most importantly, how to parse their key-value based information. SeqIO does all of this for you, so that you can focus on the things you want to do with the information, rather than worrying about how to get the information.

Formats

BioPerl's SeqIO system understands lot of formats and can interconvert all of them. Here is a current

listing of formats, as of version 1.6.

Name	Description	File extension	Module
abi	ABI tracefile	ab[i1]	Bio::SeqIO::abi
асе	Ace database	ace	Bio::SeqIO::ace
agave	AGAVE XML		Bio::SeqIO::agave
alf	ALF tracefile	alf	Bio::SeqIO::alf
asciitree	write-only, to visualize features		Bio::SeqIO::asciitree
bsml	BSML, using XML::DOM (http://search.cpan.org /search?query=XML::DOM& mode=all)	bsml	Bio::SeqIO::bsml
bsml_sax	BSML, using XML::SAX (http://search.cpan.org /search?query=XML::SAX& mode=all)		Bio::SeqIO::bsml_sax
chadoxml	CHADO sequence format		Bio::SeqIO::chadoxml
chaos	CHAOS sequence format		Bio::SeqIO::chaos
chaosxml	Chaos XML		Bio::SeqIO::chaosxml
ctf	CTF tracefile	ctf	Bio::SeqIO::ctf
embl	EMBL database	ebl emb dat	Bio::SeqlO::embl
entrezgene	Entrez Gene ASN1		Bio::SeqIO::entrezgene
excel	Excel		Bio::SeqlO::excel
exp	Staden EXP format	ехр	Bio::SeqlO::exp
fasta	FASTA	fast seq fa fsa nt aa	Bio::SeqlO::fasta
fastq	quality score data in FASTA-like format	fastq	Bio::SeqIO::fastq
flybase_chadoxml	variant of Chado XML		Bio::SeqIO::flybase_chadoxml
game	GAME XML		Bio::SeqIO::game
gcg	GCG	gcg	Bio::SeqIO::gcg
genbank	GenBank	gbank genbank	Bio::SeqIO::genbank
interpro	InterProScan XML		Bio::SeqIO::interpro
kegg	KEGG		Bio::SeqIO::kegg
largefasta	Large files, fasta format		Bio::SeqIO::largefasta
lasergene	Lasergene format		Bio::SeqIO::lasergene
locuslink	LocusLink LL_tmpl		Bio::SeqIO::locuslink

metafasta			Bio::SeqIO::metafasta
phd	Phred	phred	Bio::SeqIO::phd
pir	PIR database	pir	Bio::SeqIO::pir
pln	PLN tracefile	pln	Bio::SeqIO::pIn
qual	Phred		Bio::SeqIO::qual
raw	plain text	txt	Bio::SeqIO::raw
scf	Standard Chromatogram Format	scf	Bio::SeqIO::scf
seqxml	SeqXML sequence format (http://seqxml.org) using XML::LibXML (http://search.cpan.org /search?query=XML::LibXML& mode=all) and XML::Writer (http://search.cpan.org /search?query=XML::Writer& mode=all)	xml	Bio::SeqIO::seqxmI
strider	DNA Strider format		Bio::SeqIO::strider
swiss	SwissProt	sp	Bio::SeqIO::swiss
tab	tab-delimited		Bio::SeqIO::tab
table	Table		Bio::SeqIO::table
tigr	TIGR XML		Bio::SeqIO::tigr
tigrxml	TIGR Coordset XML		Bio::SeqIO::tigrxml
tinyseq	NCBI TinySeq XML		Bio::SeqIO::tinyseq
ztr	ZTR tracefile	ztr	Bio::SeqIO::ztr

Note: Bio::SeqIO needs the bioperl-ext package and the io_lib library from the Staden (http://staden.sourceforge.net/) package in order to read the scf, abi, alf, pln, exp, ctf, ztr formats.

For some one of the initial perplexities of BioPerl is the variety of different sequence objects, and this gives rise to questions like "*How do I convert a PrimarySeq object into a RichSeq object?*". The answer is that one should never have to do this, SeqIO takes care of all these conversions. The reason for these different objects in the first place has to with the information, or lack of information, inherent to the different file formats. Though we just said that the conversions are done automatically we offer this table that shows some common formats and their corresponding object types, just to satisfy the curious.

Table 2: Bio::SeqIO modules and formats supported

Format	Object Type	
fasta	Bio::Seq	
genbank	Bio::Seq::RichSeq	

pir	Bio::Seq
embl	Bio::Seq::RichSeq
raw	Bio::Seq
ace	Bio::PrimarySeq
bsml	Bio::Seq::RichSeq
swiss	Bio::Seq::RichSeq

Working Examples

The simplest script for parsing sequence files is written out below. It prints out the accession number for each entry in the file.

```
# first, bring in the SegIO module
use Bio::SeqIO;
# Notice that you do not have to use any Bio:SeqI
# objects, because SeqIO does this for you. In fact, it
# even knows which SeqI object to use for the provided
₩ format.
# Bring in the file and format, or die with a nice
# usage statement if one or both arguments are missing.
my $usage = "getaccs.pl file format\n";
my $file = shift or die $usage;
my $format = shift or die $usage;
# Now create a new SeqIO object to bring in the input
# file. The new method takes arguments in the format
# key => value, key => value. The basic keys that it
# can accept values for are '-file' which expects some
# information on how to access your data, and '-format'
# which expects one of the Bioperl-format-labels mentioned
# above. Although it is optional, it is good
# programming practice to provide > and < in front of any</pre>
# filenames provided in the -file parameter. This makes the
# resulting filehandle created by SeqIO explicitly read (<)</pre>
# or write(>). It will definitely help others reading your
# code understand the function of the SeqIO object.
my $inseq = Bio::SeqIO->new(
                            -file => "<$file",</pre>
                            -format => $format,
                            ):
# Now that we have a seq stream,
# we need to tell it to give us a $seq.
We do this using the 'next_seq' method of SeqIO.
while (my $seq = $inseq->next seq) {
    print $seq->accession_number,"\n";
```

This script takes two arguments on the commandline, and input filename and the format of the input file. This is the basic way to access the data in a Genbank file. It is the same for fasta, swissprot, ace, and all the others as well, provided that the correct Bioperl-format-label is provided.

Notice that SeqIO naturally works over sets of sequences in files, not just one sequence. Each call to next_seq will return the next sequence in the 'stream', or undef if the end of the file/stream has been reached. This allows you to read in the contents of your data one sequence at a time, which conserves

memory, in contrast with pulling everything into memory first. The undef that is returned at the end of file/stream is important, as it allows you to wrap successive calls to next_seq in a while loop. This code snippet would load up all the sequences in a EMBL file into an array:

```
use strict;
'use Bio::SeqI0;
my $input file = shift;
my $seq_in = Bio::SeqIO->new(
                              -format => 'embl',
                              -file => $input file,
                              );
# loads the whole file into memory - be careful
# if this is a big file, then this script will
# use a lot of memory
imy $seq;
my @seq_array;
while( $seq = $seq in->next seq() ) {
    push(@seq_array,$seq);
.
# now do something with these. First sort by length,
# find the average and median lengths and print them out
@seq array = sort { $a->length <=> $b->length } @seq array;
my $total = 0;
my scount = 0;
foreach my $seq ( @seq_array ) {
    $total += $seq->length;
    $count++;
print "Mean length ",$total/$count," Median ",$seq_array[$count/2]->length,"\n";
```

Now, what if we want to convert one format to another? When you create a Bio::SeqIO object to read in a flat file, the magic behind the curtains is that each call to next_seq is a complex parsing of the next sequence record into a SeqI object - not a single line, but the entire record!! It knows when to start parsing, and when to stop and wait for the next call to next_seq. It knows how to get at the DIVISION information stored on the LOCUS line *etc*. To get that Bio::SeqI information back out to a new file, of a different format (or of the same format, but with sequences grouped in a new way), Bio::SeqIO has a second method called write_seq that reverses the process done by next_seq. It knows how to write all of the data contained in the SeqI object into the right places, with the correct labels, for any of the supported formats. Let's make this more concrete by writing a universal format translator:

```
);
# write each entry in the input file to the output file
while (my $inseq = $seq_in->next_seq) {
    $seq_out->write_seq($inseq);
}
```

You can think of the two variables, <code>\$seq_in</code> and <code>\$seq_out</code> as being rather special types of filehandles which "know" about sequences and sequence formats. However, rather than using the <F> operator to read files you use the <code>next_seq()</code> method and rather than saying <code>print F \$line"</code> you say <code>\$seqio->write_seq(\$seq_object)</code>.

Note: Bio::SeqIO actually allows you to make use of a rather scary/clever part of Perl that can "mimic" filehandles, so that the <F> operator returns sequences and the print F operator writes sequences. However, for most people, including us, this looks really really weird and leads to probably more confusion.

Notice that the universal formatter only required a few more lines of code than the accession number lister and mean sequence length analyzer (mostly to get more command-line args). This is the beauty of using the BioPerl system. It doesn't take a lot of code to do some really complex things.

Now, let's play around with the previous code, changing aspects of it to exploit the functionality of the SeqIO system. Let's take a stream from STDIN, so that we can use other programs to stream data of a particular format into the program, and write out a file of a particular format. Here we have to make use of two new things: one Perl-specific, and one SeqIO-specific. Perl allows you to 'GLOB' a filehandle by placing a '*' in front of the handle name, making it available for use as a variable, or as in this case, as an argument to a function. In concert, Bio::SeqIO allows you to pass a GLOB'ed filehandle to it using the -fh parameter in place of the -file parameter. Here is a program that takes a stream of sequences in a given format from STDIN, meaning it could be used like this:

```
>cat myseqs.fa | all2y.pl fasta newseqs.gb genbank
```

The code for all2y.pl is:

```
use Bio::SeqI0;
# get command-line arguments, or die with a usage statement
my $usage = "all2y.pl informat outfile outfileformat\n";
my $informat = shift or die $usage;
my $outfile = shift or die $usage;
my $outformat = shift or die $usage;
# create one SeqIO object to read in, and another to write out
# *STDIN is a 'globbed' filehandle with the contents of Standard In
my $seqin = Bio::SeqIO->new(
                           -fh
                                  \Rightarrow \land *STDIN,
                           -format => $informat,
                           );
my $seqout = Bio::SeqIO->new(
                            -file => ">$outfile",
                            -format => $outformat,
                            ):
.
# write each entry in the input file to the output file
while (my $inseq = $seqin->next_seq) {
    $seqout->write_seq($inseq);
```

Why use files at all? We can pipe STDIN to STDOUT, which could allow us to plug this into some other

pipeline, something like:

```
cat *.seq | in2out.pl EMBL Genbank | someother program
The code for in2out.pl could be:
use Bio::SeqI0;
```

```
# get command-line arguments, or die with a usage statement
           = "in2out.pl informat outformat\n";
my $usage
my $informat = shift or die $usage;
my $outformat = shift or die $usage;
# create one SeqIO object to read in, and another to write out
my $seqin = Bio::SeqIO->new(
                              -fh
                                      \Rightarrow \land *STDIN,
                              -format => $informat,
                              );
my $outseq = Bio::SeqIO->new(
                               -fh
                                      \Rightarrow \ \mathsf{STDOUT},
                               -format => $outformat,
                               );
# write each entry in the input to the output
while (my $inseq = $seqin->next_seq) {
    $outseq->write_seq($inseq);
```

To and From a String

A popular question many people have asked is "*What if I have a string that has a series of sequence records in some format, and I want to make it a Bio::Seq object?*" You might want to do this if you allow users to paste in sequence data into a web form, and then do something with that sequence data. This can be accomplished by turning the contents of a string into a standard globbed perl handle (since Perl 5.8.0 this can be done with open. The IO::String (http://search.cpan.org/search?query=IO::String& mode=all) module can be used in other cases) and using the -fh parameter to supply a filehandle rather than a filepath.

This isn't a complete program, but gives you the most relevant bits. Assume that there is some type of CGI form processing, or some such business, that pulls a group of sequences into a variable, and also pulls the format definition into another variable. Since Bio::seqI0 uses the file extension to determine the file format when it's not specified, and because there's no file extension when using filehandles, the format needs to be supplied.

```
use IO::String;
                  # only needed for Perl versions previous to 5.8.0
use Bio::SeqI0;
## get a string into $string somehow, with its format in $format, say from a web form.
my $string = ">SEQ1\nacgt\n>revseq1\ntgca\n";
            = "fasta";
my $format
my $stringfh = I0::String->new($string);
                                                                                 # Use this for Perl BEFORE 5.8.0
open($stringfh, "<", \$string) or die "Could not open string for reading: $!"; # Use this for Perl AFTER 5.8.0 (in
my $seqio = Bio::SeqIO-> new(
                             -fh
                                    => $stringfh,
                             -format => $format,
                             ):
while( my $seq = $seqio->next_seq ) {
```

```
# process each seq
print $seq->id . ' = '.$seq->seq()."\n";
}
```

Naturally you can also take a sequence object and write it, in some format, to a string. The code would look something like this (note the direction of the less/greater than sign on the open function):

And more examples...

The -file parameter in Bio::SeqIO can take more than a filename. It can also take a string that tells it to pipe something else into it. This is of the form '-file' => 'command |'. Notice the vertical bar at the end, just before the single quote. This is especially useful when you are working with large, gzipped files because you just don't have enough disk space to unzip them (e.g. a Genbank full release file), but can make FASTA files from them. Here is a program that takes a gzipped file of a given format and writes out a FASTA file, used like:

```
gzip2fasta.pl gbpril.seq.gz Genbank gbpril.fa
```

Let the the code begin...

```
iuse Bio::SeqI0;
# get command-line arguments, or die with a usage statement
my $usage = "gzip2fasta.pl infile informat outfile\n";
my $infile = shift or die $usage;
my $informat = shift or die $usage;
my $outfile = shift or die $usage;
# create one SegIO object to read in, and another to write out
my $seqin = Bio::SeqIO->new(
                          -file => "/usr/local/bin/gunzip -c $infile |",
                          -format => $informat,
                          );
my $seqout = Bio::SeqIO->new(
                           -file => ">$outfile",
                           -format => 'Fasta',
                           );
# write each entry in the input to the output file
while (my $inseq = $seqin->next seq) {
    $seqout->write_seq($inseq);
```

Bioperl also allows a 'pipe - out' to be given as an argument to -file. This is of the form '-file' => "| command". This time the vertical bar is at the beginning, just after the first quote. Let's write a program to

take an input file, and write it directly to a WashU Blastable Database, without ever writing out a FASTA file, like:

```
- - - - -
    any2wublastable.pl myfile.gb Genbank mywublastable p
And the code for any2wublastable.pl is:
!use Bio::SeqI0;
# get command-line arguments, or die with a usage statement
            = "any2wublastable.pl infile informat outdbname outdbtype\n";
mv $usage
my $infile
             = shift or die $usage;
my $informat = shift or die $usage;
my $outdbname = shift or die $usage;
my $outdbtype = shift or die $usage;
# create one SegIO object to read in, and another to write out
my $seqin = Bio::SeqIO->new(
                                   => "<$infile",
                             -file
                            -format => $informat,
                            );
my $seqout = Bio::SeqIO->new(
                             -file => "| /usr/local/bin/xdformat -o $outdbname -${outdbtype} -- -",
                             -format => 'Fasta',
                             ):
# write each entry in the input to the output
while (my $inseq = $seqin->next_seq) {
    $seqout->write_seq($inseq);
```

Some of the more seasoned Perl hackers may have noticed that the new method returns a reference, which can be placed into any of the data structures used in Perl. For instance, let's say you wanted to take a GenBank file with multiple sequences, and split the human sequences out into a human.gb file, and all the rest of the sequences into the other.gb file. In this case, we will use a hash to store the two handles where 'human' is the key for the human output, and 'other' is the key to other, so the usage would be:

```
splitgb.pl inseq.gb
```

Here's what splitgb.pl looks like:

```
use Bio::SeqI0;
# get command-line argument, or die with a usage statement
my $usage = "splitgb.pl infile\n";
my $infile = shift or die $usage;
my $inseq = Bio::SeqIO->new(
                                   => "<$infile",
                             -file
                             -format => 'Genbank',
                             );
my %outfiles = (
                 'human' => Bio::SegIO->new(
                                            -file
                                                   => '>human.gb',
                                            -format => 'Genbank',
                                            ),
                'other' => Bio::SeqIO->new(
                                                   => '>other.gb',
                                            -file
                                            -format => 'Genbank',
                                            ),
```

```
);
while (my $seqin = $inseq->next_seq) {
    # here we make use of the species attribute, which returns a
    # species object, which has a binomial attribute that
    # holds the binomial species name of the source of the sequence
    if ($seqin->species->binomial =~ m/Homo sapiens/) {
        $outfiles{'human'}->write_seq($seqin);
    } else {
        $outfiles{'other'}->write_seq($seqin);
    }
}
```

Now, let's use a multidimensional hash to hold a GenBank output and a FASTA output for both splits.

```
use Bio::SeqI0;
# get command-line argument, or die with a usage statement
my $usage = "splitgb.pl infile\n";
my $infile = shift or die $usage;
my $inseq = Bio::SeqIO->new(
                             -file => "<$infile",</pre>
                             -format => 'Genbank'.
                             ):
my %outfiles = (
                human =>
                          ł
                           Genbank => Bio::SeqIO->new(
                                                       -file => '>human.gb',
                                                       -format => 'Genbank',
                                                       ),
                           Fasta => Bio::SeqIO->new(
                                                       -file => '>human.fa',
                                                       -format => 'Fasta',
                                                       ).
                           }.
                other =>
                           Genbank => Bio::SeqIO->new(
                                                       -file => '>other.gb',
                                                       -format => 'Genbank',
                                                       ),
                                   => Bio::SeqIO->new(
                           Fasta
                                                       -file => '>other.fa'.
                                                       -format => 'Fasta',
                                                       ).
                           }
                );
while (my $seqin = $inseq->next_seq) {
    if ($seqin->species->binomial =~ m/Homo sapiens/) {
        $outfiles{'human'}->{'Genbank'}->write_seq($seqin);
        $outfiles{'human'}->{'Fasta'}->write_seq($seqin);
    } else {
        $outfiles{'other'}->{'Genbank'}->write_seq($seqin);
        $outfiles{'other'}->{'Fasta'}->write_seq($seqin);
    }
```

And finally, you might want to make use of the SeqIO object in a Perl one-liner. Perl one-liners are Perl programs that make use of flags to the Perl binary allowing you to run programs from the command-line without actually needing to write a script into a file. The -e flag takes a quoted string, usually single quoted, and attempts to execute it as code, while the -M flag takes a module name and tells the one-liner to use that module. When using a single quote to enclose the string to -e, you also have to make use of perl's string modifier q(string) to single quote a string without confusing the shell. Let's find out how many GSS sequences are in gbpril.seq.gz. Note that we have placed new-line characters in this to make it easier to read, but in practice you wouldn't actually hit the return key until you were ready to run the

program.

Caveats

Because BioPerl uses a single, generalized data structure to hold sequences from all formats, it does impose its own structure on the data. For this reason, a little common sense is necessary when using the system. For example, a person who takes a flat file pulled directly from GenBank, and converts it to another GenBank file with BioPerl, will be surprised to find subtle differences between the two files - try "diff_origfile_newfile" to see what we are talking about. Just remember when using BioPerl that it was never designed to "round trip" your favorite formats. Rather, it was designed to store sequence data from many widely different formats into a common object framework and make that framework available to other sequence manipulation tasks in a programmatic fashion.

Error Handling

If you gave an impossible filename to the first script, it would have in fact died with an informative error message. In programming jargon, this is called "throwing an exception". An example would look like:

These exceptions are very useful when errors occur because you can see the full route, or "stack trace", of where the error occurred and right at the end of this is the line number of the script which caused the error, which in this case we called t.pl.

The fact that these sorts of errors are automatically detected and by default cause the script to stop is a good thing, but you might want to handle these yourself. To do this you need to "catch the exception" as follows:

- - - - - - - - - -

```
use strict;
use Bio::SeqIO;
my $input_file = shift;
my $output_file = shift;
# we have to declare $seq_in and $seq_out before
# the eval block as we want to use them afterwards
my $seq_in;
my $seq_out;
eval {
    $seq_in = Bio::SeqIO->new(
```

The use of eval { ... } accompanied by testing the value of the \$@ variable (which is set on an error) is a generic Perl approach, and will work with all errors generated in a Perl program, not just the ones in BioPerl. Notice that we have to declare \$seq_in and \$seq_out using my before the eval block - a common gotcha is to wrap a eval block around some my variables inside the block - and now my localizes those variables only to that block. If you use strict this error will be caught. And, of course, you are going to use strict right?

Speed, Bio::Seq::SeqBuilder

If you are processing large volumes of complex sequence data and only need to extract a few parameters (for example, if you only need the sequences from genbank files) you can use Bio::Seq::SeqBuilder to restrict what parts of your data Bio::SeqIO will parse, saving lots of time and speeding up your program.

For example, it can be 6 times faster to parse only 3 fields out of genbank files:

```
#!/usr/bin/perl
use strict;
use Bio::SeqI0;
!use Benchmark qw(:all);
my $file = "gbbct10.seq";
timethis(1, sub {
  my $in = Bio::SeqIO->new(-file => $file, -format => "genbank");
   for (1..1000) {
      my $seq = $in->next seq;
   }
});
timethis(1, sub {
  my $in = Bio::SeqIO->new(-file => $file, -format => "genbank");
   my $builder = $in->sequence_builder();
   $builder->want none();
   $builder->add wanted slot('display id','desc','seq');
   for (1..1000) {
      my $seq = $in->next_seq;
   }
});
rtimethis 1: 10 wallclock secs ( 9.64 usr +  0.02 sys =  9.66 CPU) @  0.10/s (n=1)
            (warning: too few iterations for a reliable count)
timethis 1: 1 wallclock secs ( 1.63 usr +  0.00 sys =  1.63 CPU) @  0.61/s (n=1)
```

(warning: too few iterations for a reliable count)

See HOWTO: Feature-Annotation for more discussion.

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