Introduction to Biopython

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(based on a slides by Stuart Brown, NYU)

Learning Goals

- Biopython as a toolkit
- Seq objects and their methods
- SeqRecord objects have data fields
- SeqIO to read and write sequence objects
- Direct access to GenBank with Entrez.efetch
- Working with BLAST results

Modules

- Python functions are divided into three sets
    - A small core set that are always available
    - Some built-in modules such as **math** and **os** that can be imported from the basic install (ie. >>> import math)
    - An number of optional modules that must be downloaded and installed before you can import them: code that uses such modules is said to have “dependencies”
    - Most are available in different Linux distributions, or via pypy.org using pip (the Python Package Index)
- Anyone can write new Python modules, and often several different modules are available that can do the same task

Object Oriented Code

- Python implements oject oriented programming
- Classes bundle data and functionality

magic method”

private

```
class MyClass:
    """A simple example class"""
    def __init__(self):
        self.data = []
        self._priv = “fuggetaboutit”

    def say_hi(self):
        return 'hello world'
    def __str__(self):
        return ”yoohoo”+str(self.data[:3]) +”...”
```

instantiation

```
z = MyClass()
z.data = [1,”foo”,”bar”,-5,9]
print(z) #???
```

The Seq object

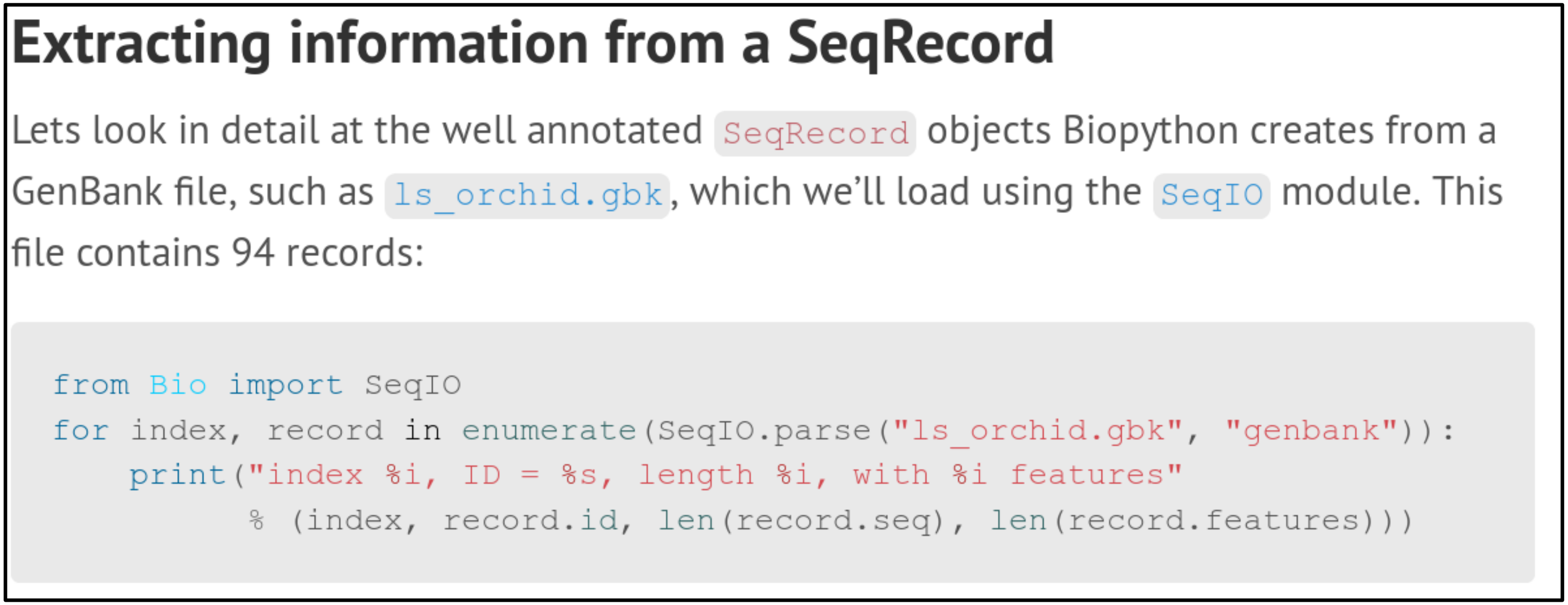
- The Seq object class is simple and fundamental for a lot of Biopython work. A Seq object can contain DNA, RNA, or protein.
- It contains a string and a defined alphabet for that string.
- The alphabets are actually defined objects such as `IUPACAmbiguousDNA` or `IUPACProtein`
  - Which are defined in the Bio.Alphabet module
  - A Seq object with a DNA alphabet has some different methods than one with an Amino Acid alphabet

```
>>> from Bio.Seq import Seq
>>> from Bio.Alphabet import IUPAC
>>> my_seq = Seq('AGTACACTGGT', IUPAC.unambiguous_dna)
>>> my_seq
Seq('AGTACACTGGT', IUPAC.unambiguous_dna())
>>> print(my_seq)
AGTACACTGGT
```

This command creates the Seq object

SeqRecord Example

http://biopython.org/wiki/SeqRecord



Extracting information from a SeqRecord

Lets look in detail at the well annotated `SeqRecord` objects Biopython creates from a GenBank file, such as `ls_orchid.gbk`, which we'll load using the `SeqIO` module. This file contains 94 records:

```python
from Bio import SeqIO
for index, record in enumerate(SeqIO.parse("ls_orchid.gbk", "genbank")):
    print("index %i, ID = %s, length %i, with %i features"
          % (index, record.id, len(record.seq), len(record.features)))
```

SeqIO and FASTA files

- **SeqIO** is the all purpose file read/write tool for SeqRecords
  - SeqIO can read many file types: http://biopython.org/wiki/SeqIO
- SeqIO has .read() and .write() methods
  - (do not need to “open” file first)
- It can read a text file in FASTA format
- In Biopython, fasta is a type of SeqRecord with specific fields
  - grab the file: NC_005816.fna, and saved it as a text file in your current directory

```
>>> from Bio import SeqIO
>>> gene = SeqIO.read("NC_005816.fna", "fasta")
>>> gene.id
'gi|45478711|ref|NC_005816.1|'
>>> gene.seq
Seq('TGTAACGAACGGTGCAATAGTGATCCACACCCAACGCCTGAAATCAGATCCAGG...CTG',
SingleLetterAlphabet())
>>> len(gene.seq)
9609
```

Multiple FASTA Records in one file

- The FASTA format can store many sequences in one text file
- SeqIO.parse() reads the records one by one
- This code creates a list of SeqRecord objects:

```
>>> from Bio import SeqIO
>>> handle = open("example.fasta", "rU")
                  # “handle” is a pointer to the file
>>> seq_list = list(SeqIO.parse(handle, "fasta"))
>>> handle.close()
>>> print(seq_list[0].seq)    #shows the first sequence in the list
```

Seq-ing deeper

Seq: an object for containing sequence information. Basic sequence operations.

```
>>> from Bio.Seq import Seq
>>> dir(Seq)
['__add__', '__class__', '__contains__', '__delattr__',
'__dict__', '__dir__', '__doc__', '__eq__',
'__format__', '__ge__', '__getattribute__',
'__getitem__', '__gt__', '__hash__', '__init__',
'__le__', '__len__', '__lt__', '__module__', '__ne__',
'__new__', '__radd__', '__reduce__', '__reduce_ex__',
'__repr__', '__setattr__', '__sizeof__', '__str__',
'__subclasshook__', '__weakref__',
'_get_seq_str_and_check_alphabet', 'back_transcribe',
'complement', 'count', 'endswith', 'find', 'lower',
'lstrip', 'reverse_complement', 'rfind', 'rsplit',
'rstrip', 'split', 'startswith', 'strip', 'tomutable',
'tostring', 'transcribe', 'translate', 'ungap', 'upper']
```

Seq Source Code

```
def __init__(self, data, alphabet=Alphabet.generic_alphabet):

        # Enforce string storage
        if not isinstance(data, basestring):
            raise TypeError("The sequence data given to a Seq object should "
                            "be a string (not another Seq object etc)")
        self._data = data
        self.alphabet = alphabet
```

```
 def __repr__(self):
        """Returns a (truncated) representation of the sequence for debugging."""
        if len(self) > 60:
            # Shows the last three letters as it is often useful to see if there
            # is a stop codon at the end of a sequence.
            # Note total length is 54+3+3=60
            return "{0}('{1}...{2}', {3!r})".format(self.__class__.__name__,
                                                    str(self)[:54],
                                                    str(self)[-3:],
                                                    self.alphabet)
        else:
            return '{0}({1!r}, {2!r})'.format(self.__class__.__name__,
                                              self._data,
                                              self.alphabet)
```

Seq Source Code

```
def transcribe(self):
        base = Alphabet._get_base_alphabet(self.alphabet)
        if isinstance(base, Alphabet.ProteinAlphabet):
            raise ValueError("Proteins cannot be transcribed!")
        if isinstance(base, Alphabet.RNAAlphabet):
            raise ValueError("RNA cannot be transcribed!")

        if self.alphabet == IUPAC.unambiguous_dna:
            alphabet = IUPAC.unambiguous_rna
        elif self.alphabet == IUPAC.ambiguous_dna:
            alphabet = IUPAC.ambiguous_rna
        else:
            alphabet = Alphabet.generic_rna
        return Seq(str(self).replace('T', 'U').replace('t', 'u'), alphabet))
```

Create your own sequence class

Base class

```
from Bio.Seq import Seq
class MySeq(Seq):
    def __repr__(self):
        if len(self) > 60:
            # Shows the last three letters as it is often useful to see if there
            # is a stop codon at the end of a sequence.
            # Note total length is 54+3+3=60
            return "{0}('{1}---{2}', {3!r})".format(self.__class__.__name__,
                                                    str(self)[:54],
                                                    str(self)[-3:],
                                                    self.alphabet)

        else:
            return '{0}({1!r}, {2!r})'.format(self.__class__.__name__,
                                              self._data,
                                              self.alphabet)
```

Derived class

Was “...”

- The new __repr__ overrides the old __repr__
- Everything else remains the same!
- MySeq is a *derived class* of Seq
- Also, MySeq is *inherited* from Seq

Direct Access to GenBank

- BioPython has modules that can directly access databases over the Internet
- The Entrez module uses the NCBI Efetch service
- Efetch works on many NCBI databases including protein and PubMed literature citations
- The ‘gb’ data type contains much more annotation information, but rettype=‘fasta’ also works
- With a few tweaks, this code could be used to download a list of GenBank ID’s and save them as FASTA or GenBank files:

```
  >>> from Bio import Entrez
  >>>Entrez.email = "you@iastate.edu"
  >>> handle = Entrez.efetch(db="nucleotide", id="186972394",  rettype="gb",
retmode="text")
>>> record = SeqIO.read(handle, "genbank")
>>> print(record)
ID: EU490707.1
Name: EU490707
Description: Selenipedium aequinoctiale maturase K (matK) gene, partial cds;
chloroplast.
Number of features: 3
/sequence_version=1
/source=chloroplast Selenipedium aequinoctiale
/taxonomy=['Eukaryota', 'Viridiplantae', 'Streptophyta', 'Embryophyta',
'Tracheophyta', 'Spermatophyta', 'Magnoliophyta', 'Liliopsida', 'Asparagales',
'Orchidaceae', 'Cypripedioideae', 'Selenipedium']
/keywords=['']
/references=[Reference(title='Phylogenetic utility of ycf1 in orchids: a plastid gene
more variable than matK', ...), Reference(title='Direct Submission', ...)]
/accessions=['EU490707']
/data_file_division=PLN
/date=15-JAN-2009
/organism=Selenipedium aequinoctiale
/gi=186972394
Seq('ATTTTTACGAACCTGTGGAAATTTTTGGTTATGACAATAAATCTAGTTTAGTA...GAA',
IUPACAmbiguousDNA())
```

These are sub-fields of the .annotations field

BLAST

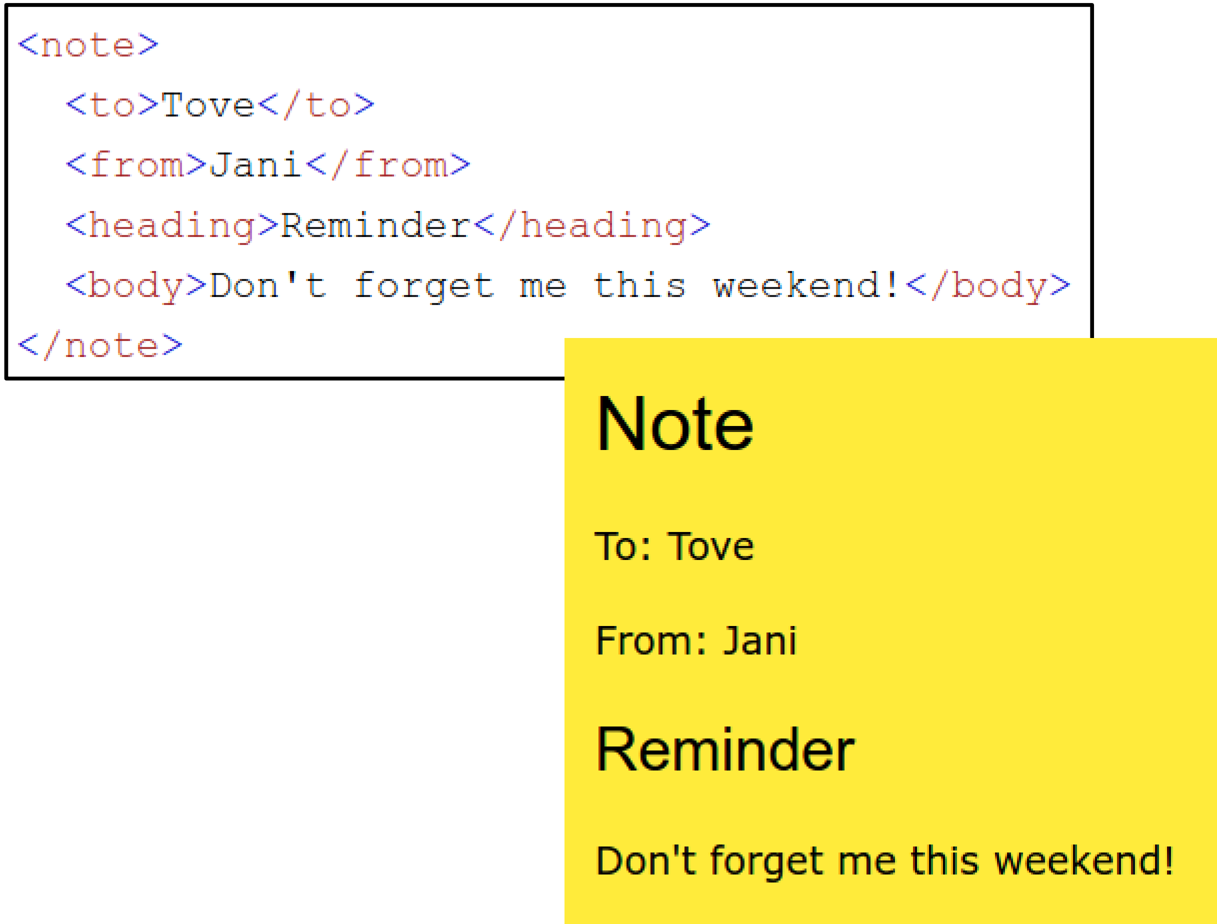
- BioPython has several methods to work with the popular NCBI BLAST software
- NCBIWWW.qblast() sends queries directly to the NCBI BLAST server. The query can be a Seq object, FA file, or a GenBank ID.

Do this now

```
>>> from Bio.Blast import NCBIWWW
>>> from Bio import SeqIO
>>> query = SeqIO.read("test.fasta", format="fasta")
>>> result_handle = NCBIWWW.qblast("blastn", "nt", query.seq)
>>> blast_result = result_handle.read()
>>> blast_file = open("my_blast.xml", "w") # create an xml output file
>>> blast_file.write(blast_result)
>>> blast_file.close()     # tidy up
>>> result_handle.close()
```

XML

Extensible Markup Language
Used to encode documents in a form that is human readable and machine readable



```
<note>
  <to>Tove</to>
  <from>Jani</from>
  <heading>Reminder</heading>
  <body>Don't forget me this weekend!</body>
</note>
```

Note

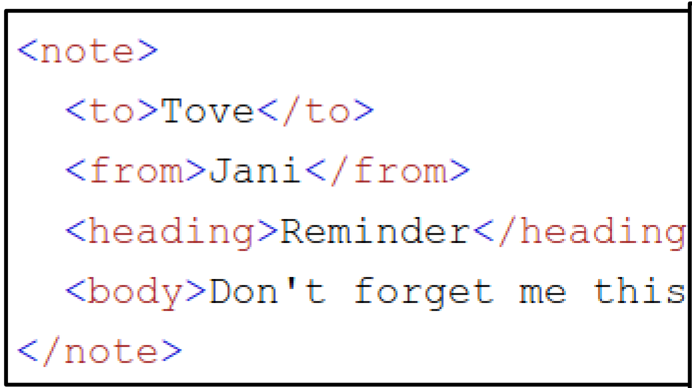
To: Tove

From: Jani

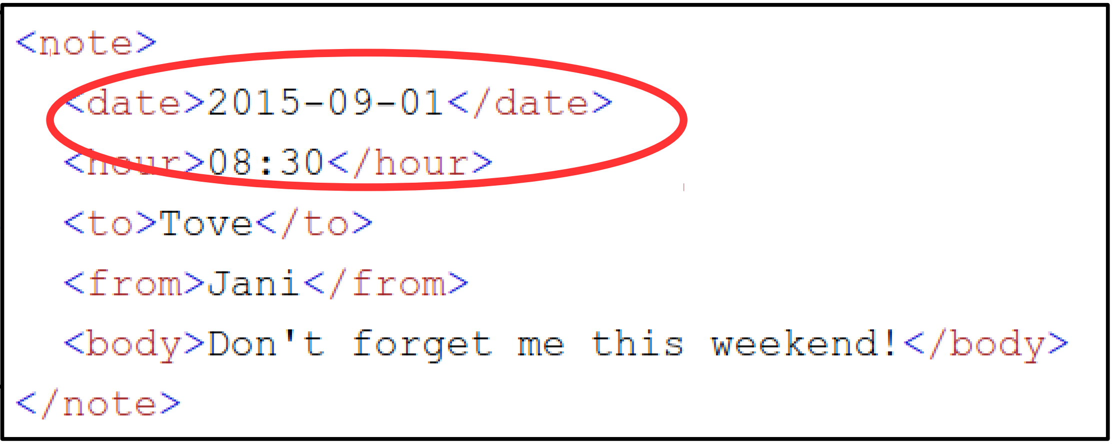
Reminder

Don't forget me this weekend!

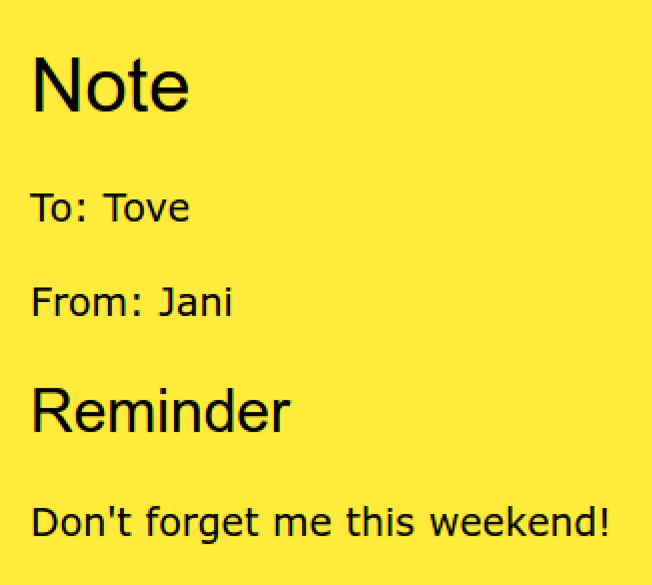
XML is Extensible



```
<note>
  <to>Tove</to>
  <from>Jani</from>
  <heading>Reminder</heading
  <body>Don't forget me this
</note>
```



```
<note>
  <date>2015-09-01</date>
  <hour>08:30</hour>
  <to>Tove</to>
  <from>Jani</from>
  <body>Don't forget me this weekend!</body>
</note>
```



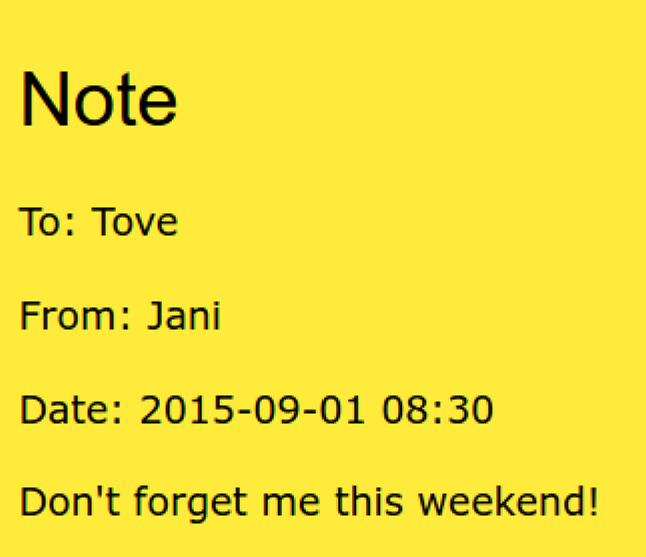
Note

To: Tove

From: Jani

Reminder

Don't forget me this weekend!



Note

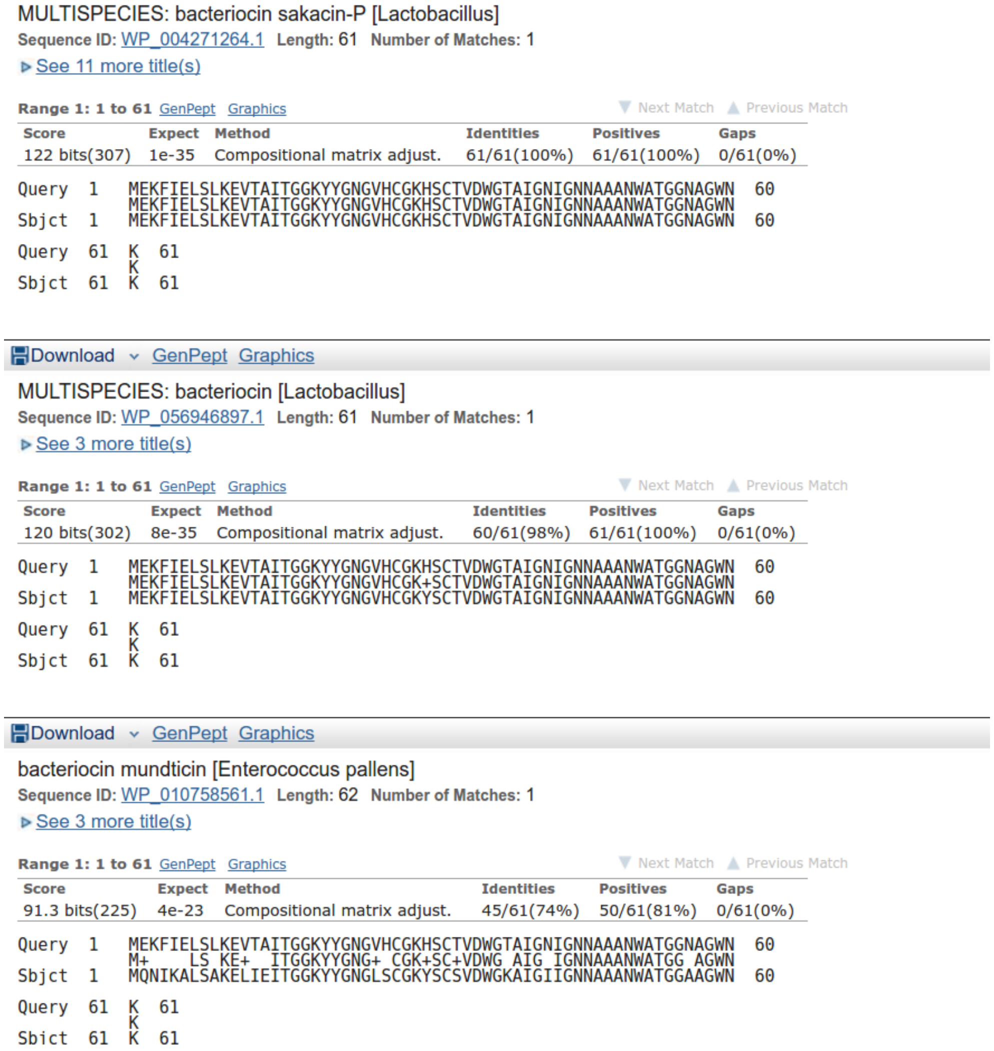
To: Tove

From: Jani

Date: 2015-09-01 08:30

Don't forget me this weekend!

BLAST XML



MULTISPECIES: bacteriocin sakacin-P [Lactobacillus]

Sequence ID: WP_004271264.1 **Length:** 61 **Number of Matches:** 1

▸ See 11 more title(s)

Range 1: 1 to 61 GenPept Graphics ▼ Next Match ▲ Previous Match

Score	Expect	Method	Identities	Positives	Gaps
122 bits(307)	1e-35	Compositional matrix adjust.	61/61(100%)	61/61(100%)	0/61(0%)

```
Query  1    MEKFIELSLKEVTAITGGKYYGNGVHCGKHSCTVDWGTAIGNIGNNAAANWATGGNAGWN  60
            MEKFIELSLKEVTAITGGKYYGNGVHCGKHSCTVDWGTAIGNIGNNAAANWATGGNAGWN
Sbjct  1    MEKFIELSLKEVTAITGGKYYGNGVHCGKHSCTVDWGTAIGNIGNNAAANWATGGNAGWN  60

Query  61   K  61
            K
Sbjct  61   K  61
```

Download ⌄ GenPept Graphics

MULTISPECIES: bacteriocin [Lactobacillus]

Sequence ID: WP_056946897.1 **Length:** 61 **Number of Matches:** 1

▸ See 3 more title(s)

Range 1: 1 to 61 GenPept Graphics ▼ Next Match ▲ Previous Match

Score	Expect	Method	Identities	Positives	Gaps
120 bits(302)	8e-35	Compositional matrix adjust.	60/61(98%)	61/61(100%)	0/61(0%)

```
Query  1    MEKFIELSLKEVTAITGGKYYGNGVHCGKHSCTVDWGTAIGNIGNNAAANWATGGNAGWN  60
            MEKFIELSLKEVTAITGGKYYGNGVHCGK+SCTVDWGTAIGNIGNNAAANWATGGNAGWN
Sbjct  1    MEKFIELSLKEVTAITGGKYYGNGVHCGKYSCTVDWGTAIGNIGNNAAANWATGGNAGWN  60

Query  61   K  61
            K
Sbjct  61   K  61
```

Download ⌄ GenPept Graphics

bacteriocin mundticin [Enterococcus pallens]

Sequence ID: WP_010758561.1 **Length:** 62 **Number of Matches:** 1

▸ See 3 more title(s)

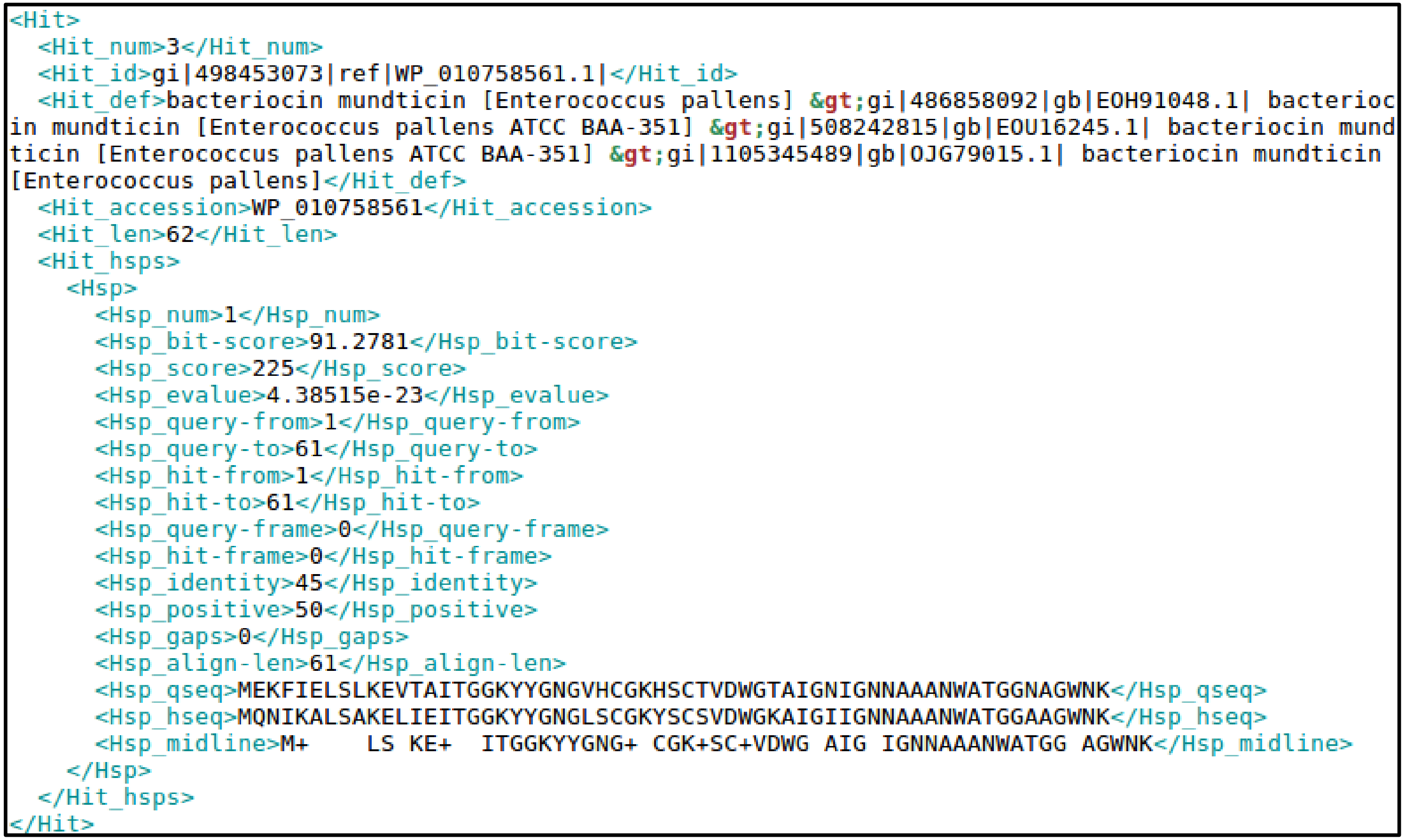
Range 1: 1 to 61 GenPept Graphics ▼ Next Match ▲ Previous Match

Score	Expect	Method	Identities	Positives	Gaps
91.3 bits(225)	4e-23	Compositional matrix adjust.	45/61(74%)	50/61(81%)	0/61(0%)

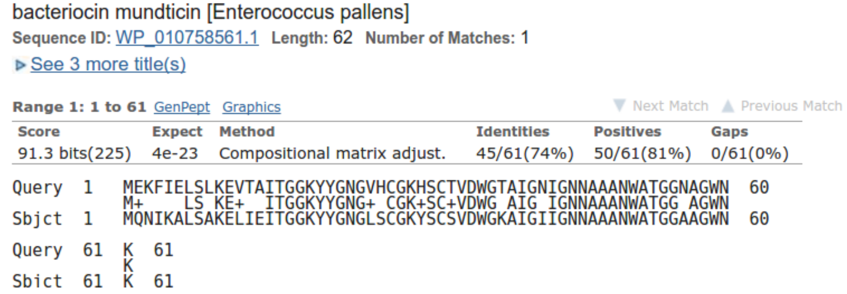
```
Query  1    MEKFIELSLKEVTAITGGKYYGNGVHCGKHSCTVDWGTAIGNIGNNAAANWATGGNAGWN  60
            M+    LS KE+  ITGGKYYGNG+ CGK+SC+VDWG AIG IGNNAAANWATGG AGWN
Sbjct  1    MQNIKALSAKELIEITGGKYYGNGLSCGKYSCSVDWGKAIGIIGNNAAANWATGGAAGWN  60

Query  61   K  61
            K
Sbjct  61   K  61
```

BLAST XML



```
<Hit>
  <Hit_num>3</Hit_num>
  <Hit_id>gi|498453073|ref|WP_010758561.1|</Hit_id>
  <Hit_def>bacteriocin mundticin [Enterococcus pallens] &gt;gi|486858092|gb|EOH91048.1| bacteriocin mundticin [Enterococcus pallens ATCC BAA-351] &gt;gi|508242815|gb|EOU16245.1| bacteriocin mundticin [Enterococcus pallens ATCC BAA-351] &gt;gi|1105345489|gb|OJG79015.1| bacteriocin mundticin [Enterococcus pallens]</Hit_def>
  <Hit_accession>WP_010758561</Hit_accession>
  <Hit_len>62</Hit_len>
  <Hit_hsps>
    <Hsp>
      <Hsp_num>1</Hsp_num>
      <Hsp_bit-score>91.2781</Hsp_bit-score>
      <Hsp_score>225</Hsp_score>
      <Hsp_evalue>4.38515e-23</Hsp_evalue>
      <Hsp_query-from>1</Hsp_query-from>
      <Hsp_query-to>61</Hsp_query-to>
      <Hsp_hit-from>1</Hsp_hit-from>
      <Hsp_hit-to>61</Hsp_hit-to>
      <Hsp_query-frame>0</Hsp_query-frame>
      <Hsp_hit-frame>0</Hsp_hit-frame>
      <Hsp_identity>45</Hsp_identity>
      <Hsp_positive>50</Hsp_positive>
      <Hsp_gaps>0</Hsp_gaps>
      <Hsp_align-len>61</Hsp_align-len>
      <Hsp_qseq>MEKFIELSLKEVTAITGGKYYGNGVHCGKHSCTVDWGTAIGNIGNNAAANWATGGNAGWNK</Hsp_qseq>
      <Hsp_hseq>MQNIKALSAKELIEITGGKYYGNGLSCGKYSCSVDWGKAIGIIGNNAAANWATGGAAGWNK</Hsp_hseq>
      <Hsp_midline>M+    LS KE+  ITGGKYYGNG+ CGK+SC+VDWG AIG IGNNAAANWATGG AGWNK</Hsp_midline>
    </Hsp>
  </Hit_hsps>
</Hit>
```



bacteriocin mundticin [Enterococcus pallens]

Sequence ID: WP_010758561.1 Length: 62 Number of Matches: 1

▷ See 3 more title(s)

Range 1: 1 to 61 GenPept Graphics ▼ Next Match ▲ Previous Match

Score	Expect	Method	Identities	Positives	Gaps
91.3 bits(225)	4e-23	Compositional matrix adjust.	45/61(74%)	50/61(81%)	0/61(0%)

```
Query  1   MEKFIELSLKEVTAITGGKYYGNGVHCGKHSCTVDWGTAIGNIGNNAAANWATGGNAGWN  60
           M+    LS KE+  ITGGKYYGNG+ CGK+SC+VDWG AIG IGNNAAANWATGG AGWN
Sbjct  1   MQNIKALSAKELIEITGGKYYGNGLSCGKYSCSVDWGKAIGIIGNNAAANWATGGAAGWN  60

Query  61  K  61
           K
Sbjct  61  K  61
```

BLAST

- BioPython has several methods to work with the popular NCBI BLAST software
- NCBIWWW.qblast() sends queries directly to the NCBI BLAST server. The query can be a Seq object, FASTA file, or a GenBank ID.

Are we there yet?

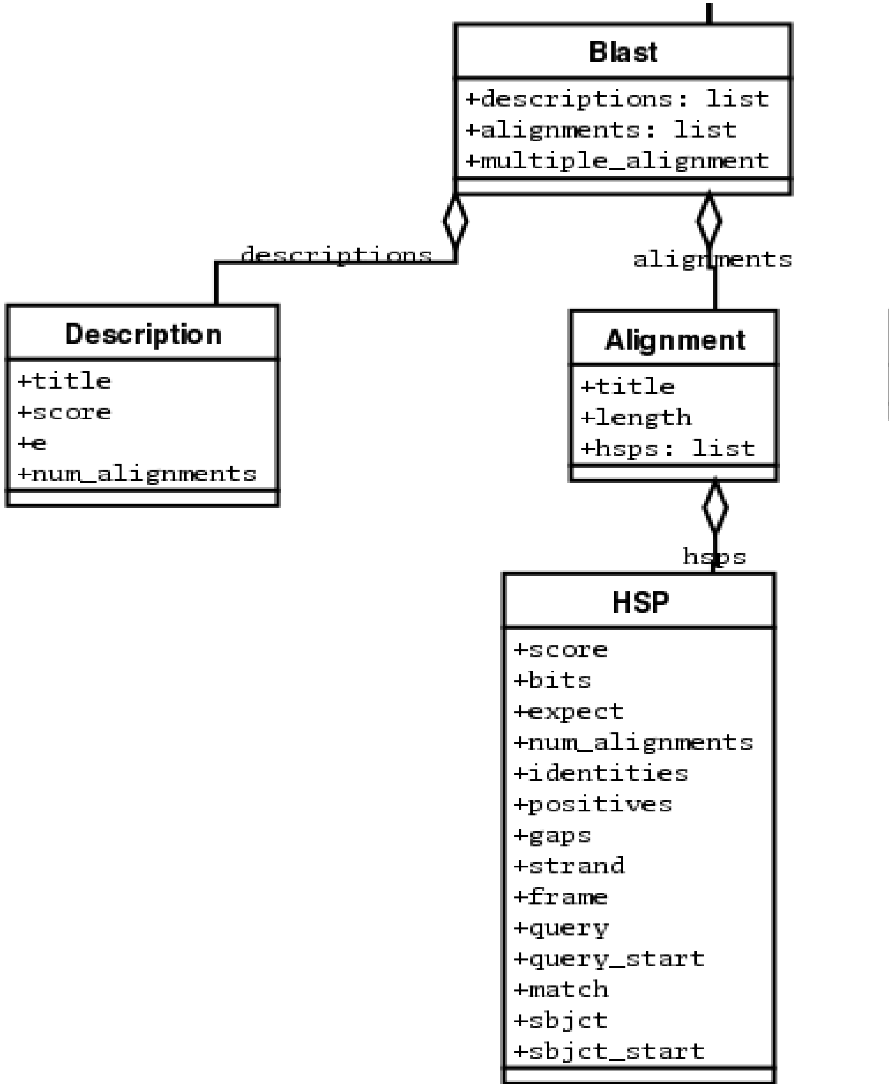
```
>>> from Bio.Blast import NCBIWWW
>>> from Bio import SeqIO
>>> query = SeqIO.read("test.fasta", format="fasta")
>>> result_handle = NCBIWWW.qblast("blastn", "nt", query.seq)
>>> blast_result = result_handle.read()
>>> blast_file = open("my_blast.xml", "w") # create an xml
output file
>>> blast_file.write(blast_result)
>>> blast_file.close()     # tidy up
>>> result_handle.close()
```

Parse BLAST Results

- It is often useful to obtain a BLAST result directly (local BLAST server or via Web browser) and then parse the result file with Python.
- Save the BLAST result in XML format
  - NCBIXML.read() for a file with a single BLAST result (single query)
  - NCBIXML.parse() for a file with multiple BLAST results (multiple queries)

```
>>> from Bio.Blast import NCBIXML
>>> handle = open("my_blast.xml")
>>> blast_record = NCBIXML.read(handle)
>>> for hit in blast_record.descriptions:
        ...     print(hit.title)
        ...     print(hit.e)
>>> handle.close()
```

BLAST Record Object



View Aligned Sequence

```
>>> from Bio.Blast import NCBIXML
>>> handle = open("my_blast.xml")
>>> blast_record = NCBIXML.read(handle)
>>> for hit in blast_record.alignments:
        for hsp in hit.hsps:
            print hit.title
            print hsp.expect
            print (hsp.query[0:75] + '...')
            print(hsp.match[0:75] + '...')
            print(hsp.sbjct[0:75] + '...')
```

```
gi|731383573|ref|XM_002284686.2| PREDICTED: Vitis vinifera cold-regulated 413
plasma membrane protein 2 (LOC100248690), mRNA
2.5739e-53
ATGCTAGTATGCTCGGTCATTACGGGTTTGGCACT-CATTTCCTCAAATGGCTCGCCTGCCTTGCGGCTATTTAC...
|||| | || ||| |||  |   || ||||||||| |||||| | |  ||| | || |   |||| || ||||| ...
ATGCCATTAAGCTTGGTGGTCTGGGCTTTGGCACTACATTTCTTGAG-TGGTTGGCTTCTTTTGCTGCCATTTAT...
```

Many Matches

- Often a BLAST search will return many matches for a single query **(save as an XML format file)**
- NCBIXML.parse() can return these as BLAST record objects in a list, or deal with them directly in a for loop.

```
from Bio.Blast import NCBIXML
E_VALUE_THRESH = 1e-20
for record in NCBIXML.parse(open("my_blast.xml")):
    if record.alignments : #skip queries with no matches
        print("QUERY: %s" % record.query[:60])
    for align in record.alignments:
        for hsp in align.hsps:
            if hsp.expect < E_VALUE_THRESH:
                print("MATCH: %s " % align.title[:60])
                print(hsp.expect)
```

Get a file by FTP in Python

```
>>> from ftplib import FTP
>>> host="ftp.sra.ebi.ac.uk"
>>> ftp=FTP(host)
>>> ftp.login()
'230 Login successful.‘
ftp.cwd('vol1/fastq/SRR020/SRR020192')
'250 Directory successfully changed.‘
>>> ftp.retrlines('LIST')
-r--r--r--    1 ftp      ftp       1777817 Jun 24 20:12 SRR020192.fastq.gz
'226 Directory send OK.'
>>> ftp.retrbinary('RETR SRR020192.fastq.gz',  open('SRR020192.fastq.gz', 'wb').write)
'226 Transfer complete.'
>>> ftp.quit()
'221 Goodbye.'
```

Learning Objectives

- Biopython is a toolkit
- Seq objects and their methods
- SeqRecord objects have data fields
- SeqIO to read and write sequence objects
- Direct access to GenBank with Entrez.efetch
- Working with BLAST results