Introduction to Biopython

Iddo Friedberg (based on a lecture by Stuart Brown, NYU)

Associate Professor

College of Veterinary Medicine

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

Download a file

- urllib is a module that lets Python download files from the internet with the request.urlretrieve method

```
>>> import urllib
>>>urllib.request.urlretrieve('https://raw.githubusercontent.com/biopython/biopython/master/Tests/GenBank/NC_005816.fna', 'yp.fasta')
```

BIOPYTHON

- Biopython is an integrated collection of modules for “biological computation” including tools for working with DNA/protein sequences, sequence alignments, population genetics, and molecular structures
- It also provides interfaces to common biological databases (e.g. GenBank) and to some common locally installed software (e.g. BLAST)

Biopython Tutorial

- Biopython has a “Tutorial & Cookbook” :

  http://biopython.org/DIST/docs/tutorial/Tutorial.html

  by: Jeff Chang, Brad Chapman, Iddo Friedberg, Thomas Hamelryck, Michiel de Hoon, Peter Cock, Tiago Antao, Eric Talevich, Bartek Wilczyński

  from which, most of the following examples are drawn

Object Oriented Code

- Python uses the concept of Object Oriented Code.
- Data structures (known as classes) can contain complex and well defined forms of data, and they can also have built in methods
- For example, many classes of objects have a “print” method
- Complex objects are built from other objects

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 (the sequence) 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

This command creates the Seq object

```
>>> 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
```

Seq objects have string methods

- Seq objects have methods that work just like string objects
- You can get the len() of a Seq, slice it, and count() specific letters in it:

```
>>> my_seq = Seq('GATCGATGGGCCTATATAGGATCGAAAATCGC', 
IUPAC.unambiguous_dna)
>>> len(my_seq)
32
>>> print(my_seq[6:9])
TGG
>>> my_seq.count("G")
9
```

Turn a Seq object into a string

- Sometimes you will need to work with just the sequence string in a Seq object using a tool that is not aware of the Seq object methods
- Turn a Seq object into a string with str()

```
>>> my_seq
Seq('GATCGATGGGCCTATATAGGATCGAAAATCGC',
IUPACUnambiguousDNA())
>>> seq_string=str(my_seq)
>>> seq_string
'GATCGATGGGCCTATATAGGATCGAAAATCGC'
```

Seq Objects have special methods

- DNA Seq objects can .translate() to protein
    - With optional translation table and to_stop=True parameters

```
>>>coding_dna=Seq("ATGGCCATTGTAATGGGCCGCTGAAAGGGTGCCCGATAG", IUPAC.unambiguous_dna)
>>> coding_dna.translate()
Seq('MAIVMGR*KGAR*', HasStopCodon(IUPACProtein(), '*'))
>>> print(coding_dna.translate(table=2, to_stop=True))
MAIVMGRWKGAR
```

Seq objects with a DNA alphabet have the reverse_complement() method:

```
>>> my_seq = Seq('TTTAAAATGCGGG', IUPAC.unambiguous_dna)
>>> print(my_seq.reverse_complement())
CCCGCATTTTAAA
```

- The Bio.SeqUtils module has some useful methods, such as GC() to calculate % of G+C bases in a DNA sequence.

```
>>> from Bio.SeqUtils import GC
>>> GC(my_seq)
46.875
```

Protein Alphabet

- You could re-define my_seq as a protein by changing the alphabet, which will change the methods that will work on it.
    - (‘G’,’A’,’T’,’C’ are valid protein letters)

```
>>> from Bio.SeqUtils import molecular_weight
>>> my_seq
Seq('AGTACACTGGT', IUPACUnambiguousDNA())
>>> print(molecular_weight(my_seq))
3436.1957

>>> my_seq.alphabet = IUPAC.protein
>>> my_seq
Seq('AGTACACTGGT', IUPACProtein())
>>> print(molecular_weight(my_seq))
912.0004
```

SeqRecord Object

- The SeqRecord object is like a database record (such as GenBank). It is a complex object that contains a **Seq** object, and also annotation fields, known as "attributes".

  .seq
  .id
  .name
  .description
  .letter_annotations
  .annotations
  .features
  .dbxrefs

- You can think of attributes as slots with names inside the SeqRecord object. Each one may contain data (usually a string) or be empty.

SeqRecord Example

```
>>> from Bio.Seq import Seq
>>> from Bio.SeqRecord import SeqRecord
>>> test_seq = Seq('GATC')
>>> test_record = SeqRecord(test_seq, id='xyz')
>>> test_record.description= 'This is only a test'
>>> print(test_record)
ID: xyz
Name: <unknown name>
Description: This is only a test
Number of features: 0
Seq('GATC', Alphabet())
>>> print(test_record.seq)
GATC
```

- Specify fields in the SeqRecord object with a **.** (dot) syntax

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
  - Lets assume you have already downloaded a FASTA file from GenBank, such as: 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
```

Database as a FASTA file

- Entire databases of sequences (DNA or protein) can be downloaded as a single FASTA file (e.g. human proteins, *Drosophila* coding CDS, Uniprot UniRef50)

FTP directory /pub/databases/uniprot/uniref/uniref50/ at ftp.uniprot.org

```
07/22/2015 02:00PM 7,171         README
07/22/2015 02:00PM 4,422         uniref.xsd
07/22/2015 02:00PM 1,755         uniref50.dtd
07/22/2015 02:00PM 3,050,098,524 uniref50.fasta.gz
07/22/2015 02:00PM 310           uniref50.release_note
```

Grab sequence from FASTA file

- If you have a large local FASTA file, and a list of sequences ('my_gene_list.txt') that you want to grab:

```
>>> from Bio import SeqIO
>>> output =open('selected_seqs.fasta', 'w')
>>> list =open('my_gene_list.txt').read().splitlines()
>>> for test in SeqIO.parse('database.fasta','fasta'):
      for seqname in list:
name = seqname.strip()
if test.id == name:
SeqIO.write(test, output, 'fasta')
 >>> output.close()
```

SeqIO for FASTQ

- FASTQ is a format for Next Generation DNA sequence data (FASTA + Quality)
- SeqIO can read (and write) FASTQ format files

```
from Bio import SeqIO
count = 0
for rec in SeqIO.parse("SRR020192.fastq", "fastq"):
        count += 1
print(count)
```

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 = "stu@nyu.edu"
# NCBI requires your identity
>>> 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, FASTA file, or a GenBank ID.

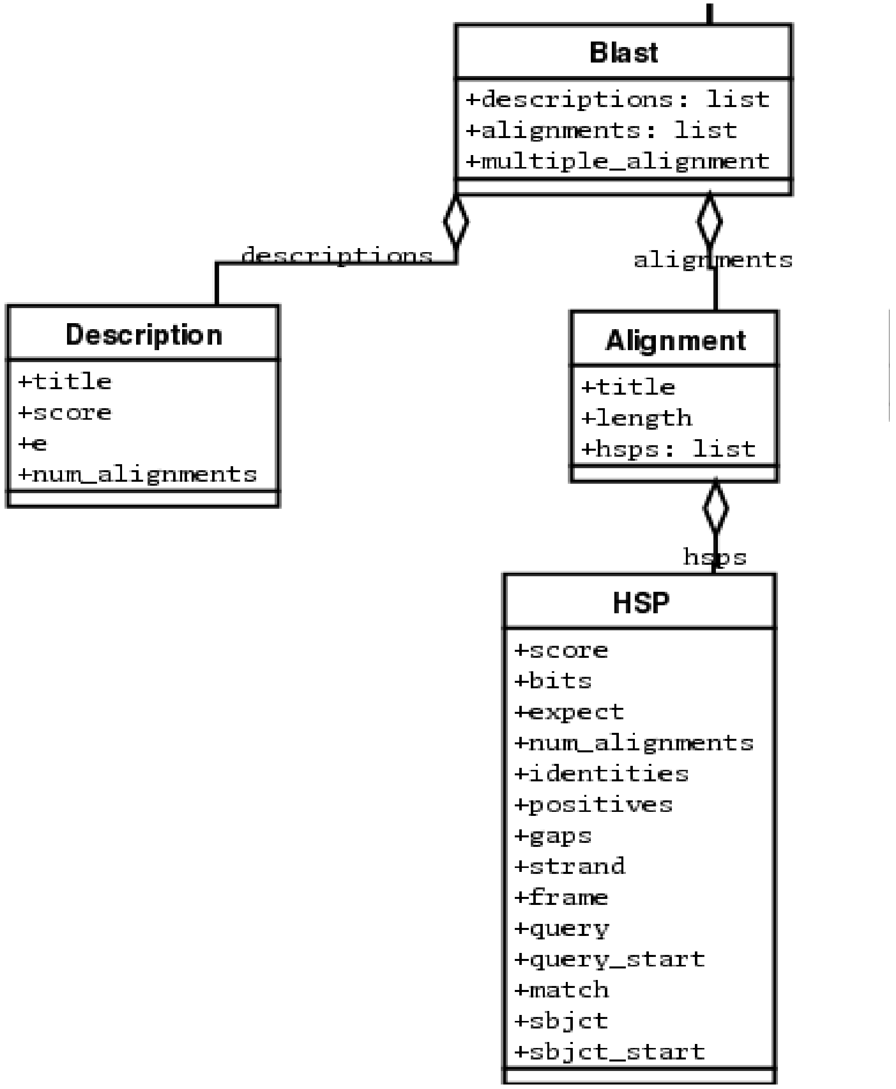
```
>>> from Bio.Blast import NCBIWWW
>>> query = SeqIO.read("test.fasta", format="fasta")
>>> result_handle = NCBIWWW.qblast("blastn", "nt", query.seq)
>>> blast_file = open("my_blast.xml", "w")
#create an xml output file
>>> blast_file.write(result_handle.read())
>>> blast_file.close()      # tidy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

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
```

Illumina Sequences

- Illumina sequence files are usually stored in the FASTQ format. Similar to FASTA, but with an additional pair of lines for the quality annotation of each base.

```
@SRR350953.5 MENDEL_0047_FC62MN8AAXX:1:1:1646:938 length=152
NTCTTTTTCTTTCCTCTTTTGCCAACTTCAGCTAAATAGGAGCTACACTGATTAGGCAGAAACTTGATTAACAGGGCTTAAGGTAA
CCTTGTTGTAGGCCGTTTTGTAGCACTCAAAGCAATTGGTACCTCAACTGCAAAAGTCCTTGGCCC
+SRR350953.5 MENDEL_0047_FC62MN8AAXX:1:1:1646:938 length=152
+50000222C@@@@@22::::8888898989::::::<<<:<<<<<<:<<<<::<<:::::<<<<<:<:<<<IIIIIGFEEGGGGGGGII@IGDGBG
GGGGGDDIIGIIEGIGG>GGGGGGDGGGGGIIHIIBIIIGIIIHIIIIGII
@SRR350953.6 MENDEL_0047_FC62MN8AAXX:1:1:1686:935 length=152
NATTTTTACTAGTTTATTCTAGAACAGAGCATAAACTACTATTCAATAAACGTATGAAGCACTACTCACCTCCATTAACATGACGTTT
TTCCCTAATCTGATGGGTCATTATGACCAGAGTATTGCCGCGGTGGAAATGGAGGTGAGTAGTG
+SRR350953.6 MENDEL_0047_FC62MN8AAXX:1:1:1686:935 length=152
+83355@@@CC@C22@@C@@CC@@C@@@CC@@@@@@@@@@@@@C?
C22@@C@:::::@@@@@@C@@@@@@@@CIGIHIIDGIGIIIIHHIIHGHHIIHHIFIIIIIHIIIIIIBIIIEIFGIIIFGFIBGDGGGGGG
FIGDIFGADGAE
@SRR350953.7 MENDEL_0047_FC62MN8AAXX:1:1:1724:932 length=152
NTGTGATAGGCTTTGTCCATTCTGGAAACTCAATATTACTTGCGAGTCCTCAAAGGTAATTTTTGCTATTGCCAATATTCCTCAGAG
GAAAAAAGATACAATACTATGTTTTATCTAAATTAGCATTAGAAAAAAAATCTTTCATTAGGTGT
+SRR350953.7 MENDEL_0047_FC62MN8AAXX:1:1:1724:932 length=152
#.,')2/@@@@@@@@@@<:<<:778789979888889:::::99999<<::<:::::<<<<<@@@@@::::::IHIGIGGGGGGDGGDG
GDDDIHIHIIIII8GGGGGIIHHIIIGIIGIBIGIIIIEIHGGFIHHIIIIIIIGIIFIG
```

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

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